

Supplemental Data

Post-Translational S-nitrosylation is an Endogenous Factor Fine-Tuning Human S100A1 Protein

Properties

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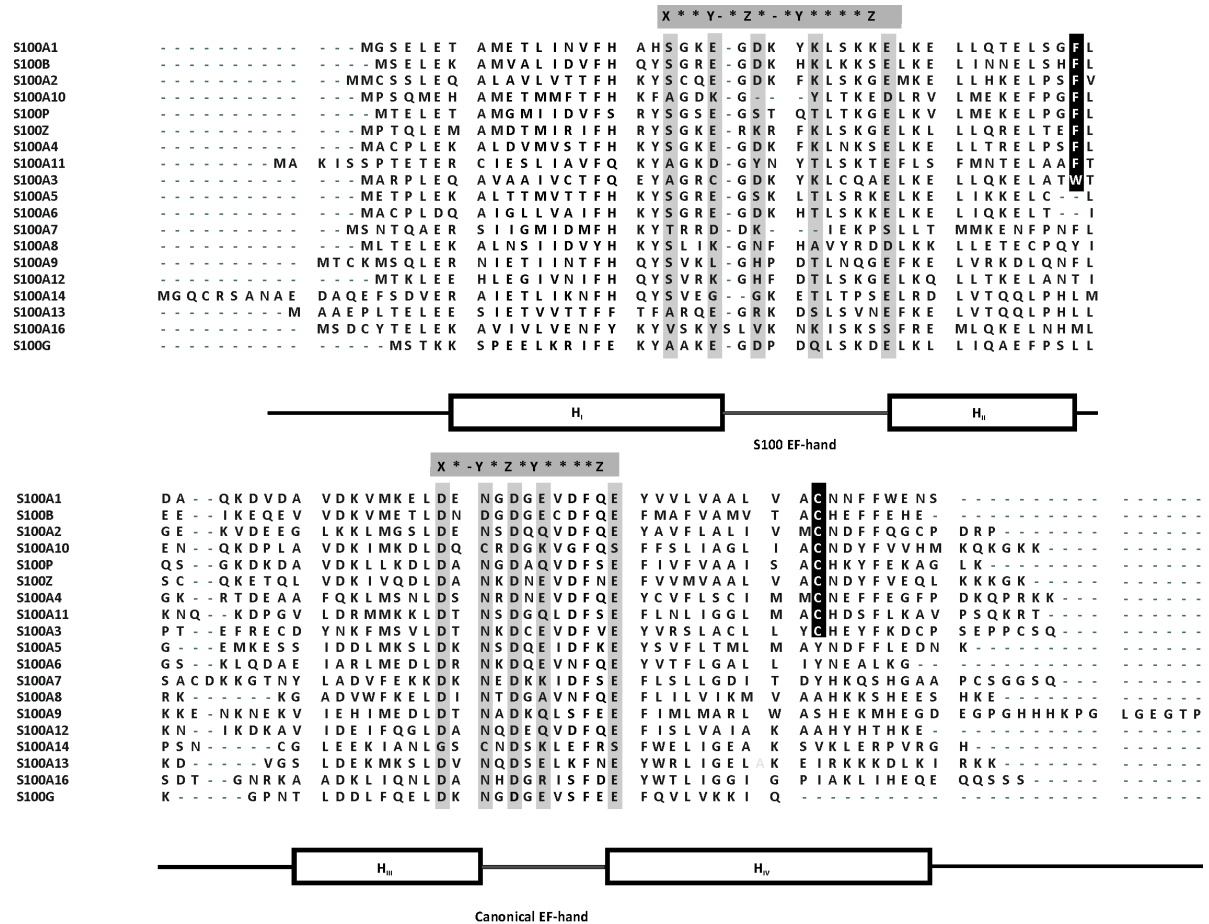


Figure S1. Alignment of human S100 proteins. Comparison of human S100A1 with other S100 family members to present the subgroup of proteins with conserved C-terminal cysteine residues and an aromatic residue in the linker region (shaded dark grey). Residues responsible for Ca²⁺ coordination in the EF-hand loops are marked light grey.

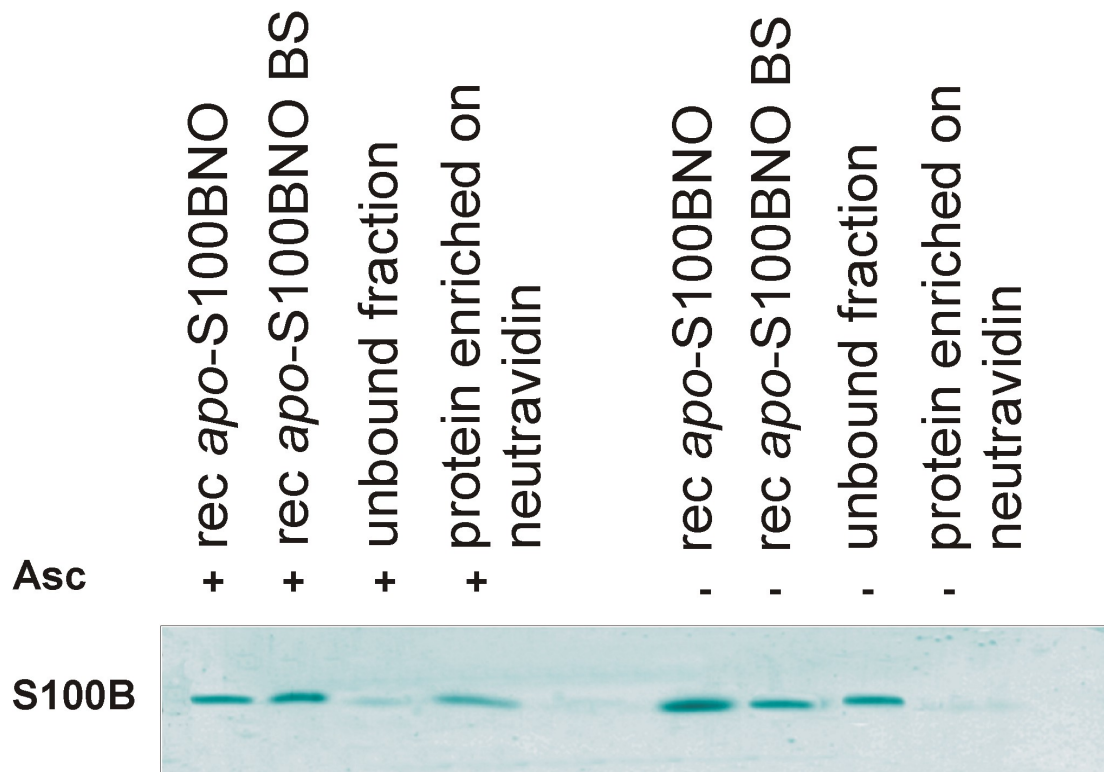


Figure S2. Biotin Switch method control experiment: Western blot detection of S-nitrosylation of recombinant chemically S-nitrosylated S100B protein. S-nitrosylated S100B protein is highly enriched on neutravidin (lane 4) using the Biotin Switch method. If the S-NO bond is not reduced using ascorbate solution the protein is found in the unbound fraction (lane 7) Lane 1: chemically S-nitrosylated, recombinant human S100B-NO protein solution before BSM (rec *apo*-S100B-NO); lane 2: rec *apo*-S100B solution after BS protocol before affinity chromatography (rec *apo*-S100B-NO BS); lane 3: rec *apo*-S100B after full BS unbound to neutravidin; lane 4: S100B-NO bound to neutravidin after BS; lane 5: rec *apo*-S100B -NO protein solution before BSM without ascorbate (Asc) reduction of S-NO bond; lane 6: rec *apo*-S100B-NO solution after BS protocol without Asc reduction; lane 7: rec *apo*-S100B-NO after BS without Asc fraction unbound to neutravidin; lane 8: S100B-NO bound to neutravidin (control BS without Asc); chemically S-nitrosylated, recombinant human S100B-NO protein eluted from neutravidin resin after full BS (rec *apo*-S100B-NO BS); lane 5: protein fraction unbound to neutravidin (full BS); lane 6: wash fraction (control BS without Asc); lane 7: wash fraction (full BS); lane 8: proteins enriched on neutravidin resins (control BS without Asc); lane 9: proteins enriched on neutravidin resins (full BS). BS, Biotin Switch method; Asc, ascorbate.

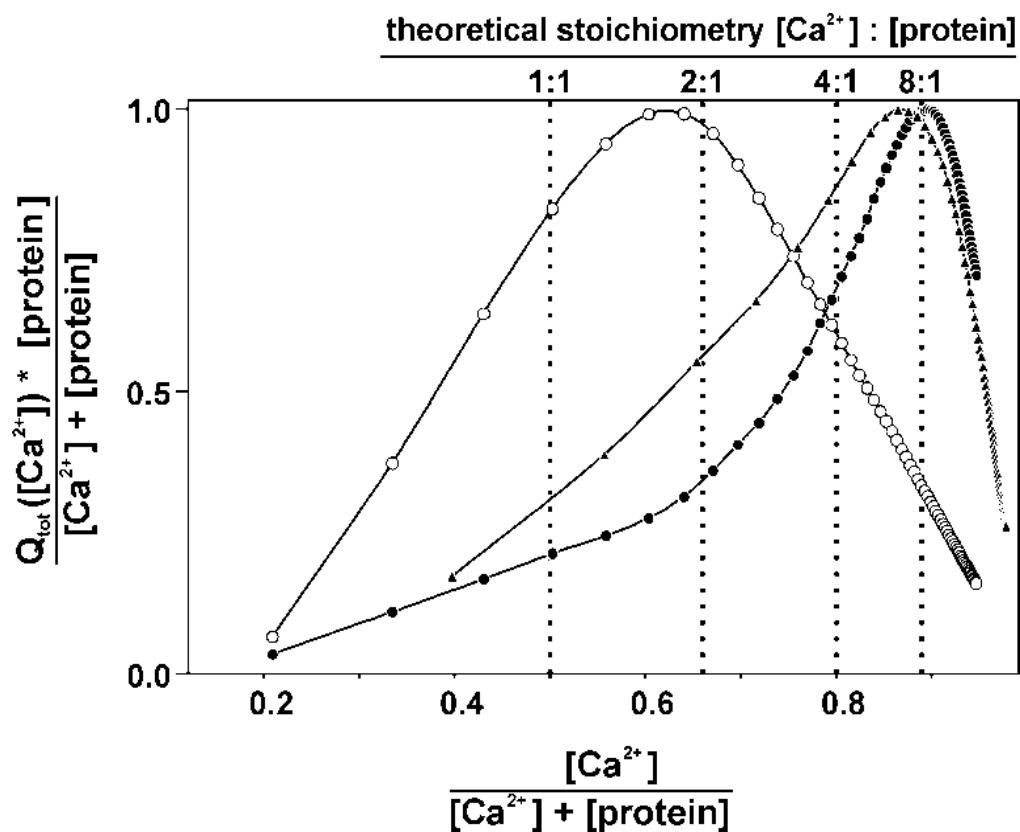


Figure S3. Stoichiometry of the S100A1 / Ca^{2+} complex. Job plots for the renormalized cumulative heat flow upon titration with Ca^{2+} ions at low-salt for 100 μM apo-S100A1 (empty circles) and at 150 mM ionic strength for 40 μM S100A1 (solid circles) and S100-NO (solid triangles). The locations of the individual maximum roughly correspond to the binding stoichiometries.

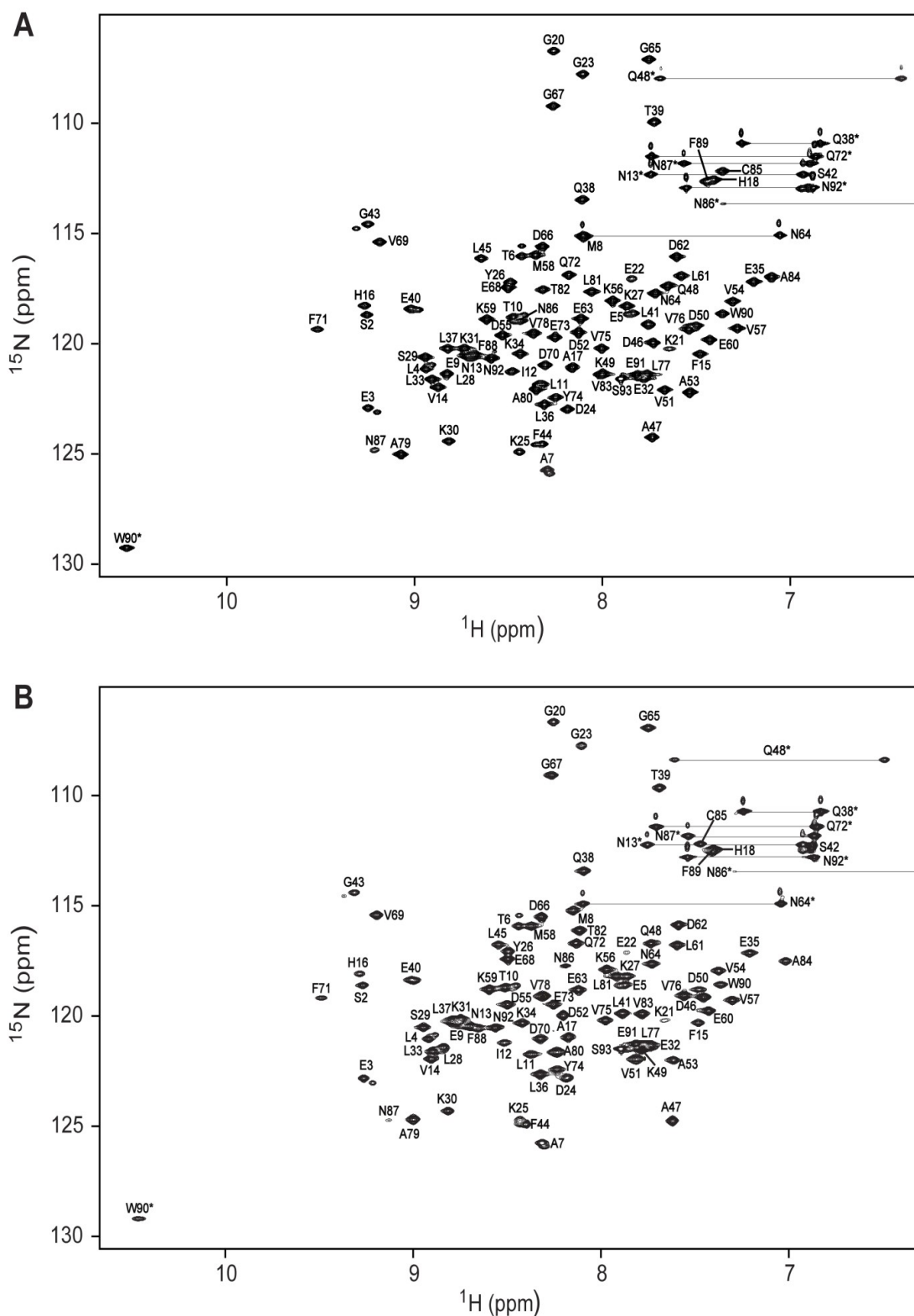


Figure S4. 2D ^1H - ^{15}N HSQC spectra for *apo*-S100A1 (A) and *apo*-S100A1-NO (B) recorded at 278 K on Varian VNMRS 800 NMR spectrometer. The backbone sequence-specific are shown by one-letter code and sequence number.

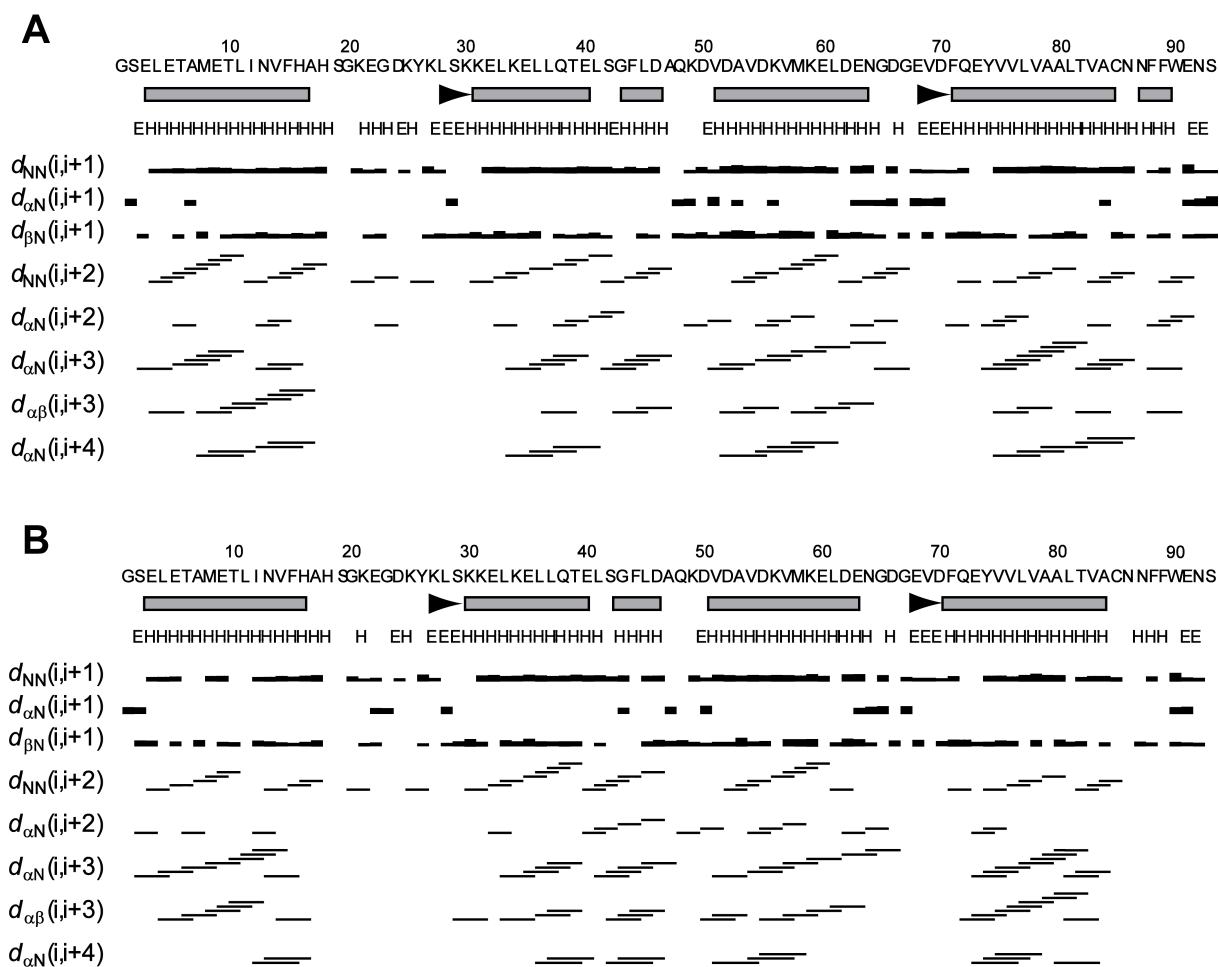


Figure S5. NOE patterns for *apo*-S100A1 (A) and *apo*-S100A1-NO (B) proteins. Grey rectangles indicate α -helical secondary structure elements, β -strands are marked with black arrowheads. The H (helix) and E (extended) shows the backbone conformation predicted as 'good' on base ^1H , ^{13}C , and ^{15}N chemical shifts with TALOS+ software.

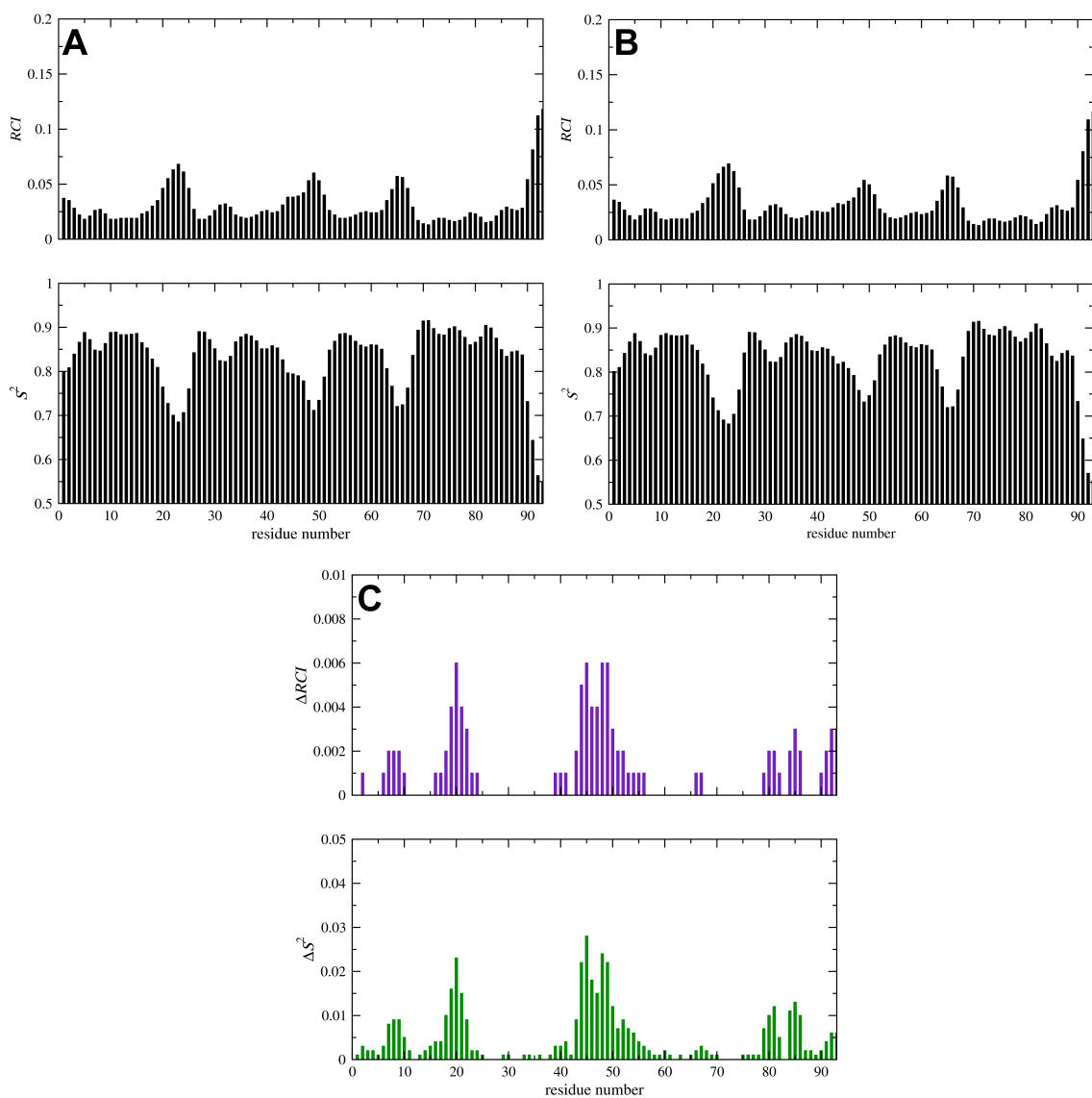


Figure S6. The Random Coil Index (RCI) analysis for reduce (A) and S-nitrosylated (B) forms of human apo-S100A1 protein. (C) presented the differences in RCI data between both variants. The data were obtained using www server: http://wishart.biology.ualberta.ca/rci/cgi-bin/rci.cgi_1_e.py

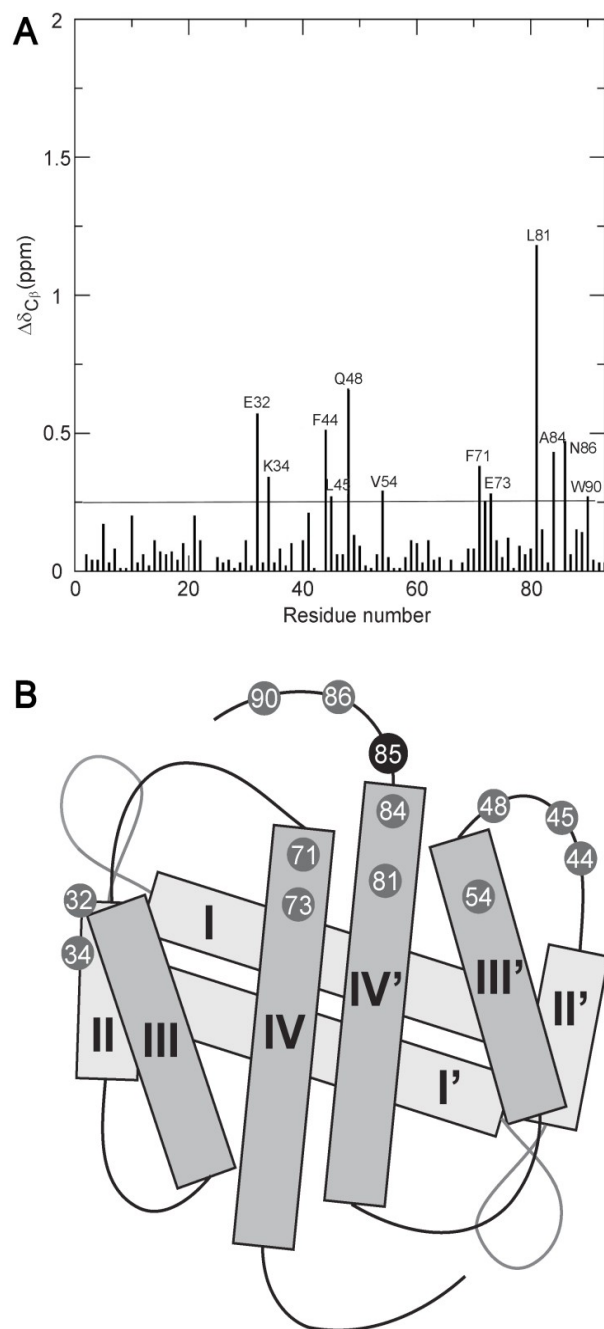


Figure S7. Chemical shift changes of $^{13}\text{C}_\beta$ atoms ($\Delta\delta_{\text{C}_\beta}$) as a consequence of Cys85 *S*-nitrosylation. **(A)** $\Delta\delta_{\text{C}_\beta}$ between *apo*-S100A1 and *apo*-S100A1-*NO* proteins were calculated as difference of chemical shifts of C_β atoms of individual residue. Only residues with $\Delta\delta_{\text{C}_\beta}$ values higher than 0.25 ppm are marked. **(B)** Schematic structure of *apo*-S100A1-*NO*. Cys85-*NO* is marked as black circle, residues with $\Delta\delta_{\text{C}_\beta} \geq 0.25$ ppm are marked with dark grey circles.

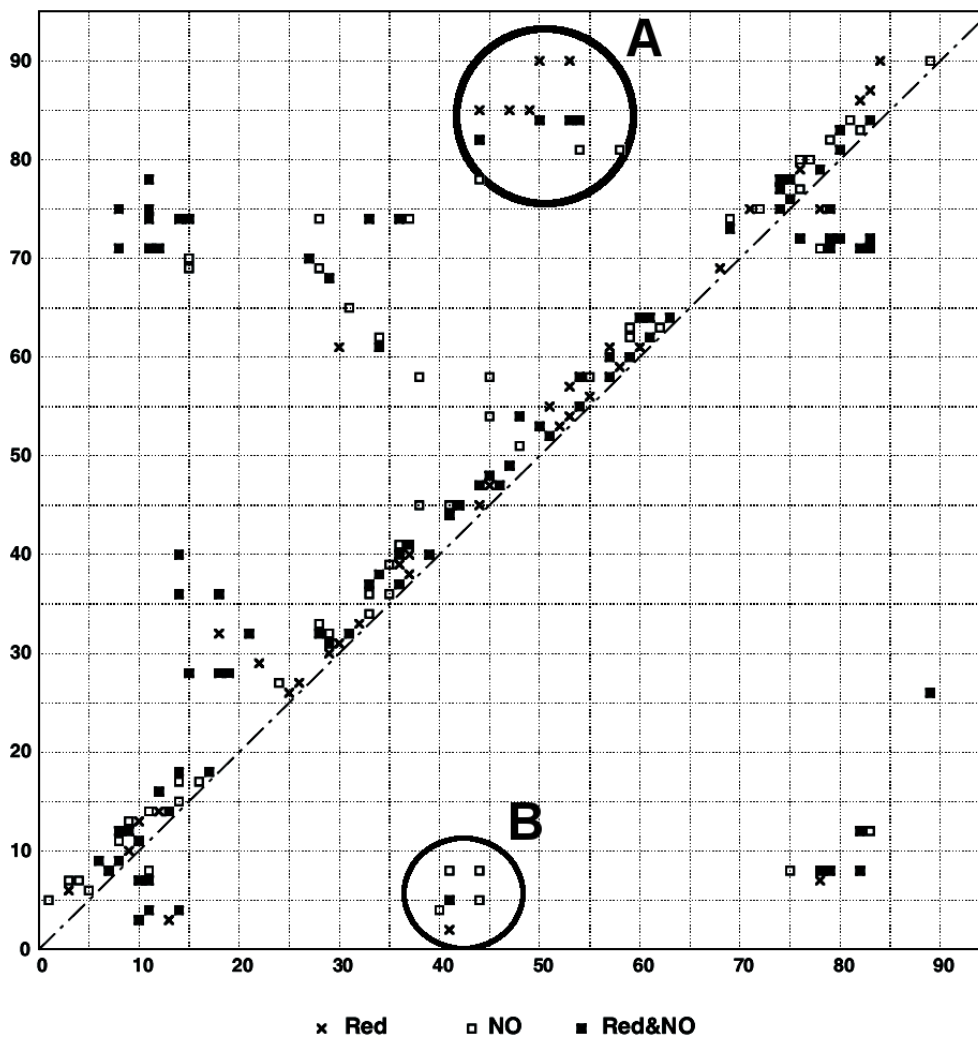


Figure S8. Map of NOE contacts observed between ^1H located in side chains observed in reduce, and S-nitrosylated forms of human *apo*-S100A1 protein. The regions exhibited notable differences in detected NOE contacts are noted as **A** and **B**.

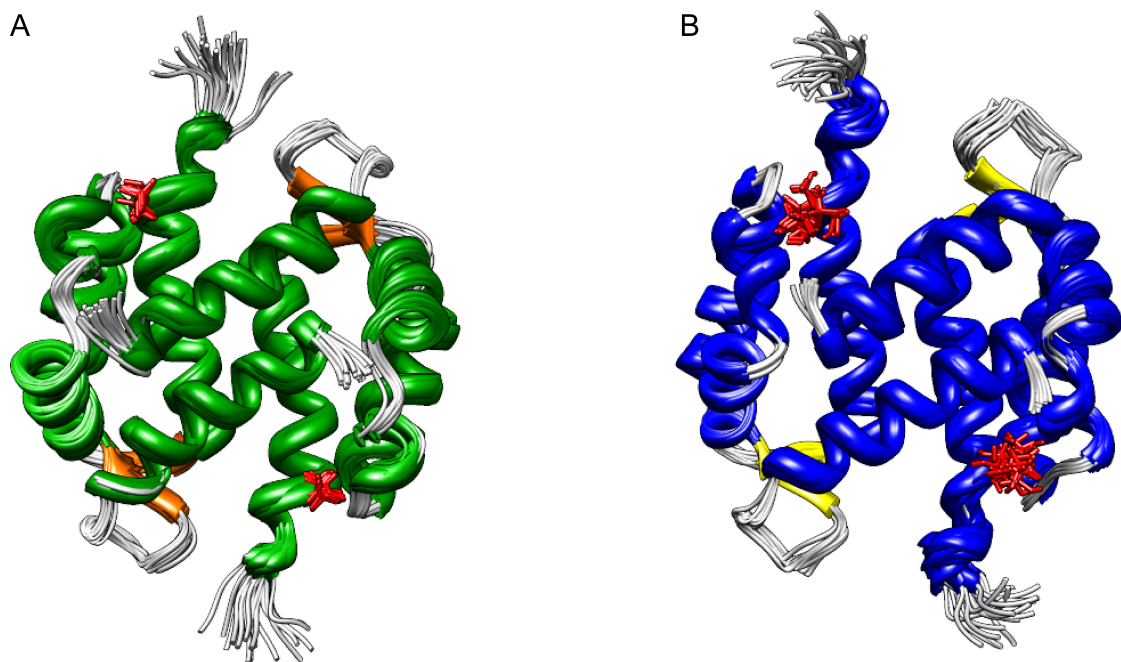


Figure S9. Ribbon presentation of 20 lowest energy structures of *apo*-S100A1 (A) and *apo*-S100A1-NO (B). The side-chain C-terminal Cys85 / Cys85-NO are marked with red.

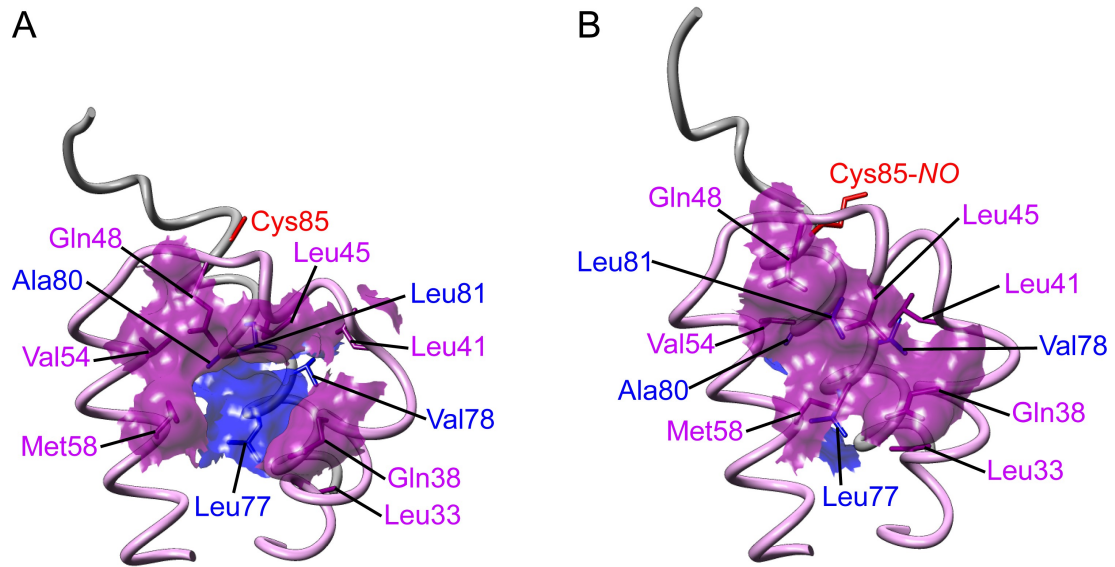


Figure S10. Surface presentation of helix II – helix III region for reduce (A) and S-nitrosylated (B) forms of human *apo*-S100A1 protein. The reorganization of side chains for residues Gln 38, Leu 45, and Met 58 are clearly visible.