

# Crystal structure of sialylated IgG Fc: Implications for the mechanism of IVIg therapy

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Intravenous immunoglobulin (IVIg) consists of pooled human serum IgG and is widely used as an anti-inflammatory. The fraction of IgG that is  $\alpha$ 2,6-sialylated exhibits anti-inflammatory activity and sialylation is proposed to enable binding to the cell surface lectin, DC-SIGN (1). In a recent article in this journal, Sonderrmann *et al.* propose a mechanism to explain the putative sialic acid-dependent binding of IgG Fc to DC-SIGN as well as to the IgE receptor, CD23 (1). The core of their hypothesis is that sialylation of IgG Fc leads to a conformational change which triggers DC-SIGN binding. To directly assess this hypothesis, we have generated  $\alpha$ 2,6-sialylated IgG1 Fc (sFc) which we both chemically verified by HPLC analysis (Fig. 1A) and structurally characterized by X-ray crystallographic analysis to a resolution of 2.3 Å (Fig. 1B, 1C, and Table 1).

We observe no conformational changes to the Fc upon sialylation. In particular, we find no evidence of a ‘closed’ state resembling the structure of IgE in complex with CD23 that could account for the reported interaction with DC-SIGN or CD23 (1). Instead, the tertiary and quaternary structure of sFc is strikingly similar to that of a previously reported IgG structure bearing non-sialylated, but still galactosylated, complex-type glycans (e.g. root-mean-square-deviation of 0.8 Å over 405 equivalent C $\alpha$  positions with PDB ID 1H3Y) (Fig. 1B) (2). We also determined the structure of an alternative low-resolution sFc crystal form at pH 7.5 which also exhibits a typical IgG Fc conformation (data not shown). Whilst large alterations in Fc conformations are conceivable, our data suggest that lattice or pH effects do not preclude their observation by X-ray crystallography.

Our crystallographic analysis also shows that Fc sialylation does not perturb the classical protein–carbohydrate interactions observed of asialylated IgG Fc (Fig. 1C). We note that these observations are consistent with solution-state NMR measurements (3). We observe extensive electron density corresponding to 20 monosaccharide residues (Fig. 1C). There are two sialic acid attachment sites per biantennary N-linked glycan on the so-called ‘3-arm’ and ‘6-arm’ (Fig. 1C). There is observable electron density for sialic acid on the 6-arm and, in contrast with the proposed model by Sondermann *et al.* (1), this glycan moiety projects in a solvent-exposed manner away from the protein surface. Similarly, the 3-arm is not, as proposed, tightly immobilised to the protein surface but is instead entirely solvent exposed.

We note that the experimental support for the proposed conformational changes includes circular dichroism, which gives exquisitely sensitive readouts to changes in local but not tertiary structure. In addition, the melting temperature shift observed by Sondermann *et al.* is well within the range of other Fc glycoforms, whereas deglycosylation leads to a more significant change in stability (4).

In light of these results, the mechanism by which sialylated IgG Fc mediates anti-inflammatory activity must remain an open question. Indeed, whilst DC-SIGN may well function in the immunological signalling pathway, there is still some uncertainty whether there is a direct interaction between sialylated IgG Fc and DC-SIGN (5).

## REFERENCES

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## FIGURE LEGEND

Fig. 1. Crystal structure of sialylated IgG1 sFc. (A) HPLC of fluorescently-labelled N-linked glycans released from recombinant, hypersialylated IgG Fc. Hypersialylation and HPLC analysis was performed as previously described (5). (B) Overlay of sFc (gray) with the asialylated glycoforms (blue; PDB 1H3Y). (C) Structure of sFc with monosaccharide residues colored according to Panel A with close-up views of the glycans with a  $2F_o - F_c$  electron density map contoured around the glycans at  $1\sigma$ . For crystallographic analysis, sFc (6.5 mg/mL) was crystallized at 25 °C in 4.0 M NaCl, 0.1 M citrate pH 4.0 using the sitting-drop vapour diffusion method using 100 nL protein plus 100 nL precipitant equilibrated against 95  $\mu$ L reservoirs. Crystals were flash frozen by immersion in a cryoprotectant containing the mother liquor diluted in 5 M NaCl and then rapidly transferred to a gaseous nitrogen stream. X-ray diffraction data were recorded at Beamline I04 at Diamond Light Source, Oxfordshire, England. Data were processed and scaled using XIA2 and the structure was solved using Phaser with native Fc (PDB accession number 3AVE) as a search model. Model building was performed with Coot and iteratively refined in the CCP4 supported program REFMAC5. Coordinates and structure factors of sFc have been deposited in the Protein Data Bank (PDB ID XXX).

TABLE 1

**Table 1.** Crystallographic data and refinement statistics of sFc.

<b>Data collection</b>	
Beamline	Diamond I04
Resolution range (Å)	66.0–2.30 (2.36–2.30) <sup>a</sup>
Space group	<i>P</i> 6 <sub>1</sub> 22
Cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	153.0, 153.0, 111.9
$\alpha$ , $\beta$ , $\gamma$ (°)	90.0, 90.0, 120.0
Wavelength (Å)	0.979
Unique reflections	34,456 (2,513)
Completeness (%)	99.5 (99.3)
<i>R</i> <sub>merge</sub> (%) <sup>b</sup>	7.3 (76.6)
<i>I</i> / $\sigma$ <i>I</i>	17.7 (2.8)
Ave redundancy	8.7 (8.5)
<b>Refinement</b>	
Resolution range (Å)	66.0–2.30 (2.36–2.30)
Number of reflections	32,683 (2,390)
<i>R</i> <sub>work</sub> (%) <sup>c</sup>	22.1
<i>R</i> <sub>free</sub> (%) <sup>d</sup>	25.8
r.m.s.d. <sup>e</sup>	
Bonds (Å)	0.009
Angles (°)	1.5
Homodimers per a.s.u. <sup>f</sup>	1
Atoms per a.s.u.	3,359/251/161
(protein/carbohydrate/water)	
Average <i>B</i> -factors (Å <sup>2</sup> )	66.5/78.5/47.7
(protein/carbohydrate/water)	
Model quality (Ramachandran plot)	
Most favored region (%)	97.1
Allowed region	2.9

<sup>a</sup>Numbers in parentheses refer to the relevant outer resolution shell.

<sup>b</sup> $R_{\text{merge}} = \sum_{\text{hkl}} \sum_i |I(\text{hkl}; i) - \langle I(\text{hkl}) \rangle| / \sum_{\text{hkl}} \sum_i I(\text{hkl}; i)$

where  $I(\text{hkl}; i)$  is the intensity of an individual measurement and  $\langle I(\text{hkl}) \rangle$  is the average intensity from multiple observations.

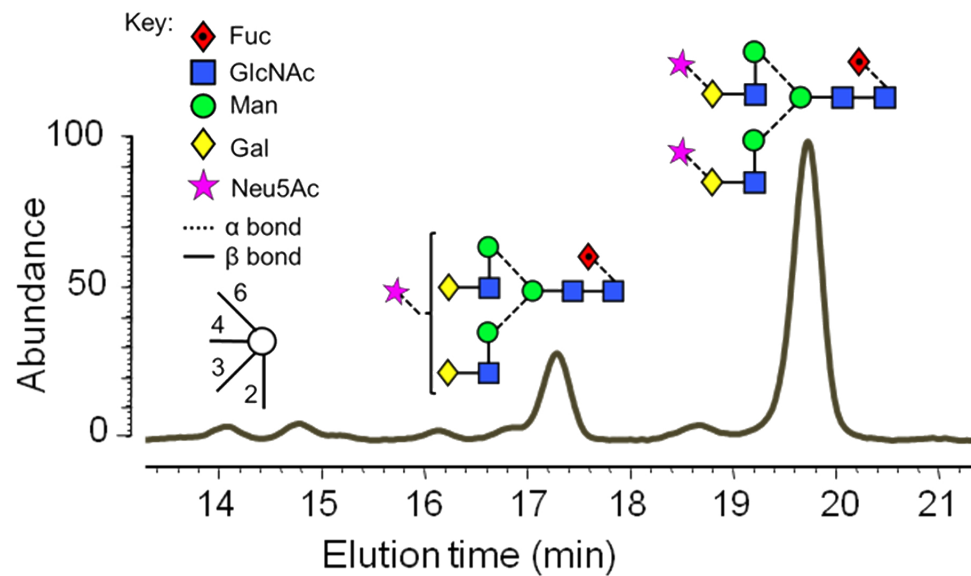
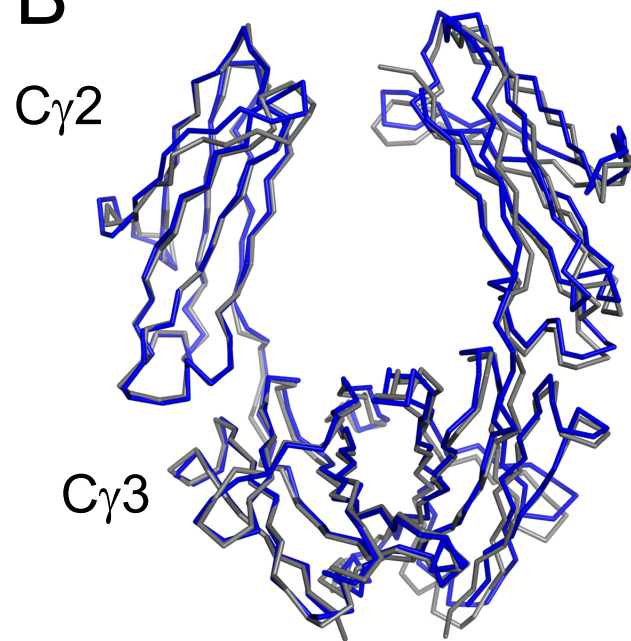
<sup>c</sup> $R_{\text{work}} = \sum_{\text{hkl}} ||F_{\text{obs}}| - k|F_{\text{calc}}|| / \sum_{\text{hkl}} |F_{\text{obs}}|$

<sup>d</sup>*R*<sub>free</sub> is calculated as for *R*<sub>work</sub>, but using only 5% of the data which were sequestered prior to refinement.

<sup>e</sup>r.m.s.d.: root mean square deviation from ideal geometry.

<sup>f</sup>a.s.u.: asymmetric unit.



**A****B****C**