Supporting Information

Thiol-Blocking Electrophiles Interfere with Labeling and Detection of Protein Sulfenic Acids

Julie A. Reisz[1], Erika Bechtold[2], S. Bruce King[2], Leslie B. Poole[3], and Cristina M. Furdui[1*]

[1]Section on Molecular Medicine, Department of Internal Medicine, Wake Forest School of Medicine, Winston-Salem, NC 27157, USA

[2]Department of Chemistry, Wake Forest University, Winston-Salem, NC 27109, USA

[3]Department of Biochemistry, Winston-Salem, NC 27157, USA

SUPPLEMENTARY FIGURES

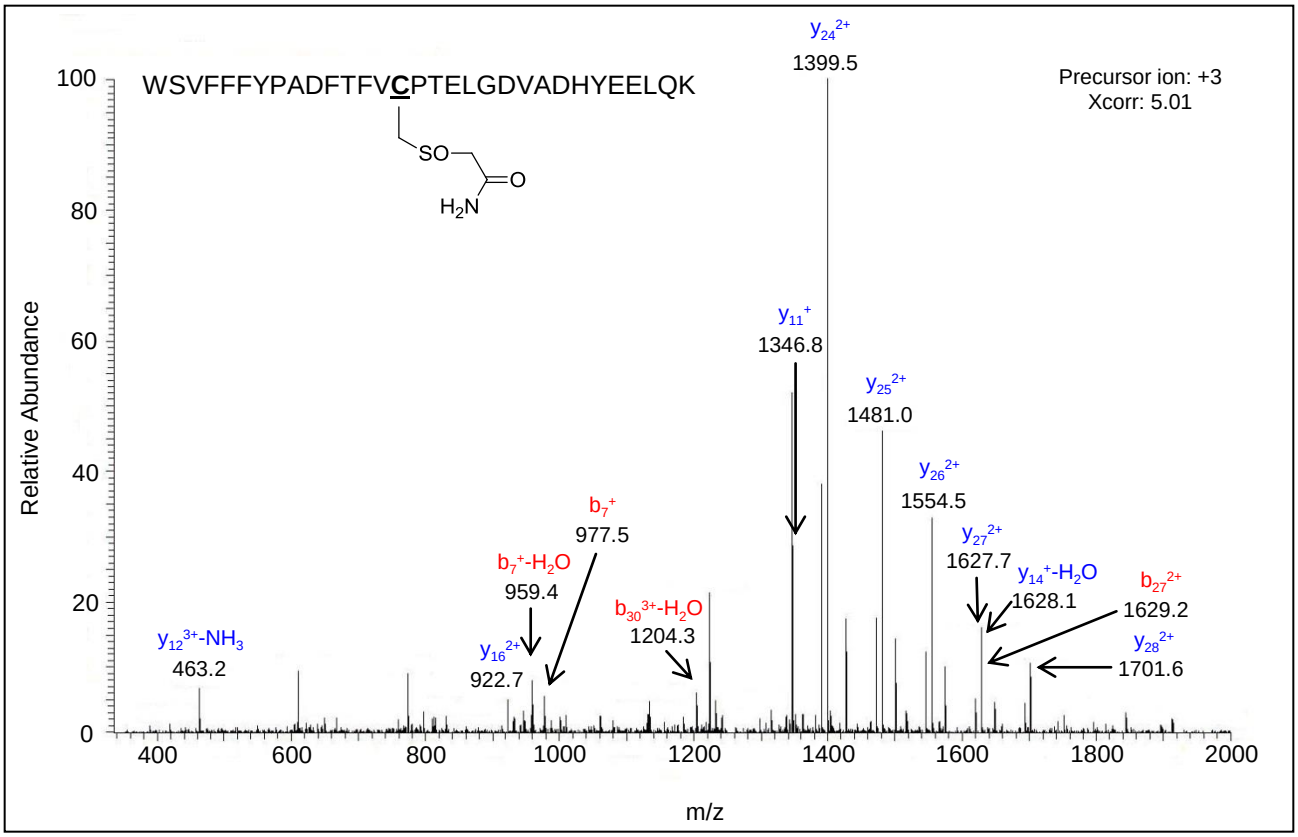


Figure S1: Positive ion MS^2 (CID) spectrum of the Cys-containing peptide of C165S AhpC resulting from the reaction of AhpC-SOH (30 μM) with IAM (5 mM) at pH 7.5. Protein species were digested with trypsin and the resulting peptides separated by nanoLC.

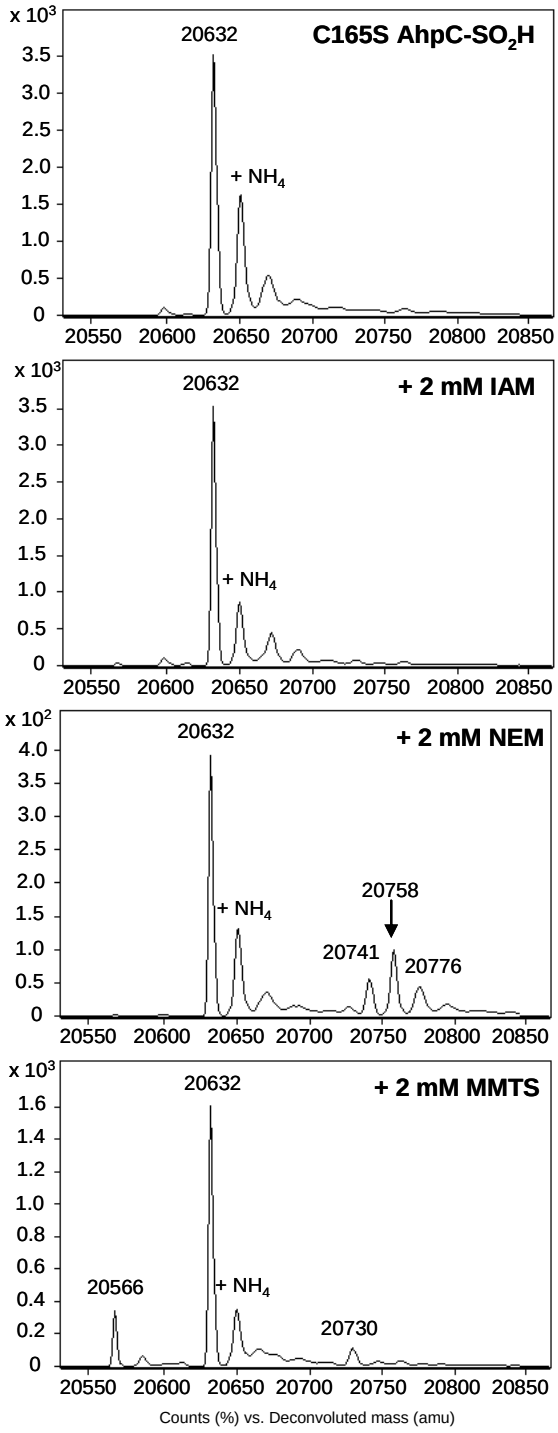


Figure S2: ESI-TOF mass spectra for the incubation of C165S AhpC-SO_2H (30 μM) with electrophiles IAM, NEM, or MMTS (5 mM) for 2 hours at r.t. and pH 7.5. The signals at 20,566 a.m.u. and 20,632 a.m.u. correspond to dehydroalanine and sulfinic acid (-SO_2H), respectively. The adduct at 20,758 a.m.u., corresponding to the addition of one equivalent of NEM, was not found to contain a NEM-modified cysteine peptide as determined via tryptic digest and nanoLC-MS/MS.

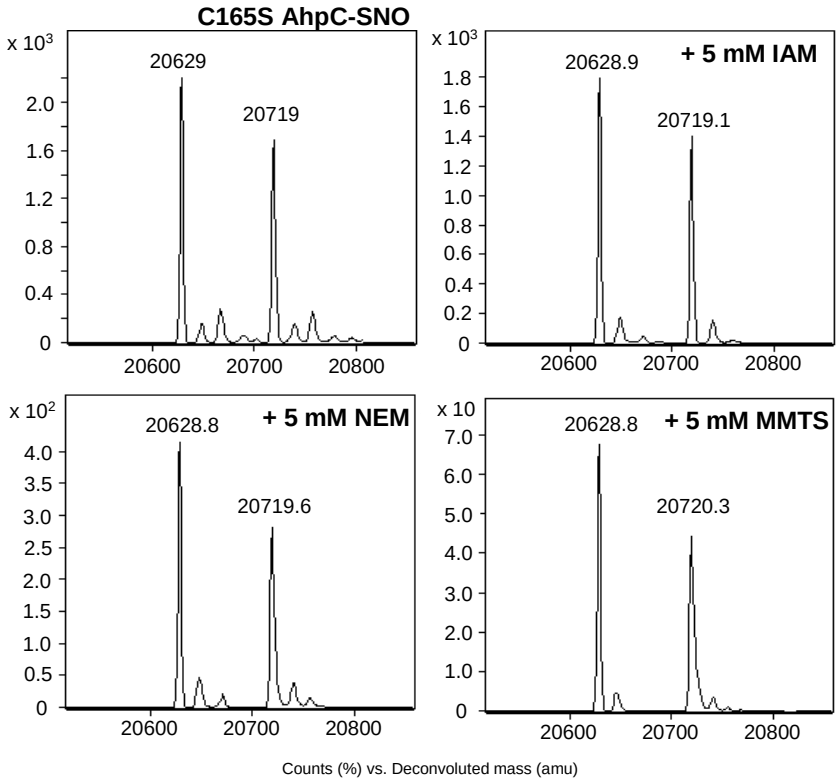


Figure S3: ESI-TOF mass spectra for the incubation of C165S AhpC-SNO (40 μM) with electrophiles IAM, NEM, or MMTS (5 mM) for 2 hours at r.t. and pH 7.5. The signals at 20,629 and 20,719 a.m.u correspond to nitrosothiol (AhpC-SNO) and mixed disulfide (AhpC-S-S-Cys), respectively.

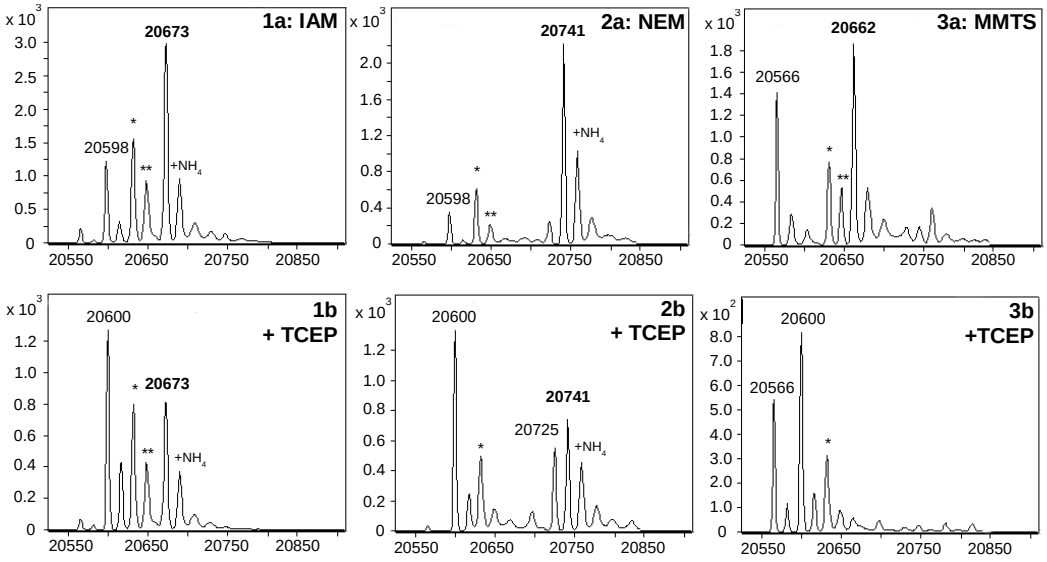


Figure S4: ESI-TOF mass spectra demonstrating the reduction of sulfenic acid-electrophile adducts (**a**) by TCEP (10 mM) over 1 h at pH 7.5 (**b**). Adducts shown in upper panels were generated via incubation of C165S AhpC-SOH (30 μM) with electrophile (5 mM): IAM (**1**), NEM (**2**), or MMTS (**3**) for 3 hours at r.t. Excess electrophile was removed prior to TCEP treatment. The signals at 20,566 and 20,598 a.m.u correspond to dehydroalanine and sulfenamide (-SN), respectively. Signals labeled with * and ** indicate 20,632 ($-SO_2H$) and 20,648 ($-SO_3H$) a.m.u, respectively. Ammonium ($+NH_4$) adducts result from the NH_4HCO_3 reaction buffer. Evidence for thiol regeneration is indicated at 20,600 a.m.u with concomitant loss of signal corresponding to the covalent adducts.

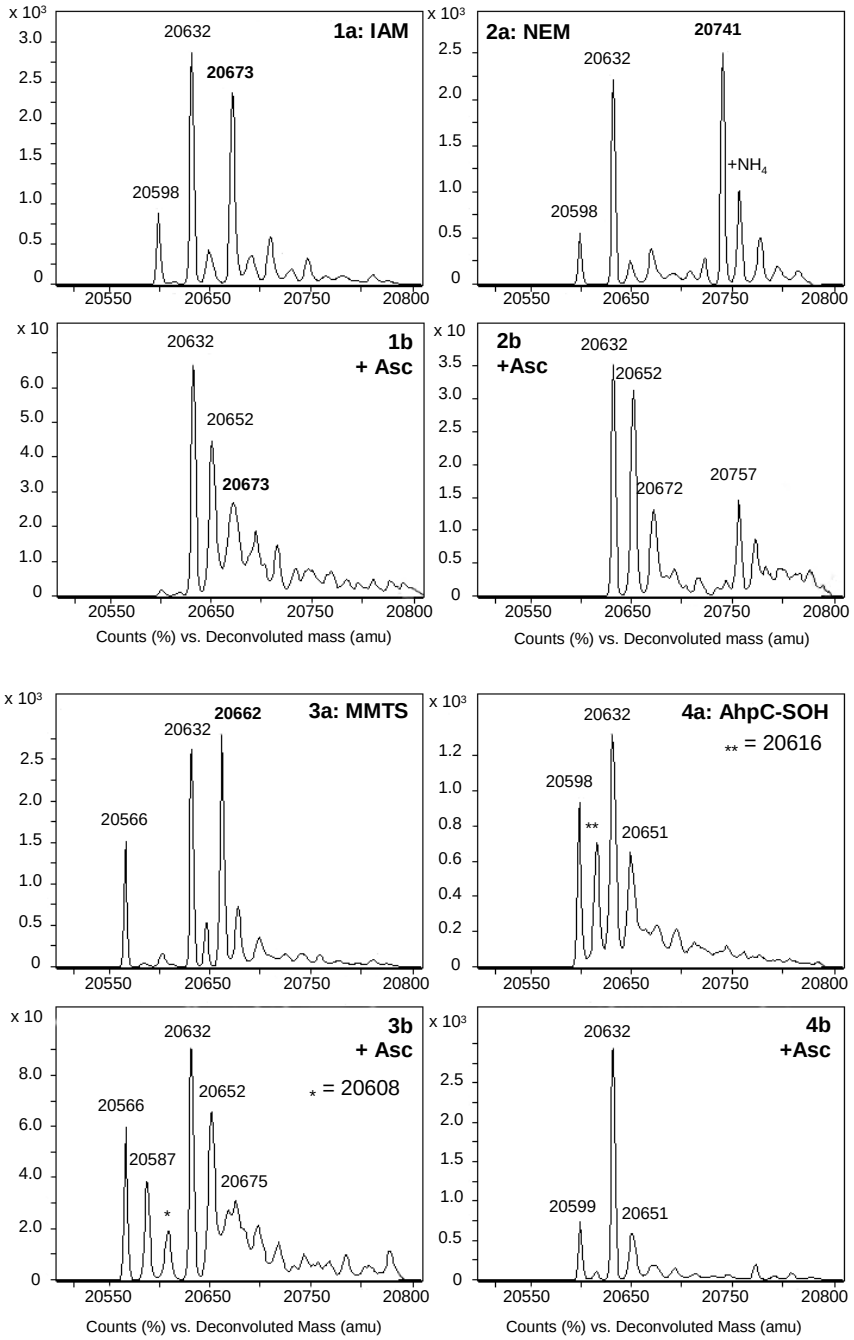


Figure S5: ESI-TOF mass spectra demonstrating the reduction of sulfenic acid-electrophile adducts (**a**) by sodium ascorbate (10 mM) over 1 h at pH 7.5 (**b**). Adducts shown in **a** panels were generated via incubation of C165S AhpC-SOH (30 μM, **4a**) with electrophile (5 mM) IAM (**1**), NEM (**2**), or MMTS (**3**) for 2 hours at r.t. Excess electrophile was removed prior to ascorbate treatment. The signals at 20,566, 20,598, 20,616, and 20,632 a.m.u correspond to dehydroalanine, sulfenamide (-SN), sulfenic acid (-SOH) and sulfinic acid ($-SO_2H$), respectively.

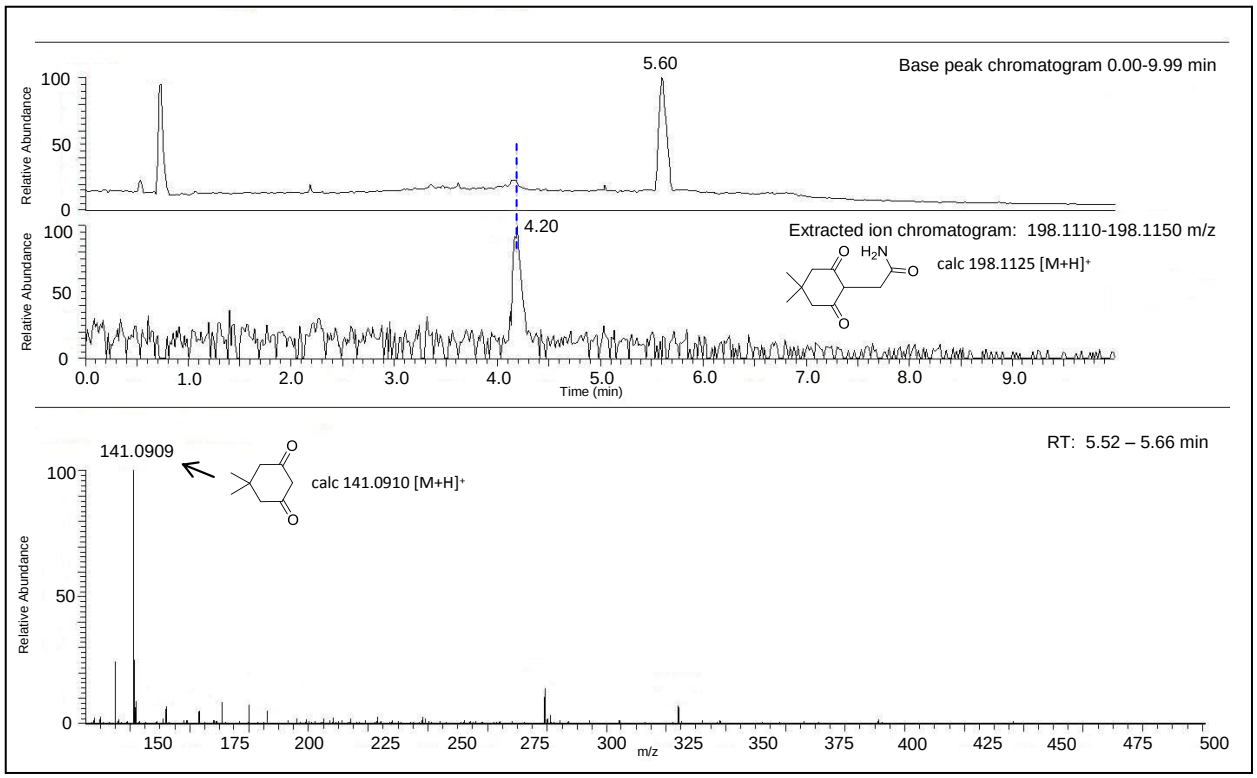
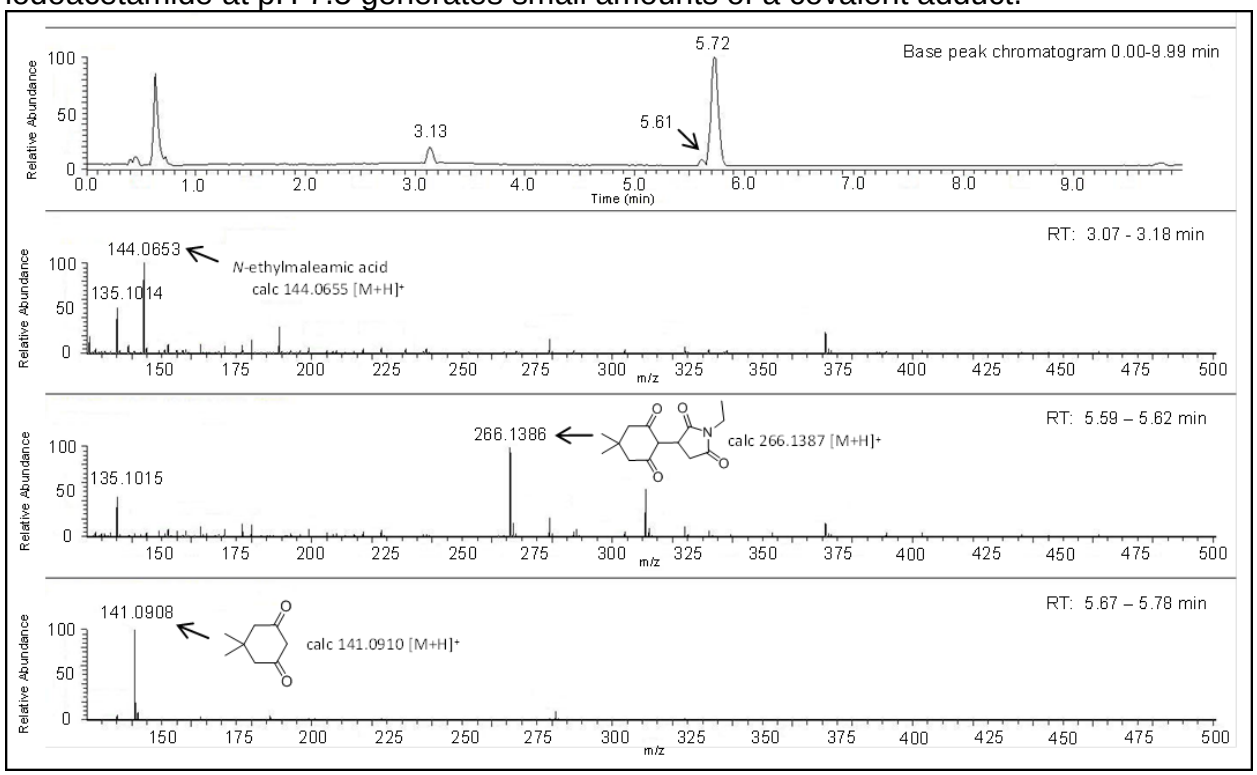
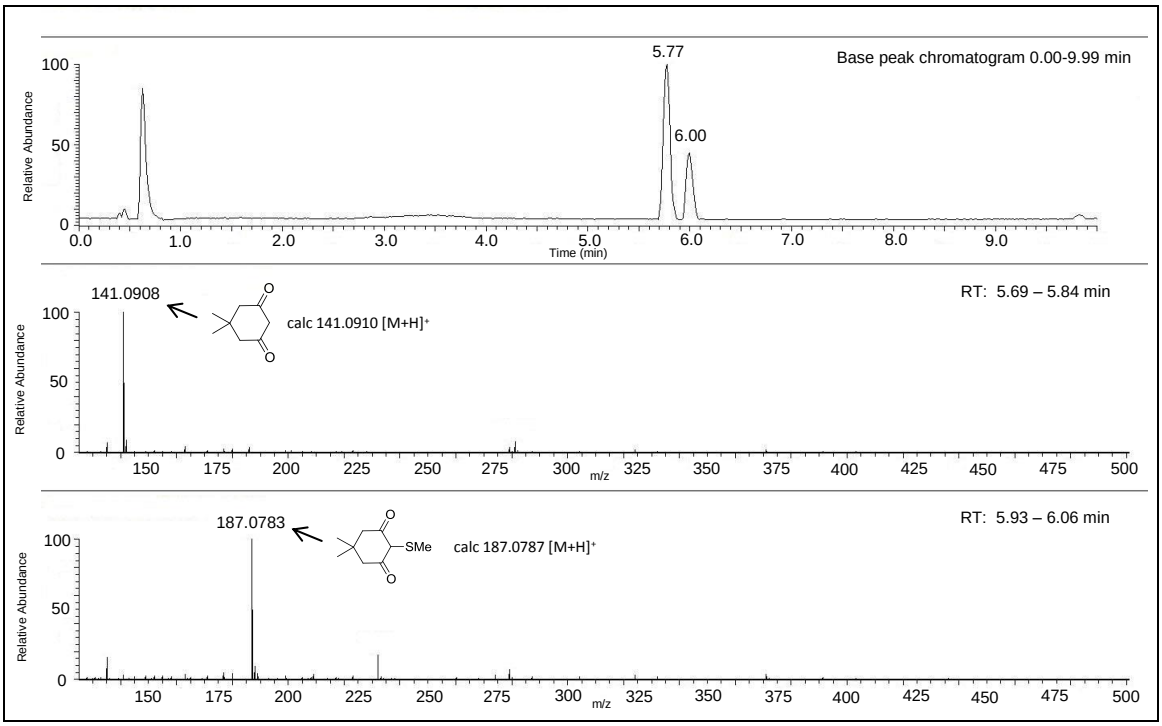


Figure S6: **a**: LC-MS spectrum indicating that the equimolar reaction of dimedone and iodoacetamide at pH 7.5 generates small amounts of a covalent adduct.



b: LC-MS spectrum resulting from the equimolar reaction of dimedone and NEM at pH 7.5.



c: LC-MS spectrum resulting from the equimolar reaction of dimedone and MMTS at pH 7.5.

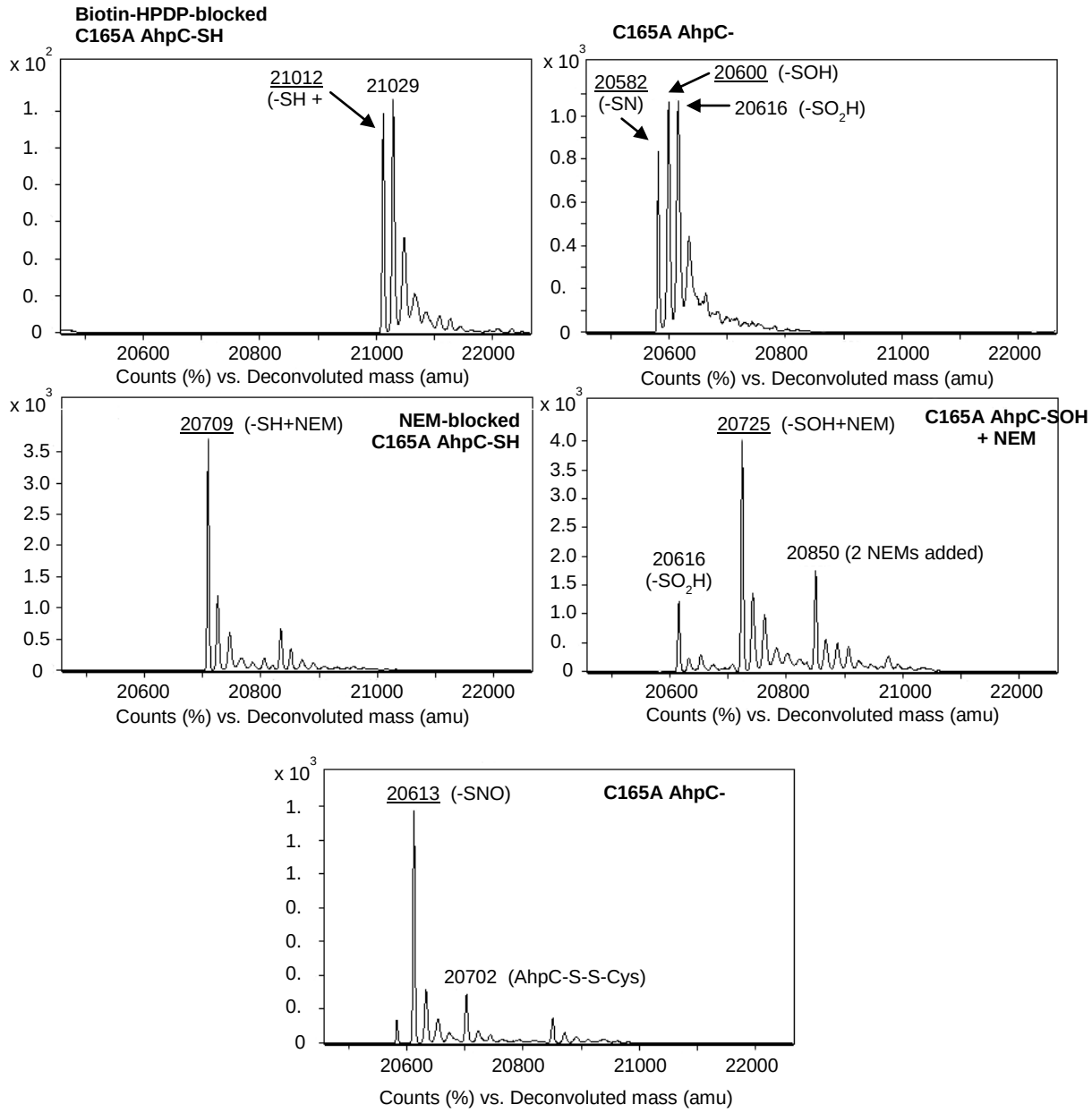


Figure S7: ESI-TOF mass spectra identifying cysteine oxoforms of C165A AhpC: biotin-HPDP-treated thiol, NEM-treated thiol, -SOH, -SOR (R = succinimide), and -SNO prior to biotin-switch assay. The observed mass of reduced C165A AhpC is 20,584 a.m.u., representing the $[M+H]^+$ ion.

EXPERIMENTAL DETAILS FOR MASS SPECTROMETRY ANALYSES:

LC-MS analysis for dimedone reactions with electrophiles

Dimedone (1 mM) and IAM, NEM, or MMTS (1 mM) were incubated in ammonium bicarbonate buffer (50 mM, pH 7.5) for 75 minutes at r.t. then an aliquot was diluted for LC with 0.1% formic acid in water. Samples were analyzed on an Accela Open UPLC coupled to a Thermo Orbitrap LTQ XL high-resolution mass spectrometer. Separations were achieved using a Thermo Hypersil GOLD C18 column (50 x 2.1 mm, 1.9 μm) at ambient temperature with a gradient of buffer A (0.1% formic acid in water) and buffer B (MeOH) at a flow rate of 250 μL/min. The column was conditioned using a linear gradient of 100% MeCN to 95% A and 5% B over 5 minutes, and held for 5 minutes. Analytes were separated using a gradient elution of 5% to 95% B over 10 minutes, followed by a decrease to 5% B over 0.1 minutes, and holding at 5% B for 4.9 minutes for a total run time of 15 minutes. One blank (95/5 H_2O/MeOH) was injected using this method between each analysis. Eluant was introduced to the mass spectrometer via positive ESI with the following settings: sheath gas of 64, a spray voltage of 4.0 kV, a capillary temperature of 325^{o}C, a capillary voltage of 6.0 V, and the tube lens held at 70.0 V. Mass spectra were acquired at a resolution of 60,000 over the range of 90-1000 m/z. The acquired raw data were analyzed using Xcalibur v 2.1 (Thermo).

nanoLC-MS/MS analysis

Digestions were performed at pH 7.5-8.0 using Trypsin Gold (Promega) overnight at 37^{o}C. The resulting peptides were analyzed on a Dionex UltiMate3000 nanoLC system coupled to a Thermo Orbitrap Velos Pro high-resolution mass spectrometer. Peptides were separated using a gradient of buffer A (0.1% formic acid/2% acetonitrile/98% water) and buffer B (0.1% formic acid/20% water/80% acetonitrile) over 60 minutes (2 to 85% B) at a flow rate of 300 nL/min with the column held at 35^{o}C. Eluant was introduced to the mass spectrometer via positive nanospray ESI with the following settings: capillary temperature 200^{o}C, spray voltage 1.8 kV, spray current 100 mA. The mass spectrometer was operated in data-dependent acquisition mode using Xcalibur v 2.1 (Thermo). After a full scan (150-2000 m/z range) at high resolution (60,000), the top 15 most intense precursor ions were isolated and fragmented using either collision-

induced dissociation (CID) or electron-transfer dissociation (ETD) depending on the mass and charge of the precursor ion (decision tree). Dynamic exclusion was enabled with a repeat duration of 30 seconds and an exclusion duration of 9.5 seconds. For CID, the normalized collision energy was set at 35%, activation Q at 0.25, and activation time at 10 ms. The ETD reagent ion (fluoranthene) reaction time was 66 ms and supplemental activation was enabled. The acquired raw data were processed using Proteome Discoverer v 1.3 (Thermo).