

# Review of: "Glycan shield of the ebolavirus envelope glycoprotein GP"

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The envelope protein GP of ebolaviruses is heavily glycosylated with N-linked, O-linked, and C-linked mannosylation sites. In this paper, the authors used MS-based glycoproteomics to develop a comprehensive overview of ebolavirus GP glycosylation (two species, EBOV and BDBV) in samples derived from HEK293 cells and S2 insect cells. They noted that two conserved sites, N257 and N563, showed an enrichment of unprocessed (high mannose) glycans, and these two sites may be primarily responsible for interaction of cell surface lectins (DC-SIGN/L-SIGN) in a high-mannose dependent manner. They further detected up to 16 unique site-specific O-glycans in the head and glycan cap domains of the GP1 subunit that binds to the receptor. They also confirmed the presence of C-mannosylation at W288, and found heterogeneous and complex N-linked glycosylation at the majority of the predicted sites. The authors conclude that all combined, the heterogeneous N-, O-, and C-linked glycosylation patterns add up to significant complexity in the GP composition, "perhaps contributing to immune evasion by 'blurring' the molecular identity of the envelope glycoproteins."

This was a very thorough MS analysis of the ebolavirus GPs, and a few notes are highlighted below:

1. The finding that the glycans at conserved sites N257 and N563 in ebolavirus GP is interesting. The fact that this observation is made in both expression systems, for both ebolavirus species tested, and in VLPs derived from HEK203 cells is compelling.
2. The significance of the C-mannosylation at tryptophan W288 is not clear. It was only observed in HEK-derived material (not S2 cells), and it is not clear how relevant it is to the virus, and its role in ebolavirus replication and pathogenesis.
3. The results are somewhat restricted without being able to correlate this to the glycoprofile of the native virus.
4. The claim that glycans influence mAb binding could be improved. The authors do not include their own experimental evidence such as affinity measurements, nor are there any literature references in that part of the results section. There are two references mentioned in the discussion but if the authors are going to make a claim like this, the hypothesis and experimental data should be tied together in a manner that is clear and obvious. It is not that way in the manuscript as written.

