***Molecular control of irreversible bistability during trypanosome developmental commitment***

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**Abstract**

The life-cycle of *Trypanosoma brucei* involves developmental transitions that allow survival, proliferation and transmission of these parasites. One of these, the differentiation of growth-arrested stumpy forms in the mammalian blood into insect-stage procyclic forms can be induced synchronously in vitro with cis-aconitate (CA). Here, we show that this transition is an irreversible bistable switch and map the point of commitment to differentiation after exposure to CA. This irreversibility implies positive feedback mechanisms operate to allow commitment: i.e. the establishment of “memory” of exposure to the differentiation signal. Using the reversible translational inhibitor cycloheximide, we show that this signal memory requires new protein synthesis. We further performed SILAC analysis of synchronised parasite populations, establishing the protein and phosphorylation profile of parasites pre- and post-commitment, thereby defining the ‘commitment proteome’. Functional interrogation of this dataset identified TbNRK as the first-discovered protein kinase controlling the initiation of differentiation to procyclic forms.

**Proteomic data availability:**

To make our data accessible to the scientific community, we uploaded our study to TriTrypDB (<http://www.tritrypdb.org>) and deposited the Thermo RAW files and search engine output into ProteomeXchange (<http://www.proteomexchange.org>) consortium via the Pride partner repository with the dataset identifier PXD002165, enabling researchers to access the data. More detailed metadata of the proteomic files are given below

**Metadata and associated files**

The following Excel files describe the metadata for each RAW file for the proteomic and phosphoproteomic analysis.

RAW-meta.xls

The following Excel files are the processed and analysed data presented in the supplementary material given in the associated publication

Table S1 Quantitative Proteomic analysis of commitment to differentiation

Table S2 Quantitative Phosphoproteomic analysis of commitment to differentiation

Table S3 PIP39 and NOP44 Phosphorylation sites are unsuitable of LC-MS analysis

Table S4 Phosphorylation site observed only in experimental samples