Learning how to work with structure and function of *Triatoma infestans* proteins

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In the present talk we will describe our experience in participating the Structural and Molecular Biology Network (SMOLbnet) supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). Among other themes, our group focused in key molecules of *Triatoma infestans,* the insect vector of Chagas' disease, which are involved in the disease transmission. We studied a previously characterized 22 kD pore forming protein present in the insect saliva named trialysin (1). Our goal has been to understand the lytic mechanism of trialysin as well as the mechanism by which it is activated during saliva release. Trialysin is synthesized as a precursor in the salivary gland, and is activated by proteases during saliva release when the insect probes for blood vessels in the skin of the vertebrate host. We generated a recombinant pro-trialysin in fusion with GST, which was activated by a purified protease from the insect saliva. However, the amount of obtained protein precluded further structural studies. In addition, we found that the N-terminus of trialysin predicts an amphipathic α-helix and the corresponding synthetic peptides retain cytolytic activity. Therefore, we studied the lytic activity of several synthetic peptides corresponding to the N-terminus of trialysin. We found that although all peptides folded into α-helices in the presence of SDS or trifluoroethanol (TFE), only the ones close to the N-terminus of mature trialysin were lytic to *T. cruzi, E. coli,* and erythrocytes. The most active against all these targets was the peptide named P6. It is a 32mer corresponding to the N-terminus of trialysin. Peptide P7, which only differs from P6 by the absence of the last five amino acids, was less active than P6, though much less hemolytic than P6. P5, lacking the first five residues of P6, was as trypanocydal as P7, but as efficient as P6 to lyse erythrocytes. To understand the lytic mechanism and compare the different activities of these peptides, we solved their structures by NMR. We found that they fold into similar amphipathic α-helices in 30% TFE. Based on obtained energy-minimized structures, we noticed the presence of a similar central domain in P5, P6 and P7 composed by the amphipathic helix, and more flexible but similar N-terminal in P6 and P7 and C-terminal domains in P6 and P5. We concluded that presence of the flexible domains in the larger peptide confers stronger activity of P6. We also studied other molecules in the SMOLBnet program, but selected protein targets exclusively related to our current research lines. In a few cases we were successful. This is the case of the trialysin peptides solved by NMR and the serine protease inhibitors solved by diffraction studies (2). For most of the chosen targets we failed in obtaining structural data, although we still have promising results. Nevertheless, we acquired experience in all steps of the process: target selection, protein expression, crystallization, and structure resolution, and the most important achievement was to involve several students, who will keep this knowledge and experience in their scientific formation.

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2. Campos, I. T., Guimaraes, B. G., Medrano, F. J., Tanaka, A. S., and Barbosa, J. A. (2004) *Acta Crystallogr.D.Biol.Crystallogr.* **60,** 2051-2053

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