Supporting Information

MOLECULAR INSIGHTS INTO THE ROLE OF SELENOPROTEINS IN THE TOXICITY OF MERCURY

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1. Materials

Glutathione peroxidase from bovine erythrocytes (4.2 mg of lyophilized powder, 500 units/mg protein, referred to as GPx1 throughout the text) was purchased from Merck. GPx1 from Bos taurus erythrocytes shares high sequence homology (> 90 % BLAST [1] with the human variant. The Trx(488-499) peptide (Sequence: Ac-SGGDILQSGCUG-NH_2, HPLC Purity > 90 %) was synthetized in the MetMed Laboratories at the Department of Chemistry, University of Florence, following already established procedures.[2] Dithiothreitol (DTT), ammonium acetate, acetic acid, aqueous ammonia solution (35%), acetonitrile (ACN), trypsin from porcine pancreas and methyl mercury chloride (MeHgCl) were purchased from Sigma-Aldrich. Formic acid (FA) was purchased from Thermo Fisher Scientific.

Warning: *Methylmercury is highly toxic and must be handled with appropriate personal protection. This requires the use of highly-resistant laminate gloves, use of a fume hood, and approved breathing apparatus.*

2. Methods

2.1. Sample preparation

Thioredoxin reductase peptide: TrxR(488-499)

Stock solution of the TrxR(488-499) peptide at 0.9 mM was prepared by dissolving the sample in ultrapure water. Ammonium acetate buffer solution (2 mM, pH 7.0) was prepared by weighing ammonium acetate and dissolving it in ultrapure water, pH adjustment was carried out with acetic acid and ammonia commercial solutions (35% NH_3). For the peptide pre-reduction, an aliquot of its stock solution was diluted with 2 mM ammonium acetate solution at pH 7.0 to 0.1 mM peptide concentration. Then, an aliquot of DTT solution at 0.2 M in water was added to achieve peptide to reducing agent ratio of 1:10 (DTT at 1 mM) and the mixture was left under stirring for 30 minutes at 37 °C in a water bath. Aliquots of MeHgCl solution at 10 mM in water (0.1 % HCl) were added to achieve reduced peptide to MeHgCl ratios of 1:0.5 and 1:3 (MeHgCl at 0.05 and 0.3 mM, respectively). These mixtures were then left incubating for 5 min or 18 h at 37 °C. After the incubation, all the reaction media were sampled and diluted to a final peptide concentration of 6 µM, using 2 mM ammonium acetate at pH 7.0, 5 % (v/v) of acetonitrile (ACN) and 0.1 % (v/v) formic acid (FA) in order to carry out the LC-MS/MS analysis.

Bovine Glutathione peroxidase 1 (GPx1)

The GPx1 protein was dissolved in 1 mL of Milli Q water. Aliquots of Gpx1 protein were prepared by diluting the GPx1 stock solution to 0.1 mM with 2 mM ammonium acetate solution at pH 7.0. Then, an aliquot of DTT solution at 0.2 M in water was added to achieve protein to reducing agent ratio of 1:10 (DTT at 1 mM) and the mixture was left under stirring for 30 minutes at 37 °C in a water bath. Finally, aliquots of MeHgCl solution at 10 mM in water (0.1 % HCl) were added to achieve reduced protein to MeHgCl ratios of 1:0.5 and 1:3 (MeHgCl at 0.05 and 0.3 mM, respectively). These mixtures were then left incubating for 5 min or 3 h at 37 °C. After the incubation, all the reaction media were sampled and diluted to a final protein concentration of 7 µM using Milli Q water, 20% (v/v) of ACN and 0.2 % (v/v) of FA, and injected for LC-MS analysis.

Tryptic digestion of GPx1 + MeHg

The commercial trypsin was solubilized in 20 µL of HCl (1mM) to a final concentration of 1 µg/µL. An aliquot of this trypsin solution was then added to reduced GPx1 incubated with 0.5 equiv. of for 5 min at 37 °C as described above. After the incubation, the solution was sampled and diluted to a final protein concentration of 6 µM using H_2O, 20 % (v/v) ACN and 0.2 % (v/v) FA used for LC-MS analysis.

2.2. LC-MS/MS analysis

TrxR(488-499) and GPx1 tryptic peptides

LC separations were performed using Dionex ultimate 3000 series UHPLC (Thermo Fisher Scientific) coupled to an Orbitrap Q-Exactive Plus Mass Spectrometer (Thermo Fisher Scientific). The used column was AcclaimTM 120 (C18, 5 µm, 120 Å, 4.6 mm × 100 mm) (Thermo Fisher Scientific). The mobile phases were: (A) H_2O and (B) ACN, both containing 0.1% FA. The flow rate used in all LC-MS experiments was 1 $mL \cdot min^{-1}$, and sample elution was performed by using the gradient from 5% to 95% of B over 6.5 min. An injection volume of 10 µL was used. Ionization was performed using an electrospray ion source operating in positive ion mode with a capillary voltage of 3.80 kV and capillary temperature 400 °C. Sheath gas, auxiliary gas and sweep gas flow rate were set at 75, 20 and 1 (arbitrary units), respectively. Auxiliary gas temperature was set at 500 °C.

A set of MS/MS spectra were acquired using an isolation width of 1.0 and 7.0 m/z, and a screening of collision energies (from 10 to 40 values of Higher-energy collisional dissociation (HCD)) was carried

out. All MS data were analysed using Thermo Fisher Scientific Xcalibur Qual Browser and FreeStyle softwares.

GPx1

LC separations were performed using Dionex ultimate 3000 series UHPLC from Thermo Fisher Scientific coupled to Orbitrap Fusion™ Lumos™ Tribrid™ Mass Spectrometer from Thermo Fisher Scientific. A chromatographic column SUPELCO BIOshell A400 Protein C4 (3.4 µm, 400 Å, 15 cm × 1 mm) from Sigma-Aldrich was used. The mobile phases were: (A) H_2O and (B) ACN, both containing 0.1% FA. The flow rate used in all LC-MS experiments was 0.05 mL·min^{-1} and the chromatography gradient was set up as follows: 0–3 min, 20% B; 3–5 min, 20% to 40% B; 5–20 min, 40% to 80% B; 20-23 min, 80% B; 23–25 min, 80% to 20% B; 25–35 min, 20% B (column equilibration). 60°C for column oven and 2 µL of sample injection were applied. Ionization was performed using an electrospray ion source operating in positive ion mode with a capillary voltage of 3.5 kV and capillary temperature 300 °C was used. Sheath gas, auxiliary gas and sweep gas flow rate were set at 25, 5 and 1 (arbitrary units), respectively. Auxiliary gas temperature was set at 150 °C.

Using the above-described ESI parameters and instrument, diluted reaction media after 5 min of incubation of Gpx1 with 0.5 equiv. of MeHgCl were also investigated by CID-MS/MS. The ionization was performed on the m/z 990.4590 (z=23) and 1035.3500 (z=22) ions (corresponding to GPx1 + MeHg adduct). The scanning *m/z* range was performed in the automatic mode, the quadrupole isolation was applied with an isolation window of 3, MS2 CID activation time was set at 10 ms, different CID collision energies (30-60 % CID) were screened.

All MS and MS/MS data were processed using Thermo Fisher Scientific Xcalibur Qual Browser and FreeStyle softwares. Top-Down sequence coverage was carried out employing the Protein-Prospector software (from University of California San Francisco) which required to pinpoint manually the position of the custom modification that is here + MeHg (+ 217.0015 Da) for MeHgCl-induced metalation.

2.3. DFT calculations

Computational details

All calculations were performed using the Gaussian 16 package[3] and the B3LYP [4] functional with D3 dispersion correction of Grimme with Becke–Johnson damping (B3LYP-D3(BJ)).[5] All stationary points involved were fully optimized in gas phase. The mercury atom was described with the relativistic electron core potential SDD and associated basis set.[6] The 6-31G** basis set was employed for all other atoms. Frequency calculations were undertaken to confirm the nature of the stationary points, yielding

one imaginary frequency for transition states (TS), corresponding to the expected process, and all of them positive for *minima*. The connectivity of the transition states and their adjacent *minima* was confirmed by intrinsic reaction coordinate (IRC) calculations.[7] Standard thermodynamic corrections (T = 298 K, 1 atm) have been considered to express reaction paths in terms of standard Gibbs free energies.

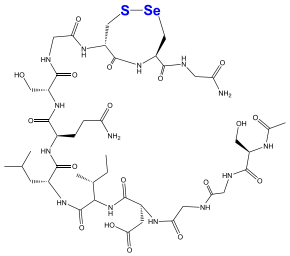
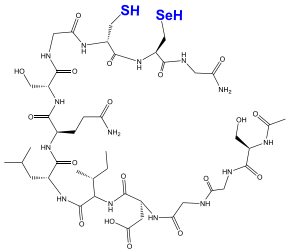
Further energy refinement was performed through single point calculations including solvent effects (water: H_2O) by means of the universal Solvation Model based on Density (SMD)[8] on the geometry optimized in gas phase. This level of theory has been named SMD(H_2O)-B3LYP-D3(BJ)/SDD(Hg), 6-31G**(other atoms)//B3LYP-D3(BJ)/ SDD(Hg), 6-31G**(other atoms). To further support our results, single-point calculations were also performed using the M06[9] functional, for comparison. This level of theory has been named SMD(H_2O)-M06/SDD(Hg), 6-31G**(other atoms)//B3LYP-D3(BJ)/SDD(Hg), 6-31G**(other atoms). Finally, the demethylation step from the mono-metalated complex, was also computed by optimizing the corresponding *minima* and transition state in solvent using SMD model at SMD(H_2O)-B3LYP-D3(BJ)/SDD(Hg), 6-31G**(other atoms) level of theory. The molecular orbitals (MO) and the geometrical structures were plotted with Chemcraft 1.8.[10]

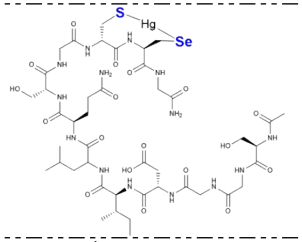
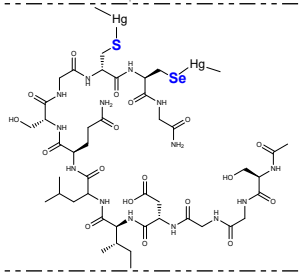
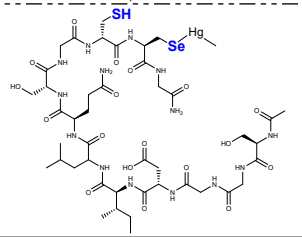
3. Results

3.1 LC-MS Analysis of TrxR(488-499) reactivity

3.1.1 List of identified species

Table S1. TrxR(488-499) peptide and formed adducts identified by mass spectrometry after incubation with MeHgCl.

Peptides	Chemical structure	Formula	Theoretical mass of the most abundant isotope ($Se^{79.9160}$ and $Hg^{201.9701}$) Da	Experimental mass of the most abundant isotope ($Se^{79.9160}$ and $Hg^{201.9701}$) Da	$\Delta M_{theor-exp}$ Da	Observed most abundant ions m/z	
						z=1 $[M+H]^+$	z=2 $[M+2H]^{2+}$
TrxR(488-499)		$C_{43}H_{70}N_{14}O_{18}SSe$	1182.3878	1182.3866	0.0012	1183.3939	592.1998
Reduced TrxR(488-499)		$C_{43}H_{72}N_{14}O_{18}SSe$	1184.4035	1184.4011	0.0024	1185.4089	593.2081

Hg-bridged TrxR(488-499)		$C_{43}h_{70}HgN_{14}O_{18}SSe$	1382.3580	1382.3566	0.0014	1383.3639	692.1835
Bis-metalated TrxR(488-499)		$C_{45}H_{76}Hg_2N_{14}O18SSe$	1614.3755	1614.3723	0.0032	1615.3796	807.6931
Mono-metalated TrxR(488-499)		$C_{44}h_{74}HgN_{14}O_{18}SSe$	1398.3894	1398.3864	0.0030	1399.3937	700.1991

3.1.2 LC-MS chromatograms

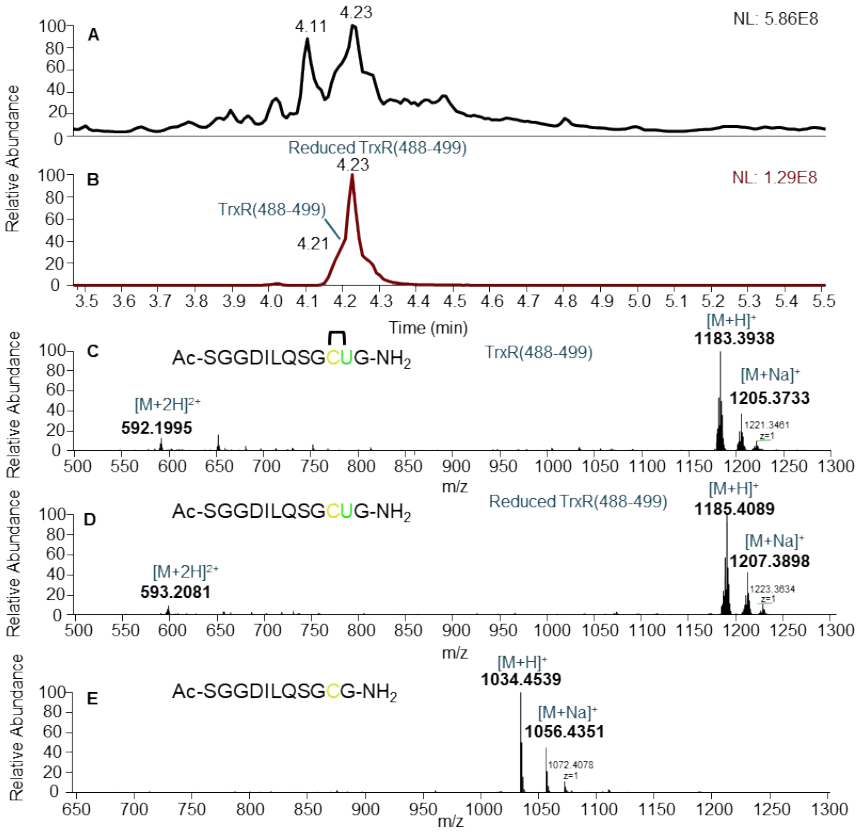


Figure S1. LC-MS of TrxR(488-499) peptide incubated (30 min) with 10 equivalents of reducing agent DTT at 37°C and pH 7.0. (A) TIC. (B) XIC chromatogram of ions m/z 1183.3938 (TrxR(488-499), t_R=4.21 min), 1185.4089 (reduced TrxR(488-499), t_R=4.23 min) (C) MS spectrum of peak at t_R=4.20 min. (D) MS spectrum of peak at t_R=4.23 min. (E) MS spectrum of peak at t_R=4.11 min.

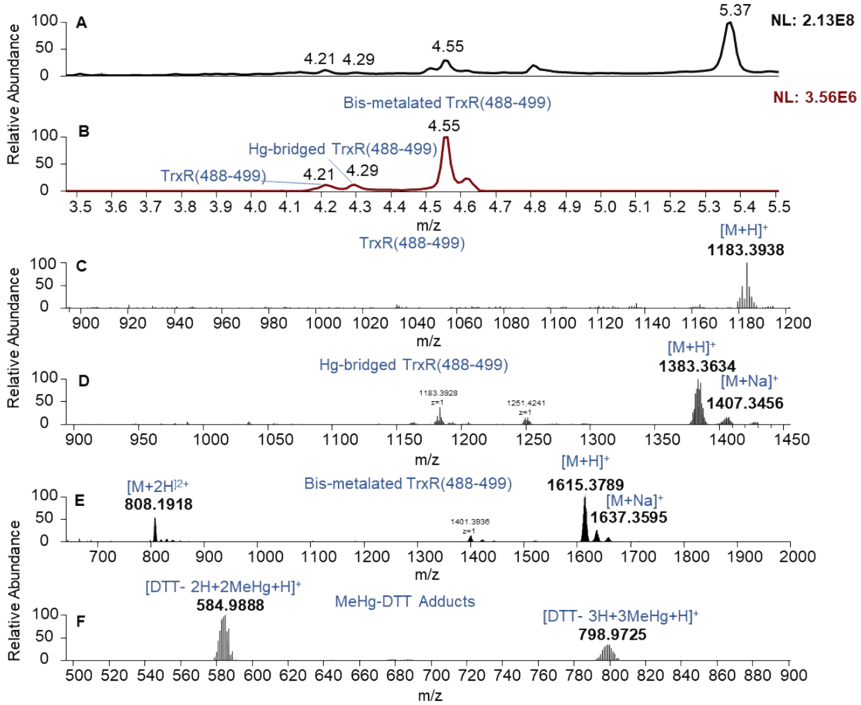


Figure S2. LC-MS of TrxR(488-499) peptide incubated (5 min) with MeHgCl (3 equiv.) at 37°C and pH 7.0, preceded by a 30 min. reduction step of the peptide with 10 equiv. of DTT. (A) TIC. (B) XIC chromatogram of ions m/z 1183.3938 (TrxR(488-499), t_R=4.21 min), 1383.3634 (Hg-bridged TrxR(488-499), t_R=4.29 min) and 1615.3789 (bis-metalated TrxR(488-499), t_R=4.55 min) (C) MS spectrum of peak at t_R=4.21 min. (D) MS spectrum of peak at t_R=4.29 min. (E) MS spectrum of peak at t_R=4.55 min. (F) MS spectrum of peak at t_R=5.37 min: DTT-Hg adducts (due to the competitive reactivity of DTT with MeHgCl).

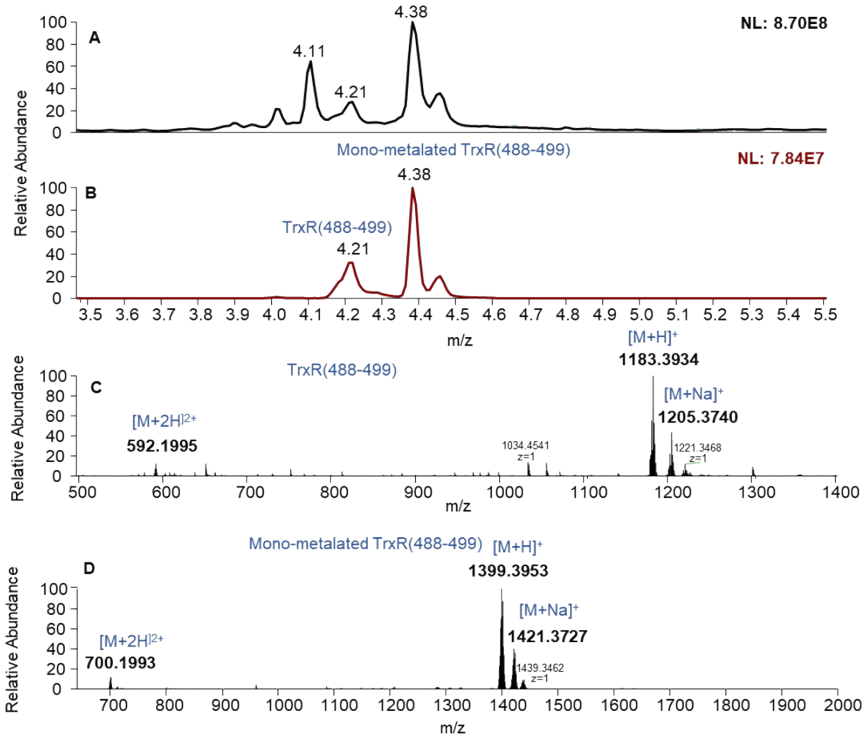


Figure S3. LC-MS of TrxR(488-499) peptide incubated (5 min) with MeHgCl (0.5 equiv.) at 37°C and pH 7.0, preceded by a 30 min. reduction step of the peptide with 10 equiv. of DTT. (A) TIC. (B) XIC chromatogram of ions m/z 1183.3934 (TrxR(488-499), t_R =4.21 min) and 1399.3953 (mono-metalated TrxR(488-499), t_R =4.38 min) (C) MS spectrum of peak at t_R=4.21 min. (D) MS spectrum of peak at t_R=4.38 min.

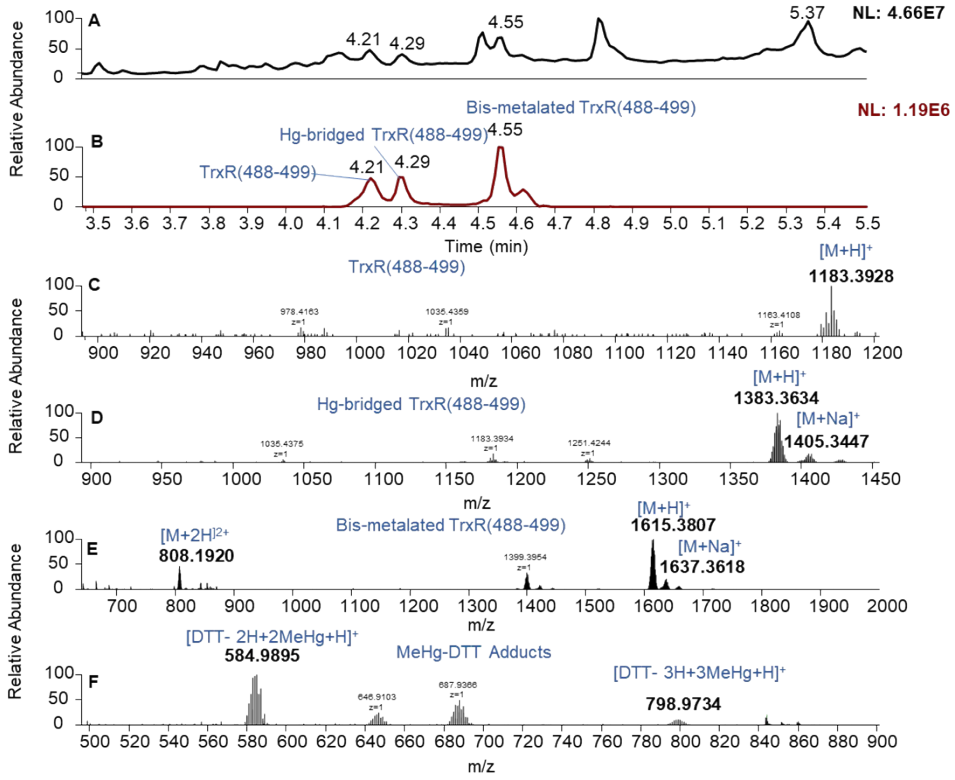


Figure S4. LC-MS of TrxR(488-499) peptide incubated (18 h) with MeHgCl (0.5 equiv.) at 37°C and pH 7.0, preceded by a 30 min. reduction step of the peptide with 10 equiv. of DTT. (A) TIC. (B) XIC chromatogram of ions m/z 1183.3928 (TrxR(488-499), t_R =4.21 min), 1383.3634 (Hg-bridged TrxR(488-499), t_R =4.29 min) and 1615.3807 (bis-metalated TrxR(488-499), t_R =4.55 min) (C) MS spectrum of peak at t_R=4.21 min. (D) MS spectrum of peak at t_R=4.29 min. (E) MS spectrum of peak at t_R=4.55 min. (F) MS spectrum of peak at t_R=5.37 min: DTT-Hg adducts (due to the competitive reactivity of DTT with MeHgCl).

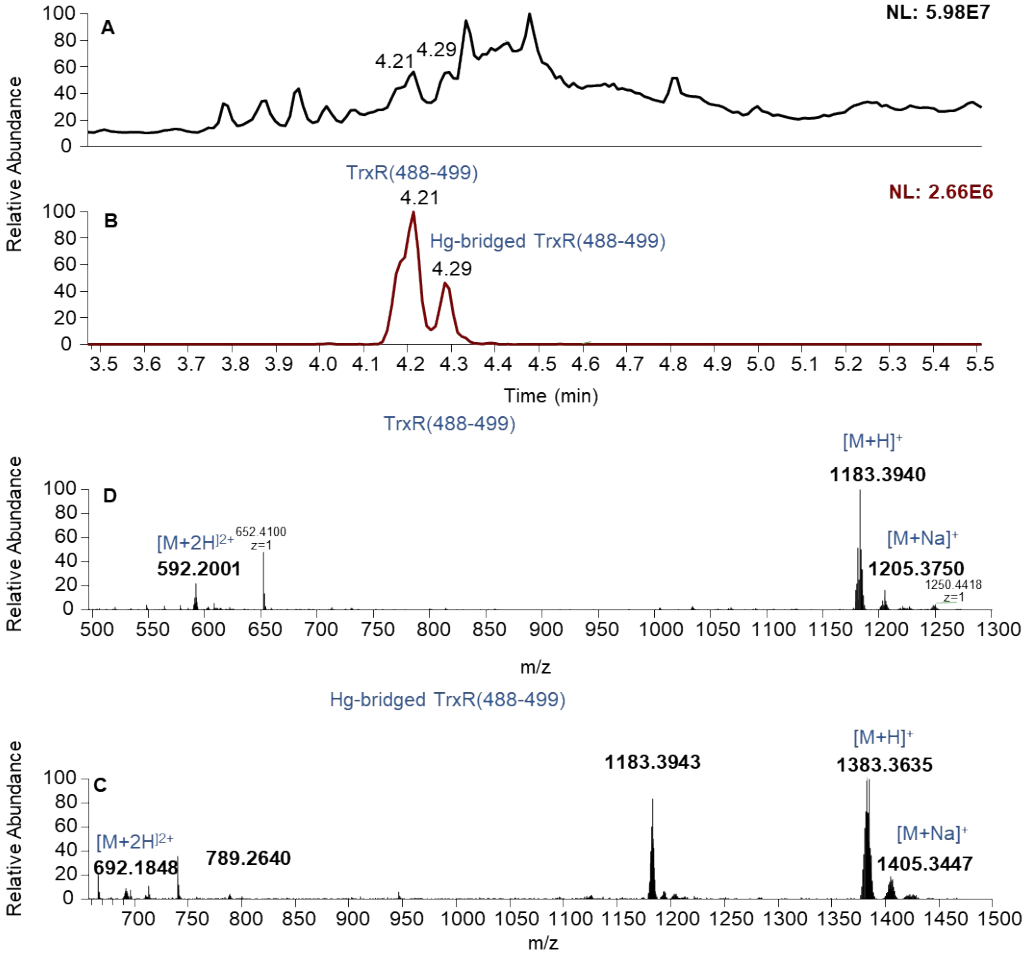


Figure S5. LC-MS of TrxR(488-499) peptide incubated (18 h) with MeHgCl (3 equiv.) at 37°C and pH 7.0 in the absence of DTT. (A) TIC. (B) XIC chromatogram of ions m/z 1183.3940 (TrxR(488-499), t_R =4.21 min) and 1383.3635 (Hg-bridged TrxR(488-499), t_R =4.29 min) (C) MS spectrum of peak at t_R=4.21 min. (D) MS spectrum of peak at t_R=4.29 min.*

*Note that when TrxR(488-499) was reacted 18 h with 3 equiv. of MeHgCl in the absence of DTT, a significant amount of the peptide remained unreacted and only a small amount of the Hg-bridged adduct was formed.

3.1.3 Comparison of theoretical and experimental isotopic patterns

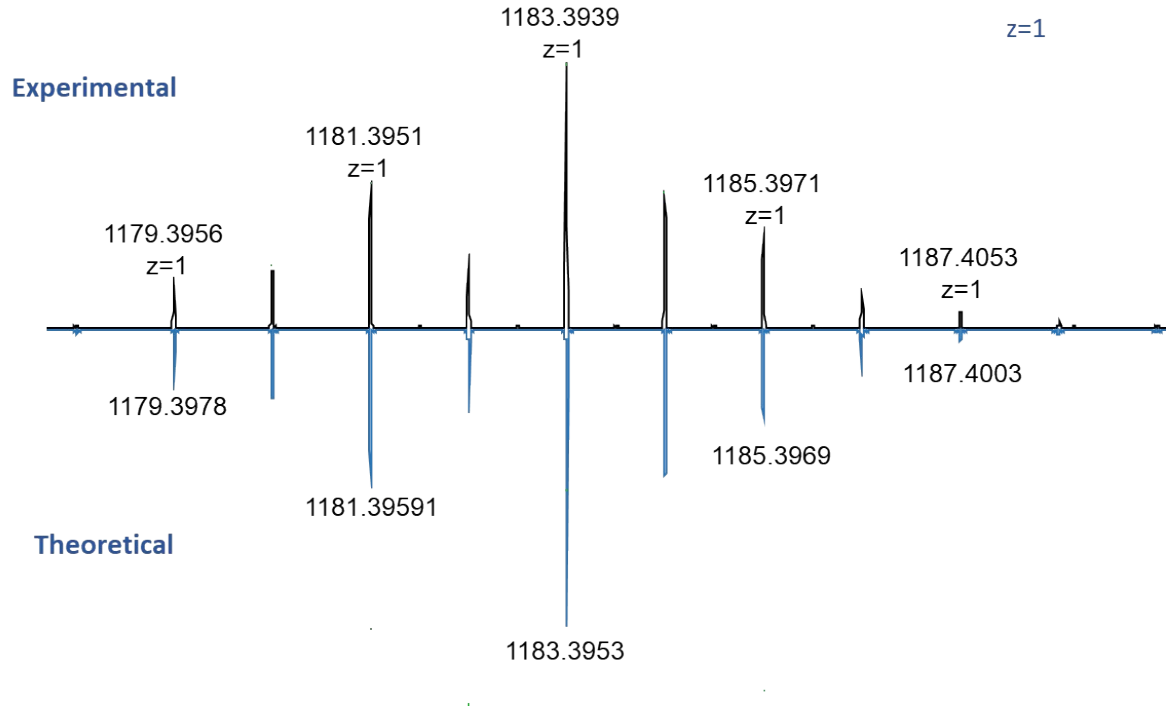


Figure S6. Unreacted TrxR(488-499) peptide, 1183.3939 ion (m/z): comparison of experimental and theoretical (calculated as $[M+H]^+$, $C_{43}H_{71}N_{14}O_{18}SSe$) isotopic patterns.

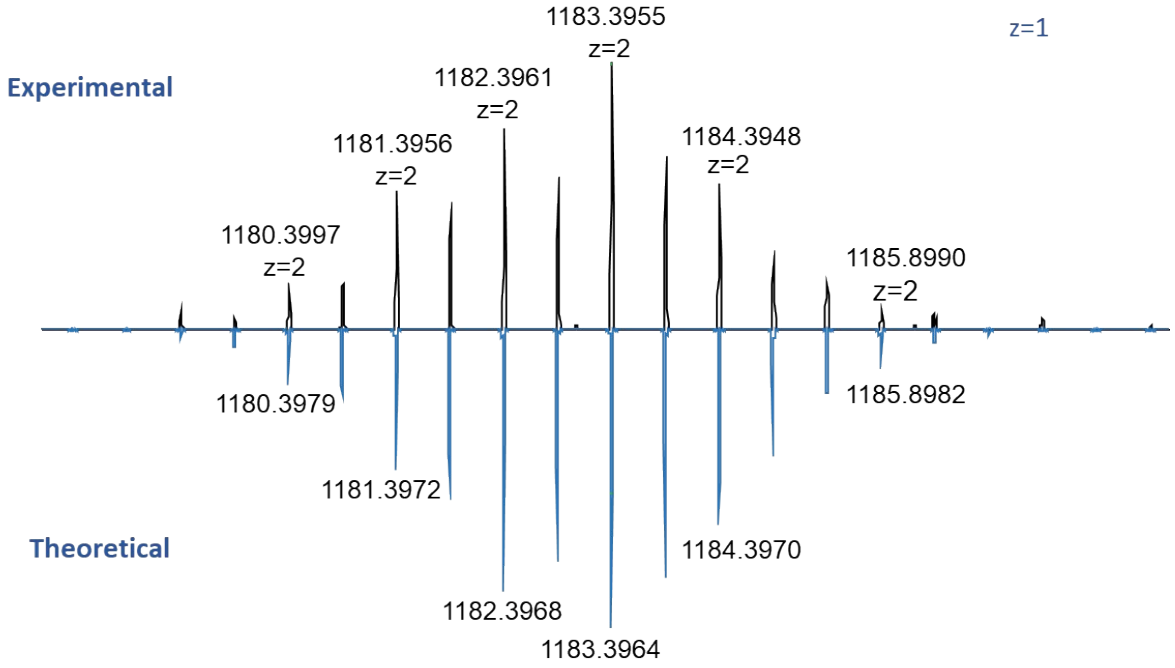


Figure S7. TrxR(488-499) dimer, 1183.3955 ion (m/z): comparison of experimental and theoretical (calculated as $[M+2H]^{2+}$, $C_{86}H_{141}N_{28}O_{36}S_2Se_2$) isotopic patterns.

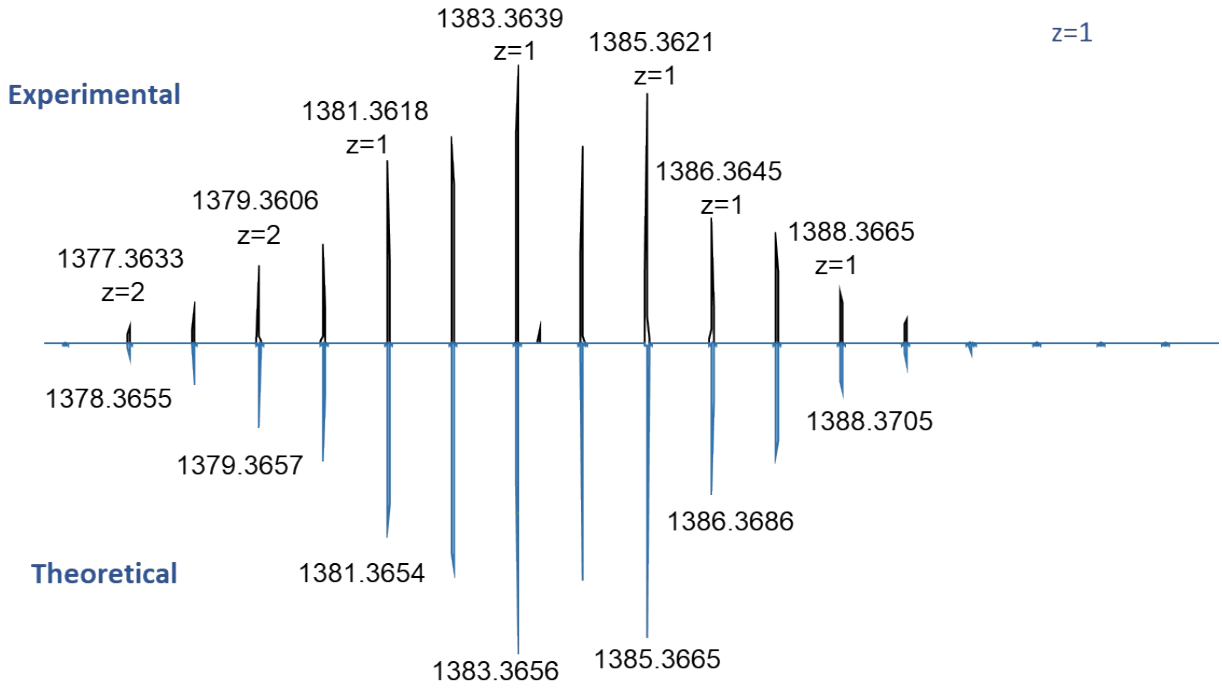


Figure S8. Hg-bridged TrxR(488-499) peptide, 1383.3639 ion (m/z): comparison of experimental and theoretical (calculated as $[M+H]^+$, $C_{43}H_{71}HgN_{14}O_{18}SSe$) isotopic patterns.

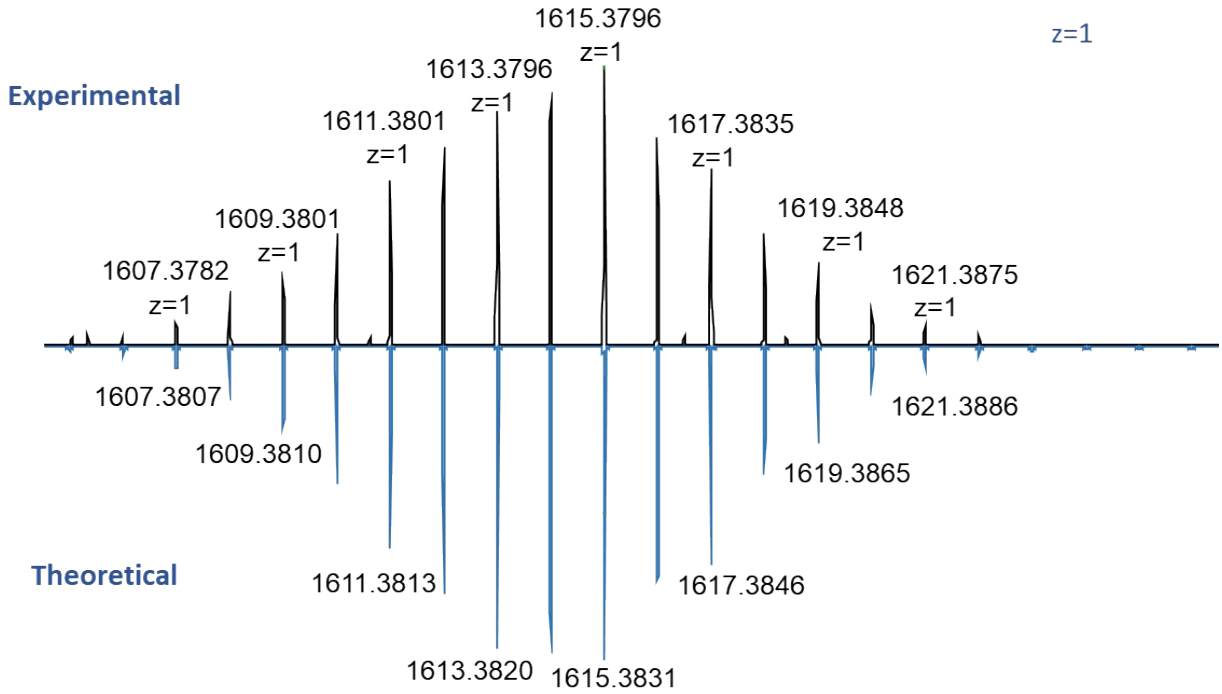


Figure S9. Bis-metalated TrxR(488-499) peptide, 1615.3796 ion (m/z): comparison of experimental and theoretical (calculated as $[M+H]^+$, $C_{45}H_{77}Hg_2N_{14}O_{18}SSe$) isotopic patterns.

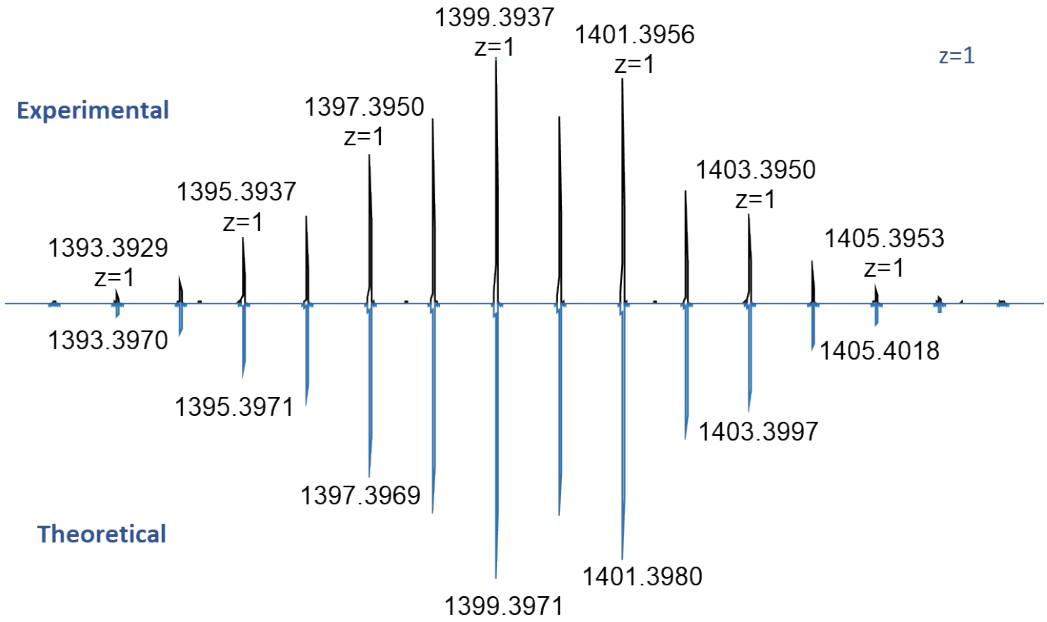


Figure S10. Mono-metalated TrxR(488-499) peptide, 1399.3937 ion (m/z): comparison of experimental and theoretical (calculated as $[M+H]^+$, $C_{44}H_{75}HgN_{14}O_{18}SSe$) isotopic patterns.

3.1.4 MS/MS of Hg-TrxR(488-499) adducts

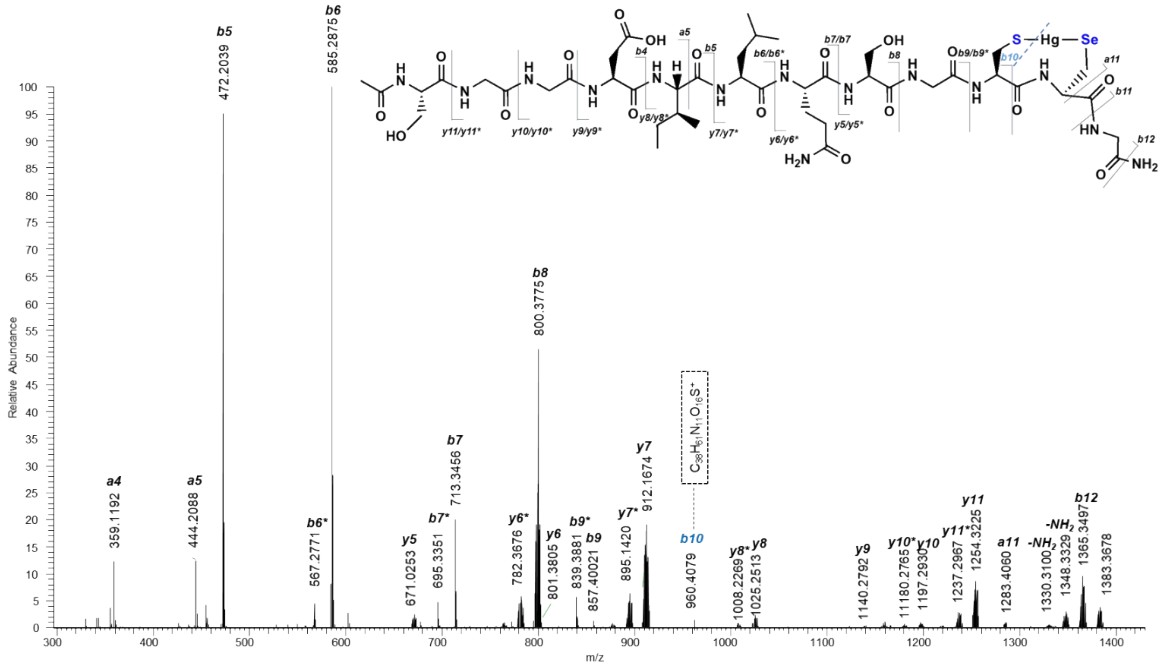


Figure S11. MS/MS of Hg-bridged TrxR(488-499) at m/z 1383.3627 (z=1). Principal fragments at higher collision dissociation energy of 15.

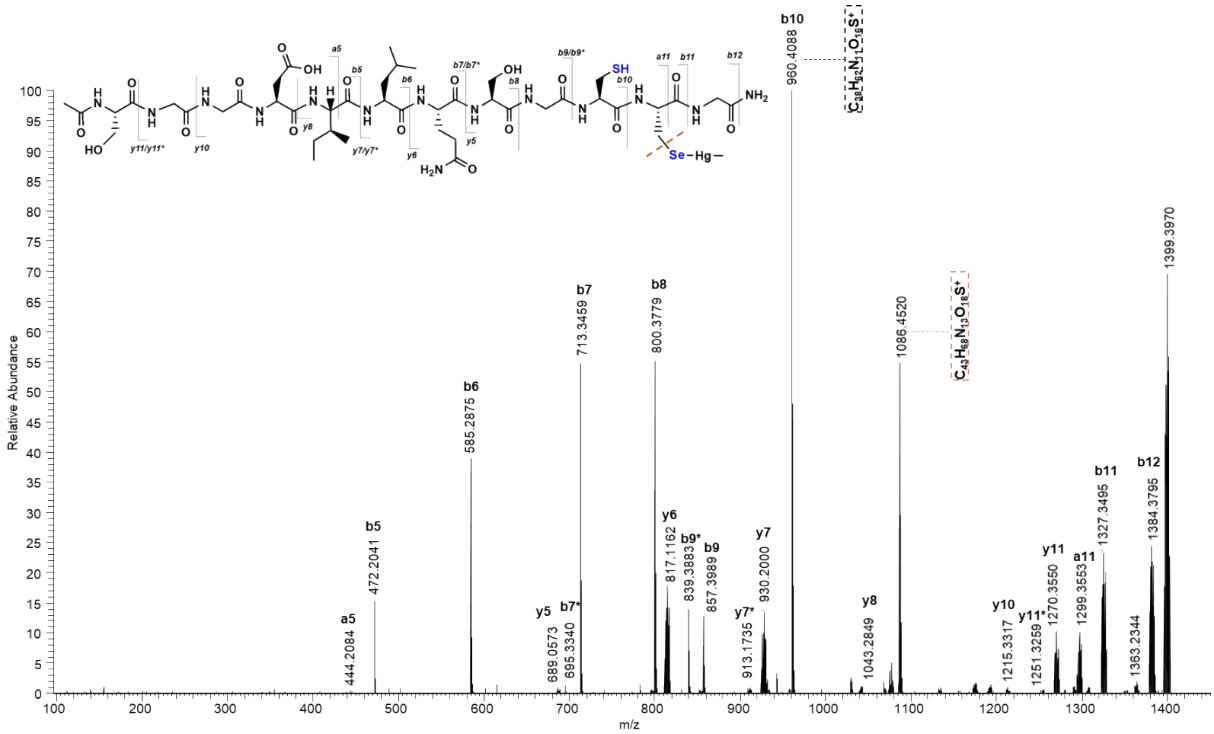
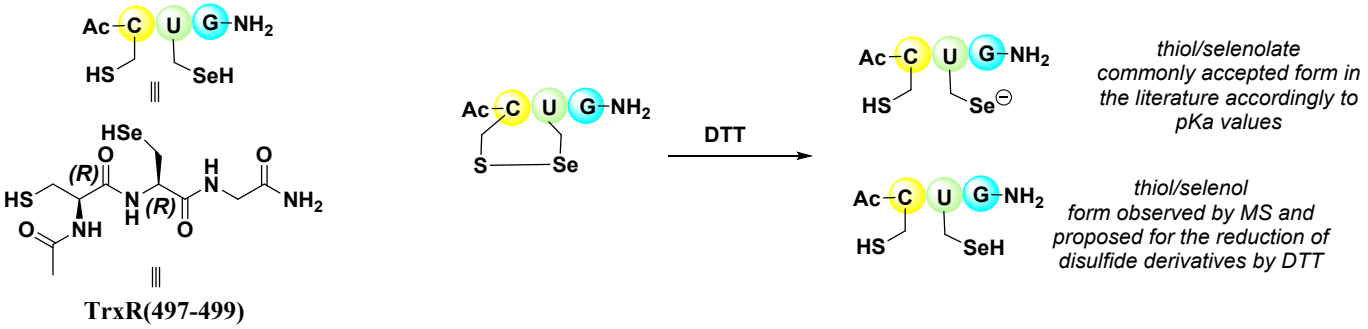


Figure S12. MS/MS of mono-metalated TrxR(488-499) at m/z 1399.3922 (z=1). Principal fragments at higher collision dissociation energy of 30.

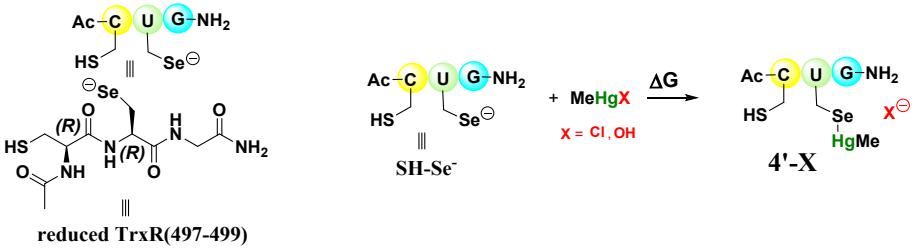
3.2. DFT Analysis of TrxR(497-499) reactivity

Scheme S1. Plausible reduced forms for TrxR(497-499) after addition of DTT : free thiol/selenolate and free thiol/selenol forms.



To perform the calculations, two different reduced forms of TrxR(497-499) following treatment with DDT were considered : (i) the free thiol-selenolate form, consistent with the pKa values of Cys and SeCys, 8.3 and 5.2, respectively in aqueous solution at pH=7.0. (ii) the free thiol/selenol form, in agreement with the experimental evidences obtained (**Figure S1** and **Table S1**) in this study and previous literature reports on the reduction of disulfide derivatives by DTT. This form also enables a direct comparison between the Cys–Se and Cys moieties within a similar chemical environment.

Table S2. Reactivity of the free thiol/selenolate form toward MeHgCl and MeHgOH, leading to adduct **4'-X** (X : Cl^-, OH^-). Gibbs free energies (ΔG in kcal/mol). Optimization carried out at B3LYP-D3(BJ)/SDD(Hg), 6-31G**(other atoms) level of theory. Energy values refined at different levels of theory.



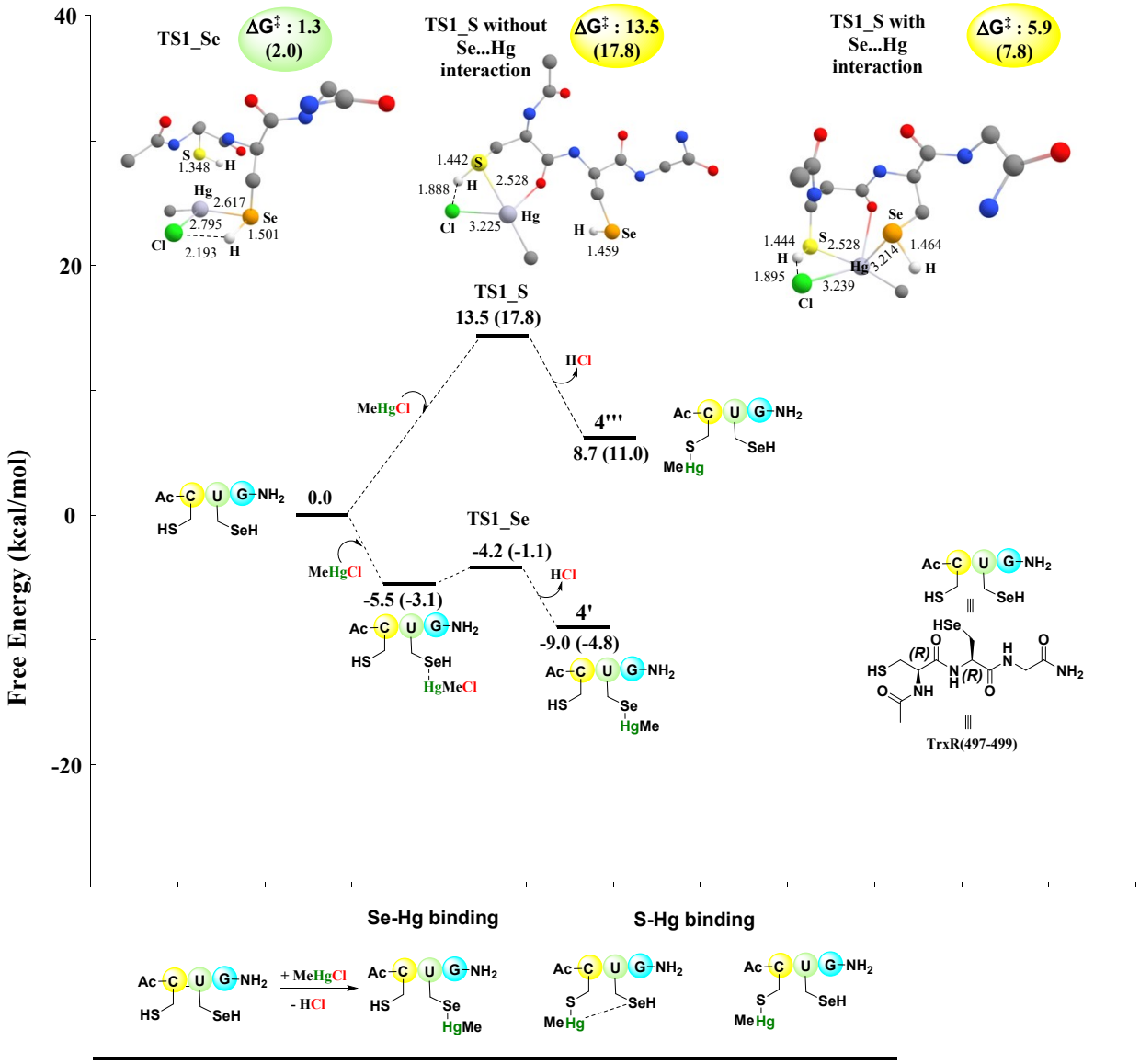
X^-	Cl^-	OH^-
Single point calculations	**ΔG**	**ΔG**
SMD(H_2O)-B3LYP-D3(BJ)/SDD(Hg), 6-31G (other atoms)**	-34.3	-5.0
SMD(H_2O)-B3LYP-D3(BJ)/SDD(Hg), 6-31+G (other atoms)**	-24.4	0.4
SMD(H_2O)-M06/SDD(Hg), 6-31G (other atoms)**	-33.0	-5.4
SMD(H_2O)-M06/SDD(Hg), 6-31+G (other atoms)**	-26.7	-0.4
Model reaction SH-Se^- + $MeHg^+$ → 4'		
SMD(H_2O)-B3LYP-D3(BJ)/SDD(Hg), 6-31G (other atoms)**	-48.0 (-48.4)[&]	
SMD(H_2O)-B3LYP-D3(BJ)/SDD(Hg), 6-31+G (other atoms)**	-33.9 (-36.7) [&]	

[&] optimization carried out in solvent at SMD(H_2O)-B3LYP-D3(BJ)/SDD(Hg), 6-31G**(other atoms) level

To validate our computational methodology, we first examined a model reaction in the absence of a counter-anion ($SH\text{-}Se^-$ + $MeHg^+$ → **4'**). Geometry optimizations were performed in the gas phase at the B3LYP-D3(BJ) level of theory, followed by single-point (SP) energy calculations including solvation effects using the SMD model (H_2O). The SDD basis set was employed for Hg, and either the 6-31G** or 6-31+G** basis sets were used for the remaining atoms. These results were compared with those obtained from geometry optimizations performed directly in solvent using the SMD(DCM) model, providing comparable results.

Consequently, for the reaction involving OH^- or Cl^- as counter-anions, geometry optimizations were carried out in the gas phase at the B3LYP-D3(BJ)/SDD(Hg), 6-31G**(other atoms) level of theory. Solvation effects were subsequently included through single-point calculations at various levels (B3LYP-D3(BJ), M06). The SDD basis set was employed for Hg, and either the 6-31G** or 6-31+G** basis sets were used for the remaining atoms. All methodologies led to consistent conclusions: the formation of adduct **4'-X** from the free thiol/selenolate form upon reaction with MeHgOH or MeHgCl is thermodynamically favored. The reaction is strongly exergonic with MeHgCl, whereas it is only slightly exergonic with MeHgOH. These results are consistent with the experimental data, which show the formation of the Se-adduct **4'** after 5 min at 37°C in water, following reduction of TrxR(497-499) by DTT and subsequent reaction with MeHgCl.

Based on the experimental results obtained in this work, particularly the MS characterization of the free thiol/selenol form after reduction of TrxR(497-499) by DTT (**Figure S1**), we also investigated the possible formation of adducts **4** from this reduced species. Considering the experimental conditions *i.e.* the presence of MeHgCl in aqueous solution, the reactivity of the free thiol/selenol form was examined toward both MeHgCl (**Figure S13**) and MeHgOH (**Figure S14**) substrates. These reactions were further used to assess the relative affinities of selenium and sulfur in comparable chemical environments (SH *vs* SeH).



Relative stability (ΔG in kcal/mol)	4'	4''	4'''
SMD(H_2O)-B3LYP-D3(BJ)	0	7.8	17.7
SMD(H_2O)-M06	0	5.4	15.8

Figure S13. Energy profiles (ΔG values in kcal/mol) for the formation of the different mono-metalated complexes **4'** (Se-adduct) and **4'''** (S-adduct) from reduced TrxR(497-499), involving thiol and selenol moieties, in reaction with MeHgCl. Calculations carried out at SMD(H_2O)-B3LYP-D3(BJ)/SDD(Hg), 6-31G**(other atoms)//B3LYP-D3(BJ)/SDD(Hg), 6-31G**(other atoms) level of theory. Key bond distances in Å, in the transition states. Into brackets are reported the values at SMD(H_2O)-M06/SDD(Hg), 6-31G**(other atoms)//B3LYP-D3(BJ)/SDD(Hg), 6-31G**(other atoms) level of theory, for comparison. Relative stability (ΔG) of the S-adducts involving Se→Hg interaction (**4''**) or without Se→Hg interaction (**4'''**) are reported

with respect to **4'**. The transition state associated to the formation of **4''** (TS1_S with Se…Hg interaction) is also presented. In the transition states, hydrogen atoms have been omitted for clarity, except for the hydrogens attached to the sulfur and selenium atoms.

DFT calculations (SMD(H_2O)-B3LYP-D3(BJ)//B3LYP-D3(BJ) level) show that the formation of the mono-metalated complex involving a Se-Hg bond (**4'**) is favored, both kinetically and thermodynamically, compared to its analogue involving a S-Hg bond (**4'''**). Initially, the selenol moiety coordinates with MeHgCl to form a stabilized adduct (ΔG : -5.5 kcal/mol). Then, the release of HCl through a four-membered transition state is highly favorable, with an associated activation barrier of $\Delta G^{\neq}$ = 1.3 kcal/mol (**TS1_Se**). The reaction is exergonic (ΔG : -3.5 kcal/mol). The nucleophilic attack of the sulfur atom at the metal center, accompanied by the release of HCl, is reversible (ΔG = +8.7 kcal/mol) and less favored kinetically ($\Delta G^{\neq}$ = 13.5 kcal/mol, **TS1_S without Se…Hg interaction**). Optimised structures of the different transition states reveal that the Se-H and S-H bonds are weakly activated and that **TS1_Se** occurs earlier than **TS1_S** (H…Cl : 2.193 for **TS1_Se** *vs* 1.888/1.895 Å, for **TS1_S** without and with Se→Hg interaction; Hg…Cl : 2.795 for **TS1_Se** *vs* 3.225/3.239 Å for **TS1_S** without and with Se→Hg interaction).The important energy difference between the two activation barriers shown in the energy profile Figure S13 perfectly matches the Se over S selectivity, which supports the preferential involvement of Se in the binding to MeHg in the mono-metalated adduct **4**.

Two levels of theory were considered to refine the energy values, SMD(H_2O)-B3LYP-D3(BJ) and SMD(H_2O)-M06 levels, on the geometry optimized at B3LYP-D3(BJ)/SDD(Hg), 6-31G**(other atoms). Similar results were obtained with both methods when comparing the formation of **4'** and **4'''** ($\Delta\Delta G^{\neq}$: 15.8 kcal/mol and ΔΔG : 15.8 kcal/mol at M06 level, *vs* $\Delta\Delta G^{\neq}$: 12.2 kcal/mol and ΔΔG : 17.7 kcal/mol at B3LYP-D3(BJ)).

For the formation of the mono-metalated peptide MeHg-S—, an alternative energy profile was considered, involving a Se→Hg interaction in the transition state (**TS1_S without Se…Hg interaction**) and leading to the final product (**4''**). DFT calculations indicate that this Se→Hg interaction has a significant effect on the reaction, lowering the activation barrier and stabilizing the final product, with the two levels of theory. These results further support the strong affinity of selenium for mercury and highlight the stabilizing role of the Se→Hg interaction in directing the reactivity.

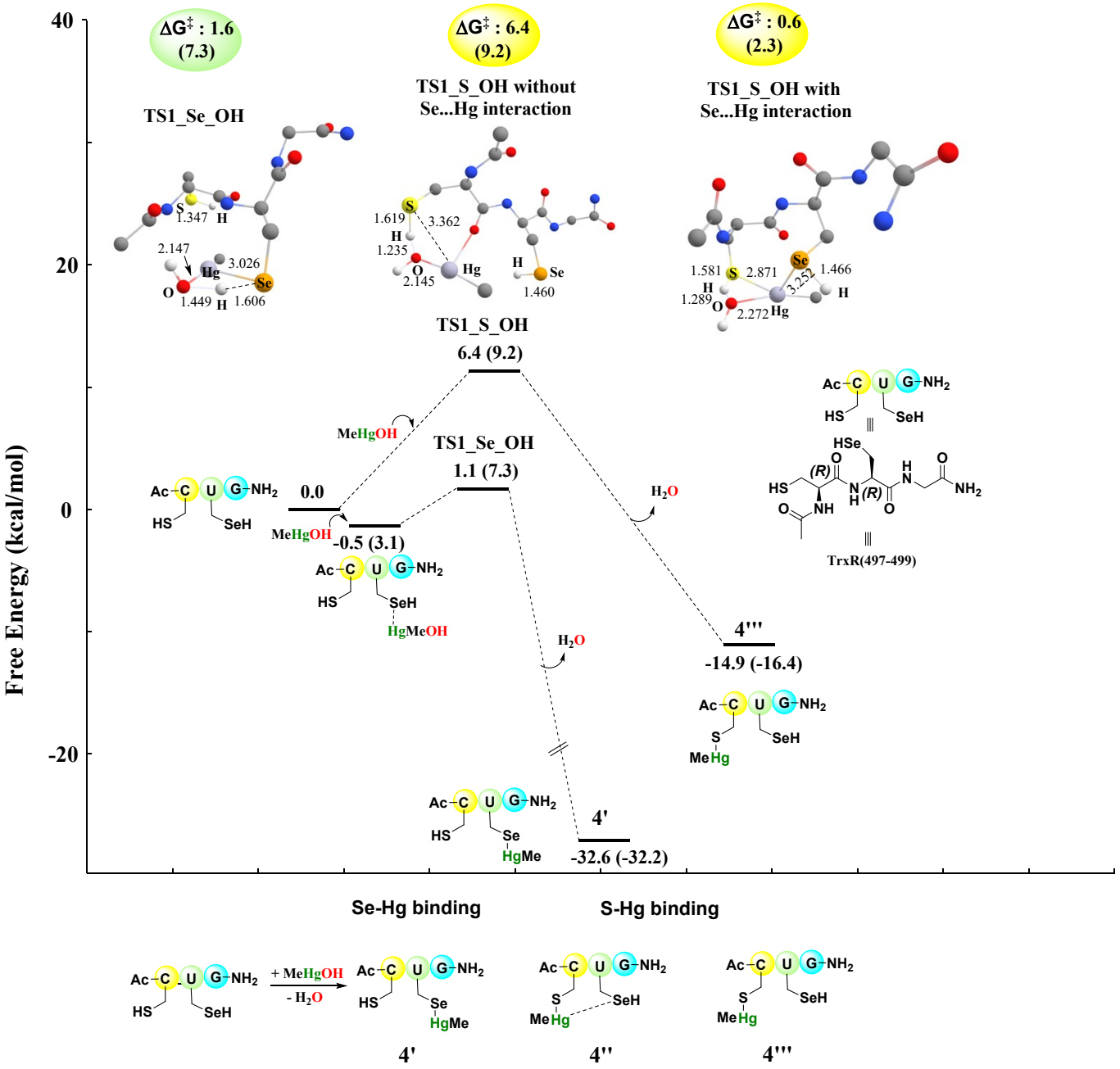


Figure S14. Energy profiles (ΔG values in kcal/mol) for the formation of the different mono-metalated complexes **4'** (Se-adduct) and **4'''** (S-adduct) from reduced TrxR(497-499), involving thiol and selenol moieties, in reaction with MeHgOH. Key bond distances in Å in the transition states. Into brackets are reported the values at SMD(H_2O)-M06/SDD(Hg), 6-31G**(other atoms)//B3LYP-D3(BJ)/SDD(Hg), 6-31G**(other atoms) level of theory, for comparison. The transition state associated to the formation of **4''** (TS1_S_OH with Se…Hg interaction) is also presented. In the transition states, hydrogen atoms have been omitted for clarity, except for the hydrogens attached to the sulfur and selenium atoms and the hydroxyl group (OH).

Similar reaction pathways were obtained for MeHgOH and MeHgCl. A pre-adduct is formed between the reduced TrxR(497-499) with MeHgOH, followed by a transition state leading to the S-bound product **4'''** that is higher in energy than the transition state leading to the Se-bound product **4'**, with $\Delta\Delta G^{\neq}$: 4.8 kcal/mol at B3LYP-D3(BJ) level and 1.9 kcal/mol at M06 level. The difference between the two transition states aligns with the Se over

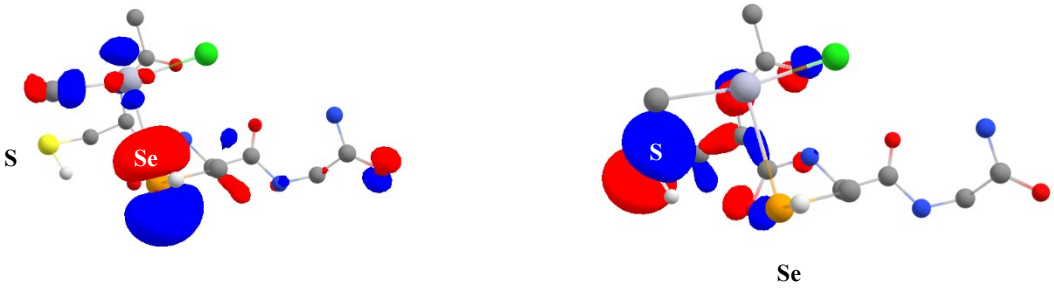
S selectivity, confirming the preferential involvement of selenium in binding to MeHg in the mono-metalated adduct **4**.

The main difference between profiles with MeHgCl and MeHgOH arises from the thermodynamics of the process. The formation of all adducts is thermodynamically favored due to the driving force associated with water formation. In addition, the transition state leading to **4''** (S-moiety with assistance of Se) becomes slightly lower in energy than that leading to **4'** ($\Delta\Delta G^{\neq}$ = 1.0 kcal·mol^{-1} at B3LYP-D3(BJ)), while the formation of **4'** remains thermodynamically preferred. The comparison of the two transition states involving the thiol moiety (with and without Se→Hg interaction) leads to a similar conclusion as for MeHgCl. The strong affinity of selenium for mercury results in a reduced activation barrier and stabilization of the final product ($\Delta\Delta G^{\neq}$ ~ 7.0 kcal/mol at B3LYP-D3(BJ) and M06 levels). Once again, both functionals give consistent results, with nucleophilic attack at Se preferred to nucleophilic attack at sulfur, both kinetically and thermodynamically.

Reduced TrxR(497-498)

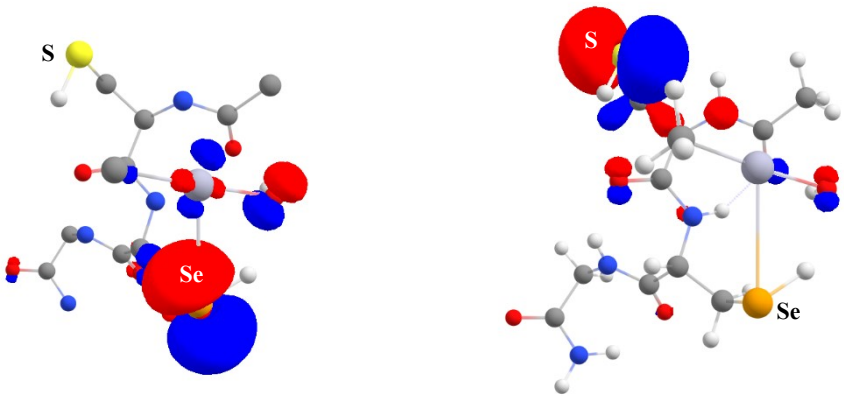
HOMO (n_{Se}) : -6.35 eV HOMO-1 (n_S): -6.69 eV

Reduced TrxR(497-499)…MeHgCl



HOMO (n_{Se}) : -6.59 eV HOMO-2 (n_S): -6.84 eV

Reduced TrxR(497-499)…MeHgOH



HOMO (n_{Se}) : -6.23 eV HOMO-4 (n_S): -7.18 eV

Figure S15. Plot (cutoff : 0.05) and energetic positions (in eV) of the orbitals associated to selenium and sulfur lone pairs for the non-activated and activated forms of reduced TrxR(497-499) containing thiol and selenol moieties, in interaction with MeHgCl or MeHgOH. Calculations performed at B3LYP-D3(BJ)/SDD(Hg), 6-31G**(other atoms) level of theory. Hydrogen atoms have been omitted for clarity, except for the hydrogens attached to the sulfur and selenium atoms.

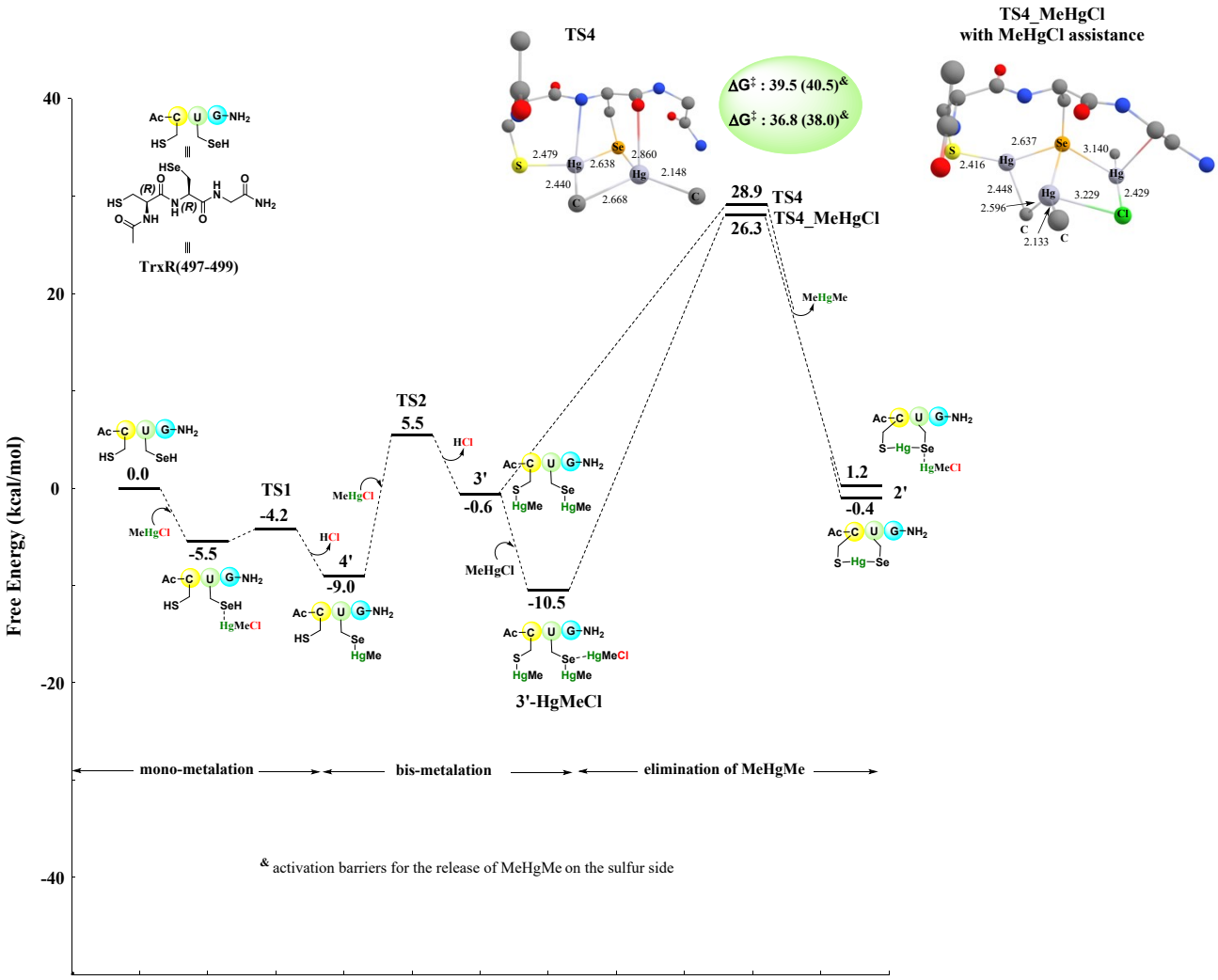


Figure S16. Energy profiles (ΔG values in kcal/mol) for the formation of the bridged Se-Hg-S complex (**2'**) from the bis-metalated complex (**3'**) without and with assistance of MeHgCl, computed at SMD(H_2O)-B3LYP-D3(BJ)/SDD(Hg), 6-31G**(other atoms)//B3LYP-D3(BJ)/SDD(Hg), 6-31G** (other atoms) level of theory. Release of MeHgMe occurs in selenium side. Values into brackets correspond to the release of MeHgMe in sulfur side. Key bond distances in Å in the transition states. In the transition states, hydrogen atoms have been omitted for clarity.

The formation of the bridged species **2'** has also been computed from the bis-metalated complex **3'**, without and with assistance of MeHgCl (from **3'-HgMeCl**). Two orientations were considered for the release of $HgMe_2$ on selenium side or in sulfur side. Relatively similar results were obtained from the two sides. The activations barriers remain high compared to that computed from the mono-metalated species **4'**, indicating that the formation of **2'** cannot occur from the bis-metalated complex, whether assisted by MeHgCl or not.

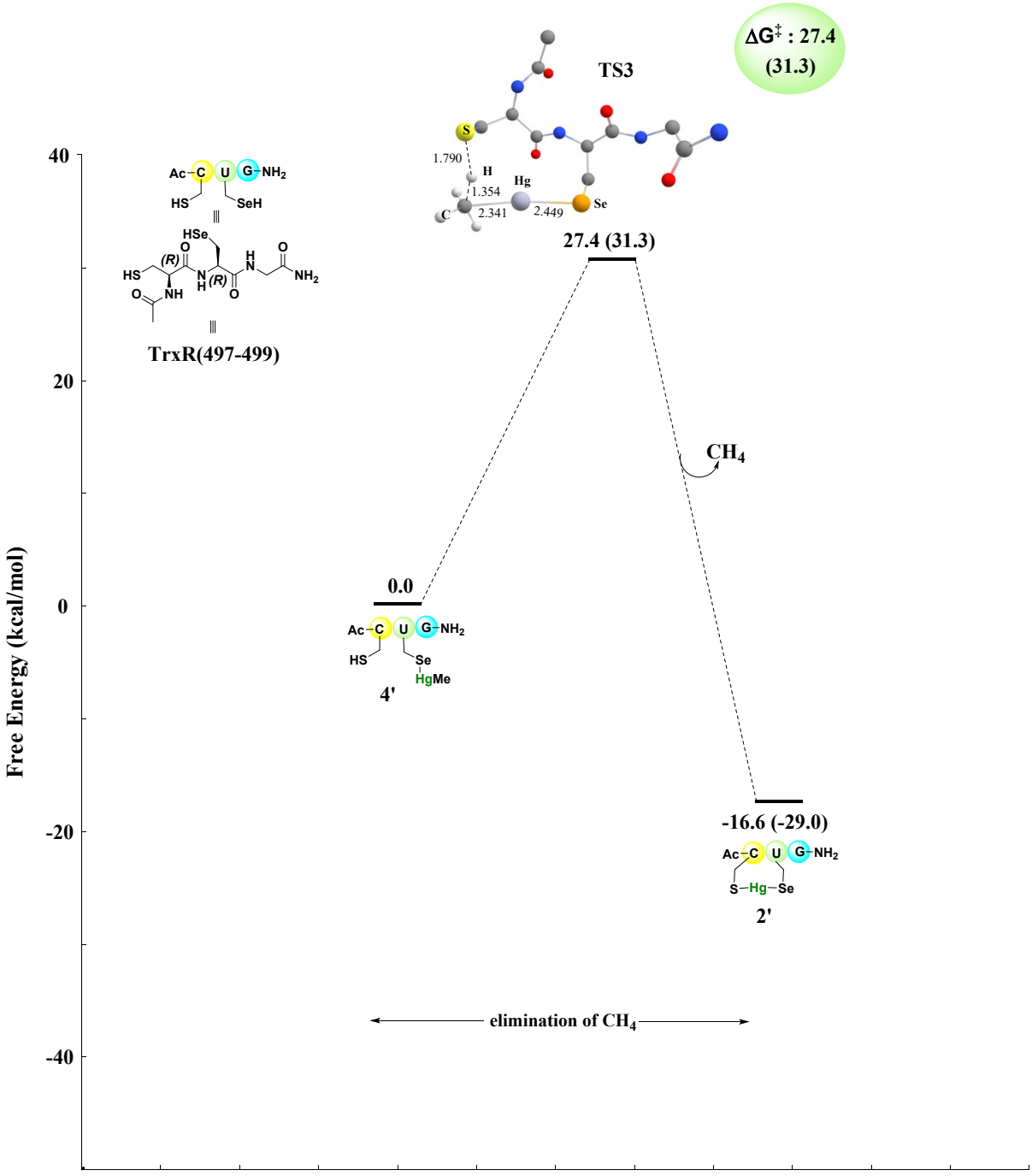


Figure S17. Energy profile (ΔG values in kcal/mol) for the formation of the bridged Se-Hg-S (**2'**) complex from the mono-metalated complex **4'**, computed in solvent at SMD(H_2O)-B3LYP-D3(BJ)/SDD(Hg), 6-31G**(other atoms) level of theory. For comparison, into brackets, the values computed at SMD(H_2O)-B3LYP-D3(BJ)/SDD(Hg), 6-31G**(other atoms)//B3LYP-D3(BJ)/SDD(Hg), 6-31G** (other atoms) from the geometries optimized in gas phase. Key bond distances in Å in the transition states. In the transition state, hydrogen atoms have been omitted for clarity, except for the hydrogens attached to the sulfur atom and the methyl substituent (CH_3) attached to mercury.

Cartesian coordinates and energies in au.

Optimization in gas phase

38
TrxR(497-499)

C	-1.283660000	-1.239906000	-0.007929000
N	-2.915135000	0.202192000	-1.110390000
C	-2.466673000	-1.180303000	-0.995628000
C	-3.624219000	-2.088197000	-0.574649000
O	-1.404157000	-1.591787000	1.166200000
N	-0.113713000	-0.802142000	-0.542295000
H	-3.579320000	0.493475000	-0.401615000
H	-2.110892000	-1.490815000	-1.983671000
H	-4.351983000	-2.147924000	-1.386309000
H	-3.247384000	-3.091778000	-0.372347000
C	-2.216265000	1.147187000	-1.796444000
O	-1.236124000	0.871142000	-2.493822000
C	-2.692334000	2.567211000	-1.599012000
H	-2.206696000	2.923324000	-0.684045000
H	-2.372411000	3.177901000	-2.442732000
H	-3.775329000	2.636498000	-1.469277000
H	-0.159089000	-0.369696000	-1.466989000
C	0.958520000	-0.356174000	0.325397000
H	0.844954000	-0.884237000	1.275431000
C	0.887644000	1.161130000	0.558002000
H	1.722672000	1.488934000	1.179503000
H	0.903427000	1.679920000	-0.401087000
C	2.298820000	-0.668132000	-0.340158000
O	2.497316000	-0.386411000	-1.523166000
N	3.240291000	-1.217226000	0.460644000
H	3.026388000	-1.374760000	1.433764000
C	4.586722000	-1.519121000	-0.015508000
H	5.039486000	-2.253902000	0.648542000
H	4.501542000	-1.938355000	-1.022522000
C	5.511310000	-0.287100000	-0.038453000
O	6.491084000	-0.205980000	0.687434000
N	5.129482000	0.666957000	-0.926214000
H	4.299792000	0.541631000	-1.495564000
H	5.678253000	1.510907000	-0.984290000
S	-4.577167000	-1.495277000	0.887000000
H	-3.521787000	-1.547246000	1.725530000
Se	-0.791811000	1.613669000	1.504437000
H	-0.466003000	3.042062000	1.545710000

Sum of electronic and zero-point Energies= -3709.266971
Sum of electronic and thermal Free Energies= -3709.322545

6
MeHgCl

Hg	-0.190003000	-0.000003000	0.000014000
Cl	2.167005000	0.000006000	-0.000033000
C	-2.286750000	0.000013000	-0.000069000
H	-2.639464000	0.917574000	-0.472576000
H	-2.639468000	-0.867987000	-0.558421000
H	-2.639434000	-0.049551000	1.030828000

Sum of electronic and zero-point Energies= -653.656896
Sum of electronic and thermal Free Energies= -653.686512

2
HCl

Cl	0.000000000	0.000000000	0.071463000
H	0.000000000	0.000000000	-1.214873000

Sum of electronic and zero-point Energies= -460.794611
Sum of electronic and thermal Free Energies= -460.812499

5
CH4

H	0.000000000	0.000000000	-1.091736000
C	0.000000000	0.000000000	0.000013000
H	0.000000000	1.029464000	0.363886000
H	-0.891542000	-0.514732000	0.363886000
H	0.891542000	-0.514732000	0.363886000

Sum of electronic and zero-point Energies= -40.480904
Sum of electronic and thermal Free Energies= -40.499533

9
MeHgMe

Hg	0.000000000	-0.000007000	-0.000009000
C	2.125887000	0.000032000	0.000041000
H	2.506926000	-0.295082000	-0.981589000
H	2.506894000	0.997715000	0.235280000
H	2.506915000	-0.702531000	0.746432000
C	-2.125886000	0.000033000	0.000041000
H	-2.506909000	-0.702497000	0.746466000
H	-2.506899000	0.997725000	0.235232000
H	-2.506923000	-0.295132000	-0.981575000

Sum of electronic and zero-point Energies= -233.292526
Sum of electronic and thermal Free Energies= -233.323898

44
Reduced TrxR(497-499) stabilized by MeHgCl

C	-0.350859000	2.161758000	-0.337744000
N	-1.889896000	1.492979000	1.495018000
C	-1.346515000	2.616528000	0.745757000
C	-2.458561000	3.486831000	0.162445000
O	-0.538115000	2.375425000	-1.540272000
N	0.741504000	1.523345000	0.131216000
H	-2.805087000	1.159530000	1.221713000
H	-0.773893000	3.215762000	1.464475000
H	-3.032907000	3.935162000	0.975814000
H	-2.019567000	4.284550000	-0.436811000
C	-1.257250000	1.018198000	2.610275000
O	-0.143762000	1.428914000	2.931605000
C	-1.992455000	-0.039325000	3.401213000
H	-1.407470000	-0.961775000	3.362594000
H	-2.045355000	0.280848000	4.444543000
H	-3.002594000	-0.233553000	3.031167000
H	0.891169000	1.422053000	1.137638000
C	1.823035000	1.168079000	-0.761221000
H	1.835194000	1.892073000	-1.583348000
C	1.702567000	-0.249819000	-1.342509000
H	2.505107000	-0.436774000	-2.056911000
H	1.720527000	-0.990025000	-0.542341000
C	3.129059000	1.239305000	0.043020000
O	3.124031000	1.248836000	1.272698000
N	4.267619000	1.242827000	-0.689430000
H	4.210631000	1.147644000	-1.692358000
C	5.581277000	1.138020000	-0.059882000

H	6.337613000	1.497337000	-0.756189000
H	5.575094000	1.767189000	0.834124000
C	5.941175000	-0.314280000	0.308717000
O	6.841689000	-0.913615000	-0.260567000
N	5.167094000	-0.841479000	1.291751000
H	4.411977000	-0.308966000	1.707088000
H	5.307527000	-1.808148000	1.542863000
S	-3.688039000	2.583397000	-0.866429000
H	-2.795848000	2.241036000	-1.817391000
Se	-0.000498000	-0.465811000	-2.329614000
H	0.283391000	-1.878843000	-2.613712000
Hg	-1.589154000	-1.561738000	0.074548000
C	-3.504969000	-1.351655000	-0.786720000
H	-3.518249000	-1.912189000	-1.723122000
H	-4.265573000	-1.740148000	-0.109298000
H	-3.670777000	-0.292491000	-0.982137000
Cl	0.384881000	-2.065884000	1.351166000

Sum of electronic and zero-point Energies= -4362.959575
Sum of electronic and thermal Free Energies= -4363.026846

44

TS1_Se

C	-0.147474000	1.905315000	-0.628216000
N	-1.831388000	1.948161000	1.186770000
C	-1.073923000	2.771377000	0.254097000
C	-1.983135000	3.677185000	-0.573725000
O	-0.304564000	1.761804000	-1.842546000
N	0.846248000	1.313705000	0.076720000
H	-2.761758000	1.667823000	0.906525000
H	-0.421328000	3.406581000	0.864879000
H	-2.479758000	4.389568000	0.088292000
H	-1.380210000	4.235164000	-1.291192000
C	-1.293644000	1.522827000	2.369327000
O	-0.128010000	1.789746000	2.671215000
C	-2.182174000	0.676033000	3.245022000
H	-1.925198000	-0.376371000	3.072054000
H	-1.965289000	0.907552000	4.288652000
H	-3.246078000	0.825913000	3.046018000
H	0.913204000	1.500822000	1.081972000
C	1.885829000	0.534789000	-0.547722000
H	1.800008000	0.678177000	-1.628690000
C	1.789357000	-0.951282000	-0.178315000
H	2.724534000	-1.473003000	-0.391097000
H	1.512557000	-1.086305000	0.869117000
C	3.246383000	1.031470000	-0.036516000
O	3.386670000	1.402330000	1.127459000
N	4.263210000	0.963676000	-0.926038000
H	4.089627000	0.593937000	-1.848807000
C	5.642231000	1.254938000	-0.544497000
H	6.216415000	1.490650000	-1.439212000
H	5.629257000	2.122805000	0.121125000
C	6.336225000	0.068561000	0.151529000
O	7.311635000	-0.478909000	-0.338900000
N	5.753914000	-0.301250000	1.322799000
H	4.995615000	0.239450000	1.721422000
H	6.175581000	-1.054195000	1.845034000
S	-3.340945000	2.823359000	-1.473632000
H	-2.515804000	2.092935000	-2.249811000
Se	0.423680000	-1.923479000	-1.239971000
H	0.233068000	-2.878533000	-0.098110000
Hg	-1.849367000	-1.069831000	-0.264799000
C	-3.884072000	-0.638136000	0.125010000
H	-4.471035000	-1.466588000	-0.271375000
H	-3.973114000	-0.608961000	1.210159000
H	-4.172537000	0.300205000	-0.343728000
Cl	-0.784807000	-2.593228000	1.823072000

Sum of electronic and zero-point Energies= -4362.948017
Sum of electronic and thermal Free Energies= -4363.012744

44

TS1_S

C	-0.388733000	1.223209000	-1.679402000
N	-0.609199000	2.408925000	0.481659000
C	-1.066717000	2.369295000	-0.894570000
C	-2.585898000	2.337342000	-1.007178000
O	-1.033443000	0.315269000	-2.218657000
N	0.961081000	1.282358000	-1.660290000
H	-1.014823000	1.775602000	1.176389000
H	-0.726822000	3.308719000	-1.349362000
H	-2.995678000	3.280559000	-0.641911000
H	-2.876745000	2.202465000	-2.049329000
C	0.540722000	3.073233000	0.802237000
O	1.157777000	3.732552000	-0.036019000
C	1.030917000	2.859259000	2.213615000
H	0.222709000	2.619921000	2.907582000
H	1.718735000	2.006695000	2.187884000
H	1.585520000	3.738671000	2.540922000
H	1.419450000	2.098322000	-1.239780000
C	1.837081000	0.146949000	-1.898560000
H	1.963671000	-0.042923000	-2.973530000
C	1.302263000	-1.126618000	-1.224247000
H	0.406534000	-1.479524000	-1.728252000
H	2.050015000	-1.917730000	-1.198569000
C	3.196986000	0.543919000	-1.276514000
O	3.370591000	1.648827000	-0.782875000
N	4.148271000	-0.426632000	-1.288855000
H	3.953447000	-1.308761000	-1.736763000
C	5.398788000	-0.289263000	-0.565857000
H	6.250307000	-0.540324000	-1.201878000
H	5.487526000	0.761728000	-0.279397000
C	5.518264000	-1.146090000	0.701439000
O	6.621726000	-1.417262000	1.147662000
N	4.351917000	-1.565629000	1.270546000
H	3.442744000	-1.196904000	1.021083000
H	4.440975000	-2.012573000	2.171419000
S	-3.518796000	1.033541000	-0.084326000
H	-2.961708000	1.113409000	1.245773000
Se	0.832373000	-0.609465000	0.628915000
H	0.798724000	-1.998716000	1.088138000
Hg	-2.241534000	-1.147868000	-0.140313000
C	-1.667909000	-3.186220000	-0.191490000
H	-0.923604000	-3.321119000	-0.976832000
H	-1.249317000	-3.432863000	0.784242000
H	-2.542073000	-3.803591000	-0.396774000
Cl	-1.924652000	0.423835000	2.674173000

Sum of electronic and zero-point Energies= -4362.950108
Sum of electronic and thermal Free Energies= -4363.011201

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TS1_S without Se-Hg interaction

C	0.200255000	1.167828000	0.963934000
N	0.709839000	3.240990000	-0.357937000
C	1.016011000	2.470247000	0.840807000
C	2.513142000	2.192745000	0.938618000
O	0.741350000	0.100819000	1.290419000

N	-1.117738000	1.248265000	0.713266000
H	1.364623000	3.193335000	-1.125814000
H	0.728075000	3.089749000	1.700797000
H	3.054458000	3.128112000	1.089283000
H	2.732874000	1.505323000	1.755359000
C	-0.367085000	4.073071000	-0.432928000
O	-1.206280000	4.118858000	0.465426000
C	-0.464812000	4.927238000	-1.678620000
H	-1.447355000	4.775276000	-2.131081000
H	-0.399804000	5.978335000	-1.384533000
H	0.312206000	4.716047000	-2.417061000
H	-1.592349000	2.151491000	0.637314000
C	-1.919905000	0.044995000	0.809263000
H	-1.655538000	-0.477820000	1.734990000
C	-1.608400000	-0.887545000	-0.385266000
H	-2.205490000	-0.625601000	-1.261420000
H	-0.552575000	-0.815529000	-0.632043000
C	-3.395658000	0.460720000	0.856243000
O	-3.722592000	1.646181000	0.752142000
N	-4.275015000	-0.549475000	1.011214000
H	-3.927436000	-1.502014000	0.932993000
C	-5.716162000	-0.327491000	1.021568000
H	-6.198867000	-1.185944000	1.486460000
H	-5.917674000	0.568695000	1.616154000
C	-6.330936000	-0.163693000	-0.381371000
O	-7.183663000	-0.930526000	-0.801333000
N	-5.835303000	0.890529000	-1.083917000
H	-5.202738000	1.549936000	-0.645692000
H	-6.239106000	1.084561000	-1.987475000
S	3.231929000	1.448048000	-0.602027000
H	4.355845000	0.894202000	0.110776000
Se	-2.006580000	-2.781702000	-0.040281000
H	-1.387417000	-2.789795000	1.280674000
Hg	2.477830000	-0.961280000	-0.462202000
C	1.871598000	-2.976939000	-0.634410000
H	2.363988000	-3.518762000	0.173141000
H	0.787280000	-3.027578000	-0.534470000
H	2.188737000	-3.361127000	-1.603557000
Cl	5.113733000	-0.364783000	1.296748000

Sum of electronic and zero-point Energies= -4362.921131
Sum of electronic and thermal Free Energies= -4362.986729

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4'

C	-0.002768000	1.750077000	-0.407600000
N	-1.947186000	2.043655000	1.144792000
C	-0.814117000	2.696398000	0.503430000
C	-1.263702000	3.947735000	-0.247861000
O	0.242433000	2.003284000	-1.581904000
N	0.427111000	0.618276000	0.224899000
H	-2.833087000	2.110375000	0.659758000
H	-0.124531000	2.994073000	1.304071000
H	-1.677649000	4.670321000	0.458113000
H	-0.407626000	4.395939000	-0.751203000
C	-1.826725000	1.272961000	2.255977000
O	-0.729520000	1.025958000	2.767468000
C	-3.112515000	0.715163000	2.825809000
H	-3.110471000	-0.371446000	2.699285000
H	-3.133742000	0.920024000	3.898332000
H	-4.009191000	1.127940000	2.358761000
H	0.359395000	0.644135000	1.246050000
C	1.522124000	-0.144346000	-0.336821000
H	1.325875000	-0.303381000	-1.400246000
C	1.651398000	-1.485354000	0.387936000
H	2.572496000	-1.986726000	0.086214000
H	1.690004000	-1.324833000	1.467773000
C	2.830007000	0.658735000	-0.181903000
O	2.950045000	1.543616000	0.666610000
N	3.830267000	0.292724000	-1.015041000
H	3.675153000	-0.458271000	-1.670910000
C	5.143385000	0.928800000	-0.985191000
H	5.642417000	0.753169000	-1.937032000
H	4.994644000	2.003493000	-0.842539000
C	6.058425000	0.375336000	0.123161000
O	7.084024000	-0.236603000	-0.136382000
N	5.615668000	0.638147000	1.379783000
H	4.741409000	1.130089000	1.526959000
H	6.144031000	0.273416000	2.157325000
S	-2.592806000	3.648907000	-1.486413000
H	-1.817258000	2.910148000	-2.304659000
Se	0.208124000	-2.788392000	-0.028248000
Hg	-1.747257000	-1.314873000	-0.334395000
C	-3.543922000	-0.279885000	-0.746963000
H	-4.136432000	-0.871345000	-1.446612000
H	-4.108643000	-0.141628000	0.175441000
H	-3.303956000	0.683778000	-1.197460000

Sum of electronic and zero-point Energies= -3902.154245
Sum of electronic and thermal Free Energies= -3902.216513

42

4''

C	0.555107000	1.238234000	1.494849000
N	0.715932000	2.497318000	-0.623323000
C	1.242902000	2.391389000	0.726595000
C	2.765655000	2.213094000	0.692314000
O	1.192347000	0.321469000	2.025068000
N	-0.797210000	1.290862000	1.482997000
H	1.255936000	2.011869000	-1.332942000
H	0.995432000	3.323278000	1.249911000
H	3.231980000	3.178461000	0.484157000
H	3.106593000	1.859529000	1.664256000
C	-0.444338000	3.136759000	-0.919737000
O	-1.116198000	3.700166000	-0.053877000
C	-0.898115000	3.021851000	-2.358605000
H	-0.064583000	2.960436000	-3.063225000
H	-1.492570000	2.105068000	-2.443013000
H	-1.536666000	3.870475000	-2.602680000
H	-1.269696000	2.113657000	1.092555000
C	-1.643452000	0.148253000	1.767447000
H	-1.694484000	-0.056332000	2.846652000
C	-1.124934000	-1.111896000	1.058974000
H	-0.181961000	-1.430162000	1.491605000
H	-1.846469000	-1.926921000	1.084375000
C	-3.047886000	0.520758000	1.243730000
O	-3.279946000	1.622226000	0.768656000
N	-3.977499000	-0.470347000	1.320310000
H	-3.735778000	-1.347581000	1.754679000
C	-5.284384000	-0.348990000	0.703407000
H	-6.077305000	-0.622705000	1.402892000
H	-5.414754000	0.703424000	0.438525000
C	-5.492211000	-1.191555000	-0.561496000
O	-6.623437000	-1.469246000	-0.927888000
N	-4.368145000	-1.591226000	-1.222121000
H	-3.445294000	-1.220653000	-1.032245000
H	-4.520351000	-2.033854000	-2.116392000
S	3.403617000	1.099031000	-0.641241000

Se	-0.820326000	-0.614101000	-0.833195000
H	-0.808625000	-2.013676000	-1.265755000
Hg	2.476567000	-1.063457000	-0.095013000
C	1.882857000	-3.072552000	0.231109000
H	1.195172000	-3.117507000	1.077352000
H	1.391048000	-3.456559000	-0.665060000
H	2.759353000	-3.683979000	0.451695000

Sum of electronic and zero-point Energies= -3902.146341
Sum of electronic and thermal Free Energies= -3902.205972

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4'''

C	-0.497531000	1.063915000	-1.164268000
N	-1.289405000	2.936162000	0.272768000
C	-1.393846000	2.310689000	-1.036335000
C	-2.859001000	1.978620000	-1.343564000
O	-0.942469000	-0.033524000	-1.535159000
N	0.799144000	1.230311000	-0.842858000
H	-2.034579000	2.702672000	0.920929000
H	-1.026728000	3.036290000	-1.774086000
H	-3.395472000	2.907065000	-1.551882000
H	-2.903647000	1.342593000	-2.226125000
C	-0.283166000	3.777831000	0.615579000
O	0.674728000	3.993426000	-0.131719000
C	-0.404798000	4.438798000	1.973208000
H	0.497369000	4.223872000	2.551128000
H	-0.449420000	5.521726000	1.829633000
H	-1.283580000	4.117168000	2.536828000
H	1.182853000	2.161410000	-0.657028000
C	1.675393000	0.079777000	-0.857511000
H	1.483712000	-0.489464000	-1.773312000
C	1.355792000	-0.826779000	0.355744000
H	1.890193000	-0.500114000	1.250525000
H	0.285906000	-0.804039000	0.545285000
C	3.127248000	0.571644000	-0.852065000
O	3.392320000	1.774168000	-0.781280000
N	4.063925000	-0.398109000	-0.922575000
H	3.761074000	-1.363264000	-0.817433000
C	5.489798000	-0.100405000	-0.866515000
H	6.041068000	-0.949625000	-1.268038000
H	5.677503000	0.781031000	-1.487188000
C	6.018766000	0.152536000	0.558004000
O	6.884856000	-0.551581000	1.055141000
N	5.436071000	1.208548000	1.185780000
H	4.785764000	1.808754000	0.691164000
H	5.772175000	1.455003000	2.104051000
S	-3.797862000	1.196901000	0.048233000
Se	1.870011000	-2.708315000	0.109026000
H	1.316120000	-2.800226000	-1.237452000
Hg	-2.786969000	-0.995364000	0.246797000
C	-2.038246000	-2.950628000	0.554825000
H	-2.452187000	-3.606871000	-0.213455000
H	-0.949397000	-2.949207000	0.483398000
H	-2.341892000	-3.307660000	1.540426000

Sum of electronic and zero-point Energies= -3902.133458
Sum of electronic and thermal Free Energies= -3902.195957

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TS2

C	0.882639000	-0.748924000	-1.398573000
N	2.401419000	-1.212046000	-3.268323000
C	2.315728000	-1.098890000	-1.819521000
C	2.745306000	-2.389903000	-1.135413000
O	0.028023000	-1.631895000	-1.294722000
N	0.658166000	0.554442000	-1.142112000
H	1.997631000	-2.029734000	-3.704076000
H	2.994311000	-0.286936000	-1.548931000
H	2.025193000	-3.183940000	-1.344899000
H	3.736733000	-2.689066000	-1.478828000
C	2.534811000	-0.060132000	-4.007829000
O	2.721899000	1.027629000	-3.473024000
C	2.443757000	-0.227354000	-5.509464000
H	3.257028000	0.334867000	-5.972302000
H	2.492646000	-1.270098000	-5.831880000
H	1.502450000	0.209138000	-5.856990000
H	1.336707000	1.268370000	-1.389101000
C	-0.606150000	0.966902000	-0.567608000
H	-0.778299000	0.308909000	0.288342000
C	-1.792318000	0.836408000	-1.547647000
H	-1.987388000	1.770311000	-2.079388000
H	-1.586096000	0.048348000	-2.267242000
C	-0.444974000	2.385531000	-0.033240000
O	0.605231000	3.025715000	-0.205586000
N	-1.516119000	2.866601000	0.617427000
H	-2.356219000	2.288226000	0.634351000
C	-1.553233000	4.201539000	1.199151000
H	-2.342643000	4.232668000	1.948430000
H	-0.589455000	4.394098000	1.681786000
C	-1.844020000	5.324093000	0.185141000
O	-2.794147000	6.079595000	0.321553000
N	-0.953271000	5.390864000	-0.839772000
H	-0.133632000	4.794957000	-0.853729000
H	-1.069698000	6.127291000	-1.518573000
S	2.839228000	-2.270258000	0.705357000
H	1.409878000	-2.057268000	1.192116000
Se	-3.460075000	0.365971000	-0.572419000
Hg	-2.642865000	-1.844815000	0.265664000
C	-2.103181000	-3.763681000	0.953674000
H	-2.857648000	-4.499660000	0.673759000
H	-1.143214000	-4.004132000	0.496609000
H	-1.994370000	-3.710931000	2.036430000
Hg	2.812383000	0.191883000	1.364997000
Cl	0.226691000	-1.204591000	2.131647000
C	2.971914000	2.205997000	1.979807000
H	4.006882000	2.529017000	1.872013000
H	2.301302000	2.776243000	1.337911000
H	2.656549000	2.246156000	3.022737000

Sum of electronic and zero-point Energies= -4555.815996
Sum of electronic and thermal Free Energies= -4555.889814

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3'

C	-0.714170000	1.536236000	0.678545000
N	-1.343648000	3.305662000	2.227865000
C	-1.652442000	2.726497000	0.921605000
C	-3.114509000	2.299865000	0.843211000
O	-1.048038000	0.372012000	0.972367000
N	0.480086000	1.829783000	0.146216000
H	-1.834491000	2.958866000	3.038574000
H	-1.448310000	3.499660000	0.178028000
H	-3.285174000	1.431196000	1.482284000
H	-3.740023000	3.125053000	1.192980000
C	-0.285910000	4.157516000	2.379145000

O	0.429282000	4.476967000	1.432398000
C	-0.058894000	4.693044000	3.778281000
H	-0.089667000	5.784755000	3.740833000
H	-0.792305000	4.339895000	4.507271000
H	0.943010000	4.405586000	4.107449000
H	0.762787000	2.795288000	-0.006486000
C	1.408335000	0.761030000	-0.145476000
H	0.835685000	-0.043619000	-0.613115000
C	2.075193000	0.195917000	1.131530000
H	3.040726000	0.668962000	1.323273000
H	1.425095000	0.358711000	1.987990000
C	2.450119000	1.269469000	-1.140711000
O	2.507926000	2.459699000	-1.462861000
N	3.293349000	0.320666000	-1.597958000
H	3.244039000	-0.602689000	-1.173078000
C	4.407238000	0.632330000	-2.484325000
H	4.728174000	-0.282392000	-2.980688000
H	4.055259000	1.347175000	-3.234678000
C	5.637348000	1.214935000	-1.763088000
O	6.731568000	0.675331000	-1.825156000
N	5.384924000	2.357977000	-1.070315000
H	4.487224000	2.822121000	-1.148629000
H	6.162881000	2.816095000	-0.620903000
S	-3.689361000	1.954408000	-0.874875000
Se	2.422853000	-1.753758000	0.967169000
Hg	0.033755000	-2.442547000	1.045452000
C	-1.949065000	-3.143089000	1.225425000
H	-2.136659000	-3.422000000	2.263138000
H	-2.616942000	-2.332539000	0.932946000
H	-2.088308000	-4.008854000	0.577077000
Hg	-2.475940000	-0.025105000	-1.514990000
C	-1.449319000	-1.738433000	-2.216579000
H	-1.722995000	-1.940648000	-3.253516000
H	-0.372388000	-1.565795000	-2.153515000
H	-1.722537000	-2.587972000	-1.589928000

Sum of electronic and zero-point Energies= -4095.015676
Sum of electronic and thermal Free Energies= -4095.087024

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3'-stabilized by MeHgCl

C	2.672373000	0.670573000	1.737887000
N	3.382099000	2.434950000	0.101346000
C	3.821881000	1.361231000	0.970042000
C	4.622544000	0.322069000	0.173393000
O	2.863941000	-0.378190000	2.357601000
N	1.486240000	1.307935000	1.675578000
H	3.127400000	2.165341000	-0.843328000
H	4.487835000	1.770315000	1.738002000
H	5.478893000	0.819714000	-0.287048000
H	4.989574000	-0.443397000	0.855560000
S	3.647375000	-0.446106000	-1.197912000
C	3.315352000	3.782562000	0.361273000
O	2.930674000	4.568530000	-0.494673000
C	3.720760000	4.232326000	1.753862000
H	3.155957000	3.701780000	2.526855000
H	4.785692000	4.049882000	1.932885000
H	3.525181000	5.300733000	1.830131000
H	1.407061000	2.160696000	1.131022000
C	0.242363000	0.854673000	2.256993000
H	0.299547000	0.910418000	3.354423000
C	-0.114322000	-0.596316000	1.909942000
H	0.675130000	-1.243805000	2.285760000
H	-1.054448000	-0.875508000	2.389420000
C	-0.822653000	1.858154000	1.772715000
O	-0.515629000	2.788122000	1.022340000
N	-2.076944000	1.655179000	2.210710000
H	-2.338812000	0.808083000	2.703080000
C	-3.193110000	2.388778000	1.656362000
H	-3.409606000	3.295973000	2.234564000
H	-2.957548000	2.686276000	0.630724000
C	-4.410750000	1.471833000	1.673921000
O	-4.369405000	0.374851000	2.231730000
N	-5.521044000	1.964166000	1.076255000
H	-5.426165000	2.685650000	0.375882000
H	-6.293561000	1.322478000	0.963652000
Se	-0.325578000	-1.004320000	-0.024817000
Hg	2.613991000	-2.281815000	0.022398000
Hg	0.285857000	1.090939000	-1.310453000
C	0.760878000	2.713366000	-2.572034000
H	1.323990000	3.451603000	-1.999179000
H	1.351930000	2.339519000	-3.408578000
H	-0.180294000	3.135467000	-2.924607000
C	1.895669000	-3.979435000	1.064276000
H	2.697141000	-4.703978000	1.214784000
H	1.516018000	-3.646271000	2.032098000
H	1.088791000	-4.430432000	0.484292000
Hg	-3.387224000	-1.016366000	-0.353629000
Cl	-3.267629000	1.058226000	-1.609761000
C	-3.784941000	-2.816747000	0.669368000
H	-3.194128000	-3.611367000	0.212737000
H	-3.481591000	-2.664201000	1.705773000
H	-4.847960000	-3.051395000	0.620905000

Sum of electronic and zero-point Energies= -4748.725022
Sum of electronic and thermal Free Energies= -4748.804442

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TS3

C	1.421250000	0.897505000	1.228743000
N	2.379140000	2.459560000	-0.468265000
C	2.679473000	1.467310000	0.541408000
C	3.549916000	0.338204000	-0.021386000
O	1.511031000	-0.100361000	1.964860000
N	0.288277000	1.564228000	0.966331000
H	2.219171000	2.111109000	-1.405832000
H	3.229446000	1.986224000	1.335925000
H	4.542258000	0.732034000	-0.249506000
H	3.646502000	-0.432755000	0.746986000
C	2.477505000	3.807459000	-0.228401000
O	2.772181000	4.256930000	0.871234000
C	2.122537000	4.684287000	-1.414841000
H	1.033852000	4.797286000	-1.462160000
H	2.569624000	5.667981000	-1.272727000
H	2.458984000	4.256621000	-2.363674000
H	0.325362000	2.255655000	0.223044000
C	-1.049038000	1.351956000	1.481213000
H	-1.169327000	1.910095000	2.424157000
C	-1.427838000	-0.106783000	1.771194000
H	-0.687842000	-0.561188000	2.422631000
H	-2.399259000	-0.153991000	2.267212000
C	-1.984384000	1.987710000	0.426819000
O	-1.536160000	2.600993000	-0.536073000
N	-3.315522000	1.828709000	0.657338000
H	-3.594055000	1.128504000	1.335946000
C	-4.245617000	1.885364000	-0.450314000
H	-5.044260000	2.615717000	-0.270177000
H	-3.700096000	2.193713000	-1.345408000

C	-4.873435000	0.493119000	-0.601315000
O	-4.831331000	-0.316745000	0.318301000
N	-5.507619000	0.257732000	-1.775442000
H	-5.401486000	0.871665000	-2.567206000
H	-5.898829000	-0.660488000	-1.925175000
S	2.857882000	-0.412670000	-1.559978000
H	2.675300000	-1.956907000	-0.817203000
Se	-1.650288000	-1.220949000	0.141353000
Hg	0.729582000	-1.803432000	-0.113342000
C	2.711566000	-3.173641000	-0.131874000
H	2.126172000	-3.816083000	0.536915000
H	2.946253000	-3.756381000	-1.024287000
H	3.612062000	-2.892305000	0.417789000

Sum of electronic and zero-point Energies= -3902.097683
Sum of electronic and thermal Free Energies= -3902.159830

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2'

C	1.713356000	0.693091000	1.527578000
N	3.016292000	1.450705000	-0.481051000
C	3.056252000	0.715478000	0.763057000
C	3.579319000	-0.715316000	0.540971000
O	1.641837000	0.231573000	2.661250000
N	0.641841000	1.138475000	0.818206000
H	2.981091000	0.907582000	-1.335297000
H	3.744833000	1.223563000	1.446233000
H	4.647478000	-0.674702000	0.314332000
H	3.440681000	-1.282251000	1.461519000
C	3.051679000	2.821909000	-0.503093000
O	3.116346000	3.479619000	0.527090000
C	2.927929000	3.444670000	-1.880174000
H	1.868224000	3.634679000	-2.083150000
H	3.453110000	4.400186000	-1.884542000
H	3.319437000	2.802430000	-2.673787000
H	0.792123000	1.577108000	-0.084600000
C	-0.699683000	1.306604000	1.334098000
H	-0.682223000	1.986909000	2.198086000
C	-1.359619000	-0.008040000	1.797414000
H	-0.708233000	-0.505014000	2.513166000
H	-2.320504000	0.188086000	2.276179000
C	-1.500843000	1.940134000	0.181533000
O	-0.980756000	2.169635000	-0.907067000
N	-2.808283000	2.192603000	0.457036000
H	-3.182999000	1.785708000	1.307754000
C	-3.780638000	2.143429000	-0.616531000
H	-4.435491000	3.022880000	-0.604457000
H	-3.243579000	2.129612000	-1.567184000
C	-4.637298000	0.886238000	-0.406274000
O	-4.737996000	0.377569000	0.704408000
N	-5.302615000	0.436737000	-1.498005000
H	-5.068418000	0.755901000	-2.424563000
H	-5.833674000	-0.416709000	-1.404612000
S	2.852803000	-1.638865000	-0.900038000
Se	-1.815803000	-1.260953000	0.306943000
Hg	0.525897000	-1.480097000	-0.361009000

Sum of electronic and zero-point Energies= -3861.702726
Sum of electronic and thermal Free Energies= -3861.760133

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TS4 on selenium side

C	2.379646000	-2.098949000	-0.309199000
N	3.329589000	-0.431424000	1.320681000
C	3.598695000	-1.306980000	0.201534000
C	4.208424000	-0.520897000	-0.982385000
O	2.499356000	-2.929527000	-1.201886000
N	1.175735000	-1.797695000	0.255117000
H	3.251923000	0.557597000	1.098779000
H	4.331336000	-2.073297000	0.479806000
H	5.255777000	-0.304246000	-0.757313000
H	4.166660000	-1.166033000	-1.860036000
S	3.464149000	1.129089000	-1.342197000
C	3.116665000	-0.760256000	2.636072000
O	2.771749000	0.091565000	3.446554000
C	3.325107000	-2.214851000	3.018725000
H	2.662935000	-2.877048000	2.452243000
H	4.354986000	-2.529012000	2.820018000
H	3.113457000	-2.318695000	4.081806000
H	1.110780000	-1.214103000	1.081144000
C	-0.031524000	-2.483908000	-0.142610000
H	0.151780000	-3.567226000	-0.148147000
C	-0.493915000	-2.102100000	-1.564816000
H	0.336532000	-2.250986000	-2.252467000
H	-1.335712000	-2.722737000	-1.875503000
C	-1.115678000	-2.127654000	0.871076000
O	-0.916319000	-1.274182000	1.741738000
N	-2.303867000	-2.752151000	0.696716000
H	-2.411532000	-3.300266000	-0.150899000
C	-3.537376000	-2.067335000	1.043593000
H	-4.230552000	-2.744705000	1.554100000
H	-3.301359000	-1.246006000	1.719751000
C	-4.180205000	-1.604842000	-0.273460000
O	-4.032872000	-2.267862000	-1.292435000
N	-4.944282000	-0.486769000	-0.200466000
H	-4.845041000	0.154379000	0.571899000
H	-5.306938000	-0.121552000	-1.070012000
Se	-1.148064000	-0.235617000	-1.780769000
Hg	1.038375000	0.926695000	-0.871891000
Hg	-1.424856000	1.218874000	0.665578000
C	-3.101493000	1.857163000	1.846460000
H	-3.491329000	1.001627000	2.401018000
H	-2.752734000	2.613071000	2.550191000
H	-3.865907000	2.285304000	1.195781000
C	0.941671000	2.374707000	1.089707000
H	1.163781000	1.795334000	1.987108000
H	1.867828000	2.779728000	0.678114000
H	0.263909000	3.200692000	1.304669000

Sum of electronic and zero-point Energies= -4094.966842
Sum of electronic and thermal Free Energies= -4095.032565

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TS4 with MeHgCl assistance on selenium side

C	-3.028004000	0.592857000	-2.186939000
N	-4.126715000	1.002560000	0.054148000
C	-4.287378000	0.492221000	-1.293408000
C	-4.799150000	-0.963471000	-1.285279000
O	-3.010817000	0.080027000	-3.303313000
N	-1.999777000	1.300978000	-1.673800000
H	-4.063227000	0.293466000	0.780414000
H	-5.044624000	1.077960000	-1.828861000
H	-5.872938000	-0.965759000	-1.081763000
H	-4.626777000	-1.376367000	-2.278528000
S	-4.111908000	-2.080908000	0.014918000
C	-4.137076000	2.291779000	0.527987000
O	-3.986379000	2.506765000	1.726501000

C	-4.269950000	3.423289000	-0.472996000
H	-3.268604000	3.724536000	-0.798242000
H	-4.854447000	3.163793000	-1.358365000
H	-4.728877000	4.269307000	0.038525000
H	-2.071852000	1.716913000	-0.756139000
C	-0.751626000	1.525995000	-2.368029000
H	-0.952799000	2.030780000	-3.324796000
C	-0.018616000	0.214910000	-2.673114000
H	-0.673756000	-0.430784000	-3.254639000
H	0.893711000	0.387675000	-3.248862000
C	0.051736000	2.480868000	-1.466885000
O	-0.507469000	3.136899000	-0.587926000
N	1.381804000	2.546307000	-1.687563000
H	1.845970000	1.858846000	-2.269786000
C	2.254477000	3.139900000	-0.700812000
H	2.347326000	4.224539000	-0.830946000
H	1.858578000	2.958938000	0.305138000
C	3.621785000	2.477265000	-0.816581000
O	3.819323000	1.552233000	-1.608113000
N	4.585760000	2.983948000	-0.017620000
H	4.336786000	3.558670000	0.773806000
H	5.448051000	2.461977000	0.048409000
Se	0.468760000	-0.737447000	-1.002365000
Hg	-1.731062000	-1.677583000	0.106115000
Hg	-0.322499000	0.485059000	1.583896000
C	-0.631832000	2.369285000	2.533830000
H	-1.710702000	2.512774000	2.593338000
H	-0.170307000	2.327183000	3.520281000
H	-0.175027000	3.121296000	1.895081000
C	-0.405731000	-2.051986000	2.129436000
H	-0.574494000	-3.106557000	1.879842000
H	0.677260000	-1.914313000	2.185899000
H	-0.881204000	-1.858555000	3.090830000
Hg	3.480158000	-0.909241000	-0.128636000
Cl	2.895906000	0.400376000	1.831833000
C	4.161781000	-2.086900000	-1.732124000
H	3.577296000	-3.006596000	-1.755499000
H	3.989303000	-1.500287000	-2.634892000
H	5.222568000	-2.306939000	-1.613574000

Sum of electronic and zero-point Energies= -4748.663219
Sum of electronic and thermal Free Energies= -4748.740821

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TS4 on sulfur side

C	0.743373000	2.671286000	1.048245000
N	2.857205000	1.874184000	-0.071981000
C	2.240765000	2.327949000	1.158805000
C	2.467329000	1.293363000	2.277067000
O	0.221342000	3.407979000	1.878318000
N	0.087599000	2.086638000	0.016979000
H	3.160571000	0.908694000	-0.102738000
H	2.705162000	3.259942000	1.500122000
H	3.533581000	1.272206000	2.518171000
H	1.920079000	1.630024000	3.159171000
S	1.972283000	-0.444527000	1.912999000
C	3.168948000	2.616502000	-1.183072000
O	3.703586000	2.098081000	-2.157311000
C	2.811373000	4.089058000	-1.138686000
H	1.740766000	4.233006000	-0.964964000
H	3.345870000	4.599267000	-0.330728000
H	3.088656000	4.534551000	-2.092796000
H	0.606376000	1.508733000	-0.632845000
C	-1.304769000	2.293338000	-0.313496000
H	-1.458775000	3.334301000	-0.634549000
C	-2.279862000	2.032100000	0.858276000
H	-1.878313000	2.489894000	1.758297000
H	-3.249836000	2.479632000	0.630727000
C	-1.610800000	1.343133000	-1.479951000
O	-0.797862000	0.488747000	-1.840735000
N	-2.840167000	1.486314000	-2.026975000
H	-3.493148000	2.100063000	-1.547001000
C	-3.522421000	0.327810000	-2.573707000
H	-3.875108000	0.515204000	-3.594796000
H	-2.821356000	-0.508120000	-2.600632000
C	-4.739418000	0.061165000	-1.677327000
O	-5.290814000	0.984744000	-1.092373000
N	-5.191603000	-1.218746000	-1.659691000
H	-4.584836000	-1.976808000	-1.931031000
H	-5.935534000	-1.427877000	-1.009008000
Se	-2.727480000	0.122270000	1.199485000
Hg	-0.493598000	-0.882478000	1.012647000
Hg	1.791884000	-1.448121000	-0.732238000
C	2.838165000	-1.168579000	-2.568335000
H	3.257628000	-0.162227000	-2.555154000
H	3.618553000	-1.927705000	-2.630049000
H	2.116178000	-1.286923000	-3.375768000
C	0.397663000	-3.090793000	0.643236000
H	-0.398934000	-3.366107000	1.343662000
H	0.231366000	-3.663007000	-0.269823000
H	1.342682000	-3.356680000	1.120683000

Sum of electronic and zero-point Energies= -4094.966569
Sum of electronic and thermal Free Energies= -4095.034319

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TS4 with MeHgCl assistance on sulfur side

C	-0.975767000	-2.368274000	2.022738000
N	-0.828216000	-3.788427000	-0.058092000
C	-1.653948000	-3.348387000	1.046166000
C	-2.953587000	-2.699619000	0.532649000
O	-1.583122000	-1.961863000	3.008547000
N	0.289420000	-1.975931000	1.720968000
H	-1.032373000	-3.367650000	-0.959100000
H	-1.951145000	-4.202986000	1.665297000
H	-3.607236000	-3.482703000	0.140921000
H	-3.440642000	-2.231004000	1.387734000
S	-2.784212000	-1.480950000	-0.847494000
C	0.209962000	-4.686094000	-0.054276000
O	0.889965000	-4.857134000	-1.059884000
C	0.463536000	-5.434268000	1.241226000
H	0.671353000	-4.746528000	2.066574000
H	-0.408307000	-6.034172000	1.522059000
H	1.320644000	-6.089144000	1.092383000
H	0.791743000	-2.365270000	0.932331000
C	1.001854000	-1.007433000	2.522559000
H	1.068951000	-1.366368000	3.558833000
C	0.305034000	0.370959000	2.556478000
H	-0.733406000	0.229162000	2.846814000
H	0.796718000	1.023085000	3.279882000
C	2.396156000	-0.842013000	1.920776000
O	2.685759000	-1.328144000	0.822840000
N	3.236629000	-0.068219000	2.647585000
H	2.833673000	0.401295000	3.453616000
C	4.198284000	0.789881000	1.980155000
H	5.184825000	0.721981000	2.452328000
H	4.293869000	0.469457000	0.943237000
C	3.681782000	2.231612000	2.119011000

O	2.994439000	2.547174000	3.082785000
N	4.099345000	3.105023000	1.170976000
H	4.457759000	2.774495000	0.288483000
H	3.707718000	4.035917000	1.200296000
Se	0.375955000	1.389564000	0.856084000
Hg	-0.471963000	-0.516920000	-0.804260000
Hg	2.121916000	0.279655000	-1.422580000
C	3.938990000	1.293372000	-1.907035000
H	4.722339000	0.974402000	-1.217653000
H	4.223680000	1.027610000	-2.925043000
H	3.761405000	2.367621000	-1.841805000
C	0.674521000	-1.598762000	-2.600161000
H	0.956487000	-2.558917000	-2.169013000
H	-0.282668000	-1.645273000	-3.119415000
H	1.427325000	-1.257735000	-3.311131000
Hg	-2.753646000	1.745960000	0.165903000
Cl	-1.848853000	2.112026000	-2.068172000
C	-3.565919000	1.448288000	2.084667000
H	-3.196715000	2.229474000	2.749917000
H	-4.654237000	1.472525000	2.035409000
H	-3.222865000	0.467800000	2.419979000

Sum of electronic and zero-point Energies= -4748.658070
Sum of electronic and thermal Free Energies= -4748.735230

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SH-Se(minus) – selenol/selenolate form

C	0.347756000	-1.418738000	-0.152471000
N	2.748660000	-1.012282000	0.425245000
C	1.815668000	-1.880405000	-0.264158000
C	2.154927000	-1.980075000	-1.760800000
O	-0.550792000	-2.076885000	-0.689887000
N	0.146131000	-0.235329000	0.474381000
H	2.775764000	-0.035077000	0.111028000
H	1.904583000	-2.872765000	0.187243000
H	3.207229000	-2.258911000	-1.852767000
H	1.534783000	-2.762162000	-2.201275000
C	3.151083000	-1.253763000	1.710568000
O	2.961246000	-2.318862000	2.292066000
C	3.878985000	-0.074445000	2.338879000
H	3.443426000	0.874028000	2.000442000
H	3.820830000	-0.163149000	3.424551000
H	4.934396000	-0.089440000	2.044421000
H	1.004165000	0.234562000	0.766803000
C	-0.758304000	0.778069000	-0.087365000
H	-0.824707000	0.599995000	-1.169990000
C	-0.139664000	2.160634000	0.161678000
H	-0.648125000	2.891930000	-0.466629000
H	-0.297275000	2.450317000	1.202087000
C	-2.181291000	0.648586000	0.476297000
O	-2.797970000	1.607237000	0.964826000
N	-2.715097000	-0.586060000	0.354327000
H	-2.127963000	-1.307596000	-0.078545000
C	-3.996117000	-0.944392000	0.939472000
H	-4.045814000	-2.028345000	1.039676000
H	-4.072439000	-0.484623000	1.932563000
C	-5.218789000	-0.512453000	0.109677000
O	-6.039751000	-1.318874000	-0.313769000
N	-5.307714000	0.830685000	-0.065298000
H	-4.555127000	1.429946000	0.273102000
H	-6.036540000	1.181028000	-0.667018000
S	1.824073000	-0.468000000	-2.782927000
H	1.899062000	0.554156000	-1.819873000
Se	1.820216000	2.235764000	-0.216945000

Sum of electronic and zero-point Energies= -3708.750837
Sum of electronic and thermal Free Energies= -3708.804995

5

$MeHg^+$

Hg	0.229456000	0.000000000	0.000000000
C	-1.965718000	0.000008000	0.000000000
H	-2.187403000	-0.533169000	-0.922329000
H	-2.187403000	-0.532235000	0.922868000
H	-2.187374000	1.065374000	-0.000539000

Sum of electronic and zero-point Energies= -193.114925
Sum of electronic and thermal Free Energies= -193.141445

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4'-Cl^-

C	0.734122000	0.531237000	-2.134035000
N	0.084045000	2.356594000	-0.498216000
C	0.352204000	2.004650000	-1.875095000
C	-0.824114000	2.358793000	-2.790094000
O	0.775012000	0.126881000	-3.294688000
N	1.002420000	-0.220379000	-1.047006000
H	-0.835150000	2.144523000	-0.133788000
H	1.224456000	2.595668000	-2.175528000
H	-1.028904000	3.430860000	-2.750153000
H	-0.573130000	2.064911000	-3.808018000
C	0.971956000	3.045162000	0.281180000
O	2.094198000	3.353581000	-0.116744000
C	0.480675000	3.363116000	1.676781000
H	0.673211000	2.491675000	2.311647000
H	1.032222000	4.224155000	2.055739000
H	-0.593983000	3.564684000	1.702021000
H	0.868234000	0.159812000	-0.112033000
C	1.292089000	-1.641253000	-1.163835000
H	1.219702000	-1.879680000	-2.224493000
C	0.313356000	-2.499376000	-0.353973000
H	0.448825000	-3.550157000	-0.619409000
H	0.453404000	-2.384061000	0.718968000
C	2.770622000	-1.868931000	-0.775050000
O	3.612097000	-2.163802000	-1.615203000
N	3.058229000	-1.665941000	0.545250000
H	2.309721000	-1.334989000	1.156418000
C	4.416754000	-1.361370000	0.950017000
H	4.563153000	-1.659730000	1.993550000
H	5.106614000	-1.929455000	0.326718000
C	4.820697000	0.121803000	0.810283000
O	6.002742000	0.432482000	0.697690000
N	3.797625000	1.006312000	0.842117000
H	2.848384000	0.728034000	1.052071000
H	3.967065000	1.989682000	0.693801000
S	-2.406934000	1.545735000	-2.299587000
H	-1.956707000	0.266423000	-2.313150000
Se	-1.593625000	-2.076021000	-0.747252000
Hg	-2.036476000	-0.294102000	0.922969000
C	-2.680490000	1.220676000	2.263575000
H	-1.846033000	1.399010000	2.940479000
H	-2.932547000	2.128261000	1.711323000
H	-3.555102000	0.879682000	2.820856000
Cl	0.756645000	-0.113202000	2.200791000

Sum of electronic and zero-point Energies= -4362.501701
Sum of electronic and thermal Free Energies= -4362.565905

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4'-OH$^-$

C	1.346512000	1.521507000	-1.025333000
N	-0.537438000	2.655091000	0.162040000
C	0.316437000	2.656884000	-1.004982000
C	-0.523050000	2.605452000	-2.303162000
O	2.304175000	1.578696000	-1.810518000
N	1.129193000	0.480785000	-0.201116000
H	-1.476361000	2.287585000	0.034154000
H	0.913916000	3.573128000	-0.998602000
H	-0.947541000	3.595548000	-2.490236000
H	0.141764000	2.358873000	-3.133155000
C	-0.117165000	3.156849000	1.359705000
O	1.003236000	3.632619000	1.516546000
C	-1.123716000	3.044179000	2.490310000
H	-0.863662000	2.177595000	3.106400000
H	-1.051966000	3.936066000	3.115585000
H	-2.149423000	2.909127000	2.141558000
H	0.259287000	0.467021000	0.317000000
C	1.824817000	-0.794098000	-0.367878000
H	2.041574000	-0.899797000	-1.438055000
C	0.949568000	-1.952840000	0.090695000
H	1.477753000	-2.885075000	-0.113589000
H	0.771171000	-1.904304000	1.165037000
C	3.176498000	-0.761882000	0.373866000
O	3.431631000	-1.508847000	1.327277000
N	4.055473000	0.134121000	-0.125049000
H	3.720354000	0.729223000	-0.885959000
C	5.308050000	0.453342000	0.543939000
H	5.646638000	1.431075000	0.203528000
H	5.134639000	0.487017000	1.626276000
C	6.443807000	-0.545060000	0.257589000
O	7.496176000	-0.203056000	-0.269236000
N	6.173734000	-1.808867000	0.673176000
H	5.256971000	-2.026680000	1.059694000
H	6.843382000	-2.530827000	0.458168000
S	-1.984890000	1.479798000	-2.217120000
H	-1.297510000	0.327705000	-2.008367000
Se	-0.772096000	-2.001525000	-0.903311000
Hg	-2.443949000	-0.753694000	0.544980000
C	-3.478950000	0.215645000	2.148514000
H	-4.248319000	-0.479177000	2.493633000
H	-2.845871000	0.507234000	2.986489000
H	-3.968892000	1.092181000	1.717804000
O	-3.828297000	-0.625341000	-1.242608000
H	-3.571463000	-1.303223000	-1.883921000

Sum of electronic and zero-point Energies= -3978.016052
Sum of electronic and thermal Free Energies= -3978.082463

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Reduced TrxR(497-499) stabilized by MeHgOH

C	-0.476059000	1.527997000	-1.405683000
N	-1.199124000	2.159451000	0.867476000
C	-1.286596000	2.515876000	-0.541992000
C	-2.725265000	2.673045000	-1.026866000
O	-0.991284000	0.746816000	-2.205697000
N	0.858436000	1.574119000	-1.163535000
H	-1.768219000	1.381674000	1.236159000
H	-0.797535000	3.493049000	-0.633428000
H	-3.174474000	3.547772000	-0.554130000
H	-2.718625000	2.832028000	-2.106236000
C	-0.126534000	2.563796000	1.599684000
O	0.757351000	3.293563000	1.142172000
C	-0.059476000	2.027211000	3.014453000
H	-0.999758000	1.581561000	3.343068000
H	0.728616000	1.267755000	3.046173000
H	0.232649000	2.835103000	3.687626000
H	1.241615000	2.269597000	-0.515642000
C	1.778377000	0.584126000	-1.679844000
H	1.810425000	0.615970000	-2.778450000
C	1.349964000	-0.828381000	-1.254373000
H	0.408283000	-1.090572000	-1.727139000
H	2.103959000	-1.584048000	-1.471647000
C	3.164520000	0.950412000	-1.111609000
O	3.336157000	1.972389000	-0.464363000
N	4.154609000	0.049918000	-1.363120000
H	3.959870000	-0.756758000	-1.935515000
C	5.469967000	0.163676000	-0.763474000
H	6.256740000	0.085469000	-1.517289000
H	5.528932000	1.158516000	-0.314426000
C	5.784747000	-0.871726000	0.323269000
O	6.945209000	-1.114308000	0.616218000
N	4.715924000	-1.476004000	0.915873000
H	3.762517000	-1.155332000	0.801608000
H	4.925696000	-2.055206000	1.715294000
S	-3.864016000	1.247549000	-0.777240000
H	-4.069810000	1.423255000	0.542848000
Se	1.085319000	-0.750773000	0.705609000
H	1.173747000	-2.206069000	0.849720000
Hg	-2.145050000	-1.325929000	0.169117000
C	-1.918556000	-2.546222000	-1.514418000
H	-1.109378000	-3.263978000	-1.367145000
H	-2.850571000	-3.083294000	-1.696608000
H	-1.699661000	-1.889318000	-2.356936000
O	-2.516542000	-0.139872000	1.839524000
H	-2.104157000	-0.547258000	2.613246000

Sum of electronic and zero-point Energies= -3978.541410
Sum of electronic and thermal Free Energies= -3978.604102

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MeHgOH

Hg	0.013679000	0.001478000	0.000012000
C	-2.073468000	0.001047000	-0.000048000
H	-2.437993000	1.030210000	-0.001808000
H	-2.440423000	-0.513441000	-0.890337000
H	-2.440497000	-0.510420000	0.891950000
O	2.032532000	-0.113778000	-0.000052000
H	2.405175000	0.779330000	-0.000067000

Sum of electronic and zero-point Energies= -269.226376
Sum of electronic and thermal Free Energies= -269.256781

3

H2O

O	0.000000000	0.000000000	0.119209000
H	0.000000000	0.759337000	-0.476834000
H	0.000000000	-0.759337000	-0.476834000

Sum of electronic and zero-point Energies= -76.398948
Sum of electronic and thermal Free Energies= -76.416605

45

TS1_Se_OH

C	0.606240000	1.428205000	0.144799000
N	-1.561654000	2.248800000	1.141873000
C	-0.140852000	2.508500000	0.962998000
C	0.063602000	3.899455000	0.354911000
O	1.133965000	1.693061000	-0.941746000
N	0.693693000	0.217324000	0.738559000
H	-2.184923000	2.753760000	0.522365000
H	0.311911000	2.497173000	1.962502000
H	-0.260388000	4.657783000	1.070761000
H	1.120338000	4.051881000	0.138225000

C	-2.051665000	1.405039000	2.080040000
O	-1.305317000	0.679605000	2.755620000
C	-3.553504000	1.369568000	2.236516000
H	-4.067911000	2.111805000	1.622468000
H	-3.903578000	0.368208000	1.970030000
H	-3.795856000	1.538931000	3.288479000
H	0.270863000	0.122920000	1.662826000
C	1.580954000	-0.837040000	0.230561000
H	1.518525000	-0.805698000	-0.860806000
C	1.181934000	-2.213889000	0.747370000
H	2.027141000	-2.886862000	0.617782000
H	0.960181000	-2.184684000	1.815530000
C	3.040343000	-0.515755000	0.638375000
O	3.637188000	-1.167944000	1.498046000
N	3.591467000	0.528559000	-0.018372000
H	3.026598000	1.008238000	-0.711395000
C	4.915937000	1.042430000	0.310417000
H	4.989916000	2.070769000	-0.041027000
H	5.038748000	1.016391000	1.398360000
C	6.063212000	0.255133000	-0.350510000
O	6.815303000	0.775759000	-1.161318000
N	6.153573000	-1.033618000	0.066537000
H	5.482075000	-1.408980000	0.727466000
H	6.859086000	-1.620892000	-0.350494000
S	-0.916475000	4.217077000	-1.168855000
H	-0.285757000	3.283028000	-1.906997000
Se	-0.334706000	-3.053286000	-0.207585000
H	-1.467778000	-2.545682000	0.810376000
Hg	-2.061913000	-0.607495000	-0.647156000
C	-1.911578000	0.519737000	-2.411816000
H	-0.851012000	0.657281000	-2.624393000
H	-2.393601000	-0.030507000	-3.220816000
H	-2.395541000	1.487446000	-2.277955000
O	-2.500981000	-1.607486000	1.201065000
H	-2.150920000	-1.083910000	1.943303000

Sum of electronic and zero-point Energies= -3978.525960
Sum of electronic and thermal Free Energies= -3978.589397

45

TS1_S_OH without Se…Hg interaction

C	-0.468954000	1.290500000	-1.534077000
N	-0.893601000	2.323031000	0.663116000
C	-1.206380000	2.404528000	-0.758037000
C	-2.710346000	2.451149000	-1.034257000
O	-1.044777000	0.387647000	-2.142790000
N	0.883477000	1.369180000	-1.430643000
H	-1.427125000	1.685725000	1.249954000
H	-0.779822000	3.355403000	-1.100254000
H	-3.090974000	3.402954000	-0.657261000
H	-2.856684000	2.428725000	-2.115514000
C	0.242984000	2.870322000	1.169906000
O	1.028050000	3.524009000	0.480249000
C	0.513659000	2.565028000	2.628248000
H	-0.369772000	2.202679000	3.158624000
H	1.286708000	1.790401000	2.664551000
H	0.910126000	3.457071000	3.115393000
H	1.316252000	2.165340000	-0.950412000
C	1.768215000	0.278262000	-1.786293000
H	1.816242000	0.143364000	-2.876632000
C	1.294230000	-1.041521000	-1.157155000
H	0.367972000	-1.370101000	-1.617986000
H	2.045101000	-1.828167000	-1.213366000
C	3.162360000	0.674925000	-1.254803000
O	3.367264000	1.775364000	-0.765668000
N	4.119840000	-0.289295000	-1.346593000
H	3.904192000	-1.165513000	-1.795923000
C	5.425548000	-0.135805000	-0.734841000
H	6.222654000	-0.385552000	-1.438572000
H	5.529240000	0.918415000	-0.465408000
C	5.660332000	-0.976015000	0.526749000
O	6.799490000	-1.212616000	0.897481000
N	4.549042000	-1.419724000	1.180522000
H	3.613435000	-1.090810000	0.979242000
H	4.711143000	-1.859207000	2.074444000
S	-3.808547000	1.126532000	-0.357574000
H	-3.232587000	0.879470000	1.093695000
Se	0.944763000	-0.639379000	0.749710000
H	0.999931000	-2.053657000	1.132257000
Hg	-2.205560000	-1.196654000	0.165886000
C	-1.921605000	-2.925104000	-1.020470000
H	-1.129130000	-3.546468000	-0.602688000
H	-2.857972000	-3.481757000	-1.053333000
H	-1.660105000	-2.570118000	-2.017031000
O	-2.445232000	0.252528000	1.899807000
H	-2.869118000	-0.066920000	2.709102000

Sum of electronic and zero-point Energies= -3978.525944
Sum of electronic and thermal Free Energies= -3978.588150

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TS1_S_OH with Se…Hg interaction

C	0.374491000	1.263326000	0.992868000
N	1.174881000	3.012326000	-0.527674000
C	1.148009000	2.585977000	0.864424000
C	2.571598000	2.517024000	1.435175000
O	0.908419000	0.173006000	1.252351000
N	-0.954695000	1.371796000	0.799039000
H	2.028810000	2.783552000	-1.027798000
H	0.575317000	3.339948000	1.417396000
H	2.884044000	3.532902000	1.686416000
H	2.553204000	1.927137000	2.352634000
C	0.146456000	3.670017000	-1.115829000
O	-0.922057000	3.884631000	-0.534813000
C	0.387070000	4.137147000	-2.537203000
H	-0.408546000	3.747147000	-3.176846000
H	0.319064000	5.228122000	-2.560936000
H	1.357573000	3.832333000	-2.935463000
H	-1.366663000	2.272273000	0.541142000
C	-1.772286000	0.182267000	0.801432000
H	-1.446306000	-0.447648000	1.634382000
C	-1.568200000	-0.602646000	-0.516956000
H	-2.247486000	-0.258059000	-1.299966000
H	-0.541618000	-0.476953000	-0.851233000
C	-3.237026000	0.586718000	0.994447000
O	-3.583011000	1.769617000	0.980427000
N	-4.097714000	-0.443838000	1.150493000
H	-3.750253000	-1.382946000	0.977603000
C	-5.538664000	-0.237622000	1.227046000
H	-5.997985000	-1.119323000	1.671985000
H	-5.722730000	0.629929000	1.867784000
C	-6.208425000	-0.016438000	-0.142408000
O	-7.082004000	-0.763154000	-0.557339000
N	-5.733700000	1.060351000	-0.825078000
H	-5.090072000	1.707783000	-0.384140000
H	-6.175181000	1.291819000	-1.701859000
S	3.845297000	1.818161000	0.294087000
H	4.003350000	0.404762000	1.068440000
Se	-1.916435000	-2.531870000	-0.352011000
H	-1.098566000	-2.684904000	0.847301000
Hg	2.615600000	-1.275502000	-0.174502000
C	1.567716000	-1.974520000	-1.846305000
H	2.138607000	-2.777384000	-2.313155000
H	0.593455000	-2.344703000	-1.527302000
H	1.454254000	-1.144968000	-2.545967000
O	3.882040000	-0.753918000	1.476850000
H	4.750795000	-1.181045000	1.425362000

Sum of electronic and zero-point Energies= -3978.514218
Sum of electronic and thermal Free Energies= -3978.579669

Optimization in solvent

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4'

C	0.590493000	1.719805000	0.872499000
N	0.380135000	3.708609000	-0.615522000
C	1.257729000	2.648335000	-0.157401000
C	1.818579000	1.865476000	-1.354254000
O	1.244410000	1.259881000	1.822711000
N	-0.696290000	1.436037000	0.633922000
H	-0.000093000	3.645489000	-1.551814000
H	2.108490000	3.087952000	0.370285000
H	2.437584000	2.536447000	-1.953362000
H	2.453564000	1.055505000	-0.991291000
C	-0.128252000	4.617173000	0.250506000
O	0.204298000	4.626465000	1.446373000
C	-1.114645000	5.605999000	-0.316453000
H	-1.253425000	5.501692000	-1.394159000
H	-2.076604000	5.463113000	0.185074000
H	-0.770927000	6.618904000	-0.090160000
H	-1.125705000	1.789956000	-0.215207000
C	-1.518969000	0.493082000	1.364296000
H	-1.732401000	0.878512000	2.369256000
C	-0.901467000	-0.908029000	1.537119000
H	-0.065657000	-0.863278000	2.228436000
H	-1.656887000	-1.582051000	1.941913000
C	-2.832200000	0.392473000	0.584154000
O	-2.922350000	0.791068000	-0.584574000
N	-3.869384000	-0.154747000	1.250086000
H	-3.684264000	-0.581324000	2.148610000
C	-5.026194000	-0.618316000	0.517768000
H	-5.802922000	-0.902595000	1.230836000
H	-5.422564000	0.188426000	-0.102392000
C	-4.700127000	-1.831374000	-0.360723000
O	-3.654505000	-2.478694000	-0.227287000
N	-5.650354000	-2.139907000	-1.258880000
H	-6.487825000	-1.583030000	-1.354211000
H	-5.531784000	-2.951843000	-1.848977000
S	0.565557000	1.192525000	-2.524651000
H	0.085534000	0.203110000	-1.711855000
Se	-0.277919000	-1.713176000	-0.165703000
Hg	2.181461000	-1.382610000	0.097111000
C	4.282359000	-1.145442000	0.179470000
H	4.741003000	-2.040119000	0.605949000
H	4.659153000	-0.985008000	-0.833210000
H	4.521775000	-0.278322000	0.798850000

Sum of electronic and zero-point Energies= -3902.225272
Sum of electronic and thermal Free Energies= -3902.286502

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TS3

C	0.999582000	1.498730000	1.245418000
N	1.172050000	3.310068000	-0.468039000
C	1.880987000	2.541093000	0.533751000
C	3.167512000	1.935764000	-0.053430000
O	1.424818000	0.934027000	2.268727000
N	-0.222217000	1.296300000	0.734262000
H	1.250976000	2.972351000	-1.422891000
H	2.170186000	3.219408000	1.342777000
H	3.903843000	2.737192000	-0.148769000
H	3.557080000	1.214793000	0.672564000
C	0.240941000	4.241179000	-0.147780000
O	0.042706000	4.596863000	1.025075000
C	-0.575399000	4.775233000	-1.298243000
H	-0.076923000	4.649218000	-2.261643000
H	-1.522101000	4.223263000	-1.322944000
H	-0.800015000	5.829829000	-1.128730000
H	-0.481777000	1.772773000	-0.121736000
C	-1.327981000	0.651907000	1.414143000
H	-1.509624000	1.164511000	2.369068000
C	-1.126031000	-0.831337000	1.748750000
H	-0.277179000	-0.949526000	2.415876000
H	-2.014700000	-1.217825000	2.250196000
C	-2.553628000	0.866124000	0.516105000
O	-2.445301000	1.337939000	-0.625529000
N	-3.743205000	0.537122000	1.050538000
H	-3.767937000	0.065433000	1.944098000
C	-4.929023000	0.497515000	0.224995000
H	-5.799071000	0.375135000	0.873978000
H	-5.041765000	1.442088000	-0.311388000
C	-4.905319000	-0.668122000	-0.768403000
O	-4.181025000	-1.657208000	-0.604378000
N	-5.758378000	-0.539764000	-1.798545000
H	-6.325254000	0.287962000	-1.915741000
H	-5.847065000	-1.293022000	-2.466655000
S	2.965003000	1.115798000	-1.689919000
H	3.173173000	-0.489021000	-0.923881000
Se	-0.876572000	-1.998341000	0.164458000
Hg	1.525245000	-1.609607000	-0.114949000
C	3.852552000	-1.507445000	-0.344375000
H	3.742677000	-2.351370000	0.352818000
H	4.267271000	-1.906436000	-1.270621000
H	4.529535000	-0.810254000	0.152436000

Sum of electronic and zero-point Energies= -3902.174786
Sum of electronic and thermal Free Energies= -3902.235360

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2'

C	1.625217000	0.889312000	1.544966000
N	2.829429000	1.619226000	-0.537604000
C	2.948230000	0.968739000	0.748825000
C	3.625167000	-0.408313000	0.620575000
O	1.630589000	0.415979000	2.691816000
N	0.532838000	1.375466000	0.933649000
H	2.714914000	1.021180000	-1.348685000
H	3.600032000	1.580613000	1.380679000
H	4.687971000	-0.252117000	0.423721000
H	3.528117000	-0.927404000	1.573655000
C	2.751523000	2.967616000	-0.673881000
O	2.863618000	3.732143000	0.295566000
C	2.458844000	3.465142000	-2.066938000
H	1.378685000	3.634068000	-2.145542000
H	2.965689000	4.418219000	-2.227231000
H	2.753237000	2.750898000	-2.838729000
H	0.606133000	1.667542000	-0.035332000
C	-0.831748000	1.315545000	1.423872000
H	-0.932896000	1.948175000	2.314482000
C	-1.330698000	-0.092284000	1.812273000
H	-0.620434000	-0.561297000	2.487910000
H	-2.289598000	-0.005021000	2.324149000
C	-1.706536000	1.880231000	0.296351000
O	-1.232231000	2.122877000	-0.821669000
N	-3.006865000	2.069589000	0.610293000
H	-3.318425000	1.709137000	1.505146000
C	-4.024940000	2.043951000	-0.425926000
H	-4.805331000	2.779357000	-0.208379000
H	-3.566356000	2.305182000	-1.378198000

C	-4.678436000	0.663536000	-0.469853000
O	-4.822245000	-0.003663000	0.564599000
N	-5.106806000	0.263050000	-1.675765000
H	-4.947245000	0.818934000	-2.503729000
H	-5.595975000	-0.617918000	-1.761935000
S	3.060188000	-1.512231000	-0.764352000
Se	-1.695017000	-1.318917000	0.279665000
Hg	0.665391000	-1.535269000	-0.360738000

Sum of electronic and zero-point Energies= -3861.771968
Sum of electronic and thermal Free Energies= -3861.827999

5

CH4

H	0.000000000	0.000000000	-1.092685000
C	0.000000000	0.000000000	0.000011000
H	0.000000000	1.030184000	0.364206000
H	-0.892165000	-0.515092000	0.364206000
H	0.892165000	-0.515092000	0.364206000

Sum of electronic and zero-point Energies= -40.477548
Sum of electronic and thermal Free Energies= -40.496179

37

SH-Se(minus) – selenol/selenolate form

C	1.306996000	-1.322101000	0.024469000
N	2.859887000	0.250584000	1.136600000
C	2.507512000	-1.157317000	0.982444000
C	3.718142000	-1.971712000	0.534560000
O	1.402357000	-1.903566000	-1.065107000
N	0.156653000	-0.802793000	0.505581000
H	3.420572000	0.643870000	0.388199000
H	2.198251000	-1.524632000	1.965855000
H	4.474452000	-1.961335000	1.321176000
H	3.417204000	-3.004155000	0.356946000
C	2.169630000	1.112378000	1.916749000
O	1.261019000	0.735697000	2.682742000
C	2.543714000	2.563368000	1.772577000
H	1.998107000	2.950356000	0.902934000
H	2.241119000	3.111803000	2.664961000
H	3.612374000	2.696543000	1.588882000
H	0.222313000	-0.301425000	1.391084000
C	-0.929391000	-0.393392000	-0.369224000
H	-0.834592000	-0.941755000	-1.306790000
C	-0.877088000	1.122816000	-0.641250000
H	-1.749493000	1.399273000	-1.238164000
H	-0.938771000	1.650232000	0.314355000
C	-2.255311000	-0.708367000	0.310467000
O	-2.432603000	-0.460446000	1.518204000
N	-3.223532000	-1.200570000	-0.481650000
H	-3.027868000	-1.322208000	-1.466510000
C	-4.573418000	-1.474127000	-0.009141000
H	-5.040461000	-2.186997000	-0.685849000
H	-4.512386000	-1.921215000	0.987748000
C	-5.480329000	-0.242906000	0.068314000
O	-6.593133000	-0.233964000	-0.475350000
N	-4.989292000	0.790717000	0.775124000
H	-4.115017000	0.696461000	1.280699000
H	-5.555210000	1.620557000	0.888906000
S	4.572609000	-1.324827000	-0.967124000
H	3.511144000	-1.471193000	-1.785052000
Se	0.764902000	1.691879000	-1.602020000

Sum of electronic and zero-point Energies= -3708.840378
Sum of electronic and thermal Free Energies= -3708.895306

5

$MeHg^+$

Hg	-0.223826000	-0.000002000	0.000000000
C	1.888960000	-0.000009000	0.000035000
H	2.190603000	0.883325000	0.559511000
H	2.191031000	-0.926238000	0.484948000
H	2.190650000	0.043125000	-1.044707000

Sum of electronic and zero-point Energies= -193.280233
Sum of electronic and thermal Free Energies= -193.306628

3.3. LC-MS Analysis of GPx1 reactivity

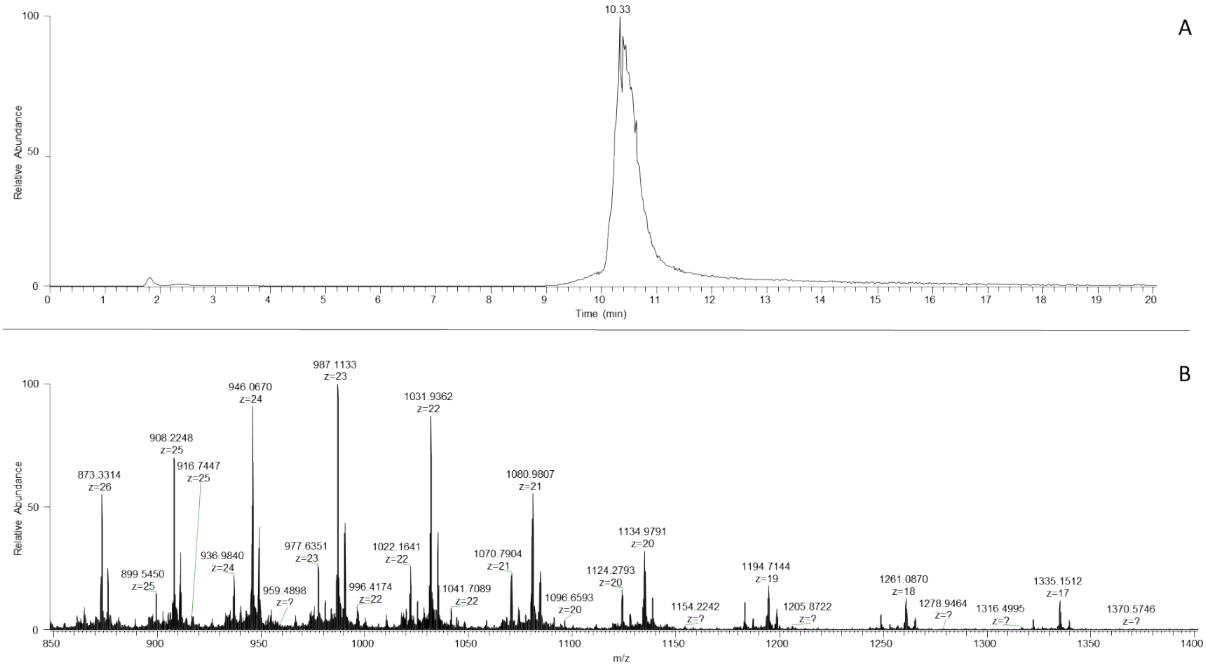

Figure S18. LC-MS of GPx1 protein incubated (5min) with MeHgCl (0.5 equiv.) at 37°C and pH 7.0 preceded by a 30 min. reduction step of the protein with 10 equiv. of DTT. **(A)** TIC **(B)** MS spectrum of peak at t_R= 10.33 min.

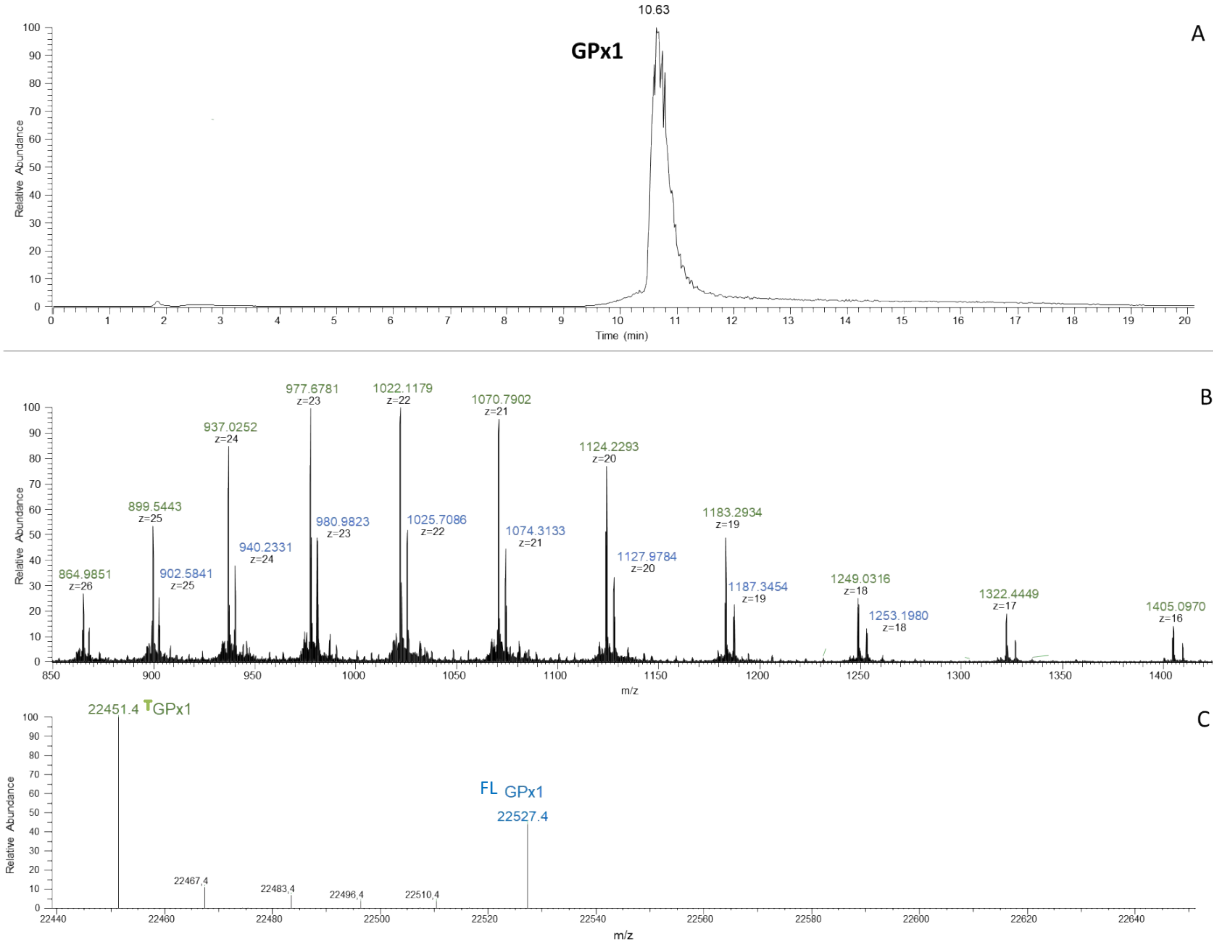

Figure S19. LC-MS of GPx1. **(A)** TIC. **(B)** MS spectrum of peak at t_R= 10.63 min. **(C)** Deconvoluted MS spectrum of peak at t_R= 10.63 min.

Bottom-Up results

1	CAAQRSAAAL	AAAAPRTVYA	FSARPLAGGE	PFNLSSLRGK	VLLIENVASL	UGTTVRDYTQ	MNDLQRRLGP	RGLVVLGFPC
81	NQFGHQENAK	NEEILNCLKY	VRPGGGFEPN	FMLFEKCEVN	GEKAHPLFAF	LREVLPTPSD	DATALMTDPK	FITWSPVCRN
161	DVSWNFEKFL	VGPDGVPVRR	YSRRFLTIDI	EPDIETLLSQ	GASA			

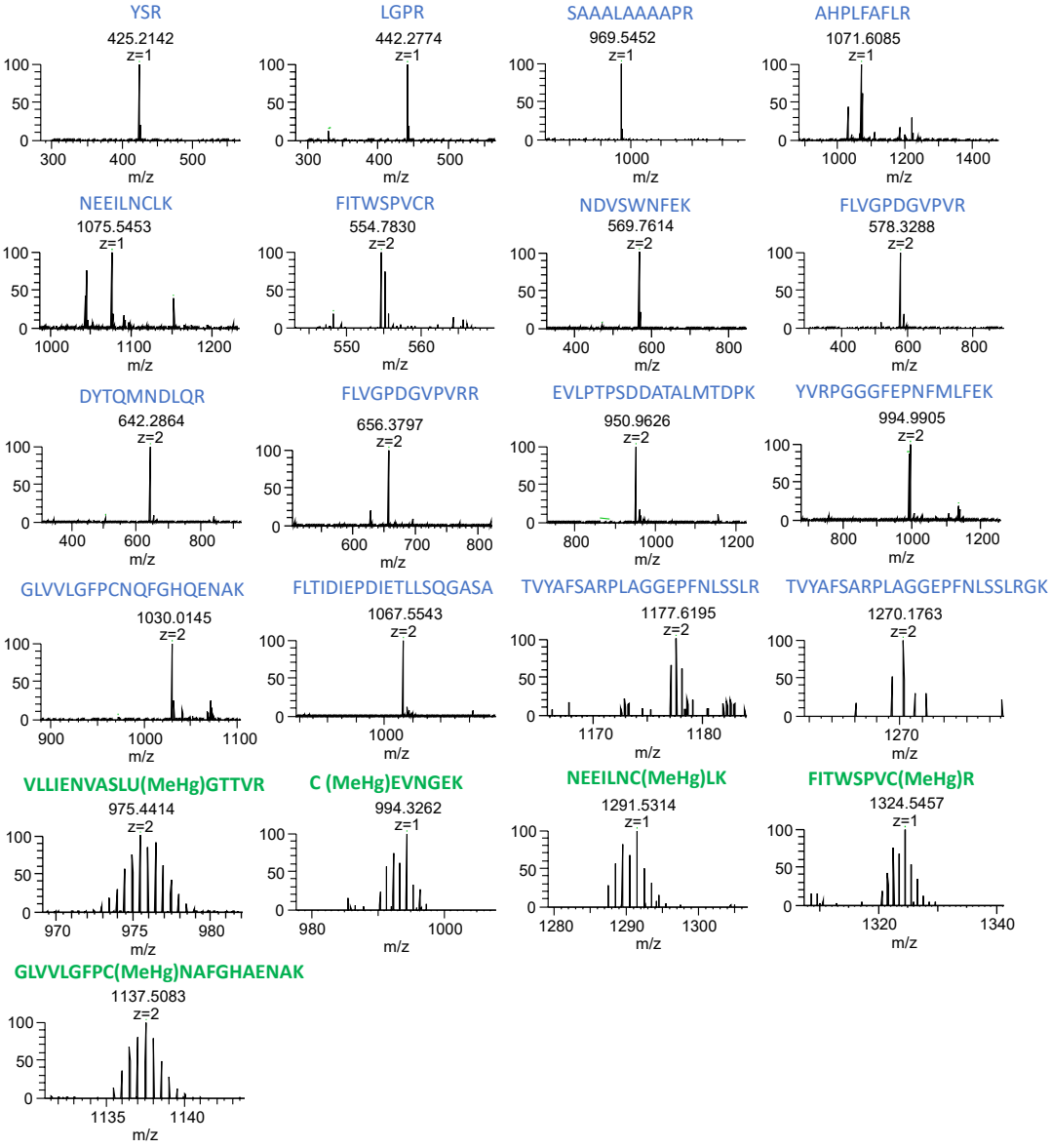

Figure S20. LC-MS of GPx1 protein reduced with DTT (10 equiv., 30 min), incubated with MeHg (0.5 equiv., 5 min) at 37 °C and pH =7 and digested by trypsin.21 peptides were identified in the MS covering 97 % of GPx1 sequence: unmodified peptides are indicated in blue and metalated peptides in green.

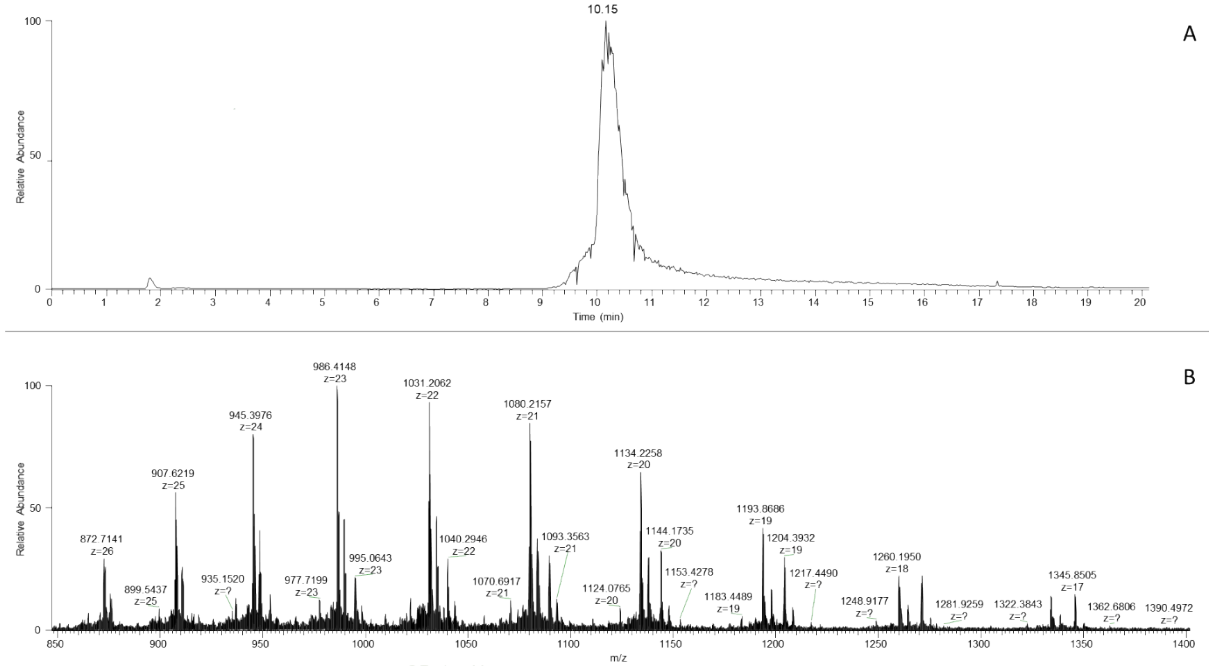

Figure S21. LC-MS of GPx1 protein incubated (3 h) with MeHgCl (3 equiv.) at 37°C and pH 7.0 preceded by a 30 min. reduction step of the protein with 10 equiv. of DTT. **(A)** TIC **(B)** MS spectrum of peak at t_R= 10.15 min.

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