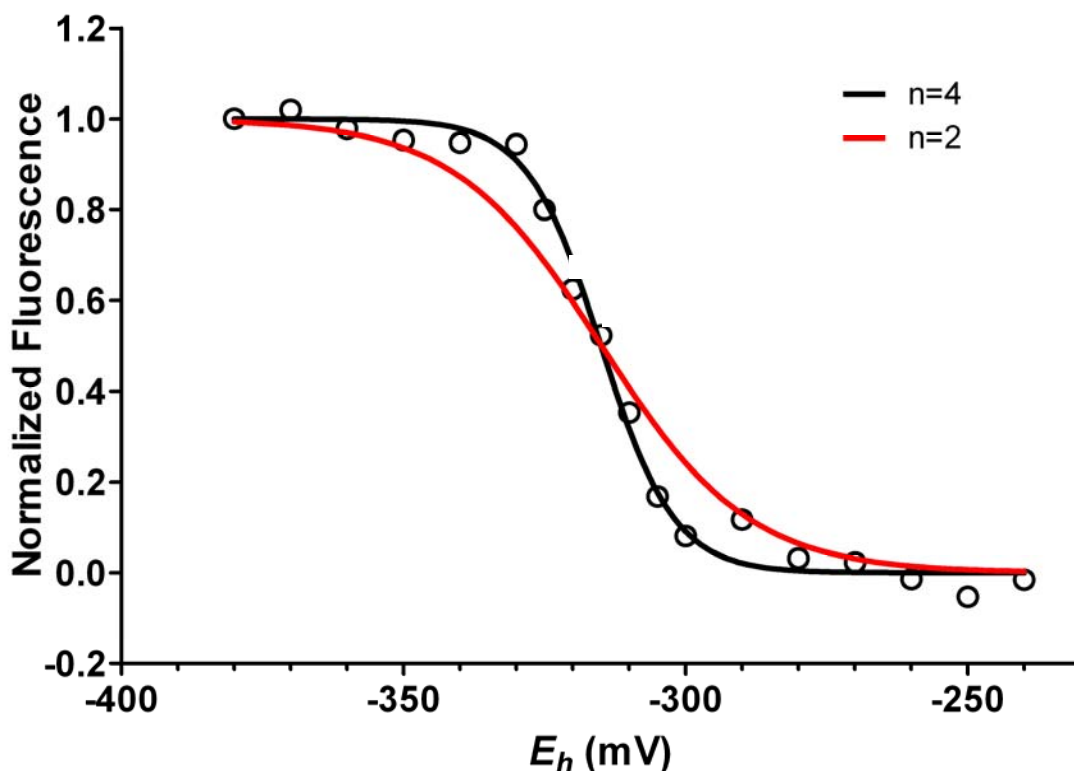


Supplementary Figures

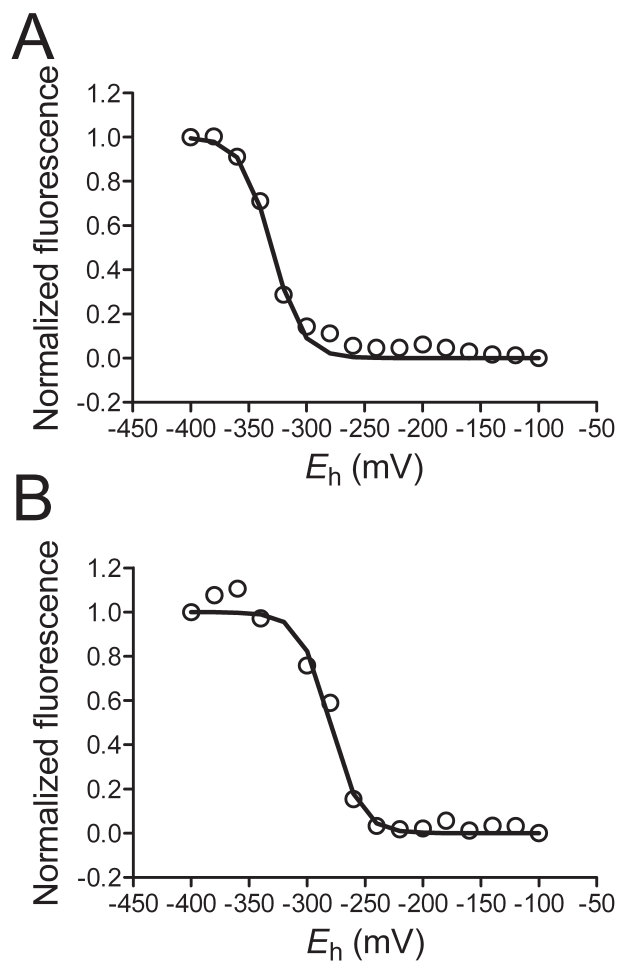
Reconstitution of the Mia40-Erv1 oxidative folding pathway for the small Tim proteins

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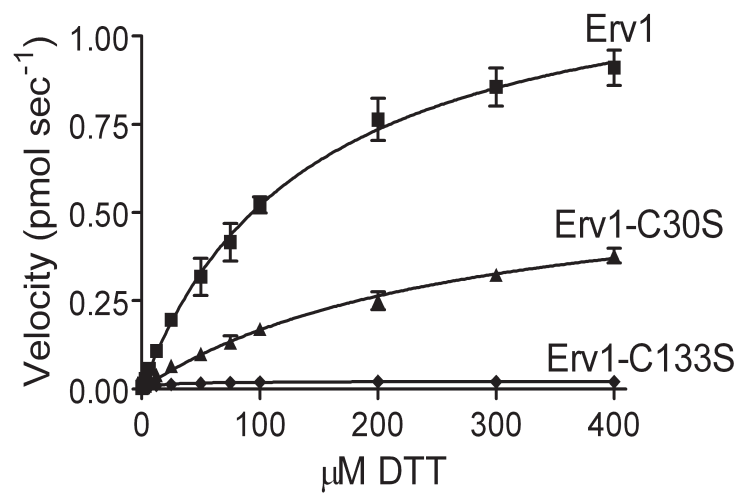
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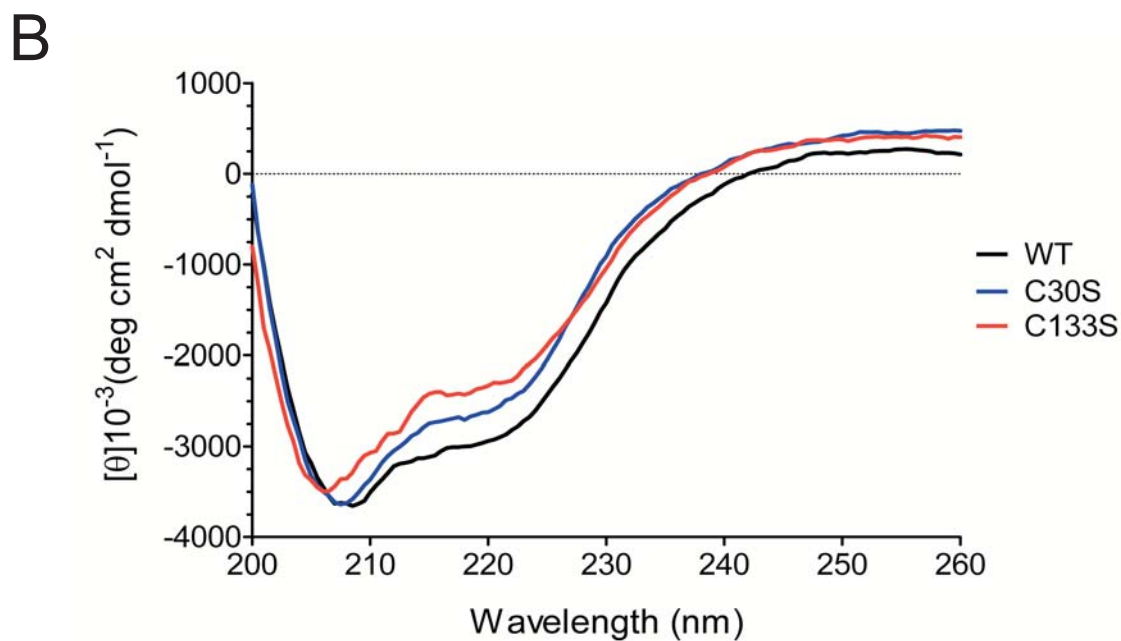
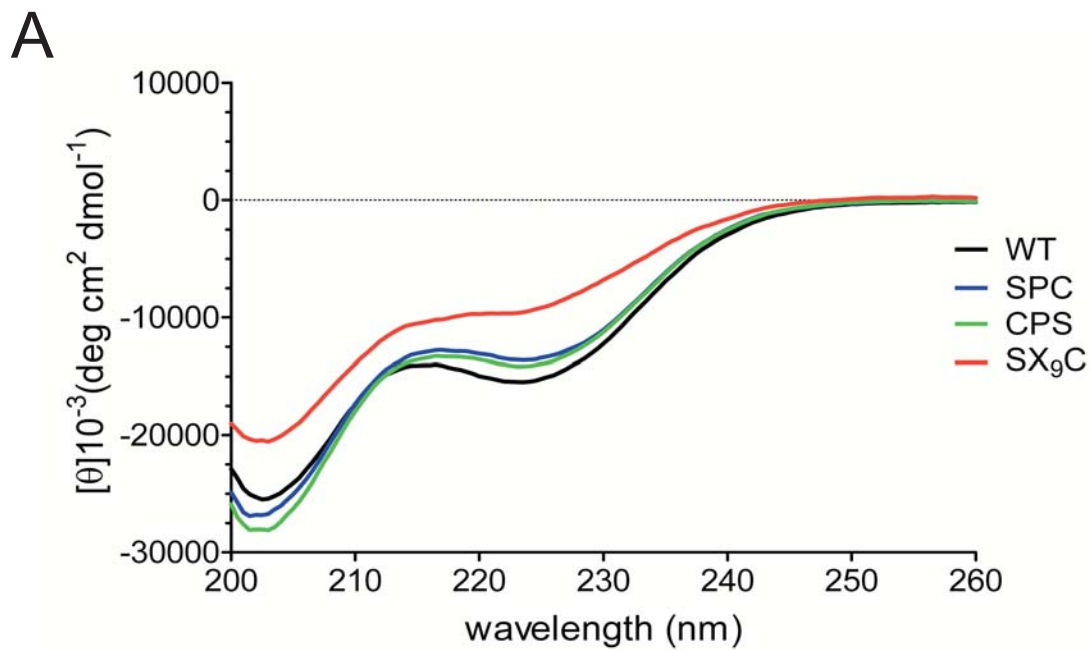
Supplementary Figure 1 mBBR titrations for Tim13 yield a similar E_m as CD studies. As in Fig. 1, redox titrations of dithiol/disulfide couples in Tim13 were carried out using DTT redox buffers. Redox equilibration was performed for 2.0 h at pH 7.0 with total redox buffer concentrations of 2.0 mM under anaerobic conditions. Data in all titrations were fit to the Nernst equation for a four-electron carrier (black line) and two-electron carrier (red line). The likelihood of the data to fit a curve for a four electron carrier vs. a two electron carrier was calculated using the informational theory approach Akaike's criterion with Prism (Akaike, 1974). The probability was higher (98 %) that the data fit a curve for $n = 4$ electrons than $n = 2$ electrons (2 %) ($n = 4$)



Supplementary Figure 2 Oxidation-reduction titrations of Mia40 under aerobic conditions give a similar E_m as those under anaerobic conditions. As in Figure 2, Mia40 was incubated over a range of redox potentials in aerobic conditions and the midpoint potential was measured with (A) mBBr titration and (B) intrinsic tryptophan fluorescence. Values of -322 mV and -289 mV were obtained, respectively. ($n = 4$)



Supplementary Figure S3 Erv1 and the C30S Erv1 mutant oxidize the nonphysiologic substrate DTT. 1 μM Erv1, C30S Erv1, and C133S Erv1 were incubated with 0-400 μM DTT over a 50 min time course. Aliquots were removed during the time course and the concentration of H₂O₂ was measured. The velocity (pmol/sec) of H₂O₂ produced was plotted vs. the concentration of DTT in the assay.



Supplementary Figure S4. CD analysis of the secondary structure content for the Mia40 and Erv1 mutants. (A) CD analyses of the Mia40 mutants SPC and CPS show similar spectra to WT, while analyses of the SX₉C mutant shows a slight decrease in alpha-helical content, as shown by the shallow peak at 225 nm. (B) CD analyses of the Erv1 mutants C30S and C133S indicate that the structures do not differ markedly from the WT protein.