Saturday, March 19, Session VII

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THE CENTRE OF MOLECULAR STRUCTURE (IBT, CAS) AT BIOCEV

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Starting end of 2015 - beginning of 2016, the "Centre of Molecular Structure" (CMS) has become fully operational. This is a technical platform of the Institute of Biotechnology (Czech Academy of Sciences) located at Biocev in Vestec. CMS is a part of the Czech infrastructure for integrative structural biology (CIISB).

The CMS welcomes guests to use its facilities, in order to help them solve their problems using the available technologies. These are divided into three large sectors: 1) biophysical measurements; 2) advanced structural mass spectrometry 3) macromolecular crystallization and X-ray diffraction, with the associated state-of-the-art scientific instrumentation.

The available technologies will be presented by means of the story of a beginning scientist, just embarking on a scientific career in Biochemistry and Molecular Biology (story telling): how the CMS facilities are used in order to help solve the problems encountered during a research project.

The equipment available at the CMS is listed in the following table:

This work is supported by the project "BIOCEV – Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University (CZ.1.05/1.1.00/02.0109)" from the ERDF, and by the Ministry of Education, Youth and Sports under National support of research infrastructures (infrastructure CIISB, project no. LM2015043).

Instrument name	Technology	Used for
Biophysical measurements		
Specord 50 Plus	UV/Vis spectrometer	High precision UV/Vis spectroscopy
Chirascan Plus	Circular dichroism	Conformation of macromolecules
ProteOn XPR36	Surface Plasmon Resonance	Analysis of interactions
Monolith NT.115	Thermophoresis	Study of interactions
Monolith NT.LabelFree	Thermophoresis	Study of protein interactions
Prometheus NT.48	Differential Scanning Fluorimetry	Protein stability
Microcal iTC200	Calorimetry	Characterisation of interactions
Microcal DSC	Calorimetry	Thermal transitions analysis
Zetasizer Nano ZS90	Dynamic light scattering	Analysis of particle size / hydrodynamic radii
Mass spectrometry		
Bruker Solarix 15T	MALDI or electrospray ionization	Analysis of biomacromolecules and complexes by advanced mass spec
Protein Crystallography		
Spectrolight 600	In-drop dynamic light scattering	Determine mono-dispersity in drops
Art Robbins Gryphon	Automated multichannel pipetting	Crystallisation by robotics
Formulatrix RI1000	Crystallisation plate hotel	Design of crystallisation plans; storage and visualization of crystallisation plates
D8 Venture liq Ga source with Photon II detector	X-ray generation and detection	Testing of crystal diffraction (including in-situ); diffraction data collection

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FREEZING PROCESS IN HUMAN CELL NUCLEI: DETERMINATION OF CRYOPROTECTANT PARAMETERS STRONGLY INFLUENCING THE CELL CONDITION

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In this work, the freezing process and cryoprotective function of three principally different cryoprotectants (antifreeze protein, trehalose and DMSO) were investigated in living cells as well as the phase transition processes of the cryoprotectants solutions. In order to gain deeper insight to the behaviour of the complex cell nucleus, the changes in the genome and nuclear envelope integrity were investigated simultaneously with the higher-order chromatin structure. Cells were cultured in standard medium and compared to cells that were treated with cryoprotectants at the initial unfrozen state, after freezing, and after melting. Analysis was performed after results were obtained from ab-initio modelling, molecular dynamics, Raman spectroscopy, differential scanning calorimetry and X-ray diffraction for each of the cryoprotectants solutions. Our results provide valuable experimental and computational data and help to design novel cryoprotective substances and develop more efficient cryoprotection protocols.

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CHIROPTICAL PROPERTIES OF THE ANTIMICROBIAL PEPTIDE *LASIOCEPSIN* AND OF ITS ANALOGS

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We report chiroptical properties of the novel antimicrobial peptide (AMP) lasiocepsin (LAS, 27As) containing two disulfide bridges [1] and of its three analogs designed to study the influence of heterodetic disulfide-closed rings. The set of peptides included the natural LAS (H-Gly-Leu-Pro-Arg-Lys-Ile-Leu-**Cys**-Ala-Ile-Ala-Lys-Lys-Lys-Gly-Lys-**Cys**-Lys-Gly-Pro-Leu-Lys-Leu-Val-Cys-Lys-**Cys**-OH), two analogs with just one disulfide bridge and the remaining two cysteines replaced by alanine residues (Las[Cys17-Cys27, Ala8,25] – LAS 2; Las[Cys8-Cys25, Ala17,27] – LAS 3), and a linear analog having all four cysteines replaced by alanines (Las[Ala8,17,25,27] – LAS 4A). LAS 2 retains reduced activities against common pathogens while LAS 3 and LAS 4A are inactive [1]. The effect of changing the disulfide bridge pattern on secondary structure is investigated by electronic circular dichroism (ECD) and vibrational optical activity (VOA) including Raman optical activity (ROA) and vibrational circular dichroism (VCD). A combination of these techniques helps us to clarify the role of disulfide bridges in stabilization of LAS's conformation. ECD indicates similar conformation of the disulfide bridge for analogs containing one disulfide (LAS 2, LAS 3), while ROA enables us to determine sense of disulfide torsion, even in the more complicated case of natural LAS containing two disulfide groups. The experimental mainly ROA results were compared to theoretical spectral dependences which were based on known NMR structure of natural lasiocepsin [2].

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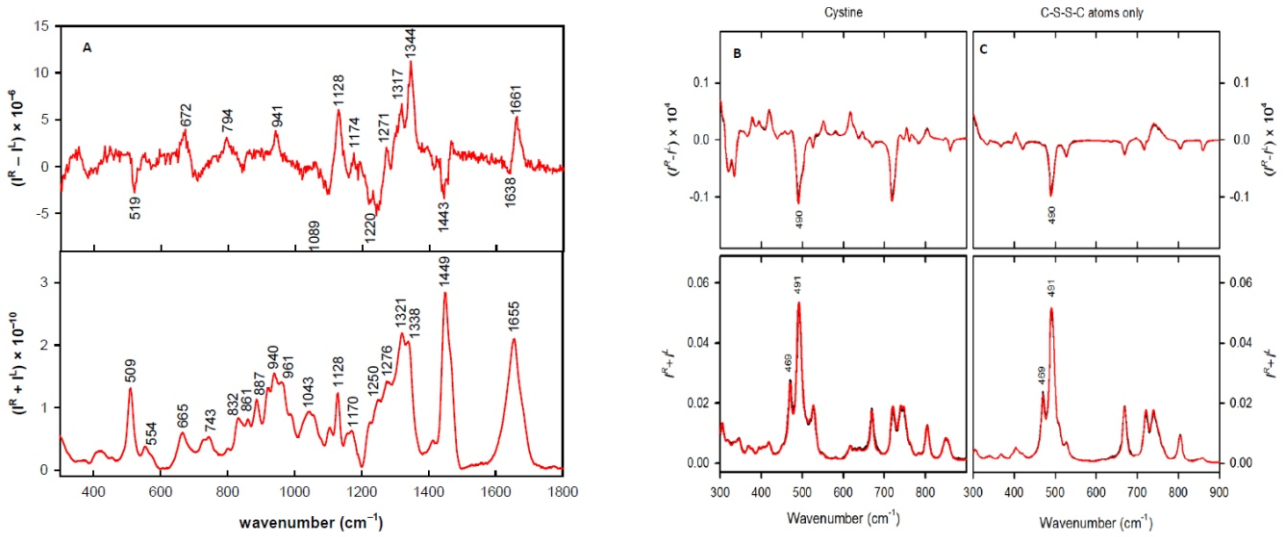

Figure 1: (A) experimental Raman/ROA spectra of lasiocepsin; (B,C) Calculated Raman/ROA signals in disulfide stretching region / for cystin atoms (B), C-S-S-C atoms involves in the calculation (C).

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STRUCTURAL INSIGHT INTO THE 14-3-3 PROTEIN-DEPENDENT INHIBITION OF PROTEIN KINASE ASK1

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Apoptosis signal-regulating kinase 1 (ASK1), a member of the mitogen-activated protein kinase kinase kinase (MAP3K) family, regulates diverse physiological processes such as apoptosis, cytokine secretion or cell differentiation. The activity of ASK1, which is triggered by various stress stimuli, is regulated through homo-oligomerization and interaction with several proteins including the 14-3-3 protein which binds to the phosphorylated motif located at the C-terminus of the kinase domain of ASK1 and suppresses its catalytic activity through unknown mechanism. In this study, we performed biophysical and structural analysis of the complex between the kinase domain of ASK1 phosphorylated at S966 (pASK1-CD) and the 14-3-3 protein using AUC, SAXS and chemical cross-linking. Our results show that the complex between 14-3-3 and pASK1-CD is dynamic and conformationally heterogeneous with both proteins sampling several mutual orientations. In addition, structural analysis together with results of phosphorus NMR and time-resolved tryptophan fluorescence measurements suggest that the 14-3-3 dimer interacts with regions from the C-lobe of the kinase domain of ASK1 and induces conformational change in its active site. Thus, our study provides new insight into the interaction between the kinase domain of ASK1 and 14-3-3 and offers a plausible structural explanation for the 14-3-3 protein-dependent inhibition of ASK1 kinase activity.

This work was supported by the Czech Science Foundation (Project 14-10061S) and The Czech Academy of Sciences (Research Projects RVO: 67985823 of the Institute of Physiology).