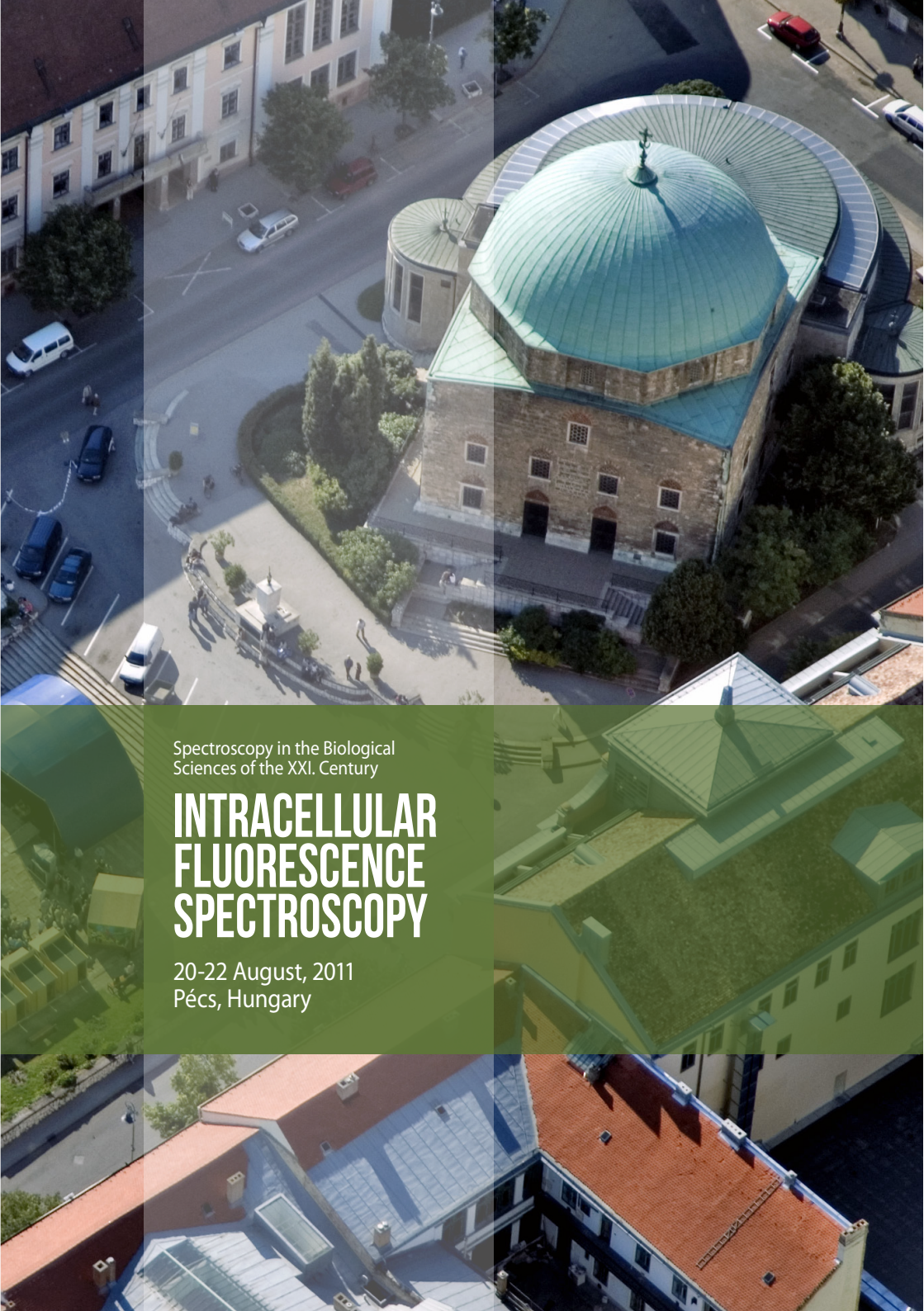

Spectroscopy in the Biological Sciences of the XXI. Century

INTRACELLULAR FLUORESCENCE SPECTROSCOPY

20-22 August, 2011
Pécs, Hungary

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A Satellite Conference of the
8th European Biophysics Congress

GENERAL INFORMATION

Organising Committee

Alexander P. Demchenko
The Palladin Institute of Biochemistry, Academy of Sciences of Ukraine, Kiev, Ukraine

Gábor Hild
University of Pécs, Medical School, Department of Biophysics, Pécs, Hungary

Beáta Bugyi
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Congress Venue

Corso Hotel Pécs

H-7626 Pécs
Koller u. 8.

www.corsohotel.hu

About Pécs

Pécs, the host city of the forthcoming Intracellular Fluorescence Spectroscopy Conference, is the fifth largest city of Hungary with a cozy, Mediterranean atmosphere and lively environment.

With its over 2000-year history, Pécs is a city of rich cultural heritage and innovations as well as of long tradition of conducting science. Pécs is a multicultural city: it has been developed on cultural layers of Latin, Slavic, Hungarian, Turkish and German origin. Both in the past and now, the city serves as a gateway between "eastern" and "western" cultures; between the Balkan and Western Europe. These features were appreciated with the title of the Cultural Capital of Europe in 2010.

Visit the home page of Pécs for more details: **http://en.pecs.hu**

Poster preparation

Poster presentations will take place from 16:00-18:00, Sunday (21 August, 2011).

The posters should be prepared for standard stand A0 size (84 x 119 cm - 1m^2).

On Monday, 22 August, 2011, young scientists and the winners of the poster prizes will present their work in 20 minutes (including 5 minutes for discussion).

Conference website

http://ebsa-ifs.aok.pte.hu

Contact information

You may contact us at:

ebsa-ifs@aok.pte.hu

PROGRAM

August 20, Saturday

12:00-16:00	Arrival, registration

Opening ceremony and talks

16:00-16:30	Welcome speeches
16:30-17:15	*Keynote lecture 1* – **Dr Michael Ferenczi**: What can we learn about molecular mechanisms using Fluorescence Lifetime Imaging in organised cellular systems: the example of muscle
17:15-18:00	*Keynote lecture 2* – **Dr Alexander Demchenko**: From two-dimensional to three-dimensional sensing and imaging in biological membranes: fluorescence and MD simulations
18:00	Welcome reception

August 21, Sunday

Session 1 – Technical issues

8:00-8:30	*Talk 1* – **Dr Axel Bergmann**: Probing Molecular Interactions by Fluorescence Lifetime Imaging
8:30-9:00	*Talk 2* – **Dr Róbert Szipőcs**: Tunable, Low Repetition Rate, Femtosecond Pulse Ti:Sapphire Laser comprising a 2.6 W pump for in vivo Nonlinear Microscopy
9:00-9:30	*Talk 3* – **Nadezhda Kudryasheva**: Spectral components of coelenterate bioluminescence and photoluminescence
9:30-10:00	*Talk 4* – **Dr András Kengyel**: Regulation and kinetic characterization of a GFP-fused non-muscle myosin IIA
10:00-10:30	Tea & coffee break

Session 2 – Cell biology applications

10:30-11:00	*Talk 5* – **Dr Péter Lénárt**: A contractile actin meshwork drives chromosome transport in starfish oocytes
11:00-11:30	*Talk 6* – **Dr Gábor Csúcs**: Fluorescence Correlation Spectroscopy - studies from yeast

11:30-12:00	*Talk 7* – **Anna Wypijewska**: Fluorescence detection of enzyme-ligand interactions in the process of mRNA cap degradation by DcpS enzyme
12:00-12:30	*Talk 8* – **Dr Balázs Visegrády**: The role of the I-BAR domain in the filopodia formation
12:30-14:00	Lunch

Session 3 – Biochemistry and biophysics

14:00-14:30	*Talk 9* – **Dr András Lukács**: Ultrafast dynamics of the BLUF mutant dAppA Q63E revealed by fluorescence upconversion and transient infrared spectroscopy
14:30-15:00	*Talk 10* – **Dr Beáta Bugyi**: Tropomyosin isoform specific regulation of nucleation factors
15:00-15:30	*Talk 11* – **Zoltán Ujfalusi**: The effect of tropomyosin and heavy meromyosin on the flexibility of formin-bound actin filaments
15:30-16:00	Tea & coffee break
16:00-18:00	Poster session, Poster prize competition
18:00	Social event (Announcing the winner of the Poster prize competition, dinner)

August 22, Monday

Session 4 – Young scientists' talks

9:00-9:20	*Talk 12* – Poster prize winner #1
9:20-9:40	*Talk 13* – Poster prize winner #2
9:40-10:00	*Talk 14* - Poster prize winner #3
10:00-10:20	*Talk 15* – **Roland Kardos**: Conformation and dynamics of actin in the presence of actin binding proteins
10:30	Closing remarks

TALKS

Probing Molecular Interactions by Fluorescence Lifetime Imaging

Axel Bergmann, Wolfgang Becker

Becker & Hickl GmbH, Nahmitzer Damm 30, 12277 Berlin, Germany. e-mail: bergmann@becker-hickl.com

Fluorescence imaging techniques have found broad application in live sciences because they are non-destructive, extremely sensitive, and deliver information about biochemical interactions on the molecular scale. Of all fluorescence parameters, it is the fluorescence decay function that yields the most direct insight into molecular processes within live cells and tissues. A fluorescence lifetime imaging (FLIM) technique for biological imaging has to combine high photon efficiency, high lifetime accuracy, resolution of multi-exponential decay profiles, simultaneous recording in several wavelength intervals and optical sectioning capability. We will show that the combination of multi-dimensional time-correlated single photon counting (TCSPC) with confocal or two-photon laser scanning meets these requirements almost ideally. Multi-dimensional TCSPC is based on the excitation of the sample by a high-repetition rate laser and the detection of single photons of the fluorescence signal. Each photon is characterised by its time in the laser period, its wavelength, and the coordinates in the scanning area. The recording process builds up a photon distribution over these parameters. We will demonstrate the application of the technique to ion concentration measurements, FRET experiments, and autofluorescence imaging.

Tropomyosin isoform specific regulation of nucleation factors

Andrea Vig, Tamás Huber and **Beáta Bugyi**

University of Pécs, Medical School, Department of Biophysics, Szigeti str. 12, Pécs H-7624, Hungary

In the cohesive structure of the cytoskeleton functionally distinct actin arrays orchestrate fundamental cell functions in a spatiotemporally controlled manner. Emerging evidences emphasize that protein isoforms are essential for the functional polymorphism of the actin cytoskeleton. The generation of diverse actin networks is catalysed by different nucleation factors, like formins and Arp2/3 complex. These actin arrays also exhibit qualitative and quantitative differences in the associated tropomyosin (Tm) isoforms. How the molecular composition and the function of actin networks are coupled is not completely understood.

We investigated the effects of different Tm isoforms (skeletal muscle, cytoskeletal 5NM1 and Br3) on the activity of mDia1 formin and Arp2/3 complex using fluorescence spectroscopic approaches. The results show that the studied Tm isoforms have different effects on the mDia1-, and Arp2/3 complex-mediated actin assembly. The activity of the Arp2/3 complex is inhibited by skTm and Tm5NM1, while TmBr3 does not have any effect. All three Tm isoforms inhibited the activity of mDia1.

These results contribute to the understanding of the mechanisms by which tropomyosin isoforms regulate the functional diversity of the actin cytoskeleton.

Fluorescence Correlation Spectroscopy – studies from yeast

Mathias Bayer[1,2], Yves Barral[2], **Gabor Csucs**[1]

[1]*Light Microscopy Centre, ETH Zurich*
[2]*Institute of Biochemistry, ETH Zurich*

Fluorescence Correlation Spectroscopy (FCS) and Fluorescence Cross-Correlation Spectroscopy (FCCS) are powerful biophysical techniques that can be used not only to investigate the mobility and absolute concentration but also the interaction of various proteins (measuring even binding constants) both in vitro and in vivo. From a theoretical point of view budding yeast (S. Cerevisiae) would be well suited as experimental system both for FCS and for FCCS measurements. In my talk I want to introduce FCS and FCCS as techniques and discuss the difficulties to use them in "real" *in vivo* measurements. I will especially concentrate on the specific problems coupled to measurements in yeast and introduce some strategies to overcome them. Furthermore I'll report about our results on understanding the asymmetric cell division of yeast and the role of specific proteins (Kar9, Bim1 etc.) by means of FCS and FCCS.

From two-dimensional to three-dimensional sensing and imaging in biomembranes: fluorescence and MD simulations

Alexander P. Demchenko

Palladin Institute of Biochemistry, The National Academy of Sciences of Ukraine. Leontovicha street 9, Kiev-01030, Ukraine. e-mail: alexdem@ukr.net

Essential changes are recently observed in the scientists' views on the functioning and connected structural and dynamic properties of biological membranes. Hierarchical cluster-type structure of membranes has got further confirmation that allows clarifying the role of protein and lipid components. An established fact on dramatic difference in lipid composition between two monolayers (external and internal) of plasma membranes result in differences in surface charge and potential, ion binding, interaction with protein molecules, etc. Glycolipid component of outer monolayer and interaction of inner monolayer with cytoskeleton allow the membrane by expanding the asymmetry to attain its important functional properties. In the attempts to describe cellular membranes and provide their realistic modeling there is a timely necessity to shift from two-dimensionality (which reduces the analysis to membrane plane only) to more realistic three-dimensional models. This requires acquiring two new types of primary data: fluorescence probes of new generation that allow variable well-defined location and orientation in the membrane with multiparametric reporting and extended-time MD simulations. The results obtained in this line will be discussed.

Fluorescence detection of enzyme-ligand interactions in the process of mRNA cap degradation by DcpS enzyme

Anna Wypijewska, Elzbieta Bojarska, Maciej Lukaszewicz, Janusz Stepinski, Jacek Jemielity and Edward Darzynkiewicz

Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Warsaw, Poland

Fluorescence methods benefit to examination of various cell disorders and find adoption for medical needs. Our interest is the mRNA cap degradation by Decapping Scavenger (DcpS) enzyme. Basic aims of DcpS investigation refer to finding its efficient inhibitors. Recently it has been shown that DcpS inhibition may improve Spinal Muscular Atrophy treatment. It can also be a target for anticancer therapy, where cap analogues may be used to trap eIF4E in order to prevent the initiation of translation. By the inhibition of both, eIF4E and DcpS, we can assure the stability of a therapeutic cap analogue and therefore reduce protein over-expression.

Our research enabled us to propose 7-methylgoanosine diphosphate (m^7GDP) as a potential DcpS inhibitor. As DcpS enzymes were previously thought to degrade this cap structure, we inspected 3 recombinant DcpS proteins, human, *A. suum* and *C. elegans*, and applied HPLC analysis to prove that DcpS has no ability to hydrolyse m^7GDP. Using Time Synchronized Titration fluorescence technique the high binding affinity of m^7GDP to DcpS proteins has been shown. On the basis of our findings we propose a new model of the mRNA degradation, in which m^7GDP stability in the mRNA decay machinery makes it a potential inhibitor of cap binding proteins (eIF4E and DcpS).

POSTERS

P1

Characterization of the biochemical properties and biological function of the formin homology domains of Drosophila DAAM

Szilvia Barkó[1], Beáta Bugyi[2], Marie-France Carlier[2], Rita Gombos[3], Tamás Matusek[3], József Mihály[3] and Miklós Nyitrai[1]

[1]*University of Pécs, Medical School, Department of Biophysics, Pécs, Szigeti str. 12, H-7624, Hungary.*
[2]*Cytoskeleton Dynamics and Motility, Laboratoire d'Enzymologie et Biochemie Structurales, Centre National de la Recherche Scientifique, 1 Avenue de la Terrasse, 91198,Gif-sur-Yvette, France.*
[3]*Biological Research Center of the Hungarian Academy of Sciences, Institute of Genetics, Szeged, Temesvári krt. 62, H-6726, Hungary.*

We characterised the properties of Drosophila melanogaster DAAM-FH2 and DAAM-FH1-FH2 fragments and their interactions with actin and profilin by using various biophysical methods and in vivo experiments. The results show that while the DAAM-FH2 fragment does not have any conspicuous effect on actin assembly in vivo, in cells expressing the DAAM-FH1-FH2 fragment a profilin-dependent increase in the formation of actin structures is observed. The trachea specific expression of DAAM-FH1-FH2 also induces phenotypic effects leading to the collapse of the tracheal tube and lethality in the larval stages. In vitro, both DAAM fragments catalyse actin nucleation but severely decrease both the elongation and depolymerisation rate of the filaments. Profilin acts as a molecular switch in DAAM function. DAAM-FH1-FH2, remaining bound to barbed ends drives processive assembly of profilin-actin, while DAAM-FH2 forms an abortive complex with barbed ends that does not support profilin-actin assembly. Both DAAM fragments also bind to the sides of the actin filaments and induce actin bundling. These observations show that the Drosophila melanogaster DAAM formin represents an extreme class of barbed end regulators gated by profilin.

P2

The effect of Toxofilin on the structure of monomeric actin

Lívia Czimbalek, Veronika Kollár, Roland Kardos, Gábor Hild

University of Pécs, Medical School, Department of Biophysics, 7624 Pécs, Szigeti str. 12. Hungary

Actin is one of the main components of the intracellular cytoskeleton. It plays an essential role in the cell motility, intracellular transport processes and cytokinesis as well.

Toxoplasma gondii is an intracellular parasite, which can utilise the actin cytoskeleton of the host cells for their own purposes. One of the expressed proteins of *T. gondii* is the 27 kDa-sized toxofilin. The long protein is an actin monomer binding protein which is involved in the host invasion.

In our work we studied the effect of the actin-binding site of toxofilin$_{69\text{-}196}$ on the G-actin. We determined the affinity of toxofilin to the actin monomer. The fluorescence of the actin bound ε-ATP was quenched with acrylamide in the presence or absence of toxofilin. In the presence of toxofilin the accessibility of the bound ε-ATP decreased, which indicates that the nucleotide binding cleft is shifted to a more closed conformational state.

The results of the completed experiments can help us to understand in more details what kind of cytoskeletal changes can be caused in the host cell during the invasion of the host cells by intracellular parasites.

P3

The role of the I-BAR domain in the filopodia formation

Kinga Futó[1], Laura M. Machesky[2], Balázs Visegrády[1]

[1]*University of Pécs, Medical School, Department of Biophysics, Szigeti str. 12, Pécs H-7624, Hungary*
[2]*Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD, U.K.*

The Inverse BAR (I-BAR) domain is part of the superfamily of the membrane-deforming protein is Bin-amphiphysin-Rvs (BAR) proteins which induce either positive or negative membrane curvature both *in vitro* and in cells. Generation of membrane curvature by these membrane deforming proteins often works together with actin dynamics. I-BAR shares its function between actin bundling and membrane binding but it is still obscured what molecular mechanisms are responsible for these functions. The aim of our project is to investigate the detailed membrane binding properties of the I-BAR of IRSp53 and its relations to the actin cytoskeleton. *In vitro* FRET experiments and fluorescence quenching studies were carried out between the I-BAR and liposomes made up from different lipid constructs. We have found that the I-BAR has preference to bind to the negatively charged lipids however it can also bind to the uncharged lipids. The fluorescence quenching studies reflected that the accessibility of the I-BAR surface was higher toward the negatively charged lipids than for the uncharged ones. TNS fluorescence assay reflected that the I-BAR domain binds to the surface of the micelles rather than penetrating into its core.

P4

Fluorescence lifetime as a tool to study protein-protein interactions of GroEL and PRD1 viral proteins

Jenni Karttunen[1], Sari Mäntynen[1], , Hanna M. Oksanen[2], Janne Ihalainen[1] and Jaana K.H. Bamford[1]

[1]*Department of Biological and Environmental Science and NanoScience Center, University of Jyväskylä, Finland*
[2]*Institute of Biotechnology and Department of Biological and Environmental Science, University of Helsinki, Finland.*

We have established a set of vectors to promote easy cloning of eCFP and eYFP fusions with any protein of interest. We exploit these fluorescent fusion proteins to study protein-protein interactions by fluorescence lifetime of eCFP. The decrease of eCFP lifetime reveals FRET between eCFP and eYFP and hence the interaction between proteins in question.

GroEL-GroES chaperonin complex is required for the proper folding of *Eschericia coli* proteins. Bacteriophage T4 and its distant relative coliphage RB49 encode co-chaperon proteins (respectively gp31 and CocO) that can replace GroES in the chaperonin complex. GP31 is also required in the folding of the major capsid protein of the phage. PRD1 is a large membrane-containing bacteriophage infecting gram-negative bacteria such as *E. coli* and *Salmonella enterica*. It has 15 kb long linear dsDNA genome and the capsid has an icosahedral symmetry. The GroEL-GroES chaperonin complex is needed in the assembly of PRD1. We have found evidence that PRD1 protein P33 can work similar way as other viral co-chaperones and substitute GroES in chaperonin complex. Fluorescence lifetime studies between proteins GroEL and P33 reveals an interaction that backs up the theory.

P13

Tropomyosin isoform specific regulation of nucleation factors

Andrea Vig, Tamás Huber and Beáta Bugyi

University of Pécs, Medical School, Department of Biophysics, Pécs, Hungary

In the cohesive structure of the cytoskeleton functionally distinct actin arrays orchestrate fundamental cell functions in a spatiotemporally controlled manner. Emerging evidences emphasize that protein isoforms are essential for the functional polymorphism of the actin cytoskeleton. The generation of diverse actin networks is catalyzed by different nucleation factors, like formins and Arp2/3 complex. These actin arrays also exhibit qualitative and quantitative differences in the associated tropomyosin (Tm) isoforms. How the molecular composition and the function of actin networks are coupled is not completely understood.

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These results contribute to the understanding of the mechanisms by which tropomyosin isoforms regulate the functional diversity of the actin cytoskeleton.

PARTICIPANTS

List of participants

T: talk, P: poster, number: page number, **boldface**: presenting author

Almudena Albillos Martínez (-)

Szilvia Barkó (**P 28**)

Axel Bergmann (**T 12**)

Emőke Bódis (-)

Beáta Bugyi (**T 13**, P 28, P 40)

Gábor Csúcs (**T 14**)

Lívia Czimbalek (P 29)

Alexander P. Demchenko (**T 15**)

Réka Dudás (-)

Nelli Farkas (P 36, P 37)

Michael Ferenczi (**T 16**)

Kinga Futó (T 25, **P 30**)

László Grama (-)

Gábor Hild (P 32)

Tamás Huber (P 13, P 40)

Roland Kardos (**T 17**, P 32)

Jenni Karttunen (**P 31**)

András Kengyel (**T 18**)

Ágnes Kokas (P 34)

Veronika Kollár (P 32)

Nadezhda Kudryasheva (**T 19**, P 35)

Péter Lénárt (**T 20**)

Dénes Lőrinczy (-)

András Lukács (**T 21**)

Laura Nagy (-)

Miklós Nyitrai (P 34, P 24, P 28, P 33, P 36, P 39)

Katalin Raics (-)

Dávid Szatmári (**P 33**)

Róbert Szipőcs (**T 22**)

Gábor Csaba Talián (**P 34**)

Mónika Tóth (P 34)

Anna Tugarova (P 35)

Kata Türmer (P 34, **P 36**, **P 37**, **P 38**)

Zoltán Ujfalusi (**T 24**, **P 39**)

Andrea Vig (T 13, **P 40**)

Balázs Visegrády (**T 25**, P 30)

Anna Wypijewska (**T 26**)

Conference organized by the
Department of Biophysics
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