Supporting Information

Compiled statements in the literature attributing the broad substrate specificity of mammalian TR to the presence of the Sec residue in the enzyme.

1. Holmgren, A.:

"The essential role of Sec in thioredoxin reductase explains the very broad substrate specificity including reduction of thioredoxin, selenite, dehydroascorbic acid and ascorbyl free radical, hydrogen peroxide and lipid hydroperoxides."

"Selenoproteins of the thioredoxin system." *Selenium: Its Molecular Biology and Role in Human Health* (Hatfield, D. L., Berry, M. J., and Gladyshev, V. N., Ed.), 2006, Springer, New York. Pages 183-194.

2. Bjornstedt and Holmgren:

"The discovery of selenocysteine in mammalian TR may explain the broad substrate specificity of the enzyme and the requirement of selenium for cell proliferation."

Biomedical and environmental sciences. (1997) 10, 271-279.

3. Mustacich and Powis:

"The broad substrate specificity of mammalian TrxRs is due to a second redox-active site, a C-terminal –Cys-SeCys- (where SeCys is selenocysteine), that is not found in glutathione reductase or *Escherichia coli* TrxR."

Biochemical Journal. (2000) 346, 1-8.

4. Novoselov and Gladyshev:

"Because of the presence of Sec, animal TRs have broad substrate specificity and can reduce a variety of proteins and low molecular weight compounds"

Protein Science (2003) 12, 372-378.

5. Behne and Kyriakopoulos:

"In addition to thioredoxin, mammalian thioredoxin reductases are able to use other substrates, including hydroperoxides, dehydroascorbate, and various enzymes and proteins. This broad substrate specificity has been attributed to the presence of selenocysteine situated in the flexible C-terminal extension."

Annual Review of Nutrition (2001) 21, 453-473.

6. Powis and coworkers:

"Thioredoxin reductase-1 is a selenocysteine-containing flavoprotein with broad substrate specificity because of the ready accessibility of its COOH-terminal redox active site, which contains an essential selenocysteine residue."

Molecular Cancer Therapeutics (2006) 5, 630-636.

7. Nalvarte and Spyrou:

"The combination of a low pK_a of Sec (typically 5.25) and an easily accessible C-terminal localization of the Sec-containing active site should explain why reduced TrxRs are highly reactive at physiological pH and display a broad substrate specificity."

Journal of Biological Chemistry (2004) 279, 54510-54517.

8. Arner E, and coworkers:

"This formed selenolthiol motif reduces the active site disulfide of Trx, the disulfide of the synthetic model substrate, 5,5'-dithiobis-(2-nitrobenzoate) (DTNB), or any of the many other substrates of the enzyme, such as lipoic acid, selenite, and several quinone compounds; the wide substrate specificity of mammalian TrxR is attributed to the easy access and reductive capacity of the C-terminal selenolthiol (1, 2, 7, 9, 11, 12)."

Journal of Biological Chemistry (2006) 281(9), 5593-5603

9. Stadtman, TC and coworkers:

"In contrast to *Escherichia coli* and yeast thioredoxin reductases, the human placental enzyme contains an additional redox center consisting of a cysteine-selenocysteine pair that precedes the C-terminal glycine residue. This reactive selenocysteine-containing center imbues the enzyme with its unusually wide substrate specificity."

Free Radicals in Biology and Medicine (2001) 30, 51-61.

10. Stadtman, TC and coworkers:

"The presence of a selenol group that is fully ionized at physiological pH and is highly reactive undoubtedly explains the unusual, wide substrate specificity of the mammalian enzyme as contrasted to TRs from *E. coli*, yeast, and some other lower eukaryotes that are not selenoproteins."

Proc. Natl. Acad. Sci. U. S. A. (1998) 95, 8520–8525.

11. Holmgren, A and coworkers:

"Unlike their small bacterial counterparts, these enzymes are large selenoproteins. The homodimeric flavoenzymes contain a penultimate C-terminal selenocysteine in their GCSG active sites [13–15], which is also the reason for TrxRs' wide substrate specificity, including non-disulphide compounds [16]."

Biochemical Society Transactions (2005) 33, 1375–1377.

12. Arner, E and coworkers:

"Furthermore, in contrast with the bacterial enzyme, mammalian TrxR has a remarkably broad substrate specificity and reduces not only protein disulphides but also low-molecular-mass disulphide compounds such as 5,5′-dithiobis-(2-nitrobenzoic acid) ('DTNB') [9] and lipoic acid [12], low-molecular-mass non-disulphide compounds such as selenite [13] or alloxan [14] and also functions as a peroxidase with H_2O_2 or lipid hydroperoxides [15]. …The selenocysteine residue in the C-terminal motif Gly-Cys-Sec-Gly (in which Sec represents selenocysteine) is conserved in the human, rat and bovine enzymes and is essential for its catalytic activity [18]. The presence of this presumably easily accessible redox-active motif should explain the wide substrate specificity."

Biochemical Journal (2000) 347, 661–668.

13. Holmgren, A and coworkers:

"The selenocysteine (Sec) (U) in the open C terminus is essential for the reducing activity of TrxR (20). Selenol has a low pKa (5.3) value, and thus selenolate is the predominant form under physiological conditions. This property and the accessible location of C-terminal active site account for the wide substrate specificity of TrxR (21)."

Proc. Natl. Acad. Sci. U. S. A. (2007) 104, 12288–12293.

14. Holmgren, A and coworkers:

"Mammalian TrxRs have a remarkably wide substrate specificity explained by their easily accessible C-terminal active site redox center, which contains an essential selenocysteine residue (21-23)."

Journal of Biological Chemistry (2005) 280, 25284-25290.

15. Gladyshev and Hatfield:

"The broad substrate specificity of TR has been attributed to the presence of Sec in the enzyme."

Journal of Biomedical Science (1999) 6, 151-160.

16. Gromer and Becker:

“Our hypothesis is supported by the fact that hTrxR has a very broad substrate specificity, including such bulky molecules as thioredoxin and NK-lysin. A flexible C-terminal extension, carrying reducing equivalents, could explain the ability of the enzyme to serve so many different substrates.”

Biochemical Journal (1998) 332, 591-592.

Table S1: Enzyme Assay Concentrations for Lipoic Acid/Lipoamide Assays

Substrate	Enzyme	Concentration of Enzyme (nM)
Lipoic Acid	mTR-GCUG	54.3
Lipoic Acid	mTR-GCCG	91.1
Lipoic Acid	mTRΔ8	48.2
Lipoic Acid	mTRΔ3	81.1
Lipoic Acid	mTRΔ2	80.0
Lipoic Acid	DmTR-SCCS	64.0
Lipoic Acid	DmTRΔ8	55.7
Lipoic Acid	CeTR2-GCCG	330.0
Lipoamide	mTR-GCUG	54.3
Lipoamide	mTR-GCCG	364.0
Lipoamide	mTRΔ8	96.0
Lipoamide	mTRΔ3	122.0
Lipoamide	mTRΔ2	80.0
Lipoamide	DmTR-SCCS	165.0
Lipoamide	DmTRΔ8	167.0
Lipoamide	CeTR2-GCCG	330.0

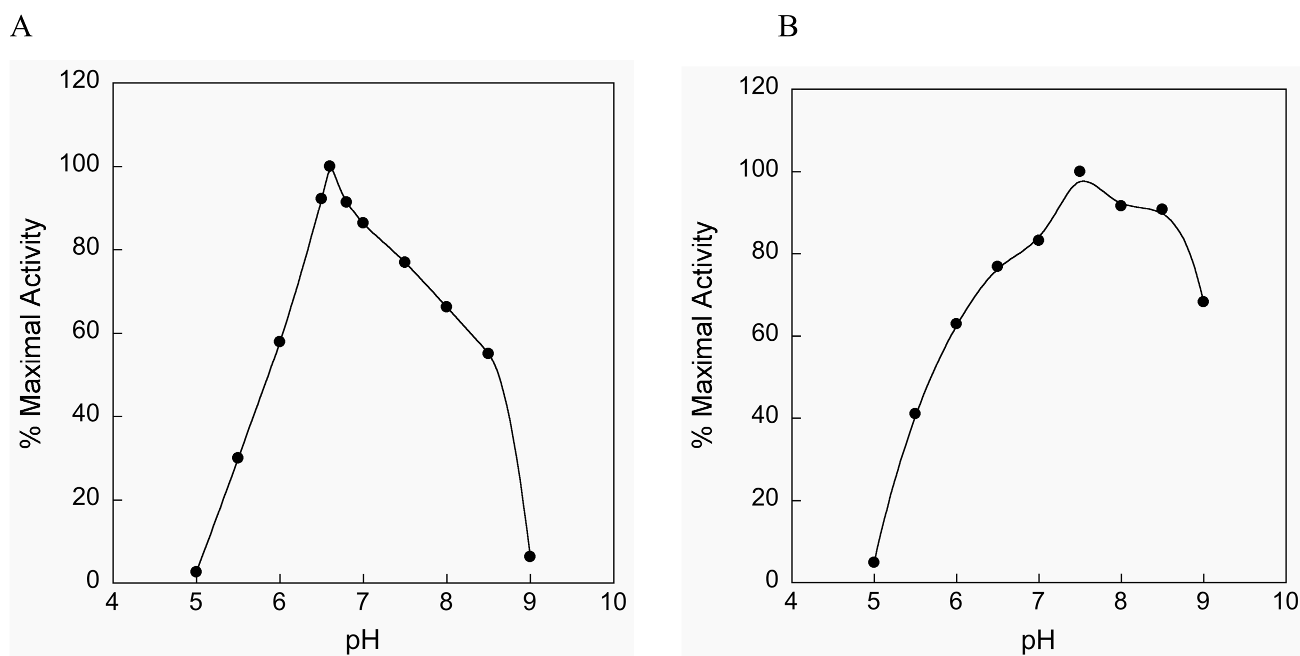

Figure S1: (A) Activity of selenite as a function of pH for mTR-GCUG. (B) Activity of selenocystine as a function of pH for mTR-GCUG. While both profiles are broad, the optimum for selenite is decidedly more acidic.

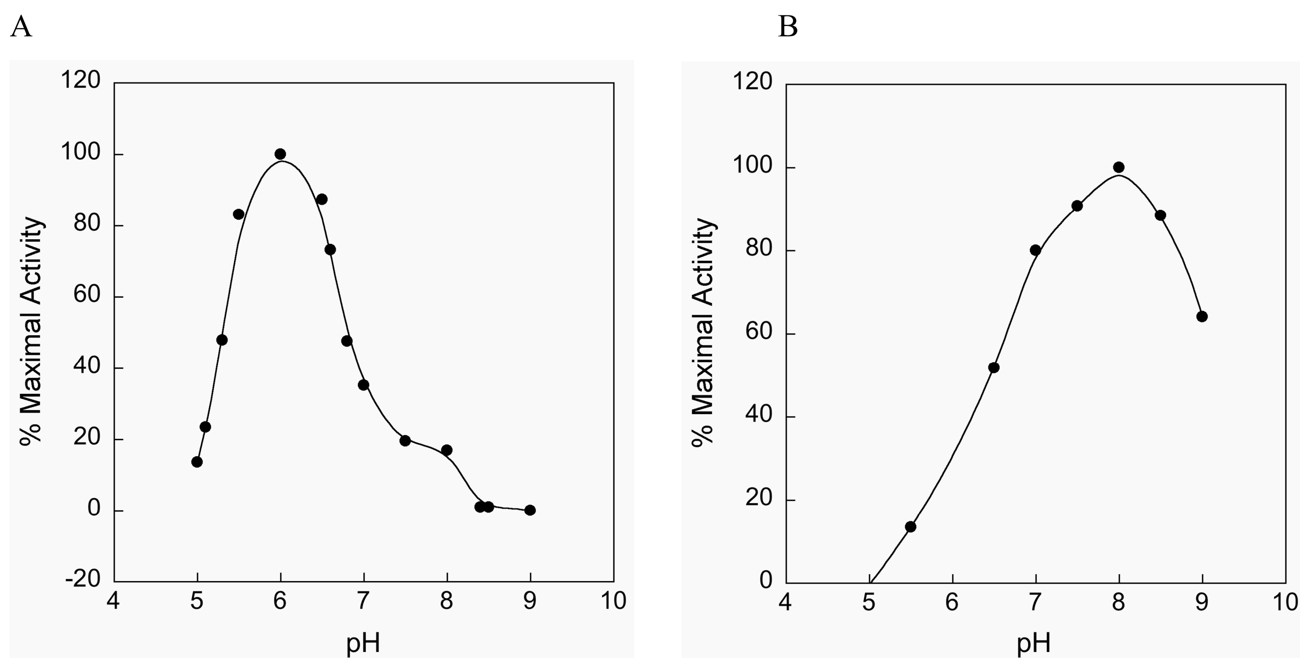

Figure S2: (A) Activity of selenite as a function of pH for mTRΔ8. (B) Activity of selenocystine as a function of pH for mTRΔ8. For the truncated enzyme, the profile for selenite is sharp with a pH optimum near 6.0, while for selenocystine the profile is broad with an optimum near 8.0.

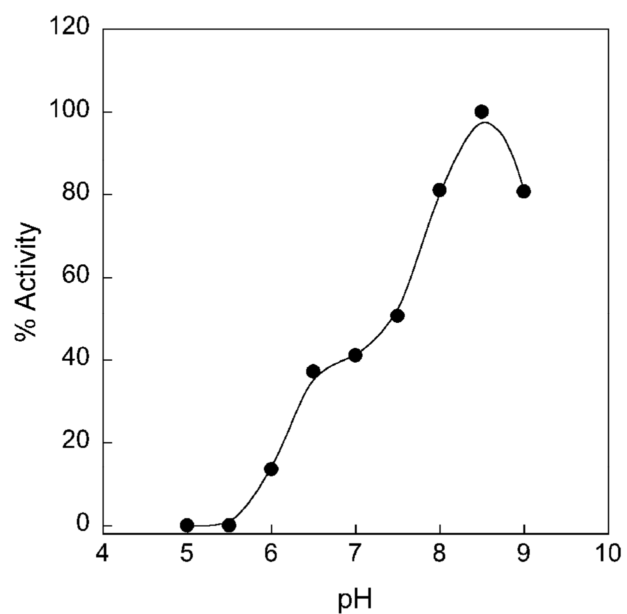

Figure S3: Activity of mTRΔ8 with lipoamide as a substrate as a function of pH. The plot clearly shows an optimum near 8.5, in contrast to the same plot with lipoic acid.