Conditional Protein Complex Formation in *Leishmania donovani*: A BromoTAG-dTAG Proximity-Inducing Toolbox

Luciana S Paradela[1], Simone Montagna[1], Ria Spallek[2], Gopal Sapkota[2], Mark Field[3,4] & Manu De Rycker[1]

[1]Drug Discovery Unit, Biological Chemistry and Drug Discovery, Faculty of Life Sciences, University of Dundee; [2]Medical Research Council Protein Phosphorylation and Ubiquitylation Unit, Faculty of Life Sciences, University of Dundee; [3]Biological Chemistry and Drug Discovery, Faculty of Life Sciences, University of Dundee; [4]Biology Centre, Czech Academy of Sciences, Institute of Parasitology, České Budějovice, Czech Republic.

Proximity-inducing tools, including bifunctional molecules that enable conditional protein complex formation, have emerged in mammalian systems as powerful strategies to modulate protein function and cell signalling. BDPIC (BromoTag–dTAG Proximity-Inducing Chimera) is one such molecule, designed to promote ternary complex formation between two tagged proteins via simultaneous engagement of orthogonal affinity tags. While BDPIC has been applied for targeted dephosphorylation of phosphoproteins and is increasingly being explored for protein degradation in mammalian cells, its use in protozoan parasites remains largely unexplored. To enable control over parasite protein-protein interactions as well as new therapeutic approaches, such as targeted protein degradation, we sought to explore the applicability of BDPIC in *Leishmania donovani*. Here, we established a live-cell NanoBRET proximity assay to evaluate BDPIC permeability and its ability to mediate complex formation of BromoTag (BrTag) and dTAG fusion proteins. Eight transgenic parasite lines were generated, expressing all combinations of NanoLuc donor and HaloTag acceptor fused to BrTag or dTAG at either the N- or C-terminus. Immunoblotting confirmed expression of fusion proteins at predicted molecular weights, validating construct integrity and enabling systematic comparison of fusion orientations. Using NanoBRET as a quantitative readout of induced proximity, we evaluated BDPIC for its ability to promote BrTag–dTAG ternary complex formation in living parasites. BDPIC treatment produced robust and reproducible increases in energy transfer across multiple construct orientations relative to vehicle controls. The magnitude of NanoBRET induction depended on tag positioning, demonstrating the importance of spatial geometry for optimal energy transfer, and more widely, for the orientation of ternary complex components. Strong ligand-dependent signals in intact parasites confirm that BDPIC efficiently permeates *L. donovani* and promotes intracellular induced proximity of tagged proteins. This work provides a powerful new tool for chemically inducing protein proximity in *L. donovani*, expanding the toolbox for proximity-driven functional studies. It represents a key step towards developing targeted protein degradation as a new therapeutic modality for kinetoplastid diseases.