

Supplemental Figure 1: Rate of reduction (ring opening) of oxidized tripeptide Cys-Sec-Gly for truncated mTR3. A linear increase in activity is observed as the concentration of tripeptide increases in the assay. The truncated enzyme could not be saturated with tripeptide due to solubility limits of the peptide under the assay conditions. The rates of peptide turnover were calculated by measuring the slope of a plot of NADPH consumption (in min^{-1}) vs peptide concentration (in mM).

Supplemental Figure 2: Rate of reduction (ring opening) using oxidized tetrapeptides as substrates for the truncated enzymes *Dm*TR (λ) and mTR3 (\square). (A) Peptide Ac-GCUG(ox) as substrate; (B) Peptide Ac-SCUS(ox) as substrate; (C) Peptide Ac-GCCG(ox) as substrate; and (D) Peptide Ac-SCCS(ox) as substrate. A more complete analysis of this data is given in Table 3.

Supplemental Figure 3: Crystal of *Dm*TR. Crystals were grown at 20 °C using the hanging drop diffusion method with a reservoir solution containing 200 mM Succinate pH 5.5 and 22% PEG 6000. Hanging drops were 8 μL : 5 μL protein, 2 μL reservoir and 1 μL of 10 mM NADP. Crystals (280 x 100 x 100 μm) were fully developed in 5 to 7 days.

Supplemental Figure 4: Electrostatic surface potential calculated using GRASP (1) for *Dm*TR showing a close-up of the tetrapeptide binding pocket where thiol-disulfide exchange occurs between the conserved N-terminal dithiol and the C-terminal tetrapeptide of the adjacent subunit containing the 8-membered ring that must be opened during redox cycling. The tetrapeptide

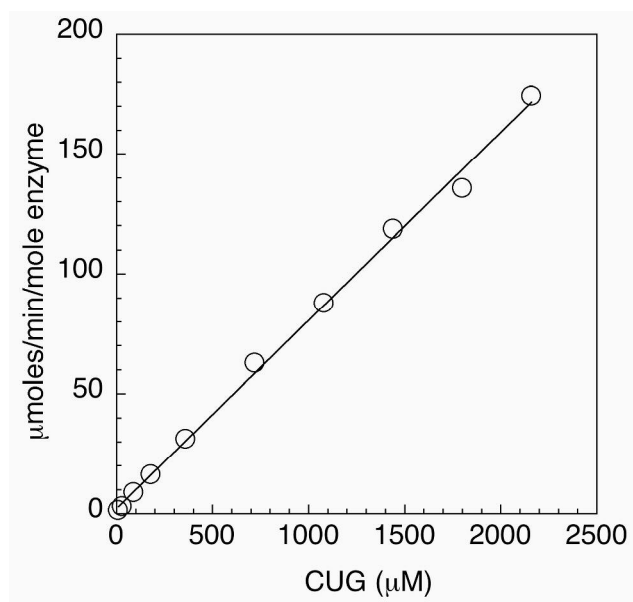
SCCS(ox) in the C+ conformation with Cys489' in the interchange position is shown in space-filling mode. Figure generated with PyMOL (2).

Supplemental Figure 5: Electrostatic surface potential calculated for mTR3 (PDB 1ZKQ) showing a close-up of the tetrapeptide binding pocket where thiol-disulfide exchange occurs between the conserved N-terminal dithiol and the C-terminal tetrapeptide of the adjacent subunit containing the 8-membered ring that must be opened during redox cycling. The tetrapeptide GCUG(ox) in the T- with Cys522' in the interchange position is shown in space-filling mode.

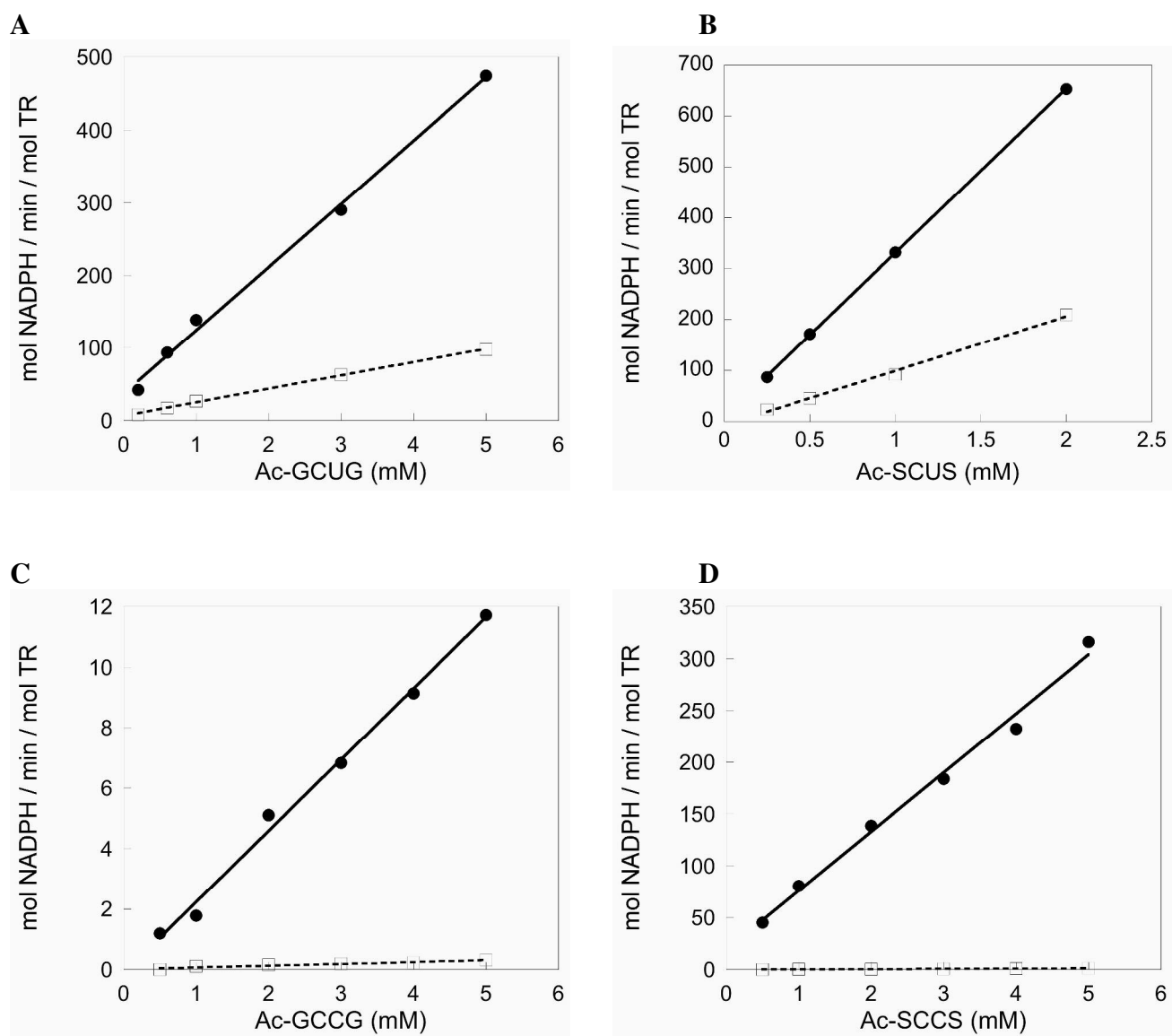
Supplemental Figure 6: Stereo diagram for mTR3 (PDB 1ZKQ) showing residues from helix 3 along with the tetrapeptide GCUG(ox) in the C+ conformation with Cys522' in the interchange position.

- (1) Occena, L. G. a. S., D.L. (1995) GRASP - A Prototype Interactive Graphic Sawing Program - (MU-IE Technical Report). *MU-IE Technical Report 019501*, 1-17.
- (2) DeLano, W. L. (2002) in *The PyMOL Molecular Graphics System*, DeLano Scientific, San Carlos.

Supplemental Figure 1.



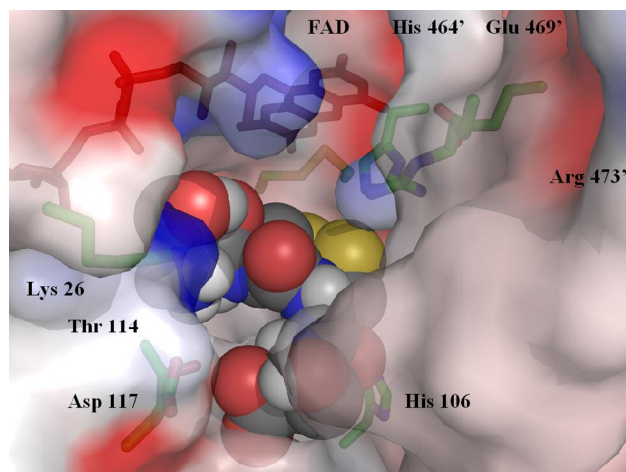
Supplemental Figure 2



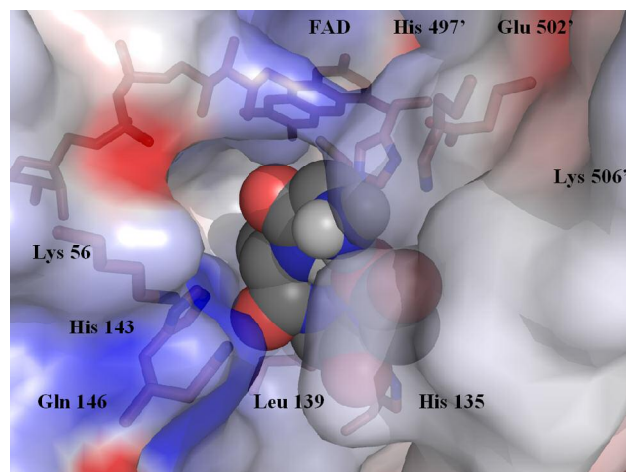
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6

