Supporting Information

Molecular mechanism of ISC iron-sulfur cluster biogenesis revealed by high-resolution native mass spectrometry

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Table S1. Predicted and observed mass of IscU intermediates during cluster formation.

Monomeric IscU species	Predicted mass shift from apo (Da)	Average observed mass shift from apo (Da)
M+OH	17.0	17.0 ± 0.4
M+S	32.1[1]	32.2 ± 0.4
M+S+OH	49.1[1]	49.1 ± 0.4
M+Fe	53.8[2]	53.7 ± 0.6
M+2S	64.1[1]	65.0 ± 0.6
M+2S+OH	81.1[1]	81.8 ± 0.7
M+FeS	85.9[1, 2]	85.9 ± 0.6
M+3S	96.2[1]	97.0 ± 0.2
M+2Fe	107.6[2]	110.0 ± 2.3
M+4S	128.3[1]	129.8 ± 0.9
M+2Fe1S	141.7[2, 4]	143.3 ± 0.4
M+Cys+S	151.2[1]	151.0 ± 0.5
M+[2Fe−2S]	173.8[3]	176.0 ± 0.5
M+2Fe3S	209.8[2, 4]	208.6 ± 0.2
M+GSH	305.3	304.8 ± 0.6
M+GSH+S	337.4[1]	337.1 ± 0.7
M+GSH+2S	369.4[1]	370.1 ± 0.4
D+S	32.1[1]	31.2 ± 1.0
D+2S	64.1[1]	64.3 ± 1.0
D+3S	96.2[1]	96.1 ± 1.8
D+2Fe6S	308.0	307.7 ± 0.5
D+4Fe6S	415.6[2, 4]	418.1 ± 0.5
D+4Fe7S	449.7[2, 4]	450.5 ± 0.8
D+4Fe8S	483.8[2, 4]	482.6 ± 0.6

Predicted mass shifts from apo were estimated using the following assumptions: [1]S is annotated as S^0, [2]Fe is annotated as Fe^{2+}; [3][2Fe−2S] is annotated as $[2Fe-2S]^{2+}$; [4]S is annotated as S^{2-}. Errors are replicate errors (n = 3). A lower overall charge of the protein-associated small molecule will lead to more protons bound to the protein ion to compensate the overall charge of the protein ion, and these protons are also accounted for in the mass shifts.[1-2]

Table S2. Mass shifts observed for IscU species during cluster formation using ^{34}S-L-cysteine.

Assigned Intermediate	Mass shift from apo (using natural Cys)	Mass shift from apo (using [^{34}S]-Cys)	Number of S (experimental)	Number of S (theoretical)
M+S*	32.2	33.6	0.7	1
M+2S*	64.4	67.0	1.4	2
M+3S*	96.6	100.5	2.1	3
M+4S*	129.2	135.1	3.1	4
M+Cys+S	150.3	154.4	2.2	2
M+[2Fe-2S]	175.6	179.5	2.1	2
D+S*	32.7	34.4	0.9	1
D+2S*	65.3	68.4	1.6	2
D+3S*	96.9	102.0	2.7	3
D+4Fe6S	418.3	429.9	6.1	6
D+4Fe7S	451.1	464.4	7.0	7

*The experimental calculated numbers of sulfur atoms for M+nS (n = 1-4) and D+mS (m = 1-3) are ~70-80 % of that of theoretical values. This might be due to the formation of low abundance sulfinic acid modifications that would have the same mass as persulfide containing species.

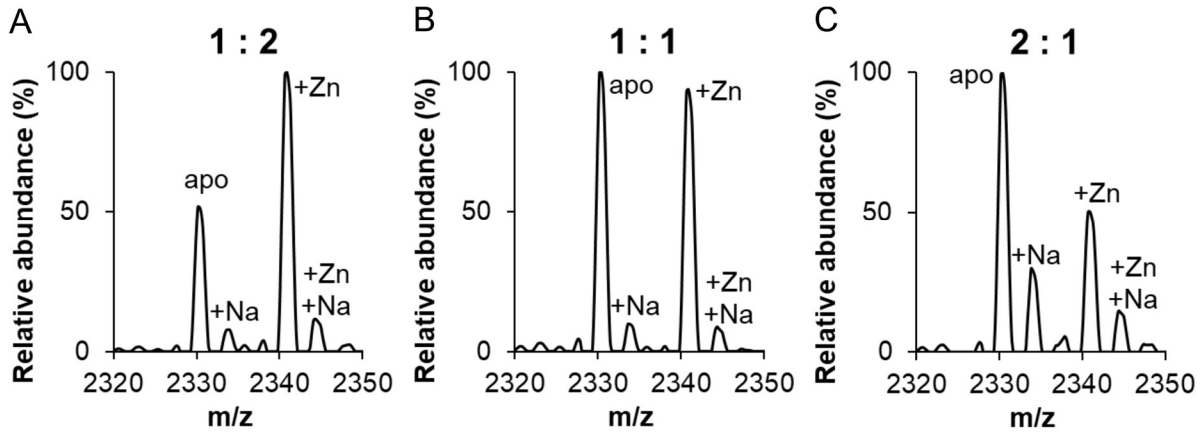

Figure S1. Mixtures of apo-IscU and Zn-IscU monitored by native MS. Native MS spectra for **(A)** 1:2, **(B)** 1:1, and **(C)** 2:1 ratios of apo-IscU and Zn-IscU mixtures are shown for monomeric IscU with a +6 charge state.

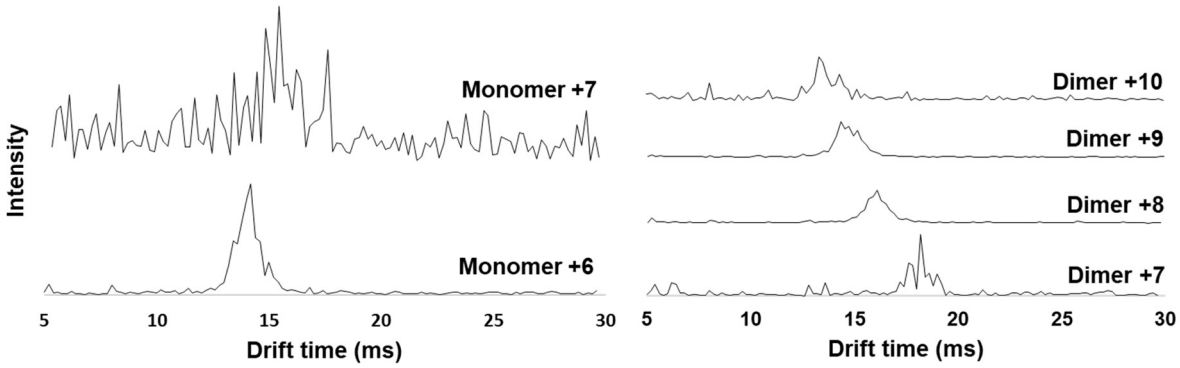

Figure S2. Drift time distribution of apo-IscU in monomeric and dimeric forms. An apo-IscU sample (10 μM) was analyzed by ion mobility MS with a frequency sweep of 5-5005 Hz in 8 mins at approximately 1.725 Torr He in the drift tube. The monomeric form of apo-IscU (left panel) showed a single compact peak for the +6 charge state and a broadened peak with higher drift time in the +7 charge state that has a slightly unfolded conformation. The +6 charge state was used for further analysis of monomeric apo-IscU. The dimeric form of apo-IscU (right panel) shows a compact distribution and follows a single drift time trend. The ion mobility experiment was performed on a FT-IMS-EMR instrument, as described in the Methods.

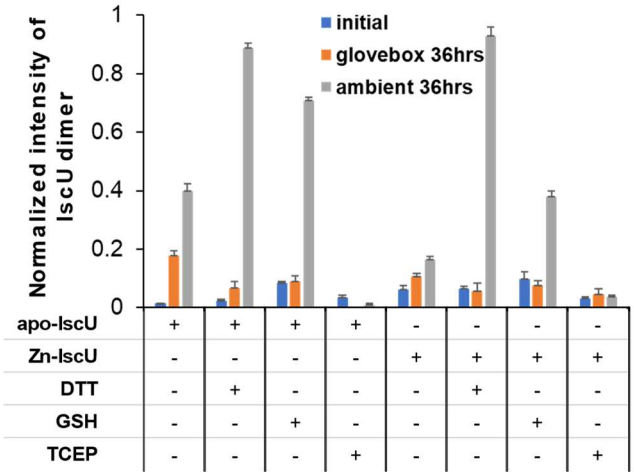

Figure S3. Probing IscU dimerization with native MS. Apo/Zn-IscU (10 μM) was mixed with different reducing agents for 36 hours at 25 °C and then probed by native MS. Reagents included 1 mM D-L-DTT, 200 μM GSH, or 1 mM TCEP.

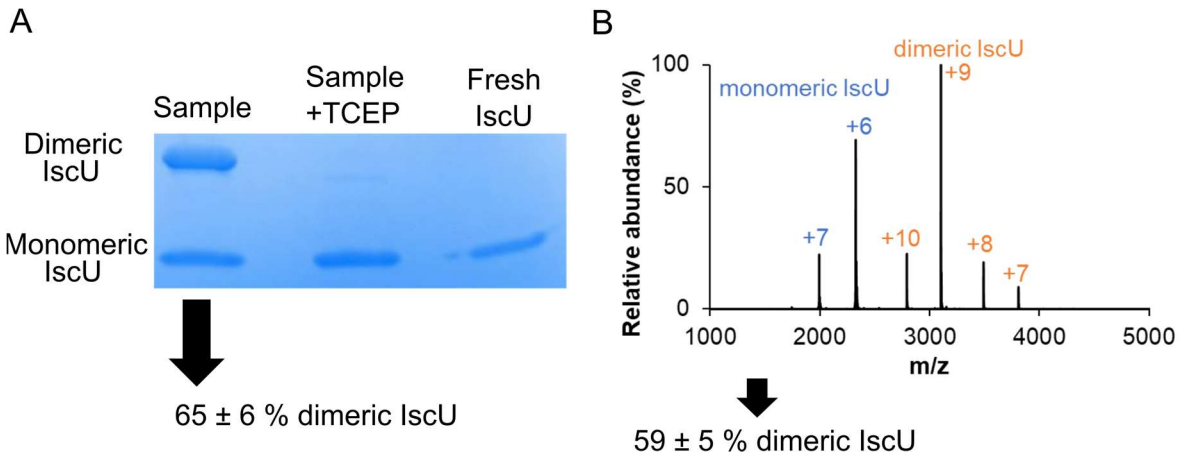

Figure S4. Analysis of an oxidized IscU sample by non-denaturing SDS-PAGE and native MS. An oxidized apo-IscU sample (prepared by a 3 day incubation under aerobic conditions) was analyzed by non-denaturing SDS-PAGE **(A)** and native MS **(B)**. The dimeric IscU percentage in (A) was quantitated by densitometry analysis using software ImageJ, and was quantitated in (B) as described in Methods.

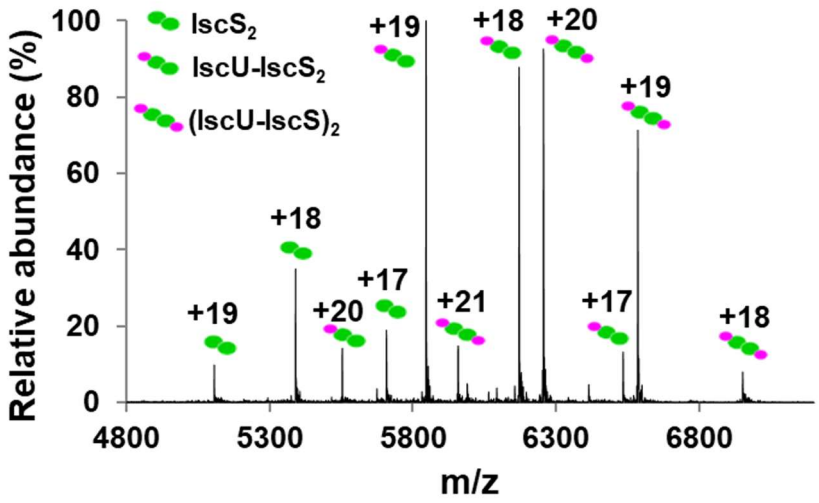

Figure S5. Representative native MS spectra upon combining Zn-IscU and IscS. The charge states for different complex stoichiometries that are generated upon addition of 50 μM Zn-IscU to 50 μM IscS (Fig. 2).

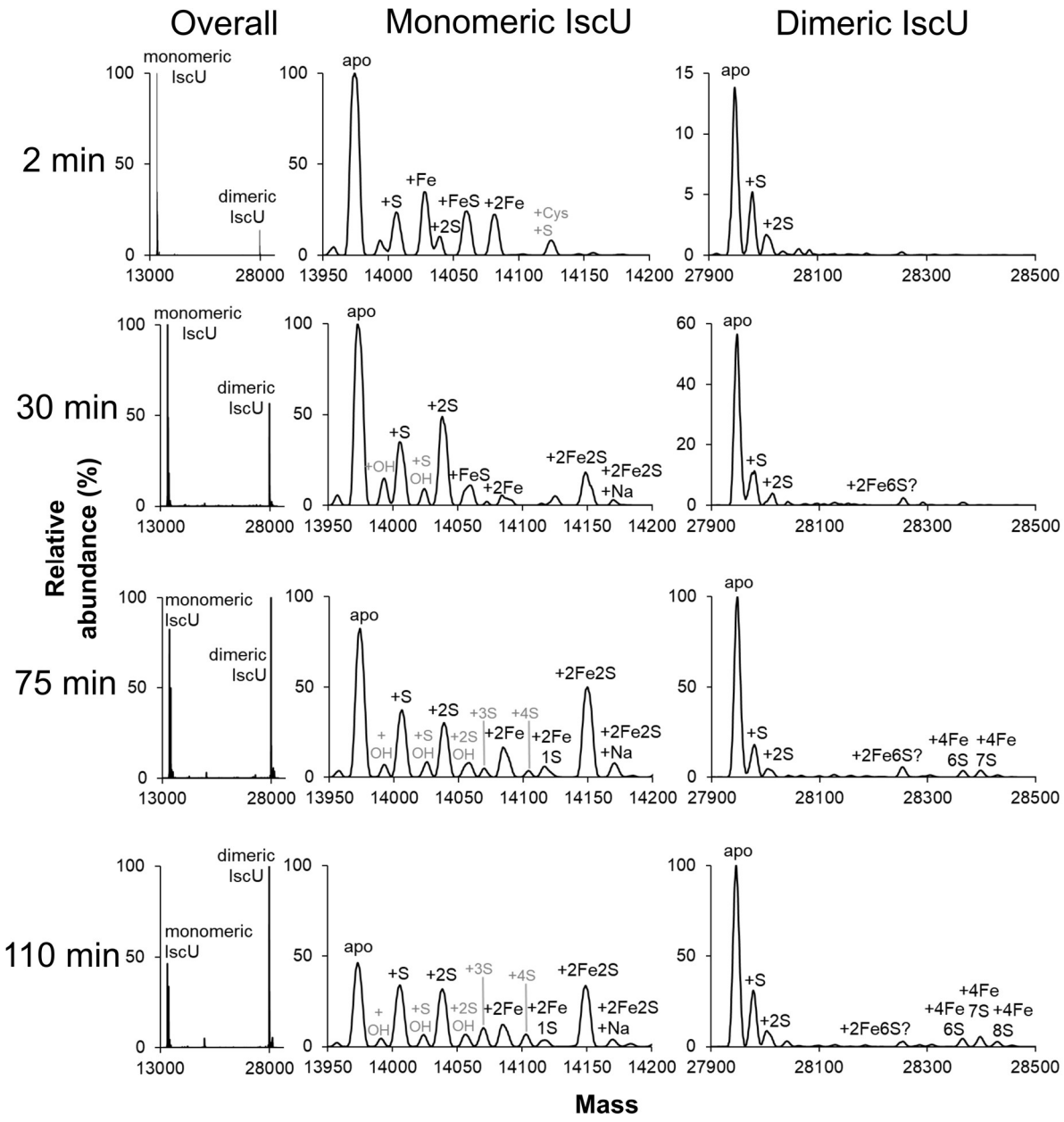

Figure S6. Time-dependent native MS spectra. Native MS spectra for the 2, 30, 75, 110 min time points in Fig. 5D are shown. Species mentioned as potential intermediates or products (Fe-IscU, 2Fe-IscU, S-IscU, 2Fe1S-IscU, [2Fe-2S]-IscU, 4Fe6S-IscU, 4Fe7S-IscU, 4Fe8S-IscU) are highlighted (darker labels) in these spectra.

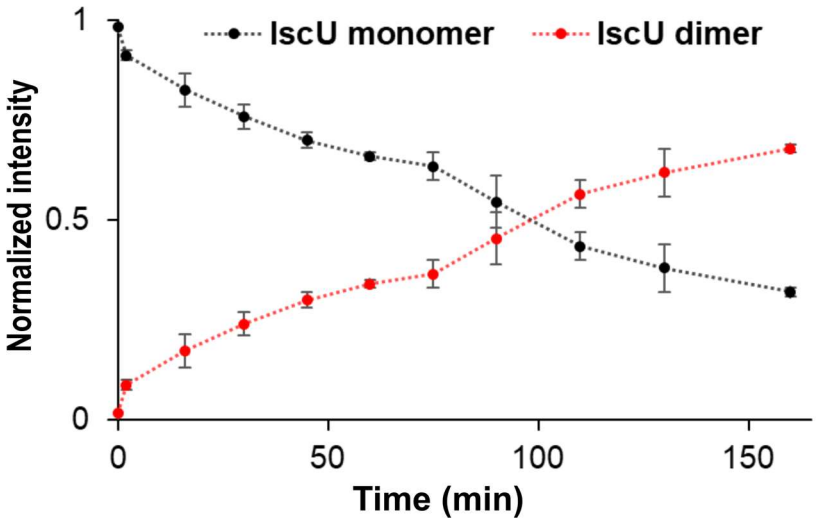

Figure S7. The sum of monomeric IscU species versus dimeric IscU species as a function of time in the time-dependent tracking experiment (Fig. 5).

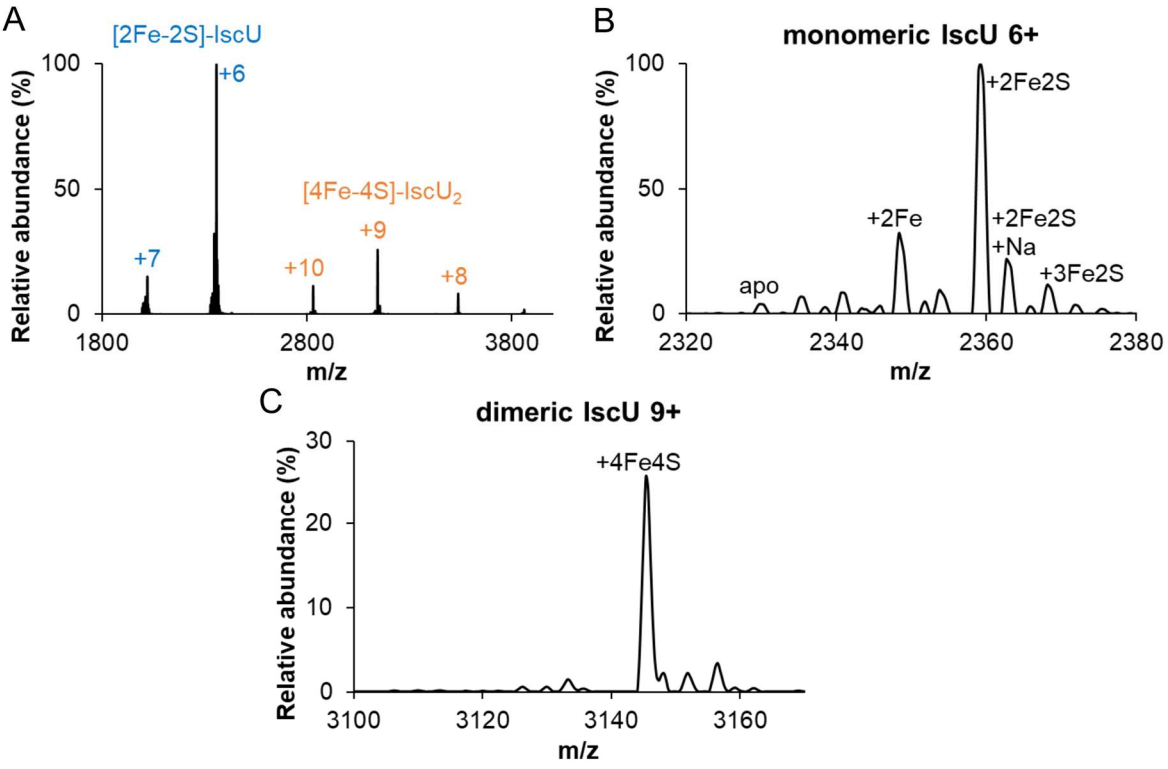

Figure S8. Native MS spectra for Fe-S cluster assembly reaction using excess substrates and non-physiological reductant. An assay containing 50 μM IscU, 3 μM IscS, 500 μM, 500 μM and 1 mM dithiothreitol (DTT) and was reacted for 30 min and analyzed by native MS. The 1800-4000 m/z **(A)**, 2320-2380 m/z **(B)**, and 3100-3170 m/z **(C)** ranges are shown for overall, monomeric IscU with a +6 charge state, and dimeric IscU with +9 charge state, respectively.

References

(1) Crack, J. C.; Thomson, A. J.; Le Brun, N. E., Mass spectrometric identification of intermediates in the O2-driven [4Fe-4S] to [2Fe-2S] cluster conversion in FNR. *Proc Natl Acad Sci U S A* **2017,** *114* (16), E3215-E3223.

(2) Johnson, K. A.; Verhagen, M. F.; Brereton, P. S.; Adams, M. W.; Amster, I. J., Probing the stoichiometry and oxidation states of metal centers in iron-sulfur proteins using electrospray FTICR mass spectrometry. *Analytical chemistry* **2000,** *72* (7), 1410-8.