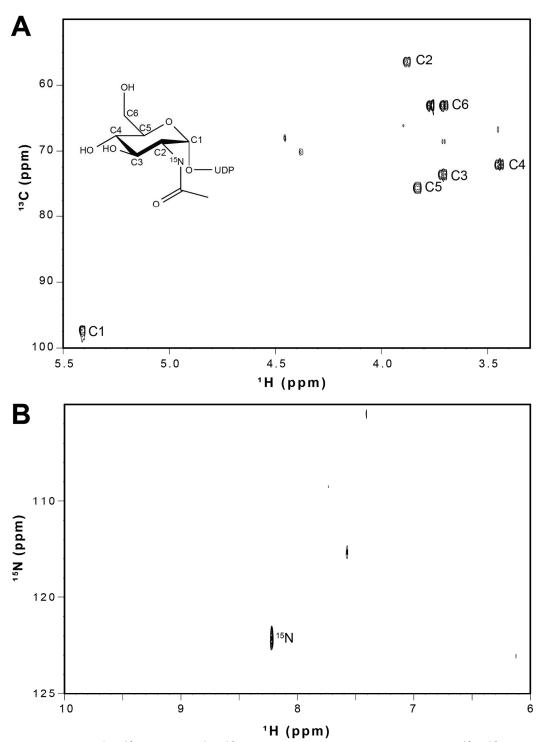
Supplement for:

Intramolecular N-glycan/polypeptide interactions observed at multiple N-glycan remodeling steps through [¹³C,¹⁵N]-*N*-acetylglucosamine labeling of immunoglobulin G1

Adam W. Barb*

Roy J. Carver Department of Biochemistry, Biophysics and Molecular Biology

Iowa State University, Ames, IA 50011



¹H (ppm) Figure S1. 2d ¹H-¹³C (A) and ¹H-¹⁵N HSQC (B) spectra of UDP- α -D-[¹³C, ¹⁵N]-GlcNAc following a partial purification. Resonances in (A) were assigned using COSY spectra (data not shown). The resonance assignment in (B) was confirmed with an ¹H-¹⁵N HSQC of *N-Man5-Fc (Fig. **3**C).

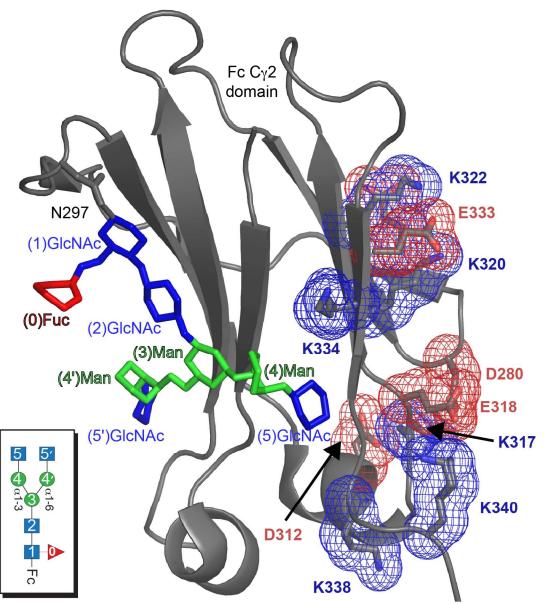


Figure S2. Basic lysine residues are poised to influence the resonance frequency of atoms of the (5)GlcNAc residue. K334 is \sim 8 Å from the (5)GlcNAc and despite the appearance in the figure, D312 is \sim 25 Å away. Carbohydrate residues are colored according to Fig 1. This figure was created using pdb 4ku1¹ and PyMol (Schrödinger).

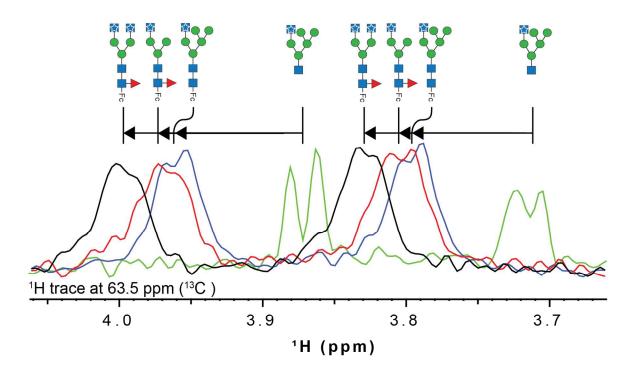


Figure S3. Representative lineshapes and peak positions from (5)-GlcNAc H6 peaks extracted from the same data shown in Figures 5&6, except these spectra were processed without applying a line-broadening window function in the ¹H dimension. Spectra are colored according to the convention adopted in Figures 5-7. Note the clearly resolved ${}^{2}J_{\text{HH}}$ couplings in the EndoF1 treated spectra (green lines).

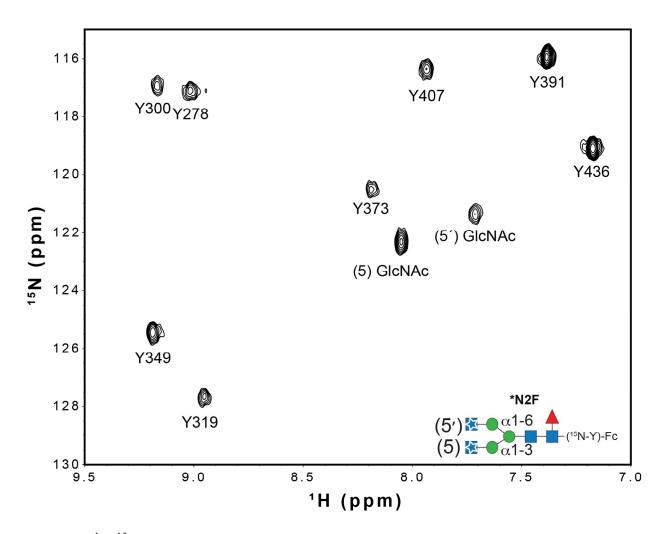


Figure S4. ¹H-¹⁵N-HSQC-TROSY showing selective amino acid and carbohydrate amide resonances. (¹⁵N-Y)-IgG1 Fc was remodeled to the *N2F with [¹³C, ¹⁵N]-GlcNAc. Resonances were assigned based on ² and ³.

Supplemental References

(1) Frank, M.; Walker, R. C.; Lanzilotta, W. N.; Prestegard, J. H.; Barb, A. W. *Journal of molecular biology* **2014**, *426*, 1799.

(2) Matsumiya, S.; Yamaguchi, Y.; Saito, J.; Nagano, M.; Sasakawa, H.; Otaki, S.; Satoh, M.; Shitara, K.; Kato, K. *Journal of molecular biology* **2007**, *368*, 767.

(3) Yamaguchi, Y.; Walchli, M.; Nagano, M.; Kato, K. *Carbohydrate research* **2009**, *344*, 535.