Supporting Information

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SI Methods

Production and Purification of IscS. A colony of BL21(DE3) competent cells (Novagen) transformed by the IscS expression plasmid (1), a gift from Dr. Patricia Kiley (University of Wisconsin–Madison, Madison, WI), was used to inoculate 10 mL terrific broth (TB) liquid medium containing 100 μg/mL ampicillin. The cells were grown for 6–8 h at 37 °C, and a 10 μL inoculum was transferred to 100 mL TB liquid medium containing 100 μg ampicillin per milliliter, which was subsequently grown for approximately 16 h at 37 °C. Cells from this 100-mL culture were used to inoculate 1 L TB medium containing 100 μg/mL ampicillin. Gene expression was induced at $Abs_{600} \approx 1$ by adding IPTG to a final concentration of 0.4 mM. Cells were harvested after approximately 12 h incubation and stored at −80 °C. Protein purification was carried out as described previously (2), except that a HiLoad 16/60 Superdex 200 gel filtration column (GE Healthcare) was employed for the final purification step instead of reversed phase chromatography. The elution buffer for this step consisted of 20 mM Tris·HCl (pH 8.0), 1 mM DTT, 0.5 mM EDTA, and 150 mM NaCl. Only the fractions showing the characteristic yellow color were taken to avoid collecting IscS not containing pyridoxal-5′-phosphate (PLP) as proposed in a recent study (3). The combined fractions were further treated with 100 μM PLP. Excess PLP was removed by ultrafiltration, and the IscS sample was concentrated, frozen with liquid nitrogen, and stored in −80 °C until used.

NMR Spectroscopy. To assign signals of the structured (S) and disordered (D) states of wild-type apo-IscU, a sample of [U-^{13}C, U-^{15}N]-apo-IscU was prepared in 50 mM Tris·HCl buffer at pH 7.5 containing 0.5 mM EDTA and 5 mM DTT. This sample was used for the collection of 2D ^{15}N-heteronuclear single-quantum correlation (HSQC), 2D ^{13}C-CT-HSQC, 3D HNCO, 3D CBCA(CO)NH, 3D HNCACB, 3D C(CO)NH, 3D HBHA(CO)NH, and 3D N_Z-exchange ^{15}N-HSQC datasets. The chemical shift perturbation profile of wild-type apo-IscU upon adding IscS was investigated by collecting 3D HNCO spectra of two samples: 1.0 mM [U-^{13}C, U-^{15}N]-apo-IscU and the same sample mixed with 1.5 mM unlabeled IscS in 50 mM Tris·HCl buffer at pH 7.5 containing 0.5 mM EDTA and 5 mM DTT. The ^{1}H-^{15}N perturbation profile was calculated from $\Delta\delta_{NH} = [(\Delta\delta_N/6)^2 + (\Delta\delta_H)^2]^{1/2}$. The exchange rates between the S and D states of wild-type apo-IscU in the absence or presence of 0.1 equivalent IscS were determined from the initial slopes of the exchange cross peak volumes, normalized by the volumes of their diagonal peaks, as a function of the mixing time in 2D N_Z-exchange ^{15}N-HSQC spectra (4). Data were acquired first with a sample of 0.7 mM [U-^{15}N]-apo-IscU in 50 mM Tris·HCl buffer at pH 7.5 containing 0.5 mM EDTA and 5 mM DTT. Subsequently, unlabeled IscS was added to a final concentration of 0.07 mM, and a second dataset was collected. The hydrogen exchange rates of [U-^{15}N]-apo-IscU(N90A) in the absence or presence of unlabeled IscS were measured by (*i*) concentrating a [U-^{15}N]-apo-IscU(N90A) sample dissolved in H_2O, (*ii*) diluting the concentrated sample by 20-fold with a buffer made up in D_2O, and (*iii*) taking a series of 2D ^{15}N-band-selective optimized flip-angle short transient-HMQC spectra (5). The buffer used for this experiment contained 50 mM Tris·HCl and pH 7.5, containing 150 mM NaCl, 0.5 mM EDTA, and 5 mM DTT. To estimate the effect of IscS on the hydrogen exchange rate of apo-IscU(N90A), four identical samples were prepared: One of these was diluted with the buffer made up in H_2O to obtain zero-time data, and the other three were mixed with none, 0.11 equivalent, or 0.22 equivalent of IscS along with the buffer in D_2O. After collecting all data, the samples were exchanged back to the H_2O buffer. Two-dimensional ^{15}N-HSQC spectra of these samples were similar, confirming that the hydrogen rate changes were due neither to degradation of protein samples nor to direct spectral perturbations by the added IscS.

The titration experiments involving IscU variants were initiated by taking the 2D ^{15}N-HSQC spectra of 0.6 mM [U-^{15}N]-apo-IscU variants, including WT, D39A, K89A, and N90A in 20 mM Tris·HCl buffer at pH 8.0 containing 150 mM NaCl, 0.5 mM EDTA, and 5 mM DTT. Subsequently, 0.6 mM unlabeled IscS was added to the IscU samples, and 2D ^{15}N-HSQC spectra were collected. To the solutions of WT and N90A, unlabeled IscS was further added to the 1:1 mixture of [U-^{15}N]-apo-IscU and unlabeled IscS to obtain a 1:2 mixture, and a second 2D ^{15}N-HSQC spectrum was collected. To test whether the unfolding of IscU was reversible, we added two equivalents of unlabeled apo-IscU to the 1:2 mixture of [U-^{15}N]-apo-IscU:IscS so as to partially displace [U-^{15}N]-apo-IscU.

The samples of Zn^{2+}:IscU and Fe^{2+}:IscU studied by 2D ^{1}H-^{15}N HSQC spectroscopy were prepared by adding one equivalent of $ZnCl_2$ or $(NH_4)_2Fe(SO_4)_2$, respectively, to [U-^{15}N]-apo-IscU in 50 mM Tris·HCl buffer at pH 7.5 containing 5 mM DTT. The sample of Fe^{2+}:IscU complex was prepared in an anaerobic chamber (Coy Laboratory).

The [U-^{15}N]-apo-IscU sample mimicking a physiological ionic condition was prepared in 50 mM Hepes·NaOH buffer at pH 7.5 containing 150 mM KCl, 10 mM $MgCl_2$, and 5 mM DTT. Macromolecular crowding was mimicked by including either 100 mg/mL Ficoll® PM 70 (Sigma-Aldrich) or 100 mg/mL BSA in the physiological solution (50 mM Hepes·NaOH buffer at pH 7.5 containing 150 mM KCl, 10 mM $MgCl_2$, and 5 mM DTT). Ficoll PM 70 has been widely used to investigate excluded volume effects on proteins under crowded conditions because of its nonionic and inert characters (6, 7).

Gel Filtration. The gel filtration study was conducted with a HiLoad 16/60 Superdex 200 column (GE Healthcare). The elution buffer contained 50 mM Tris•HCl at pH 8.0, 150 mM NaCl, 0.5 mM EDTA, and 1 mM DTT. Five protein standards were used to calibrate the column: γ-globulin (158 kDa), conalbumin (75 kDa), ovalbumin (43 kDa), ribonuclease A (13.7 kDa), and aprotinin (6.5 kDa). The calibration curve (Fig. S7) was obtained from $K_{av} = (V_e - V_o)/(V_c - V_o)$, where V_e is the protein elution volume, V_c is the geometric column volume, and V_o is the column void volume as measured with blue dextran. To estimate the molecular weight of the IscU:IscS complex, a 3:1 mixture of IscU:IscS was injected. The IscU:IscS complex eluted at 73.7 mL (corresponding to 101.5 kDa); IscS eluted at 76.02 mL (corresponding to 83.4 kDa); and IscU eluted at 91.94 mL (corresponding to 21.6 kDa). The effective molecular weight of IscU in the gel filtration study was consistent with a previous report (8).

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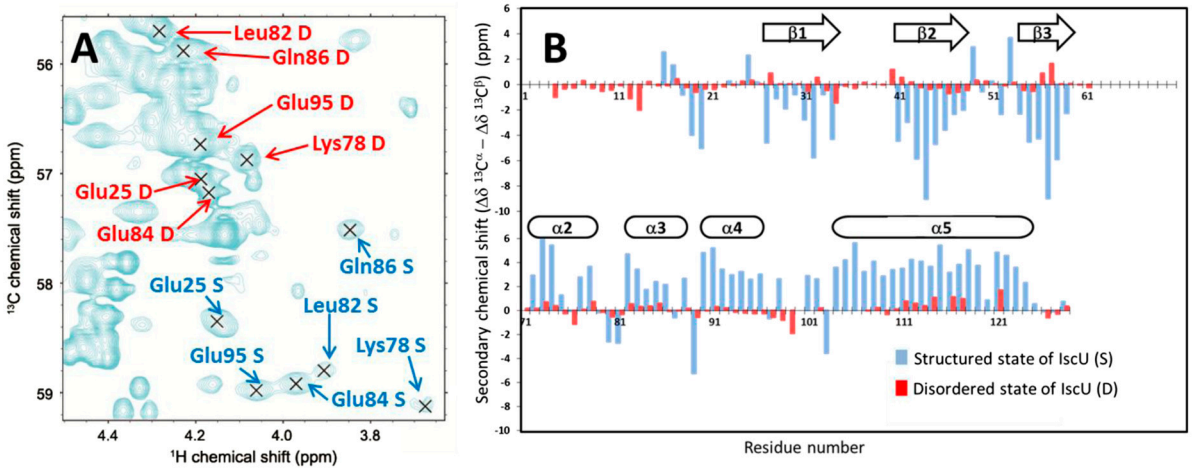

Fig. S1. (*A*) Portion of a two-dimensional ^{1}H-^{13}C NMR spectrum of apo-IscU labeled uniformly with carbon-13 and nitrogen-15 at pH 7.5 and 25 °C. The spectrum contains peaks assigned to the two conformational states (S and D) of the protein. The assignments were made by conventional triple-resonance methods. (*B*) Secondary NMR chemical shifts of the two forms of apo-IscU show that one is structured (S, blue bars) and the other largely disordered (D, red bars). The symbols above the bar graph indicate the secondary structure of the S state of apo-IscU as determined from NMR analysis. The N-terminal 15 residues appear to be disordered in both states, and only a single set of peaks was observed for these residues (shown in red).

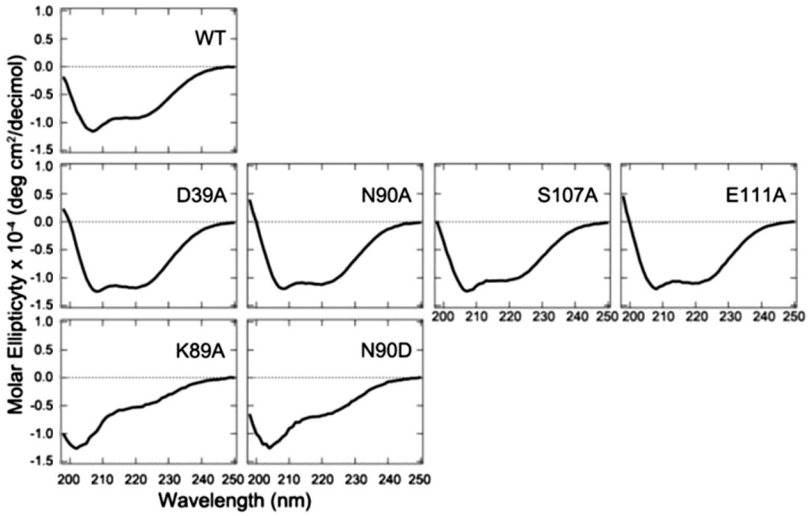

Fig. S2. Far-UV circular dichroism (CD) spectra of apo-IscU variants. All spectra were taken at 25 °C with protein samples at pH 8.0. Note that the CD spectrum of wild-type IscU (first row) is intermediate between those of the more structured IscU variants (second row) and the unstructured variants (third row).

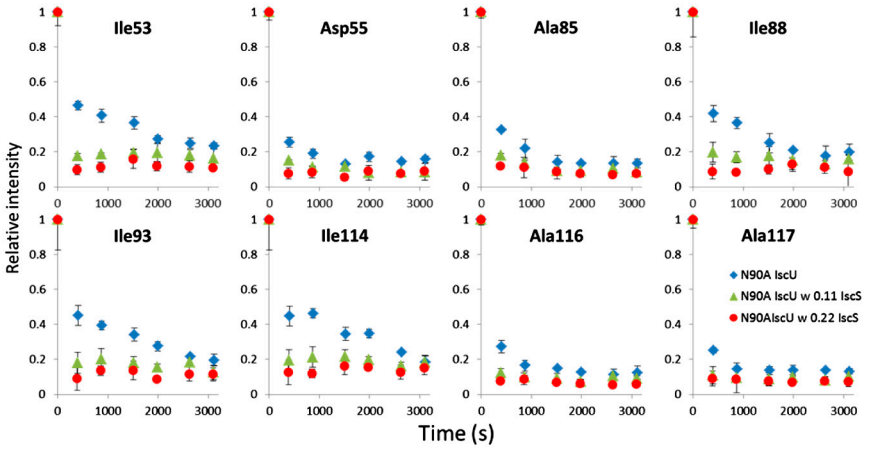

Fig. S3. Addition of the desulfurase (IscS) greatly increases H → D exchange rates of protected backbone amides of apo-IscU(N90A). Shown are the intensities of two-dimensional ^{1}H-^{15}N NMR peaks as a function of time following transfer of the solvent from H_2O to D_2O. Peak intensities were normalized to those of the spectrum of the protein in H_2O (red symbols at zero time at unit intensity). Blue symbols indicate exchange in the absence of IscS. In the presence of 0.11 equivalent of IscS (green) or 0.22 equivalent of IscS (red), exchange was nearly complete within the dead time of the experiment.

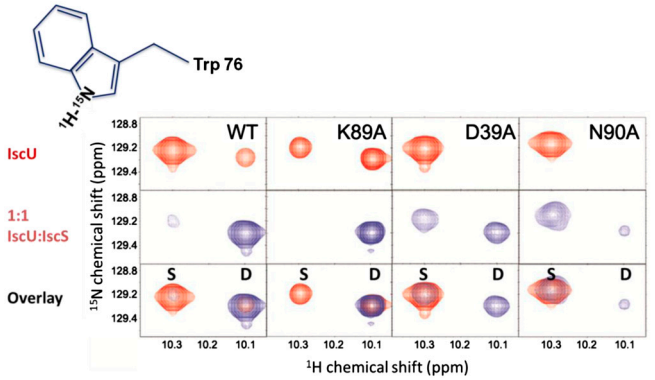

Fig. S4. The ability of IscS to unfold an apo-IscU variant depends on the position of the equilibrium between its S and D states. Shown are ^{1}H-^{15}N NMR cross peaks from the tryptophan-76 side chain of [U-^{15}N]-apo-IscU that report on the conformational status of the variants. The top row shows signals from the apo-IscU variants alone; the middle row shows the signals following the addition of a stoichiometric amount of IscS; and the bottom row shows the two spectra overlaid, with signals from the S and D states indicated. These spectra show that whereas K89A, a variant that is more disordered than WT, became fully disordered by IscS, the more structured variants (D39A and N90A) became only partially disordered by IscS, with the degree of disorder inversely correlated with their thermal stabilities (Fig. 1*D*).

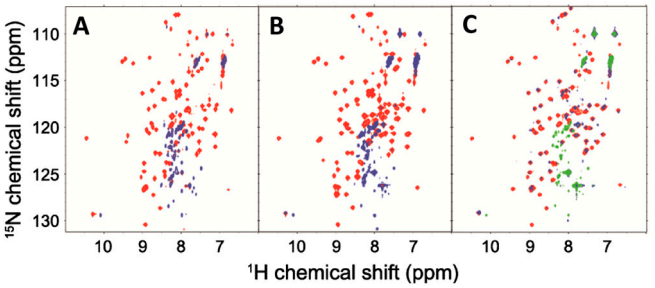

Fig. S5. Like WT apo-IscU, apo-IscU variants that are fully structured (D39A and N90A) became disordered upon addition of the desulfurase IscS. Shown are two-dimensional ^{1}H-^{15}N NMR spectra, acquired on a 600-MHz NMR spectrometer at 25 °C, of ^{15}N-labeled apo-IscU variants at pH 8.0: (*A*) wild-type, (*B*) D39A, and (*C*) N90A. Each panel shows the spectrum of the indicated apo-IscU variant in red and the spectrum of its 1:1 (stoichiometric) mixture with IscS in blue. With variant N90A, a 1:2 mixture of apo-IscU:IscS was required to shift the equilibrium to the disordered state (green).

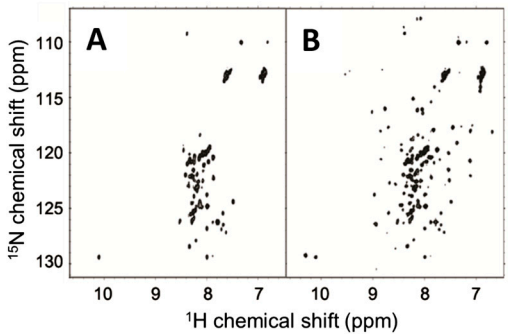

Fig. S6. Unfolding of IscU by IscS is reversible; when competed off by unlabeled IscU, [U-^{15}N]-IscU regains structure. (*A*) 1 : 2 [U-^{15}N]-IscU:IscS mixture. (*B*) 1 : 2 [U-^{15}N]-IscU:IscS mixture after the addition of two equivalents of unlabeled IscU.

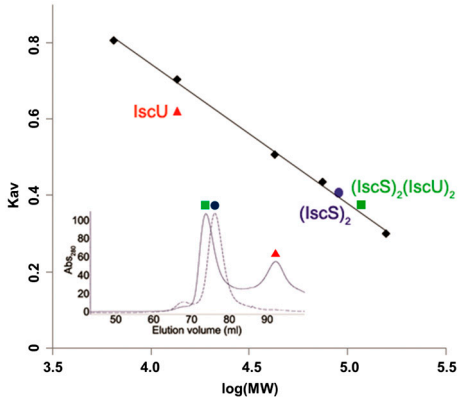

Fig. S7. Analytical gel filtration study to determine the size of the complex between IscU and IscS. The elution volume and the actual molecular weight of IscU (red triangle), IscS (blue circle), and IscU:IscS complex (green square) are marked on the calibration curve (black line) that was obtained with five protein standards (black diamonds; see *SI Methods* for details). The inset shows the elution profile from a 3 : 1 mixture of IscU and IscS (solid line) and an elution profile from IscS (dotted line). The effective molecular weights calculated from the elution volume were 21.6 kDa for IscU, 83.4 kDa for IscS, and 101.5 kDa for the IscU:IscS complex. The results are consistent with the stoichiometry $(IscU)_2(IscS)_2$ for the complex.

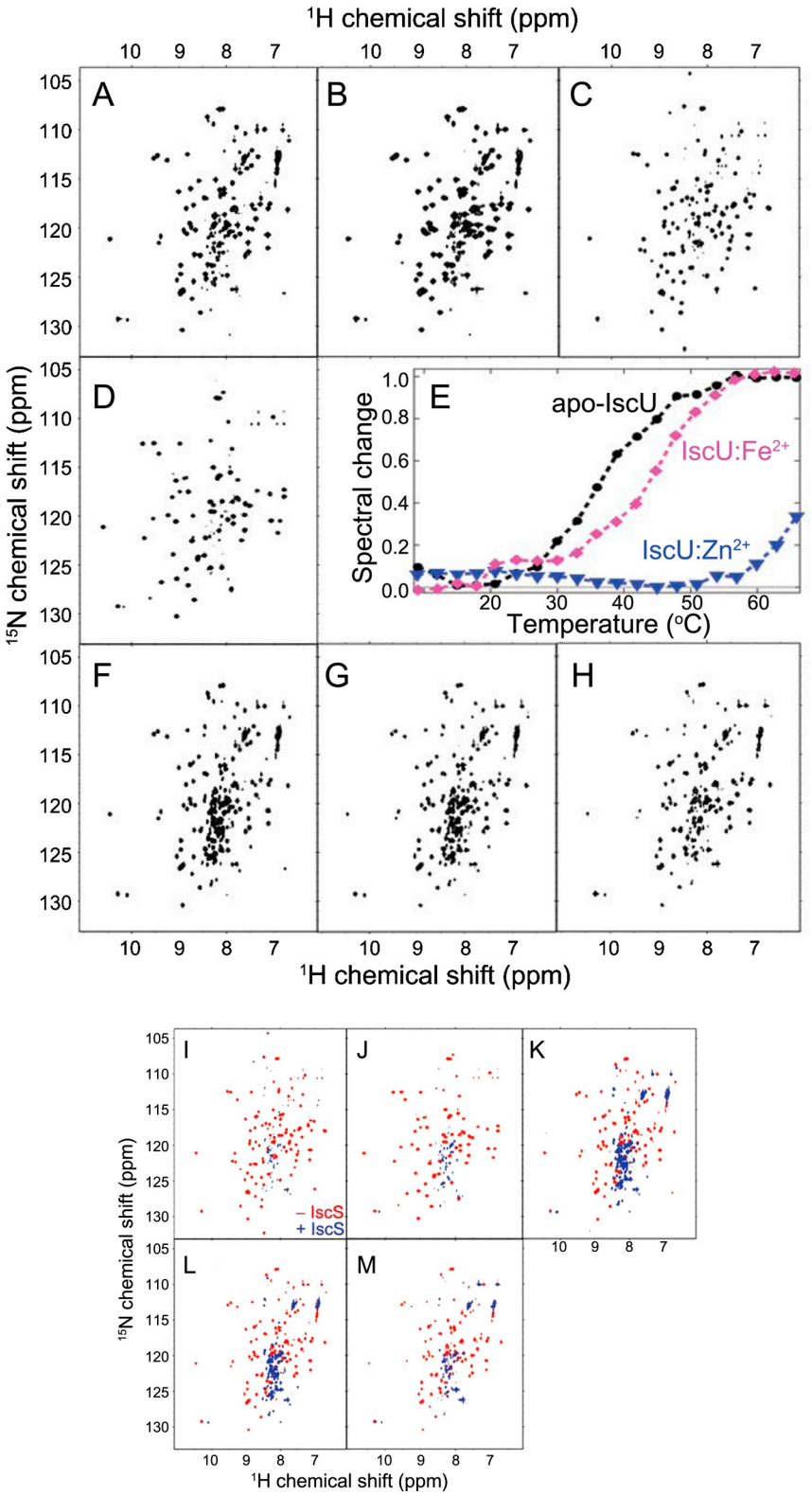

Fig. S8. Two-dimensional ^{1}H-^{15}N NMR spectra of ^{15}N-labeled IscU under different conditions in the absence (*A–D* and *F–H*) and presence (*I–M*) of unlabeled IscS. (*A*) Recombinant apo-IscU prepared from normal M9 *Escherichia coli* growth medium. (*B*) Recombinant apo-IscU prepared from M9 medium supplemented with 8.4 μM $ZnSO_4$. In both *A* and *B*, metals were removed from apo-IscU by treatment with EDTA followed by gel filtration to remove EDTA. (*C*) IscU : Zn^{2+} complex. (*D*) IscU : Fe^{2+} complex. (*E*) Comparison of the thermal stabilities of the IscU : Zn^{2+} (blue) and IscU : Fe^{2+} (pink) complexes with that of apo-IscU (black). (*F*) IscU in 50 mM Hepes buffer at pH 7.5 containing 150 mM KCl and 10 mM $MgCl_2$ (HMK buffer). (*G*) IscU in 100 mg/mL Ficoll PM 70. (*H*) IscU in 100 mg/mL bovine serum albumin. (*I*) IscU : Zn^{2+} complex upon the addition of one equivalent IscS (blue) overlaid on the spectrum prior to adding IscS (red). (*J*) IscU : Fe^{2+} complex upon the addition of one equivalent IscS (blue) overlaid on the spectrum prior to adding IscS (red). (*K*) IscU in HMK buffer upon the addition of one equivalent IscS (blue) overlaid on the spectrum prior to adding IscS (red). (*L*) IscU in 100 mg/mL Ficoll PM 70 upon the addition of one equivalent IscS (blue) overlaid on the spectrum prior to adding IscS (red). (*M*) IscU in 100 mg/mL BSA upon the addition of one equivalent IscS (blue) overlaid on the spectrum prior to adding IscS (red).

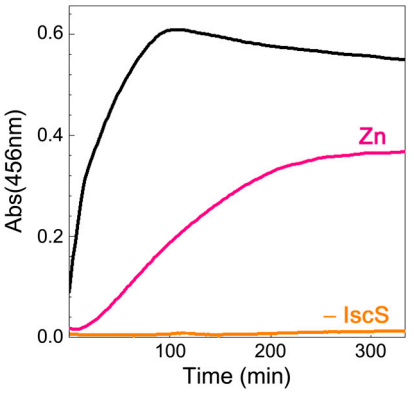

Fig. S9. Time course of iron-sulfur cluster assembly of wild-type IscU in the presence of zinc (scarlet) or in the absence of IscS (orange). The result of wild-type apo-IscU (black; Fig. 3) is also shown as a positive control. The cluster assembly in the presence of zinc was even slower than those of IscU variants favoring the S state. The iron-sulfur cluster was not assembled in the absence of IscS.