Functionally mapping the diversification of African trypanosomes using spatial proteomics

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African trypanosomes are dixenous unicellular parasites that cause disease with devastating impact in sub-Saharan Africa. Diversity between species and life-cycle stages is concomitant with distinct host and tissue tropisms within this group. Understanding the molecular biology that underpins the diversity in cell biology requires a comprehensive functional map of each organism which, collectively, is currently lacking.

Protein function is often intimately linked with localisation and as a consequence the subcellular distribution of a protein provides information on its role in the cell. We have optimised a method for resolving subcellular compartments in *Trypanosoma brucei* and *Trypanosoma congolense* and implemented it in the spatial proteomics strategy of hyperLOPIT (hyperplexed localisation of organelle proteins by isotope tagging). Between the insect and vertebrate stages, represented by procyclic and bloodstream forms respectively, we have detected over 7000 in both *T. brucei* and *T. congolense*. Of these, 6171 *T. brucei* proteins (n = 3) and 6324 *T. congolense* proteins (n = 3) are included in a spatial proteome characterisation and classified to over 15 subcellular compartments using a machine learning approach based on a t-augmented Gaussian mixture model.

This work provides a comprehensive map of the *T. brucei* and *T. congolense* spatial proteomes. Individually, these data sets guide the determination of uncharacterised protein function, particularly for *T. congolense*, where high-throughput functional analysis lags behind that of *T. brucei*. Further, comparative analysis yields insight into the evolutionary diversification of these species and the effects of speciation on the molecular biology and architecture of the parasite cell.