Supplemental Table 1

Kinetic parameters of Arabidopsis and Human GSNORs								
	AtGSNOR					HsGSNOR		
	WT	C10A	C271A	$C_3 \rightarrow A_3$	C177A	WT	C195A	C268A
k_{cat} (sec^{-1})	123.8 ± 3.0	95.8 ± 4.4	75.9 ± 8.9	75.5 ± 8.2	N.D.	17.4 ± 0.5	19.9 ± 4.1	17.1 ± 2.7
K_m (µM)	68.7 ± 5.1	49.8 ± 8.1	84.7 ± 19.4	34.4 ± 7.3	N.D.	18.2 ± 0.6	15.6 ± 3.0	18.6 ± 2.3
k_{cat}/K_m ($M^{-1}sec^{-1}$)	1.80E+06	1.92E+06	9.00E+05	2.20E+06	N.D.	9.56E+05	1.28E+06	9.14E+05

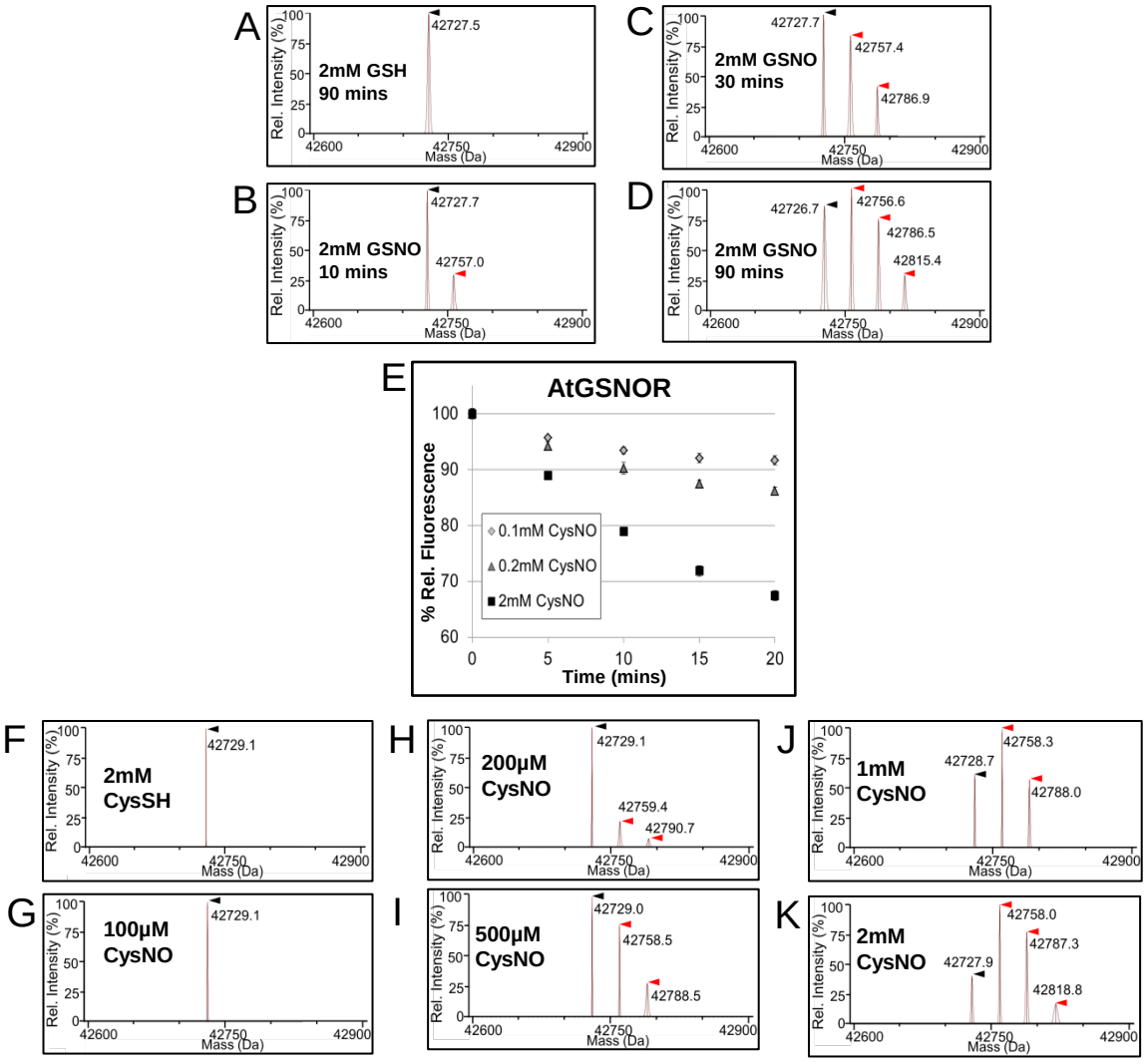
Supplemental Table S1: Kinetic parameters of Arabidopsis and Human GSNORs. Catalytic rate constants and Km values obtained from three or more assays (depicted in Supplemental Figure S3 A-B), plus or minus two times standard error about the mean. N.D.: Not determined. Kinetic parameters were obtained using Origin Pro 2015 software.

Supplemental Table 2

	Amino acid range	Topological position	Relevance	% D uptake CysSH	% D uptake CysNO
	AtGSNOR				
(i)	E367-C370	T23, coil	Contains Cys370.	25.3 ± 2.8	34.6 ± 1.4
	C373-L375	E18	5Å from Gly204, which contacts the NADH ribose moiety.	10.5 ± 2.3	2 ± 0.4
(ii)	S305-Q310	E15, H10	Dimer interface	21.8 ± 1.0	24.8 ± 2.0
(iii)	I294-A298	E14	Contacts NADH phosphates, 5-6Å from Cys271.	30 ± 2.1	42 ± 3.7
(iv)	A280-L282	H9	6-8Å from **(iii).**	17.5 ± 4.1	2.4 ± 1.4
	HsGSNOR				
(i)	G365-T371	T24, coil, E18	5Å from Gly201, which contacts the NADH ribose moiety.	22 ± 4.0	7 ± 1.1
(ii)	A301-Q306	E15, H10	Dimer interface	23 ± 2.5	29 ± 1.0
(v)	P57-V63	T4, coil	Comprises part of roof of substrate-binding cavity.	23 ± 1.6	18 ± 2.3

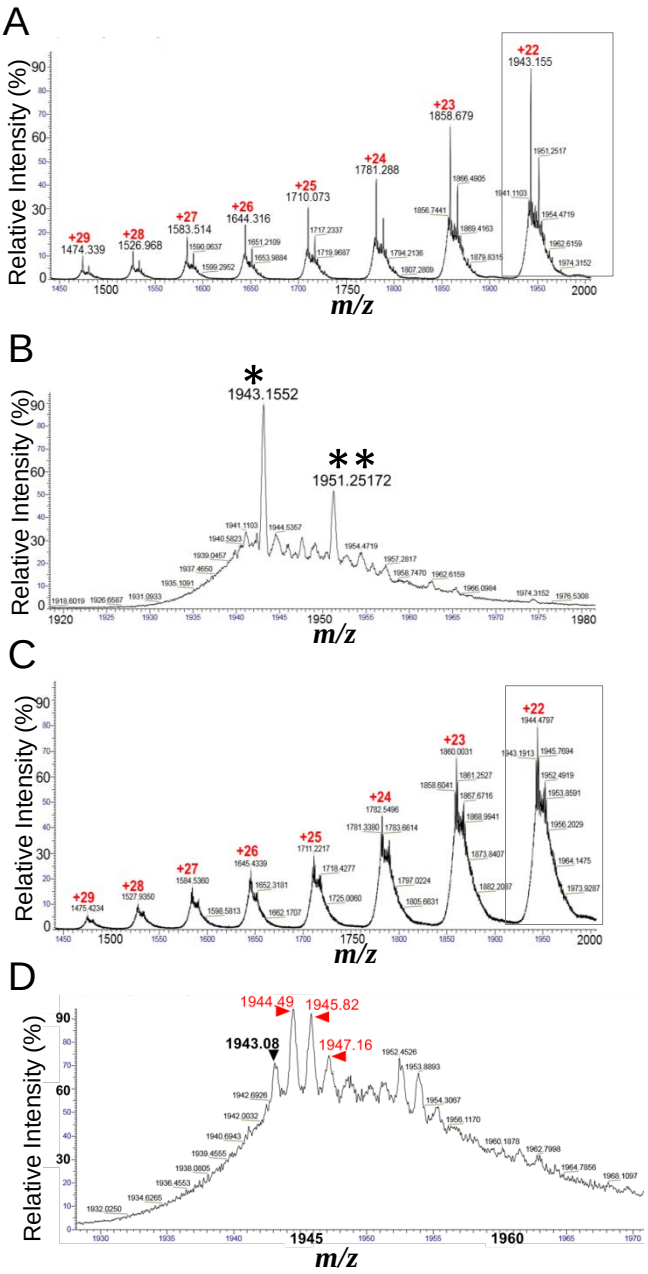
Supplemental Table S2: Deuterium incorporation of regions of AtGSNOR and HsGSNOR exhibiting differential HDX upon nitrosation. The same Roman numeral designates orthologous regions shared by AtGSNOR and HsGSNOR. T: Turn. E: Beta strand. H: Alpha helix. %D: Average percent deuterium incorporation from three experiments calculated from peptide masses of D_2O-free mapping runs, plus or minus two times standard error about the mean calculated for the smallest overlapping peptide.

Supplemental Figure 1



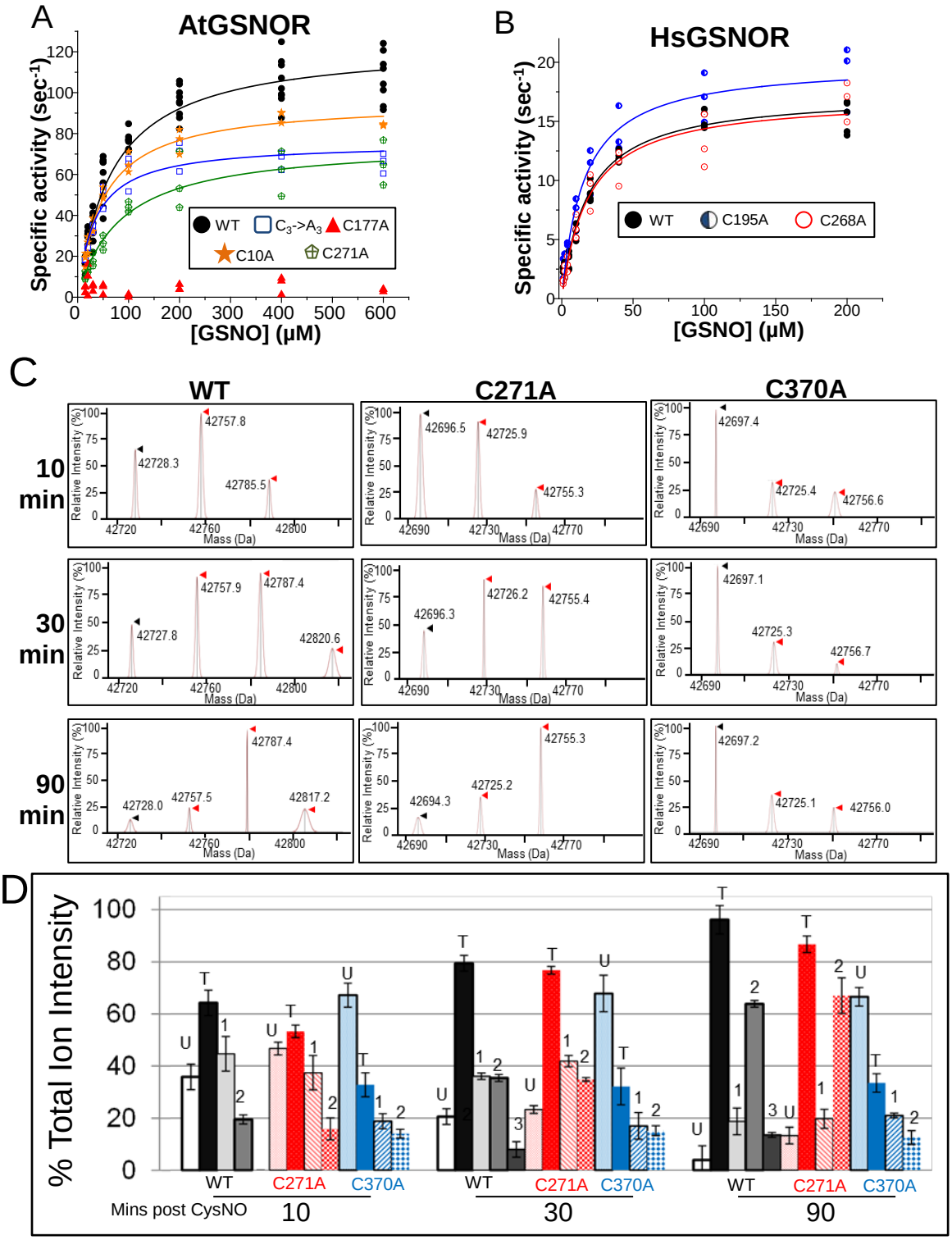
Supplemental Figure S1: Time and concentration dependence of AtGSNOR S-nitrosation. A-D: Time course of AtGSNOR nitrosation in the presence of 2mM GSNO (or GSH as a control) as assessed by mass spectrometry. Black and red arrowheads designate unmodified proteins and nitrosated adducts (+29 Da), respectively. E. Loss of tryptophan fluorescence is detectable following incubation in 100µM CysNO F-K: Nitrosated AtGSNOR adducts can be detected by mass spectrometry upon treatment with 200µM CysNO (H).

Supplemental Figure 2



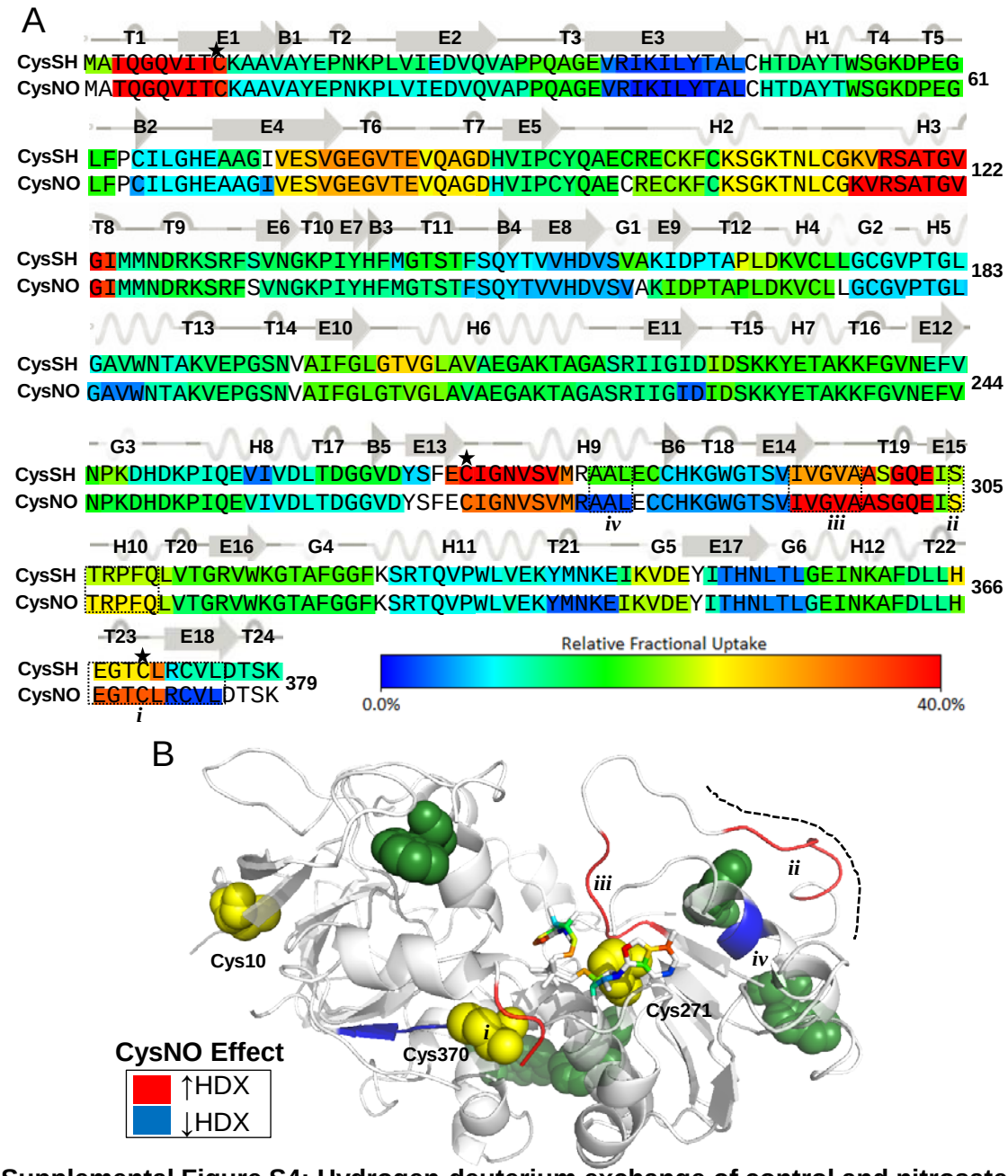
Supplemental Figure S2: Raw mass spectra of His6-tagged AtGSNOR treated with CysSH or CysNO. A, C: Mass spectra of AtGSNOR from 1450-2000 m/z treated with CysSH (A) or CysNO (C). Red numbers above each cluster of peaks indicate the charge state. *:Peak consistent with the predicted molecular mass of His6-tagged AtGSNOR expressed from the pET28 vector. **: Peak consistent with gluconation of His6-AtGSNOR. B,D: Magnification of the +22 charge states from A and C, respectively. Note the appearance of adducts at 1944.48, 1945.77, and 1947.13 m/z in D not present in B, indicative of nitrosation.

Supplemental Figure 3



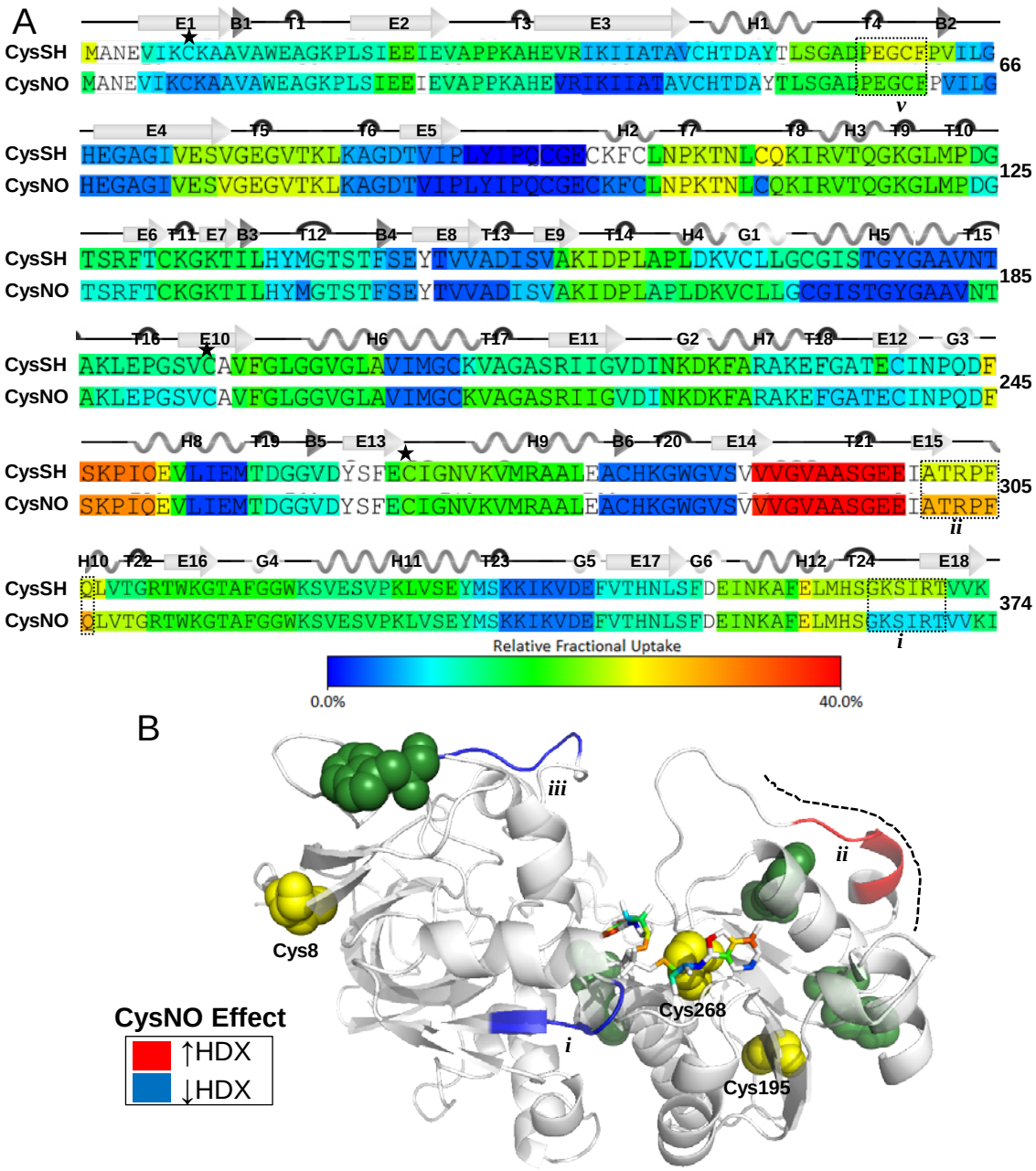
Supplemental Figure S3: Enzyme kinetics and nitrosation of recombinant wild-type and mutant His$_6$-tagged Arabidopsis and human GSNORs. A-B: Michealis-Menten plots of wild-type and mutant Arabidopsis (A) and human (B) GSNOR proteins. Graphs were drawn using Origin Pro 2015 software. C. Time course of mutant and wild-type AtGSNOR nitrosation in the presence of 2mM CysNO as assessed by mass spectrometry. D. Quantification of time courses depicted in (C) from two biological replicates. Error bars indicate one standard deviation. Letters above each bar denote the number of nitrosations. U: Unmodified. T: Total nitrosated species. 1,2,3: Mono-, di- and tri-nitrosated adducts, respectively.

Supplemental Figure 4



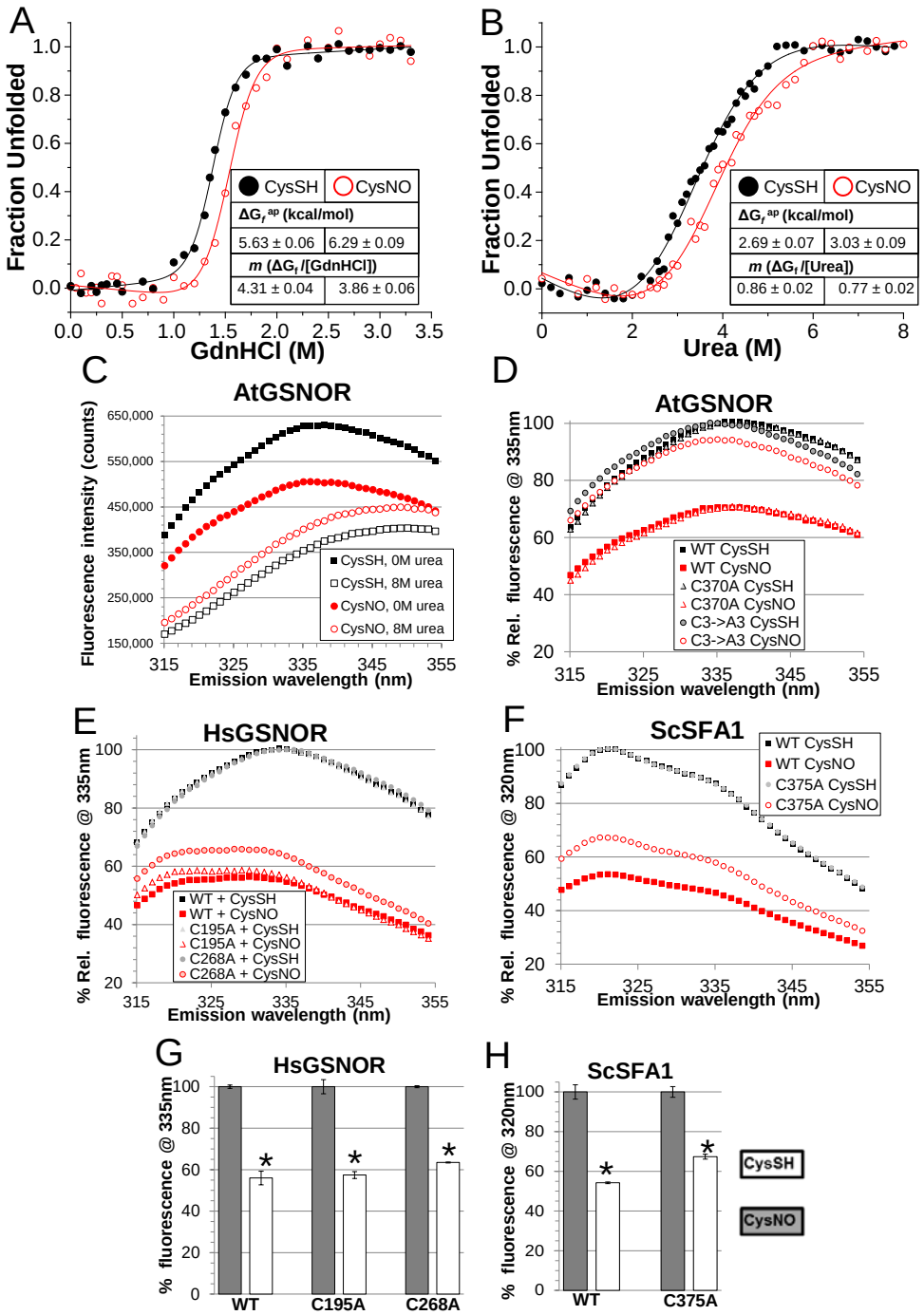
Supplemental Figure S4: Hydrogen-deuterium exchange of control and nitrosated AtGSNOR. A: Heat map and protein topology (PDB code 3UKO) of AtGSNOR treated with CysSH or CysNO colored according to the extent to which deuterons replaced amide backbone protons at a particular group of residues. For the nitrosated protein, sequence coverage and average coverage depth were 96% and 5.1x, respectively. Putative nitrosated cysteines are denoted by black stars. For protein topology, B: Beta bridge. E: Beta strand. G: 3_{10} helix. H: Alpha helix. T: Turn. B: AtGSNOR crystal structure (PDB code 3UKO) colored to illustrate regions of differential hydrogen-deuterium exchange. Tryptophans and putative nitrosated cysteines are depicted with green and yellow spheres, respectively. A dotted line indicates the dimer interface. For simplicity, only one monomer per dimer is shown. Roman numerals denote regions exhibiting the greatest difference in HDX between the CysSH and CysNO-treated samples. **i**. Turn 23, coil, and beta strand 18 (E367-L375) containing cysteine 370 and located 5Å from coil residues N-terminal to helix 6 that bind NADH. **ii**. Beta strand 15 and alpha helix 10 (S305-Q310) at the dimer interface. **iii**. Beta strand 14 and coil (I294-A298) containing NADH-binding residues and located 5Å from cysteine 271. **iv**. Helix 9 (A280-L282) located 6 Å from beta strand 14.

Supplemental Figure 5



Supplemental Figure S5: Hydrogen deuterium exchange of control and nitrosated HsGSNOR. A: Heat map and protein topology (PDB code 1MP0) of HsGSNOR treated with CysSH or CysNO colored according to the extent to which deuterons replaced amide backbone protons at a particular group of residues. For the non-nitrosated protein, sequence coverage and average coverage depth were 94.9% and 4.3x, respectively. Putative nitrosated cysteines are denoted by black stars. For protein topology, B: Beta bridge. E: Beta strand. G: 3_{10} helix. H: Alpha helix. T: Turn. B: HsGSNOR crystal structure (PDB code 1MP0) colored to illustrate regions of differential hydrogen-deuterium exchange. Tryptophans and putative nitrosated cysteines are depicted with green and yellow spheres, respectively. A dotted line indicates the dimer interface. For simplicity, only one monomer per dimer is shown. Roman numerals denote regions exhibiting the greatest difference in HDX between the CysSH and CysNO-treated samples. **i**. Turn 24, coil, and beta strand 18 (G365-V372) 5Å from helix 6 that binds NADH. **ii**. Beta strand 15, coil, and helix 10 (A301-G306) at the dimer interface. **v**. Turn 4 and coil (P57-V63) comprising part of the roof of the substrate-binding cavity.

Supplemental Figure 6



Supplemental Figure S6: Nitrosation alters GSNOR tryptophan fluorescence but not global protein stability. A-B: Nitrosation does not appreciably affect the apparent folding free energy or folding cooperativity of AtGSNOR. Control and modified proteins were incubated at 25 ºC with guanidine hydrochloride (A) or urea (B) for three hours, after which tryptophan fluorescence was measured. Emission at 335nm was normalized for the CysSH and CysNO treated samples, and regression baselines and parameters ΔG_{fold} and $m_{\Delta G}$ were estimated using a two-state model [32] with Origin Pro 2015. C. Fluorescence emission spectra of folded (solid shapes) and unfolded (open shapes) AtGSNOR following CysSH (black) or CysNO treatment (red). D-F: Emission spectra of wild-type (squares) and cysteine mutant (triangles and circles) AtGSNORs (D), HsGSNOR (E), and ScSFA1 (F) treated with CysSH (black) or CysNO (red). G-H: Normalized maximal emission intensities of HsGSNOR (G) and ScSFA1 (H) treated with CysSH (gray bars) or CysNO (open bars). Protein solutions were excited at 280nm for fluorescence experiments. Error bars represent 2 times standard error from three or more experiments.