Impact of the N-glycosylation on full-length IgG2 and IgG4 antibodies: a comparative study using molecular dynamics simulations

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Monoclonal antibodies (mAbs) are key therapeutic and diagnostic tools whose function depends on their structural integrity and post-translational modifications. N-glycosylation at the conserved Asn-297 residues of the Fc domain is known to modulate Fcγ receptor binding and Fc stability. However, the influence of glycans on the conformational dynamics of the full-length antibody remains poorly characterised. Most computational studies are limited to isolated Fc domains or rely on homology-modelled structures with reconstructed glycan chains, potentially misrepresenting the native ensemble.

In this work, we investigate two full-length mAbs with experimentally resolved native glycan chains retrieved from the PDB: Pembrolizumab, an inhibitor targeting PD-1, and Mab231, an anti-canine lymphoma antibody. Each system was simulated in its glycosylated and aglycosylated forms using all-atom molecular dynamics for a total of 1.5 µs across three independent replicates. Conformational sampling was characterised by RMSD, RMSF, conformational entropy, dynamic cross-correlation matrices and PCA. Glycan–protein contacts and allosteric communication networks were computed using MDAnalysis and the allopath tool (Delemotte et al.) (Figure 1).

Our results reveal that N-glycosylation has a limited effect on local residue flexibility but significantly reshapes the inter-domain dynamic cross-correlation landscape in an isoform-specific manner. Contrary to the prevailing assumption that glycans primarily stabilise the Fc region, we demonstrate that their mechanical influence extends to the Fab domains. Allosteric network analysis confirms that both glycans propagate conformational information into the Fab arms, with the magnitude differing markedly between PMB and Mab231. PCA reveals no clear separation between glycosylation states, suggesting that glycosylation restricts conformational sampling rather than selecting qualitatively distinct conformations.

This study provides the first comprehensive, atomistic characterisation of N-glycosylation dynamics in full-length IgG2 and IgG4 antibodies using native PDB structures with intact glycan chains. Our findings underscore the importance of considering the complete antibody architecture in studies of glycosylation, and provide a mechanistic basis for understanding how glyco-engineering strategies may modulate both effector functions and antigen-binding properties. The preprint is available at https://doi.org/10.64898/2026.04.14.718417.

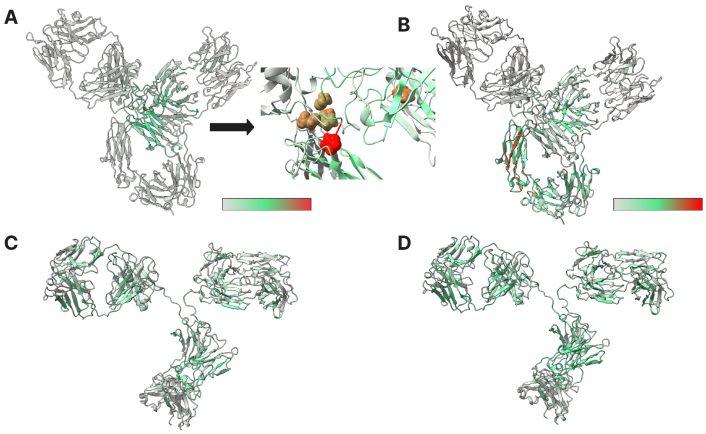

Figure 1. *Allosteric networks propagated from glycan chains (source) to the antibody residues (sink) for PMB and Mab231. Colour intensity reflects the degree of residue involvement. A) CARA on PMB; B) CARB on PMB; C) CARC_1 on Mab231; D) CARC_2 on Mab231. Note the significant involvement of Fab-domain residues, particularly for CARA in PMB.*

References

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