**////Title: Using Intrabodies to Induce Cell Death in Trypanosome Parasites**

**////Maintext:**

Trypanosomes are single-celled parasites that cause life-threatening diseases in humans, domestic livestock and wild animals. In sub-Saharan Africa, infection with a species called *Trypanosoma brucei* or *T.brucei* causes African sleeping sickness, which results in organ failure and eventually fatal coma if left untreated. There are limited diagnostic tests and treatments available and much of trypanosome biology remains undiscovered.

Professor Derrick Robinson and his team from the CNRS and the University of Bordeaux study the biology of these single-celled pathogens. They specialise in characterising novel proteins essential to the function of the cytoskeleton with a focus on understanding the role of these proteins in cell organisation, function motility, and structure.

The cytoskeleton in trypanosomes is an internal, sub-pellicular, structure of proteins and microtubules that work together to perform essential cell functions. Trypanosomes are motile pathogens and can swim using a cytoskeleton-linked structure called a flagellum.

The flagellum is physically connected to the cytoskeleton, and near its base, it passes through an organelle called the flagellar pocket (FP). The FP is an infolding of the pellicular membrane and the site where molecules move in or out of the cell. At the point where the flagellum exits the FP, it is encircled by a ring-like cytoskeletal structure called the flagellar pocket collar (FPC), which is essential for FP biogenesis and subsequently cell viability.

Professor Robinson and his team set out to understand the structural importance of an FPC component protein called BILBO1 in *T.brucei* by blocking its function in a novel knockdown experiment.

Instead of using RNA interference technology, where RNA molecules are used to knock down or stop the gene expression of a protein, or CRISPR/Cas9 to edit or delete the BILBO1 gene, they explored the use of intrabodies which have a longer lifespan than RNA molecules and are able to target protein variants.

Intrabodies are a type of nanobody – an antibody with a single variable site, they are stable when expressed inside a cell and will bind tightly to a specific region of a target molecule. Intrabody binding can disrupt the protein’s behaviour and essentially knocks down its function.

By testing and developing nanobodies against BILBO1 in *T.brucei*,Professor Robinson discovered that a particular nanobody, Nb48, was highly successful at binding to the BILBO1 protein and could also function as an intrabody. This study confirmed that Nb48 is an excellent diagnostic probe for *T.brucei* and a novel immune tool capable of specifically targeting the cytoskeletal protein BILBO1.

The researchers then used the gene encoding the Nb48 nanobody to develop an intrabody expression system that would lead to the inducible expression of the Nb48 nanobody protein within live *T.brucei* parasites, and thus, knock down the function of BILBO1 protein from within the cell.

Importantly, knocking down the BILBO1 protein led to the disruption of FPC formation, which resulted in the rapid killing of *T.brucei* parasites in an effect similar to RNA interference. This result indicates that the BILBO1 protein plays a vital structural role in the FPC and that using an intrabody expression system is as effective as RNA interference methods.

This work demonstrates the value of using nanobodies not only as diagnostic markers but also as intrabodies which can be used to explore protein function in the trypanosome cytoskeleton and, potentially, many other cell types. Furthermore, intrabodies may represent an exciting alternative to RNA interference or CRISPR/Cas9 for targeting protein function within a cell.

This is the first time the trypanosome cytoskeleton has been successfully targeted using intrabodies as a knockdown tool. We are now a step closer to new methods of understanding the biology of disease-causing agents and organisms and this could ultimately lead to novel developments in the fight against infectious pathogens.

This video is a summary of ‘Intrabody-Induced Cell Death by Targeting the *T. brucei* Cytoskeletal Protein TbBILBO1’ published in the journal Microbiology Spectrum. DOI: https://doi.org/10.1128/Spectrum.00915-21

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