*Supplementary*

# Origin of Elevated S-Glutathionylated GAPDH in Chronic Neurodegenerative Diseases

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**Keywords:** glutathione; oxidative stress; redox signaling; glyceraldehyde−3−phosphate dehydrogenase; hydrogen peroxide; neurodegenerative disease; molecular dynamic simulation

| [A] NAD+ Interaction Energies [Native Subunit] | | | | |
|---|---|---|---|---|
| NAD+ | *h*-GAPDH (1U8F) | Type | Bond Length Å | E kcal/mol |
| O2β 13 | Oδ2 ASP 35 | H-donor | 2.59 | -3.4 |
| O2β 17 | Oδ1 ASP 35 | H-donor | 3.16 | -0.5 |
| O2β 17 | Oδ2 ASP 35 | H-donor | 2.79 | -3.8 |
| C1β 19 | Oδ2 ASP 35 | H-donor | 3.24 | -0.6 |
| N6α 27 | $H_2O$ | H-donor | 3.04 | -0.6 |
| N6α 27 | $H_2O$ | H-donor | 2.92 | -2.1 |
| O2δ 52 | $H_2O$ | H-donor | 2.64 | -2 |
| N7N 62 | $H_2O$ | H-donor | 2.99 | -2.6 |
| O1α 2 | $H_2O$ | H-acceptor | 2.61 | -2.8 |
| O1α 2 | $H_2O$ | H-acceptor | 2.72 | -3.2 |
| O1α 2 | $H_2O$ | H-acceptor | 2.74 | -3.2 |
| O2α 3 | N ARG 13 | H-acceptor | 3.51 | -1 |
| O2α 3 | $H_2O$ | H-acceptor | 2.61 | -3 |
| O2α 3 | $H_2O$ | H-acceptor | 2.68 | -3.1 |
| O3β 13 | $H_2O$ | H-acceptor | 2.76 | -1.6 |
| O2β 17 | $H_2O$ | H-acceptor | 2.67 | -1.4 |
| N7α 24 | $H_2O$ | H-acceptor | 2.81 | -2.7 |
| N1A 30 | $H_2O$ | H-acceptor | 2.77 | -1.8 |
| O1N 37 | $H_2O$ | H-acceptor | 2.74 | -3.1 |
| O1N 37 | $H_2O$ | H-acceptor | 2.63 | -3 |
| O1N 37 | $H_2O$ | H-acceptor | 2.85 | -2.8 |
| O2N 38 | N ILE 14 | H-acceptor | 2.80 | -4.8 |
| O7N 61 | N CYS 152 | H-acceptor | 2.82 | -5.3 |
| O7N 61 | Sγ CYS 152 | H-acceptor | 3.05 | -0.5 |
| 6-ring Cα | ASP 35 | π-H | 4.90 | -0.6 |
| 6-ring Cα | ALA 123 | π-H | 4.75 | -1 |
| Summed Binding Energies (kcal/mol) | | | | -60.5 |

| [B] NAD+ Interaction Energies [GSH →C152(SOH)] | | | | |
|---|---|---|---|---|
| NAD+ | *h*-GAPDH (1U8F) | Type | Bond Length Å | E kcal/mol |
| O3β 13 | Oδ2 ASP 35 | H-donor | | -1.9 |
| O2β 17 | Oδ2 ASP 35 | H-donor | 2.82 | -4.1 |
| O2β 17 | Oδ2 ASP 35 | H-donor | 3.25 | -0.5 |
| C1β 19 | O THR 99 | H-donor | 3.28 | -0.7 |
| O3D 48 | $H_2O$ | H-donor | 2.73 | -1.9 |
| N7N 62 | O ASN 316 | H-donor | 3.17 | -1.9 |
| N7N 62 | O GLU 317 | H-donor | 3.31 | -2.7 |
| O1α 2 | $H_2O$ | H-acceptor | 2.67 | -3.1 |
| O1α 2 | $H_2O$ | H-acceptor | 2.61 | -2.7 |
| O1α 2 | $H_2O$ | H-acceptor | 2.68 | -3 |
| O2α 3 | $H_2O$ | H-acceptor | 2.57 | -2.5 |
| O2α 3 | $H_2O$ | H-acceptor | 2.62 | -2.9 |
| O2α 3 | $H_2O$ | H-acceptor | 2.58 | -2.8 |
| O3β 13 | O GLY 12 | H-acceptor | 3.07 | -1.3 |
| O3β 13 | $H_2O$ | H-acceptor | 2.77 | -1.5 |
| O2β 17 | $H_2O$ | H-acceptor | 2.87 | -1.2 |
| N7A 24 | $H_2O$ | H-acceptor | 2.78 | -2.8 |
| N1A 30 | $H_2O$ | H-acceptor | 2.80 | -0.9 |
| N3A 33 | Cα GLY 10 | H-acceptor | 3.27 | -0.5 |
| O1N 37 | N GLY 100 | H-acceptor | 3.37 | -1 |
| O1N 37 | Nζ LYS 194 | H-acceptor | 2.74 | -15.6 |
| O1N 37 | $H_2O$ | H-acceptor | 2.61 | -2.5 |
| O2N 38 | O THR 182 | H-acceptor | 2.59 | -2.9 |
| O2N 38 | $H_2O$ | H-acceptor | 3.39 | -0.8 |
| O2N 38 | $H_2O$ | H-acceptor | 2.51 | -1.4 |
| O7N 61 | N ALA 183 | H-acceptor | 3.40 | -0.8 |
| O7N 61 | $H_2O$ | H-acceptor | 2.67 | -1.8 |
| O1N 37 | O LYS 194 | Ionic | 2.74 | -6.5 |
| O2N 38 | Nζ LYS 194 | Ionic | 4.00 | -0.5 |
| 6-ring | Cα ASP 35 | π-H | 4.19 | -0.5 |
| Summed Binding Energies (kcal/mol) | | | | -73.20 |

| [C] NAD+ Interaction Energies [C152(SSG)] | | | | |
|---|---|---|---|---|
| NAD+ | *h*-GAPDH (1U8F) | Type | Bond Length Å | E kcal/mol |
| O3β 13 | Oδ2 ASP 35 | H-donor | 2.61 | -3.1 |
| O2β 17 | Oδ1 ASP 35 | H-donor | 2.97 | -1.0 |
| O2β 17 | Oδ2 ASP 35 | H-donor | 2.77 | -4.0 |
| N6α 27 | O ARG 80 | H-donor | 3.25 | -1.4 |
| N6α 27 | $H_2O$ | H-donor | 3.03 | -1.6 |
| O3δ 48 | Oγ SER 122 | H-donor | 2.72 | -1.9 |
| C1δ 54 | O SER 122 | H-donor | 3.43 | -0.7 |
| C6N 69 | O SER 122 | H-donor | 3.63 | -0.7 |
| O1α 2 | $H_2O$ | H-acceptor | 2.62 | -2.8 |
| O2α 3 | N ARG 13 | H-acceptor | 2.86 | -5.8 |
| O3β 13 | $H_2O$ | H-acceptor | 3.00 | -1.0 |
| O2β 17 | $H_2O$ | H-acceptor | 2.73 | -1.6 |
| N7α 24 | $H_2O$ | H-acceptor | 2.77 | -2.8 |
| N1α 30 | $H_2O$ | H-acceptor | 2.89 | -1.6 |
| O2N 38 | N ILE 14 | H-acceptor | 2.84 | -5.7 |
| O1α 2 | $H_2O$ | H-acceptor | 2.81 | -1.1 |
| O1α 2 | $H_2O$ | H-acceptor | 2.96 | -0.8 |
| O7N 61 | Nε2 HIS 179 | H-acceptor | 3.06 | -3.3 |
| O7N 61 | Nδ2 ASN 316 | H-acceptor | 2.91 | -2.1 |
| Summed Binding Energies (kcal/mol) | | | | -43.0 |

| [D] NAD+ Interaction Energies [C152SH→ (GSSG)] | | | | |
|---|---|---|---|---|
| NAD+ | *h*-GAPDH (1U8F) | Type | Bond Length Å | E (kcal/mol) |
| O3β 13 | Oδ1 ASP 35 | H-donor | 2.81 | -1.8 |
| O3β 13 | Oδ1 ASP 35 | H-donor | 2.75 | -3.6 |
| O2β 17 | Oδ1 ASP 35 | H-donor | 2.73 | -2.9 |
| C1β 19 | O THR 99 | H-donor | 3.3 | -0.7 |
| N6α 27 | O ARG 80 | H-donor | 2.89 | -1.4 |
| O3δ 48 | O SER 122 | H-donor | 2.59 | -0.6 |
| C5N 67 | Sγ CYS 152 | H-donor | 3.89 | -2 |
| O1α 2 | Oγ1 THR 184 | H-acceptor | 2.72 | -3.2 |
| O1α2 | $H_2O$ | H-acceptor | 2.74 | -3.1 |
| O1α 2 | $H_2O$ | H-acceptor | 3.03 | -1.7 |
| O2α 3 | N ARG 13 | H-acceptor | 2.77 | -5.2 |
| O2α 3 | $H_2O$ | H-acceptor | 2.56 | -2.3 |
| O5β 4 | $H_2O$ | H-acceptor | 2.81 | -1.4 |
| O3β 13 | $H_2O$ | H-acceptor | 2.71 | -1.5 |
| O2β 17 | $H_2O$ | H-acceptor | 2.72 | -1.5 |
| N7α 24 | $H_2O$ | H-acceptor | 2.84 | -1.8 |
| O3 35 | $H_2O$ | H-acceptor | 2.84 | -1.2 |
| O2N 38 | $H_2O$ | H-acceptor | 2.61 | -3 |
| O3δ 48 | Oγ1 THR 103 | H-acceptor | 3.13 | -0.8 |
| O7N 61 | N ALA 183 | H-acceptor | 2.97 | -1.6 |
| O7N 61 | $H_2O$ | H-acceptor | 2.89 | -2.1 |
| 6-ring | Cα ASP 35 | pi-H | 4.83 | -0.7 |
| Summed Binding Energies (kcal/mol) | | | | -44.1 |

**Supplementary Table S1. Panel [A].** Interaction energies between $NAD^+$ and the subunit matrix in the native enzyme. **Panel [B]** Interaction energies between $NAD^+$ and the subunit matrix after MDS docking of G(SH) within the active site region of the *h*–GAPDH subunit with its catalytic cysteine C152(SH) modified to C152(SOH). **Panel [C]** Interaction energies between $NAD^+$ and the subunit matrix of the S–glutathionylated subunit. **Panel [D]** Interaction energies between $NAD^+$ and the subunit matrix after a second molecule of glutathione displaces S–glutathione from the subunit by thiol–disulfide exchange with G(SS)G tightly bound within the active site region.

| [A] Top Panel: Interaction Docking of 1st GSH<br>Lower Panel: S-Glutathionylated GAPDH | | | | |
|---|---|---|---|---|
| Ligand Interaction Energies [C152(SOH) ←G(SH)] | | | | |
| G(SH) | *h*-GAPDH (1U8F) | Type | Bond (Å) | E kcal/mol |
| N2 17 | Oδ2 ASP 198 | H-donor | 2.91 | -3.7 |
| N3 28 | Oδ2 ASP 198 | H-donor | 3.09 | -2.8 |
| N3 28 | Oδ1 ASP 198 | H-donor | 2.89 | -5.1 |
| O12 8 | Oγ1 THR 153 | H-acceptor | 2.82 | -2.8 |
| O13 8 | N GLY 212 | H-acceptor | 2.85 | -5 |
| O31 34 | Nζ LYS 194 | H-acceptor | 2.81 | -13.2 |
| O32 34 | $H_2O$ | H-acceptor | 2.67 | -1.7 |
| O32 35 | NH1 ARG 197 | H-acceptor | 2.88 | -9.9 |
| O32 35 | NH2 ARG 197 | H-acceptor | 2.75 | -9.7 |
| O32 35 | $H_2O$ | H-acceptor | 2.73 | -2.6 |
| O 36 | Oγ1 THR 153 | H-acceptor | 3.06 | -0.9 |
| O31 34 | Nζ LYS 194 | Ionic | 2.81 | -5.9 |
| O32 35 | NH1 ARG 197 | Ionic | 2.88 | -5.4 |
| O32 35 | NH2 ARG 197 | Ionic | 2.75 | -6.4 |
| N1 1 | 5-ring HIS 179 | cation-π | 3.45 | -2.1 |
| Summed Binding Energies (kcal/mol) | | | | -77.2 |

| Ligand Interaction Energies [C152(SS)G)] | | | | |
|---|---|---|---|---|
| R(SS)G | *h*-GAPDH (1U8F) | Type | Bond (Å) | E kcal/mol |
| N1 1 | O ALA 213 | H-donor | 2.81 | -2.2 |
| Sγ2 26 | Oγ SER 151 | H-donor | 3.99 | -0.6 |
| O12 8 | Cα THR 154 | H-acceptor | 3.37 | -1.1 |
| O12 8 | N LEU 157 | H-acceptor | 2.76 | -5 |
| O2 22 | Oγ1 THR 211 | H-acceptor | 2.68 | -2.4 |
| O31 33 | Nε ARG 234 | H-acceptor | 3.00 | -2.5 |
| O31 33 | NH2 ARG 234 | H-acceptor | 2.70 | -5.2 |
| O31 33 | $H_2O$ | H-acceptor | 2.73 | -3.6 |
| O31 33 | $H_2O$ | H-acceptor | 2.59 | -2.2 |
| O32 34 | $H_2O$ | H-acceptor | 2.57 | -1.1 |
| O32 34 | $H_2O$ | H-acceptor | 2.57 | -1.7 |
| O 35 | Cα THR 154 | H-acceptor | 3.21 | -0.6 |
| O 35 | Oγ1 THR 154 | H-acceptor | 2.65 | -2.9 |
| Sγ2 26 | Oγ SER 151 | H-acceptor | 3.99 | -0.6 |
| Sγ2 26 | Oγ SER 151 | H-donor | 3.99 | -0.6 |
| O31 33 | Nε ARG 234 | Ionic | 3.00 | -4.5 |
| O31 33 | NH1 ARG 234 | Ionic | 2.70 | -6.8 |
| O31 34 | NH2 ARG 234 | Ionic | 3.91 | -0.7 |
| Summed Binding Energies (kcal/mol) | | | | -44.3 |

| [B] Top Panel: Docking of 2nd GSH<br>Lower Panel: S-Glutathionylated GAPDH | | | | |
|---|---|---|---|---|
| Ligand Interaction Energies [C152(SSG) ←G(SH)] | | | | |
| G(SH) | *h*-GAPDH (1U8F) | Type | Bond (Å) | E kcal/mol |
| N1 1 | Sγ CYS 152 | H-donor | 3.25 | -7.2 |
| N1 1 | $H_2O$ | H-donor | 2.79 | -7.5 |
| N3 27 | $H_2O$ | H-donor | 3.16 | -1.1 |
| O12 8 | Oγ SER 151 | H-acceptor | 2.62 | -2.1 |
| O12 8 | N G LY 212 | H-acceptor | 2.71 | -6.5 |
| Oε1 16 | $H_2O$ | H-acceptor | 2.72 | -2 |
| Sγ2 26 | Cβ SER 151 | H-acceptor | 4.02 | -0.5 |
| Sγ2 26 | N CYS 152 | H-acceptor | 3.07 | -2.6 |
| O31 33 | $H_2O$ | H-acceptor | 2.63 | -1.5 |
| O31 33 | $H_2O$ | H-acceptor | 2.59 | -3.2 |
| O32 34 | Nε2 GLN 185 | H-acceptor | 2.83 | -3.5 |
| O32 34 | $H_2O$ | H-acceptor | 2.64 | -2.1 |
| O32 34 | $H_2O$ | H-acceptor | 2.63 | -2.7 |
| O 35 | N THR 153 | H-acceptor | 2.71 | -7.4 |
| O 35 | Oγ1 THR 153 | H-acceptor | 2.62 | -0.9 |
| O32 34 | NH1 ARG 234 | Ionic | 3.03 | -4.3 |
| Cα2 19 | 5-ring HIS 179 | π-H | 4.77 | -0.5 |
| Summed Binding Energies (kcal/mol) | | | | -55.6 |

| Ligand Interaction Energies [C152(SSG) ←G(SH)] | | | | |
|---|---|---|---|---|
| R(SS)G<br>G(SH) | *h*-GAPDH (1U8F) | Type | Bond (Å) | E kcal/mol |
| N2 17 | Oδ2 ASP 198 | H-donor | 3 | -4.8 |
| O12 8 | $H_2O$ | H-acceptor | 2.57 | -1.2 |
| O12 8 | $H_2O$ | H-acceptor | 2.57 | -1.7 |
| O2 22 | $H_2O$ | H-acceptor | 2.68 | -0.8 |
| O2 22 | $H_2O$ | H-acceptor | 2.71 | -2.1 |
| Sγ2 26 | $H_2O$ | H-acceptor | 2.98 | -5.3 |
| O31 33 | Nε1 TRP 196 | H-acceptor | 2.88 | -3.7 |
| O31 33 | NH1 ARG 197 | H-acceptor | 2.79 | -8.9 |
| O31 33 | Nζ LYS 215 | H-acceptor | 2.94 | -6.8 |
| O31 33 | $H_2O$ | H-acceptor | 2.62 | -1.9 |
| O32 34 | NH2 ARG 194 | H-acceptor | 2.72 | -5.2 |
| O32 34 | Nζ LYS 195 | H-acceptor | 3 | -0.5 |
| O32 34 | $H_2O$ | H-acceptor | 2.68 | -3.7 |
| O 35 | Cα LYS 197 | H-acceptor | 3.31 | -0.5 |
| O 35 | N LEU 197 | H-acceptor | 2.86 | -6.3 |
| O 35 | $H_2O$ | H-acceptor | 2.57 | -2.5 |
| O31 33 | NH1 ARG 197 | Ionic | 2.79 | -6.1 |
| O31 33 | NH2 ARG 197 | Ionic | 3.48 | -2 |
| O31 33 | Nζ LYS 215 | Ionic | 2.94 | -4.9 |
| O32 34 | NH1 ARG 197 | Ionic | 3.34 | -2.6 |
| O32 34 | NH2 ARG 197 | Ionic | 2.72 | 7.9 |
| O32 34 | Nζ LYS 215 | Ionic | 3 | -4.5 |
| Summed Binding Energies (kcal/mol) | | | | -68.1 |

| [C] Interaction Energies of Docked GSSG<br>within active site regionof GAPDH | | | | |
|---|---|---|---|---|
| Ligand Interaction Energies [C152(SH) → G(SS)G] | | | | |
| G(SS)G | *h*-GAPDH (1U8F) | Type | Bond (Å) | E kcal/mol |
| N1 1 | Sγ CYS 152 | H-donor | 2.97 | -4.7 |
| N1 1 | Oγ1 THR 153 | H-donor | 3.11 | -4.9 |
| N2 17 | Oδ1 ASP 198 | H-donor | 2.96 | -4.5 |
| Cβ2 23 | Sγ CYS 152 | H-donor | 3.51 | -1.3 |
| N3 27 | Oδ1 ASP 198 | H-donor | 2.74 | -6 |
| N3 62 | $H_2O$ | H-donor | 3.20 | -0.6 |
| O12 8 | $H_2O$ | H-acceptor | 2.60 | -1.4 |
| O12 8 | $H_2O$ | H-acceptor | 2.57 | -2.8 |
| O2 22 | Oγ1 THR 182 | H-acceptor | 2.61 | -1.4 |
| O32 34 | $H_2O$ | H-acceptor | 2.64 | -1.2 |
| O32 34 | $H_2O$ | H-acceptor | 2.72 | -3.7 |
| O32 34 | $H_2O$ | H-acceptor | 2.58 | -0.5 |
| O32 34 | $H_2O$ | H-acceptor | 2.66 | -3.3 |
| O 35 | Oγ SER 151 | H-acceptor | 2.58 | -2.8 |
| O12 43 | $H_2O$ | H-acceptor | 2.56 | -1.4 |
| O12 43 | $H_2O$ | H-acceptor | 2.55 | -2.1 |
| O 51 | $H_2O$ | H-acceptor | 2.70 | -1.3 |
| O2 57 | NH1 ARG 197 | H-acceptor | 3.04 | -0.6 |
| O2 57 | NH2 ARG 197 | H-acceptor | 3.04 | -1.6 |
| Sγ2 61 | N SER 151 | H-acceptor | 4.23 | -1.8 |
| O31 68 | Cα SER 125 | H-acceptor | 3.64 | -0.5 |
| O31 68 | Nζ LYS 215 | H-acceptor | 2.81 | -2.3 |
| O31 68 | $H_2O$ | H-acceptor | 2.60 | -1.1 |
| O31 68 | $H_2O$ | H-acceptor | 2.58 | -2.6 |
| O32 69 | NH1 ARG 197 | H-acceptor | 2.76 | -2.6 |
| O32 69 | $H_2O$ | H-acceptor | 2.63 | -3.1 |
| O 70 | N GLY 100 | H-acceptor | 2.83 | -4.1 |
| N1 1 | Sγ CYS 152 | Ionic | 2.97 | -4.7 |
| O31 68 | NH1 ARG 197 | Ionic | 3.37 | -2.4 |
| O31 68 | Nζ LYS 215 | Ionic | 2.81 | -5.8 |
| O32 69 | NH1 ARG 197 | Ionic | 2.76 | -6.3 |
| O32 69 | NH2 ARG 197 | Ionic | 3.49 | -1.9 |
| Summed Binding Energies (kcal/mol) | | | | -85.3 |

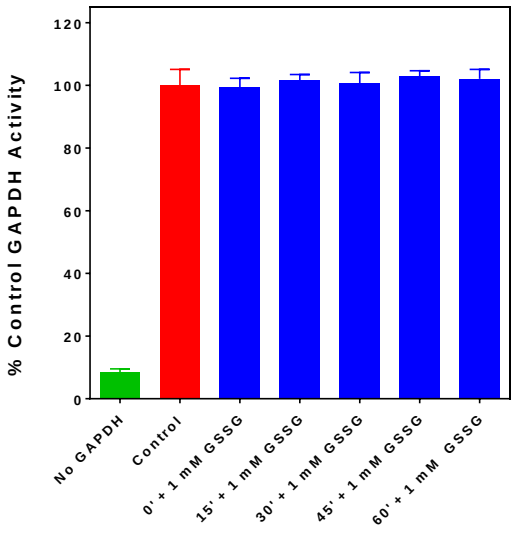
**Supplementary Table S2. [A]. Top Panel** Interaction energy calculations after MDS docking of G(SH) within the active site region of the *h*–GAPDH subunit with its catalytic cysteine C152(SH) modified to C152(SOH). The docked G(SH) ligand aligns poised for nucleophilic attack with its sulfur atom 4.4 Å from the C152(SOH) sulfur atom. **[A]. Bottom Panel** Following formation of the interaction energies between C152S–glutathione moiety and the enzyme weaken. **[B]. Top Panel** Interaction energies of a second molecule of glutathione aligned and docks in an antiparallel aspect to the first C152S–glutathione moiety with its sulfur center within range (4.44 Å) for thiol–disulfide exchange). **[B]. Bottom Panel** the presence of the second docked glutathione molecule weakens the interaction energies between the C152S–glutathione moiety and the enzyme. **[C]** After nucleophilic attack by the docked G(SH) molecule and the C152S–glutathione disulfide, the resulting molecular interactions of the G(SS)G ligand with residues within the active site pocket of the subunit indicate that the G(SS)SG remains tightly bound to the GAPDH inhibiting its dissociation from the enzyme active site. Enhanced residency time of G(SS)G within the active site pocket is likely to be enhanced by the proximity of the sulfur atom of G(SS)G proximal (3.56 Å) to the catalytic sulfur cysteine (see Manuscript Figure 3).

| [A] Interaction Energies of docked Cysteine within active site region of GAPDH | | | | |
|---|---|---|---|---|
| Ligand Interaction Energies [C152(SOH) ←C(SH)] | | | | |
| C(SH) | *h*-GAPDH (1U8F) | Type | Bond (Å) | E kcal/mol |
| N1 | Sγ CYS 156 | H-donor | 3.3 | -5.9 |
| N1 | O SER 292 | H-donor | 2.78 | -6.4 |
| Sγ 10 | O HIS 291 | H-donor | 3.25 | -1.4 |
| OXT 13 | Oγ1 THR 294 | H-acceptor | 2.63 | -3.4 |
| O 14 | Cα TYR 314 | H-acceptor | 3.36 | -0.9 |
| O 14 | N ASP 315 | H-acceptor | 3.03 | -3.4 |
| O 14 | $H_2O$ | H-acceptor | 2.78 | -1.2 |
| Summed Binding Energies (kcal/mol) | | | | -22.6 |

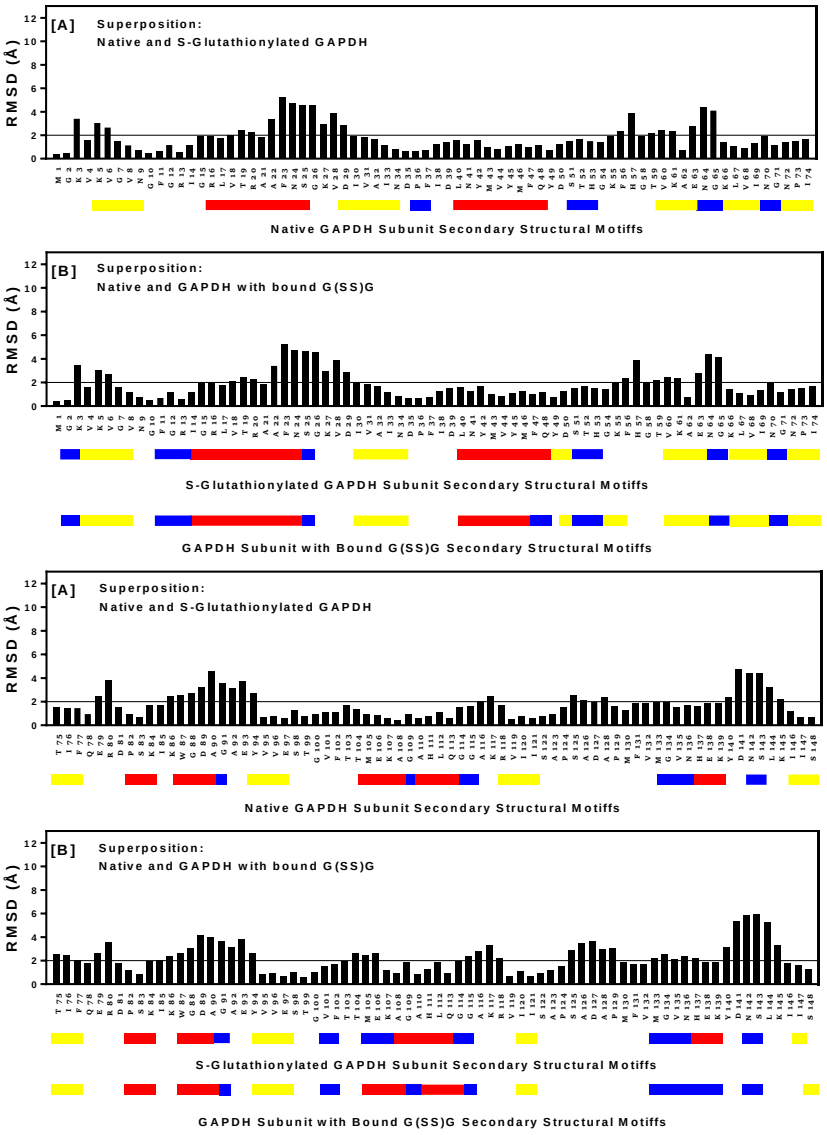
| [B] Interaction Energies of S-cysteinylated GAPDH | | | | |
|---|---|---|---|---|
| Ligand Interaction Energies [C152(SS)C | | | | |
| C(SH) | *h*-GAPDH (1U8F) | Type | Bond (Å) | E kcal/mol |
| N1 | O HIS 291 | H-donor | 2.73 | -7 |
| N1 | $H_2O$ | H-donor | 2.91 | -6.7 |
| Sγ 10 | Sγ CYS 156 | H-donor | 4.3 | -0.6 |
| OXT 12 | N ASP 315 | H-acceptor | 2.87 | -6.0 |
| O 13 | N His 291 | H-acceptor | 3.05 | -3.5 |
| Summed Binding Energies (kcal/mol) | | | | -23.8 |

| [C] Interaction Energies of Docked CSSC within active site region of GAPDH | | | | |
|---|---|---|---|---|
| Ligand Interaction Energies [C152(SH) → C(SS)C] | | | | |
| C(SS)C | *h*-GAPDH (1U8F) | Type | Bond (Å) | E kcal/mol |
| N1 | O MET 133 | H-donor | 2.69 | -11.6 |
| N 1 | O ASN 155 | H-donor | 2.75 | -7.4 |
| N 1 | Oδ1 ASN 155 | H-donor | 2.76 | -8.8 |
| Sγ 10 | Sγ CYS 156 | H-donor | 3.51 | -0.8 |
| N 14 | SγCYS 156 | H-donor | 3.3 | -1.4 |
| N 14 | Oδ2 ASP 315 | H-donor | 2.71 | -18.1 |
| N 14 | $H_2O$ | H-donor | 2.9 | -5.3 |
| Sγ 10 | Cα CYS 156 | H-acceptor | 3.68 | -0.6 |
| OXT 12 | $H_2O$ | H-acceptor | 2.62 | -3.4 |
| O 13 | NH1 ARG 323 | H-acceptor | 2.72 | -7.1 |
| O 13 | $H_2O$ | H-acceptor | 2.77 | -2.3 |
| O 13 | $H_2O$ | H-acceptor | 2.62 | -2.4 |
| Sγ 23 | Cα TYR 320 | H-acceptor | 3.66 | -0.6 |
| OXT 25 | N ASP 289 | H-acceptor | 2.83 | -3 |
| OXT 25 | Cα GLY 319 | H-acceptor | 3.39 | -0.9 |
| OXT 25 | $H_2O$ | H-acceptor | 2.56 | -2 |
| O 26 | N ASP 289 | H-acceptor | 2.95 | -4 |
| O 26 | $H_2O$ | H-acceptor | 2.64 | -3.1 |
| O 13 | NH1 ARG 323 | Ionic | 2.72 | -6.7 |
| O 13 | NH2 ARG 323 | Ionic | 3.86 | -0.8 |
| N 14 | Oδ2 ASP 315 | Ionic | 2.71 | -6.8 |
| Summed Binding Energies (kcal/mol) | | | | -97.1 |

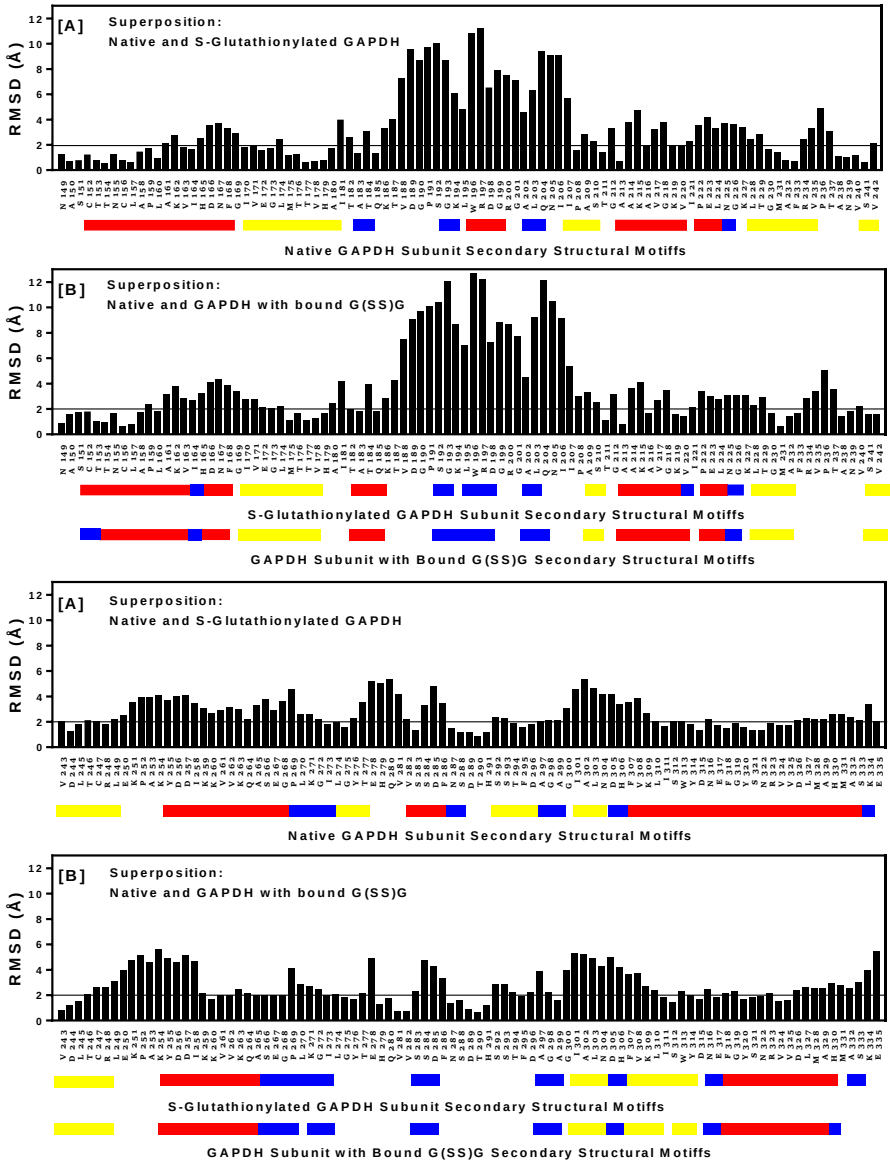
**Supplementary Table S3. Panel [A].** Interaction energy calculations after MDS docking of cysteine within the active site region of the *h*–GAPDH subunit with its catalytic cysteine C152(SH) modified to C152(SOH). The docked Cysteine ligand aligns poised for nucleophilic attack with its sulfur atom 3.69 Å from the C152(SOH) sulfur atom. **Panel [B].** Interaction energies between S–cysteinylated GAPDH and the subunit polypeptide residues **Panel [C].** After docking a second cysteine molecule within the vicinity of the S–cysteinylated C152 residue, and thiol–disulfide exchange, the resulting strong molecular interactions of the oxidized cystine dipeptide ligand, C(SS)C with residues within the active site pocket subunit indicate that the C(SS)C would remain tightly bound to the GAPDH inhibiting its dissociation from the enzyme active site.




**Supplementary Figure S1**. Incubation with G(SS)G does not influence GAPDH activity. *r*–GAPDH was incubated with 1 mM G(SS)G for 60 min and then assayed for enzyme activity with 1 mM G(SS)G in the assay buffer (blue bars). The % enzyme activity was not influenced by the presence of GAPDH in the assay buffer compared to activity measured in its absence (red bars).




**Supplementary Figure S2**. *Analysis of residue alignment comparison parameters on the perturbations induced by glutathione binding after MDS* within *the NAD⁺ binding domain (M1-S148) of an h-GAPDH subunit.* The protein sequence alignment and structure superposition tool in MOE is used to align the native *h*-GAPDH subunit structure with the output from MDS, and the energy minimizes subunit structures for C152S-glutathionylated subunit (Panels **[A]**) and the native *h*-GAPDH subunit structure with the output from MDS and the energy minimizes subunit structure for G(SS)G subunit docked within the active site (Panels **[B]**). The root mean square distance (RMSD) for each alignment column (i.e., residue pair) used during the superposition is represented by a black vertical bar above the pairwise aligned sequences. Closely matching RMSD values are highlighted by a horizontal line cutoff below 2.0 Å, while a larger RMSD excursion (5–13 Å) indicates poor atomic coordinate superposition. The linearized secondary structural features for the three structure superpositions are shown below Panels **[A]** (native) and Panels **[B]** (C152S-glutathionylated subunits and subunits with G(SS)G docked within the active site) are color coded as follows: Red $\alpha$-helix; yellow $\beta$-sheet; and blue 2-5 residue turns; while no color is random coil is shown below Panel **[B]** of the RMSD values.




**Supplementary Figure S3**. *Analysis of residue alignment comparison parameters on the perturbations induced by glutathione binding after MDS* within *the catalytic domain (N149-E335) of an h-GAPDH subunit.* The protein sequence alignment and structure superposition tool in MOE is used to align the native *h*-GAPDH subunit structure with the output from MDS, and the energy minimizes subunit structures for C152S-glutathionylated subunit (Panels **[A]**) and the native *h*-GAPDH subunit structure with the output from MDS and the energy minimizes subunit structure for G(SS)G subunit docked within the active site (Panels **[B]**). The root mean square distance (RMSD) for each alignment column (i.e., residue pair) used during the superposition is represented by a black vertical bar above the pairwise aligned sequences. Closely matching RMSD values are highlighted by a horizontal line cutoff below 2.0 Å, while a larger RMSD excursion (5–13 Å) indicates poor atomic coordinate superposition. The linearized secondary structural features for the three structure superpositions are shown below Panels **[A]** (native enzyme subunits) and Panels **[B]** (C152S-glutathionylated subunits and subunits with G(SS)G docked within the active site) are color coded as follows: Red $\alpha$-helix; yellow $\beta$-sheet; and blue 2-5 residue turns; while no color is random coil is shown below Panel **[B]** of the RMSD values. The region of greatest perturbation and includes all residues in the S-loop region of the subunit (residues 180–203, see text for details).