

Abstracts

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Plenary talks

O-001

View into the ribosomal exit tunnel

Ada Yonath

*Department of Structural Biology, Weizmann Institute,
Rehovot 76100, Israel*

Ribosomes, the universal polymerases for the translation of the genetic code into proteins, possess an elongated tunnel through which the nascent protein progress until they emerge into the cell. While traveling through the tunnel the nascent proteins can interact with specific tunnel wall components capable of imposing transient or a relatively long elongation arrest. Furthermore, internal architecture and flexibility allow response to cellular signals, thereby provides a mechanism for gene regulation.

O-002

The interplay between actin dynamics and membrane tension determines the shape of moving cells

Kinneret Keren

*Physics Department, Technion-Israel Institute of Technology,
Haifa 32000, Israel*

A central challenge in cell motility research is to quantitatively understand how numerous molecular building blocks self-organize to achieve coherent shape and movement on cellular scales. We focus on one of the classic examples of such self-organization, namely lamellipodial motility, in which forward translocation is driven by a treadmilling actin network. We combine detailed measurements of lamellipodial morphology, spatio-temporal actin dynamics and membrane tension, with mathematical modelling to explain how global shape and speed of the lamellipodium emerge from the

underlying assembly and disassembly dynamics of the actin network within an inextensible membrane bag.

O-003

Structure determination of dynamic macromolecular complexes by single particle cryo-EM

Holger Stark

*Max-Planck-Institute for biophysical Chemistry, Goettingen,
Germany*

Macromolecular complexes are at the heart of central regulatory processes of the cell including translation, transcription, splicing, RNA processing, silencing, cell cycle regulation and repair of genes. Detailed understanding of such processes at a molecular level requires structural insights into large macromolecular assemblies consisting of many components such as proteins, RNA and DNA. Single-particle electron cryomicroscopy is a powerful method for three-dimensional structure determination of macromolecular assemblies involved in these essential cellular processes. It is very often the only available technique to determine the 3D structure because of the challenges in purification of complexes in the amounts and quality required for X-ray crystallographic studies.

In recent years it was shown in a number of publications that it is possible to obtain near-atomic resolution structures of large and rigid macromolecules such as icosahedral viruses. Due to a number of methodological advances there are now also great perspectives for high-resolution single particle cryo-EM studies of large and dynamic macromolecules. Successful high-resolution structure determination of dynamic complexes requires new biochemical purification strategies and protocols as well as state of the art electron microscopes and high-performance computing. In the future cryo-EM will thus be able to provide structures at near-atomic

FLIM, for real-time analysis of molecular mobility and reaction inside biofilms. By means of non-biological or biological (virus, biocides and antibiotics) reactive compounds, significant advances to understand the roles of the extracellular matrix and bacteria physiological properties were obtained, an important step to improve pathogenic biofilm inactivation.

P-334

Towards skin cancer diagnostics by two-photon microscopy

Albrecht Haase¹, Francesco Tessarolo¹, Claudia Caldini¹, Paolo Bauer², Giuseppe Zumiani², Sebastiana Boi³, Renzo Antolini¹

¹BIOtech Research Centre and Physics Department, University of Trento, Mattarello, Italy, ²Department of Dermatology, APSS, S. Chiara Hospital, Trento, Italy, ³Department of Histopathology, APSS, S. Chiara Hospital, Trento, Italy

Here we present a feasibility study to develop two-photon microscopy (2PM) into a standard diagnostic tool for non-invasive, skin cancer diagnosis. The goal is defining experimental parameters to maximize image quality of optical biopsies in human skin, while avoiding tissue damage. Possible diagnostic indicators will be compared for healthy tissue, benign, and malignant melanocytic lesions.

We report on preliminary results of a study on 2PM imaging of “ex-vivo” biopsy samples, were autofluorescence intensity and contrast between lesion and surrounding tissues were optimised varying excitation wavelength, detection band, and dispersion pre-compensation. Moreover, we determined modulation functions for laser power and detector gain to compensate losses in deep tissue imaging. As the main process of photo-damage, thermo-mechanical modifications were quantified and damage threshold powers were determined.

In order to image structural changes in ordered tissue like collagen fibres, second-harmonic generation signals were recorded and optimised.

P-335

In-vivo two-photon imaging of the honeybee antennal lobe

Albrecht Haase¹, Elisa Rigosi^{2,3}, Gianfranco Anfora³, Claudio Vinegoni⁴, Giorgio Vallortigara², Renzo Antolini¹

¹BIOtech Research Centre and Physics Department, University of Trento, Mattarello, Italy, ²CIMeC, Centre for Mind/Brain Sciences, University of Trento, Rovereto (TN), Italy, ³IASMA Research and Innovation Center, Fondazione E. Mach, San Michele all'Adige, Italy, ⁴Center for Systems Biology and Center for Molecular Imaging Research, Massachusetts General Hospital, Harvard Medical School, Boston, USA

We adapted a two-photon microscope for in-vivo imaging of the honeybee olfactory system, focusing on its primary centres, the antennal lobes. The setup allowed obtaining both 3D-tomographic measurements of the antennal lobe

morphology and time-resolved in-vivo calcium imaging of its neuronal activity.

The morphological data were used to precisely measure the glomerular volume in both sides of the brain, investigating the question of volumetric lateralization. Functional calcium imaging allowed to record the characteristic glomerular response maps to external odour stimuli applied to the bees' antennae. Compared to previous neural imaging experiments in the honeybee, this work enhanced spatial and temporal resolution, penetration depth, and it minimized photo-damage. Final goal of this study is the extension of the existing functional atlases of the antennal lobe to 3D and into the temporal dimension by investigating the time-resolved activity pattern.

P-336

Fluorescent analysis of apoptosis induced by *Pseudomonas aeruginosa* in endothelial cells

F. Iordache¹, C. Iordache², M. Lixandru, ²A. Holban³, C. Bleotu³, V. Lazar³, C. Chifiriuc³, H. Maniu¹

¹Institute of Cellular Biology and Pathology “Nicolae Simionescu”, Bucharest, Romania, ²National Institute for Research and Development in Microbiology and Immunology Cantacuzino, Romania, ³Faculty of Biology, University of Bucharest, Romania

A consequence of invasion by an intracellular pathogen is apoptosis of the host cell, usually observed in phagocytic cells. The aim of this study was to evaluate if *Pseudomonas (P.) aeruginosa*, although not considered a classic intracellular pathogen, could induce apoptosis in endothelial cells (EC). Strains were evaluated for virulence factors expression: pore forming toxins (haemolysins, lecithinases, lipases), proteases (caseinases, gelatinase), DNA-ses, mucinases. The adherence and invasion capacity of *P. aeruginosa* to EC was done using Cravioto's method. For detection of apoptosis was used acridine orange/ethidium bromide staining. The qualitative assay of bacterial adherence to cellular substrate revealed that all tested strains adhered to EC, exhibiting a diffuse, aggregative or mixed diffuse-aggregative pattern and 20-70% adherence rates. Cell free culture of *P. aeruginosa* did not determine a cell death response, but in case of whole bacterial culture, acridine orange/ethidium bromide staining showed the capacity to induce apoptosis of EC, fluorescent microscopy revealed the chromatin condensation, fragmented nuclei, membrane blebbing, and apoptotic bodies. *P. aeruginosa* have the ability to adhere and invaded EC and to induce morphological changes, including apoptosis.

P-337

Photo-toxicity of near-infrared voltage sensitive fluorescent dyes

Giedrius Kanaporis¹, Jonas Jurevicius¹, Irma Martisiene¹, Ruta Vosyliute¹, Antanas Navalinskas¹, Rimantas Treinys¹, Arvydas Matiukas², Arkady M. Pertsov²

¹Lithuanian University of Health Sciences, Kaunas, Lithuania, ²SUNY Upstate Medical University, Syracuse, NY, USA

The use of voltage sensitive fluorescent dyes (VSD) for noninvasive measurement of the action potential (AP) in