

Fig 1. Full IgY Sequence: (A) The complete amino acid sequence of IgY upsilon heavy chain including leader sequence and rearranged VDJ sequences and (B) The amino acid sequence chicken λ light chain. Numbering for chicken IgY heavy chain is based on the deduced amino acid sequences from cDNA, starting from the first alanine in the V_H region [42,43] Numbering for chicken light chain immunoglobulin is derived from the nucleotide sequence from recombinant cDNA plasmids constructed from chicken spleen poly(A)-containing RNA [43].

Supplementary Information: Generation of IgY model:

Avian IgY	AVTLDESGGGLQTPGRALSLVCLASGFTFSSYDMGWVQQAPGLGLEFVPGIONTGR YTGYGSAVLGRATISRDNQSTVAIQLNLRRAEDTGTTTCAKAAGSGYCGWGTAGS IDAWGHGTEVIVSSASPTSPRLYLPLSACCSDSAVPPAVGCLLSPSSAGGISWEGS
hIgE	GGTAVAGRVSGTPVKLSFVRLSPGEEKRKSFVCSAAPGGALLKKEVQVCRVDPVPPV DF APEVQVLHASSCT--PSQSEVELLCLVTFGSPASAEVEWLVDGVGLLVASQSPA TPPTVKILQSSCDGGGHFPPTIQLLCLVSGYTPGTINITWLEDGQVMDVDLSTAST VRSGSTYSLSSRVNVS GTDWREGKSYS CRVRHPATNTVVEDHVKGCPDGAQSCSPI TQEGELASTQSELTLSQKHWSDRITYTCQVTYQGH--TFEDSTKKCADS--NPRGV <i>QLYAIPPSPGELYISLDAKLRCLVVNL-PSDSSLVTWTREKSGNLRPDPMLQEH</i> <i>SAYLSRPSFDLFIKRSPTITCLVVDLAPSKGTVNLTWSRASGKPVNHSTRKEEKQ</i> <i>FNGT</i> YASASSAVPVSTQDWLSGERFTCTVQHEELPLPLSKSVYRNTGPTTPPLIYPF <i>RNGT</i> LTVTSTLPLVGTRDWIEGETYQCRVTHPHLPRALMRSTTKTSGPRAAPEVYAF APHPEELSLSRVTLSCLVRGFRPRDIEIRWLRDHRAVPATEFVTTAVLPEERTANG ATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKTK----G DGDTEFFVYSKMSVETAKW-NGGTVACMAVHEAL-PMRFSQRTLQKQAGK SG--FFVFSRLEVTRAWEQKDEFICRAVHEAASPSQTVQRAVSVNPG

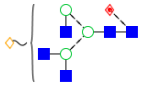
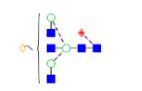
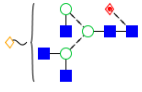
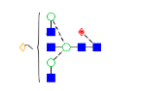
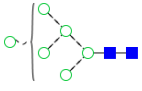
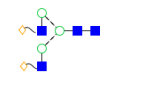
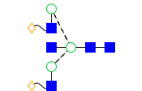
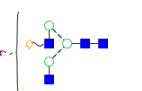
Avian IgY Sequence (Supplementary Figure 1): The section in italics corresponds to the sequence from the crystal structure 2w59.pdb- The crystal structure of avian IgY-Fc-3-4 Fragment.

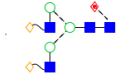

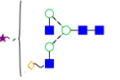
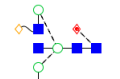


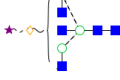
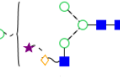
hIgE – Sequence from the crystal structure 1o0v.pdb - The crystal structure of human IgE Fc.

The sequence alignment for the region PVAPEVQV ... PDGAQSCS of IgY to hIgE was based on the Clustal alignment of the two sequences.

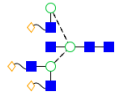
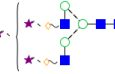
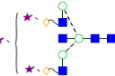


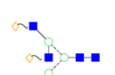

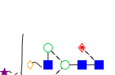
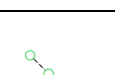

The sequence alignment for the region PIQLYAIP ... RTLQKQAG of IgY to hIgE was performed by alignment of the three-dimensional structures (2w59 and 1o0v) in pymol.

The homology model was generated using: (i) a hIgG Fab domain crystal structure (for the IgY light chain and the IgY heavy chain sequence AVTLDESG ... LKKEVQVC) which was linked to; (ii) a random coil sequence RVDVPP (for the IgY heavy chain linker region between domains Cu1 and Cu2) which was linked to; (iii) the hIgE Fc crystal structure (for the IgY heavy chain sequence PVAPEVQV ... RTLQKQAG).


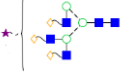
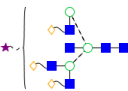
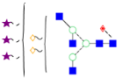
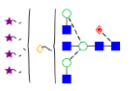

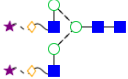
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12	FA3G1		2.26	6.90	972.8573	972.8654	[M-2H] ⁻²
	FA2BG1						
13	FA3G1		1.22	7.04	972.8509	972.8654	[M-2H] ⁻²
	FA2BG1						
14	M6		0.44	7.14	757.2596	757.2702	[M-2H] ⁻²
	A2G2				879.3184	879.3232	[M-2H] ⁻²
15	A2BG2		0.92	7.32	980.8661	980.8629	[M-2H] ⁻²
	A2[6]G1S1*				943.8293	943.8445	[M-2H] ⁻²



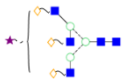


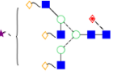



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16	FA2G2		0.59	7.52	952.3262	952.3521	[M-2H] ⁻²
	M5A1G1				858.7934	858.8099	[M-2H] ⁻²
	A2[3]G1S1*				943.8293	943.8445	[M-2H] ⁻²
17	FA2BG2		5.14	7.69	1053.8828	1053.8918	[M-2H] ⁻²
	FA3G2				---	1045.3842	[M-2H] ⁻²
	A3G1S1*				---	1045.3842	[M-2H] ⁻²
	A2BG1S1*				923.3214	923.3312	[M-2H] ⁻²
	M4A1G1S1*						

Peak	Structure		Peak area (%)	GU	Experimental mass (m/z)	Theoretical mass (m/z)	Ion Specie
18	FA3'G2		1.60	7.93	1053.8828	1053.8918	[M-2H] ⁻²
	FA2BG1S1*				1118.4037	1118.4131	[M-2H] ⁻²
	FA3G1S1*						
19	M7		0.51	8.02	838.2908	838.2966	[M-2H] ⁻²
	FA2BG1S1*				1118.4243	1118.4131	[M-2H] ⁻²
	FA3G1S1*						
20	A3G3		16.38	8.36	---	1061.8893	[M-2H] ⁻²
	A2G2S1*				1024.8506	1024.8709	[M-2H] ⁻²
	A2G2S3*				---	1315.9663	[M-2H] ⁻²

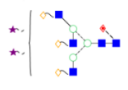
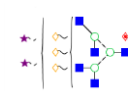

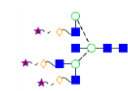

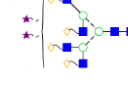
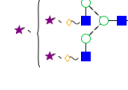
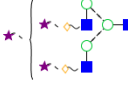
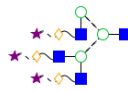
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21	A3BG3		0.65	8.51	---	1163.4290	[M-2H] ⁻²
	A2G2S3*				---	1315.9663	[M-2H] ⁻²
	A2BG2S3*				---	1417.5060	[M-2H] ⁻²
22	FA3G3		2.12	8.64	1134.9095	1134.9182	[M-2H] ⁻²
	A2BG2S1*				1126.4175	1126.4106	[M-2H] ⁻²
	A3'G3				---	1061.8893	[M-2H] ⁻²
23	M8		4.00	8.78	919.3203	919.3230	[M-2H] ⁻²
	FA2G2S1*				1097.8785	1097.8998	[M-2H] ⁻²
	M5A1G1S1*				1004.3476	1004.3576	[M-2H] ⁻²
	FA2G2S3*				---	1388.9953	[M-2H] ⁻²

Peak	Structure		Peak area (%)	GU	Experimental mass (m/z)	Theoretical mass (m/z)	Ion Specie
24	FA3'G3		8.68	9.00	1134.9095	1134.9182	[M-2H] ⁻²
	FA2BG2S1*				1199.4524	1199.4395	[M-2H] ⁻²
	FA3G2S1*				1199.4524	1199.4395	[M-2H] ⁻²
25	FA4G3		3.93	9.23	---	1236.4579	[M-2H] ⁻²
	FA3'G2S1*				1199.4240	1199.4395	[M-2H] ⁻²
	A2G2S2*				1170.4113	1170.4186	[M-2H] ⁻²
	FA2BG2S3*				---	1490.5349 993.3542	[M-2H] ⁻² [M-3H] ⁻³
	M5A1G1S3*				---	1295.4530 863.2996	[M-2H] ⁻² [M-3H] ⁻³
	FA3G2S3*				---	1490.5349 993.3542	[M-2H] ⁻² [M-3H] ⁻³

Peak	Structure		Peak area (%)	GU	Experimental mass (m/z)	Theoretical mass (m/z)	Ion Specie
26	M9		5.11	9.51	1000.3368	1000.3495	[M-2H] ⁻²
	A3G3S1*				1207.4227	1207.4370	[M-2H] ⁻²
27	A3BG3S1*		24.92	9.65	---	1308.9767	[M-2H] ⁻²
	FA3'G2S3*				---	1490.5349 993.3542	[M-2H] ⁻² [M-3H] ⁻³
	FA2BG1S4*				---	1555.0562 1036.3684	[M-2H] ⁻² [M-3H] ⁻³
	FA3G1S4*				---	1555.0562 1036.3684	[M-2H] ⁻² [M-3H] ⁻³
	A2G2S2*				1170.4043	1170.4186	[M-2H] ⁻²

Peak	Structure		Peak area (%)	GU	Experimental mass (m/z)	Theoretical mass (m/z)	Ion Specie
28	FA3G3S1*		0.67	9.82	---	1280.4659	[M-2H] ⁻²
	A4G4				1244.4453	1244.4554	[M-2H] ⁻²
	A3'G3S1*				1207.4299	1207.4370	[M-2H] ⁻²
	A2BG2S2*				---	1271.9583	[M-2H] ⁻²
	A4G4S4*				---	1826.6462 1217.4284	[M-2H] ⁻² [M-3H] ⁻³
29	FA3'G3S1*		0.24	9.90	---	1280.4659	[M-2H] ⁻²
	FA2G2S2*				1243.4315	1243.4476	[M-2H] ⁻²
30	FA4G4		1.19	10.06	---	1317.4843	[M-2H] ⁻²
	FA2BG2S2*				1344.9840	1344.9872	[M-2H] ⁻²

Peak	Structure		Peak area (%)	GU	Experimental mass (m/z)	Theoretical mass (m/z)	Ion Specie
31	M9Glc		10.11	10.24	1081.3684	1081.3759	[M-2H] ⁻²
	FA3'G2S2*				1344.9840	1344.9872	[M-2H] ⁻²
	FA2G2S4*				---	1534.5430 1022.6929	[M-2H] ⁻² [M-3H] ⁻³
	A3G3S2*				1352.9702	1352.9847	[M-2H] ⁻²
32	A3BG3S2*		0.97	10.67	---	1454.5244	[M-2H] ⁻²
33	A3'G3S2*		1.11	10.80	1352.9626	1352.9847	[M-2H] ⁻²
34	FA3G3S2*		0.65	11.17	---	1426.0137	[M-2H] ⁻²
	A3G3S3*				1498.5284 998.6880	1498.5324 998.6858	[M-2H] ⁻² [M-3H] ⁻³

Peak	Structure		Peak area (%)	GU	Experimental mass (m/z)	Theoretical mass (m/z)	Ion Specie
35	FA3'G3S2*		0.06	11.33	---	1426.0137	[M-2H] ⁻²
	FA4G3S2*				---	1527.5533	[M-2H] ⁻²
	A3G3S3*				1498.5126 998.6880	1498.5324 998.6858	[M-2H] ⁻² [M-3H] ⁻³
	A3BG3S3*				---	1673.1010 1115.0649	[M-2H] ⁻² [M-3H] ⁻³
36	A3G3S3*		0.10	11.46	1498.5284 998.6880	1498.5324 998.6858	[M-2H] ⁻² [M-3H] ⁻³
	A4G4S2*				1535.5466	1535.5508	[M-2H] ⁻²
	A2G2S3*				---	1315.9663 876.9757	[M-2H] ⁻² [M-3H] ⁻³
37	A2G2S3*		2.81	11.59	---	1315.9663 876.9757	[M-2H] ⁻² [M-3H] ⁻³
	A3G3S3*				1498.5284 998.6880	1498.5324 998.6858	[M-2H] ⁻² [M-3H] ⁻³

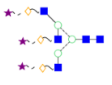
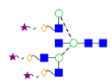
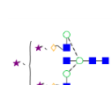
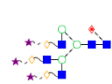
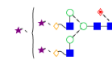
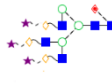
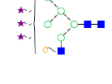
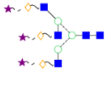
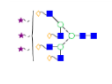
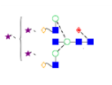
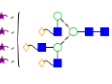
Peak	Structure		Peak area (%)	GU	Experimental mass (m/z)	Theoretical mass (m/z)	Ion Species
38	A3'G3S3*		0.13	11.92	---	1498.5324 998.6858	[M-2H] ⁻² [M-3H] ⁻³
	A3BG3S3*				---	1600.0721 1066.3790	[M-2H] ⁻² [M-3H] ⁻³
	A2BG2S3*				---	1417.5060 944.6682	[M-2H] ⁻² [M-3H] ⁻³
	FA3G3S3*				---	1571.5614 1047.3718	[M-2H] ⁻² [M-3H] ⁻³
39	FA2G2S3*		0.52	12.09	---	1388.9953 925.6611	[M-2H] ⁻² [M-3H] ⁻³
	FA3G3S3*				---	1571.5614 1047.3718	[M-2H] ⁻² [M-3H] ⁻³
40	M5A1G1S3*		0.10	12.66	---	1295.4530 863.2996	[M-2H] ⁻² [M-3H] ⁻³
	A3'G3S3*				1498.5284 998.6880	1498.5324 998.6858	[M-2H] ⁻² [M-3H] ⁻³
	A4G4S3*				---	1681.0985 1120.3966	[M-2H] ⁻² [M-3H] ⁻³
	FA2BG2S3*				---	1490.5349 993.3542	[M-2H] ⁻² [M-3H] ⁻³
	A3G3S4*				---	1644.0801 1095.7177	[M-2H] ⁻² [M-3H] ⁻³

Table of *N*-glycans released from IgY purified from serum. The HILIC-chromatogram was separated into 40 peaks and structural assignment carried out using established methods (Royle et al., 2008) and the software tool GlycoBase (<https://glycobase.nibrt.ie>). *Sialic acid linkages; WAX fractions were separated out into 5 fractions; S1: Monosialylated, S2: Disialylated, S3: Trisialylated, S3J: Trisialylated and S4: Tetrasialylated. All monosialylated glycans are linked by α 2-3 and α 2-6; Disialylated glycans have all combinations of α 2-3 and α 2-6 linkages [i.e. (3, 3), (3, 6) and (6, 6)]; Trisialylated glycans have all combinations of α 2-3 and α 2-6 linkages except (6, 6, 6) [i.e. (3, 3, 3), (3, 3, 6) and (3, 6, 6)]; and Tetrasialylated glycans have all combinations of α 2-3 and α 2-6 linkages [i.e. (3,3,3,3), (3,3,3,6), (3,3,6,6) and (6,6,6,6)]. Structure abbreviations: all *N*-glycans have two core GlcNAcs; F at the start of the abbreviation indicates a core-fucose α 1,6-linked to the inner GlcNAc; M_x, number (x) of mannose on core GlcNAcs; A_x, number of antenna (GlcNAc) on trimannosyl core; A2, biantennary with both GlcNAcs as β 1,2-linked; A3, triantennary with a GlcNAc linked β 1,2 to both mannose and the third GlcNAc linked β 1,4 to the α 1,3 linked mannose; A3', isomer with the third GlcNAc linked β 1-6 to the α 1-6 linked mannose; A4, GlcNAcs linked as A3 with additional GlcNAc β 1,6 linked to α 1,6 mannose; B, bisecting GlcNAc linked β 1,4 to β 1,3 mannose; G_x, number (x) of β 1,4 linked galactose on antenna; F(x), number (x) of fucose linked α 1,3 to antenna GlcNAc; S_x, number (x) of sialic acids linked mostly to galactose (Structures with more sialic acids than galactoses may have some sialic acids linked to GlcNAc or another sialic acid in form of polysialic acid.).

