Program & Abstract Booklet

Second Symposium on Super-resolution and Advanced Fluorescence Microscopy and István Ábrahám Memorial Workshop

April 1-2, 2022 - Pécs, Hungary

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National Research, Development and Innovation Office
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Contents

Welcome

Dear Participant,

It is our pleasure to welcome you at the Second Symposium on Super-resolution and Advanced Fluorescence Microscopy and István Ábrahám Memorial Workshop.

The Symposium is a forum for Hungarian and international scientists, who develop and use super-resolution microscopy and other advanced fluorescent microscopy methods, to present their latest technical innovations and scientific results especially in the field of life sciences. The Symposium is also an opportunity for researchers working in various specialized microscopy facilities to build collaborations and to learn about good practices in managing those facilities.

The program includes the István Ábrahám Memorial Workshop., where colleagues from all over the world come together to present scientific talks and to remember István Ábrahám, former Head of the Institute of Physiology, founder of the Nano-Bio-Imaging Core Facility in Pécs and President of the Hungarian Neuroscience Society, who passed away last year.

Thanks to over one hundred registered participants and 28 oral presentations from single-molecule imaging over nanotubes to expansion microscopy (to name a few), we expect an engaging scientific program and plenty of opportunity for discussions as well as for socializing.

We wish you a great time at the meeting.

Péter Buzás, PhD

Institute of Physiology, Medical School, University of Pécs

András Lukács, PhD

Institute of Biophysics, Medical School, University of Pécs

István Hernádi, PhD

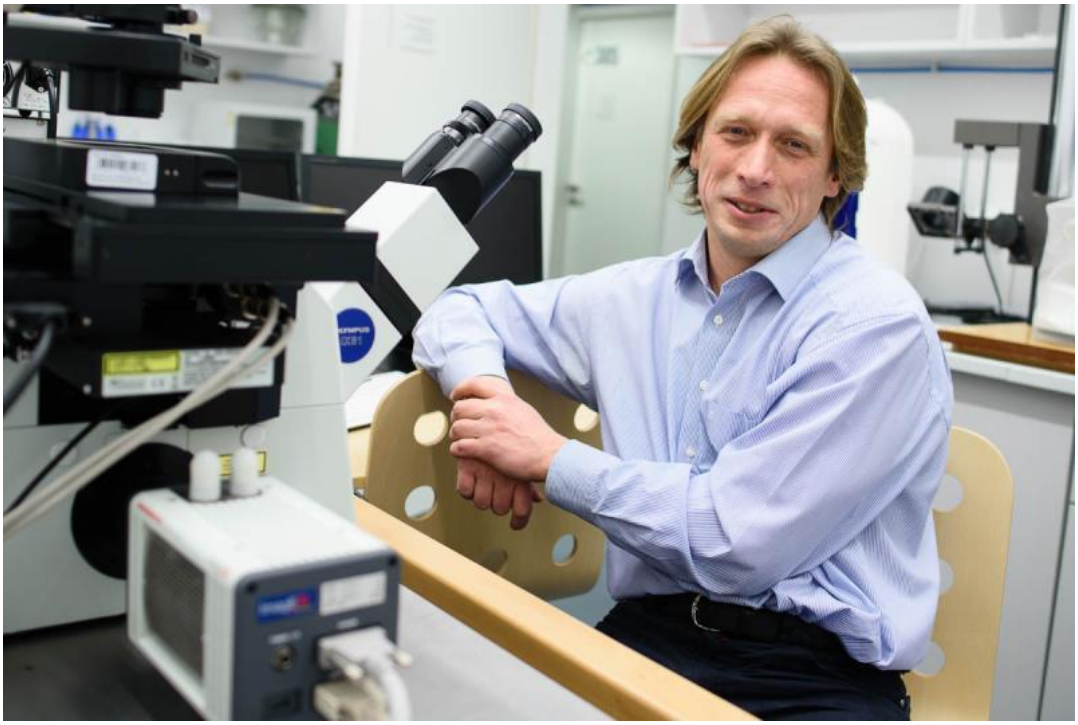
on behalf of the Centre for Neuroscience, University of Pécs

György Vámosi, PhD

on behalf of Cellular Imaging Hungary

Prof. Dr. István Miklós Ábrahám

(1967-2021)



István Ábrahám graduated summa cum laude from the University of Pécs, Medical School, in 1993. As a student, he began research at the Institute of Physiology, Neurophysiology Research Group of the Hungarian Academy of Sciences, under the guidance of Professor László Lénárd. During his undergraduate years, Professor Ábrahám achieved outstanding results in which he was awarded the Fellowship of the Republic in three separate instances. Additionally, he was awarded a Demonstrator Fellowship, and in 1993, he was awarded the Pro Scientia Gold Medal.

Uniquely, in 1993, he presented two lectures at the National Conference of the Undergraduate Research Society, in which he was first author. One lecture referenced HPLC measurements of brain neurotransmitters for which he was awarded Second Prize, while the other one was entitled “Non-linear dynamical approach of neuronal activity: chaos and single unit activity in the globus pallidus”, inspired by the emerging chaos theories of the time, for which he was awarded First Prize.

Following graduation, he continued his PhD studies at the Institute of Experimental Medicine in Budapest, under the supervision of Dr. Krisztina Kovács. During this time, he broadened his professional knowledge in the research group of world-renowned neuro-endocrinologist Béla Bohus, at the University of Groningen in the Netherlands. He

defended his thesis summa cum laude at the Doctoral School of Semmelweis University of Medicine in 1998.

After earning his PhD, he spent two more years at the Molecular Neuroendocrinology Research Group of the Institute of Experimental Medicine, where his research focused on stress-related neuronal networks.

Between 2000 and 2002, he was a Marie Curie Fellow at the Babraham Institute in Cambridge, England, where he developed a lifelong professional relationship with Professors Allan Herbison and Seong Kyu Han. His interest then shifted towards studying the non-genomic effects of estrogen in the brain.

Following his return home, he became one of the leading researchers in the Neurobiology Research Group of the MTA at-ELTE for a period lasting four years, in which he continued studying the effects of estrogen in the brain. During this time, two PhD students obtained their doctoral degrees under his professional supervision.

In 2007, he was offered the opportunity to set up and manage his own research group at the University of Otago in New Zealand, where he achieved considerable professional success. During the six years he spent in New Zealand, two other students completed their PhDs under his guidance. While in Otago, he developed a close collaboration with Professor Akihiro Kusumi in the field of single molecule detection. It was this collaboration which gradually shifted his interest towards super-resolution microscopy.

Despite his success abroad, his heart always remained in Hungary, where he envisioned a future for his children and his family. Eventually, he returned to his Alma Mater in 2011.

With the support of the Albert Szent-Györgyi Scholarship, among others, Professor Ábrahám began implementing his innovative ideas in 2013. Following his appointment as Professor, he founded the Molecular Neuroendocrinology Research Group, which has consistently undergone expansion, and evolved into a professionally diverse and exceptionally cohesive group in the following years.

He was instrumental in founding and chairing the first Centre for Neuroscience in the country, at the University of Pécs. Professor Ábrahám served on several editorial boards of international scientific journals and scientific societies. In early 2021, he was elected President of the Hungarian Neuroscience Society.

Following his appointment as Director of the Institute of Physiology in 2019, István immersed himself into the task of reforming the institute with his characteristic drive and determination. Additionally, he exerted immense energy in seeing one of his greatest dreams take flight, which was the creation of a facility accommodating a wide range of super-resolution and advanced fluorescence microscopes. In Spring 2021, the equipment was about to be set up at its new premises, designed by himself; but tragically, he never saw this completed. The centre was launched at its final location and named István Ábrahám Nano-Bio-Imaging Core Facility in December 2021.

Organizers

Organizing committee

Péter Buzás
Symposium president, Institute of Physiology, Medical School, University of Pécs

András Lukács
Institute of Biophysics, Medical School, University of Pécs

Dóra Zelena
Institute of Physiology, Medical School, University of Pécs

Klaudia Barabás
Institute of Physiology, Medical School, University of Pécs

István Hernádi
Institute of Biology, Faculty of Science, University of Pécs

Miklós Erdélyi
Department of Optics and Quantum Electronics, University of Szeged, Hungary

György Vámosi
Department of Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen, Hungary

Local organizers

Tímea Dévai

Dávid Ernszt

László Grama

Tibor Jánosi

Kitti Komé

Tamás Kovács

Géza Makkai

General Information

Web page

The address of the symposium web page is https://superresolution.hu.

Contact

Institute of Physiology
Medical School
University of Pécs

Szigeti út 12.
7624 Pécs
Hungary

Email: symposium@superresolution.hu

Phone: +36 72 536001 ext. 38500

Travel and accommodation inquiries for invited speakers

Ms. Tímea Dévai

Institute of Biophysics
Medical School
University of Pécs

Email: timea.marki@aok.pte.hu

Venue

The Symposium will take place in the new Endre Grastyán Building of the Medical School of the University of Pécs, Hungary. The talks will be in lecture hall SIOT 0032 (“green side”).

Accommodation

Accommodation is available in nearby Hotel Makár.

Catering

Meals and refreshments will be served in the foyer outside the lecture hall for registered participants. Coffee, refreshments, and lunch will be provided on Friday and Saturday. The conference dinner will be on Friday.

Conference WiFi

WiFi access is provided for participants during the conference. Log-in details are available at the reception desk.

Acknowledgement

This event is supported by the 2021 Science Patronage program of the National Research, Development and Innovation Office, Hungary (grant no. MEC_SZ_21 140882) and by the Medical School of the University of Pécs.

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Invited Speakers

Allan Herbison

Department of Physiology, Development and Neuroscience, Cambridge University, United Kingdom

Allan is currently Professor of Neuroendocrinology at the University of Cambridge. After graduating from the University of Otago, NZ and time in clinical practice, Allan undertook a PhD in Neuroendocrinology at the University of Cambridge. Allan then spent a further 12 years as a Principal Investigator at The Babraham Institute and Fellow of Pembroke College, University of Cambridge before returning to the University of Otago, NZ where he founded the Center for Neuroendocrinology. In 2019, Allan moved back to Cambridge supported by the Wellcome Trust. Allan has received multiple international fellowships and prizes and was elected Fellow of the Royal Society of New Zealand in 2007. Allan has always been interested in adapting the latest neuroscience approaches to the study of neuroendocrine circuits and has pioneered the use of genetically manipulated rodent models and methodologies at molecular, cellular, and whole animal levels. His studies have focused on elucidating the neural circuits and mechanisms underlying the episodic pulse and surge modes of GnRH secretion in mammals. Allan has published over 230 papers and has an H-index of 83.

Deepak Srivastava

Department of Basic and Clinical Neuroscience, Institute of Psychiatry Neuroscience and Psychology and MRC Centre for Neurodevelopmental Disorders, King's College London, United Kingdom

Deepak is a Reader In Molecular Neuroscience in the Department of Basic and Clinical Neuroscience and a Goup Leader in the MRC Centre For Neurodevelopmental Disorders at King's College London. He obtained his PhD in Neuroscience of the University of Cambridge and carried out his post-doctoral training the laboratory of Peter Penzes at Northwestern University. He moved to London in 2012. Deepak's group investigates molecular and cellular aspects of synaptic biology with a translational focus. They use also use advanced imaging technologies to investigate these questions. Deepak is also the founder and Director of the Wohl Cellular Imaging Centre, which is an advance light microscopy facility focused on the study of neuroscience.

Gerhard Schütz

Institute of Applied Physics, TU Wien, Austria

Gerhard Schütz is interested in single molecule biophysics and its application to live cell biology. His group engaged in the development of a toolbox of technique devised to understand plasma membrane-proximal signaling processes. This includes single molecule tracking, single molecule localization microscopy, and molecular association analysis. Particularly, his work focuses on understanding the molecular mechanisms behind T cell antigen recognition.

György Vámosi

Department of Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen

György Vámosi graduated as a physicist at the University of Debrecen, and made his PhD at the MPI BPC in Göttingen and the University of Debrecen. He was a visiting scientist at the DKFZ, Heidelberg and a Fulbright scholar at the Sanford-Burnham-Prebys Institute, Orlando. He is interested in the function and interactions of proteins in the membrane and the nucleus. His group focuses on interleukin-2/15 receptors and nuclear receptors, they apply and develop FRET and fluorescence correlation microscopy methods for studying protein-protein and protein-DNA interactions. He represents Hungary in the Euro-BioImaging Board.

Antje Keppler

Euro-BioImaging Bio-Hub, European Molecular Biology Laboratory, Heidelberg, Germany

Antje Keppler is Director of the Euro-BioImaging Bio-Hub at the European Molecular Biology Laboratory (EMBL) in Heidelberg. She leads an international team to coordinate and operate the pan-European research infrastructure services for biological imaging in Euro-BioImaging. She studied biochemistry at the Ruhr-University in Bochum, before she moved to Lausanne for conducting her PhD in chemical biology in the laboratory of Prof. Kai Johnsson at the EPFL. She continued to work in the field of imaging applications at the EMBL during her postdoc, before starting her career track in science management first at the University Hospital in Heidelberg, and since 2009 for Euro-BioImaging at the EMBL. Antje also is coordinator of the international Global BioImaging network, which promotes open science and open access to imaging research infrastructure services.

Claudia Pfander

Euro-BioImaging: Opportunities for researchers, core facilities and industry

Claudia Pfander is the Coordinator of the Euro-BioImaging Industry Board and External Relations Manager, acting as the liaison person for companies in the imaging sector and commercial users of infrastructure services.

Claudia originally studied Chemistry at the Westfälische Wilhelms-Universität Münster before completing a PhD in Biology at the Max-Planck-Institute of Biochemistry. After several years as scientific coordinator for technology pipeline development in malaria research at the Wellcome Trust Sanger Institute, she work as a funding and innovation consultant for the Life Science sector, managing several public-private partnerships in Health research. Since 2020 she is based at the BioHub of Euro-BioImaging at EMBL.

Péter Horváth

Synthetic and Systems Biology Unit, Biological Research Centre, Szeged, Hungary and Institute for Molecular Medicine Finland, HiLIFE, University of Helsinki, Finland

Peter Horvath (1980) is currently the director and a group leader in the Biological Research Center in Szeged and holds a Finnland Distinguished Professor (FiDiPro) Fellow position in the Institute for Molecular Medicine Finland (FIMM), Helsinki. He graduated as a software engineer and received his Ph.D. from INRIA and University of Nice, Sophia Antipolis, France in satellite image analysis. Between 2007 and 2013 he was a senior scientist at the ETH Zurich, in the Light Microscopy Centre. Peter Horvath is interested in solving computational cell biology problems related to light microscopy and is involved in four main research fields; 2/3D biological image segmentation and tracking; development of microscopic image correction techniques; machine learning methods applied in high-throughput microscopy and the development of single-cell isolation methods. He is the co-founder of the European Cell-based Assays Interest Group and the councilor of the Society of Biomolecular Imaging and Informatics.

Program

Friday, 1 April 2022

9:20 – 10:20	ARRIVAL, REGISTRATION
10:20 – 10:45	OPENING

István Ábrahám Memorial Workshop

10:45 – 11:30	Allan Herbison **Rapid estrogen actions in the brain; the Hungary-UK-NZ pathway**
11:30 – 12:15	Andrea Kwakowsky **Sex differences in aging and neurodegenerative diseases**
12:15 – 13:00	Akihiro Kusumi **Metastable signalling platforms as revealed by single molecule imaging: the mutual interest between Istvan and me**
13:00 – 14:15	LUNCH BREAK

Second Symposium on Super-resolution and Advanced Fluorescence Microscopy Young Investigators Session

14:15 – 14:45	Keynote lecture Deepak Srivastava **Illuminating the synapses - using advanced imaging to understand the actions of estrogens on cortical neurons**
14:45 – 14:50	TECHNICAL BREAK
14:50 – 15:05	Dávid Csabai **Ultrastructural mitochondrial alterations in rodent model of depression and potential clinical relevance**
15:05 – 15:20	Domonkos Nagy-Herczeg **Comparison of popular fluorescent actin markers to measure actin dynamics in dendritic spines**
15:20 – 15:35	Henriett Halász **Fundamental growth determinants and transport functions of B cell membrane nanotubes**

15:35 – 15:50	**Tamás Madarász** **How membrane sculpturing proteins influence the growth and morphology of membrane nanotubes?**
15:50 – 16:00	COFFEE BREAK
16:00 – 16:30	Keynote lecture János Szabadics **Optical recording of synaptic connections using Voltron**
16:30 – 16:45	Szilárd Szőcs **Feedback inhibition in the entorhinal cortex mediated by neurogliaform cells**
16:45 – 17:00	Boglárka Balogh **LED light-induced microglia activation in the mouse retina**
17:00 – 17:10	COFFEE BREAK
17:10 – 17:25	Khai Huynh Ngoc **TRPV1 is selectively expressed in glutamatergic neurons of the mouse supramammillary nucleus**
17:25 – 17:40	Evelin Albitz **Develop of bioorthogonally applicable fluorogenic probes for multicolor imaging**
17:40 – 17:55	Attila Ignácz **Use of Expansion microscopy to reveal sub-synaptic protein organization**
18:30	DINNER

Saturday, 2 April 2022

9:00 – 9:40	Gerhard Schütz **Following T cell antigen recognition molecule by molecule**
9:40 – 10:05	György Vámosi **Studying intracrine IL-2 signaling and IL-15 trans-presentation by modern microscopy**
10:05 – 10:25	Zsombor Kőszegi **Single-molecule microscopy of GPCR signalling**
10:25 – 10:40	COFFEE BREAK

10:40 – 11:00	**Edina Szabó-Meleg** **Microscopic examination of motoproteins in the mitochondrial transport via membrane nanotubes**
11:00 - 11:55	Antje Keppler , Claudia Pfander **Euro-BioImaging: Opportunities for researchers, core facilities and industry**
11:55 – 12:25	Live Demonstration by Femtonics **Benefits of Acousto-Optical Two-Photon Scanning** .
12:25 – 13:25	LUNCH BREAK
13:25 – 13:40	IMAGE CONTEST AWARD PRESENTATION
13:40 – 14:10	Péter Horváth **Life beyond the pixels: machine learning in single cell microscopy**
14:10 – 14:40	Gábor Csúcs **Super-resolution via pixel re-assignment**
14:40 – 15:00	Dániel Varga **Quantitative analysis of SMLM data**
15:00 – 15:20	Krisztina Németh **Large Stokes-shift bioorthogonal probes for STED, 2P-STED and multi-color STED nanoscopy**
15:20 – 15:35	COFFEE BREAK
15:35 – 15:50	András Lukács **Presentation of the István Ábrahám Nano-Bio-Imaging Core Facility of the University of Pécs**
15:50 – 16:05	Beáta Longauer **Application of fluorescence vancomycin as a novel bacterial cytoskeleton marker**
16:05 – 16:20	Soma Godó **Single-Molecule Imaging Reveals Rapid Estradiol Action on the Surface Movement of AMPA Receptors in Live Neurons**
16:20 – 16:35	Dávid Ernszt **The role of lipid rafts in the signal transduction of TrkA-receptors**
16:35 – 16:50	CLOSING REMARKS

Abstracts

Develop of bioorthogonally applicable fluorogenic probes for multicolor imaging

Evelin Albitz[1,2], Krisztina Németh[1] and Péter Kele[1]

[1] *Chemical Biology Research Group, Institute of Organic Chemistry, Research Centre for Natural Sciences, Budapest, Hungary*
[2] *Hevesy György PhD School of Chemistry, Eötvös Loránd University*

Advances in microscopy techniques enabled investigation of cellular structures in the sub-diffraction range (<200 nm). When examining several biomolecules or biological processes at the same time, a number of problems may arise. The biggest concern in multicolor fluorescent imaging is posed by chromatic aberration i.e., different wavelength light beams cannot be focused into the same plane, which causes fringes of color along the boundaries of the dark and bright parts of the image. This problem can be addressed by the use of dyes, which can either be excited at the same wavelength or can be detected in the same window. In both case, probes with large Stokes-shift are necessary, often in combination with small Stokes-shift dyes. The number of such large Stokes-shift dyes and dye pairs are limited. Further problems may arise during imaging due to auto- and background fluorescence, both impairing the achievable signal-to-noise ratio. While autofluorescence is easily handled by the use of large Stokes-shift probes or probes excitable toward the red edge of the spectrum, background fluorescence of non-specifically bound probes is best addressed by the use of fluorogenic probes. Tetrazine modulated fluorogenic probes are remarkable examples due to the combined feature of the tetrazine moiety enabling specific targeting through bioorthogonal ligation and being the modulator (quencher) of fluorescence at the same time.

We have developed a series of tetrazine modulated fluorogenic probes with large Stokes-shifts for multicolor live-cell imaging of intracellular proteins. These probes, either in combination with each other or with other small Stokes-shift probes allow imaging schemes using one detection window upon different excitation wavelengths [1] or single source excitation with multiple emission [2]. While the former combination addresses emission derived chromatic aberration the latter reduces excitation derived chromatic errors. At the same time, the large Stokes-shifts and the fluorogenic nature keeps auto- and background fluorescence at a low level.

Acknowledgment: LP2013-55/2013, NKFIH-K-131439, NKFIH-FK-137589, NKFIH-PD-135121, KEP-6, FIKU, ÚNKP-21-3

References:

[1] E. Németh; G. Knorr; K. Németh; P. Kele. A Bioorthogonally Applicable, Fluorogenic, Large Stokes-Shift Probe for Intracellular Super-Resolution Imaging of Proteins. *Biomolecules*. 2020, *10*, 397.

[2] E. Albitz; D. Kern; A. Kormos; M. Bojtár; Gy. Török; A. Biró; Á. Szatmári; K. Németh; P. Kele. Bioorthogonal Ligation-Activated Fluorogenic FRET Dyads. *Angew. Chem. Int. Ed.* 2022, *61*, e202111855.

LED light-induced microglia activation in the mouse retina

Boglárka Balogh[1,2,3], Gergely Szarka[1,2,3], Ádám J Tengölics[1,2,3], Gyula Hoffmann[1,2,3], Béla Völgyi[1,2,3,4], Tamás Kovács-Öller[1,2,3,4]

[1] *János Szentágothai Research Centre, University of Pécs, Pécs, Hungary*
[2] *Retinal Electrical Synapses Research Group, National Brain Research Program (NAP 2.0), Hungarian Academy of Sciences, Budapest, Hungary*
[3] *Institute of Biology, Faculty of Sciences, University of Pécs, Pécs, Hungary*
[4] *Medical School, University of Pécs, Pécs, Hungary*

Constant exposure to different wavelengths and intensities of light promoted by Light Emitting Diodes (LEDs), could produce retinal degeneration and neuronal cell death. Accumulated over years, damage induced by chronic phototoxic reactions occurring in the retina has been suggested to be involved in the etiology of many debilitating ocular conditions. We proposed to show how LED light affects our vision by showing the changes in the expression of death and survival factors and microglial activation in LED-induced damage (LID).

To this end, we created a mouse model where the LED exposure of the treated animals matched that of an average office worker. After the treatment, the primary inflammatory processes were examined by microglia and Casp-3 activation using immunohistochemical markers and confocal laser-scanning microscopy in the retina.

We found that after the LED treatment, the primary inflammatory processes, the microglial and Casp-3 activation increased in the retinas of the treated animals. Overall, our results showed that LED light exposure induces damage and widespread microglial activation within the inner retina. These findings warn against the prolonged use of LED-based light sources.

Ultrastructural mitochondrial alterations in rodent model of depression and potential clinical relevance

Dávid Csabai [1,2], Abigél Tornai [1], Ove Wiborg [3], Craig A. Stockmeier [4,5] and Boldizsár Czéh [1,2]

[1] *Neurobiology of Stress Research Group, Szentágothai János Research Centre, University of Pécs, Pécs, Hungary*
[2] *Instute of Laboratory Medicine, Medical School, University of Pécs, Pécs, Hungary*
[3] *Department of Health Science and Technology, Aalborg University, Aalborg, Denmark*
[4] *Department of Psychiatry, Case Western Reserve University, Cleveland, Ohio, USA*
[5] *Department of Psychiatry and Human Behavior, Division of Neurobiology and Behavior Research, Translational Research Center (TR415), University of Mississippi Medical Center, Jackson, Mississippi, USA*.

Objective: Chronic stress models represent valid animal models for major depressive disorder (MDD) and can mimic core symptoms of the disease, e.g. anhedonia. It is well documented that chronic stress alters the functional and morphological integrity of neurons in limbic areas such as the hippocampus (HC) and prefrontal cortex (PFC). Typical stress-induced structural changes are the dendritic debranching, loss of dendritic spines and synapses. Recent theories suggest that stress may also alter mitochondrial structure and function. The purpose of our investigations is to investigate putative changes of synaptic and mitochondrial morphology and number in neurons of chronically stressed rats and depressed patients.

Methods: We carried out standard transmission electron microscopic analysis to quantify the number and morphological features of synapses and mitochondria in the HC and medial PFC of control and stressed rats. A random systematic sampling was performed to take ultrastructural images at 40 000x magnification. Images were analysed with an unbiased stereology protocol.

Results: Structures were investigated on thousands of images taken from areas of interest. We found significant reduction in the number and morphology of synapses, also of mitochondria in the infralimbic cortex of rats, yet there were no changes in mitochondrial morphology. Ultrastructural parameters were also analysed in samples collected from the hippocampi of human patients.

Conclusion: In a rodent chronic stress model, we found significant reduction of synaptic and mitochondrial numbers, but mitochondrial morphology was not altered. Further investigations on human samples are needed for clinical conclusions.

Acknowledgment: We are grateful to Prof. László Seress (Central Electron Microscope Laboratory, University of Pécs, Medical School, Pécs, Hungary) for his helpful suggestions regarding the electron microscopic analysis.

Super resolution via pixel re-assignment

Dorothea Pinotsi[1], Joachim Hehl[1], Tobias Schwarz and **Gabor Csucs[1]**

[1] *ETH Zurich, Scientific Center for Optical and Electron Microscopy, Zurich, CH*

Image Scanning Microscopy (ISM) is a super resolution light microscopy method first proposed by Colin Sheppard in 1988. At that time, due to technical difficulties (missing hardware and computational capacity) it was not possible to realize such a setup. So it was in 2010 when Müller *et al.* published the first experimental realization of the system. ISM is often referred as pixel re-assignment as the later term describes the basic idea behind the method more precisely. During the past few years numerous experimental and commercial implementations of the technique were introduced. Interestingly, despite the common theoretical background, those implementations are significantly different from each-other, some of them (like the Zeiss Airy scan) are rather software based, others (like re-scan microscopy or the SoRa) are purely hardware based ones. In ScopeM we started a project aiming the quantitative comparison of those various commercial implementations. In my presentation I will give an overview of the various methods and report our preliminary results about that comparative study.

The role of lipid rafts in the signal transduction of TrkA receptor

Dávid Ernszt[1,2], Soma Godó[1,2], Tamás Kovács[1,2], Klaudia Barabás[1,2], István Ábrahám[1,2]

[1] *University of Pécs Medical School, Institute of Physiology, Pécs, Hungary*
[2] *University of Pécs Szentágothai Research Centre, Molecular Neuroendocrinology Research Group, Pécs, Hungary*

The signaling process of eukaryotic cells is a chain of complex molecular events that can be regulated at several points. There is more and more evidence that the localization of receptors has an impact on signaling activity. The activity of receptor tyrosine kinases depends on their association with lipid raft regions in the cell membrane. Lipid rafts are specific sphingolipid and cholesterol rich regions in the cell membrane where the accumulation of signaling molecules has been observed. Furthermore, these regions are considered to function as signaling nodes. The aim of our research project is to understand the role of lipid rafts in the signal transduction of tropomyosin receptor kinase A (TrkA) receptors.

Our investigations were performed on a CHO cell line stably expressing TrkA receptor, conjugated with HaloTag reporter molecule. The amount of lipid rafts was modified by using methyl beta-cyclodextrin (MβCD) treatment. Lipid rafts were labelled with the cholera toxin β subunit and TrkA receptors were visualized with HaloTag technique. STED and TIRF microscopy were used for imaging. NGF-induced TrkA activity was examined by measuring ERK phosphorylation, using western blot technique.

We have observed that under control conditions, TrkA receptors are found in lipid raft regions and also in other areas of the membrane. After five minutes of NGF treatment, a significant proportion of TrkA molecules leaves the lipid raft regions. With MBCD treatment we reduced the membrane cholesterol level and thus the amount of lipid raft regions. We found that NGF-induced ERK phosphorylation is greater in MBCD-threated cells than in MBCD-untreated condition. From our results we conclude that TrkA receptors are rather located outside the lipid raft regions during signaling. Moreover, the lipid raft regions reduce the signaling activity of TrkA receptors.

This work was supported by the ÚNKP-21-4-II new national excellence program of the ministry for innovation and technology from the source of the national research, development and innovation fund. Project no. TKP2021-EGA-16 has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the TKP2021-EGA funding scheme. This project was supported by EFOP-3.6.1.-16-2016-00004 (Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs)

Single-Molecule Imaging Reveals Rapid Estradiol Action on the Surface Movement of AMPA Receptors in Live Neurons

Soma Godó[1], Klaudia Barabás[1], Ferenc Lengyel[1], Dávid Ernszt[1], Tamás Kovács[1], Miklós Kecskés[2], Csaba Varga[2], Tibor Z. Jánosi[1], Géza Makkai[1], Gergely Kovács[1], Barbara Orsolits[3], Takahiro Fujiwara[4], Akihiro Kusumi[5] and István M. Ábrahám[1]

[1] *PTE-NAP Molecular Neuroendocrinology Research Group, Centre for Neuroscience, Szentágothai Research Center, Medical School, Institute of Physiology, University of Pécs, Pécs, Hungary*
[2] *PTE-NAP Cortical Microcircuits Research Group, Institute of Physiology, Medical School, Centre for Neuroscience, Szentágothai Research Institute, Pécs, Hungary*
[3] *Laboratory of Neuroimmunology, Institute of Experimental Medicine of the Hungarian Academy of Sciences, Budapest, Hungary*
[4] *Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University, Kyoto, Japan*
[5] *Membrane Cooperativity Unit, Okinawa Institute of Science and Technology Graduate University (OIST), Onna, Japan*

Gonadal steroid 17β-estradiol (E2) exerts rapid, non-genomic effects on neurons and strictly regulates learning and memory through altering glutamatergic neurotransmission and synaptic plasticity. However, its non-genomic effects on AMPARs are not well understood. Here, we analyzed the rapid effect of E2 on AMPARs using single-molecule tracking and super-resolution imaging techniques. We found that E2 rapidly decreased the surface movement of AMPAR via membrane G protein-coupled estrogen receptor 1 (GPER1) in neurites in a dose-dependent manner. The cortical actin network played a pivotal role in the GPER1 mediated effects of E2 on the surface mobility of AMPAR. E2 also decreased the surface movement of AMPAR both in synaptic and extrasynaptic regions on neurites and increased the synaptic dwell time of AMPARs. Our results provide evidence for understanding E2 action on neuronal plasticity and glutamatergic neurotransmission at the molecular level.

Project no. TKP2021-EGA-16 has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the TKP2021-EGA funding scheme. This project was supported by EFOP-3.6.1.-16-2016-00004 (Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs).

Fundamental growth determinants and transport functions of B cell membrane nanotubes

Henriett Halász[1], Tamás Madarász[1], Miklós Nyitrai[1], János Matkó[2] and Edina Szabó-Meleg[1]

[1] *University of Pécs, Medical School, Department of Biophysics, Pécs, Hungary*
[2] *Eötvös Loránd University, Faculty of Science, Department of Immunology, Budapest, Hungary*

Direct communication between cells is essential in multicellular organisms to exchange information with each other during e.g. cell repair, activation, or cell survival. Membrane nanotubes (NTs) are thin membranous bridges connecting two or more cells located even in far distance from each other. These cell protrusions are long, F-actin-based cytoplasmic extensions promoting the intercellular spreading of e.g. cell organelles, drug resistance, Aβ peptides, prions and a diverse collection of viruses. Different studies have been performed in the last two decades on a variety of cells that underlined the diversity and heterogeneity of these structures amongst different cell types, including immune cells.

Objective: Antibody-producing B lymphocytes are key cellular components of the adaptive humoral immune response where NTs may play important roles. However, in contrast to T lymphocytes and macrophages, NTs between B cells are largely unexplored and poorly characterized. Our main goal was to determine basic control mechanisms of nanotube growth as well as the transport properties of B cell NTs.

Methods: Mature and immature B cells were measured to reveal their NT forming potential. Cells were labeled fluorescently for some cytoskeletal proteins and cell organelles (mitochondria, lysosomes and vesicles). Confocal and superresolution SIM microscopy were used to follow NT growth and transport processes. Collected images were analyzed, and statistical analysis was performed.

Results: Only mature B lymphocytes form spontaneously extensive NT networks under conditions resembling the physiological environment. Length- and width-distribution of B cell NTs showed large diversity. NTs contain not only F-actin – the essential element in NT-growth and the skeleton of B cell NTs –, but in contrast to T cells, their majority also contain microtubules, which were found, however, not essential for NT formation. Bidirectional transport of membranous vesicles was observed inside NTs and mitochondria transfer is also suggested. In addition, intercellular exchange of immunoregulatory molecules through NTs was demonstrated which may represent unexplored pathways of intercellular communication and immunoregulation.

Conclusion: Despite the continuously accumulating knowledge about membrane nanotubes, several fundamental questions about the molecular and genetic mechanisms controlling their growth as well as their *in vivo* functional significance remained open.

Life beyond the pixels: machine learning in single cell microscopy

Peter Horvath

Synthetic and Systems Biology Unit, Biological Research Centre, Szeged, Hungary
Institute for Molecular Medicine Finland, HiLIFE, University of Helsinki, Finland

In this talk I will give an overview of the computational steps in the analysis of a single cell-based large-scale microscopy experiments. First, I will present a novel microscopic image correction method designed to eliminate illumination and uneven background effects which, left uncorrected, corrupt intensity-based measurements. New single-cell image segmentation methods will be presented using differential geometry, energy minimization and deep learning methods (www.nucleAIzer.org). I will discuss the Advanced Cell Classifier (ACC) (www.cellclassifier.org), a machine learning software tool capable of identifying cellular phenotypes based on features extracted from the image. It provides an interface for a user to efficiently train machine learning methods to predict various phenotypes. For cases where discrete cell-based decisions are not suitable, we propose a method to use multi-parametric regression to analyze continuous biological phenomena. To improve the learning speed and accuracy, we propose an active learning scheme that selects the most informative cell samples.

Our recently developed single-cell isolation methods, based on laser-microcapturing and patch clamping, utilize the selection and extraction of specific cell(s) using the above machine learning models. I will show that we successfully performed DNA and RNA sequencing, proteomics, lipidomics and targeted electrophysiology measurements on the selected cells.

TRPV1 is selectively expressed in glutamatergic neurons of the mouse supramammillary nucleus

Huynh Ngoc Khai[1], Angéla Kecskés[2]

[1] *Faculty of Sciences, University of Pécs, Pécs, Hungary*
[2] *Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Pécs, Hungary*

It is well known that the transient receptor potential vanilloid 1 (TRPV1) ion channel is predominantly expressed in primary sensory neurons located in the dorsal root and trigeminal sensory ganglia. This non-selective cation channel plays a significant role in pain and neurogenic inflammation. *Trpv1* expression was also demonstrated in the mouse supramammillary nucleus potentially involved in memory, learning, and social interactions, but the neuronal subtype is unknown. Therefore, we characterized the *Trpv1* mRNA-expressing neurons in the mouse supramammillary nucleus.

Brains of 3-4 month-old male C57Bl/6 mice (N = 3) were sectioned coronally and used for the experiment. Since specific anti-TRPV1 antibody is not available, *Trpv1*mRNA expression in the mouse supramammillary nucleus was detected with RNAscope *in situ* hybridization technology. In addition, we co-localized *Trpv1* mRNA-positive neurons with specific neuronal markers, such as *Vglut2* mRNA for glutamatergic and tyrosine hydroxylase immunopositivity for dopaminergic neurons. Fluorescent images (z-stacks with 1 µm intervals) of the sections were acquired using a Zeiss LSM 710 confocal laser scanning microscope and analyzed with Fiji.

Trpv1 mRNA expression in the mouse supramammillary nucleus was successfully detected. We found a strong co-expression of *Trpv1* and *Vglut2* mRNA, but no overlap between *Trpv1* mRNA and tyrosine hydroxylase signal.

Our result suggests that glutamatergic neurons specifically express *Trpv1* in the mouse supramammillary nucleus. We propose that our finding may serve as the basis for narrowing down the scope of investigation of the operation and function of TRPV1 channel in this brain region.

Use of Expansion microscopy to reveal sub-synaptic protein organization

Attila Ignácz, Domonkos Nagy-Herczeg and Katalin Schlett

Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary

Fluorescence microscopy is a fundamental tool for neuroscience, but the diffraction limit of light prevents the investigation of sub-synaptic cellular structures with traditional fluorescence microscopy. Within the last decades, numerous superresolution technologies have emerged which allowed breaking the diffraction limit for optical microscopy, but their widespread use is hindered by the high cost of the specific instruments or by technical limitations including e.g. the type of fluorescent labels or photobleaching.

In contrast, expansion microscopy (ExM) is a physical form of magnification that increases the effective resolving power of any traditional widefield or confocal microscope. In ExM, a swellable gel is synthesized throughout the sample. During gelation, specific biomolecules such as proteins and RNA can be covalently linked into the hydrogel network. After gelation, the structure of the sample is disrupted by breaking crosslinks and bonds in proteins; however, the anchored molecules (e.g., antibody tags, RNA, fluorescent proteins) are retained and isotropically expanded by dialysis in water. Thus, ExM enables nanoscale-resolution imaging of fixed cells and tissues on conventional diffraction-limited microscopes.

In the present study we used murine hippocampal cell cultures to test expansion microscopy. The cells were transfected with EGFP to outline individual cells, followed by traditional fixation and immunolabelling for synaptic markers. Nuclear size before and after expansion was compared, as a unit of measurement for the scale of expansion. Our results confirmed a 4.5-5.5-fold increase in size in every axes. When the morphology of EGFP expressing cells was analysed, no evident distortions in the shape of dendrites or dendritic spines were observed upon expansion. Using immunolabelling against pre- and postsynaptic proteins we also confirmed that expanded samples are suitable for studying sub-synaptic structures with conventional confocal microscopy.

Taken together, we confirm that expansion microscopy is a feasible yet reliable method to increase spatial resolution in fluorescence confocal imaging of neurons.

Acknowledgements:

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Euro-BioImaging: Opportunities for researchers, core facilities and industry

Antje Keppler and **Claudia Pfander**

Bio-Hub, Euro-BioImaging ERIC, EMBL Heidelberg

Open, coordinated, quality-managed and sustainable access to cutting-edge imaging instrumentation and services in the life sciences creates trust and impact at many levels: For the scientists using the services; for the staff working at the imaging platform; for reviewers, publishers and consumers of research results; for research and infrastructure funders; for the hosting academic institutions; for image data consumers; and last but not least for the politicians taking into account scientific outcome for their fact-based decision making for society.

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Companies in the imaging sector can engage with the Euro-BioImaging network via its Industry Board to exchange on new imaging trends and real-life applications, while biotech and pharma can make use of Euro-BioImaging services.

Through Euro-BioImaging, life scientists can access imaging instruments, expertise, training opportunities and data management services that they might not find at their home institutions or among their collaboration partners. All scientists, regardless of their affiliation, area of expertise or field of activity can benefit from these pan-European open access services, which are provided with high quality standards by leading imaging facilities.

Single-molecule microscopy of GPCR signalling

Zsombor Koszegi, Jak Grimes, Yann Lanoiselee, Davide Calebiro

University of Birmingham, Institute of Metabolism and Systems Research, Birmingham, UK

Objective
G protein-coupled receptors (GPCRs), comprising hundreds of different types, play a fundamental role in living cells. They mediate the effects of various hormones and neurotransmitters and are major pharmacological drug targets. GPCRs function through two classes of signalling molecules, G proteins and arrestins. GPCRs recruit arrestins, which mediate signal desensitization and receptor internalization. Recent structural studies have provided details about receptor–arrestin interactions, however, the spatio-temporal details and mechanisms that govern these interactions remain poorly understood. In this study, we set out to investigate individual receptor and arrestin molecules at high spatial and temporal detail as they interact at the plasma membrane in living cells.

Methods
We employed super-resolution microscopy to examine receptor–arrestin interactions. To achieve single-molecule resolution, we used total internal reflection fluorescence microscopy in combination with advanced trajectory analysis in the MATLAB environment. We used live CHO-K1 cells to transiently express wild-type and mutant GPCR and arrestin molecules, as well as other structural elements of the plasma membrane. Labelling was done using SNAP and Halo tags that allow the attachment of bright, organic fluorophores. Image acquisition was performed on a custom-built microscope, equipped with 100x oil-immersion objective, diode lasers, an iLas2 illuminator, and four EMCCD cameras. Complementary experiments in this project were done using molecular biology, bioluminescence resonance energy transfer, and molecular dynamics simulations.

Results
We show with high spatial and temporal detail that arrestin molecules spontaneously bind to the plasma membrane where they transiently interact with GPCRs via lateral diffusion. Following receptor interaction, the plasma membrane stabilizes arrestin in a membrane-bound, active-like conformation, allowing it to reach clathrin-coated pits without the activating receptor.

Conclusion
Our results challenge the conventional view on the role of arrestins and describe the complex sequence of events involved in arrestin interaction with GPCRs and the lipid bilayer. Overall, with the use of single-molecule microscopy, we highlight the importance of arrestin molecules in GPCR signalling and hope to contribute to future alternatives to current pharmacological approaches that achieve their desired effects via targeting arrestin-mediated mechanisms.

Metastable signalling platforms as revealed by single molecule imaging: the mutual interest between Istvan and me

Akihiro Kusumi

Membrane Cooperativity Unit
Okinawa Institute of Science and Technology Graduate University (OIST)
Okinawa 904-0495, Japan

Signalling is one of the most important functions of the cellular plasma membrane (PM). A variety of extracellular signalling molecules bind to their specific receptors in the PM, and the engaged receptors in turn trigger various cytoplasmic signalling cascades. These signalling pathways are intertwined and affect each other, in a process called crosstalk, which enables the cells to fine tune the overall signal. The crosstalk of different receptor signalling pathways has been examined quite extensively, but the platform responsible for signal integration has never been discovered.

Istvan and I were both interested in such platforms for signal transduction and integration, and both of us considered that single-molecule imaging would be the best way to find these platforms and reveal the mechanisms by which such platforms form and function. This way, we started collaboration. Istvan focused on neurons, and Prof. Fujiwara in my lab and I participated in his project. Meanwhile, I paid attention to more ubiquitous platforms in cell lines. Istvan's work will be presented by his lab members, and so, I will report the studies done in my lab.

Using single-molecule imaging, we found a nanometer-scale (50-80 nm) liquid-like protein assembly on the PM cytoplasmic surface (at a density of ~2-μm apart from each other on average, with a lifetime of ~10 s), working as the signal transduction and integration platform for receptors, including GPI-anchored receptors (GPI-ARs), receptor-type tyrosine kinases (RTKs), and GPCRs. The platform consists of integrin, talin, RIAM, VASP, and zyxin, and is thus termed iTRVZ. These molecules are known as focal-adhesion constituents, but iTRVZ is distinct from focal adhesions, because iTRVZ exists on both the apical and basal PMs and lack vinculin. The iTRVZ formation is driven by specific protein-protein interactions, liquid-liquid phase separation, and interactions with actin filaments and raft domains via PI(4,5)P2. iTRVZ integrates and amplifies the GPI-AR and RTK signals in a strongly non-linear fashion, and thus works as an AND gate (coincidence detector) and noise filter. These findings greatly advance our understanding of the mechanism for crosstalk between signalling pathways.

Sex differences in aging and neurodegenerative diseases

Andrea Kwakowsky

National University of Ireland Galway, Pharmacology and Therapeutics, Galway, Ireland
University of Auckland, Department of Anatomy and Medical Imaging, Auckland, New Zealand

After completing my PhD studies in Hungary I have been working as an early career researcher in the field of neuroendocrinology with Professor István Ábrahám and Professor Alan Herbison at the Centre for Neuroendocrinology, University of Otago. This research focused on the interaction between the brain and hormonal activity in the body with a particular focus on non-classical estrogen signaling. While in dementias like Alzheimer's disease (AD), sex differences have been well documented, the underlying mechanisms are not understood. My research has started to explore the role of sex hormones and their therapeutic potential in AD, particularly the female sex hormones that are most likely to be involved and account for some of these sex differences. Here I provide an overview of the exciting research I conducted with István that aimed to explore the potential and mechanism of action of estren, a selective non-classical estrogen-like signaling activator on beta-amyloid-induced cholinergic neurotoxicity and behavioral deficit *in vivo*. I also present evidence of sex-specific changes affecting the neurotrophin and the main inhibitory neurotransmitter system in the aging brain. AD is the world's leading cause of dementia and sex hormones might influence disease prevalence, progression and are promising therapeutic targets for the disease. Importantly, AD is a complex and multifactorial disease and gender has to be considered when designing new preventive and therapeutic options for these conditions.

Application of fluorescence vancomycin as a novel bacterial cytoskeleton marker

Beata Longauer[1,2], Miklós Nyitrai[1,2] and Szilvia Barko[1]

[1] *Department of Biophysics, Medical School, University of Pécs, Hungary*
[2] *Nuclear Mitochondrial Interactions Research Group, Eötvös Loránd Research Network (ELKH), University of Pécs, Hungary*

The antibiotic resistance is a growing threat in the human medicine. A very large proportion of antibiotics act on the bacterial cell wall by inhibiting cell wall synthesis. The MreB is present in almost all of the non-coccoid bacteria. It plays a key role in the cell wall formation. The gene of this protein has chromosomal origin and it is essential to bacteria. Lack or malfunction of MreB causes a loss of rod shape, the cells widen and their growths stops, finally the bacteria may die during cell lysis.

The peptidoglycan (PG) layer of the bacterial cell wall is composed of long sugar molecules that intersect with peptide bridges to form a three-dimensional network-like formation. PG helps maintain cell shape, provides physical integrity and mechanical strength to the bacterium, making it vital for bacterial survival. The widely used antibiotic vancomycin acts also on the cell wall: studies have shown that fluorescent vancomycin can inhibit PG synthesis by binding to the lipid II precursor.

By the advent of fluorescently labelled antibiotics and the rapid development of fluorescent technologies, it has become possible to investigate the mechanism of action of antibiotics within a bacterial cell. We have examined the ability of fluorescently labelled vancomycin to bind to the MreB protein by different fluorescence spectroscopic and SIM microscopic methods. Our anisotropy measurements data show that fluorescent vancomycin is able to bind to MreB. Neither the rate of polymerization, nor the amount of filaments is affected. Our microscopic experiments suggest that vancomycin alters the intracellular localization of the protein.

Our results suggest that fluorescence derivative of vancomycin binds MreB *in vivo* and also *in vitro*, and the antibiotic changes the intracellular localization of MreB. Further measurements are needed to confirm our experimental results.

How membrane sculpturing proteins influence the growth and morphology of membrane nanotubes?

Tamás Madarász[1], Henriett Halász[1], Katalin Szeiliné Türmer[1], János Matkó[2], Miklós Nyitrai[1,3] and Edina Szabó-Meleg[1,3]

[1] *University of Pécs, Medical School, Department of Biophysics, Pécs, Hungary*
[2] *Eötvös Loránd University, Faculty of Science, Department of Immunology, Budapest, Hungary*
[3] *University of Pécs, Szentágothai Research Center, Pécs, Hungary*

Actin driven membrane protrusions, such as lamellipodia, filopodia and membrane ruffles have a wide variety of functions, from cell migration to neurological synapses and wound healing. Although these structures exist in several forms, similar molecular mechanisms are responsible for their growth. Membrane nanotubes are thin membrane protrusions that show great similarity with filopodia, however they are able to physically connect two, even distant cells. Membrane nanotubes were identified in 2004 as a new form of intercellular communication and matter transport and were described in vitro in several cell lines and in vivo in mouse, chicken and zebrafish embryos. They were shown to be involved in the transport of e.g. calcium ions, different cell organelles, lipid molecules, various proteins, prions, vesicles, DNA and RNA molecules, in the effective dissemination of bacteria and viruses (HIV) among cells. Despite membrane nanotubes show similarity in several properties with the previously mentioned membrane protrusions, they also show characteristic differences from them, they differ for instance not only in their length and diameter, but are hovering in the medium and providing continuity between the cytoplasm of otherwise isolated cells.

Two possible pathways for the formation of nanotubes are reported, one of them is when filopodia-like formations develop and fuse with an adjacent cell. It is well known that IBAR domain proteins, such as insulin receptor substrate 53 protein (IRSp53) promotes filopodia formation. In this work laser-scanning confocal and superresolution microscopy (SIM) were applied to investigate the effect of IBAR and IRSp53 proteins on the formation and morphology of filopodia and membrane nanotubes. The effect of latrunculin A was studied in filopodia and nanotubes in the absence and presence of the overexpressed IRSp53. Effect of IRSp53 and its domain IBAR on individual actin filament were measured with total internal reflection fluorescence microscopy (TIRF).

Our results suggest that although membrane nanotubes are similar in several aspects with filopodia, they also show characteristic differences from them in the basic mechanism of their inception.

This work was supported by the GINOP-2.3.2-15-2016-00036 and EFOP 3.6.1-16.2016.00004.

Comparison of popular fluorescent actin markers to measure actin dynamics in dendritic spines

Domonkos Nagy-Herczeg, Attila Ignácz, Katalin Schlett

Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest

Excitatory synapses in the central nervous system are mainly localized on dendritic spines, highlighting the importance of these protrusions in learning and memory. Changes in synaptic activity induces rapid remodelling in the actin cytoskeleton leading to morphological changes of the dendritic spines. FRAP (fluorescent recovery after photobleaching) is widely used to study the dynamics of actin cytoskeleton, based on photobleaching fluorescently labelled actin-bound signals within a small area, followed by measuring the return of fluorescent signal intensity within the bleached regions. This technique provides tools to calculate the kinetics of the actin remodelling and determine the proportion of stable and rapidly rearranging microfilaments within certain cellular areas.

Within the last decades, numerous actin labelling fluorescent markers have been developed. To select the most suitable for FRAP experiments, murine embryonic hippocampal cell cultures were transfected with three different actin labelling fluorescent markers. The EGFP-Actin fusion protein is covalently labelled with EGFP and incorporates into the F-actin network. Actin-Chromobody-GFP is a monomeric camelid antibody, while LifeAct-GFP has an actin binding domain which can bind to filamentous and monomeric actin. Neurons expressing the freely diffusible EGFP protein only were used as controls.

Actin-FRAP experiments were performed in dendritic protrusions under control conditions and after F-actin stabilization by Jasplakinolide. In addition, we compared how the different labelling methods affected the motility of dendritic protrusions and general neuronal morphology. There was no significant difference between the motility of the filopodia expressing Actin-Chromobody-GFP, LifeAct GFP and EGFP, while EGFP-actin expression reduced motility. Sholl analysis of dendritic arborisation revealed that cells expressing Actin-Chromobody-GFP have shorter dendrites and lower number of branches. Fluorescence recovery of the covalently labelled EGFP-Actin was completely blocked by F-actin stabilization. On the other hand, both indirect actin labelling constructs recovered almost completely after bleaching, indicating that free, diffusible fusion proteins mask the detection of actual actin dynamics. Thus, only the covalently labelled EGFP-actin method is suitable for FRAP experiments.

Large Stokes-shift bioorthogonal probes for STED, 2P-STED and multi-color STED nanoscopy

György Török[1,2,3], Gergely B. Cserép[1], András Telek[1], Dóra Arany[1], Zsófia László[1], Melinda Váradi[1], Éva Bakos[4], Csilla Özvegy-Laczka[4], László Homolya[3], Miklós Kellermayer[2], Péter Kele[1] and **Krisztina Németh[1]**

[1] *Chemical Biology Research Group, Institute of Organic Chemistry, Research Centre for Natural Sciences, Budapest, Hungary*
[2] *Department of Biophysics and Radiation Biology, Semmelweis University, Budapest, Hungary*
[3] *Laboratory of Molecular Cell Biology, Institute of Enzymology, Research Centre for Natural Sciences, Budapest, Hungary*
[4] *Membrane protein research group, Institute of Enzymology, Research Centre for Natural Sciences, Budapest, Hungary*

Four, red-emitting (610-670 nm), tetrazine-functionalized fluorescent probes (CBRD=Chemical Biology Research Group Dye 1-4) with large Stokes-shift were designed and synthesized for super-resolution microscopy. The large Stokes-shifts and the wide spectral bands of the probes enabled the use of two common depletion lasers (660 nm and 775 nm). More improved spatial resolution could be achieved by our probes proved to be suitable for STED in combination with two-photon excitation (2P-STED). Multiple STED imaging applications are demonstrated by immunostaining of cytoskeletal protein keratin-19, and mitochondrial protein TOMM20; as well as through bioorthogonal labeling scheme of actin using bioorthogonalized phalloidin in fixed cells. In addition, live-cell (bioorthogonal) labeling of insulin receptors through a genetically encoded cyclooctynylated non-canonical amino acid in the extracellular region is also presented. The non-permeable sulfonic acid bearing probes were also successfully used in the labeling and super-resolution imaging of intracellular TOMM20-HaloTag fusion protein in live cells, in combination with anionic transporter polypeptides (OATP 3A1) enabling the transport of the negatively charged probes through the membrane. Multi-color labeling and three-color STED imaging of intracellular structures using one of the dyes together with two commercial dyes is also presented.

Euro-BioImaging: Opportunities for researchers, core facilities and industry

Antje Keppler and **Claudia Pfander**

Bio-Hub, Euro-BioImaging ERIC, EMBL Heidelberg

Open, coordinated, quality-managed and sustainable access to cutting-edge imaging instrumentation and services in the life sciences creates trust and impact at many levels: For the scientists using the services; for the staff working at the imaging platform; for reviewers, publishers and consumers of research results; for research and infrastructure funders; for the hosting academic institutions; for image data consumers; and last but not least for the politicians taking into account scientific outcome for their fact-based decision making for society.

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Following T cell antigen recognition molecule by molecule

Gerhard Schütz

T-cells readily detect the presence of even a single antigenic peptide/MHC complex (pMHC) among thousands of endogenous pMHCs via T-cell receptors (TCRs) on the surface of antigen-presenting cells. The mechanisms underlying this phenomenal sensitivity have remained elusive. Recent studies suggest that the topography of the immunological synapse formed between the T cell and the antigen-presenting cell is of pivotal importance for these processes. We hence were first interested, how the TCR is distributed within the immunological synapse. For this, we used single molecule localization microscopy in combination with supercritical angle detection to localize single TCR molecules at an isotropic precision below 15nm. Second, researchers speculated that mechanical forces could be instrumental for the high specificity and sensitivity of the T cell response. Force magnitude, spread, and temporal behavior, however, are still poorly defined. We developed a calibrated FRET-based sensor equipped either with a TCR-reactive single chain antibody fragment or peptide-loaded MHC. The sensor was tethered to planar glass-supported lipid bilayers and informed most directly on the magnitude and kinetics of TCR-imposed forces at the single molecule level. From the single molecule FRET signals we quantified the magnitude of tensile forces exerted by T cells via single TCR molecules. In addition, the data allowed us to draw conclusions on the directionality of the observed forces, as well as on the pulling speed.

Optical recording of synaptic connections using Voltron

János Szabadics, János Brunner, Ádám Szatai, Bálint Tamás, Antónia Arszovszki, Eszter Sipos, Gergely Tarcsay

Institute of Experimental Medicine, Budapest, Hungary

Recent developments in the design of genetically encoded voltage indicators made some of them suitable for detecting both small subthreshold voltage changes and fast action potentials. We apply one of these new GEVIs, the Voltron in *in vitro* hippocampal slices to map synaptic connections in a large number of neurons simultaneously. The talk will discuss the applicability of this new experimental system and show examples of connections detected by Voltron imaging between hippocampal neurons.

Microscopic examination of motoproteins in the mitochondrial transport via membrane nanotubes

Henriett Halász[1], Viktória Tárnai[2], Tamás Madarász[1], Miklós Nyitrai[1], János Matkó[3] and **Edina Szabó-Meleg**[1,4]

[1] *University of Pécs, Medical School, Department of Biophysics, Pécs, Hungary*
[2] *University of Pécs, Faculty of Natural Sciences, Institute of Biology, Pécs, Hungary*
[3] *Eötvös Loránd University, Department of Immunology, Budapest, Hungary*
[4] *University of Pécs, Szentágothai Research Centre, Pécs, Hungary*

Membrane nanotubes (NTs) are new type of tube-like, dynamic communication channels, which physically connect spatially separated cells over long distances. They are extremely diverse, thin (diameter: 50-1000 nm) and fragile structures. NTs have been revealed in prokaryotes, in wide range of mammalian cells (e.g.: immune cells) and in embryonic tissues. They are typically even 100 µm long, actin-driven cellular protrusions, but some cases the microtubules are also their constituents. NTs have important role in some material transport processes, for instance in the delivery of different cell components (e.g.: mitochondria), plasma membrane proteins, nucleic acids, but they are also involved in the development of resistance against antibiotics or chemotherapeutic agents, in the intercellular spread of different pathogens and in the deterioration of some neurological disorders.

The function and the composition of NTs of B cells are less studied and characterized compared to the NTs of other immune cells. Our aims were i.) to identify the role of the motorprotein(s) in the mitochondrial transport processes mediated by NTs with specific inhibitors and gene silencing technique, ii.) to examine the possible colocalization of mitochondria with the measured motorproteins and iii.) to reveal the role of the microtubules in the biological function of NTs of mouse originated B cells with laser scanning and structured illumination (SIM) microscopic techniques.

We revealed the functional importance of B cell NTs, since as active channels, NTs can control intensive even bidirectional mitochondrial transport. The delivery of mitochondria is promoted by the cooperation of two different cytoskeletal motorproteins. Furthermore, we suppose that microtubules have crucial role in the stability of NTs of B-cells.

Our results contribute to a better understanding of the molecular background of some diseases, and as a consequence can help in the development of new therapeutic approaches targeting disorders associated with mitochondrial origin, because if a healthy cell can transplant mitochondria to a damaged cell via NTs it may restore the energetic profile of the recipient cell.

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Feedback inhibition in the entorhinal cortex mediated by neurogliaform cells

Szilárd Szőcs, Nóra Henn-Mike, Ágnes Agócs-Laboda, Zoltán Petykó, Csaba Varga

University of Pécs, Szentágothai Research Centre, Department of Physiology, Cortical Microcircuits Research Group, HU

The role of local GABAergic inhibitory neurons in generating the entorhinal specific cell activities is still not entirely known. Several studies focused on the function of parvalbumin+ fast spiker interneurons, and only limited data has been published on the connectivity-matrix of many other GABAergic cell types. Many interneurons are localized in the layerI, where apical dendrites of layerII-V pyramidal and stellate cells are located. The majority of these critically positioned interneurons are neurogliaform cells. Neurogliaform cells have been shown to elicit elongated GABAA and GABAB receptor mediated inhibition in the neocortex and hippocampus in virtually all cell types which are located within the range of the rich axonal clouds of the neurogliaform cells. They are generally supposed to perform feed-forward inhibition: in the somatosensory cortex thalamic input; in the dentate gyrus entorhinal input; in the CA1 entorhinal and CA3 inputs give excitatory synapses on neurogliaform cells. The feedback inhibition, however, has not been linked with neurogliaform cells.

In the present work we aimed to shed light on the involvement of layerI GABAergic interneurons in the local microcircuits. Specifically, we investigated whether these cells receive excitatory inputs from the layerII pyramidal and stellate cells and whether neurogliaform cells show correlation to the island-like “patchy” structures which is a hallmark of MEC superficial layers. Our results showed strong, monosynaptic excitatory connection between layerII pyramidal cells and neurogliaform cells. Therefore, we hypothesize that neurogliaform cells are involved in effective feedback inhibition of the entorhinal cortex microcircuits. Moreover, we found that the neurgliaform cells are evenly distributed in layerI, therefore, they can elicit inhibition in all cell types sending dendrites to layerI.

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Studying intracrine IL-2 signaling and IL-15 trans-presentation by modern microscopy

György Vámosi

Department of Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen

Interleukin-2 and -15 are important regulators of T cell activation, proliferation, survival and apoptosis. Overexpression or lack of these cytokines or their receptors are involved in autoimmune and malignant lymphoid disorders. Anti-IL-2R antibody therapies have been used to prevent renal transplant rejection and treat multiple sclerosis and adult T cell lymphoma. To understand why the efficiency of these therapies is often limited, we studied intracellular IL-2R subunit assembly and signaling. We have shown by (FLIM-)FRET microscopy that heterotrimeric IL-2 receptors are partially preassembled already in the Golgi. In cells also producing IL-2, ligand binding and signaling can already take place in an intracrine manner before receptors reach the cell surface rendering antibody therapies targeting membrane-localized receptors inefficient. Interleukin-15 is the only member of the interleukin family requiring cell-cell interaction for its function. During IL-15 trans-presentation (TP), a process essential for long-term survival of memory T cells, an antigen presenting cell (APC) expressing IL-15Rα presents the IL-15 ligand to the IL-15Rβγ heterodimer on a T cell. The first step of T cell activation is antigen presentation (AP) also taking place between an APC and a T cell. We have shown that the two processes can take place alone or simultaneously, and that signaling by the TCR or IL-15R do not each amplify other. Thus, IL-15 TP can be considered an antigen independent process.

Quantitative analysis of SMLM data

Dániel Varga, Tibor Novák, Péter Bíró, Bálint H. Kovács, Miklós Erdélyi

University of Szeged, Department of Optics and Quantum Electronics, Hungary

Recent developments in super-resolution imaging techniques opened the way to study macromolecular structures below the diffraction limit. Among these techniques localization microscopy (SMLM) provides the highest spatial resolution (<10nm) [1]. However, the raw data of this approach is a list of localization coordinates and the analysis and interpretation of such point clouds requires new approaching methods. In this presentation I would like to review the localization coordinate based image analysis methods we used to quantify and interpret SMLM data, including molecule counting, clusterization, geometrical parameter determintaion and object segmentation.

Since in SMLM a localization coordinate represents a single fluorophore, the technique aroused interest in terms of the quantitative characterization of target molecules. The determination of the number of molecules is extremely relevant, but the stochastic photophysical behavior of the fluorophores and the often unknown labeling stoichiometry makes this task difficult. We approached this issue by determining the probability mass function of the localization numbers corresponding to a single target molecule during dSTORM measurements by modeling the fluorescent molecules with a three state system [2]. The point clouds generated by SMLM also gave new opportunities to cluster analysis. Detecting clusterization is an important task on its own, but the quantitative characterization of the clusters can lead to further findings. We developed an algorithm based on the DBSCAN method to determine the distribution of different parameters of the clusters [3]. The determination of physical dimensions is usually straightforward in conventional images, such as fitting a theoretical curve to the cross sectional profile of the examined structure. Such pixelated images can be created from SMLM data, but the generation of this image can result in information loss, so we used the localization coordinates directly to measure physical parameters [4-5].

The SMLM imaging modality is relatively new and is creating exciting opportunities, but there is still a need to develop analysis methods to interpret the pointillist data.

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Benefits of Acousto-Optical Two-Photon Scanning

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The presentation will include a live online broadcast, demonstrating new possibilities in calcium imaging enabled by acousto-optical scanning.