Supporting Information

Mechanism of activation of the human cysteine desulfurase complex by frataxin

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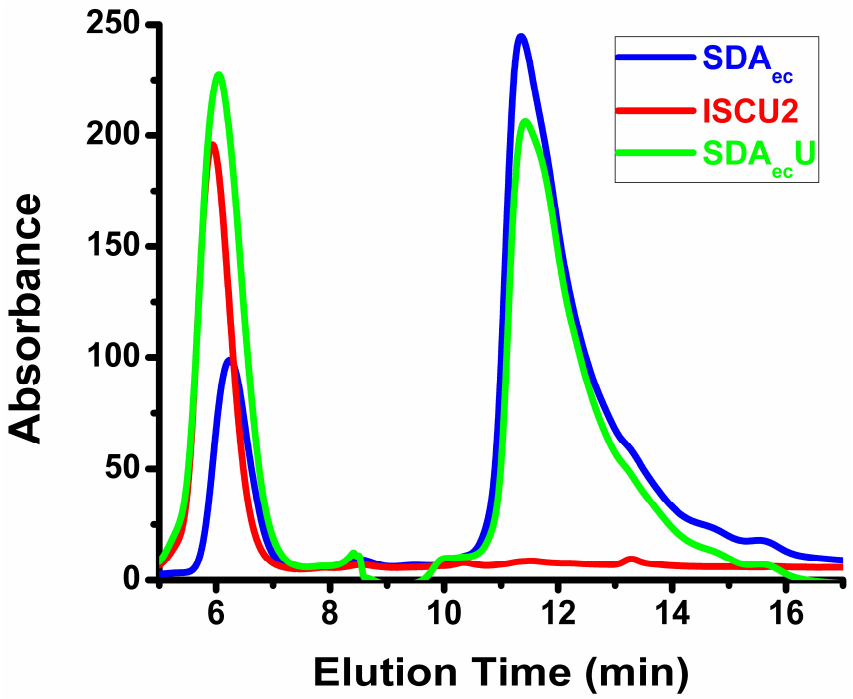

Figure S1. Chromatographic separation of subunits for the $SDA_{ec}U$ complex. Assembly complex subunits were separated on a reversed-phase column (gradient composed of CH_3CN, isopropanol and TFA; see Methods). ISCU2 (140 μM, red) eluted with a retention time of ~6 min. The SDA_{ec} sample (45 μM, blue) had two peaks with retention times of ~6 min and ~12 min. The $SDA_{ec}U$ sample (45 μM SDA_{ec} plus 140 μM ISCU2, green) had increased peak intensity with a retention time at ~6 min consistent with the presence of ISCU2.

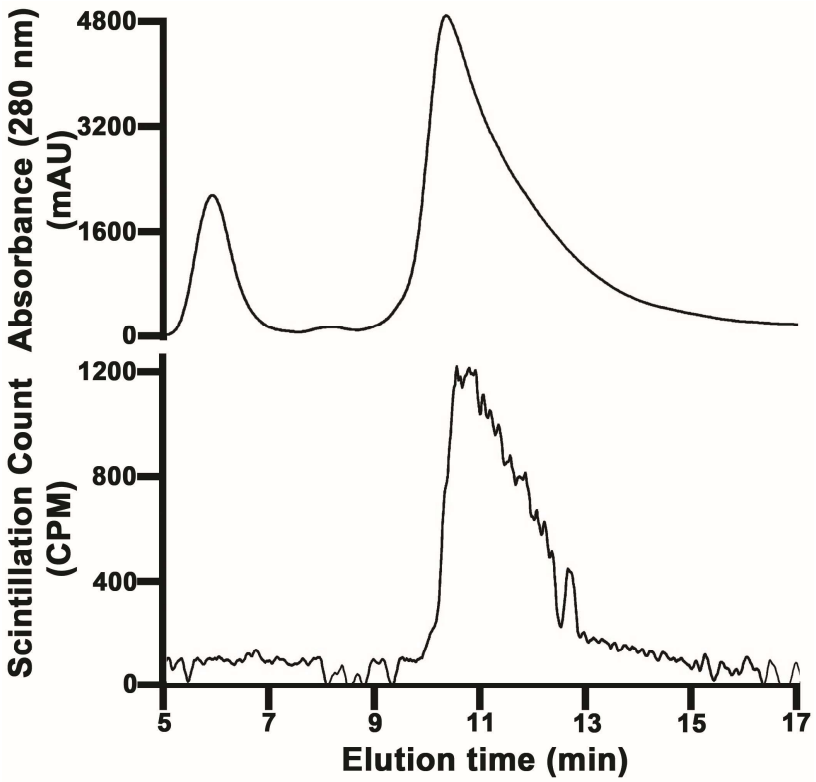

Figure S2. Separation and detection of [^{35}S]-labeled NFS1. The SDA_{ec} complex was reacted with L-[^{35}S]-cysteine, quenched with acid, and the proteins were separated on a reversed-phase column under quench conditions. The retention time for NFS1 (~11 min) was identified by the radioactivity peak (bottom panel). The absorbance band at ~6 min (top panel) was assigned to ISD11 and/or ACP_{ec}, which do not contain cysteine residues and cannot be labeled through persulfide formation.

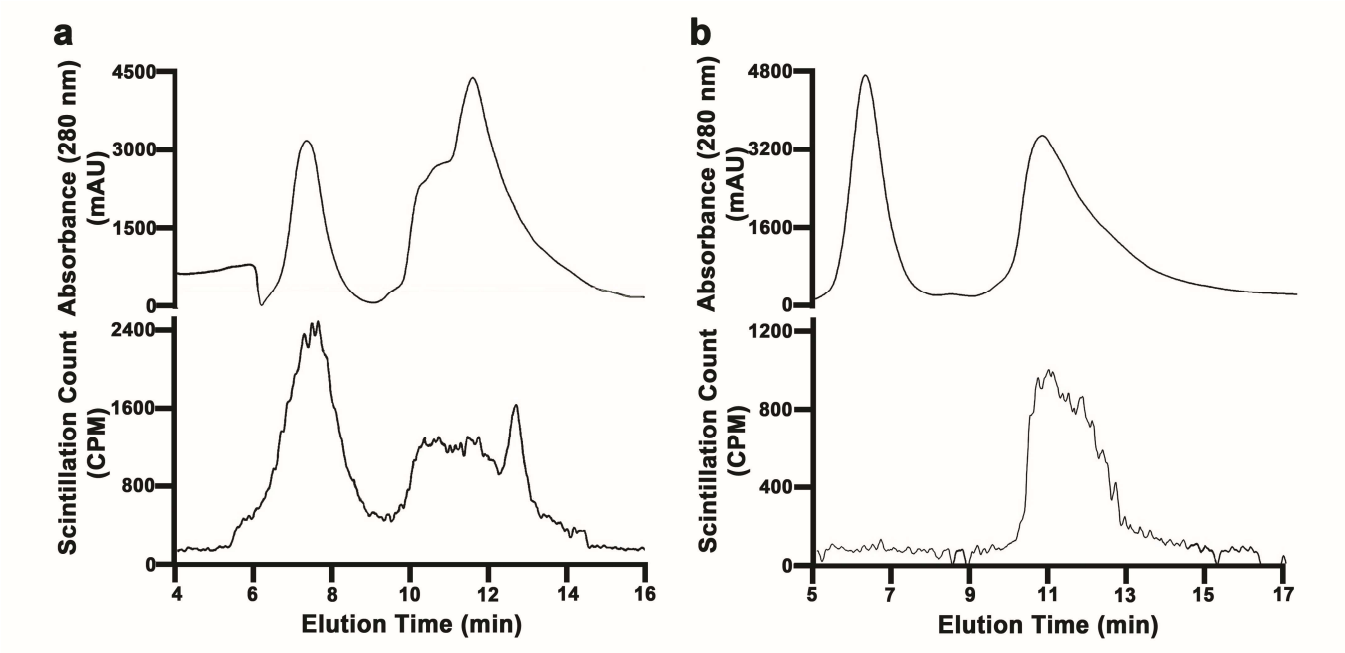

Figure S3. The interprotein sulfur transfer reaction is quenched by acid. NFS1 was labeled by combining the SDA_{ec} complex with L-[^{35}S]-cysteine for 30 min. (**a**) Labeled NFS1 (SDA_{ec}) was incubated with ISCU2 for 30 min, followed by the addition of the quenching solution. The [^{35}S] label was associated with both the NFS1 (peak at ~12 min) and ISCU2 (peak at ~7 min). (**b**) Labeled NFS1 (SDA_{ec}) was first combined with quenching solution and then incubated with ISCU2 for 30 min. The [^{35}S] label was associated with NFS1 (peak at ~12 min) but not ISCU2 (peak at ~7 min). Samples were analyzed by a reversed-phase chromatography similar to Figs. S1 and S2. Note that the peaks for ISD11/ACP_{ec} coincides with ISCU2 but have no cysteine residues and cannot form a persulfide species.

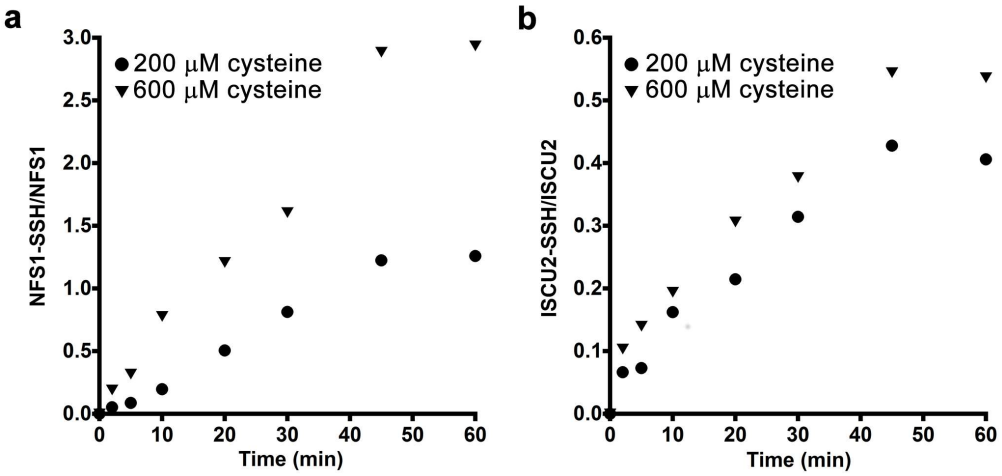

Figure S4. Reactions of the $SDA_{ec}U$ complex with excess cysteine result in the generation of multiple persulfide species on NFS1 and ISCU2. The $SDA_{ec}U$ complex (30 μM SDA_{ec} and 30 μM ISCU2) was reacted with 200 or 600 μM L-[^{35}S]-cysteine at 37 °C and quenched at various times. The protein subunits were separated by reversed-phase chromatography and the amount of radioactivity was quantitated for (**a**) NFS1 and (**b**) ISCU2.

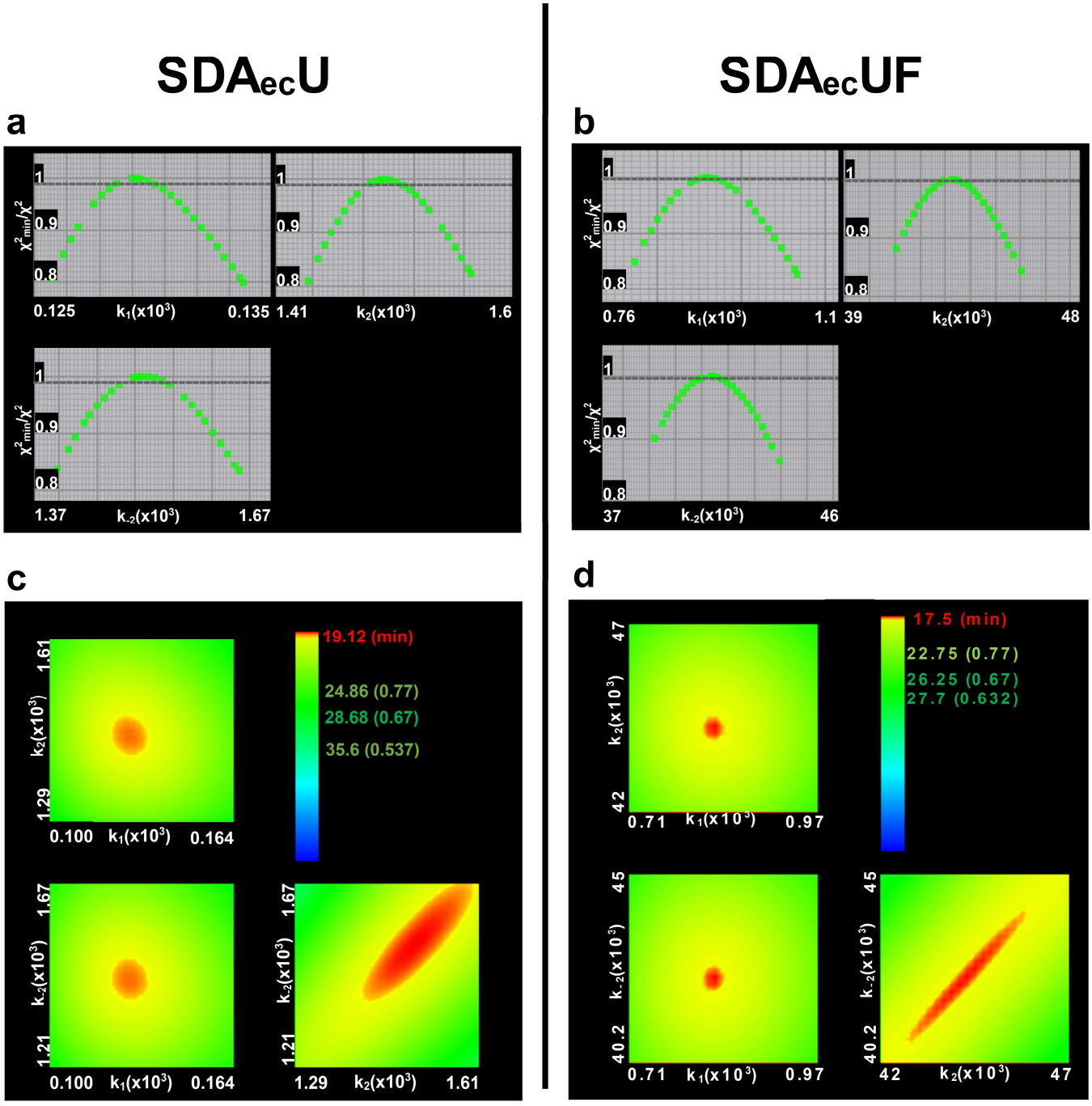

Figure S5. FitSpace analysis of the goodness of fit for the equilibrium persulfide formation model. Calculation of the (χ^2_{min}/χ^2) when k_1, k_2 and k_{-2} were varied individually for the (**a**) $SDA_{ec}U$ and (**b**) $SDA_{ec}UF$ complexes. The (χ^2_{min}/χ^2) value approaches one for the best fit parameter value. Deviation from the best fit parameter value increase χ^2 for the fit and, in turn, decrease the (χ^2_{min}/χ^2) value. A heatmap describing the 2D confidence contour (see Materials and Methods) for the (**c**) $SDA_{ec}U$ and (**d**) $SDA_{ec}UF$ complexes indicate that all the parameters are well constrained.

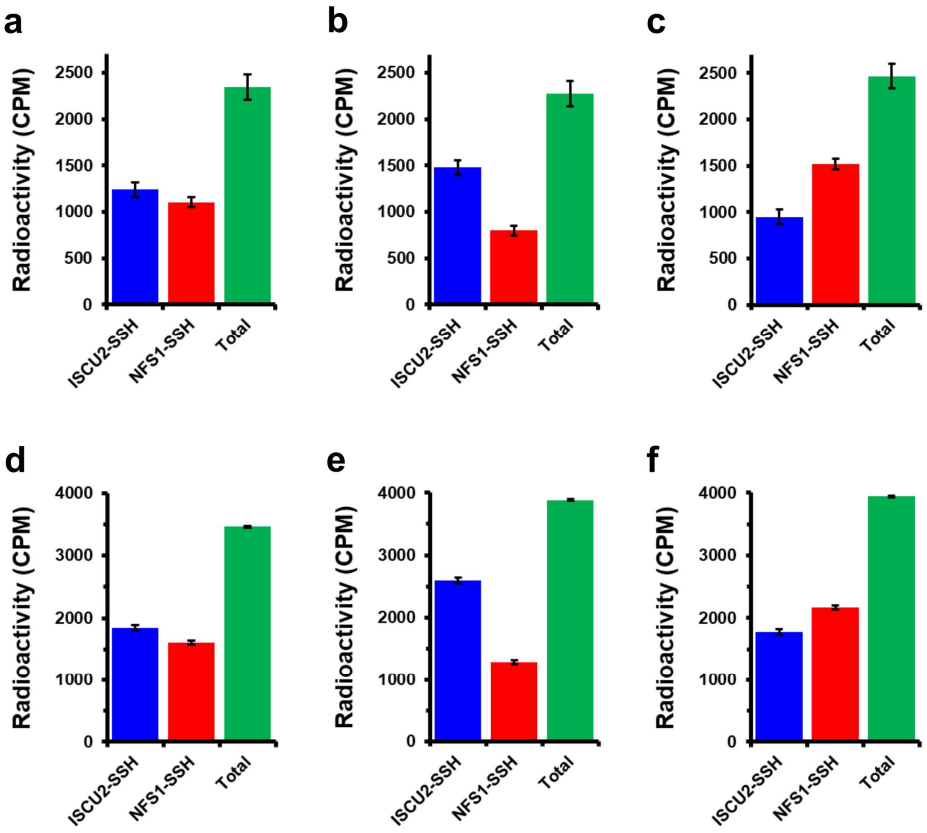

Figure S6. Sulfur transfer between NFS1 and ISCU2 is reversible. The $SDA_{ec}U$ and $SDA_{ec}UF$ complexes were labeled by incubating the samples with a stoichiometric amount of [^{35}S]-cysteine for 40 min at 37 °C. A portion of the samples were then spiked with either two-fold excess unlabeled ISCU2 or SDA_{ec}, incubated for an additional 30 min, and then the reaction was quenched with acid. NFS1 and ISCU2 were separated by HPLC and the radioactivity was measured using a scintillation counter for the $SDA_{ec}U$ complex before (**a**) and after spiking with unlabeled ISCU2 (**b**) or SDA_{ec} (**c**). A similar experiment was performed for the $SDA_{ec}UF$ complex and the radioactivity counts are shown before (**d**) and after spiking with unlabeled ISCU2 (**e**) or SDA_{ec} (**f**). Replicate errors (n = 3) are shown for persulfide labels on ISCU2 (blue), NFS2 (red), and the sum of ISCU2 and NFS1 (green).

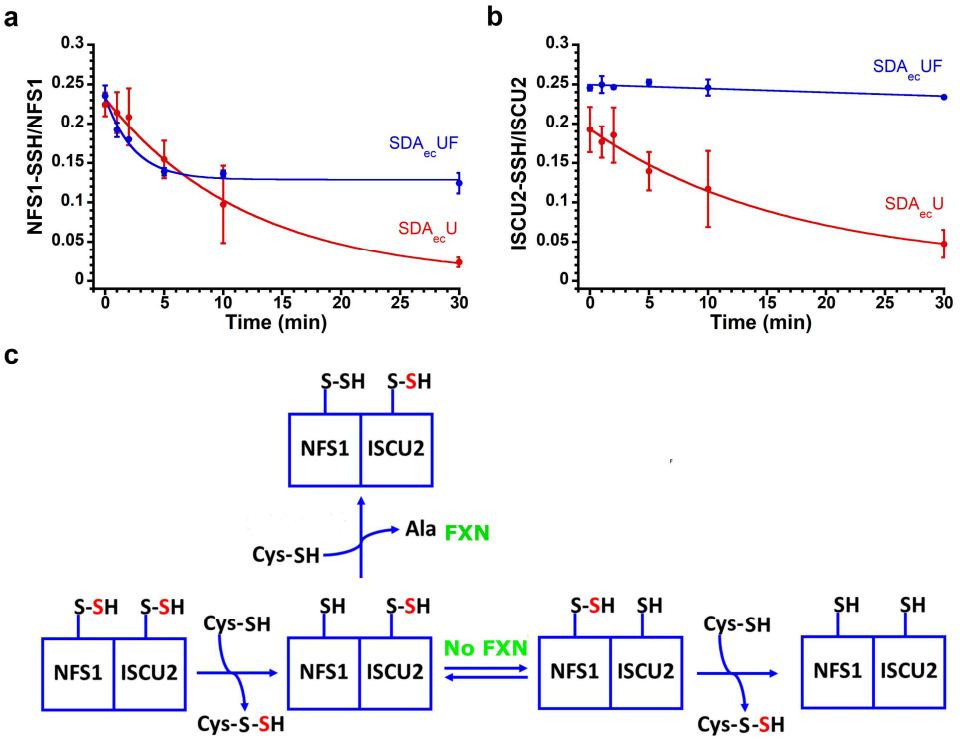

Figure S7. **FXN affects the cysteine-dependent persulfide decay kinetics on NFS1 and ISCU2.** SDA$_{ec}$U (red) and SDA$_{ec}$UF (blue) complexes were reacted with stoichiometric amounts (30 μM) L-[^{35}S]-cysteine for 40 min and then chased with 1 mM non-radioactive L-cysteine. Samples were then quenched with acid at various times and the amount of remaining [^{35}S]-label on (**a**) NFS1 and (**b**) ISCU2 were determined. The data were fit to an exponential decay or linear (loss of ISCU2 label from SDA$_{ec}$UF complex) equation. (**c**) Model for the effect of FXN on persulfide decay from the SDA$_{ec}$U and SDA$_{ec}$UF complexes. The NFS1 persulfide is proposed to be the primary species cleaved during the chase experiment. In the presence of FXN, the persulfide on NFS1 is rapidly regenerated by turnover with non-radioactive L-cysteine. In the absence of FXN, slower cysteine desulfurase turnover results in re-equilibration (transfer) of the ISCU2 radiolabeled sulfur (atom colored red) to NFS1 and subsequent cleavage.

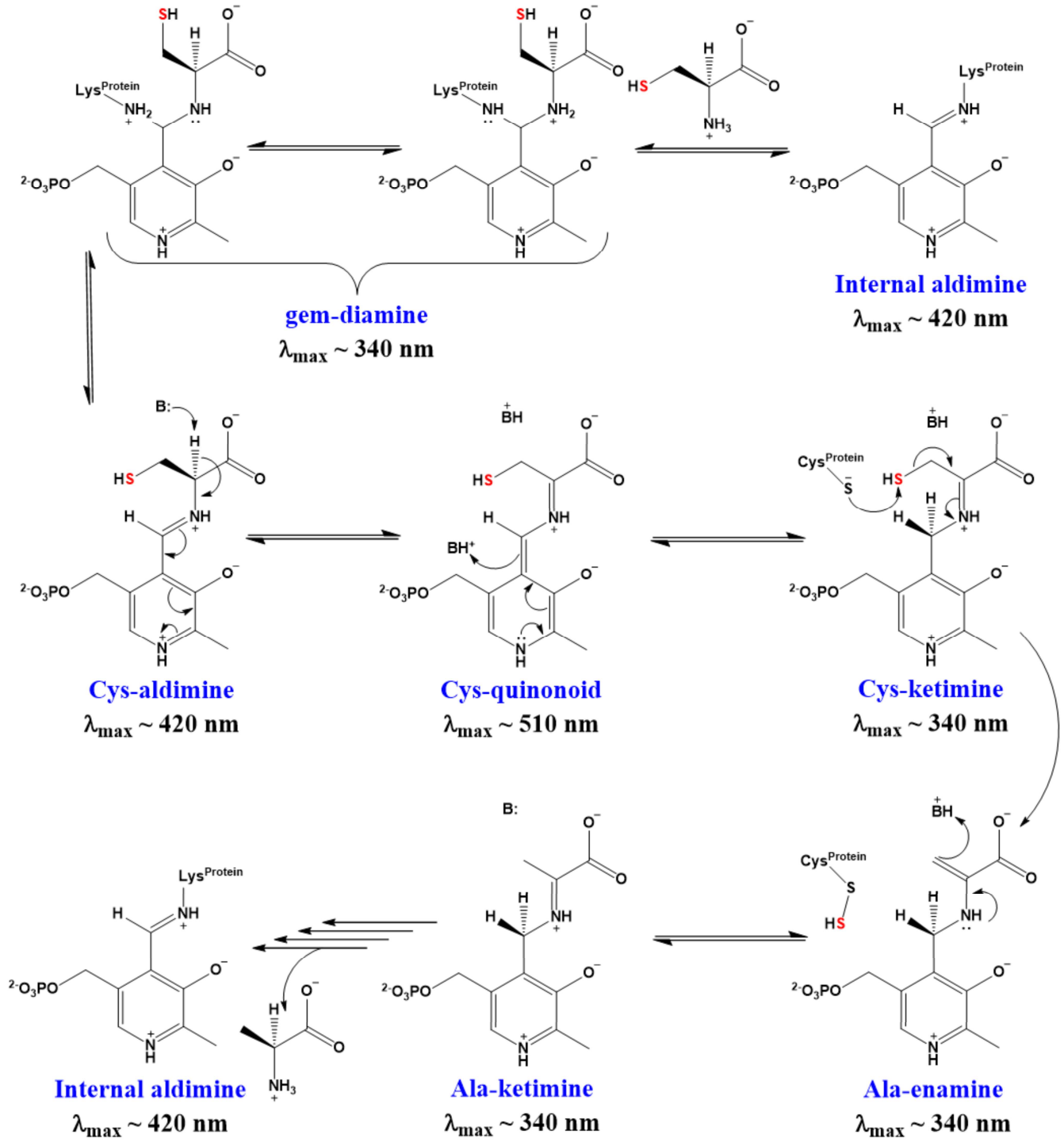

Figure S8. Currently accepted mechanism of cysteine desulfurase (adapted from Zheng et al. Biochemistry 1994, 33, 4714-4720).

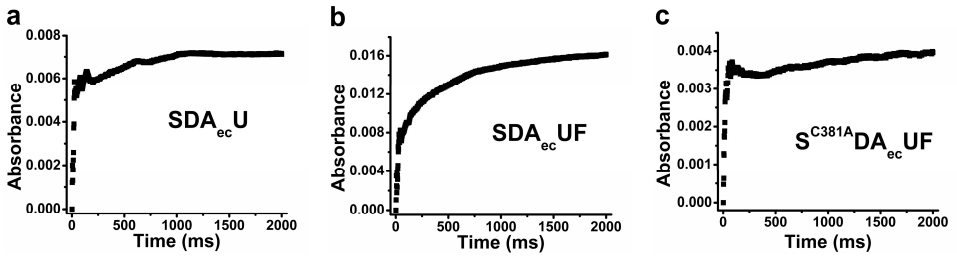

Figure S9. Formation of Cys-ketimine intermediates for Fe-S assembly complexes. The Cys-ketimine intermediates of $SDA_{ec}UF$, $SDA_{ec}U$ and $S^{C381A}DA_{ec}UF$ complexes were followed at 340 nm using stopped-flow kinetics. The shapes of the kinetic profiles are very similar but the maximum amount of intermediate formed is much higher for $SDA_{ec}UF$ complex compared to both $SDA_{ec}U$ and $S^{C381A}DA_{ec}UF$ complexes. The traces are an average of three independent experiments. The kinetics of ketimine formation could not be adequately fit to obtain rate constants.

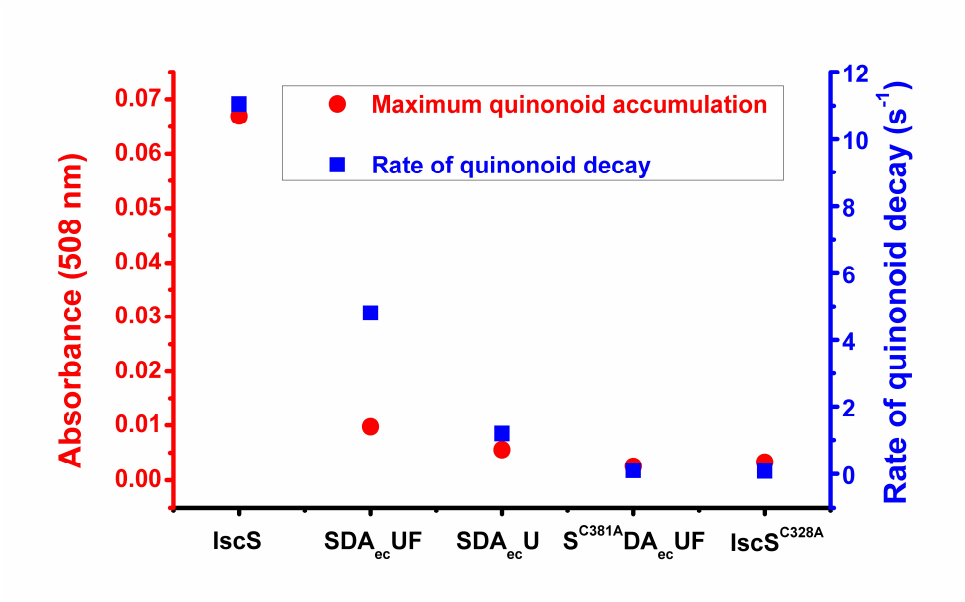

Figure S10. Maximum quinonoid accumulation is inversely correlated with the rate of quinonoid decay. Faster quinonoid decay correlates with greater accumulation of quinonoid intermediate.

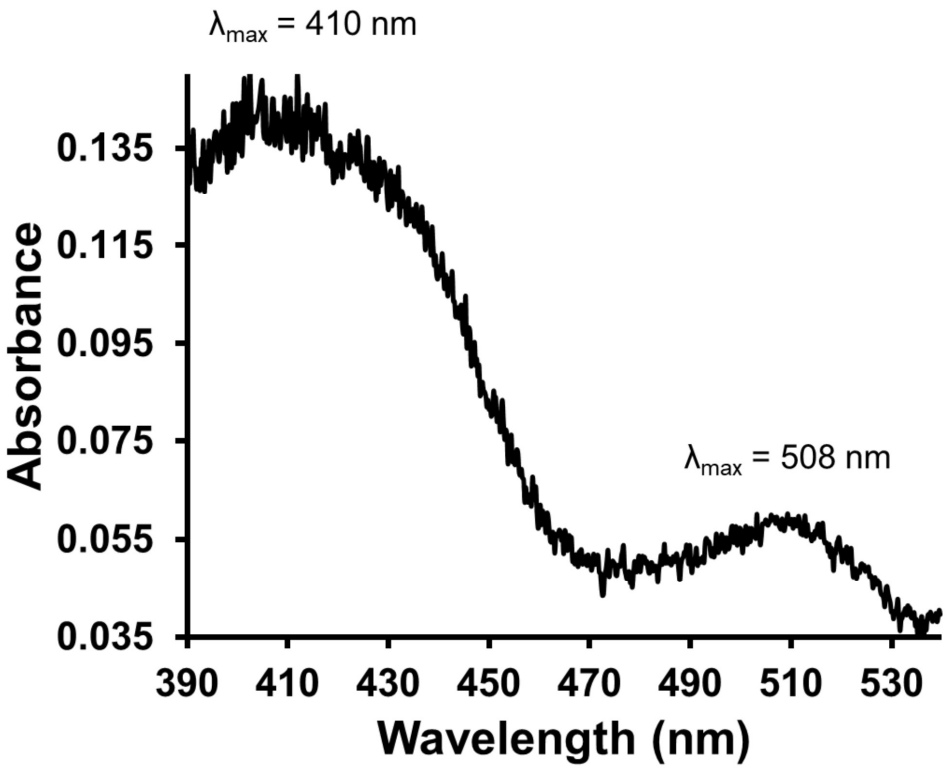

Figure S11. Identification of wavelength of maximum absorbance (λ_{max}) for the cys-aldimine and cys-quinonoid intermediates. Reaction contained 50 μM IscS and 5 mM cysteine (final concentration) and was measured after 20 ms. These wavelengths were used to determine the kinetics of intermediates for all the complexes.

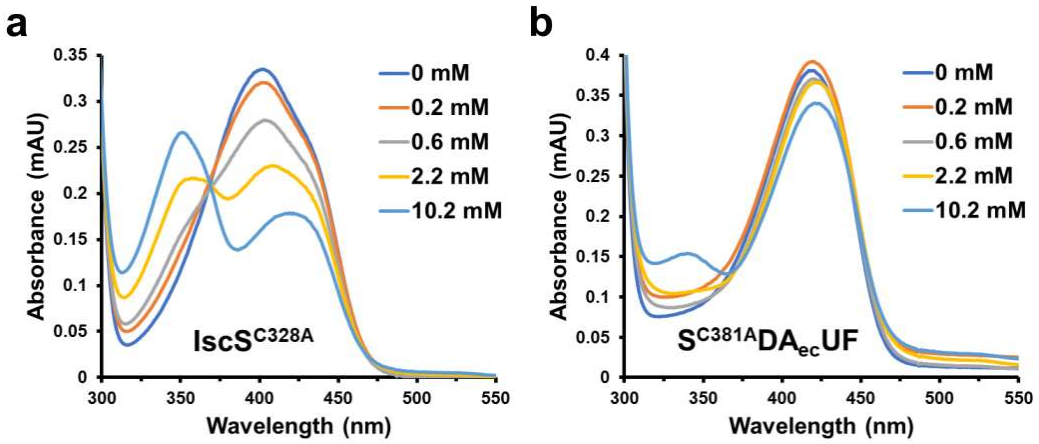

Figure S12. Addition of substrate changes the PLP absorbance spectra for IscS^{C328A} and S^{C381A}DA$_{ec}$UF variant enzymes. Increasing concentrations of L-cysteine (0, 0.2, 0.6, 2.2 and 10.2 mM) were mixed with 50 µM of **(a)** IscS^{C328A} and **(b)** S^{C381A}DA$_{ec}$UF in 50 mM HEPES, 250 mM NaCl, pH 7.5 at room temperature and the UV-Vis absorption spectra were recorded within 30 s. **(a)** Addition of cysteine to the IscS^{C328A} variant leads to a decrease in the absorbance peak at 405 nm and increase in absorbance peaks at both 350 and 420 nm. (**b**) Addition of cysteine to the S^{C381A}DA$_{ec}$UF variant only showed the formation of a peak at ~340 nm at the highest concentration of cysteine.