# Supporting Information

# Fast and Selective Modification of Thiol Proteins/Peptides by *N*-(Phenylseleno)phthalimide

Zhengfang Wang,[1] Yun Zhang,[1] Hao Zhang,[2] Peter de B. Harrington[1*], and Hao Chen[1*]

[1]*Center for Intelligent Chemical Instrumentation, Department of Chemistry and Biochemistry, Ohio University, Athens, OH 45701*

[2]*Department of Chemistry, Washington University, St. Louis, MO 63130*

Additional and supporting mass spectra are included in this Supporting Information section.

A 330 μL of 0.2 mM hemoglobin in $H_2O$ was first reacted with 330 μL of 2 mM NPSP in ACN to obtain 660 μL of 0.1 mM derivatized hemoglobin. The derivatized protein solution was dried by vacuum evaporation at 60 °C. The resulting protein was redissolved in 660 μL of FA/$H_2O$ (10/90, v/v) and half of the solution was added with 2 μL of 1 mM pepsin in $H_2O$ for digestion. Figure 1S-a shows the ESSI spectrum of the digest, in which two derivatized peptide ions $[KLLSHC^*LL+H]^+$ and $[KLLSHC^*LL+2H]^{2+}$ were observed at *m/z* 1082 and 542, respectively. Figures 1S-b and c further show the CID data of these ions, confirming that the tags are located in cysteine residues. This result also confirms that the NPSP derivatization is highly selective to cysteine residues, even in protein substrates.

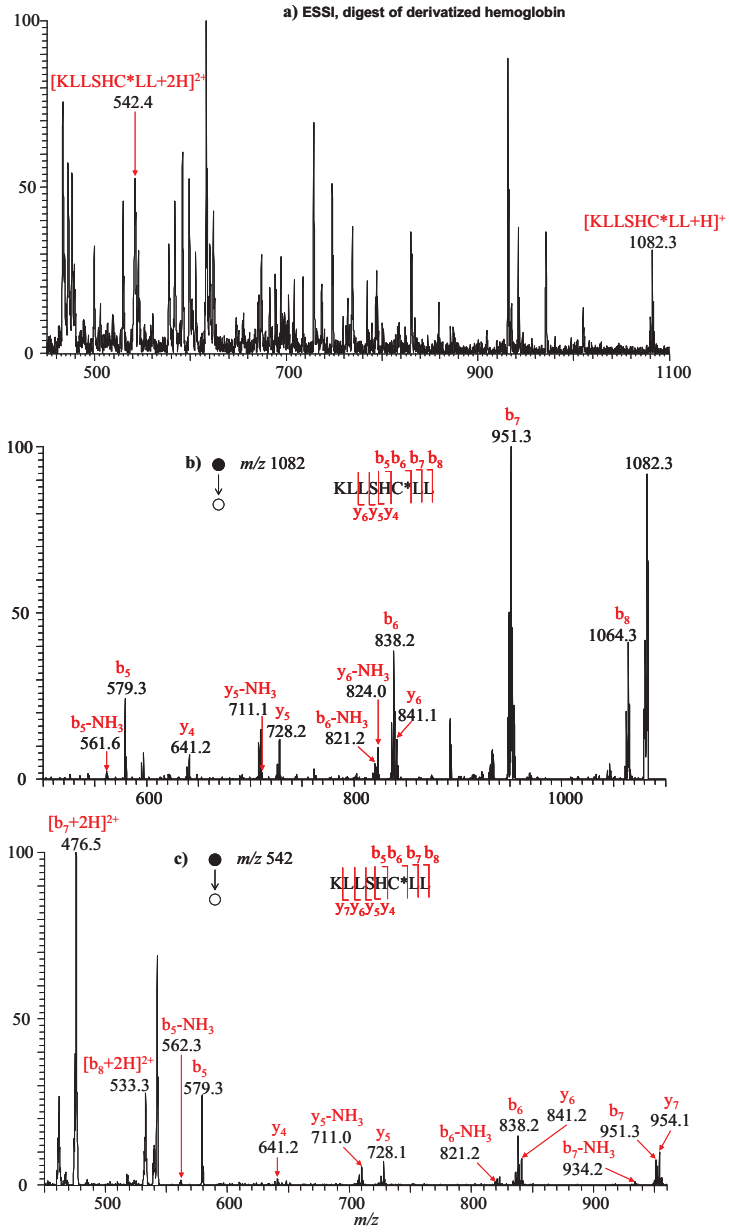


**Figure 1S.** ESSI-MS spectrum of peptic digest of NPSP-derivatized hemoglobin. CID spectra of derivatized peptide ions, b) $[KLLSHC^*LL+H]^+$ of *m/z* 1082 and c) $[KLLSHC^*LL+2H]^{2+}$ of *m/z* 542.

Figure 2S shows the CID spectra of protonated ions of the tagged peptides from insulin generated by NPSP derivatization. All the spectra show the preservation of the tags upon CID.

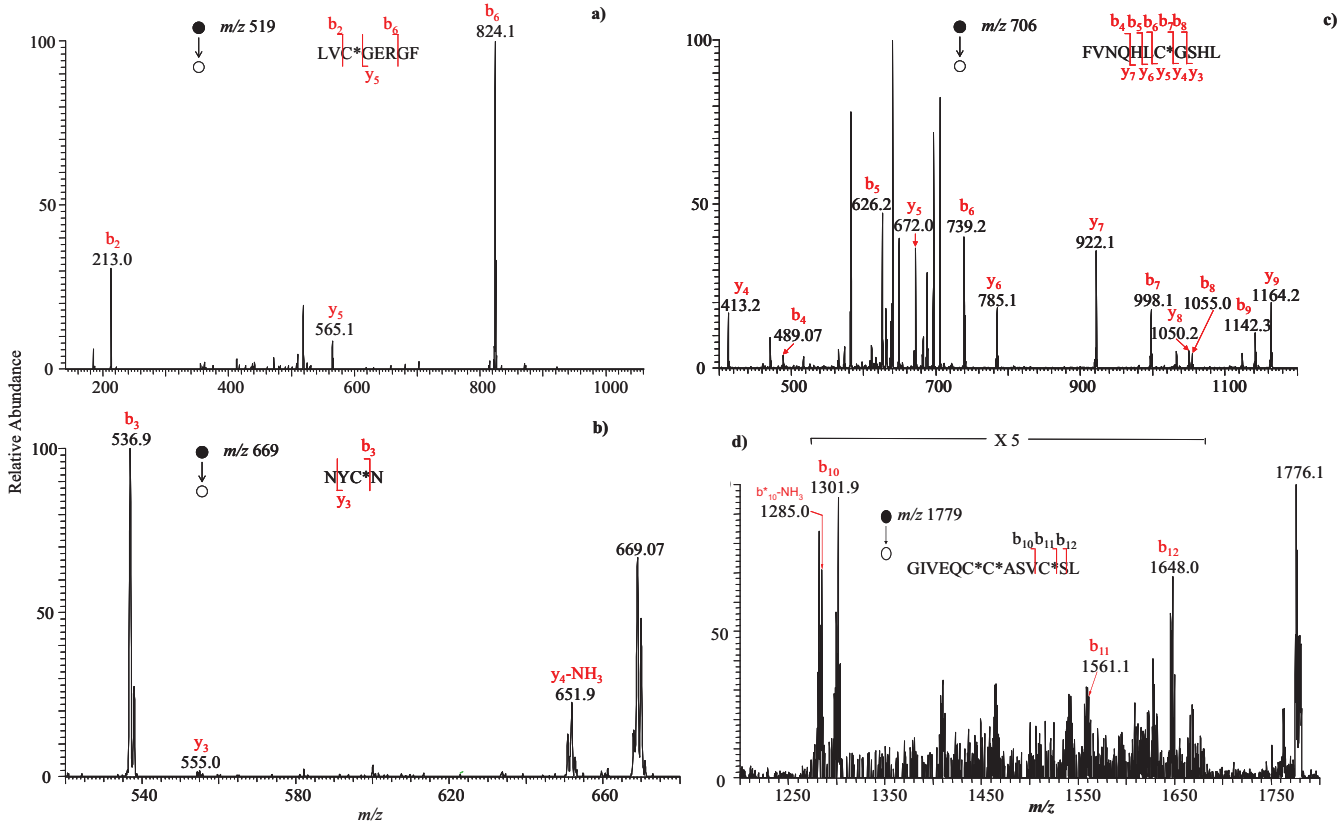


**Figure 2S.** CID spectra of the derivatized peptide ions: a) $[LVC^*GERGF+2H]^{2+}$ (*m/z* 519), b) $[NYC^*N+H]^+$ (*m/z* 669), c) $[FVNQHLC^*GSHL+2H]^{2+}$ (*m/z* 706), and d) $[GIVEQC^*C^*ASVC^*SL+H]^+$ (*m/z* 1779).

Figure 3S-a shows the MS spectrum of trypsin-digested [$Arg^8$]-conopression G. The digest peptide contains an interpeptide disulfide bond. The +1 and +2 ions of the peptide were observed at *m/z* 1024.5 and 513.0, respectively. After TBP reduction and subsequent NPSP derivatization, the reduced peptide products were successfully modified, as evidenced by the appearance of $[NC^*PR+H]^+$ (*m/z* 645.1) and $[C^*FIR+H]^+$ (*m/z* 694.2) in Figure 3S-b. CID spectra were also collected for confirming the peak assignment.

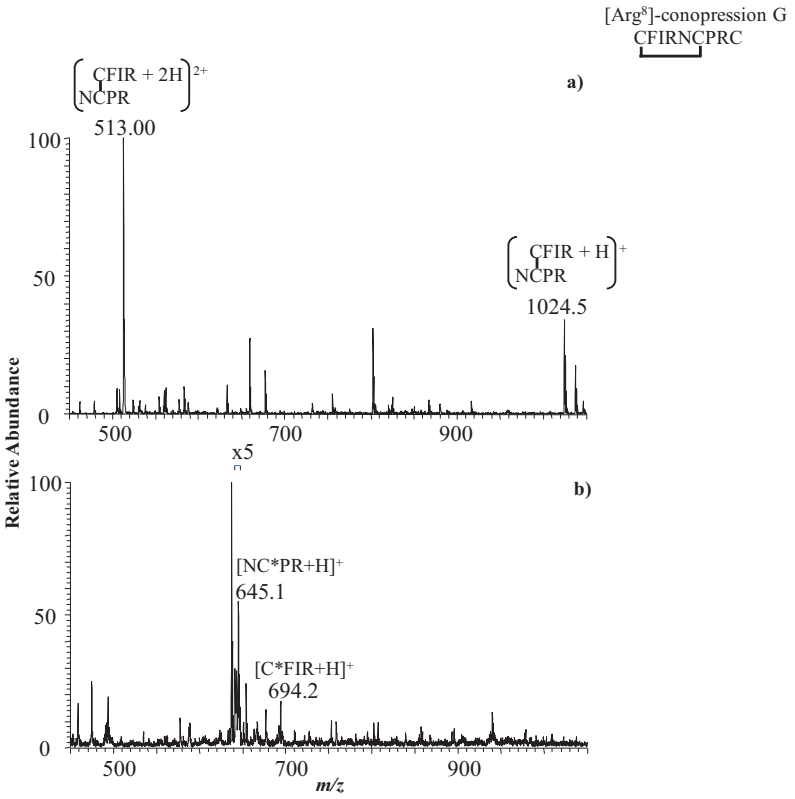


**Figure 3S**. ESSI-MS spectra of a) trypsin-digested [Arg$^8$]-conopression G and b) the peptide digest after TBP reduction and NPSP derivatization.

In a preliminary test, the insulin peptic digest also underwent electrochemical reduction by flowing the digest through a thin-layer electrochemical flow cell (Antec Leyden, Netherlands) consisting of a magic diamond working electrode. The reduced peptides underwent NPSP derivatization. Figure 4S-a shows the protonated ion of a reduced peptide FVNQHLCGSHL from electrochemical reduction and Figures 2S-b displays the corresponding ion of the peptide derivatized by NPSP.

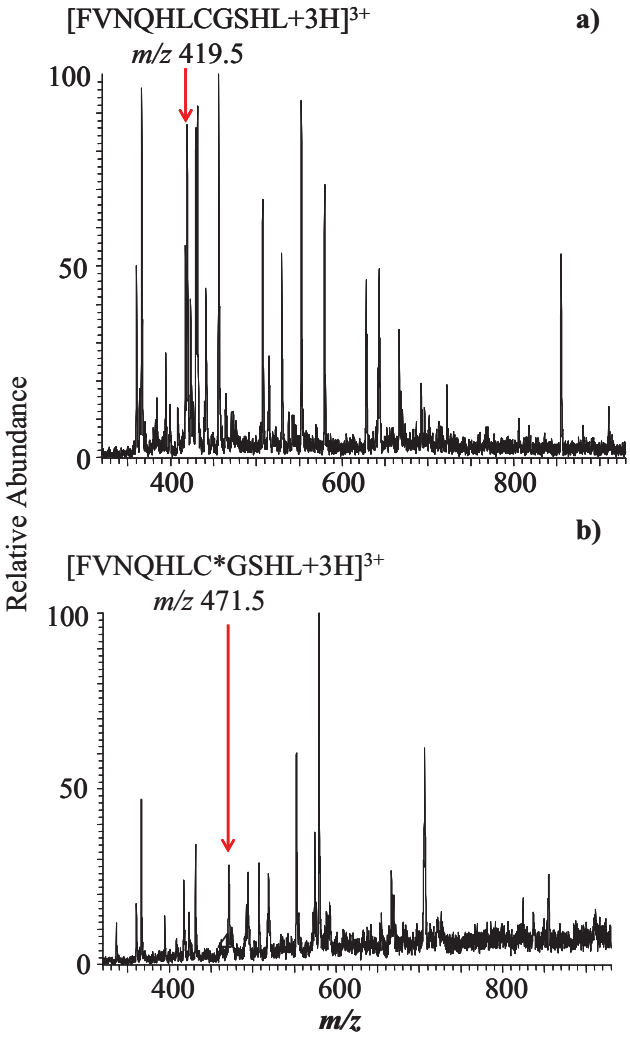


**Figure 4S.** ESSI-MS spectra of a) the insulin digest after EC reduction, and b) the insulin digest after EC reduction followed with NPSP derivatization.