

Supporting information for

**De-sialylation of glycopeptides by acid treatment:
enhancing sialic acid removal without reducing identification**

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The supporting materials include:

Figures S1 – S6

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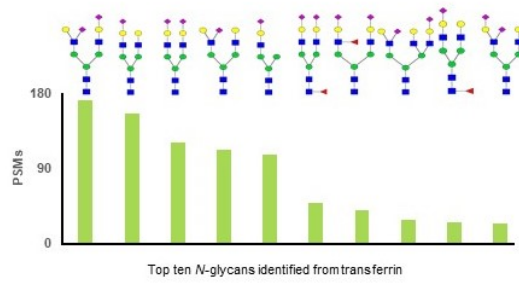


Figure S1. Top ten sialylated glycan structures identified from transferrin glycopeptides.

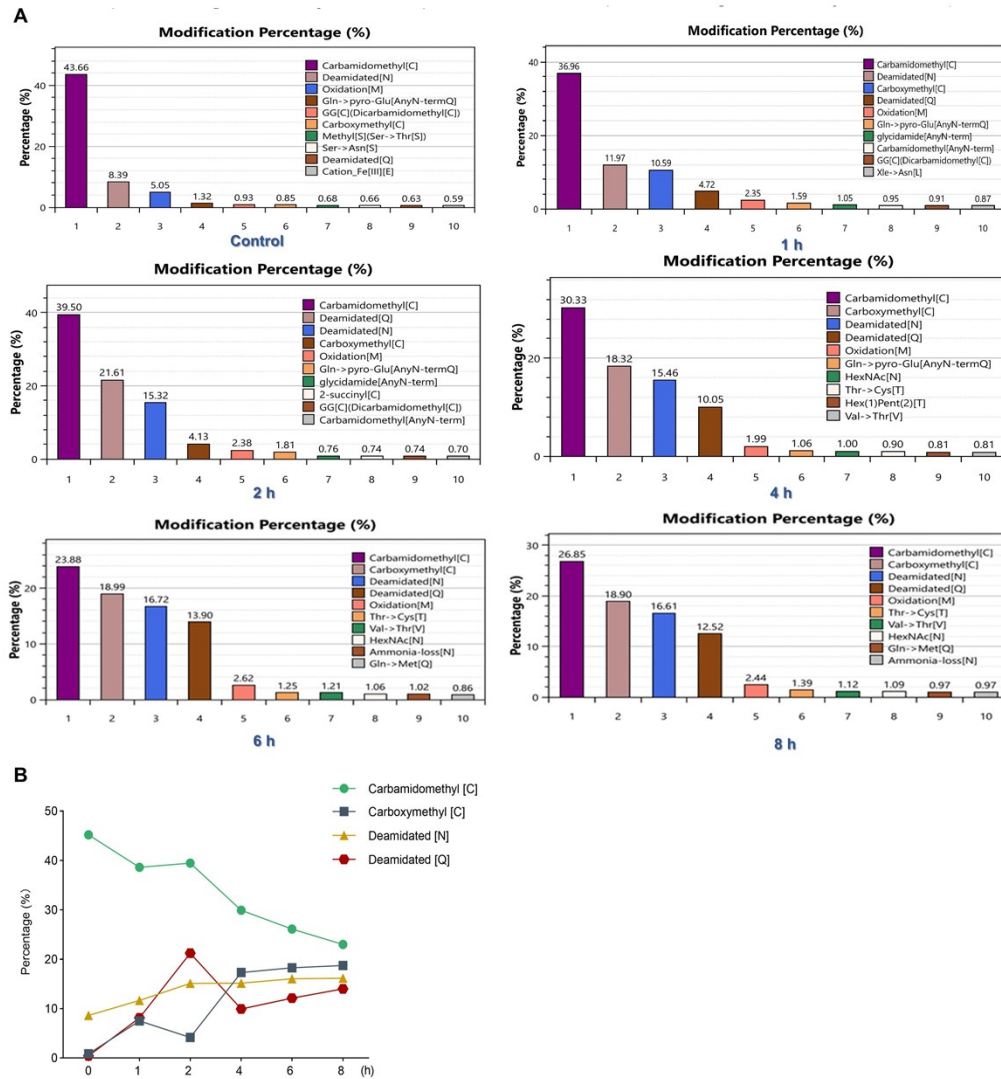


Figure S2. Peptide modifications detected from pooled human sera glycopeptide samples treated with 1% TFA with different treatment time. (A) Modifications identified by open-pFind at six different TFA-treatment time points. (B) Dynamic changes of each modification with treatment time.

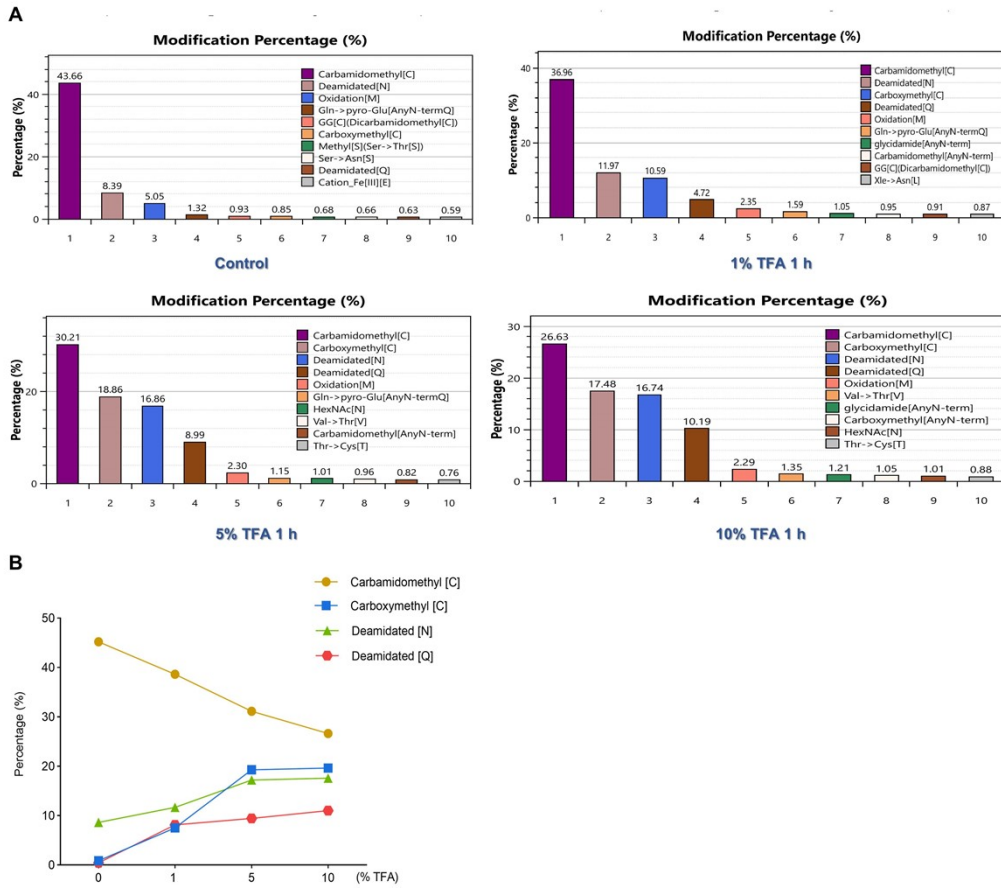


Figure S3. Peptide modifications detected from pooled human sera glycopeptide samples treated with different concentrations of TFA. (A) Modifications identified sera glycopeptide samples treated with four different TFA concentrations by using open-pFind. (B) Dynamic changes of each modification in samples treated with different TFA concentrations.

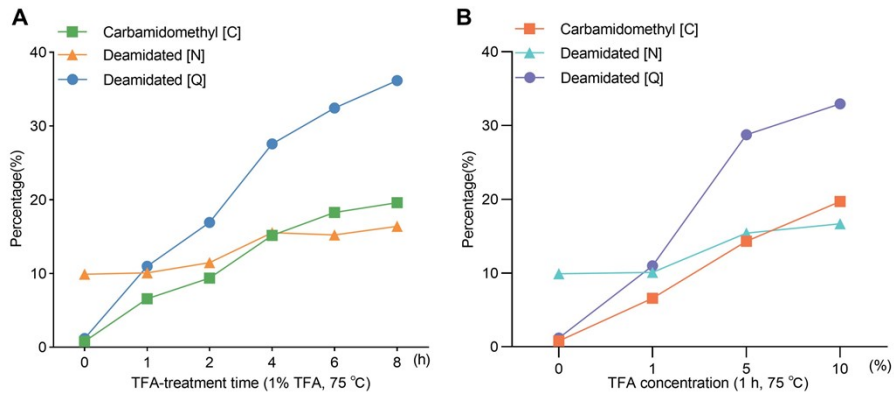


Figure S4. Dynamic changes of deamidated glycopeptides in pooled human sera with (A) different incubation time and (B) TFA concentrations. The proportions of deamidated glycopeptides were monitored using StrucGP.

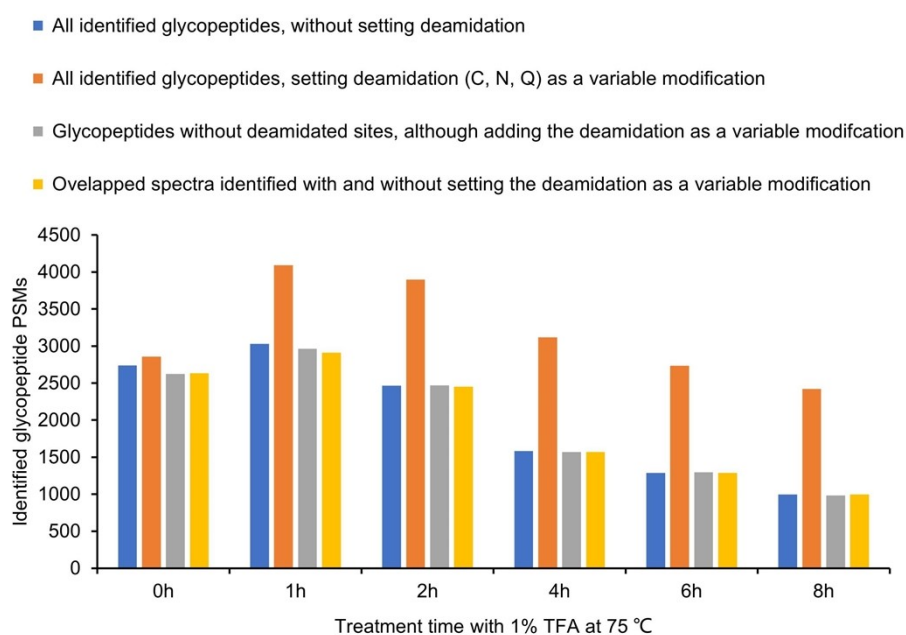


Figure S5. Effects on the number of glycopeptide identification with and without adding the deamidation as a variable modification. Once adding the deamidation (C, N, Q) as a variable modification, the number of totally identified glycopeptides were increased significantly, while the number of glycopeptides without any deamidation sites were almost the same as the glycopeptide identifications without setting the deamidation as a variable modification (based on PSMs). In addition, the majority of spectra identified from normal database search (without setting deamidation) could still be identified after adding the variable modification, and the majority of identified glycopeptides were also the same as the normal database search.

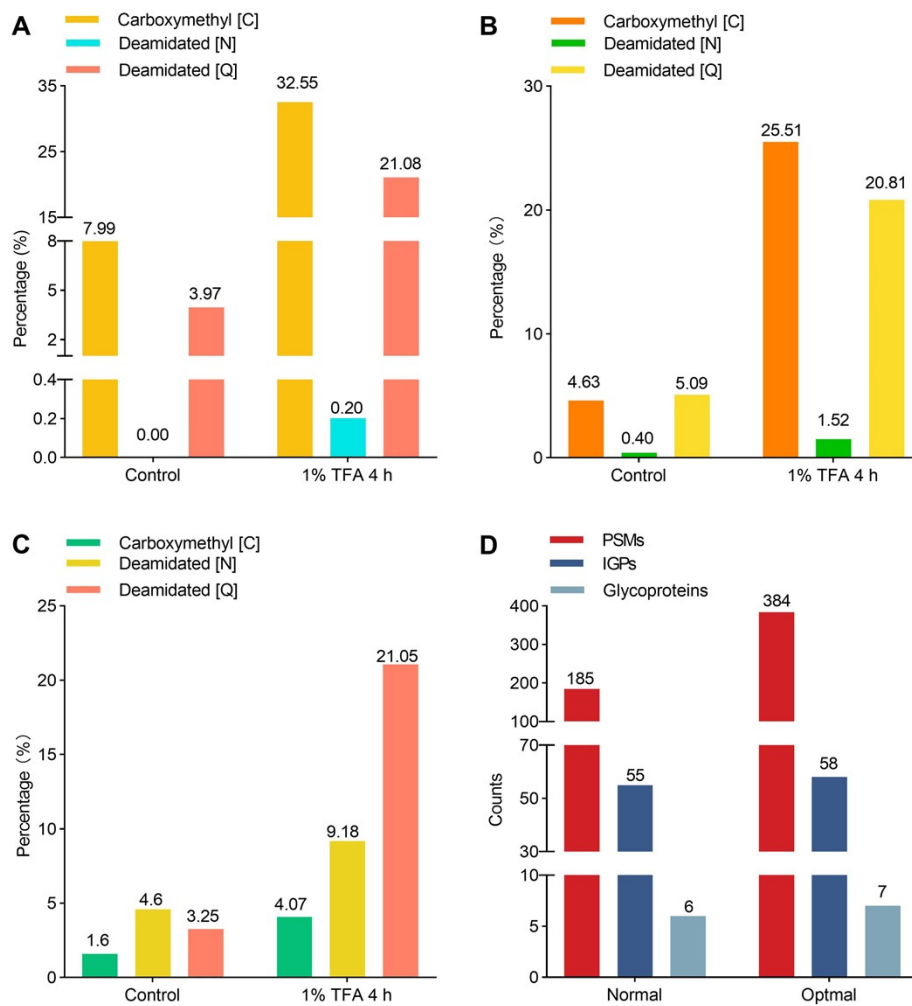


Figure S6. Verification of the optimal de-sialylation method in a mixture of standard glycopeptides (including bovine fetuin). (A-C) Variations in peptide modifications identified by (A) pGlyco3, (B) StrucGP, and (C) open-pFind. (D) Different database search methods identified counts of PSMs, IGPs, and glycoproteins.