

ST8Sia2: The host's enchanted armor protecting against *T. cruzi* infection

Bruno Rafael Barboza¹, Janaina Macedo da Silva¹, Lays Adrienne Mendonça Trajano Silva¹, Vinícius de Moraes Gomes¹, Deivid Martins Santos¹, Antônio Moreira Marques Neto¹, Simon Ngao Mule¹, Claudia Blanes Angeli¹, Juliana Borsoi², Carolina Borsoi Moraes³, Cristiane Moutinho Lagos de Melo^{4,5}, Martina Mühlenhoff⁶, Walter Colli⁷, Suely Kazue Nagahashi Marie⁸, Lygia da Veiga Pereira², Maria Julia Manso Alves⁷, Giuseppe Palmisano^{1,9}

¹. GPLab, GlycoProteomics Laboratory, Department of Parasitology, Institute of Biomedical Sciences, University of São Paulo, Brazil, Av. Prof. Lineu Prestes, nº 1374 – Butantã – São Paulo/SP – CEP 05508-000 (ICB II);

². IB/USP, Department of Genetics and Evolutionary Biology, Institute of Biosciences, University of São Paulo, Brazil, R. do Matão, 277 - Butantã, São Paulo - SP, 05508-090;

³. FCF/USP, Department of Clinical and Toxicological Analyses, School of Pharmaceutical Sciences, University of São Paulo, Brazil, Av. Professor Lineu Prestes, 580 - São Paulo/SP, CEP 05508-000;

⁴. LDV, Laboratory of Vaccine Development, Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo, Brazil, Av. Prof. Lineu Prestes, nº 1374 – Butantã – São Paulo/SP – CEP 05508-000 (ICB II);

⁵. UFPE, Laboratory of Immunological and Antitumor Analysis, Department of Antibiotics, Bioscience Center, and Keizo Asami Immunopathology Laboratory, Federal University of Pernambuco, Recife, Brazil; Av. Prof. Artur de Sá, s/n, Cidade Universitária, 50740-525 Recife, PE, Brazil.;

⁶. MHH, Institute of Clinical Biochemistry, Hannover Medical School, Germany., Carl-Neuberg-Str. 1 D-30625 Hannover, Germany;

⁷. IQ/USP, Department of Biochemistry, Institute of Chemistry, University of São Paulo, Brazil, Av. Prof. Lineu Prestes, 748 - Butantã, São Paulo - SP, 05508-900;

⁸. FMUSP, Laboratory of Molecular and Cellular Biology (LIM 15), Department of Neurology, School of Medicine, University of São Paulo, Brazil, Av. Dr Arnaldo 455 - Cerqueira César - São Paulo/SP, 01246-903;

⁹. Macquarie University, School of Natural Sciences, Macquarie University, Sydney, Australia, Macquarie University Wallumattagal Campus Macquarie Park NSW 2109;

Glycosylation is a highly diverse co- and/or post-translational modification process crucial for the structural and biological functions of a cell. It involves the dynamic addition and removal of sugars, known as glycans, to proteins, lipids, and small RNAs in eukaryotic cells, facilitated by an elegant and sophisticated repertoire of enzymes. Among these modifications, polysialic acid (polySia) stands out due to its unique polymeric structure, formed by the repeated enzymatic addition of sialic acid units by polysialyltransferases such as ST8Sia2. While the importance of polySia in the nervous system is well-documented, its role in host-pathogen interactions remains largely unexplored. Our study reveals the protective role of ST8Sia2-mediated polysialylation in the context of *T. cruzi* infection, the causative agent of Chagas disease, and demonstrates for the first time the interplay between polysialylation and oxidative stress. To unravel the host protective mechanisms mediated by ST8Sia2, we employed a combination of *in silico* and experimental tools to assess host polysialylation levels during *T. cruzi* infection. Our findings indicate that *T. cruzi* infection significantly downregulates ST8Sia2 expression and reduces polysialylation in host cells. Inhibition of ST8Sia2, either genetically or chemically, increases *T. cruzi* load within host cells, suggesting a critical defensive role of polySia against the parasite. Intriguingly, we observed that modulation of polysialylation induces oxidative stress, evidenced by increased reactive oxygen species (ROS) production. This oxidative microenvironment appeared to favor *T. cruzi* survival and replication, indicating a complex interplay between host polysialylation and pathogen evasion mechanisms. Our study highlights ST8Sia2 as a pivotal component of the host defense system,

conferring protection against *T. cruzi* infection. These insights open new avenues for therapeutic interventions aimed at enhancing host polysialylation to strengthen cellular defenses against *T. cruzi* and potentially against other pathogens. Further research into the mechanistic details of ST8Sia2's protective role could pave the way for innovative treatments for Chagas disease.

Agradecimentos: We thank Celia Ludio Braga at the Department of Biochemistry, Institute of Chemistry, University of São Paulo, Brazil for helping with the parasite cell culture. We thank the Vaccine Development Laboratory, Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo, Brazil, for providing the flow cytometry platform (LSR Fortessa cell analyzer flow cytometer, BD Biosciences). We are grateful for the financial support provided by São Paulo Research Foundation (FAPESP), grants n° 2018/18257-1 (GP), 2018/15549-1 (GP), 2020/04923-0 (GP), 2022/09915-0 (BRB), 2021/00140-3 (JMDS), 2022/00796-9 (LAMTS), 2021/00507-4 (VdMG), 2021/14179-9 (DMS), 2021/14751-4 (SNM), 2020/02988-7 (SKNM), 23/02095-0 (CMM); by Conselho Nacional de Desenvolvimento Científico e Tecnológico (“Bolsa de Produtividade”) (SKNM, CMM, and GP); by Fundação Faculdade de Medicina (FFM-SKNM); by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (AMMN).