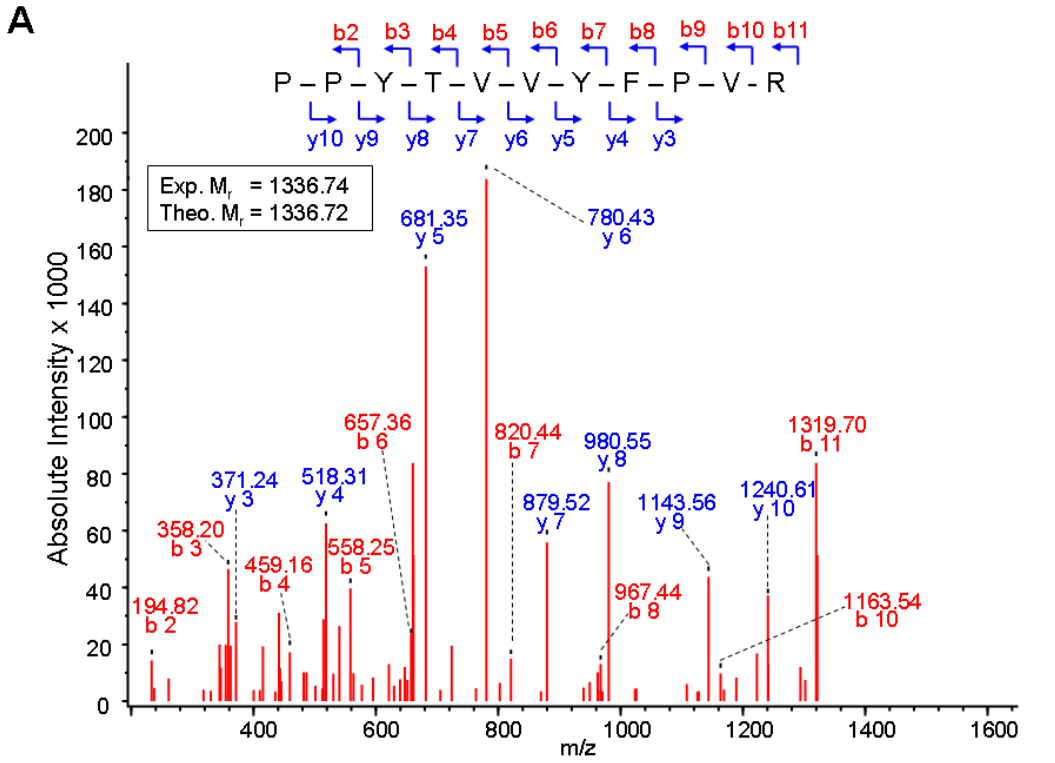
Supplement: Ion trap mass spectrometry of unmodified, recombinant GSTP1a-1a.

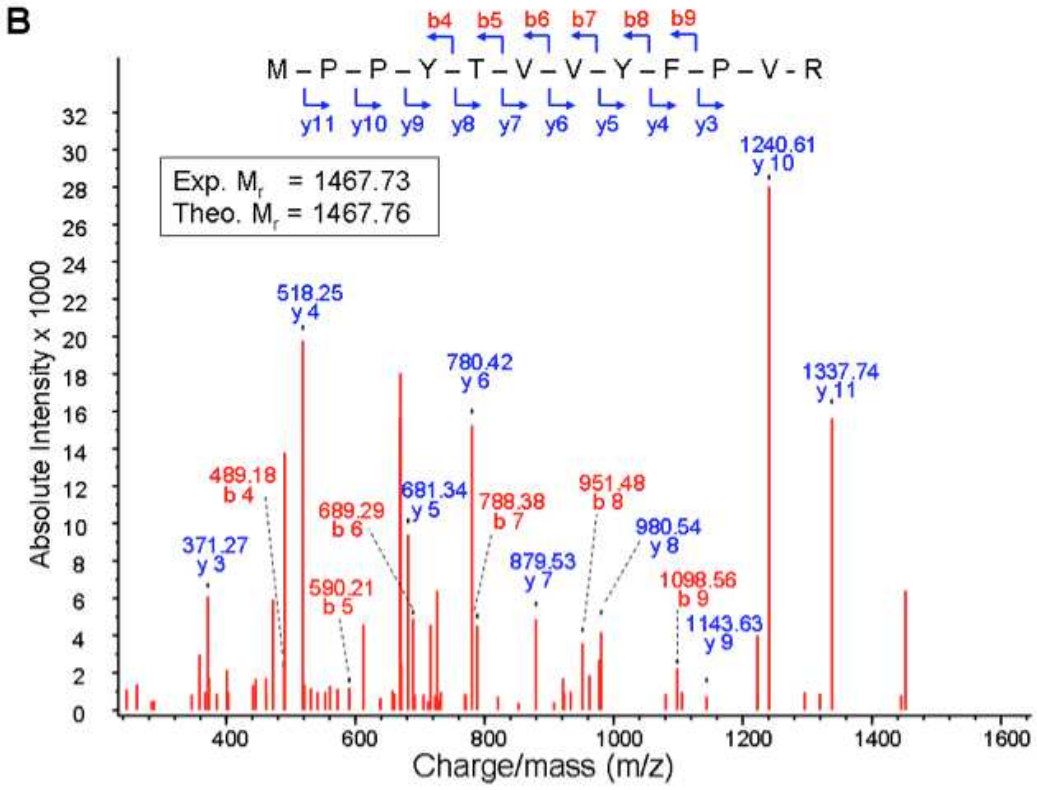
For ion-trap mass spectrometry of unmodified, recombinant GSTP1a-1a, 200 pmol GSTP1a-1a (untreated with nitroalkene) was prepared as described in Experimental Procedures and resuspended in 50 μL of tryptic digest buffer (40 mM ammonium bicarbonate, 10% acetonitrile, 1mM $CaCl_2$). Sequence grade trypsin was added and samples incubated at 37°C overnight. The sample (1 μl) was applied to a precolumn (0.3 mm × 1.0 mm, 100 Å, PepMap C-18) for desalting and concentration at a flow rate of 35 μL min^{-1} using mobile phase A (5% acetonitrile containing 0.1% formic acid). Peptides were eluted and separated on a nano-analytical column (75 μm × 15 cm, 100 Å, PepMap C-18) at a flow rate of 200 nL min^{-1} using a linear gradient from 5–40% mobile phase B (95% acetonitrile containing 0.08 % formic acid) over 40 min. The peptides were introduced into the mass spectrometer by nano-electrospray ionization and subjected to MS/MS analysis in a Bruker Esquire HCT ion trap mass spectrometer. MS survey data from *m/z* 375 to 1500 were acquired. The MS/MS data were searched against human sequences in the MSDB protein sequence database (Imperial College School of Medicine, London) using MASCOT (Matrix Science, London, UK). The MS/MS data were matched to the human GSTP1a (GenBank Accession Number CAA00533) sequence with high statistical significance (MOWSE score 808). The sequence coverage was 70%.

Supplemental Figure. Recombinant GSTP1a consists of a mixture of peptides with and without N-terminal methionine. MS/MS analysis of tryptic digestions of GSTP1a-1a showed that the recombinant protein contained a mixture of peptides that retained the N-terminal methionine and peptides from which the N-terminal methionine had been removed. The peptides were observed in the +2 charge state in both cases. Panel A) representative MS/MS spectrum of

the N-terminal peptide without the N-terminal methionine (experimental mass = 1336.74; calculated mass = 1336.72); Panel B) representative MS/MS spectrum of the N-terminal tryptic peptide with the methionine retained (experimental mass = 1467.73; calculated mass = 1467.76). The amino acid sequence of each peptide is shown above each spectrum using the one-letter abbreviations. The MS/MS fragment ions are indicated using standard nomenclature for the peptide ions from the C-terminus (y-series ions) and for the peptide ions from the N-terminus (b-series ions) (1). Coverage was almost complete in both series for both peptide forms.

1. Johnson, R. S., Martin, S. A., Biemann, K., Stults, J. T., and Watson, J. T. (1987) *Anal Chem* **59**, 2621-2625


A
b2 b3 b4 b5 b6 b7 b8 b9 b10 b11
P – P – Y – T – V – V – Y – F – P – V - R
y10 y9 y8 y7 y6 y5 y4 y3
Exp. Mr = 1336.74
Theo. Mr = 1336.72
681.35 y 5
780.43 y 6
657.36 b 6
820.44 b 7
980.55 y 8
1319.70 b 11
371.24 y 3
518.31 y 4
879.52 y 7
1143.56 y 9
1240.61 y 10
358.20 b 3
459.16 b 4
558.25 b 5
967.44 b 8
194.82 b 2
1163.54 b 10
Absolute Intensity x 1000
m/z


B
b4 b5 b6 b7 b8 b9
M – P – P – Y – T – V – V – Y – F – P – V - R
y11 y10 y9 y8 y7 y6 y5 y4 y3
Exp. Mr = 1467.73
Theo. Mr = 1467.76
1240.61 y 10
518.25 y 4
780.42 y 6
1337.74 y 11
489.18 b 4
681.34 y 5
788.38 b 7
951.48 b 8
689.29 b 6
980.54 y 8
371.27 y 3
879.53 y 7
1098.56 b 9
590.21 b 5
1143.63 y 9
Absolute Intensity x 1000
Charge/mass (m/z)