Poster Presentation

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Crystallization and preliminary X-ray diffraction analysis of nepenthesin-1

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Carnivorous pitcher plants of the genus Nepenthes secrete their own aspartic proteases, nepenthesins, to digest prey. Nepenthesins differ significantly in sequence from other plant aspartic proteases. This difference, which brings more cysteine residues into the structure of nepenthesins, in conjunction with putative N-glycosylation, can contribute to uniquely high temperature and pH stabilities of these proteases [1, 2]. In continuation of our previous study of the expression and biochemical and enzymatic characterization of a recombinant form of nepenthesin-1 (rNep-1) from Nepenthes gracilis [3], we report its crystallization and preliminary X-ray analysis. Crystals of rNep-1 in complex with the pepstatin A inhibitor have been grown using the hanging-drop vapour-diffusion technique. Diffraction data were collected to 2.9 Å resolution using synchrotron radiation at Bessy II of HZB, Berlin. The crystals belong to space group P21, with unit-cell parameters a = 54.4 Å, b = 86.6 Å, c = 95.8 Å, β = 106°. The self-rotation function combined with solvent-content calculations and Matthews coefficient suggest the presence of two molecules of rNep-1 in the asymmetric unit. This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic (grants No. EE2.3.30.0029 and No. LG14009), by BIOCEV CZ.1.05/1.1.00/02.0109 from the European Regional Development Fund, and by the Grant Agency of the Czech Technical University in Prague, grant No. SGS13/219/OHK4/3T/14.

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