The RNA-bound Proteome of *Trypanosoma cruzi*

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We present the first quantitative RBPome and Whole Cell proteome comparison between the 3 main lifecycle stages of *Trypanosoma cruzi* parasites. Lifecycle progression and bespoke adaptation to distinct host environments demand precise gene expression that is predominantly dependent upon post-transcriptional control. Constitutive transcription elevates the importance of RNA binding proteins for gene regulation in these parasites, yet strikingly few *T.cruzi trans*-regulators are characterized relative to other kinetoplastids. Using optimized crosslinking and deep, quantified mass spectrometry, we present a comprehensive analysis of the stage-specific RBPomes and whole cell proteomes of the main *T.cruzi* lifecycle stages. Remarkably, while the whole cell proteomes display characteristically distinct surface proteins between these lifecycle stages as expected, the RBPomes of the human-infectious trypomastigote and amastigote stages bear striking similarities.

The presence of basal translational machinery, expected stage-specific markers and near-identical proteomes derived from crosslinked and non-crosslinked cells in all sample sets support the validity of these proteomes. These represent the most in depth T.cruzi proteomes to date with outstanding coverage. Comparisons between these datasets provides unique insight into key candidate regulators that may prove essential to parasite survival, longevity and virulence. These proteomes can further enable in depth comparisons between Kinetoplastid species toward common essential regulators and host-adaptative mechanisms. Outcomes provide novel insight into the *trans*-regulatory mRNA:Protein (mRNP) complexes that drive *T.cruzi* parasite lifecycle progression and human infection.

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