Supporting Information

Tandem MS Analysis of Selenamide-Derivatized Peptide Ions

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Figures S-1a, b, c display the ESI-MS spectra showing the reactions of ebselen with HCKFWW, NRCSQGSCWN, the insulin chain B, respectively. In Figure S-1a, the protonated ebselen was seen at *m/z* 276.1 and the doubly charged reaction product $[HC^*KFWW+2H]^{2+}$ was observed at 591.2. In Figure S-1b, *m/z* 1704.4, 852.7 and 1429.4 correspond to $[NRC^*SQGSC^*WN+H]^+$, $[NRC^*SQGSC^*WN+2H]^{2+}$, and $[NRC^*SQGSCWN+H]^+$ (the exact position of the ebselen tag is uncertain), respectively. In Figure S-1c, the +2, +3 and +4 ions of the insulin chain B containing two ebselen tags were seen at *m/z* 1975.4, 1317.3 and 988.3, respectively. Also, *m/z* 267.1 was from the excess amount of TCEP.

Figures S-1d, e, f show the ESI-MS spectra for the reactions of NPSP with HCKFWW, NRCSQGSCWN and the insulin chain B, respectively. As shown in Figure S-1d, $[HC'KFWW+H]^+$ and $[HC'KFWW+2H]^{2+}$ were observed at *m/z* 1062.4 and 531.7, respectively. The peak at *m/z* 191.0 probably corresponds to the protonated PhSe(=O)OH. In Figure S-1e, $[NRC'SQGSC'WN+2H]^{2+}$ was observed at *m/z* 733.6. Also, +1 and +2 ions of the oxidized peptide (probably due to air oxidation) were seen at *m/z* 1152.4 and 576.7, respectively. In Figure S-1f, *m/z* 1237.7 and 928.6 correspond to $[FVNQHLC'GSHLVEALYLVC'GERGFFYTPKA+3H]^{3+}$ and

[FVNQHLC'GSHLVEALYLVC'GERGFFYTPKA+4H]$^{4+}$ respectively. In this case, +2, +3 and +4 ions of the oxidized chain B were also observed at *m/z* 1699.4, 1133.3 and 850.3, respectively.

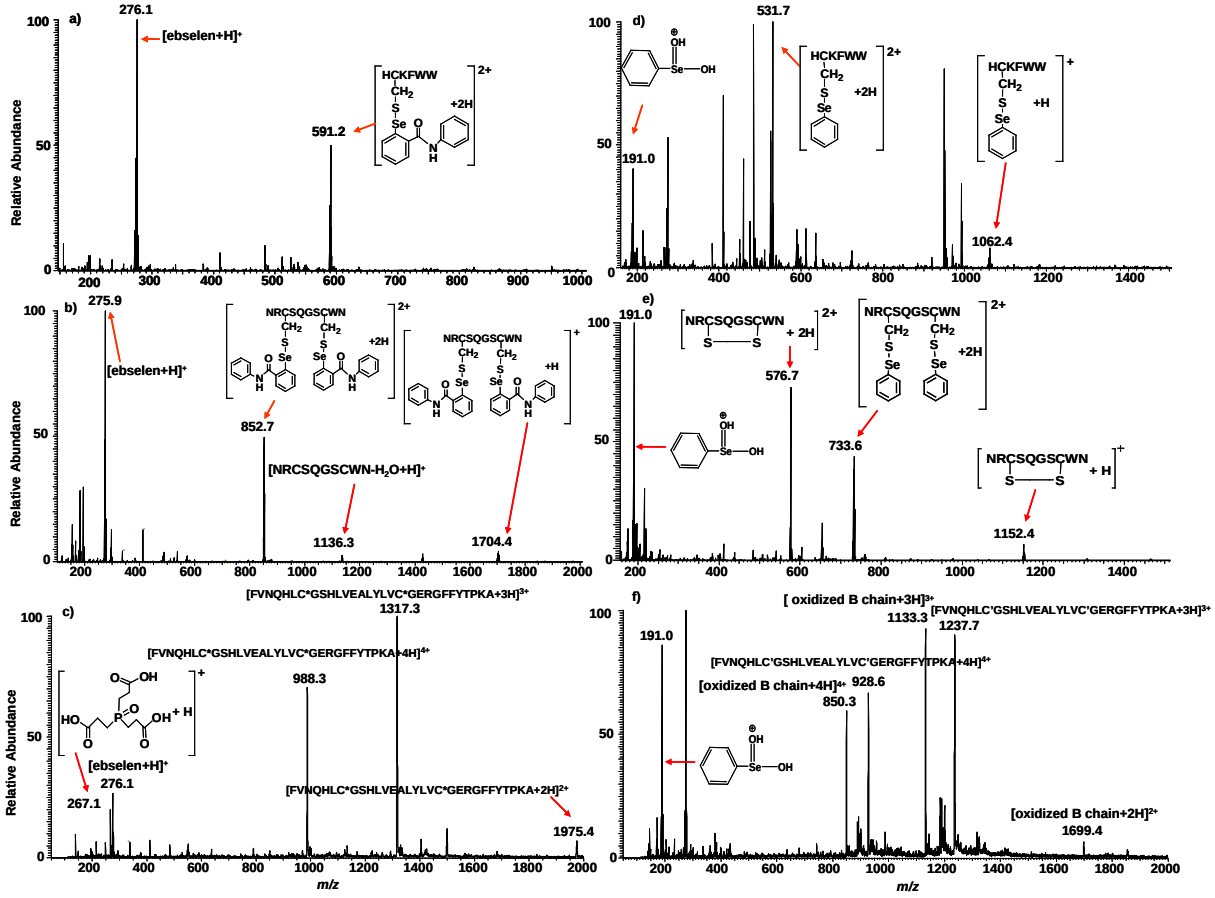

Figure S-1. ESI-MS spectra showing (a) the reaction of HCKFWW with ebselen; (b) the reaction of NRCSQGSCWN with ebselen; (c) the reaction of the insulin chain B with ebselen; (d) the reaction of HCKFWW with NPSP; (e) the reaction of NRCSQGSCWN with NPSP; (f) the reaction of the insulin chain B with NPSP.

Figure S-2 shows the MS/MS/MS mass spectrum of the singly charged ion of NRCSQGSCWN with one ebselen tag (*m/z* 1429.4) generated via CID of [NRC*SQGSC*WN+2H]$^{2+}$ (*m/z* 852.8) by loss of one protonated ebselen. This MS/MS/MS mass spectrum shows the presence of a group of fragment ions b_5*, b_6*, b_7*, and b_7*-NH_3 from

dissociation of [NRC*SQGSCWN+H]$^+$ and another group of fragment ions $\underline{b_4}$, $\underline{b_5}$, $\underline{b_5\text{-}NH_3}$, $\underline{b_6}$, $\underline{b_7}$, $\underline{b_7\text{-}NH_3}$, $\underline{y_3}$*, $\underline{y_6}$* from dissociation of [NRCSQGSC*WN+H]$^+$. Thus, it can be seen that the loss of ebselen tag from [NRC*SQGSC*WN+2H]$^{2+}$ (*m/z* 852.8) has no regioselectivity, which can occur either to Cys-3 or to Cys-8.

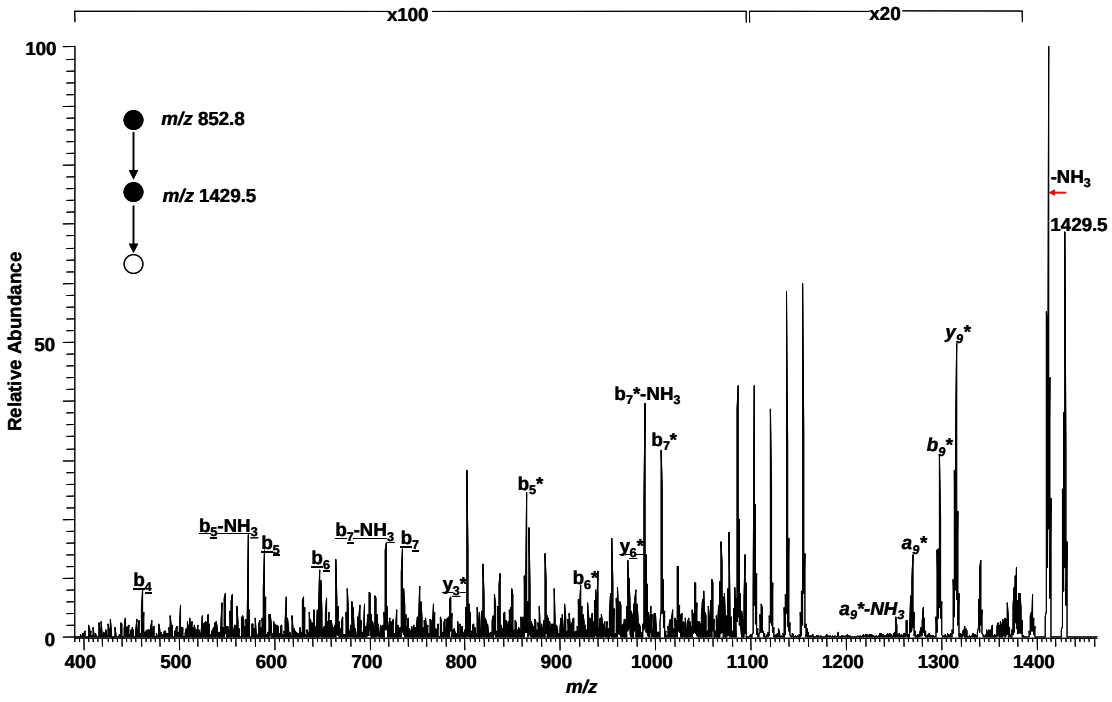

Figure S-2. CID-MS/MS/MS mass spectrum of singly charged ion of *m/z* 1429.5 (a mixed [NRC*SQGSCWN+H]$^+$ and [NRCSQGSC*WN+H]$^+$)

Figure S-3 shows ETD MS/MS mass spectra of [C*QDSETRTFY+2Na]$^{2+}$ (*m/z* 784.8) and [C'QDSETRTFY+2Na]$^{2+}$ (*m/z* 725.3) generated by ESI of a mixture of peptide CQDSETRTFY (MW 1248.5 Da), NPSP and NaCl in ACN/H_2O/HOAc (50:50:1 by volume). Both Figure S-3a and S-3b show the facile loss of the selenium tag.

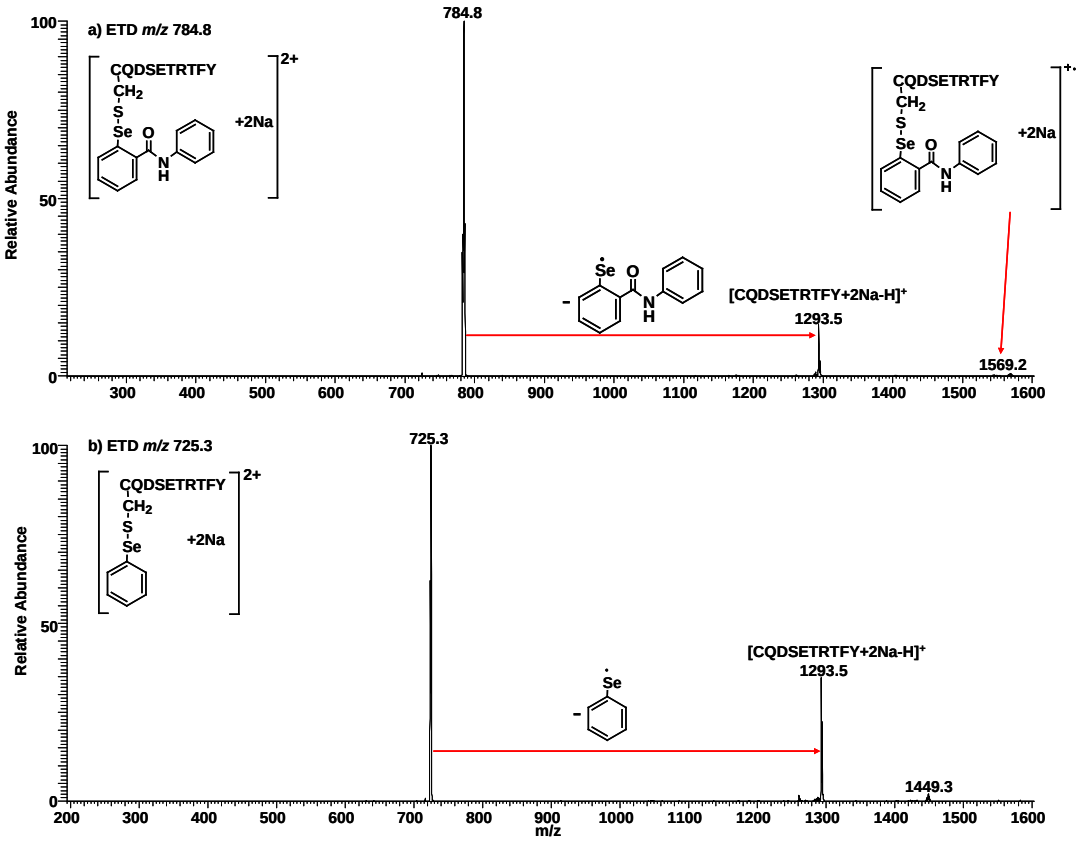

Figure S-3. ETD MS/MS mass spectra of (a) of $[C^{*}QDSETRTFY+2Na]^{2+}$ (*m/z* 784.8) and $[C'QDSETRTFY+2Na]^{2+}$ (*m/z* 725.3).