Mechanistic Investigation of the High Reactivity and Specificity of Peroxiredoxins with Peroxides

Supplemental Data

Péter Nagy[1], Amir Karton[2], Andrea Betz[1], Alexander V. Peskin[1], Paul Pace[1], Robert O'Reilly[2], Mark B. Hampton[1], Leo Radom[2], and Christine C. Winterbourn[1]

[1]Department of Pathology, University of Otago Christchurch, P.O. Box 4345, Christchurch, New Zealand.

[2]School of Chemistry and ARC Centre of Excellence for Free Radical Chemistry and Biotechnology, University of Sydney, Sydney, NSW 2006, Australia.

Supporting Methods

Reagents. All chemicals were A.C.S. certified grade or better. Water was purified by running through a Milli–Q system (Millipore) so that its resistivity was greater than 18 MΩ-cm. All reagents and enzymes were purchased from Sigma–Aldrich (St Louis) unless otherwise indicated. Prx3 was purchased from Abfrontier (Seoul, Korea). The buffer solutions were prepared from $NaH_2PO_4 \cdot 2H_2O$, Na_2HPO_4 and the pH was adjusted with ~6M HCl or freshly prepared ~6M NaOH (mostly free of CO_3^{2-} contamination). Unless stated otherwise, reactions were carried out at 20 ^oC with diethylenetriamine–penta–acetic acid (DTPA; 50 μM) present to chelate contaminating trace metal ions. The concentrations of stock solutions of H_2O_2 were determined iodometrically. The concentration of H_2O_2 in solutions prepared from the stock solution were confirmed spectrophotometrically ($\varepsilon(H_2O_2)^{240nm} = 43.6\ M^{-1}cm^{-1}$).

Preparation of recombinant Prxs and site-directed mutagenesis of Arg residues. *Cloning, expression and purification of bacterially expressed human Peroxiredoxin 3.* Human Prx3 cDNA (Origene) was amplified using primers to encode a TEV protease cleavage site immediately before amino acid 62 [forward primer: 5'–GCG GAA TTC GAA AAC CTG TAT TTT CAG TCG GCA CCT GCT GTC ACC CAG CAT GC–3'; reverse primer: 5'–GCG CTC GAG TCA CTG ATT TAC CTT CTG AAA GTA C–3'], and sub–cloned EcoRI–XhoI into pET28a vector (Novagen) in frame to express an amino terminal 6 x Histidine tagged Prx2 recombinant protein. Note that aa 1 of all recombinant Prx3 protein constructs used in this study corresponds to aa 62 of the hPrx 3 preprotein. This plasmid was then used as a template for site–directed mutagenesis using the GeneTailor Site–Directed Mutagenesis System (Life Technologies) to generate mutants Prx3–R123G, Prx3–R146G, Prx3–R146A, Prx3–R146H, Prx3–R146K and the double mutant Prx3–R123G–R146G using relevant altered codon primer pairs (following the manufacturer's instructions). Wild type and mutant sequences were verified by DNA sequencing and transformed into BL21(DE3)pLysS *E.coli* for protein expression.

His–Prx3 and mutants were purified in an identical manner. Briefly BL21(DE3)pLysS carrying His–Prx3 plasmids were cultured to early log phase, induced with 0.75mM IPTG, cultured for a further 4 hours at 25 ºC, then harvested into lysis buffer (50 mM Na–phosphate pH8 / 200 mM NaCl / 10 mM imidazole / 0.2 mM PMSF) and sonicated. Clarified extracts were applied to His Select Cobalt Affinity Gel (Sigma) and His–Prx3 proteins were purified according to manufacturer's instructions (Sigma). Histidine tags were cleaved off using TEV protease expressed and purified from pRK793 [1] and removed with His Select Cobalt Affinity Gel (Sigma). Recombinant Prx3 proteins were stored at –80 ºC.

Cloning, expression and purification of bacterially expressed human Peroxiredoxin 2. Human Prx2 cDNA was amplified using primers to encode a Factor Xa cleavage site immediately before the amino terminal methionine [forward primer: 5'–GCGGAATTCATCGAAGGTCGTATGGCCTCCGGTAACGCGCGCATCGGA3';
reverse primer: 5'–CGCTCGAGCTAATTGTGTTTGGAGAAATATTC3'], cloned into TOPOblunt vector (Invitrogen; according to manufacturer's instructions), and further sub–cloned EcoRI–XhoI into pET28a vector (Novagen) in frame to express an amino

terminal 6 x Histidine tagged Prx2 recombinant protein. This plasmid was then used as a template for site–directed mutagenesis using Pfu polymerase (according to manufacturer's instructions) to generate mutants Prx2–R127K, Prx2–R150K and the double mutant Prx2–R127K–R150K, using relevant altered codon primer pairs. The double mutant Prx2–R127K–R150K was created using R127K mutagenesis primers on the Prx2–R150K template. Wild type and mutant sequences were verified by DNA sequencing and transformed into BL21(DE3)pLysS E.coli for protein expression.

His–Prx2 and mutants were purified in an identical manner. Briefly BL21(DE3)pLysS carrying His–Prx2 plasmids were cultured to early log phase, induced with 1 mM IPTG, cultured for a further 4 hours at 37 ºC, then harvested into lysis buffer (20 mM Tris.Cl pH7.4 / 500 mM NaCl / 10 % glycerol / 0.1 mg/ml lysosyme / 1 mM PMSF) and sonicated. Clarified extracts were applied to His Select Cobalt Affinity Gel (Sigma) and His–Prx2 proteins were purified according to manufacturer's instructions (Sigma). Histidine tags were cleaved off using Factor Xa protease (Roche, according to manufacturer's instructions), and removed with His Select Cobalt Affinity Gel (Sigma). Full–length recombinant Prx2 proteins were stored at –80 ºC.

Full length Prx2 and Prx3 proteins were analysed by coomassie stained SDS–PAGE, circular dichroism (CD) and mass spectrometry (see Figures S1 and S3).

Biophysical characterization of the recombinant WT and Arg mutants of Prx.
CD spectroscopy. WT and mutant proteins were treated with 2 molar equivalents of H_2O_2 to produce the dimeric forms. Protein solutions were desalted by passing through a Micro Bio–Spin 6 Chromatography column (Bio–Rad, Hercules, CA, USA) and the CD spectra were recorded. The spectra were collected using a 0.1–cm path length cuvette containing 0.1 mg/mL protein in 20 mM sodium phosphate buffer (pH 7.4). The assays were carried out at 20 °C in a Jasco J–720 spectropolarimeter (Jasco, Easton, MD). Spectra are presented as an average of three scans recorded from 195 to 260 nm.

Thermal stability measurements. The melting temperature of wild–type and mutant Prx 2 and Prx3 was determined by differential scanning fluorimetry (DSF) [2]. Protein samples in 20 mM Na–phosphate buffer pH 7.4 (0.1 mg/mL, 64 μL) were mixed with SYPRO orange fluorescent dye (Invitrogen) (16 μL, diluted 100–fold from supplied concentration with dH_2O) in a 96–well microplate (total volume 25 μL). After sealing the plate, a stepped thermal melt program scanning from 20 °C to 100 °C in increments of 1 °C, with a dwell time of 10 s after each temperature rise, was run on a Bio–Rad iQ5 Multicolor Real–Time PCR Detection System. Excitation and emission wavelengths were 490 and 575 nm, respectively. Melting temperatures (Tm) were determined as the point of inflection (dRFU/dt). Measurements were carried out in triplicate for each condition.

Computational Details. Unless otherwise indicated, all the calculations were carried out using Gaussian 09 Revision A.02 [3]. Geometry optimizations were performed using the B3-LYP [4–6] density functional theory procedure in conjunction with the 6-31+G(2df,p) basis set, and were carried out in the gas phase. All geometry optimizations and single-point energy calculations were performed using the "ultrafine" pruned (99,590) grid, as recommended by Martin et al. [7]. Equilibrium structures were verified to have all real

harmonic frequencies, and transition structures were confirmed to have one imaginary frequency. The connectivities of the transition structures were confirmed by performing intrinsic reaction coordinate (IRC) calculations [8–10]. Bulk solvent effects were approximated by single-point energy calculations using the conductor-like polarizable continuum model (CPCM) [11] as implemented in Gaussian 03 Revision E.01 [12] at the HF/6-31+G(d) level of theory in conjunction with UAHF atomic radii, as recommended by Takano and Houk [13]. Two dielectric constants were used: $\varepsilon = 78.4$ for water, and the standard value of $\varepsilon = 4.0$ for modeling the protein-like environment of the active site [14, 15].

Reaction enthalpies and Gibbs free energies in the gas phase were calculated at the G4 level at 298 K [16]. G4 theory aims at reproducing the basis-set-limit CCSD(T) (coupled cluster theory with singles, doubles, and quasiperturbative triple excitations) energy and includes zero-point energy, first-order spin-orbit, and temperature corrections. It is capable of calculating thermochemical properties (such as reaction energies, bond dissociation energies, and enthalpies of formation) with mean absolute deviations from experimental energies of 3.0 kJ mol^{-1}. As all of the reactions considered in the present work are isogyric and do not involve atomic species, the G4 protocol becomes nonempirical.

The combination of a CPCM solvation correction on top of a gas-phase G4//B3-LYP/6-31+G(2df,p) energy is denoted CPCM-G4//B3-LYP/6-31+G(2df,p).

Computational Results. Table S2 displays the enthalpies (ΔH_{298}) and Gibbs free energies (ΔG_{298}) in the gas-phase (calculated at the G4//B3-LYP/6-31+G(2df,p) level) and in the protein-like environment (calculated at the CPCM-G4//B3-LYP/6-31+G(2df,p) level) associated with the reactions in Figure 4 of the main text and Figure S2.

Table S3 presents the absolute gas-phase G4//B3-LYP/6-31+G(2df,p) vibrationless energies (E_e), absolute energies at 0 K (E_0), enthalpies at 298 K (H_{298}), and Gibbs free energies at 298 K (G_{298}). Table S3 also lists the CPCM-G4//B3-LYP/6-31+G(2df,p) enthalpies at 298 K, and the Gibbs free energies at 298 K, both in the aqueous phase (H_{298}(aq), G_{298}(aq), $\varepsilon = 78.4$) and in the protein-like phase (H_{298}(enz), G_{298}(enz), $\varepsilon = 4.0$).

Table S4 gives selected bond distances and bond angles for the species associated with the reactions in Figure 4 of the main text and Figure S2. We note that in nearly all the catalyzed transition structures, the partial S•••O_a bond is longer by 0.068–0.224 Å, the partial O_a•••O_b bond is shorter by 0.008–0.160 Å, and the $\angle SO_aO_b$ angle is reduced by 0.4–14.0° relative to values in the uncatalyzed transition structure.

Table S5 presents structures optimized at the B3-LYP/6-31+G(2df,p) level of theory (in Cartesian coordinates, Å).

The reaction profiles computed in the protein-like environment are all qualitatively similar, and a typical example ([Gua+•••S,O_a,O_b], see Figure 4 of the main text) is displayed schematically in Figure S4. It shows the reactants, reactant complex (RC), transition structure (TS), product complex (PC), and products at the various stages of the reaction.

In the main text, we noted that the hydrogen-bond interactions in the catalyzed model systems stabilize the transition structures more than they stabilize the reactant complexes. The estimated stabilization energies are reported here. Regarding the

reactions depicted in Figure S2, the hydrogen bonds at the various chalcogen centers are estimated to stabilize the S_N2 transition structures by 43.1 (O_a), 44.2 (O_b), 65.2 ($O_a + O_b$), 77.5 ($S + O_b$), and 89.9 ($S + O_a + O_b$) kJ mol^{-1}, respectively.[1] The stabilizing effects in the corresponding reactant complexes are found to be 29.3(O_a), 29.2 (O_b), 46.6 ($O_a + O_b$), 54.6 ($S + O_b$), and 68.9 ($S + O_a + O_b$) kJ mol^{-1}, respectively. Regarding the [Gua^+•••S,O_a,O_b] and [Gua^+•••S,O_a] reactions in Figure S2, the interaction energies between the guanidinium cation and the [^-SH•••O_bH•••O_aH] moiety are estimated to be 131.4 ([Gua^+•••S,O_a,O_b]-TS) and 128.0 kJ mol^{-1} ([Gua^+•••S,O_a]-TS) in the transition structures, and 104.1 ([Gua^+•••S,O_a,O_b]-RC) and 105.6 kJ mol^{-1} ([$Guan^+$•••S,O_a]-RC) in the reactant complexes. Thus, it is evident that in all cases the hydrogen bonds stabilize the transition structures more than they stabilize the reactant complexes.

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[1]Obtained by taking the difference in enthalpies between the transition structures for the catalyzed reactions and the sum of the enthalpies for the uncatalyzed transition structure and the appropriate number of HF molecules.

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Table S1. Melting points of the WT and mutant proteins of Prx2 and Prx3.

Prx 3	Melting point (^{o}C)
recombinant WT	68
Commercial	~71
R146K	~71
R146G	~73
R123G	~69
R123G/R146G	~71
R146A	~77
R146H	~84

Prx 2	Melting point (^{o}C)
recombinant WT	~52
R150K	~49
R127K	~49
R127K/R150K	~50

Table S2. Enthalpies (ΔH_{298}) and Gibbs free energies (ΔG_{298}) associated with the reactions in Figure 4 of the main text and Figure S2 (kJ mol^{-1}).[a] Calculations were performed in the gas-phase (calculated at the G4//B3-LYP/6-31+G(2df,p) level) and in the enzyme-like environment (calculated at the CPCM-G4//B3-LYP/6-31+G(2df,p) level)

		ΔH_{298}[b]	ΔG_{298}[b]	ΔH_{298}[c]	ΔG_{298}[c]
Uncat[d]	RC	5.2	37.1		
	TS	67.2	99.7		
	PC	–176.7	–145.7		
	$\Delta H^{\ddagger}$, $\Delta G^{\ddagger}$	67.2[e]	99.7[e]		
Uncat	RC	–21.2	10.6	–100.3	–68.5
	TS	49.4	81.9	–7.4	25.0
	PC	–195.2	–164.2	–256.0	–225.0
	$\Delta H^{\ddagger}$, $\Delta G^{\ddagger}$	70.6	81.9[d]	92.9	93.5
[FH•••O_a]	RC	–50.5	18.5	–172.2	–103.2
	TS	6.3	74.4	–81.1	–13.1
	PC	–269.5	–208.7	–365.7	–304.9
	$\Delta H^{\ddagger}$, $\Delta G^{\ddagger}$	56.8	55.9	91.1	90.1
[O_b•••HF]	RC	–50.5	18.6	–172.2	–103.2
	TS	5.3	72.4	–80.9	–13.7
	PC	–246.3	–178.6	–350.7	–283.1
	$\Delta H^{\ddagger}$, $\Delta G^{\ddagger}$	55.7	53.9	91.3	89.4
[FH•••S]	RC	–51.1	10.1	–176.5	–115.3
	TS	14.0	78.9	–83.0	–18.1
	PC	–223.7	–160.2	–327.5	–264.1
	$\Delta H^{\ddagger}$, $\Delta G^{\ddagger}$	65.1	68.8	93.5	97.2
[FH•••O_a,O_b•••HF]	RC	–67.8	35.1	–227.8	–124.9
	TS	–15.7	89.6	–152.7	–47.3
	PC	–303.3	–206.6	–445.5	–348.9
	$\Delta H^{\ddagger}$, $\Delta G^{\ddagger}$	52.1	54.5	75.1	77.5
[FH•••S,O_a•••HF]	RC	–70.0	36.9	–243.1	–136.2
	TS	–13.5	88.9	–154.8	–52.5
	PC	–268.0	–169.2	–422.9	–324.1
	$\Delta H^{\ddagger}$, $\Delta G^{\ddagger}$	56.6	52.0	88.3	83.8
[FH•••S,O_b•••HF]	RC	–75.9	22.8	–240.8	–142.1
	TS	–28.1	71.2	–156.5	–57.2
	PC	–264.3	–164.4	–410.1	–310.3
	$\Delta H^{\ddagger}$, $\Delta G^{\ddagger}$	47.8	48.4	84.3	84.9
[2FH•••S,O_a,O_b•••HF]	RC	–90.1	41.3	–289.5	–158.0
	TS	–40.4	96.2	–214.9	–78.3
	PC	–303.6	–168.1	–494.5	–359.1
	$\Delta H^{\ddagger}$, $\Delta G^{\ddagger}$	49.7	54.8	74.6	79.8
[Gua^+•••S,O_a,O_b]	RC	–126.8	–52.3	–528.1	–453.6
	TS	–82.0	–1.7	–466.9	–386.6
	PC	–321.7	–245.4	–769.4	–693.1
	$\Delta H^{\ddagger}$, $\Delta G^{\ddagger}$	44.8	50.6	61.2	67.1

[H_2C=NH•••S,O_a,O_b•••Gua^+]	RC	–362.5	–246.6	–806.1	–690.1
	TS	–322.1	–200.8	–751.6	–630.3
	PC	–548.2	–436.6	–1029.9	–918.4
	$\Delta H^‡$, $\Delta G^‡$	40.4	45.8	54.5	59.8
[H_2NH•••S,O_a,O_b•••Gua^+]	RC	–348.9	–233.5	–785.4	–670.1
	TS	–310.6	–189.3	–731.9	–610.6
	PC	–540.5	–437.8	–1008.7	–906.0
	$\Delta H^‡$, $\Delta G^‡$	38.3	44.3	53.6	59.5
[HOH•••S,O_a,O_b•••Gua^+]	RC	–129.4	–8.5	–578.7	–457.8
	TS	–97.3	22.9	–519.8	–399.6
	PC	–316.5	–203.8	–793.6	–680.9
	$\Delta H^‡$, $\Delta G^‡$	32.2	31.5	58.9	58.2
[FH•••S,O_a,O_b•••Gua^+]	RC	–141.5	–21.9	–594.4	–474.8
	TS	–115.1	5.2	–535.5	–415.2
	PC	–320.5	–207.2	–799.0	–685.7
	$\Delta H^‡$, $\Delta G^‡$	26.4	27.1	58.9	59.6
[Gua^+•••S,O_a]	RC	–126.8	–52.3	–528.1	–453.6
	TS	–78.6	2.0	–476.3	–395.7
	PC	–321.7	–245.4	–769.4	–693.1
	$\Delta H^‡$, $\Delta G^‡$	48.2	54.3	51.9	58.0
[Gua^+•••S,O_a,O_b•••HOH]	RC	–134.7	–24.3	–572.8	–462.3
	TS	–94.7	25.8	–526.0	–405.5
	PC	–324.4	–217.4	–802.0	–695.0
	$\Delta H^‡$, $\Delta G^‡$	40.1	50.1	46.8	56.8
[Gua^+•••S,O_a,O_b•••HF]	RC	–143.1	–30.2	–585.6	–472.7
	TS	–115.3	3.6	–544.5	–425.7
	PC	–371.5	–257.8	–822.7	–709.0
	$\Delta H^‡$, $\Delta G^‡$	27.8	33.8	41.0	47.0

[a]RC = reactant complex, TS = transition state, PC = product complex, $\Delta H^‡$, $\Delta G^‡$ = barrier relative to the RC unless otherwise specified. [b]In enzyme-like medium (ε = 4.0), unless otherwise noted. [c]In the gas-phase. [d]In aqueous solution (ε = 78.4). [e]Relative to the free reactants (see main text).

Table S3. Gas-phase G4//B3-LYP/6-31+G(2df,p) vibrationless energies (E_e), energies at 0 K (E_0), enthalpies at 298 K ($H_{298}(g)$), and Gibbs free energies at 298 K ($G_{298}(g)$), as well as aqueous-phase (ε = 78.4) enthalpies at 298 K ($H_{298}(aq)$) and Gibbs free energies at 298 K ($G_{298}(aq)$), and enzyme-like phase (ε = 4.0) enthalpies at 298 K ($H_{298}(enz)$) and Gibbs free energies at 298 K ($G_{298}(enz)$) calculated at the CPCM-G4//B3-LYP/6-31+G(2df,p) level for the species in Figure 4 of the main text and Figure S2 (Hartree).

	(E_e)	(E_0)	$H_{298}(g)$	$H_{298}(aq)$	$H_{298}(enz)$	$G_{298}(g)$	$G_{298}(aq)$	$G_{298}(enz)$
HS^-	–398.704223	–398.698343	–398.695039	–398.807181	–398.779851	–398.716086	–398.828227	–398.800897
H_2O	–76.418287	–76.397288	–76.393442	–76.405457	–76.402063	–76.414749	–76.426765	–76.423371
H_2O_2	–151.520679	–151.494513	–151.490268	–151.510810	–151.505121	–151.515916	–151.536457	–151.530768
HSOH	–474.454332	–474.432219	–474.427989	–474.439097	–474.435957	–474.455521	–474.466629	–474.463489
$H_2C{=}NH$	–94.612138	–94.572929	–94.568906	–94.577225	–94.574277	–94.594549	–94.602867	–94.599919
$CH_6N_3^+$	–205.726510	–205.639575	–205.632877	–205.748366	–205.720573	–205.666054	–205.781542	–205.753750
HF	–100.431554	–100.422380	–100.419061	–100.429850	–100.426631	–100.438669	–100.449458	–100.446239
NH_3	–56.551556	–56.517788	–56.513841	–56.521873	–56.519642	–56.536606	–56.544637	–56.542406
Uncat-RC	–550.264153	–550.230050	–550.223527	–550.316004	–550.293056	–550.258088	–550.350565	–550.327617
Uncat-TS	–550.226034	–550.194929	–550.188141	–550.292378	–550.266148	–550.222474	–550.326711	–550.300480
Uncat-PC	–550.322710	–550.289419	–550.282817	–550.385301	–550.359325	–550.317706	–550.420190	–550.394215
[FH•••O_a]-RC	–650.724748	–650.678243	–650.669951	–650.751798	–650.730843	–650.709973	–650.791820	–650.770864
[FH•••O_a]-TS	–650.687910	–650.643930	–650.635263	–650.735549	–650.709191	–650.675644	–650.775929	–650.749571
[FH•••O_a]-PC	–650.797768	–650.752433	–650.743645	–650.838814	–650.814241	–650.786788	–650.881958	–650.857385
[O_b•••HF]-RC	–650.724747	–650.678239	–650.669949	–650.751765	–650.730825	–650.709962	–650.791777	–650.770837
[O_b•••HF]-TS	–650.687525	–650.643701	–650.635192	–650.735987	–650.709597	–650.675906	–650.776702	–650.750311
[O_b•••HF]-PC	–650.788789	–650.746125	–650.737961	–650.828701	–650.805402	–650.778504	–650.869243	–650.845945
[FH•••O_a,O_b•••HF]-RC	–751.179221	–751.120670	–751.110182	–751.183710	–751.164061	–751.156896	–751.230425	–751.210775
[FH•••O_a,O_b•••HF]-TS	–751.148595	–751.091954	–751.081582	–751.167238	–751.144227	–751.127366	–751.213022	–751.190011
[FH•••O_a,O_b•••HF]-PC	–751.262080	–751.203610	–751.193117	–751.275618	–751.253737	–751.242228	–751.324729	–751.302849
[FH•••S]-RC	–650.726217	–650.680554	–650.671588	–650.751682	–650.731061	–650.714571	–650.794665	–650.774044
[FH•••S]-TS	–650.687797	–650.644892	–650.635976	–650.730747	–650.706270	–650.677556	–650.772327	–650.747849
[FH•••S]-PC	–650.783140	–650.737984	–650.729120	–650.820593	–650.796816	–650.771242	–650.862715	–650.838938
[FH•••S,O_a•••HF]-RC	–751.184748	–751.126241	–751.116035	–751.183142	–751.164911	–751.161225	–751.228331	–751.210100
[FH•••S,O_a•••HF]-TS	–751.149241	–751.093093	–751.082391	–751.165641	–751.143362	–751.129318	–751.212568	–751.190289
[FH•••S,O_a•••HF]-PC	–751.252979	–751.195391	–751.184505	–751.260743	–751.240313	–751.232797	–751.309035	–751.288605
[FH•••S,O_b•••HF]-RC	–751.184296	–751.125948	–751.115132	–751.186222	–751.167131	–751.163462	–751.234552	–751.215461
[FH•••S,O_b•••HF]-TS	–751.149790	–751.093849	–751.083040	–751.173015	–751.148936	–751.131141	–751.221115	–751.197036
[FH•••S,O_b•••HF]-PC	–751.247279	–751.190145	–751.179643	–751.260343	–751.238893	–751.227510	–751.308210	–751.286760

[2FH•••S,O_a,O_b•••HF]-RC	–851.636392	–851.565877	–851.552744	–851.617461	–851.599182	–851.608199	–851.672915	–851.654637
[2FH•••S,O_a,O_b•••HF]-TS	–851.606014	–851.537135	–851.524328	–851.601953	–851.580264	–851.577817	–851.655441	–851.633753
[2FH•••S,O_a,O_b•••HF]-PC	–851.714235	–851.643541	–851.630841	–851.699414	–851.680482	–851.684783	–851.753356	–851.734424
[Gua^+•••S,O_a,O_b]-RC	–756.152970	–756.032593	–756.019343	–756.068267	–756.053845	–756.070839	–756.119763	–756.105341
[Gua^+•••S,O_a,O_b]-TS	–756.128395	–756.008900	–755.996022	–756.053152	–756.036786	–756.045286	–756.102416	–756.086050
[Gua^+•••S,O_a,O_b]-PC	–756.247642	–756.124140	–756.111248	–756.136188	–756.128076	–756.162059	–756.186999	–756.178887
[FH•••S,O_a,O_b•••Gua^+]-RC	–856.612438	–856.478349	–856.463635	–856.497196	–856.486073	–856.517559	–856.551120	–856.539997
[FH•••S,O_a,O_b•••Gua^+]-TS	–856.588492	–856.455750	–856.441208	–856.491088	–856.476012	–856.494854	–856.544734	–856.529658
[FH•••S,O_a,O_b•••Gua^+]-PC	–856.691929	–856.556656	–856.541551	–856.561965	–856.554237	–856.597880	–856.618294	–856.610565
[HOH•••S,O_a,O_b•••Gua^+]-RC	–832.595104	–832.447602	–832.432049	–832.468686	–832.456909	–832.487169	–832.523806	–832.512030
[HOH•••S,O_a,O_b•••Gua^+]-TS	–832.570182	–832.425157	–832.409604	–832.459898	–832.444664	–832.464990	–832.515284	–832.500049
[HOH•••S,O_a,O_b•••Gua^+]-PC	–832.677281	–832.530132	–832.513895	–832.536540	–832.528158	–832.572145	–832.594790	–832.586408
[H_2C=NH•••S,O_a,O_b•••Gua^+]-RC	–850.874555	–850.711424	–850.694114	–850.730465	–850.717907	–850.755467	–850.791817	–850.779259
[H_2C=NH•••S,O_a,O_b•••Gua^+]-TS	–850.852237	–850.690197	–850.673358	–850.716831	–850.702505	–850.732682	–850.776155	–850.761828
[H_2C=NH•••S,O_a,O_b•••Gua^+]-PC	–850.961112	–850.796803	–850.779373	–850.796409	–850.788616	–850.842401	–850.859437	–850.851644
[H_2NH•••S,O_a,O_b•••Gua^+]-RC	–812.806219	–812.647766	–812.631179	–812.670748	–812.658063	–812.689889	–812.729458	–812.716773
[H_2NH•••S,O_a,O_b•••Gua^+]-TS	–812.784314	–812.626822	–812.610779	–812.658061	–812.643480	–812.667215	–812.714497	–812.699915
[H_2NH•••S,O_a,O_b•••Gua^+]-PC	–812.892554	–812.733422	–812.716201	–812.739468	–812.731054	–812.779724	–812.802990	–812.794576
[Gua^+•••S,O_a]-RC	–756.152970	–756.032593	–756.019343	–756.068267	–756.053845	–756.070839	–756.119763	–756.105341
[Gua^+•••S,O_a]-TS	–756.132072	–756.012405	–755.999585	–756.049863	–756.035489	–756.048753	–756.099031	–756.084657
[Gua^+•••S,O_a]-PC	–756.247642	–756.124140	–756.111248	–756.136188	–756.128076	–756.162059	–756.186999	–756.178887
[Gua^+•••S,O_a,O_b•••HF]-RC	–856.610174	–856.475545	–856.460277	–856.499320	–856.486667	–856.516776	–856.555819	–856.543166
[Gua^+•••S,O_a,O_b•••HF]-TS	–856.592375	–856.459350	–856.444647	–856.489810	–856.476073	–856.498857	–856.544019	–856.530283
[Gua^+•••S,O_a,O_b•••HF]-PC	–856.701733	–856.565484	–856.550605	–856.584469	–856.573681	–856.606782	–856.640646	–856.629857
[Gua^+•••S,O_a,O_b•••HOH]-RC	–832.592433	–832.446292	–832.429793	–832.472693	–832.458924	–832.488902	–832.531802	–832.518033
[Gua^+•••S,O_a,O_b•••HOH]-TS	–832.572499	–832.427472	–832.411969	–832.457593	–832.443665	–832.467267	–832.512892	–832.498964
[Gua^+•••S,O_a,O_b•••HOH]-PC	–832.680353	–832.533397	–832.517085	–832.539395	–832.531156	–832.577518	–832.599829	–832.591590

Table S4. Selected bond lengths (Å) and angles (degrees) for the gas-phase transition structures in Figure 4 of the main text and Figure S2. Calculations were performed at the B3-LYP/6-31+G(2df,p) level of theory.

Reaction	S–O_a	O_a–O_b	∠SO_aO_b	O_a•••H[a]	O_b•••H[b]
Uncat	2.373	1.804	170.5		
[FH•••O_a]	2.441	1.771	165.9	1.607	
[O_b•••HF]	2.597	1.666	159.4		1.515
[FH•••O_a,O_b•••HF]	2.573	1.684	156.5	1.574	1.542
[FH•••S,O_a•••HF]	2.261	1.893	169.3	1.559	
[FH•••S,O_b•••HF]	2.529	1.678	165.9		1.528
[2FH•••S,O_a,O_b•••HF]	2.530	1.687	162.9	1.615	1.555
[Gua^+•••S,O_a,O_b]	2.543	1.655	170.1	2.026	1.799
[FH•••S,O_a,O_b•••Gua^+]	2.557	1.666	171.9	1.745	1.783
[HOH•••S,O_a,O_b•••Gua^+]	2.583	1.652	168.6	1.781	1.774
[Gua^+•••S,O_a]	2.365	1.796	174.9	1.612	
[Gua^+•••S,O_a,O_b•••HF]	2.585	1.644	168.7	1.934	1.497
[Gua^+•••S,O_a,O_b•••HOH]	2.500	1.699	171.7	1.780	1.745

[a]Length of the hydrogen bond to O_a. [b]Length of the hydrogen bond to O_b.

Table S5. Reference gas-phase geometries. Geometries were optimized at the B3-LYP/6-31+G(2df,p) level of theory for all of the species considered in the theoretical investigation (Cartesian coordinates, Å).

HS^-			
H	0.000000	0.000000	–1.272201
S	0.000000	0.000000	0.079513
H_2O			
O	0.000000	0.000000	0.116673
H	0.000000	0.766022	–0.466693
H	0.000000	–0.766022	–0.466693
H_2O_2			
H	0.793783	0.906388	0.465504
O	0.000000	0.724255	–0.058188
O	0.000000	–0.724255	–0.058188
H	–0.793783	–0.906388	0.465504
HSOH			
S	–0.581447	–0.090054	0.009229
H	–0.867378	1.233607	0.017845
O	1.089774	0.023713	–0.117812
H	1.452347	0.017548	0.776984
$H_2C{=}NH$			
H	–0.897316	–1.054395	0.000000
N	0.056070	–0.683562	0.000000
C	0.056070	0.585598	0.000000
H	1.012861	1.113073	0.000000
H	–0.844458	1.212671	0.000000
$CH_6N_3^+$			
H	0.389036	2.006815	–0.151204
N	–0.311337	1.297256	–0.000002
H	–1.257452	1.611665	0.151229
C	0.000000	0.000000	–0.000005
N	1.279126	–0.379002	–0.000002
H	2.024468	0.283153	0.151229
H	1.543434	–1.340321	–0.151204
N	–0.967788	–0.918255	–0.000002
H	–1.932470	–0.666492	–0.151204

H –0.767016 –1.894817 0.151229
==========
HF
==========
F 0.000000 0.000000 0.092373
H 0.000000 0.000000 –0.831361
==========
NH_3
==========
N 0.000000 0.111122 0.000000
H –0.945753 –0.259088 0.000000
H 0.472877 –0.259384 0.818974
H 0.472877 –0.259384 –0.818974
==========
==========
==========
Uncat–RC
==========
O 1.547853 –0.731266 0.005801
O 1.547851 0.731266 0.005802
H 0.566898 0.896901 –0.019656
H 0.566901 –0.896901 –0.019653
S –1.514028 0.000000 –0.081942
H –1.674991 0.000000 1.257553
==========
Uncat–TS
==========
O –0.690851 –0.038291 –0.144520
O –2.484570 –0.063460 0.048525
H –2.548838 0.880857 –0.132104
H –0.578922 –0.391603 0.744995
S 1.678282 –0.058008 –0.009258
H 1.678619 1.252884 0.303203
==========
Uncat–PC
==========
O 0.102090 0.892248 0.031709
O –2.274403 –0.187394 0.019792
H –2.037269 –1.093238 –0.198999
H –1.364481 0.279669 0.033811
S 1.209118 –0.260043 –0.090896
H 1.434376 –0.664570 1.207507
==========
==========
==========
[FH•••O_a]–RC
==========
O 0.735862 0.332350 0.619168
O 0.376586 1.408541 –0.295580
H –0.549275 1.109270 –0.524030
H –0.117922 –0.219592 0.574728

H	2.018941	–0.351009	0.073786
S	–2.078983	–0.473692	–0.121999
H	–2.627500	0.190865	0.915406
F	2.848877	–0.786399	–0.186289

[$FH•••O_a$]–TS

O	–0.402768	–0.542484	0.089762
O	–2.024710	–1.222705	–0.118727
H	–1.977795	–1.704370	0.715509
H	–0.090715	–0.846989	–0.772070
H	–0.922231	0.977697	0.041358
S	1.975481	–0.007085	–0.044359
H	2.190804	–1.120373	0.683411
F	–1.265326	1.880989	0.030362

[$FH•••O_a$]–PC

O	0.735356	–0.401670	–0.631591
O	–3.264394	–0.443562	0.195556
H	–2.466746	0.159374	0.124955
H	–2.916361	–1.294261	–0.086150
H	–0.078937	0.268081	–0.347532
S	2.110967	–0.040597	0.173686
H	1.905613	–0.671245	1.364855
F	–1.109638	0.994384	–0.038536

[$O_b•••HF$]–RC

O	–0.376338	1.408733	–0.295901
O	–0.736066	0.333028	0.619280
H	0.117576	–0.219108	0.574996
H	0.549666	1.109392	–0.523722
S	2.078621	–0.473858	–0.121972
H	2.627295	0.189929	0.915941
H	–2.018438	–0.351208	0.073726
F	–2.848311	–0.786818	–0.186269

[$O_b•••HF$]–TS

O	0.131920	0.784627	–0.150912
O	–1.517787	0.986291	–0.040697
H	–1.509916	1.379030	0.840009
H	0.252537	–0.176394	–0.009338
S	2.451632	–0.377054	–0.044009
H	2.574088	0.024775	1.237229
H	–2.382142	–0.257569	–0.022193
F	–3.008193	–1.011591	0.021256

[O_b•••HF]–PC

O	0.849854	0.801501	0.207381
O	–1.168179	–0.568447	0.256284
H	–1.019913	–1.318041	–0.326814
H	–0.094502	0.169063	0.211761
S	2.133028	–0.117889	–0.196881
H	2.468976	–0.681233	1.000578
H	–2.407689	–0.104170	0.020297
F	–3.392080	0.217352	–0.162783

[FH•••O_a,O_b•••HF]–RC

O	0.196894	–0.727033	0.741671
O	0.197196	0.725465	0.742063
H	–0.723348	0.877602	0.327848
H	–0.723506	–0.878488	0.326941
H	1.421156	–1.449978	–0.012318
S	–2.474663	–0.000035	–0.393040
H	–3.068397	–0.000845	0.817475
F	2.118492	–1.990765	–0.390857
H	1.420470	1.449796	–0.011899
F	2.116565	1.992433	–0.390174

[FH•••O_a,O_b•••HF]–TS

O	–0.049446	–0.089895	0.062454
O	1.457636	0.141972	0.777726
H	1.517605	1.092096	0.596531
H	–0.602242	–0.413867	0.807459
H	–0.291911	1.437060	–0.232427
S	–2.539915	–0.726899	–0.038304
H	–2.144620	–1.884297	–0.602798
F	–0.125817	2.390962	–0.310117
H	2.530366	–0.639623	–0.006589
F	3.277366	–1.099584	–0.431077

[FH•••O_a,O_b•••HF]–PC

O	–1.729300	–0.440765	–0.598089
O	2.283249	–0.631560	–0.361710
H	1.416275	–0.581950	0.218789
H	1.978929	–0.509061	–1.265421
H	–0.937646	–0.479589	0.086974
S	–2.997553	0.384665	0.039567
H	–2.626805	1.668543	–0.217745
F	0.189384	–0.539843	0.905454
H	3.463534	0.227906	–0.092637
F	4.281167	0.772967	0.018476

[FH•••S]–RC			
O	–1.981048	1.111742	0.138317
O	–2.575225	–0.184913	–0.176736
H	–1.763282	–0.745764	–0.218753
H	–1.022863	0.864264	0.173536
H	2.249465	0.239539	–0.059355
S	0.540970	–0.837326	–0.012240
H	0.572721	–1.090836	1.310838
F	3.084292	0.746154	–0.078119
[FH•••S]–TS			
O	–1.262367	0.029536	–0.056144
O	–3.041470	0.675435	–0.051393
H	–2.881847	1.131447	0.782269
H	–1.687931	–0.803126	–0.286565
H	2.069310	0.519983	–0.036360
S	0.706390	–1.004106	–0.048842
H	0.682876	–1.131408	1.291085
F	2.771783	1.189892	–0.012074
[FH•••S]–PC			
O	–0.649511	0.784030	0.160595
O	–3.192357	0.072061	–0.087588
H	–3.088352	–0.793004	–0.493985
H	–2.239466	0.388471	0.024021
H	2.225229	0.064889	–0.075258
S	0.291646	–0.478035	–0.018347
H	0.435089	–1.009197	1.245347
F	3.192900	0.238742	–0.110070
[FH•••S,O_a•••HF]–RC			
O	–1.028736	–0.207692	–0.839366
O	–0.887332	1.225505	–0.988461
H	–0.317657	1.465742	–0.225299
H	–0.080425	–0.536130	–0.704316
H	–2.133047	–0.550020	0.220544
S	1.912489	–0.942185	–0.126376
H	1.523896	–1.640143	0.958974
F	–2.843851	–0.798856	0.828350
H	1.478122	0.738855	0.621474
F	1.094722	1.627095	0.924234

[FH•••S,O_a•••HF]–TS

O	–1.036202	–0.525611	0.213659
O	–2.861722	–0.467898	0.710616
H	–2.950970	0.484745	0.572968
H	–1.372660	–1.127449	–0.459680
H	–1.121950	0.934490	–0.324673
S	1.046777	–0.969879	–0.543790
H	1.168707	–1.844144	0.471288
F	–1.295013	1.848137	–0.612309
H	2.399877	0.360484	0.312078
F	3.107451	0.891642	0.693915

[FH•••S,O_a•••HF]–PC

O	–0.487498	–0.406042	–0.629714
O	–3.185947	–0.394640	0.298624
H	–2.963711	0.545043	0.368949
H	–2.344053	–0.743855	–0.051112
H	–0.806254	0.870570	–0.305048
S	0.846593	–1.028354	0.012010
H	0.609825	–0.978810	1.357909
F	–1.194835	1.797594	–0.034155
H	2.450983	0.274498	0.076870
F	3.294310	0.745925	0.146265

[FH•••S,O_b•••HF]–RC

O	0.961156	1.224766	–0.674592
O	1.627670	–0.069332	–0.692585
H	0.859097	–0.670441	–0.452042
H	0.035085	0.929247	–0.470828
S	–1.239759	–0.951173	–0.071518
H	–1.568146	–1.111811	–1.368371
H	2.813946	–0.030100	0.348555
F	3.583212	–0.061380	0.932398
H	–2.833225	0.240818	0.380780
F	–3.603348	0.796667	0.583559

[FH•••S,O_b•••HF]–TS

O	0.586159	–0.149055	0.385905
O	2.148742	0.082317	0.952394
H	1.964042	0.931666	1.370558
H	0.445097	–1.029510	0.764196
S	–1.722431	–1.029046	–0.153044
H	–1.356901	–0.980464	–1.447010
H	3.129619	0.273992	–0.203856
F	3.835926	0.413418	–0.865602

H –2.801470 0.689117 –0.120445
F –3.358226 1.488119 –0.092301
==========
[FH•••S,O_b•••HF]–PC
==========
O –0.135139 –0.437911 0.408517
O 2.121623 0.580879 0.126796
H 2.009029 1.178610 –0.617833
H 1.168125 0.116115 0.256235
S –1.252422 0.556461 –0.148369
H –1.559276 1.360466 0.921279
H 3.367840 –0.197867 –0.034821
F 4.238577 –0.654461 –0.159474
H –3.078475 –0.352279 –0.095383
F –3.989726 –0.695779 –0.100313
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[2HF•••S,O_a,O_b•••HF]–RC
==========
O –0.763677 0.516990 0.908868
O –1.260014 –0.823615 0.661001
H –0.418351 –1.250674 0.313544
H 0.202028 0.389556 0.635309
H –1.577912 1.722991 0.161532
S 1.689289 –0.992027 –0.045078
H 2.053914 –1.348042 1.202004
F –2.012932 2.497364 –0.189861
H –2.564334 –0.981496 –0.319397
F –3.331800 –1.211949 –0.838643
H 3.271364 0.300652 –0.346660
F 4.032976 0.880412 –0.469723
==========
[2HF•••S,O_a,O_b•••HF]–TS
==========
O –0.642020 –0.117638 –0.357396
O –2.277835 0.237175 –0.571243
H –2.216996 1.178638 –0.348193
H –0.448104 –0.439099 –1.253349
H –0.218625 1.429674 –0.175063
S 1.733116 –0.901877 –0.740642
H 1.398767 –1.966463 0.011625
F –0.329138 2.382450 –0.084179
H –3.128922 –0.550685 0.464834
F –3.742839 –1.008642 1.061190
H 3.078124 –0.130347 0.625472
F 3.756948 0.176416 1.240106
==========
[2HF•••S,O_a,O_b•••HF]–PC
==========
O 0.288016 0.029770 –0.826081

O	–2.352719	0.417709	–0.525984
H	–2.045655	1.234239	–0.099465
H	–1.488245	–0.014344	–0.736653
H	0.138675	1.227887	–0.200121
S	1.347723	–1.031252	–0.236158
H	0.908824	–1.234915	1.040595
F	–0.184633	2.105496	0.251604
H	–3.404375	–0.376252	0.243983
F	–4.106796	–0.863531	0.714297
H	3.147383	–0.147858	0.323763
F	4.035589	0.117083	0.592204

$[Gua^{+}\bullet\bullet\bullet S,O_a,O_b]$–RC

C	1.862532	–0.005458	0.011890
N	3.223479	0.082660	–0.056821
N	1.257844	–1.150325	–0.233416
N	1.178403	1.080123	0.363792
O	–1.582112	1.271612	0.565190
O	–1.840175	1.989203	–0.668106
H	–2.769326	2.234143	–0.554239
H	–1.794239	0.291189	0.325954
H	0.185366	–1.309285	–0.126566
H	1.803563	–1.943821	–0.531307
H	1.671952	1.920569	0.612761
H	0.141987	1.100256	0.437663
H	3.737454	–0.679792	–0.466553
H	3.650109	0.990763	–0.147122
S	–1.765273	–1.669773	–0.036004
H	–1.797470	–2.028641	1.262566

$[Gua^{+}\bullet\bullet\bullet S,O_a,O_b]$–TS

O	–1.267625	1.035044	–0.124388
O	–0.692839	2.580359	0.016434
H	–0.956594	2.891103	–0.859683
H	–2.072842	1.079302	0.417402
S	–2.378290	–1.247453	0.020418
H	–2.229143	–1.429276	1.349571
C	1.763604	–0.343583	–0.013314
N	3.022262	–0.860948	0.094860
H	3.813893	–0.254889	–0.047388
H	3.171140	–1.831939	–0.124685
N	1.611529	0.965191	0.167878
H	2.388245	1.505653	0.509926
H	0.703489	1.449378	0.093631
N	0.750025	–1.145088	–0.274991
H	0.924848	–2.122004	–0.450596
H	–0.275027	–0.903887	–0.185580

[Gua^{+}•••S,O_a,O_b]–PC			
C	2.168086	–0.226579	–0.035794
N	3.461676	–0.734584	–0.020844
N	1.205154	–1.167775	0.192220
N	1.865448	1.015954	–0.231364
O	–1.629356	–0.184096	0.538800
O	–0.579961	2.180733	0.019492
H	–0.555574	2.802444	0.753497
H	–1.404314	0.765840	0.331365
H	0.242023	–0.855266	0.290582
H	1.457437	–1.970255	0.746796
H	2.667754	1.569790	–0.515042
H	0.350046	1.810390	–0.076035
H	3.575418	–1.678499	–0.362349
H	4.183853	–0.105829	–0.336944
S	–2.978623	–0.646978	–0.329759
H	–3.918599	–0.395756	0.612616
[FH•••S,O_a,O_b•••Gua^{+}]–RC			
O	–2.146980	0.428390	0.777043
O	–2.671628	–0.464586	–0.238489
H	–3.595750	–0.183545	–0.281129
H	–1.549437	1.061506	0.243943
S	–0.009743	2.270505	–0.497837
H	0.052107	1.738820	–1.734090
H	1.512002	1.549009	0.106062
F	2.348020	1.034100	0.515613
C	0.645412	–1.522022	0.035289
N	1.889802	–1.494933	–0.460235
H	2.081332	–1.927035	–1.348921
H	2.446726	–0.677377	–0.197970
N	–0.316882	–2.255180	–0.555539
H	–0.166375	–2.569908	–1.500571
H	–1.280193	–2.034470	–0.322426
N	0.384456	–0.876836	1.168002
H	1.081332	–0.186862	1.439538
H	–0.583278	–0.594772	1.334679
[FH•••S,O_a,O_b•••Gua^{+}]–TS			
O	–0.800423	1.622015	–0.135259
O	0.416493	2.756576	–0.057307
H	0.226190	3.187511	–0.901365
H	–1.399110	1.979748	0.539076
S	–2.763333	–0.003848	0.074956
H	–2.777114	–0.160080	1.415349

H	–1.888786	–1.720253	–0.186045
F	–1.319801	–2.537279	–0.274381
C	1.960536	–0.467653	0.060831
N	2.846862	–1.494553	0.128473
H	3.788368	–1.356840	–0.199781
H	2.494787	–2.437902	0.092627
N	2.422001	0.787698	0.006131
H	3.382725	0.977829	0.236234
H	1.753625	1.577912	–0.033426
N	0.669503	–0.699486	0.070879
H	0.244803	–1.620035	–0.041348
H	0.005716	0.090766	0.085972

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[FH•••S,O_a,O_b•••Gua^+]–PC

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O	–1.482773	0.674966	–0.719746
O	–0.018958	2.706473	–0.049017
H	0.150697	3.393722	–0.700859
H	–1.090807	1.570038	–0.478567
S	–2.530325	0.156500	0.457283
H	–1.671444	0.042383	1.502375
H	–1.925314	–1.904054	0.028528
F	–1.415262	–2.687014	–0.128380
C	2.134752	–0.223129	–0.004809
N	3.156258	–1.057881	0.417477
H	4.005683	–0.609661	0.722760
H	3.307532	–1.900742	–0.117242
N	2.108987	1.060522	0.181810
H	2.973418	1.410549	0.582909
H	0.821360	2.146322	0.021885
N	1.114076	–0.864716	–0.643137
H	0.923481	–1.827725	–0.407149
H	0.278037	–0.319926	–0.829825

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[HOH•••S,O_a,O_b•••Gua^+]–RC

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O	–2.121273	0.396551	0.842016
O	–2.725327	–0.501975	–0.123433
H	–3.646045	–0.208620	–0.113107
H	–1.562965	1.042120	0.256865
S	–0.140984	2.228500	–0.554625
H	–0.133334	1.695077	–1.792230
H	1.718763	1.494775	0.127144
C	0.652021	–1.494433	0.004149
N	1.900117	–1.526594	–0.488976
H	2.053006	–1.920407	–1.402979
H	2.503174	–0.747044	–0.211004
N	–0.346696	–2.146187	–0.617101
H	–0.211613	–2.426033	–1.575206

H	–1.300941	–1.897382	–0.368483
N	0.421199	–0.880303	1.160247
H	1.151082	–0.243906	1.470487
H	–0.535822	–0.552991	1.324483
O	2.512044	1.012751	0.532089
H	3.162421	1.687986	0.748575

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[HOH•••S,O_a,O_b•••Gua^+]–TS

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O	–0.701629	1.709733	–0.186425
O	0.510364	2.829124	–0.106573
H	0.342539	3.249928	–0.960261
H	–1.332158	2.057361	0.466525
S	–2.724739	0.139799	0.153379
H	–2.623863	–0.013765	1.490599
H	–1.889733	–1.917477	–0.106624
C	1.895521	–0.508870	0.049487
N	2.760400	–1.545783	0.210367
H	3.732893	–1.418898	–0.015584
H	2.402781	–2.485245	0.145864
N	2.378358	0.740439	0.058717
H	3.304390	0.907972	0.415068
H	1.736677	1.549148	–0.028103
N	0.612781	–0.732404	–0.099590
H	0.195283	–1.660910	–0.207441
H	–0.053393	0.054889	–0.077871
O	–1.323951	–2.728728	–0.221958
H	–1.731758	–3.223352	–0.939976

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[HOH•••S,O_a,O_b•••Gua^+]–PC

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O	–1.582448	–0.685340	0.724544
O	–0.106127	–2.759647	0.106040
H	0.047153	–3.425453	0.783528
H	–1.194287	–1.579123	0.491445
S	–2.518960	–0.124460	–0.531103
H	–1.551738	0.093893	–1.457703
H	–1.663491	2.200777	–0.006279
C	2.077103	0.129360	0.002488
N	3.068403	0.969085	–0.478369
H	3.915129	0.528047	–0.800688
H	3.218863	1.832077	0.023029
N	2.059844	–1.157737	–0.162488
H	2.907199	–1.504115	–0.601413
H	0.745058	–2.221527	0.022441
N	1.077410	0.767762	0.675707
H	0.844392	1.721791	0.428852
H	0.257576	0.210514	0.893359
O	–1.022626	2.918788	0.131483
H	–1.435101	3.514146	0.765670

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[H_2C=NH•••S,O_a,O_b•••Gua^+]–RC
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O	–2.554959	–0.954167	–0.579915
O	–3.307487	–1.068813	0.653215
H	–3.879427	–1.823829	0.455415
H	–1.698614	–1.482558	–0.386354
S	0.190360	–2.183429	–0.103316
H	0.022658	–2.316957	1.227153
H	2.518529	–0.971856	0.005248
C	–0.347398	1.918472	–0.029604
N	0.046251	3.206368	0.186869
H	–0.524470	3.955350	–0.170785
H	1.032772	3.398265	0.253170
N	–1.645850	1.680049	–0.235920
H	–2.322065	2.388269	–0.004219
H	–1.990797	0.714494	–0.379015
N	0.531643	0.945274	–0.018553
H	1.537592	1.100026	0.030376
H	0.268612	–0.081681	–0.041787
N	3.179264	–0.178754	0.044980
C	4.385958	–0.565852	0.050859
H	5.191509	0.172155	0.090894
H	4.686998	–1.619266	0.017397

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[H_2C=NH•••S,O_a,O_b•••Gua^+]–TS
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O	–1.197233	–1.737713	–0.228179
O	–2.838246	–1.617367	–0.198534
H	–3.004576	–1.982979	–1.077583
H	–1.027823	–2.497450	0.357042
S	1.296628	–2.266763	0.205208
H	1.284368	–2.033353	1.534950
H	2.490585	–0.130855	–0.091954
C	–1.201469	1.634955	0.082512
N	–1.043817	2.978590	0.248476
H	–1.782158	3.595659	–0.047902
H	–0.109962	3.354122	0.200835
N	–2.442293	1.140344	0.016139
H	–3.217169	1.721099	0.289645
H	–2.613394	0.122573	–0.083347
N	–0.148747	0.856891	0.010394
H	0.812631	1.211657	–0.038145
H	–0.256286	–0.168130	–0.002878
N	2.704529	0.883086	–0.150977
C	3.945219	1.107426	–0.270521
H	4.310577	2.135626	–0.342921
H	4.700780	0.314223	–0.307531

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[H_2C=NH•••S,O_a,O_b•••Gua^+]–PC

O	0.268338	–1.789549	–0.705793
O	–2.279019	–2.095730	–0.129483
H	–2.850528	–2.420740	–0.832006
H	–0.659127	–2.093173	–0.484313
S	1.324568	–2.268825	0.488886
H	0.972585	–1.394006	1.464529
H	2.545881	0.322200	–0.108363
C	–1.667512	1.461190	0.014348
N	–1.747319	2.770099	0.467574
H	–2.663833	3.094532	0.733153
H	–1.202004	3.455652	–0.034327
N	–2.603130	0.574464	0.166613
H	–3.445979	0.964758	0.576053
H	–2.470258	–1.108438	–0.029526
N	–0.497619	1.154127	–0.610562
H	0.350213	1.657167	–0.369883
H	–0.333602	0.171827	–0.805763
N	2.542273	1.347562	–0.125778
C	3.718163	1.821177	–0.082260
H	3.858050	2.904582	–0.092886
H	4.627636	1.211112	–0.034099

$[H_2NH{\bullet\bullet\bullet}S,O_a,O_b{\bullet\bullet\bullet}Gua^+]$–RC

O	–2.419477	0.117683	–0.580173
O	–3.137710	0.363706	0.654299
H	–3.992745	–0.045210	0.459521
H	–1.905363	–0.749017	–0.388354
S	–0.564665	–2.250062	–0.106142
H	–0.791743	–2.295983	1.221487
H	2.215808	–2.237812	0.040893
C	0.866465	1.628533	–0.030536
N	1.820932	2.580189	0.186592
H	1.674106	3.505643	–0.183346
H	2.780733	2.281420	0.250793
N	–0.389682	2.032273	–0.246634
H	–0.652393	2.976248	–0.017648
H	–1.148299	1.341650	–0.384842
N	1.180852	0.356709	–0.010543
H	2.139966	0.005539	0.045683
H	0.465294	–0.426016	–0.036727
N	3.136229	–1.786267	0.082565
H	3.602663	–2.137621	0.914575
H	3.666987	–2.120481	–0.717404

$[H_2NH{\bullet\bullet\bullet}S,O_a,O_b{\bullet\bullet\bullet}Gua^+]$–TS

O	0.608699	–1.793030	–0.199401

O	–0.592283	–2.909655	–0.101572
H	–0.455026	–3.316315	–0.967515
H	1.297400	–2.162722	0.381672
S	2.691368	–0.288167	0.141084
H	2.544473	–0.114090	1.471986
H	1.936086	2.137371	–0.206949
C	–1.825558	0.538324	0.065808
N	–2.693482	1.581772	0.206005
H	–3.649170	1.459428	–0.086895
H	–2.322951	2.514448	0.114134
N	–2.322530	–0.704319	0.043192
H	–3.273708	–0.857140	0.333834
H	–1.702911	–1.531162	–0.027232
N	–0.538959	0.765548	–0.023764
H	–0.125227	1.707027	–0.103933
H	0.132082	–0.016834	–0.020553
N	1.310353	2.953333	–0.282991
H	1.582793	3.608625	0.444799
H	1.508620	3.399233	–1.174848

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$[H_2NH•••S,O_a,O_b•••Gua^+]$–PC

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O	1.868659	0.412007	–0.525890
O	1.766011	–2.192349	–0.176863
H	1.955242	–2.731274	–0.951145
H	2.003920	–0.571648	–0.405714
S	3.055522	1.252300	0.286309
H	2.524747	1.234530	1.534022
H	–2.144849	3.456620	–0.229325
C	–1.656681	–1.027665	0.067900
N	–3.014806	–1.013361	0.370487
H	–3.477043	–1.908935	0.348611
H	–3.558305	–0.267385	–0.039181
N	–0.903015	–2.079248	0.182648
H	–1.421804	–2.892638	0.497431
H	0.762678	–2.211127	–0.047158
N	–1.160389	0.165241	–0.338705
H	–1.732648	1.000861	–0.270531
H	–0.154965	0.282517	–0.407053
N	–2.902188	2.785967	–0.123703
H	–3.316806	2.949184	0.789914
H	–3.603008	3.021027	–0.821271

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$[Gua^+•••S,O_a]$–RC

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C	1.862532	–0.005458	0.011890
N	3.223479	0.082660	–0.056821
N	1.257844	–1.150325	–0.233416
N	1.178403	1.080123	0.363792

O	–1.582112	1.271612	0.565190
O	–1.840175	1.989203	–0.668106
H	–2.769326	2.234143	–0.554239
H	–1.794239	0.291189	0.325954
H	0.185366	–1.309285	–0.126566
H	1.803563	–1.943821	–0.531307
H	1.671952	1.920569	0.612761
H	0.141987	1.100256	0.437663
H	3.737454	–0.679792	–0.466553
H	3.650109	0.990763	–0.147122
S	–1.765273	–1.669773	–0.036004
H	–1.797470	–2.028641	1.262566

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$[Gua^{+}•••S,O_a]$–TS

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C	1.818487	–0.065567	–0.006336
N	3.164441	0.132202	0.070220
N	1.331507	–1.299297	0.064997
N	1.006286	0.967153	–0.126454
O	–1.637909	0.741476	0.050353
O	–1.472483	2.529363	–0.000374
H	–1.998691	2.681889	0.794488
H	–2.179513	0.736529	–0.749668
H	0.290790	–1.471704	0.058115
H	1.949197	–2.087123	0.167188
H	1.356340	1.910781	–0.112723
H	–0.038214	0.859055	–0.111280
H	3.784625	–0.651950	–0.045239
H	3.540071	1.029910	–0.188391
S	–1.819909	–1.612589	–0.090809
H	–2.129490	–1.579679	1.217292

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$[Gua^{+}•••S,O_a]$–PC

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C	2.168086	–0.226579	–0.035794
N	3.461676	–0.734584	–0.020844
N	1.205154	–1.167775	0.192220
N	1.865448	1.015954	–0.231364
O	–1.629356	–0.184096	0.538800
O	–0.579961	2.180733	0.019492
H	–0.555574	2.802444	0.753497
H	–1.404314	0.765840	0.331365
H	0.242023	–0.855266	0.290582
H	1.457437	–1.970255	0.746796
H	2.667754	1.569790	–0.515042
H	0.350046	1.810390	–0.076035
H	3.575418	–1.678499	–0.362349
H	4.183853	–0.105829	–0.336944
S	–2.978623	–0.646978	–0.329759
H	–3.918599	–0.395756	0.612616

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[Gua^{+}•••S,O_a,O_b•••HF]–RC
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C –1.976990 –0.225747 0.072552
N –2.875305 –1.228992 –0.081082
N –2.287812 1.018455 –0.282649
N –0.795828 –0.462674 0.620556
O 2.572081 0.009167 0.474198
O 2.383960 –0.822519 –0.699235
H 3.288803 –0.907606 –1.029698
H 2.023187 0.828304 0.232948
H –1.517608 1.723331 –0.249163
H –3.211405 1.264077 –0.595360
H –0.395956 –1.396444 0.627562
H –0.143878 0.347227 0.627053
H –3.647316 –1.115356 –0.716700
H –2.589004 –2.173719 0.120623
S 0.611701 2.348530 –0.073041
H 0.645619 2.880691 1.163811
F 0.903643 –2.710419 0.085303
H 1.553779 –2.059434 –0.212945
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[Gua^{+}••• S,O_a,O_b•••HF]–TS
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C –1.951897 –0.493969 0.014688
N –2.843028 –1.516906 0.080089
N –2.386028 0.764891 –0.043776
N –0.658464 –0.737018 0.028091
O 1.886465 0.494605 0.109791
O 2.980255 –0.720291 –0.061060
H 3.433167 –0.611700 0.786417
H 2.204903 1.107031 –0.574185
H –1.663456 1.520595 –0.033164
H –3.353659 0.984941 0.121817
H –0.242867 –1.663474 –0.015035
H 0.006211 0.046827 0.037821
H –3.794759 –1.361449 –0.207955
H –2.502361 –2.462205 0.007244
S 0.302470 2.526426 –0.097048
H 0.504585 2.742773 1.215833
F 1.389258 –2.626258 –0.072246
H 2.055668 –1.897295 –0.064626
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[Gua^{+}••• S,O_a,O_b•••HF]–PC
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C 1.938557 –0.853371 0.062840
N 2.782221 0.052949 –0.403623
N 1.994897 –2.148333 –0.334717
N 1.051644 –0.477347 0.988999
O –1.659928 –0.396267 0.062338

O	–0.670676	1.935453	–0.686378
H	–1.177014	2.746700	–0.589929
H	–1.373994	0.516379	–0.241771
H	1.293743	–2.788470	0.001674
H	2.425320	–2.376071	–1.215579
H	1.061392	0.545836	1.141386
H	0.137533	–0.917766	0.994675
H	3.551446	–0.203528	–0.998442
H	2.510901	1.057404	–0.158501
S	–3.314911	–0.528953	–0.072771
H	–3.667336	–0.084141	1.158421
F	1.584519	2.076891	0.464876
H	0.228073	2.090741	–0.250807

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[Gua^{+}••• S,O_a,O_b•••HOH]–RC

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C	2.012168	0.303838	0.027699
N	2.933915	1.300951	0.073998
N	2.398745	–0.945672	–0.239924
N	0.740977	0.550067	0.265048
O	–2.564949	–0.179218	0.624551
O	–2.679193	0.615885	–0.582188
H	–3.589737	0.433624	–0.851119
H	–1.985931	–0.944364	0.317805
H	1.645807	–1.670847	–0.239653
H	3.366439	–1.211875	–0.166761
H	0.334485	1.485328	0.247210
H	0.113159	–0.280480	0.264193
H	3.813682	1.183834	–0.400829
H	2.618593	2.246181	0.220598
S	–0.462622	–2.397520	–0.105567
H	–0.411827	–2.806985	1.177413
H	–1.749773	2.181748	–0.103476
O	–1.032770	2.822392	0.076643
H	–1.126116	3.511236	–0.588404

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[Gua^{+}••• S,O_a,O_b•••HOH]–TS

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C	–1.897842	–0.339275	0.013538
N	–2.908418	–1.240753	0.145586
N	–2.172409	0.964021	0.021739
N	–0.656701	–0.759015	–0.109036
O	1.805556	0.478665	0.123450
O	2.769163	–0.920450	0.098208
H	3.174379	–0.784217	0.964404
H	2.257813	0.953676	–0.589579
H	–1.370190	1.641573	–0.007075
H	–3.088668	1.289452	0.281323
H	–0.391860	–1.743493	–0.158727

H	0.135771	–0.098714	–0.093400
H	–3.852500	–0.957141	–0.056504
H	–2.701112	–2.220035	0.033817
S	0.547976	2.621369	–0.151386
H	0.782847	2.824366	1.157704
H	1.715333	–2.309170	0.012081
O	1.022863	–3.013658	–0.077606
H	1.339655	–3.608773	–0.763528

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[Gua^{+}••• S,O_a,O_b•••HOH]–PC

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C	2.026435	0.086011	0.012045
N	3.118815	0.919359	0.179229
N	2.353785	–1.238336	–0.173514
N	0.798505	0.467215	0.028397
O	–1.436071	–0.817510	–0.019310
O	–2.892965	1.557072	–0.023523
H	–3.438677	1.606344	0.767693
H	–2.566690	0.635344	–0.062674
H	1.562063	–1.871853	–0.172685
H	3.170948	–1.579786	0.310013
H	0.650098	1.476163	0.030323
H	–0.506076	–0.342042	0.004192
H	3.958316	0.674264	–0.325495
H	2.917071	1.907829	0.185894
S	–1.198120	–2.451589	–0.070453
H	–1.222518	–2.727162	1.258770
H	–1.419257	2.671951	–0.000384
O	–0.585639	3.188353	–0.010821
H	–0.674310	3.797328	–0.750226

Figure S1. Characterization of the recombinant proteins by CD-spectroscopy. (**a**) CD spectra of the dimeric forms of the Prx3 WT and R146A, R146K, R146H, R146K, R146G, R123G and R123G/R146G mutants. (**b**) CD spectra of the dimeric forms of the Prx2 WT and R127K, R150K, R127K/R150K mutants. Bar graphs represent the analysis of the SD spectra using the CDSSTR, CONTINLL and SELCON3 algorithms. 'Tight' and 'loose' helices (H(r) and H(d)) and beta sheets (S(r) and S(d)), as well as turns and unordered bits were calculated by averaging the data obtained using the different algorithms.

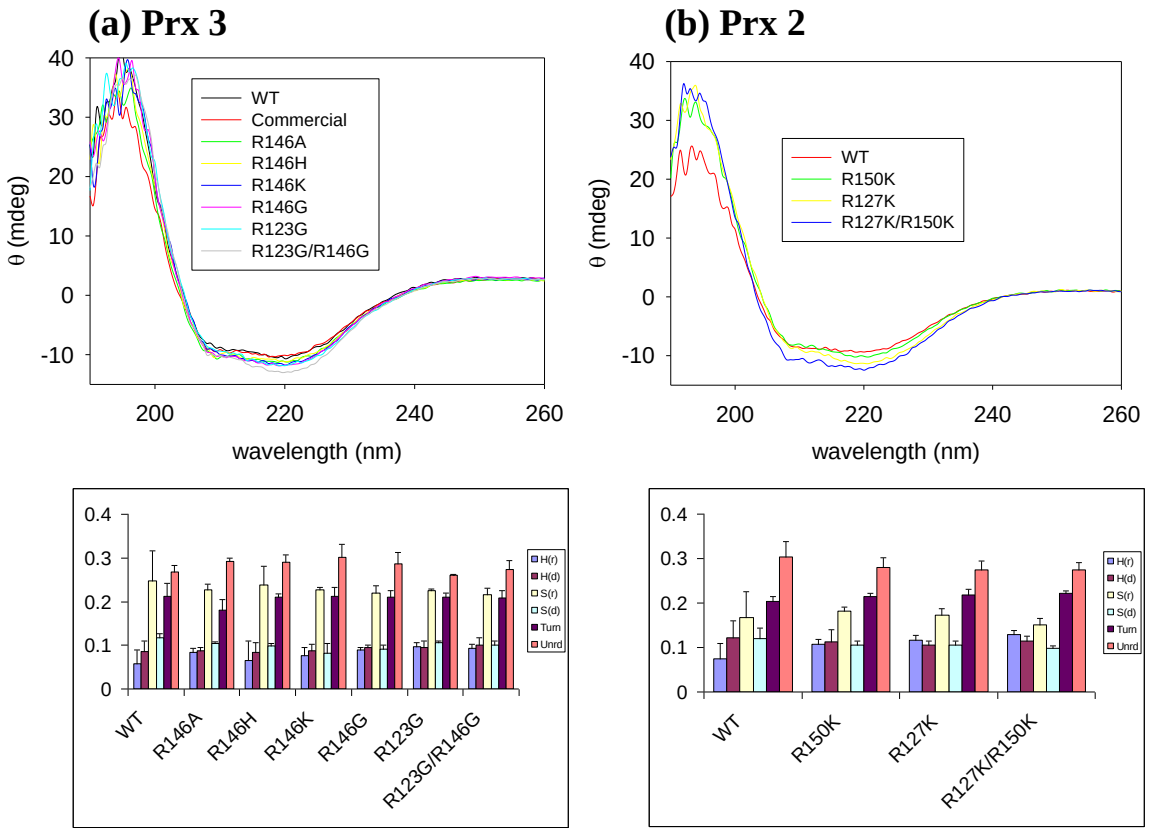

Figure S2. Ab initio calculations for the catalytic role of H-bonding interactions (by HF) in the reaction of HS^- with H_2O_2. Calculated reactant complexes (RC), transition structures (TS), and product complexes (PC) located on the potential energy surface for the uncatalyzed two-electron reduction of H_2O_2 by HS^- and the reactions catalyzed by HF moieties. Hydrogen bonds are shown as dashed lines, and partial bonds in the S_N2 transition structures as half dashed/half solid lines. (Atomic color scheme: H, white; O, red; F, cyan; S, yellow).

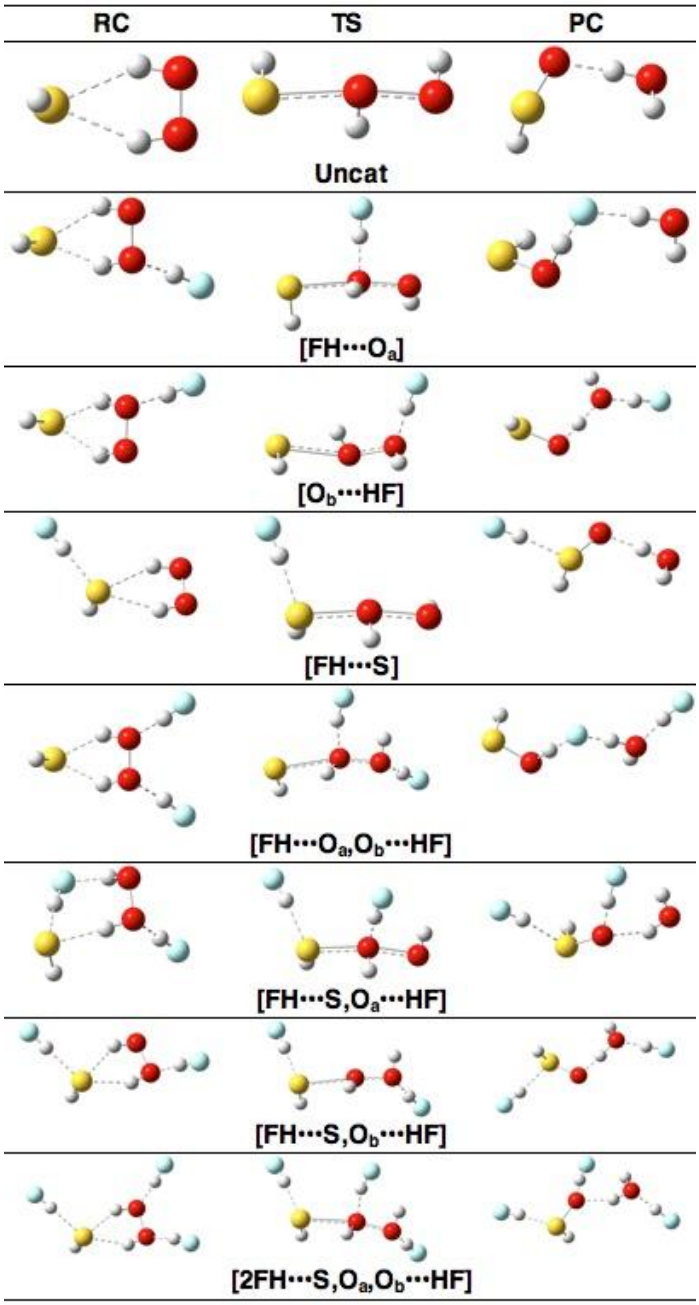

Figure S3. Purity of (a) Prx2 and (b) Prx3 WT and mutant proteins used in experiments. Prx proteins were purified as described and approximately 2 μg of each protein was separated by SDS–PAGE followed by Coomassie staining.

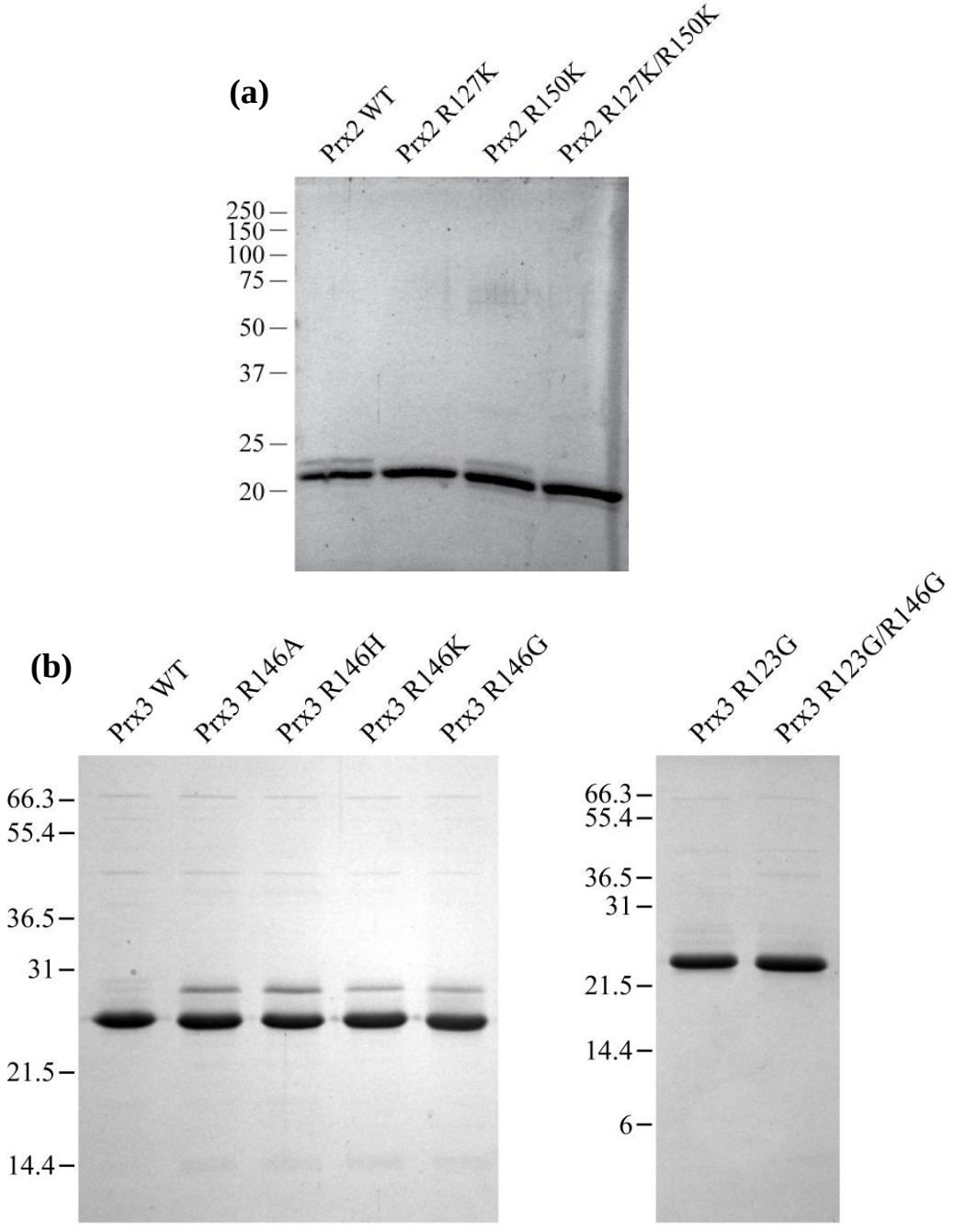

Figure S4. Schematic energy profile for the [Gua^{+}•••S,O$_a$,O$_b$] reaction. The energy profile (ΔH_{298}, kJ mol^{-1}, CPCM-G4//B3-LYP/6-31+G(2df,p)) is shown for the reactants, reactant complex (RC), transition structure (TS), product complex (PC) and products.