p197 as the missing link between the outer mitochondrial membrane and the basal body in *T.brucei*

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Basal bodies (BB) are highly conserved essential structures throughout the eukaryotic tree of life. In trypanosomes the basal body not only serves as the microtubule organization center but also as the base of the flagellum and the anchoring point of the tripartite attachment complex (TAC). The TAC is a unique structure essential for the correct segregation of the replicated kinetoplast DNA (kDNA). It reaches via the exclusion zone filaments (EZFs) from the BB to the mitochondrial outer membrane (OM), spans both mitochondrial membranes and finally connects to the kDNA in the mitochondrial matrix.

How this complex attaches itself to the BB, how its assembly is orchestrated as well as its precise architecture are not fully understood yet. In order to investigate these questions, we focused on the TAC component p197, which is the subunit most proximally localized to the BB known so far. We generated a cell line which exclusively expresses a p197 variant that is both C-terminally tagged with an HA-epitope and N-terminally with a c-Myc epitope. The cell line was viable indicating that the double tagged p197 is functional. Using super resolution expansion microscopy this allows us to precisely localize both the N- and the C-termini of the p197 molecules within the TAC structure. The result shows that p197 binds to both the mature BB and the pro BB with its C-terminus. More precisely it localizes adjacent to SAS6, the cartwheel protein of the mature and the pro BB. Interestingly however the N-terminus of p197 does not localize to the BB but to an area close to the mitochondrial OM. p197 is predicted to have an alpha helical structure. Should this be case the protein could theoretically span a distance of more than 250nm, which is much more than would be required to connect the basal body to the OM in trypanosomes. To further investigate whether p197 indeed forms this connection several deletion mutants of p197 were generated. Both C- and N-terminal truncations are nonfunctional, yet able to correctly localize. Moreover, the N-terminal truncation of p197 binds to both the mBB and the pBB, as expected, whereas the C-terminal truncation of the protein behaves like a mitochondrial protein in biochemical assays, and therefore most probably binds to one of the OM components of the TAC. Altogether, our results demonstrate that surprisingly the exclusion zone filaments of the TAC are made up of a single large molecule, p197.