Structural and functional studies of Trypanosoma brucei MORN1 protein

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Membrane occupation and recognition nexus repeat (MORN)-containing proteins are found throughout the tree of life. The MORN1 protein in *Trypanosoma brucei* is composed of 15 MORN repeats and is essential for the viability of the parasite`s bloodstream form. It localizes to a hook-shaped complex that wraps around the neck of the flagellar pocket membrane. Our biophysical characterization of TbMORN1 including circular dichroism, electron microscopy, and solution small angle X-ray scattering (SAXS) suggest that it is an all-beta protein, existing in a rod-shaped dimer. These data are consistent with a recently solved crystal structure for TbMORN1 homologue from *Plasmodium falciparum*, the construct missing the first 6 MORN repeats. Mutational, chemical cross-linking, and SAXS data indicate that TbMORN1 dimerizes via its C-terminal repeats. Lipid blots, native electrophoresis, and fluorescence anisotropy assays showed that TbMORN1 binds to the lipid phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) with low micromolar affinity. Based on a computational model, putative $PI(4,5)P_2$ binding sites were predicted and $PI(4,5)P_2$ binding mutants have been generated and assayed. With the ongoing structural and *in vivo* studies we are trying to understand whether $PI(4,5)P_2$-binding is essential for correct protein assembly and/or localization in the parasite.