

Structural and functional studies of *Trypanosoma brucei* TbMORN1 protein

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TbMORN1 is an essential protein in BSF *T. brucei*. It is present in the hook complex at the flagellar pocket neck of the parasite, lying just below its plasma membrane. TbMORN1 is composed of 15 tandem Membrane Occupation and Recognition Nexus (MORN) repeats. MORN repeat proteins are found throughout the tree of life, examples including a family of lipid kinases in plants, the junctophilin proteins found at triad junctions in mammalian muscle, and TbMORN1's closest homologue, the TgMORN1 in *T. gondii*. Although the function of MORN repeats remains elusive, they have been proposed to interact with lipids, in particular phosphatidic acid, PtdIns(4)P and PtdIns(4,5)P₂.

We are investigating the TbMORN1 capacity to bind phosphoinositides. Lipid blots indicated that TbMORN1 interacts with different phosphoinositide species in a phosphorylation-dependent manner. Based on the TbMORN1 3D model and bioinformatics analysis, 4 PtdIns(4,5)P₂ binding sites were predicted in MORN repeats 5, 6, 13 and 14. Sequence alignment showed that these binding sites are conserved among TbMORN1 and TgMORN1. Affinity of PtdIns(4,5)P₂ to TbMORN1 and TgMORN1 is being investigated by the fluorescence anisotropy. Preliminary determinations of K_D values indicate that TbMORN1 binds to PtdIns(4,5)P₂ with micromolar affinity. Crystallization experiments yielded diffraction quality crystals and diffraction data of TbMORN1 construct encompassing MORN repeats 7-15, and first crystallization hits of TgMORN1 constructs encompassing repeats 2-15 and 7-15. Optimization of crystallization conditions and of diffraction data is in progress.