

Title: Biophysical and biochemical characterisation of the interaction between *Leishmania braziliensis* PRMT1 and PRMT3

Arginine methylation is a key post-translational modification that can alter the structure, dynamics and interaction profiles of proteins. Protein arginine methyltransferases (PRMTs) catalyse the transfer of a methyl group from a S-adenosylmethionine molecule onto the arginine side chain guanidino group. Mammalian PRMTs are classified into subtypes – PRMT1, 2, 3, 4, 6 and 8 catalyse asymmetric dimethylation (ADMA); PRMT5 and 9 catalyse symmetric dimethylation (SDMA); and PRMT7 catalyses monomethylation (MMA). Kinetoplastids possess five homologues: PRMT1, 3, 5, 6 and 7. *T. brucei* PRMT3 has been shown to be a pro-enzyme (prozyme) which lacks key conserved motifs including in the catalytic double E loop. *T. brucei* PRMT1 is only active in complex with the PRMT3 prozyme. In *Leishmania*, however, PRMT3 retains the conserved double E loop, which raises questions about its role in this organism. Here we use recombinant protein samples to investigate *L. braziliensis* (*Lbr*) PRMT1 and 3 *in vitro*. Activity assays show that methylation of a substrate peptide only occurs when PRMT1 and 3 are both present. Analytical size exclusion chromatography (SEC) and SEC-MALLS show that *Lbr*PRMT1 and 3 form a heterotetrameric complex in solution. Mutation of double E loop residues revealed that *Lbr*PRMT1 is the active component of the complex. Previous work suggested *Lbr*PRMT3 could interact with and modulate the activity of other *Lbr*PRMTs, however methyltransferase assays showed that *Lbr*PRMT3 had no effect on the activities of *Lbr*PRMT5 and 7 *in vitro*. Moreover, *Lbr*PRMT3 could not methylate a peptide substrate previously monomethylated with PRMT7. Our data suggests that *Lbr*PRMT1 and 3 form a similar complex to *T. brucei* PRMT1-3. However, the retention of the conserved double E loop in *Leishmania* PRMT3 enzymes suggests an as of yet undiscovered functional difference between the two trypanosomatids.