An octadecameric *O*-glucosyltransferase generates diversity in antibody epitopes on trypanosome variant surface antigens

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Antigenic variation in African trypanosomes is classically understood as a genetic process, driven by monoallelic expression and switching among ~2,500 variant surface glycoprotein (VSG) genes. However, recent work has revealed an additional layer of chemical variation whereby Class B VSGs are *O*-glycosylated within cysteine-flanked surface-exposed loops, and loss of this modification alters antibody recognition and influences infection outcome. Despite its clear immunological importance, the enzyme responsible for VSG *O*-glycosylation has remained unknown.

Here, we identify expression site–associated gene 3 (ESAG3) as the long-sought VSG *O*-glycosyltransferase. Depletion of ESAG3 abolishes recognition by infection-derived monoclonal antibodies that specifically require *O*-glucose on VSG3, while genetic complementation restores binding *in vivo*. Using purified recombinant protein, we show that ESAG3 catalyses *O*-glucosylation of VSG-derived peptides *in vitro* with strict specificity for UDP-glucose and manganese. These data establish ESAG3 as the first *O*-glycosyltransferase characterised in *Trypanosoma brucei*.

Single-particle cryo-electron microscopy reveals that recombinant ESAG3 assembles into an unprecedented 18-subunit ring with C3 symmetry, representing the first structurally resolved nucleotide-sugar-dependent glycosyltransferase from kinetoplastids. Disrupting ESAG3 inter-subunit interfaces collapses the octadecamer into smaller oligomers with increased catalytic turnover, suggesting that the assembled 18-subunit architecture constrains activity rather than maximising it.

Collectively, our work establishes ESAG3 as the third ESAG to be functionally characterised in African trypanosomes and provides a framework for understanding how VSG *O*-glycosylation integrates with genetic variation to reshape antibody recognition of the parasite surface.