

## Biochemical characterization of a potential *Trypanosoma cruzi* nitric oxide synthase

Jeniffer Ellen Calisto de Amorim<sup>1,2</sup>, Lays Adrienne Mendonça Trajano-Silva<sup>2</sup>, Simon Ngao Mule<sup>2</sup>, Walter Colli<sup>1</sup>, Maria Julia Manso Alves<sup>1</sup>, Giuseppe Palmisano<sup>2</sup>

<sup>1</sup> IQ-USP, <sup>2</sup>University of Sao Paulo – Institute of chemistry – Biochemistry Department - University of Sao Paulo, Av. Lineu Prestes, 748, Butantã São Paulo;

<sup>2</sup> ICB-USP, <sup>1</sup>University of Sao Paulo – Institute of Biomedical Science II – Parasitology Department - Glycoproteomic Laboratory, University of Sao Paulo, Av. Lineu Prestes, 1374, Butantã São Paulo;

Post-translational modifications (PTMs) decorate proteins influencing several physicochemical properties and playing a key role in numerous biological processes. More than 450 PTMs have been described from prokaryotes to eukaryotes. Among these PTMs is protein S-nitrosylation (SNO) which is generated by the addition of nitric oxide (NO) to specific cysteine thiols in target proteins. Nitric oxide (NO) is a highly diffusible and short-lived cellular messenger produced by the nitric oxide synthase (NOS) family of proteins. The sites and stoichiometry of SNO regulates a variety of important pathophysiological responses aiming to maintain cellular homeostasis. Recently, our group identified S-Nitrosylated proteins in trypomastigote forms of *Trypanosoma cruzi*, the etiological agent of Chagas disease, and host proteins following the incubation of the parasites with the host extracellular matrix (ECM), suggesting the presence of NOS and roles of NO in the host's extracellular environment. Based on previous results from our laboratory, we identified a single copy of the putative oxido-reductase protein (NOS putative gene) located on chromosome 9 in *T. cruzi* Y strain, encoded by 1878 bp. In this context, the study aims to characterize the nitric oxide synthase enzyme from *T. cruzi*. We have designed specific primers containing restriction enzyme sites (HindIII and XbaI) to amplify the putative *T. cruzi* NOS gene using *T. cruzi* genomic DNA as the template. The first step was to optimize the amplification conditions by performing a primer gradient of annealing temperatures, and we chose 65°C to proceed to the next steps. The NOS gene was amplified by PCR and purified using the Wizard® SV gel and PCR clean-up system (Promega). The NOS putative gene was inserted into the pTREXn vector containing HA tagging, and the construct was cloned in *E. coli* DH5 $\alpha$  with subsequent miniprep plasmid purification using the Wizard® Plus SV Minipreps DNA Purification System (Promega). The pTREXn-NOS was confirmed by sequencing analysis. These results will direct the NOS localization and functional characterization in *T. cruzi* providing detailed information about the SNO pathway in this organism. In the future, this could contribute to the targeting of the putative TcNOS for the development of new drugs to control Chagas disease.

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