Supporting Information for:

Envelope glycans of immunodeficiency virions are almost entirely oligomannose antigens

Katie J. Doores[*1,2], Camille Bonomelli[*3], David J. Harvey[3], Snezana Vasiljevic[3], Raymond A. Dwek[3], Dennis R. Burton[1,2], Max Crispin[3], Christopher N. Scanlan[3†]

[*] These two authors contributed equally to this work.

[†]To whom correspondence should be addressed: chris.scanlan@bioch.ox.ac.uk

[1]Department of Immunology and Microbial Science and IAVI Neutralizing Antibody Center,The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

[2]Ragon Institute of MGH. MIT and Harvard, Boston, MA 02114, USA

[3]Oxford Glycobiology Institute, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK

FIGURE S1

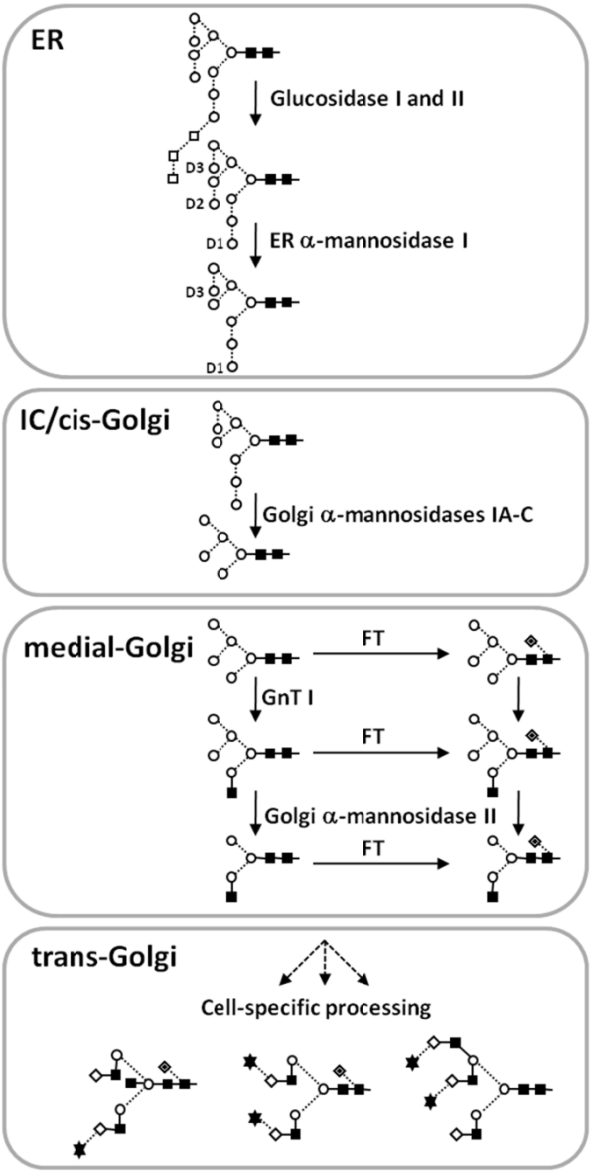


Figure S1. Glycan processing in the secretory system. The processing of N-linked glycans from $Glc_3Man_9GlcNAc_2$ follows a mostly linear pathway in the ER, intermediate compartment (IC), and cis-Golgi compartments, prior to the diversification in the medial and trans Golgi compartments. The first step in N-linked glycosylation is the addition of a glucosylated oligomannose precursor, $Glc_3Man_9GlcNAc_2$, to the nascent polypeptide. This structure is trimmed by ER α-glucosidase I and II, ER α-mannosidase I, and Golgi α-mannosidases IA–C to form the $Man_5GlcNAc_2$ in the IC/cis-Golgi compartment. $Man_5GlcNAc_2$ is the substrate for the medial-Golgi-resident enzyme UDP-*N*-acetyl-D-glucosamine:α-3-D-mannoside β1→2-*N*-acetylglucosaminyltransferase I (GnT I), which catalyses the addition of a single β1→2-linked GlcNAc residue to form $GlcNAc\beta1\rightarrow2Man_5GlcNAc_2$. This structure is then the obligate biosynthetic intermediate

for all subsequent hybrid and complex-type glycosylation in the Golgi apparatus. The tissue-specific expression of post-GnT I glycosidases and glycosyltransferases ensures that cell type, not protein structure, is the dominant factor determining glycoforms at a given glycosylation site. For this reason, glycoproteins from the same cell will usually display similar glycosylation. The position of the terminal D1, D2 and D3 mannoses of the three arms of $Man_9GlcNAc_2$ are indicated.

FIGURE S2

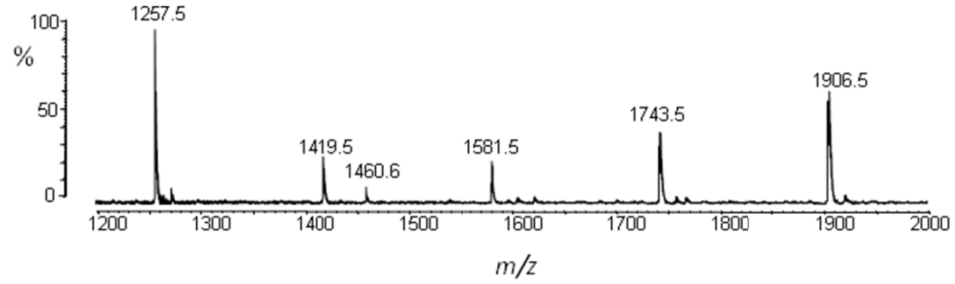


Figure S2. Glycosylation of HIV native env trimer derived from cells treated with swainsonine. MALDI-TOF MS of PNGase F-released N-linked glycans from native envelope of HIV-1_{JRFL} from HEK 293T cells expressed in the presence of 20 μM swainsonine. Masses of glycans correspond to $[M+Na]^+$ ions ($[M+K]^+$ ions are also detected).

FIGURE S3

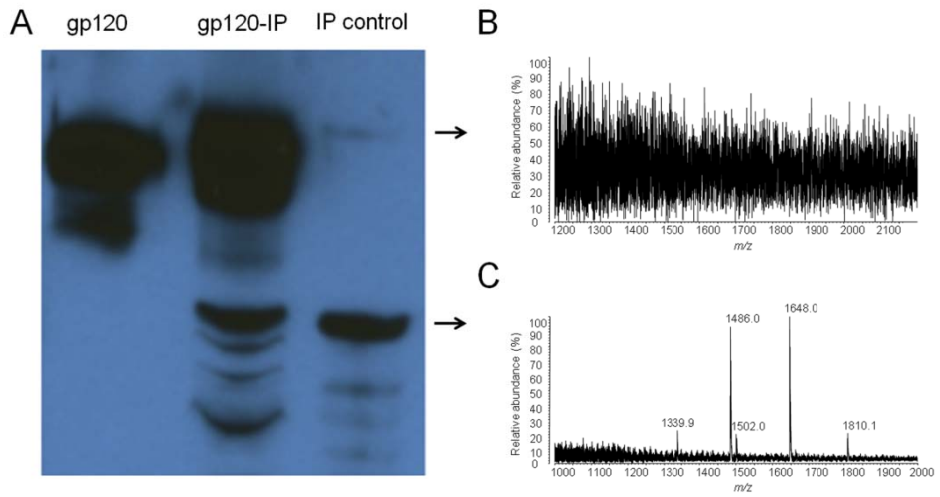


Figure S3. Immunoprecipition of HIV particles. (A) Western blot of recombinant gp120, viral gp120 obtained by immunoprecipitation (IP) with anti-HIV antibodies, and immunoprecipitated supernatant from control, mock-treated cells. MALDI-TOF MS analysis of PNGase F-treated SDS-PAGE bands from the IP control corresponding to (B) the region where gp120 usually migrates, and of (C) the anti-HIV antibodies.

FIGURE S4

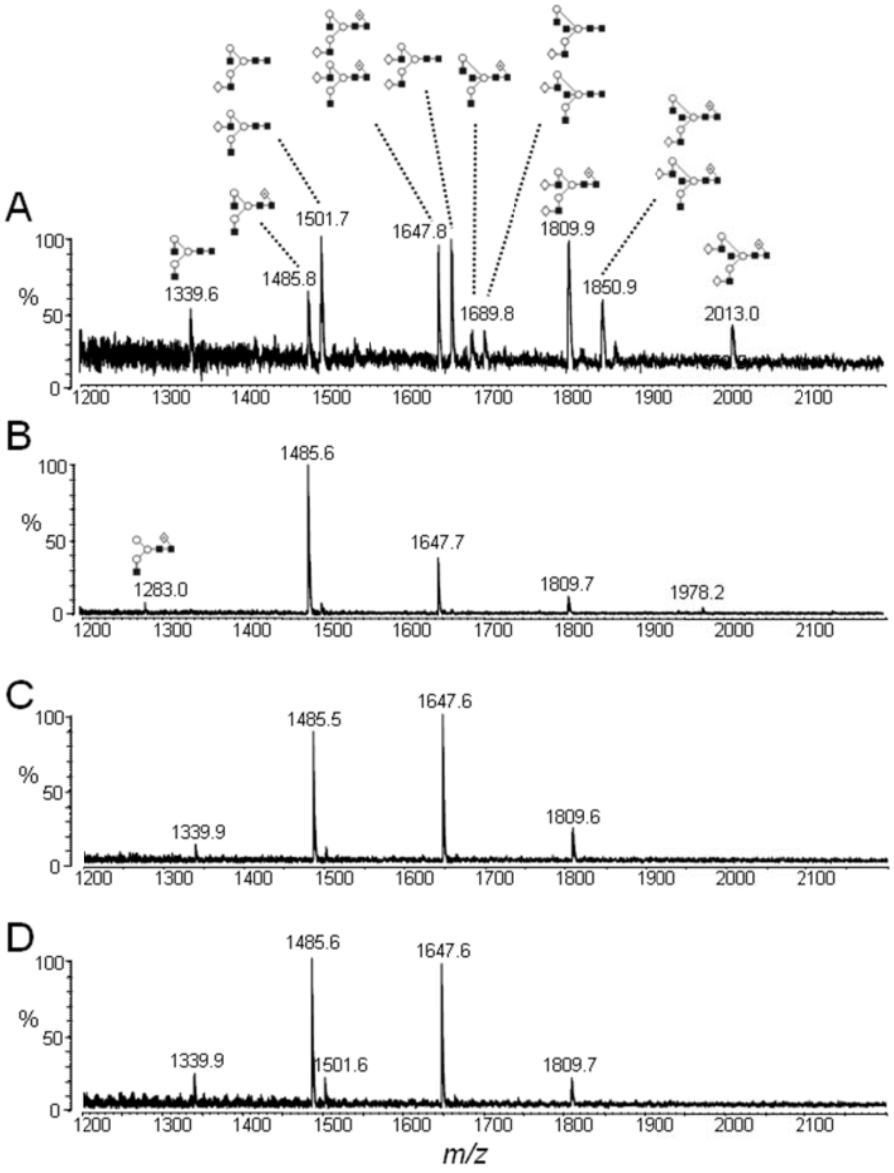


Figure S4. Glycosylation of anti-HIV antibodies. MALDI-TOF MS analysis of PNGase F-released N-linked glycans of anti-HIV antibodies. (A) D7324, (B) F425B4e8 (C) b6 and (D) b12. Masses of glycans correspond to $[M+Na]^+$ ions.

FIGURE S5

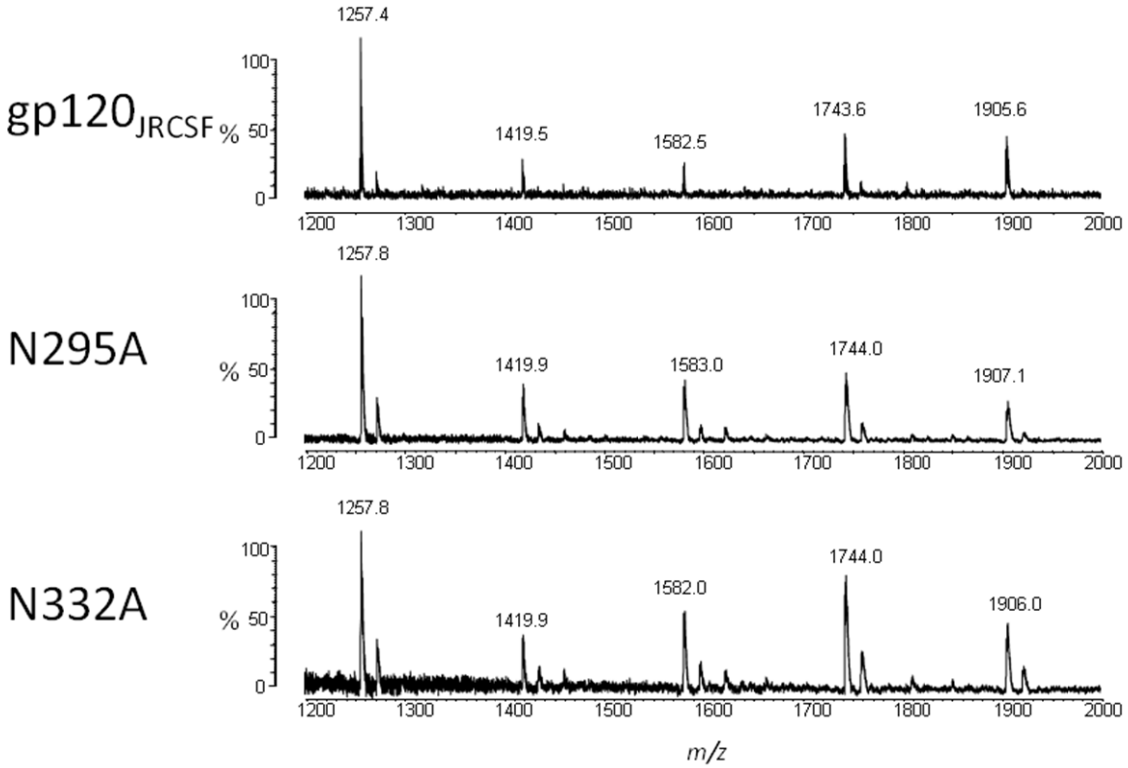
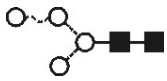
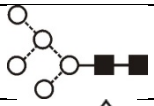
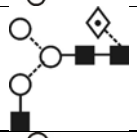
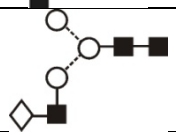
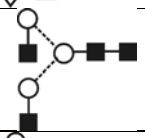
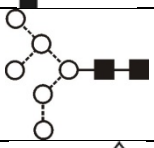
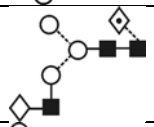
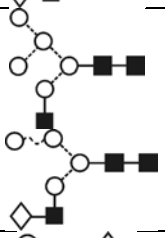
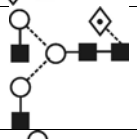
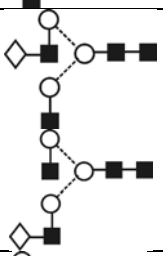
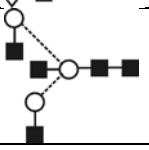
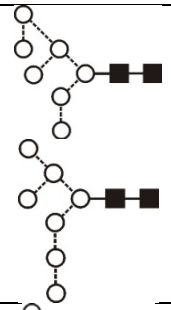
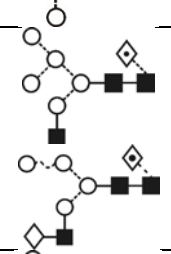
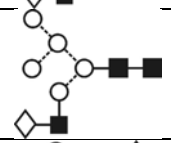
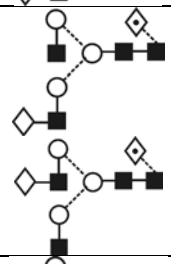
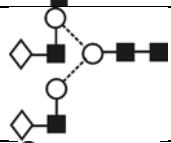
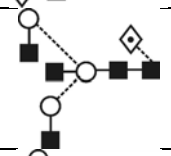
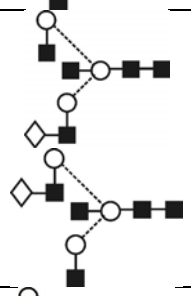
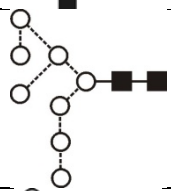
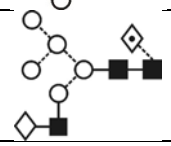
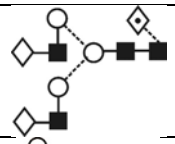
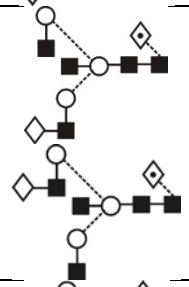
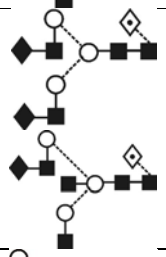
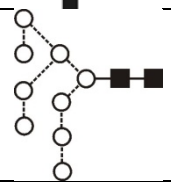
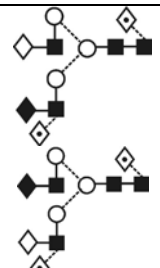
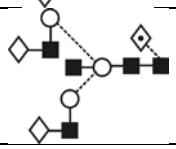
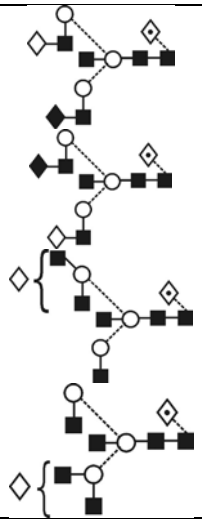


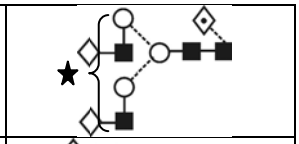
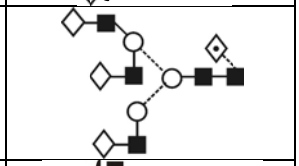
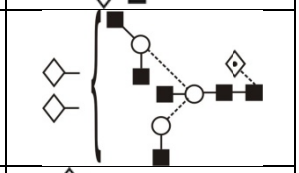
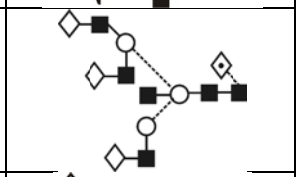
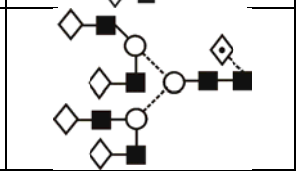
Figure S5. Glycosylation of HIV native env trimer. MALDI-TOF MS of PNGase F-released N-linked glycans from native envelope of HIV-1_{JRCSF} from HEK 293T cells, that of two glycosylation mutants, N295A and N332A, of the same strain. Masses of glycans correspond to $[M+Na]^+$ ions ($[M+K]^+$ ions are also evident).

Table S1. Compositions and masses of the N-linked glycans released from recombinant $gp120_{JRCSF}$ (R-gp120) expressed in HEK 293T and from shed $gp120_{JRCSF}$ (S-gp120), isolated from culture supernatant, both as obtained after desialylation. Symbols refer to the following monosaccharides: ■ = GlcNAc; ○ = Man; ◇ = Gal; ◆ = GalNAc; ◈ = Fuc.

MALDI, *m/z* ([M+Na]$^+$)			Composition			Proposed structure[b]
Found[a]		Calculated[a]	Hex	HexNAc	dHex	
R-gp120	S-gp120					
1095.7	-	1095.4	4	2	0	
1257.8	1257.4	1257.4	5	2	0	
1282.8	-	1282.5	3	3	1	
1298.8	-	1298.5	4	3	0	
1339.8	-	1339.5	3	4	0	
1419.9	1419.3	1419.5	6	2	0	
1444.9	1444.4	1444.5	4	3	1	
1460.8	1460.3	1460.5	5	3	0	
1485.9	1485.4	1485.5	3	4	1	
1501.8	1501.3	1501.5	4	4	0	
1542.9	-	1542.6	3	5	0	

1581.9	1581.3	1581.5	7	2	0	
1606.9	1606.4	1606.6	5	3	1	
1622.9	1622.3	1622.6	6	3	0	
1647.9	1647.4	1647.6	4	4	1	
1663.9	1663.3	1663.6	5	4	0	
1688.9	1688.3	1688.6	3	5	1	
1704.9	-	1704.6	4	5	0	
1743.9	1743.3	1743.6	8	2	0	
1768.9	1768.3	1768.6	6	3	1	

1810.0	1809.3	1809.7	5	4	1	
1850.9	1850.3	1850.7	4	5	1	
1892.0	-	1891.7	3	6	1	
1905.9	1905.5	1905.6	9	2	0	
1956.0	-	1955.7	5	4	2	-
1997.1	-	1996.7	4	5	2	
2013.0	2012.4	2012.7	5	5	1	
2029.0	2028.3	2028.7	6	5	0	-
2054.0	-	2053.7	4	6	1	

2122.9	-	2122.7	5	4	1	
2175.0	2174.3	2174.8	6	5	1	
2216.0	-	2215.8	5	6	1	
2378.0	-	2377.9	6	6	1	
2540.9	-	2539.9	7	6	1	

a) Monoisotopic mass except where indicated.
b) Proposed structures are based on previously published fragmentation data of glycans from HEK 293T cells and represent the most likely isomer.