Influence of the intramolecular disulfide Cys46-Cys55 bridge on the interaction of human neuroglobin with SDS.

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SUPPLEMENTARY INFORMATION

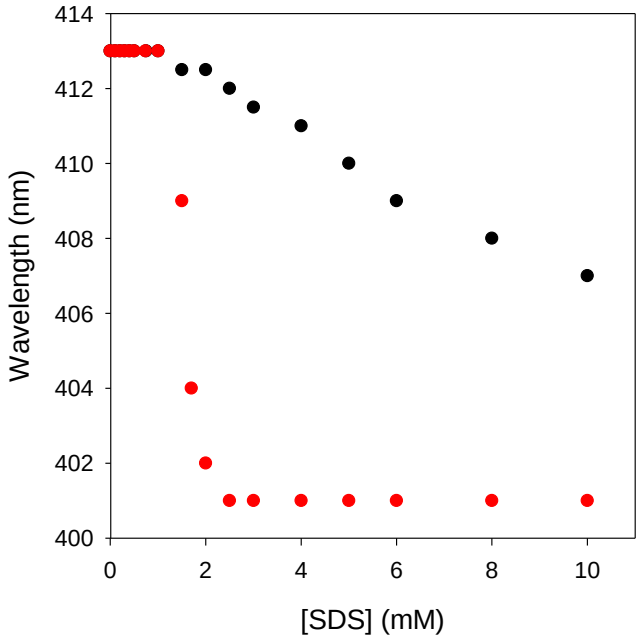

Figure S1. Changes in the wavelength of the Soret band of hNgb wt (black) and its C46AC55A mutant (red) in the presence of increasing concentration of SDS. Protein concentration is 7.2 µM in 50 mM phosphate buffer plus 0.1 M NaCl solution (pH=7.3), T=25°C. The error associated with the reported data is of the same order of magnitude or smaller than the size of the points in the graph.

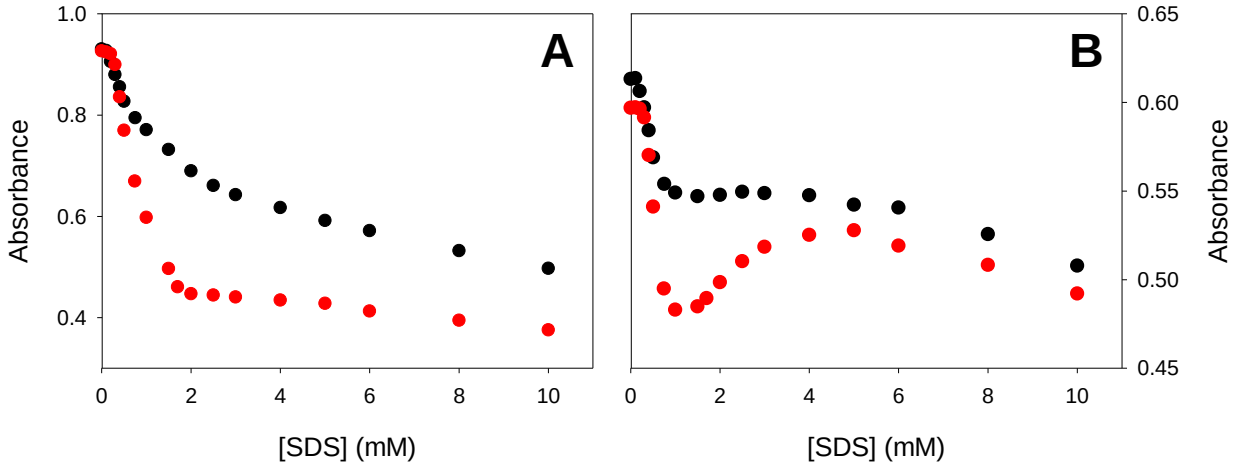

Figure S2. Changes in the absorbance at 413 nm (A) and 401 (B) of hNgb wt (black) and its C46AC55A mutant (red) in the presence of increasing concentration of SDS. Protein concentration is 7.2 µM in 50 mM phosphate buffer plus 0.1 M NaCl solution (pH=7.3), T=25°C. The error associated with the reported data is of the same order of magnitude or smaller than the size of the points in the graph.

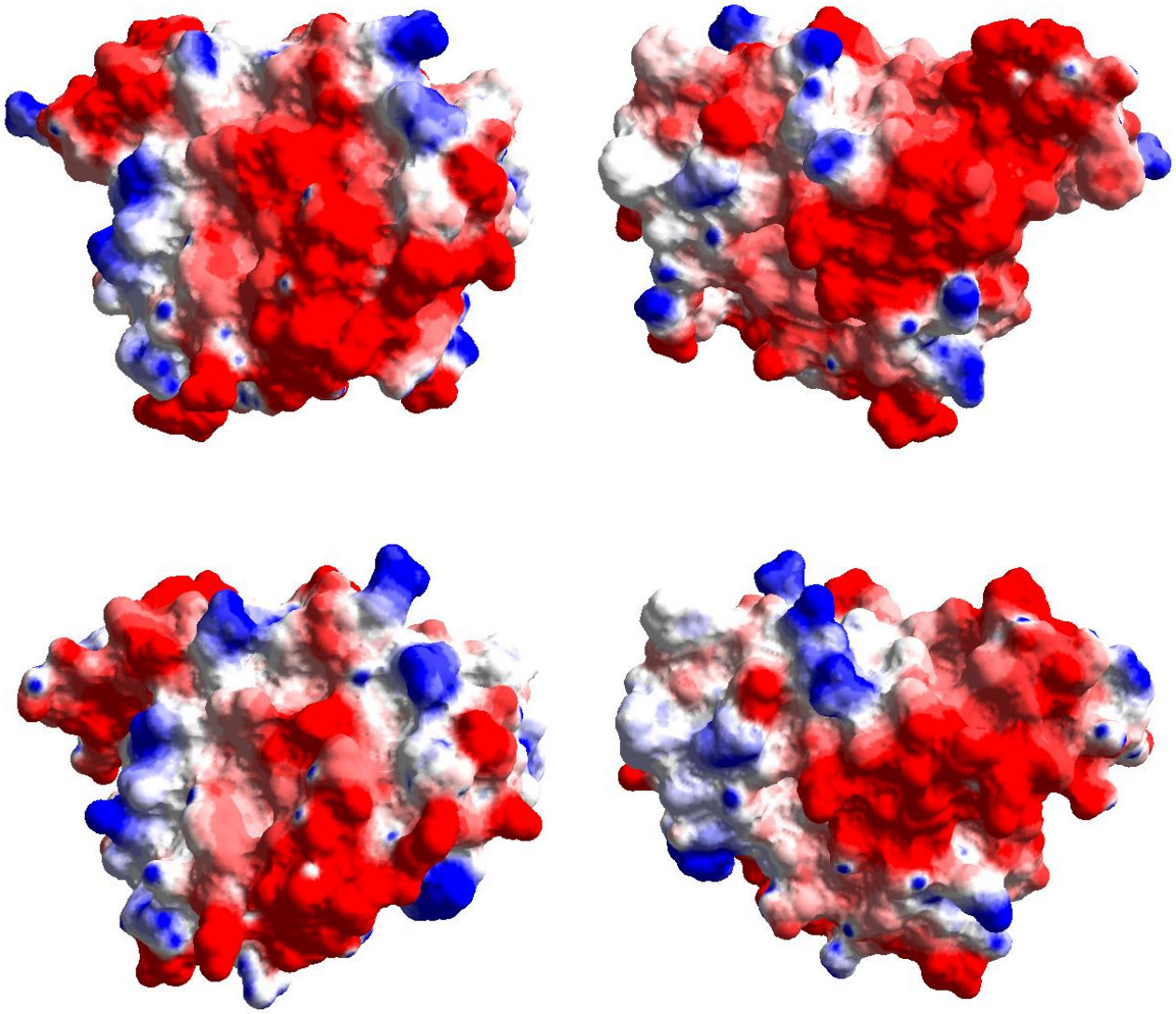

Figure S3: Effect of the Cys46-Cys55 disulfide bond on the overall surface charge distribution in hNgb: wild type hNgb (above, pdb code 4mpm, chain B) and its C46G/C55A/C120S mutant lacking all cysteines (below, pdb code 1oj6, chain A).

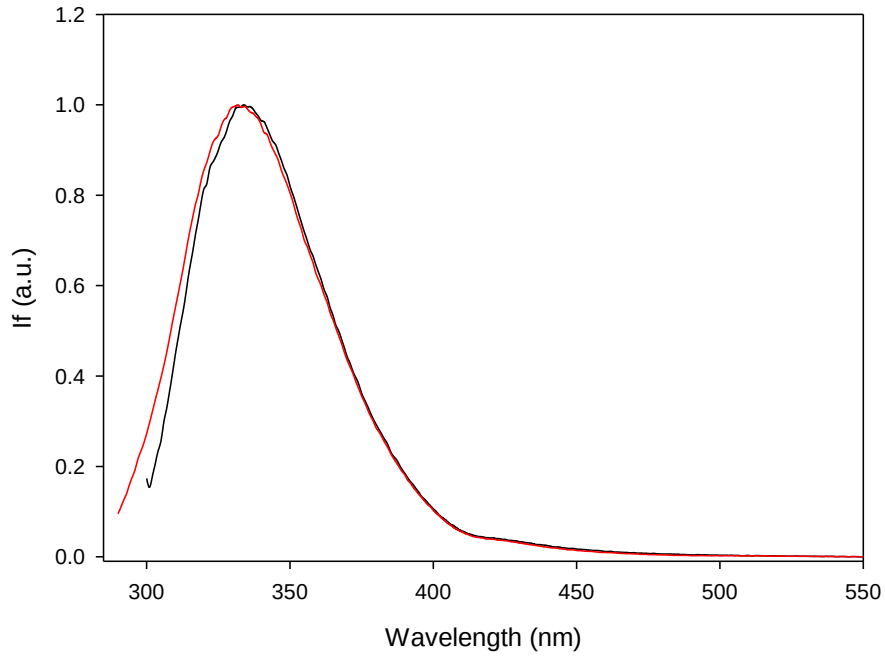

Figure S4. Normalized fluorescence spectra of hNgb wt upon excitation at 295 nm (black) and 280 nm (red). 50 mM phosphate buffer plus 0.1 M NaCl solution (pH=7.3), T=25°C.

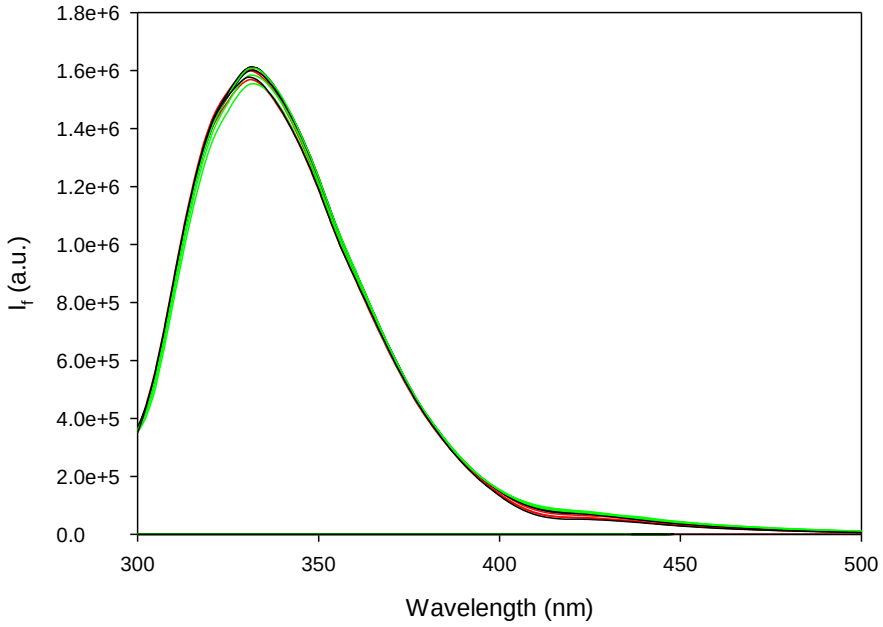

Figure S5. Fluorescence spectra of hNgb wt at increasing SDS concentrations (1 -10 mM): [SDS] = 1 mM, black; 1.5 ≤ [SDS] ≤ 2.5 mM, red; 2.5 ≤ [SDS] ≤ 5.0 mM, blue; 6.0 ≤ [SDS] ≤ 10.0 mM, green. 50 mM phosphate buffer plus 0.1 M NaCl solution (pH=7.3), T=25°C.

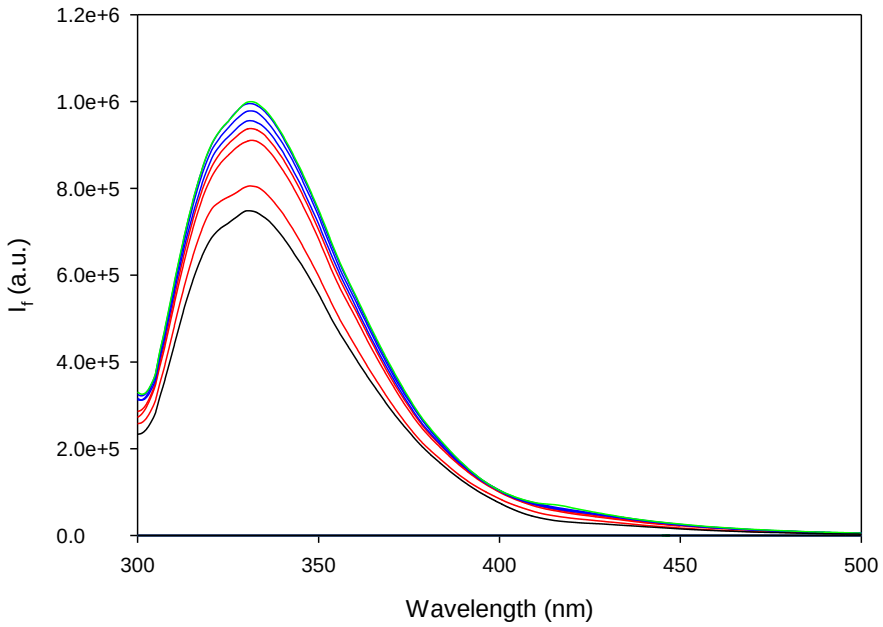

Figure S6. Fluorescence spectra of C46AC55A at increasing SDS concentrations (1-5 mM). [SDS] = 1 mM, black; 1.5 ≤ [SDS] ≤ 2.0 mM, red; 2.5 ≤ [SDS] ≤ 4.0 mM, blue; 6.0, green. 50 mM phosphate buffer plus 0.1 M NaCl solution (pH=7.3), T=25°C.

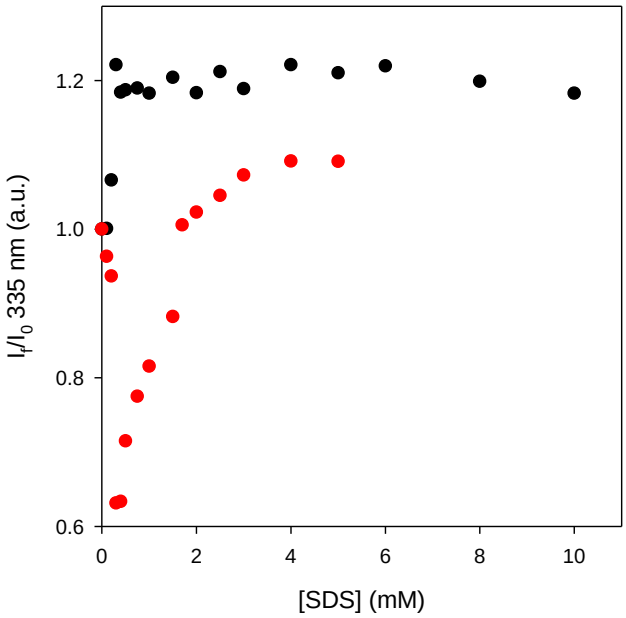

Figure S7. Changes of fluorescence intensity of hNgb in wild type form (black) and C46AC55A (red) as a function of SDS concentration. λ_{ex}=295 nm. 50 mM phosphate buffer plus 0.1 M NaCl solution (pH=7.3), T=25°C. The error associated with the reported data is of the same order of magnitude or smaller than the size of the points in the graph.

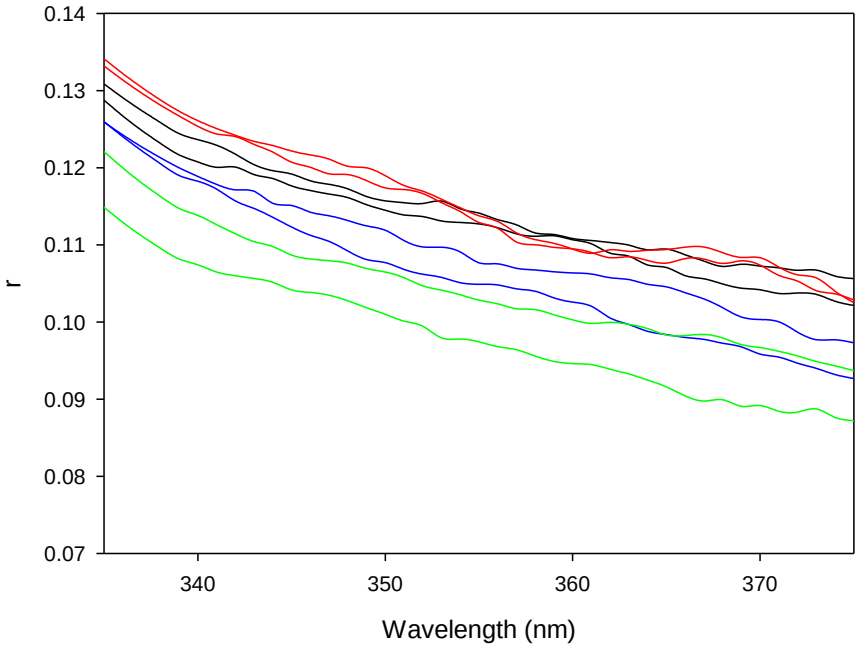

Figure S8. Fluorescence emission anisotropy spectra of 7.0 µM hNgb wt at increasing SDS concentrations. [SDS] = 0 and 0.2 mM, black; [SDS] = 0.4 and 0.5 mM, red; [SDS] = 1 and 2 mM, blue; [SDS] = 5 and 10 mM, green. 50 mM phosphate buffer plus 0.1 M NaCl solution (pH=7.3), T=25°C.

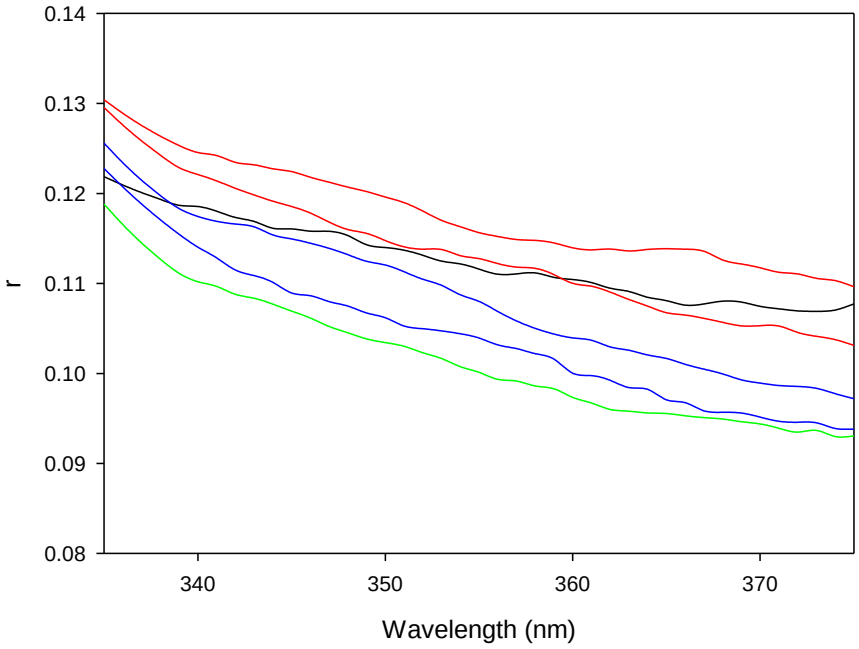

Figure S9. Fluorescence emission anisotropy spectra of 7.0 µM C46AC55A at increasing SDS concentrations. [SDS] = 0 mM, black; [SDS] = 0.2 and 0.5 mM, red; [SDS] = 1 and 2 mM, blue; [SDS] = 5 mM, green. 50 mM phosphate buffer plus 0.1 M NaCl solution (pH=7.3), T=25°C.

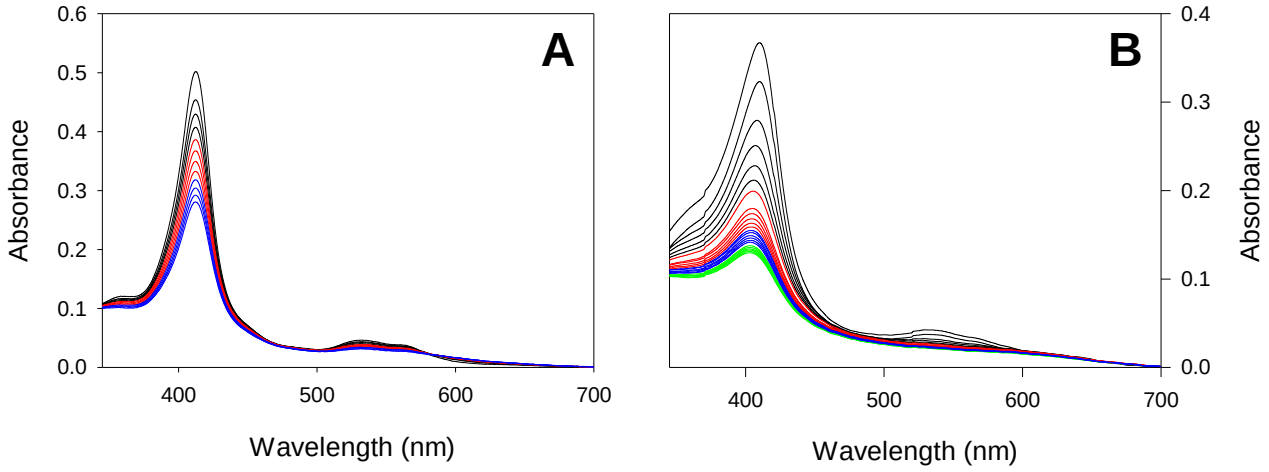

Figure S10. Electronic spectra of hNgb wt recorded in SDS 0.2 mM (A) and 2 mM (B) plus 200 μM H_2O_2 as a function of time, 50 mM phosphate buffer plus 0.1 M NaCl solution (pH=7.3), T=25°C. A: 0 min ≤ t ≤ 35 min, black; 45 min ≤ t ≤ 75 min, red; 85 min ≤ t ≤ 105 min, blue. B: 0 min ≤ t ≤ 5 min, black; 6 min ≤ t ≤ 12 min, red; 13 min ≤ t ≤ 18 min, blue; 19 min ≤ t ≤ 25 min, green.

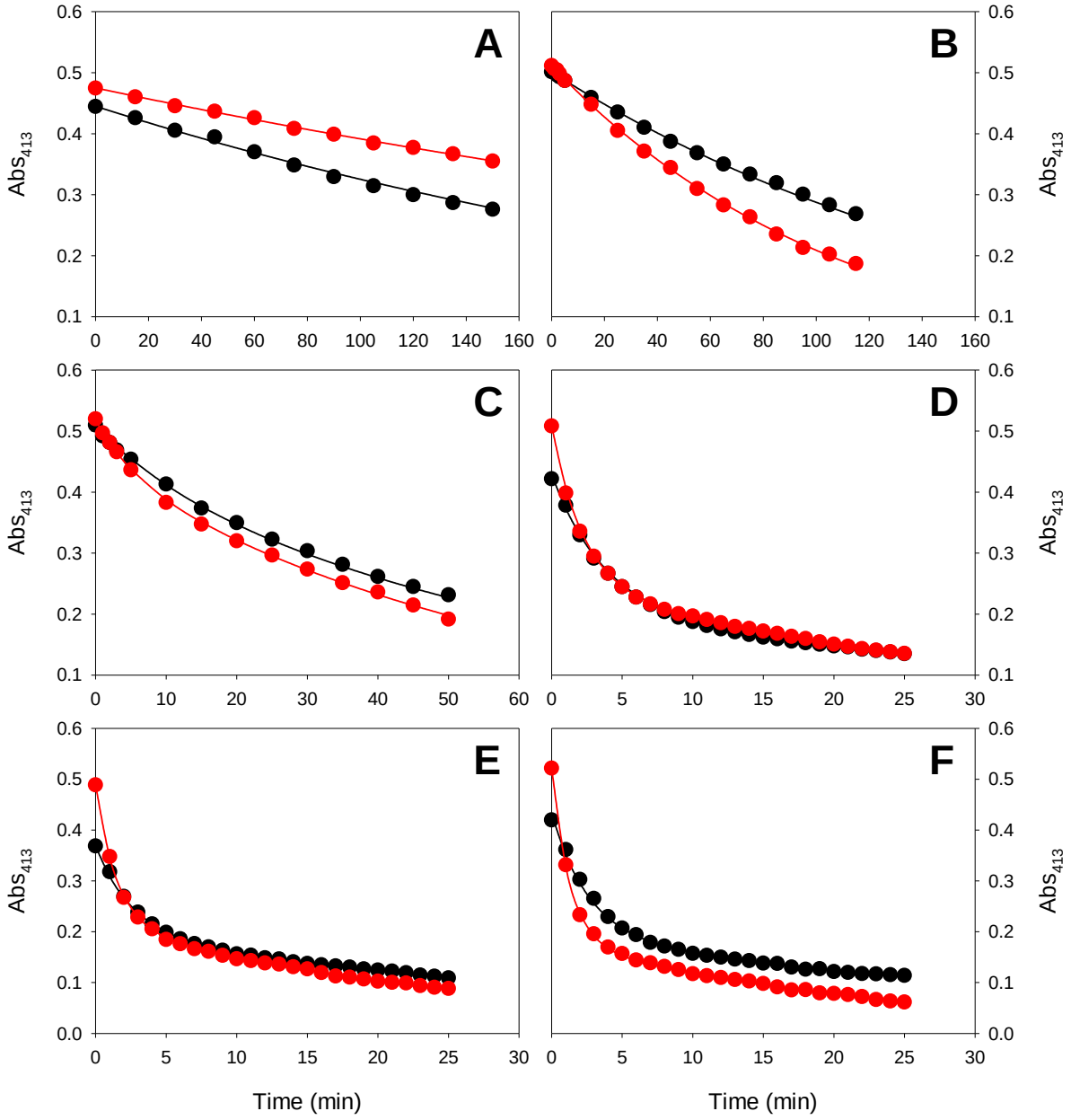

Figure S11. Absorbance of the Soret band as a function of time for wt hNgb (black) and its C46AC55A mutant (red) exposed to 200μM H_2O_2 at different SDS concentrations: [SDS] = 0 mM, A; [SDS] = 0.2 mM, B; [SDS] = 0.5 mM, C; [SDS] = 1.0 mM, D; [SDS] = 2.0 mM, E; [SDS] = 3.0 mM. Continuous lines are best fit curves interpolating the reported data $A = A_1 e^{k1t}$ for [SDS] ≤ 0.2 mM, $A = A_1 e^{k1t} + A_2 e^{k2t}$ when [SDS] ≥ 0.5 mM. 50 mM phosphate buffer plus 0.1 M NaCl, pH=7.4, T=25°C. The error associated with the reported data is of the same order of magnitude or smaller than the size of the points in the graph.

Table S1. Wavelengths (λ, nm) of the relevant spectral bands in the UV-Vis spectra and the second derivative UV-Vis spectra of hNgb wt and its C46AC55A mutant at selected SDS concentrations.

	Uv-VIS			2nd der		
	Soret	α, β	CT	Soret	α, β	CT
hNgb wt						
[SDS] = 0 mM	413	532, 564		413	526, 538_{sh}, 565	
[SDS] = 0.5 mM	413	535, 565	640	414	528, 536, 566	
[SDS] = 1.0 mM	414	535, 565	640	414	527, 537, 567	647
[SDS] = 2.0 mM	412	533, 566	640	414	526, 538, 567	645
[SDS] = 2.5 mM	412	532, 566	640	414	526, 537, 567	610, 645
[SDS] = 5.0 mM	410, 400_{sh}	490, 530, 566	630	413	490, 526, 537, 567	612, 645
[SDS] = 10.0 mM	407, 400_{sh}	490, 525, 568	610	411, 400_{sh}	490, 528, 541, 567	611
hNgb C44AC55A						
[SDS] = 0 mM	413	531, 562		414	526, 564	
[SDS] = 0.5 mM	413	535, 563		413	526, 538, 566	
[SDS] = 1.0 mM	413, 360_{sh}	537, 565	640	414	528, 540, 568	640
[SDS] = 2.0 mM	402, 414_{sh}, 365_{sh}	540	610, 633	410, 400_{sh}	542	640
[SDS] = 5.0 mM	401, 360_{sh}	490, 534	600	404	490, 532	611
[SDS] = 10.0 mM	401, 360_{sh}	490, 534	604	403	490, 532	610

Table S2. Fluorescence lifetimes, percentage amplitude, average lifetime of 7.1 μM hNgb wt, 50 mM phosphate buffer plus 0.1 M NaCl solution (pH=7.3), T=25°C. λ_{ex}= 295 nm. The estimated errors are ± 0.1 ns on the τ_1, τ_2 and < τ > values, ± 0.3 ns on the τ_3 value, ± 2% on the α values.

	τ_1 (ns)	α_1 (%)	τ_2 (ns)	α_2 (%)	τ_3 (ns)	α_3 (%)	χ^2	<τ> (ns)
λ_{em}= 320 nm	0.3	21	2.2	41	6.1	38	1.19	1.1
λ_{em}= 340 nm	0.4	17	2.2	39	6.5	44	1.25	1.6

Table S3. Fluorescence lifetimes of 7.1 μM C46AC55A, 50 mM phosphate buffer plus 0.1 M NaCl solution (pH=7.3), T=25°C. λ_{ex}= 295 nm. The estimated errors are ± 0.1 ns on the τ_1, τ_2 and < τ > values, ± 0.3 ns on the τ_3 value, ± 2% on the α values.

	τ_1 (ns)	α_1 (%)	τ_2 (ns)	α_2 (%)	τ_3 (ns)	α_3 (%)	χ^2	<τ> (ns)
λ_{em}= 320 nm	0.3	18	2.5	38	6.6	44	1.16	1.1
λ_{em}= 340 nm	0.5	19	2.5	40	6.7	41	1.24	1.6

Table S4. Average emission anisotropy (335-350 nm) values of hNgb wt calculated by spectra shown in Figure S7. The estimated errors are ± 0.005.

SDS concentration (mM)	0	0.2	0.4	0.5	1	2	5	10
average r 335 - 350 nm	0.120	0.122	0.125	0.124	0.118	0.116	0.112	0.106

Table S5. Average emission anisotropy (335-350 nm) values of C46AC55A calculated by spectra shown in Figure S8. The estimated errors are $\pm$ 0.005.

SDS concentration (mM)	0	0.2	0.4	0.5	1	2	5
average r (335-350 nm)	0.117	0.124	0.125	0.121	0.117	0.113	0.109