

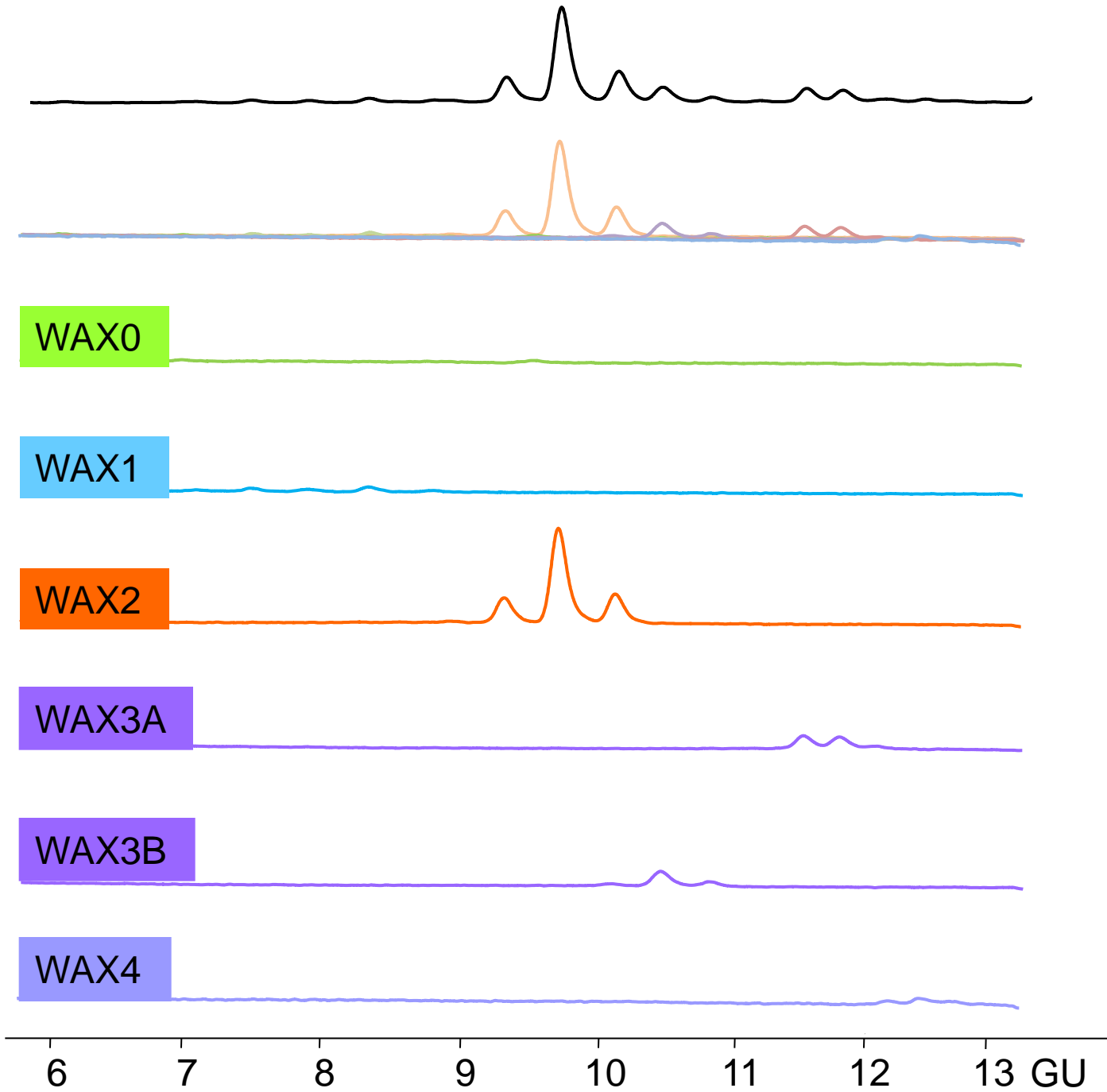
**Figure S1: Detailed N-glycan analysis of mouse serum of individual WAX fractions.**

The whole mouse N-glycome contained monosialylated, disialylated biantennary, trisialylated triantennary and biantennary, and tetrasialylated triantennary glycans. Galactose was linked  $\beta$ 1-3 or  $\beta$ 1-4 based on digestions with bovine testes  $\beta$ -galactosidase (BTG) and *Streptococcus pneumoniae*  $\beta$ -galactosidase (SPG). BTG digests non-reducing terminal galactose  $\beta$ 1-3 and  $\beta$ 1-4 linkages, whereas SPG digests non-reducing terminal galactose  $\beta$ 1-4 linkages. *Arthrobacter ureafaciens* sialidase (ABS) releases  $\alpha$ 2-3,6 and 8 linked non-reducing terminal sialic acids. ABS digestions on some samples required higher amount of the enzyme suggesting less accessible linkage of sialic acid on GlcNAc. *Streptococcus pneumoniae* sialidase (NAN1) releases  $\alpha$ 1-3 linked non-reducing terminal sialic acids; almond meal  $\alpha$ -fucosidase (AMF) releases  $\alpha$ 1-3 and 4 linked non-reducing terminal fucose residues, whereas *Xanthomonas* sp. alpha-fucosidase (XMF) removes  $\alpha$ 1,2 linked fucose and bovine kidney  $\alpha$ -fucosidase (BKF) releases  $\alpha$ 1-2 and  $\alpha$ 1-6 fucose linked non-reducing terminal fucose residues more efficiently than  $\alpha$ 1-3 and 4 linked fucose and also digests core  $\alpha$ 1-6 fucose; coffee bean  $\alpha$ -galactosidase (CBG) hydrolyses  $\alpha$ 1-3 and  $\alpha$ 1-4 galactose; jack bean  $\beta$ -N-acetylhexosaminidase (JBH) releases non reducing terminal  $\beta$  (1-2,3,4,6) linked N-acetylglucosamine (GlcNAc) and N-acetylgalactosamine (GalNAc) residues; jack bean  $\alpha$ -mannosidase (JBM) removes mannose linked  $\alpha$ 1-2,6 >3;  $\beta$ -N-acetylglucosaminidase cloned from *Streptococcus pneumoniae*, expressed in *E. coli* (GUH) digests  $\beta$ GlcNAc, but not a bisecting GlNAc  $\beta$ 1-4 linked to mannose.

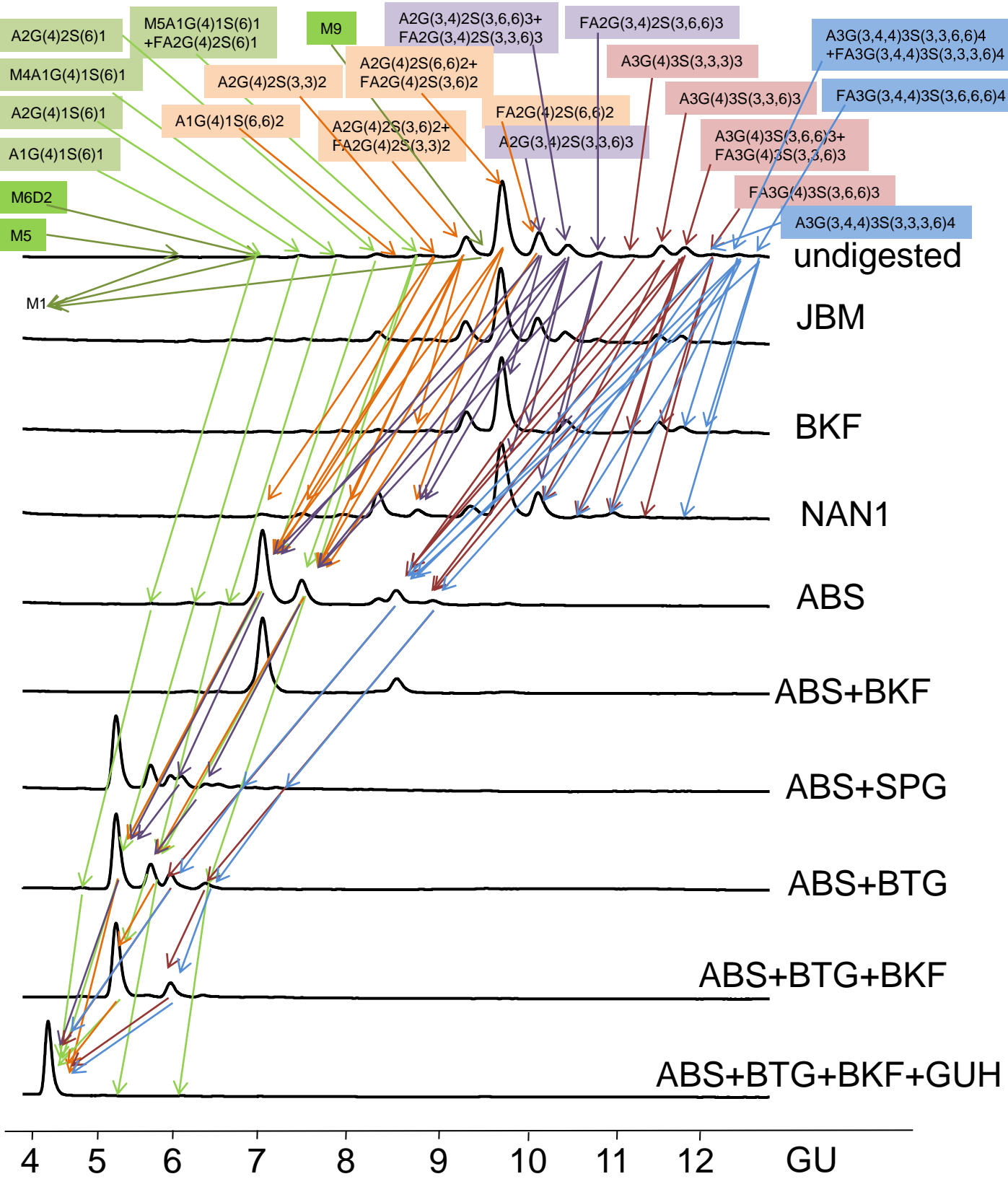
For structural abbreviations see legend of Figure 1.

# A) HILIC-HPLC of unfractionated and WAX fractionated fractions of whole mouse serum *N*-glycome

WAX0 = neutral, S1 = monosialylated, S2 = disialylated, S3A = trisialylated triantennary, S3B = trisialylated biantennary and S4 = tetrasialylated fraction.

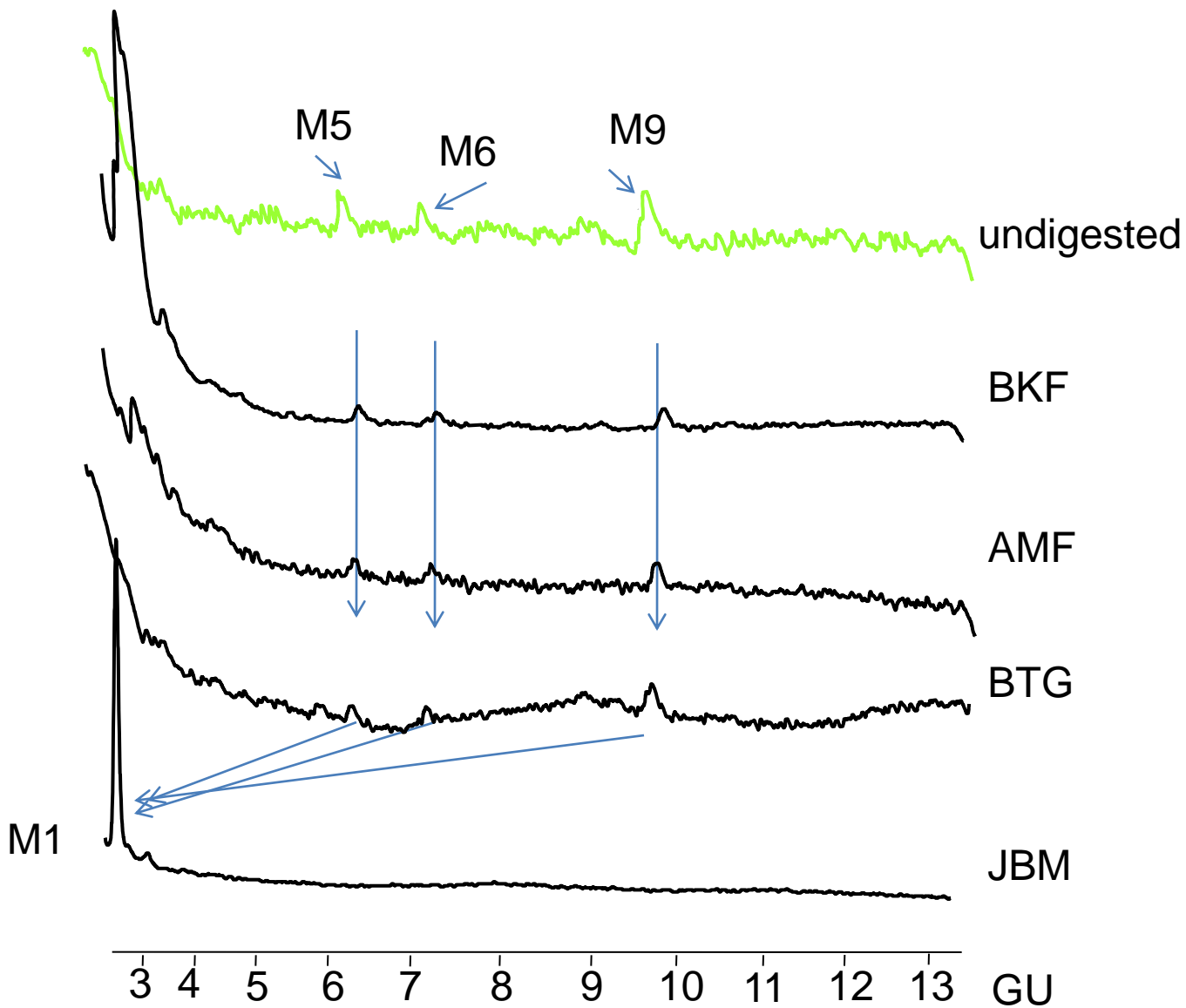


**B) Exoglycosidase digests of unfractionated mouse serum N-glycome**



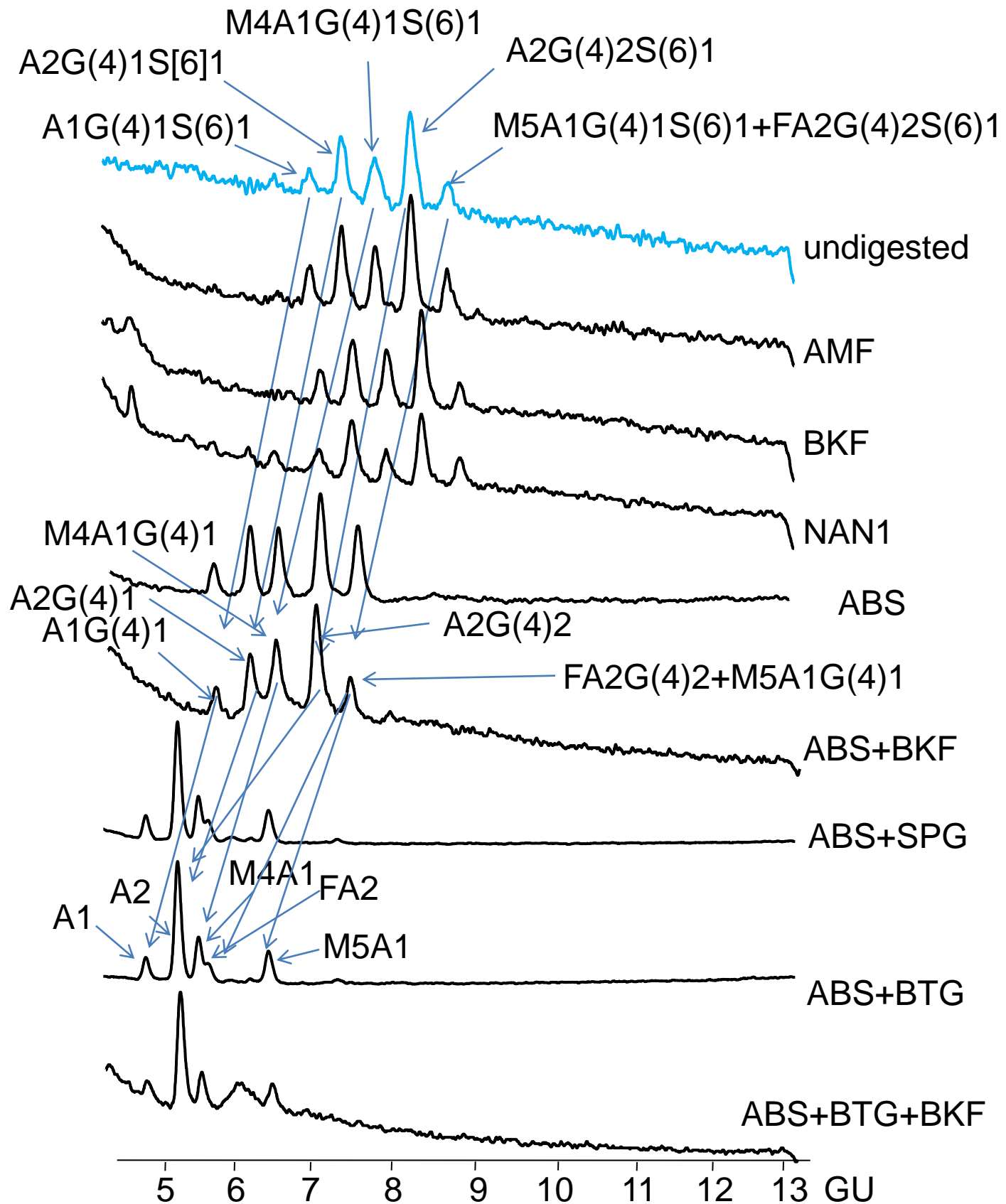
XMF, AMF, JBH and CBG did not make difference to the profiles – no outer arm fucose, no GalNAc and no alpha-galactose present in mouse serum

Neutral (S0)

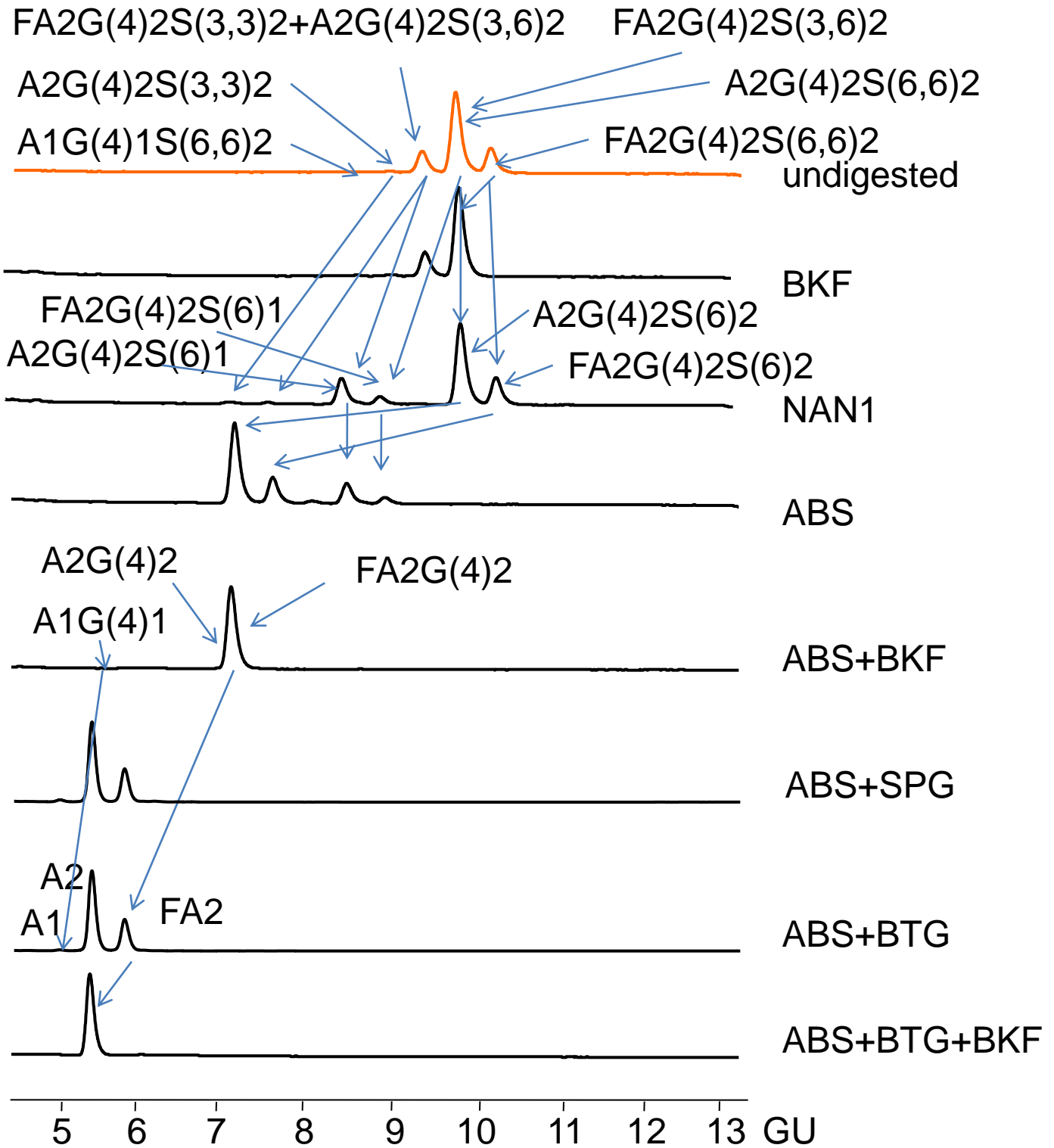


Neutral fraction contains only high mannosylated glycans

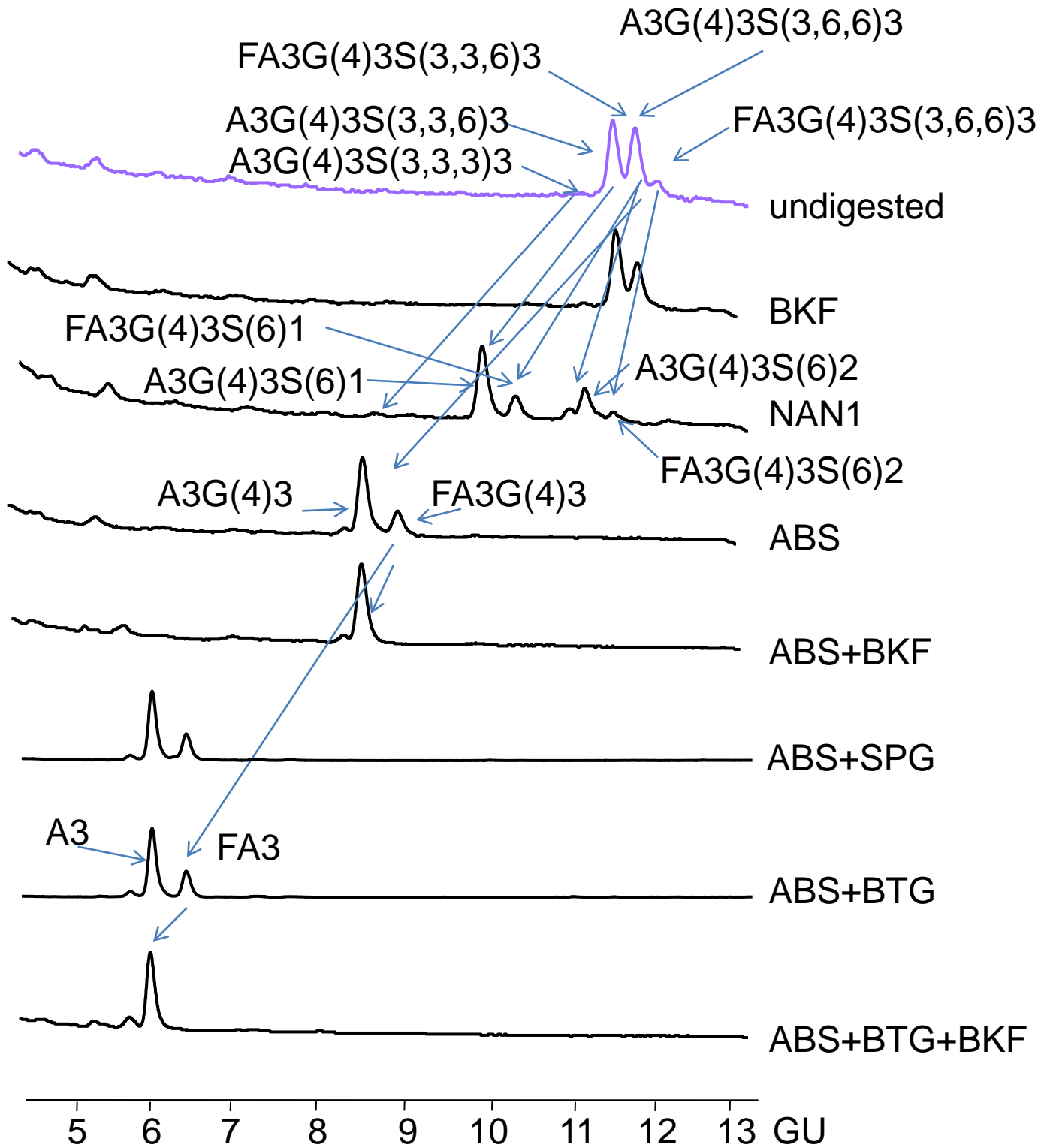
# Monosialylated (S1)



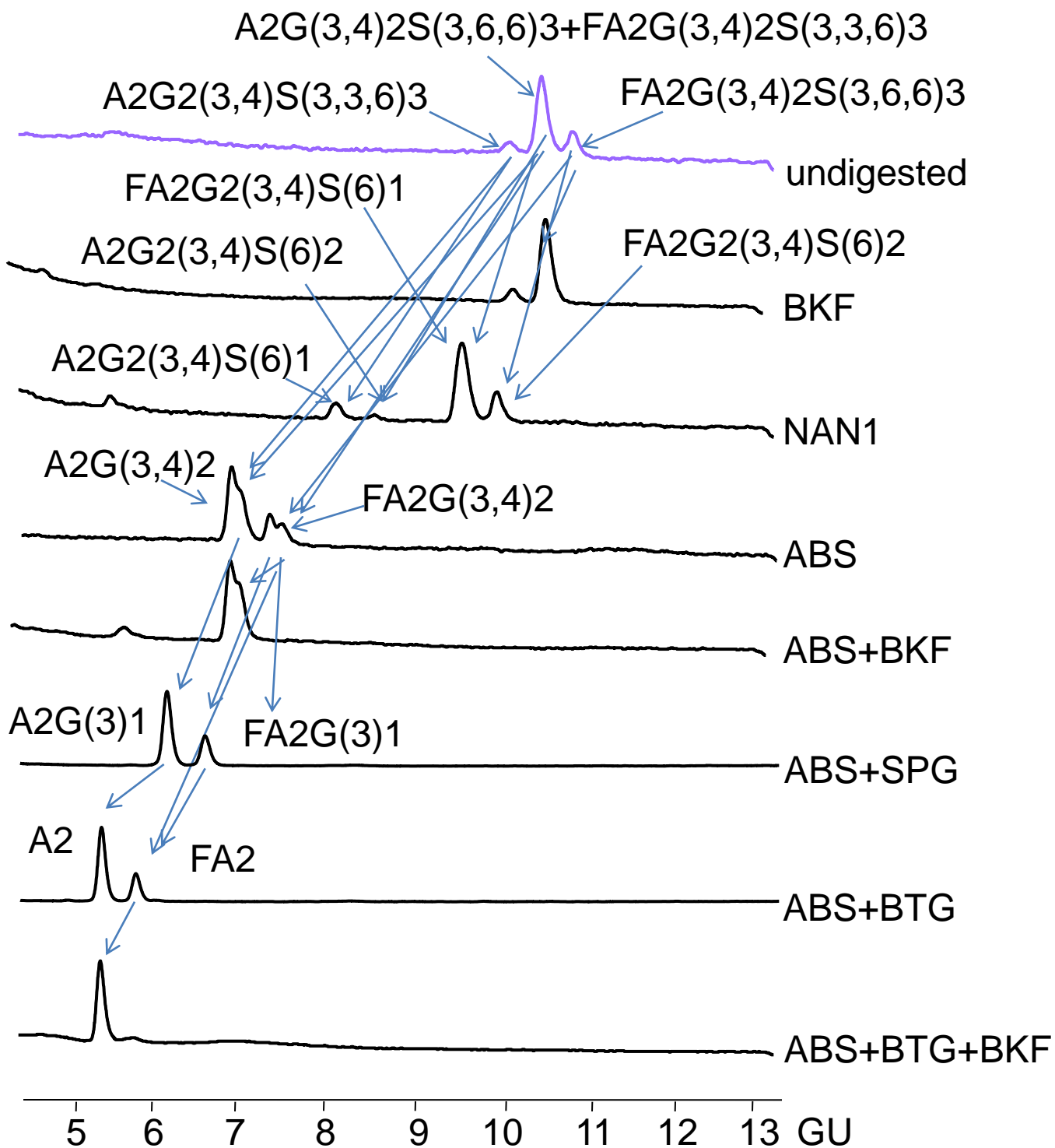
# Disialylated (S2)



# Trisialylated triantennary (S3A)



# Trisialylated biantennary (S3B)





# Tetrasialylated (S4)

