**SUPPORTING INFORMATION**

**Analysis of IgA1 *N*-glycosylation and its contribution to FcαRI binding**

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## Experimental Procedures

*Enzymatic removal of IgA1 N-glycans for FT-ICR MS analysis—N*-glycans were released by treatment with PNGase F as previously described (1) with modifications. The reaction was optimized for release of *N*-glycans from 100 $\mu$g of IgA1 with denatured protein per manufacturer's protocol for glycerol-free PNGase F (New England Biolabs). The reaction was incubated for 28 h at 37° C and glycan removal was verified by monitoring the shift in migration of IgA1 heavy chain on SDS-PAGE under reducing conditions. Released IgA1 *N*-glycans were analyzed by use of a hybrid linear quadrupole ion trap FT-ICR mass spectrometer (LTQ FT MS, Thermo Electron). A Triversa NanoMate was used as the source of electrospray ions. Individual candidate IgA1 *N*-glycans were confirmed by use of collision induced dissociation (CID) in the LTQ and detection of fragment ions in either the ion trap or FT-ICR cell. All identified PNGase F-released IgA1 *N*-glycans were observed with mass accuracies ≤4 ppm.

## Reference

1. Moore, J. S., Wu, X., Kulhavy, R., Tomana, M., Novak, J., Moldoveanu, Z., Brown, R., Goepfert, P. A., and Mestecky, J. (2005) *AIDS* **19**, 381-389

## Figure Legends

**Figure S1: PNGase F-released *N*-glycans.** NanoMate ESI FT-ICR mass spectrum of PNGase F-released IgA1 *N*-glycans from mIgA1κ. The identified series of*N*-glycans were compared to the identified IgA1 tailpiece *N*-glycopeptides to identify putative $C_H2$ domain *N*-glycans. All PNGase F-removed *N*-glycans identified in both mIgA1κ and mIgA1λ analysis were calculated as potential N459 *N*-glycans. Those that were not observed in the IgA1 *N*-glycopeptide LC FT-ICR MS analysis were assigned as $C_H2$ *N*-glycans.

**Figure S2: Representative structural analysis of a triantennary IgA1 *N*-glycan.** The LTQ MS/MS spectrum of PNGase F-released mIgA1λ *N*-glycans demonstrates the structural analysis of a triantennary mIgA1λ *N*-glycan (observed as an *N*-glycopeptide in figure 6A), similar to that shown in Figure 4B. The precursor mass in the FT-ICR MS spectrum (not shown) corresponded to the IgA1 *N*-glycan $[(NeuAc)_1(Gal)_2(GlcNAc)_3(Man)_3(GlcNAc)_2]^{2+}$. Nomenclature for the monosaccharides is the same as in Figure 4.

**TABLE S1**
**Sedimentation coefficients of the species observed in IgA1 samples**
Data was analyzed using the c(s) analysis routine in the program Sedfit.

| Sample | Monomer (Svedberg) | Dimer (Svedberg) | Trimer (Svedberg) | Polymer or high MW aggregate (Svedberg) |
|---|---|---|---|---|
| IgA1κ | 6.2 ± 0.3 | 9.1 ± 0.5 | 12.2 ± 0.6 | 19.7 ± 0.2 |
| IgA1λ | 6.1 ± 0.3 | 9.2 ± 0.3 | 11.6 ± 0.4 | 19.9 ± 0.4 |
| $IgA1_{Mce}$ | 6.3 ± 0.2 | 9.5 ± 0.3 | 12.3 ± 0.5 | 15.5 ± 0.7, 19.9 ± 0.1 |

**Figure S1.**

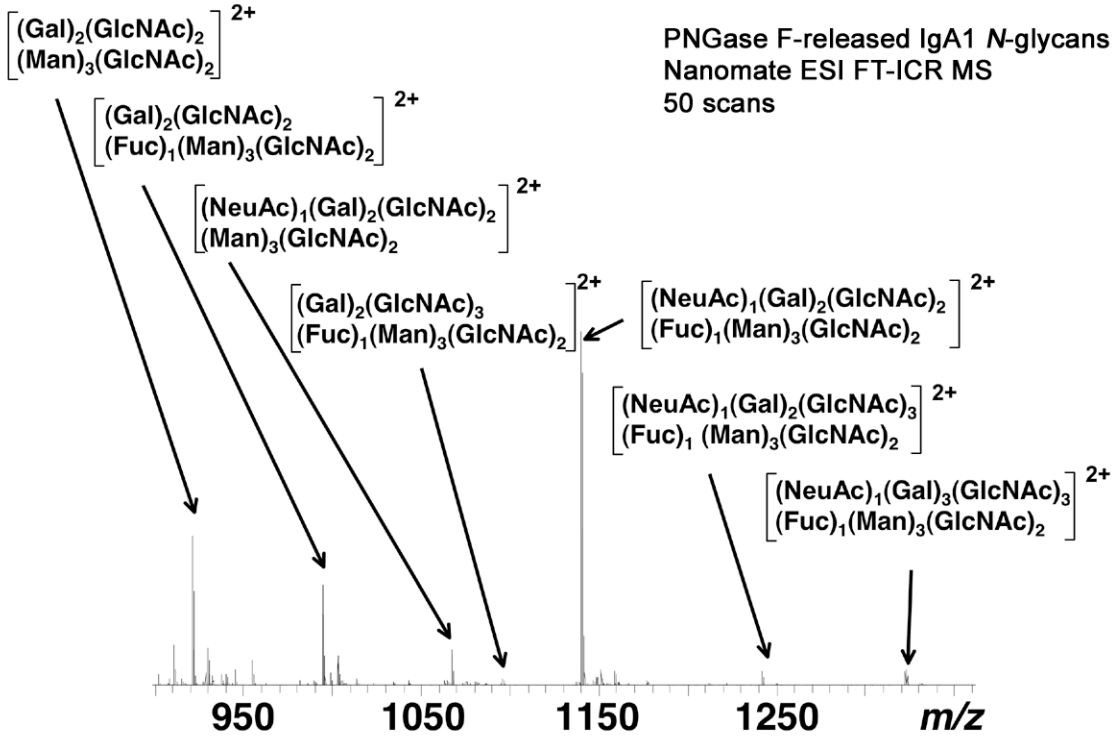
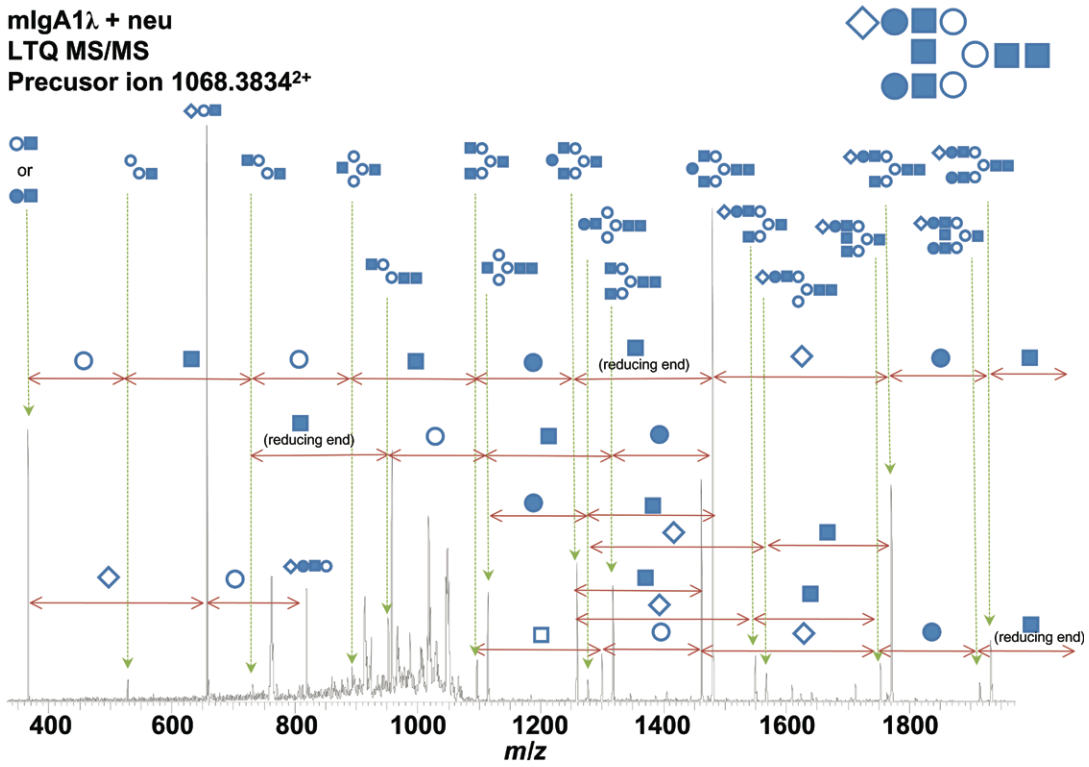


**Figure S2.**


mIgA1λ + neu
LTQ MS/MS
Precusor ion 1068.3834²⁺
or
(reducing end)
(reducing end)
(reducing end)
400
600
800
1000
1200
1400
1600
1800
m/z