Supporting Information

Synthetic Seleno-Glutaredoxin 3 Analogs are Highly Reducing Oxidoreductases with Enhanced Catalytic Efficiency

(Norman Metanis *et al.*)

Supporting Materials and Methods.

Buffers for kinetic measurements were prepared using de-ionized water. KH_2PO_4 and K_2HPO_4 were purchased from Fisher Biotech. Recombinant *E. coli* Trx1 and Grx1 were purchased from Promega Corporation (Madison, WI) and American Diagnostica Inc. (Greenwich, CT), respectively. Deuterated solvents (DMSO-d_6, $CDCl_3$) were purchased from Aldrich Chem. Co. ^{1}H NMR spectra were recorded on a Varian Mercury-300, Bruker DRX-500 spectrometers, using CDCl3 as a solvent.

All Boc-amino acids were obtained from Midwest Biotech (Fishers, IN), with the following side chain protecting groups: Arg(Tos), Asp(OcHxl), Asn(Xan), Cys(MeBzl), Glu(OcHxl), Ser(Bzl), Sec(MeBzl), Thr(Bzl), Tyr(Br-Z), Lys(2ClZ), His(Bom) (OcHxl = cyclohexyl; Bzl = benzyl; MeBzl = 4-methylbenzyl; 2ClZ = 2-chlorobenzyloxycarbonyl; Br-Z = 2-Bromobenzyloxycarbonyl; Bom = benzyloxymethyl; Xan = N-Xanthyl). 1*H*-Benzotriazolium-1-[bis(dimethylamino)methylene]-5-chloro,hexafluorophosphate(1-),3-oxide (HCTU), *S*-Trityl-β-mercaptopropionic acid-Leu-OCH_2-Pam (TMPAL-Pam) resin, were obtained from Peptides International (Louisville, KY). All solvents; HPLC-grade, *N,N*-dimethylformamide (DMF), dichloromethane, and acetonitrile (ACN), were purchased from Fisher. Trifluoroacetic acid (TFA) was obtained from Halocabon Products (River Edge, NJ). Anhydrous HF was purchased from Matheson Gas (Cucamonga, CA). Anisole was purchased from Sigma-Aldrich (St. Louis, MO). *N,N*-diisopropylethyl amine (DIEA), and Boc-Lys(2ClZ)-OCH_2-Pam resin from Applied Biosystems (Foster, CA). All other chemicals were

obtained from Fisher or Sigma-Aldrich, Inc.

High Performance Liquid Chromatography (HPLC). Analytical reversed-phase HPLC was performed on a Hewlett-Packard HPLC 1050 with 214 nm UV detection using Vydac C_{18} column (5 μm, 0.46 × 15 cm) or Varian system using a Phenomenex C_{18} column (Jupiter 5 μm, 300 Å, 150 × 4.6 mm) or C_4 (Jupiter 10 μm, 90 Å, 150 × 4.6 mm). Semi-preparative reversed-phase HPLC was performed on a Gilson HPLC system using a Vydac C_{18} column (10 μm, 1.0 × 25 cm). Preparative reversed-phase HPLC was performed on Waters HPLC system using Vydac C_{18} column (10 μm, 2.5 × 25 cm). Linear gradients of acetonitrile in water with 0.1% TFA were used for all systems to elute bound peptides. The flow rates were 1 mL/min (analytical), 5 mL/min (Semi-preparative), and 30 mL/min (preparative). Buffer A is MilliQ water containing 0.1% TFA; buffer B is acetonitrile with 10% water and 0.09% TFA.

Mass Spectrometry. Electrospray ionization MS was performed on an API-III triple quadruple mass spectrometer (Sciex, Thornhill, ON, Canada). Peptide masses were calculated from the experimental mass to charge (m/z) ratios from all of the observed multiply charged species of a peptide by using MacSpec software (Sciex). Theoretical masses and the $\varepsilon_{280\ nm}$ values of peptides and proteins were calculated by using *Sherpa*$_{\text{Lite}}^{4.0}$ for Mac.

Supporting Figure S1

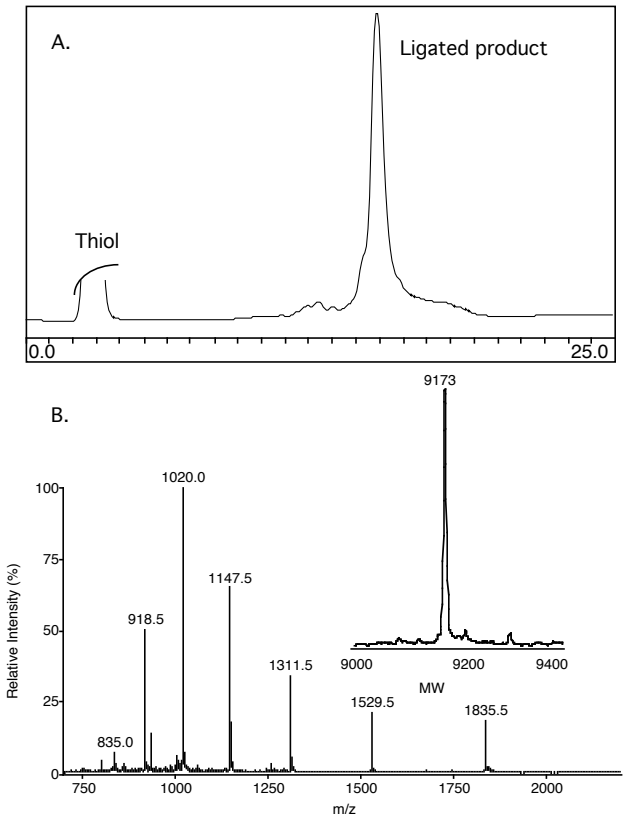


Figure S1: A. NCL between Grx3(1-37)(C11U-C14U)-COSR peptide and Grx3(C38-82) peptide as followed by analytical HPLC and ESI-MS. The reaction was complete in 4 hr. B. The expected mass of 9173 Da observed by ESI-MS reflects formation of the desired protein Grx3(C11U-C14U-A38C) having the diselenide bond in the active site (oxidized).

Supporting Figure S2.

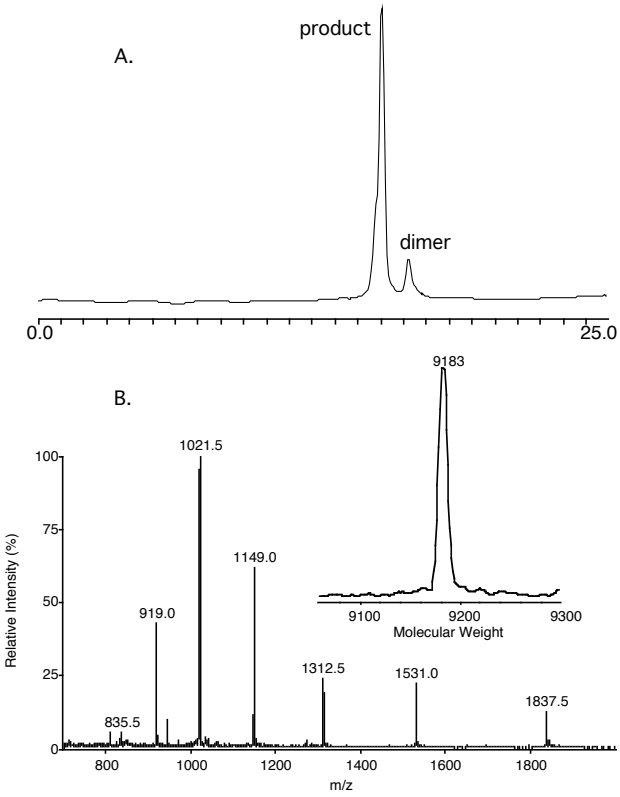
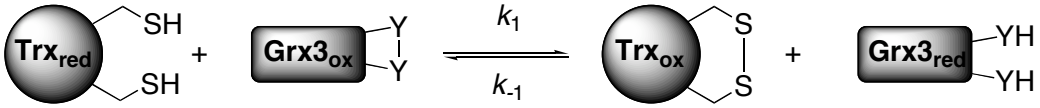


Figure S2: A. The ligated Grx3(C14U-A38C) was oxidized to form the selenenylsulfide bond in the active site and Cys38 alkylated with iodoacetamide to form the desired alkylated oxidized Grx3(C14U-A38X) or abbreviated Grx3(C14U) analog, as followed by analytical HPLC and ESI-MS. B. The expected mass of 9183 Da observed by ESI-MS reflects formation of the desired protein in the oxidized form.

Supporting rate equation.



Y = S, Se

$$A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C + D$$

$$\begin{bmatrix} [C]_0 = [D]_0 = 0 \\ [A]_0 = [B]_0 \\ [A]_t + [C]_t = [A]_0 \\ [A]_t = [A]_0 - [C]_t \end{bmatrix}$$

$$\frac{d[C]_t}{dt} = k_1[A]_t[B]_t - k_{-1}[C]_t[D]_t$$

$$\frac{d[C]_t}{dt} = k_1[A]_t^2 - k_{-1}[C]_t^2$$

$$\frac{d[C]_t}{dt} = k_1([A]_0 - [C]_t)^2 - k_{-1}[C]_t^2$$

$$\frac{d[C]_t}{dt} = k_1([A]_0^2 - 2[A]_0[C]_t + [C]_t^2) - k_{-1}[C]_t^2$$

$$\frac{d[C]_t}{dt} = k_1[A]_0^2 - 2k_1[A]_0[C]_t + (k_1 - k_{-1})[C]_t^2$$

$$\frac{d[C]_t}{dt} + 2k_1[A]_0[C]_t + (k_{-1} - k_1)[C]_t^2 = k_1[A]_0^2$$

$$[C]_t = \frac{[A]_0(e^{2[A]_0\sqrt{k_1k_{-1}}\cdot t} - 1)}{(e^{2[A]_0\sqrt{k_1k_{-1}}\cdot t} - 1) + \sqrt{\frac{k_{-1}}{k_1}}(e^{2[A]_0\sqrt{k_1k_{-1}}\cdot t} + 1)}$$

The background oxidation (which has a linear term: mt) is added to the end of the rate equation to give:

$$[C]_t = \frac{[A]_0 (e^{2[A]_0 \sqrt{k_1 k_{-1}} \cdot t} - 1)}{(e^{2[A]_0 \sqrt{k_1 k_{-1}} \cdot t} - 1) + \sqrt{\frac{k_{-1}}{k_1}} (e^{2[A]_0 \sqrt{k_1 k_{-1}} \cdot t} + 1)} + mt$$