## **Supplemental Figures**

Fig. S1. Stereo view of the electron density map of the full-length CusC channel at a resolution of 2.09 Å. This is a  $2F_o - F_c$  electron density map (white mesh) contoured at 1.2  $\sigma$ . The C $\alpha$  traces of CusC are colored red.

Fig. S2. Secondary structural topology of the CusC monomer. The topology was constructed based on the crystal structure of CusC. The  $\alpha$ -helices and  $\beta$ -strands are colored blue and red, respectively.

Fig. S3. Stereo view of the experimental electron density map of the  $\Delta$ C1 mutant at a resolution of 2.53 Å. Anomalous maps of the 10 selenium sites (contoured at 3  $\sigma$ ), corresponding to the five methionines from each protomer, are colored green. The electron density (colored white) is contoured at the 1.2  $\sigma$  level and superimposed with the C $\alpha$  traces of the two  $\Delta$ C1 protomers (red and blue) in the asymmetric unit.

Fig. S4. Crystal packing of  $\Delta$ C1. Packing diagram of  $\Delta$ C1viewed along the (a) *a* axis. (b) Packing diagram of  $\Delta$ C1viewed orthogonal to (a). The  $\Delta$ C1 molecules are colored red and green.

Fig. S5. Stereo view of the electron density map of the C1S CusC mutant at a resolution of 2.69 Å. The electron density (colored white) is contoured at the 1.2  $\sigma$  level and superimposed with the C $\alpha$  traces of two C1S protomers (blue and green) in the asymmetric unit.

Fig. S6. Representative gel filtration experiment. The experiment demonstrated that the C1S mutant is monomeric. The *y* axis values were defined as:  $K_{av} = (V_e - V_0)/(V_T - V_0)$ , where  $V_T, V_e$ , and  $V_0$  are the total column volume, elution volume, and void volume of the column, respectively. Standards used were: A, cytochrome C (M<sub>r</sub> 12,400); B, carbonic anhydrase (M<sub>r</sub> 29,000); C, albumin bovine serum (M<sub>r</sub> 66,000); D, alcohol dehydrogenase (M<sub>r</sub> 150,000); and E,  $\beta$ -Amylase (M<sub>r</sub> 200,000). The void volume was measured using blue dextran (M<sub>r</sub> 2,000,000). The experiment suggested an average molecular weight of 48.1 ± 4.0 kDa for the C1S mutant. This value is in good agreement with the theoretical value of 48.4 kDa for one C1S molecule, indicating that the C1S mutant is monomeric in form.











## **Figure S4b**





