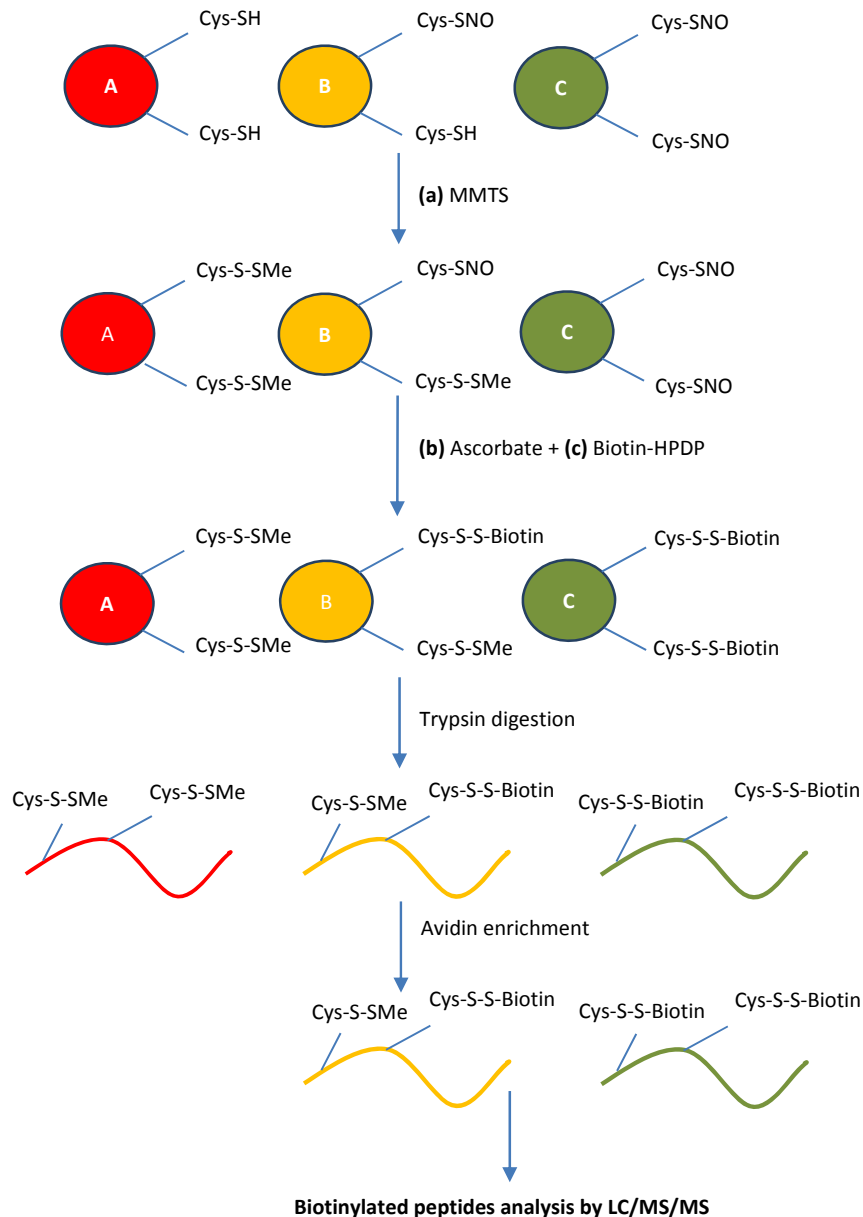


**Supplemental Fig. S1. Overview of the biotin switch technique.** The free thiols in the cell lysates with different degree of S-nitrosation were firstly blocked with MMTS (a). The SNO-Cys on MMTS-modified proteins were then reduced by ascorbate (b) so as to react with biotin-HPDP (c) followed by the in-solution trypsin digestion. The biotinylated peptides were enriched by avidin cartridge and subjected to LC/MS/MS analysis. A, B, C: different Cysteine containing proteins. Cys-SH: Cysteine with free thiols. Cys-SNO: S-nitrosated cysteine. Cys-S-SMe: MMTS modified cysteine. Cys-S-S-Biotin: Biotinylated Cysteine.

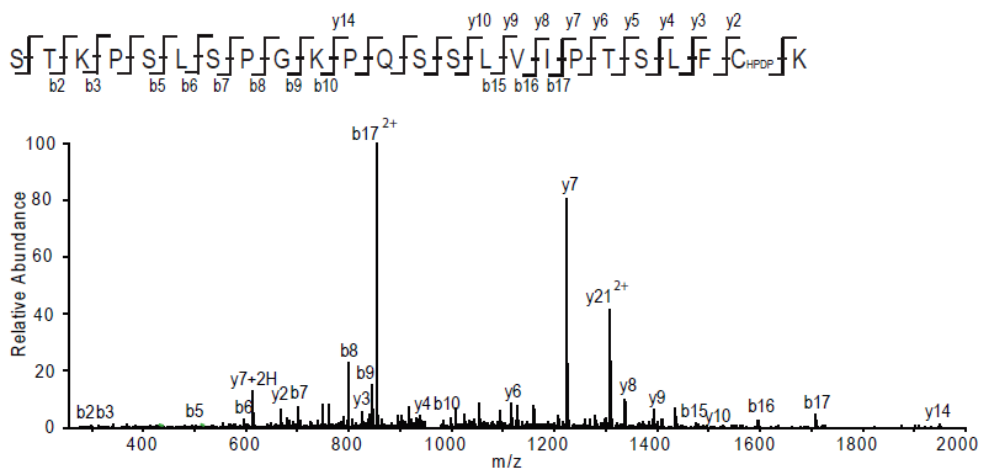


**Supplemental Fig. S2. Identification of biotinylated-Cys sites in sGC alpha subunit by MS/MS.**

- (a) CID MS/MS spectrum of the triply-charged biotinylated-peptide ion at m/z 977.52 of the peptide STKPSLSPGKPKQSSLVIPITSLFCK (Cys282)
- (b) CID MS/MS spectrum of the doubly-charged biotinylated-peptide ion at m/z 936.92 of the peptide FDOQCGELDVYK (Cys516)
- (c) CID MS/MS spectrum of the doubly-charged biotinylated-peptide ion at m/z 942.96 of the peptide YCLFGNNVTLANK (Cys594)
- (d) CID MS/MS spectrum of the doubly-charged biotinylated-peptide ion at m/z 676.81 of the peptide FESCSVPR (Cys609)
- (e) CID MS/MS spectrum of the doubly-charged biotinylated-peptide ion at m/z 783.87 of the peptide DCPGFVFTPR (Cys628)

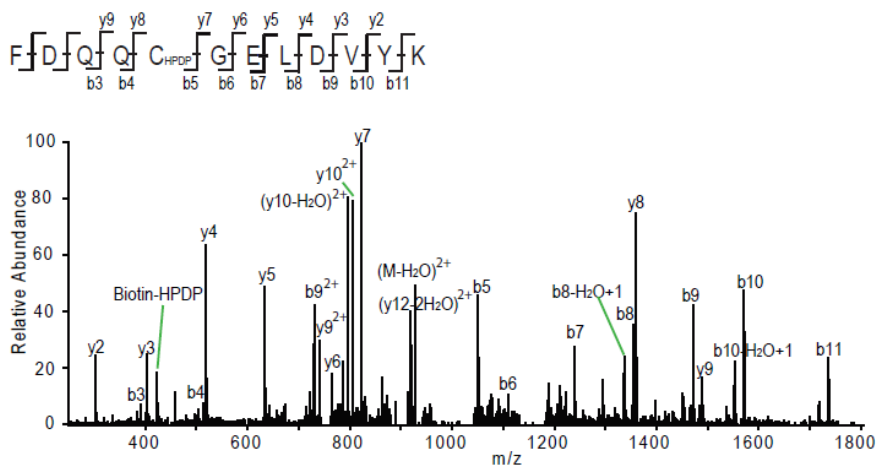
**Supplemental Fig. S2. Identification of biotinylated-Cys sites in sGC alpha subunit by MS/MS.**

(a) CID MS/MS spectrum of the triply-charged biotinylated-peptide ion at  $m/z$  977.52 of the peptide STKPSLSPGKPQSSLVLPSTSLFCH<sub>2</sub>DPK (Cys282)



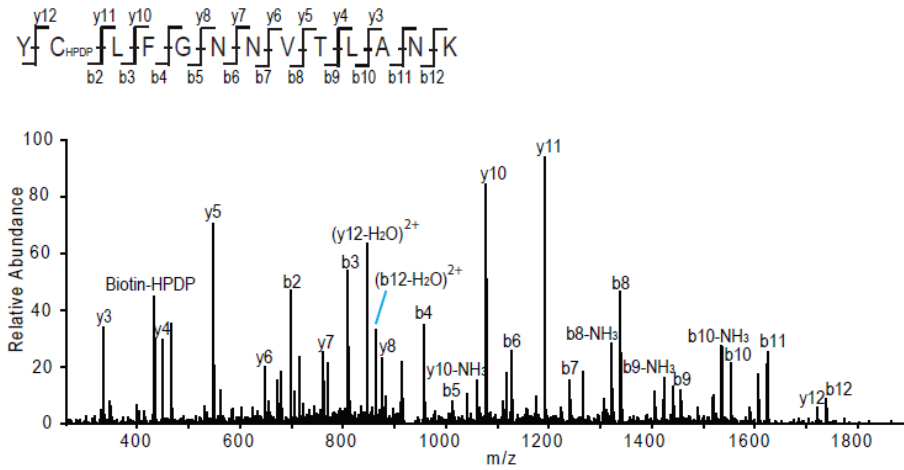
**Supplemental Fig. S2. Identification of biotinylated--Cys sites in sGC alpha subunit by MS/MS.**

(b) CID MS/MS spectrum of the doubly-charged biotinylated-peptide ion at  $m/z$  936.92 of the peptide FDQQCGELDVYK (Cys516)



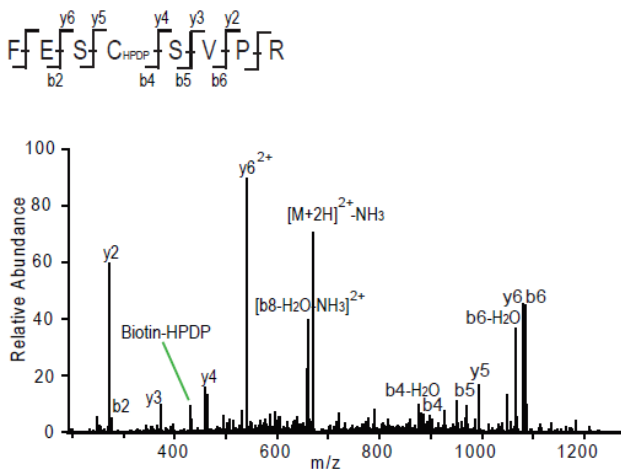
**Supplemental Fig. S2. Identification of biotinylated-Cys sites in sGC alpha subunit by MS/MS.**

(c) CID MS/MS spectrum of the doubly-charged biotinylated-peptide ion at  $m/z$  942.96 of the peptide YCLFGNNVTLANK (Cys594)



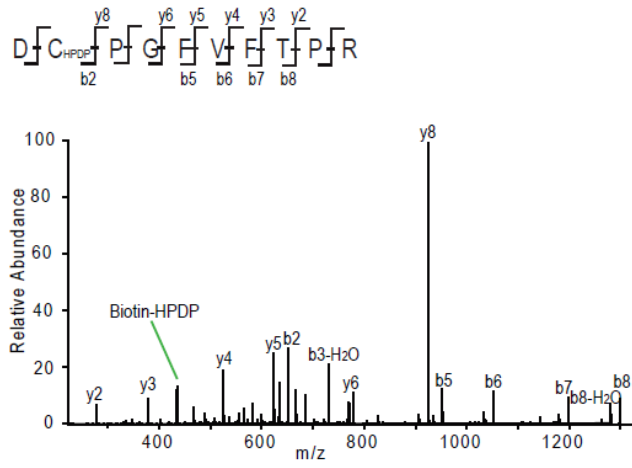
**Supplemental Fig. S2. Identification of biotinylated-Cys sites in sGC alpha subunit by MS/MS.**

(d) CID MS/MS spectrum of the doubly-charged biotinylated-peptide ion at  $m/z$  676.81 of the peptide FESCSVPR (Cys609)



**Supplemental Fig. S2. Identification of biotinylated-Cys sites in sGC alpha subunit by MS/MS.**

(e) CID MS/MS spectrum of the doubly-charged biotinylated-peptide ion at m/z 783.87 of the peptide DCPGFVFTPR (Cys628)



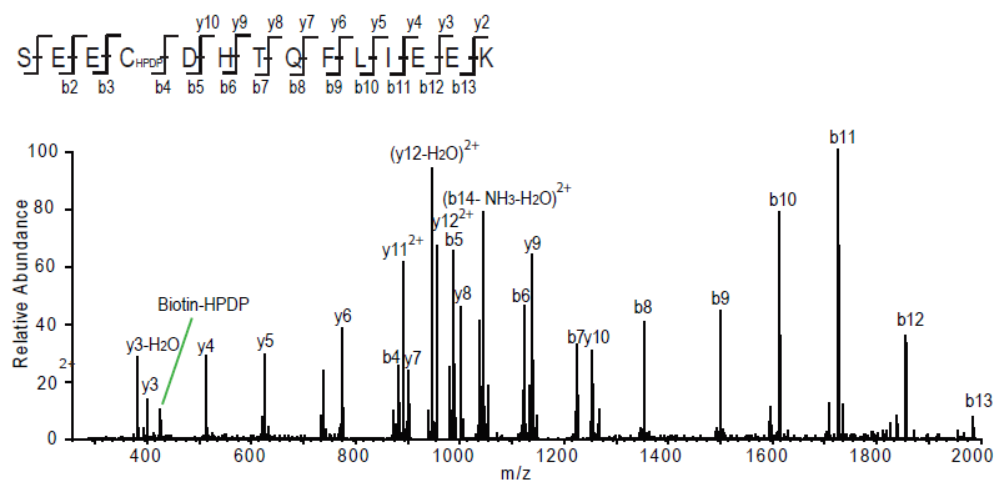
**Supplemental Fig. S3. Identification of biotinylated-Cys sites in sGC beta subunit by MS/MS.**

- (a) CID MS/MS spectrum of the doubly-charged biotinylated-peptide ion at m/z 1068.48 of the peptide SEECDHTQFLIEEK (Cys174)
- (b) CID MS/MS spectrum of the doubly-charged biotinylated-peptide ion at m/z 693.84 of the peptide SEECDHTQFLIEEK (Cys214)
- (c) CID MS/MS spectrum of the doubly-charged biotinylated-peptide ion at m/z 940.46 of the peptide DLVVTQCGNAIYR (Cys232)
- (d) CID MS/MS spectrum of the doubly-charged biotinylated-peptide ion at m/z 958.47 of the peptide YCLFGNTVNLTSR (Cys541)
- (e) CID MS/MS spectrum of the triply-charged biotinylated-peptide ion at m/z 823.37 of the peptide CLMSPENSDPQFHLEHR (Cys571)



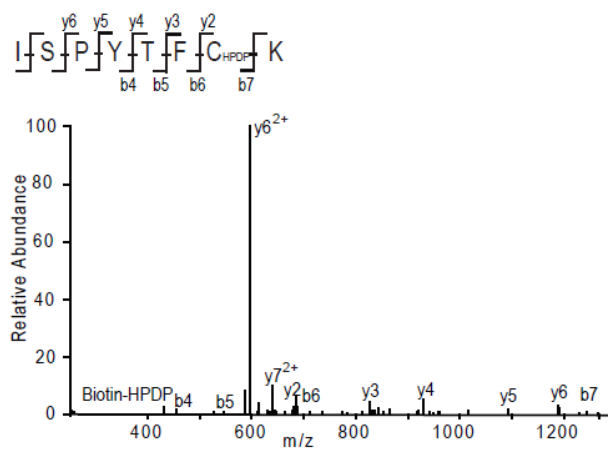
**Supplemental Fig. S3. Identification of biotinylated-Cys sites in sGC beta subunit by MS/MS.**

(a) CID MS/MS spectrum of the doubly-charged biotinylated-peptide ion at  $m/z$  1068.48 of the peptide SEECDHTQFLIEEK (Cys174)



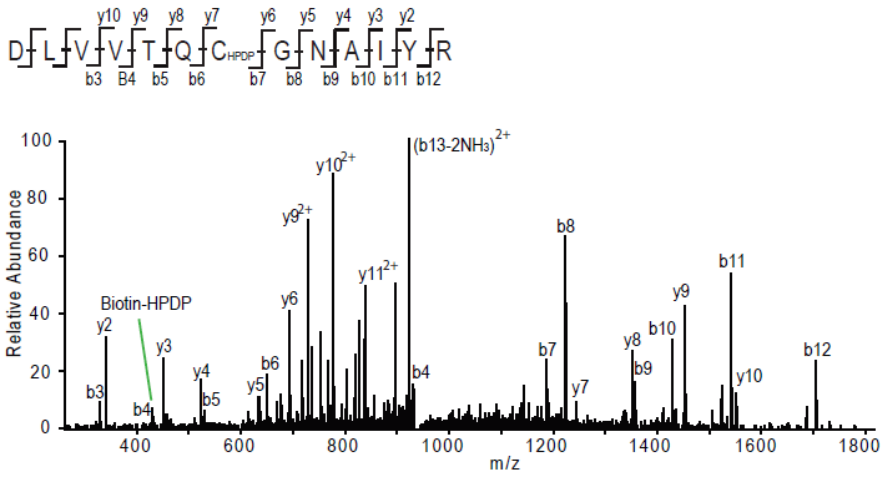
**Supplemental Fig. S3. Identification of biotinylated-Cys sites in sGC beta subunit by MS/MS.**

(b) CID MS/MS spectrum of the doubly-charged biotinylated-peptide ion at  $m/z$  693.84 of the peptide SEECDHTQFLIEEK (Cys214)



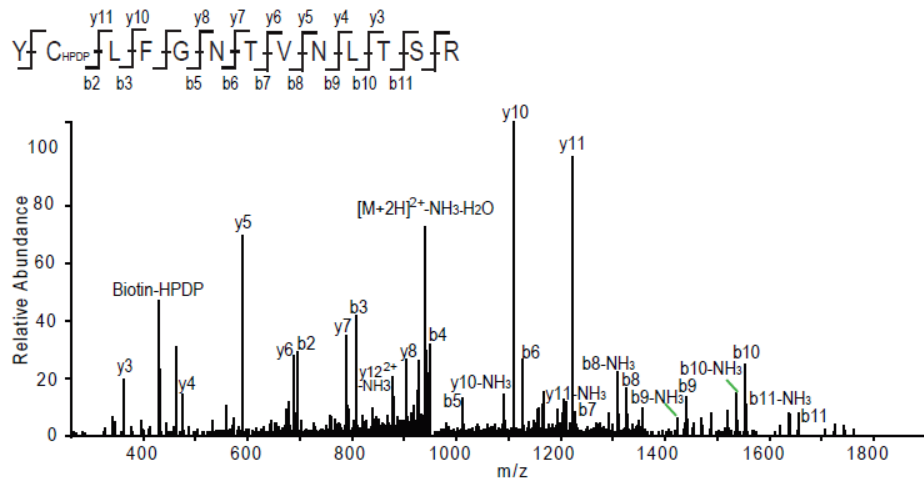
**Supplemental Fig. S3. Identification of biotinylated-Cys sites in sGC beta subunit by MS/MS.**

(c) CID MS/MS spectrum of the doubly-charged biotinylated-peptide ion at m/z 940.46 of the peptide DLVVTQCGNAIYR (Cys232)



**Supplemental Fig. S3. Identification of biotinylated-Cys sites in sGC beta subunit by MS/MS.**

(d) CID MS/MS spectrum of the doubly-charged biotinylated-peptide ion at  $m/z$  958.47 of the peptide YCLFGNTVNLTSR (Cys541)



**Supplemental Fig. S3. Identification of biotinylated-Cys sites in sGC beta subunit by MS/MS.**

(e) CID MS/MS spectrum of the triply-charged biotinylated-peptide ion at  $m/z$  823.37 of the peptide CLMSPENS DPQFHLEHR (Cys571)

