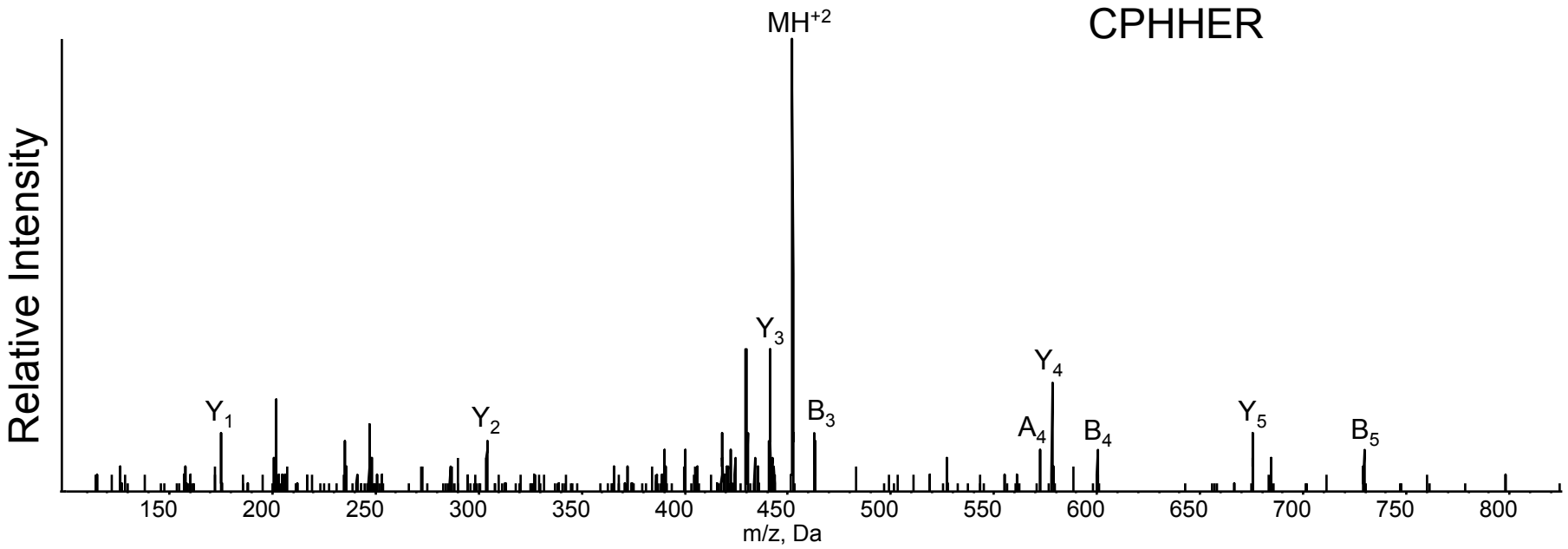
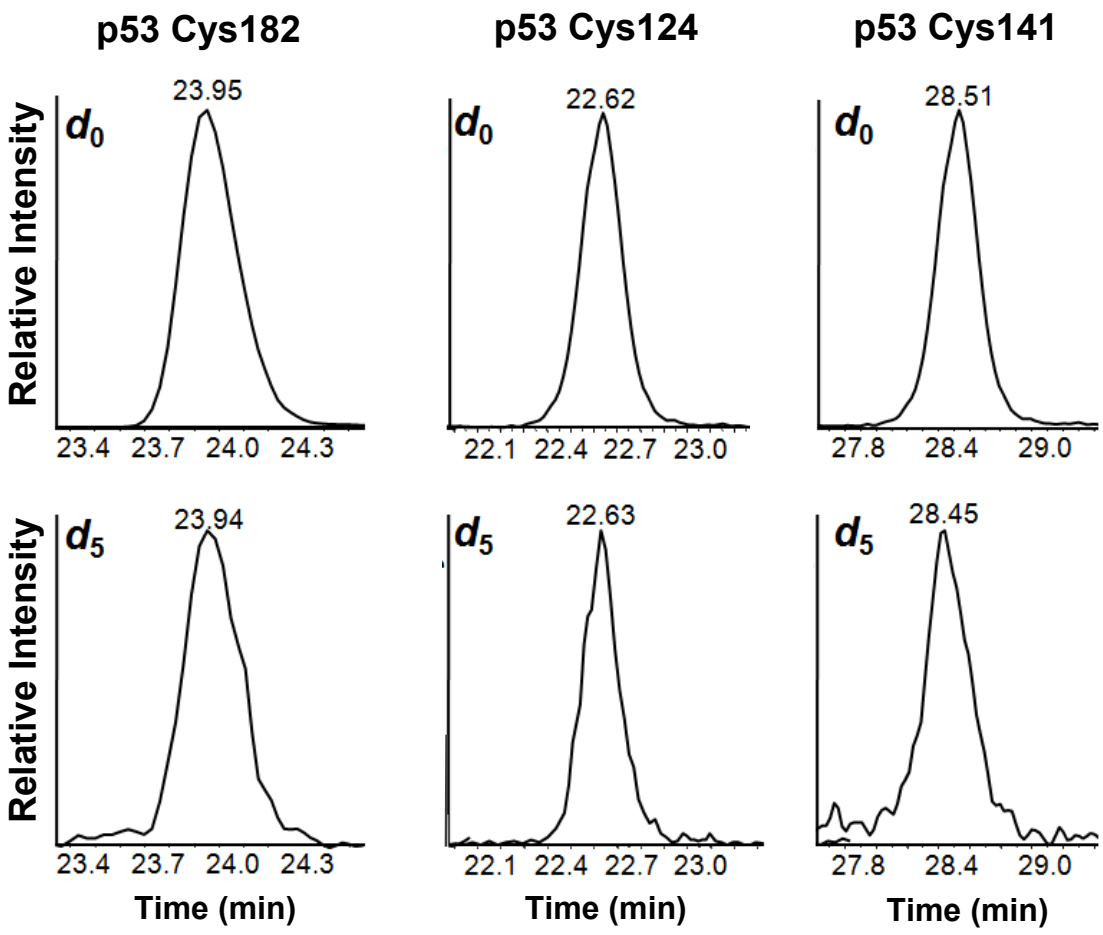
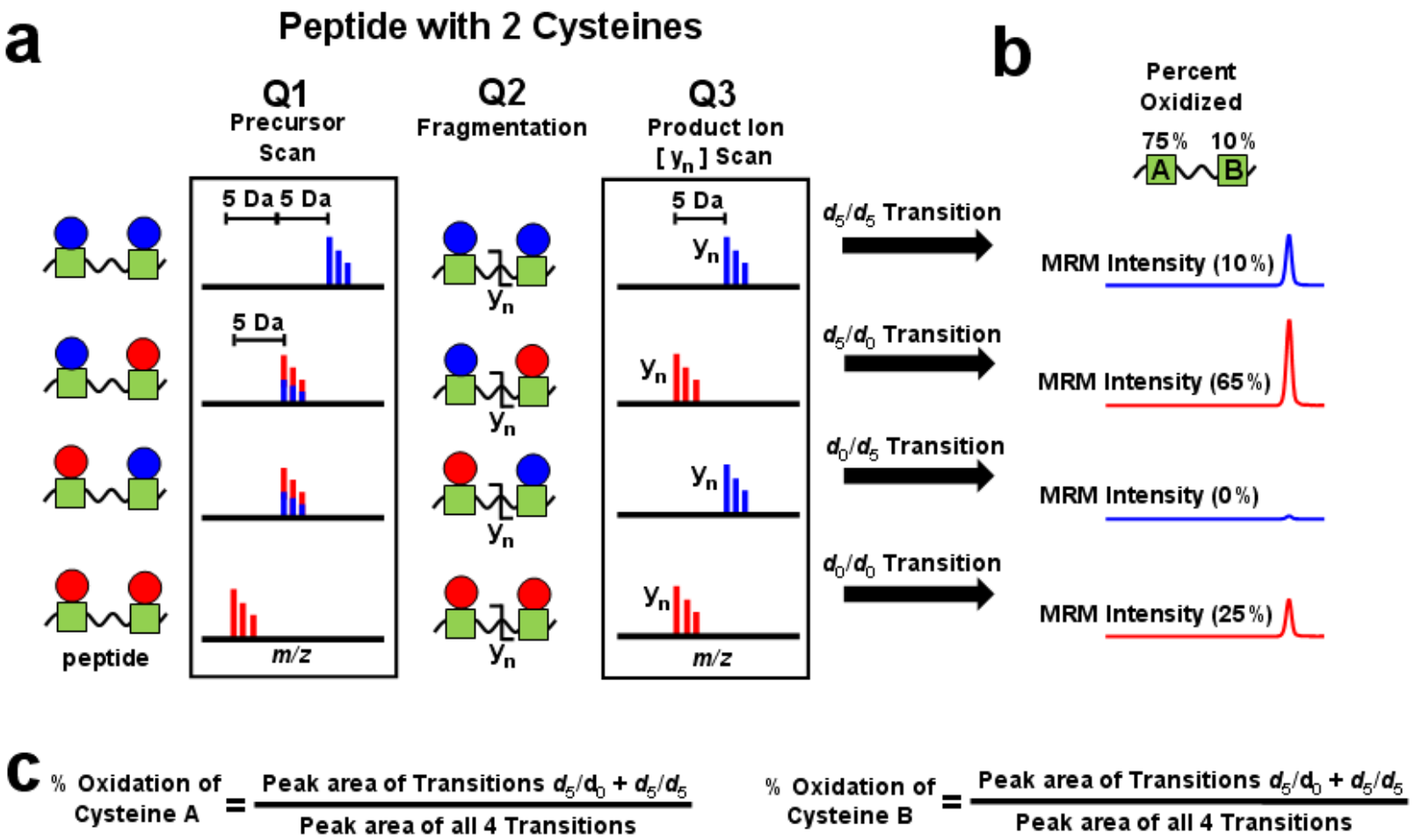


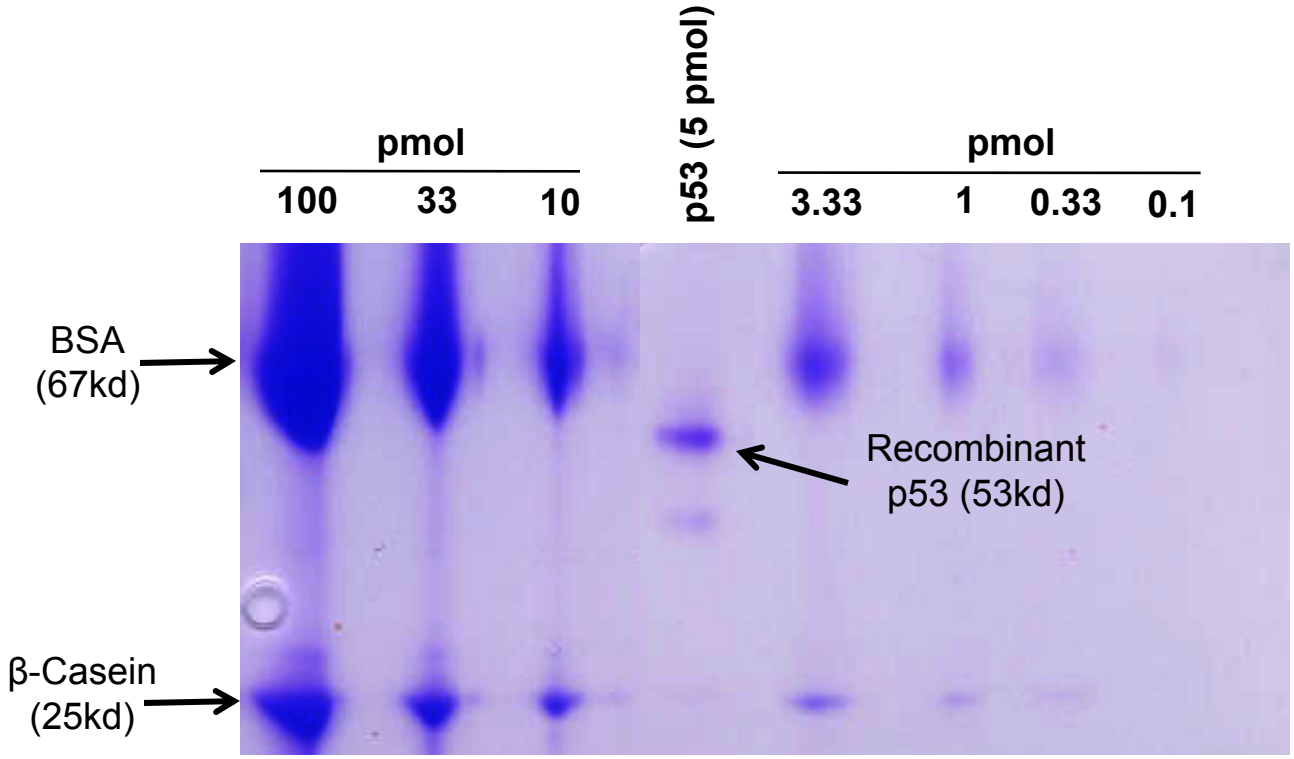

**Supplementary Figure 1. Manual Assignment of p53 peptide CPHHER.** Only observed when direct injection (i.e. no trap column) is used, this peptide observed after tryptic digestion of recombinant p53 has an observed *m/z* 452.30 which is Δm of 0.11. All assigned fragment ions are within 0.2 Da and there is both a y and b ion series that includes three consecutive ions.



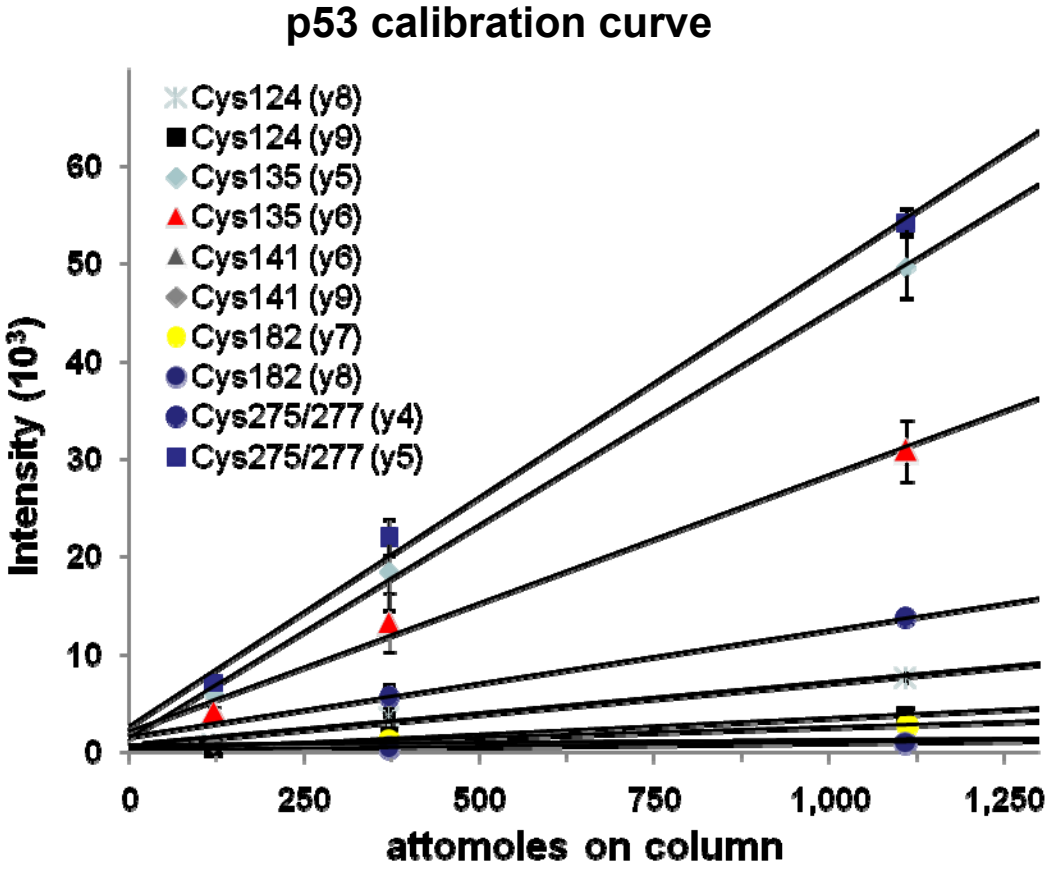

**Supplementary Figure 2. Relative retention of $d_0$ and $d_5$ NEM alkylated p53 peptides.** Three representative chromatograms for $d_0$ and $d_5$ NEM alkylated p53 peptides. The time corresponding to maximum height is labeled.



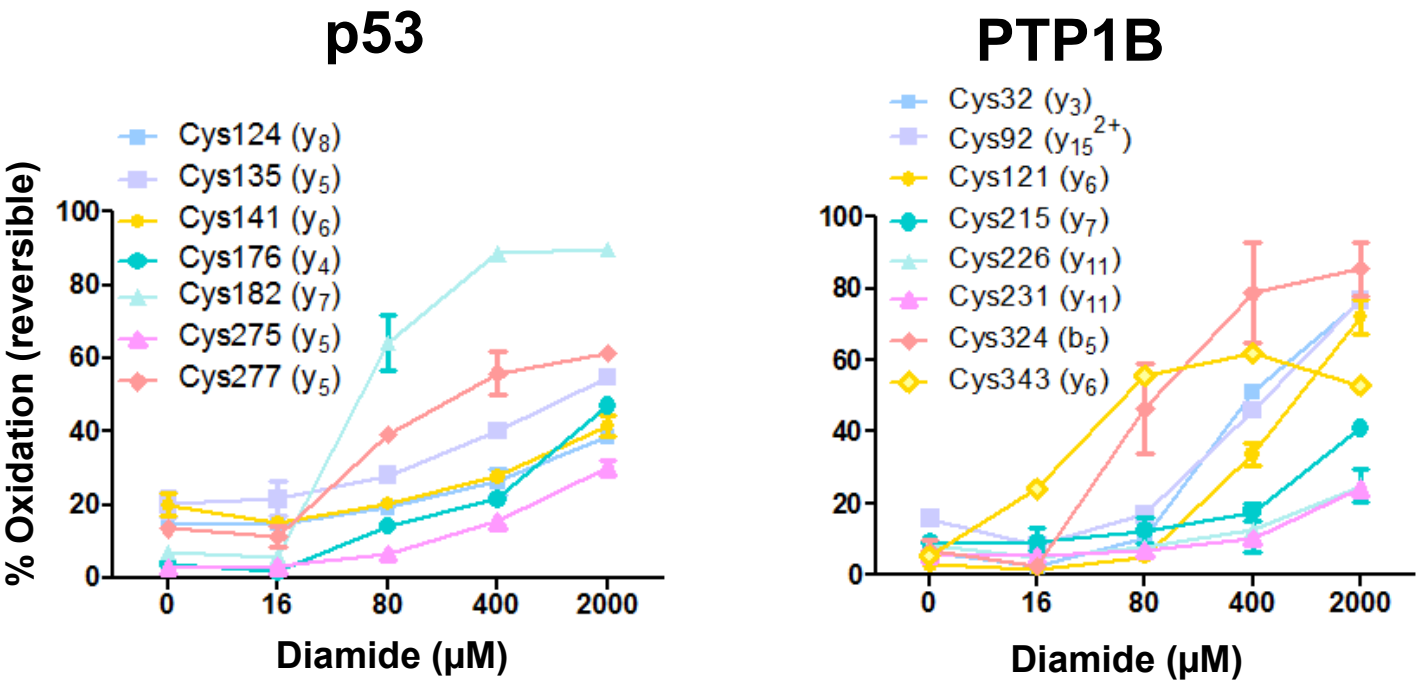

**Supplementary Figure 3. Differentiating oxidation of individual cysteines in a peptide containing 2 cysteines.** Proteins with zinc finger or Fe-S domains often have two proximately located cysteines that are commonly found on a single peptide after proteolysis. Tryptic digestion of both p53 and PTP1B generates a peptide containing two cysteines. (**a**) When the cysteines in the peptide have differential susceptibility to oxidation and only a single cysteine is oxidized, the $d_0/d_5$ and $d_5/d_0$ NEM alkylated peptides ($d_0$, red circle; $d_5$, blue circle) have the same mass and which cysteine is oxidized cannot be determined at the intact peptide level. However, measuring a product ion containing a single cysteine in Q3 allows determination of which cysteine is oxidized. (**b**) A hypothetical two cysteine-containing peptide with differentially oxidized cysteines and the expected MRM intensities for the four transitions required to measure all four possible permutations of $d_0$ and $d_5$ alkylation. (**c**) Calculations used to determine the percent oxidation of each cysteine in the peptide.



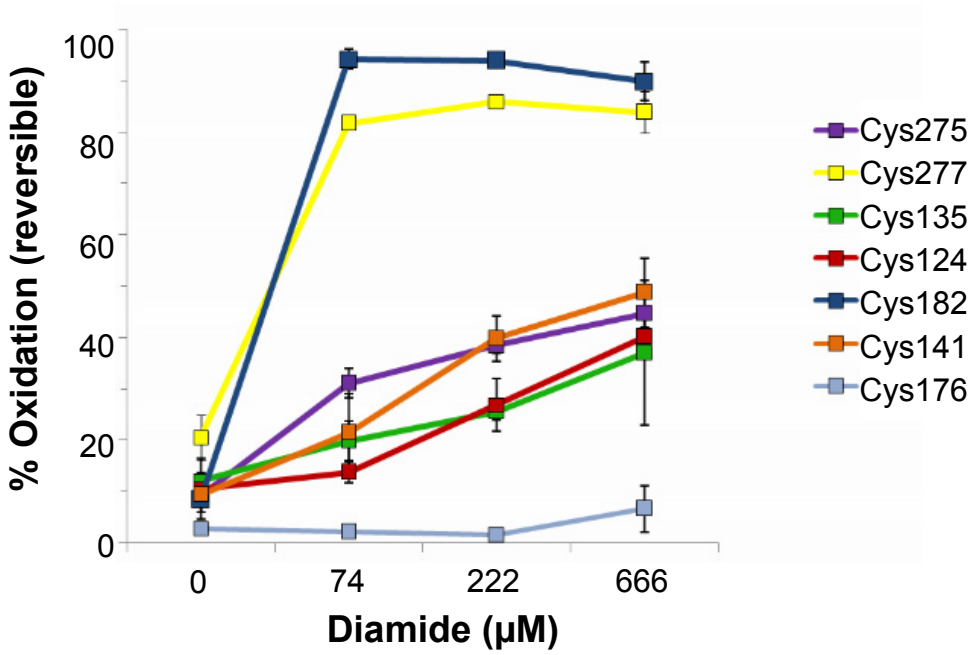

**Supplementary Figure 4. Verification of recombinant p53 protein amount.** Coomassie stained 1D-SDS-PAGE gel in which known amounts of standard proteins β-casein and bovine serum albumin (BSA) are serially diluted. 5 pmol of recombinant p53 was loaded between 10 pmol and 3.3 pmol of the standards. The intensity of staining is consistent with an amount of 5 pmol p53 loaded, confirming that the values in the Figure 2 concentration curve are accurate.



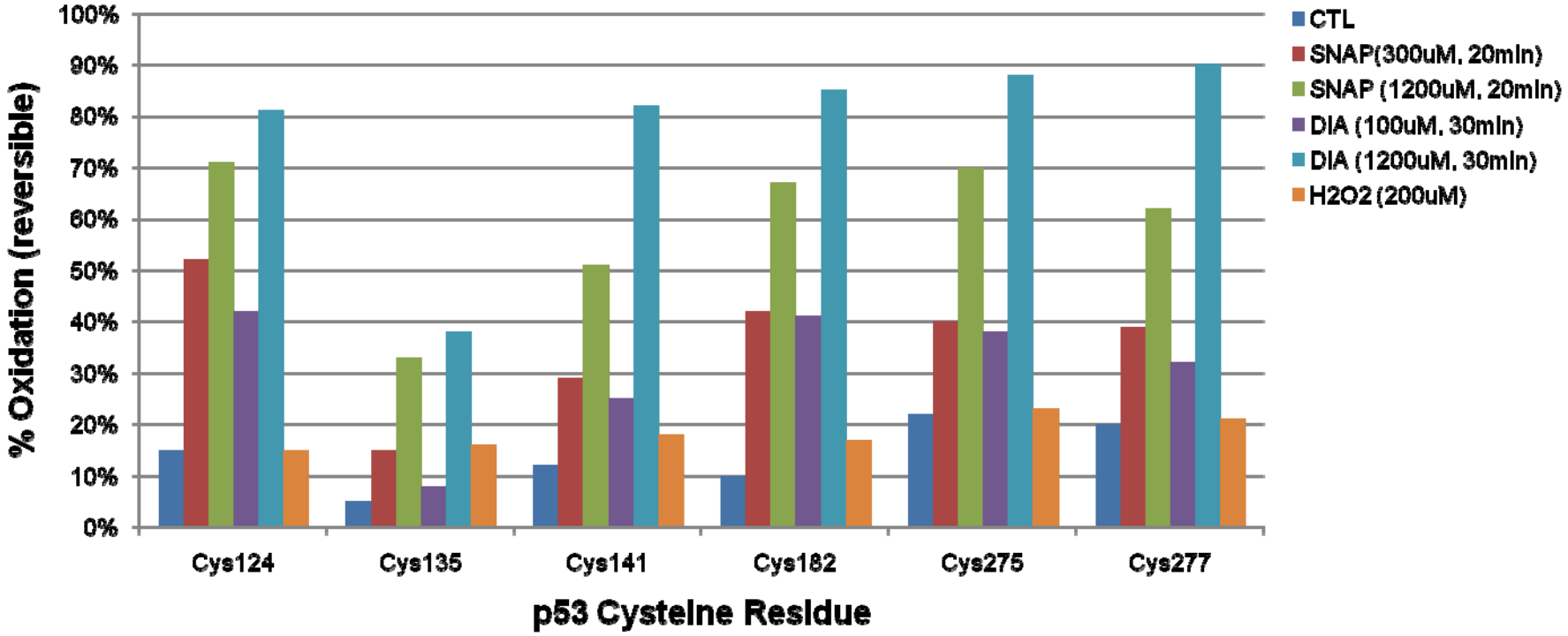

**Supplementary Figure 5. Sensitivity and reproducibility of OxMRM analysis of p53.** Calibration curve up to 1.11 fmol recombinant p53 for all MRM transitions for 6 cysteines. Error bars represent the standard of deviation from 3 technical replicates. The full transition list in is detailed in Supplementary Table 2.



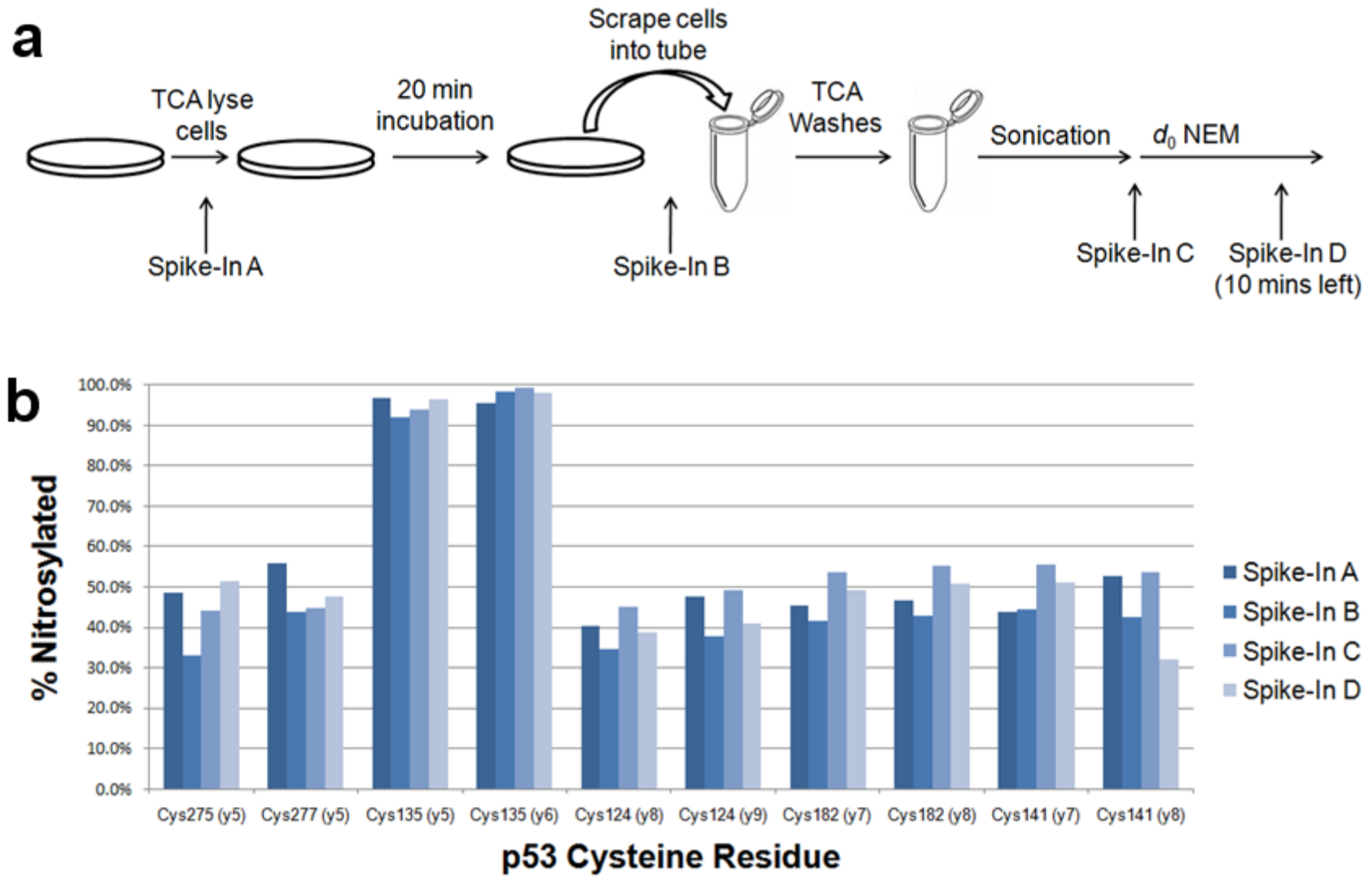

**Supplementary Figure 6. Oxidation of endogenous p53 and PTP1B in MCF7 cells.** Complete diamide dose response curve for p53 and PTP1B isolated from MCF7 cells. Error bars represent standard deviation of three biological replicates. The full transition list in is detailed in Supplementary Table 2.




**Supplementary Figure 7. Oxidation of endogenous p53 in HCA2 primary fibroblasts.** Diamide dose response curve for p53 isolated from HCA2 cells. Error bars represent standard deviation of three biological replicates. The full transition list in is detailed in Supplementary Table 2.




**Supplementary Figure 8. Oxidation of isolated p53 using multiple oxidants.** 5 pmol of isolated p53 was treated with either diamide, SNAP, or $H_2O_2$ for the times and doses indicated demonstrating little cysteine specificity for these oxidants.




**Supplementary Figure 9. Stability of SNO modified recombinant p53 when spiked into different points of sample preparation.** Four 15 cm plates of MCF7 cells were lysed in parallel under control condition. A vast excess of isolated p53 was freshly nitrosylated for 30 min using 900 μM SNAP and then spiked in at various points (1 spike-in per plate) through the sample preparation from the initial TCA lysis through to the final stages of the $d_0$ NEM alkylation. Since there is no difference in percent oxidation for any other SNO-oxidized cysteines of p53 between any of the time points it confirms that no loss of reversibly oxidization, including nitrosylation, occurred during sample preparation.

## Supplementary Table 1

| Protein | Peptide | Cys# | Alkylation | MH+ (mono) | Charge (Q1) | MRM Transitions Q1 | MRM Transitions Q3 | DP | CE | Fragment Ion Type |
|---|---|---|---|---|---|---|---|---|---|---|
| p53 | SVTCTYSPALNK | 124 | $d_0$ NEM | 1408.7 | 2 | 704.8 | 893.5 | 100.0 | 33.0 | y8 |
| | | | | | | 704.8 | 1121.5 | 100.0 | 33.0 | y9 |
| | | | $d_5$ NEM | 1413.7 | 2 | 707.4 | 893.5 | 100.0 | 33.0 | y8 |
| | | | | | | 707.4 | 1126.6 | 100.0 | 33.0 | y9 |
| | | | Sulfinic Acid | 1315.6 | 2 | 658.3 | 893.5 | 100.0 | 33.0 | y8 |
| | | | | | | 658.3 | 792.4 | 100.0 | 33.0 | y7 |
| | | | | | | 658.3 | 542.3 | 100.0 | 33.0 | y5 |
| | | | Sulfonic Acid | 1331.6 | 2 | 666.3 | 893.5 | 100.0 | 33.0 | y8 |
| | | | | | | 666.3 | 792.4 | 100.0 | 33.0 | y7 |
| | | | | | | 666.3 | 542.3 | 100.0 | 33.0 | y5 |
| p53 | MFCQLAK | 135 | $d_0$ NEM | 965.5 | 2 | 483.2 | 834.4 | 80.0 | 22.0 | y6 |
| | | | | | | 483.2 | 687.3 | 80.0 | 22.0 | y5 |
| | | | $d_5$ NEM | 970.5 | 2 | 485.7 | 839.4 | 80.0 | 22.0 | y6 |
| | | | | | | 485.7 | 692.4 | 80.0 | 22.0 | y5 |
| | | | Sulfinic Acid | 872.4 | 2 | 436.7 | 602.3 | 80.0 | 22.0 | y5 |
| | | | | | | 436.7 | 459.3 | 80.0 | 22.0 | y4 |
| | | | Sulfonic Acid | 888.4 | 2 | 444.7 | 610.3 | 80.0 | 22.0 | y5 |
| | | | | | | 444.7 | 459.3 | 80.0 | 22.0 | y4 |
| p53 | TCPVQLWVDSTPPPGTR | 141 | $d_0$ NEM | 1979.0 | 2 | 990.0 | 624.3 | 100.0 | 50.0 | y6 |
| | | | | | | 990.0 | 927.4 | 100.0 | 50.0 | y9 |
| | | | $d_5$ NEM | 1984.0 | 2 | 992.5 | 624.3 | 100.0 | 50.0 | y6 |
| | | | | | | 992.5 | 927.5 | 100.0 | 50.0 | y9 |
| | | | Sulfinic Acid | 1885.9 | 2 | 943.5 | 1649.9 | 100.0 | 50.0 | y15 |
| | | | | | | 943.5 | 1505.8 | 100.0 | 50.0 | y14 |
| | | | | | 2 | 943.5 | 1026.5 | 100.0 | 50.0 | y10 |
| | | | | | | 943.5 | 624.3 | 100.0 | 50.0 | y6 |
| | | | Sulfonic Acid | 1901.9 | 2 | 951.5 | 1649.9 | 100.0 | 50.0 | y15 |
| | | | | | | 951.5 | 1505.8 | 100.0 | 50.0 | y14 |
| | | | | | 2 | 951.5 | 1026.5 | 100.0 | 50.0 | y10 |
| | | | | | | 951.5 | 624.3 | 100.0 | 50.0 | y6 |
| p53 | CPHHER | 176 | $d_0$ NEM | 903.4 | 2 | 452.2 | 578.3 | 70.0 | 32.0 | y5 |
| | | | | | | 452.2 | 441.2 | 70.0 | 32.0 | y4 |
| | | | $d_5$ NEM | 908.4 | 2 | 454.7 | 578.3 | 70.0 | 32.0 | y5 |
| | | | | | | 454.7 | 441.2 | 70.0 | 32.0 | y4 |
| | | | Sulfinic Acid | 810.3 | 2 | 405.9 | 675.3 | 70.0 | 32.0 | y5 |
| | | | | | | 405.9 | 578.3 | 70.0 | 32.0 | y4 |
| | | | Sulfonic Acid | 826.3 | 2 | 413.9 | 675.3 | 70.0 | 32.0 | y5 |
| | | | | | | 413.9 | 578.3 | 70.0 | 32.0 | y4 |
| p53 | CSDSDGLAPPQHLIR | 182 | $d_0$ NEM | 1733.8 | 2 | 867.4 | 860.5 | 75.0 | 47.0 | y7 |
| | | | | | | 867.4 | 931.5 | 80.0 | 47.0 | y8 |
| | | | $d_5$ NEM | 1738.9 | 2 | 869.9 | 860.5 | 75.0 | 47.0 | y7 |
| | | | | | | 869.9 | 931.5 | 80.0 | 47.0 | y8 |
| | | | Sulfinic Acid | 1640.8 | 2 | 820.9 | 1505.8 | 75.0 | 47.0 | y14 |
| | | | | | | 820.9 | 860.5 | 80.0 | 47.0 | y7 |
| | | | Sulfonic Acid | 1656.8 | 2 | 828.9 | 1505.8 | 75.0 | 47.0 | y14 |
| | | | | | | 828.9 | 860.5 | 80.0 | 47.0 | y7 |
| p53 | VCACPGR | 275 : 277 | $d_0$ NEM : $d_0$ NEM | 955.4 | 2 | 478.2 | 557.3 | 80.0 | 24.0 | y4 |
| | | | | | | 478.2 | 628.3 | 80.0 | 24.0 | y5 |
| | | | $d_5$ NEM : $d_0$ NEM | 960.5 | 2 | 480.7 | 557.3 | 80.0 | 24.0 | y4 |
| | | | | | | 480.7 | 628.3 | 80.0 | 24.0 | y5 |
| | | | $d_0$ NEM : $d_5$ NEM | 960.5 | 2 | 480.7 | 562.3 | 80.0 | 24.0 | y4 |
| | | | | | | 480.7 | 633.3 | 80.0 | 24.0 | y5 |
| | | | $d_5$ NEM : $d_5$ NEM | 965.5 | 2 | 483.2 | 562.3 | 80.0 | 24.0 | y4 |
| | | | | | | 483.2 | 633.3 | 80.0 | 24.0 | y5 |
| | | | 2 $d_0$ NEM | 955.4 | 2 | 478.2 | 856.3 | 80.0 | 24.0 | y6 |
| | | | 1 $d_0$, 1 $d_5$ NEM | 960.5 | 2 | 480.7 | 861.3 | 80.0 | 24.0 | y6 |
| | | | 2 $d_5$ NEM | 965.5 | 2 | 483.2 | 866.3 | 80.0 | 24.0 | y6 |
| | | | Sulfinic Acid : $d_0$ NEM | 862.3 | 2 | 431.7 | 628.3 | 80.0 | 24.0 | y5 |
| | | | Sulfinic Acid : $d_5$ NEM | 867.3 | 2 | 434.2 | 633.3 | 80.0 | 24.0 | y5 |
| | | | Sulfonic Acid : $d_0$ NEM | 878.3 | 2 | 439.7 | 628.3 | 80.0 | 24.0 | y5 |
| | | | Sulfonic Acid : $d_5$ NEM | 883.3 | 2 | 442.2 | 633.3 | 80.0 | 24.0 | y5 |
| | | | $d_0$ NEM : Sulfinic Acid | 862.3 | 2 | 431.7 | 535.2 | 80.0 | 24.0 | y5 |
| | | | $d_5$ NEM : Sulfinic Acid | 867.3 | 2 | 434.2 | 535.2 | 80.0 | 24.0 | y5 |
| | | | $d_0$ NEM : Sulfonic Acid | 878.3 | 2 | 439.7 | 551.2 | 80.0 | 24.0 | y5 |
| | | | $d_5$ NEM : Sulfonic Acid | 883.3 | 2 | 442.2 | 551.2 | 80.0 | 24.0 | y5 |
| | | | Sulfinic Acid : Sulfinic Acid | 769.3 | 2 | 385.2 | 535.2 | 80.0 | 24.0 | y5 |
| | | | Sulfinic Acid : Sulfonic Acid | 785.3 | 2 | 393.2 | 551.2 | 80.0 | 24.0 | y5 |
| | | | Sulfonic Acid : Sulfinic Acid | 785.3 | 2 | 393.2 | 535.3 | 80.0 | 24.0 | y5 |
| | | | Sulfonic Acid : Sulfonic Acid | 801.3 | 2 | 401.2 | 551.2 | 80.0 | 24.0 | y5 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| PTP1B | HEASDFPCR | 32 | $d_0$ NEM | 1186.5 | 3 | 396.2 | 540.2 | 60.0 | 16.7 | b5 |
| | | | | | | 396.2 | 500.2 | 60.0 | 16.7 | y3 |
| | | | $d_5$ NEM | 1191.5 | 3 | 397.8 | 540.2 | 60.0 | 16.7 | b5 |
| | | | | | | 397.8 | 505.2 | 60.0 | 16.7 | y3 |
| | | | Sulfinic Acid | 1093.4 | 3 | 365.2 | 540.2 | 60.0 | 16.7 | b5 |
| | | | | | | 365.2 | 407.2 | 60.0 | 16.7 | y3 |
| | | | Sulfonic Acid | 1109.4 | 3 | 370.5 | 540.2 | 60.0 | 16.7 | b5 |
| | | | | | | 370.5 | 423.2 | 60.0 | 16.7 | y3 |
| PTP1B | CAQYWPQK | 121 | $d_0$ NEM | 1148.5 | 2 | 574.8 | 777.3 | 73.0 | 34.0 | b5 |
| | | | | | | 574.8 | 721.4 | 73.0 | 34.0 | y5 |
| | | | $d_5$ NEM | 1153.5 | 2 | 577.3 | 782.3 | 73.0 | 34.0 | b5 |
| | | | | | | 577.3 | 721.4 | 73.0 | 34.0 | y5 |
| | | | Sulfinic Acid | 1055.5 | 2 | 528.2 | 684.2 | 73.0 | 34.0 | b5 |
| | | | | | | 528.2 | 721.4 | 73.0 | 34.0 | y5 |
| | | | Sulfonic Acid | 1071.5 | 2 | 536.2 | 700.2 | 73.0 | 34.0 | b5 |
| | | | | | | 536.2 | 721.4 | 73.0 | 34.0 | y5 |
| PTP1B | CAQYWPQKEEK | 121 | $d_0$ NEM | 1534.7 | 2 | 767.9 | 944.5 | 80.0 | 42.0 | y7 |
| | | | | | | 767.9 | 758.4 | 60.0 | 42.0 | y6 |
| | | | $d_5$ NEM | 1539.7 | 2 | 770.4 | 944.5 | 80.0 | 42.0 | y7 |
| | | | | | | 770.4 | 758.4 | 60.0 | 42.0 | y6 |
| | | | Sulfinic Acid | 1441.6 | 2 | 721.3 | 944.5 | 80.0 | 42.0 | y7 |
| | | | | | | 721.3 | 758.4 | 60.0 | 42.0 | y6 |
| | | | Sulfonic Acid | 1457.6 | 2 | 729.3 | 944.5 | 80.0 | 42.0 | y7 |
| | | | | | | 729.3 | 758.4 | 60.0 | 42.0 | y6 |
| PTP1B | ESGSLSPEHGPVVVHCSAGIGR | 215 | $d_0$ NEM | 2300.1 | 3 | 767.4 | 1024.5 | 90.0 | 47.0 | y9 |
| | | | | | | 767.4 | 925.4 | 100.0 | 44.0 | y8 |
| | | | $d_5$ NEM | 2305.1 | 3 | 767.4 | 788.4 | 90.0 | 47.0 | y9 |
| | | | | | | 767.4 | 560.3 | 100.0 | 44.0 | y8 |
| | | | Sulfinic Acid | 2207.1 | 3 | 736.4 | 931.4 | 90.0 | 47.0 | y9 |
| | | | | | | 736.4 | 560.3 | 100.0 | 42.0 | y6 |
| | | | Sulfonic Acid | 2223.1 | 3 | 741.7 | 947.4 | 90.0 | 47.0 | y9 |
| | | | | | | 741.7 | 560.3 | 100.0 | 42.0 | y6 |
| PTP1B | CREFFPNHQWVK | 324 | $d_0$ NEM | 1715.8 | 3 | 572.6 | 808.3 | 90.0 | 30.0 | b5 |
| | | | | | | 572.6 | 1019.4 | 80.0 | 32.0 | b7 |
| | | | $d_5$ NEM | 1720.8 | 3 | 574.3 | 813.3 | 90.0 | 30.0 | b5 |
| | | | | | | 574.3 | 1024.4 | 80.0 | 32.0 | b7 |
| | | | Sulfinic Acid | 1622.8 | 3 | 541.6 | 715.3 | 90.0 | 30.0 | b5 |
| | | | | | | 541.6 | 926.4 | 80.0 | 32.0 | b7 |
| | | | Sulfonic Acid | 1638.8 | 3 | 546.9 | 731.3 | 90.0 | 30.0 | b5 |
| | | | | | | 546.9 | 942.4 | 80.0 | 32.0 | b7 |
| PTP1B | DCPIKEEK | 343 | $d_0$ NEM | 1086.5 | 3 | 362.8 | 372.2 | 57.0 | 16.7 | y6 (2+) |
| | | | | | | 362.8 | 533.3 | 57.0 | 21.7 | y4 |
| | | | $d_5$ NEM | 1091.5 | 3 | 364.5 | 372.2 | 57.0 | 16.7 | y6 (2+) |
| | | | | | | 364.5 | 533.3 | 57.0 | 21.7 | y4 |
| | | | Sulfinic Acid | 983.5 | 3 | 331.8 | 372.2 | 57.0 | 16.7 | y6 (2+) |
| | | | | | | 331.8 | 372.2 | 57.0 | 21.7 | y4 |
| | | | Sulfonic Acid | 1009.5 | 3 | 337.2 | 372.2 | 57.0 | 16.7 | y6 (2+) |
| | | | | | | 337.2 | 372.2 | 57.0 | 21.7 | y4 |
| PTP1B | SGTFCLADTCLLLMDKR | 226 : 231 | $d_0$ NEM : $d_0$ NEM | 2137.0 | 3 | 713.0 | 1403.7 | 83.0 | 38.5 | y11 |
| | | | | | | 713.0 | 1332.7 | 83.0 | 38.5 | y10 |
| | | | $d_5$ NEM : $d_0$ NEM | 2142.0 | 3 | 714.7 | 1403.7 | 83.0 | 38.5 | y11 |
| | | | | | | 714.7 | 1332.7 | 83.0 | 38.5 | y10 |
| | | | $d_0$ NEM : $d_5$ NEM | 2142.0 | 3 | 714.7 | 1408.7 | 83.0 | 38.5 | y11 |
| | | | | | | 714.7 | 1337.7 | 83.0 | 38.5 | y10 |
| | | | $d_5$ NEM : $d_5$ NEM | 2147.0 | 3 | 716.4 | 1408.7 | 83.0 | 38.5 | y11 |
| | | | | | | 716.4 | 1337.7 | 83.0 | 38.5 | y10 |
| | | | Sulfinic Acid : $d_0$ NEM | 2043.9 | 3 | 682.0 | 1332.7 | 83.0 | 38.5 | y11 |
| | | | Sulfinic Acid : $d_5$ NEM | 2048.9 | 3 | 683.6 | 1337.7 | 83.0 | 38.5 | y11 |
| | | | Sulfonic Acid : $d_0$ NEM | 2059.9 | 3 | 687.3 | 1332.7 | 83.0 | 38.5 | y11 |
| | | | Sulfonic Acid : $d_5$ NEM | 2064.9 | 3 | 689.0 | 1337.7 | 83.0 | 38.5 | y11 |
| | | | $d_0$ NEM : Sulfinic Acid | 2043.9 | 3 | 682.0 | 1239.6 | 83.0 | 38.5 | y11 |
| | | | $d_5$ NEM : Sulfinic Acid | 2048.9 | 3 | 683.6 | 1239.6 | 83.0 | 38.5 | y11 |
| | | | $d_0$ NEM : Sulfonic Acid | 2059.9 | 3 | 687.3 | 1255.6 | 83.0 | 38.5 | y11 |
| | | | $d_5$ NEM : Sulfonic Acid | 2064.9 | 3 | 689.0 | 1255.6 | 83.0 | 38.5 | y11 |
| | | | Sulfinic Acid : Sulfinic Acid | 1950.9 | 3 | 651.0 | 1239.6 | 83.0 | 38.5 | y11 |
| | | | Sulfinic Acid : Sulfonic Acid | 1966.9 | 3 | 656.3 | 1255.6 | 83.0 | 38.5 | y11 |
| | | | Sulfonic Acid : Sulfinic Acid | 1966.9 | 3 | 656.3 | 1239.6 | 83.0 | 38.5 | y11 |
| | | | Sulfonic Acid : Sulfonic Acid | 1982.9 | 3 | 661.6 | 1255.6 | 83.0 | 38.5 | y11 |
| PTP1B | SYILTQGPLPNTCGHFWEMVWEQK | 92 | $d_0$ NEM | 2012.0 | 3 | 997.1 | 1008.9 | 104.0 | 53.0 | y15 (2+) |
| | | | | | | 997.1 | 1114.0 | 104.0 | 53.0 | y17 (2+) |
| | | | $d_5$ NEM | 2017.0 | 3 | 998.8 | 1011.4 | 104.0 | 53.0 | y15 (2+) |
| | | | | | | 998.8 | 1116.5 | 104.0 | 53.0 | y17 (2+) |
| | | | Sulfinic Acid | 1918.9 | 3 | 966.1 | 962.4 | 104.0 | 53.0 | y15 (2+) |
| | | | | | | 966.1 | 1476.6 | 104.0 | 53.0 | y11 |
| | | | Sulfonic Acid | 1934.9 | 3 | 971.5 | 970.4 | 104.0 | 53.0 | y15 (2+) |
| | | | | | | 971.5 | 1476.6 | 104.0 | 53.0 | y11 |