STRUCTURAL ANALYSES OF *BLASTOCLADIELLA EMERSONII* CENTRIN (BeCen1)

<u>Camargo, A.I¹</u>, Araújo, A.P.U¹, Garcia, W¹, Ribichich, K. F², Beltramini, L.M¹ ¹Instituto de Física de São Carlos, ²Instituto de Química de São Paulo, Universidade de São Paulo, São Paulo, Brazil.

Centrin is an EF-Hand calcium binding protein, usually located at microtubuleorganizing centers (MTOCs), such as basal bodies, spindle pole bodies (SBPs), or centrossomes. Two divergent subfamilies were found in the aquatic fungi Blastocladiella emersonii, one related to Chlamydomonas reinhardii centrin, CrCenp, and other represented by Saccharomyces cerevisiae, ScCdc31p. In fungi, only genes encoding proteins similar to ScCdc31p were described until now. BeCen1, a centrin similar to CrCenp, was previously cloned and expressed as a fusion with maltose binding protein. To explore the structure and function of this centrin it was overexpressed in pET28a vector. After purification on nickel-affinity chromatography, BeCen1 was characterized by spectroscopies methods. Analyses of the Circular Dichroism spectrum showed minima at 207 and 222 nm, which is typical of alpha helix predominant structures. When an external fluorescence probe (ANS) was used, in Ca²⁺ presence, increase of BeCen1 fluorescence emission and a blue shift from 508 nm to 472 nm was observed. This result reflects the exposition of a hydrophobic region probably leading to conformational changes in BeCen1 due to Ca²⁺ binding activity, as found in calcium sensor proteins.

Supported by Brazilian agencies: FAPESP, CNPq

Keywords: Blastocladiella emersonii, Centrin, EF-Hand.