Supporting Information

Metamorphic Protein IscU Changes Conformation by *cis-trans* Isomerizations of Two Peptidyl-Prolyl Peptide Bonds

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Contents

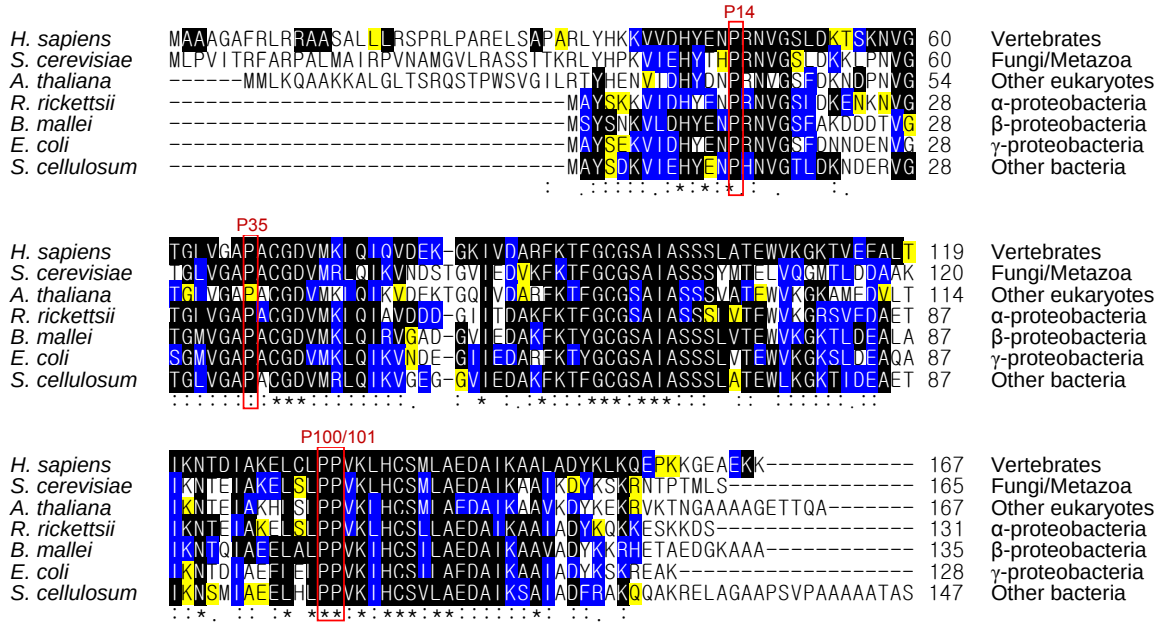

Figure S1. Alignment of the sequences of 131 IscU proteins (9 vertebrates, 20 fungi/metazoa, 11 other eukaryotes, 11 alpha-proteobacteria, 28 beta-proteobacteria, 46 gamma-proteobacteria, and 6 other prokaryotes). The results show that prolyl residues 14, 100, and 101 are absolutely conserved. Black boxes indicate identical conservation within the group. Blue boxes indicate conserved substitution within the group. Yellow boxes indicate semi-conserved substitution within the group. "*" Indicates identical conservation in all sequences. ":" Indicates 90% similarity. "." Indicates 80% similarity. Figure adapted from (*1*).

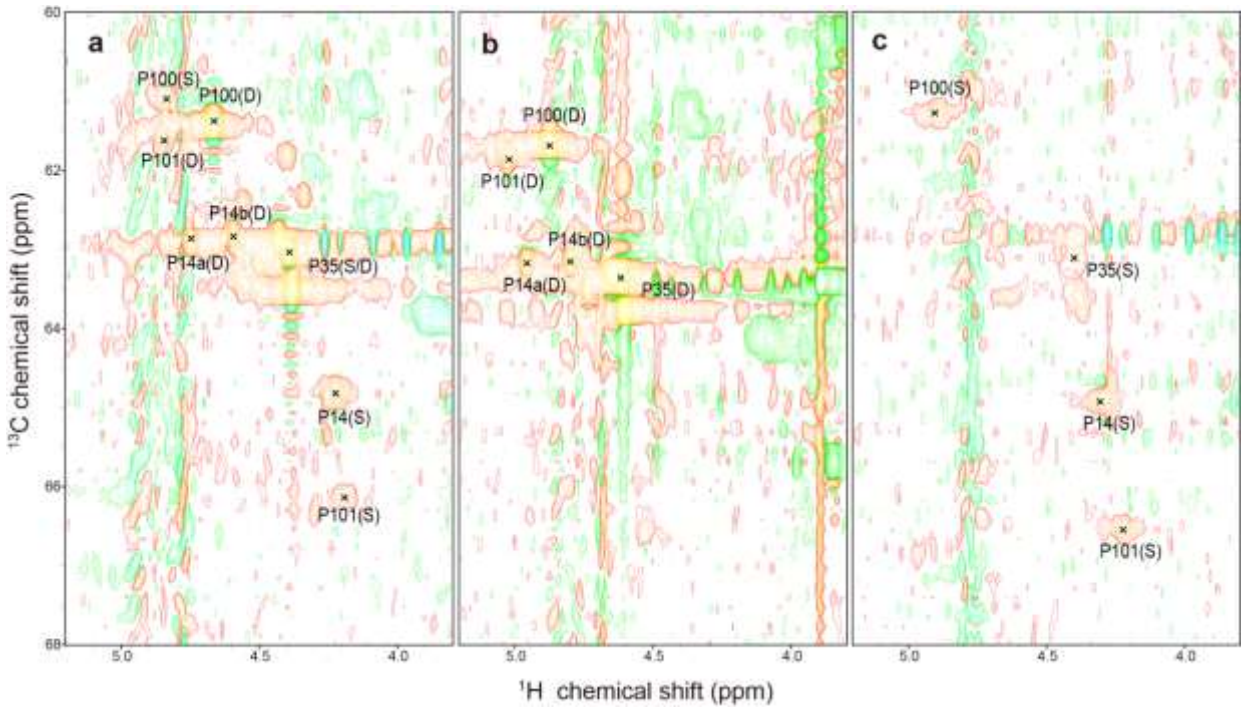

Figure S2. Aliphatic regions of 2D ^{1}H-^{13}C HSQC spectra of 1 mM [U-^{13}C, ^{15}N-Pro]-IscU acquired at 600 MHz (^{1}H) under the conditions specified. These spectra are the same as those shown in Figure 1 but plotted at lower contour levels to verify that no additional prolyl signals are present. (**a**) Spectrum of apo-IscU acquired at pH 8.0 and 25 °C, where the protein is a mixture of the S- and D-states. (**b**) Spectrum of apo-IscU acquired at 45 °C, where the protein is in the D-state. (**c**) Spectrum of the Zn-bound form of IscU acquired at pH 8.0 and 25 °C, where the protein is in the S-state. Assignments to individual prolyl residues were determined as described in the text. Each NMR sample in a and c contained 1 mM [U-^{13}C, ^{15}N-Pro]-IscU, 50 mM Tris-HCl (pH 8.0), 0.5 mM EDTA, 5 mM DTT, 150 mM NaCl, 50 μM DSS, and 50 μM NaN_3 in 10% D_2O. The NMR sample in b contained 1 mM [U-^{13}C, ^{15}N-Pro]-IscU:Zn^{2+}, 50 mM Tris-HCl (pH 8.0), 5 mM DTT, 150 mM NaCl, 50 μM DSS, and 50 μM NaN_3 in 10% D_2O.

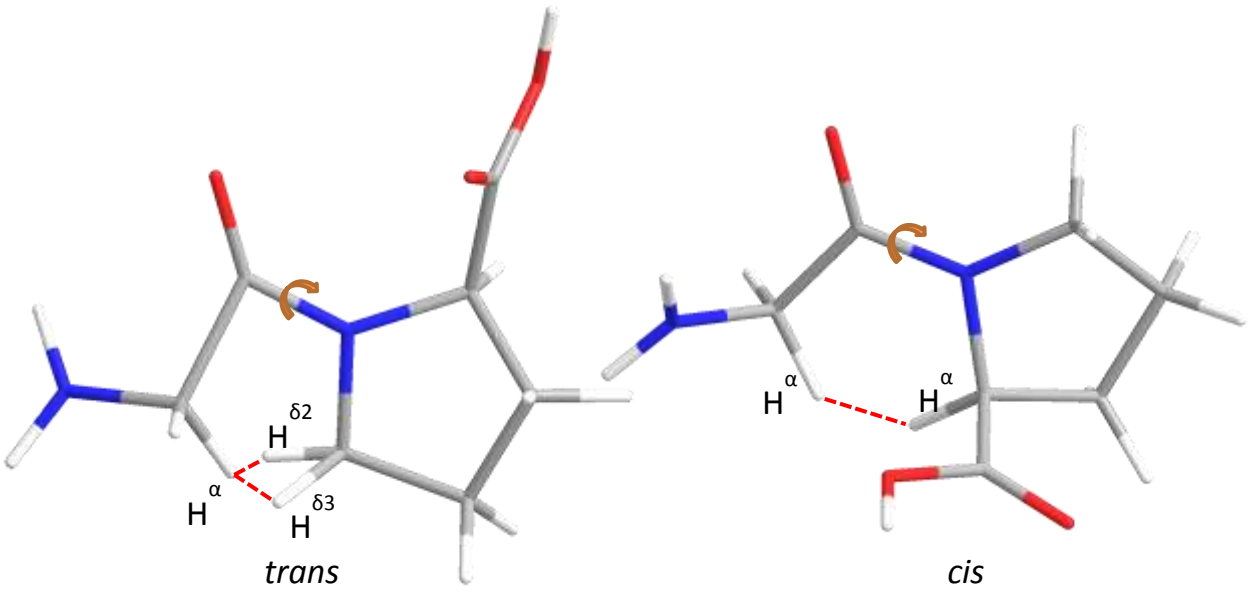

Figure S3. Illustration of the NOE signals expected depending on the configuration (*trans* or *cis*) of the peptidyl-prolyl peptide bond. The dashed lines connect protons that are near in space in each configuration. **Left**. When the peptide bond is *trans*, NOEs are expected between the $^{1}H^{\alpha}$ of the preceding residue and one or both of the prolyl $^{1}H^{\delta 2}$ or $^{1}H^{\delta 3}$. **Right**. When the peptide bond is *cis*, an NOE is expected between $^{1}H^{\alpha}$ of the previous residue and the prolyl $^{1}H^{\alpha}$.

REFERENCE

1. Kim, J. H., Füzéry, A. K., Tonelli, M., Ta, D. T., Westler, W. M., Vickery, L. E., and Markley, J. L. (2009) Structure and dynamics of the iron-sulfur cluster assembly scaffold protein IscU and its interaction with the cochaperone HscB, *Biochemistry 48*, 6062-6071.