Three-Dimensional Structure and Determinants of Stability of the Iron-Sulfur Cluster Scaffold Protein IscU from *Escherichia coli*

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Supporting Information

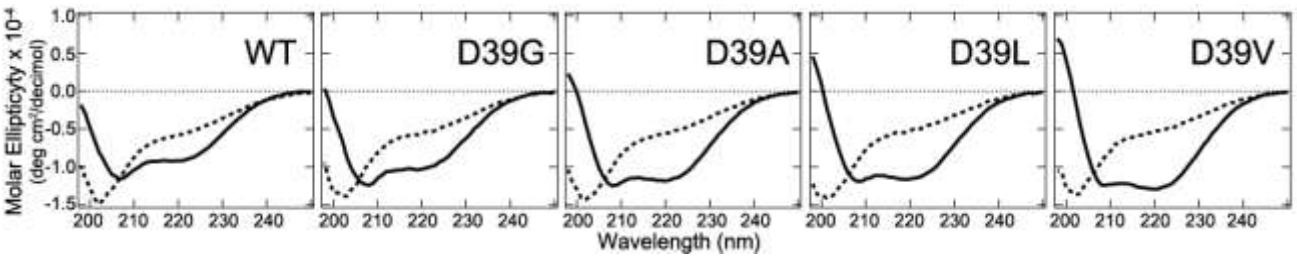

Figure S1. Far-UV circular dichroism (CD) spectra of apo-IscU variants taken at 25 ºC (solid line) and 69 ºC (dotted line). The CD signals at the wavelength of 197−200 nm and 222 nm indicate that apo-IscU variants wild-type (WT) and D39G IscU have less-ordered structures than D39A, D39L, and D39V. Note that the spectra taken at 69 ºC are nearly indistinguishable between variants, indicating that spectral differences at 25 ºC were not caused by an experimental artifact.

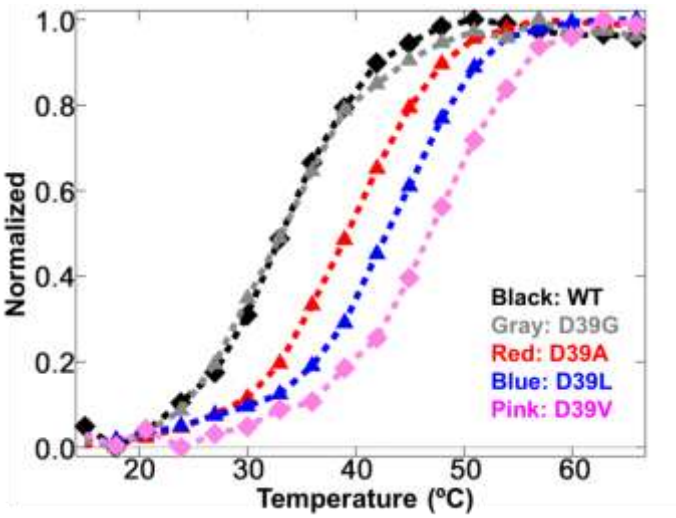

Figure S2. Thermal stabilities of the S-states of the apo-IscU variants (WT, black; D39G, gray; D39A, red; D39L, blue; D39V, pink) as measured by circular dichroism (CD) spectroscopy. CD signals at 222 nm were monitored as a function of increasing the temperature from 9 ºC to 69 ºC. The *y*-axis was normalized so that '0' indicates maximal S-state and '1' indicates minimal S-state state. The curves simply follow the experimental points. Apo-IscU(D39V) showed the highest S-state thermal stability, and apo-IscU(WT) and apo-IscU(D39G) showed the lowest (equivalent) thermal stability. Unpublished studies from our laboratory (Dai Z, Tonelli M, Markley JL, submitted) have shown that increasing temperature converts the S-state to the D-state and that the D-state is not fully unfolded in that it strongly stabilizes two *cis* peptidyl-prolyl peptide bonds. True unfolding probably occurs as a second transition at higher temperature. The unfolding transition may not be picked up by CD signals at 222 nm because the D-state of apo-IscU lacks secondary structure.[1]

REFERENCE

1. Kim, J. H., Tonelli, M., and Markley, J. L. (2012) Disordered form of the scaffold protein IscU is the substrate for iron-sulfur cluster assembly on cysteine desulfurase, *Proc. Natl. Acad. Sci. U.S.A. 109*, 454-459.