Supporting Information

Glutathione-Complexed Iron-Sulfur Clusters. Reaction Intermediates and Evidence for a Template Effect Promoting Assembly and Stability.

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Experimental Methods

Synthesis of glutathione cluster complex - Ferric chloride (1 mM) and sodium sulfide (1 mM) were added to 1 mL 10 mM glutathione solution, pH 8.6. This reaction mixture was then either applied directly for mass spectrometric experiments or subjected to ethanol precipitation by addition of 4 mL ethanol with mixing by vortexing. The precipitate was collected by centrifugation at 13,000 rpm for 10 min, washed twice with ethanol and dried under vacuum.

ESI-Mass spectrometry experiments - Mass spectra of both the reaction mixture and a 10 mM glutathione solution were obtained on a Bruker Micro-TOF (ESI) spectrometer and data was analyzed by use of DataAnalysis software (Bruker).

Mössbauer - A 10 mg sample of ^{57}Fe metal was dissolved in 250 µL of a 1:1 mixture of concentrated HCl and HNO_3. The suspension was stirred for ~ 10 min until all of the solids had dissolved and gas evolution had ceased. A solution of 5 M NaOH was then slowly added in aliquots of 20 µL and the pH of the solution checked after each addition. Additions continued until a pH ~ 7.4 was obtained; typically ~ 300-340 µL 5 M NaOH total. The color of the mixture turned light yellow, and then dark orange. This ^{57}Fe stock salt solution was subsequently used to synthesize ^{57}Fe-labeled clusters. The solution was centrifuged to discard any precipitate, and then 0.077 g GSH in 4 mL, pH 7.4 was added. A 500 µL solution of 200 mM Na_2S was added and reaction continued for 10 min prior to precipitation with a ten-fold volume excess of ethanol with stirring. The resulting solution was centrifuged at 14,000 rpm and the supernatant removed by decanting. The solid was resuspended two additional times in ethanol with stirring and was collected by centrifugation prior to final drying by speedvac for a period of up to 4 h to obtain the ^{57}Fe-S glutathione cluster complex. The Mössbauer absorber was prepared by distributing uniformly 82 mg of this sample in a 2 cm-diameter holder.

Conventional Mössbauer effect (ME) experiments at constant acceleration at room temperature were carried out in transmission geometry using a nominal 50 mCi ^{57}Co source in a Rh matrix. Instrumentation consisted of ORTEC pulse processing modules (142pc, 572A, 551-TSCA), a LND-4045 proportional counter and CMTE MA-250 linear velocity transducer with MR-350 drive. Recently developed equipment was used in the acquisition of spectra and for velocity reference wave generation.[1] Isomer shifts are referred to α-Fe at room temperature. Mössbauer spectra were fit taking into account sample´s Mössbauer effective thickness µ by considering the Mössbauer

transmission integral.[2] The mean absorber nuclear cross-section was assumed as a sum of three Voigtian (normal distribution of Lorentzian lines) doublets. Each doublet was associated to a given quadrupolar interaction, characterized by δ (mean isomer shift), ΔE_Q (mean quadrupolar splitting) and σ (standard deviation of the normal distribution). The same absorber Lorentzian width γ was assumed for the three interactions and the emitter Lorentzian width was assumed as the natural linewidth 0.095 mm/s. Interaction I and III were well fitted by considering pure Lorentzian shapes (σ= 0 mm/s), while interaction II needed a wider profile (σ = 0.27(1)mm/s). δ and ΔE_Q fitted values are presented on Table 1 of the manuscript, while μ and γ were fitted to 11.4(2) and 0.32(4) mm/s respectively.

Synthesis of N-acetylglutathione - N-acetylglutathione was prepared according to previously published literature by Anderson et al.[3] In brief, glutathione (GSH, 1.00 g, 3.25 mmol) was dissolved in formic acid (3 mL, 3.66 g) and stirred at room temperature. Acetic anhydride (1.5 mL, 1.62 g) was added dropwise to the stirred solution. The resulting mixture was allowed to react at room temperature for 2 h. Diethyl ether (45 mL) was added to the reaction mixture and the resulting precipitate was separated by gravity filtration. The precipitate was washed with petroleum ether (3 x 10 mL) and dried under vacuum. The crude product was recrystallized from a 1:1 mixture of ethyl acetate and dimethyl formamide to yield N-acetylglutathione as a white solid (0.90 g, 2.57 mmol, 79%). Characterization of N-acetylglutathione was confirmed by ESI-MS and NMR (Figure S2).

Figures

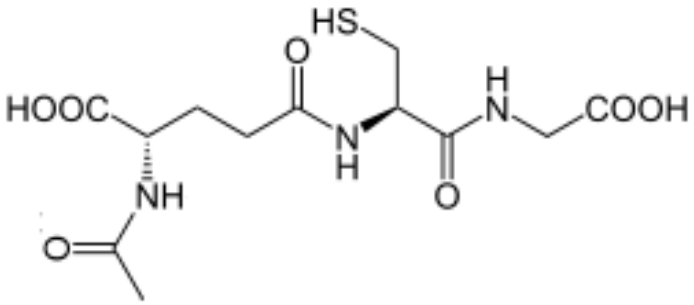


Figure S 1 Structure of N-acetylglutathione

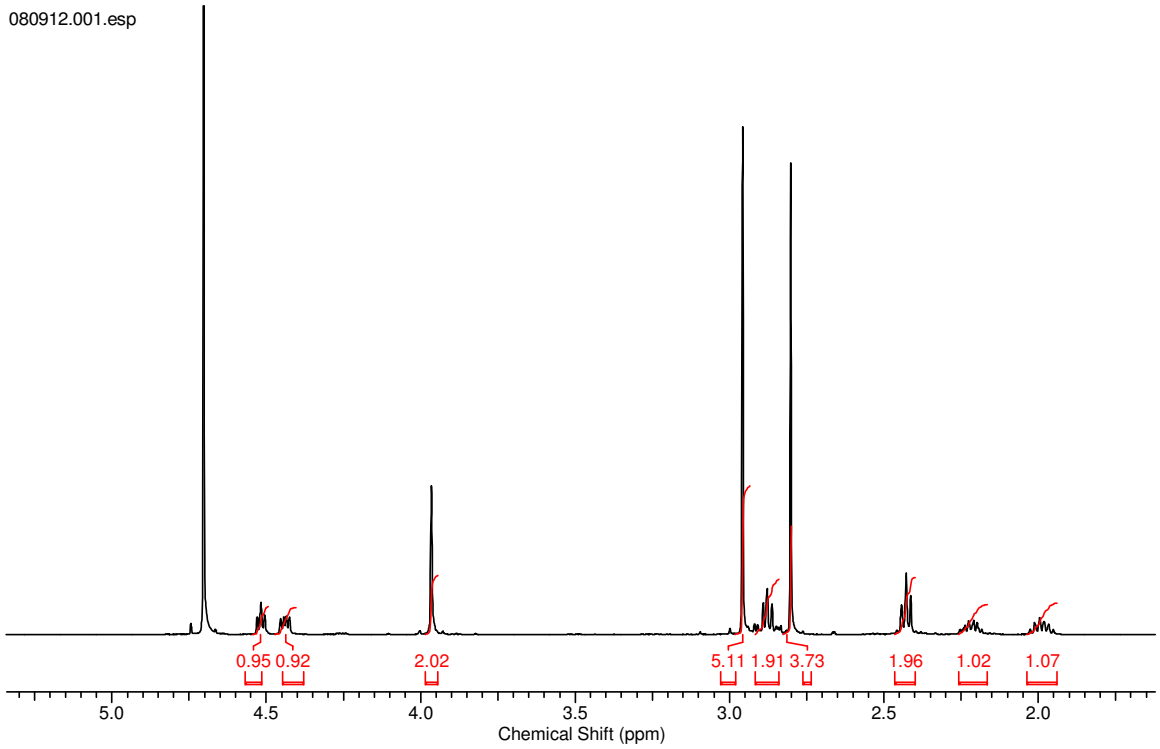
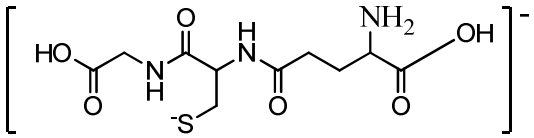
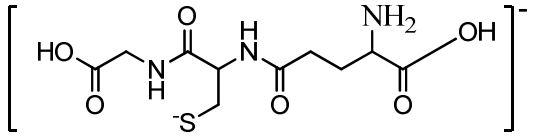
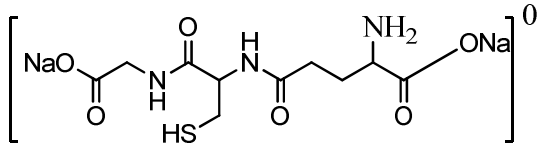
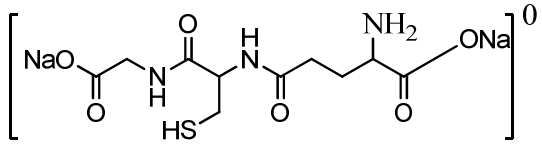
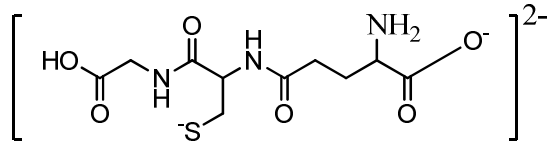
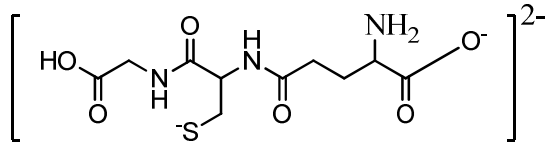


Figure S2. NMR spectrum of N-acetylglutathione

A

		m/z
4 	$+ 2Fe^{3+} + 2S^{2-} + 2H^{+} + Na^{+}$	1425.3
4 	$+ Fe^{3+} + Fe^{2+} + 2S^{2-} + 3H^{+} + Na^{+}$	1426.3
4 	$+ H^{+}$	1405.4
4 	$+ Na^{+}$	1427.4
4 	$+ 2Fe^{3+} + S^{2-} + 3H^{+} + 2Na^{+}$	1413.3
4 	$+ 2Fe^{3+} + S^{2-} + 2H^{+} + 3Na^{+}$	1435.3

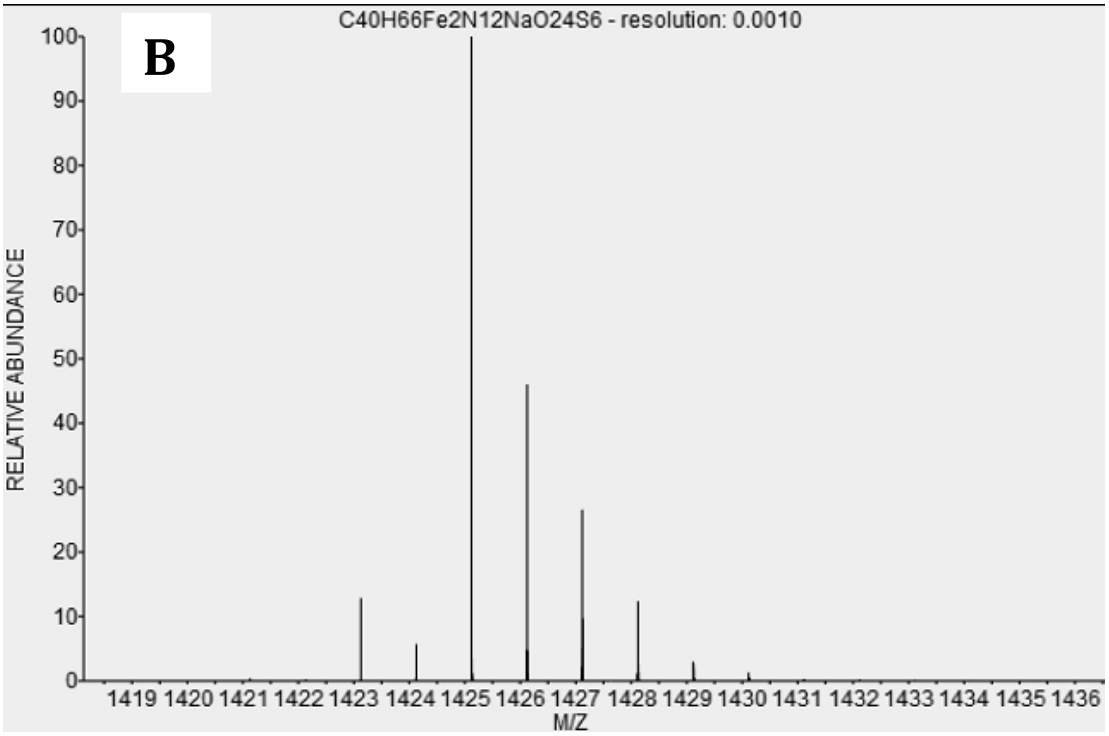


Figure S3. Analysis of $[Fe_2S_2](GS)_4$ formation by ESI mass spectrometry. **(A)** List of species and their corresponding observed m/z values. **(B)** Simulated mass spectrum of $[(GS^-)_4[Fe_2S_2]^{2+}+2H^+ + Na^+]$

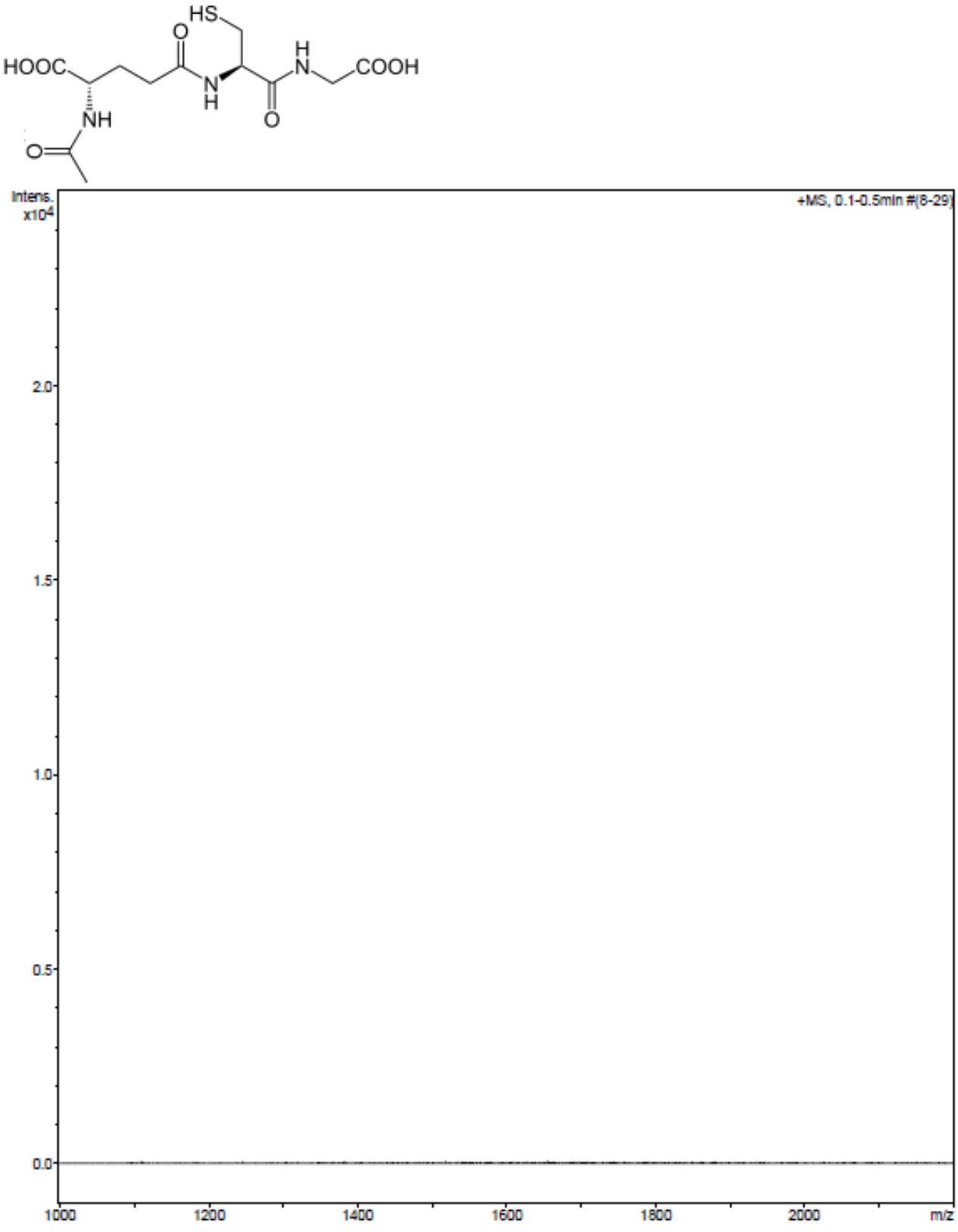


Figure S4. No oligomers were observed in the solution of N-acetylglutathione.

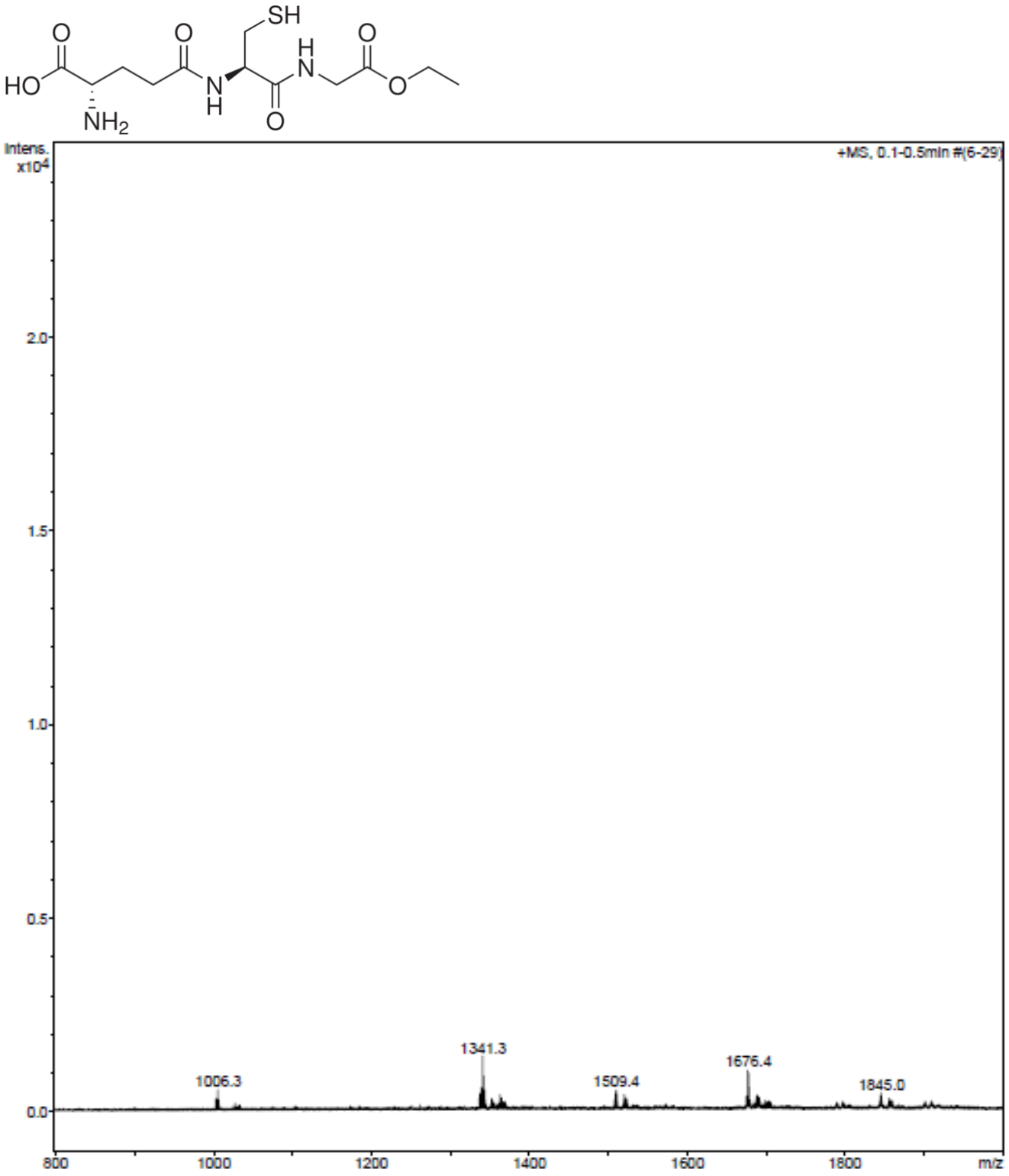


Figure S5. Minor oligomerization was observed in the solution of glutathione ethyl ester, most likely reflecting hydrogen bonding between the ester and protonated amine

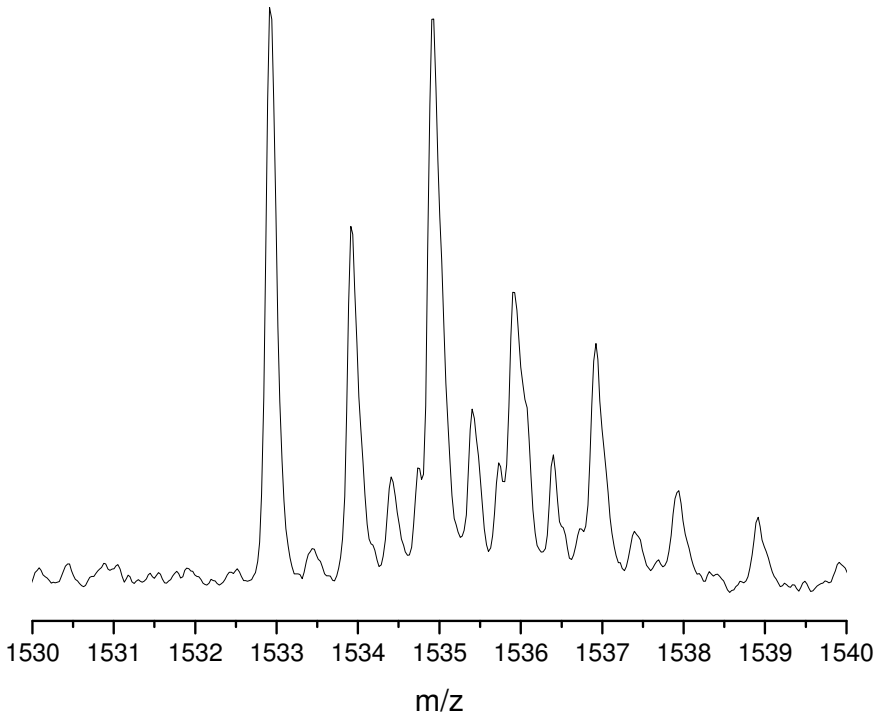


Figure S6. The template effect of pre-assembled glutathione tetramer was also observed by ESI-MS with K^+ as counter ions. The base peak at 1533.0 corresponds to $[C_{40}H_{61}K_8N_{12}O_{24}S_4]^+$, and the supplementary peaks are due to the natural distribution of potassium isotopes and agrees with calculated masses at: 1533.0 (100.0%), 1535.0 (94.3%), 1534.0 (52.6%), 1536.0 (44.6%), 1537.0 (41.3%), 1538.0 (17.6%), 1539.0 (11.1%), 1540.0 (4.2%).

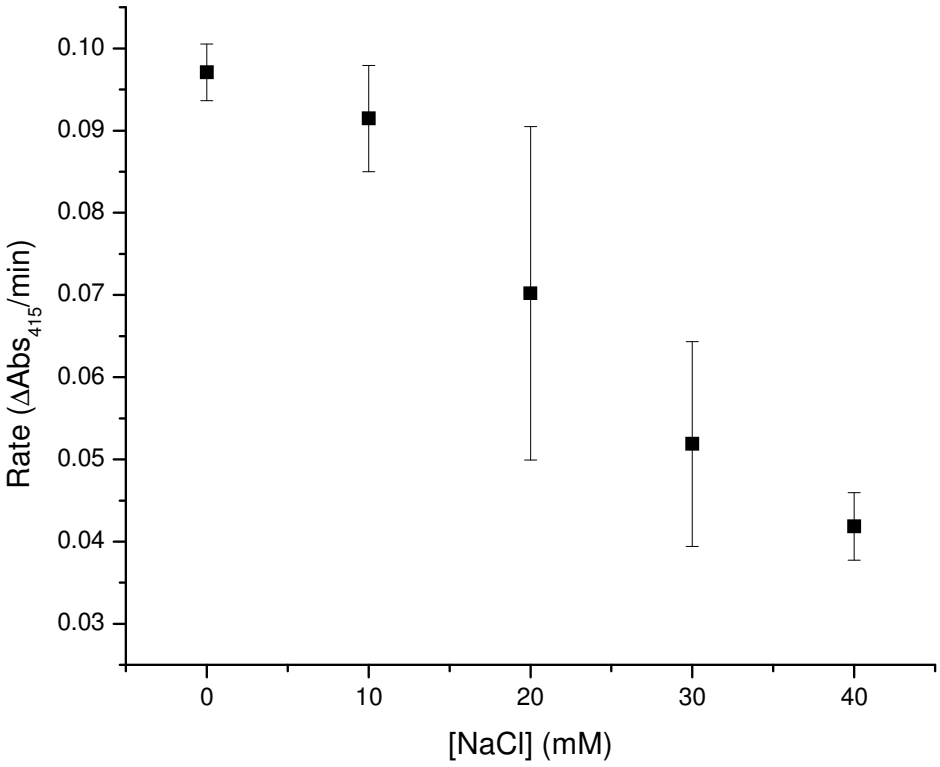


Figure S7. The dependence of cluster stability on ionic strength was evaluated from the time-dependence for cluster degradation reactions monitored at 415 nm in the presence of varying solution concentration of NaCl. Rates were calculated from the initial change in absorbance over the first 10 min of incubation at 20 ºC. The positive correlation between ionic strength and cluster degradation rates supports the essential role for salt-bridge formation in stabilizing the macrocyclic chelate that protects the cluster from hydrolysis.

References

1. A. Veiga, M. A. Mayosky, N. Martínez, P. M. Zélis, G. A. Pasquevich and F. H. Sánchez, *Hyperfine Interact.*, 2011, 202, 8.
2. Philipp Gütlich, Eckhard Bill, Alfred X. Trautwein, Mössbauer Spectroscopy and Transition Metal Chemistry, Fundamentals and Applications, Springer, Heidelberg, 2011.
3. M. E. Anderson, F. Powrie, R. N. Puri and A. Meister, *Archiv. Biochem. Biophys.*, 1985, 239, 538-548.