Limits of Flagellar Pocket Access in *Trypanosoma brucei*

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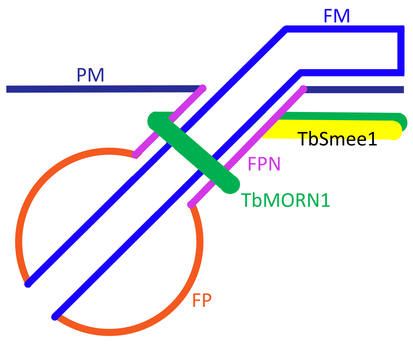
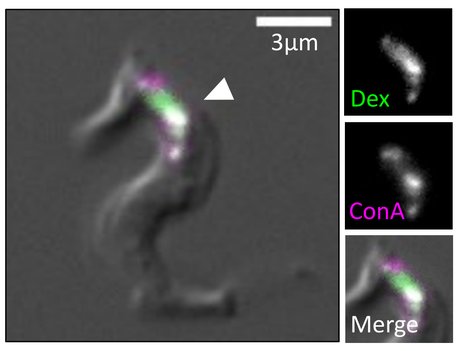
Abstract: *Trypanosoma brucei* is an extracellular parasite which lives in the bloodstream of infected mammalian hosts and is in continuous exposure to the immune system. Its surface is covered in a dense glycoprotein coat which is continually endo- and exocytosed in order to remove any bound antibodies. Nutrients such as LDL (low density lipoprotein) and transferrin are scavenged by endocytosis. Remarkably, all endo- and exocytic activity is restricted to a single small subdomain of the plasma membrane - an invagination called the flagellar pocket. Even though trypanosomes are capable of internalising very large macromolecular complexes such as LDL, the exact size limit for macromolecule entry to the flagellar pocket is unknown. Cytoskeleton-associated protein complexes that are coiled around the neck of the flagellar pocket, such as the hook complex, are suspected to influence the size limit and endocytic activity. In this study, the size limits for fluid phase cargo entry were systematically measured. Depletion of specific protein components of the hook complex did not influence the defined size limit, suspecting a different role of the hook complex in the uptake mechanism than previously assumed.

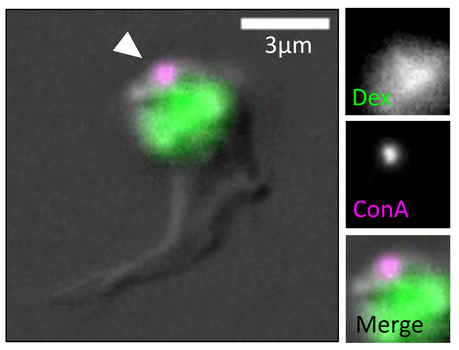
1. The physical limits of flagellar pocket access are unknown



Internalised material must pass through the flagellar pocket neck in order to access the flagellar pocket. Components of the hook complex localise to the flagellar pocket neck region. The size limits of the neck region are unkown.

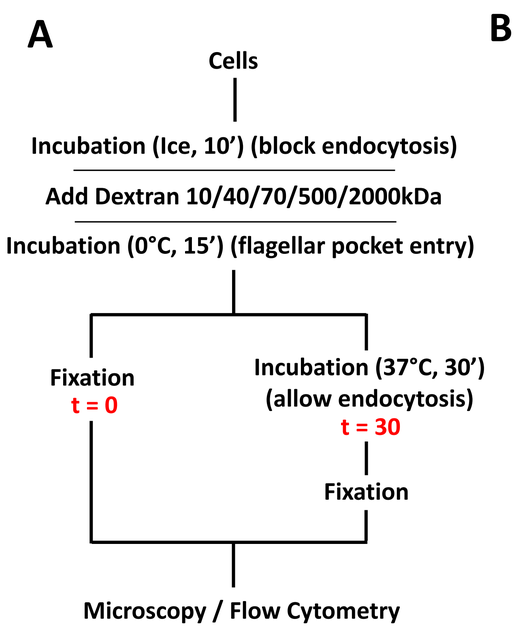


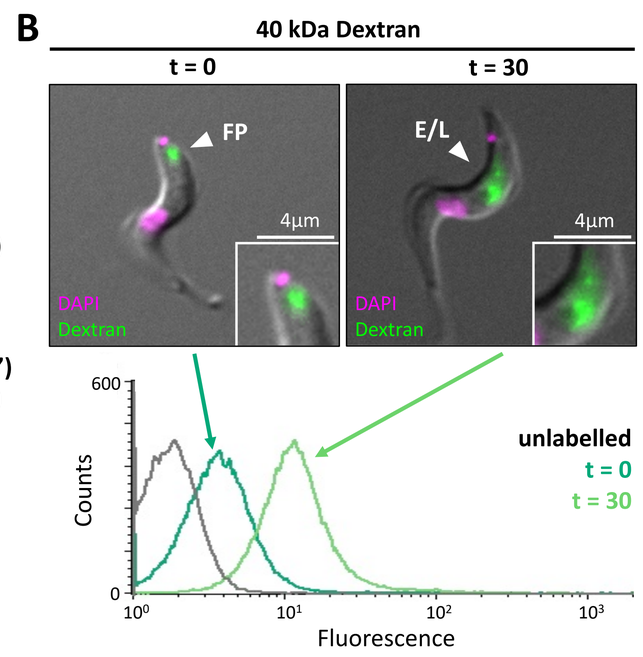
Concanavalin A (ConA) and 10 kDa Dextran can be used to report on flagellar pocket access and subsequent internalisation. After depletion of specific hook complex proteins, ConA accumulates at the flagellar pocket entry, whereas dextran enters the flagellar pocket.

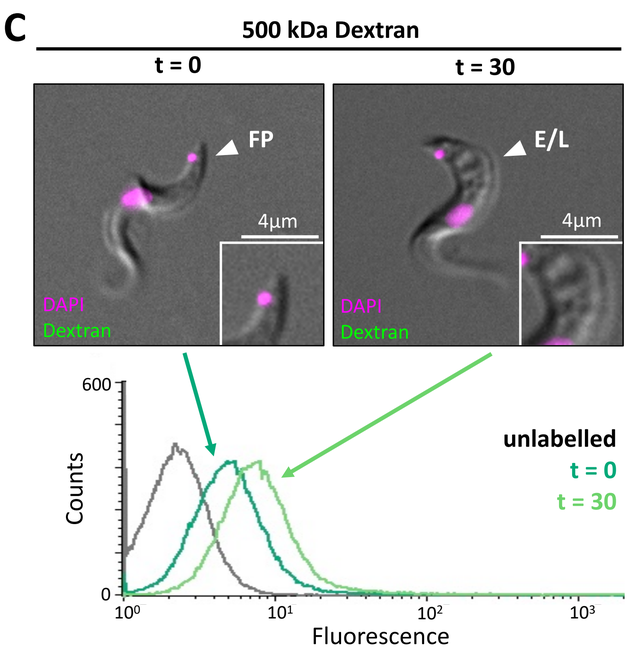


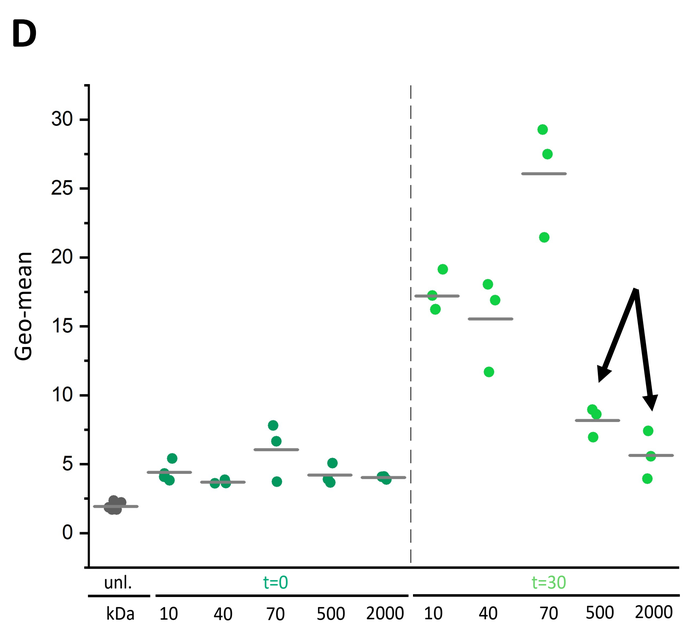
What is the size limit for flagellar pocket access? Does the hook complex influences the size limit?

2. Flagellar pocket access and cargo internalisation can be monitored using microscopy and flow cytometry









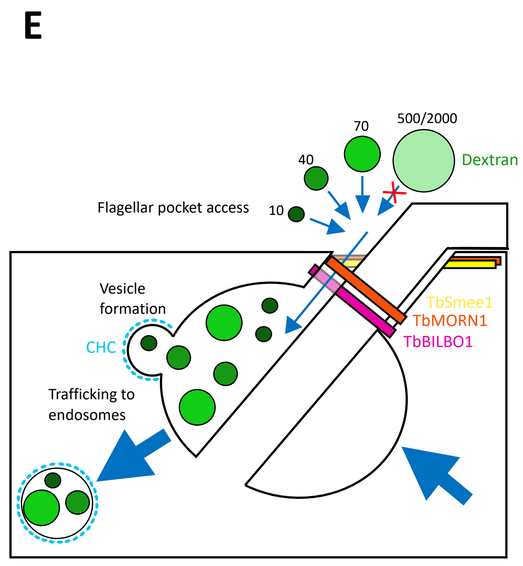
(A) Schematic of uptake assay using different dextran sizes and two different timepoints.

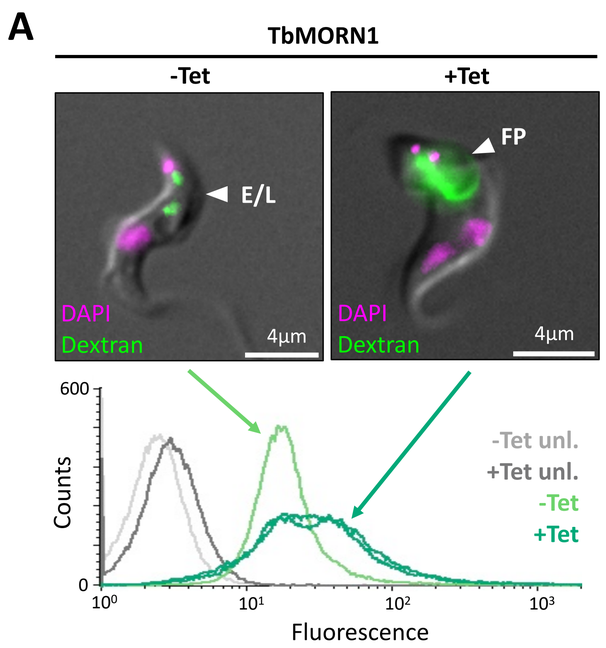
(B) 10, 40 and 70 kDa dextran can enter the flagellar pocket (FP) and are then internalised and trafficked to endosomes/lysosomes (E/L).

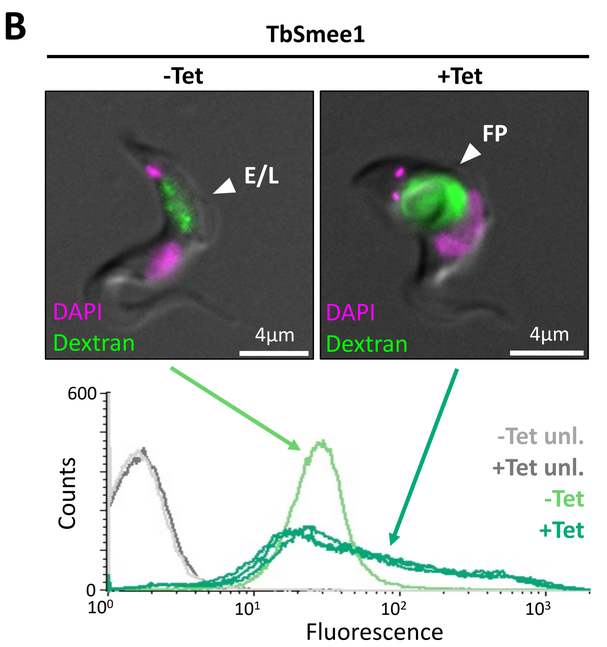
(C) 500/2000 kDa dextran shows low/no flagellar pocket access or internalisation.

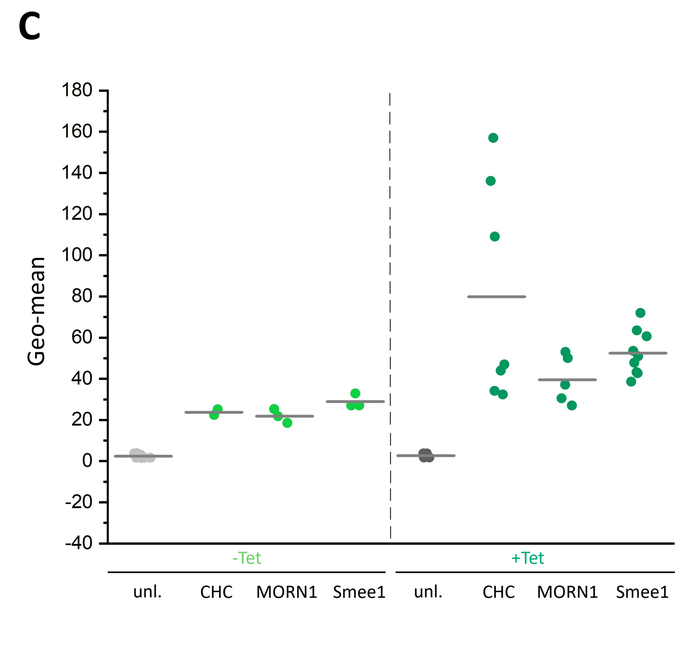
(D) Geo-means (n=3) of flow cytometry suggest strongly reduced access of 500/2000 kDa dextran to flagellar pocket (arrows).

3. The hook complex does not influence the size limit for fluid-phase cargo









(E) Schematic of possible size-exclusion mechanism during flagellar pocket entry of different dextran sizes.

(A) 70 kDa dextran can still enter the flagellar pocket upon depletion of TbMORN1 (+Tet).

(B) 70 kDa dextran can still enter the flagellar pocket upon depletion of TbSmee1 (+Tet).

(C) Clathrin (CHC) knockdown was used as a control for an endocytosis defect. 70 kDa dextran can enter the flagellar pocket after CHC, TbMORN1 or TbSmee1 depletion (+Tet).

4. Depletion of the hook complex does not influence the size limit

Dextran with a diameter of 13 nm (70 kDa) can be taken up by *Trypanosoma brucei*, whereas 32 nm diameter or higher have a strongly reduced access or are not able to pass the flagellar pocket neck at all. As previously shown, LDL is able to enter the flagellar pocket with a diameter of ~22 nm. This suggests that there is a defined exclusion limit of macromolecular access in the fluid phase at around 32 nm diameter. After depletion of specific hook complex proteins, 13 nm dextran was still able to enter the flagellar pocket. This was unexpected, since previous experiments indicated an accumulation of smaller markers like ConA (8 nm) at the flagellar pocket entry. ConA is a surface-binding marker, whereas dextran travels in the fluid phase. Surface-binding fluorescent microspheres revealed similar accumulation at the flagellar pocket entry after hook complex depletion (data not shown). This would favor the idea that an endocytosis defect resulting from disturbed membrane flow influences the uptake of different markers, as clathrin knockdown indicated the exact same phenotype as TbMORN1 and TbSmee1.