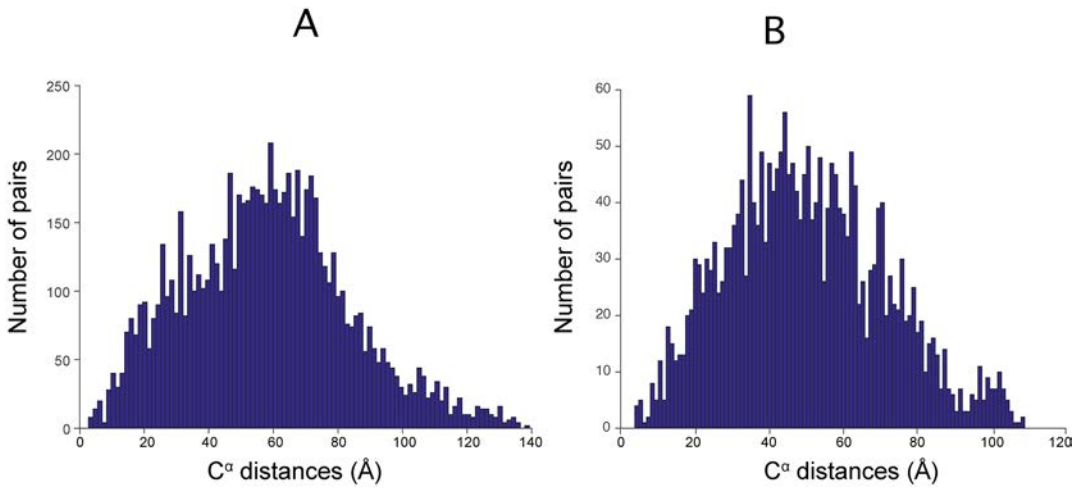

Figure S1. Related to Figure 2 and Figure 6. (A) Random C^{α}–C^{α} distance distribution of lysine-lysine pairs within the structure of $(NIAU)_2$ (PDB: 5WLW). (B) Random C^{α}–C^{α} distance distribution of lysine-cysteine pairs within the structure model of $(NIAUF)_2$.

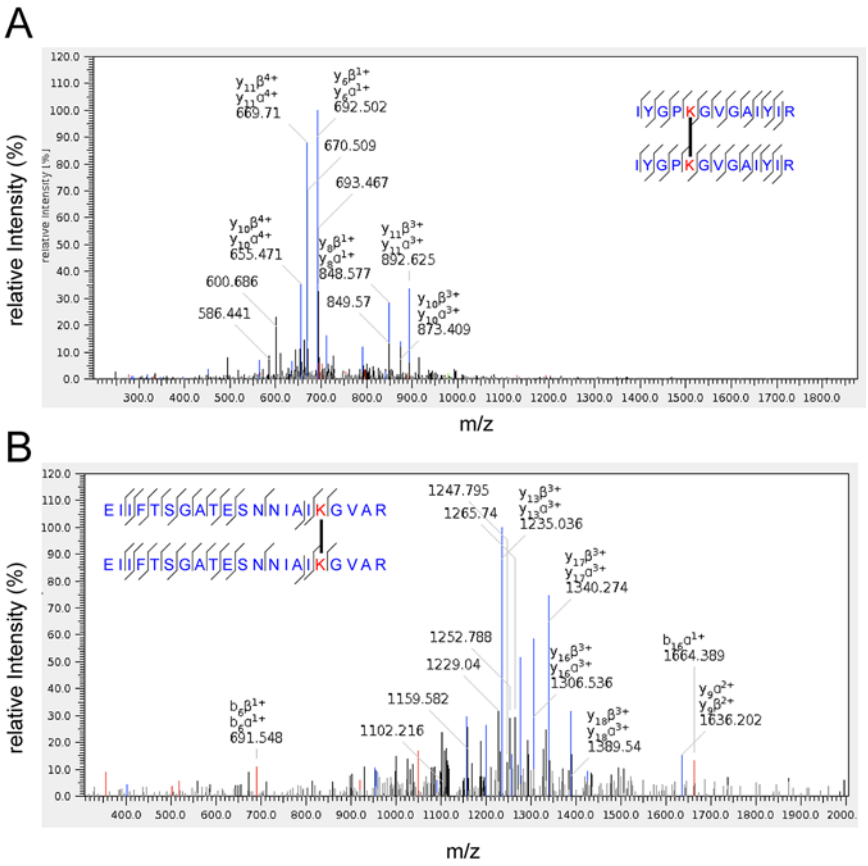

Figure S2. Related to Figure 3. (A) MS/MS fragmentation spectrum of the crosslinked K211–K211 peptide. (B) MS/MS fragmentation spectrum of the crosslinked K84–K84 peptide.

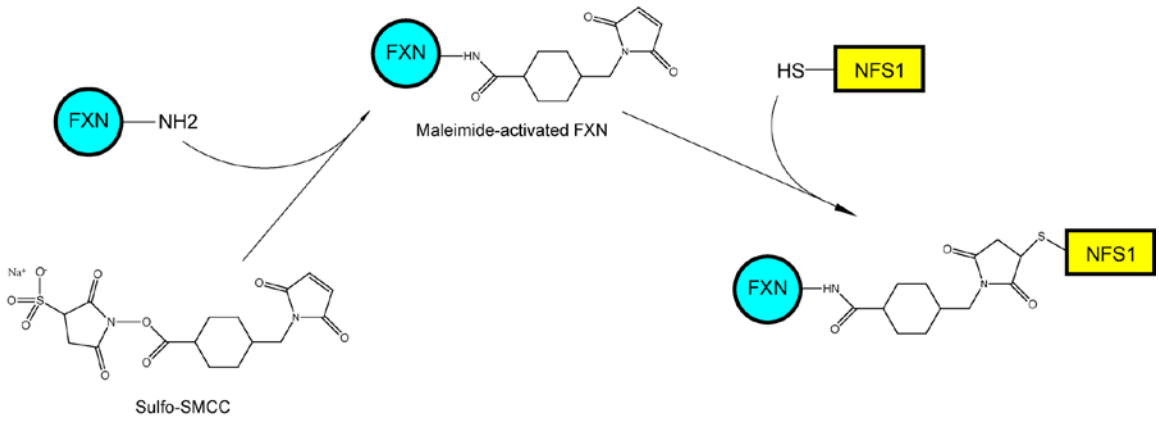

Figure S3. Related to Figure 6. Two-step reaction scheme for conjugating FXN and NFS1 with sulfo-SMCC. The crosslinker is first reacted with FXN to produce a maleimide-activated protein. After excess non-reacted crosslinker is removed, the maleimide-activated protein is reacted with the sulfhydryl groups on NFS1.

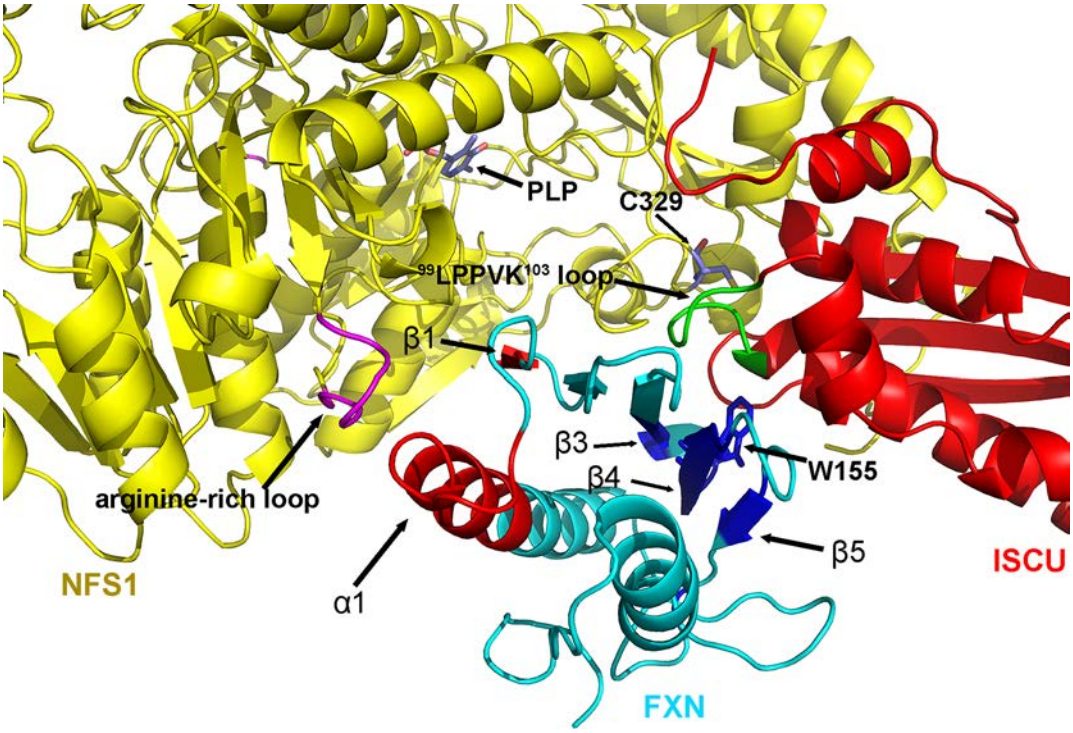

Figure S4. Related to Figure 6C. Expanded view of the structural model of $(NIAUF)_2$ showing the interaction interfaces of NFS1, ISCU, and FXN in half of the complex. The regions of the FXN model colored red and blue indicate the binding interfaces to NFS1 and ISCU, respectively, identified by NMR studies. The arginine-rich loop on NFS1 (magenta) and the '^{99}LPPVK^{103}' loop on ISCU (green) are involved in the interaction with FXN.

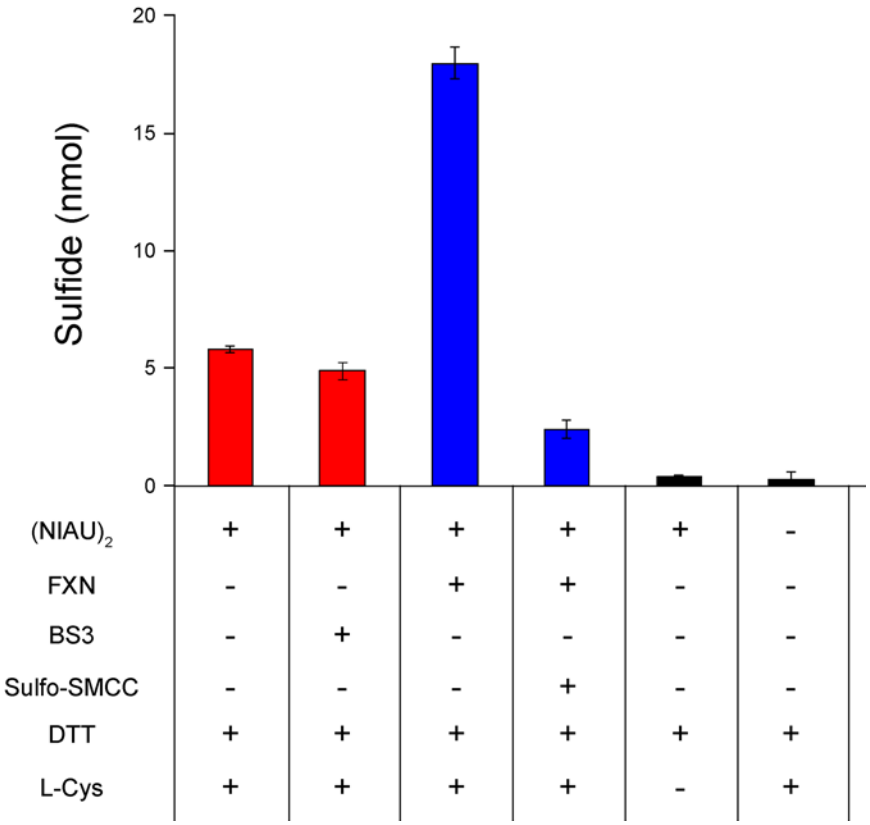

Figure S5 Related to Figure 2 and Figure 6. Cysteine desulfurase activity assay of non-crosslinked or crosslinked $(NIAU)_2$ or $(NIAUF)_2$ using L-cysteine as the substrate and DTT as the reducing agent. The composition of each reaction mixture is denoted below the x-axis.

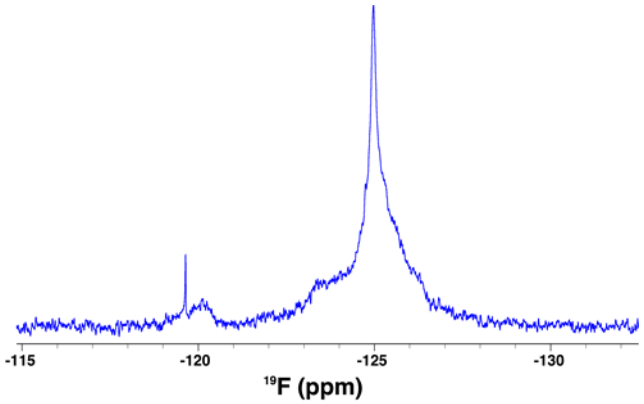

Figure S6. Related to Discussion and STAR Methods. ^{19}F NMR spectrum of ([^{19}F-Trp]NIA)$_2$ collected on a 600 MHz NMR spectrometer. Signals are from the three [^{19}F-Trp] residues of NFS1. The small sharp signal at -119.7 ppm is from an impurity. Although NFS1 contains only three tryptophan residues (W97, W440, and W454), the spectrum can be fitted by more than three ^{19}F signals. ([^{19}F-Trp]NIA)$_2$ was prepared from *E. coli* overexpressing NFS1 and ISD11 in a medium containing 4F-indole. Neither ISD11 nor acyl carrier protein (Acp) contain tryptophan. The spectrum suggests the existence of more than one conformational state in solution because the number of peaks present (at least 4) exceeds the number of labelled tryptophan residues (3).