The MISP family of surface glycoproteins from *Trypanosoma brucei* is co-expressed with VSG and BARP in the metacyclic trypomastigote stage, adopts a triple helical bundle structure, and is not essential for the colonization of the tsetse salivary glands

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Trypanosoma brucei spp. develop into mammalian-infectious metacyclic trypomastigotes inside the tsetse salivary glands. Besides acquiring a variant surface glycoprotein (VSG) coat, little is known about the expression of invariant surface antigens by the metacyclic stage. Proteomics analyses of saliva from *T. brucei*-infected flies identified, in addition to VSG and Brucei Alanine-Rich Protein (BARP) peptides, a family of GPI-anchored surface proteins herein named Metacyclic Invariant Surface Proteins (MISP). The MISP family is encoded by five paralog genes with >80% protein identity, which are exclusively expressed by salivary gland stages of the parasite, and peaks in metacyclic stage as shown by confocal microscopy and immuno-high resolution scanning electron microscopy. Crystallographic analysis of MISP and a high confidence model of BARP reveal a triple helical bundle architecture commonly found in other trypanosome surface proteins. Molecular modelling combined with live fluorescent microscopy suggests that MISP expose immunogenic N-terminal epitopes above the VSG coat, although vaccination with a recombinant MISP isoform did not protect mice against a *T. brucei* infectious bite. Lastly, both using RNAi and CRISPR-Cas9-driven knock out of all MISP paralogues suggests they are not essential for parasite development in the tsetse vector.