Modifications of superoxide dismutase (SOD1) in human erythrocytes: a possible role in amyotrophic lateral sclerosis (ALS)

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Running head: SOD1 modification pattern in erythrocytes

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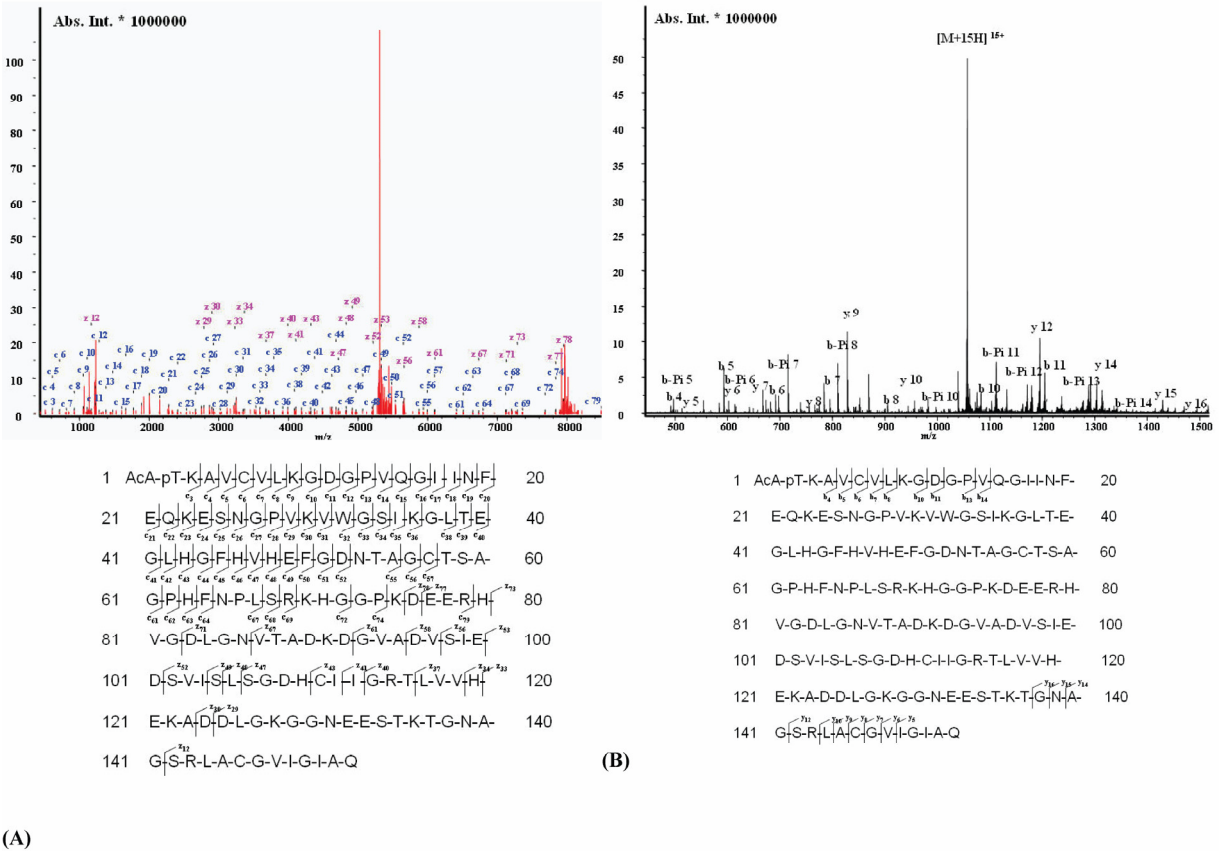
Supplemental Figure 1:

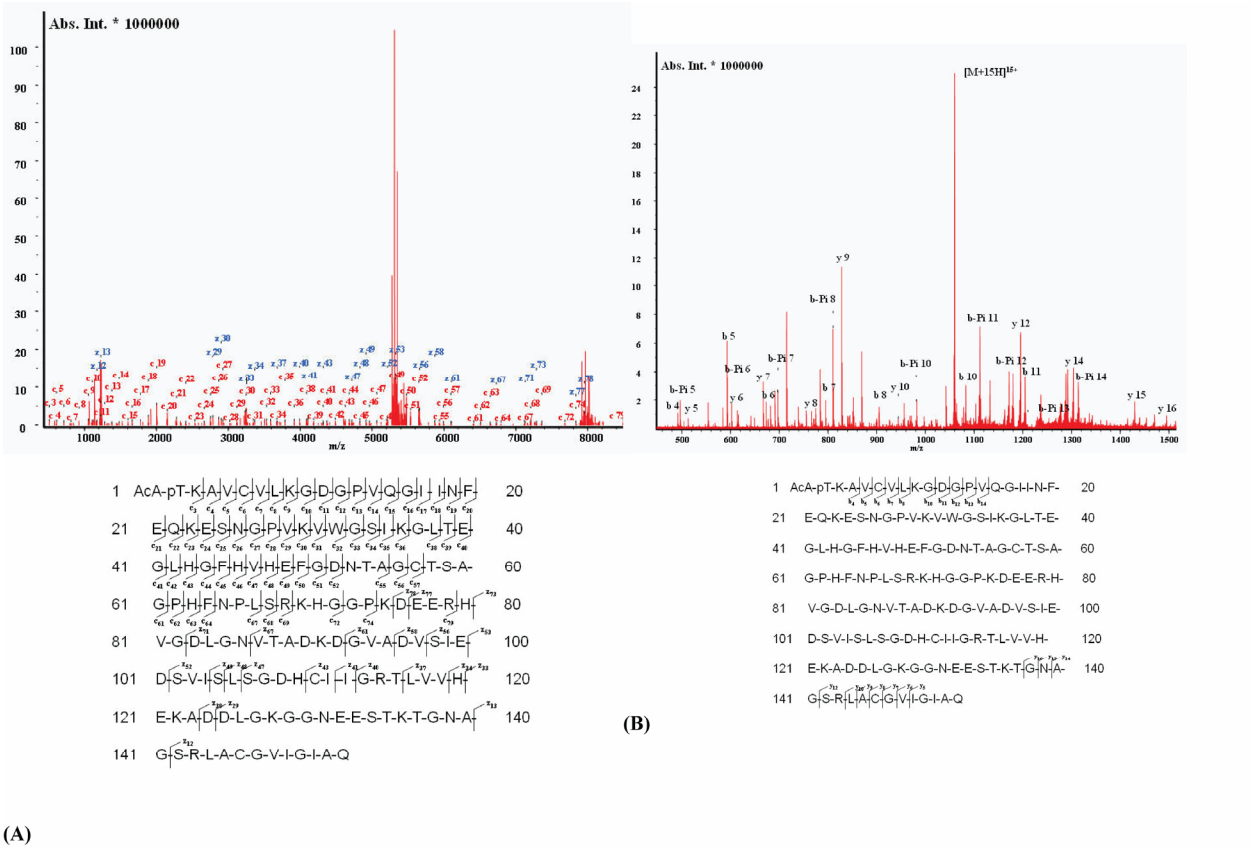


(A) (B)

Supplemental Figure 1 – MS/MS identification of singly-phosphorylated SOD1.

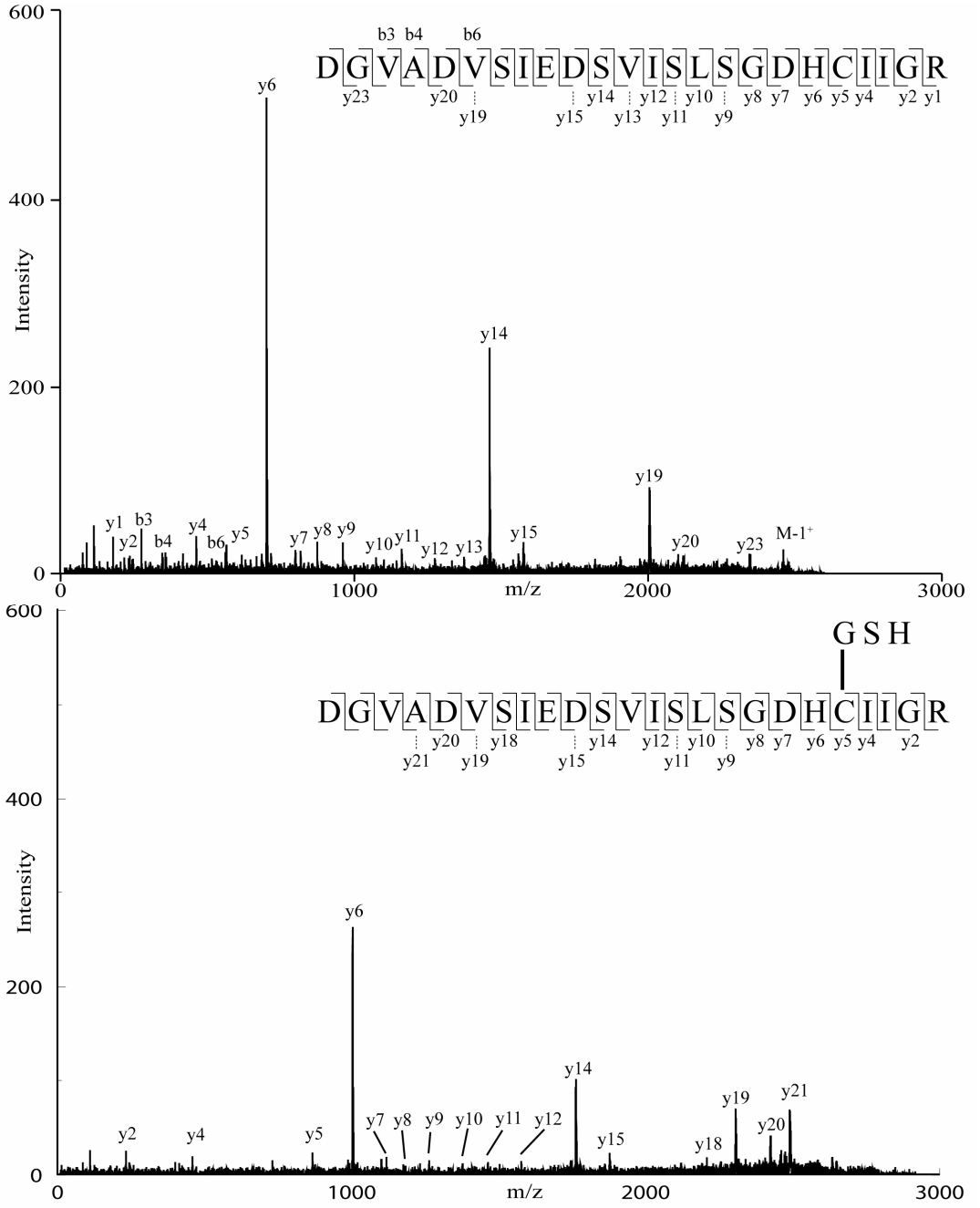
MS/MS analysis showing ions resulting from the fragmentation of SOD1 containing phosphorylated Thr-2. Ion signals corresponding to singly phosphorylated human SOD1 (precursor: m/z 1063.8 Da, 15+ charge state), are isolated for top-down experiments by μESI-FTICR-MS with (A) electron-capture dissociation (ECD) and (B) collision-induced dissociation (CID) respectively. Inspection of the fragment ions from the CID MS/MS spectrum of singly-phosphorylated human SOD1 (Figure 2 B) reveals the neutral losses of 80 or 98 Da in b_5, b_6, b_7, b_8, b_{10} and b_{11}. The ECD MS/MS spectrum of singly-phosphorylated SOD1 indicates 100% amino acid sequence coverage. 50 scans.

Supplemental Figure 2:



Supplemental Figure 2 – MS/MS identification of hydrated singly-phosphorylated SOD1.
MS/MS analysis showing ions resulting from the fragmentation of hydrated SOD1 containing phosphorylated Thr-2. Ion signals corresponding to hydrated singly-phosphorylated human SOD1 (precursor: m/z 1139.6 Da, 14+ charge state), are isolated for top-down experiments by µESI-FTICR-MS with (A) electron-capture dissociation (ECD) and (B) collision-induced dissociation (CID) respectively. Inspection of the fragment ions from the CID MS/MS spectrum of hydrated singly-phosphorylated human SOD1 (Figure 2 B) reveals the neutral loss of 80 or 98 Da in b_5, b_6, b_7, b_8, b_{10} and b_{11}. The ECD MS/MS spectrum of hydrated singly-phosphorylated SOD1 indicates 100% amino acid sequence coverage. 50 scans.

Supplemental Figure 3:

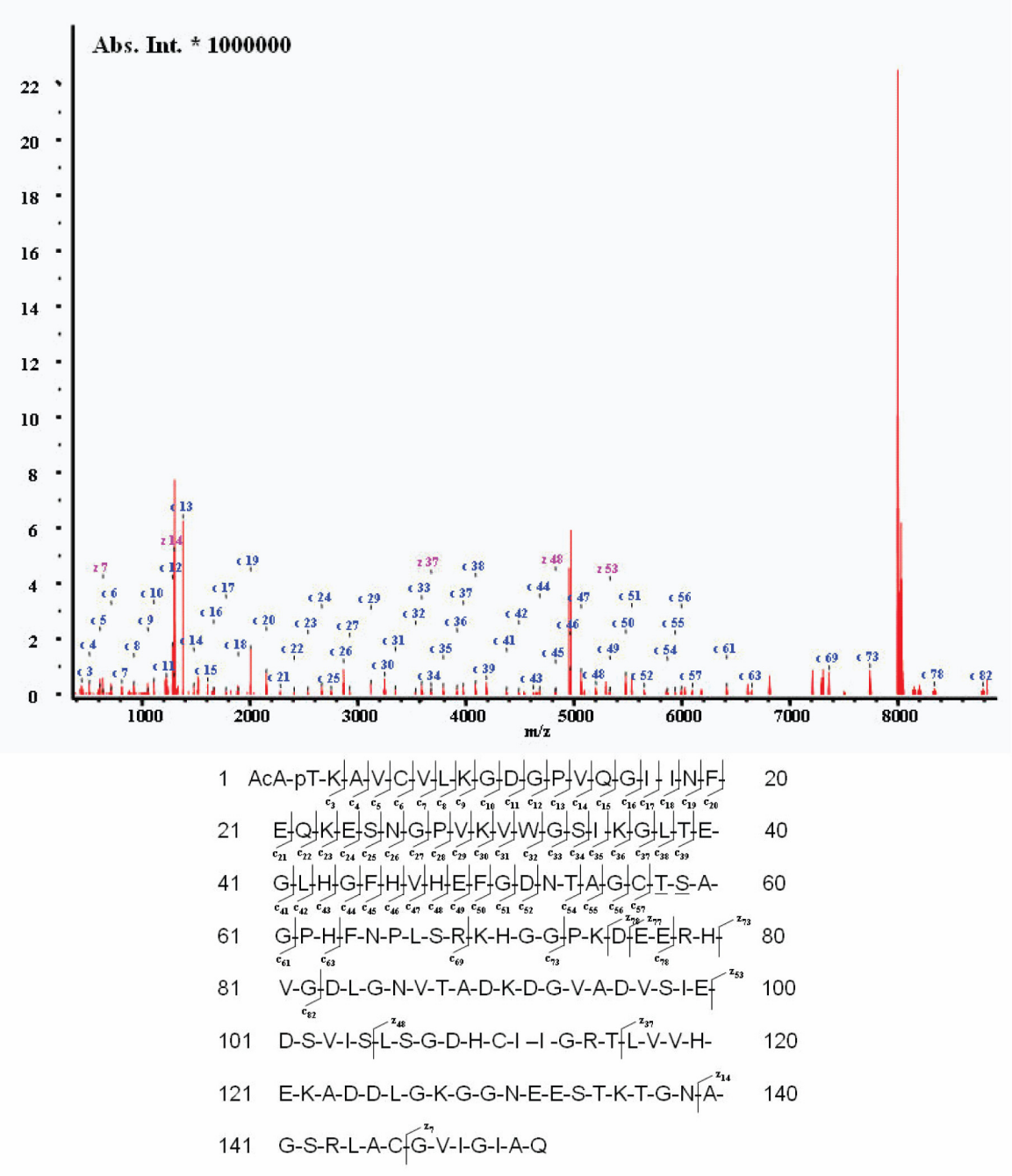


Supplemental Figure 3. MS/MS spectrum showing the fragmentation products of the D92-R115 parent peptide ± glutathione.

Upper panel: Observed y- and b-ions are identified in the spectrum and the sequence diagram. The parent peptide, DGVADVSIEDSVISLSGDHCIIGR, has a mass of 2457.24 Da.

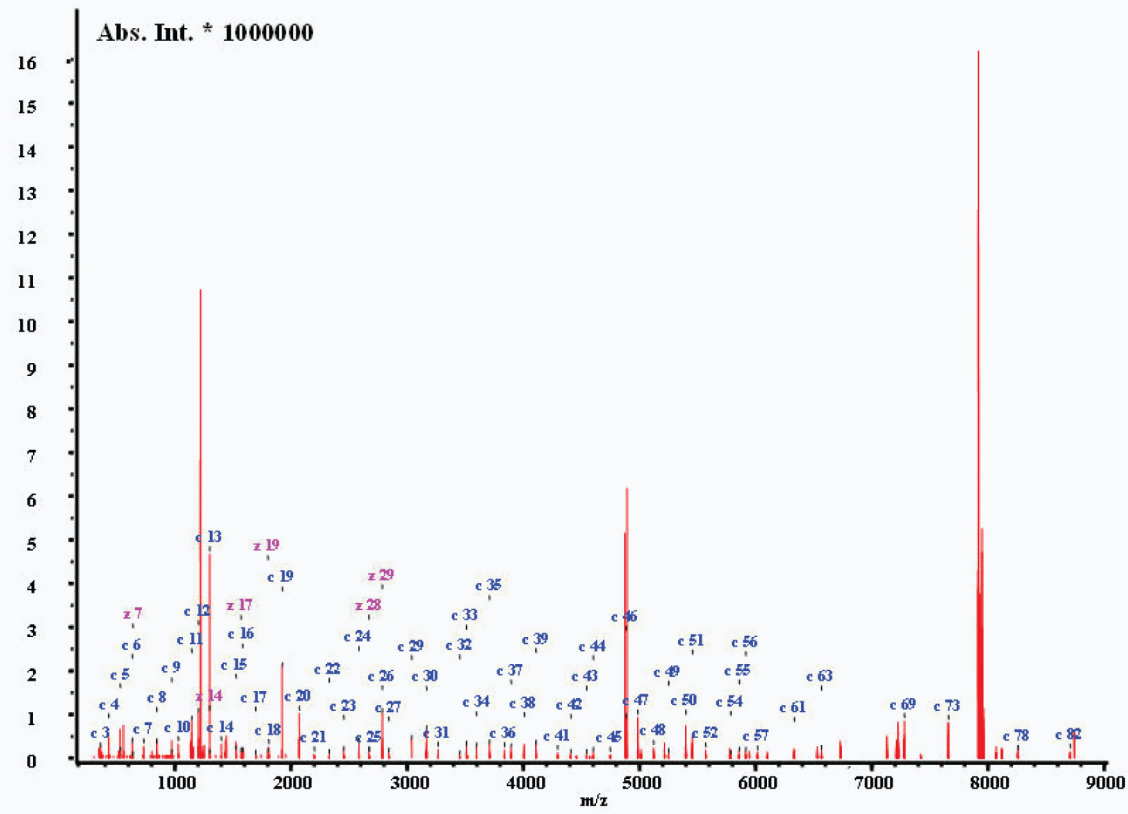
Lower panel: Observed y- and b-ions are identified in the spectrum and the sequence diagram. The parent peptide DGVADVSIEDSVISLSGDHCIIGR, (glutathionylated at Cys-111), has a mass of 2762.29 Da.

Supplemental Figure 4:



Supplemental Figure 4 – MS/MS identification of hydrated doubly-phosphorylated SOD1.
MS/MS analysis showing ions resulting from the fragmentation of hydrated SOD1 containing phosphorylated Thr-2 and Thr-58/Ser-59. Ion signals corresponding to hydrated singly-phosphorylated human SOD1 (Precursor: m/z 1145.6 Da, 14+ charge state.), are isolated for top-down experiments by µESI-FTICR-MS with electron-capture dissociation (ECD). The ECD MS/MS spectrum of hydrated singly-phosphorylated SOD1 indicates 100% amino acid sequence coverage. 50 scans.

Supplemental Figure 5:



1 AcA-T-K-A-V-C-V-L-K-G-D-G-P-V-Q-G-I - I-N-F- 20

21 E-Q-K-E-S-N-G-P-V-K-V-W-G-S-I -K-G-L-T-E- 40

41 G-L-H-G-F-H-V-H-E-F-G-D-N-T-A-G-C-T-S-A- 60

61 G-P-H-F-N-P-L-S-R-K-H-G-G-P-K-D-E-E-R-H- 80

81 V-G-D-L-G-N-V-T-A-D-K-D-G-V-A-D-V-S-I-E- 100

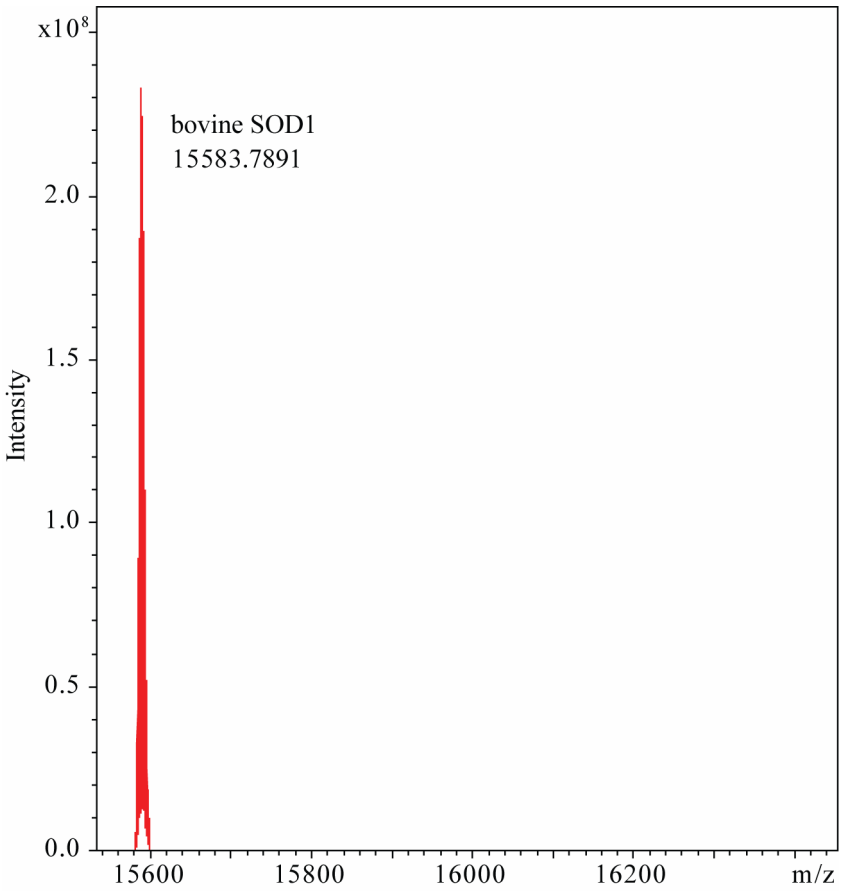
101 D-S-V-I-S-L-S-G-D-H-C-I –I -G-R-T-L-V-V-H- 120

121 E-K-A-D-D-L-G-K-G-G-N-E-E-S-T-K-T-G-N-A- 140

141 G-S-R-L-A-C-G-V-I-G-I-A-Q

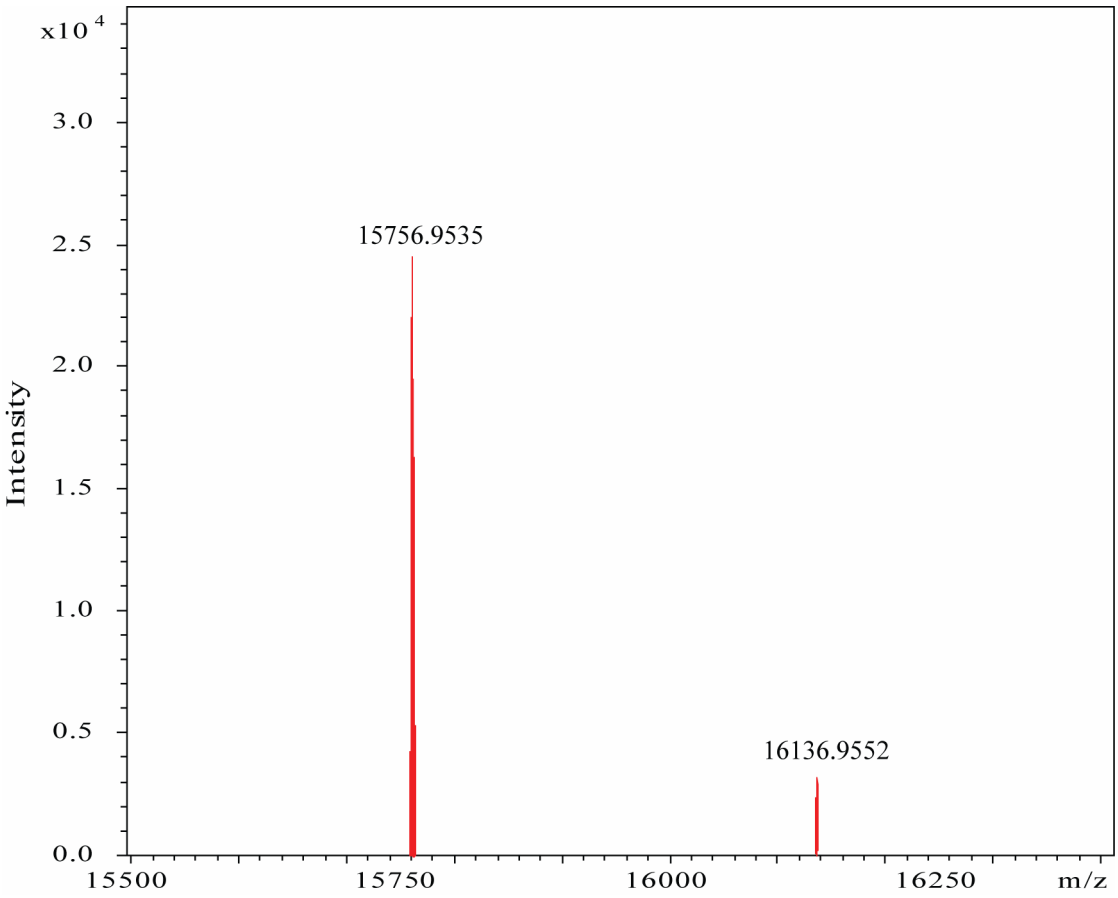
Supplemental Figure 5 – MS/MS identification of glutathionylated SOD1.
MS/MS analysis showing ions resulting from the fragmentation of Glutathionylated SOD1. Ion signals corresponding to hydrated singly-phosphorylated human SOD1 (Precursor: m/z 1794.1 Da, 9+ charge state.), are isolated for top-down experiments by μESI-FTICR-MS with electron-capture dissociation (ECD). The ECD MS/MS spectrum of hydrated singly-phosphorylated SOD1 indicates 73% amino acid sequence coverage. 50 scans.

Supplemental Figure 6:



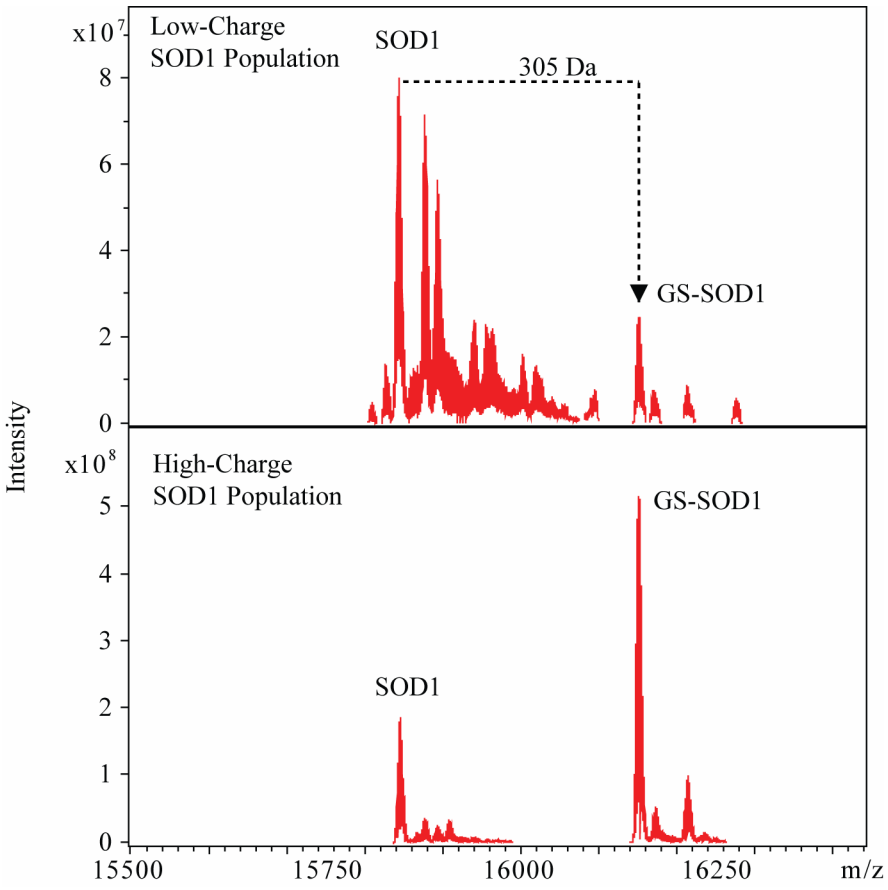
Supplemental Figure 6. Mass spectrum of bovine erythrocyte SOD1. Deconvoluted μESI-FTICR mass spectrum of bovine erythrocyte SOD1. We observe a single peak at a monoisotopic mass of 15583.7891 Da (calculated mass is 15583.7930 Da).

Supplemental Figure 7:



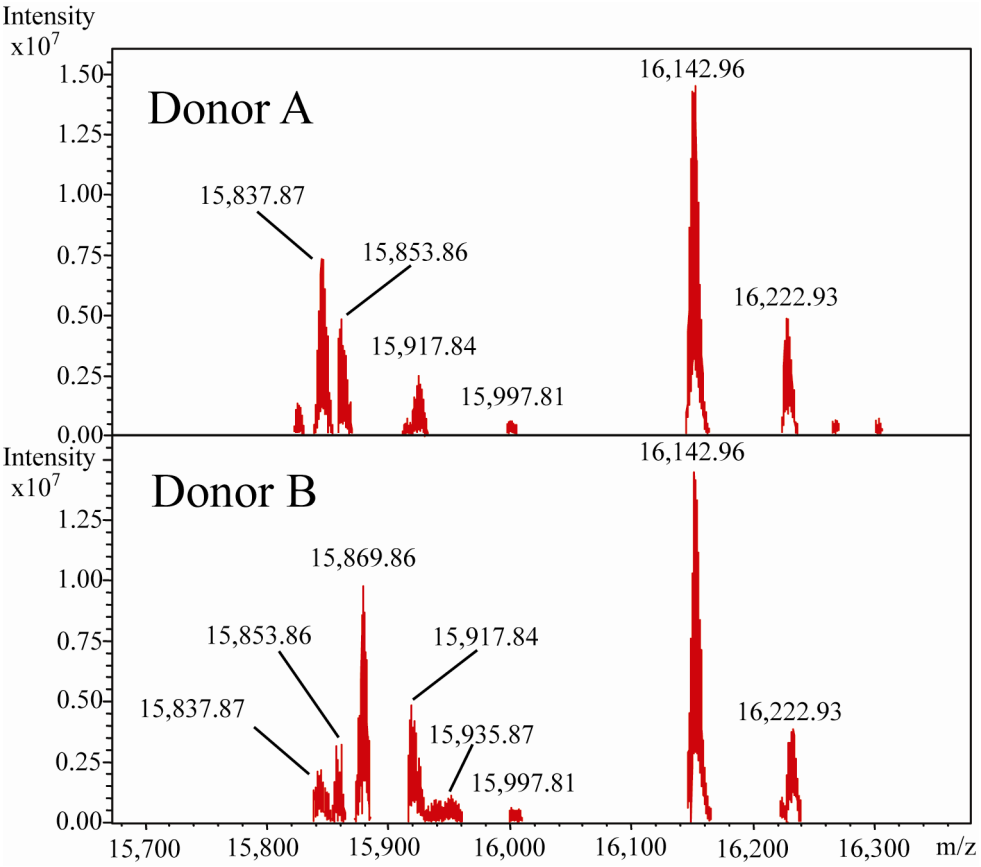
Supplemental Figure 7. Mass spectrum of endogenous SOD1 from *S. cerevisiae*. Deconvoluted μESI-FTICR mass spectrum of endogenous SOD1 isolated from *S. cerevisiae*. We observe monoisotopic mass peaks of 15756.9535 Da (calculated mass is 15756.8339 Da).

Supplemental Figure 8:



Supplemental Figure 8. Mass spectra of high/low charge SOD1 populations in yeast-isolated SOD1. Deconvoluted μESI-FTICR mass spectra of human SOD1 isolated from *S. cerevisiae* separated into high- and low-charge populations using anion exchange chromatography. We observe a shift due to glutathionylation in the highly-charge SOD1 population relative to the low-charge SOD1 population as in Fig. 1E. We do not observe phosphorylation.

Supplemental Figure 9:



Supplemental Figure 9. Mass spectra of SOD1 isolated from freshly-drawn human erythrocytes. Deconvoluted FTICR-MS spectra of SOD1 isolated from human erythrocytes processed immediately following removal from donors.