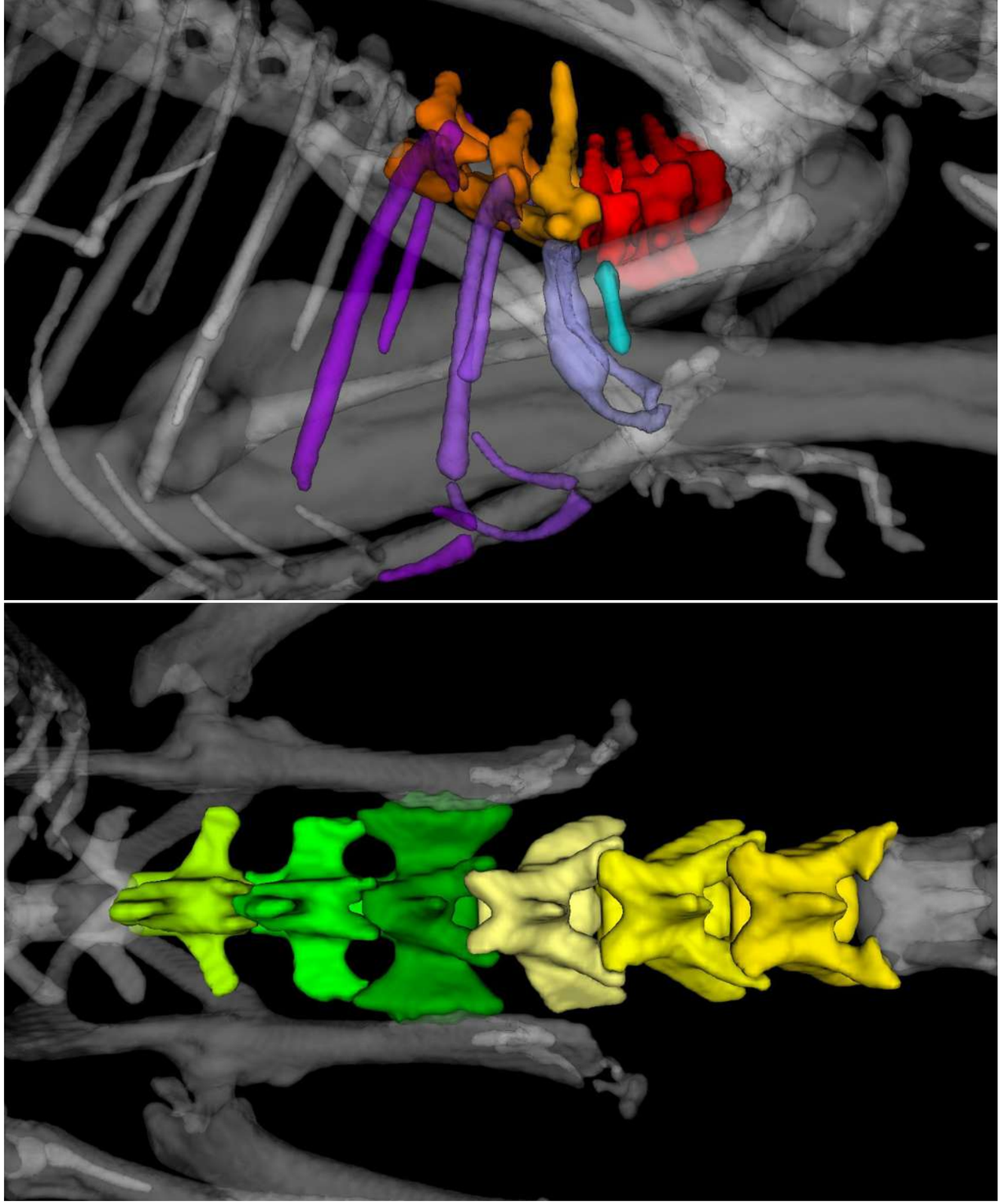
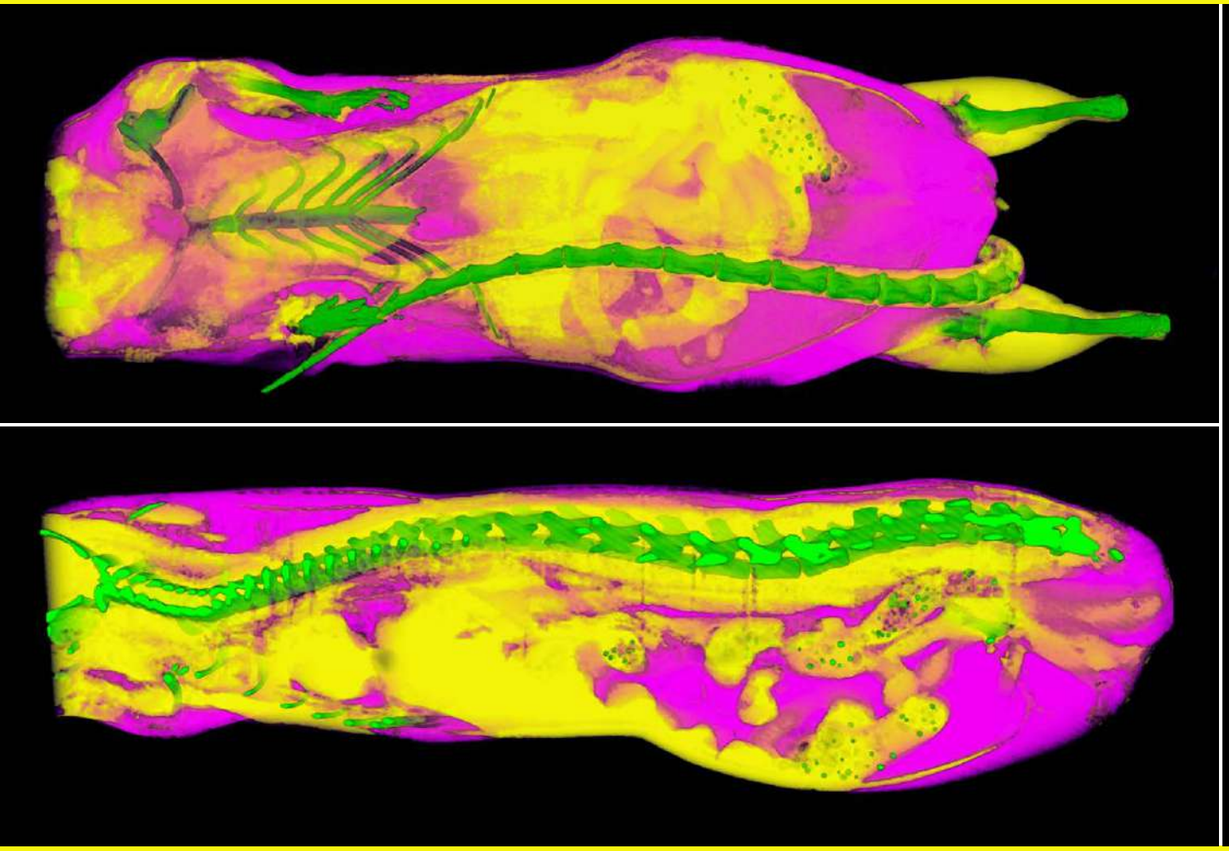
BIOCEV: Annual Brochure 2020

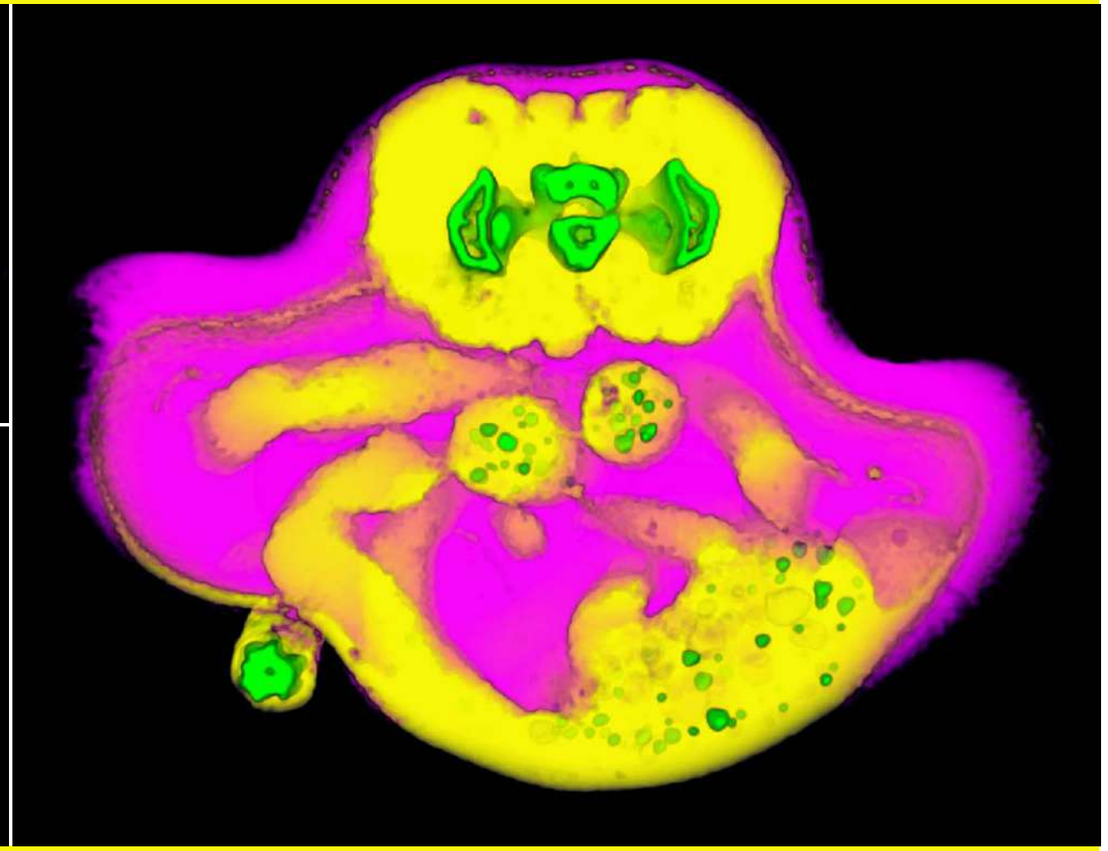
Research
& Development:

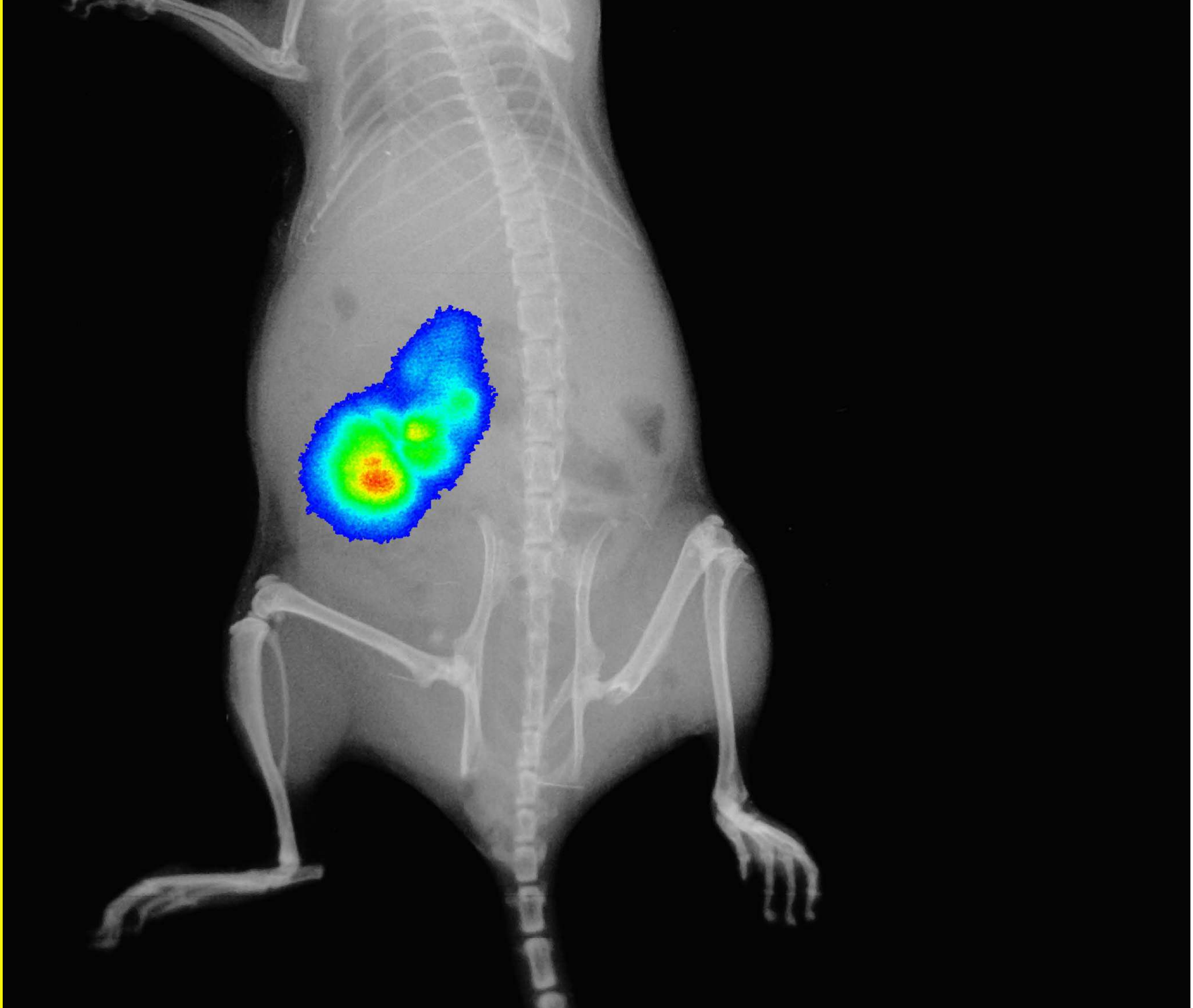
Research Infrastructures
and Core Facilites:

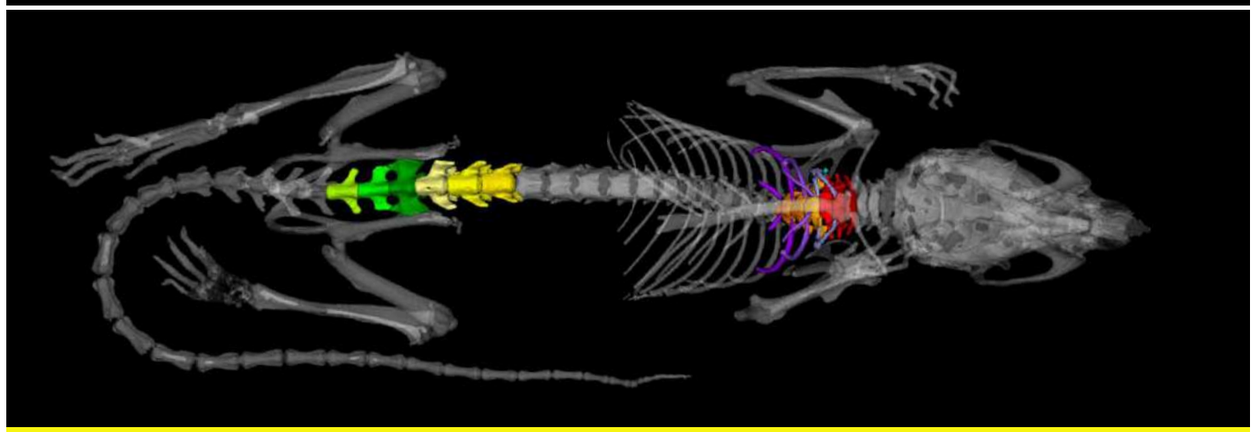
BIOCEV
Biotechnology and Biomedicine Centre of the Academy
of Sciences and Charles University in Vestec











Foreword

Foreword by BIOCEV Scientific Director:
Prof. MUDr. Pavel Martásek, DrSc.

Dear Ladies and Gentlemen,

Two important institutions in our country, the Czech Academy of Sciences and Charles University in Prague, have teamed up to build a temple of science at the gates of Prague. Does that sound a bit exaggerated? Maybe it does and maybe it does not. I am convinced that BIOCEV has started to assert its position as a first class science and research centre and meets all prerequisites to continue to play this role in the future. Endowed with superb instruments and equipment, BIOCEV brings together enthusiastic teams of well-trained scientists from six institutes of the Czech Academy of Sciences and two faculties of Charles University, all working together under one roof.

CV:	
	First Faculty of Medicine, Charles University
	Institut Jacques Monod, Paris, France
	Université Paris VII, France
	The University of Texas at San Antonio, USA
	New York Medical College, Valhalla, USA
	Tohoku University, Sendai, Japan
	BIOCEV

Today, significant discoveries are often being made on the boundaries of traditional scientific disciplines. Current biomedicine and biotechnology are becoming increasingly interdisciplinary – in an effort to not only understand the molecular basis of diseases, but also to diagnose the disease process as early as possible.

All that is reflected in the design of new treatments and the development of new drugs. BIOCEV's five main scientific programmes have been designed to be significantly interdisciplinary and as complementary as possible. Mutual direct communication between the various scientists within BIOCEV has brought about significant added value. In addition, BIOCEV also plays a vital role in educating young scientists and researchers, which is inconceivable without the direct participation of students of doctoral and Master's programmes in high-quality research projects.

I am convinced that it is worthwhile to invest considerable resources in quality biomedical and biotechnology research. BIOCEV is a centre of excellence for basic research. Experience from elsewhere clearly shows that sooner or later, basic research leads to outcomes that can be called 'applied', or perhaps 'practically usable', and this has been happening. One example is the development of a promising substance that directly targets cancer cell mitochondria and which has successfully passed phase I of clinical trials. Another is the definition of a new cancer medication category known as migrastatics that targets metastases.

I am glad that there are some smaller biotechnology firms in BIOCEV's immediate vicinity which have already become quite successful. Support for the setting up of small biotechnology firms, the building of science and technology parks along with fiscal support for research and development, as well as other possible types of support have not been introduced to a sufficient extent or quickly enough in the Czech Republic and need to be systematically cultivated over the long term.

I wish BIOCEV a fruitful journey
in the years to come.

Foreword by Rector of Charles University:
Prof. Tomáš Zima, MD, DSc.

Dear Ladies and Gentlemen,

2020 will mark four years since the opening ceremony of BIOCEV, one of the most important biotechnology and biomedical research centres of the Academy of Sciences of the Czech Republic and Charles University. I am delighted that you are now holding in your hands the second publication summarising the major achievements and reliving the most significant moments from BIOCEV's relatively short history. Although the centre only opened in 2016, when measured by the successes it has achieved in research, it has found its rightful place among the leading research centres in the Czech Republic. BIOCEV has been actively developing five research programmes with the participation of the best researchers in molecular and cellular biology, phenogenomics, structural biology, tissue engineering and biomedicine. First-class equipment and instrumentation make BIOCEV a centre of excellent basic research, and facilitate its active involvement in the tuition and training of students in Master's and doctoral degree programmes, with a strong emphasis on engaging in collaboration with biotechnology companies.

I am truly happy to see the wonderful results produced by the BIOCEV research team to date - results that can be used in applied research and in the development of new medical procedures to combat severe health problems. The end results of BIOCEV's research work include drugs targeted at the exact location of damaged metabolism and protein and tissue engineering. BIOCEV also enjoys a great international reputation among researchers, both in the Czech Republic and abroad. This has helped to attract over 500 researchers and engineers to the research teams, of whom almost one third come from countries such as Australia, Canada, China, France, Ukraine, Poland, and Germany. The research teams have published more than 800 research outputs, including articles in prestigious international journals. The foundation of this important research centre has brought together traditionally strong branches of the technical and natural sciences.

I am delighted that, through two of its faculties, the First Faculty of Medicine and the Faculty of Science, Charles University plays a part in BIOCEV and its excellent research projects. Thanks to our involvement in BIOCEV, we have been able to develop new degree programmes for Master's and doctoral students, and organise specialised international courses for both Czech and international students and young researchers. BIOCEV has helped to take our university training and research to the next level. BIOCEV is becoming a fully-fledged member of the European biotechnology and biomedicine research community and contributes towards increasing the competitiveness of the Czech Republic and Europe.

It goes without saying that high-quality science and research are one of the key factors that can help to ensure long-term prosperity for our country. I am delighted that, in just a short period of time, BIOCEV has become one of the key centres in the Czech Republic and has achieved a number of concrete results in research.

Foreword by President of the Czech Academy of Sciences:
Prof. RNDr. Eva Zažímalová, CSc.

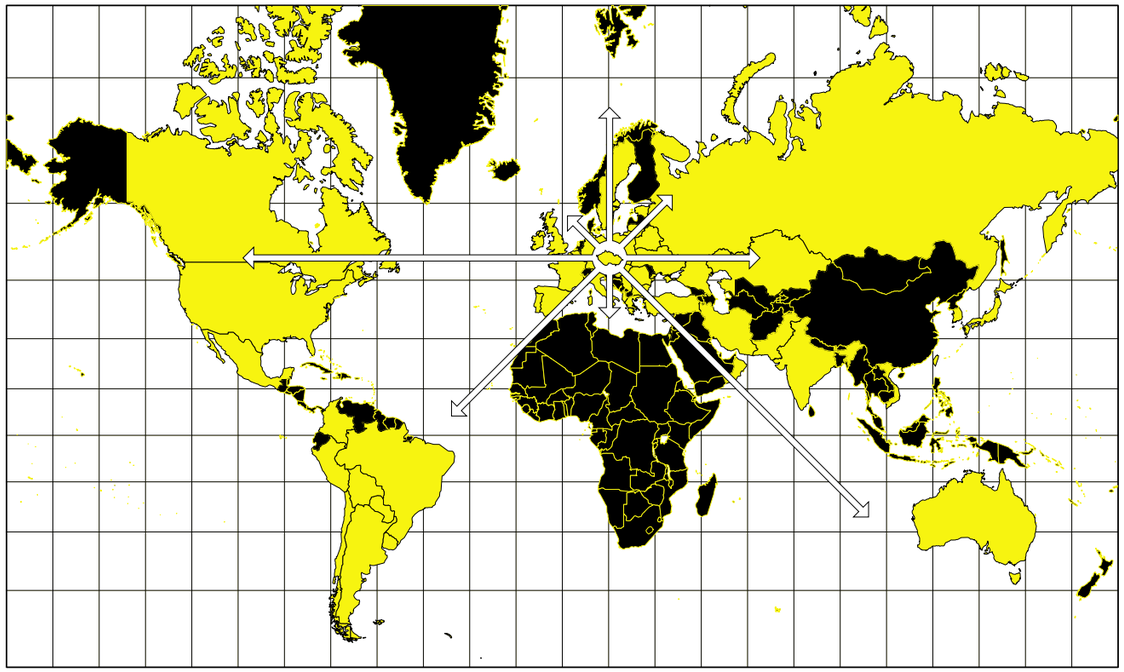
Dear Ladies and Gentlemen,

I consider the integration of Czech science into the international context through joint research projects as one of the key tasks of the Czech Academy of Sciences. I am therefore very pleased to say a few words on the occasion of the publication of this new brochure. It comprehensively presents BIOCEV Centre's research and scientific activities, and its research teams. Above all, it must be said that within the context of Czech and European science, today's BIOCEV is a top platform for the development of modern biotechnologies and biomedicine. Thanks to the establishment of this centre, it has been possible to interconnect traditionally strong fields of Czech science, such as microbiology, molecular biology and chemistry, together with cutting edge service laboratories and research infrastructures.

One of the most tangible pieces of evidence that BIOCEV is a successful project is the fact that, in its relatively short existence, more than 800 high-quality publications have been produced. The greatest successes of its research include the antitumor agent called MitoTam, which might have far-reaching effects in the treatment of cancer. The first phase of its pre-clinical testing, supported by SmartBrain and KKCG, has already been completed. Its results are very promising; therefore, later this year the second phase of its clinical trials will commence. Nevertheless, the fact that BIOCEV is a centre of top basic and applied research is evidenced by more than just its excellent scientific results. Personally, I consider it equally important that the centre's specialised programmes and top-ranking instrumentation are accessible for the teaching and education of undergraduate and doctoral students and, last but not least, collaboration with the business community is accentuated. This is precisely what makes BIOCEV an advanced scientific centre and what brings benefits to society as a whole.

I sincerely hope that BIOCEV continues to attract many talented and enthusiastic young scientists and establishes fruitful collaboration with the best scientific institutions as well as with progressive and innovative companies.

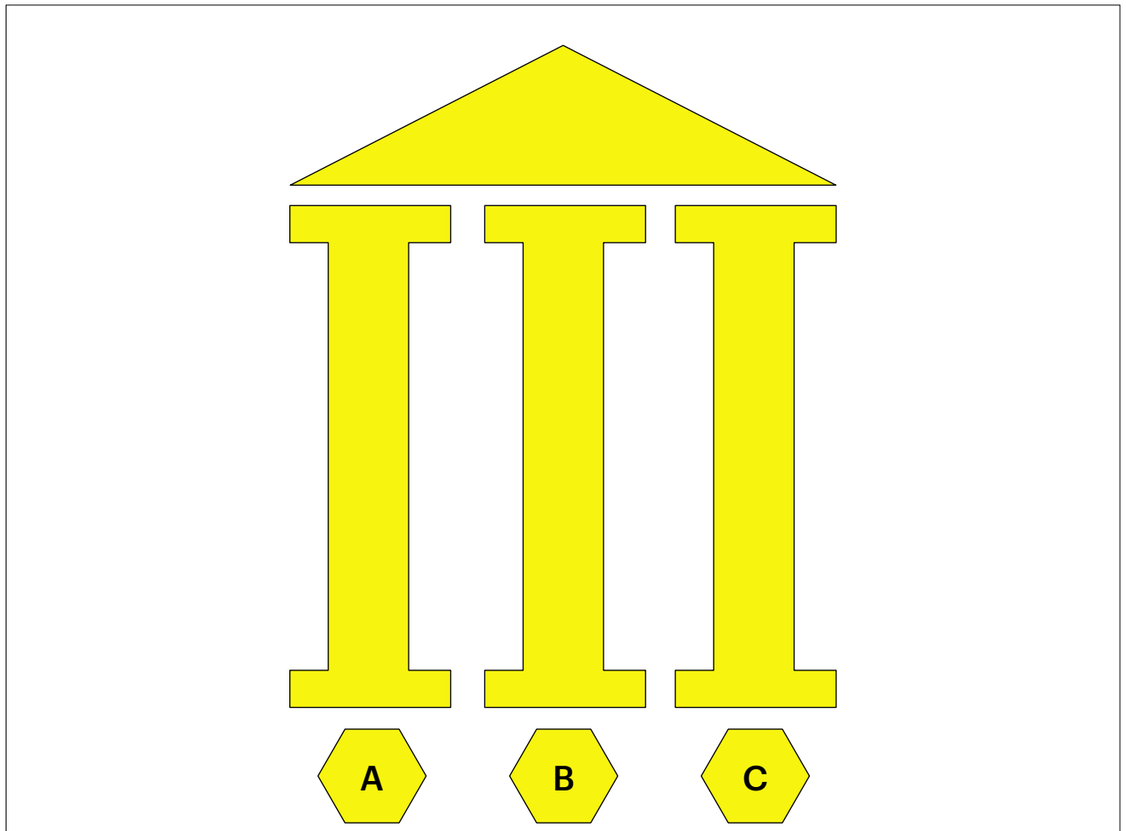
BIOCEV is a European scientific centre of excellence in biotechnology and biomedicine, whose outputs lead to a better quality of life and the development and growth of both the knowledge economy and competitiveness of the Czech Republic.



Thanks to the BIOCEV, Czech and foreign experts on biomedicine, virology, parasitology, genetics, tissue engineering, molecular biology, and medicinal chemistry are able to come together under one roof.

Pillars

BIOCEV builds upon three pillars of the knowledge triangle: teaching and education (A), research and development (B), and the transfer of research results into practice (C). The end results of BIOCEV research include drugs specifically targeted to the site of impaired metabolism, polymer vaccines, novel antibiotics, and protein and tissue engineering.



A	Teaching and Educaion
	A wide range of educational activities and the development of new graduate and postgraduate programmes of study
	Popularisation and media coverage of biotechnology and biomedical fields
	Training for private sector employees
B	Technology Transfer
	The transfer of the results of basic research into practice (human and veterinary medicine)
	Intensive cooperation between companies and BIOCEV research facilities
	The protection of intellectual property
C	Research and Development
	5 biotechnology and biomedical research programmes
	Fully-equipped facilities with cutting-edge instruments
	Integration into the European Research Area

BIOCEV offers a unique learning environment to students of all levels ranging from secondary schools to Ph.D. students, which is attractive not only in the Czech Republic, but also on an international scale.

27 000	5.5
of modern laboratories (m²)	site area (ha)
6	5
partner institutions	research programmes
6	56
core facilites and research infrastructures	research groups
543	307
researchers, students and technical stuff	researchers

Students who have worked or participated in research at BIOCEV workplaces can easily find jobs in their specialisation, both in the Czech Republic and abroad.

150	80
women researchers	PhD graduates
862	21
publications	patents
168	12
on-going projects	international on-going projects
1	3
ERC Grant	EMBO installation grant

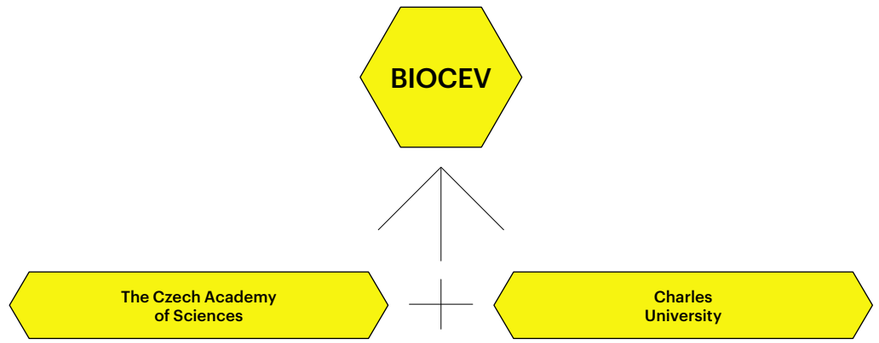
Partners and Organizational Structure

BIOCEV is a joint project of six institutes of The Czech Academy of Sciences (Institute of Molecular Genetics, Institute of Biotechnology, Institute of Microbiology, Institute of Physiology, Institute of Experimental Medicine, and Institute of Macromolecular Chemistry) and two faculties of Charles University in Prague (Faculty of Science and 1st Faculty of Medicine). The project's goal is to establish European Centre of Excellence in biomedicine and biotechnology.

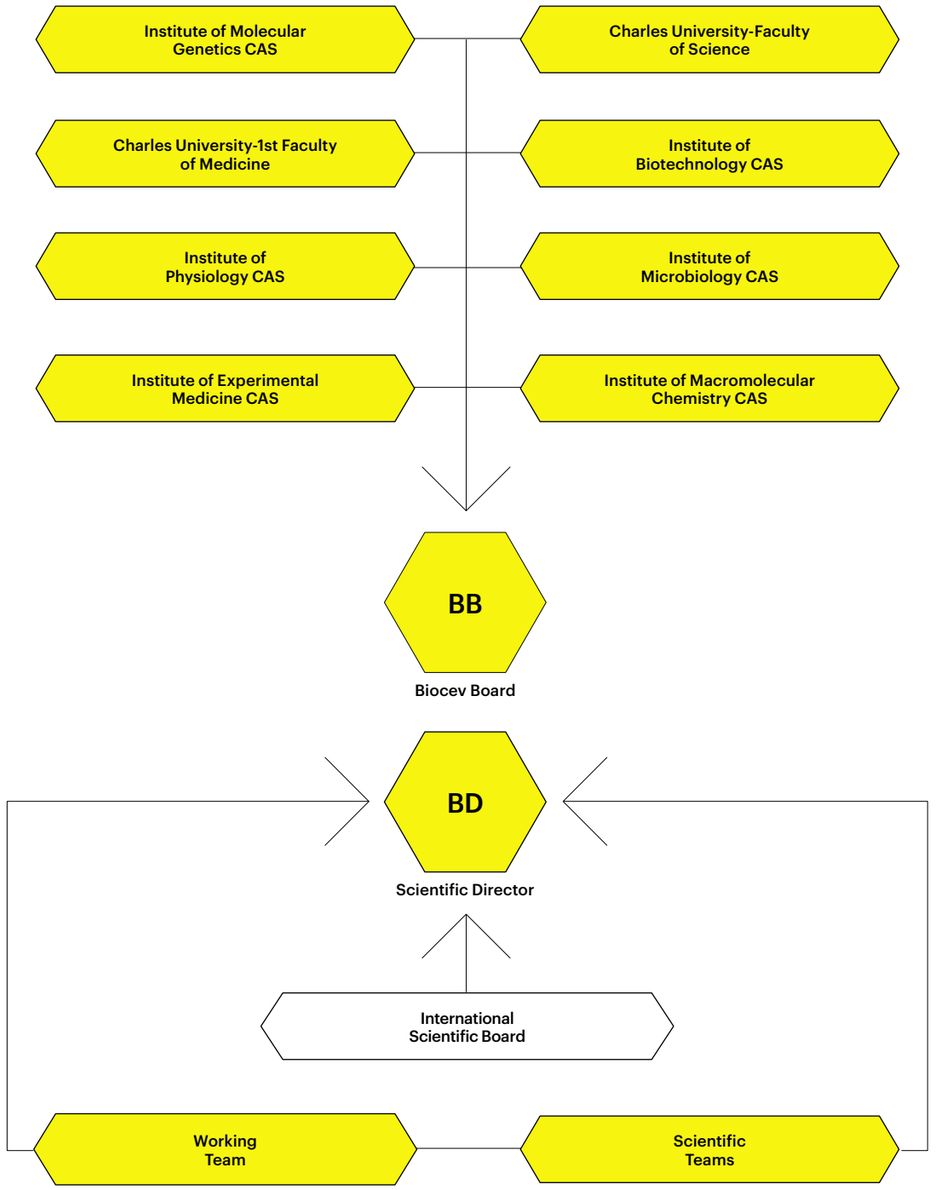
National and International Cooperation

Cooperation with institutions and entities at both the national and international levels is a key part of the concept and functioning of the BIOCEV centre. Emphasis is placed on integrating into the European Research Area and on working with other projects implemented in the Czech Republic and abroad, commercial entities and, last but not least, regional and local authorities. Emphasis is placed is on establishing new partnerships that, among other things, improve the centre's competitiveness.

Partners



Organizational structure



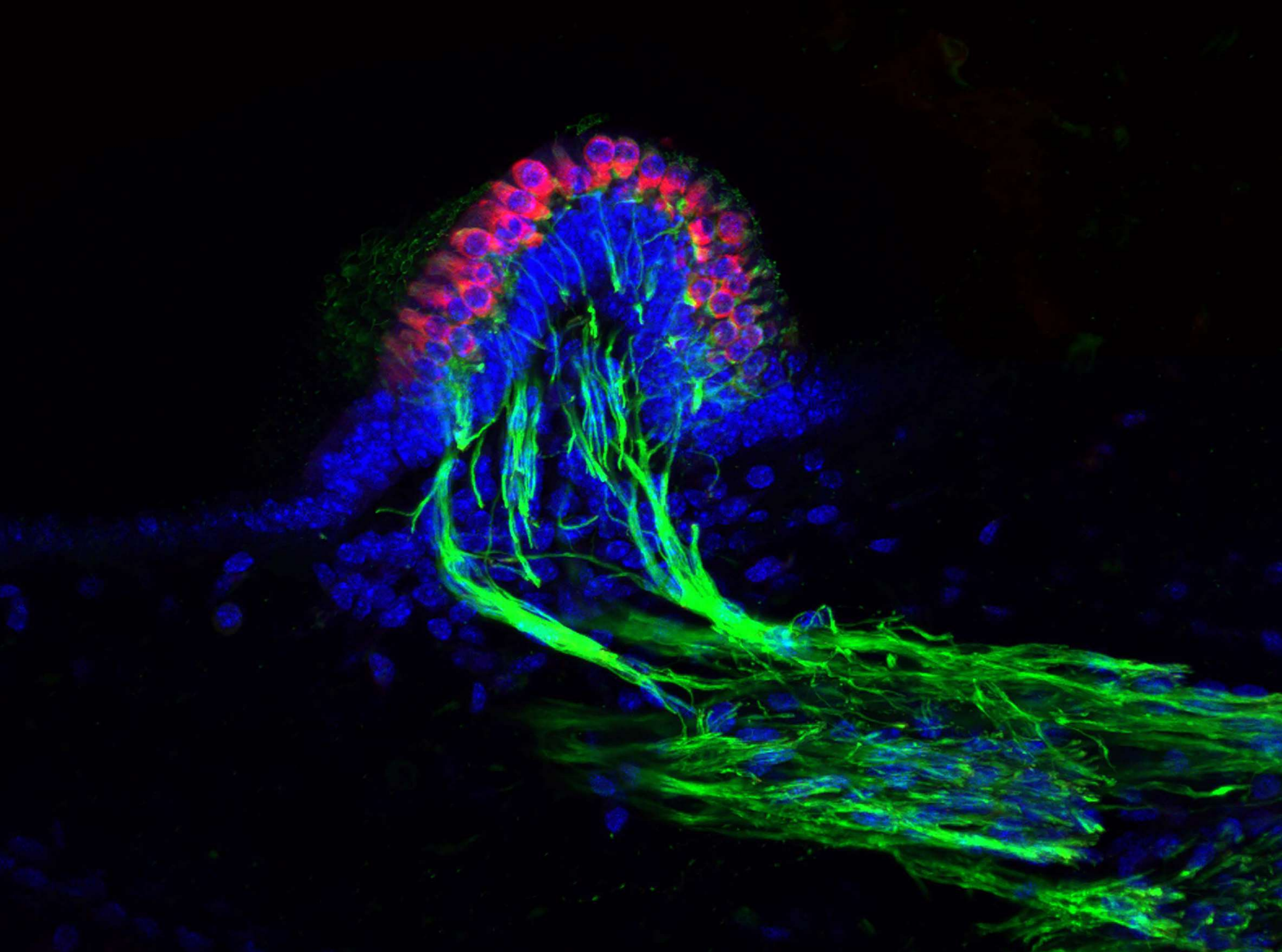
The Research Programmes

No. of research programmes: 05

The Research Programmes

30

Research & development represents one of the three basic pillars of project BIOCEV. Research & development performed in BIOCEV is focused on the selected areas of biotechnologies and biomedicine. The scientific scope of BIOCEV has been divided into five research programmes, each of them dealing with a number of separate research projects. The programmes and projects have been designed to form a mutually integrated system of synergistic links inside BIOCEV.



Functional Genomics

No. of research teams: 07

Pages: 34–53

01

Functional Genomics

Radislav Sedláček, PD, Dr. rer. nat.
Head of the Research Programme, Head of the Czech Centre for Phenogenomics
The Institute of Molecular Genetics of the Czech Academy of Sciences

Research Directions

Functional Genomics

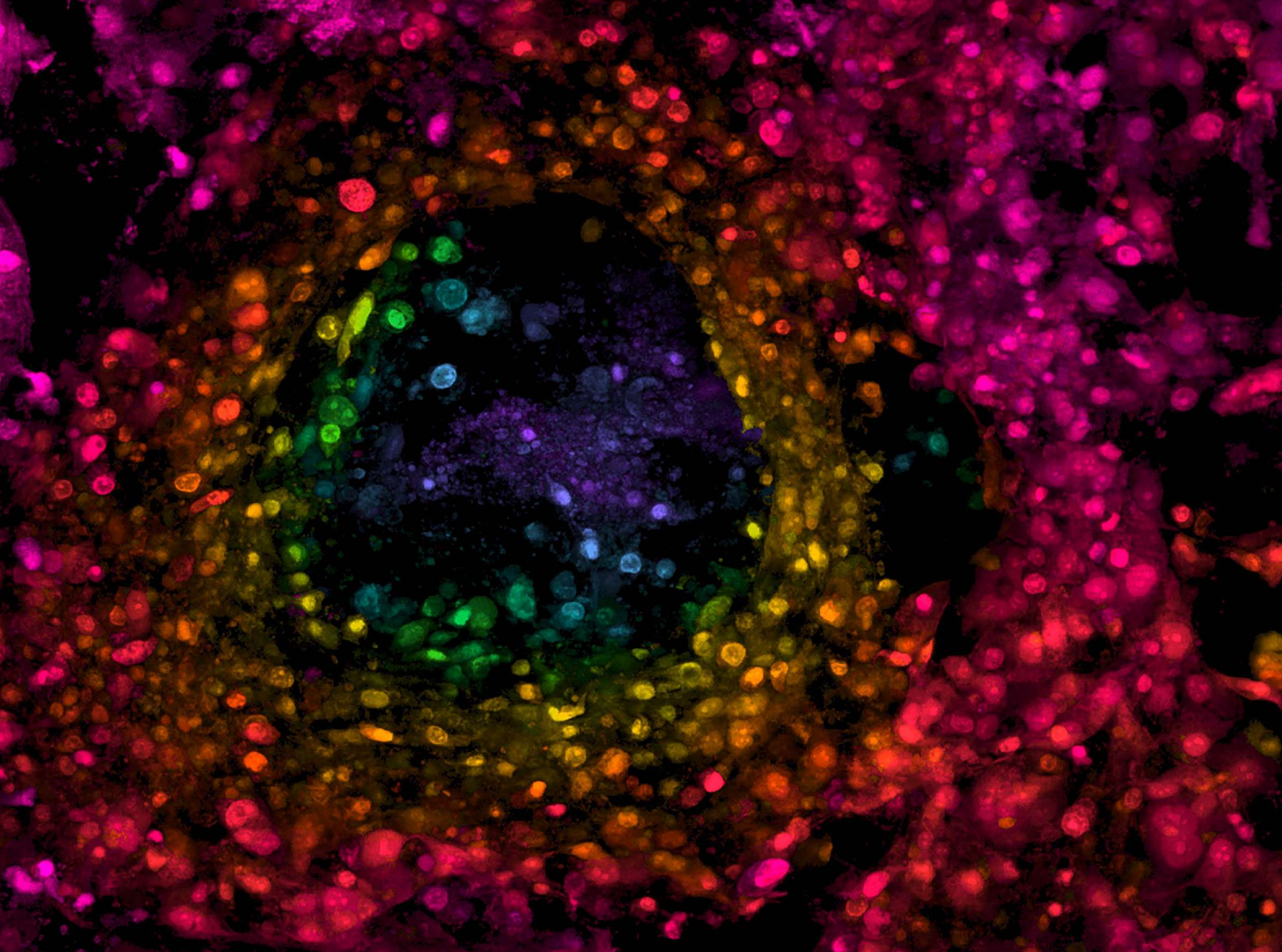
1.	Functional genomics based on mouse and rat models	2.	The effect of functional gene ablation/mutation on physiologic functions, participation in disease	3.	Description of gene functions in individual physiological systems

Supported from a robust and standardized phenotyping platform offering a functional screening of almost all physiologic body systems, the groups of this programme aim to identify and characterize genes representing new potential targets to treat various human diseases. The main effort focuses on metabolic syndrome and physiologic functions interrelated with complex human diseases. Identified genes and genetic determinants will then be scrutinized for their potential to be employed as therapeutic targets. In addition to the liver and metabolic disorders, research projects will also focus on visual and auditory systems, whose correct functioning is of enormous importance for humans' quality of life and well-being. Genes important for the physiologic systems will be identified, examined, and prove whether they could serve as diagnostic markers or therapeutic targets. Once the therapeutic potential is proved, the researchers will, together with drug developing groups or companies, study the drugs' efficacy employing the know-how, capacity, and experimental models of the Czech Centre for Phenogenomics.

The genomes of humans, mice and many other species have been completely sequenced; nevertheless the knowledge of genome sequences as such does not shed light on questions concerning the functions of these sequences. Among the unanswered questions are those regarding the functions of most of the genes that encode proteins; the number of these genes is estimated to be 20 000. In order to describe the biological functions of a gene, an informative modification (mutation, for instance a conditional deletion) must be inserted into the genes.

In order to annotate the function of human genes, functional (pheno)genomics needs to be combined with comparative genomics – the function of the human genome should be inferred from the function of an orthologous genetic product, e.g. from a mouse.

In recent years, mouse and rat models have been considered excellent models in the search for the functions of genes within complex organisms as most of their physiological functions are very similar to those of humans and, also, their genetic differences are minimal (in comparison to other non-mammalian models). Although characterization of the function of a particular gene product (e.g. protein) in vitro delivers important information about molecular mechanisms, verifying their real functions cannot be done without intensive research at the level of complex organisms and their distinct physiological systems. The functional genomics research programme is based on the previous work by a few research groups that have built up substantial expertise and have already attained considerable scientific results in this field.



Transgenic Models of Diseases Laboratory
Radislav Sedláček, PD, Dr. rer. nat., Head of the Lab.

The Institute of Molecular Genetics of the
Czech Academy of Sciences

Ubiquitination-mediated processes in health and disease

Using mutant mouse models, we are addressing the role of several ubiquitin ligases. The major focus of these studies is to understand the role of ubiquitination in the regulation of intestinal barrier function and immunity. In our current work, we are focusing on cullin ligases involved in GIT homeostasis and pathological processes since the cullin family has been largely associated with different types of cancer in GIT and thus represents a promising pharmacological target.

Functional redundancy of the kallikrein locus

The mammalian kallikrein gene cluster exists as a gene cluster (on chromosome 19 in humans) and comprises a class of extracellular proteases that mediate tissue homeostasis. They are often dysregulated during cancer and can serve as excellent diagnostic markers. We are addressing the functional redundancy of kallikreins by introducing more concurrent gene deletions. We expect these experiments not only to resolve the role of kallikreins in important physiological functions such as wound healing, but also to yield insight into their epigenetic co-regulation.

Molecular mechanisms of craniofacial development and biomineralization

Our focus is to investigate the molecular mechanism driving the complex process of craniofacial development. We showed that Wnt modulator Trabd2d, which is transmembrane metalloproteinase, is involved during neural tube closure and head development. In another project, we are focused on unveiling the basic processes of epithelial morphogenesis and its regulation by ubiquitination networks. We are also interested in studying the molecular regulation of biomineralization processes. We discovered Fam46A loss of function leads to biomineralization defects, and importantly, similar mutations have been found in patients with osteogenesis imperfecta. We thus aim to reveal the molecular mechanisms responsible for this type of osteogenesis imperfecta in patients.

Potential for Cooperation

We provide expertise in gene targeting and genome editing using CRISPR/Cas9 and TALEN technology, and embryonic stem cells. In addition, we offer services in the comprehensive and standardized phenotyping of mouse models.

2021 De Gasparo, R., Pedotti, M., Simonelli, L. et al. Bispecific IgG neutralizes SARS-CoV-2 variants and prevents escape in mice. Nature (2021)

2020 Bautista-Niño PK, Portilla-Fernandez E, Rubio-Beltrán E, van der Linden JJ, de Vries R, van Veghel R, de Boer M, Durik M, Ridwan Y, Brandt R, Essers J, Menzies RI, Thomas R, de Bruin A, Duncker DJ, van Beusekom HMM, Ghanbari M, Hoeijmakers JHJ, Sedlacek R, Touyz RM, Montezano AC, van der Pluijm I, Danser AHJ, Haanes KA, Roks AJM: Local endothelial DNA repair deficiency causes aging-resembling endothelial-specific dysfunction. Clin Sci (Lond) 2020 134(7): 727-746.2020

2020 Bayram J, Malcova I, Sinkovec L, Holubova J, Streparola G, Jurnecka D, Kucera J, Sedlacek R, Sebo P, Kamanova J: Cytotoxicity of the effector protein BteA was attenuated in Bordetella pertussis by insertion of an alanine residue. PLoS Pathog 2020 16(8): e1008512.

2020 Budinska E, Gojda J, Heczkova M, Bratova M, Dankova H, Wohl P, Bastova H, Lanska V, Kostovcik M, Dastych M, Senkyrik M, Krizova J, Mraz M, Hradecky J, Hajslova J, Lenicek M, Podzimkova K, Chalupsky K, Sedlacek R, Cahova M: Microbiome and Metabolome Profiles Associated With Different Types of Short Bowel Syndrome: Implications for Treatment. JPEN J Parenter Enteral Nutr 2020 44(1): 105-118.

2020 Cacheiro P, Muñoz-Fuentes V, Murray SA, Dickinson ME, Bucan M, Nutter LMJ, Peterson KA, Haselimashhadi H, Flenniken AM, Morgan H, Westerberg H, Konopka T, Hsu CW, Christiansen A, Lanza DG, Beaudet AL, Heaney JD, Fuchs H, Gailus-Durner V, Sorg T, Prochazka J, Novosadova V, Lelliott CJ, Wardle-Jones H, Wells S, Teboul L, Cater H, Stewart M, Hough T, Wurst W, Sedlacek R, Adams DJ, Seavitt JR, Tocchini-Valentini G, Mammano F, Braun RE, McKerlie C, Herault Y, de Angelis MH, Mallon AM, Lloyd KCK, Brown SDM, Parkinson H, Meehan TF, Smedley D: Human and mouse essentiality screens as a resource for disease gene discovery. Nat Commun 2020 11(1): 655.

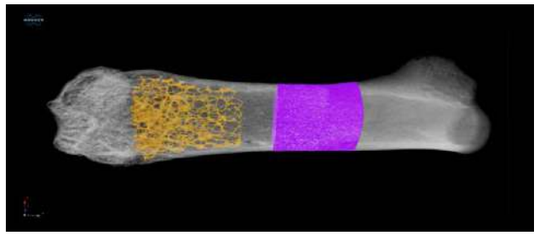
2020 Desideri F, Cipriano A, Petrezselyova S, Buonaiuto G, Santini T, Kasparek P, Prochazka J, Janson G, Paiardini A, Calicchio A, Colantoni A, Sedlacek R, Bozzoni I, Ballarino M: Intronic Determinants Coordinate Charme lncRNA Nuclear Activity through the Interaction with MATR3 and PTBP1. Cell Rep 2020 33(12): 108548.

2020 Jenickova I, Kasparek P, Petrezselyova S, Elias J, Prochazka J, Kopkanova J, Navratil M, Barinka C, Sedlacek R: Efficient allele conversion in mouse zygotes and primary cells based on electroporation of Cre protein. Methods 2020 S1046-2023(20): 30114-6.

Contact person:	Assoc. Prof. Radislav Sedláček, Ph.D.
	e-mail: radislav.sedlacek@img.cas.cz

Basic keywords:	01	Gene
	02	Genome
	03	Transgenesis
	04	Phenotyping
	05	Mouse
	06	Ubiquitin ligase
	07	Metalloproteinase
	08	Serine protease
	09	Inflammation
	10	Intestine

Research areas:	01	Gene targeting and genome editing
	02	Animal models of disease
	03	Ubiquitin ligases
	04	Proteolytic enzymes
	05	Stem cell pluripotency and early embryonic development
	06	Inflammatory processes
Main objectives:	01	The development of technologies for gene targeting and genome editing
	02	Generation of animal models and their phenotyping
	03	The role of ubiquitin ligases



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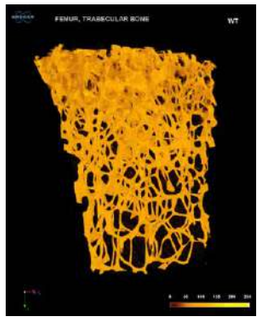
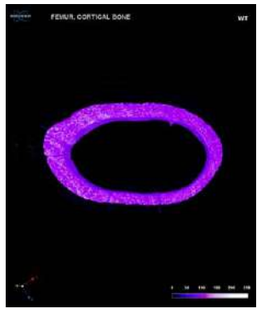


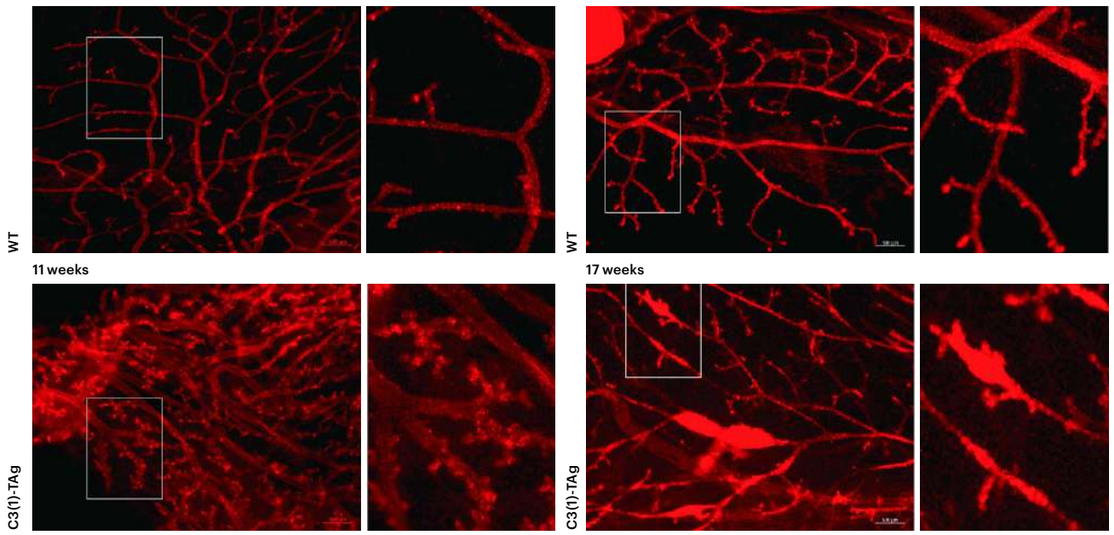
Fam46a KO



3D µCT of sections of femur metaphyses, trabecular and cortical bone.

11 weeks

17 weeks



Mouse Molecular Genetics
Prof. MUDr. Jiří Forejt, DrSc., Head of the Lab.

The Institute of Molecular Genetics of the
Czech Academy of Sciences

Content of the Research

Our laboratory focuses on four main research topics. The first topic involves the identification and function of the *Prdm9* and *Hstx2* major hybrid sterility genes. Our laboratory studies genetic incompatibilities in inter-subspecific hybrids, more specifically C57BL6/J and PWD/Ph inbred strains as model representatives of *Mus m. musculus* and *Mus m. domesticus*. The candidates for hybrid sterility genes were evaluated by positional cloning and transgenic rescue for the *Hst1* locus and by expression profiling of sorted testicular cells for X-linked *Hstx1* and *Hstx2* loci. We identified the Hybrid sterility 1 (Hst1) locus with the *Prdm9* gene encoding the meiotic histone H3 lysine-4 and lysine-36 tri-methyltransferase as the first hybrid sterility gene in vertebrates. We localized the second major hybrid sterility locus/gene, *Hstx2*, to a 2.7 megabase interval on the X chromosome. The *Hstx2* locus plays a dual role; besides interacting with *Prdm9* to control hybrid sterility, it carries a major genetic factor, designated *Meir1*, which regulates the genome-wide rate of meiotic recombination. The relation between *Hstx2* and *Meir1* will be clarified after identification of their causative genes. We pioneered the use of a powerful genetic polymorphism that occurred during the evolution of two mouse subspecies; *Mus m. musculus* and *Mus m. domesticus* to create a panel of chromosome substitution (consomic) strains as a powerful tool for phenogenomic studies.

Our second topic involves cis and trans-control of meiotic chromosome asynapsis. Both hybrid sterility genes, *Prdm9* and *Hstx2* control the ability of homologous chromosomes to recognize each other at the pachytene stage of primary spermatocytes. The third topic involves the genetic factor within the *Hstx2* locus controlling the meiotic recombination rate, particularly the possible relationship between the frequency and positioning of crossovers and noncrossovers to the sterility of inter-subspecific hybrids. The last topic, male sex chromosome inactivation, involves the meiotic X-chromosome inactivation in germ cells of hybrid mice by applying genome-wide expression profiling, monitoring transcription profiles and histone modifications in meiotic and postmeiotic testicular cells.

Potential for Cooperation

We are looking for expert bioinformatics analysis of ChIP-seq data and genome targeting by CRISPR/Cas9 or TALEN technology. We offer our service in the genetics of complex traits using mouse chromosome substitution strains, and in male and female gametogenesis.

2019 Lustyk D, Kinský S, Ullrich KK, Yancoskie M, Kašíková L, Gergelits V, Sedlacek R, Chan YF, Odenthal-Hesse L, Forejt J, Jansa P.: Genomic structure of Hstx2 modifier of *Prdm9*-dependent hybrid male sterility in mice. Genetics 213 (3), 1047-1063 Nov 2019. DOI: /10.1534/genetics.119.302554.

2019 Papanikos F, Clément JAJ, Testa E, Ravindranathan R, Grey C, Dereli I, Bondarieva A, Valerio-Cabrera S, Stanzione M, Schleiffer A, Jansa P, Lustyk D, Fei JF, Adams IR, Forejt J, Barchi M, de Massy B, Toth A,: ANKRD31 regulates spatiotemporal patterning of meiotic recombination initiation and ensures recombination between heterologous sex chromosomes in mice Mol Cell. Jun 6;74(5):1069-1085.e11, 2019.

2018 Gregorova, S., Gergelits, V. Chvatalova, I., Bhattacharyya, T., Valiskova B., Fotopulosova V., Jansa, P., Wiatrowska, D., Forejt, J.,: Modulation of *Prdm9*-controlled meiotic chromosome asynapsis overrides hybrid sterility in mice. Elife Mar 14;7. pii: e34282, 2018.

2018 Wang, L., Valiskova, B., Forejt, J.,: Cisplatin-induced DNA double-strand breaks promote meiotic chromosome synapsis in PRDM9-controlled mouse hybrid sterility. Elife. 2018 Dec 28;7. pii: e42511.

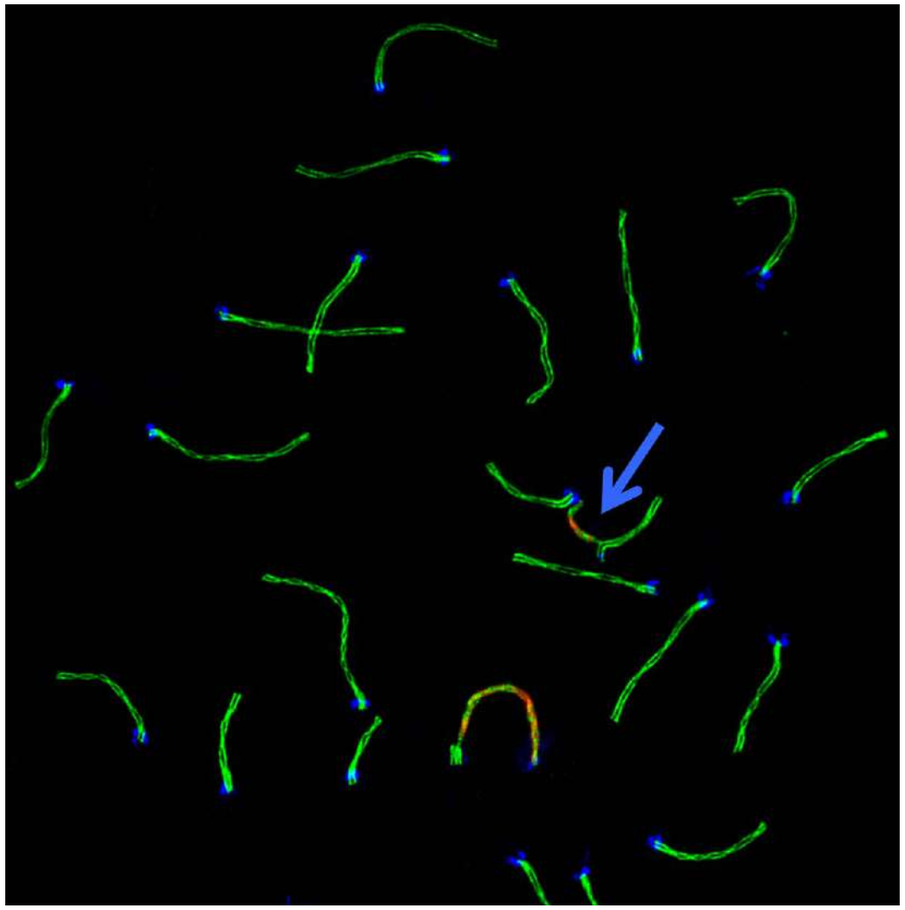
2016 M, Faltusova B, Gergelits V, Bhattacharyya T, Mihola O, Trachtulec Z, Knopf C, Fotopulosova V, Chvatalova I, Gregorova S, Forejt J. Hybrid Sterility Locus on Chromosome X Controls Meiotic Recombination Rate in Mouse. PLoS Genet. 2016 Apr 22;12(4):e1005906

2016 Forejt J. Genetics: Asymmetric breaks in DNA cause sterility. Nature 530:167-8, 2016.

Contact person:	Prof. MUDr. Jiří Forejt, DrSc.
	e-mail: jiri.forejt@img.cas.cz

Basic keywords:	01	Hybrid sterility
	02	Mammalian meiosis
	03	*Prdm9*
	04	Rad21l
	05	Crossing-over
	06	Synaptonemal
	07	Complex
	08	Meiotic silencing of unsynapsed chromatin
	09	Chromosome substitution strains
	10	Complex trait genetics

Research areas:	01	Mammalian meiosis
	02	Hybrid sterility
	03	Reproduction
	04	Meiotic recombination
	05	Mouse chromosome substitution strains
	06	Complex trait genetics
Main objectives:	01	Genes involved in the sterility of inter-species hybrids
	02	Homolog chromosome recognition in meiosis
	03	Genome-wide recombination rate



Incomplete chromosome 17 synapsis in a pachytene spermatocyte spread. Visualization of partial Ts(16:17)43H trisomy (arrow) by super-resolution immunofluorescence microscopy. Blue -centromeric regions, Green - SYCP3 protein of lateral elements of synaptonemal complexes (Jansa et al. Biol. Reprod. 2014).

Auditory Function in Mutant Mice

RNDr. Jiří Popelář, CSc., Head of the Lab.

The Institute of Experimental Medicine
of the Czech Academy of Sciences

Content of the Research

Hearing impairment is one of the most frequent inborn sensory defects. One out of every 500 children is born deaf. A large percentage of the hearing losses are monogenic. As the population is aging, the progressive hearing loss that is attacking the majority of the population in old age is becoming an even more serious problem and it is based in the pathological changes of the genome. In both cases, mice models are of significant use when studying the genetic principles of the pathological conditions. The main research aims are oriented towards investigating the structure and function of the auditory system in various animal models under normal and pathological conditions and during ontogeny and ageing. Pathologies of the peripheral and central parts of the auditory system, appearing as a consequence of noise exposure or in conjunction with aging, are investigated in experimental rodents including mutant mice using electrophysiological, behavioral, and morphological methods. Studies on the hearing changes in some strains of laboratory rats show that the defects are related to the inner ear and easily detectable by the examination of auditory evoked potentials (Auditory Brainstem Responses - ABRs) and otoacoustic emissions (DPOAE – Distortion Product Otoacoustic Emissions). It is also possible to perform the screening with the help of shock-induced reaction behavior (Startle Reflex) and its modification by preceding sound (PPI – Prepulse Inhibition).

The experimental results have the potential to be used for the prevention and treatment of hearing disorders in humans. This goal has been previously realized through our participation within the framework of TARGEAR, the European project developing innovative integrated strategies for the healing of age-related hearing loss. In cooperation with the Transgenic and Archival Module of the Czech Centre for Phenogenomics (CCP), it will be possible to create targeted deletions of mouse mutants for certain genes suspected in playing a role in inherited hearing loss and hearing impairment during the organism's aging. In case of suspected hearing loss in mice passing through phenotypization in the mouse clinic, it will be possible to complete the "classic" examinations (ABR, DPOAE, startle) even with the detailed morphological and histochemical studies.

Potential for Cooperation

Presently, our laboratory is coordinated through a common research project with Gabriela Pavlínková, Ph.D., head of Laboratory of Molecular Pathogenetics. We are open to collaborating with any research team or institution working in the field of Neuroscience.

2019 Macova I., Pysanenko K., Chumak T., Dvorakova M., Bohuslavova R., Syka J., Fritzsch B. and Pavlinkova G. (2019): Neurod1 is essential for the primary tonotopic organization and related auditory information processing in the midbrain. Journal of Neuroscience 2019 Feb 6, 39(6): 984-1004. DOI: https://doi.org/10.1523/JNEUROSCI.2557-18.2018

2015 Chumak T., Bohuslavova R., Macova I., Dodd N., Buckiova D., Fritzsch B., Syka J., Pavlinkova G. (2015) Deterioration of the Medial Olivocochlear Efferent System Accelerates Age-Related Hearing Loss in Pax2-Isl1 Transgenic Mice. Mol Neurobiol 2016 May; 53(4): 2368-83. doi: 10.1007/s12035-015-9215-1. Epub 2015 May 20.

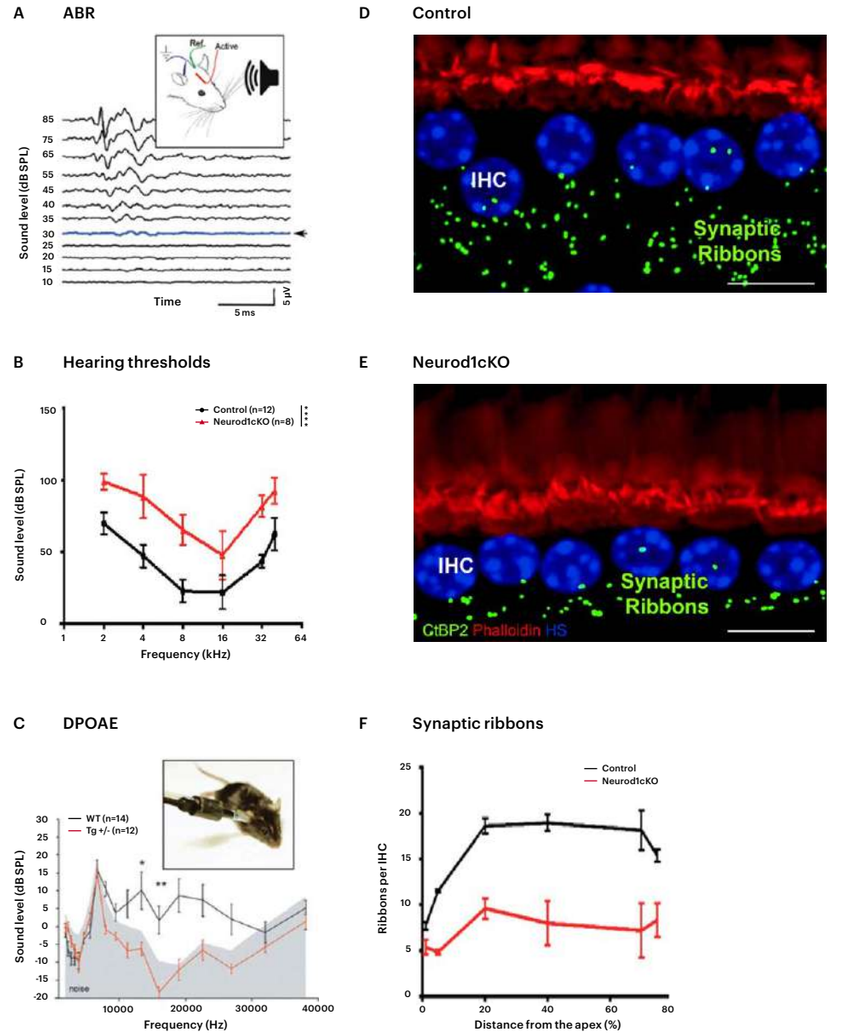
2015 Suta D., Rybalko N., Shen D-W., Popelar J., Poon P.W.F., Syka J. (2015): Frequency discrimination in rats exposed to noise as juveniles. Physiology & Behavior, 144, 60-65.

2013 Popelář J, Rybalko N, Burianová J, Schwaller B, Syka J. (2013) The effect of parvalbumin deficiency on the acoustic startle response and prepulse inhibition in mice. Neurosci Lett. 553, 216–220.

Contact person:	RNDr. Jiří Popelář, CSc.
	e-mail: jiri.popelar@iem.cas.cz

Basic keywords:	01	Auditory evoked responses
	02	Otoacoustic emissions
	03	Noise exposure
	04	Aging
	05	Acoustical startle response
	06	Prepulse inhibition
	07	Immunostaining

Research areas:	01	Auditory Neuroscience
Main objectives:	01	To analyze changes in the hearing function in various animal models under normal and pathological conditions
	02	To characterize in detail the changes in the structure and function of auditory systems in mutant mice demonstrating a hearing deficit



(A) - An example of electrical potentials (Auditory Brainstem Responses - ABRs) evoked by short sound stimuli of decreasing intensity. Potentials were recorded by three subdermal needle electrodes placed on the head and the neck of an anaesthetized mouse (see inserted schema). The arrow indicates the hearing threshold, the lowest sound intensity that the animal could hear. (B) – Hearing thresholds based on ABR recording to stimulation with short tones of 2-40 kHz are significantly increased in Neurod1cKO mice in comparison with control mice in the whole frequency range. (C) - Otoacoustic emissions, of which the Distortion Product Otoacoustic Emissions (DPOAEs) represents a specific subtype, are faint sounds produced by active vibration of the cochlear outer hair cells. They can be recorded in the outer ear canal by a probe with a sensitive microphone (see inserted picture) during sound stimulation. The plot demonstrates missing DPOAE in transgenic animals with non-functional outer hair cells (red line) in comparison with a normal level of DPOAE measured in the controls (black line). The grey area depicts the level of non-specific background noise. (D), (E) - Confocal analysis of immunostaining for a synaptic ribbon protein (CtBP2) in the inner hair cell (IHC) area shows a reduction of ribbons in mutant adult mice (E) compared to the controls (D). Phalloidin labels F-actin in stereocilia of hair cells, HS - Hoechst nuclear staining. Scale bar, 10 µm. (F) – The number of synaptic ribbons is significantly lower in the IHC of Neurod1cKO mice in comparison with control mice in the whole cochlear length.

Lipocalins in the Modulation of Mammalian Reproduction

Doc. Mgr. Pavel Stopka, Ph.D., Head of the Lab.

Faculty of Science,
Charles University

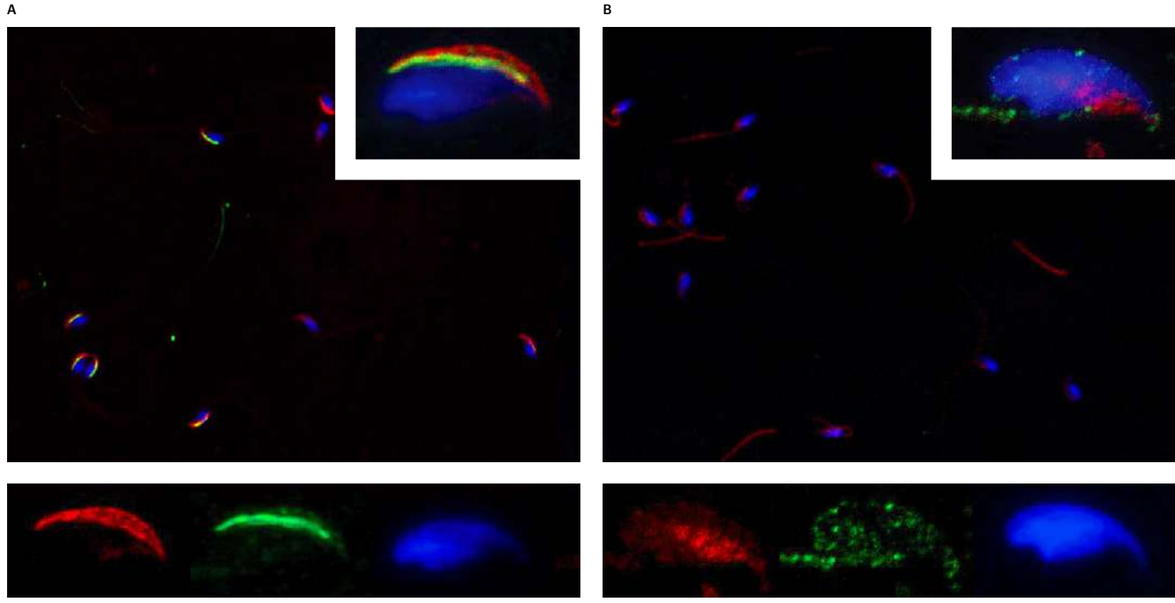
Content of the Research

We concentrate on gene functions, and their expression profiles in wild-living, wild-derived and inbred mouse lines. Therefore, we provide know-how and services with our expertise, which among others, includes qPCR profiling, transcriptome profiling (NGS), deep proteomic analyses, as well as behavioural analysis of mouse phenotype differences. At the moment, we are mainly focused on lipocalins, which is a family of globular proteins involved in many biological processes. Over the past 10 years, dozens of new genes belonging to the protein family of lipocalins (58 in mice – e.g. the main urinary, odorant binding proteins, probasin, Lcn and others) have been discovered and described. All lipocalins which have so far been described have a similar size (approx. 18 – 25 kDa) and tertiary structure, but they differ in their primary and secondary structures. These proteins participate in a whole series of important biological processes including detoxification. Detoxification in this context refers to the extraction of metals, free radicals, etc. The proteins are also involved in pheromonal communication and the transport of important molecules (e.g. steroids), in the process of the gamete ripening.

Originally, these proteins were mostly described from the point of view of chemical communication and nutritional immunity. Although this (monophyletic) family of proteins is one of the most numerous gene clusters in mammals, it is at the same time the least investigated. Our current research proved that this family of proteins is an extraordinarily universal communication medium by which the information transfer happens both between tissues and cells (i.e. by hormone transfer) and between individuals (i.e. by pheromone transfer). Our recent discovery shows that they are essential in the process of olfaction and detoxification of many tissues and their mucosa. Many odorant-binding proteins seem to be involved in odorant internalization, odorant transport towards chemosensory receptors and potentially also in the process of removing free radicals. Following our recent discoveries, we are working on precisely detecting the function of these proteins.

Potential for Cooperation

As a result of our team having been recently involved in characterizing the potential functions of lipocalins in behavioural processes, we are now moving ahead towards coordinating our efforts together with other research teams to uncover the potential roles of lipocalins in reproduction, immunity and as markers of parasitic and pathogenic invasions.



Localization of selected proteins PNA ● and LCN5 ● in mouse epididymal acrosome-intact (A) and acrosome-reacted sperm (B). Nuclei are stained with DAPI ●.

2018 Kuntová B., Stopková R., Stopka P. 2018: Transcriptomic and Proteomic Profiling Revealed High Proportions of Odorant Binding and Antimicrobial Defense Proteins in Olfactory Tissues of the House Mouse. Frontiers in Genetics 9.

2017 Cerna, M., Kuntova, B., Talacko, P., Stopkova, R., And Stopka, P. 2017. Differential regulation of vaginal lipocalins (OBP, MUP) during the estrous cycle of the house mouse. Sci Rep 7, 11674.

2016 Stopka P., Kuntová, B., Klempt, P., Havrdová, L., Černá, M. & Stopková, R. 2016. On the saliva proteome of the Eastern European house mouse (Mus musculus musculus) focusing on sexually dimorphic chemical signals and immunity. Scientific Reports 6, 32481.

2016 Stopková, R., Vinkler, D., Dudková, B., Šedo, O, Albrecht, T., Suchan J., Dvořáková-Hortová K., Zdráhal Z. & Stopka P. 2016. Mouse lipocalins (MUP, OBP, LCN) are co-expressed in tissues involved in chemical communication. Front. Ecol. Evol. 4:47.

Contact person:	Doc. Mgr. Pavel Stopka, Ph.D.
	e-mail: pstopka@natur.cuni.cz

Basic keywords:	01	Mouse
	02	Lipocalins
	03	Reproduction
	04	Behaviour
Research areas:	01	Functional genomics
	02	Proteomics
	03	Behaviour
	04	Evolution
Main objectives:	01	To reconstruct lipocalin evolution with the use of the interbreed comparison method on the genome level and on the level of expression profiling
	02	to create a systematic biological model of the function of the individual lipocalins on the level of biological processes (e.g. hormone transport, spermatogenesis, reproduction, pheromonal communication)
	03	To clarify the mechanism of gene duplication within the individual gene clusters of lipocalins on the molecular level

Laboratory of Cancer Biology
Mgr. Lukáš Čermák, Ph.D., Head of the Lab.

The Institute of Molecular Genetics of the
Czech Academy of Sciences

Content of the Research

Our laboratory was established in 2018. We focus on elucidating the role of proteasome-dependent protein degradation in various intracellular processes, especially within the context of cancer progression.

Protein degradation via the proteasome-ubiquitin system (UPS) plays a crucial role in cellular homeostasis. UPS is involved in the cell cycle, differentiation, or stress, and immune response. The ubiquitination process is achieved by triggering an enzymatic cascade. The ubiquitin moiety is activated by covalent linkage to E1 – the ubiquitin-activating enzyme and transferred to E2 – the ubiquitin-conjugating enzyme. Under physiologic conditions, the UPS is required for precise temporal and spatial expression of a diverse protein repertoire. Defects in this system are often associated with pathologic states such as cancer or developmental abnormalities. E3-ubiquitin ligases are responsible for substrate recognition and subsequent degradation. Despite this fact, many are "orphans" as they have not been paired with any specific substrate yet. In our projects, we focus on Cullin-dependent ubiquitin ligases.

To discover novel substrates of these multisubunit enzymes, we perform state-of-art affinity purification of protein complexes associated with these enzymes. Detailed biochemical analysis of the interaction between potential substrates and ubiquitin ligases reveals novel mechanisms of substrate recognition and signaling pathways involved in cellular growth, survival, and stress response. Besides the cancer cell line environment, we aspire to confirm the novel roles of selected ubiquitin ligases in a physiologic context.

Potential for Cooperation

In collaboration with the Czech Center for Phenogenomics (CCP), we are developing mouse models of their deficiency and dysregulation. Our group has previously cooperated with groups within the Functional Genomics Programme headed by Drs. R. Sedláček and T. Stopka and IMG groups led by Dr. V. Kořínek and Dr. Filipp. We have collaborated with external foreign partners, including Prof. Olle Sangfelt from Karolinska Institute and Prof. Michele Pagano from NYU in New York, USA

2019 Ubiquitin Ligases Involved in the Regulation of Wnt, TGF-β, and Notch Signaling Pathways and Their Roles in Mouse Development and Homeostasis. Baloghova N, Lidak T, Cermak L. Genes. 2019 Oct 16;10(10):815

2014 Horn M, Geisen C, Cermak L, Becker B, Nakamura S, Klein C, Pagano M, Antebi A. DRE-1/FBXO11-dependent degradation of BLMP-1/BLIMP-1 governs C. elegans developmental timing and maturation. Developmental Cell. 2014 Mar 31;28(6):697-710

2012 FBXO11 targets BCL6 for degradation and are inactivated in diffuse large B-cell lymphomas. Duan S, Cermak L, Pagan JK, Rossi M, Martinengo C, di Celle PF, Chapuy B, Shipp M, Chiarle R, Pagano M. Nature. 2012 Jan 5

2011 Duan S*, Cermak L*, Pagan JK, Rossi M, Martinengo C, di Celle PF, Chapuy B, Shipp M, Chiarle R, Pagano M. FBXO11 targets BCL6 for degradation and is inactivated in diffuse large B-cell lymphomas. Nature. 2011 Nov 23

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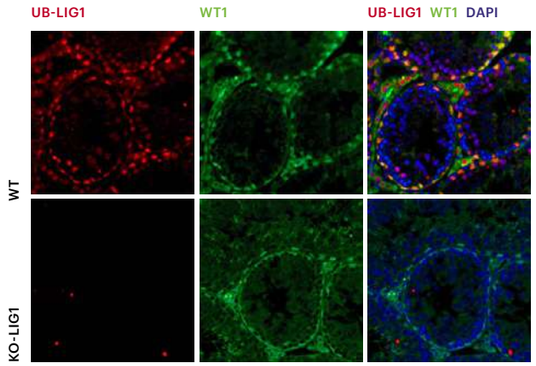
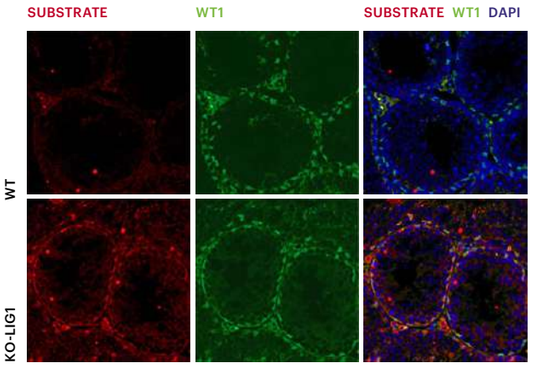
	e-mail: lukas.cermak@img.cas.cz

Basic keywords:	01	Proteasome
	02	Ubiquitin
	03	Cancer
	04	Stress
	05	Development

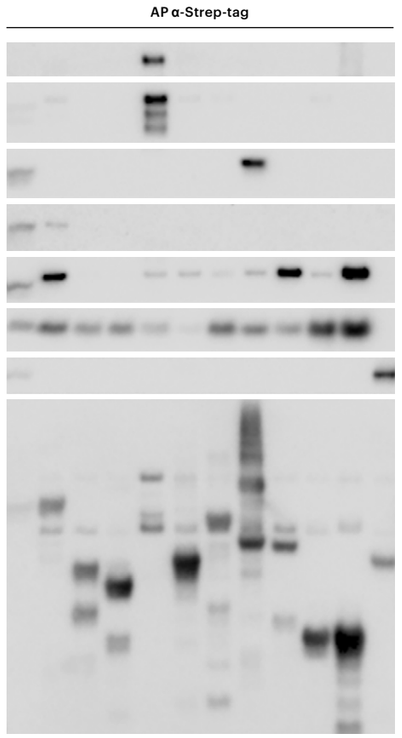
Research areas:	01	Ubiquitin ligases
	02	Protein stability
	03	Stress response
	04	Cancer biology
	05	Mouse models
Research areas:	01	Generation of novel transgenic mouse strains expressing DNA recombinases in cells of the gastrointestinal tissues
	02	Identification and characterization of genes that encode tumor suppressors or oncogenes responsible for initiation or progression of gastrointestinal neoplasia
	03	Generation and analysis of mice harboring conditional alleles of the selected gene(s)
	04	Alternatively, generation and analysis of mice harboring an activated oncogene in selected tissues.



A: Immunofluorescence images from testicular tubules of mouse model deficient in ubiquitin ligase (LIG1).

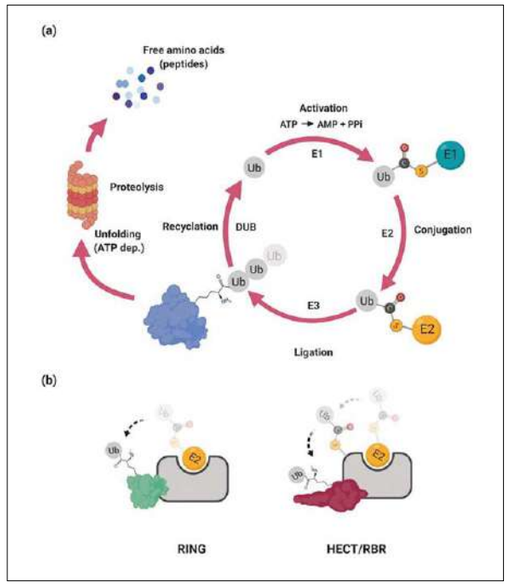


B: The potential substrate is stabilized in the epithelium of testicular tubules from KO mice.



(HEK293T cells)

Affinity purification of various Strep-FLAG-tagged F-box proteins from HEK293T cells.



The ubiquitin–proteasome system. (a) The mature free ubiquitin monomer protein is either recycled from the ubiquitinated substrate or cleaved from the polyubiquitin precursor. Both of these reactions are catalyzed by deubiquitinases (DUBs). Ubiquitin is then activated (E1), conjugated (E2), and finally ligated to the cognate substrate via ubiquitin ligases (E3). The polyubiquitinated substrate is later transferred to the proteasome, unfolded, and proteolytically degraded to small peptides or free amino acids. For more details see the text. (b) RING E3s catalyze the direct transfer of ubiquitin from E2-ubiquitin to the substrate. HECT (homologous to E6AP C-terminus), and RBR (RING-between-RING) E3s accept ubiquitin from E2 to form an E3-ubiquitin thioester intermediate via transthiolation reaction. For more details see the text.

Molecular Mechanisms of Germ Cell Development
Ing. Zdeněk Trachtulec, Ph.D., Head of the Lab.

The Institute of Molecular Genetics of the
Czech Academy of Sciences

Content of the Research

Genetic recombination is the quintessence of gametogenesis; it ensures not just the reshuffling of parental alleles and thus higher variability among the offspring, but first of all the proper segregation of chromosomes during meiotic cell divisions and thereby fertility. The sites of recombination are determined in many mammals by the PRDM9 (PR/SET-domain carrying 9) protein, an epigenetic factor that carries histone-3-lysine-4-methyltransferase and DNA-binding activities. Our earlier research focused on the PRDM9 protein, which is essential for fertility in laboratory mice, but no effect has been found in dogs. It has been found that some, but not all mice heterozygous for certain *Prdm9* mutations display sex-specific sterility, however it is unknown whether the difference in fertility is caused by the variation in *Prdm9* mutations or in the genetic background. We participated in the production of *Prdm9* mutants harboring deletions in one of the exons encoding the catalytic PR/SET and were able to analyze the precise genomic distribution of recombination sites along with the fertility of heterozygous and homozygous animals on precisely defined genetic backgrounds.

The current focus of our research has changed, as the fertility studies in model mammals have moved from the effects of a single gene to genetic interactions important for human reproductive medicine that occur during spermatogenesis and oogenesis. The *Prdm9* gene (also called *Meisetz*) is necessary for both male and female meiosis and fertility in the classical laboratory mouse. The biochemical function of the PRDM9 protein is to methylate histones. The mouse and human PRDM9 proteins specify the sites of meiotic recombination. However, PRDM9 is dispensable for fertility in dogs. PRDM9 polymorphisms have been revealed in sterile human patients and PRDM9 contribute to the instability of the human genome. We have identified *Prdm9* as the first vertebrate hybrid sterility gene. Different *Prdm9* mutations display different stages and degrees of spermatogenic arrest on various backgrounds, indicating that the resulting phenotype is dependent on genetic interactions of *Prdm9*.

Potential for Cooperation

We offer our expertise in special fertility phenotyping methods, such as the preparation and immunostaining of meiotic cells and testicular sections. In addition, we also offer access to various unique mouse fertility models affected by the *Prdm9* gene, including some of which are sensitized and thus able to enhance the phenotyping of other fertility genes and loci.

2020 Mihola O, Kobets T, Krivankova K, Linhartova E, Gasic S, Schimenti JC, Trachtulec Z: Copy-number variation introduced by long transgenes compromises mouse male fertility independently of pachytene checkpoints. Chromosoma 2020 129: 69–82.

2020 Kusari F, Mihola O, Schimenti JC, Trachtulec Z: The Meiotic Epigenetic Factor PRDM9 Impacts Sperm Quality of Hybrid Mice. Reproduction 2020 160:53-64.

2019 Mihola O, Pratto F, Brick K, Linhartova E, Kobets T, Flachs P, Baker CL, Sedlacek R, Paigen K, Petkov PM, Camerini-Otero RD, Trachtulec Z: Histone methyltransferase PRDM9 is not essential for meiosis in male mice. Genome Research 2019 29:1078-1086

2015 Baker CL, Petkova P, Walker M, Flachs P, Mihola O, Trachtulec Z, Petkov PM, Paigen K: Multimer Formation Explains Allelic Suppression of *Prdm9* Recombination Hotspots. PLoS Genetics 2015 11: e1005512.

2014 Flachs P, Bhattacharyya T, Mihola O, Piálek J, Forejt J, Trachtulec Z: *Prdm9* incompatibility controls oligospermia and delayed fertility but no selfish transmission in mouse intersubspecific hybrids. PLoS One 2014 9: e95806.

2012 Flachs P, Mihola O, Simecek P, Gregorova S, Schimenti JC, Matsui Y, Baudat F, de Massy B, Pialek J, Forejt J, Trachtulec Z: Interallelic and Intergenic Incompatibilities of the *Prdm9* (Hst1) Gene in Mouse Hybrid Sterility. PLoS Genetics 2012 8: e1003044.

2012 Forejt J, Pialek J, Trachtulec Z: Hybrid male sterility genes in the mouse subspecific crosses. In: Evolution of the House Mouse. Cambridge University Press 2012, 482-503.

2009 Mihola O, Trachtulec Z, Vlcek C, Schimenti JC, Forejt J: A mouse speciation gene encodes a meiotic histone H3 methyltransferase. Science 2009 323: 373-5.

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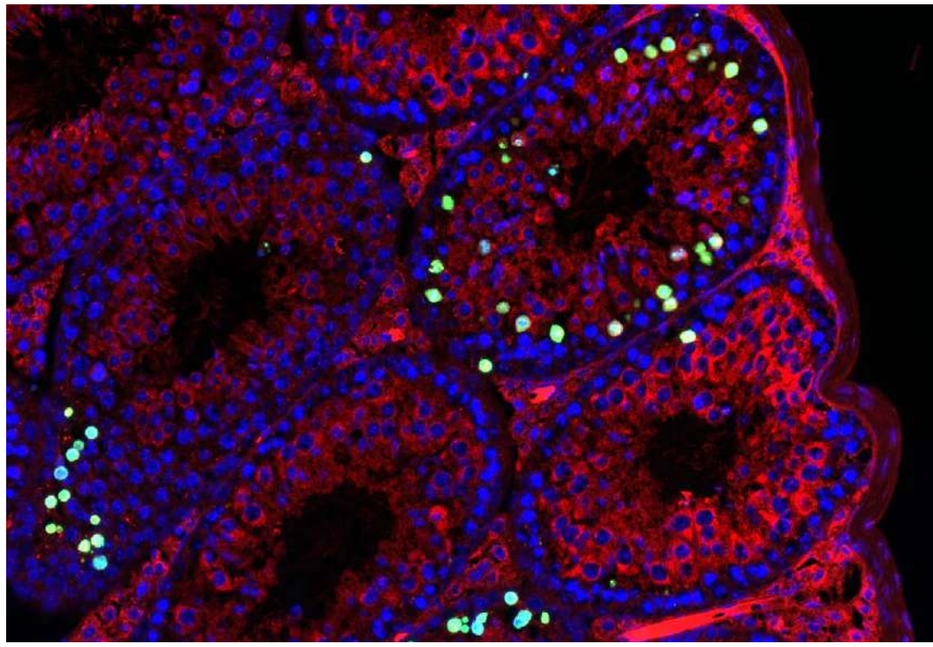
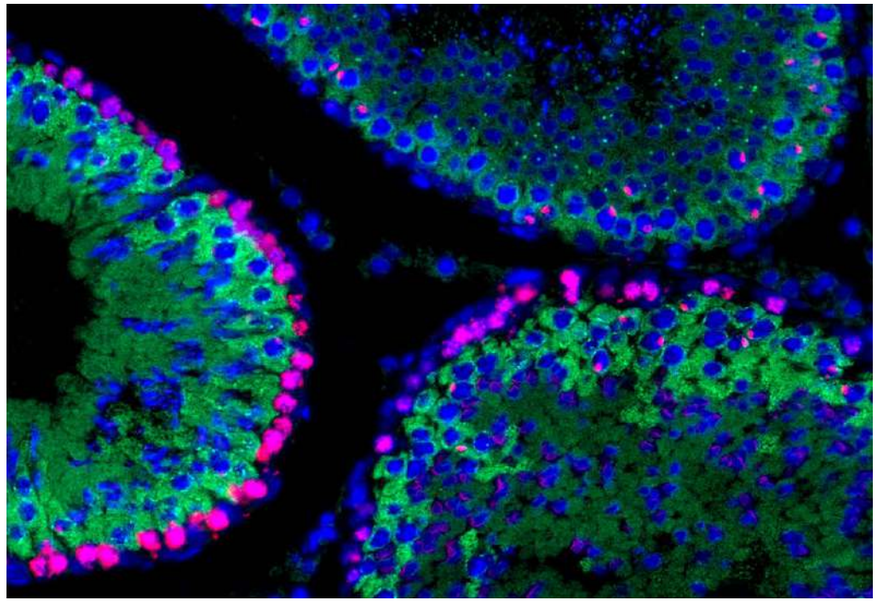
	e-mail: trachtul@img.cas.cz

Basic keywords:	01	spermatogenesis
	02	Meiosis
	03	*Prdm9*
	04	Epigenomics
	05	Oogenesis
	06	Mouse
	07	Rat

Research areas:	01	Mouse genetics
	02	Rat genetics
	03	Fertility
	04	Meiosis
Main objectives:	01	Analyses of genes regulating germ cell development in mouse and rat testes and ovaries, interspecific differences important for translation studies
	02	Analyses of interactions and incompatibilities of genes expressed in testes and ovaries
	03	Analyses of models of germ cell development defects that are affected by the *Prdm9* gene, including complete meiotic arrest (azoospermia), limited fertility (reduced sperm count - oligospermia), sperm head malformations (teratozoospermia), and reproductive age defects (time-dependent arrest of germ cell development)



A cross-section of adult mouse testes. The cytoplasm of developing germ cells (pachytene spermatocytes and round spermatids) is immunolabeled with the antibody against PIWIL1 ●. DNA breaks of apoptotic cells are visualized by the TUNEL staining (●●●●). All cell nuclei are in blue (DAPI labelling).



A section of adult rat testes immunostained using antibodies against γH2AX (● marking chromatin surrounding DNA breaks under repair) and against PIWIL1 ●. All cell nuclei are stained with DAPI ●.

Mechanisms Involved in the Remodeling of the Chromatin Structure During Cell Fate Decisions

Prof. MUDr. Tomáš Stopka, Ph.D., Head of the Lab.

First Faculty of Medicine,
Charles University

Content of the Research

The focus of StopkaLab is conducting research on hematopoietic differentiation and blood lineage determination in both normal and pathologic states such as myelodysplastic syndrome (MDS) and acute leukemia (AML). These stem cell disorders are currently incurable and their pathobiology is not understood. By using primary patient samples and transgenic mouse models, we have found that genetic and epigenetic mechanisms work together during normal processes of blood development as well as upon clonal selection of malignant cells. Our primary goal is to provide knowledge of blood cell disorders. Specifically, how gene transcription is regulated during blood cell development and during the state when most of these processes are blocked during leukemogenesis. Individual research projects specialize on a particular role of key lineage determinants. Among the chromatin-remodeling proteins, we focus on the role of ATPase ISWI homologue Smarca5, involved in chromatin assembly as well as its transcription-dependent sliding of hematopoietic stem cells that undergo rapid genetic changes in order to commit into an avalanche of steps forming a mass of specialized blood elements.

Mechanisms which guide lineage predetermination at the level of hematopoietic stem and progenitor cells are currently unknown. We utilize a vast number of genetic models of the key lineage transcription factors GATA-1 and PU.1, whose gene expression is required for both installation of a particular genetic programme as well as removal of the alternative lineage programmes in a particular fate-decision step. Once lineage commitment occurs at an early progenitor level, the cell enters coordinated steps of cell differentiation and the process can be blocked upon somatic mutations observed in MDS or AML patients. We apply genetic mouse models and modern global technologies such as next generation sequencing to determine and predict genetic associations between hematologic diseases and genomic data in order to develop and adopt biomarkers useful in hematology and oncology.

Potential for Cooperation

We participate in research projects to improve our understanding of how to unblock the leukemic progenitors from the leukemic state and how to restore their differentiation; one example of this can be found in our collaboration with the Albert Einstein College of Medicine, NY. Additionally we aim to coordinate our activities with the American Society of Hematology and the MDS Foundation. We are recruiting and training talented students who are in Diploma and Dissertation Theses programs.

2020 Zikmund T, Paszekova H, Kokavec J, Kerbs P, Thakur S, Turkova T, Tauchmanova P, Greif PA, Stopka T. Loss of ISWI ATPase SMARCA5 (SNF2H) in Acute Myeloid Leukemia Cells Inhibits Proliferation and Chromatid Cohesion. Int J Mol Sci. 2020 Mar 18;21(6):2073.

2019 Zikmund T, Kokavec J, Turkova T, Savvulidi F, Paszekova H, Vodenkova S, Sedlacek R, Skoultchi AI, Stopka T. ISWI ATPase Smarca5 Regulates Differentiation of Thy-mocytes Undergoing β-Selection. J Immu-nol. 2019 Jun 15;202(12):3434-3446.

2017 Vargova K, Pesta M, Obrtlikova P, Dusilkova N, Minarik L, Vargova J, Berkova A, Zemanova Z, Michalova K, Spacek M, Trneny M, Stopka T. MiR-155/miR-150 network regulates progression through the disease phases of chronic lymphocytic leukemia. Blood Cancer J. 2017 Jul 21;7(7):e585.

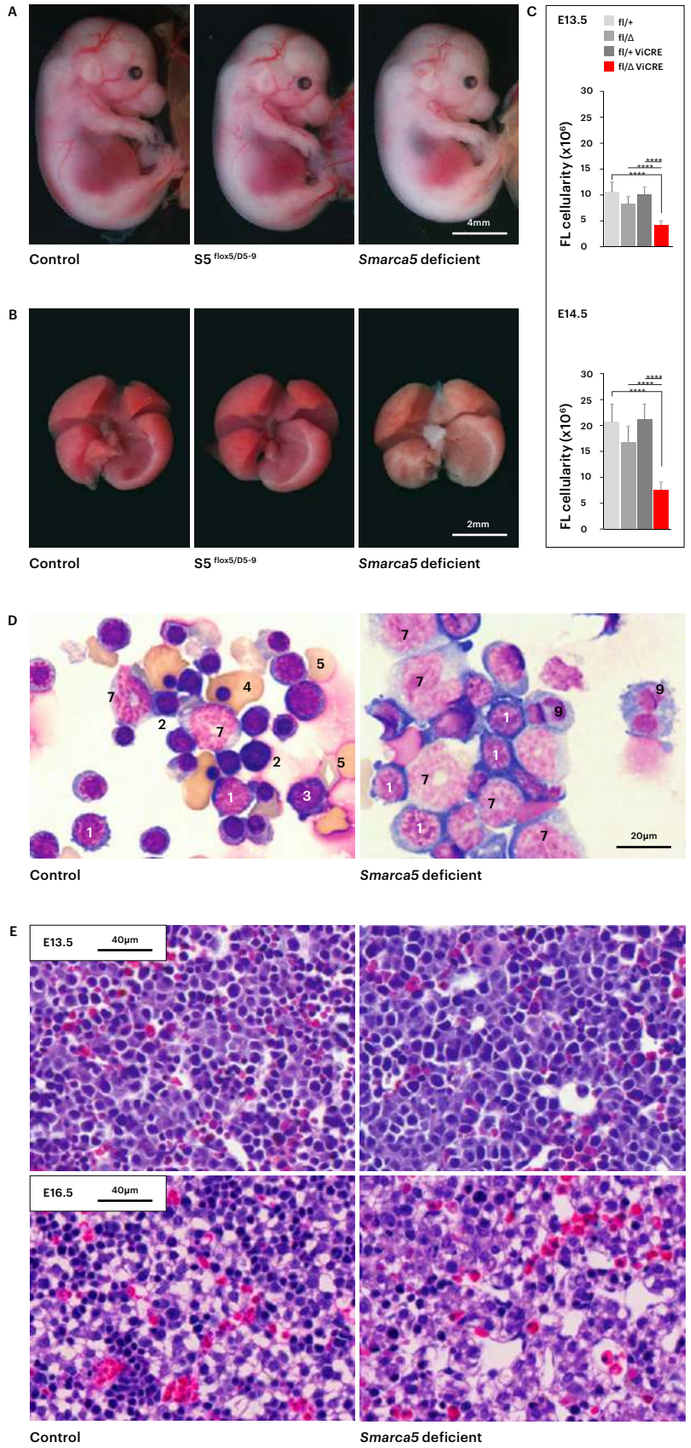
2017 Kokavec J, Zikmund T, Savvulidi F, Kulvait V, Edelmann W, Skoultchi AI, Stopka T. The ISWI ATPase Smarca5 (Snf2h) Is Required for Proliferation and Differentiation of He-matopoietic Stem and Progenitor Cells. Stem Cells. 2017 Jun;35(6):1614-1623.

2016 Vargova J, Vargova K, Dusilkova N, Kulvait V, Pospisil V, Zavadil J, Trneny M, Klener P, Stopka T. Differential expression, local-ization and activity of MARCKS between mantle cell lymphoma and chronic lym-phocytic leukemia. Blood Cancer J. 2016 Sep 23;6(9):e475.

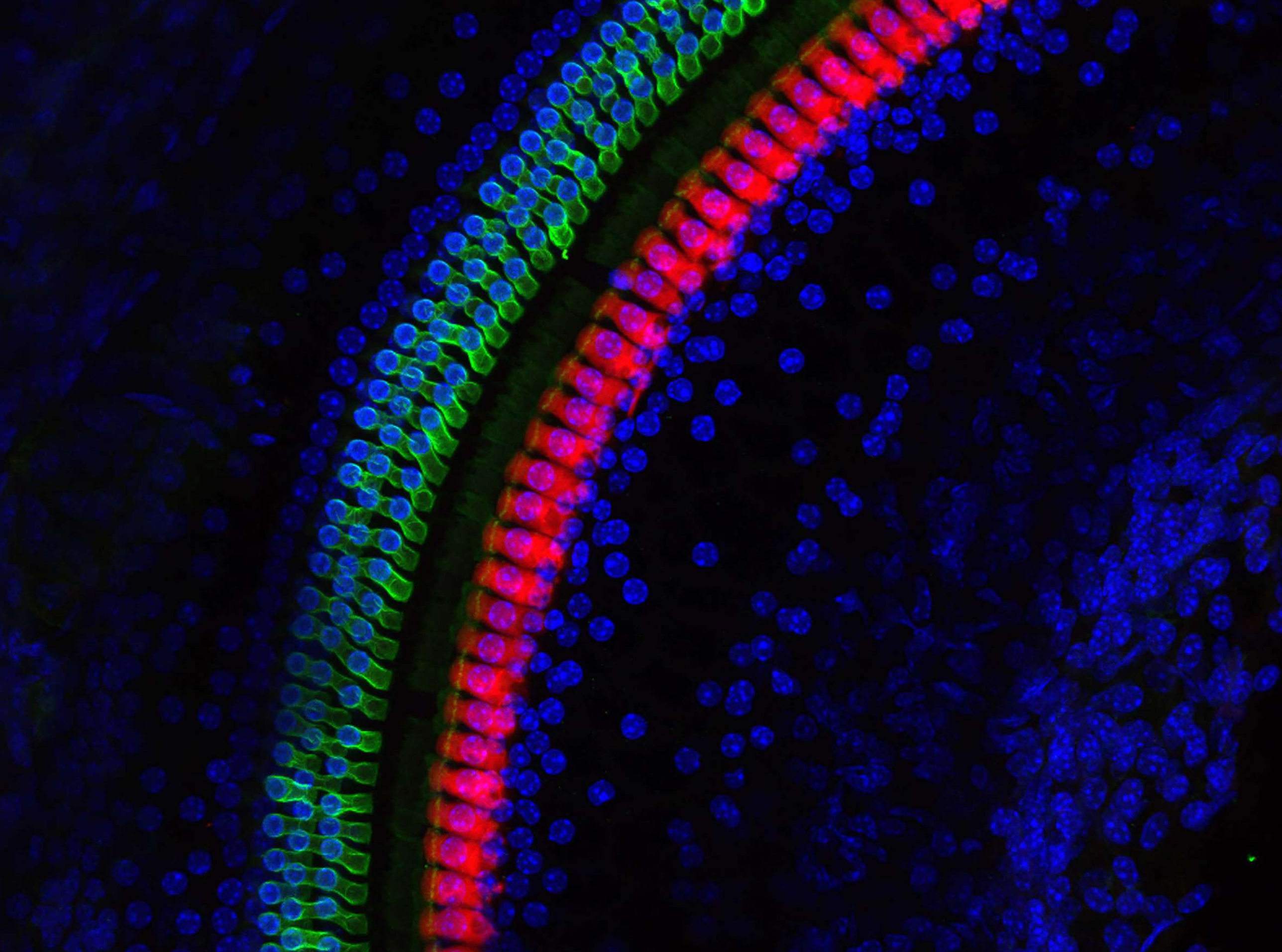
Contact person:	Prof. MUDr. Tomáš Stopka, Ph.D.
	e-mail: tomas.stopka@lf1.cuni.cz

Basic keywords:	01	Hematopoiesis
	02	Leukemia
	03	Differentiation
	04	Pu.1
	05	Gata-1
	06	Smarca5
	07	Chromatin remodeling
	08	Microrna

Research areas:	01	The pathophysiology of leukemias (molecular hematology)
	02	Stem cell biology of myelodysplasia
	03	Lineage specific transcription factor biology
	04	Chromatin biology of lineage determination
Main objectives:	01	To determine chromatin remodeling mechanisms during hematopoietic development
	02	To elucidate the role of ISWI factors in hematopoiesis using conditional genetic models in mouse
	03	To elucidate epigenetic mechanisms during leukemogenesis utilizing mouse models of AML
	04	To advance our understanding of genetic and epigenetic interactions in human leukemias



Smarca5 deletion (Vav1-iCre) results in anemia. (A): Phenotypic appearance of control Smarca5fl8x5/1 (left), heterozygous Smarca5fl8x5/D529 (middle), and Smarca5 deficient Smarca5fl8x5/D5-9 Vav1-iCre (right) sibling embryos at embryonic day (E)15.5. (B): E15.5 fetal livers (FLs) of the same genotypes. (C): FL cellularity (E13.5 upper, E14.5 lower) of control Smarca5fl8x5/1, Smarca5fl8x5/D529, and mutant Smarca5fl8x5/D5-9 Vav1-iCre embryos. (D): Cytology (May-Gr€unwald-Giemsa) of E14.5 FL and (E) histology (H&E) of E13.5 (top) and E16.5 FLs (bottom). (F): Cytology (May–Gr€unwald–Giemsa) of E14.5 peripheral blood. Cell subtypes: (1) proerythroblast, (2) basophilic normoblast, (3) polychromatic n., (4) orthochromatic n., (5) reticulocyte, (6) erythrocyte, (7) myeloid precursor, (8) embryonic erythrocyte, and (9) atypical double-nucleated cell. Similar results were obtained in at least six repeat experiments. Two-tailed Student's t test (****, p<.00001). Abbreviation: E13.5/14.5, embryonic day 13.5/14.5.



Cellular Biology and Virology

No. of research teams: 20

Pages: 56–101

02

Cellular Biology and Virology

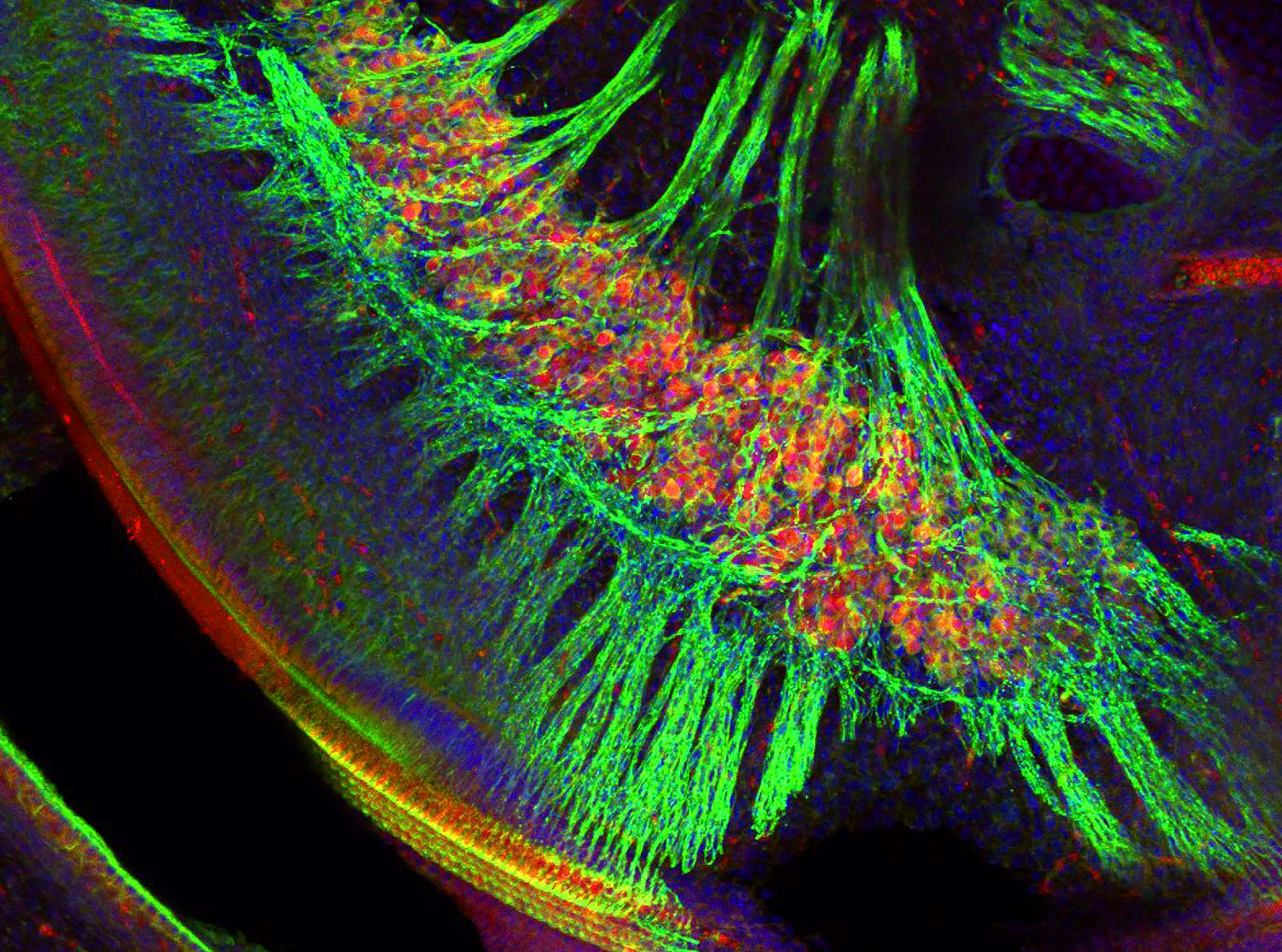
Prof. RNDr. Jan Tachezy, Ph.D.
Vice-Chairman of the BIOCEV Board, Head of the Research Programme
Faculty of Science, Charles University

This programme includes four synergic and mutually complementing sub-programmes: Eukaryotic Microbiology, the Biology of Cancer Cells, Virology and the Structure and Differentiation of Mammalian Cells. These sub-programmes cover a large spectrum of eukaryotic cells ranging from unicellular pathogens (parasitic protists) and unicellular eukaryotes forming multi-cellular assemblies (yeasts) to mammalian cells and tissues, as well as interactions between eukaryotic cellular structures and simple intracellular parasites – viruses. The project involves the establishment of laboratories for studying eukaryotic pathogens, viruses and tumour cells using the top imaging technology of the future centre of European infrastructure Euro-BioImaging. In addition to scientific goals, the accreditation of new doctoral programmes in the fields of Eukaryotic Microbiology and Biomedicine will be prepared.

Cellular biology research represents a leading discipline of modern science and as such it has great potential for innovation in biomedicine and biotechnology. The programme will include a study of the association of tumour diseases with viral infections, molecular mechanisms of microorganism interactions and pathogen-host signalling and potential for gene therapy and vaccination, namely against viral infections. Expected application outputs include antiparasitic, antimycotic and antitumour agents, antiviral vaccines, novel treatment approaches and biomodulators.

Research Directions — Cellular Biology and Virology

1.	Identification of unique cellular functions related to the pathogenesis and parasitic way of life of selected parasitic protists and a comparison with their free-living relatives	2.	Identification of unique cellular functions related to multicellular development in the pathogenic and nonpathogenic yeasts, their interaction with the host and the functional characterization of specific metabolites and signalling molecules	3.	Discovery of new molecules that will be applicable as cell biology tools with a possible pharmacological potential
4.	Understanding the mechanisms of the invasiveness of cancer cells and the characterization of membrane proteases in relation to oncogenesis	5.	Understanding the interactions between viruses and cellular structures during viral infections, the mechanisms of stress and defence responses, the mechanisms of deregulation of cellular processes including malignant transformation	6.	Development of effective carriers for the introduction of heterologous DNA into target cells
7.	Development of preventive antiviral and anticancer vaccines	8.	Identification of the structural elements of the cell nucleus and the function of these elements during the regulation of gene expression	9.	Understanding of the mitochondrial structure in relation to its specific gene expression
10.	Characterization of selected receptors across eukaryotic organisms				



Biogenesis and Functions of Cellular Organelles in Pathogenic Protists

Prof. RNDr. Jan Tachezy, Ph.D., Head of the Lab.

Faculty of Science,
Charles University

Content of the Research

Our research group is interested in parasitic and free living anaerobic protists. We study the evolution, biogenesis and the functioning of their unusual organelles, particularly mitochondria and peroxisomes. The specific character of these organelles reflects (i) adaptation of protists for their life in oxygen-poor environments, and (ii) their specific adaptation to parasitism. Our discovery of the FeS cluster assembly machinery (ISC) of a mitochondrial type in the hydrogenosomes of *Trichomonas vaginalis* and the mitosomes of *Giardia intestinalis* provided strong arguments for the mitochondrial origin of these organelles. Today, ISC components are widely used as markers for tracing various mitochondria-related organelles in protists. We further supported the mitochondrial origin of hydrogenosomes by indentifying the remnant respiratory complex I and characterization of protein import machinery in hydrogenosomes. Using high resolution electron microscopy, we demonstrated that the structure of the hydrogenosomal protein translocase TOM is similar to mitochondrial counterparts and we proposed that such a structure was likely present in a common ancestor to all eukaryotes.

Perioxsomes are known as organelles that compartmentalize pathways producing toxic products, typically hydrogen peroxide, and also enzymes that catalyze converting hydrogen peroxide into non-toxic water and oxygen. It is generally believed that peroxisomes are absent in anaerobes. We have recently discovered a new type of perioxisome that is present in hydrogenosome-bearing anaerobic free-living *Mastigamoeba balamuthi* and parasitic *Entamoeba histolytica* that lack catalase but can metabolize *myo*-inositol. These unique organelles may represent a suitable target for the development of new antiparasitic drugs.

The third direction of our research is investigating the virulence factors of *Trichomonas vaginalis* that are critical for the establishment of infection and disease development. We are focused on analyzing *T. vaginalis* exosomes and their role in interacting with the host cells and the role played by the dsRNA virus in *T. vaginalis* virulence.

Potential for Cooperation

We collaborate with several international counterparts in the fields of genomics, proteomics, bioinformatics and advanced bioimaging with a specific focus on parasitic protists. Withing the framework of the MiCoBion project under H2020 we collaborate with Catholic University of Leuven, European Molecular Biology Laboratory in Heidelberg and Institute Jacques Monod - University de Paris in France.

2021 Rout S., Oeljeklaus S., Makki A., Tachezy J., Warscheid B. and Schneider B. 2021. Determinism and contingencies shaped the evolution of mitochondrial protein import receptors. Proc Natl Acad Sci U S A. 118:e2017774118. doi: 10.1073/pnas.2017774118. PMID: 33526678.

2020 Le T., Žárský V., Nývltová E., Rada P., Harant K., Vancova M., Verner Z., Hrdý I., Tachezy J. 2020. Ananerobic peroxisomes in *Mastigamoeba balamuthi*. Proc Natl Acad Sci U S A. 117:2065-2075. doi: 10.1073/pnas.1909755117. Epub 2020 Jan 13.

2021 Žárský V., Klimeš V., Pačes J., Vlček Č, Hradilová M., Beneš V., Nývltová E., Hrdý I., Pyrih J, Mach J., Barlow L., Stairs C.W., Eme L., Hall N., Eliáš M., Dacks BJ, Roger A., Tachezy J. 2021. The *Mastigamoeba balamuthi* genome and the nature of the free-living ancestor of *Entamoeba*. Mol Biol Evol doi: 10.1093/molbev/msab020. Online ahead of print. PMID: 33528570

2019 Makki A., Rada P, Žárský V., Kereiche S., Kovacik L., Novotny M., Jores T., Rapaport D., Tachezy J. 2019. Triplet-pore structure of a highly divergent TOM complex of hydrogenosomes in *Trichomonas vaginalis*. PLoS Biology. 17(1):e3000098. doi: 10.1371/journal.pbio.3000098.

2015 Vojtěch Žárský, Jan Tachezy, 2015. Evolutionary loss of peroxisomes – not limited to parasites, Biol Direct. 10:74. doi: 10.1186/s13062-015-0101-6.

2015 Nývltová E., Stairs C.W., Hrdý I., Rídl J.,Mach J., Pačes J., Roger A.J., Tachezy J. 2015. Lateral gene transfer and gene duplication played a key role in the evolution of *Mastigamoeba balamuthi* hydrogenosomes. Mol Biol Evol 32:1039-55. doi: 10.1093/molbev/msu408. Epub 2015 Jan 7. PMID: 25573905

2013 Nývltová E., Šuták R, Šedinová M., Harant K, Hrdý I., Pačes, J., Vlček, Č., Tachezy, J. 2013. NIF-type iron-sulfur cluster assembly system is duplicated and distributed in the mitochondria and cytosol of *Mastigamoeba balamuthi*. Proc Natl Acad Sci U S A, 110: 7371-6. doi: 10.1073/pnas.1219590110. Epub 2013.

2004 Hrdý I., Hirt R., Doležal P., Bardoňová L.,Tachezy J., Embley M. 2004. Trichomonas hydrogenosomes contain the NADH dehydrogenase module of mitochondrial complex I. Nature, 432: 618-622.

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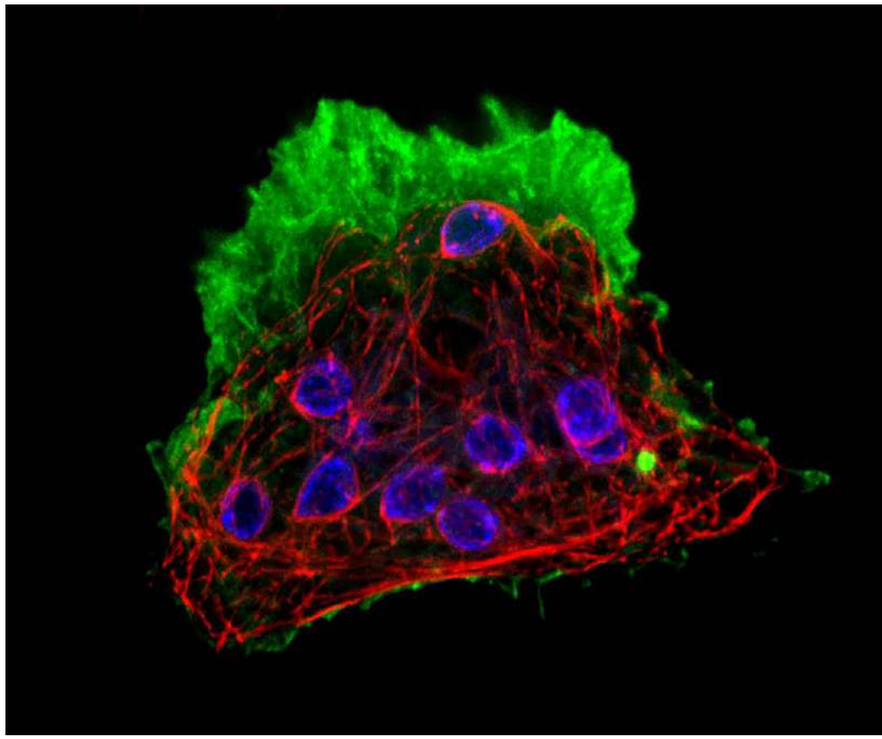
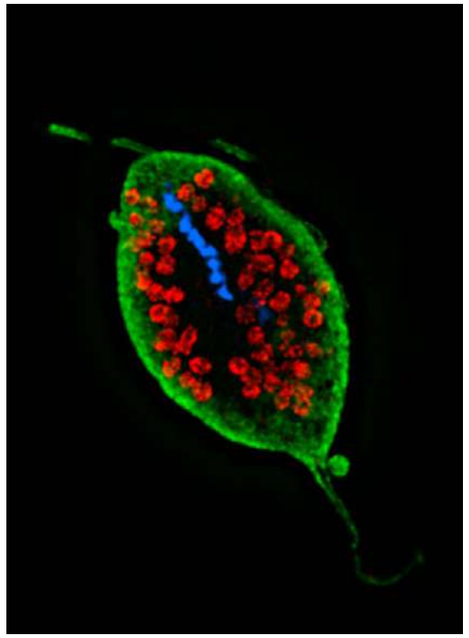
	e-mail: jan.tachezy@natur.cuni.cz

Basic keywords:	01	Parasite
	02	Protists
	03	Mitochondria
	04	Hydrogenosome
	05	Mitosome
	06	Trichomonas vaginalis
	07	Giardia intestinalis
	08	Entamoeba histolytica
	09	Mastigamoeba balamuthi
	10	FeS assembly
	11	dsRNA virus
	12	Virulence
	12	Exosome

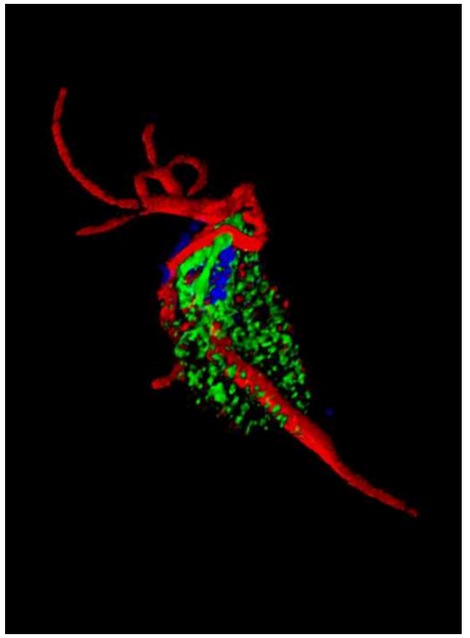
Research areas:	01	Parasitology
	02	Molecular biology and biochemistry of pathogenic protists
	03	Cell biology
Main objectives:	01	Factors affecting the pathogenicity and virulence of parasitic protists
	02	Functions of anaerobic mitochondria and peroxisomes
	03	Adaptation to anaerobiosis and the evolution of parasitism



Free living protists Mastigamoeba balamuthi (tubulin ●, actin ●, nuclei ●).



Human parasite Trichomonas vaginalis with labelled hydrogenosomes (malic enyme ●, membrane C-tail anchored protein ●, nucleus ●).



Human parasitic protist *Trichomonas vaginalis* with labelled cytoskeleton (●) and secretory pathway (●).

Mechanisms for Transporting Proteins through Mitochondrial and Bacterial Membranes

Mgr. Pavel Doležal, Ph.D., Head of the Lab.

Faculty of Science,
Charles University

Content of the Research

Our lab studies mitochondrial biogenesis and protein transport across biological membranes. We have three areas of interest. Firstly, we are interested in the extremely reduced mitochondrial forms known as mitosomes and hydrogenosomes. All eukaryotic organisms have mitochondria. The simplest forms of mitochondria are called mitosomes, miniature organelles containing a handful of proteins, which remotely resemble the complex threadlike mitochondria of animals and fungi (see the figure). We are interested in the biogenesis, the inheritance and the function of mitosomes in the human pathogen Giardia intestinalis. The second area of interest involves protein transport across mitochondrial and bacterial membranes. Proteins pass membranes via specialized protein translocases or using membrane fusions. Different cellular membranes contain distinct molecular machines that must specifically recognize and transport individual proteins. We are interested in mitochondrial protein import and also in the pathogen's strategies in diverting these pathways in the host cell.

The last topic is to understand the events that enable the transformation of the bacterial endosymbiont into mitochondria. Mitochondria developed from endosymbiotic bacteria through a gradual transformation. As mitochondrial genes had been transferred to the host cell nucleus, the proteins synthesized on the cytosolic ribosomes needed to pass both mitochondrial membranes. Specialized molecular machines were installed into the mitochondrial membranes to allow for substrate specific protein transport. Using the comparative bioinformatics combination with functional analyses, we endeavor to understand the early events that led to the creation and the evolution of mitochondria.

Potential for Cooperation

Our experimental approaches include a broad range of cell biology and biochemistry techniques. We are introducing new molecular tools for experimental work with poorly studied organisms such as the unicellular human pathogen Giardia intestinalis and the free living Naegleria gruberi. We have previously collaborated with the University of Birmingham, Alabama, USA and Jacques Monod Institute investigating the Trichomonas vaginalis hydrogenosome proteome. Our work is largely dependent on the use of heterologous and in vitro expression systems, as it allows us to study the protein of interest down to molecular details. We rely on and welcome collaboration with specialists in membrane protein biology and advanced fluorescence microscopy.

2019 Voleman L, Doležal P. Mitochondrial dynamics in parasitic protists. PLoS Pathog. 2019
2015 Martincová E, Voleman L, Pyrih J, Žárský V, Vondráčková P, Kolísko M, Tachezy J, Doležal P. Probing the Biology of Giardia intestinalis Mitosomes Using In Vivo Enzymatic Tagging. Mol Cell Biol. 2015
2006 Dolezal P, Likic V, Tachezy J, Lithgow T. Evolution of the molecular machines for protein import into mitochondria. Science. 2006

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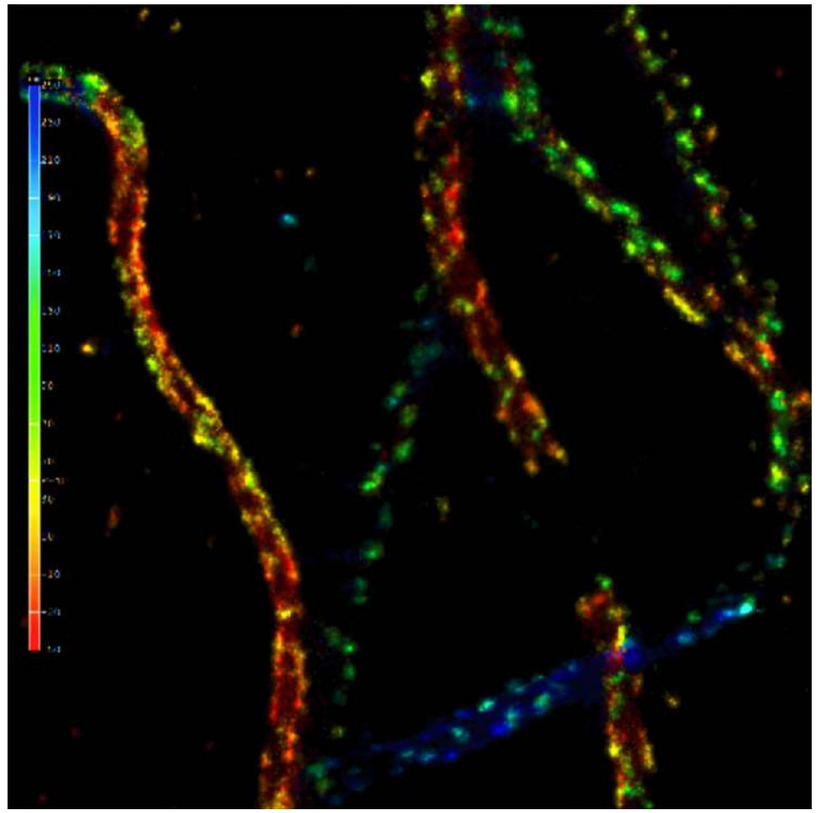
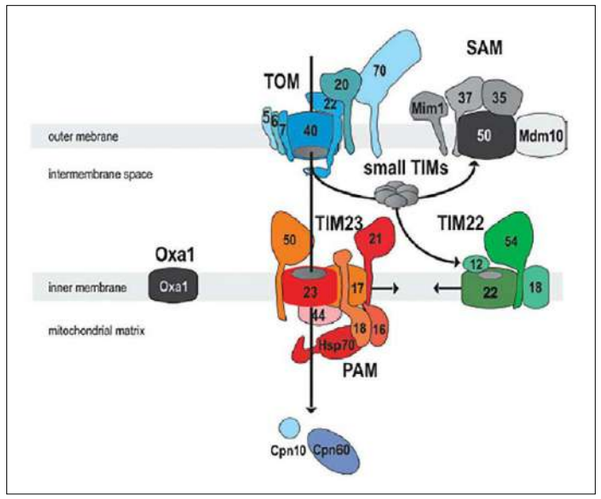
e-mail: pavel.dolezal@natur.cuni.cz

Basic keywords:	01	Mitochondria
	02	Evolution
	03	Protein transport
	04	Organelle dynamics
	05	Endoplasmic reticulum
	06	Mitosomes
	07	Microsporidia

Research areas:	01	Protein transport
	02	Mitochondrial biogenesis
	03	Iron-sulfur cluster formation
	04	Invasion apparatus of Microsporidia
	05	Encystation of Giardia intestinalis
Main objectives:	01	To characterize the protein transport pathways into highly reduced mitochondria of anaerobic eukaryotes
	02	To describe the transformation steps of the bacterial endosymbiont into the current mitochondria
	03	To characterize the structure and function of the invasion apparatus of Microsporidia
	04	To characterize the formation of infectious cysts by Giardia intestinalis



STORM imaging of the invasion apparatus of microsporidian parasites. Through these tubes parasites directly invade the cytosol of the host cell. We study the ejection, composition and the structure of these biological nanotubes.



Schematic representation of the protein import pathways into mitochondria. There are four core complexes in the two mitochondrial membranes that transport proteins into mitochondrial subcompartments. We study the function of these complexes and how they evolved from the bacterial protein transport machines.

The Uptake and Intracellular Metabolism of Metals

RNDr. Róbert Šuťák, Ph.D., Head of the Lab.

Faculty of Science,
Charles University

Content of the Research

The importance of metals for the survival of microorganisms is best demonstrated with iron, a crucial nutrient for all organisms. The oceans' waters are one example of a nutrient-limited environment in which iron is scarce. Although oceanic phytoplankton, similar to other photosynthetic organisms, are highly demanding on iron availability, they contribute to nearly 50% of the primary production. Iron also plays an important role in host-pathogen relationships. The acquisition of iron from the host environment can be especially challenging for parasitic protists that rely solely on the host for their available nutrients. One of the host's defense mechanisms is to starve parasites by keeping the crucial iron in a form unreachable by pathogens. The activation and expression of microbial iron acquisition systems are linked to their pathogenicity and proliferation.

Our project is focused on the characterization of nutrition requirements, the mechanism of the uptake and intracellular metabolism of metals, mainly iron and copper, the identification of the molecules involved in these processes, and the effect of chelators. The research includes metabolism specific for pathogenic organisms (parasitic protists) and strategies necessary for supporting life in a nutritionally limited environment (unicellular algae). Recently, our research has been focused on screening chelators and ionophores as potential chemotherapeutics against a wide range of parasitic protists. The projects involve intensive collaboration with the "Mitochondria, metals and oxidative stress" laboratory at the Institute Jacques Monod in France and the Institute of Biotechnology CAS.

Potential for Cooperation

The laboratory is open for new opportunities to collaborate on research of the metabolism of metals in different organisms, including projects focused on pathological mechanisms of iron/copper-related diseases and the search for antiparasitic chemotherapeutic targets. We can offer expertise in biochemical and molecular biology methods with a focus on detection and functional analysis of metals and metal-containing proteins in a wide variety of unicellular organisms ranging from algae, to parasitic protists and fungi. We can test novel antiparasitic and antimycotic compounds and observe their effect on cells on different levels (mRNA, proteins, metabolic products).

Our project offers students the opportunity to conduct supervised laboratory experiments on the biochemistry of unicellular eukaryotes. Students can join our laboratory in the study of pathogenic protists important in human medicine (trypanosomes, leishmanias, *Naegleria fowleri, Acanthamoeba castellanii, Candida albicans, Cryptococcus neoformans*).

2020 Arbon D, Ženíšková K, Mach J, Grechnikova M, Malych R, Talacko P, Sutak R. Adaptive iron utilization compensates for the lack of an inducible uptake system in Naegleria fowleri and represents a potential target for therapeutic intervention. PLoS Negl Trop Dis. 2020

2018 Mach J, Bíla J, Ženíšková K, Arbon D, Malych R, Glavanakovová M, Nývltová E, Sutak R. Iron economy in Naegleria gruberi reflects its metabolic flexibility. Int J Parasitol. 2018

2018 Kazamia E, Sutak R, Paz-Yepes J, Dorrell RG, Vieira FRJ, Mach J, Morrissey J, Leon S, Lam F, Pelletier E, Camadro JM, Bowler C, Lesuisse E. Endocytosis-mediated siderophore uptake as a strategy for Fe acquisition in diatoms. Sci Adv. 2018

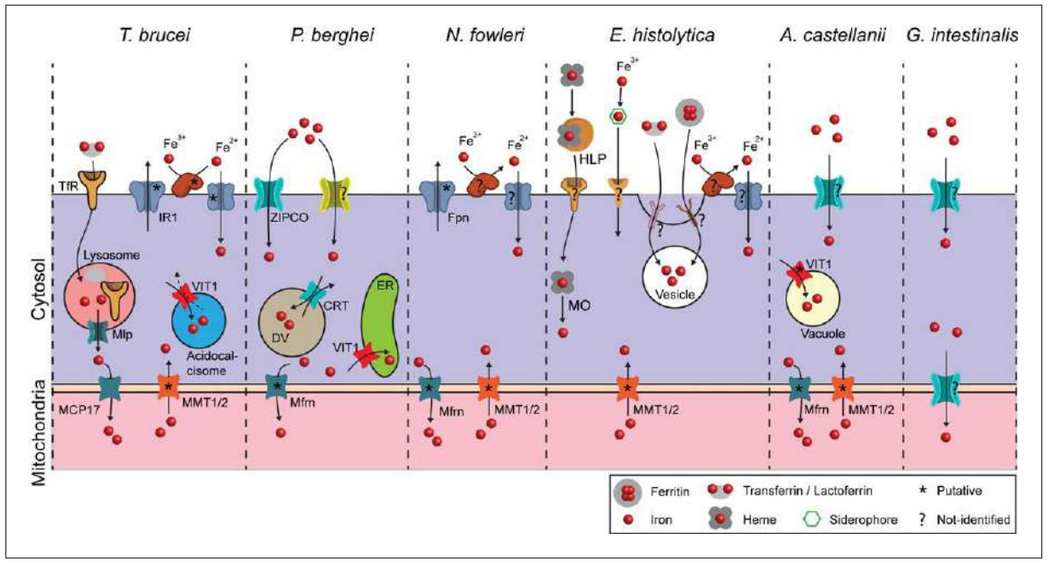
2012 Sutak R, Botebol H, Blaiseau PL, Léger T, Bouget FY, Camadro JM, Lesuisse E. A comparative study of iron uptake mechanisms in marine microalgae: iron binding at the cell surface is a critical step. Plant Physiol. 2012

2010 Sutak R, Slapeta J, San Roman M, Camadro JM, Lesuisse E. Nonreductive iron uptake mechanism in the marine alveolate Chromera velia. Plant Physiol. 2010

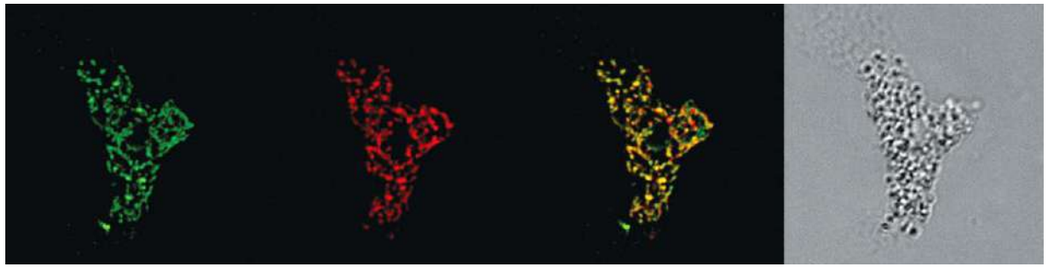
Contact person: RNDr. Róbert Šuťák, Ph.D.
e-mail: robert.sutak@natur.cuni.cz

Basic keywords:	01	Metals
	02	Iron
	03	Copper
	04	Marine microalgae
	05	Parasitic protists

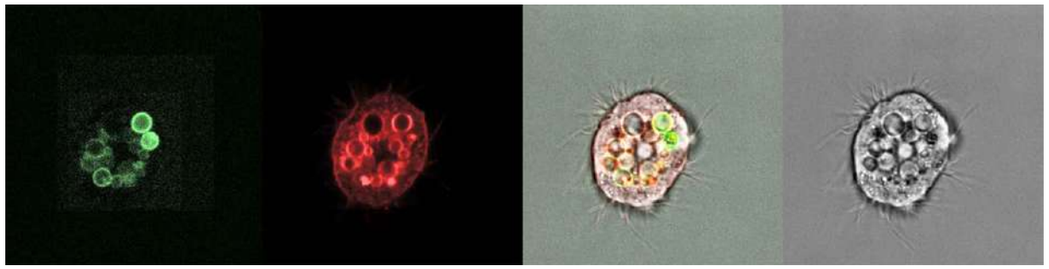
Research areas:	01	Biochemistry
	02	Phycology
	03	Parasitology
	04	Microbiology
Main objectives:	01	The characterization of the uptake and intracellular metabolism of metals in marine microalgae and parasitic protists.
	02	The characterization of the EECT of the depletion of essential metals on cell metabolism.



Representation of iron uptake and transport within the cells of selected parasitic protists.



Immunolocalization of putative mitochondrial iron storage protein ferritin in the amoeba Naegleria gruberi (●) and mitochondria stained by mitotracker (●).



Immunolocalization of putative NRAMP protein, the metal-ion transporter to vacuoles of Acanthamoeba castellanii (●) and FM4-64 membrane dye (●).

Molecular and Cellular Biology of Yeast Populations: Interaction, Signaling and Differentiation

Prof. RNDr. Zdena Palková, CSc., Head of the Lab.

Faculty of Science,
Charles University

Content of the Research

The research of the group focuses on unique molecular and cellular mechanisms involved in the signaling, development and differentiation of yeast multicellular populations. Yeast cells growing on solid surfaces form structured populations, colonies and biofilms with characteristic morphologies and organization. During population development, yeast cells differentiate into specifically localized cell subpopulations that perform specific tasks within the structure. Yeast colonies and biofilms thus behave like primitive multicellular organisms, in which cells communicate, synchronize development and differentiate into primitive "tissues". Cell differentiation often contributes to the longevity of the entire population.

The main research topics include: (1) the function and regulation of processes involved in cell-cell interactions and the development of specialized colony cells, including processes coordinating cell differentiation and the balance between cell cooperation and competition, important for community evolution; (2) regulation of the formation, development and dispersal of structured biofilms of natural yeast strains, including yeast pathogens. In addition to various biochemical, cellular and molecular biology techniques, the research uses unique techniques developed by the group to manipulate and investigate cell differentiation in situ in yeast colonies and biofilms. Group goals also include further development of specific techniques for studying structured populations, particularly state of-the-art microscopy methods for in situ analyses and methods for the separation of differentiated cells for OMICS analyses.

The group closely collaborates with team 2.1.6 focusing on yeast stress response and ageing. A recently established collaboration with T. Cooper's group at the University of Tennessee, USA, within the framework of INTER-EXCELENCE program LTAUSA18, focuses on nitrogen signaling and regulations in yeast populations.

Potential for Cooperation

We offer considerable expertise in yeast molecular and cellular biology, with emphasis on investigating the environmental resistance of biofilms and other populations of natural and laboratory strains and using immobilized yeast cell populations to detect water contaminants (our patent No 305223). Our group includes molecular biology experts and is currently validating an optimized RT-LAMP assay we developed in response to the COVID pandemic, for rapid testing on SARS-CoV-2 in saliva.

2020 Marsikova J, Pavlickova M, Wilkinson D, Vachova L, Hlavacek O, Hatakova L, Palkova Z. (2020) The Whi2p-Psr1p/Psr2p complex regulates interference competition and expansion of cells with competitive advantage in yeast colonies. Proc Natl Acad Sci USA 117:15123-15131

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2018 Nguyen PV, Hlavacek O, Marsikova J, Vachova L, Palkova Z. (2018) Cyc8p and Tup1p transcription regulators antagonistically regulate Flo11p expression and complexity of yeast colony biofilms. PloS Genet 14: e1007495

2018 Wilkinson D., Maršíková J., Hlaváček O., Gilfillan GD., Ježková E., Aaløkken R., Váchová L., Palková Z. (2018) Transcriptome remodeling of differentiated cells during chronological ageing of yeast colonies: New insights into metabolic differentiation. Oxid Med Cell Longev, Vol 2018, Article ID 4932905, 17 pages

2016 Palkova Z, Vachova L. (2016) Yeast cell differentiation: Lessons from pathogenic and non-pathogenic yeasts. Semin Cell Dev Biol. 57: 110-119

2015 Vopalenska, I, Vachova, L, Palkova, Z. (2015) New biosensor for detection of copper ions in water based on immobilized genetically modified yeast cells. Biosens Bioelectron 72:160-167

2012 Cap M, Stepanek L, Harant K, Vachova L, Palkova Z (2012) Cell differentiation within a yeast colony: Metabolic and regulatory parallels with a tumor-affected organism. Mol Cell 46: 436-448.

2011 Vachova L, Stovicek V, Hlavacek O, Chernyavskiy O, Stepanek L, Kubinova L, Palkova Z (2011) Flo11p, drug efflux pumps, and the extracellular matrix cooperate to form biofilm yeast colonies. J Cell Biol 194: 679-87.

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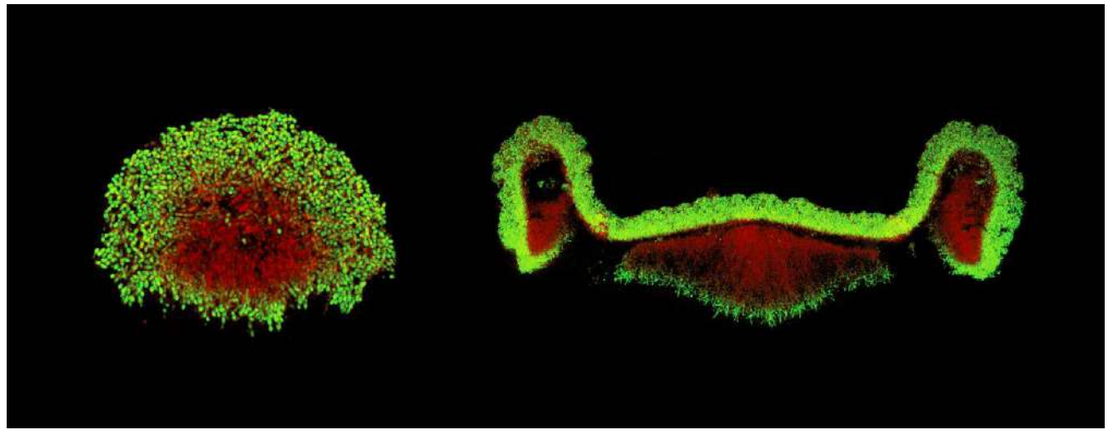
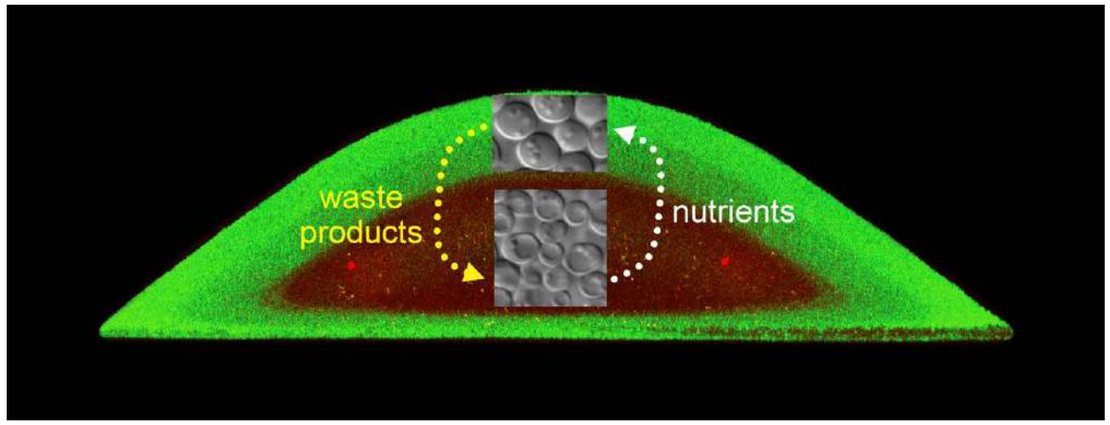
	e-mail: zdenap@natur.cuni.cz

Basic keywords:	01	Yeast colonies and biofilms
	02	Signaling
	03	Differentiation
	04	Cell-cell interaction
	05	Multicellularity
	06	Yeast pathogens

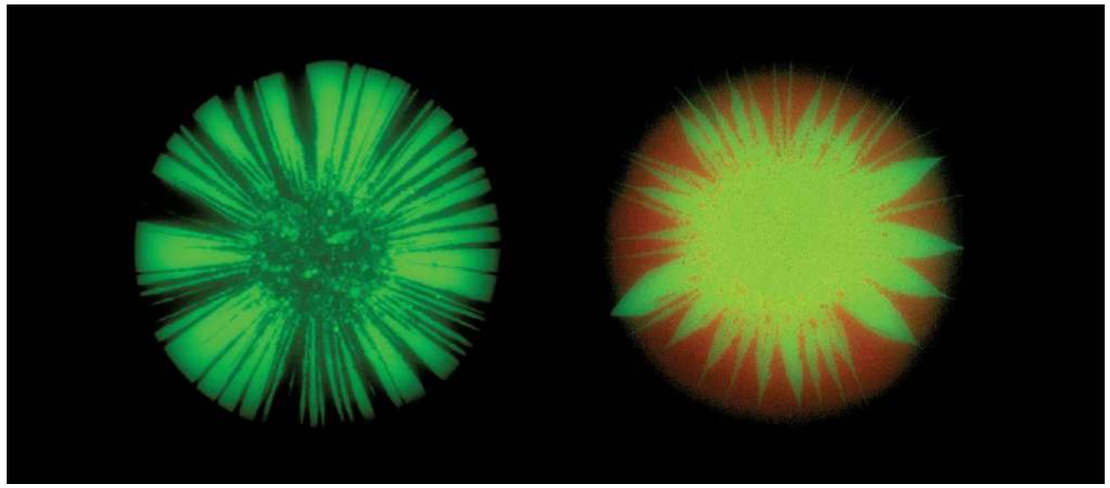
Research areas:	01	The molecular and cellular biology of yeasts
	02	Cell-cell signaling and regulation
	03	The development and differentiation of microbial multicellular structures
Main objectives:	01	Signaling, development and differentiation of structured yeast populations. Investigation of the interactions, signaling and regulations involved in cell cooperation and competition during formation of specialized cell subpopulations and evolution of cells with beneficial mutations. 301
	02	Formation, development and dispersal of biofilms formed by yeasts, including yeast pathogens, at solid-liquid interfaces. Cell-cell interactions and signaling involved in changes in multicellular lifestyle under different environmental conditions.



Vertical cross-sections of the yeast colony biofilm (35-hrs-old, left; 3-day-old, right), visualized by 2-PE confocal microscopy, showing an area (●) in which a protective extracellular matrix (ECM) is produced.



Vertical cross-section shows the nutrient flow between two cell subpopulations of a differentiated yeast colony. This flow resembles the Cori cycle and glutamine/ammonium cycle between tumor cells and some other tissues of tumor affected mammalian organism.



Spatial competition of the two yeast strains in mixed giant colonies. The green and black strain do not compete (left), the red strain (●) has advantage over the green strain (●), which is outcompeted at the colony margin (right).

The Role of Metabolism, Signaling Molecules and Cellular Structures in the Process of Aging, Stress and Adaptation

RNDr. Libuše Váchová, CSc., Head of the Lab.

The Institute of Microbiology of the
Czech Academy of Sciences

Content of the Research

The research of the group focuses on molecular processes and regulatory mechanisms involved in the adaptation, aging and cell death of yeast populations and on the response of populations to different stresses. In the natural environment, yeast cells form multicellular communities that are better protected from environmental attacks and have developed a number of mechanisms that allow them to adapt to starvation and other environmental stresses. Many of these mechanisms are specific to structured multicellular populations and do not exist in individual cells.

The first research topic of our laboratory is focused on specific metabolic processes and defense strategies important for the long-term development and survival of colonies and biofilms. It involves identifying the effect of various external or internal stresses or other factors (including metabolites or waste products produced by community cells) on vitality, aging, long-term survival and evolution of the community. In particular, we are investigating the response and adaptation of cells to stress and are developing methods for determining the metabolic status and aging of stress-exposed cells. The second topic involves research into the roles of cellular organelles, such as mitochondria, including identification of differences in mitochondria composition and activity among differentiated cells of the populations. Various approaches are used in the studies, which include, in addition to a wide range of methods in microbiology, biochemistry, and cell and molecular biology, also unique techniques developed for yeast population research in our laboratory. These techniques include, for example, specific approaches of two photon confocal microscopy and specialized analyses of cell subpopulations isolated from the structure.

The research of the group takes advantage of the close collaboration with team 2.1.5. Molecular and Cellular Biology of Yeast Populations, and of the collaboration with the T. Cooper group at the University of Tennessee, USA.

Potential for Cooperation

We offer expertise in the physiology and cell biology of yeast populations, including analyses of selected stress defense processes and analyses of selected proteins and their modifications. We also offer cooperation in the field of immobilized yeast populations covered by our patent No 305223.

2020 Marsikova J, Pavlickova M, Wilkinson D, Vachova L, Hlavacek O, Hatakova L, Palkova Z.(2020) The Whi2p-Psr1p/Psr2p complex regulates interference competition and expansion of cells with competitive advantage in yeast colonies. Proc Natl Acad Sci USA 117:15123-15131

2020 Van Nguyen P, Plocek V, Vachova L, Palkova Z (2020) Glucose, Cyc8p and Tup1p regulate biofilm formation and dispersal in wild *Saccharomyces cerevisiae*. NPJ Biofilms Microbiomes 6(1):7. doi: 10.1038/s41522-020-0118-1

2018 Nguyen PV, Hlavacek O, Marsikova J, Vachova L, Palkova Z. (2018) Cyc8p and Tup1p transcription regulators antagonistically regulate Flo11p expression and complexity of yeast colony biofilms. PloS Genet 14: e1007495

2018 Vachova L., Palkova Z. (2018) How structured yeast multicellular communities live, age and die? FEMS Yeast Res 18, 2018, foy033, 1-9

2018 Aouacheria A, Cunningham, KW., Hardwick, JM., Palkova, Z., Powers, T., Severin, FF., Vachova, L. (2018), Comment on "Sterilizing immunity in the lung relies on targeting fungal apoptosis-like programmed cell death", Science 360 (6395): eaar6910, DOI: 10.1126/science.aar6910

2016 Podholova K, Plocek V, Resetarova S, Kucerova H, Hlavacek O, Vachova L, Palkova Z. (2016) Divergent branches of mitochondrial signaling regulate specific genes and the viability of specialized cell types of differentiated yeast colonies. Oncotarget 7:15299-314.

2013 Paiva S, Strachotova D, Kucerova H, Hlavacek O, Mota S, Casal M, Palkova Z, Vachova L (2013) The transport of carboxylic acids and the important role of the Jen1p transporter during the development of yeast colonies. Biochem J. 454 (3): 551-8.

2011 Vachova L, Stovicek V, Hlavacek O, Chernyavskiy O, Stepanek L, Kubinova L, Palkova Z (2011) Flo11p, drug efflux pumps, and the extracellular matrix cooperate to form biofilm yeast colonies. J Cell Biol 194: 679-87.

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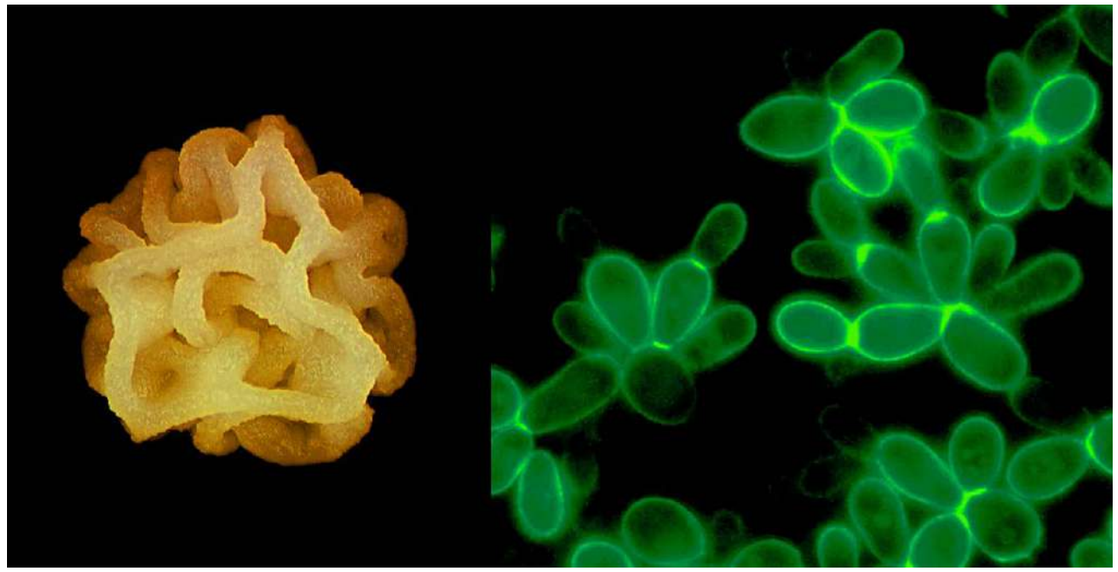
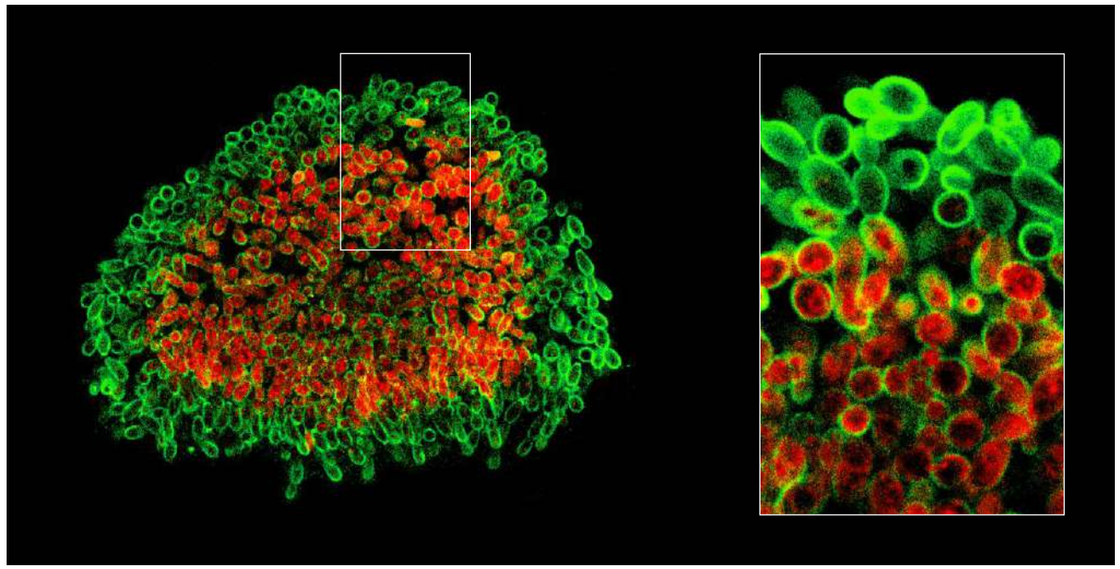
	e-mail: vachova@biomed.cas.cz

Basic keywords:	01	Yeast
	02	Multicellular communities
	03	Stress response and adaptation
	04	Ageing
	05	Biofilms
	06	Organelles/mitochondria
	07	Proteolytic mechanisms

Research areas:	01	Cell and molecular biology
	02	The physiology and biochemistry of yeast populations
	03	Stress defense
Main objectives:	01	Processes and regulations specific to the development and aging of yeast populations
	02	Processes involved in the stress-adaptation of specifically localized cell subpopulations
	03	The role of specific metabolic processes and organelles in the development, stress-defense and survival of cell subpopulations of differentiated yeast colonies
	04	Defense mechanisms against intrinsic and extrinsic stresses involved in survival of colonies and biofilms



The morphology of a structured biofilm colony formed by a natural yeast strain (left). Cells within this colony produce adhesin Flo11p (●) (right) that is indispensable for specific properties of biofilm colonies.



Defense strategies of biofilm colonies against chemical threats. Surface cell layers (●) activate multidrug resistance pumps that are able to expel toxic compounds. Vertical colony cross-section shown by 2-PE confocal microscopy (left), detail of upper part of the colony (right).

Medicinal Chemistry Lab
Ing. Milan Jakubek, Ph.D., Head of the Lab.

First Faculty of Medicine,
Charles University

Content of the Research

The scope of our group is the molecular design, synthesis, separation, characterization and bioanalytical testing of biologically active organic compounds and their formulations. From the medical application point of view, we focus on theranostics, cellular probes, enzymatic as well as epigenetic modulators and drugs. Molecular design is based on organic substances utilizing several privileged structural motifs such as hydrazones, methinium salts, coumarins, curcuminoids, tryptanthrins, Tröger's bases and fused heterocycles. The medical potential of the prepared molecules is tested by our group members or in cooperation with excellent colleagues from both the Czech Republic and abroad. Further detailed information on scientific research activities can be found in our projects and publications.

Potential for Cooperation

We offer collaboration in the field of chemical synthesis, testing of biological activity, intracellular localization, analytical studies and formulation of API. Exchange of research fellowships is welcome. Student positions at all levels are available. Cooperation with company partners and further valorisation of research outputs.

2020 Antonyova et al.: Role of mtDNA disturbances in the pathogenesis of Alzheimer's and Parkinson's disease. DNA Repair 91-92 (2020) 102871
2020 Abramenko et al.: Spectroscopic study of in situ-formed metallocomplexes of proton pump inhibitors in water Chem Biol Drug Des. (2020) 10.1111/cbdd.13782.
2020 Talianová et al.: Coumarin Tröger's base derivatives with cyanine substitution as selective and sensitive fluorescent lysosomal probes. Bioorg Chem. 94 (2020) 103447.
2020 Krčová et al.: Highly selective mitochondrial probes based on fluorinated pentamethinium salts: On two-photon properties and microscopic applications. Dyes and Pigments 172 (2020) 107802.
2019 Jakubek et al.: Strategy for improved therapeutic efficiency of curcumin in the treatment of gastric cancer. Biomed Pharmacother. 118 (2019) 109278.
2019 Hromádka et al.: Pigments from Filamentous Ascomycetes for Combination Therapy. Curr Med Chem. 26 (2019) 3812-3834
2019 Jakubek et al.: Hydrazones as novel epigenetic modulators: Correlation between TET 1 protein inhibition activity and their iron(II) binding ability. Bioorg Chem. 88 (2019) 102809
2019 Bříza et al.: Pentamethinium salts as ligands for cancer: Sulfated polysaccharide co-receptors as possible therapeutic target. Bioorg Chem. 82 (2019) 74-85
2019 Havlík et al.: Versatile fluorophores for bioimaging applications: π-expanded naphthalimide derivatives with skeletal and appendage diversity. Chem Commun. 55 (2019) 2696-2699
2018 D'Acunto et al.: Metallomics for Alzheimer's disease treatment: Use of new generation of chelators combining metal-cation binding and transport properties. Eur J Med Chem. 150 (2018) 140-155
2018 Kejík et al.: Epigenetic agents in combined anticancer therapy. Future Med. Chem. 10 (2018) 1113-1130

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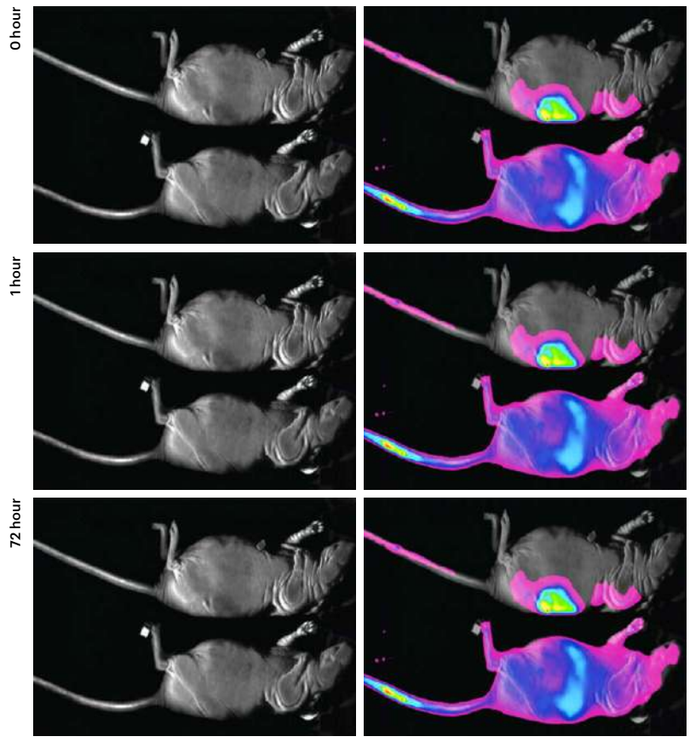
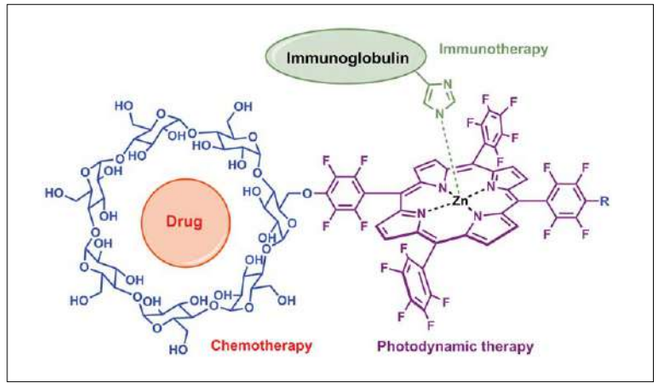
Basic keywords:		
	01	Epigenetics
	02	Theranostics
	03	Anti-cancer agents
	04	Combination therapy
	05	Polymethinium salts
	06	Metal chelators
	07	Fluorescent cellular probes
	08	Curcumin derivatives
	09	Nanoformulation
	10	Medicinal chemistry
	11	Synthetic organic chemistry
	12	Bioanalytical chemistry
	13	Biochemistry

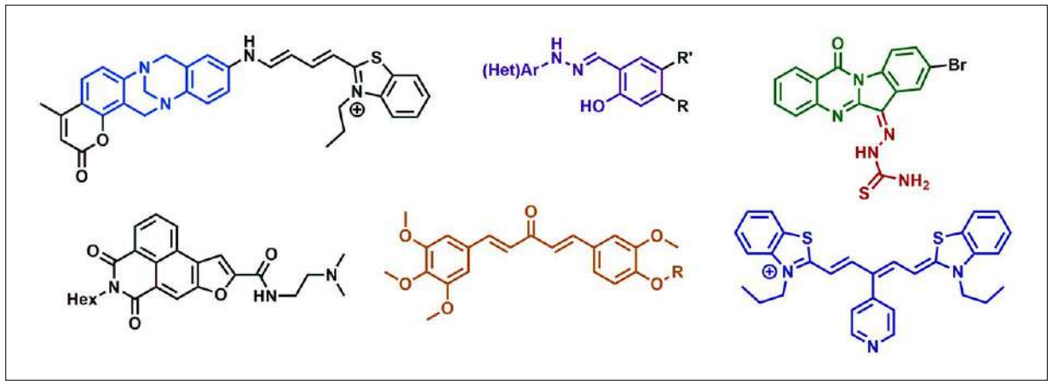
Research areas:	01	Design, synthesis, separation and characterization of small biologically active organic molecules
	02	Derivatives of bioactive natural substances, e.g. tryptanthrins, ancaflavin and monascin, curcumin derivatives
	03	Metal chelators for bioanalytical and medical applications
	04	Tröger's base derivatives for medical applications
	05	New fluorescent substances for labelling cell organelles (e.g. coumarin, benzofuran, naphthalimide or pyrrolopyrrole derivatives; pentamethinium salts)
	06	Formulations of solid and liquid API, including pharmaceutical forms
	07	Nanomedicine
	08	Theranostics
Main objectives:	01	Preparation of novel bioactive compounds and derivatives of natural substances
	02	Preparation of selective fluorescent probes
	03	Determination of the mechanism of the action, inhibition activity toward epigenetically important enzymes
	04	Formulation of APIs



Two-photon fluorescent probes for bioimaging.



Multimodal therapeutic system based on porphyrin-cyclodextrin conjugates.



Novel compounds from our lab: fluorescent Tröger's base derivatives, metal chelators based on hydrazones, derivatives of tryptanthrin, fused naphthalimides, curcumine derivatives, cyanine dyes.

Laboratory of Proteases
Mgr. Klára Grantz Šašková, Ph.D., Head of the Lab.

Faculty of Science,
Charles University

Content of the Research

Our junior research group was established in 2016 at the Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University (BIOCEV). We are a proud member of the Department of Genetics and Microbiology, Faculty of Science, at Charles University in Prague. Our research team has particular interest in the family of DDI1-like proteins involved in DNA repair and transcription regulation. In brief, we focus on Ddi1-like proteins, called DNA damage-inducible protein 1, that have been largely understudied by the scientific community at large. However Dd1-like proteins have become more in general focus in the past couple of years. The reason for this surge in interest is simple, Ddi1-like proteins are newly discovered players involved in DNA repair and also in transcription regulation. The complete mechanism of both Ddi1 activities however still remains to be uncovered. In light of this fact we would like to further our contribution to the understanding of the particular mechanism.

We are also investigating hepatitis B (HBV), a viral infection attacking the liver which can lead to chronic disease. According to the World Hepatitis Alliance, one-half of new positive liver cancer diagnoses and one in twelve cancer deaths can be traced to hepatitis B and hepatitis C infection. Unlike other infectious diseases such as HIV, tuberculosis and malaria, investment in research for HBV has been overlooked and research has stagnated. As of this writing, around 350 million people worldwide are infected with most being unaware of their infection. This is because symptoms seldom manifest in the individuals affected until the disease has progressed to an advanced stage. Therefore, finding a cure to HBV is vital, and is one of our focuses. In particular, we are interested in the biology of hepatitis B virus, specifically the covalently closed circular DNA (cccDNA), which resides in the nucleus of infected hepatocytes as a non-integrated plasmid-like molecule and serves as a transcriptional template for HBV. The elimination of cccDNA is a key step towards finding a possible HBV cure. Our team has elected to focus our attention on various strategies for cccDNA degradation, such as the development of nanoparticles transporting CRISPR/Cas9 system targeted to cccDNA.

Potential for Cooperation

We are always seeking motivated students and post doctorates. If you would like to know more, have any inquiries or questions, we encourage you to contact our research team leader Klára Grantz Šašková by email.

2020 Fassmannová, D., Sedlák, F., Sedláček, J., Špička, I., Grantz Šašková*, K. Nelfinavir inhibits the TCF11/Nrf1-mediated proteasome recovery pathway in multiple myeloma. Cancers 2020, 12(5), 1065, https://doi.org/10.3390/cancers12051065

2019 Svoboda, M.; Trempe, J.-F.; Konvalinka, J.; Grantz Šašková*, K. The yeast proteases Ddi1 and Wss1 are both involved in the DNA replication stress response. DNA Repair 2019, 50, 45-51, DOI: doi.org/10.1016/J.DNAREP.2019.06.008

2019 Srb, P.; Svoboda, M.; Benda, L.; Lepšík, M.; Tarábek, J.; Šícha, V.; Grüner, B.; Grantz Šašková, K.; Brynda, J.; Řezáčová, P.; Konvalinka, J.; Veverka V. Capturing a dynamically interacting inhibitor by paramagnetic NMR spectroscopy. Physical Chemistry Chemical Physics 2019, 21(10), 5661-5673. DOI: 10.1039/C9CP00416E

2019 Ramirez, J.; Lectez, B.; Osinalde, N.; Siva, M.; Elu, N.; Aloria, K.; Prochazkova, M.; Perez, C.; Martinez-Hernandez, J.; Barrio, R.; Grantz Saskova, K.; Arizmendi, JM.; Mayor, U. Quantitative proteomics reveals neuronal ubiquitination of Rngo/Ddi1 and several proteasomal subunits by Ube3a, accounting for the complexity of Angelman syndrome. Human Molecular Genetics 2019, 27 (11),1955-1971. DOI: 10.1093/hmg/ddy103

2016 Trempe, J.-F.; Saskova, K. G.; Siva, M.; Ratcliffe, C. D. H.; Veverka, V.; Hoegl, A.; Menade, M.; Feng, X.; Shenker, S.; Svoboda, M.; Kozisek, M.; Konvalinka, J.; Gehring, K., Structural studies of the yeast DNA damage-inducible protein Ddi1 reveal domain architecture of this eukaryotic protein family. Sci Rep 2016, 6, 33671. DOI: 10.1038/srep33671

2016 Siva, M.; Svoboda, M.; Veverka, V.; Trempe, J.-F.; Hofmann, K.; Kozisek, M.; Hexnerova, R.; Sedlak, F.; Belza, J.; Brynda, J.; Sacha, P.; Hubalek, M.; Starkova, J.; Flaisigova, I.; Konvalinka, J.; Grantz Saskova, K.*, Human DNA-Damage-Inducible 2 Protein Is Structurally and Functionally Distinct from Its Yeast Ortholog. Sci Rep 2016, 6, 30443. DOI: 10.1038/srep30443

2016 Machara, A.; Lux, V.; Kozisek, M.; Grantz Saskova, K.; Stepanek, O.; Kotora, M.; Parkan, K.; Pavova, M.; Glass, B.; Sehr, P.; Lewis, J.; Muller, B.; Krausslich, H.-G.; Konvalinka, J., Specific Inhibitors of HIV Capsid Assembly Binding to the C-Terminal Domain of the Capsid Protein: Evaluation of 2-Arylquinazolines as Potential Antiviral Compounds. J Med Chem 2016, 59 (2), 545-58. DOI: 10.1021/acs.jmedchem.5b01089

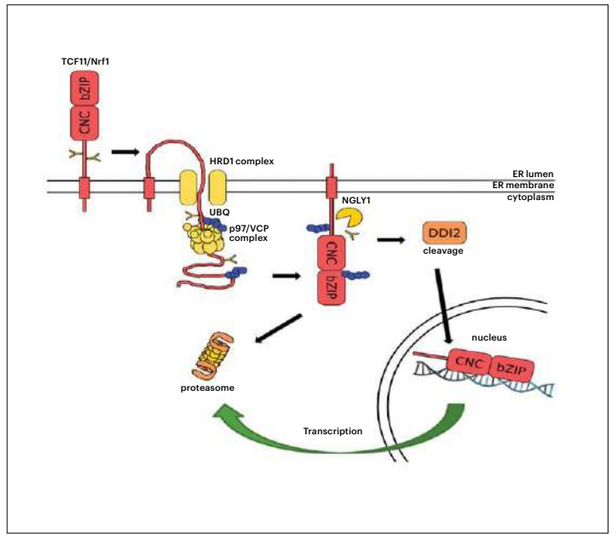
Contact person:	Mgr. Klára Grantz Šašková, Ph.D.
	e-mail: saskova2@natur.cuni.cz

Basic keywords:	01	Ddi1-like proteins
	02	Ubiquitin
	03	Proteasome
	04	DNA repair
	05	HBV
	06	cccDNA
	07	Nanoparticles

Research areas:	01	Ubiquitin-proteasome pathway
	02	Ddi1-like proteins
	03	Hepatitis B virus
Main objectives:	01	Ddi1-like proteins in DNA repair and transcription regulation
	02	Development of nanoparticles for mRNA targeted delivery
	03	Development of antivirals targeting HBV

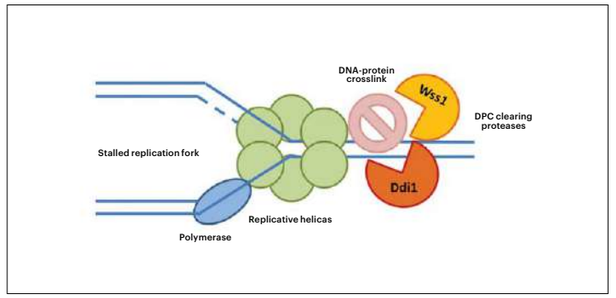
Two distinct roles of Ddi1-like proteins in DNA repair and transcription regulation (A, B, C)

A) Human DDI2 activates TCF11/Nrf1.



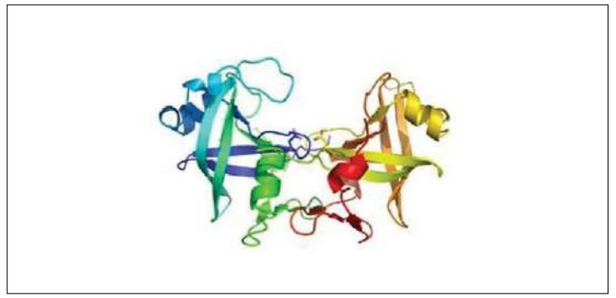
Under normal conditions TCF11/Nrf1 undergoes Hrd1-dependent ubiquitylation, and is retrotranslocated by p97 and degraded by the proteasome. When proteasome is impared, DDI2 is recruited to the ER membrane. TCF11/Nrf1 is in turn deglycosylated by NGLY1, extracted from the membrane by p97 and cleaved by DDI2 to translocate into the nucleus, where TCF11/Nrf1 transactivates proteasome gene expression.

B) Yeast Ddi1 proteolytically degrades covalent DNA-protein crosslinks.



Yeast Ddi1 aspartic protease together with Wss1 metalloprotease proteolytically degrades covalently trapped proteins on DNA.

C) 3D structure of the DDI2 aspartic protease domain.



The overall fold of the proteolytic domain of Ddi1-like proteins is highly similar to retropepsins, including HIV protease. Moreover, Ddi1-like proteins contain an additional N-terminal ubiquitin-like domain, a helical domain and a C-terminal ubiquitin-associated domain.

Molecular and Cellular Mechanisms of the Invasiveness of Tumour Cells

Assoc. Prof. Jan Brábek, Ph.D., Assoc. Prof. Daniel Rosel, Ph.D., Heads of the Lab.

Faculty of Science,
Charles University

Content of the Research

The focus of our laboratory is on molecular and cellular mechanisms of cancer cell motility and invasiveness and its plasticity, including the subcellular structures involved. Our laboratory discovered and characterized amoeboid invasiveness and metastasis in cancer cells of a mesenchymal origin and proved the increased generation of traction forces in amoeboid cells and the plausibility of amoeboid cell invasiveness in vivo. Our laboratory was also the first to elucidate the structure of invadopodia in a complex 3D environment. We also proved the role of CAS SH3 domain tyrosine phosphorylation in the migration and invasiveness of Src-transformed cells and the role of the newly discovered direct CAS-vinculin interaction in mechanosensing and CAS-PKN3 interaction in invasion and metastasis. We also uncovered the role of tyrosine phosphorylation within SH3 domains as a novel general regulatory mechanism for cell signaling. Later, we concentrated more on the plasticity of cancer cell invasiveness and succeeded in identifying several important signaling molecules involved in this process. We declared and defined a novel category of anti-cancer drugs, targeting invasion and metastasis – migrastatics.

Potential for Cooperation

We are members of the Invadosome Consortium and benefit from our collaboration with laboratories within the Consortium as well as many other national and international joint projects. We offer our expertise to our partners in quantitative analyses of cancer cell invasion in 3D collagen, including a vertical invasion assay and outgrowth of cell spheroids. In addition, we can offer analyses of invasive behavior on a unique life-like matrix - acellular dermis and analyses of cell signaling after mechanical stretching on our cell stretcher. In addition we can provide access to the following services: a wide range of basic and advanced genetic, molecular biology and biochemistry techniques (including qPCR, CRISPR/Cas9, shRNA, affinity purifications, kinase assays and many others); Cell culture in 2D and 3D conditions on various matrices (including Matrigel, 3D collagen, cell-based matrices, acellular dermis); transcriptomic and proteomic analyses in 3D collagen. We also provide expertise on qualitative and quantitative cell adhesion, proliferation, migration, ECM degradation, invadopodia formation and invasion assays in 2D and 3D environments, microchannel assays, mechanoreception analyses and advanced microscopic analyses of migrating cancer cells as well as signaling proteins and structures involved in cell adhesion, migration and invasiveness.

2019 Rosel D, Fernandes M, Sanz-Moreno V, Brábek J. Migrastatics: Redirecting R&D in Solid Cancer Towards Metastasis? Trends Cancer. 2019, 5(12):755-756.

2019 Gemperle J, Dibus M, Koudelková L, Rosel D, Brábek J. The interaction of p130Cas with PKN3 promotes malignant growth. Molecular Oncology 2019, 3(2):264-289

2019 Koudelkova L, Pataki AC, Tolde O, Pavlik V, Nobis M, Gemperle M, Anderson K, Brábek J, Rosel D. Novel FRET based Src biosensor reveals mechanisms of Src activation and its dynamics in focal adhesions. Cell Chemical Biology, 2019, 26(2):255-268.

2018 Čermák V, Gandalovičová A, Merta L, Fučíková J, Špíšek R, Rösel D, Brábek J.RNA-seq of macrophages of amoeboid or mesenchymal migratory phenotype due to specific structure of environment.Sci Data. 2018, 5:180198.

2018 Tolde O, Gandalovičová A, Křížová A, Veselý P, Chmelík R, Rosel D, Brábek J.Quantitative phase imaging unravels new insight into dynamics of mesenchymal and amoeboid cancer cell invasion. Sci Rep. 2018, 8(1):12020.

2018 Fernandes M, Brábek J. Cancer, checkpoint inhibitors, and confusion. Lancet Oncol. 2018, 18, 11: E632-E632.

Contact person: Assoc. Prof. Jan Brábek, Ph.D., brabek@natur.cuni.cz
Assoc. Prof. Daniel Rosel, Ph.D., rosel@natur.cuni.cz

Basic keywords:

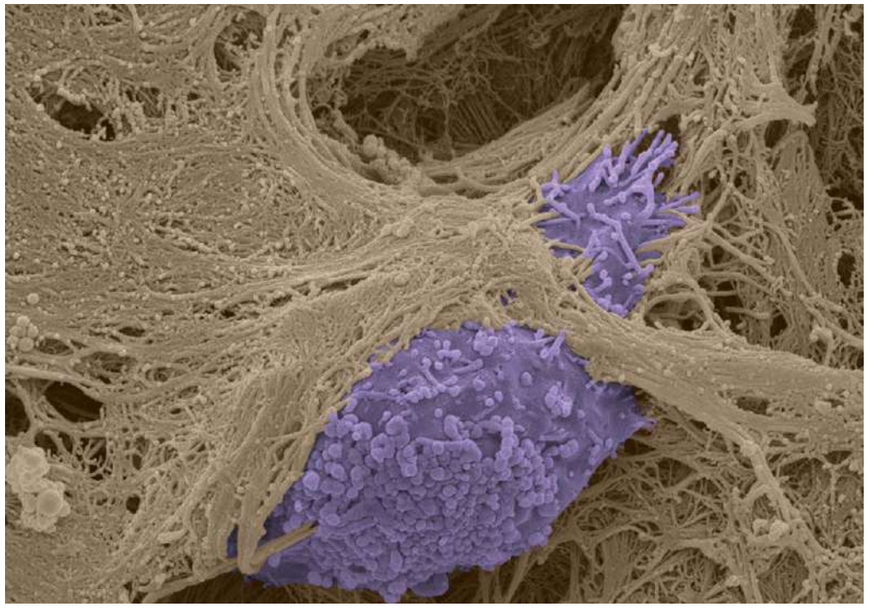
01	Invasiveness
02	Amoeboid
03	Mesenchymal
04	Metastasis
05	Invadopodia
06	SRC
07	P130cas
08	Motility
09	Integrin-mediated signaling
10	Mechanosensing
11	Migrastatics

Research areas:

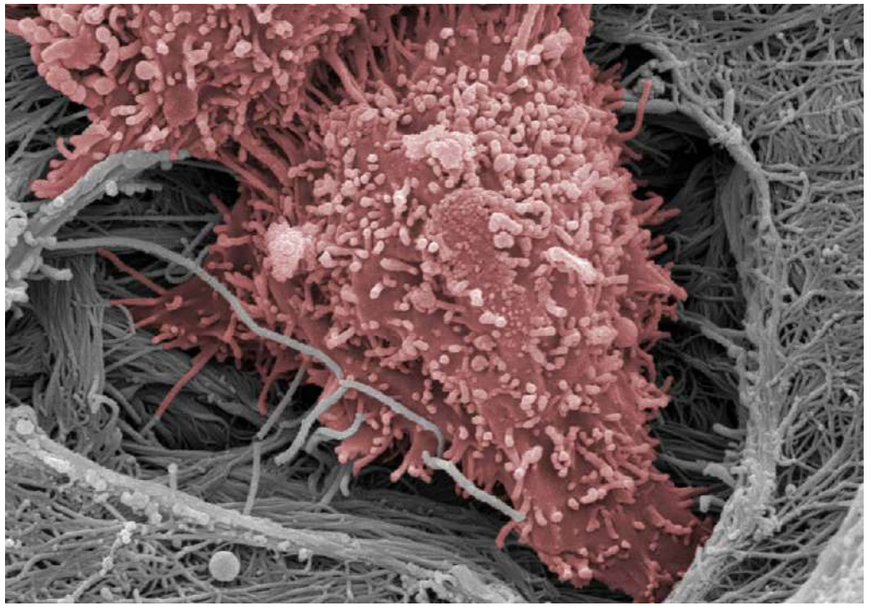
01	Identification and understanding of proteins and signaling pathways involved in amoeboid and mesenchymal cancer cell invasiveness
02	Elucidation of the mechanisms of plasticity of cancer cell invasiveness
03	Functional analysis of subcellular structures involved in cancer cell invasiveness
04	Analysis of general mechanisms of cell migration and mechanosensing

Main Capabilities:

01	A wide range of basic and advanced genetic, molecular biology and biochemistry techniques (including qPCR, CRISPR/Cas9, shRNA, affinity purifications, kinase assays and many others)
02	Cell culture in 2D and 3D conditions on various matrices (including Matrigel, 3D collagen, cell-based matrices, acellular dermis)
03	Transcriptomic and proteomic analyses in 3D collagen
04	Qualitative and quantitative cell adhesion, proliferation, migration, ECM degradation, invadopodia formation and invasion assays in 2D and 3D environments, microchannel assays, mechanoreception analyses
05	Advanced microscopic analyses of migrating cancer cells as well as signaling proteins and structures involved in cell adhesion, migration and invasiveness



Amoeboid migration of a sarcoma cell in the acellular dermis.



Mesenchymal migration of a sarcoma cell in the acellular dermis.

Interaction between Normal and Tumour Haematopoietic Stem Cells with Their Specific Microenvironment (Niche)

Prof. MUDr. Emanuel Nečas, DrSc., Head of the Lab.

First Faculty of Medicine,
Charles University

Content of the Research

The focus of our research group is on bone marrow regeneration. The advantage in studying hematopoiesis and bone marrow is the well established steady-state structure and function of the tissue, easy accessibility, advanced research methods, and the well-defined embryonic, fetal, and adult stages of development. Our project targets basically adult hematopoietic stem cells. These stem cells are used for stem cell transplants to patients with hematopoietic and some other disorders. The use of stem cells for treating diseases requires that the transplanted cells induce tissue regeneration. While the role and function of hematopoietic stem cells in undisturbed steady state hematopoiesis has been intensively explored, significantly less attention has been paid to their function in regenerating hematopoiesis.

Our current research provided an original snapshot of intensively regenerating bone marrow and suggested its similarity to the stage in embryonic hematopoiesis preceding the emergence of hematopoietic stem cells (Faltusová et al., 2020; Nečas and Faltusová, 2020). Due to lack of stem cells, the intensively regenerating bone marrow cannot be transplanted, although it gives rise to a significant number of mature blood cells. The driving force of regeneration appears to be in the activated progenitor cells committed to erythroid and myeloid (granulocytic-macrophage) development which temporarily substitute the lack of hematopoietic stem cells. We are trying to understand what makes progenitor cells self-renew and multiply more than differentiate in damaged and regenerating bone marrow. Self-renewal is the basic feature of stem cells but normally the ability to self-renew is reduced in progenitor cells in favor of their differentiation. This seems to be reversed in regenerating bone marrow. There must be some yet unknown external cues acting on the progenitor cells in regenerating tissues such as bone marrow. The external cues must then activate the regenerative pattern of the gene activity in target cells. This is a significant research challenge regarding stem cell biology as well as understanding the general principles underlying the regenerative potential of damaged tissues.

Potential for Cooperation

The Potential for Cooperation is in the identification and analysis of the gene response occurring in cells activated by tissue injury and also in the analysis of the tissue microenvironmental factors governing the regenerative process.

2019 Současný pohled na krvetvornou tkáň. Nečas E Faltusová K. Československá fyziologie. 2019; 62/(2):57-67.

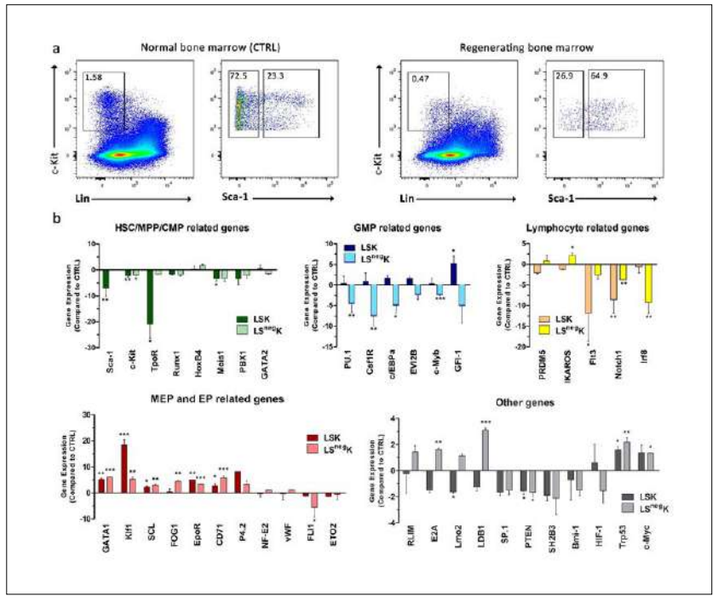
2020 T-lymphopoiesis is Severely Compromised in Ubiquitin-Green Fluorescent Protein Transgenic Mice. Faltusová K, Báječný M, Heizer T, Páral P, Nečas E. Folia Biol (Praha). 2020;66(2):47-59.

2020 Altered Erythro-Myeloid Progenitor Cells Are Highly Expanded in Intensively Regenerating Hematopoiesis. Faltusová K, Chen CL, Heizer T, Báječný M, Szikszai K, Páral P, Savvulidi F, Renešová N, Nečas E. Front Cell Dev Biol. 2020 Feb 25;8:98. doi: 10.3389/fcell.2020.00098.

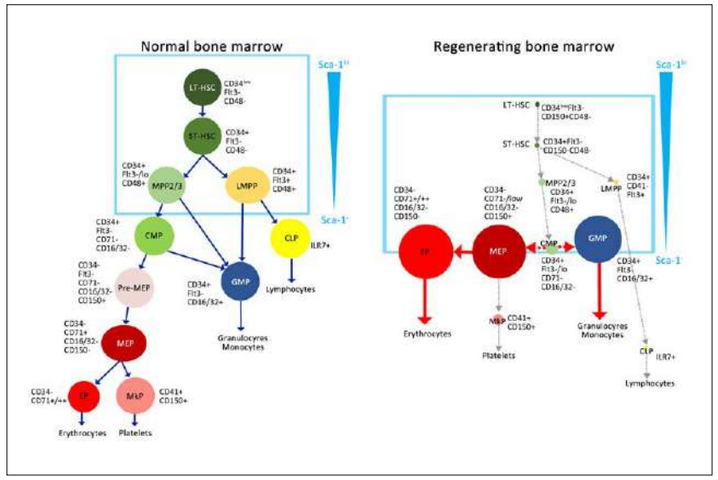
2020 Regenerující krvetvorba se podobá embryonální krvetvorbě nezávislé na kmenových buňkách. Nečas E, Faltusová K. Transfuze Hematol Dnes. 2020; 26(3): 157-166.

Contact person:	Prof. MUDr. Emanuel Nečas, DrSc.
	e-mail: emanuel.necas@lf1.cuni.cz

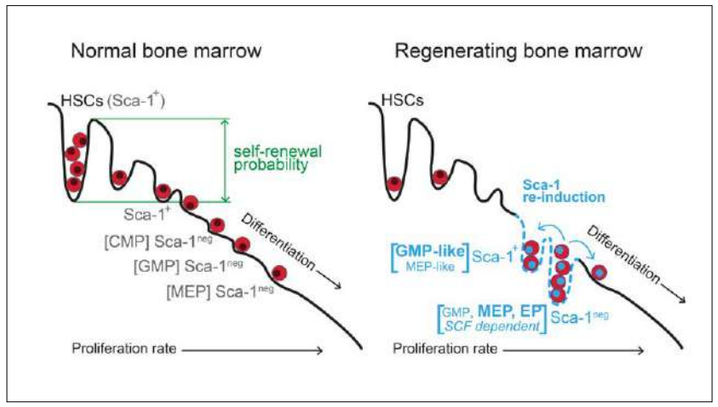
Basic keywords:	01	Hematopoiesis
	02	Regeneration
	03	Stem cells
	04	Progenitor cells
	05	Bone marrow
	06	Microenvironment
	07	Transplantation
Research areas:	01	Experimental hematology
	02	Stem cell research
	03	Molecular imaging - flow cytometry and gene expression
Main objectives:	01	Tissue regeneration is generally thought to begin with the activation of stem cells. Based on our current results, we want to challenge this view by demonstrating the essential role of progenitor cells and control of their differentiation in early response of hematopoietic tissue to acute damage



Changes in the expression of genes related to hematopoiesis in regenerating bone marrow. mRNAs for genes related to hematopoietic stem and progenitor cells were determined in Sca-1$^+$ and Sca-1$^-$ immature c-Kit positive cells in normal and intensively regenerating bone marrow.



Populations of hematopoietic stem and progenitor cells and their developmental hierarchy are significantly altered in regenerating bone marrow by expansion of myeloid progenitor cells at the expense of stem cells.



A model of progenitor cells activation by tissue microenvironment in regenerating bone marrow.

Interactions of Viral and Cellular Structures during Viral Infection and the Development of Nanostructures for Medical and Veterinary Purposes

Doc. RNDr. Jitka Forstová, CSc., Head of the Lab.

Faculty of Science,
Charles University

Context of the Research

Our laboratory of molecular virology conducts basic research to understand the functional meanings of interactions between viral and cellular structures during viral infection, the mechanisms of stress-related and defensive reactions of cells, and the mechanisms of deregulation of cellular processes including oncogenic transformation. Simultaneously, the results of the research are reflected in the exploitation of artificial viral nanostructures for the development of vaccines, diagnostic kits and vehicles for transporting therapeutic compounds into cells. After conducting studies of the very early phase of polyomaviral infection - virion movements in the cytoplasm, the role of various endosomal and cytoskeletal structures in viral trafficking, we turned our attention to the mechanism of virus translocation into the cell nucleus, the establishment of viral infection in the cell nucleus, and the identification of cellular factors positively or negatively (restriction factors) affecting virus replication.

Among the ongoing projects are i) the study of the role of nuclear lamina and associated proteins in establishing and sustaining MPyV and BKPyV infection, ii) studies of the dynamics of changes in nuclear structures with the progress of polyomavirus infection and iii) research of activation and deregulation of innate (including intrinsic) immunity responses by small DNA viruses replicating in the cell nucleus - the mechanism by which DNA viruses interact with different restriction factors, DNA sensors and how the pathways of IFN induction (activated by the sensed virus) are connected.

Potential for Cooperation

Our virology team is open to further collaboration in both basic and applied research. Based on our recently published work, we have established cooperation with Dr. Atherton from King´s College, London, on the structural basis of VP1's microtoubule binding/stabilisation activity. The virology team has collaborated over the years with several foreign laboratories, for example with Prof. Cheng at the University of California in Davis, USA, Prof. Oppenheim from Hebrew University, Israel, Prof. Dilworth from Middlesex University London, Prof. Amati, University Sapienza, Roma, Prof. Dalianis, Karolinska Institute, Stockholm. We have performed application research in collaboration with the Czech company Dyntec spol. s r.o. on developing veterinary prophylactic vaccines against porcine circovirus 2 and bovine papillomavirus, based on recombinant polyomavirus nanostructures. We have collaborated also with another Czech company, Vidia spol. s r.o. to prepare a prototype of a diagnostic kit for human JCV polyomavirus and a diagnostic kit for BKV polyomavirus subtypes.

2020 Horníková L, Bruštíková K, Ryabchenko B, Zhernov I, Fraiberk M, Mariničová Z, Lánský Z, Forstová: The Major Capsid Protein, VP1, of the Mouse Polyomavirus Stimulates the Activity of Tubulin Acetyltransferase 1 by Microtubule Stabilization. Viruses. 2020; 18;12(2):227.

2020 Horníková L, Bruštíková K, Forstová J.: Microtubules in Polyomavirus Infection.Viruses. 2020; 18;12(1):121.

2018 Soldatova I, Prilepskaja T, Abrahamyan L, Forstová J, Huérfano S.: Interaction of the Mouse Polyomavirus Capsid Proteins with Importins Is Required for Efficient Import of Viral DNA into the Cell Nucleus.: Viruses. 2018 Mar 31;10(4):165.

2017 Fraiberk M, Hájková M, Krulová M, Kojzarová M, Drda Morávková A, Pšikal I, Forstová J.Exploitation of stable nanostructures based on the mouse polyomavirus for development of a recombinant vaccine against porcine circovirus 2. ; PLoS One. 2017 Sep 18;12(9):e0184870.

2017 Huérfano S, Ryabchenko B, Španielová H, Forstová J.: Hydrophobic domains of mouse polyomavirus minor capsid proteins promote membrane association and virus exit from the ER.; FEBS J. 2017; 284(6):883-902.

2017 Horníková L, Fraiberk M, Man P, Janovec V, Forstová J.: VP1, the major capsid protein of the mouse polyomavirus, binds microtubules, promotes their acetylation and blocks the host cell cycle. FEBS J. 2017; 284(2):301-323.

2016 Henke P, Kirakci K, Kubát P, Fraiberk M, Forstová J, Mosinger: Antibacterial, Antiviral, and Oxygen-Sensing Nanoparticles Prepared from Electrospun Materials. J.ACS Appl Mater Interfaces. 2016; 28;8(38):25127-36.

Contact person:	Doc. RNDr. Jitka Forstová, CSc.
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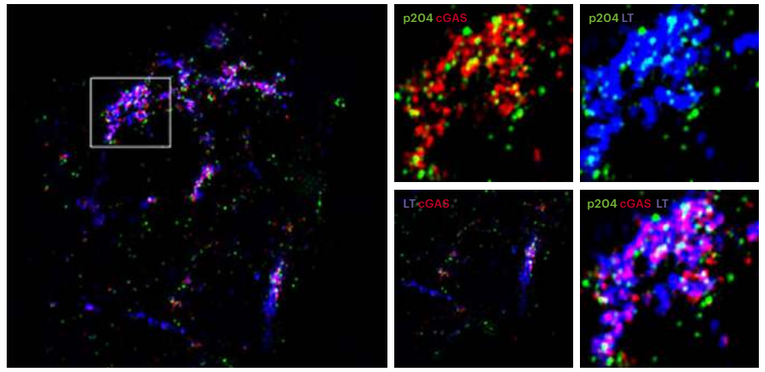
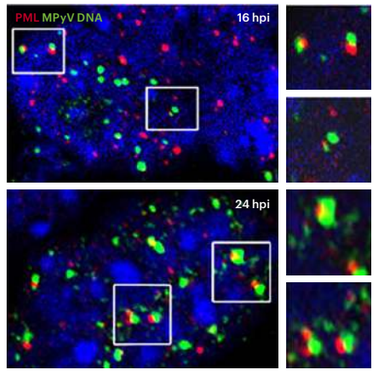
Basic keywords:	01	Small tumorogenic viruses
	02	Polyomaviruses
	03	Trafficking
	04	Endocytosis
	05	Protein-protein
	06	Interactions
	07	Vlps
	08	Nanostructures
	09	Vaccines

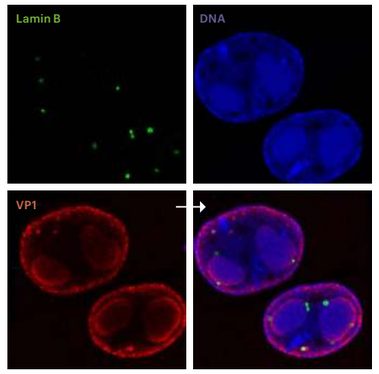
Research areas:	01	Virology
	02	Cell biology
	03	Biotechnology
Main objectives:	01	Understanding molecular mechanisms of individual steps of polyomavirus infection
	02	Revealing the mechanisms of cell defence against DNA virus infections
	03	Development of nanostructures for medical and veterinary exploitation



Mutual position of DNA sensors p204, cGAS and murine polyomavirus (MPyV) LT antigen in a region of viral DNA replication. Mouse fibroblasts were infected with MPyV and stained 24 hours post infection. Left: a section of the nucleus of infected cells, the pictures on the right show the enlarged viral DNA replication region in the square. SIM microscopy. The bar = 5μm.

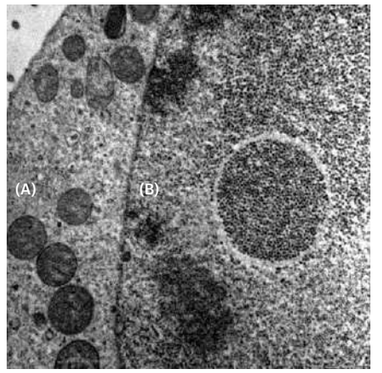


Nucleus PML bodies cumulate around replicating viral DNA patches with progressing infection. Confocal sections through the centres of the nuclei of MEF cells 16 and 24 post infection. The right panel represents enlarged arias pointed by white rectangles. PML (●), MPyV DNA (●), Host cell DNA (●). Images were obtained by Leica Laser Confocal Microscope.



BK polyomavirus (BKPyV) progeny virions travel from the cell nucleus towards plasma membrane to be released from infected cells. Human Renal Proximal Tubular Epithelial Cells infected by human BKPyV 3 days post infection. Lamin (●), VP1 virus capsid protein (●) Host cell DNA (●). Confocal microscopy of a cell section

(A) Cytoplasm (B) Nucleus



Detail of the nucleus of BKPyV infected cell

Electron microscopy of Human Renal Proximal Tubular Epithelial Cells five days post infection with human BK polyomavirus (BKPyV).

Immunization against Tumors Caused by Human Viruses

RNDr. Michal Šmahel, Ph.D., Head of the Lab.

Faculty of Science,
Charles University

Content of the Research

Our project is focused on the optimization of DNA immunization against tumors induced by human papillomaviruses (HPVs). To enhance the efficacy of vaccination, we construct various fusion genes that express antigens with favorable characteristics, e.g. with inserted strong universal helper epitopes or with cellular localization that targets antigens into pathways with MHC class I and/ or class II presentation. Furthermore, we attempt to augment the anti-tumor effect of DNA immunization by combining it with other immunotherapeutic approaches - blockading immune checkpoint receptors with monoclonal antibodies and activating toll-like receptors with their ligands. DNA vaccines that are delivered by a gene gun are mainly aimed against the viral oncoprotein E7 which represents a tumor specific antigen.

We examine the efficacy of immunotherapy in mouse tumor models, including derivation of tumor cell lines with a reduced expression of MHC class I molecules. As this reduction is one of the most frequent mechanisms of tumor escape from host immunity, it could contribute to the limited therapeutic effect of cancer immunotherapy in clinical trials. To study factors that influence the effect of cancer immunotherapy, we modify mouse oncogenic cell lines with the CRISPR/ Cas9 system and analyze immune reactions induced by immunotherapy. We particularly characterize immune cells that infiltrate tumors with various MHC class I expressions and contribute to an anti-tumor effect. Moreover, we analyze spatial and temporal heterogeneity of immune cells in the tumor microenvironment. Finally, we investigate the interaction of some human oncogenic viruses with plasmacytoid dendritic cells.

Potential for Cooperation

We can provide our mouse models of tumors with downregulated MHC class I expression and analyze immune reactions by ELISPOT and ELISA assays, immunohistochemical staining of tumors, and flow cytometric phenotyping of tumor-infiltrating immune cells. We can collaborate with research laboratories studying tumor immunology, immunotherapy of malignant diseases, and tumor escape mechanisms. We cooperate with clinical institutions that are interested in immune reactions in patients with malignancies or clinical trials of cancer immunotherapy. We are also interested in working with pharmacological companies in preclinical testing of combined anti-tumor therapy that includes immunotherapy, especially immune checkpoint blockades.

2020 Vackova J, Piatakova A, Polakova I, Smahel M. Abrogation of IFN-γ Signaling May not Worsen Sensitivity to PD-1/PD-L1 Blockade. Int J Mol Sci 2020; 21: pii: E1806.

2019 Polakova I, Pelak O, Thurner D, Pokryvkova B, Tachezy R, Kalina T, Smahel M. Implementation of Mass Cytometry for Immunoprofiling of Patients with Solid Tumors. J Immunol Res 2019; 2019: 6705949.

2019 Lhotakova K, Grzelak A, Polakova I, Vackova J, Smahel M. Establishment and characterization of a mouse tumor cell line with irreversible downregulation of MHC class I molecules. Oncol Rep 2019; 42: 2826-2835.

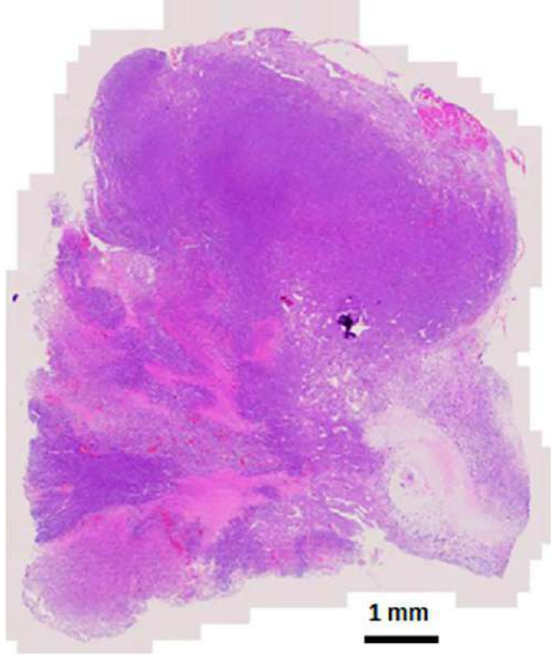
2018 Grzelak A, Polakova I, Smahelova J, Vackova J, Pekarcikova L, Tachezy R, Smahel M. Experimental Combined Immunotherapy of Tumours with Major Histocompatibility Complex Class I downregulation. Int J Mol Sci 2018; 19: pii: E3693.

2017 Smahel M. PD-1/PD-L1 Blockade Therapy for Tumors with Downregulated MHC Class I Expression. Int J Mol Sci 2017; 18: pii: E1331.

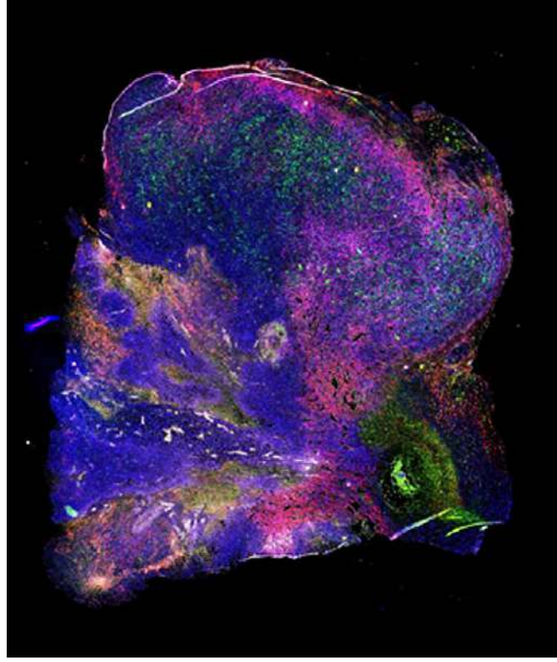
Contact person:	RNDr. Michal Šmahel, Ph.D.
	e-mail: michal.smahel@natur.cuni.cz

Basic keywords:	01	Human papillomavirus
	02	Cancer immunotherapy
	03	DNA vaccine
	04	Gene gun
	05	Tumor escape mechanism
	06	MHC class I downregulation
	07	CRISPR/Cas9 system
	08	Immune checkpoint blockade
	09	Fusion gene
	10	Intratumoral heterogeneity
	11	Plasmacytoid dendritic cell

Research areas:	01	Cancer immunotherapy
	02	Tumor escape mechanisms
	03	Intratumoral heterogeneity
	04	Viruses and plasmacytoid dendritic cells
Main objectives:	01	Experimental immunotherapy for tumors associated with human papillomaviruses
	02	Enhancement of combined immunotherapy of tumors with reduced MHC class I expression
	03	Development of mouse tumor models for analysis of various aspects of anti-tumor immunity
	04	Examination of spatial and temporal heterogeneity of immune reactions in tumor microenvironment

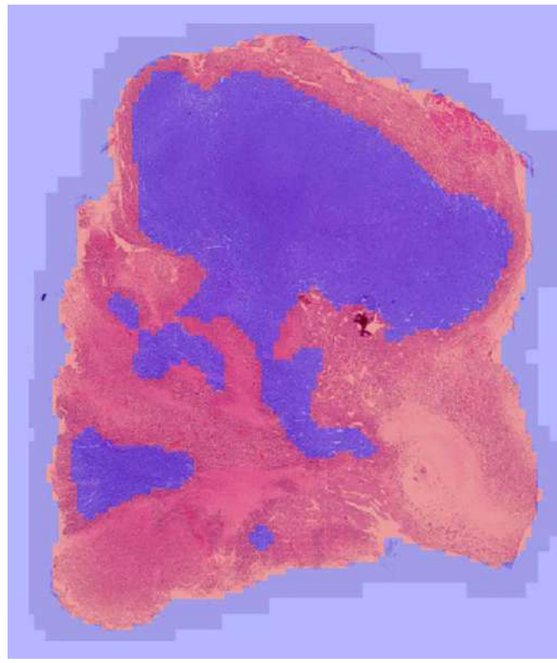


part A



part B

Histological examination of tumor heterogeneity in infiltration by immune cells.
From a mouse inoculated with TC-1/A9 cells and immunized with the PADRE.E7GGG gene, the tumor was taken 17 days after inoculation of the cells, fixed in formalin and embedded in paraffin. Tissue sections were stained with hematoxylin-eosin (part A) or specific antibodies using the Opal 5-Color Fluorescent IHC Kit (part B; blue – DAPI, green – NK/NKT cells, red/violet – macrophages). Necrotization of the tumor was evaluated by the inForm software (part C; red – necrotic tissue, blue – vital tissue).



part C

Identification of Targets for the Diagnostics and Therapy of Tumor Diseases-Associated with Human Viruses

RNDr. Ruth Tachezy, Ph.D., Head of the Lab.

Faculty of Science,
Charles University

Content of the Research

Our projects are mostly focused on the epidemiology and molecular biology of HPVs and the diagnosis and immunoprofiling of tumors induced by HPV or by non-viral factors. Our laboratory has developed several approaches to characterize the viral etiology of tumors. Recently, we have been focusing on head and neck carcinoma (HNC) in which HPV is etiologically involved. In contrast to cervical cancer, which is nearly always high risk HPV-positive, HPV-associated and HPV-independent HNC represents a unique model to study a cancer that is caused by distinct, virus-associated and virus-independent molecular mechanisms. In this "model", the miRNA expression patterns are evaluated in relation to the etiology of the disease and prognosis with the aim to select those miRNAs, that can be considered as possible treatment and diagnostics targets for specific groups of tumors. Furthermore, characterization of the immune response has been shown to be an important prognostic tool in a wide range of carcinomas, potentially even more relevant than the current cancer staging system. The other focus of our research team is on immunoprofiling peripheral blood and HNC-infiltrating immune cells, in addition to HPV status by mass cytometry (CyTOF). This method allows us to detect and quantify a broad spectrum of immune cells both in patients' tumor and peripheral blood samples. Moreover, FFPE tumor samples are characterised by the multispectral immunohistochemistry method, which give us the opportunity to detect immune cells and tumor-associated factors in the microenvironment The results of our studies might help to identify novel targets for therapeutic strategies, including cancer immunotherapy, and they can improve the understanding of the pathophysiology of these virally induced tumors.

We study BKPyV infection in kidney recipients, especially the role of polymorphism, genotypes and type-specific antibody response, together with other clinical characteristics, to BKPyV reactivation and development of BKPyV associated diseases. The research might help to improve patient's management and long-term survival. Furthermore, our interest is on Merkel cell polyomavirus, which is associated with a rare aggressive skin neuroendocrine tumor Merkel cell carcinoma.

Potential for Cooperation

For our work, it is essential that we work very closely with clinicians. We have long experience with analyzing a variety of clinical samples both for consequent molecular based methods as well as for serological assays. We have experience with running clinical trials. Recently, we have adopted new methods for tumor immunoprofiling. The results of our studies can be possibly used for the development of diagnostic assays and potentially as targets for therapeutic strategies including immunotherapy.

2018 The role of miRNAs in virus-mediated oncogenesis. Vojtěchová Z, Tachezy R. Int J Mol Sci, 2018, 19 (4): E1217.

2014 High level of Tregs is a positive prognostic marker in patients with HPV-positive oral and oropharyngeal squamous cell carcinomas. Lukesova E, Boucek J, Rotnaglova E, Salakova M, Koslabova E, Grega M, Eckschlager T, Rihova B, Prochazka B, Klozar J, Tachezy R. Biomed Res Int. 2014: 303929, 2014.

2013 Human papillomavirus Type-Specific Prevalence in the Cervical Cancer Screening Population of Czech Women. Tachezy R, Smahelova J, Kaspirkova J, Salakova M. Plos One, 2013, 8, 11, e79156.

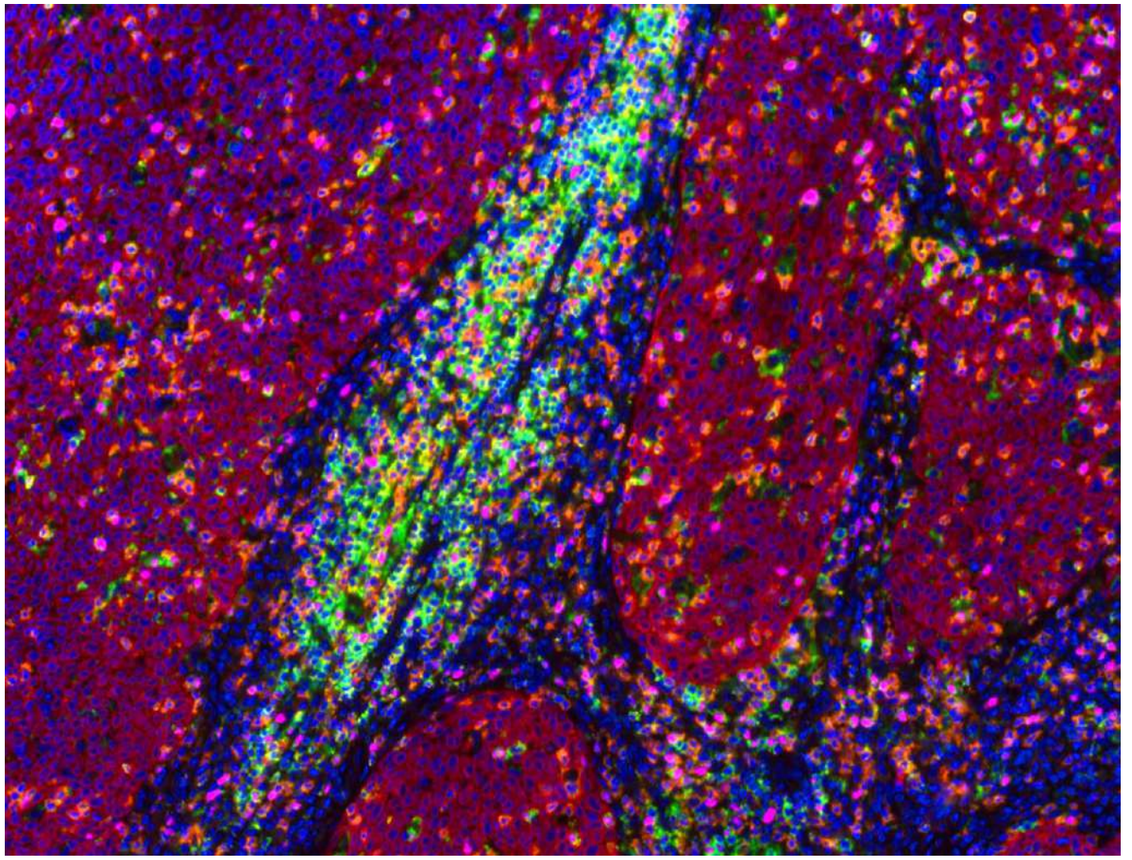
2013 Markers of HPV infection and survival in patients with head and neck tumors. Koslabova E, Hamsikova E, Salakova M, Klozar J, Foltynova E, Salkova E, Rotnaglova E, Ludvikova V, Tachezy R. Int J Cancer, 133, 8, 1832-9, 2013.

2011 HPV infection in tonsillar cancer: prognostic significance and clinically relevant markers. Rotnaglova E, Tachezy R, Salakova M, Prochazka B, Koslabova E, Vesela E, Ludvikova V, Hamsikova E, Klozar J. Int J Cancer, 129, 101, 2011.

Contact person:	RNDr. Ruth Tachezy, Ph.D.
	e-mail: ruth.tachezy@natur.cuni.cz

Basic keywords:	01	Virus
	02	Cancer
	03	DNA
	04	RNA
	05	Antibodies
	06	Immunity
	07	Tumor
	08	Microenvironment
	09	Prognosis
	10	Integration
	11	miRNA
	12	Diversity
	13	Next generation sequencing
	14	Virome

Research areas:	01	The association of viruses with human neoplasia's: virology, epidemiology, molecular biology, immunology
	02	Virome analyses
Main objectives:	01	Identification of the markers of virally induced tumors with a focus on miRNAs
	02	Identification of prognostic immune markers specific for tumors of viral and non-viral etiology
	03	Characterization of tumor microenvironment by expression profiling of mRNAs and detection of tumor-infiltrating lymphocytes
	04	Study of the role of variability of human polyomaviruses in the severity of disease
	05	Analyses of honeybee *(Apis mellifera)* virome and interaction of the virome with other pathogens and environmental factors



Analysis of tumor infiltrating cells by fluorescent multispectral Immunohistochemistry method (fIHC) in FFPE tumor tissue.

Study of Vaccinia Virus Interactions with the Host and Reactivation of Latent HIV-1

MUDr. Zora Mělková, Ph.D., Head of the Lab.

First Faculty of Medicine,
Charles University

Content of the Research

The primary focus of our research group is to study virus-host interactions, namely interactions of HIV-1, vaccinia virus and SARS-CoV-2 with the host cell and organism. The results of our research should provide the basis for new therapeutic approaches towards selected viral infections.

HIV/AIDS is an incurable infection affecting over 35 million people world-wide. The available antiretroviral drugs can only control this infection, but they are unable to provide a cure, i.e. to eliminate HIV-1 from the human organism. The main obstacle in curing HIV-1 is the presence of the reservoir cells harboring latent HIV-1. To address this issue, we aim to identify, characterize and develop redox-modulating agents with the ability to reverse HIV-1 latency and to decrease the size of the HIV-1 latent reservoir. We have described the HIV-1 latency-reversing properties of heme arginate and determined the effects of its metabolites on HIV-1. Furthermore, we study the effects of heme arginate in vivo, after its administration to HIV+ patients. Based on our results, we propose that heme argiante can serve as a new agent for curing HIV-1.

Since the beginning of SARS-CoV-2 pandemic, we have provided grounds for and participated in the laboratory diagnostics of this infection. Simultaneously, we have started to explore new possibilities to inhibit virus growth and to treat COVID-19 in patients, with the main focus on heme arginate as well as on searching for new antivirals effective against SARS-CoV-2.

The evergreen topic of our laboratory is vaccinia virus-related research. Contrary to common belief, the need to understand the pathogenesis of poxvirus infection and post-vaccination complications, as well as to develop safe vaccination vectors and to provide drugs effective against poxviruses, still remains. In recent years, we have been focused on responses of the atopic organism towards infection with vaccinia virus. We have developed our own model of eczema vaccinatum in atopic Nc/Nga mice and used it to characterize immune responses towards vaccinia virus strain WR, Dryvax and non-replicating MVA. Within this atopic model, we are continuing to study the dysregulated immune responses using recombinant vaccinia viruses expressing distinct genes.

Potential for Cooperation

We are open to prospective Ph.D. students and to any type of collaboration with researchers interested in the study of virus-host interactions and in the development of strategies to treat and cure viral infections.

2020 Transcripts of vaccinia virus postreplicative genes do not contain a 5' methylguanosine cap. Vopalensky V, Sykora M, Melkova Z, Masek T, Pospisek M. bioRxiv preprint, version posted July 15, 2020. doi: 10.1101/2020.07.15.204867

2018 Expression of TIM-3 on Plasmacytoid Dendritic Cells as a Predictive Biomarker of Decline in HIV-1 RNA Level during ART. Font-Haro A, Janovec V, Hofman T, Machala L, Jilich D, Melkova Z, Weber J, Trejbalova K, Hirsch I. Viruses 2018; 10(4), 154-167.

2017 Effects of heme degradation products on reactivation of latent HIV-1. Shankaran P, Madlenakova M, Hajkova V, Jilich D, Svobodova I, Horinek A, Fujikura Y, Melkova Z., Acta Virol. 2017;61(1):86-96.

2017 Current views on HIV-1 latency, persistence, and cure. Melkova Z., Shankaran P, Madlenakova M, Bodor J. Folia Microbiol (Praha). 2017 Jan;62(1):73-87.

2016 Protein expression from unintegrated HIV-1 DNA introduces bias in primary in vitro latency models. Bonczkowski P, De Scheerder MA, Malatinkova E, Borch A, Melkova Z, Koenig R, De Spiegelaere W, Vandekerckhove L. Sci Rep. 2016 Dec 2;6:38329.

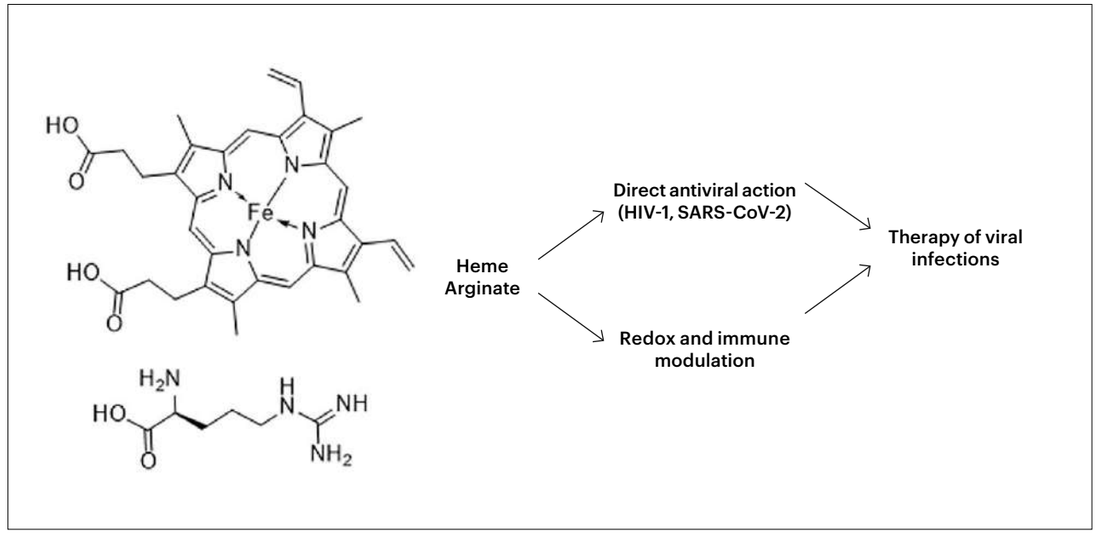
2014 Development of Eczema Vaccinatum in Atopic Mouse Models and Efficacy of MVA Vaccination against Lethal Poxviral Infection. Knitlova J, Hajkova V, Voska L, Elsterova J, Obrova B, Melkova Z. PLoS One. 2014 Dec 8;9(12):e114374.

2011 Heme arginate potentiates latent HIV-1 reactivation while inhibiting the acute infection. Shankaran P, Vlkova L, Liskova J, Melkova Z. Antiviral Res. 2011 Dec;92(3):434-46.

Contact person:	MUDr. Zora Mělková, Ph.D.
	e-mail: zora.melkova@lf1.cuni.cz

Basic keywords:	01	Virus-host interactions
	02	HIV-1
	03	SARS-CoV-2
	04	Vaccinia virus
	05	Redox-modulation
	06	Antiviral therapy
	07	Heme arginate

Research areas:	01	Virus-host interactions
	02	HIV-1, SARS-CoV-2, vaccinia virus
	03	Redox-modulating and antiviral agents
Main objectives:	01	To characterize the effects of heme arginate on the reactivation of latent HIV-1 and on the size of the latent reservoir
	02	To define new, redox-based approaches towards an HIV-1 cure
	03	To find and develop new agents for therapy of SARS-CoV-2
Patent applications:	01	Use of heme arginate for the manufacture of a medicinal product for the treatment of infection with beta coronaviruses. Mělková Z., Martásek P. Appl. No. PV 2020-437, submitted July 30, 2020.
	02	Use of heme arginate for the manufacture of a medicinal product to reduce the size of the HIV-1 latent reservoir in vivo. Mělková Z, Jilich D, Madleňáková M, Martásek P. Appl. No. PV 2017-839, submitted Dec 22, 2017.



Heme arginate, a human hemin containing compound approved for treatment of acute attacks of porphyria, reveals a potential for therapeutic use in distinct viral infections.

Genomics of Eukaryotes and Lateral Gene Transfer
Doc. Mgr. Vladimír Hampl, Ph.D., Head of the Lab.

Faculty of Science,
Charles University

Content of the Research

We are a research team based at Charles University in Prague. We are evolutionary protistologists, which means our interest is nothing less than the origin and evolution of eukaryotic life. The central theme of our research group is the study of organelles, in particular mitochondria and plastids, their formation by endosymbiosis, simplification and loss. The research is focused on two types of organelles. The first is plastids of euglenophytes, derived from prasinophyte alga and has three envelope membranes. In euglenophytes, a light perceiving eyespot of an unclear evolutionary origin is present in these organisms. However, euglenophytes are still able to survive in darkness by switching temporarily to heterotrophy; this feature has enabled the origin of several secondarily osmotrophic species with non-photosynthetic colorless plastids. Our laboratory is focused on understanding their metabolic functions, protein import, origin of membranes and the role of horizontal gene transfer in their establishment.

The second is mitochondrial derivatives in microaerophilic protists from groups of Preaxostyla, archamoebae and retortamonads. Preaxostyla, living exclusively in oxygen-depleted environments, are one of the least studied protist lineages. Here we are interested in the function of these organelles, the evolution of their anaerobic metabolism and the role of horizontal gene transfer in it, the type and mechanisms of FeS cluster biogenesis and the circumstances of mitochondrial loss in oxymonads. Both of these areas of research provide insight into organelle origin and evolution of the organelles' structure, molecular biology, transport, targeting, biogenesis, genome composition, molecular genetics mechanisms and biochemical pathways. We also study the phenomenon of lateral gene transfer, which plays an important role in some of these processes.

Potential for Cooperation

The team works closely with the following researchers at domestic and foreign laboratories outside BIOCEV; Dr. Marek Eliáš at the University of Ostrava, Dr. Ivan Čepička at Charles University, Dr. Julius Lukeš and Dr. Martin Kolínsko at the University of South Bohemia, Dr. Anna Karnkowska at the University of Warsaw, Dr. Andrew Roger and Dr. Alastair Simpson at Dalhousie University, Dr. Joel Dacks at the University of Edmonton, Dr. Juraj Krajčovič at Komenskeho University in Bratislava, Dr. Patrick Keeling at the University of British Columbia, Dr. Thomas Richards at the University of Exeter, Dr. Frederic Barras at the University of Marseiles, and Dr. Roland Lill at the University of Marburg.

2020 Tomečková L, Tomčala A, Oborník M, Hampl V. The Lipid Composition of Euglena gracilis Middle Plastid Membrane Resembles That of Primary Plastid Envelopes. Plant Physiol. 2020 Dec;184(4):2052-2063. doi: 10.1104/pp.20.00505.

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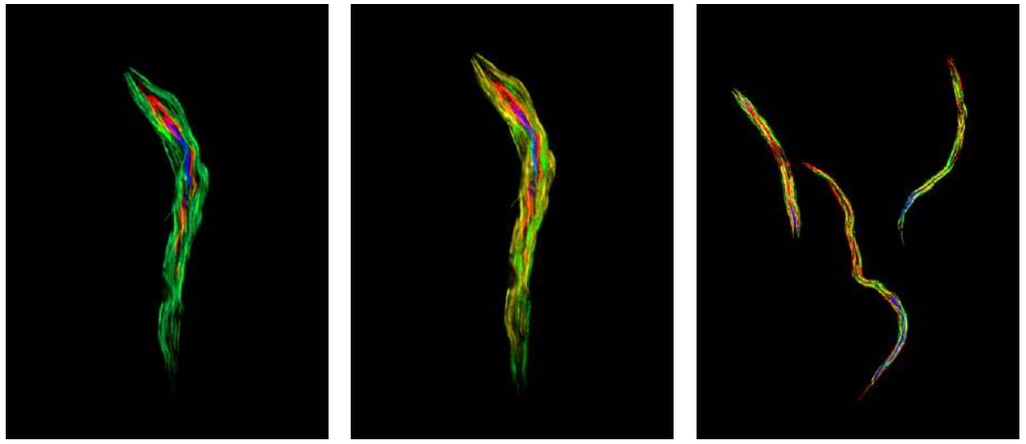
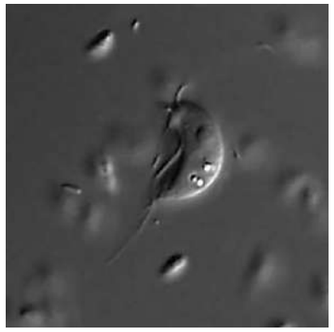
Contact person:	doc. Mgr. Vladimír Hampl, Ph.D.
	e-mail: vladimir.hampl@natur.cuni.cz

Basic keywords:	01	Mitochondrion
	02	Plastid
	03	Endosymbiosis
	04	Protists
	05	Metamonada
	06	Euglenida
	07	Genomics
	08	Transcriptomics
	09	Phylogenetics

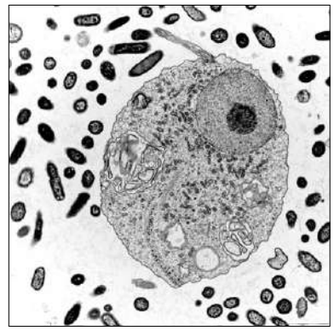
Research areas:	01	Genomes of protists
	02	Cell biology of protists focused on reduced mitochondria and plastids
	03	Phylogeny and diversity of protists
Main objectives:	01	Function of mitochondrial derivates in anaerobic eukaryotes and the circumstances of their loss in oxymonads
	02	Function, biogenesis and origin of plastids in euglenophytes



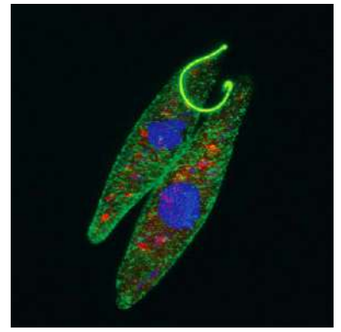
Protozoan *Streblomastix strix* thriving in the gut of a termite *Zootermopsis angusticollis* grows on its surface a population of rod-shaped *Bacteroidetes bacteria*, which help to digest wood biomass. Bacterial species are distinguished by specific FISH probes with different colours.



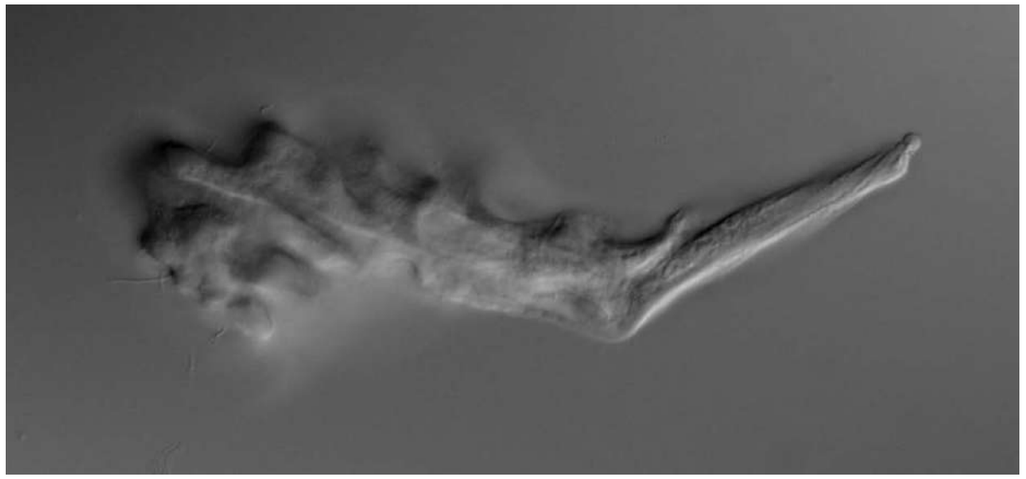
A heterotrophic free-living flagellate *Paratrimastix pyriformis*, which grazes on bacteria using a conspicuous ventral grove through which passes a flagellum.



Electron micrograph of a microaerophilic flagellate *Monocercomonoides* sp. showing nucleus, digestive vacuoles, endoplasmic reticulum, flagellum and microtubular cytoskeleton but no mitochondrion, which was lost in this group.



Fluorescence staining of two cells of a freshwater alga *Euglena gracilis* with an elaborated cytoskeleton under its surface (microtubules in green), a flagellum (green), chloroplasts (red) and spiralised chromosomes (blue).



Termite *Reticulitermes flavipes* hosts a giant protozoan *Pyrsonympha major,* whose role in the gut has yet to be elucidated.

Structure and Function of Membrane Receptors
Prof. Ladislav Vyklický, Head of the Lab.

The Institute of Physiology
of the Czech Academy of Sciences

Content of the Research

Ion channels are transmembrane proteins that allow the flow of ions across membranes and play a key role in neuronal excitability. We combine state-of-the-art electrophysiology, molecular biology with biochemistry, and structural biology to study the structure and molecular mechanisms of the activation of ionotropic glutamate receptors (iGluRs) and transient receptor potential cation channels (TRP).

The topic of iGluRs involves mainly the subfamily of N-methyl-D-aspartate receptors (NMDAR), focusing on the structure-function relationship and the analysis how NMDAR activity is regulated by neurosteroids. The results could help elucidate the role of de-novo mutations in genes encoding NMDARs in the emergence of the pathogenesis of neurodevelopmental disorders and to search for effective ways of treatment. The NMDARs are studied on the genetic level (genetic variation of the NMDAR gene complex, analysis of regulatory elements of genes), the receptor level (single-channel and whole-cell recording), the cellular level (receptor expression, trafficking and synaptic localization), and the system level (knock-in mouse model with inserted mutations in the GRIN2B gene and knock-out zebrafish model with deleted NMDAR subunits.

The main research interest of the pain group is to study molecular and cellular mechanisms of pain. Our goal is to improve analgesic therapy especially for chronic pain conditions, neuropathic, chemotherapy-induced and cancer-related pain. Our experimental work is concentrated on the study of the modulation of nociceptive synaptic transmission at the spinal cord level, which is the first relay center between the periphery and the higher brain areas. Our focus is on the role of TRPV1 and purinergic P2X receptors, cytokines and neuroinflammation. In our research, we use mainly electrophysiological, optogenetic, immunohistochemical and behavioral methods. For more information, contact Jiri Palecek (palecek@fgu.cas.cz).

Potential for Cooperation

We are interested in cooperation directed towards understanding the mechanisms and treatment of neurodevelopmental disorders and pain.

2020 Hrcka Krausova B, Kysilov B, Cerny J, Vyklicky V, Smejkalova T, Ladislav M, Balik A, Korinek M, Chodounska H, Kudova E, Vyklicky L (2020) Site of Action of Brain Neurosteroid Pregnenolone Sulfate at the N-Methyl-D-Aspartate Receptor. Journal of Neuroscience 40:5922-5936.

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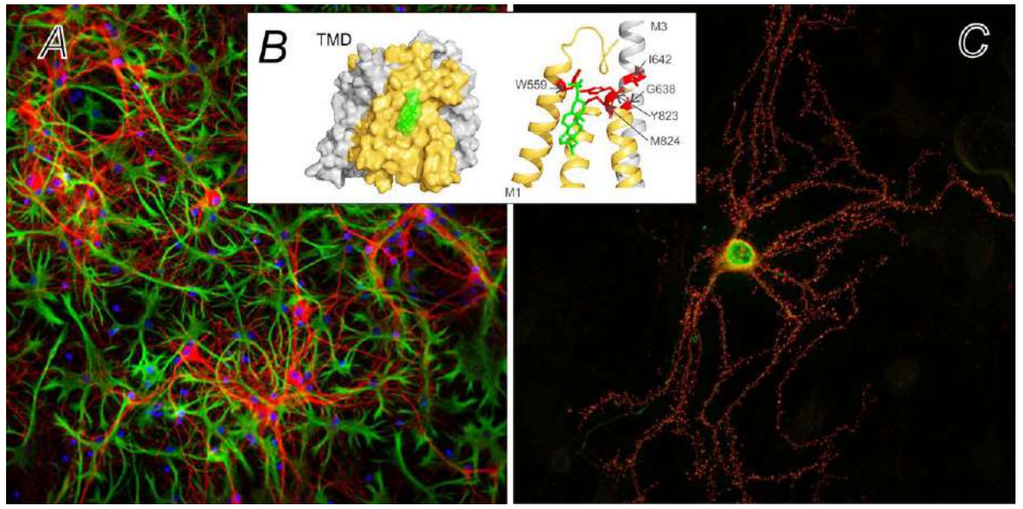
2018 Vyklicky V, Krausova B, Cerny J, Ladislav M, Smejkalova T, Kysilov B, Korinek M, Danacikova S, Horak M, Chodounska H, Kudova E, Vyklicky L (2018) Surface Expression, Function, and Pharmacology of Disease-Associated Mutations in the Membrane Domain of the Human GluN2B Subunit. Frontiers in Molecular Neuroscience 11:110.

2018 Nerandžič, Vladimír - Mrózková, Petra - Adámek, Pavel - Špicarová, Diana - Nagy, I.- Paleček, Jiří. Peripheral inflammation affects modulation of nociceptive synaptic transmission in the spinal cord induced by N-arachidonoylphosphatidylethanolamine. British Journal of Pharmacology 2018, 175, 12, p. 2322-2356.

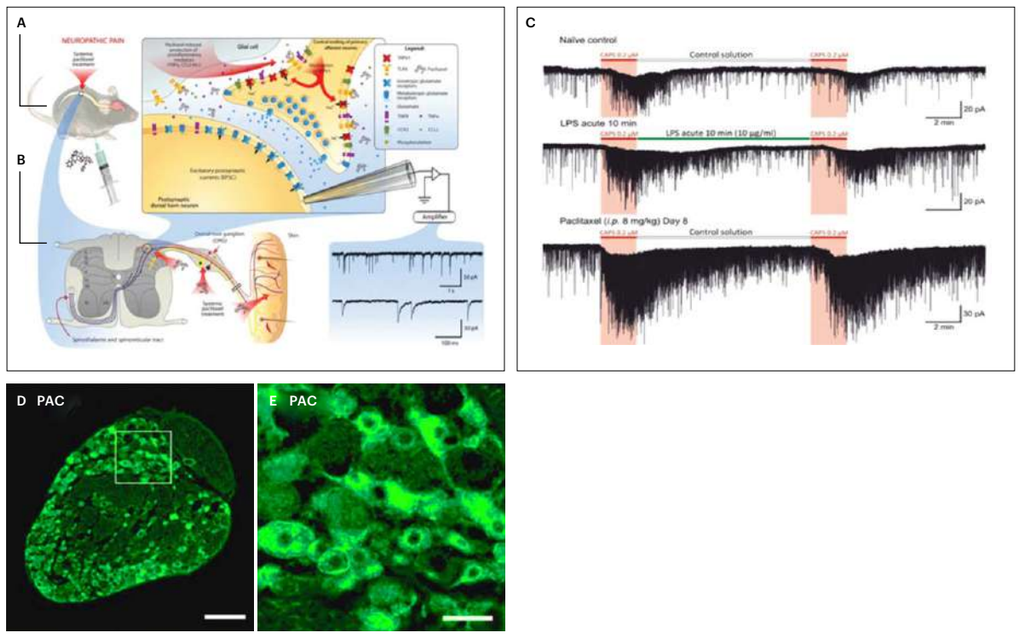
Contact person:	Prof. Ladislav Vyklický
	e-mail: ladislav.vyklicky@fgu.cas.cz

Basic keywords:	01	NMDA receptor
	02	*De-novo* mutations
	03	Neurodevelopmental disorders
	04	NMDAR gene complex
	05	Pain
	06	Neuropathy
	07	TRPV1
	08	Neuroinflammation

Research areas:	01	Pharmacology and the structure of NMDA receptors
	02	Mutations and biogenesis of ionotropic glutamate receptors
	03	The role of TRP receptors in pain modulation
Main objectives:	01	Characterize the molecular mechanism(s) that control the activity of neurosteroids at NMDA receptors
	02	Study synthetic analogs for their prospective effect to prevent NMDAR channelopathy
	03	Understand molecular and system consequences of mutations in GRIN genes
	04	Characterize genetic variations of the glutamate receptor gene complex presented in neurodevelopmental disorders
	05	Study structure-function mechanisms controlling NMDAR opening and closing
	06	Study peripheral and central mechanisms of neuropathic pain in different models
	07	Analyse the role of TRPV1 receptors in the modulation of nociceptive synaptic transmission in the spinal cord
	08	Characterize the role of spinal cord inhibitory system for the development of chronic pain conditions
	09	Define neuroinflammatory changes involved in neuropathic pain



A, Cell culture of primary hippocampal neurons (14 days in vitro). Neurons are immunostained with tubulin (red) and glia with GFAP (green). B, Structure of the transmembrane domain (TMD) of the NMDA receptor and PE¬S (20-oxo-pregn-5-en-3β-yl sulfate) binding. C, Confocal image of a primary hippocampal neuron expressing GluN2A subunits (red) in synapses. The green signal represents intracellularly localized GluN2A.



Chemotherapy (paclitaxel) induced neuropathic pain is partially mediated by spinal TRPV 1 receptors (A, B, C), leading to their increased responsiveness (C) that is prevented by the inhibition of increased PI3K phosphorylation in DRG neurons (D, E).

Mitochondrial Structure and Gene Expression

RNDr. Petr Ježek, DrSc., Head of the Lab.

The Institute of Physiology
of the Czech Academy of Sciences

Content of the Research

Our laboratory focuses on the role of mitochondria in physiological and pathophysiological processes of the cell and organism. The main objective is to elucidate the structure and physiology of mtDNA nucleoids using 3D super-resolution microscopy. The major methodology used is 3D immunocytochemistry by direct stochastic optical reconstruction microscopy (dSTORM) or the expression of nucleoid protein conjugates with photoconvertible fluorescent proteins. Moreover, dSTORM probes are developed against specific mtDNA sequences that will be visualized. For example, the visualization of D-loop sequences counts the number of mtDNA molecules per nucleoid. Since mtDNA is reduced in numerous pathologies, studies of e.g. diabetic pancreatic beta cells with reduced mtDNA down to 25% will help to elucidate type-2 diabetes development.

We are trying to develop the mitochondrial import of exogenous RNA for sequence-specific silencing of mitochondrially encoded genes. Alternatively, we want to employ the mitochondrial RNA import of wild-typed RNA for the substitution of mutated mtDNA in cybrid cell lines derived from patient cells with mtDNA mutations. The above mentioned goal of our department is to substitute mtDNA loss in pancreatic beta cells during the development of diabetes. We are using super-resolution microscopy for calculating mitochondrial nucleoid volume together with RT-PCR for mtDNA copy number quantification. The most recent project addressed in our department was focused on mtDNA replication. We are investigating several nucleoid proteins and their impact on replication.

Potential for Cooperation

We would like to establish external collaboration within the microscopic field. We have mastered PALM–dSTORM microscopy and we want to continue working on STED microscopy. The department possesses a Bi-PLANE FPALM super-resolution microscope at the FGU CAS. Concerning applied research, we have also developed novel drug carriers to transport specific anticancer drugs called photosensitizers into cancer tissues. Our findings have been patented in the Industrial Property Office of the Czech Republic. Our laboratory has been working with several professors on different research topics; with Prof. MD František Saudek DrSc, from IKEM Diabetes Centre on the diabetes project; Prof. Daniel Bogenhagen MD, from Stony Brook University NY, USA, on the mitochondrial DNA replication project and with Dr. Joerg Bewersdorf, from the Department of Cell Biology, Yale University, USA, on super-resolution microscopy development.

2020 Kahancová A, Sklenář F, Ježek P, Dlasková A. Overexpression of native IF1 downregulates glucose-stimulated insulin secretion by pancreatic INS-1E cells Sci.Rep. 2020; Jan 31;10(1):1551.

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2020 Plecitá-Hlavatá L, et al., Jezek P. Mitochondrial superoxide production decreases upon glucose-stimulated insulin secretion in pancreatic β-cells due to decreasing mitochondrial matrix NADH/NAD+ ratio. Antioxid Redox Signal. 2020 Oct 20;33(12):789-815.

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2019 Dlasková A, Špaček T, Engstová H, Špačková J, Schröfel A, Holendová B, Smolková K, Plecitá-Hlavatá L, Ježek P. Mitochondrial cristae narrowing upon higher 2-oxoglutarate load Biochim Biophys Acta. 2019; 1860(8):659-678. doi: 10.1016/j.bbabio.2019.06.015.

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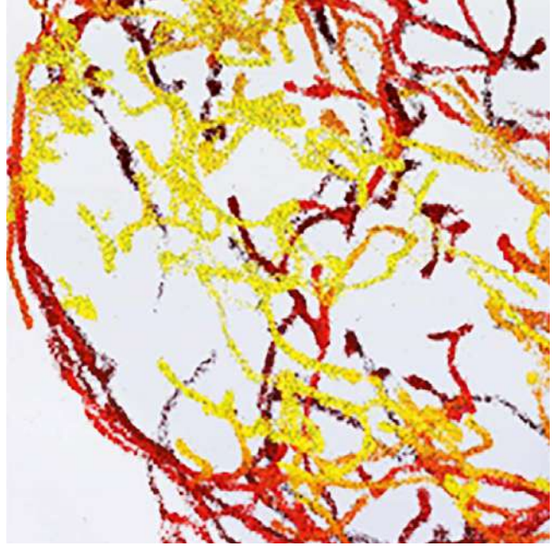
2019 Špaček T, Pavluch V, Alán L, Capková N, Engstová H, Dlasková A, Berková Z, Saudek F, Ježek P. Nkx6.1 decline accompanies mitochondrial DNA reduction but subtle nucleoid size decrease in pancreatic islet beta-cells of diabetic Goto Kakizaki rats. Sci.Rep. 2017 Nov 15;7(1):15674. doi: 10.1038/s41598-017-15958-6.

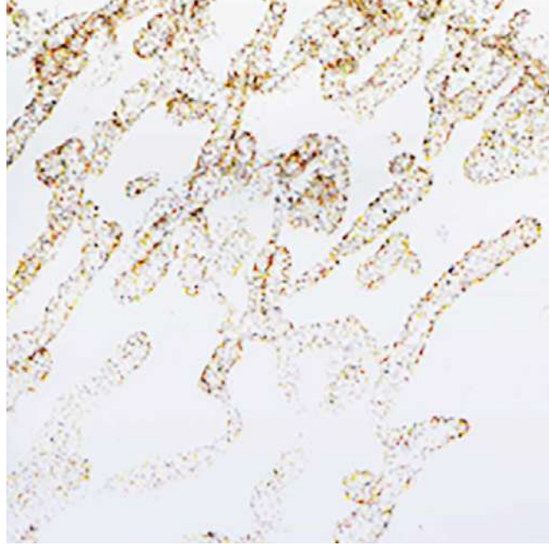
Contact person:	RNDr. Petr Ježek, DrSc.
	e-mail: petr.jezek@fgu.cas.cz

Basic keywords:	01	Mitochondria
	02	Mitochondrial nucleoids
	03	Tfam
	04	Mtssb
	05	Diabetes
	06	Mtdna copy number
	07	Mitophagy
	08	Fish
	09	Rna mitochondrial import

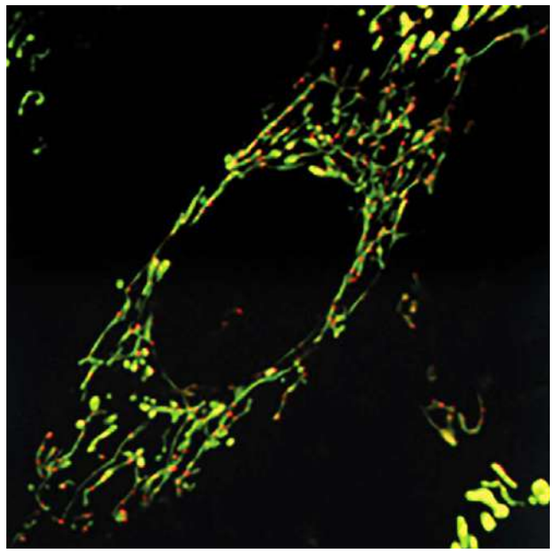
Research areas:	01	Mitochondrial DNA biology
Main objectives:	01	Study of the mitochondrial network and DNA nucleoid biology using 3D superresolution microscopy, study of mitochondrial DNA in diabetes mellitus



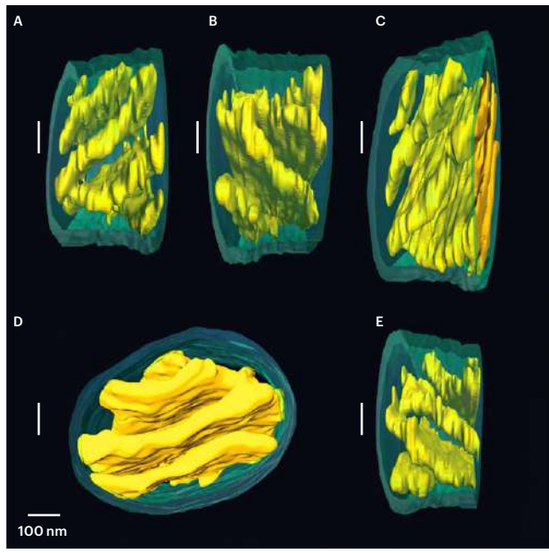
3D super-resolution image of the mitochondrial network in HepG2 cells, with the matrix stained by Eos2



3D super-resolution image of the mitochondrial network in HepG2 cells, with the outer membrane having overexpressed FIS1-Eos2



2D confocal-microscopic image of matrix-addressed GFP with mtDNA nucleoids visualized by TFAM immunocytochemistry



Focused ion beam/scanning electron microcopy of mitochondrial cristae in HepG2 cells (Dr. A. Dlasková, imaged in Biocev core facility)

Immunity and Cell Communication
Mgr. Peter Dráber, Ph.D., Head of the Lab.

First Faculty of Medicine,
Charles University

Content of the Research

The immune system has evolved to protect the body from invading pathogens and tumor growth. Yet aberrant activation of immune responses can lead to severe autoimmune diseases. In order to properly regulate the activation, propagation and termination of immune responses, individual cells of the immune system must communicate with each other and provide information about ongoing inflammation. A major means of cellular communication is the production of cytokines. Cytokines are small soluble proteins that are secreted by one cell and detected by specialized receptors present on target cells. Proper sensing of these cytokines enables immune cells to activate an adequate immune reaction. For example, cytokine TNF is critical in orchestrating the immune system to fight infection, but in pathological situations, it is responsible for the progression of rheumatoid arthritis. Similarly, cytokine IL-17 can organize the immune system to protect the body against yeast infections, but is also highly involved in the progression of psoriasis.

Our research aims to uncover molecular mechanisms that show how crucial pro-inflammatory cytokines signal and identify new therapeutic targets to shape immune responses. We employ mass-spectrometry to analyze the composition of selected cytokine receptors in order to identify new components of these signaling complexes. We use the CRISPR/Cas9 approach to prepare cell lines deficient in selected proteins. We study cell signaling responses using biochemical methods, flow cytometry, microscopy and RNA sequencing. Proteins, which are important regulators of the immune system, are further studied using in vivo mouse models. The goal of our research is to identify new molecular mechanisms on how different cytokines enable communication between different immune cells and based on this knowledge, identify new possible approaches to modulate the activity of the immune system.

Potential for Cooperation

Our expertise is in the analysis of signaling complexes via mass-spectrometry and biochemical methods, the preparation of knockout cell lines and analysis of in vivo models of TNF- and IL-17-mediated autoimmunity.

2020 Draberova H, Janusova S, Knizkova D, Semberova T, Pribikova M, Ujevic A, Harant K, Knapkova S, Hrdinka M, Fanfani V, Stracquadanio G, Drobek A, Ruppova K, Stepanek O, Draber P. Systematic analysis of the IL-17 receptor signalosome reveals a robust regulatory feedback loop. EMBO J. 2020 Sep 1;39(17):e104202.

2018 Peltzer N, Darding M, Montinaro A, Draber P, Draberova H, Kupka S, Rieser E, Fisher A, Hutchinson C, Taraborrelli L, Hartwig T, Lafont E, Haas TL, Shimizu Y, Böiers C, Sarr A, Rickard J, Alvarez-Diaz S, Ashworth MT, Beal A, Enver T, Bertin J, Kaiser W, Strasser A, Silke J, Bouillet P, Walczak H. LUBAC is essential for embryogenesis by preventing cell death and enabling haematopoiesis. Nature. 2018 May;557(7703):112-117.

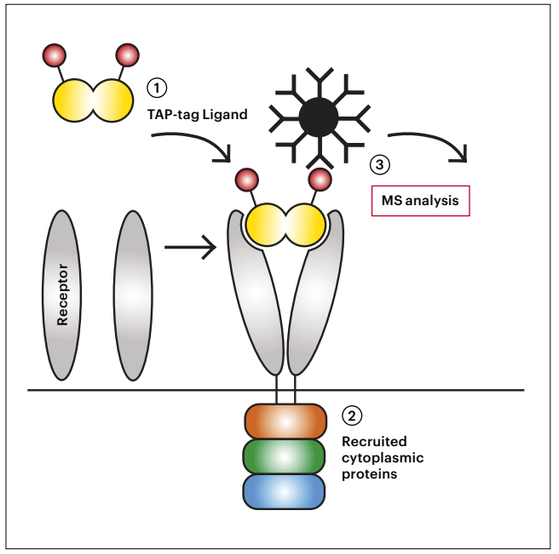
2018 Lafont E*, Draber P*, Rieser E*, Reichert M*, Kupka S, de Miguel D, Draberova H, von Mässenhausen A, Bhamra A, Henderson S, Wojdyla K, Chalk A, Surinova S, Linkermann A, Walczak H. M1-ubiquitin- and NEMO-recruited TBK1/IKKε prevent TNF-induced cell death by RIPK1 phosphorylation. Nature Cell Biology. 2018 Dec;20(12):1389-1399.

2015 Draber P*, Kupka S*, Reichert M*, Draberova H, Lafont E, de Miguel D, Spilgies L, Surinova S, Taraborrelli L, Hartwig T, Rieser E, Martino L, Rittinger K, Walczak H. LUBAC-Recruited CYLD and A20 Regulate Gene Activation and Cell Death by Exerting Opposing Effects on Linear Ubiquitin in Signaling Complexes. Cell Rep. 2015 Dec 15;13(10):2258-72.

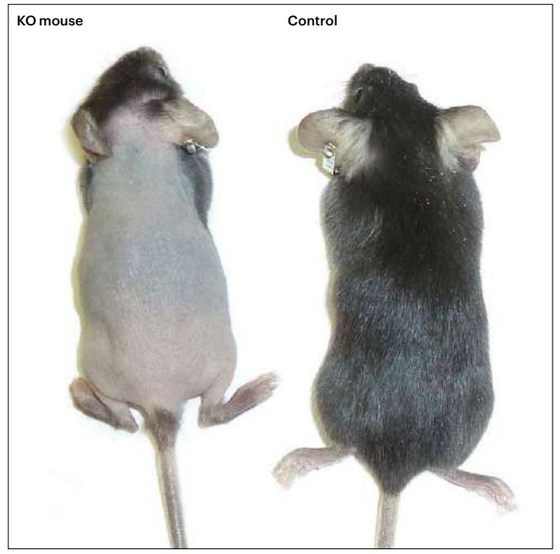
Contact person:	Mgr. Peter Dráber, Ph.D.
	e-mail: peter.draber@lf1.cuni.cz

Basic keywords:	01	Immunity
	02	Inflammation
	03	Autoimmune disorders
	04	Cell communication
	05	Cytokines
	06	Receptor signaling complexes
	07	Signal transduction

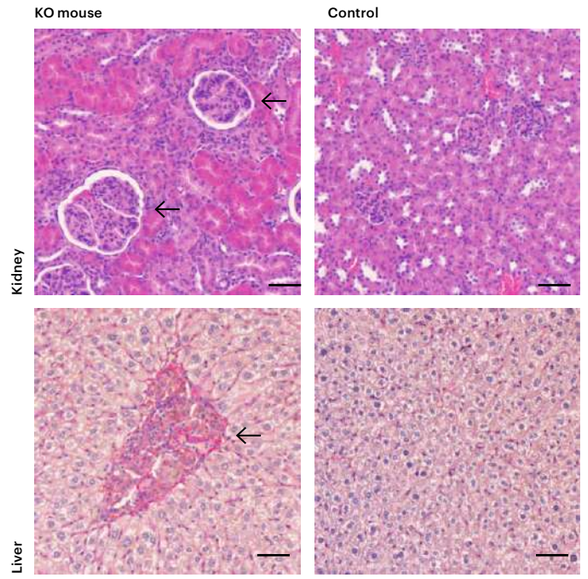
Research areas:	01	Proinflammatory cytokines
	02	Molecular analysis of receptor signaling complexes
	03	Propagation and regulation of cell signaling
	04	In vivo models of autoimmunity and inflammation
Main objectives:	01	Elucidate how different cytokines activate cell responses upon binding to their receptors
	02	Identify potential therapeutic targets that can modulate cellular responses to these cytokines
	03	Identify new approaches how to target these proteins in order to modulate the immune system in vivo



A) Mass-Spec based discovery of new signaling proteins



B) Preparation of animal models



C) Detailed study of phenotypes

Leukocyte Motility
Mgr. Miroslav Hons, Ph.D., Head of the Lab.

First Faculty of Medicine,
Charles University

Content of the Research

An efficient immune response requires cells of the immune system to be at the right place at the right time and depends on their migration and correct positioning in tissues. Our research team examines the mechanisms which enable immune cells to establish motile behavior and we explore how defects in leukocyte motility impact immunity on the system level.

Locomotion of leukocytes is driven by molecules distributed in the environment, such as chemokines or molecules from damaged cells or bacteria. Those chemical signals are recognized by specific receptors on the surface of leukocytes and trigger signaling cascades resulting in the rapid reorganization of the cytoskeleton, morphological changes and motility. Nevertheless, leukocytes migrate in tissues with diverse physical properties. Some tissues might be porous, while others can be dense and difficult to crawl through. Tissues are also often not homogeneous and migrating leukocytes have to be able to avoid physical obstacles to find their way. Thus, leukocytes scanning through the body must be able to read two kinds of signals: chemical - coming from biologically active molecules, and mechanical - from surrounding tissues. We focus on how leukocytes recognize mechanical inputs and what mechano-receptors they use. We also want to understand how mechanical stress influences leukocytes behavior and how leukocyte integrate mechanical and chemical signals.

We have adopted a number of experimental approaches. We use custom-designed mechanical devices and microfabrication to create artificial environments and microfluidic chips with precise geometries to challenge cells with defined mechanical inputs. This we combine with various types of imaging to record and analyze leukocyte behavior and their morphological and cytoskeletal dynamics. To complement our reductionistic approaches, we study the migratory behavior of leukocytes in lymphoid organs and peripheral tissues with intravital imaging.

Potential for Cooperation

We seek for collaborations with theoreticians, biophysicists and chemists to improve our quantitative understanding of our model systems and to develop new ones.. We are also open to collaboration with clinical and preclinical researchers.

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2007 L-selectin-negative CCR7- effector and memory CD8+ T cells enter reactive lymph nodes and kill dendritic cells. Guarda G, Hons M, Soriano SF, Huang AY, Polley R, Martín-Fontecha A, Stein JV, Germain RN, Lanzavecchia A, Sallusto F. Nat Immunol. 2007 Jul;8(7):743-52.

Contact person: Mgr. Miroslav Hons, Ph.D.
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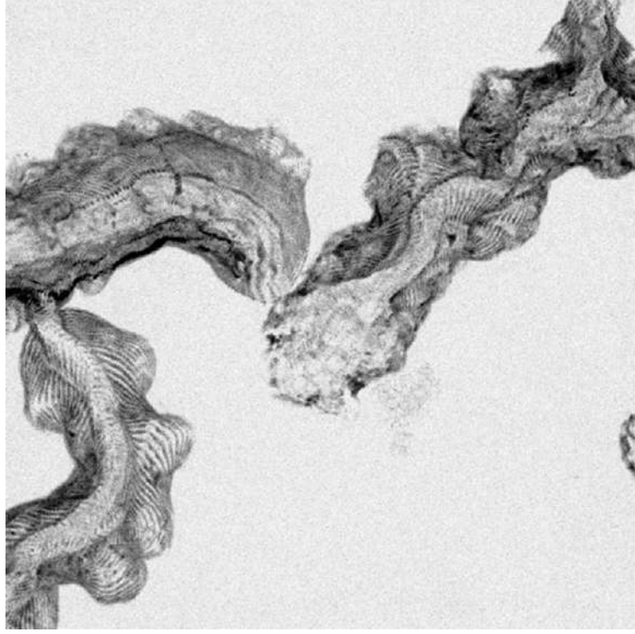
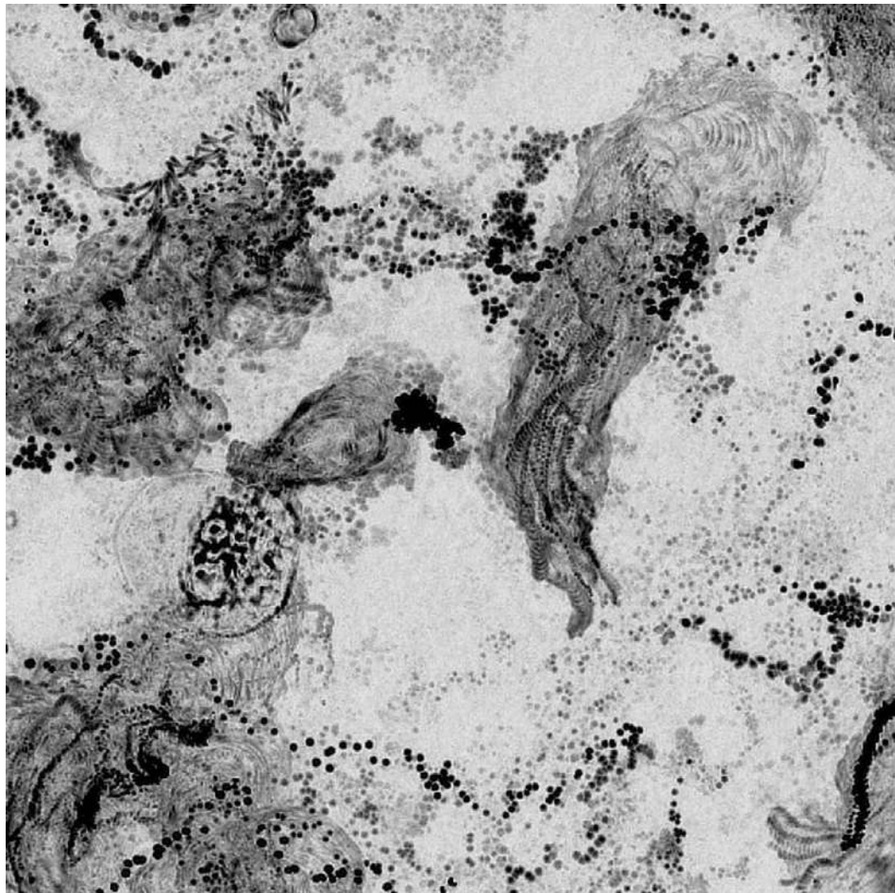
Basic keywords:	01	leukocyte
	02	lymphocyte
	03	motility
	04	migration
	05	mechanobiology
	06	immunity
Research areas:	01	cell migration
	02	mechanobiology
	03	immunology
Main objectives:	01	Determine the role of mechanical stress in leukocyte motility
	02	Determine molecular mechanisms of recognition of mechanical stress in leukocytes.



A time-stack of outlines of migrating T cells

A time-stack of outlines of migrating T cells



Molecular Mechanisms of Axon Guidance
Daniel Rozbesky, Ph.D., Head of the Lab.

Faculty of Science,
Charles University

Content of the Research

We are a newly established group at Biocev. Our research focuses on signalling molecules and cell surface receptors that play central roles in neurobiology and have relevance to translational medicine. Currently, we have a particular interest in studying signalling molecules and mechanisms underlying cell guidance and nervous system wiring. Our efforts are focused on the structural biology and molecular mechanisms of MICAL signalling in cytoskeletal dynamics. MICALs (Molecules Interacting with CasL) are a family of unique signalling molecules that directly bind and disassemble actin filaments and are known to play essential roles in cell processes requiring discrete changes in the cytoskeleton. In the collapsing axon growth cone, MICALs provide a direct link between semaphorins and F-actin collapse. The semaphorins are one of the largest families of axon guidance cues which exert repulsive or attractive effects on axon growth cones through interaction with the plexin family of cell surface receptors.

A growing number of studies implicate MICALs and their signalling pathways in a range of psychiatric and neurological disorders. Although the field has made enormous advances in understanding MICAL function at the level of genetic and cellular experiments, our knowledge of the molecular-level mechanisms of MICAL signalling in cytoskeletal dynamics is still lacking.

Questions that are currently of high interest in the lab are how MICALs precisely turn their activity on and off, and how MICAL activity sculpts the actin cytoskeleton. To address these questions, we employ a hybrid approach of protein crystallography and cryoEM to visualize the high-resolution architecture of these signalling molecules and their supramolecular assemblies. This hybrid approach is also combined with a wide range of methodologies, including protein engineering, advanced light microscopy, mass spectrometry, and cell-based functional assays. Our studies are highly collaborative, with strong links to various local and international collaborators.

Potential for Cooperation

We are always open to new collaborations. Particularly useful would be a collaboration with cellular neuroscientists to support our findings and hypothesis in vivo or in vitro. Also, we are looking for experts with experience in cytoskeletal dynamics to study interactions between MICALs and actin filaments. On the other hand, we are keen to provide our expertise in collaborative projects, in particular, we are happy to provide expertise in protein engineering, biophysics and structural biology.

2020 Rozbesky D, Verhagen MG, Karia D, Nagy GN, Alvarez L, Harlos K, Padilla-Parra S, Pasterkamp RJ, Jones EY. Structural basis of semaphorin-plexin cis interaction. EMBO J. 39: e102926

2020 Rozbesky D, Monistrol J, Jain V, Hillier J, Padilla-Parra S, Jones EY (2020). Drosophila OTK is a glycosaminoglycan-binding protein with high conformational flexibility. Structure. 28: 507-515.

2020 Mehta V, Pang KL, Rozbesky D, Nather K, Keen A, Lachowski D, Kong Y, Karia D, Ameismeier M, Huang J, Fang Y, Del Rio Hernandez A, Reader JS, Jones EY, Tzima E (2020). The guidance receptor Plexin D1 moonlights as an endothelial mechanosensor. Nature. 578: 290-295

2019 Rozbesky D, Robinson RA, Jain V, Renner M, Malinauskas T, Harlos K, Siebold C, Jones EY (2019). Diversity of oligomerization in Drosophila semaphorins suggests a mechanism of functional fine-tuning. Nat Commun. 10: 3691-3703.

2018 Walters LC, Harlos K, Brackenridge S, Rozbesky D, Barrett J, Jain V, O'Callaghan C, Borrow P, Toebes M, Hansen SG, Sacha J, Abdulhaqq S, Greene JM, Fruh K, Marshall E, Picker LJ, Jones EY, McMichael AJ, Gillespie GM (2018) Pathogen-derived HLA bound epitopes reveal broad primary anchor pocket tolerability and conformationally malleable peptide binding. Nat Commun. 9: 3137-3150

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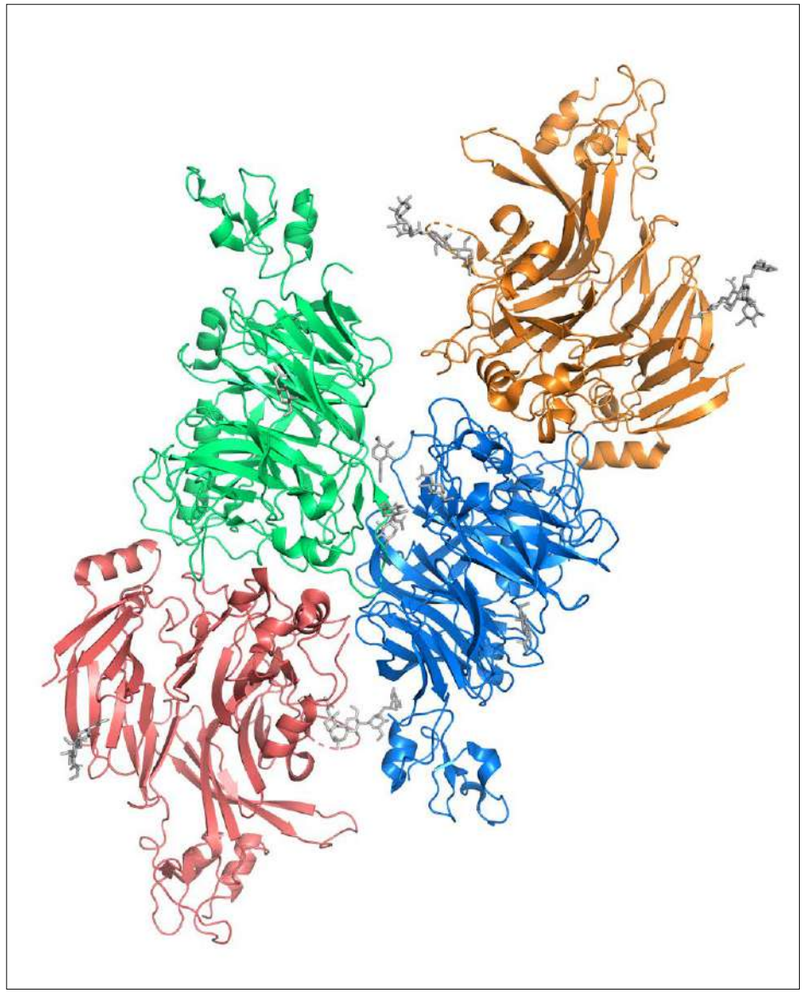
Basic keywords:	01	Axon guidance
	02	MICAL
	03	Semaphorin
	04	Plexin
	05	Guidance cues
	06	Molecular mechanisms
	07	Signal transduction
	08	Cytoskeletal dynamics
	09	Protein interactions
	10	Structural biology

Research areas:	01	Structural biology
	02	Neurobiology
Main objectives:	01	Dissect molecular mechanisms of MICAL activation and autoinhibitiom
	02	Elucidate the interplay between MICAL and F-actin in the regulation of cytoskeletal dynamics



Recently, we determined a crystal structure of semaphorin 1b in complex with its cognate plexin A receptor revealing a molecular mechanism of semaphorin-plexin cis interaction.

Synthetic Biology
Mgr. Klára Hlouchová, Ph.D., Head of the Lab.

Faculty of Science,
Charles University

Content of the Research

Our laboratory is interested in various aspects of protein evolution ranging from protein engineering and synthetic life to astrobiology and the origins of life. Synthetic biology presents tools that we use to study the vast natural and unnatural protein sequence space. We have adapted and developed our methodology to construct combinatorial peptide and protein libraries in silico, in vitro and in vivo to better understand the constraints on protein structure and function evolution.

Using bottom-up approaches of synthetic biology, we synthesize and characterize completely artificial random sequence libraries from reduced or modified amino acid alphabets. Such libraries are characterized in bulk (e.g. to compare the structural/functional propensities of the different alphabets) or used for selections of specific properties. Moreover, upon insertion into model organisms (such as bacteria or yeast), we study the response of biological systems to novel sequences, representing proxies to de novo protein emergence. Top-down approaches are used in our laboratory to reduce or modify extant biological macromolecules. Such projects help us understand what amino acid alphabets are (or were, during the course of evolution) still capable of supporting protein structure/function and the fitness landscape of biological proteins.

Potential for Cooperation

We can provide expertise in protein combinatorial library design and synthesis, cell-free expression and library display methodology. We would welcome collaborations in more applied directions and closer collaboration with peptide chemists and structural biologists. Within collaborative grants, we currently cooperate with the bioinformatics and molecular evolution group of Prof. Erich Bornberg-Bauer (University of Muenster, Germany), protein biophysics and proteomics group of Prof. Stephen Fried (Johns Hopkins University, USA), astrobiologist Prof. Kosuke Fujishima (Earth and Life Science Institute, Tokyo Tech, Japan), the protein interaction group of Prof. Ylva Ivarsson (Uppsala University, Sweden), the chemical biology group of Prof. Florian Hollfelder (University of Cambridge, UK), and the bioinformatics group of Prof. Stephen Freeland (UMBC, USA).

2020 Bornberg-Bauer E, Hlouchova K, Lange A. (2020) Structure and function of naturally evolved de novo proteins. Current Opinions in Structural Biology. In Press

2020 Tretyachenko V, Voráček V, Souček R, Fujishima K, and Hlouchová K. (2020) CoLiDe: Combinatorial Library Design tool for probing protein sequence space. Bioinformatics DOI:10.1093/bioinformatics/btaa804

2019 Vymětal J, Vondrášek J, and Hlouchová, K. (2019). Sequence Versus Composition: What Prescribes IDP Biophysical Properties? Entropy, 21(7), 654.

2017 Tretyachenko V, Vymětal J, Bednárová L, Kopecký V, Hofbauerová K, Jindrová H, Hubálek M, Souček R, Konvalinka J, Vondrášek J, and Hlouchová K. (2017) Random protein sequences can form defined secondary structure and are well-tolerated in vivo. SciRep 7, 15449.

Contact person:	Mgr. Klára Hlouchová
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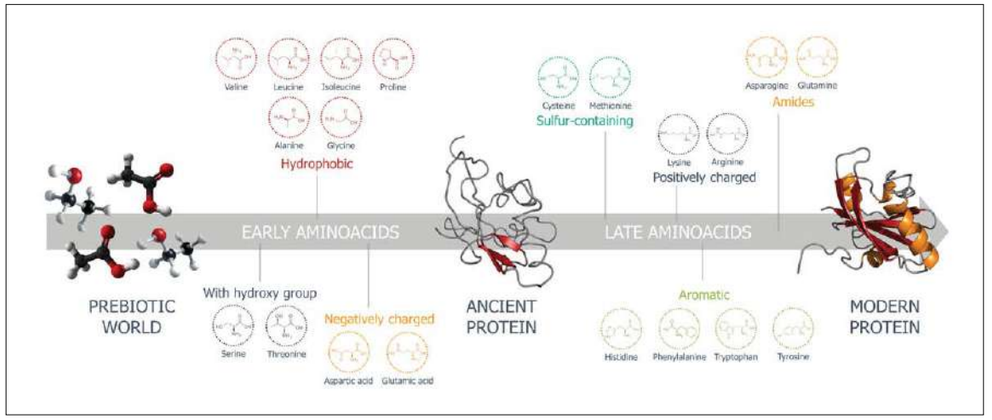
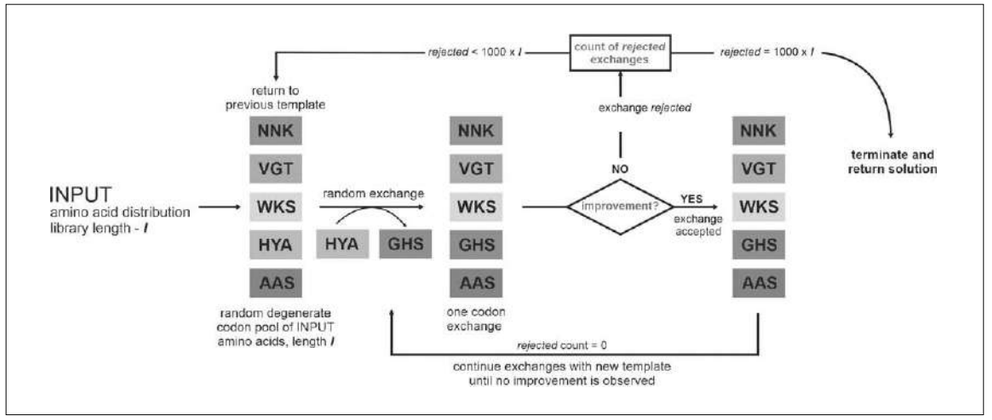
Basic keywords:	01	Protein evolution
	02	sequence space
	03	combinatorial libraries
	04	unnatural amino acids
	05	peptide-RNA interaction
	06	de novo proteins
	07	astrobiology
	08	protein engineering and design

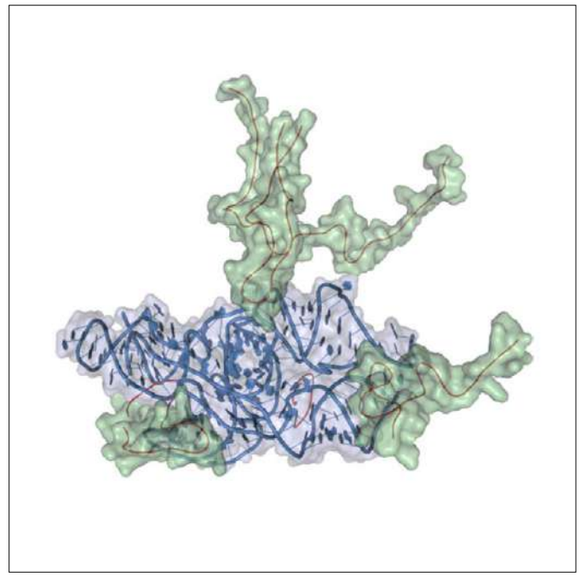
Research areas:	01	Evolution of protein structure/function
	02	Reconstruction and engineering of basic biochemical systems
Main objectives:	01	Study of the effect of genetic code evolution on protein structure and function
	02	Characterization of sequence properties important for de novo protein evolution
	03	Search of sequence space enlarged by xeno amino acids for viable proteins



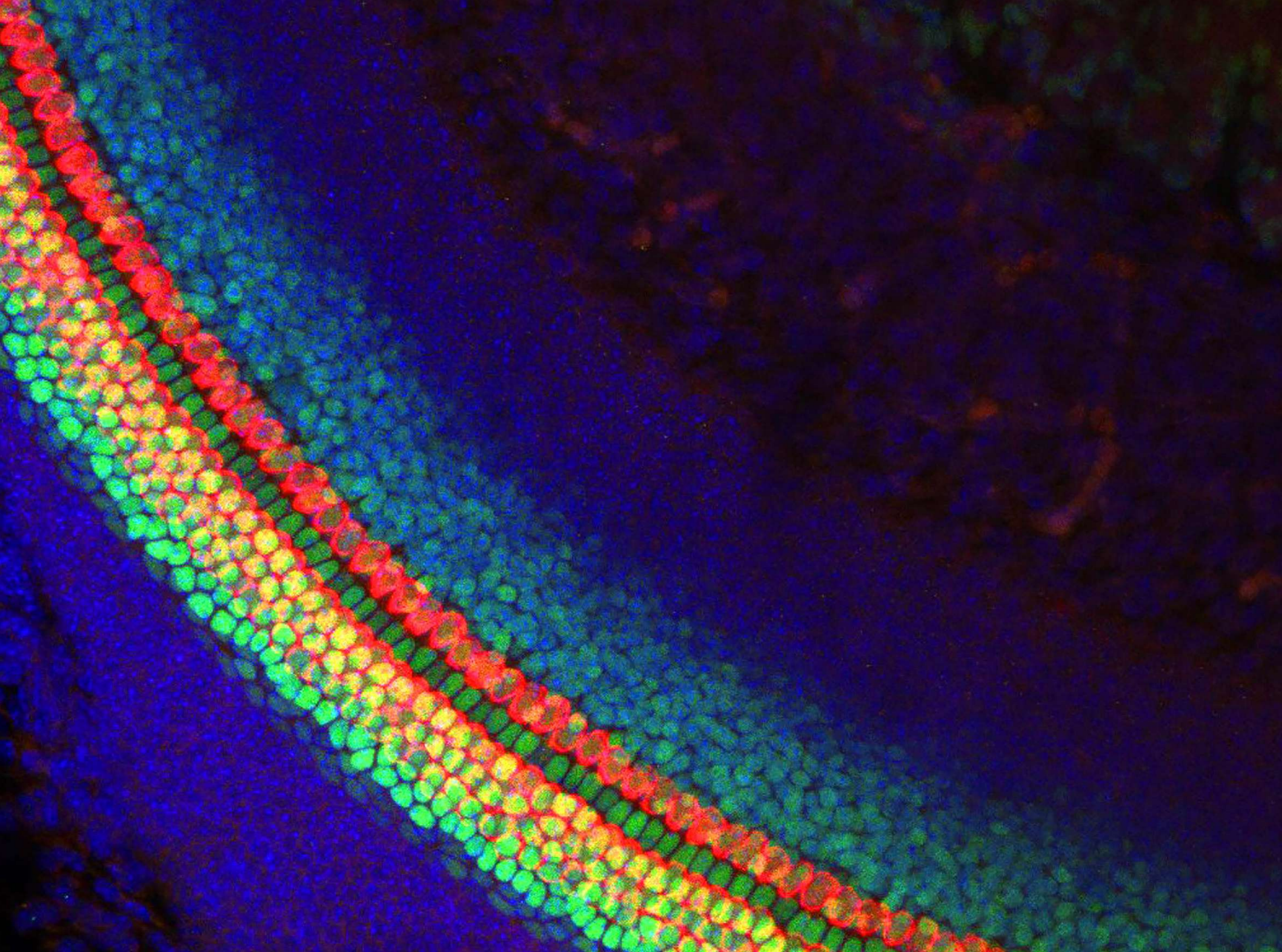
Protein structure evolution accompanied by amino acid alphabet expansion.



Scheme of the CoLiDe algorithm for the design of combinatorial protein libraries.



Reconstruction of the ribosomal peptidyl transferase center.



Structural Biology and Protein Engineering

No. of research teams: 10

Pages: 104–129

03

Structural Biology and Protein Engineering

Prof. Ing. Bohdan Schneider, CSc., DSc.
Head of the Research Programme
The Institute of Biotechnology of the Czech Academy of Sciences

Research Directions

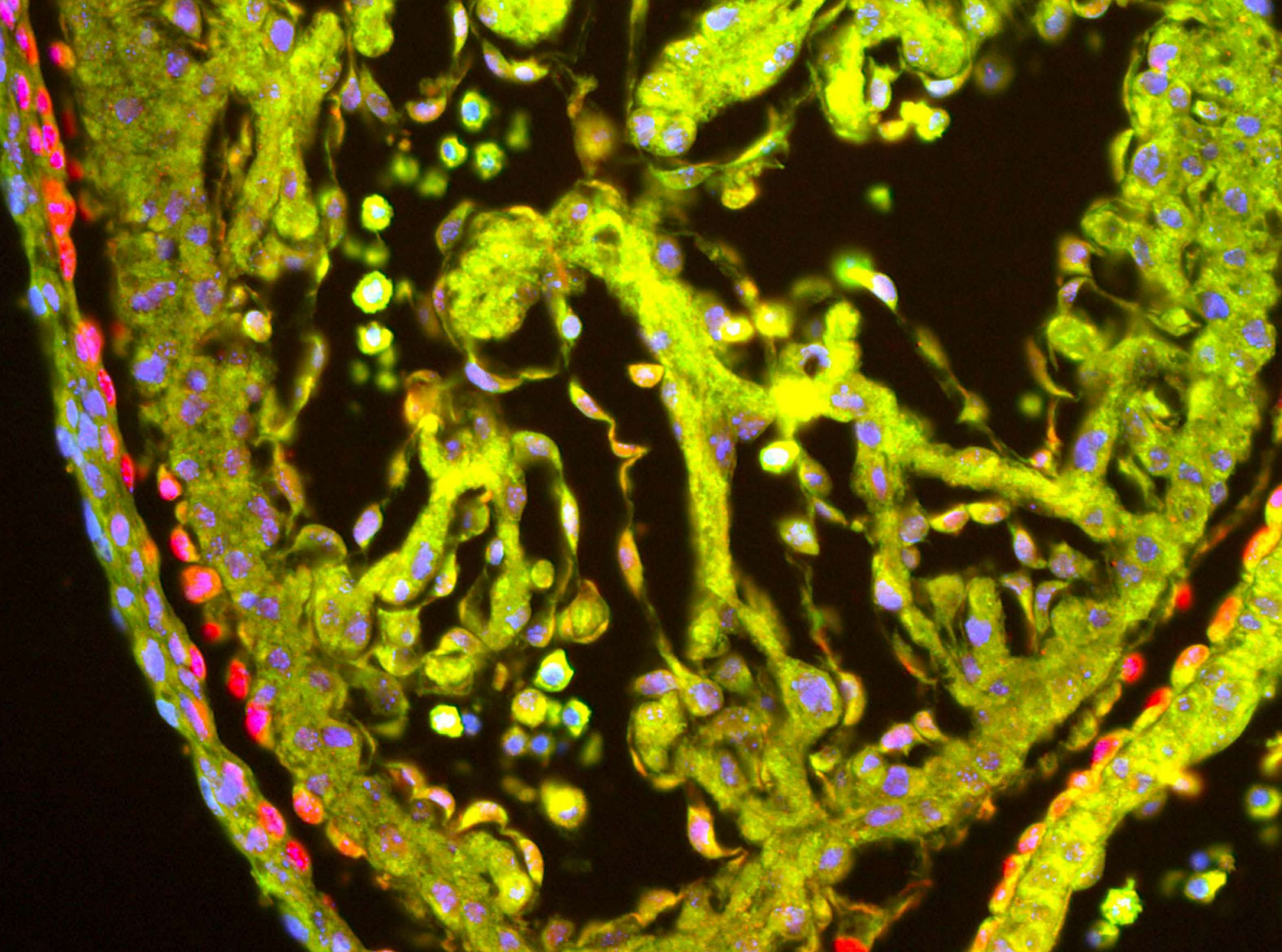
Structural Biology and Protein Engineering

1. Protein expression and purification	2. Structural and biophysical characterization of biomolecules and their complexes	3. Study of the structure and function of natural compounds
4. Research and development of high-affinity binding proteins	5. Studies of structural bioinformatics, large scale analyses of biomolecular structures	

Our main projects are protein expression, purification and characterization. A fully-equipped lab for molecular biology, >100 expression vectors is available, several expression platforms including E. coli, yeast, insect and mammalian expression, dedicated expression facilities for small/large scale expressions (including fermenters), and extensive experience in protein purification and characterization. We offer a complete service for protein expression and purification starting from the experimental design and cloning of expression vectors through the selection of the best candidates up to the large-scale production of milligram quantities of a recombinant protein.

A user can select from more than 100 expression plasmids tailored for a variety of expression systems. To maximize the chances of the successful completion of a given project, we typically screen tens of expression constructs in parallel, which is facilitated and streamlined by the use of our Gateway-based expression plasmids. Please note that a user can opt in/out of the process in any step, i.e. bring his/her own expression clones, get cell pellets for his/her own purification experiments, or get a virus for the infection of insect cells. We would, however, recommend contacting us during the early stage of your project so the most suitable experimental approach (taking into the account your planned downstream application) could be discussed and designed.

This programme is focused on the research of novel biotechnologically, diagnostically, and medically important biomolecules, proteins and nucleic acids that will be constructed using state-of-the-art methods of molecular biology and protein engineering. The structures and properties of the studied molecules will be analysed by complex biophysical methods, such as advanced mass spectrometry, and crystallography. Understanding the structures of the studied molecules and their mutual interactions will help us to modify them so that their desired biological activities improve so that they can be used for diagnosing diseases, as drugs or as advanced materials.



Structural Biology of Signaling Proteins

RNDr. Veronika Obšilová, Ph.D., Head of the Lab.

The Institute of Physiology
of the Czech Academy of Sciences

Content of the Research

Our research team has been studying the 14-3-3 proteins which are highly conserved regulatory molecules found in all eukaryotes. 14-3-3 proteins have the ability of binding the functionally different signal proteins, including kinases, phosphatases and transmembrane receptors by changing their function. Through the functional modulation of a wide range of binding partners, 14-3-3 proteins are involved in many processes, including cell cycle regulation, metabolism control, apoptosis, and the control of gene transcription. More than 300 proteins have been described as binding partners to date. We employ both biophysical (fluorescence spectroscopy, analytical ultracentrifugation, SAXS, mass spectrometry, isothermal titration calorimetry, X-ray crystallography, protein structure modeling, etc.) and biochemical (recombinant protein expression, site-directed mutagenesis, enzyme kinetics) approaches to understand the details of how the activity and function of protein-protein complexes are regulated.

The 14-3-3 proteins are a family of regulatory molecules, which specifically bind to phosphoserine (or phosphothreonine)- containing motifs (pSer/pThr) in a sequence-specific manner. Through these binding reactions, the 14-3-3 proteins play key regulatory roles in signal transduction, cell cycle control, metabolism control and apoptosis. More than 200 14-3-3 binding partners have been reported so far and some of them play prominent roles in cancer development (e.g. transcription factors p53 and FOXO), neurodegeneration (e.g. Tau protein, ASK1 kinase), cardiovascular diseases (e.g. RGS proteins, phosducin) or inflammation (e.g. NFkB, ASK1 kinase). However, the detailed mechanisms of the 14-3-3 protein-mediated regulations are mostly elusive, mainly due to a lack of structural data. The main goal of our research is to gain a mechanistic understanding of the 14-3-3 protein function in the regulation of selected 14-3-3 protein binding partners. In recent years we have been studying the 14-3-3 protein-mediated regulation of forkhead transcription factor FOXO4, tyrosine hydroxylase, and a regulator of G-protein signaling RGS3. Our current projects are focused on the regulation of caspase-2, protein kinase CaMKK2, FOXO-DBD, neutral trehalase Nth1, and protein kinase ASK1.

Potential for Cooperation

We collaborate with Assoc. Prof. Michael J. Ausserlechner, Ph.D. at the Medical University of Innsbruck, Austria, Prof. RNDr. Petr Herman, CSc. at the Faculty of Mathematics and Physics, Charles University and Doc. RNDr. Jan Vesely, Ph.D. at Faculty of Science, Charles University.

2020 Kalabova D, Filandr F, Alblova M, Petrvalska O, Horvath M, Man P, Obsil T*, Obsilova V* 14-3-3 protein binding blocks the dimerization interface of caspase-2. FEBS J. 2020 Jan 21. doi:10.1111/febs.15215

2019 Hagenbuchner J+, Obsilova V+, Kaserer T+, Kaiser N, Rass B, Psenakova K, Docekal V, Alblova M, Kohoutova K, Schuster D, Aneichyk T, Vesely J, Obexer P, Obsil T*, Ausserlechner MJ.*(2019) Modulating FOXO3 transcriptional activity by small, DBD-binding molecules. Elife. 8. pii: e48876.

2019 Psenakova K, Kohoutova K, Obsilova V, Ausserlechner MJ, Veverka V*, Obsil T*. (2019) Forkhead Domains of FOXO Transcription Factors Differ in both Overall Conformation and Dynamics. Cells. 8. pii: E966.

2018 Smidova A, Alblova M, Kalabova D, Psenakova K, Rosulek M, Herman P, Obsil T*, Obsilova V.* (2018) 14-3-3 protein masks the nuclear localization sequence of caspase-2. FEBS J. 285, 4196-4213.

2017 Alblova M, Smidova A, Docekal V, Vesely J, Herman P, Obsilova V*, Obsil T.* (2017) Molecular basis of the 14-3-3 protein-dependent activation of yeast neutral trehalase Nth1. Proc Natl Acad Sci U S A. 114, E9811-E9820.

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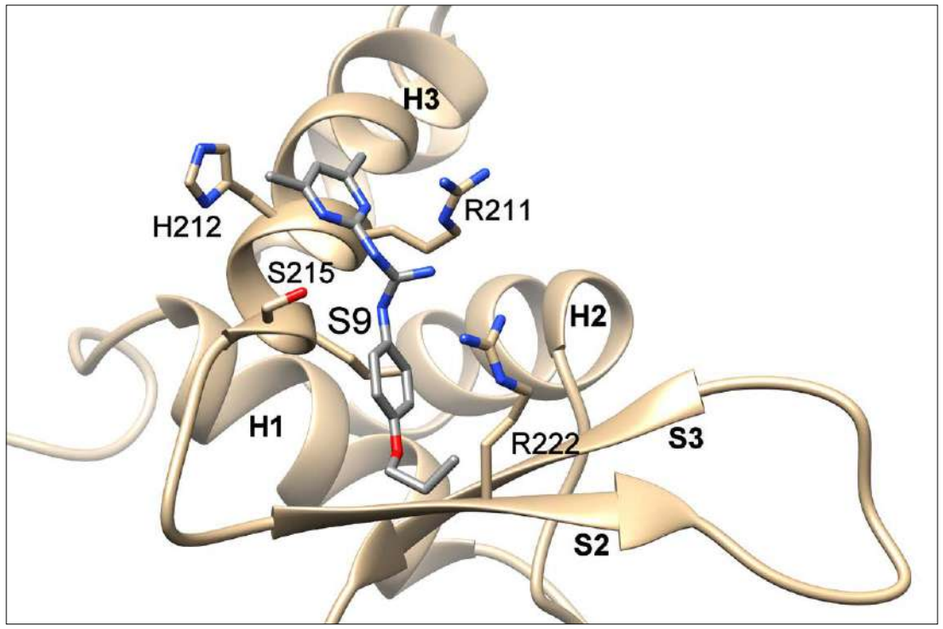
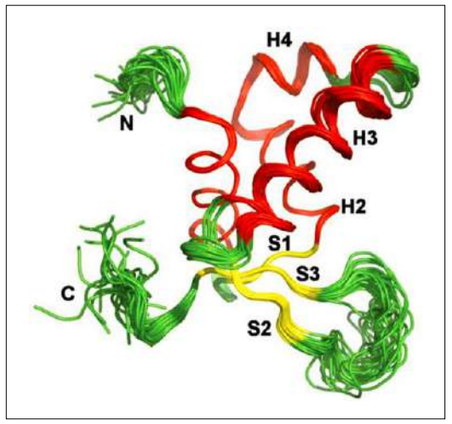
Basic keywords:	01	Proteins
	02	Structure
	03	Function
	04	Protein-protein interactions
	05	Crystallography
	06	Fluorescence

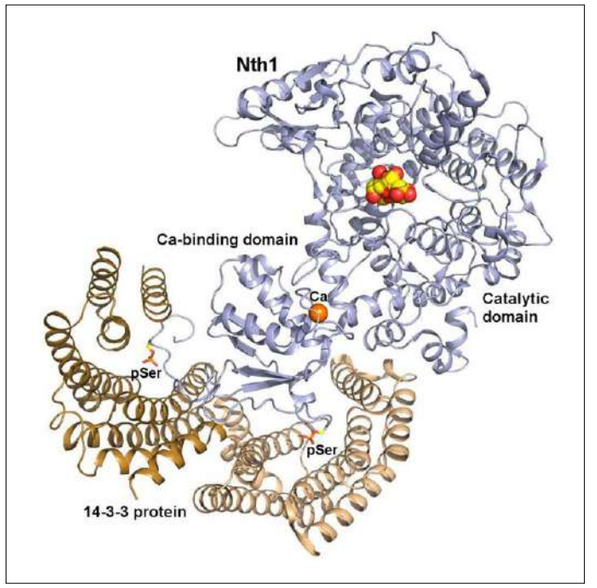
Research areas:	01	Structural biology
	02	Biochemistry
Main objectives:	01	Preparation, biochemical and biophysical characterization of selected signaling proteins
	02	Determination of binding affinities and stoichiometries of studied signaling complexes
	03	Structural studies of selected signaling protein complexes



Compound S9 blocks the DNA binding surface of Forkhead transcription factor FOXO3. The figure shows the structural model of the DNA-binding domain of FOXO3 with bound compound S9 based on data from NMR measurements and docking simulations (Hagenbuchner et al. (2019) eLife).



Solution NMR structure of the DNA-binding domain of mouse Forkhead transcription factor FOXO1 (ensemble of 30 conformers) (Psenakova et al. (2019) Cells).



Detailed insight on the structure of the complex 14-3-3:pNth1 (PDB 5N6N). The protomers of the Bmh1 homodimer are shown in yellow and brown. Nth1 is shown in blue. The phosphorylated Ser60 and Ser83 are shown as sticks. The calcium ion is shown in orange. Structural analysis revealed that the binding of phosphorylated Nth1 by 14-3-3 triggers Nth1's activity by enabling the proper 3D configuration of Nth1's catalytic and calcium-binding domains relative to each other, thus stabilizing the flexible part of the active site required for catalysis (Alblova et al. (2017) PNAS USA).

Structure of Medically and Biotechnologically Important Enzymes

Ing. Jan Dohnálek, Ph.D., Head of the Lab.

The Institute of Biotechnology of the
Czech Academy of Sciences

Content of the Research

We explain the structure-function relationship for proteins or complexes relevant for health and biotechnology. These include new enzymes or enzymes with potential for novel applications, including sugar-nonspecific nucleases, oxidoreductases, glycoside hydrolases, proteases, antibiotic destructases and complexes involved in bacterial transcription. Potential applications include the fight against bacterial infections, the food industry, dry goods, biofuel exploitation and waste remediation. We also contribute to developing macromolecular crystallography methods - especially biomolecular crystallization and computational tools. We offer expertise in experimental phasing, the determination of protein structure de novo, using the latest approaches utilizing synchrotron x-ray radiation and the most intensive in-house x-ray source for diffraction experiments, cryo-electron microscopy and electron diffraction.

Our successful projects are based on collaboration with academic researchers and with our industrial partner. The first type of projects involves the elucidation of the structure and function of novel enzymes with Novozymes A/S in Denmark and with the laboratory of Petra Lipovova at the University of Chemistry and Technology, Prague. The second is our research on natural killer cell surface receptors being done in collaboration with the team of Ondrej Vanek at Charles University in Prague. The third is an explanation of structure-function questions in bacterial transcription that can be utilized in the fight against bacteria causing human diseases in tandem with Libor Krasny at the Institute of Microbiology, Czech Academy of Sciences. Our other collaborative projects are aimed for example, at explaining the function of sensor proteins in collaboration with M. Martinkova at Charles University in Prague. We are in part responsible for the design and operation of the Centre of Molecular Structure, a part of the Czech and European research infrastructure for structural biology. We have helped the Extreme Light Infrastructure build a modern Biolab under the ELI-IBT collaborative project ELIBIO. We have also helped establish the starting European infrastructure for biophysical techniques MOSBRI.

Potential for Cooperation

Our laboratory team is open to collaboration in project-based partnerships, for example joint publications and/or patents. We are also interested in contractual research in the structural and non-structural characterization of macromolecular targets, in the development and sharing of new technologies connected mainly with x-ray diffraction and small angle x-ray scattering analysis of biological macromolecules.

Selected publications:

2020 – Kouba T, Koval T, Sudzinova P, Pospisil J, Brezovska B, Hnilicova J, Sanderova H, Janouskova M, Sikova M, Halada P, Sykora M, Barvik I, Novacek J, Trundova M, Duskova J, Skalova T, Chon U, Murakami KS, Dohnalek J, Krasny L (2020) Mycobacterial HelD is a nucleic acids-clearing factor for RNA polymerase. Nat. Commun. 11(1):6419.

– Skořepa O, Pazicky S, Kalousková B, Bláha J, Abreu C, Ječmen T, Rosůlek M, Fish A, Sedivy A, Harlos K, Dohnálek J, Skálová T, Vaněk O (2020) Natural Killer Cell Activation Receptor NKp30 Oligomerization Depends on Its N-Glycosylation. Cancers 12, 1998. doi: 10.3390/cancers12071998.

– Skalova T, Lengalova A, Dohnalek J, Harlos K, Mihalcin P, Kolenko P, Stranava M, Blaha J, Shimizu T, Martínková M (2020) Disruption of the dimerization interface of the sensing domain in the dimeric heme-based oxygen sensor AfGcHK abolishes bacterial signal transduction. Journal of Biological Chemistry 295:1587-1597.

2019 – Kovaľ T, Švecová L, Østergaard LH, Skalova T, Dušková J, Hašek J, Kolenko P, Fejfarová K, Stránský J, Trundová M, Dohnálek J (2019) Trp-His covalent adduct in bilirubin oxidase is crucial for effective bilirubin binding but has a minor role in electron transfer. Scientific Reports 9:13700.

– Kovaľová T, Kovaľ T, Benešová E, Vodičková P, Spiwok V, Lipovová P, Dohnálek J (2019) Active site complementation and hexameric arrangement in the GH family 29; a structure-function study of α-l-fucosidase isoenzyme 1 from *Paenibacillus thiaminolyticus*. Glycobiology 29:59-73.

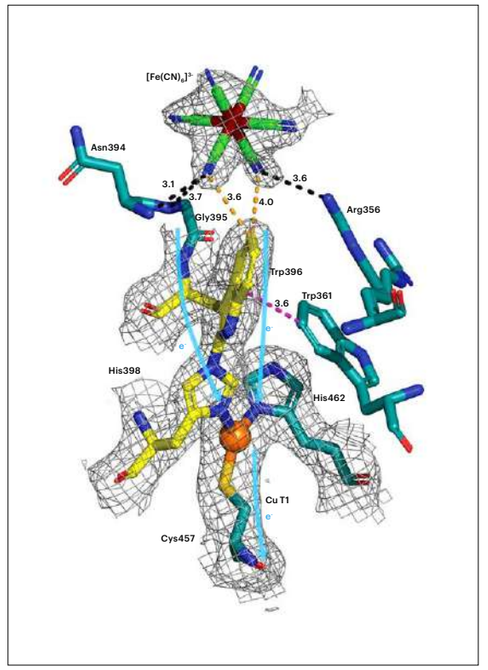
2018 – Koval T, Dohnalek J (2018) Characteristics and application of S1-P1 nucleases in biotechnology and medicine. Biotechnology Advances (2018) 36:603-612.

Contact person: Ing. Jan Dohnálek, Ph.D.
e-mail: jan.dohnalek@ibt.cas.cz

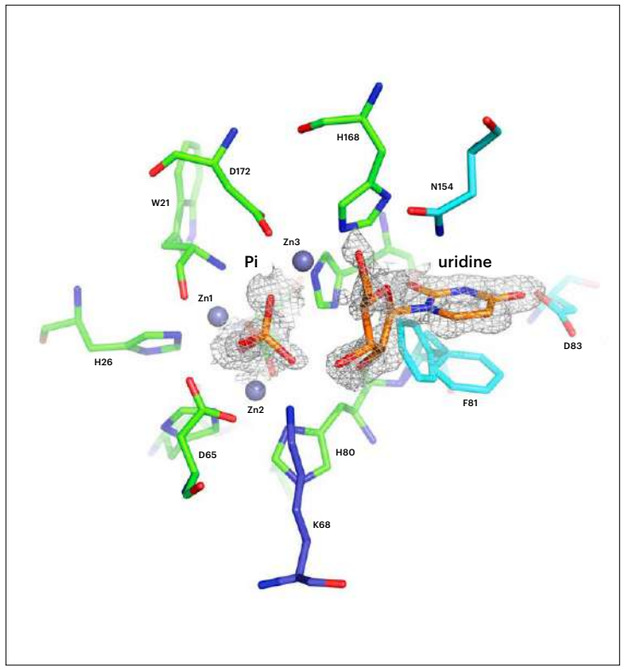
Basic keywords:
- Protein
- Enzyme
- Structure-function relationship
- X-ray diffraction
- Crystallography
- Small angle X-ray scattering
- Human pathogen
- Transcription
- Biotechnology

Research areas:
- Determination of the three-dimensional structure of biological macromolecules, mainly proteins and their complexes
- Structure-function analysis
- Novel enzymes for medicinal and biotechnological applications
- Surface receptors of an innate immune system
- Bacterial transcription mechanisms
- Development of methods of X-ray crystallography

Main objectives:
- Explanation of unknown relationships within enzymatic and receptor systems
- Identification of new inhibitors/drug candidates for human diseases
- Potentiation of enzymes for effective application in medicine or biotechnology

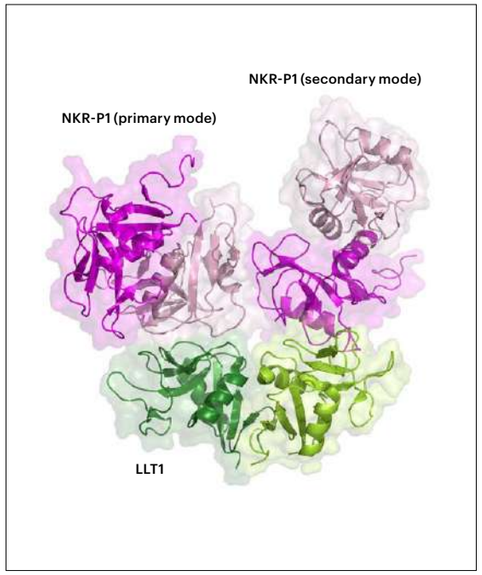


Surface active site of the enzyme bilirubin oxidase from *Myrothecium verrucaria* with an unusual covalent bond between histidine and tryptophane residues (yellow in the middle) and the substrate ferrocyanide bound at the molecular surface (brown, green and blue), as observed in a crystal structure. The first copper ion site of the enyzme is shown as an orange sphere. Contours of electron density show experimental evidence of the bond and complex formation. This active site mediates electron transfer from the substrate (top) to the first copper ion and further to the second active site of the enzyme (not shown) where molecular oxygen is reduced to water.

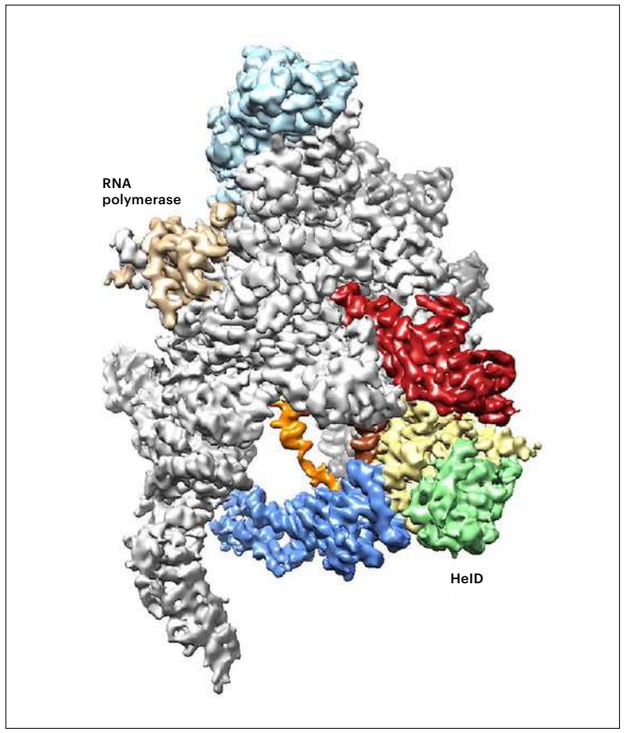


Active site of S1 nuclease in a complex with uridine and phosphate (shown in electron density contours of the crystal structure). The sugar-non-specific nuclease binds ribonucleotides (RNA) in a manner similar to 2-deoxyribonucleotides (DNA). The zinc ions are shown as grey spheres and the lysine residue important for product coordination/release is shown in purple.

X-ray structure of a complex of the extracellular part of an immune receptor NKR-P1 (two domains – magenta and pink) in complex with its ligand LLT1 from a partner cell (two domains – light and dark green). NKR-P1 was found in two binding modes in this crystal structure - primary and secondary mode. The discovery of two binding modes brings up questions of possible clusters or chains formation of these proteins between cell surfaces, to increase the stability of the cell-cell contact.



RNA polymerase from the bacterium *Mycobacterium smegmatis* in a complex with HelD protein (parts of HelD are shown in red, yellow, green, blue, orange and brown). The molecules are represented as experimental electrostatic potential from 3D cryoelectron microscopy reconstruction.



Molecular Interactions of Anti-Cancer Drugs

RNDr. Cyril Bařinka, Ph.D., Head of the Lab.

The Institute of Biotechnology of the
Czech Academy of Sciences

Content of the Research

Our laboratory strives to elucidate the molecular details of the structure and function of several pharmaceutically important zinc-dependent hydrolases, most notably members of the histone deacetylase (HDAC) and glutamate carboxypeptidase II (GCPII) families. Furthermore, we exploit protein engineering and structure- assisted drug discovery to develop macromolecules and small molecule ligands, respectively, that can be used as research tools and/or advanced into clinical studies. GCPII, also known as prostate specific membrane antigen (PSMA), has been implicated in several (patho)physiological processes. In the nervous system, GCPII exerts its peptidase activity by hydrolyzing a peptidic neurotransmitter. Accordingly, GCPII-specific inhibitors have been reported to be neuroprotective in multiple preclinical models of neurodegeneration. Over-expression of GCPII in prostate carcinoma makes the enzyme a prime marker for prostate cancer imaging in clinics and a target of future therapeutic interventions.

Lysine acetylation is a major post-translational modification found on most proteins of the human proteome and as such it impacts a broad spectrum of cellular functions, including gene expression, immune surveillance and energy metabolism. At the molecular level, the protein acetylation status is defined by opposing activities of histone acetyltransferases (writers) and histone deacetylases (HDACs; erasers). Eighteen HDACs have been identified in humans and our laboratory is interested in (i) deciphering the structure-function relationship, (ii) defining physiological functions, and (iii) designing specific inhibitors of HDAC6 and HDAC11 isoforms.

Potential for Cooperation

We offer our expertise in heterologous protein expression, purification and characterization together with the development of enzymatic assays. We have implemented a high-throughput system for the production of recombinant proteins in standard expression hosts including E.coli, yeast, insect cells (baculovirus-based or plasmid-based expressions), and mammalian cells (HEK293T, CHO). The system allows for the rapid screening of >100 combinations of purification tags/expression hosts to select the most efficient method for subsequent large-scale production. Proteins of interest can be further purified using optimized purification protocols and characterized in depth by an array of biophysical methods. Micrograms to hundreds of milligram quantities of purified proteins can then be prepared.

Selected publications:

2020 – Ustinova K, Novakova Z, Saito M, Meleshin M, Mikesova J, Kutil Z, Baranova P, Havlinova B, Schutkowski M, Matthias P, Barinka C*: The disordered N-terminus of HDAC6 is a microtubule-binding domain critical for efficient tubulin deacetylation. J Biol Chem. 2020, 295(9):2614-2628.

– Ptacek J, Zhang D, Qiu L, Kruspe S, Motlova L, Kolenko P, Novakova Z, Shubham S, Havlinova B, Baranova P, Chen SJ, Zou X, Giangrande P, Barinka C*: Structural basis of prostate-specific membrane antigen recognition by the A9g RNA aptamer. Nucleic Acids Res. 2020 48(19):11130-11145.

– Novakova Z*, Belousova N, Foss CA, Havlinova B, Gresova M, Das G, Lisok A, Prada A, Barinkova M, Hubalek M, Pomper MG, Barinka C*: Engineered Fragments of the PSMA-Specific 5D3 Antibody and Their Functional Characterization. Int J Mol Sci. 2020 21(18):6672.

2019 – Kutil Z, Skultetyova L, Rauh D, Meleshin M, Snajdr I, Novakova Z, Mikesova J, Pavlicek J, Hadzima M, Baranova P, Havlinova B, Majer P, Schutkowski M*, Barinka C*: The unraveling of substrate specificity of histone deacetylase 6 domains using acetylome peptide microarrays and peptide libraries. FASEB J, 2019, 33(3):4035-4045.

2018 – Kutil Z, Novakova Z, Meleshin M, Mikesova J, Schutkowski M, Barinka C*: Histone deacetylase 11 is a fatty-acid deacylase. ACS Chem Biol, 2018, 13(3):685-693.

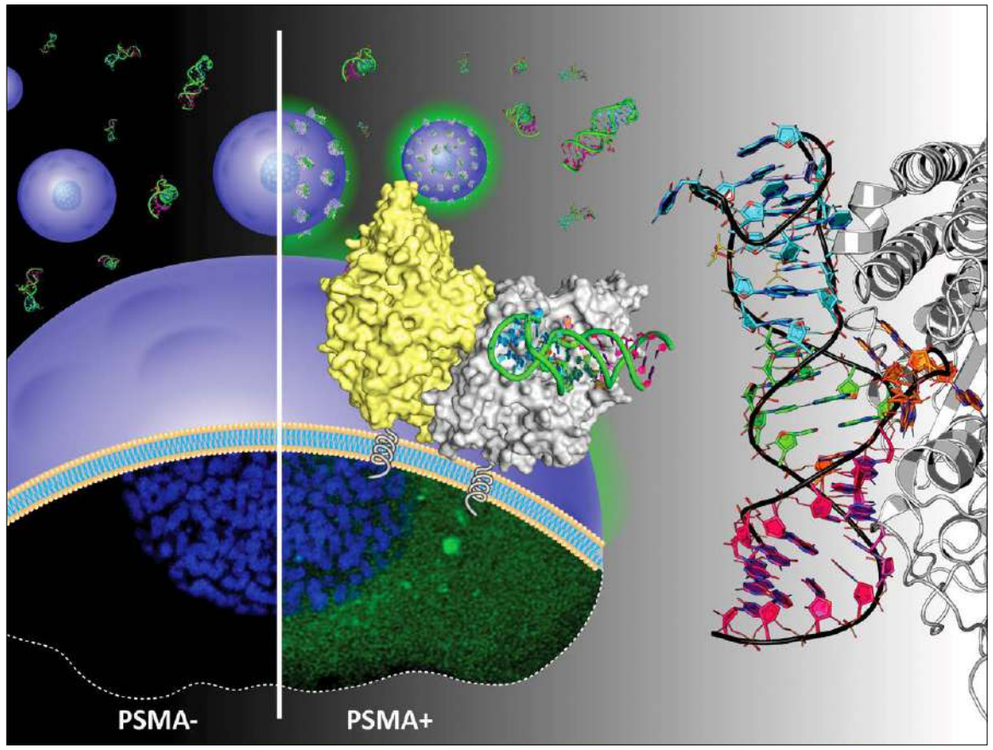
2017 – Novakova Z, Foss C, Copeland B, Morath V, Baranova P, Havlinova B, Skerra A, Pomper M, Barinka C*: Novel monoclonal antibodies recognizing human prostate-specific membrane antigen (PSMA) as research and theranostic tools. The Prostate. 2017, 77(7):749-764.

– Skultetyova L, Ustinova K, Kutil Z, Novakova Z, Pavlicek J, Mikesova J, Trapl D, Baranova P, Havlinova B, Hubalek M, Lansky Z, Barinka C*: Human histone deacetylase 6 shows strong preference for tubulin dimers over assembled microtubules. Sci Rep, 2017 7(1):11547-515.

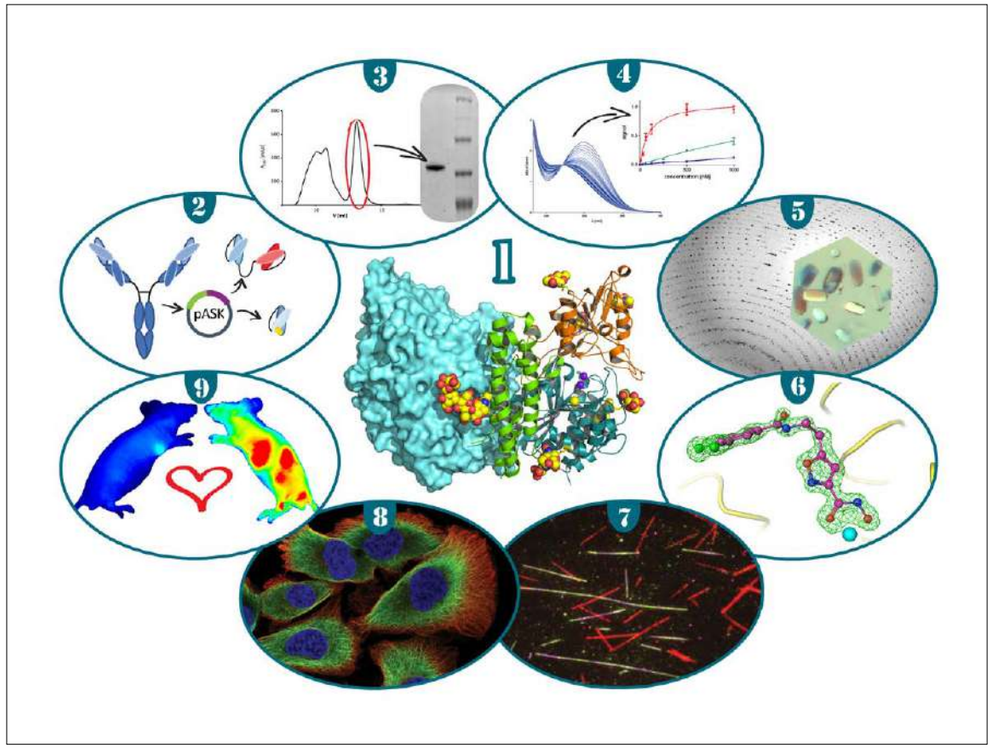
Contact person:	RNDr. Cyril Bařinka, Ph.D.
	e-mail: cyril.barinka@ibt.cas.cz

Basic keywords:	01	Histone deacetylase
	02	Glutamate carboxypeptidase ii
	03	Structure-based drug design
	04	X-ray crystallography
	05	High-throughput heterologous protein expression
	06	Enzymatic
	07	Assay development
	08	Structure-function studies
	09	Zinc-dependent hydrolases
	10	Post-translation modifications

Research areas:	01	Enzymology and structural biology
	02	Protein engineering
	03	Cancer imaging
	04	Acetylome
Main objectives:	01	Structural and functional characterization of glutamate carboxypeptidase II-related peptidases
	02	Defining the substrate specificity of histone deacetylases and acetyltransferases
	03	Development of imaging modalities for prostate cancer



Prostate-specific membrane antigen (PSMA) is an established biomarker for the imaging of prostate cancer. An X-ray structure of PSMA in a complex with A9g RNA aptamer complemented by *in silico* modeling revealed structural motifs and conformational changes critical for PSMA/A9g high affinity interactions and A9g exquisite specificity. Fluorescently labeled A9g can thus be used for imaging of and delivery of therapeutics to PSMA-positive cells *in vitro* and *in vivo*.



Our research revolves around proteins (1). We first clone and engineer a protein of interest by molecular biology approaches (2) and then we heterologously express and purify it to homogeneity (3). To unravel the structure and function of studied targets we use a variety of biochemical and biophysical techniques (4), including X-ray crystallography (5) and cryoEM. Detailed structural characterization (6) facilitates the development of specific inhibitors and provides a mechanistic understanding of protein function in vitro (7). Finally, we further corroborate and translate our findings into more complex environments including living cells (8) and whole organisms (9).

Structural Proteins and Their Complexes

RNDr. Zdeněk Lánský, Ph.D., Head of the Lab.

The Institute of Biotechnology of the
Czech Academy of Sciences

Content of the Research

Cytoskeletal networks form the internal dynamic scaffold of living cells essential for key cellular processes, such as cell division, cell motility and morphogenesis. Our aim is to understand how the individual elements of the cytoskeleton mechanically cooperate to produce a coherent behavior in the cytoskeletal network, the roles of the individual co-operating proteins and the principles that underpin their collective action. We use two main strategies, a bottom-up approach, reconstituting elements of cytoskeletal networks from individual components in vitro, and a top-down approach, deconstructing the networks in vivo. We use genetic manipulations, biochemical and biophysical methods and mathematical modeling.

We study i) neuronal pathfinding, a foundational process in ontogenetic development, ii) contractility of actin networks and their anchoring to the plasma membrane, key mechanisms in cytokinesis, the final stage of cell division, iii) regulatory roles of intrinsically disordered, microtubule-associated proteins, essential axonal factors known for their roles in a number of neurodegenerative diseases, iv) long-range intracellular transport and trafficking of organelles, key for example for the maintenance of neuronal function and v) the formation of ciliary structures, essential for sensing environmental cues such as signaling molecules, light, and mechanical stimuli. Central to our approach are imaging and force measurement techniques with single molecule resolution. We use single molecule imaging by Total Internal Reflection Fluorescence (TIRF) microscopy to track individual molecules in an experimental system in both space and time. We use optical tweezers to probe the forces exerted by the cytoskeletal systems or to manipulate these systems or perturb them physically on a single molecule level. Employing methods of physics, we quantitatively describe the studied biological systems and predict their behavior.

Potential for Cooperation

We offer our expertise in the characterization of protein-protein or protein-DNA interactions on the single molecule level by advanced biophysical methods, such as single molecule Total Internal Reflection Fluorescence microscopy and optical trapping. We are always looking for talented students and postdocs, experimentalists or theoreticians, with a background in (bio)physics, chemistry, biology or an equivalent field, motivated to work on cross-disciplinary projects. If you are interested, please contact Zdenek Lansky (zdenek.lansky@ibt.cas.cz).

2019 Siahaan V., Krattenmacher J., Hyman A. A., Diez S.,Hernandez-Vega A., Lansky Z., Braun M. Kinetically distinct phases of tau on microtubules regulate kinesin motors and severing enzymes. Nature Cell Biology, 2019, 21, 1086–1092.

2019 Schmidt-Cernohorska M, Zhernov I, Steib E, Le Guennec M, Achek R, Borgers S, Demurtas D, Mouawad L, Lansky Z, Hamel V, Guichard P. Flagellar microtubule doublet assembly in vitro reveals a regulatory role of tubulin C-terminal tails. Science, 2019, 363(6424):285-288.

2018 Lüdecke, A., Seidel, A.M., Braun, M., Lansky, Z., Diez, S. Diffusive tail anchorage determines velocity and force produced by kinesin-14 between crosslinked microtubules. Nature Communications 2018, 9(1):2214.

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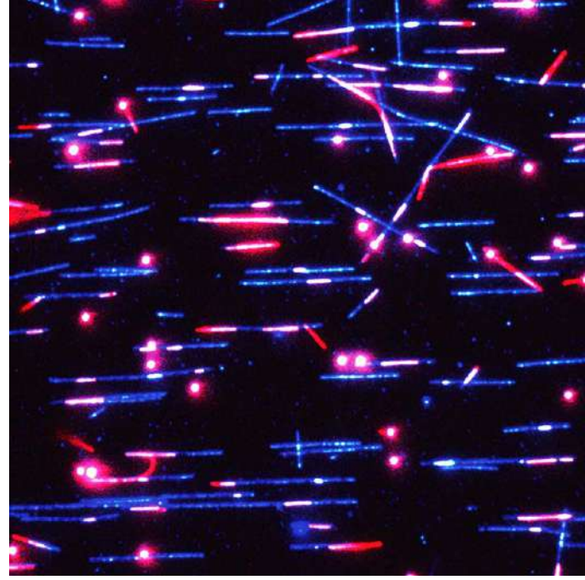
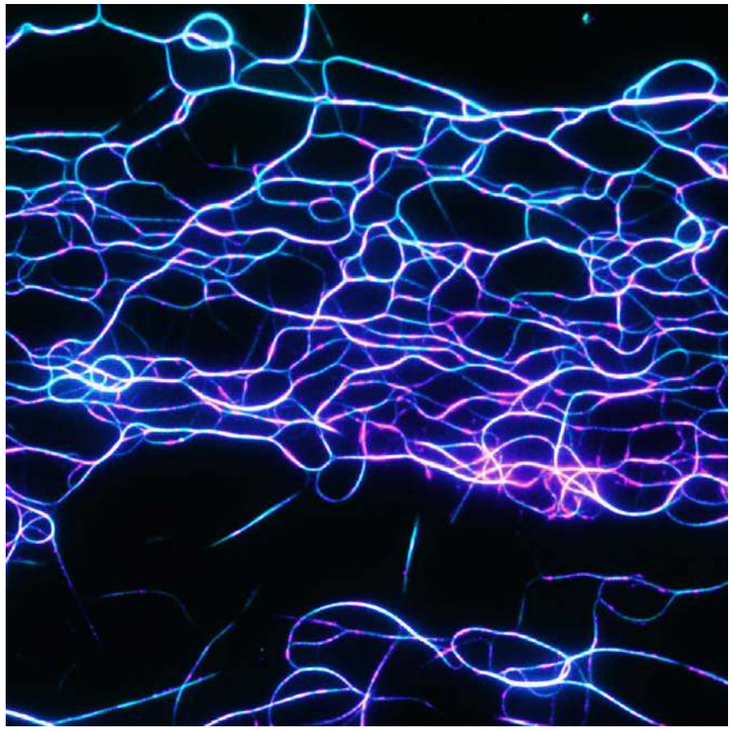
Basic keywords:	01	Cytoskeleton
	02	Microtubules
	03	Actin
	04	Molecular motors
	05	Single molecule biophysics
	06	Microscopy
	07	Optical trapping
	08	Recombinant proteins
	09	In vitro reconstitutions

Research areas:	01	Organelle transport in neurons
	02	Regulation of neuronal traffic by unstructured proteins
	03	Cytoskeletal mechanics of the axonal growth cone steering
	04	Contractile mechanisms in cytoskeletal networks
Main objectives:	01	Unravelling the molecular mechanisms underlying self-assembly and remodelling of cytoskeletal networks



Snapshot generated by multichannel TIRF microscopy. Fluorescently labeled cytoskeletal filaments (microtubules) shown in blue are attached to the coverslip surface. Shorter microtubules shown in red are cross-linked to the surface-immobilized microtubules by a microtubule cross-linking protein Ase1 (shown in white).



Multicolor micrograph showing an in vitro reconstituted contractile network composed of phalloidin-stabilized, rhodamine-labeled actin filaments (cyan) and GFP-labeled actin crosslinker anillin (magenta).

Research of Natural Substances: Structure and Function

Mgr. Gabriela Balíková Novotná, Ph.D., Project Head

The Institute of Microbiology of the
Czech Academy of Sciences

Content of the Research

The project currently has three main areas of focus. In the first, we address the problem of antibiotic resistance and the urgent need for new antimicrobial compounds. We study the molecular and biological function of ribosomal ABCF proteins, which include important determinants of antibiotic resistance but also translational factors of mostly an unknown function. The newly discovered antibiotic-signalling function of ABCF proteins in antibiotic producers has the potential to become a valuable tool for the search for new antibiotics from natural sources. In addition, in cooperation with clinical microbiologists, we are further improving the activity of the natural hybrid lincosamide antibiotic CELIN and we are constructing a CELIN producer by using a combination of CElesticetin and LINcomycin biosynthetic pathways. For more information, contact Gabriela Balíková Novotná (gnovotna@biomed.cas.cz)

The second line of research focuses on studying the diversity and ecology of microbial communities producing natural compounds in the environment. We pay special attention to the microbial enzymes involved in the degradation of biopolymers from a diverse origin in nature. To accomplish this, we use a combination of multi-omics approaches, including metagenomics, metatranscriptomics and metaproteomics. Moreover, we carry out efforts in the isolation, functional screening and characterization of new microbial strains with biotechnological potential, using genome sequencing and proteomics for exploring the regulation of the production of natural, biologically-active compounds, proteins and genes. For more information, contact Rubén López Mondéjar (rubenlopezmondejar@gmail.com)

The third topic centers on understanding the complex biology of several fungal groups of eminent importance for humankind. It spans the full spectrum of activities, from the study of diversity, ecology, evolution, biochemistry and secondary metabolites production (incl. new drug discovery). The most recent projects involve microorganisms associated with bark beetles and the taxonomy of toxinogenic and clinical fungi including the genus Aspergillus and dermatophytes. Other projects involve the diversity of fungi in highly acidic soils and the taxonomy of ergot fungi (genus Claviceps). For more information, contact Miroslav Kolařík (mkolarik@biomed.cas.cz).

2020 Koberska, M., Vesela, L., Vimberg, V., Lenart, J., Vesela, J., Kamenik, Z., Janata J., and Balikova Novotna, G. (2020). Beyond self-resistance: ABCF ATPase LmrC is a signal-transducing component of an antibiotic-1 driven signaling cascade hastening the onset of lincomycin biosynthesis, bioRxiv, 2020.10.16.343517.

2020 Algora C, Baldrian P, López-Mondéjar R (2020). Litter-inhabiting fungi show high level of specialization towards biopolymers composing plant and fungal biomass. Biology and Fertility of Soils, https://doi.org/10.1007/s00374-020-01507-3.

2020 López-Mondéjar R, Tláskal V, Větrovský T, Štursová M, Toscan R, Nunes da Rocha U, Baldrian P (2020). Metagenomics and stable isotope probing reveal the complementary contribution of fungal and bacterial communities in the recycling of dead biomass in forest soil. Soil Biology and Biochemistry 148:107875.

2020 Sklenář F, Jurjević Ž, Peterson SW, Kolařík M, Nováková A, Flieger M, Stodůlková E, Kubátová A, Hubka V (2020). Increasing the species diversity in the Aspergillus section Nidulantes: Six novel species mainly from the indoor environment. Mycologia 112: 342-370

2018 Saati-Santamaría Z, López-Mondéjar R, Jiménez-Gómez A, Díez-Méndez A, Větrovský T, Igual JM, Velázquez E, Kolarik M, Rivas R, García-Fraile P (2018). Discovery of phloeophagus beetles as a source of Pseudomonas strains that produce potentially new bioactive substances and description of Pseudomonas bohemica sp. nov.. Frontiers in Microbiology 9:913.

2017 Kadlcik, S., Kamenik, Z., Vasek, D., Nedved, M., and Janata, J. (2017). Elucidation of salicylate attachment in celesticetin biosynthesis opens the door to create a library of more efficient hybrid lincosamide antibiotics. Chem. Sci. 8, 3349–3355.

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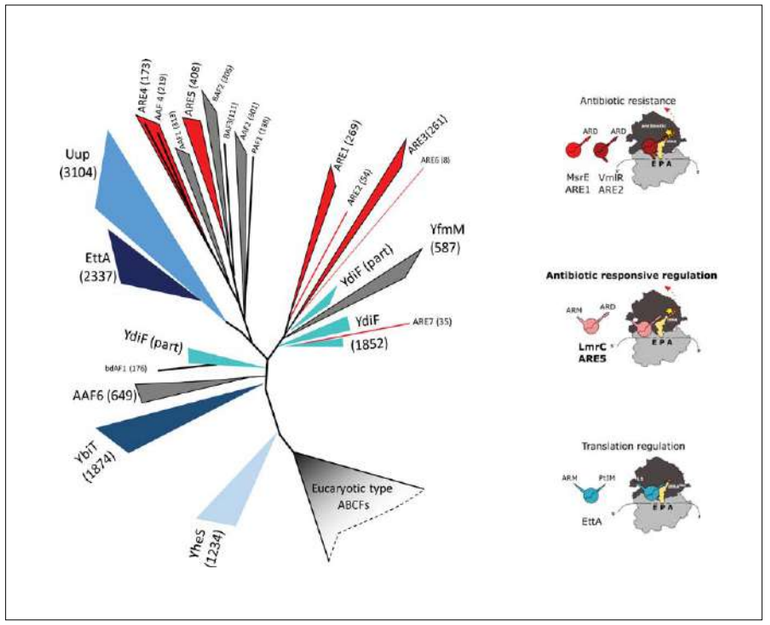
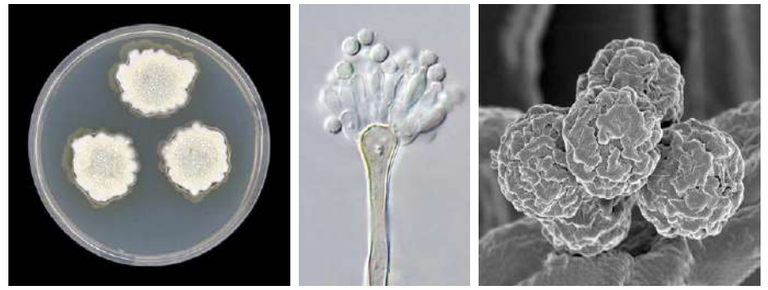
Basic keywords:	01	Structure of natural compounds
	02	Hydrolytic Enzymes
	03	Antibiotics production
	04	Mechanisms of Resistance
	05	Metagenomics
	06	Metatranscriptomics
	07	Metaproteomics
	08	Genome sequencing

Research areas:	01	Bioactive compounds from bacteria, fungi and invertebrates
	02	The structure and function of biotechnologically relevant enzymes
	03	Antibiotic mode of the action and mechanisms of resistance
	04	Characterization of bacterial human and environmental communities
Main objectives:	01	Determination of the structure and mechanism of the action of natural biologically active compounds, understanding their role in the environment as well as their interactions with target molecules
	02	Characterization of the biosynthetic,, biotransformational and biodegradational potential of bacteria, fungi and invertebrates, including the properties and structure of their enzymes
	03	Study of the metabolic activity of microbial producers of natural substances in the environment using metagenomics, metatranscriptomics and metaproteomics



Bacterial ABCF proteins contain 30 subgroups, the function of which is mostly unknown. Among them, ARE subgroups contain proteins with antibiotic resistance or antibiotic-responsive regulatory functions. All bacterial ABCFs ATPases bind to the exit site of the ribosome and exert various functions through their subdomains, which protrude from the nucleotide-binding domain dimer.



Aspergillus dipodomyus is one of tens of novel species discovered by our team. Species of the genus *Aspergillus* can cause infections in humans and animals, produce mycotoxins, spoil food, promote the development of allergies and have numerous biotechnological applications. This particular species is outstanding by its production of mycotoxin sterigmatocystine, which is an important contaminant of agricultural products.

Functional screening of bacterial isolates from soil showing cellulolytic activity (white halos).

An example of degradation of filter paper by a new bacterial strain isolated from soil (on the right) versus the control (on the left). The new strain showed a high cellulolytic potential for deconstructing paper, being a candidate for second-generation technologies using paper or lignocellulosic agricultural waste as an inexpensive and sustainable alternative for the production of value-added chemicals and biofuels.

Intermolecular Recognition of Proteins and Nucleic Acids
Prof. Bohdan Schneider, Head of the Research Programme

The Institute of Biotechnology of the
Czech Academy of Sciences

Content of the Research

At present, the research project is investigating these three main research topics:

1) Cytokines. We investigate interactions between medically important human cytokines and their cellular receptors. These protein molecules are important in the immunity response to viral and bacterial infection. Errors in their regulation cause serious autoimmune and/or allergic health disorders and may promote malignancy. We study the possible ways of generating more stable variants of these proteins, mostly related to the family of Interleukin 10, and of modulating their specificity and affinity to the receptors. An important direction of our research is the de novo development of proteins specifically binding cytokines and/or their receptors via directed evolution, ribosome display and yeast display.

2) DNA and RNA. Nucleic acids are structurally plastic molecules, and their biological roles are enabled through adaptation to their binding partners. We study structural aspects of both double and single-stranded DNA and their recognition by other molecules using x-ray crystallography and spectral methods. We have a long tradition in performing bioinformatic analysis of nucleic acid structures and developing unique bioinformatic and statistical tools. The results of the analysis are available at dnatco.datmos.org and watlas.datmos.org as freely accessible service tools and standards.

3) Solvation & hydration. We study how proteins and nucleic acids interact with their aqueous environment by analyzing the structure of the solvation shell around these biomolecules. We analyze large ensembles of protein and nucleic acid crystal structures to determine structural patterns of the first hydration shell around amino acids residues in proteins and of dinucleotide steps in DNA and RNA.

Potential for Cooperation

Our laboratory actively participates in the ELIXIR and Instruct-ERIC infrastructural projects. We have established close collaboration with laboratories at the Weizmann Institute of Science in Israel, with RCSB PDB at Rutgers University in the USA, and have a collaborative project with ELI-Beamlines in the Czech Republic. We are open to other collaborations, especially in these areas: protein production and characterization, structural bioinformatics of DNA, RNA, and proteins, and their interactions.

2020 Jiří Černý, Paulína Božíková, Jakub Svoboda & Bohdan Schneider: A unified dinucleotide alphabet describing both RNA and DNA structures. Nucleic Acids Research. 48: 6367-6381 (2020). doi: 10.1093/nar/gkaa383.

2020 Petr Kolenko, Jakub Svoboda, Jiří Černý, Tatsiana Charnavets & Bohdan Schneider: Structural variability of CG-rich DNA 18-mers accommodating double T–T mismatches. Acta Crystallographica D76: 1233-1243 (2020). doi: 10.1107/S2059798320014151

2019 Jiří Zahradník, Lucie Kolářová, Yoav Peleg, Petr Kolenko, Silvie Svidenská, Tatsiana Charnavets, Tamar Unger, Joel L. Sussman & Bohdan Schneider: Flexible regions govern promiscuous binding of IL-24 receptors IL-20R1 and IL-22R1. FEBS J. 286: 3858-3873 (2019). doi.org/10.1111/febs.14945.

2018 Jiří Zahradník, Lucie Kolářová, Hana Pařízková, Petr Kolenko, & Bohdan Schneider: Interferons type II and their receptors R1 and R2 in fish species: Evolution, structure, and function. Fish and Shellfish Immunology 79: 140-152 (2018). doi.org/10.1016/j.fsi.2018.05.008.

2018 Bohdan Schneider, Paulína Božíková, Iva Nečasová, Petr Čech, Daniel Svozil & Jiří Černý: A DNA structural alphabet provides a new insight into the DNA flexibility. Acta Crystallographica D74: 52-64 (2018). doi:10.1107/S2059798318000050

2017 Bohdan Schneider, Paulína Božíková, Petr Čech, Daniel Svozil & Jiří Černý: A DNA Structural Alphabet Distinguishes Structural Features of DNA Bound to Regulatory Proteins and in the Nucleosome Core Particle. Genes 8: 278 (2017). doi: 10.3390/genes8100278.

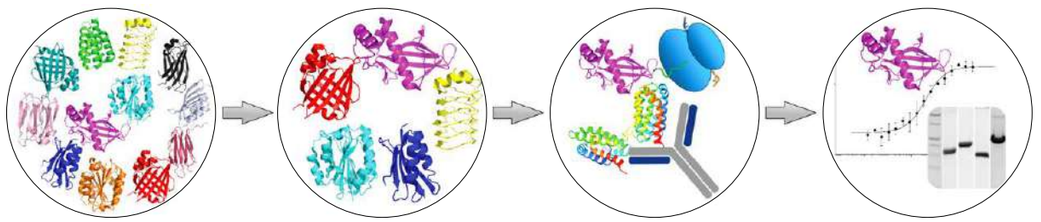
2016 Pavel Mikulecký, Jiří Zahradník, Petr Kolenko, Jiří Černý, Tatsiana Charnavets, Lucie Kolářová, Iva Nečasová, Phuong Ngoc Pham & Bohdan Schneider: Crystal structure of human interferon-gamma receptor 2 reveals the structural basis for receptor specificity. Acta Crystallographica D72: 1017-1025 (2016). doi: 10.1107/S2059798316012237.

2016 Lada Biedermannová & Bohdan Schneider: Hydration of proteins and nucleic acids: Advances in experiment and theory. A review. Biochimica et Biophysica Acta - General Subjects 1860: 1821-1835 (2016). doi: 10.1016/j.bbagen.2016.05.036.

Contact person:	Prof. Bohdan Schneider
	e-mail: bohdan.schneider@gmail.com

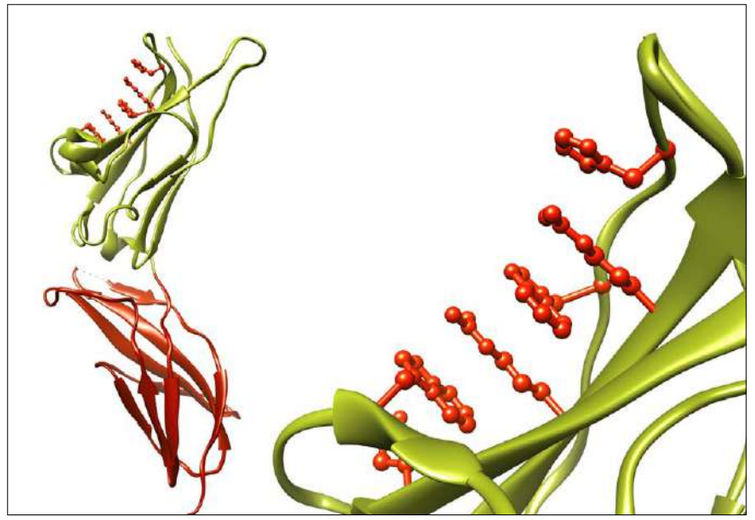
Basic keywords:	01	Molecular structure
	02	Cytokines
	03	DNA
	04	RNA
	05	Biomolecular recognition
	06	Hydration
	07	Biophysics
	08	Bioinformatics
	09	Crystallography
	10	Structural databases

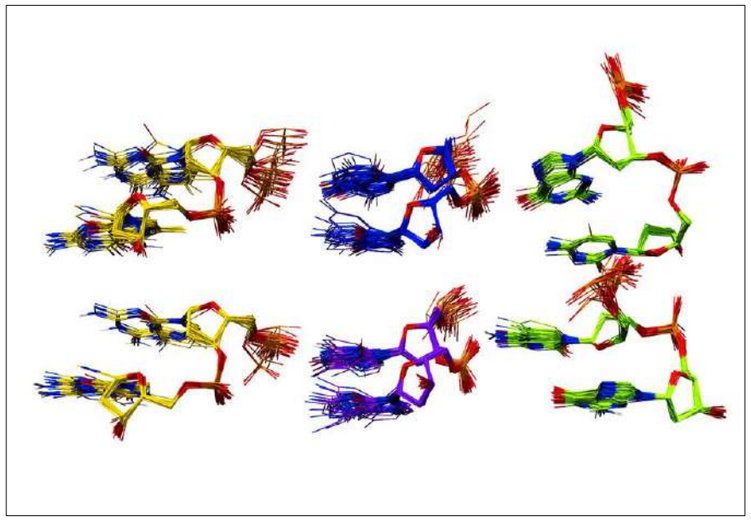
Research areas:	01	Intermolecular interactions and recognition of biomolecules
	02	Nucleic acid structure and dynamics
	03	Protein engineering of cytokines and their receptors
	04	Hydration and solvation of nucleic acids and proteins
Main objectives:	01	In general, understanding the mechanisms of specific interactions of biomolecules with potential diagnostic, medical and/or biotechnological use.
	02	Modifying properties of human cytokines and their cellular receptors (proteins of innate immune defense) and developing new protein molecules that can bind cytokines and their receptors to either block or enhance signaling.
	03	Description of the nucleic acid structure, development of tools to build their structural models, annotate, and validate them.
	04	Analysis of the structure of the hydration and solvation shell of proteins and nucleic acids and application of the acquired information to improve computer modeling and/or interpretation of biophysical experiments.



A systematic procedure of designing new scaffolds suitable for directed evolution by ribosome display or yeast display. The procedure yielded scaffold-related variants with nanomolar affinity towards human interleukin 10.

The extracellular part of human interferon-γ receptor 2 (IFNγR2) solved at 1.8 Å resolution by X-ray crystallography. A characteristic structural feature of IFNγR2: an extensive ϖ–cation motif of stacked residues KWRWRH.





Selected examples of dinucleotide conformers (NtC). A universal structural alphabet of nucleic acids was developed in the form of so called NtC dinucleotide conformers. Automatic structural analysis of any DNA or RNA molecule has been published on the publicly available server dnatco.datmos.org.

Protein Structure Characterization by Advanced Mass Spectrometry

RNDr. Petr Novák, Ph.D., Head of the Lab.

The Institute of Microbiology of the
Czech Academy of Sciences

Content of the Research

The Structural Biology and Cell Signaling Laboratory studies two intertwined topics covering structural biology and cell signaling. The structural biology group produces recombinant proteins and characterizes their protein-ligand interactions or dynamics utilizing advanced mass spectrometric techniques. The cell signaling group studies the connections between cellular signaling with the metabolism of pathologies by conventional molecular biology and biochemistry methods. Through the close collaboration of these two groups, a unique research platform has been formed, wherein the results obtained by the study of biological systems can also be verified and explained at the molecular level.

The current research interests of our laboratory are the following; characterization of proteins or protein/ligand complexes and their dynamics using chemical cross-linking, hydrogen/deuterium exchange, hydroxyl radical footprinting and ion mobility. The influence of chemical stress on the induction of senescence, apoptosis and necrosis in various pathologies and the monitoring of metabolic turnover are studied using immunochemistry, real-time quantitative PCR, RNA interference, chromatin immunoprecipitation, quantitative proteomics and metabolomics.

The laboratory is also involved in two Horizon 2020 research consortia – EU-FT-ICR MS and EPIC-XS. The first one is focused on the development and application of ultra-high resolution mass spectrometry to various research areas and the second brings together top-notch European proteomics laboratories.

Potential for Cooperation

We offer our expertise in the following fields; analysis of proteins, their modifications, higher order structure dynamics, protein-protein and protein-ligand interactions including generic protein drugs (biosimilars), and software development for mass spectrometric data interpretation and visualization. Our laboratory has previously collaborated with the following national and international academic institutions; Charles University in Prague, Czech Republic with Prof. Jan Černý, the University of Calgary in Calgary, Canada with Prof. David C. Schriemer, The University of Connecticut, Storrs, U.S.A with Prof. Daniele Fabris, and Stanford University in California, U.S.A. with Prof. Merritt Maduke. In addition, we also have experience in industrial partnerships, we have collaborated on research projects with the following companies; the TriLAB Group, CF Plus Chemicals, Biovendor, BEACTICA and Bruker Daltonics. The collaboration potential of the laboratory is strengthened via the EU H2020 project offering trans-national access.

2020 Trcka F, Durech M, Vankova P, Vandova V, Simoncik O, Kavan D, Vojtesek B, Muller P, Man P The interaction of the mitochondrial protein importer TOMM34 with HSP70 is regulated by TOMM34 phosphorylation and binding to 14-3-3 adaptors. J. Biol Chem 2020 Jul 3;295(27):8928-8944. DOI: 10.1074/jbc.RA120.012624

2019 Rahimidashaghoul K, Klimánková I, Hubálek M, Korecký M, Chvojka M, Pokorný D, Matoušek V, Fojtík L, Kavan D, Kukačka Z, Novák P, Beier P. Reductant-Induced Free Radical Fluoroalkylation of Nitrogen Heterocycles and Innate Aromatic Amino Acid Residues in Peptides and Proteins. Chemistry 2019 Dec10; 25(69): 15779-15785. DOI: 10.1002/chem.201902944

2018 Rozbesky D, Rosulek M, Kukacka Z, Chmelik J, Man P, Novak P Impact of Chemical Cross-Linking on Protein Structure and Function. Anal Chem 2018 Jan 16; 90(2): 1104-1113. DOI: 10.1021/acs.analchem.7b02863

2017 Kadek A, Kavan D, Marcoux J, Stojko J, Felice AK, Cianférani S, Ludwig R, Halada P, Man P. Interdomain electron transfer in cellobiose dehydrogenase is governed by surface electrostatics. Biochim Biophys Acta. 2017 Feb;1861(2):157-167. doi:10.1016/j.bbagen.2016.11.016,

2016 Pompach P, Nováková J, Kavan D, Benada O, Růžička V, Volný M, Novák P. Planar Functionalized Surfaces for Direct Immunoaffinity Desorption/Ionization Mass Spectrometry. Clin Chem. 2016 Jan;62(1):270-8. doi:10.1373/clinchem.2015.244004

Contact person:	RNDr. Petr Novák, Ph.D.
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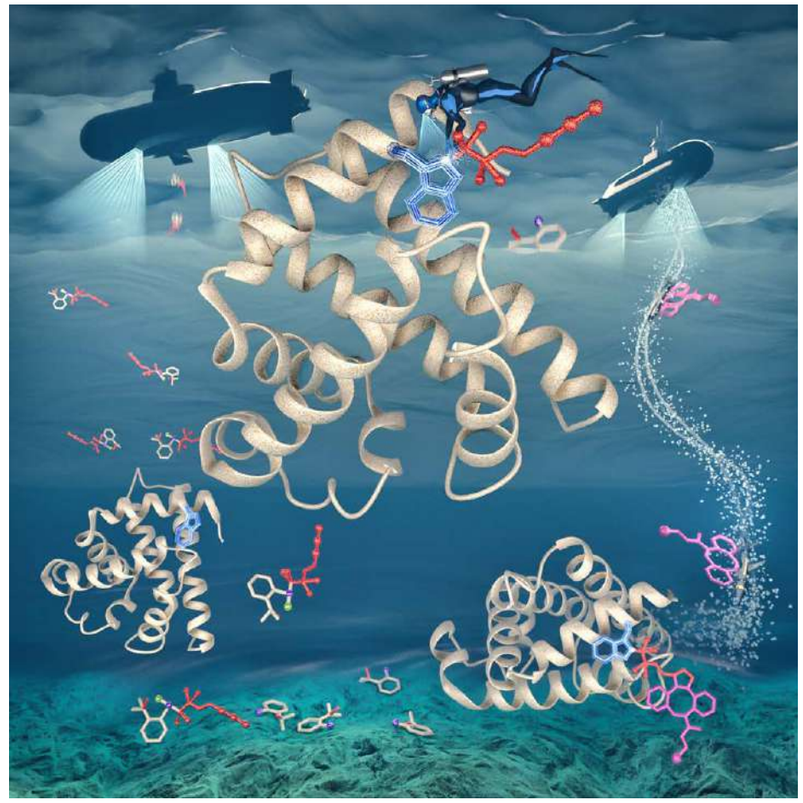
Basic keywords:		
	01	Protein covalent labeling
	02	Chemical cross-linking
	03	H/D exchange
	04	Hydroxyl radical footprinting
	05	Mass spectrometry
	06	Code writing
	07	Transcription factors
	08	Tumorgenesis and inherited pathologies
	09	Cell metabolism and signaling
	10	Cell signaling

Research areas:	01	Structural mass spectrometry, clinical proteomics, method and software development, transcriptional regulation, cell metabolism in pathologies
Main objectives:	01	Protein dynamics in solution, protein-protein and protein-ligand complex characterization, biomarker discovery, software development for mass spectrometric data interpretation and visualization, chromatin integrity, cell metabolism and signaling



Graphical illustration accompanying a joint research article with Petr Beier's group from the Institute of Organic Chemistry and Biochemistry and Vaclav Matousek from CF Plus Chemicals that was published in Chemistry: A European Journal and its topic was selected for the cover page of the respective issue. It symbolizes preparation of pharmacologically active protein bioconjugate drugs.

Research and Development of High-Affinity Binding Proteins

RNDr. Petr Malý, CSc., Head of the Lab.

The Institute of Biotechnology of the
Czech Academy of Sciences

Content of the Research

The Ligand Engineering Laboratory is focused on engineering novel binding proteins raised against a wide range of medically important targets. Those include human cytokines and their receptors, tumor markers, virus envelope proteins and virus-neutralizing antibodies. Our primary goal is to develop proteins with a required function such as high affinity, high selectivity or designed neutralization function. Moreover, we engineer mimicking proteins as potential vaccine immunogens. To reach our goals, we use methods of protein engineering including directed evolution of proteins and exploit highly complex combinatorial libraries that were designed in-house and assembled based on selected protein domain scaffolds. We use protein libraries with a three-helix bundle concept, randomized beta-sheet protein fold or targeted randomization of protein loops. This complex approach with in silico predictions and ribosome display allows us to generate unique protein binders' collections and identify variants with required properties against almost any chosen target. Tailor-made protein binders find utility in further research but also in human diagnostics and therapy as high-affinity non-immunoglobulin alternatives to monoclonal antibodies with the advantage of constructional flexibility and cheap bacterial production. As examples, we have developed three collections of immunosuppressive ABD-derived binding proteins targeting the IL-23/Th-17pro-inflammatory axis that function as blockers of human IL-23 cytokine (ILP proteins), IL-23 receptor (REX proteins) and IL-17 receptor A (ARS proteins) [Autoimmunity, 2017; 50: 102-113] [Proteins, 2014; 82: 975-989] [Int.J.Mol.Sci., 2018; 19: 3089], Czech patents No. 304514 and No. 307849 and European patent No. 2922560. We have generated D7 protein variants recognizing insoluble human fibrin fibrils. These proteins deliver liposomes to the human thrombus under flow conditions in a silicone replica of the middle cerebral artery and could serve as "surface navigators" for in vivo imaging of stroke by MRI. We have collaborated on the development of a unique glass microfluidic Y-system with a planar immunocapture channel and engineered protein ligands for selective cell immunocapture in a biosensor. The results can be exploited for the capture of circulating tumor cells or rare cell populations.

Potential for Cooperation

The design and development of tailor made proteins with a required function. The generation and characterization of protein ligands with high affinity, neutralizing potential or mimicking proteins for vaccine development.

2020 – Smejkal J., Maly P., Kuchar M., Panova N., Semeradtova A., Aubrecht P., Stofik M., Maly J. (2020). "Cell immunocapture microfluidic chip based on high-affinity recombinant protein binders." Biosens. Bioelectron **172**: 112784.

2019 – Kosztyu, P., M. Kuchar, J. Cerny, L. Barkocziova, M. Maly, H. Petrokova, L. Czernekova, V. Liskova, L. Raskova Kafkova, P. Knotigova, J. Masek, J. Turanek, P. Maly and M. Raska (2019). "Proteins mimicking epitope of HIV-1 virus neutralizing antibody induce virus-neutralizing sera in mice." EBioMedicine **47**: 247-256.

– Petrokova, H., J. Masek, M. Kuchar, A. Viteckova Wunschova, J. Stikarova, E. Bartheldyova, P. Kulich, F. Hubatka, J. Kotoucek, P. T. Knotigova, E. Vohlidalova, R. Hezova, E. Maskova, S. Macaulay, J. E. Dyr, M. Raska, R. Mikulik, P. Maly and J. Turanek (2019). "Targeting Human Thrombus by Liposomes Modified with Anti-Fibrin Protein Binders." Pharmaceutics **11**(12), 642.

– Plavec, T. V., M. Kuchar, A. Benko, V. Liskova, J. Cerny, A. Berlec and P. Maly (2019). "Engineered Lactococcus lactis Secreting IL-23 Receptor-Targeted REX Protein Blockers for Modulation of IL-23/Th17-Mediated Inflammation." Microorganisms **7**(5), 152.

2018 – Hlavnickova, M., M. Kuchar, R. Osicka, L. Vankova, H. Petrokova, M. Maly, J. Cerny, P. Arenberger and P. Maly (2018). "ABD-Derived Protein Blockers of Human IL-17 Receptor A as Non-IgG Alternatives for Modulation of IL-17-Dependent Pro-Inflammatory Axis." Int J Mol Sci **19**(10), 3089.

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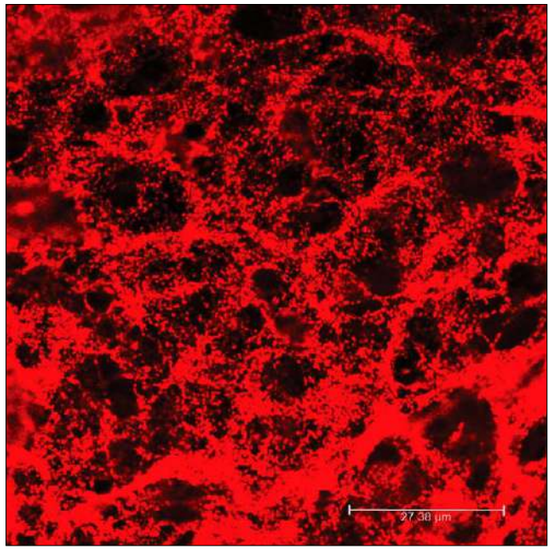
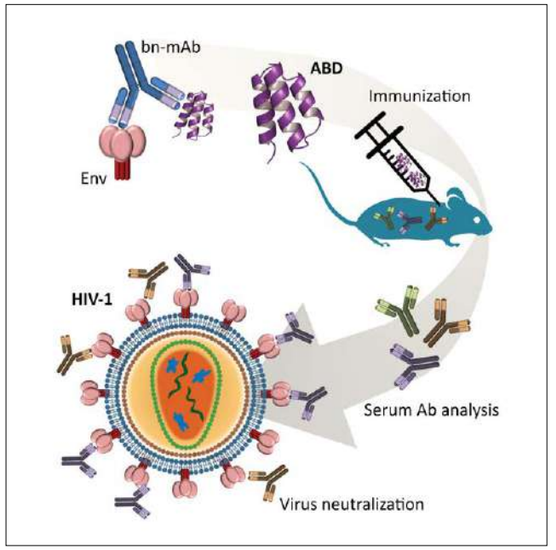
Basic keywords:	01	Protein binder
	02	Protein domain scaffold
	03	Albumin-binding domain
	04	Combinatorial library
	05	Ribosome display
	06	Receptor antagonists
	07	Cytokine–binding protein
	08	High-affinity ligand

Research areas:	01	Protein engineering
	02	Immunology
	03	Cell biology
	04	Pharmacology
Main objectives:	01	Research and development of human cell-surface receptor antagonists
	02	Cytokine-binding proteins, binders of human serum oncomarkers
	03	Capture binding proteins for biosensors

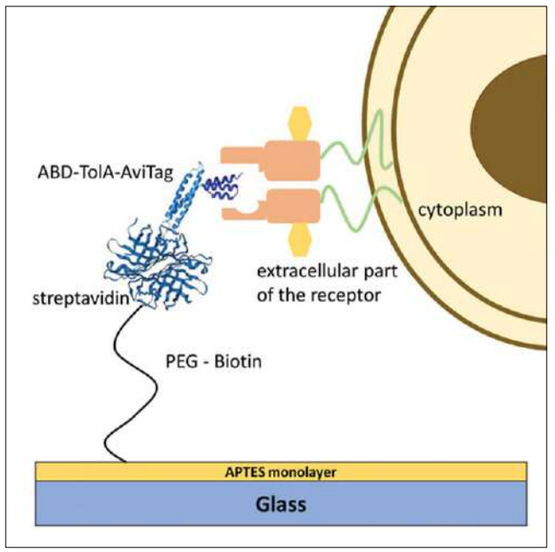


The developed anti-fibrin protein binder D7F1 was used as a specific binding component for the delivery of rhodamine-lyssamine PE-labelled liposomes to the human thrombus for its imaging. Red indicates the D7F1-mediated visualization of an insoluble fibrin network by confocal microscopy. See Petroková et al. Pharmaceutics 11(12), 642, 2019; Vítečková Wünschová et al., Pharmaceutics 12(12), 1207, 2020.

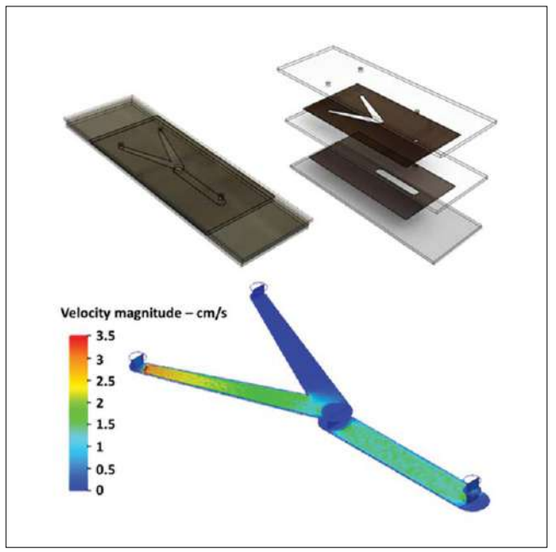


A scheme demonstrating the identification of protein variants specific for the paratope of a chosen broadly neutralizing antibody and their use as non-cognate ligand immunogens for the stimulation of preventive neutralizing antibodies production. See Kosztyu, P. et al. EBioMedicine 47: 247-256, 2019. , and comment by P. J. Klasse "Non-cognate ligands of Procrustean paratopes as potential vaccine components", EBioMedicine 47: 6-7, 2019. Mimicking VRA proteins are subject of the Czech patent reg. No. 308617 (granted 11-2020) and subject of the PCT international patent application No. PCT/CZ2020/050066 (09-2020).

Immobilization of protein binders and cell immunocapture



Y-microfluidic chip fabrication and computational fluid dynamic modeling



An illustration of cell immunocapture in the developed microfluidic biosensor. A principle of the immobilization of recombinant protein ligands to chip surface and a visualization of Y-microfluidic chip fabrication. For details see Smejkal J., Maly P., Kuchar M., Panova N., Semeradtova A., Aubrecht P., Stofik M., Maly J. "Cell immunocapture microfluidic chip based on high-affinity recombinant protein binders." Biosensors and Bioelectronics 172: 112784, 2020.

Structural Bioinformatics of Proteins
Ing. Jiří Černý, Head of the Lab.

The Institute of Biotechnology of the
Czech Academy of Sciences

Content of the Research

Biomolecules – proteins and nucleic acids – are the basis of all living organisms. Their interactions, as well as the interactions with small molecules such as drugs, are the driving force determining every moment of the life of an organism. Moreover, these interactions form the basis for understanding the principles and designing the treatments for various diseases. The international team working at the Laboratory of Structural Bioinformatics of Proteins uses computational modeling in order to improve the understanding of both the structure and the interactions of biomolecules. The project concentrates on the molecular modeling of biologically relevant molecules and their interactions by employing accurate quantum chemical calculations and empirical potential methods. The research covers the analysis of structural data and the rational design of mutations, ligands and inhibitors. It also addresses the development and testing of computational methods and procedures for modeling the structure and properties of biomolecules and their complexes. The project works closely with projects from the Structural Biology and Protein Engineering program as well as with projects from programs 2 and 5 on the analysis of structural data and the rational design of mutations, ligands and inhibitors of studied proteins.

Our laboratory has achieved notable discoveries. We have developed a new algorithm for Enriched Conformational Sampling of DNA and Proteins with a Hybrid Hamiltonian Derived from the Protein Data Bank. We have also revealed a new role of the LILI motif of M3-S2 linkers in the NMDA receptor channel gating.

Potential for Cooperation

We offer our expertise in molecular modeling and in silico mutagenesis and improving protein stability and interactions. Protein/protein and protein/small molecule docking for either the design or identification of key residues involved in the recognition and strength of interaction. We offer the following methods; homology modeling, in silico mutagenesis with respect to protein stability and interactions, protein/protein docking, identification of key residues involved in the recognition and strength of interaction, protein/small molecule docking, molecular dynamics simulations (MD) and ab initio quantum chemical calculations. Our methods and data are also available as web services, DNATCO: assignment of DNA conformers at dnatco.org and in the database, WatAA: Atlas of Protein Hydration. Exploring synergies between data mining and ab initio calculations.

2019 Kosztyu, P., M. Kuchar, J. Cerny, L. Barkocziova, M. Maly, H. Petrokova, L. Czernekova, V. Liskova, L. Raskova Kafkova, P. Knotigova, J. Masek, J. Turanek, P. Maly and M. Raska. "Proteins mimicking epitope of HIV-1 virus neutralizing antibody induce virus-neutralizing sera in mice." EBioMedicine 47: 247-256.

2018 Skrlec, K., P. Zadravec, M. Hlavnickova, M. Kuchar, L. Vankova, H. Petrokova, L. Krizova, J. Cerny, A. Berlec and P. Maly. "p19-Targeting ILP Protein Blockers of IL-23/Th-17 Pro-Inflammatory Axis Displayed on Engineered Bacteria of Food Origin." Int J Mol Sci 19(7).

2018 Ladislav M, Cerny J, Krusek J, Horak M, Balik A, Vyklicky L. "The LILI Motif of M3-S2 Linkers Is a Component of the NMDA Receptor Channel Gate." Front Mol Neurosci.

2018 Vyklicky V, Krausova B, Cerny J, Ladislav M, Smejkalova T, Kysilov B, Korinek M, Danacikova S, Horak M, Chodounska H, Kudova E, Vyklicky L. "Surface Expression, Function, and Pharmacology of Disease-Associated Mutations in the Membrane Domain of the Human GluN2B Subunit." Front Mol Neurosci.

2016 Mikulecky, P., J. Zahradnik, P. Kolenko, J. Cerny, T. Charnavets, L. Kolarova, I. Necasova, P. N. Pham and B. Schneider. "Crystal structure of human interferon-gamma receptor 2 reveals the structural basis for receptor specificity." Acta Crystallogr D Struct Biol 72(Pt 9): 1017-1025.

2016 Taherkhani, M., A. Armentano, J. Černý and K. Müller-Dethlefs. "Threshold ionization spectroscopic investigation of supersonic jet-cooled, laser-desorbed Tryptophan." Chemical Physics Letters

2015 Osicka, R., A. Osickova, S. Hasan, L. Bumba, J. Cerny and P. Sebo. "Bordetella adenylate cyclase toxin is a unique ligand of the integrin complement receptor 3." E life

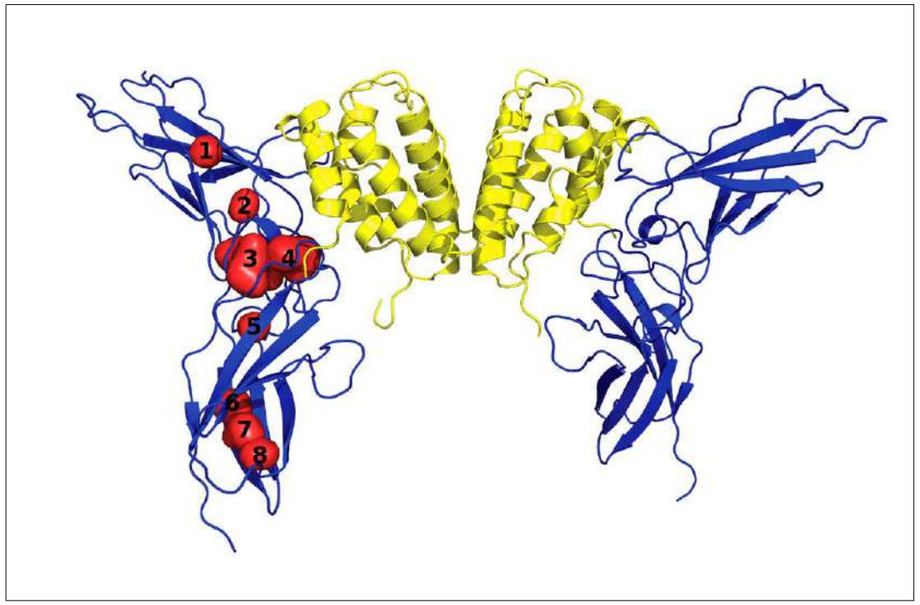
2015 Craveur P, Joseph AP, Esque J, Narwani TJ, Noël F, Shinada N, Goguet M, Leonard S, Poulain P, Bertrand O, Faure G, Rebehmed J, Ghozlane A, Swapna LS, Bhaskara RM, Barnoud J, Téletchéa S, Jallu V, Cerny J, Schneider B, Etchebest C, Srinivasan N, Gelly JC, de Brevern AG. "Protein flexibility in the light of structural alphabets." Front Mol Biosci.

2015 K. Kluckova, M. Sticha, J. Cerny, T. Mracek, L Dong, Z. Drahota, E. Gottlieb, J. Neuzil, J. Rohlena, "Ubiquinone-binding site mutagenesis reveals the role of mitochondrial complex II in cell death initiation."Cell Death & Disease

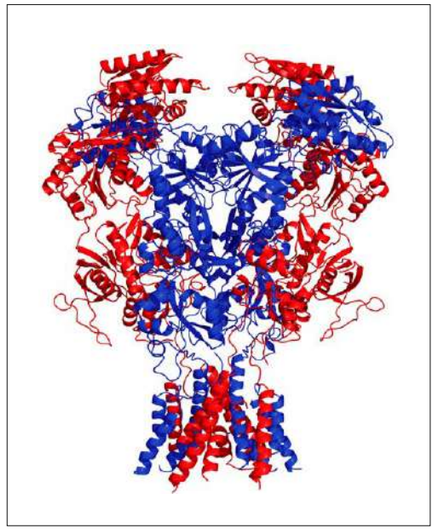
Contact person:	Ing. Jiří Černý
	e-mail: jiri.cerny@ibt.cas.cz

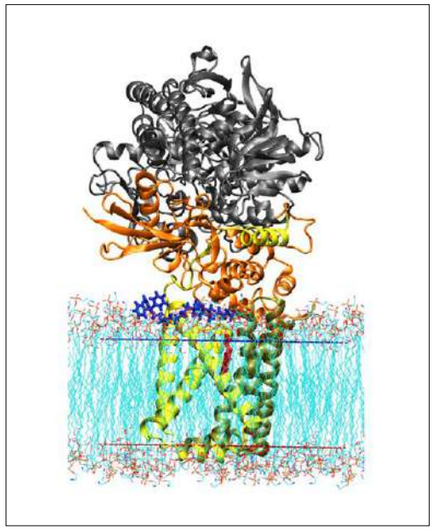
Basic keywords:	01	Structural bioinformatics
	02	Molecular modeling
	03	Rational design of ligands and inhibitors
	04	Homology modeling
	05	Docking

Research areas:	01	Structural Bioinformatics.
	02	Molecular Modeling.
	03	Computer aided design of inhibitors.
	04	Design of protein scaffolds.
Main objectives:	01	Development and testing of computational methods and procedures for modeling the structure and properties of biomolecules and their complexes.
	02	Development of novel computational methods and parameter optimization in order to describe designed ligands and inhibitors and their interactions.
	03	Analysis of the conformational behavior of oligopeptide blocks in proteins.
	04	Identification of stable protein folds suitable for mutagenesis.

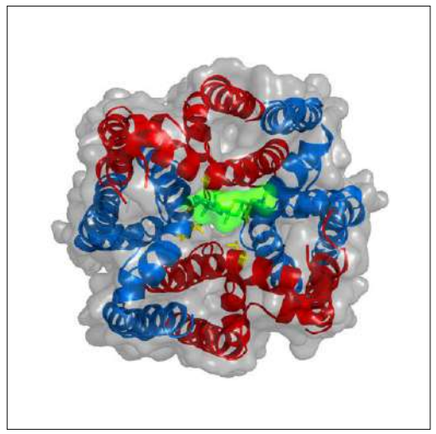


Mutations at the interface and inside cavities of the IFN/IFNγR1 complex were designed in order to increase the binding affinity.





Binding mode of potential anti-cancer compounds targeted to the mitochondrial complexes I and II was modeled for WT and cancer related variants bearing mutations at the binding site. (with Jiří Neužil)



The mechanism of opening and inhibition of the NMDA receptor channel was suggested by combining molecular modeling and docking.

Dynamics of Biological Processes
Gustavo Fuertes Vives, Ph.D., Head of the Lab.

The Institute of Biotechnology of the
Czech Academy of Sciences

Content of the Research

The aim of this project is to understand how light controls the structure, dynamics and activity of photosensitive proteins. We focus on transcription factors that interact with specific sequences of DNA in a light-dependent manner (EL222, CarH, CooA). Photon absorption by the embedded chromophores triggers conformational changes in the protein chain that ultimately alter protein/protein and protein/DNA interaction networks in the cell. Our plan is to unveil the molecular details of photoswitching between "dark" and "light" states on time scales ranging from femtoseconds to hours. The acquired knowledge will allow us to create novel photoreceptors for use in areas like synthetic biology and optogenetics.

Our team employs an integrative 4D structural biology approach combining methods with distinct spatial and temporal resolution. In the field of molecular biology, we have the capability to produce isotopically labeled proteins, and proteins containing non-canonical amino acids. As steady-state and time-resolved biophysical methods we use: Femtosecond-stimulated Raman spectroscopy (FSRS), 1D and 2D-infrared spectroscopy (2D-IR), Fluorescence spectroscopy (FRET, FCS and FA), Small-angle neutron (SANS), and X-ray scattering (SAXS). Our portfolio of computational methods includes quantum mechanics (QM), molecular dynamics (MD), and hybrid approaches.

Potential for Cooperation

Within BIOCEV, we closely collaborate with the "Intermolecular Recognition of Proteins and Nucleic Acids" and the "Structure of Medically and Biotechnologically Important Enzymes" projects. In the Czech Republic, together with the ELI Beamlines laser facility (Institute of Physics CAS, Dolni Brezany), we are part of the ELIBIO (Structural Dynamics of Biomolecular Systems) project led by Janos Hajdu, which explores new frontiers in light and optics to create breakthrough science in biology, chemistry and physics. Our overseas collaborations include: "Padhu" (IQFR CSIC, Spain); cobalamin-binding transcription factors, C.S. Raman (The University of Maryland, USA); CO-binding transcription factors, Pau Bernado (CBS, France); site-specific isotopic labeling of proteins in cell-free systems, Bernhard Brutscher (IBS, France); laser-triggered NMR spectroscopy. Moreover, our group has access to large-scale research infrastructures to perform cutting-edge experiments including SANS (Institute Laue Langevin, France), SAXS (The European Molecular Biology Laboratory, Germany) and time-resolved spectroscopy (LaserLab Amsterdam, The Netherlands)

2020 Andrikopoulos, P.C., Liu, Y., Picchiotti, A., Lenngren, N., Kloz, M., Chaudhari, A.S., Precek, M., Rebarz, M., Andreasson, J., Hajdu, J., Schneider, B., and Fuertes, G. (2020). Femtosecond-to-nanosecond dynamics of flavin mononucleotide monitored by stimulated Raman spectroscopy and simulations. Phys Chem Chem Phys. https://doi.org/10.1039/C9CP04918E.

2019 Fuertes, G., Nevola, L., and Esteban-Martin, S. (2019). Perspectives on drug discovery strategies based on IDPs in Intrinsically Disordered Proteins: Dynamics, Binding, and Function (Academic Press), pp. 275-327. https://doi.org/10.1016/B978-0-12-816348-1.00009-0

2018 Fuertes, G., Banterle, N., Ruff, K.M., Chowdhury, A., Pappu, R.V., Svergun, D.I., and Lemke, E.A. (2018). Comment on "Innovative scattering analysis shows that hydrophobic disordered proteins are expanded in water." Science 361. https://doi.org/10.1126/science.aau8230

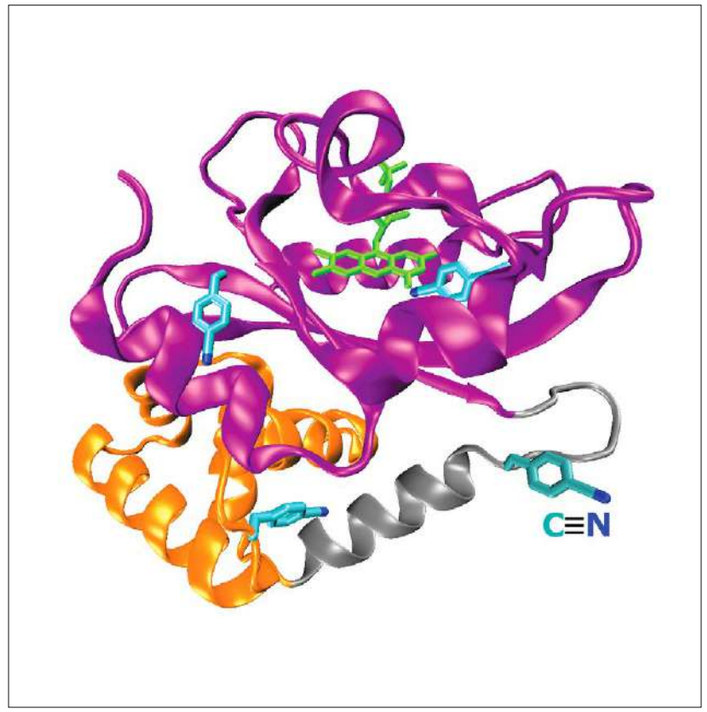
2017 Fuertes, G., Banterle, N., Ruff, K.M., Chowdhury, A., Mercadante, D., Koehler, C., et al. (2017). Decoupling of size and shape fluctuations in heteropolymeric sequences reconciles discrepancies in SAXS vs. FRET measurements. Proc. Natl. Acad. Sci. 114, E6342–E6351. https://doi.org/10.1073/pnas.1704692114

Contact person:	Gustavo Fuertes Vives, PhD.
	e-mail: gustavo.fuertes@ibt.cas.cz

Basic keywords:	01	LOV domains
	02	B12-based photoreceptors
	03	Hemeproteins
	04	Allosterism
	05	Conformational dynamics
	06	Fold-switching proteins
	07	Photochemistry & photobiology
	08	Vibrational spectroscopy
	09	Time-resolved structural biology
	10	Non-canonical amino acids

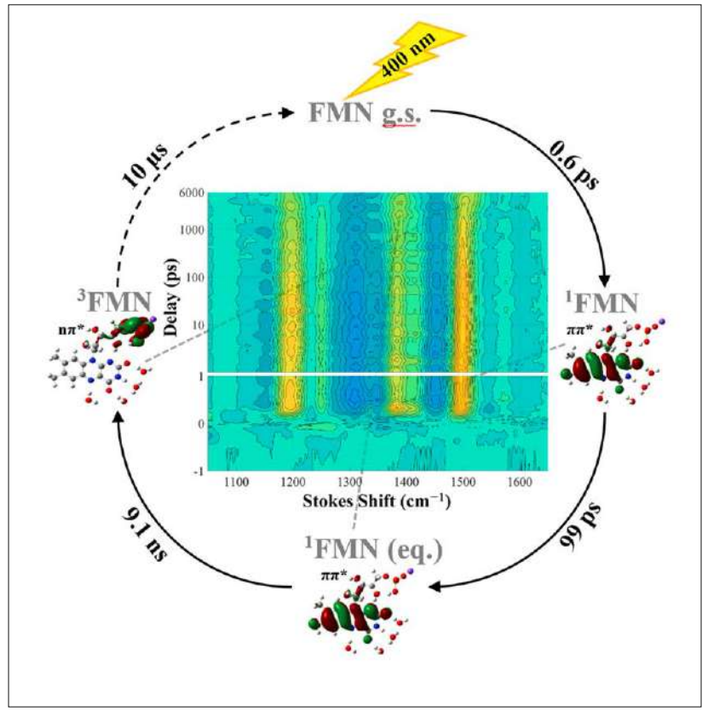
Research areas:	01	Photosensory receptors
	02	Structural dynamics of biomolecules
	03	Protein engineering
	04	Computational chemistry
Main objectives:	01	Molecular basis of photocontrolled protein-protein and protein-DNA interactions.
	02	Development of genetic code expansion technology for the site-specific labeling of proteins.
	03	Analysis of photoinduced processes in biomolecules using advanced computational tools.
	04	Elucidation of vibrational energy transfer pathways in biomolecules.

Monitoring light-induced conformational changes in EL222 with multiple genetically encoded infrared reporters.

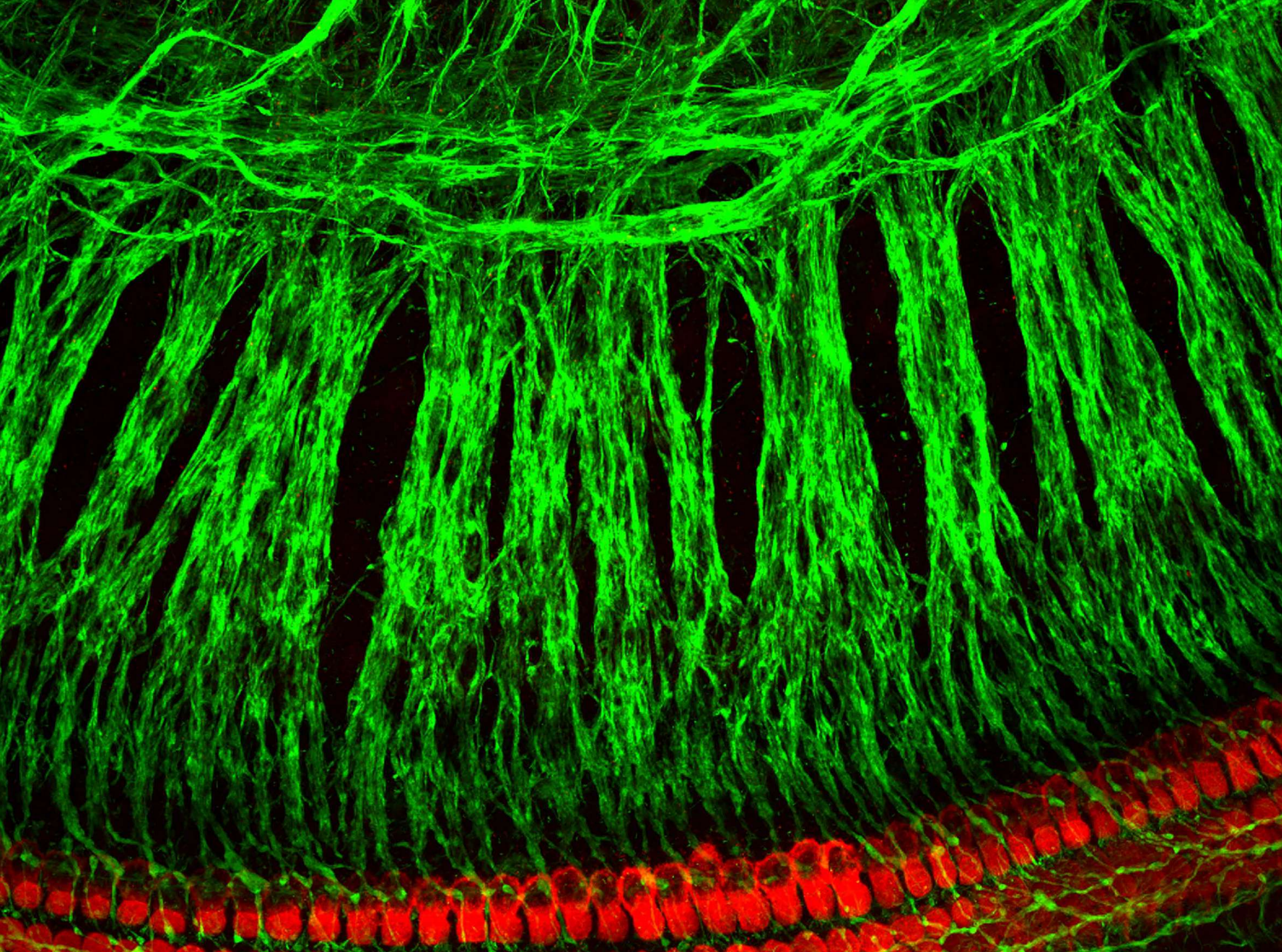


The photocontrolled transcription factor EL222 features a flavin-binding light-oxygen-voltage (LOV) domain (violet) and a DNA-binding helix-turn-helix domain (orange) joined by a linker (grey). Absorption of blue-light by the flavin mononucleotide (FMN) chromophore (green) triggers structural changes in the polypeptide chain. We are decorating EL222 with the non-canonical amino acid 4-cyanophenylalanine (cyan) bearing a triple bond as a site-specific vibrational probe of microenvironment. Photoinduced changes are tracked by time-resolved infrared spectroscopy from femtoseconds to hours with single residue resolution.

The photocycle of flavin mononucleotide (FMN) free in solution monitored by femtosecond-stimulated Raman spectroscopy (FSRS) and quantum chemistry (QM) simulations [Andrikopoulos, P.C. et al, PCCP, 2020).



Blue-light (400 nm) triggers the ultrafast (sub-picosecond) formation of the singlet state (^{1}FMN) that equilibrates in ~100 picoseconds. The intersystem crossing from singlet to triplet states (^{3}FMN) happens on the nanosecond time scale. Finally, the FMN triplet phosphoresces back to the ground-state (g.s.) within several microseconds. The photocycle of LOV proteins is more complicated and is characterized by the formation of a covalent bond between FMN and a conserved cysteine residue. Our results pave the way for the study of the photocycle of FMN embedded in LOV proteins by a joint FSRS/QM approach.



Biomaterials and Tissue Engineering

No. of research teams: 05

Pages: 132–147

04

Biomaterials and Tissue Engineering

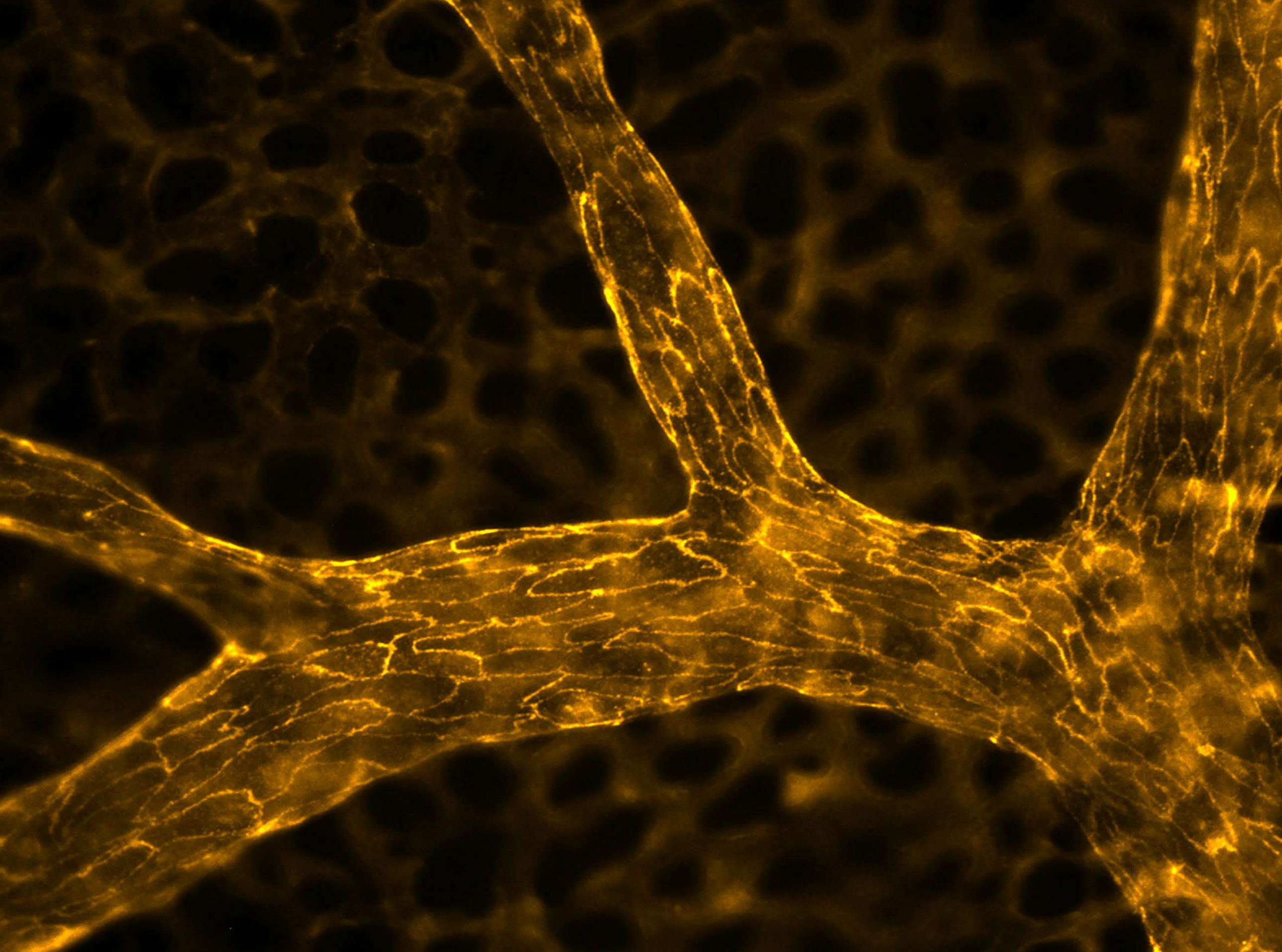
RNDr. Tomáš Etrych, DSc.
Head of the Research Programme
The Institute of Macromolecular Chemistry of the Czech Academy of Sciences

Research Directions — Biomaterials and Tissue Engineering

1.	New synthetic methods for the controlled preparation of supramolecular dendritic, hyperbranched, star-shaped, and comb-like water-soluble polymer structures	2.	High-molecular water-soluble polymer transport systems for the targeted delivery of drugs, diagnostics, and their combinations	3.	In vitro and in vivo biological evaluation of new high-molecular-weight polymer conjugates in selected animal neoplastic and cardiovascular models
4.	Magnetic carriers based on polymethacrylates and polymethacrylamides, easily accessible for various chemical modifications enabling the immobilzation of biologically active molecules	5.	Anti-fouling polymer layers with covalently attached bioreceptors for SPR biosensors detecting selected analytes in the blood serum, plasma and blood	6.	Superporous biodegradable hydrogels as scaffolds for regenerating soft tissues and replacements of cartilage and damaged spinal cords
7.	Newly constructed bioartificial tissue grafts (blood vessels, heart valve, bone, skin)	8.	Innovated grafts of blood vessels and bones prepared by modifying currently clinically used grafts	9.	Systems for targeted drug delivery
10.	Biosensors and stimulators of cell functions	11.	Protocols for stem cell differentiation into clinically relevant lines, clinical and preclinical studies to verify safety and efficacy, and tissue reconstruction methods		

The programme is focused on advanced trends in medicine aimed at the usage of sophisticated tissue prostheses systems composed of synthetic materials combined with specific biologically-active compounds and cells for the regeneration and replacement of diseased tissues and organs or for controlled drug and gene delivery targeted to specific tissues and cells in a diseased organism. The programme's feasibility is guaranteed by the close collaboration of "synthesis" teams concerned with the development and synthesis of artificial carriers for cells, therapeutics and diagnostics, and "biomedicine" teams concerned with applications of cells, including stem cells, development of bioartificial grafts for tissue engineering, and the testing of therapeutic and diagnostic systems. The basic common activities of the "synthesis" teams consisting of the synthesis of polymer materials with attached biologically-active components will be based in general on investigating and testing the interactions between cells and tissues with the biomaterials directed towards specific medical applications.

The overall aim of the applied research within this programme is to develop technologies for the preparation of the intended products and to standardize this preparation so that it is possible to transfer these technologies over to clinical practice. Planned outputs include: bioartificial blood vessel, valve, bone, and cartilage grafts, scaffolds for the therapy of spinal cord lesions, targeted drug, gene, and diagnostics delivery systems for the therapy and diagnostics of cancer and cardiovascular diseases, biosensors and protein chips, and affinity carriers for separating and purifying biological fluids and suspensions.



Polymer and Colloid Immunotherapeutics
Ing. Richard Laga, Ph.D., Head of the Research Project

The Institute of Macromolecular Chemistry
of the Czech Academy of Sciences

Content of the Research

The research project is focused on the development of new types of polymer, colloid and hybrid polymer-colloid delivery systems of various therapeutics and contrast agents for the treatment and diagnosis of serious human diseases. Special attention is paid to the controlled synthesis and detailed physicochemical characterization of biocompatible carriers of variable size, composition and morphology with regard to their biological and biophysical properties in vivo.

One of the research goals is the development of advanced macromolecular vaccines for the prophylaxis of infectious diseases or for the immunotherapy of cancers. The vaccines are based on the conjugates of protein, peptide or gene-encoded antigens and highly potent synthetic adjuvants with hydrophilic macromolecular carries, which provide immunotherapeutics with higher solubility in body fluids, more effective interaction with immune system cells, and a long-lasting effect.

The next goal includes using long-circulating 31P/1H-MRI contrast agents based on the conjugates of paramagnetic ions or superparamagnetic nanoparticles with water-soluble phosphorous polymers. The conjugates are designed to provide a high intensity MR signal distinguishable from the natural biological background, exploitable in the anatomical and functional imaging of organs, tissues (including tumors) and cells.

Another part of the research is devoted to the development of contrast agents for photoacoustic imaging based on surface-modified polypyrrole nanoparticles and core-shell nanoparticles with superhydrophobic cores with a low refractive index. The particles are designed to generate photoacoustic signals directly by their matrices or by a special NIR-absorbing dye immobilized in a superhydrophobic core.

Potential for Cooperation

We are fully open to collaboration with new partners, to whom we can offer our deep knowledge in the field of organic, polymer and colloid synthesis, bioconjugate chemistry and physical chemistry of macromolecular and colloid systems. Our expertise can be used in the development of polymer or colloid-based platforms for the delivery of various types of biologically active molecules or contrast agents with the aim of prolonging their circulation in the body, improving their solubility in body fluids, reducing their side effects and allowing their controlled release and activation at sites of action, or improving their persistence and visibility in the internal body structures, respectively.

2020 M. Paúrová, I. Šeděnková, J. Hromádková, M. Babič; Polypyrrole nanoparticles: control of the size and morphology; Journal of Polymer Research. 27(12), 366 (2020).

2019 G. M. Lynn, P. Chytil, J. R. Francica, A. Lagova, G. Kueberuwa, A. S. Ishizuka, N. Zaidi, R. A. Ramirez-Valdez, N. J. Blobel, F. Baharom, J. Leal, A. Q. Wang, M. Y. Gerner, T. Etrych, K. Ulbrich, L. W. Seymour, R. A. Seder, and R. Laga; Impact of polymer-TLR-7/8 agonist (adjuvant) morphology on the potency and mechanism of CD8 T cell induction; Biomacromolecules, 20(2), 854-870 (2019).

2019 J. R. Francica, R. Laga, G. M. Lynn, G. Mužíková, L. Androvič, B. Aussedat, W. E. Walkowicz, K. Padhan, A. Ramirez-Valdez, R. Parks, S. D. Schmidt, B. J. Flynn, Y. Tsybovsky, G. B. E. Stewart-Jones, J. M. Steichen, K. O. Saunders, F. Baharom, W. R. Schief, C. Petrovas, B. F. Haynes and R. A. Seder; Star nanoparticles delivering HIV-1 peptide minimal immunogens elicit near-native Envelope antibody responses in nonhuman primates; PLOS Biology, 17(6), e3000328 (2019).

2015 G. Lynn, R. Laga, P. Darrah, A. Ishizuka, A. Balaci, A. Dulcey, M. Pechar, R. Pola, M. Gerner, A. Yamamoto, J. Meyerson, K. Quin, M. Smelkinson, O. Vanek, R. Cawood, T. Hills, O. Vasalatiy, K. Kastenmuller, J. Francica, T. Etrych, K. Fisher, L. Seymour, R. Seder; In vivo characterization of the physicochemical properties of polymer-linked TLR agonists that enhance vaccine immunogenicity, Nature Biotechnology 33, 1201-1210 (2015).

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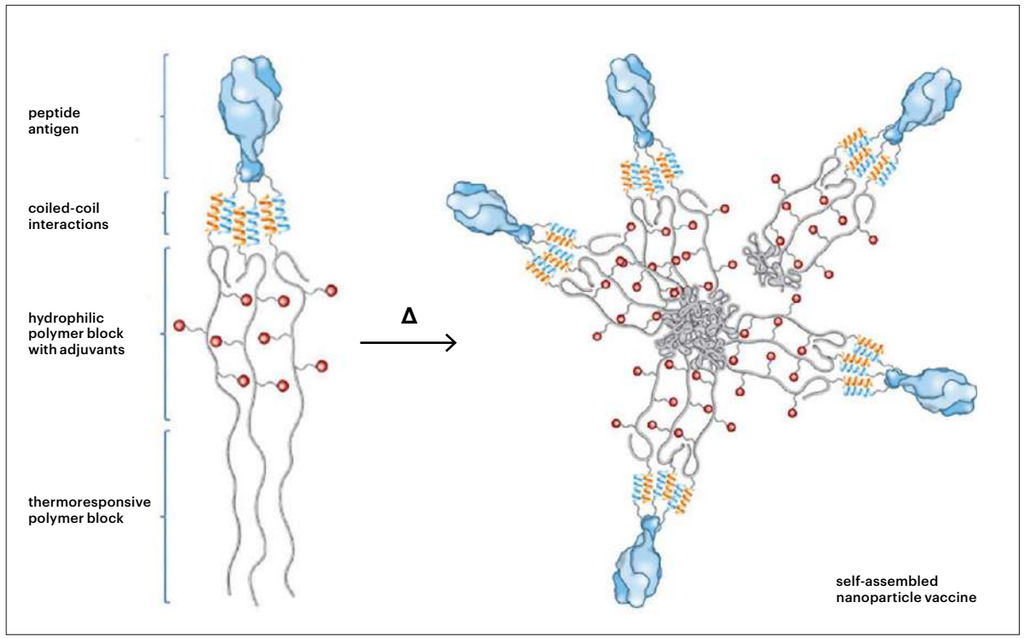
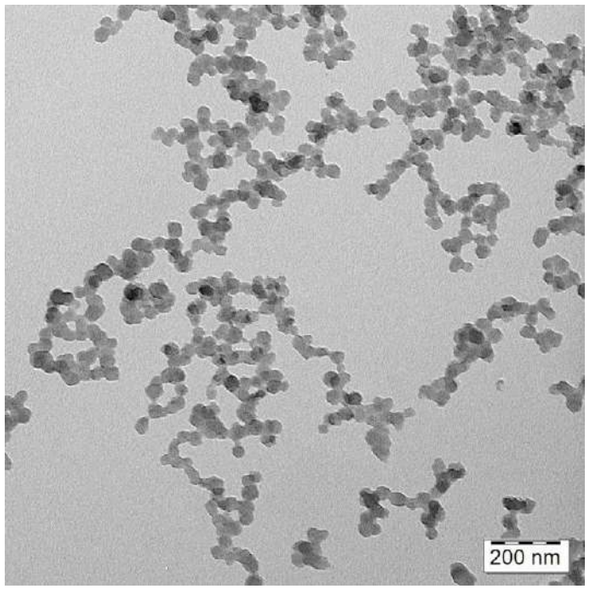
Basic keywords:	01	Polymer carrier
	02	Colloid nanoparticles
	03	Drug delivery
	04	Polymer therapeutics
	05	Polymer diagnostics
	06	Polymer vaccines
	07	Photoacoustic imaging
	08	Magnetic resonance imaging

Research areas:	01	Hybrid polymer-colloid drug delivery systems
	02	Polymer vaccines and immunostimulants
	03	Polymer and colloid diagnostics
Main objectives:	01	Development of polymer, colloid and hybrid polymer-colloid carriers of chemotherapeutics for the treatment of cancer diseases
	02	Development of polymer-based vaccines and immunostimulants for the prophylaxis of infectious diseases and immunotherapy of cancers
	03	Development of long-circulating polymer- and colloid-based diagnostics for non-invasive imaging of internal body structures



A schematic description of a self-assembled nanoparticulate polymer vaccine composed of a thermoresponsive di-block co-polymer containing adjuvant molecules (TLR-7/8a) in the side chains of its hydrophilic block and a peptide antigen (HIV-Gag) attached to the polymer end via a coiled-coil interaction of two complementary peptide chains.



TEM micrograph of polypyrrole nanoparticles for photoacoustic imaging.

Bioartificial Structures for the Replacement and Regeneration of Damaged Tissues

Doc. MUDr. Lucie Bačáková, CSc., Head of the Lab.

The Institute of Physiology of the
Czech Academy of Sciences

Content of the Research

In this project, scaffolds are prepared from synthetic and biological molecules and their combinations will be seeded with appropriate types of differentiated and stem cells. Three examples are featured here. In the first example, small-diameter blood vessels and the pericardium are subjected to decellularization using detergents and enzymes in special lab-made bioreactors. It is believed that after decellularization, a tissue loses most of its immunogenic activity, and is suitable for allogeneic and xenogeneic transplantation. The matrices are recellularized with adipose-derived stem cells (ASCs), taken from subcutaneous tissue, and differentiated towards endothelial or vascular smooth muscle cells by an appropriate composition of culture media and mechanical stimulation in dynamic bioreactors. The recellularized matrices are also tested in vivo in a pig model.

In the next example, samples of metallic materials currently used in orthopedics and stomatology, such as Ti, Ti6Al4V and stainless steel, subjected to various surface treatments, such as machining, shot peening, chemical milling, coating with diamond-like carbon, nanocrystalline diamond, ferroelectric ceramics or zeolites, are evaluated in vitro in terms of adhesion, growth and osteogenic differentiation of cells. Osseointegration of these materials in vivo is evaluated in rabbit or pig models. For the last example, in order to create a dermal/epidermal replacement, nanofibrous polylactide or polycaprolactone membranes are coated with fibrin, seeded with dermal fibroblasts, and coated with a collagen hydrogel. After the migration of fibroblast into the hydrogel, the top of the construct is seeded with keratinocytes. Another possibility is to seed the fibrin-coated membranes with adipose-derived stem cells, and to coat them with a collagen hydrogel containing endothelial cells, which form capillary-like structures. Modified nanofibrous meshes and nanocellulose materials can also be used as intelligent wound dressings delivering growth factors, drugs and cells into wounds.

Potential for Cooperation

We work with many research institutes, universities, hospitals and private companies in the Czech Republic and abroad. Examples of domestic collaboration include Czech Technical University and the Institute of Clinical and Experimental Medicine. Examples of collaboration abroad are the following; The University of Pennsylvania, Philadelphia, USA, The University of Sydney, School of Physics, Sydney, Australia, The University of Bordeaux, Bordeaux, France, Lancaster University, Lancaster, UK.

2018 Bacakova L, Zarubova J, Travnickova M, Musilkova J, Pajorova J, Slepicka P, Kasalkova NS, Svorcik V, Kolska Z, Motarjemi H, Molitor M. Stem cells: their source, potency and use in regenerative therapies with focus on adipose-derived stem cells - a review. Biotechnology Advances 36(4): 1111-1126, 2018.

2017 Matejka R, Stepanovska J, Rosina J, Hruzova D, Zarubova J. System for rotational endothelialization of vascular prostheses. Utility model, approved on 3.10.2017 under No. UV 31066. Owners: Czech Technical University, Faculty of Biomedical Engineering, Kladno; National Cell and Tissue Centre, Inc., Brno; Institute of Physiology, Acad. Sci. CR, Prague.

2018 Kopova I, Kronek J, Bacakova L, Fencl J. A cytotoxicity and wear analysis of trapeziometacarpal total joint replacement implant consisting of DLC-coated Co-Cr-Mo alloy with the use of titanium gradient interlayer. Diamond & Related Materials 97: 107456, 2019.

2018 Bacakova M, Pajorova J, Broz A, Hadraba D, Lopot F, Zavadakova A, Vistejnova L, Beno M, Kostic I, Jencova V, Bacakova L. A two-layer skin construct consisting of a collagen hydrogel reinforced by a fibrin-coated polylactide nanofibrous membrane. Int J Nanomedicine 14:5033-5050, 2019.

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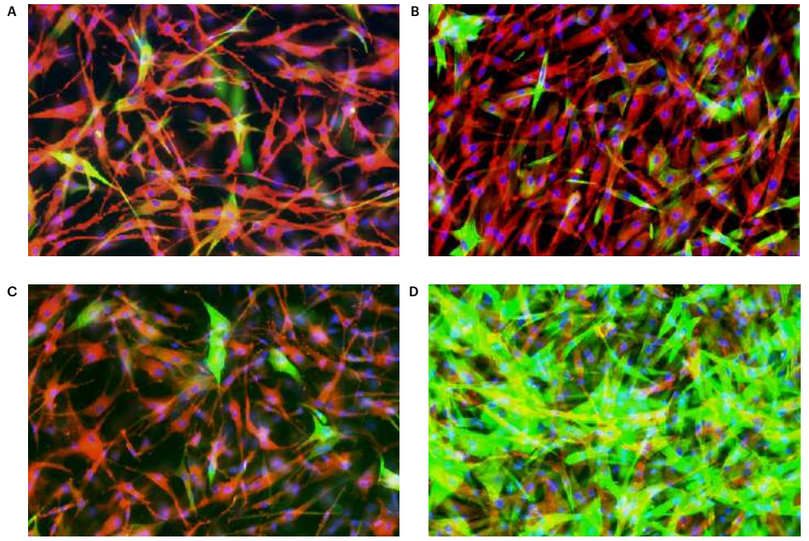
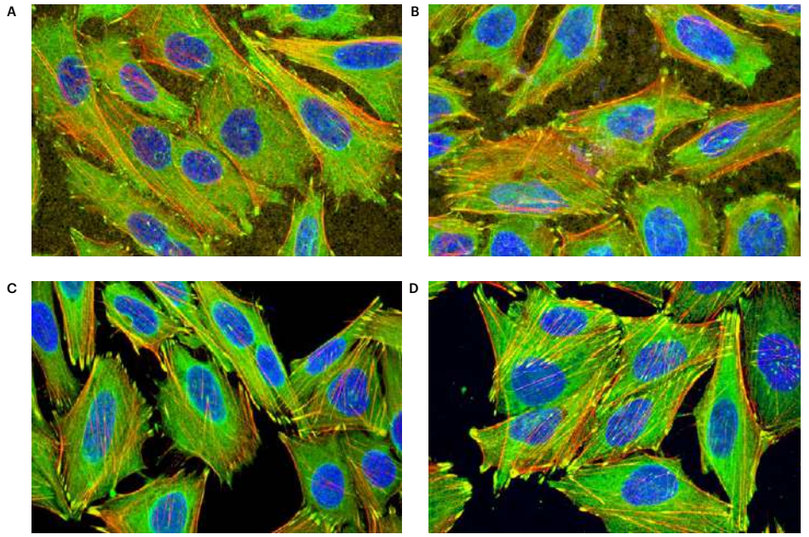
	e-mail: lucie.bacakova@fgu.cas.cz

Basic keywords:	01	Regenerative medicine
	02	Cell-material interaction
	03	Tissue engineering
	04	Tissue replacements
	05	Blood vessels
	06	Heart valves
	07	Bone
	08	Skin
	09	Stem cells

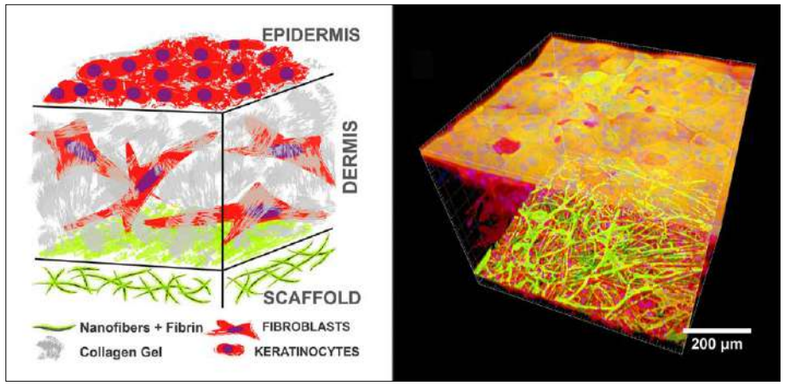
Research areas:	01	Creation of novel blood vessel replacements and vascular patches based on decellularized matrices, repopulated with mesenchymal stem cells
	02	Innovation of bone implants, currently used in clinical practice, by various surface modifications, and the construction of novel bone implants
Main objectives:	01	Reconstruction of vascular tunica intima and tunica media on decellularized tubular or planar matrices (e.g. decellularized porcine small-diameter blood vessels and pericardium), using autologous subcutaneous adipose tissue-derived stem cells
	02	Selection of the most appropriate surface modifications to metallic bone implants for their osseointegration; creation of novel bone implants based on mineralized matrices
	03	Creation of dermo-epidermal skin replacements based on nanofibrous synthetic polymeric meshes coated with fibrin and collagen, populated with fibroblasts or adipose tissue-derived stem cells and keratinocytes, and endowed with capillary-like structures formed by endothelial cells



Immunofluorescence staining of SM α-actin (red) and calponin (green), i.e. markers of differentiation towards vascular smooth muscle cells, in ASCs cultured for 3 days (A, B) and 7 days (C, D) in a fibrin gel on glass in a medium with TGF-β1 and BMP-4 under static conditions (A,C) and in a pulsatile pressure-generating bioreactor (B, D).



Human osteoblast-like Saos-2 cells on day 3 after seeding on silicalite-1 films (SFs) deposited on Si(100) (A), on SFs deposited on stainless steel (B), on bare Si(100) (C), and on bare stainless steel (D). Immunofluorescence staining of vinculin.



Developing a bilayer construct of keratinocytes and fibroblasts on a polylactide nanofibrous membrane modified with fibrin and collagen hydrogel. Left: schematic design, right: real construct.

Application of Stem Cells and Biomaterials in Cell Therapy

Doc. RNDr. Pavla Jendelová, Ph.D., Head of the Lab.

The Institute of Experimental Medicine
of the Czech Academy of Sciences

Content of the Research

Our group focuses on the research and development of new treatments of central nervous system (CNS) injury with potential translation into human medicine. We deal with the use of stem/progenitor cells (neural precursors, multipotent mesenchymal stromal cells) and polymeric hydrogels to treat brain and spinal cord injuries.

We focus on the mechanisms laying behind the therapeutic effects of cell transplantation in the treatment of nervous system injury paying special attention to extracellular vesicles produced by stem cells and microRNA. The main objectives of the projects include the detection of the role of microRNA in the pathophysiology of spinal cord injury and stroke. To ensure the maintenance of the therapeutic potential of stem cells for clinical applications, we are working on the improvement of *in vitro* cell culture parameters and optimization of hypothermic/cryogenic storage conditions.

The 3D self-organization of cells into spheroids/organoids or the application of cell-biomaterial constructs more closely mimic the microenvironment occurring in vivo, opening additional opportunities for the development of 3D *in vitro* modelling systems. We are working on the establishment of a suitable 3D environment for stem/progenitor cells to expand the scopes of their application *in vitro* and *in vivo*.

Potential for Collaboration

We currently have active collaborations with the following institutions: The Institute of Biotechnology, CAS (within the GACR project), The Institute of Physics, CAS (TACR projects), The Institute for Clinical and Experimental Medicine, New York Medical College (bilateral Program InterAction) and The Cambridge Brain Research Center. Our laboratory has expertise in the following experimental methods: cell cultures of neural spinal progenitors (SPC-01), induced pluripotent cells (iPS), iPS-NPs, MSCs, immunohistochemistry, gene expression by RT PCR, Western blot, organotypic slices of spinal cord tissue, in vitro modelling of spinal cord injury, 3D cell culture and in vitro cell-based assays and co-culturing systems.

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2019 Jirak D, Ziolkowska N, Turnovcova K, Karova K, Sykova E, Jendelova P, Romanyuk N. Metabolic Changes in Focal Brain Ischemia in Rats Treated With Human Induced Pluripotent Stem Cell-Derived Neural Precursors Confirm the Beneficial Effect of Transplanted Cells. Front Neurol. 2019 Oct 22;10:1074.

2019 Petrenko Y, Chudickova M, Vackova I, Groh T, Kosnarova E, Cejkova J, Turnovcova K, Petrenko A, Sykova E, Kubinova S. Clinically Relevant Solution for the Hypothermic Storage and Transportation of Human Multipotent Mesenchymal Stromal Cells. Stem Cells Int. 2019 Jan 20;2019:5909524.

2019 Androvic P, Romanyuk N, Urdzikova-Machova L, Rohlova E, Kubista M, Valihrach L. Two-tailed RT-qPCR panel for quality control of circulating microRNA studies Sci Rep. 2019 Mar 12;9(1):4255.

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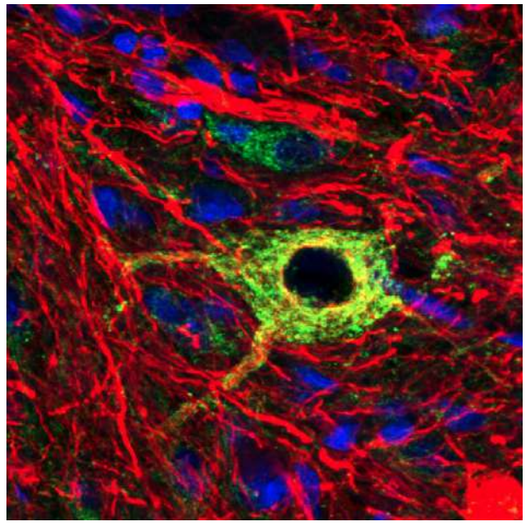
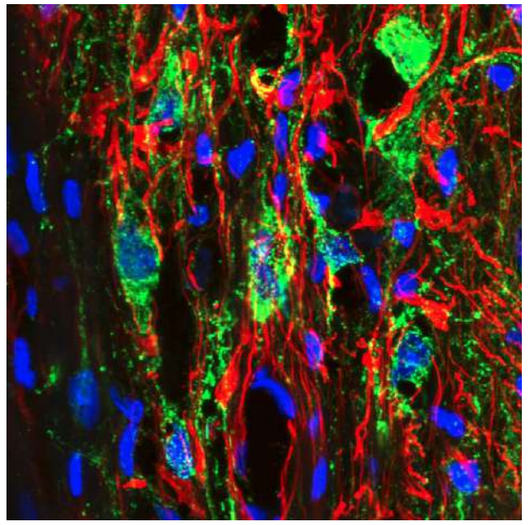
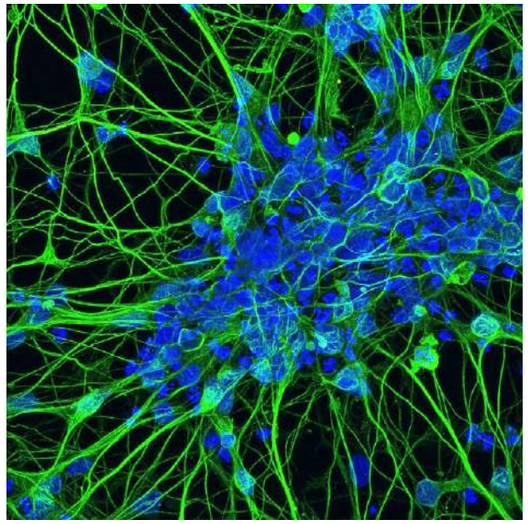
Basic keywords:	01	Tissue engineering
	02	Regenerative medicine
	03	Stem cells
	04	Spinal cord injury
	05	Magnetic nanoparticles
	06	Cell labeling
	07	Biomaterials
	08	IPS cells
	09	Mesenchymal stem cells
	10	Neural progenitors
	11	Neurodegenerative diseases

Research areas:	01	Thorough analysis of the biological properties of different stem cell populations and their extracellular derivatives
	02	Analysis of temporal expression of microRNA and mRNA in nervous tissue after CNS injury
	03	Validation of specific microRNA on relevant cell cultures and demonstration of the therapeutic effects of specific microRNAs in vivo
	04	Development of 3D cell- and cell-scaffold based constructs for regenerative medicine and disease modelling
	05	Optimization of the cell biopreservation conditions
	06	Studying the role of microRNA in central nervous system injury
Main objectives:	01	The use of stem cells and biomaterials in the treatment of spinal cord injury and neurodegenerative diseases
	02	Development and testing of artificial and natural polymers for 3D stem cell culture and spinal cord injury repair
	03	Development of non-toxic protocols for cryopreservation and hypothermic storage of stem cells
	04	Establishment of perfusion bioreactor systems for the improvement of 3D cell culture conditions
	05	Revealing the effect of stem cell derived secretome on the viability and functional properties of different cell types in vitro
	06	Revealing the role of specific microRNA in pathophysiology of spinal cord injury and ischemia uncovering their possible therapeutic implications

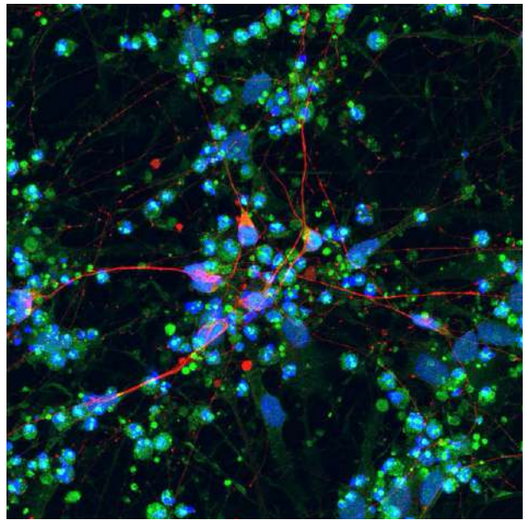




Human neural progenitors derived from induced pluripotent stem cells migrate out from laminin coated pHEMA-MOETACl hydrogel with dual porosity after transplantation into chronic spinal cord injury (SCI). Almost 6 months after grafting cell differentiate into neurons (left picture: ● – NF-200, ● – MTC02, ● – DAPI) and astrocytes (right picture: ● – GFAP, ● – MTC02, ● – DAPI).



induced pluripotent stem cell derived neural precursors differentiating into neurons in vitro (● – βIII-tubulin, ● – DAPI).



Differentiation neural precursors derived from induced pluripotent stem cell that are treated with miR-20a inhibitor (● – fluorescent labeled miR-20a inhibitor, ● – protein Sirt 1, ● – DAPI).

Stem Cells in the Epidermis and Their Use in Tissue Engineering
Prof. MUDr. Karel Smetana, DrSc., Head of the Lab.

First Faculty of Medicine,
Charles University

Content of the Research

The project is focused on the characterization of stem cells located in the human epidermis and mucosa, especially in the epidermal stem cells and highly multipotent neural crest-originated stem cells of the hair follicle. The main focus is directed at the characterization of the microenvironment necessary to correct the functioning of these cells. Especially, research on malignant tumors that originated from these stem cells is employed as a model of stem cell-niche interaction. Cancer-associated fibroblasts as the main component of this microenvironment is the center of our focus. We have characterized these cells from the morphological, functional and expression profile points of view. The chemokines (CXCL-1, IL-8), pro-inflammatory cytokines (IL-6), growth factors (TGF-β) and endogenous lectins (galectin-1) produced by these fibroblasts participate in the niche formation. We would like to fructify these findings in the development of new strategies promoting wound healing and therapeutic manipulation through the cancer cell microenvironment interaction. Our current interest is focused predominantly on studying the microenvironment of these cells under physiological and pathological conditions that are necessary for their expansion in vitro, studying the possibilities of epidermal stem cell isolation and investigating the in vitro propagation.

Potential for Cooperation

We are open and welcome collaboration in the fields of cell biology, tissue engineering, experimental oncology and glycobiology. Collaboration can be based on scientific and also commercial principles, including the fructification of our patents on cell therapy and the therapeutic manipulation of the cancer cell-microenvironment. We are interested in exchanging scientific staff and postgraduate students. We can offer expertise in cell cultivation, immunocytochemistry and lectin histochemistry. Our laboratory is included in the large project "Center for Tumour Ecology" supported by the European Community an the Ministry of Education, Youth and Sport of the Czech Republic. We have cooperated with numerous institutions in our country and abroad. An example of our national collaboration includes our work with the Institute of Molecular Genetics, Academy of Sciences of the Czech Academy of Sciences, Prague (Dr. Kolář) and with the Institute of Animal Physiology and Genetics of the Czech Academy of Sciences, Liběchov (Dr. Kupcová Skalníková). Further examples of international cooperation include our collaboration with the following institutions: the Institute of Physiological Chemistry, Ludwig-Maximilians University, Munich with Prof Gabius, Prof Kaltner, and Prof André, and the University of Strasbourg Inst. de Sciences et d' Ingénierie Supramoleculéculaires with Prof Lehn.

2019 Lacina, L., Brábek, J., Král, V., Kodet, O., Smetana K Jr.: Interleukin-6: a molecule with complex biological impact in cancer. Histol. Histopathol. 2019; 34:125–136 (2019).
2019 Plzák, J., Bouček, J., Bandúrová, V., Kolář, M., Hradilová, M., Zzabo, P., Lacina, L., Chovanec, M., Smetana, K. Jr.: The Head and Neck Squamous Cell Carcinoma Microenvironment as a Potential Target for Cancer Therapy. Cancers (Basel). 11: 440 (2019).
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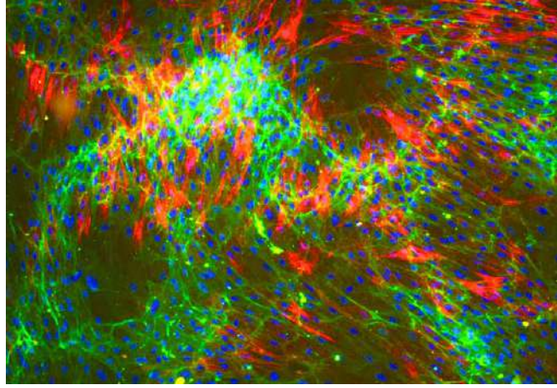
2015 Kodet O., Lacina L, Krejčí E., Dvořánková B., Grim M., Štork J., Kodetová D., Vlček Č., Šáchová J., Kolář M., Strnad H., Smetana K. Jr.: Melanoma cells influence the differentiation pattern of human epidermal keratinocytes. Mol. Cancer 14: 1 (2015).

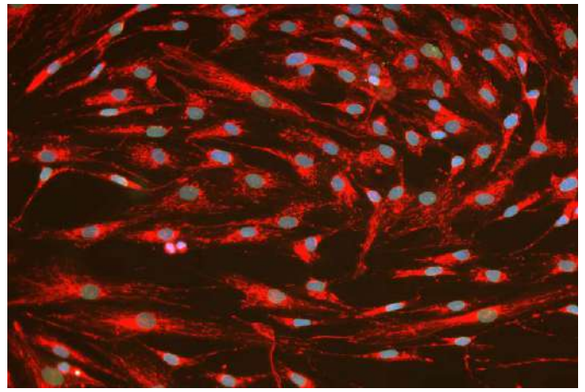
Contact person:	Prof. MUDr. Karel Smetana, DrSc.
	e-mail: karel.smetana@lf1.cuni.cz

Basic keywords:	01	Epidermal stem cell
	02	Neural crest-originated
	03	Stem cell
	04	Cancer microenvironment
	05	Wound repair
	06	Cancer-associated fibroblasts

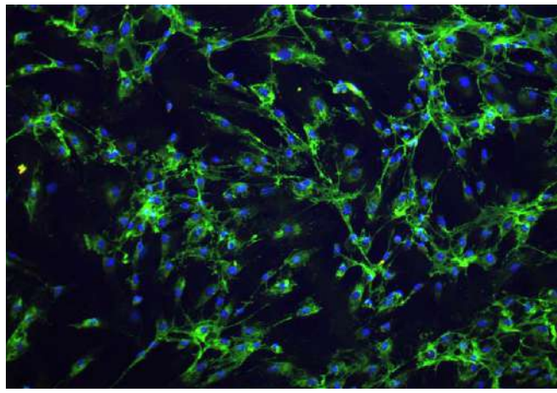
Research areas:	01	Cell biology-glycobiology
	02	Tissue engineering
	03	Developmental biology
	04	Experimental oncology
Main objectives:	01	Research on stem cells located in the human epidermis
	02	The role of epidermal stem cells in cancer
	03	The microenvironment necessary for epidermal stem cells in cancer and wound healing
	04	New strategies for improving wound healing



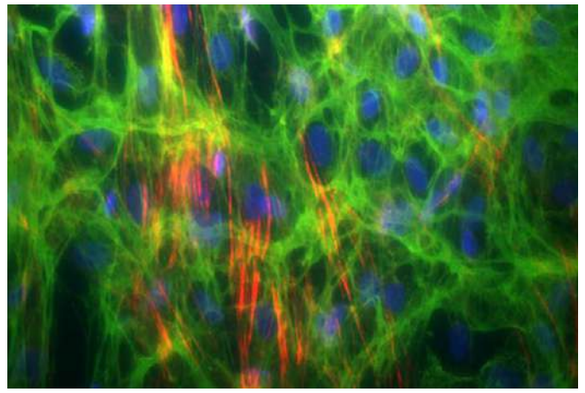
Galectin-1: Human neonatal fibroblasts stimulated by the endogenous lectin galectin-1 and TGF-β1 express smooth muscle actin (SMA, red signal). They improve wound contraction and help to facilitate wound healing. They also produce an extracellular matrix rich in galectin-1 (green signal). These cells including their function are very similar to cancer-associated fibroblasts.



Normal human dermal fibroblasts are positive for vimentin (red signal)



Right column down: Normal human dermal fibroblasts produce fibronectin (green signal)



Cancer-associated fibroblasts exhibiting signals for smooth muscle actin (red signal) are surrounded by a fibronectin-rich network of an extracellular matrix (green signal)

Clean Room Laboratory
RNDr. Jakub Širc, Ph.D., Head of the Lab.

The Institute of Macromolecular Chemistry
of the Czech Academy of Sciences

Content of the Research

The Clean Room Laboratory is intended for the manufacture of medical devices, medicinal substances and investigational medicinal products, in accordance with the requirements of the registration or authorization for a clinical trial. The laboratory has established a pharmaceutical quality system that respects guidelines of the European Community Commission 2003/94 / EC, 91/356 / EEC and 91/412 / EEC laying down the principles and guidelines for good manufacturing practice, quality assurance and quality risk management (GMP).

The pharmaceutical system at CRL IMC is fully documented and archived. The laboratory has available clean rooms corresponding to classes ranging from “D” to “A” according to GMP classification, with appropriate instrumentation, qualified staff and an established documentation system. Aseptic production takes place in "A" class clean rooms, while preparatory laboratory work is carried out in “C” class rooms. An isolator with a built-in lyophilizer is designed to handle biologically highly active substances. CRL IMC includes an analytical laboratory with an HPLC and UV-VIS spectrophotometer in GMP mode. The laboratory staff is experienced in basic and applied research in the field of macromolecular and medicinal chemistry, particularly the development of hydrogel and nanofibrous drug delivery systems for local administration of anticancer agents. The Clean Room Laboratory has been awarded ISO 9001:2015 certification issued by SGS.

Potential for Cooperation

The Clean Room Laboratory offers the following services: basic chemical synthesis and processing of products (even with active pharmaceutical substances), polymerizations, hot air or steam product sterilization, lyophilisation, final processing of products with a requirement for sterility or apyrogenicity, and analytical control of intermediates and products. The equipment available at the Clean Room Laboratory includes; an isolator with a built-in lyophilizer for aseptic production and for processing biologically highly active substances (cytostatics, immunosuppressants, hormones etc.), shaking incubator, vacuum dryer, steam autoclave, hot air sterilizer, HPLC, spectrophotometer, particle counters, aeroscopes, microbial contamination control incubators and other equipment needed to provide GMP work. In 2019, the Clean Room Laboratory participated and was awarded Honorable mention of Innovation in the preventive program for decreasing respiratory illnesses at ŠKODA AUTO and NEXARS.

2019 Hydrogel implants for transscleral drug delivery for retinoblastoma treatment. A.-I. Cocarta, R. Hobzova, J. Sirc, T. Cerna, J. Hrabeta, K. Svojgr, P. Pochop, M. Kodetova, J. Jedelska, U. Bakowsky, J.Uhlik, Materials science & engineering c-materials for biological applicationS, Volume: 103, Article Number: 109799, DOI: 10.1016/j.msec.2019.109799, Published: OCT 2019

2019 Poly(D,L-lactide)/polyethylene glycol micro/nanofiber mats as paclitaxel-eluting carriers: preparation and characterization of fibers, in vitro drug release, antiangiogenic activity and tumor recurrence prevention: R. Hobzova, Z. Hampejsova, T. Cerna, J. Hrabeta, K. Venclikova, J. Jedelska, U. Bakowsky, Z. Bosakova, M. Lhotka, S. Vaculin, M. Franek, M. Steinhart, J. Kovarova, J. Michalek, J. Sirc, Materials science & engineering c-materials for biological applications, Volume: 98, Pages: 982-993, DOI: 10.1016/j.msec.2019.01.046, Published: MAY 2019

2019 Electrospun nanofibers for local anticancer therapy: review of in vivo aktivity: L. Poláková, J. Širc, R. Hobzová, A. I. Cocarta, E. Heřmánková, International journal of pharmaceutics, Volume: 558, Pages: 268-283, DOI: 10.1016/j.ijpharm.2018.12.059, Published: MAR 10 2019

2018 Polymeric bile acid sequestrants: review of design, in vitro binding activities, and hypocholesterolemic effects: E. Heřmánková, A. Žák, L. Poláková, R. Hobzová, R. Hromádka, J. Širc, European journal of medicinal chemistry, Volume: 144, Pages: 300-317, DOI: 10.1016/j.ejmech.2017.12.015, Published: JAN 20 2018

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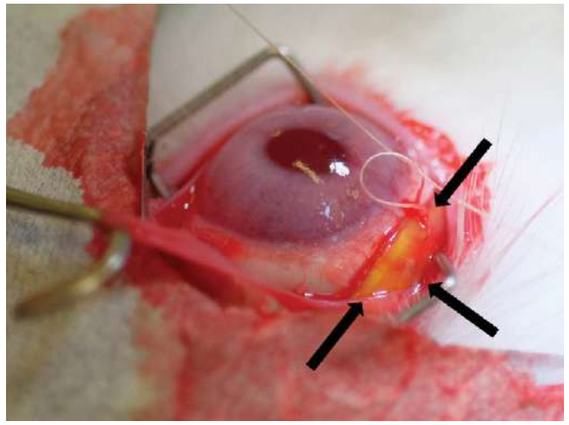
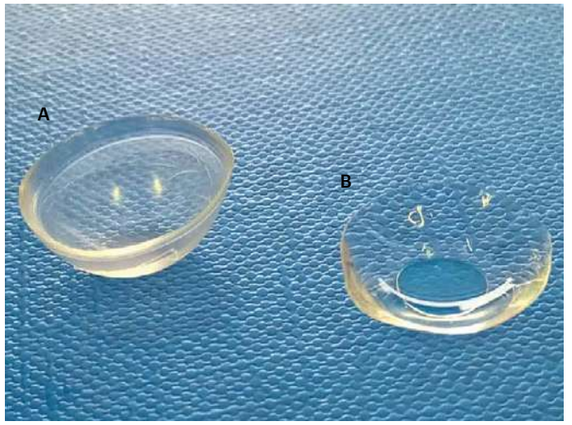
Basic keywords:	01	clean room laboratory
	02	good manufacturing practice
	03	GMP
	04	quality control
	05	quality assurance
	06	medicinal product
	07	medical device
	08	pharmaceutical formulation
	09	hydrogels
	10	drug delivery

Research areas:	01	Production of sterile products for various medicinal applications in accordance with the applicable legislation
	02	Preparation of sterile products for in vitro and in vivo experiments
	03	Development of polymeric delivery systems for local drug administration
Main objectives:	01	Production of chemical compounds
	02	Active pharmaceutical ingredients
	03	Medicinal products and medical devices in a clean room environment
	04	The laboratory collaborates and provides services to other research teams in the finalization of their applied research
	05	Preparation and production of sterile products for in vivo experiments
	06	Preclinical and clinical trials in accordance with GMP and ISO 9001 standards

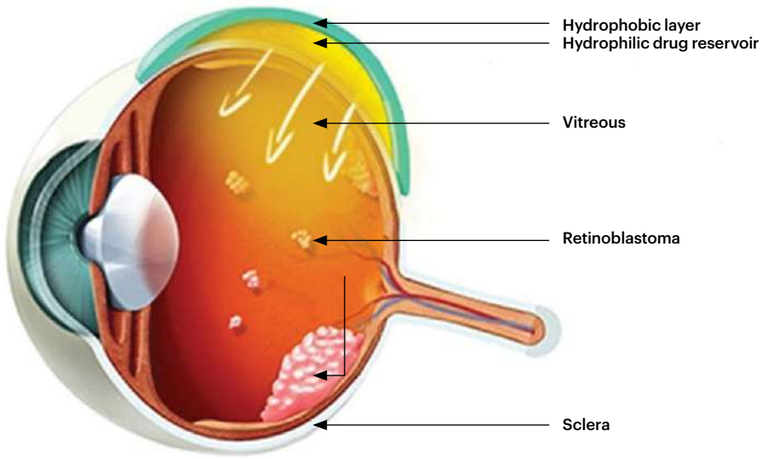
A) pEOEMA implant B) pHEMA implant



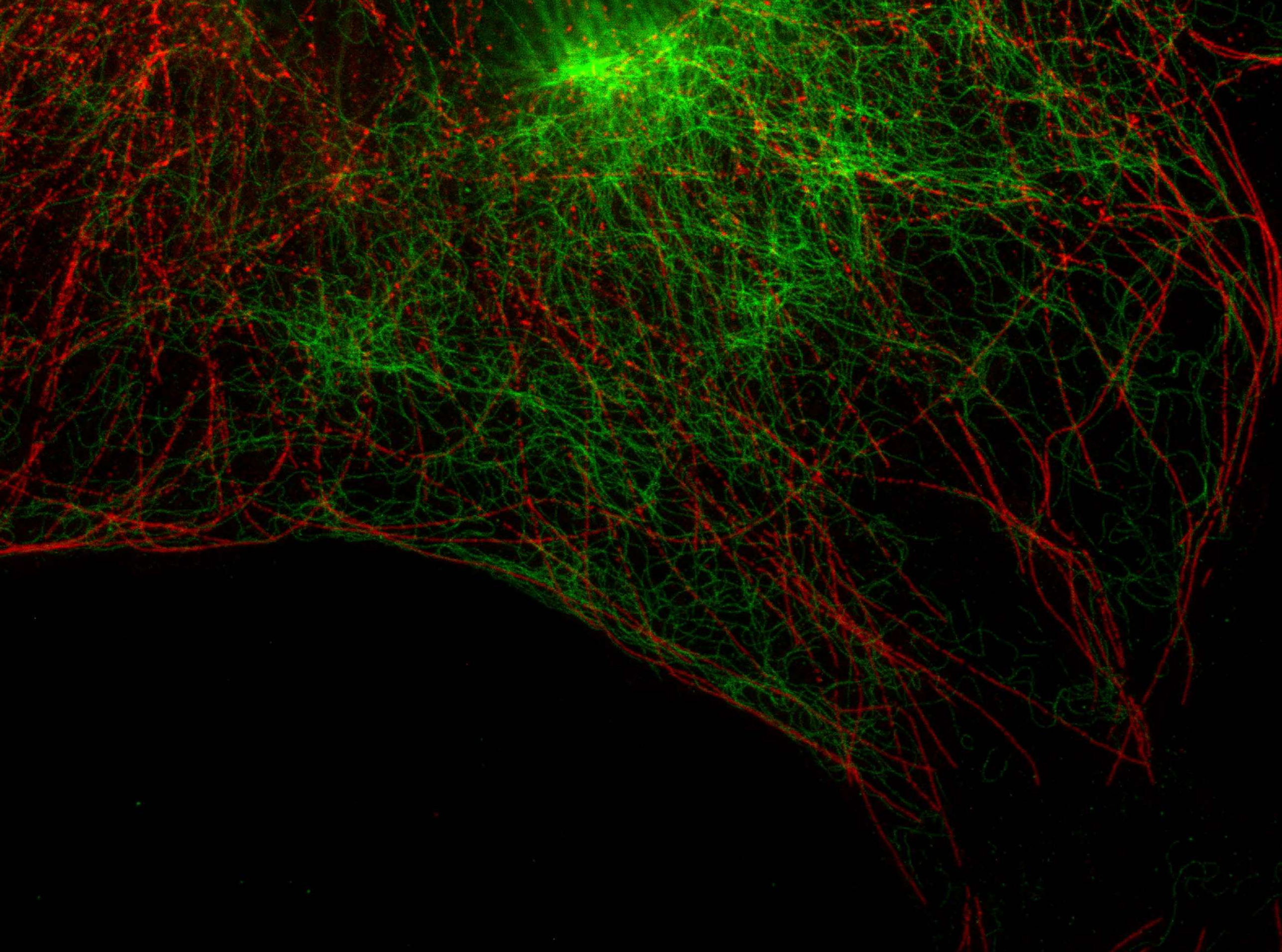
The controlled administration of an anti-cancer drug by transscleral diffusion into the eye globe may prolong and increase the drug's effect for the treatment of intraocular diseases. Hydrgoel implantation on a rabbit model.



The developed hydrogel constructs are composed of two layers – an inner hydrophilic reservoir releasing the drug into the eye globe and an outer hydrophobic part protecting the surrounding vascularized tissue against cytotoxic effects.



Hydrogel constructs for transscleral drug delivery for the treatment of retinoblastoma.



150

Development of Diagnostic and Therapeutic Procedures

No. of research teams: 09

Pages: 150–173

05

Development of Diagnostic and Therapeutic Procedures

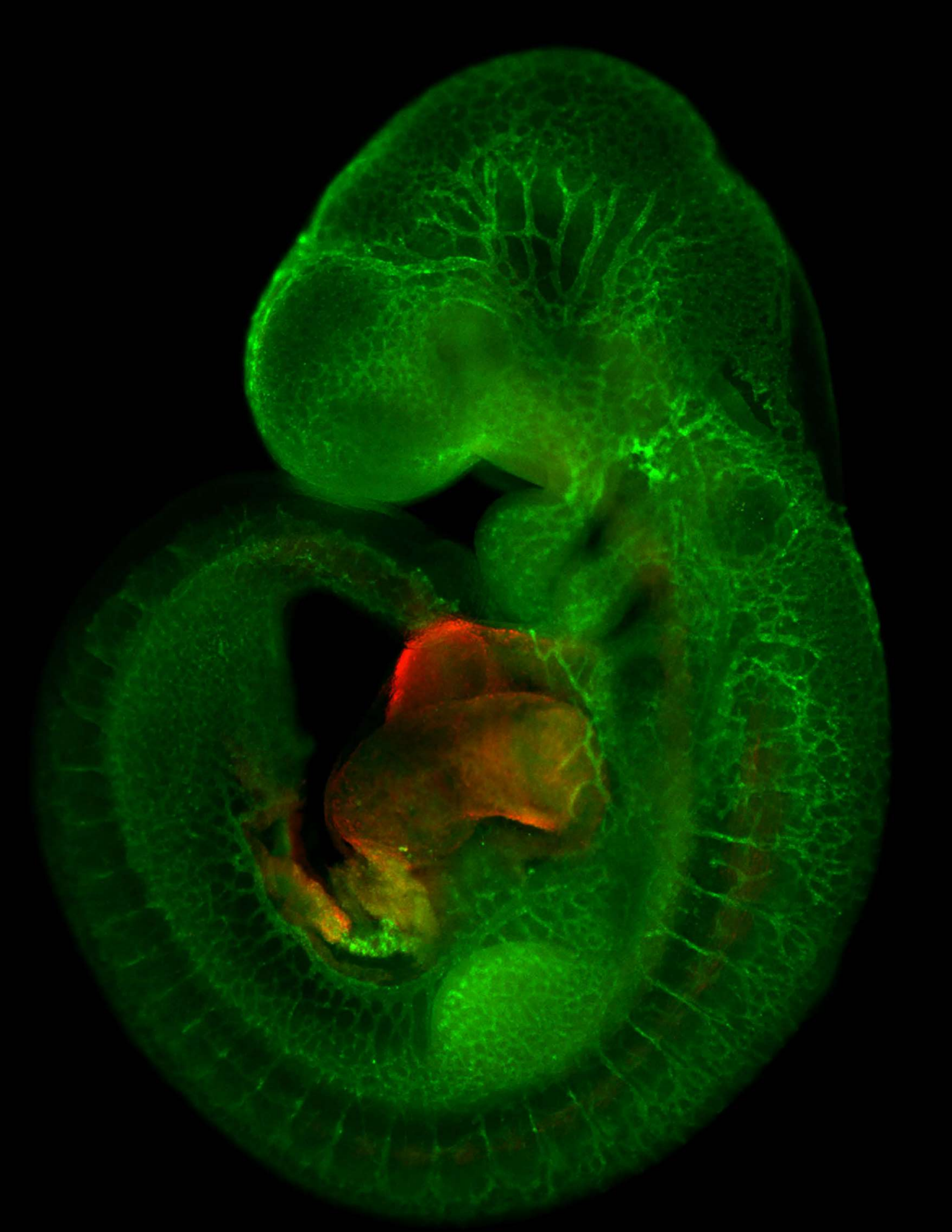
Prof. Ing. Stanislav Kmoch, CSc.
Head of the Research Programme
First Faculty of Medicine, Charles University

This programme includes a spectrum of projects covering reproductive medicine, diabetic complications, autoimmune and selected tumour diseases, inherited metabolic disorders, and the study of heme pathology and of the effect of a lack or excess of gaseous signalling molecules. The unifying element of all the projects is the study of the pathological condition of a cell, that is, finding out the causes of this condition, profiling the expression of the chosen genes, detecting changes in the localization and modification of the chosen proteins and identifying other molecules that relate to the induction of the pathology, thus furthering the development of new procedures for the prevention of the disease and creating new methods and diagnostics for monitoring the process of the disease and tools for advancing the molecular therapy of the accompanying pathological condition.

The programme has a notable application potential, namely in medicine. Insight into the preventive possibilities will impact the health and quality of life of large population subgroups. The recent clinical practice will be directly influenced by novel diagnostic approaches, with elucidation of a novel generation of biomarkers, preparation of newly designed diagnostic kits, and later with the design of novel treatment modalities. The future of therapeutic interventions lies in personalized therapy; the application outputs of the research programme unequivocally accent this direction.

Research Directions — Development of Diagnostic and Therapeutic Procedures

1.	Clarify the molecular mechanisms of fertilization, identify proteins and molecules of gametes and reproductive organs that are responsible for successful reproduction	2.	Develop antibodies against sperm and reproductive tract proteins crucial for successful fertilization and their commercialization	3.	New strategies in the treatment of autoimmune diseases in general
4.	Develop new anti-cancer substances and novel therapeutic approaches that will be transferred to the commercial sphere	5.	Identify indicator genes for diabetes-specific heart abnormalities and genes contributing to developmental heart defects in diabetic embryopathy	6.	Early diagnosis, prevention and treatment of prenatal exposure to diabetes
7.	Identify the genes participating in the development, function and disease of the liver which can influence the development of metabolic syndrome	8.	Develop a new platform for biological studies based on single-cell expression profiling	9.	Describe the role of newly-studied protein partners in the modulation of the phenotypic expression of diseases with the possibility of proposing new therapeutic methods
10.	Prepare prospective therapeutically used substances that can be transferred to the commercial sphere	11.	Identify reliable biomarkers or develop a mass spectrometry-based therapeutic test		



Reproductive Biology
RNDr. Kateřina Komrsková (Hortová), Ph.D., Head of the Lab.

The Institute of Biotechnology of the
Czech Academy of Sciences

Content of the Research

The laboratory has extensive knowledge of assessing male reproductive parameters as markers of fertility disorders. The group has been focusing on studying the molecular mechanisms of reproduction and the nature of specific sperm proteins that play a role in sperm maturation, sperm-egg interaction and early embryo development. The area of interest covers the monitoring of sperm quality in patients with testicular cancer and diabetes mellitus and sperm antibody characterization in infertile couples. Many of them are used in the Centres of Assisted Reproduction and have been commercialized. Trans-generational epigenetic deregulation of microRNA expression induced by pollutants that influence the key role in germ cell differentiation without changing the DNA has been proven. Epigenetic aberrations (selected histone modifications and DNA methylation) in spermatozoa, testicular tissues and early embryos after exposure to environmental pollutants still remain to be studied.

Potential for Cooperation

We offer; knowledge of the detailed assessment of reproductive parameters, monitoring of trans-generational epigenetic inheritance, characterization of epigenetic aberrations and microRNA deregulations, all tailored to specific areas of project interest (medical conditions, lifestyle, environmental factors, etc.). In addition, we offer; 1) knowledge of hybridoma technology, the production of specific monoclonal antibodies with high affinity and specificity; 2) transgenic mouse lines, property of the Lab and a tool for cooperation, include the following: transgenic C57BL/6 acr3-EGFP expressing green protein (EGFP) in sperm acrosome, transgenic C57BL/6 su9-DsRed2 expressing red fluorescent protein (RFP) in somatic cell mitochondria. In cooperation with commercial companies, twelve prototypes for the detection of studied molecules have been developed and two European and two Czech patents have been submitted. The lab has ongoing collaboration with scientific groups, which has resulted in number of key publications; Harry Moore at the University of Sheffield, UK, Klaus Steger, at the University of Giessen, Germany, Kate Loveland, at Hudson Inst, Monash University, Australia, Peter Johnson at the University of Liverpool, UK and Peter Sutovsky at the University of Missouri-Columbia, Jiri Neuzil at the School of Medical Science, Griffith University, Australia and IBT, CAS, Czech Republic.

2020 Jankovicova, J., et al. Expression and distribution of CD151 as a partner of alpha6 integrin in male germ cells. Sci Rep. 2020 Mar 9;10(1):4374. doi: 10.1038/s41598-020-61334-2. PMID: 32152440

2019 Frolikova, M., et al. Addressing the Compartmentalization of Specific Integrin Heterodimers in Mouse Sperm. International Journal of Molecular Sciences, 20(5): 1004, 2019. doi:10.3390/ijms20051004. ISSN: 1422-0067.

2019 Bajzikova, M., et al. Reactivation of Dihydroorotate Dehydrogenase-Driven Pyrimidine Biosynthesis Restores Tumor Growth of Respiration-Deficient Cancer Cells. Cell Metabolism, 29(2): 399–416, 2019. doi: 10.1016/j.cmet.2018.10.014. ISSN 1550-4131.

2017 Dong, L. F., et al. Horizontal transfer of whole mitochondria restores tumorigenic potential in mitochondrial DNA-deficient cancer cells. eLife, 6:e22187, 2017. doi: 10.7554/eLife.22187. ISSN 2050-084X.

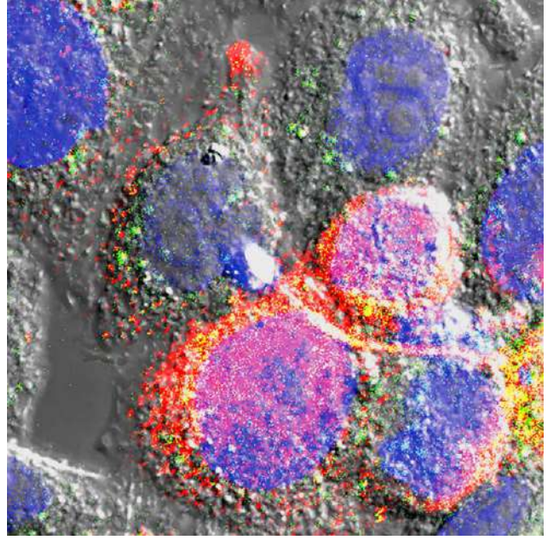
2016 Frolikova, M., et al. Characterization of CD46 and β1 integrin dynamics during sperm acrosome reaction. Scientific Reports, 6: 33714, 2016. doi: 10.1038/srep33714. eISSN 2045-2322.

2015 Vieweg, M., et al. Methylation analysis of histone H4K12ac-associated promoters in sperm of healthy donors and subfertile patients. Clinical Epigenetics, 7(1): 31, 2015. doi: 10.1186/s13148-015-0058-4. ISSN 1868-7075.

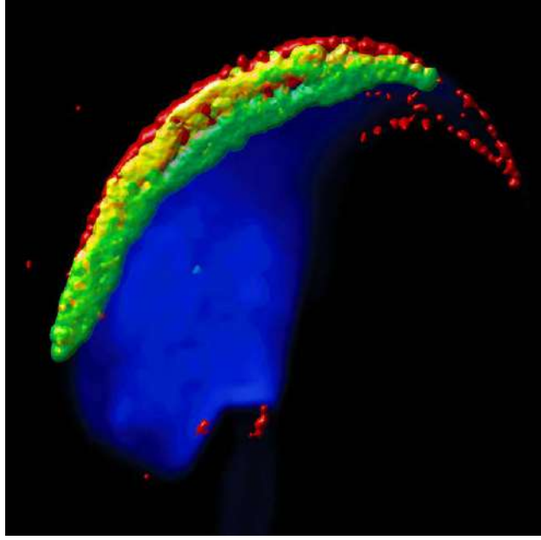
Contact person:	RNDr. Kateřina Komrsková (Hortová), Ph.D.
	e-mail: katerina.komrskova@ibt.cas.cz

Basic keywords:	01	Reproduction
	02	Endocrine disruptors
	03	Infertility
	04	Sperm epigenom
	05	Capacitation
	06	Acrosome reaction
	07	Sperm-egg interaction
	08	Estrogens
	09	Izumo 1
	10	Tetraspanin network

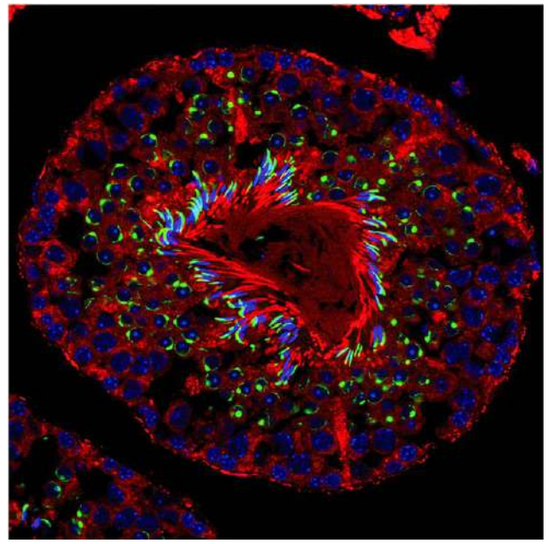
Research areas:	01	The protein network dynamics during sperm capacitation, acrosome reaction and sperm-egg interaction
	02	The effect of lifestyle and environmental factors on sperm epigenome, embryonic development and reproduction
	03	The role of estrogens and estrogenic receptors during sperm maturation
Main objectives:	01	Investigation and characterisation of molecular mechanisms responsible for Izumo1, and tetraspanin protein (CD46, CD81, CD9) dynamics during sperm maturation and sperm-egg interaction
	02	Assessment of the correlation between identified epigenetic fertility factors. Analysis of murine and human sperm epigenomes to determine specific genomic sequences susceptible to cigarette smoke/hormonal mediated defects
	03	Detection and localization of estrogen receptors (ERs) during sperm development



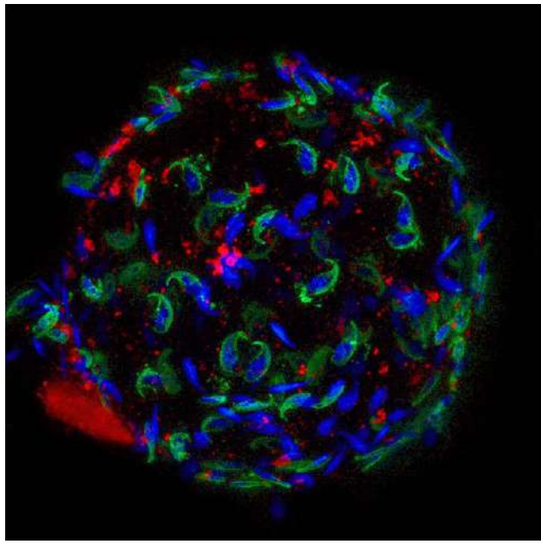
CHO cells mimic the human egg model co-transfected with both JUNO (red) and fusion protein FcRL (green) with attached human sperm, nuclei (blue).



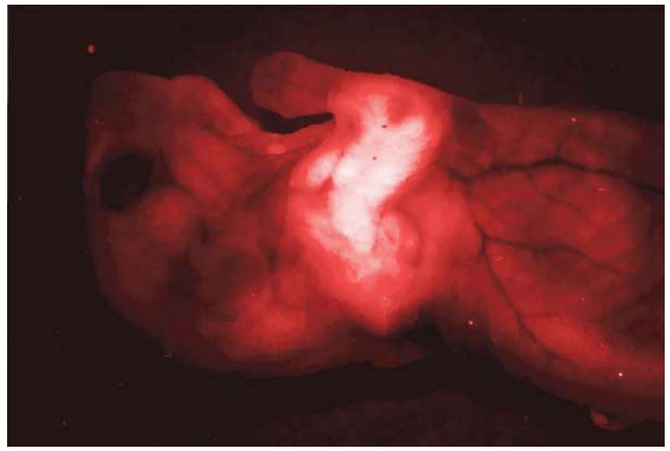
Super-resolution microscopy (SIM) shows the localization of CD46 (green) on the inner and outer acrosomal membrane and β1-integrin (red) on the plasma and outer acrosomal membrane.



Testis: cross section using transgenic mouse C57BL/6N acr3-EGFP/su9-DsRed2, acrosome in developing spermatids and sperm (green), mitochondria (red), nuclei (blue).



Super-resolution microscopy (STED) of mouse oocyte shows the localization of proteins Izumo1 (green), Fcrl (red) and nucleus (blue).



C57BL/6Nsu9-DsRed2 embryo: Red fluorescent protein expressed in mitochondria is visible under fluorescent light and confirmed by geno-typing.

Molecular Therapy of Cancer
Prof. Ing. Jiří Neužil, CSc., Head of the Lab.

The Institute of Biotechnology of the
Czech Academy of Sciences

Content of the Research

We focus on the role of mitochondria in cancer biology. Our recent discovery has showed that cancer cells with damaged mitochondrial (mt) DNA cannot form tumours, unless they 'steal' high mitochondria from the host. Targeting the mitochondrial function thus presents a unique opportunity to treat tumours. This is linked to our work, in which we design and study anti-cancer agents targeting mitochondrial respiratory complexes. Some of the agents are under pre-clinical testing. We are studying a novel phenomenon, the horizontal transfer of genes between mammalian cells in vivo, and have shown this for mtDNA with important consequences for tumour initiation and progression, as well as metastasis. A particular focus of the group is on mitocans, anti-cancer agents targeting mitochondria, especially those targeting mitochondrial respiratory complexes. Linked to this, we have discovered mitochondrial respiratory complex II as a new target for cancer therapy, and found that under stress, CII is present in cancer cells under stress in a partially assembled species that we refer to as CII-low, whose role is to help the cell conserve energy.

Our work led to the definition of a new class of anti-cancer agents, which is based on their modification by attaching the mitochondrial vector triph-enylphosphonium. Of these agents, mitochondrially targeted tamoxifen (MitoTam) has shown high anti-cancer efficacy that resulted in a Phase 1/1b clinical trial with very promising results, and we are preparing for the Phase 2 trial.

Our work on MitoTam led to the discovery that this agent also very efficiently kills senescent cells by way of blocking mitochondrial respiration and the associated dissipation of mitochondrial membrane potential. We are now testing the effect of MitoTam on pathologies that are typified by a high number of senescent cells, and have found a considerable effect on type 2 diabetes mellitus. Concerning type 2 diabetes, we are planning a clinical trial that, if successful, may have a considerable practical impact.

Potential for Cooperation

We are actively working with a number of research groups from New Zealand, Australia, Singapore, the USA and Europe. We also have very dynamic partnerships in the Czech Republic and within our Institute of Biotechnology. We have hosted PhD and undergraduate students from Portugal, Chile, Serbia or Spain at our laboratory. We are open to collaboration on research topics that concern mitochondria and cancer.

2020 Levoux J et al. Platelets enhance pro-angiogenic activity of mesenchymal stem cells via mitochondrial transfer and metabolic remodeling. Cell Metab, in press.

2019 Bajzikova M et al (2019) Reactivation of dihydroorotate dehydrogenase by respiration restores tumor growth of mitochondrial DNA-depleted cancer cells. Cell Metab 29, 399-416.

2019 Hubackova S et al (2019) Selective elimination of senescent cells by mitochondrial targeting is regulated by ANT2. Cell Death Differ 26, 276-290.

2018 Bezawork-Geleta A et al (2018) Alternative assembly of respiratory complex II connects energy stress to metabolic checkpoints. Nat Commun 9, 2221.

2017 Bezawork-Geleta A et al (2017) Mitochondrial complex II: At the crossroads. Trends Biochem Sci 42, 312-325.

2017 Rohlenova K et al (2017) Selective disruption of respiratory supercomplexes as a new strategy to suppress Her2high breast cancer. Antiox Redox Signal 26, 84-103.

2017 Dong LF et al (2017) Horizontal transfer of whole mitochondria recovers tumorigenic potential in mtDNA-deficient cells. eLife 6, e22187.

2015 Tan AS et al (2015) Mitochondrial genome acquisition restores respiratory function and tumorigenic potential in cancer cells without mitochondrial DNA. Cell Metab 21, 81-94.

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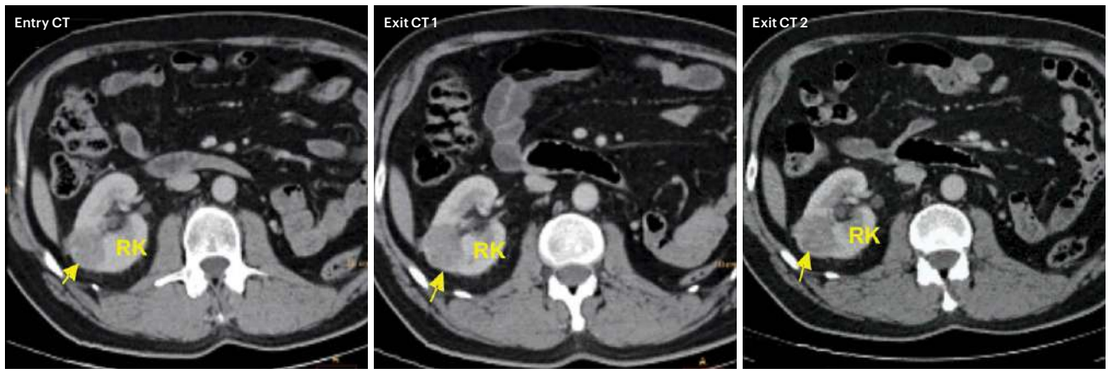
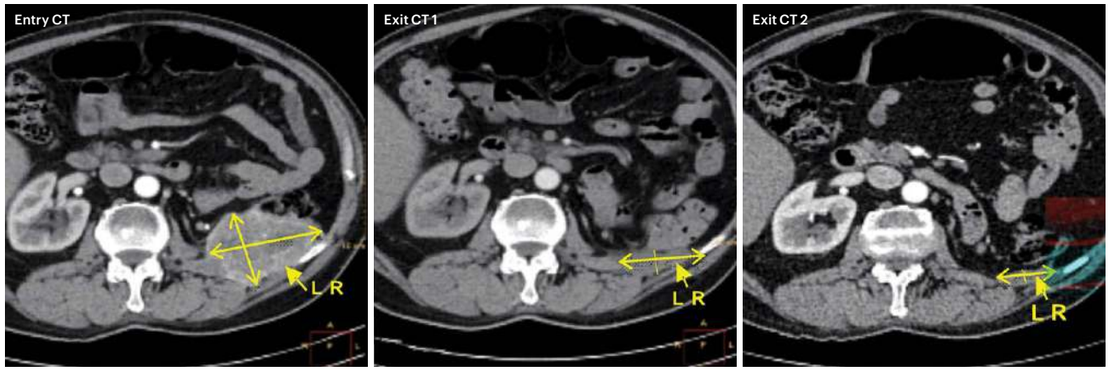
Basic keywords:	01	Cancer
	02	Tumour initiation and progression
	03	Mitochondria
	04	Respiration
	05	Mitochondrial transfer
	06	Anti-cancer agents
	07	Mitochondrial targeting

Research areas:	01	Cancer biology
	02	The role of mitochondria in cancer
	03	Design and synthesis of novel anti-cancer agents
Main objectives:	01	Understand cancer initiation and progression
	02	Understand the role of mitochondria in cancer
	03	Utilise mitochondrial targeting as an efficient target for anti-cancer drugs

Patient 1



Patient 2



The computer tomography scans show the effect of MitoTam on renal cancer in two patients that underwent left nephrectomy to remove the primary left kidney tumour. In patient 1, MitoTam stabilized the target metastatic lesion in the right kidney, in patient 2 the agent caused partial remission of the local relapse in the surgical bed. Both patients underwent several rounds of therapy.

Molecular Pathogenetics
RNDr. Gabriela Pavlínková, Ph.D., Head of the Lab.

The Institute of Biotechnology of the
Czech Academy of Sciences

Content of the Research

Our research program is focused on transcriptional regulation during embryonic development, the molecular mechanisms of developmental programming, and identification of the molecular causes of abnormal embryonic development and disease predispositions. We are particularly interested in HIF-1, ISL1, SOX2, and NEUROD1 transcription factor networks and how their dysfunction affects embryonic development and how they can increase pre-dispositions of an individual to diseases such as diabetes, heart disease or hearing loss. We are also analyzing the combinatorial effects of the environment (e.g. diabetes) and genetic mutations, and epigenetic modifications. Using mouse models, and single cell and bulk transcriptome and epigenome analyses, we are studying molecular mechanisms to identify targets for the development of preventive and diagnostic strategies.

Potential for Cooperation

We collaborate with a number of national and international partners. In Czechia, we have established fruitful collaborations with the research groups led by Professor J. Syka at the Institute of Experimental Medicine CAS; Professor M. Macek Motol Hospital, Prague; Professor F. Kolar, Physiology Institute CAS, Prague, Professor D. Sedmera, Charles University, First Faculty of Medicine, the Institute of Anatomy, Professor F. Saudek, IKEM, Prague, Professor J. Syka, and Professor M. Kubista, the Institute of Biotechnology CAS. We also work with foreign partners: Nobel laureate, Professor Greg Semenza from the Johns Hopkins University School of Medicine USA; Professor Claudia Kappen, Pennington Institute, USA; Professor Bernd Fritzsch, The University of Iowa, USA; and Professor Agnes Görlach, Technische Universität München, Germany. As a result of these partnerships, we have produced joint publications and have received collaborative grants.

Research Highlights

Our research has revealed novel molecular mechanisms associated with aberrant neuronal development and function. We have found that dysregulated HIF-1α expression may contribute to cardiac dysfunction and disease associated with defects in the cardiac sympathetic system (Bohuslavova *et al*. 2019). We have shown that the deletion of Neurod1 in cochlear neurons leads to altered characteristics of neurons and overall dysfunctional tonotopy of the auditory system. Our data provide the first insights into the limits of physiology-mediated brainstem plasticity during abnormal development (Macova *et al*. 2019).

2020 Filova I, Dvorakova M, Bohuslavova R, Pavlinek A, Elliott KL, Vochyanova S, Fritzsch B, Pavlinkova G*. Combined Atoh1 and Neurod1 Deletion Reveals Autonomous Growth of Auditory Nerve Fibers. Molecular Neurobiology. 2020, 57: 5307–5323.

2019 Bohuslavova R, Cerychova R, Papousek F, Olejnickova V, Bartos M, Gorlach A, Kolar F, Sedmera D, Semenza G, Pavlinkova G*. HIF-1α is required for development of the sympathetic nervous system. PNAS July 2, 2019 116 (27) 13414-13423.

2019 Fritzsch B, Elliott K, Pavlinkova G, Duncan J, Hansen M, Kersigo J. Neuronal Migration Generates New Populations of Neurons That Develop Unique Connections, Physiological Properties and Pathologies. Front Cell Dev Biol. 2019; 7: 59.

2019 Macova I, Pysanenko K, Chumak T, Dvorakova M, Bohuslavova R, Syka J, Fritzsch B, Pavlinkova G*. Neurod1 is essential for the primary tonotopic organization and related auditory information processing in the midbrain. Journal of Neuroscience 2019 Feb 6;39(6):984-1004.

2018 Cerychova R, Bohuslavova R, Papousek F, Sedmera D, Abaffy P, Benes V, Kolar F, Pavlinkova G*. Adverse effects of Hif1a mutation and maternal diabetes on the offspring heart. Cardiovasc Diabetol. 2018 May 12;17(1):68.

2017 Pavlinkova G*, Margaryan H, Zatecka E, Valaskova E, Elzeinova F, Kubatova A, Bohuslavova R, Peknicova J. Transgenerational inheritance of susceptibility to diabetes-induced male subfertility. Scientific Reports. 2017; 7: 4940.

2017 Bohuslavova R, Dodd N, Macova I, Chumak T, Horak M, Syka J, Fritzsch B, Pavlinkova G*. Pax2-Islet1 Transgenic Mice Are Hyperactive and Have Altered Cerebellar Foliation. Molecular Neurobiology 2017, 54(2): 1352–1368.

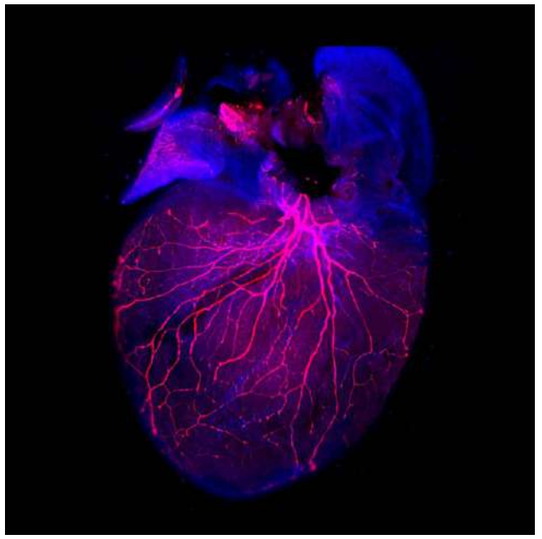
2016 Chumak T, Bohuslavova R, Macova I, Dodd N, Buckiova D, Fritzsch B, Syka J, Pavlinkova G*. Deterioration of the Medial Olivocochlear Efferent System Accelerates Age-Related Hearing Loss in Pax2-Isl1 Transgenic Mice. Molecular Neurobiology 2016 May;53(4):2368-83.

2016 Dvorakova M, Jahan I, Macova I, Chumak T, Bohuslavova R, Syka J, Fritzsch B, Pavlinkova G*. Incomplete and delayed Sox2 deletion defines residual ear neurosensory development and maintenance. Scientific Reports. 2016, 6:38253.

Contact person:	RNDr. Gabriela Pavlínková, Ph.D.
	e-mail: gabriela.pavlinkova@ibt.cas.cz

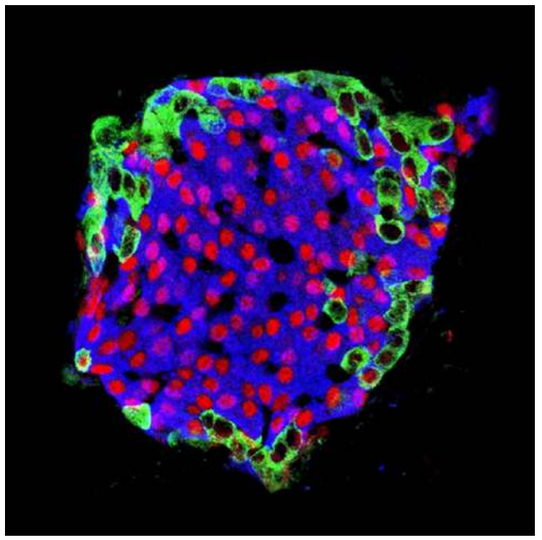
Basic keywords:	01	Transcriptional regulation
	02	Diabetes
	03	Hypoxia
	04	Inner ear
	05	Mouse models
	06	Heart
	07	Embryopathy
	08	Pancreas
	09	Embryonic development

Research areas:	01	Diabetic embryopathy
	02	Tissue reprogramming associated with diabetes mellitus
	03	Neurosensory development
	04	Pancreas development
Main objectives:	01	To identify the key molecular players in changes caused by exposure to diabetes in exposed embryos
	02	To identify the molecular mechanisms in changes associated with exposure to a diabetic environment in adult tissues
	03	To identify the genes that are crucial in generating specific cell types to understand the pathophysiology of hearing disorders
	04	To identify transcriptional and epigenetic regulation in the development of pancreatic endocrine cells

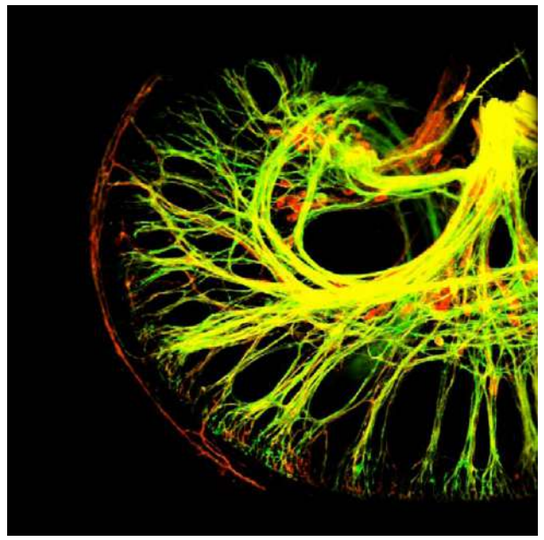


Embryonic heart with sympathetic innervation labeled by anti-TH (●)

Expression of SOX2 (●) and Foxg1-Cre/tdTomato (●) at embryonic day E9.5



Islet of Langerhans: Glucagon (●), PDX1 (●), insulin (●)



Disarrayed innervation in the cochlea of Neurod1 deletion mutant

Single-Cell Expression Profiling in Research and Diagnostics
Prof. Dr. Mikael Kubista, Ph.D., Head of the Lab.

The Institute of Biotechnology of the
Czech Academy of Sciences

Content of the Research

The Gene Expression Laboratory is the Czech Republic's leading academic laboratory specializing in high- throughput gene expression profiling and single-cell analysis using RT-qPCR and RNA-Seq. We have several research projects in the field of developmental biology and neurobiology, and applied projects in cancer research and diagnosis. Our lab is also involved in the development of methods and applications for nucleic acid analyses and standardization protocols for effective workflows.

Methodology and Quality Controls

We are very active in the areas of new method development and implementation. In the past, the laboratory pioneered single cell RT-qPCR expression profiling, multidimensional expression profiling, intracellular expression profiling by qPCR, developed a new method for miRNA analysis and introduced several tools and protocols for performing quality control of RT-qPCR experiments. Our group leader, Prof. Mikael Kubista, was actually among the pioneers who developed real-time PCR. Currently the main focus of our lab is the investigation of gene expression using a combination of up-to date techniques such as single cell expression profiling and RNA-Seq, with the aim of measuring both coding mRNA and also non-coding RNA (miRNA, lncRNA etc.).

Developmental Biology

Our group uses the African clawed frog model for three main projects – asymmetric localization of biomolecules during oogenesis and early development; regulation of wound healing and regeneration; and studying the role of nitric oxide during early embryogenesis. We have developed techniques for spatial and temporal expression profiling using RT-qPCR and more recently RNA-Seq, and we have combined them with functional experiments using microinjections and phenotype analysis such as in situ hybridization and imaging.

Neurobiology

In the field of neurobiology, we are interested in the characterization of glial cells in acute and degenerative disorders in the central nervous system. Currently, we are studying the proliferation and differentiation of NG2 glial cells in different types of brain injuries, the role of Trpv4 and Aqp4 proteins in cell volume regulation, and the mechanisms of miRNA regulation in the nervous tissue following spinal cord injury and stroke. Neurodegenerative projects involve single-cell transcriptomics analysis of amyotrophic lateral sclerosis and Alexander disease. In these projects we apply the most current approaches for performing gene expression analysis in the field, such as single-cell gene expression profiling and RNA-Seq.

2020 Decoding the Transcriptional Response to Ischemic Stroke in Young and Aged Mouse Brain. Androvic P, Kirdajova D, Tureckova J, Zucha D, Rohlova E, Abaffy P, Kriska J, Valny M, Anderova M, Kubista M, Valihrach L. Cell Rep. 2020 Jun 16;31(11):107777. doi: 10.1016/j.celrep.2020.107777.

2020 Performance Comparison of Reverse Transcriptases for Single-Cell Studies. Zucha D, Androvic P, Kubista M, Valihrach L. Clin Chem. 2020 Jan 1;66(1):217-228. doi: 10.1373/clinchem.2019.307835.

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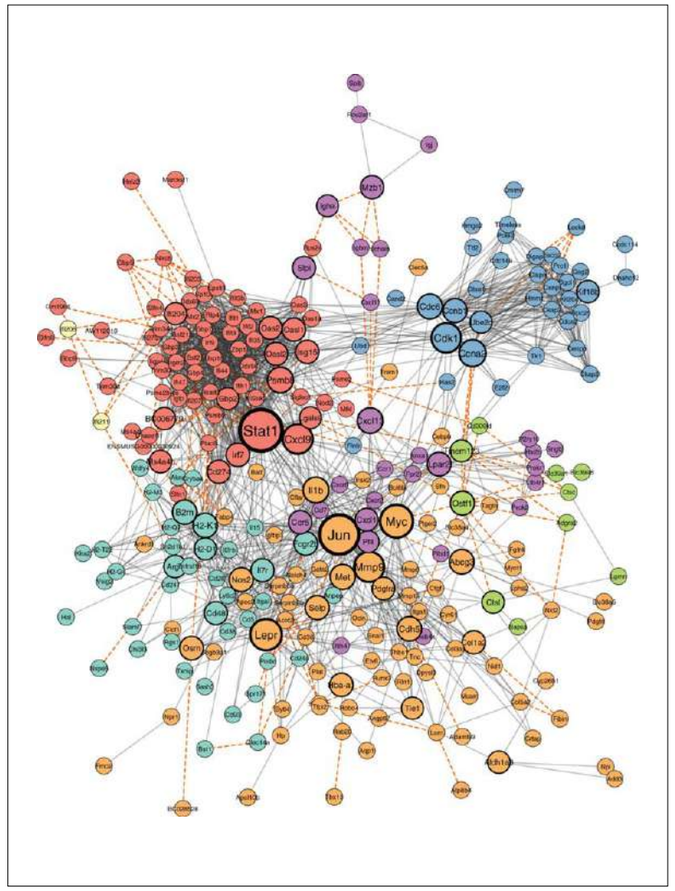
2018 Asymmetric distribution of biomolecules of maternal origin in the Xenopus laevis egg and their impact on the developmental plan. Sindelka R, Abaffy P, Qu Y, Tomankova S, Sidova M, Naraine R, Kolar M, Peuchen E, Sun L, Dovichi N, Kubista M. Sci Rep. 2018 May 29;8(1):8315. doi: 10.1038/s41598-018-26592-1.

2017 Two-tailed RT-qPCR: a novel method for highly accurate miRNA quantification. Androvic P, Valihrach L, Elling J, Sjoback R, Kubista M. Nucleic Acids Res. 2017 Sep 6;45(15):e144. doi: 10.1093/nar/gkx588.

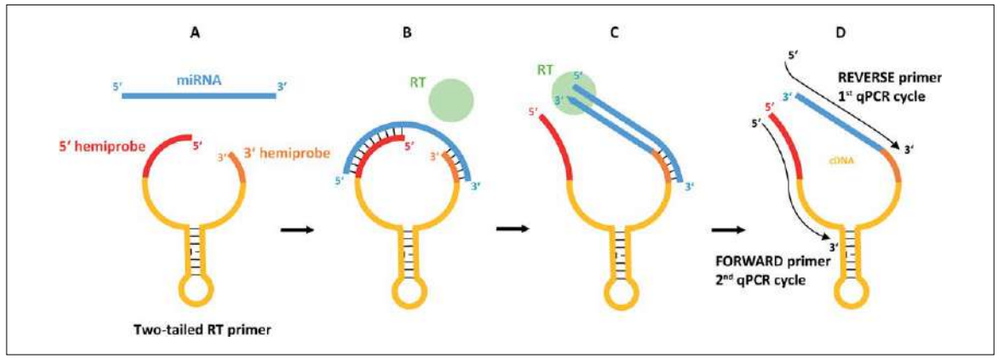
Contact person:	Prof. Dr. Mikael Kubista, Ph.D.
	e-mail: mikael.kubista@ibt.cas.cz

Basic keywords:	01	Single cell
	02	Expression profiling
	03	Early development
	04	Glial cells
	05	RNA-seq
	06	Data analysis

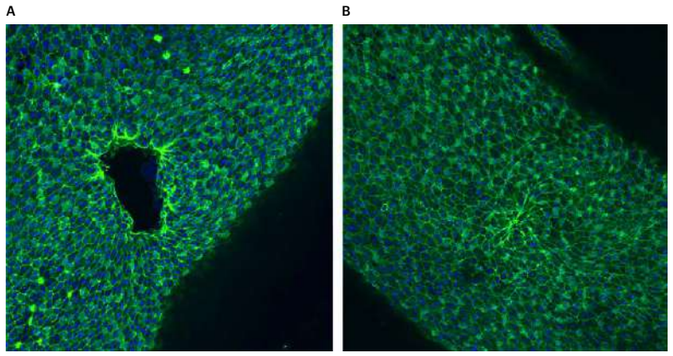
Research areas:	01	Developmental studies based on the model organism Xenopus laevis
	02	Glial cell biology and new method development/implementation
	03	Cancer research
Main objectives:	01	Mechanisms of early development, regeneration, wound healing and localization of biomolecules leading to asymmetric cell division
	02	Characterization of glial cells after brain and spinal cord injuries and in the progression of neurodegenerative diseases, especially of Alzheimer's disease and amyotrophic lateral sclerosis
	03	Development of new methods for gene expression analysis and data processing



Gene network responsible for the differential response of aged animals to ischemic stroke.



Two-tailed RT-qPCR: a novel method for highly accurate miRNA quantification.



Formation of an actin ring during wound healing in 1 (A) and 3 (B) hours after injury.

Tumour Resistance and Metabolism
Jaroslav Truksa, Ph.D., Head of the Lab.

The Institute of Biotechnology of the
Czech Academy of Sciences

Focus of the Research

The main aim of our research is to understand the molecular mechanisms of tumour resitance. In particular, we are focused on the expression and biological role of ABC transporter proteins in tumour-initiating cells (TICs) and tamoxifen-resistant cells. ABC transporters mediate so-called multi drug resistance (MDR) by exporting chemotherapeutic drugs out of the cells, thus leading to therapy failure. Our latest research also shows that proteins of the MDR family could be responsible for resistance to mitochondrially targeted drugs.

We are also focusing on iron metabolism in the biology of TICs. Iron metabolism of cancer cells has been well studied and has established a link with higher iron demand in proliferative cancer cells, where iron withdrawal induces apoptosis. We have described profound changes resulting in an "iron addiction" of TICs and their enhanced sensitivity to iron withdrawal (Rychtarcikova *et al.*, 2017)

Another research aim is to describe and identify novel targets of cancer cells and TICs which could be utilized to remove, reprogram or specifically induce apoptosis in these cells. Our research is also focused on mitochondria, including the utilization of mitochondria as targets for cancer therapy. Recently, we have described the connection between dysfunctional and fragmented mitochondria that exhibit high levels of mitochondrial superoxide and the tamoxifen-resistant phenotype (Tomkova *et al.*, 2019) and we have shown that a high expression of miR-301a directly targets estrogen receptor, thus facilitating the acquision of hormone-independent growth (Lettlova *et al.*, 2017). Furthermore, we participated in a study that described the crucial role of dihydroorotate dehydrogenase in tumorigenesis, an ezyme that is crucial for mitochondrial respiration and pyrimidine biosynthesis (Bajzikova *et al.*, 2019). Currently, we are developing promising novel compounds that are targeted to mitochondria and interfere with mitochondrial functions and iron metabolism (Figure A). These compounds show significant cytostatic, cytotoxic and migrastatic effects (Figure B).

Potential for Cooperation

We collaborate with Griffith University, Australia (Prof. Neuzil), The University of Oviedo (Prof. Carlos Lopez Otin), The University of California San Diego (Dr. Xin Du), First Medical Faculty, Charles University (Dr. Krijt), Faculty of Science, Charles University (Dr. Brabek). Ph.D. students are welcome to spend and work a short stay in the laboratory to broaden their expertise.

2019 Tomkova V, Sandoval-Acuna C, Torrealba N, Truksa J.: Mitochondrial fragmentation, elevated mitochondrial superoxide and respiratory supercomplexes disassembly is connected with the tamoxifen-resistant phenotype of breast cancer cells. Free Radical Biology and Medicine, 143: 510-521, 2019

2019 Bajzikova M, Kovarova M, Coelho AR, Boukalova S, Oh S, Rohlenova K, Svec D, Hubackova S, Endaya B, Judasova K, Bezawork-Geleta A, Kluckova K, Chatre L, Zobalova R, Novakova A, Vanova K, Ezrova Z, Maghzal GJ, Novais SM, Olsinova M, Krobova L, An YJ, Davidova E, Nahacka Z, Sobol M, Cunha-Oliveira T, Sadoval-Acuña C, Strnad H, Zhang T, Huynh T, Serafim TL, Hozak P, Sardao VA, Koopman WJH, Ricchetti M, Oliveira PJ, Kolar F, Kubista M, Truksa J, Dvorakova-Hortova K, Pacak K, Gurlich R, Stocker R, Zhou Y, Berridge MV, Park S, Dong LF, Rohlena J, Neuzil J.:Reactivation of dihydroorotate dehydrogenase by respiration restores tumor growth of mitochondrial DNA-depleted cancer cells. Cell Metabolism, 29: 399-416, 2019

2018 Lettlova S, Brynychova V, Blecha J, Vrana D, Vondrusova M, Soucek P, Truksa J.: MiR-301a-3P Suppresses Estrogen Signaling by Directly Inhibiting ESR1 in ERα Positive Breast Cancer. Cellular Physiology and Biochemistry, 46: 2601-2615, 2018

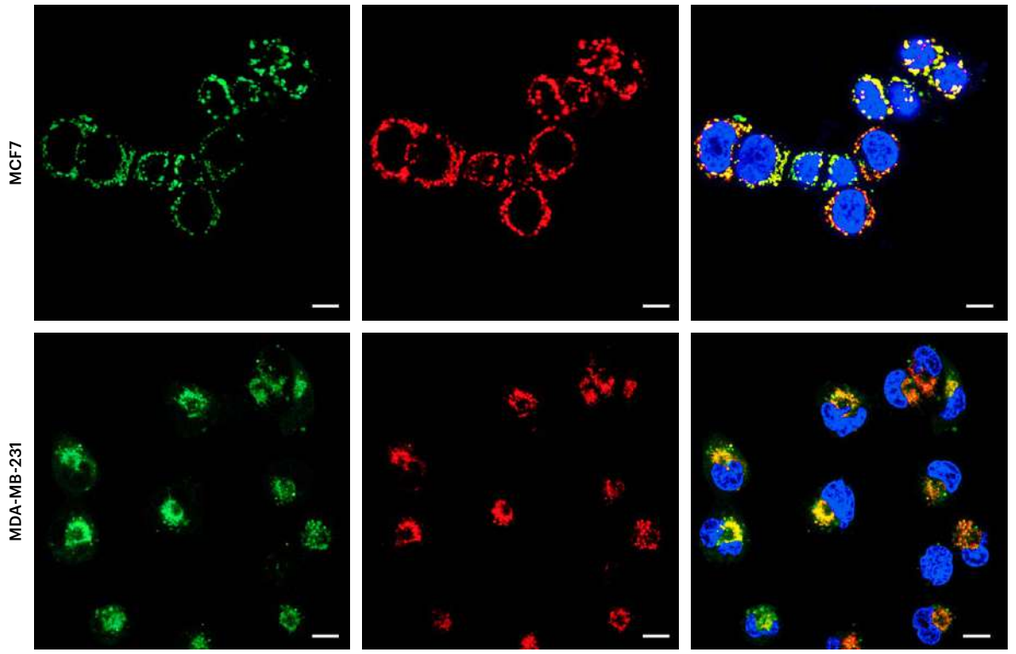
2017 Rychtarcikova Z., Lettlova S., Tomkova V., Korenkova V., Langerova L., Simonova E., Zjablovskaja P., Alberich-Jorda M., Neuzil J., Truksa J.: Tumor-initiating cells of breast and prostate origin show alterations in the expression of genes related to iron metabolism. Oncotarget, 8:6376-6398, 2017

Contact person:	Jaroslav Truksa, Ph.D.
	e-mail: jaroslav.truksa@ibt.cas.cz

Basic keywords:	01	Tumour-initiating cells
	02	ABC transporters
	03	Mitochondria
	04	Iron metabolism
	05	Tumour invasivity
	06	Cellular proliferation
	07	Tamoxifen-resistance
	08	Iron chelation

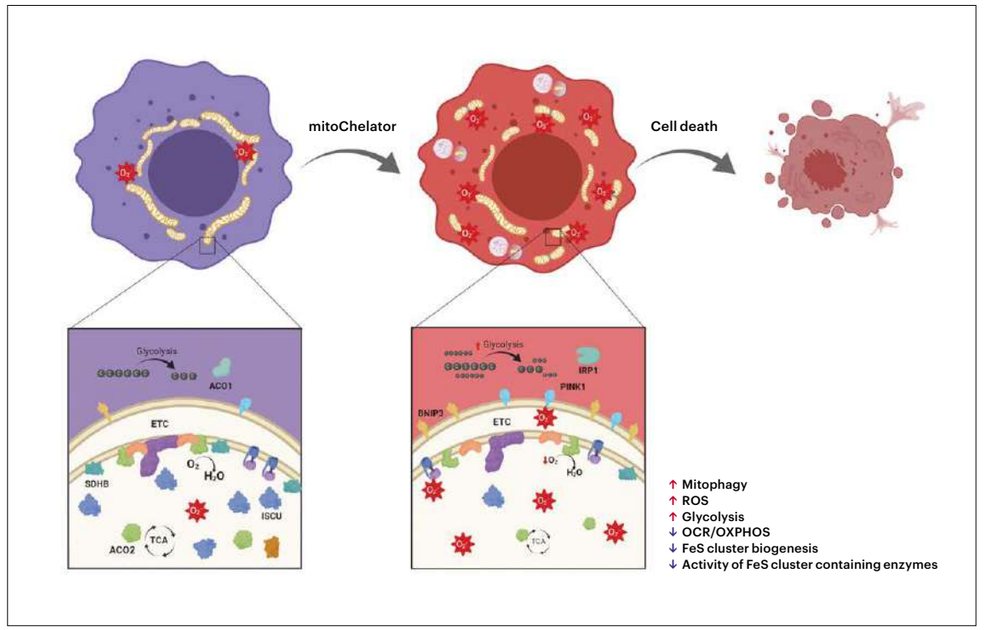
Research areas:	01	Molecular mechanisms of cancer resistance
	02	Iron metabolism
	03	ABC transporter proteins
	04	Tumour-initiating cells
	05	Mitochondrial biology
Main objectives:	01	Elucidate molecular mechanisms of tumour resistance and define differences in the expression and regulation of ABC transporter in tumour-initiating cells, tamoxifen-resistant cells
	02	Understand the role of iron metabolism in helath and disease with focus on alterations in iron metabolism between normal and malignant cells
	03	Develop novel compounds that specifically target cancer cells by tageting their mitochondria and iron metabolism

A



Panel A shows accumulation of the mitochondrially targeted chelator (mitoChelator-FITC) inside mitochondria (Mitotracker) and documents clear colocalization (Merge).

B



Panel B is a graphical abstract depicting the effects of the mitochondrially targeted chelator on cancer cells.

Clinical Proteomics
Doc. RNDr. Jiří Petrák, Ph.D., Head of the Lab.

First Faculty of Medicine,
Charles University

Content of the Research

Proteome is defined as the complete set of proteins present at a given time in an organism, tissue or cell. Proteomics aims at making a quantitative and qualitative description of proteomes and their dynamic changes. Using the most advanced proteomic strategies based on effective separation methods and high-resolution mass spectrometry, the identification and quantification of up to 10,000 proteins can be accomplished in a single experiment. This opens a new way toward understanding physiological and pathological processes on the molecular level.

Employing proteomic approaches, our team is attempting to identify the key proteins involved in the molecular mechanism of human diseases, to identify novel diagnostic or prognostic biomarkers and to discover new drug targets. Our current research is focused on three main topics. The first project aims at elucidating the molecular processes governing the development and progression of heart failure.The objective of our second project is to identify tumor markers and drug targets in rare neuroendocrine tumors – pheochromocytomas and paragangliomas. Our third mission is to develop new proteomic methods for membrane proteome analysis. Although highly effective for soluble proteins, standard proteomic approaches do not efficiently cover membrane proteins.

Potential for Cooperation

Our team employs modern proteomic analyses to study global changes of cellular proteomes in order to describe the molecular mechanisms and the proteins involved in human physiology and pathology. Using high-resolution multi-dimensional separation methods combined with high-resolution mass spectrometry, we can monitor quantitative and qualitative changes of thousands of proteins. We offer our extensive experience with a wide spectrum of quantitative (LFQ and stable isotope-based) proteomic analyses of animal and human protein samples including body fluids and other clinical specimens. In addition, our expertise also covers diverse molecular and cell biology methods, immunological methods, cell cultures and animal models.

2019 Vit O, Harant K, Klener P, Man P, Petrak J. A three-pronged "Pitchfork" strategy enables an extensive description of the human membrane proteome and the identification of missing proteins. J Proteomics. 2019 Jul 30;204:103411 .

2019 Jankovska E, Svitek M, Holada K, Petrak J. Affinity depletion versus relative protein enrichment: a side-by-side comparison of two major strategies for increasing human cerebrospinal fluid proteome coverage. Clin Proteomics. 2019 Feb 26;16:9.

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2018 Melenovsky V, Cervenka L, Viklicky O, Franekova J, Havlenova T, Behounek M, Chmel M, Petrak J. Kidney Response to Heart Failure: Proteomic Analysis of Cardiorenal Syndrome. Kidney Blood Press Res. 2018;43(5):1437-1450.

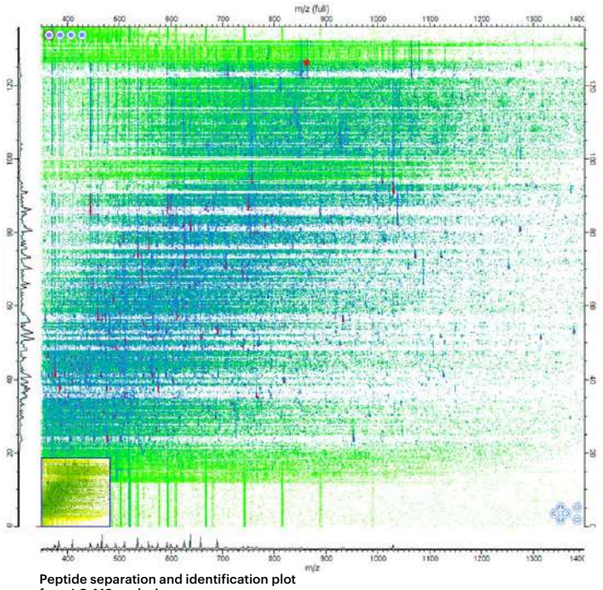
2017 Vit O, Petrak J. Integral membrane proteins in proteomics. How to break open the black box? J Proteomics. 2017 Feb 5;153:8-20.

2017 Zimmermannova O, Doktorova E, Stuchly J, Kanderova V, Kuzilkova D, Strnad H,Starkova J, Alberich-Jorda M, Falkenburg JHF, Trka J, Petrak J, Zuna J, Zaliova M. An activating mutation of GNB1 is associated with resistance to tyrosine kinase inhibitors in ETV6-ABL1-positive leukemia. Oncogene. 2017 Oct 26;36(43):5985-5994.

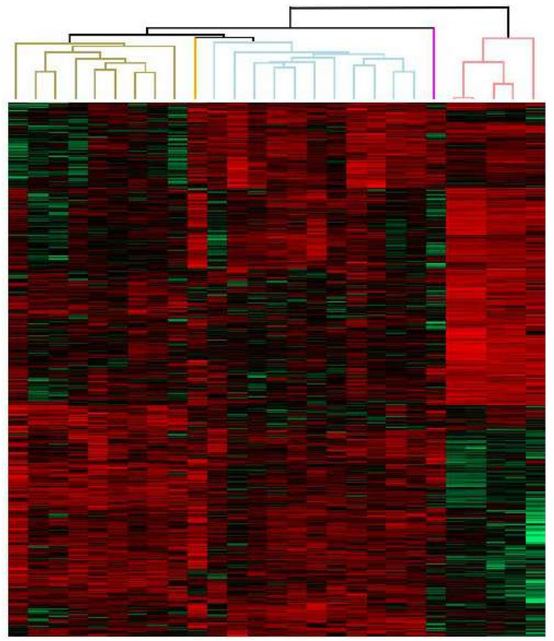
2016 Vit O, Man P, Kadek A, Hausner J, Sklenar J, Harant K, Novak P, Scigelova M, Woffendin G, Petrak J. Large-scale identification of membrane proteins based on analysis of trypsin-protected transmembrane segments. J Proteomics. 2016 Oct 21;149:15-22.

Contact person:	Doc. RNDr. Jiří Petrák, Ph.D.
	e-mail: jiri.petrak@lf1.cuni.cz

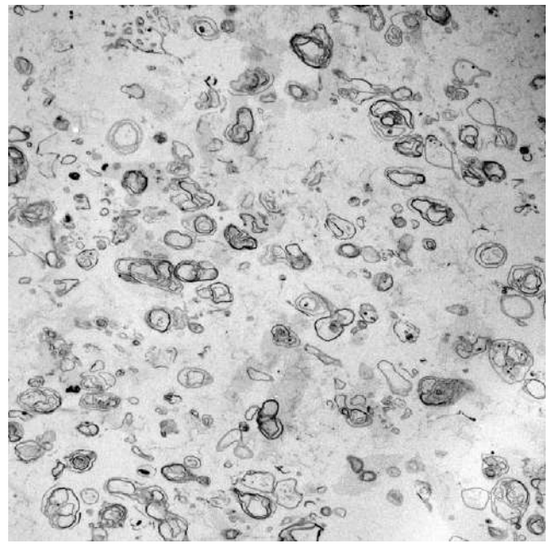
Basic keywords:	01	Proteomics
	02	Mass spectrometry
	03	Biomarkers
	03	Drug targets
Research areas:	01	Proteomic as a tool for the elucidation of molecular mechanisms of diseases and biomarker identification.
	02	The roles of individual proteins in physiology and pathology
Main objectives:	01	Identification of proteins involved in the development and progression of heart failure
	02	Identification of clinically relevant tumor biomarkers and potential drug targets
	03	Development of proteomic methods



Peptide separation and identification plot from LC-MS analysis.



Proteomic profiling of human neuroendocrine tumors – a heat map and clustering of quantitative expression data.



Electron microscopy image of membrane vesicles isolated from human lymphoma cells.

The Structure and Function of Cells in Their Normal State and Pathology

MUDr. Zdeněk Kostrouch, CSc., Head of the Lab.

First Faculty of Medicine,
Charles University

Content of the Research

The group employs advanced informatics and invertebrate model systems in order to identify and analyze regulatory cascades that direct the normal development, metabolism and reproduction of animals. We aim to uncover archetypal mechanisms. These "ancient regulatory pathways", from the evolutionary point of view are often masked by more potent newly adopted mechanisms. The organisms in our focus are Nematoda, Turbellaria and diploblastic species. We are focused on the regulation of gene expression, especially involving nuclear receptors, their ligands and interactors. We use transgenic methods and gene editing to conduct a molecular analysis of regulatory mechanisms. Our working scheme of the evolutionary tree differs from the conventional concept: we view Deuterostomes as a branch that is parallel to Diploblasts; Protostomes are thought of as a sideways developing branch. In this concept, we view vertebrate diseases, especially tumors, as an evolutionary return to states resembling or reflecting archetypal tissues. Our goal is to understand their archetypal regulations. They are likely to be present and conserved in tissues of contemporary organisms as well as in tissues that escaped from evolutionarily modern regulations and became tumors. Tumors are, in our understanding, archeplasms, not neoplasms. We are looking for archetypal mechanisms that may regulate them.

Our projects have led us to the realization that the structural state of a cell is directly connected to the regulation of gene expression by proteins with dual cytoplasmic and nuclear roles. We are searching for proteins with the potential to transmit the cytoplasmic structural signals towards the regulation of gene expression at the level of transcription and translation. We are currently working on the concept of a free proteome code, a concept that assumes the regulatory roles of proteins that cannot be assembled in cell structures and act as regulators of gene expression. We have also worked on ancient systems of gene expression regulation by nuclear receptors and came up with the concept of food constituents as the original endocrinologically active substances that still influence our lives, but are obscured by more powerful modern regulations.

Potential for Cooperation

We offer expertise in the following model systems; Caenorhabditis elegans, Schmidtea mediterranea, Tripedalia cystophora, Aurelia aurita. We collaborate with BIOCEV research groups and our colleagues from around the world on these research programs.

2019 Kostrouchová M, Kostrouchová V, Yilma P, Benda A, Mandys V, Kostrouchová M. Valproic Acid Decreases the Nuclear Localization of MDT-28, the Nematode Orthologue of MED28. Folia Biol (Praha). 2018;64(1):1-9.

2019 Novotný JP, Chughtai AA, Kostrouchová M, Kostrouchová V, Kostrouch D, Kaššák F, Kaňa R, Schierwater B, Kostrouchová M, Kostrouch Z. Trichoplax adhaerens reveals a network of nuclear receptors sensitive to 9-cis-retinoic acid at the base of metazoan evolution. PeerJ. 2017 Sep 29;5:e3789. doi: 10.7717/peerj.3789. eCollection 2017.

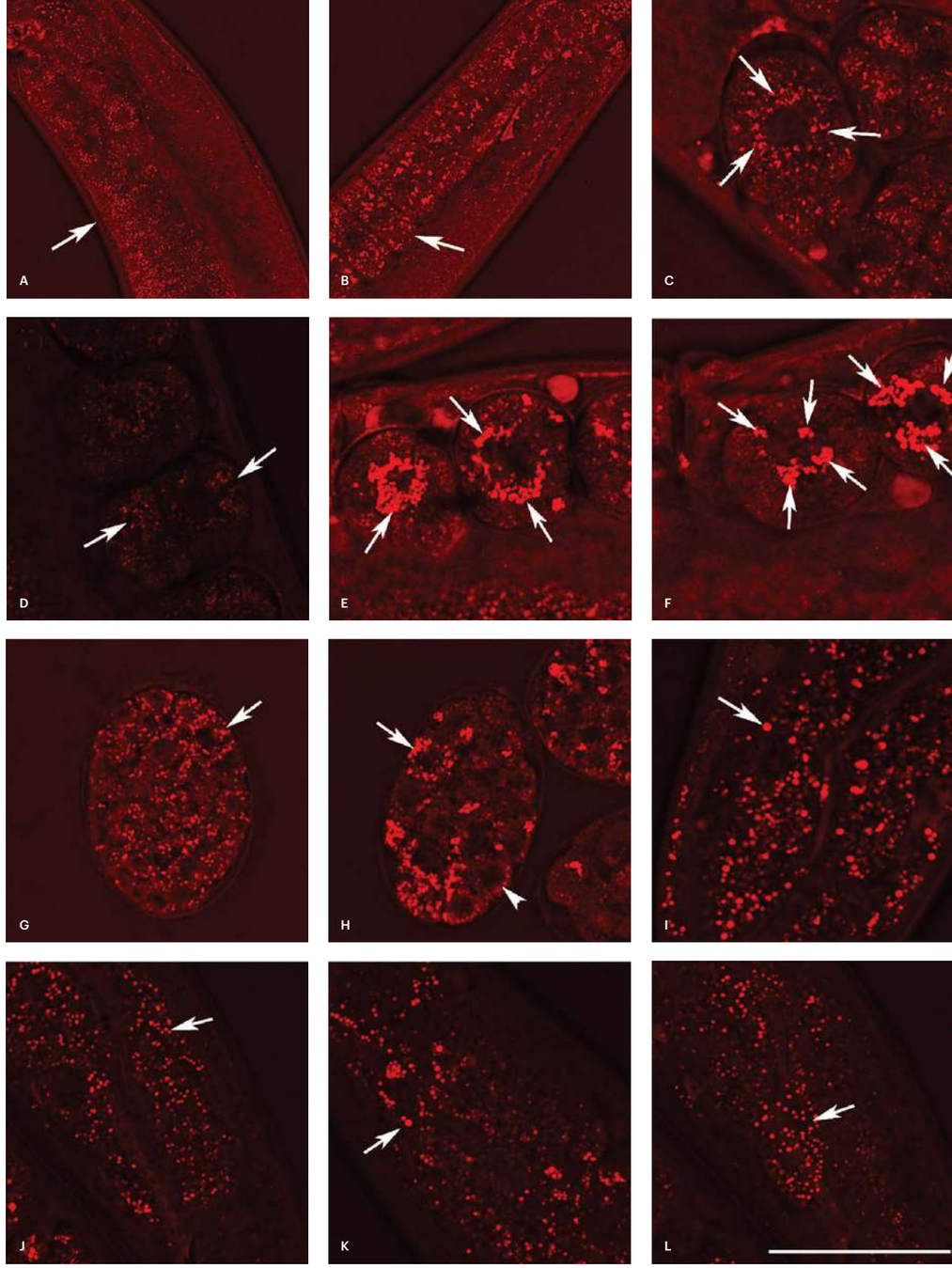
2019 Kostrouchová M, Kostrouch D, Chughtai AA, Kaššák F, Novotný JP, Kostrouchová V, Benda A, Krause MW, Saudek V, Kostrouchová M, Kostrouch Z. The nematode homologue of Mediator complex subunit 28, F28F8.5, is a critical regulator of C. elegans development. PeerJ. 2017 Jun 6;5:e3390. doi: 10.7717/peerj.3390. eCollection 2017.

2019 Kaššák F, Hána V, Saudek V, Kostrouchová M. Novel Mutation (T273R) in Thyroid Hormone Receptor β Gene Provides Further Insight into Cryptic Negative Regulation by Thyroid Hormone. Folia Biol (Praha). 2017;63(2):60-66.

Contact person:	MUDr. Zdeněk Kostrouch, CSc.
	e-mail: zdenek.kostrouch@lf1.cuni.cz

Basic keywords:	01	Nuclear receptors
	02	Gene expression
	03	Rxr
	04	Nhr-23
	05	Evolution
	06	Retinoic acid
	07	Retinoids
	08	Transcription

Research areas:	01	Regulation of metabolism and development at the base of Deuterostome evolution
	02	Regulatory networks of nuclear receptors in nematodes
	03	Nuclear receptors in human cancers
	04	Interactors of regulatory pathways involving nuclear receptors – regulatory RNAs, endocrine disruptors and functionally shared proteins
Main objectives:	01	The identification of conserved regulatory mechanisms, including nuclear receptors and proteins that transmit or modulate their function
	02	The analysis of parallel (orthologous) mechanisms in mammalian cells and human tissues, including cancers



Identification of the nematode perilipin (PLIN-1) and an analysis of its loss of function phenotype reveal a new lipid containing compartment functioning in embryonic development (arrows). The left panel shows an embryo with a disrupted plin-1 gene with an enlarged lipid containing structures detected by CARS microscopy. This compartment also exists in normal embryos (right panel) but is visible only when the brightness of the entire panel is digitally enhanced (+150 units) (Chughtai et al. 2015). The bar represents 50 μm.

Laboratory of Lymphoma Tumor Biology
Ondrej Havranek, MD Ph.D., Head of the Lab.

First Faculty of Medicine,
Charles University

Content of the Research

Non-Hodgkin lymphomas are a heterogenous group of hematologic malignancies derived more than 90% of the time from B-lymphocytes, one subtype of white blood cells. B-cells are part of the adaptive immune system and one of their main functions is to recognize antigens and differentiate them into plasmatic cells to produce antibodies. The latest developments in technology and related research have resulted in an unprecedented advancement in our understanding of mechanisms leading to lymphomagenesis, however, many mechanisms of aberrant signaling, metabolic changes, transcription regulation, and interactions of tumor cells with tumor microenvironment are still not fully understood. Using advanced methods for targeted genomic modifications, protein-protein interaction detection, high-resolution microscopy, and intracellular biosensors, the general aim of our group is to address the unknown issues of the mentioned cellular processes in lymphomas and move forward our understanding of these processes in general. We are particularly interested in: 1) looking at the details of B-cell receptor signaling initiation and differences in consequent pathway activation, 2) finding relevant and targetable sources of oncogenic PI3K/AKT pathway activation and optimal approaches for direct PI3K/AKT pathway complete and combinatorial inhibition, 3) describing novel epigenetic regulators involved in lymphoma development, 4) crosstalk between lymphoma associated mutations and general cellular processes involving cell cycle and metabolism.

Potential for Cooperation

We are open to a wide range of academic as well as industry-based collaborations. Using our expertise in model cell lines, precise genomic editing and pathway activity measurement using genetically encoded biosensors, we are actively working on many collaborative projects in the field of lymphoma signaling. Our main collaborators include Assoc. Prof. RE Davis (Department of Lymphoma and Myeloma, the UT MD Anderson Cancer Center, Houston, TX, U.S.A.), Prof. P Klener (The Institue of Pathophysiology, First Medical School, Charles University, Prague, Czech Republic), Assoc. Prof. L Seghal (The Ohio State University, Division of Hematology, U.S.A.), Prof. JH Veelkenen (Leiden University Medical Center, Department of Hematology, Holland), Prof. P Juszczynsky (The University of Hematology and Transfusion Medicine, Poland), and Prof. B Chapuy (The University of Göttingen, Department of Hematology and Oncology).

2020 Jain N, Singh S, Laliotis G, Hart A, Muhowski E, Kupcova K, Chrbolkova T, Khashab T, Chowdhury SM, Sircar A, Shirazi F, Singh RK, Alinari L, Zhu J, Havranek O, Tsichlis P, Woyach J, Baiocchi R, Samaniego F, Sehgal L. Targeting phosphatidylinositol 3 kinase-β and -δ for Bruton tyrosine kinase resistance in diffuse large B-cell lymphoma. Blood Adv 2020 Sep 22;4(18):4382-4392. (2019 IF 4.6)

2020 Vockova P, Molinsky J, Klanova M, Karban J, Spacek M, Havranek O, Kupcova K, Kazantsev D, Trneny M, Klener P. CD31/PECAM-1 impacts engraftment, growth and spread of mantle cell lymphoma cells and positively correlates with extramedullary involvement. Leuk Lymphoma 2020 Nov 25;1-8. Online ahead of print.

2019 Jain N, Harter K, Tadros S, Fiskus W, Havranek O, Ma MCJ, Bouska A, Heavican T, Kumar D, Deng Q, Moore D, Pak C, Liu CL, Gentles AJ, Hartmann E, Kridel R, Smedby E, Juliusson G, Rosenquist R, Gascoyne RD, Rosenwald A, Giancotti F, Neelapu SS, Westin J, Vose JM, Lunning MA, Greiner T, Rodig S, Iqbal J, Alizadeh AA, Davis RE, Bhalla K, Green MR. Targetable genetic alterations of TCF4 (E2-2) drive immunoglobulin expression in diffuse large B-cell lymphoma. Science Translational Medicine 2019 Jun 19;11(497) (2019 IF 16.3)

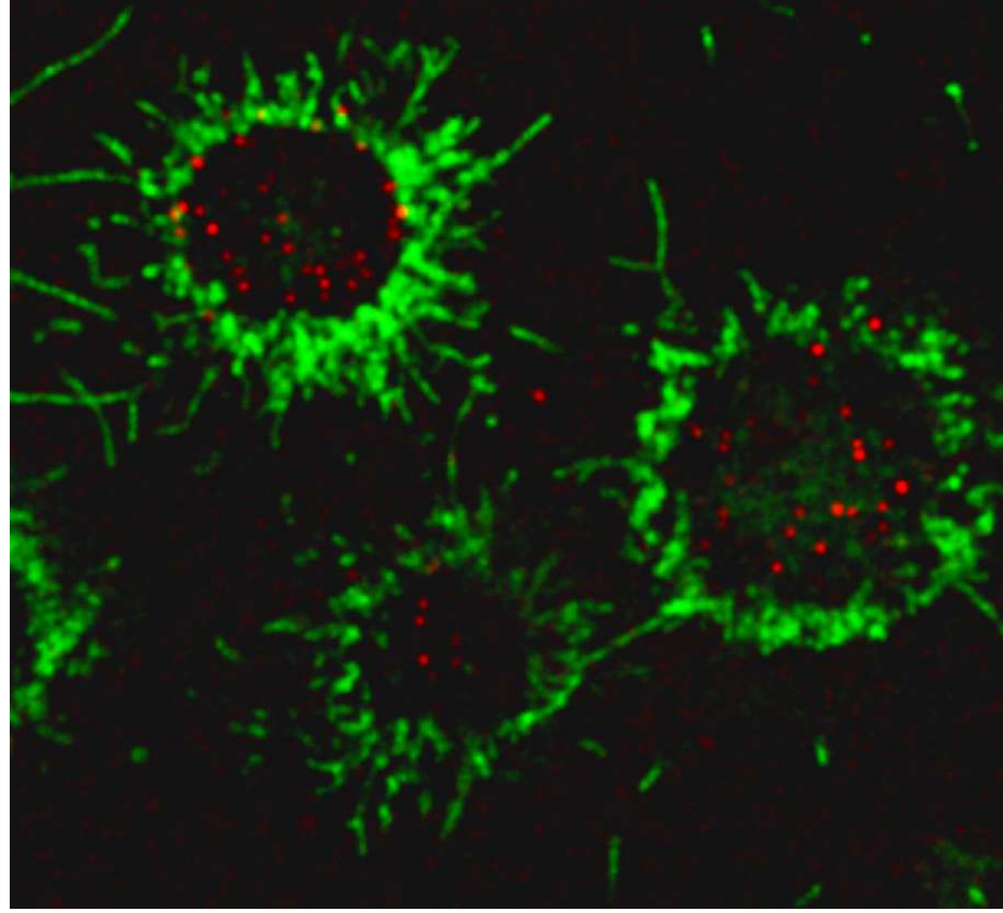
2019 Prukova D, Andera L, Nahacka Z, Karolova J, Svaton M, Klanova M, Havranek O, Soukup J, Svobodova K, Zemanova Z, Tuskova D, Pokorna E, Helman K, Forsterova K, Pacheco-Blanco M, Vockova P, Berkova A, Fronkova E, Trneny M, Klener P. Co-targeting of BCL2 with venetoclax and MCL1 with S63845 is synthetically lethal in vivo in relapsed mantle cell lymphoma. Clin Cancer Res. 2019 Jul 15;25(14):4455-4465. (2019 IF 10.1)

2019 Kleiblova P, Stolarova L, Krizova K, Lhota F, Hojny J, Zemankova P, Havranek O, Vocka M, Cerna M, Lhotova K, Borecka M, Janatova M, Soukupova J, Stribrna J, Sevcik J, Zimovjanova M, Kotlas J, Panczak A, Vesela K, Zidovska J, Cervenkova J, Schneiderova M, Burocziova M, Burdova K, Stranecky V, Foretova L, Machackova E, Tavandzis S, Kmoch S, Macurek L, Kleibl Z. Identification of deleterious germline CHEK2 mutations and their association with breast and ovarian cancer. Int J Cancer. 2019 Oct 1;145(7):1782-1797

Contact person:	Ondřej Havránek, MD PhD
	e-mail: ondrej.havranek@lf1.cuni.cz

Basic keywords:	01	Tumor biology
	02	Lymphomas
	03	B cell receptor
	04	Oncogenic signaling
	05	Cell cycle
	06	Epigenetic regulation
	07	PI3K/AKT pathway
	08	Biosensors
	09	Gene editing
	10	Metabolism

Research areas:	01	B cell receptor signaling activation
	02	Mechanisms of oncogenic PI3K/AKT pathway activation
	03	Tumor specific crosstalk between signaling, metabolism and cell cycle
	04	Circulating tumor DNA as a diagnostic and therapy prediction tool
	05	Tumor metabolism
Main objectives:	01	Description of novel mechanisms of lymphomagenesis
	02	Development of novel therapeutic approaches



SU-DHL-4 lymphoma cells with diffuse membrane distribution of the B cell receptor (●) and scattered spots of lipid rafts (●). Live cells were attached to a coverslip of a chambered slide and the attached part of the membrane was imaged.

Metabolism of Healthy and Tumor Tissues at Single-Cell Resolution

Kateřina Rohlenová, Ph.D., Head of the Lab.

Institute of Biotechnology of the
Czech Academy of Sciences

Content of the Research

Our new research group started at the Institute of Biotechnology at the BIOCEV center in October 2020. The head of the group, Katerina Rohlenova, and her research team, the Laboratory of Cellular Metabolism, are focusing on one central topic: "Metabolic crosstalk" – how cells in tissues share metabolites – and its implications for novel therapeutic strategies.

Healthy tissues as well as malignant tumors consist of multiple cell types that metabolically communicate with each other, e.g. one cell type produces metabolites that are then taken up by another cell type. This enables growth and survival in nutrient-poor environments, such as in tumors, and metabolic crosstalk can thus limit the efficacy of metabolic interventions targeted at cancer cells. One example is antimetabolite therapy that acts by interfering with nucleic acid synthesis. This therapy was established more than 70 years ago as one of the first approaches for the treatment of cancer. Despite its long and quite successful history, antimetabolite therapy suffers from resistance and is rather toxic to healthy tissue. Other therapeutic strategies, such as blocking nucleic acids at the level of de novo nucleotide synthesis lacked efficacy when tested as a cancer treatment. The reasons for this resistance remain unknown, but it could be caused at least in part by the exchange of nucleotides within tissues. Our goal is to understand how cells in tissues trade metabolites, and how healthy tissues and tumors differ in their metabolic trading patterns. Our ambition is to use this understanding to develop new therapeutic concepts and, potentially, new treatment strategies.

Potential for Cooperation

In our research, we combine the investigation of metabolism using genetically modified cellular and mouse models with bulk and single-cell omics technologies. These methods allow us to embrace tissue complexity and to thoroughly understand the metabolic interactions of cell types within tissues. We are interested the most in endothelial cells. Endothelial cells can remain quiescent for years, but when an angiogenic stimulus is present, they can rapidly start to proliferate, a switch that requires changes in their metabolism. As blood vessels are pervasive in the body and endothelial cells form the interface between circulation and tissues, their metabolic state might strongly influence the tissue environment. We are seeking collaborations across the metabolism and omics field, as well as collaboration with experts on the metabolism of different cell types in tissues.

2020 Rohlenova, K.*, Goveia, J.*, Garcia-Caballero, M.*, Subramanian, A.*, Kalucka, J., Treps, L., Falkenberg, K.D., de Rooij, L., Zheng, Y., Lin, L., Sokol, L., Teuwen, L.A., Geldhof, V., Taverna, F., Pircher, A., Conradi, L.C., Khan, S., Stegen, S., Panovska, D., De Smet, F., Staal, F.J.T., McLaughlin, R.J., Vinckier, S., Van Bergen, T., Ectors, N., De Haes, P., Wang, J., Bolund, L., Schoonjans, L., Karakach, T.K., Yang, H., Carmeliet, G., Liu, Y., Thienpont, B., Dewerchin, M., Eelen, G., Li, X., Luo, Y., and Carmeliet, P. (2020). Single-Cell RNA Sequencing Maps Endothelial Metabolic Plasticity in Pathological Angiogenesis. Cell Metab 31: 862-877 e814.

2020 Boukalova, S., Hubackova, S., Milosevic, M., Ezrova, Z., Neuzil, J., and Rohlena, J. (2020). Dihydroorotate dehydrogenase in oxidative phosphorylation and cancer. Biochim Biophys Acta Mol Basis Dis 1866: 165759.

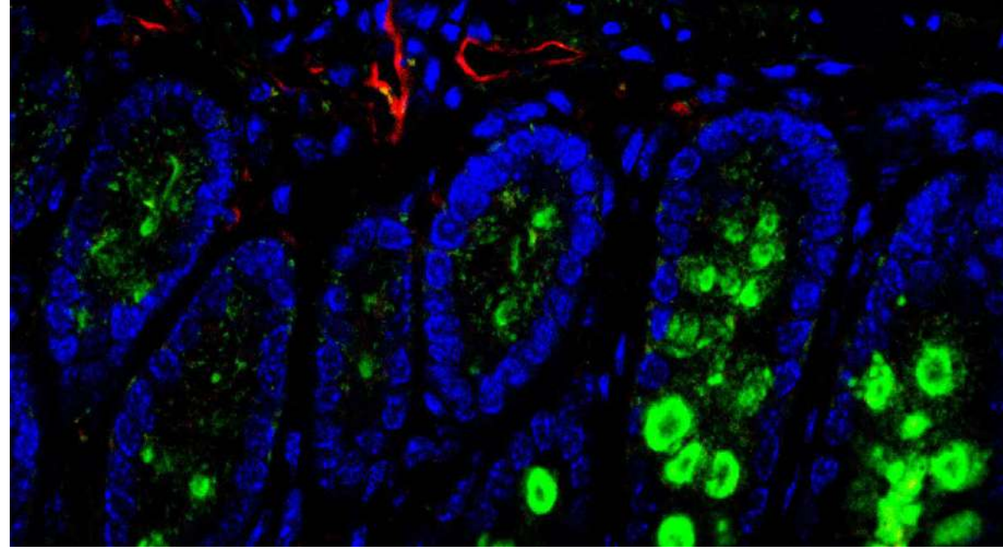
2020 Goveia, J.*, Rohlenova, K.*, Taverna, F.*, Treps, L.*, Conradi, L.C., Pircher, A., Geldhof, V., de Rooij, L., Kalucka, J., Sokol, L., Garcia-Caballero, M., Zheng, Y., Qian, J., Teuwen, L.A., Khan, S., Boeckx, B., Wauters, E., Decaluwe, H., De Leyn, P., Vansteenkiste, J., Weynand, B., Sagaert, X., Verbeken, E., Wolthuis, A., Topal, B., Everaerts, W., Bohnenberger, H., Emmert, A., Panovska, D., De Smet, F., Staal, F.J.T., McLaughlin, R.J., Impens, F., Lagani, V., Vinckier, S., Mazzone, M., Schoonjans, L., Dewerchin, M., Eelen, G., Karakach, T.K., Yang, H., Wang, J., Bolund, L., Lin, L., Thienpont, B., Li, X., Lambrechts, D., Luo, Y., and Carmeliet, P. (2020). An Integrated Gene Expression Landscape Profiling Approach to Identify Lung Tumor Endothelial Cell Heterogeneity and Angiogenic Candidates. Cancer cell 37: 21-36 e13.

2020 Kalucka, J., de Rooij, L., Goveia, J., Rohlenova, K., Dumas, S.J., Meta, E., Conchinha, N.V., Taverna, F., Teuwen, L.A., Veys, K., Garcia-Caballero, M., Khan, S., Geldhof, V., Sokol, L., Chen, R., Treps, L., Borri, M., de Zeeuw, P., Dubois, C., Karakach, T.K., Falkenberg, K.D., Parys, M., Yin, X., Vinckier, S., Du, Y., Fenton, R.A., Schoonjans, L., Dewerchin, M., Eelen, G., Thienpont, B., Lin, L., Bolund, L., Li, X., Luo, Y., and Carmeliet, P. (2020). Single-Cell Transcriptome Atlas of Murine Endothelial Cells. Cell 180: 764-779 e720.

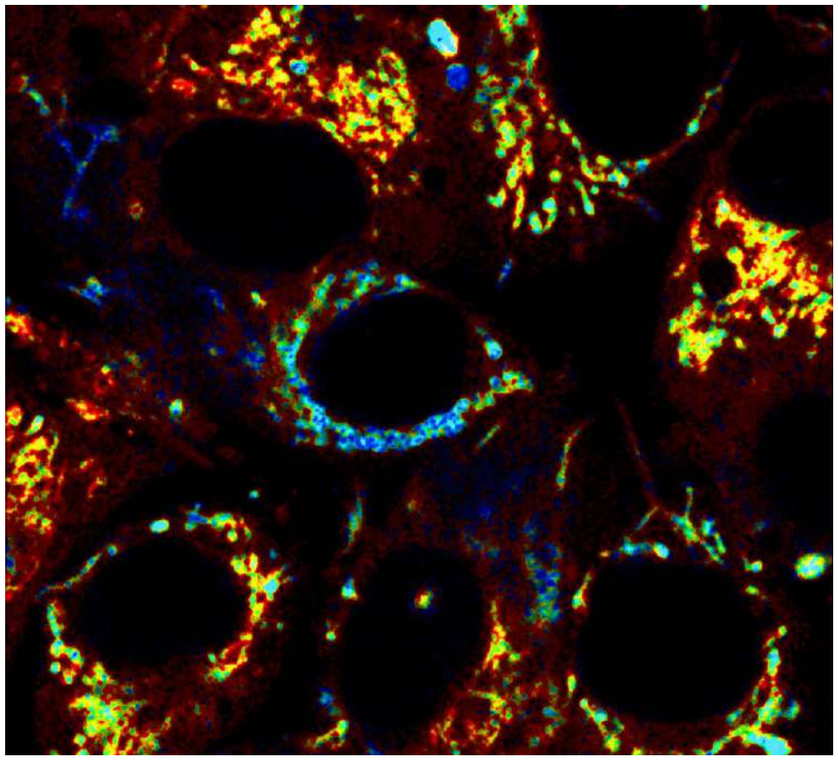
Contact person:	Kateřina Rohlenová, Ph.D.
	e-mail: katerina.rohlenova@ibt.cas.cz

Basic keywords:	01	Metabolism
	02	Cancer
	03	Oxidative phosphorylation
	04	Nucleotide biosynthesis
	05	Single-cell transcriptomics

Research areas:	01	Intercellular crosstalk of metabolites in healthy and tumor tissue
	02	Metabolism of proliferative and quiescent cells
	03	Role of endothelial cells in tissue metabolism
Main objectives:	01	Characterize metabolic crosstalk in healthy and tumor tissues for basic understanding of metabolic tissue homeostasis and to develop new therapeutic concepts



Colon section in a mouse with selective deletion of the *TFAM* gene in the endothelium (the inner lining of blood vessels). TFAM is stained ●, endothelial cells are stained ●. Nuclei are shown in ●. Such model allows us to study metabolic perturbation in endothelial cells in the context of whole tissue, and how these perturbations affect other cell types.



Endothelial cells visualized using two-photon fluorescence lifetime imaging to detect NADH. The most prominent structures are mitochondria, where concentration of NADH is the highest. Color coding represents fluorescence lifetime: ● - long lifetime (bound NADH), ● - short lifetime (free NADH).


Dw 5


Dw 5

Research Infrastructures and Core Facilities

No. of Facilities: 08

Pages: 184–219

Research Infrastructures and Core Facilities

186

The implementation of complex projects requires a high-quality methodological basis concentrated in core facilities. All are open to external users to provide them with research services. Research infrastructures within BIOCEV are: CCP - the Czech Centre for Phenogenomics, CIISB - Czech Infrastructure for Integrative Structural Biology, Czech-BioImaging - National research infrastructure for biological and medical imaging and ELIXIR-CZ - a national hub for bioinformatics and the National Center for Medical Genomics.

GeneCore – Quantitative and digital PCR

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The GeneCore - Quantitative and digital PCR Core Facility is one of Europe's leading academic service providers specialized particularly in high-throughput gene expression analysis using quantitative polymerase chain reaction (qPCR). Our ten years of experience has enabled us to participate in many various research projects, including those involving the fast-advancing field of single-cell analysis. We maintain close cooperation with the Laboratory of Gene Expression, IBT CAS v.v.i. and aim to provide a flawless experimental design, while providing the highest quality working material. We have broad experience in performing quality control of nucleic acids, in single cell analysis, high-throughput qPCR, digital PCR and NGS library preparation.

CF Services-Summary

01	Sample extraction and nucleic acid quality control
02	Assay design (qPCR and dPCR)
03	qPCR and high-throughput qPCR (gene expression, genotyping)
04	digital PCR (copy number variation, SNP or absolute quantification)
05	Single cell expression profiling
06	Library preparation, quality control and experimental design of RNA-Seq
07	miRNA analysis (Two-Tailed RT-qPCR – design and validation)
08	Elementary data analysis

CF Cooperation

01	Faculty of Fisheries and the Protection of Waters, University of South Bohemia in České Budějovice
02	IKEM – Institute for Clinical and Experimental Medicine
03	Laboratory of gene expression, Institute of Biotechnology CAS
04	Prof. MUDr. Michal Mára, CSc. – Gynekologicko-porodnická klinika 1. LF UK a VFN
05	LIFE TEST s.r.o.
06	TATAA Biocenter

CF Key Equipment

High-Throughput qPCR BioMark System (Fluidigm)
Droplet Digital QX200 System (Bio-Rad)
AVISO CellCelector (ALS) Automated cell picking
EpMotion P5073 (Eppendorf)
Fragment Analyzer (Advanced Analytical)
CFX 384 and CFX96 (Bio-Rad)
LightCycler 480 II (Roche)
Fragment Analyzer (Agilent)

CF Keywords

01	qPCR
02	Single cell analysis
03	Quality control of nucleic acids
04	RNA and DNA
05	NGS library preparation
06	miRNA
07	Digital PCR

Core Facility Services

At GeneCore, we assist researchers with their project design, biological material sampling and analysis of their results, including any necessary consultations for troubleshooting. During the experiments, we run a set of verified molecular tools, which ensure high fidelity and enable the detection of any possible reaction inhibition, contamination or sample degradation. The fast pace of advancements within the field of Molecular Biology is appreciated at GeneCore. As a result, we offer some of the most up-to-date methodologies to aid researchers in answering their biological questions while continually adding new methods to our ever-growing portfolio. This is clearly demonstrable in the rapidly progressing field of RNA-Seq, where we offer advanced assistance with library preparations, quality control and design of RNA-Seq experiments, which are essential for a successful project.

Another cutting-edge field of science that has resulted in several renowned publications, involves the assessment of the importance of small regulatory RNAs, such as miRNAs. To follow up these and similar discoveries, we offer a novel method developed by the Laboratory of Gene Expression named Two-tailed RT-qPCR. Two-tailed RT-qPCR allows for the quantification of miRNA with substantial sensitivity, while allowing discrimination between even highly similar miRNA sequences. Despite the method's complexity, the technique is cost effective. GeneCore is currently the only core facility worldwide that offers the experimental design and validation of the Two-tailed RT-qPCR method.

Potential for Collaboration

GeneCore offers collaboration both to intra-institutional and external researchers from the Czech Republic and we are always open to widen our international cooperation. Our aim is to make state-of-the-art qPCR and dPCR technologies, as well as offer expertise related to nucleic acids analysis. We can contribute to clients' workflow from the extraction of samples, nucleic acid quality control, PCR-based methods and the preparation of samples for RNA-Seq. Academic researchers are also welcome to perform their experiments at our facility under our supervision. The number of research teams seeking our services and advice is steadily growing and we are delighted to be a part of many outstanding research projects, especially as they have led to many impactful publications. We help you to tell your story.

Users´ Highlights:

2020 Gestational and pubertal exposure to low dose of di-(2-ethylhexyl) phthalate impairs sperm quality in adult mice. Dostalova P, Zatecka E, Ded L, Elzeinova F, Valaskova E, Kubatova A, Korenkova V, Langerova L, Komrskova K, Peknicova J. Reprod Toxicol. 2020 Jun 30;96:175-184. doi: 10.1016/j.reprotox.2020.06.014.

2019 Two-tailed RT-qPCR panel for quality control of circulating microRNA studies. Androvic P, Romanyuk N, Urdzikova-Machova L, Rohlova E, Kubista M, Valihrach L. Sci Rep. 2019;9(1):1-9. doi:10.1038/s41598-019-40513-w.

2018 Expression profile of miR-17/92 cluster is predictive of treatment response in rectal cancer. Kral J, Korenkova V, Novosadova V, Langerova L, Schneiderova M, Liska V, Levy M, Veskrnova V, Spicak J, Opattova A, Jiraskova K, Vymetalkova V, Vodicka P, Slyskova J. Carcinogenesis. 2018 Dec 13;39(11):1359-1367. doi: 10.1093/carcin/bgy100.

2017 Tumor-initiating cells of breast and prostate origin show alterations in the expression of genes related to iron metabolism. Rychtarcikova Z, Lettlova S, Tomkova V, Korenkova V, Langerova L, Simonova E, Zjablovskaja P, Alberich-Jorda M, Neuzil J, Truksa J. Oncotarget. 2017 Jan 24;8(4):6376-6398. doi: 10.18632/oncotarget.14093.

2016 The focus on sample quality: Influence of colon tissue collection on reliability of qPCR data. Korenkova V, Slyskova J, Novosadova V, Pizzamiglio S, Langerova L, Bjorkman J, Vycital O, Liska V, Levy M, Veskrna K, Vodicka P, Vodickova L, Kubista M, Verderio P. Sci Rep. 2016 Jul 7;6:29023. doi: 10.1038/srep29023.

Consulting complete analysis workflow. We guide you from sampling process, assay design and validation up to final data processing and result evaluation.

GeneCore realizes various range of projects. It starts from initial experiments to complex genomic studies. One gene analyses or whole genome expression profiling give us wide range of capabilities for academic, biotech and pharmaceutical partners.

State-of-art instruments allow us reach the requested results effectively. Semiautomatic and automatic pipetting systems guarantee highest precision for liquid handling. Credible data can be gain just by reliable laboratory methods.

Lab-on-chip technology in combination with high sensitive qPCR techniques leads to high throughput analyses for complex expression studies. Microfluidic technology by Fluidigm allows of 9216 real-time readouts and apply 96 gene assays simultaneously.

The Centre of Molecular Structure

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CF operators:
Tatsiana Charnavets, Ph.D.
RNDr. Jiří Pavlíček, Ph.D.
RNDr. Petr Pompach, Ph.D.
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Mgr. Pavla Vaňková, Ph.D.

CF Keywords

01	Protein structure
02	Protein function
03	Nucleic acid
04	Protein-ligand interaction
05	Structure-function relationship
06	Protein characterisation
07	X-ray structure analysis
08	Atomic structure
09	Post-translational modification
10	Crystallization of proteins and nucleic acids

CF Services-Summary

01	Biophysical characterization of biomolecules (proteins, nucleic acids) and of their interactions with ligands
02	Three-dimensional structure analysis by macromolecular crystallography, and X-ray diffraction techniques
03	Advanced analysis of molecular structure by mass spectroscopy (intact protein characterization, post-translational modifications, H/D exchange, cross-linking experiments and native electrospray, hydrocarbons and crude oil analysis)

CF Key Equipment

Dynamic light scattering (DLS) technique Zetasizer Nano ZS90 (Malvern Panalytical)
Multi-angle dynamic light scattering (MADLS) technique Zetasizer Ultra (Malvern Panalytical)
Circular dichroism (CD) spectrometer Chirascan Plus (Applied Photophysics)
Isothermal titration calorimeter MicroCal iTC200 (Malvern Panalytical)
Differential scanning calorimeter MicroCal VP-Capillary DSC (Malvern Panalytical)
Monolith microscale label free thermophoresis NT.LabelFree (Nano Temper)
Differential scanning fluorescense (DSF) assay Prometheus NT.48 (Nano Temper)
Surface Plasmon Resonance (SPR) system ProteOn XPR36 (BioRad)
UV/Vis Spectrometer Specord 50 Plus (Analytica Jena)
Monolith microscale thermophoresis (MST) NT.115 (Nano Temper)
Modular fluorescence spectrometer FLS1000 (Edinburgh Instruments)
FTIR spectrometer Vertex 70v (Bruker)
NanoDLS Spectrolight 600 (Molecular Dimensions)
Gryphon Dropsetter (Art Robbins Instruments)
Crystallization hotel Rock Imager 1000 (Formulatrix)
Crystallization hotel for low temperatures Rock Imager 182 (Formulatrix)
Glove box for inter gas with steromiscroscope (GS)
Dropsetter NT8 (Formulatrix)
D8 Venture diffractometer (Bruker)
ISX stage for D8 Venture (Bruker)
HC Lab (Arinax)
SAXSpoint 2.0 (Anton Paar)
AktaGO (GE Healthcare)
15T-SolariX XR FT-ICR mass spectrometer (Bruker Daltonics)
UPLC (Agilent Technologies)
Excimer laser (Coherent)
MALDI-TOF (Bruker Daltonics)
timsTOF Pro mass spectrometer (Bruker Daltonics)

CMS CENTRE OF MOLECULAR STRUCTURE

The Centre of Molecular Structure provides services in three key areas: biophysical characterization of biomolecules (proteins, nucleic acids) and of their interactions with ligands; three-dimensional structure analysis by macromolecular crystallography and X-ray diffraction techniques; and advanced analysis of molecular structure by mass spectrometry. We have state-of-the-art equipment for biophysical characterization enabling a complex protein or nucleic acid analysis, suitable for any molecular biology or structural biology projects. Our individual techniques are provided as full service including analysis or as the provision of experimental time to trained users.

Core Facility Services

The biophysical core research facility offers a range of services, including investigations of biomolecular interactions, structure, stability and conformation of DNA and proteins, hydrodynamic radius, zeta potential and the pre-crystallization screening of sample quality.

The protein and nucleic acid crystallization facility provides a range of necessary technologies and steps necessary for successful macromolecular crystallization and analyzing results, including sample vitrification in liquid nitrogen. The facility invites users to use either a complex approach to target crystallization with the use of all available options or individual access to the facility's equipment.

The X-ray diffraction facility focuses on single crystal X-ray diffraction (XRD) and small angle X-ray scattering (SAXS). XRD can be measured in a range of room and cryo temperatures (80-300K), in crystallization plates without disturbing the original conditions, and crystal quality can be improved using a dehydrator. SAXS can characterize biological molecules and complexes; the experiments are backed up by an autosampler, in-line SEC, and in-situ UV-Vis absorption spectroscopy. Diffraction data processing and a structure solution can be provided on request.

The structural mass spectrometry facility provides new biomolecular mass spectrometry (MS) methods in order to make the characterization of protein structure and dynamics more rapid and routine. Methods include non-denaturing mass spectrometric approaches in combination with hydrogen-deuterium exchange, chemical crosslinking and other labeling techniques together with computational approaches.

Connection to research infrastructures

The Centre of Molecular Structure is part of the Czech Infrastructure for Integrative Structural Biology (CIISB) - www.ciisb.org. The infrastructure belongs to the Instruct-ERIC pan-European research infrastructure in structural biology, making high-end technologies and methods available to all European researchers - www.instruct-eric.org. Access to the Centre of Molecular Structure is possible via proposals submitted through the above websites.

Events

Our centre participates in annual open days of the Institute of Biotechnology of the Czech Academy of Sciences and in regular CIISB user meetings. With financial support from the Instruct-ERIC consortium, the Centre of Molecular Structure organized two international workshops. In April 2018 the focus was on fragment screening using crystallography laboratory equipment and in October 2019 on the integration of computational approaches in structural biology. Additionally, we also organize tutorials aimed at teaching users new available techniques.

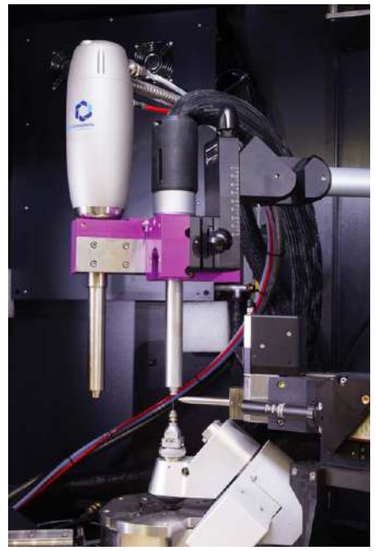
Users´ Highlights:

2020 – Krafcikova, P.; Silhan, J.; Nencka, R.; Boura, E. (2020). Structural analysis of the SARS-CoV-2 methyltransferase complex involved in RNA cap creation bound to sinefungin. Nature Communications, 11, 7.
– Steiningerova, L. et al. (2020). Different Reaction Specificities of F420H2-Dependent Reductases Facilitate Pyrrolobenzodiazepines and Lincomycin To Fit Their Biological Targets. J. Am. Chem. Soc., 142, 3440-3448.
– Kouba, T. et al. (2020). Mycobacterial HelD is a nucleic acids-clearing factor for RNA polymerase. Nature Communications, 11, 1.

2019 – Zeman, J. et al. (2019). Binding of eIF3 in complex with eIF5 and eIF1 to the 40S ribosomal subunit is accompanied by dramatic structural changes. Nucleic Acids Research, 47(15), 8282-8300.
2018 – Sharma, S. et al. (2018). Affinity switching of the LEDGF/p75 IBD interactome is governed by kinase-dependent phosphorylation. Proceedings of the National Academy of Sciences of the United States of America, 115, E7053-E7062.

SAXSpoint 2.0 (Anton Paar) with AktaGo (GE Healthcare)


Glovebox


Crystal environment control for X-ray diffraction (Arinax)

Rock Imager 1000 (Formulatrix)

Structural mass spectrometry core facility is equipped with high-end mass spectrometers including 15T solariX XR, timsToF Pro and MALDI-ToF from Bruker Daltonics

The Czech Centre for Phenogenomics

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Animal facility:	e-mail: ccp-afm@img.cas.cz
Preclinical testing:	e-mail: ccp-pm-preclinics@img.cas.cz

The CCP facilitates the effective usage of genetically-engineered rodent models for biomedical research. Through its partnership with the International Mouse Phenotyping Consortium, it participates in the coordinated effort to generate new mouse models, to provide phenotypic annotations to these mutants through the use of comprehensive and internationally-standardized testing procedures, and finally to ensure their open distribution and secure archiving. Excess capacity associated with these activities can be accessed by local and international research communities through a range of services offered on a cost-recovery basis.

CF Keywords

01	Phenotyping
02	Transgenic technologies
03	Mouse models
04	Preclinical testing

CF Services-Summary

01	Phenotyping of Rodent Models
02	Production, Archiving and Distribution of Mouse Models
03	Animal Housing in the SPF facility

CF Connections

01	EurOPDX, member
02	EOSC-Life, partner
03	INFRAFRONTIER GmbH, founding member
04	IPAD-MD project, EU Horizon 2020 project, partner
05	EMMA (The European Mouse Mutant Archive), partner
06	IMPC (International Mouse Phenotyping Consortium), full member
07	PATHBIO (Precision PathoBiology for Disease Models), Erasmus+Knowledge Alliance, partner

CF Key Equipment

Leica ST5010-CV5030 Integrated Workstation
Automated slide stainer for special stains (Ventana Benchmark Special Stains)
Automated immunohistochemistry and an in-situ hybridization system (Ventana Discovery ULTRA)
Brightfield and fluorescence slide scanner (Carl Zeiss Axio Scan.Z1)
In vivo micro-CTs (Bruker Skyscan 1176 & Skyscan 1278)
Optical and X-ray small animal imaging system (Bruker In-vivo Extreme)
Biochemistry analyzer (Beckman Coulter AU480)
In vivo respiratory mechanics (Scireq Flexivent)
ex vivo Bruker Skyscan 1272
High frequency ultrasound (VisualSonics Vevo 2100)
Non-invasive blood pressure (Kent Scientific, CODA High Throughput)
Home cage metabolic monitoring (TSE Systems Phenomaster)
Home cage behavior monitoring (TSE Systems Intellicage)
RETIanimal Electrophysiological Test Unit
Optical coherence tomography Heidelberg Engineering Spectralis
Tucker-Davis Technologies System 6 for ABR in custom-made sound booth
Advanced whole body imaging system Lago X by Spectral Instruments Imaging
Agilent 6546 LC/qTOF

About

Phenotyping

The Phenotyping Module houses a comprehensive collection of tools for the physiological and morphological assessment of experimental mice and rats in a controlled SPF (specific pathogen-free) environment. Our experienced staff offers a wide variety of standardized tests and services, including IMPReSS (International Mouse Phenotyping Resource of Standardised Screens) and phenotyping pipelines. Specific services can be requested through the different units comprising the phenotyping module: Biochemistry and Hematology, Bioimaging, Cardiovascular, Embryology, Vision, Hearing and Electrophysiology, Histopathology, Immunology, Lung function, Metabolism, Neurobiology and Behavior, Metabolomics, PDX and Cancer Models, Biostatistics/Bioinformatics.

Transgenic models and archiving

The Transgenic and Archiving Module combines mouse model generation, archiving and distribution. We provide consultation and assistance services, information on the design as well as the use of genetically modified transgenic mice. Services that can be requested include: Pronuclear Microinjection of DNA Constructs into Mouse Zygotes, Microinjection of Targeted ES Cell Lines, Mouse Archiving, Recovery of Live Mice from Cryopreserved, Embryos and Sperm, Analysis of Sperm Viability, Rederivation of Mouse Strains and Lines.

Animal facility

The Animal Facility Module is based on the latest advances in the housing, breeding, and care of laboratory mice and rats. It houses animals in state-of-the-art individually ventilated cages (IVC) or digitally ventilated cages (DVC) improving the level of animal welfare and animal facility efficiency, in accordance with the highest world standards for laboratory animals and in compliance with EU legislation. Services include: Housing and Husbandry, Colony Management, Technical and Experimental service, Health Monitoring, Import and Export of Animals, Quarantine and Training, Personnel Training, Project Licenses Administration.

Preclinical testing

The primary mission of the Centre for Preclinical Testing (CPT) is to perform preclinical testing of substances that have successfully passed through basic research, and to contribute towards the development of new pharmaceuticals to combat life-threatening diseases. Services that can be requested include: Toxicity Studies, Bioanalytical, Hematological and Biochemical Testing, Development and Validation of Bioanalytical Methods, Determination of Metabolites in Tissues and Biological Matrices, Histopathological Evaluation of Tissues, Pharmacological Studies on Xenografts, Cardiology Diagnostic Tests on Animal Models Synthesis, Characterization and Certification ofChemical Substances, Development of Formulations for Drug Applications.

Potential for Collaboration

The infrastructure is able to provide prospective collaborators with complex services related to the generation of custom-designed animal models and/or running of various customised animal model based experiments in standardised and reproducible settings. Our preclinical testing platform offers tests in a certified GLP mode.

Users´ Highlights:

2020 Jenickova I, Kasparek P, Petrezselyova S, Elias J, Prochazka J, Kopkanova J, Navratil M, Barinka C, Sedlacek R. Efficient allele conversion in mouse zygotes and primary cells based on electroporation of Cre protein. Methods. 2020 Jul 24:S1046-2023(20)30114-6. doi: 10.1016/j.ymeth.2020.07.005.

2020 Vobořil M, Brabec T, Dobeš J, Šplíchalová I, Březina J, Čepková A, Dobešová M, Aidarova A, Kubovčiak J, Tsyklauri O, Štěpánek O, Beneš V, Sedláček R, Klein L, Kolář M, Filipp D: Toll-like receptor signaling in thymic epitheliumcontrols monocyte-derived dendritic cellrecruitment and Treg generation: Nat Commun. 2020 May 12;11(1):2361. doi: 10.1038/s41467-020-16081-3.1

2020 Danek P, Kardosova M, Janeckova L, Karkoulia E, Vanickova K, Fabisik M, Lozano Asencio C, Benoukraf T, Tirado-Magallanes R, Zhou Q, Burocziova M, Rahmatova S, Pytlik R, Brdicka T, Tenen DG, Korinek V, Alberich Jorda M: β-catenin-TCF/LEF signaling promotes steady-state and emergency granulopoiesis via G-CSF receptor upregulation. Blood 2020 Nov 26;136(22):2574-2587. doi: 10.1182/blood.2019004664.

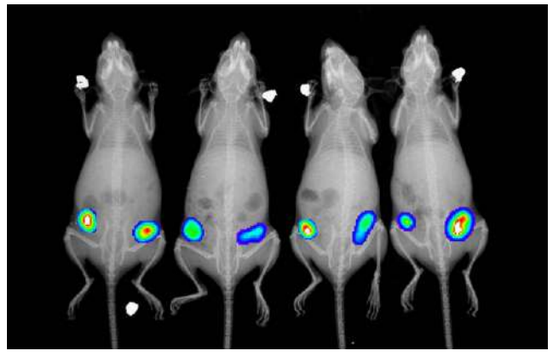
2019 Balounová J, Šplíchalová I, Dobešová M, Kolář M, Fišer K, Procházka J, Sedlacek R, Jurisicova A, Sung HK, Kořínek V, Alberich-Jorda M, Godin I, Filipp D. Toll-like receptor 2 expression on c-kit+ cells tracks the emergence of embryonic definitive hematopoietic progenitors. Nat Commun. 2019 Nov 15;10(1):5176. doi: 10.1038/s41467-019-13150-0.

2019 Kaiser K, Gyllborg D, Procházka J, Salašová A, Kompaníková P, Molina FL, Laguna-Goya R, Radaszkiewicz T, Harnoš J, Procházková M, Potěšil D, Barker RA, Casado ÁG, Zdráhal Z, Sedláček R, Arenas E, Villaescusa JC, Bryja V. WNT5A is transported via lipoprotein particles in the cerebrospinal fluid to regulate hindbrain morphogenesis. Nat Commun. 2019 Apr 2;10(1):1498. doi: 10.1038/s41467-019-09298-4.

2019 Channabasavaiah B. Gurumurthy et al. Reproducibility of CRISPR-Cas9 methods for generation of conditional mouse alleles: a multi-center evaluation Genome Biol 2019 Aug 26;20(1):171. doi: 10.1186/s13059-019-1776-2

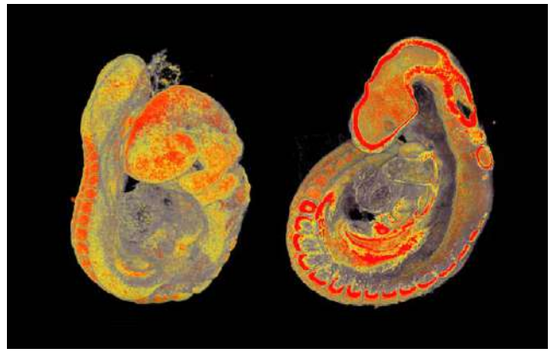
Auditory brainstem response test on mice

Virtual-reality system to rapidly quantify visuomotor behaviour



Visualization of microinvansive approach mammary fat pad xenografts

3D Image analysis Mouse skull



High resolution microCT imaging mouse embryo

Imaging Methods

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IMCF at BIOCEV (imcf.natur.cuni.cz), operated by the Biological Section of the Faculty of Science, Charles University, offers open-access services in the fields of light (mainly fluorescence) and electron microscopy, flow cytometry, and image data analysis. The team members provide consulting services on optimal experimental design and sample preparation, training in selected methods and devices. IMCF also offers assistance in data collection, data analysis, evaluation and interpretation, sample preparation and measurement as a service for complex methods. Another activity of the core facility consists of user-inspired development and implementation of new imaging methods which subsequently expand the portfolio of methods available to all users. The strength of our facility lies mainly in the application, implementation, and adaptation of advanced optical and electron microscopy techniques, especially super-resolution, functional (FLIM, FCS) and label-free imaging, FIB-SEM and CLEM approaches.

CF Services-Summary

01	Light microscopy
02	Electron microscopy
03	Flow cytometry
04	Image data analysis
05	Courses and teaching

CF Keywords

01	FIB-SEM
02	TEM
03	CLEM
04	Confocal
05	Two-photon
06	CARS
07	Super-resolution
08	STED
09	SIM
10	SMLM
11	FLIM
12	FCS
13	TIRF
14	Live cell
15	FACS
16	HPF

CF Key Equipment

FEI Helios NanoLab 660 G3 UC
JEOL JEM 2100-Plus 200kV
Abberior Instruments STED
Carl Zeiss LSM 880 NLO
Leica TCS SP8 WLL SMD-FLIM
Nikon N-SIM & N-STORM
Nikon Ti-E H-TIRF
Nikon Spinning Disk
Nanolive 3D Cell Explorer

CF Services

01	Light microscopy		Two-photon deep intravital microscopy including SHG, THG and CARS
			Laser scanning and spinning disc confocal microscopy
			3D super-resolution microscopy (STED, SMLM, SIM)
			Automated wide-field microscopy including TIRF
			Functional imaging (spectral, FLIM, FCS)
			Live cell imaging
02	Electron microscopy	In electron microscopy, we offer:	Correlative light and electron microscopy (CLEM)
			High-resolution (cryo-) SEM
			Elemental analysis (EDS)
			(cryo-) TEM 200kV
			(cryo-) Electron Tomography (ET)
			Single Particle Analysis (SPA) screening
			3D FIB-SEM
		A wide range of sample preparation methods:	High pressure freezing (HPF) or plunge-freezing followed by freeze-substitution (AFS)
			(cryo-) ultrathin sectioning
			Critical point drying
			Immunolabeling
03	Flow cytometry		Assistance with complex experiment design and data analysis (Kaluza, FlowJo)
			Flow cytometry cell counting
			Cell sorting
04	Data analysis		Access to commercial software (Amira, Imaris, Huygens, NIS-Elements)
			Assistance with image data analysis
			Access to dedicated computers

Training and teaching

Apart from standard user training, IMCF organizes several advanced practical courses. The regular courses include Single Molecule Microscopy and Manipulation, FLIM for more than just for biologists, 3D-CLEM and IR laser show for cells. In addition, we participate in Charles University courses Seeing is Believing I and II and Microscopy Technique.

Membership in large research infrastructures networks

IMCF at BIOCEV is a part of the Prague node of the Euro-BioImaging ERIC (www.eurobioimaging.eu), which grants open-access services and training to a broad range of state-of-the-art biological and medical imaging technologies. We also participate in the Czech-BioImaging national large research infrastructure for biological and medical imaging (www.czech-bioimaging.cz) that provides open access to a wide range of imaging technologies and expertise to all scientists in the Czech Republic and from abroad through a unified and coordinated logistics approach.

The Teams' Publications:

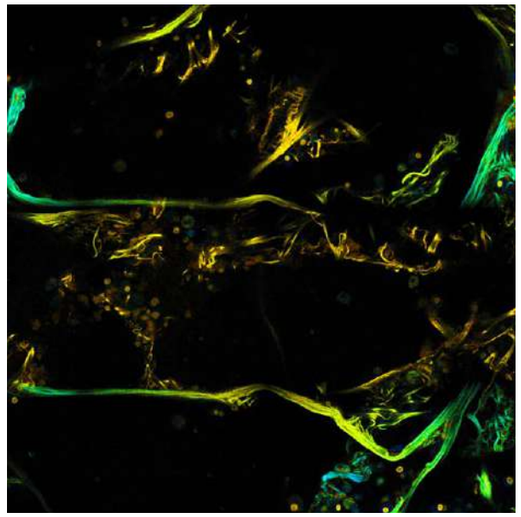
2020 Špaček, M. et al. Comparison of Different Thawing Protocols in Human Cryopreserved Venous Grafts. Ann. Vasc. Surg. 64, 347–354 (2020).

2019 Bajzikova, M. et al. Reactivation of Dihydroorotate Dehydrogenase-Driven Pyrimidine Biosynthesis Restores Tumor Growth of Respiration-Deficient Cancer Cells. Cell Metab. 29, 1–18 (2019).

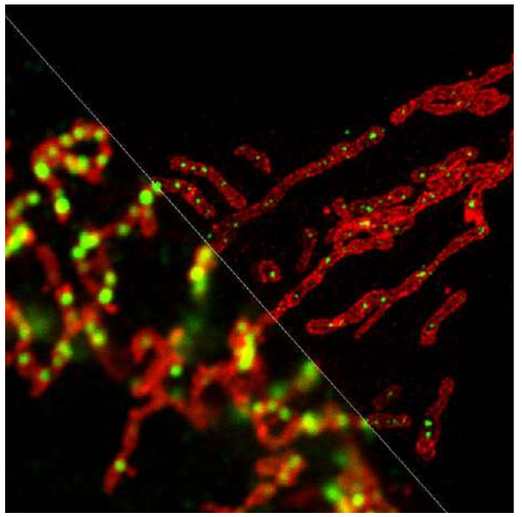
2018 Nicovich, P. R., Kwiatek, J. M., Ma, Y., Benda, A. & Gaus, K. FSCS Reveals the Complexity of Lipid Domain Dynamics in the Plasma Membrane of Live Cells. Biophys. J. 114, 2855–2864 (2018).

2017 Danko, M., Hrdlovič, P., Martinická, A., Benda, A. & Cigáň, M. Spectral properties of ionic benzotristhiazole based donor-acceptor NLO-phores in polymer matrices and their one- and two-photon cellular imaging ability. Photochem. Photobiol. Sci. 16, (2017).

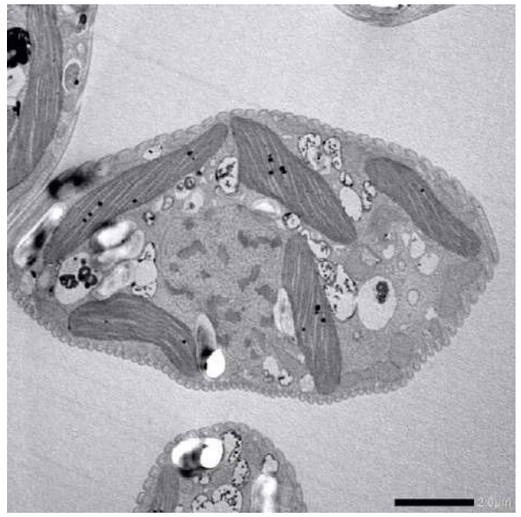
2017 Kostrouchová, M. et al. The nematode homologue of Mediator complex subunit 28, F28F8.5, is a critical regulator of C. elegans development. PeerJ 5, (2017).



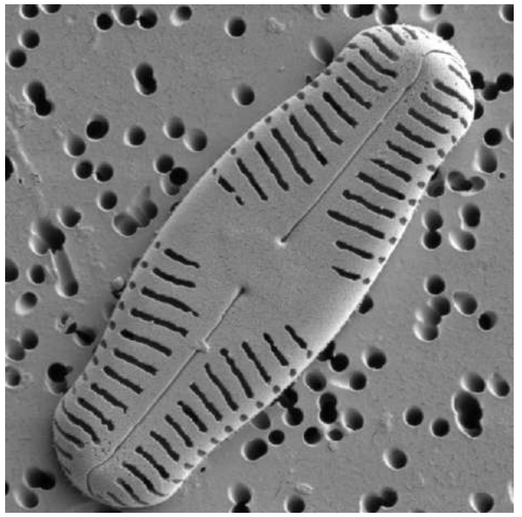
In-vitro two-photon-excited Fluorescence Lifetime Image (FLIM) of healthy lung tissue. Recorded using Carl Zeiss LSM880. Color coding represents fluorescence lifetime: ranging from blue - shorter lifetime – to red – longer lifetime.



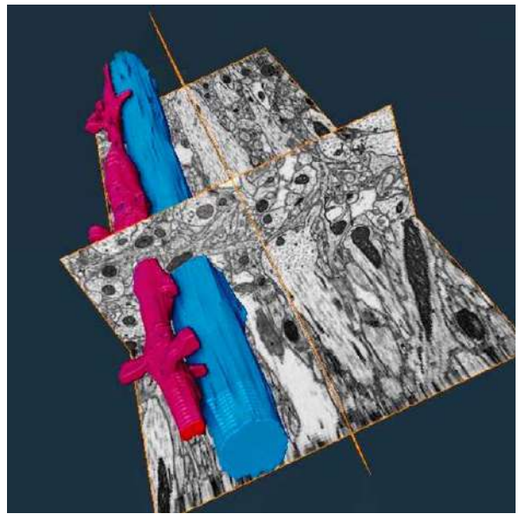
Confocal and super-resolution image of mitochondria (red) and nucleoids (green). Images were obtained using the STED method on the Abberior Instruments Expert Line microscope in cooperation with Jaromira Kovarova, IBT CAS.



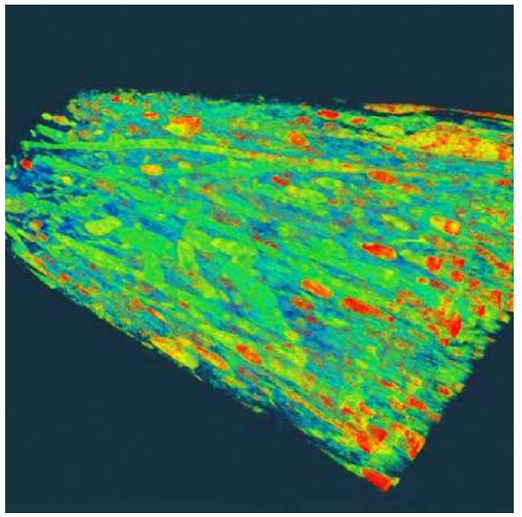
Ultrastructure of Euglena gracilis (microalgae). Samples were prepared by aldehyde-osmium fixation, Spurr's resin embedding and 80nm sections were post-contrasted with uranyl acetate and lead citrate. TEM images were obtained at 120kV using TEM JEM 2100-Plus (scale bar = 2 µm).



SEM image of benthic pennate diatom Chamaepinnularia krookiformis obtained using Dual-beam FEI Helios Nanolab 660 G3 UC at 2 kV and 0.2 nA. The sample was treated with inorganic acids.

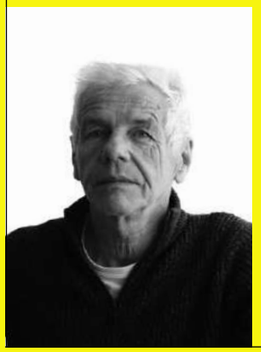


3D visualization of axon and dendrites (neurons in mouse hypothalamus). The datasets were post-processed with Amira Software 2020.2 including denoising.



3D organization of part of the mouse brain - hypothalamus. The datasets were post-processed with Amira Software 2020.2 including denoising.

Media Preparation and Washing Units



Richard Kyselý, Head of the Core Facility

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The unit offers washing of laboratory glass and plastic, provides central washing of work clothing, GMO waste decontamination and elimination of hazardous waste. The media preparation unit offers the preparation of cultivation media and solutions for tissue cultures, the preparation of bacteriological media and plates, and the preparation of “custom-made” solutions. Furthermore, the unit offers vapour sterilization of solutions and vapour or hot-air sterilization of materials, as well as dry ice supplies. The space is divided into two parts, the “dirty” corridor, where items for decontamination should be deposited, and the “clean” part, where the sterile and decontaminated items are returned to the users.

CF Services-Summary

01	Washing unit
	Washing of laboratory plastic resistant to temperatures around 100°C
	Vapour glass and plastic sterilization at 120°C and 1 atm overpressure
	Automatic washing of laboratory glass up to length/height of 50 cm
	Packaging of glass and plastic into 30 µm AL foil
	Hot-air glass and plastic sterilization at 180°C
02	The media preparation unit
	Preparation of media for tissue cultures from supplied powder media
	Preparation of solutions from chemicals provided by the laboratory
	Preparation of bacteriological media and plates
	Preparation of tissue culture solutions
	Sterilization of solutions and materials
	Sterile filtration of solutions
03	Other offered services
	Decontamination of GMO waste and other infectious waste, waste export and records
	Elimination of hazardous compounds according to the Contract on provision of services related to the waste elimination
	Organization of working clothing washing or decontamination
	Regular supplies of dry ice

CF Keywords

01	Glass and plastic washing
02	Sterilization of solutions and materials decontamination of gmo and hazardous waste
03	Preparation of tissue culture media
04	Preparation of bacteriology media and plates

CF Key Equipment

Hot-air sterilizer VENTICELL 707 - komfort
Washing and disinfectant automated instrument MIELE PG8528 EL AV
Osmomat GONOTEC 3000 Basic
Vapour sterilizers STERIVAP HP IL 9618
Water processing - Milli Q A+
Abberior Instruments STED

Core Facility Services:

The washing unit offers the following services:, automatic washing of laboratory glass in a cleaning and disinfectant machine up to a length/height of 50 cm (except for pipettes, tubes, Pasteur pipettes), washing of laboratory plastic resistant to temperatures around 100°C, vapour glass and plastic sterilization at 120°C and 1 atmosphere overpressure, hot-air glass and plastic sterilization at 180°C and packaging of glass and plastic into 30 µm aluminium foil.

The media preparation unit offers: the preparation of media for tissue cultures from supplied powder media, the preparation of tissue culture solutions (TC H2O, PBS 1x, PBS 10x, EDTA 0.02% in PBS, trypsin 0.5% in PBS, glutamine 3%, NEA 100x, HBSS 1x with Ca, Mg, HBSS 1x without Ca, Mg, and other), the prepartion of bacteriological media and plates (LB, plates with ampicillin, kanamycin, without ATB or with ATB supplied according to requirements), sterile filtration of solutions, sterilization of solutions and materials and the preparation of solutions from chemicals provided by the laboratory, measurements of osmolality, pH adjustment, and the preparation of ATB-containing plates according to laboratory specifications.

We also have other offered services, including the, decontamination of GMO waste and other infectious waste, waste export and recording,, the elimination of hazardous compounds according to the Contract on the provision of services related to the waste elimination (infectious waste, liquid and solid GMO waste, liquid and solid chemicals, oil and fat trapping), the organization of work clothing washing or decontamination, commercially supplied media, and products byrequirement and agreement.

Washing and disinfectant automated instrument MIELE PG8528 EL AV

Washing and disinfectant automated instrument MIELE PG8528 EL AV

Washing and disinfectant automated instrument MIELE PG8528 EL AV

Cryotechnologies and Biobank

Mgr. Dobromila Kumpoštová, Head of the Core Facility

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Operation of the cryobank for the long-term storage of samples in liquid nitrogen started in March 2016 and the cryobank is divided into two parts. The first part is situated in the main building of the BIOCEV Centre and is mainly intended for storing cell lines and hybridomas. The second part is located in building SO-002 as a component of the transgenic and archiving module. This part of the cryobank mainly serves for the preservation of mouse sperm and mouse embryos in liquid nitrogen or its vapours.

The storage containers (LABS40K, LABS80K – Taylor-Wharton and 24K) are connected to an external reservoir for liquid nitrogen with a capacity of 10,000 litres and are refilled automatically. The cryobank also includes three filling sites providing the possibility to draw liquid nitrogen into both pressure and non-pressure containers. The entire cryobank system is connected to a back-up power supply in case of a power outage. All operations, diagnostics and checking of the liquid nitrogen levels in the storage containers are fully automated and controlled. The parameters (temperature, humidity, O2 concentration) and safety in both the cryobank and the storage containers themselves are controlled by a monitoring system connected to a GSM network and a web interface.

The cryobank core facility also administers the production of dry ice.

CF Keywords

01	Long therm storage
02	Liquid nitrogen

CF Services-Summary

01	Access to the cryobank via chips and allocation of authorizations for handling DW canisters or manipulations with liquid nitrogen at the refilling (“tapping”) sites
02	Regular checks, maintenance, and supplies of liquid nitrogen
03	Control and administration of the entire cryobank system
04	Training of employees handling liquid nitrogen
05	Dry ice supplies

CF Key Equipment

Storage containers (DW canisters) Taylor Wharton LABS 40K and 80K
Storage containers (DW canisters) Series K - 24K
Dry ice maker

cryobank equipment

cryobank equipment

cryobank equipment

cryobank equipment

OMICS Genomics

RNDr. Štěpánka Hrdá, Ph.D., Head of the Core Facility

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Core facility OMICS - Genomics provides genomic services including Next generation sequencing, Sanger sequencing and fragment analysis. The laboratory has multiple platforms available for DNA sequencing, aiming at providing the best and most up-to-date combination of sequencing approaches for the varying needs of our customers. Service is provided for academic and nonacademic users from the Czech Republic and abroad.

CF Keywords

01	Next generation sequencing
02	Capillary sequencing, Sanger
03	Sequencing, library preparation
04	DNA shearing for next-gen sequencing
05	DNA and RNA quality control

CF Services-Summary

01	Next Generation Sequencing (Massively parallel sequencing) on the Illumina MiSeq platform (RNA-Seq, de novo sequencing, targeted resequencing, pilot projects, etc.)
02	Library preparation for NGS as a service (to a limited extent), training and assistance in the preparation of your libraries in our premises with complete equipment
03	Quality and quantity control of DNA, RNA and ready-to-run sequencing libraries by fluorometer (Quantus), bioanalyzer (Agilent) and qPCR (Roche LightCycler 480)
04	Capillary (Sanger) sequencing - single read sequencing service for plasmids and PCR fragments
05	Fragment analysis (microsatellite analysis, SNP genotyping, AFLP etc.)

CF Key Equipment

MiSeq, Illumina
3500 Genetic Analyzer, Life Technologies
3130xl Genetic Analyzer, Life Technologies
Fluorometr Quantus, Promega
LightCycler 480 Real-TimePCR, Roche
Covaris M220
Axon GenePix 4000B Microarray Scanner
Bioanalyzer 2100, Agilent
Nanodrop 2000 UV-Vis spectrophotometer

About

Core Facility Services

CF Genomics BIOCEV provides a broad array of instrumentation and services. We have more than fifteen years of experience with Sanger sequencing of plasmids and PCR products and fragment analysis (e.g. Amplified Fragment Length Polymorphism mapping, microsatellite STR analysis, SNP genotyping) on the Life Technologies ABI 3500 capillary array sequencing platform. We have gained experience with Next Generation Sequencing on the Illumina MiSeq and Oxford Nanopor platforms over the last 5 years. Free consultation on project considerations and experimental design is the first obvious step. Our facility offers library preparation from various sample types as well as training, assistance and consultation in the case of custom library preparation. Our facility has all required equipment for performing quality and quantity control of incoming material and prepared libraries. The Illumina Miseq platform is used for processing suitably large projects as well as performing DNA library quality control before an Illumina HiSeq run or other diverse pilot projects. Our staff has experience with RNA sequencing (gene expression, transcriptome sequencing), small genome de novo sequencing and resequencing, amplicon, RAD-Seq, Hyb-Seq and Chip-Seq sequencing and also 16S metagenomics.

Potential for collaborations

Sanger sequencing (formerly the main scope of work of the laboratory) now makes up about one-half of our work. The laboratory processes around 30 thousand samples from over 100 research teams per year mainly for the academic staff of Charles University as well as for other academy institutes or the private sector. The second half of the facility's business currently is the Next-generation sequencing. We provide our services (quality and quantity control, library construction, Miseq sequencing) to about 50 customers annually. We offer training and assistance in the preparation of your own project.

Users´ Highlights:

2020 Tretyachenko V, Voráček V, Souček R, Fujishima K, Hlouchová K. CoLiDe: Combinatorial Library Design tool for probing protein sequence space. Bioinformatics. 2020 Sep 21:btaa804. doi: 10.1093/bioinformatics/btaa804. Epub ahead of print. PMID: 32956450.

2019 Jones KE, Fér T, Schmickl RE, Dikow RB, Funk VA, Herrando-Moraira S, Johnston PR, Kilian N, Siniscalchi CM, Susanna A, Slovák M, Thapa R, Watson LE, Mandel JR. An empirical assessment of a single family-wide hybrid capture locus set at multiple evolutionary timescales in Asteraceae. Appl Plant Sci. 2019 Oct 25;7(10):e11295. doi: 10.1002/aps3.11295. PMID: 31667023; PMCID: PMC6814182.

2019 Treitli SC, Kolisko M, Husník F, Keeling PJ, Hampl V. Revealing the metabolic capacity of Streblomastix strix and its bacterial symbionts using single-cell metagenomics. Proc Natl Acad Sci U S A. 2019 Sep 24;116(39):19675-19684. doi: 10.1073/pnas.1910793116. Epub 2019 Sep 6. PMID: 31492817; PMCID: PMC6765251.

2018 Carlsen MM, Fér T, Schmickl R, Leong-Škorničková J, Newman M, Kress WJ. Resolving the rapid plant radiation of early diverging lineages in the tropical Zingiberales: Pushing the limits of genomic data. Mol Phylogenet Evol. 2018 Nov;128:55-68. doi: 10.1016/j.ympev.2018.07.020. Epub 2018 Jul 29. PMID: 30063997.

2017 Polgarova K, Vargova K, Kulvait V, Dusilkova N, Minarik L, Zemanova Z, Pesta M, Jonasova A, Stopka T. Somatic mutation dynamics in MDS patients treated with azacitidine indicate clonal selection in patients-responders. Oncotarget. 2017 Dec 6;8(67):111966-111978. doi: 10.18632/oncotarget.22957. PMID: 29340104; PMCID: PMC5762372.

Covaris M220. This focused ultrasonicator is designed for Next Gen Sequencing applications requiring fragment sizes between 150bp and 5kb. It is used to mechanically shear nucleic acids into smaller fragments by focused ultrasonic waves and is a recommended instrument in Illumina protocols. MicroTUBE (50ul) and miniTUBE (130 ul) are available at CF.

Illumina MiSeq - High-throughput massive parallel sequencer. This sequencer allows to access more focused applications such as targeted gene sequencing, RNA-Seq, metagenomics, small genome sequencing, amplicon sequencing and others. MiSeq reagents enable up to 15 Gb of output with 25 M sequencing reads and 2 x 300 bp read lengths. Several types of kits for library preparation are available at CF.

Applied Biosystems 3500 Genetic Analyzer. This sequencer is fluorescence-based DNA analysis instrument using capillary electrophoresis technology with 24 capillaries. Analyzer is fully automated, from sample loading to primary data analysis, for DNA sequencing and fragment analysis. CF provides the sample preparation service for standard samples as well as for difficult templates.

Agilent 2100 Bioanalyzer. This instrument provides a complete solution for DNA/RNA quality control of limiting amounts of sample on the chips. The micro-capillary electrophoresis-based technology provides high quality digital reporting of size range and quantity on a single platform. Bioanalyzer system has the resolution and power to quantify a few picograms with as little as a couple of microliters of sample. Several kits are available at CF.

Proteomics and Metabolomics

Karel Harant, Head of the Core Facility

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Our facility provides complex analyses in the fields of Proteomics and Metabolomics including sample preparation, data acquisition, processing, and evaluation. We are equipped with hi-end instrumentation capable of providing separation in gas and nano/standard flow liquid phases hyphenated with a mass spectrometer.

CF Keywords

01	Proteomics
02	Metabolomics
03	Mass spectrometry
04	Gas chromatography
05	Liquid chromatography

CF Services-Summary

A	Proteomics	
	01	Untargeted comparative analysis of complex samples
	02	Detection of interaction partners isolated by immunoprecipitation
	03	Targeted detection and quantification of selected proteins
	04	Phosphoproteomic profiling
B	Metabolomics	
	01	Targeted and untargeted analysis of volatile and non-volatile metabolites
	02	Automatic sampling of volatiles using SPME or dynamic headspace
	03	Various derivatization techniques that improve the analytical properties of the metabolites (silylation, oximation, transesterification, etc.)

CF Key Instruments

Orbitrap Fusion (Thermo Scientific)
TSQ Quantiva (Thermo Scientific)
Pegasus® 4D GCxGC-TOFMS (Leco Corporation)
MultiPurpose Sampler MPS (Gerstel)
LC Dionex Ultimate 3000 (Thermo Scientific)
Nano-LC Dionex Ultimate 3000 RSLCnano (Thermo Scientific)
Low flow fraction collector – Spider (produced in-house)
High Flow fraction collector (produced in-house)
Bioruptor pico - sonication bath (Diagenode)

CF Key Software

Proteome Discoverer
Compound Discoverer
TraceFinder
MaxQuant
Perseus
Skyline
GNPS
SIRIUS
Cytoscape
MS2LDA
Mzmine
ChromaTOF
Maestro

About

Proteomics Core Facility Services

Proteomics is an interdisciplinary domain for the detection and quantification of proteins by mass spectrometry. One of the major applications is an untargeted comparative analysis of complex samples. Typical mammalian whole-cell lysate allows for the detection and quantification of roughly 5 000 proteins in one sample. The detection and quantification of immunoprecipitated proteins to find their interaction partners is another routinely performed type of analysis. Proteomics is also suitable for the detection of various post-translational modifications such as phosphorylations, acetylations, etc.

We also offer phosphoproteomic profiling of complex samples, which includes affinity enrichment of phosphopeptides and their analysis by mass spectrometry resulting in quantitative information about roughly 5 000 – 8 000 phosphosites (depending on species, tissue, etc.).

A targeted approach is suitable when a precise relative, or absolute quantification is needed. One of the routine applications is the absolute quantification of 125 human plasma proteins. However, most targeted applications are usually developed on request.

Our services include a consultation to optimize your experimental design to be compatible with MS analysis, sample preparation, identification and quantification of proteins, data analysis, and a basic statistical evaluation. We can prepare a sample for MS analysis from a wide variety of biological samples including tissues (liver, kidney, brain, muscle, blood cells, spinal cord), cell lines (mammalian, yeast, bacterial, plant), body fluids (plasma, urine, saliva, tears), etc.

Metabolomics Core Facility Services

The field of metabolomics combines strategies to identify and quantify metabolites from biological materials using sophisticated analytical techniques and the application of software tools for data extraction and interpretation.

Our facility provides services for analyses of small molecules and metabolites using chromatographic and mass spectrometric-based instrumentation in a variety of biological matrices. We apply an individual approach to our customers to meet their specific requirements. We recognize two basic analytical approaches:

01 Targeted Profiling

The targeted approach involves the detection and relative or absolute quantification of specific compounds. This approach requires possession of analytical standards of the particular analytes. In the case of absolute quantification isotopically labeled standards are necessary.

02 Untargeted Profiling

The goal of an untargeted analysis is to detect and identify as many metabolites as possible within a specified sample preparation and analytical techniques employed. To get such a comprehensive analysis, a combination of various analytical approaches regarding separation and detection is applied. Furthermore, we offer data processing and basic statistical evaluation.

Users´ Highlights:

2021 Dienstbier A, Amman F, Petráčková D, Štipl D, Čapek J, Zavadilová J, Fabiánová K, Držmíšek J, Kumar D, Wildung M, Pouchnik D, Večerek B. (2021); Comparative Omics Analysis of Historic and Recent Isolates of Bordetella pertussis and Effects of Genome Rearrangements on Evolution.; Emerg Infect Dis; doi.org/10.3201/eid2701.191541

2020 Maršíková J, Pavlíčková M, Wilkinson D, Váchová L, Hlaváček O, Hatáková L, Palková Z (2020); The Whi2p-Psr1p/Psr2p complex regulates interference competition and expansion of cells with competitive advantage in yeast colonies.; PNAS; doi.org/10.1073/pnas.1922076117

2020 Le T, Žárský V, Nývltová E, Rada P, Harant K, Vancová M, Verner Z, Hrdý I, Tachezy J. (2020); Anaerobic peroxisomes in Mastigamoeba balamuthi; PNAS; doi: 10.1073/pnas.1909755117

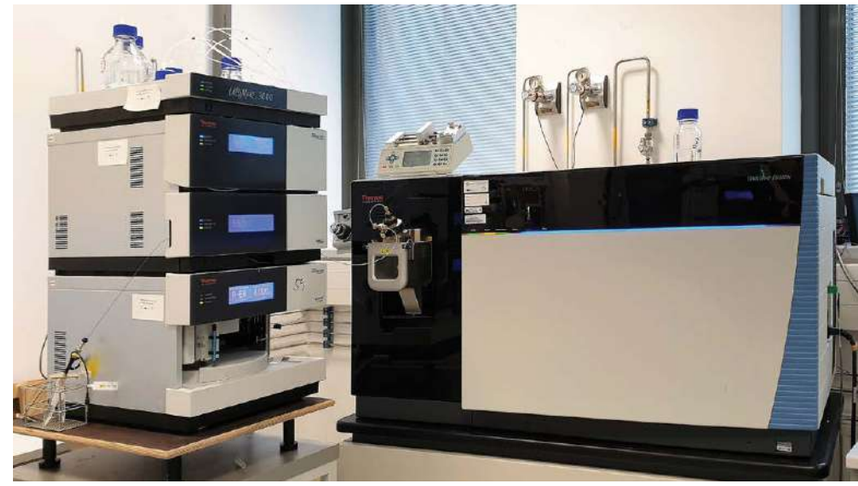
2020 Androvic P, Kirdajova D, Tureckova J, Zucha D, Rohlova E, Abaffy P, Kriska J, Valny M, Anderova M, Kubista M, Valihrach L (2020); Decoding the Transcriptional Response to Ischemic Stroke in Young and Aged Mouse Brain.; Cell Reports; doi.org/10.1016/j.celrep.2020.107777

2020 Paluchova V; Oseeva M; Brezinova M; et al.; Lipokine 5-PAHSA Is Regulated by Adipose Triglyceride Lipase and Primes Adipocytes for De Novo Lipogenesis in Mice (2020); DIABETES; doi.org/10.2337/db19-0494

2020 Čermák V, Gandalovičová A, Merta L, Harant K, Rösel D, Brábek J. (2020); High-throughput transcriptomic and proteomic profiling of mesenchymal-amoeboid transition in 3D collagen.; Sci Data; doi.org/10.1038/s41597-020-0499-2

2019 Makki A, Rada P, Žárský V, Kereiche S, Kovacik L, Novotny M, Jores T, Rapaport D, Tachezy J (2019); Triplet-pore structure of a highly divergent TOM complex of hydrogenosomes in Trichomonas vaginalis.; PLoS Biology; doi.org/10.1016/j.saa.2019.03.056

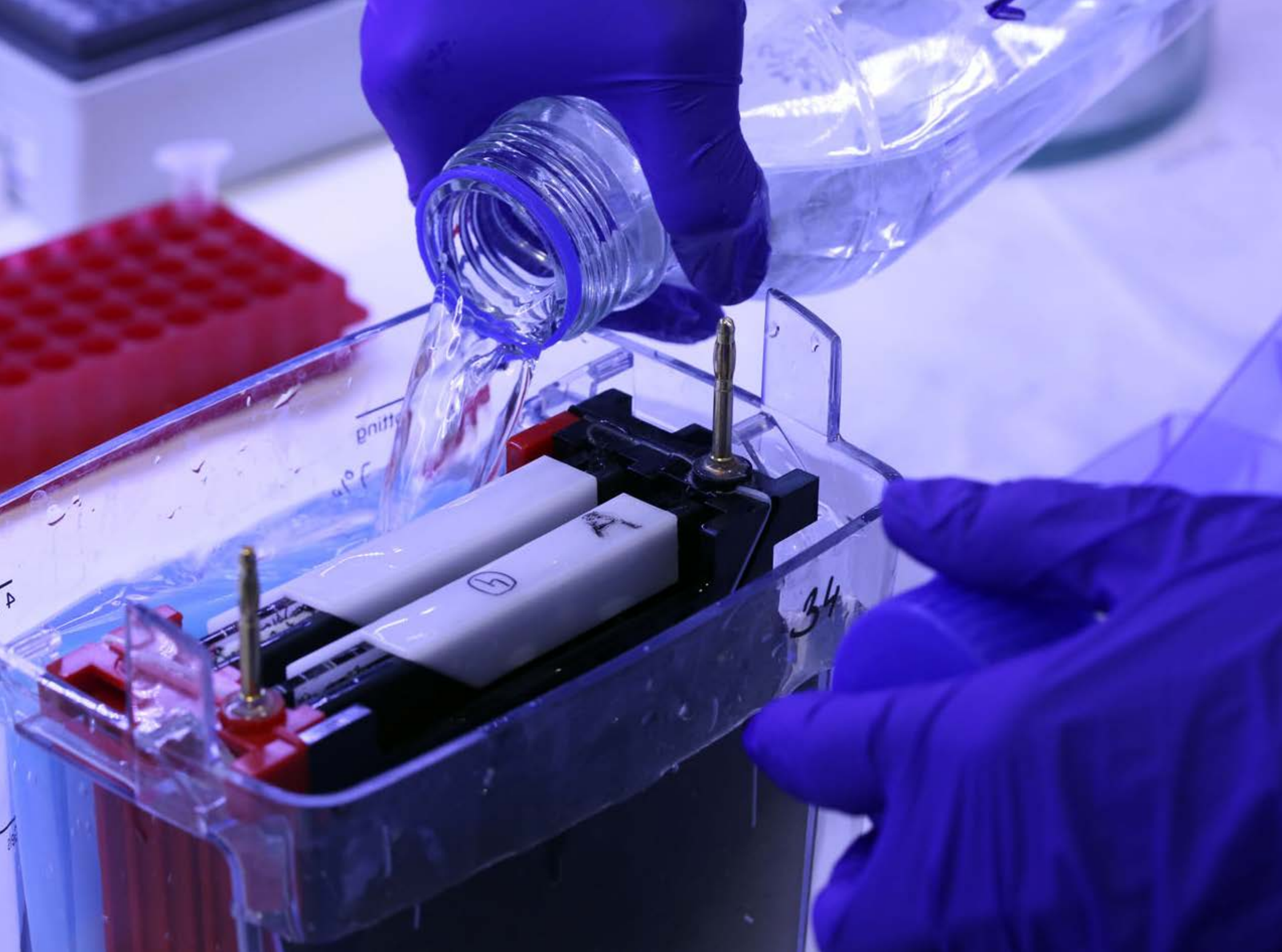
2019 Paluchova V, Oseeva M, Brezinova M, Cajka T, Bardova K, Adamcova K, Zacek P, Brejchova K, Balas L, Chodounska H, Kudova E, Schreiber R, Zechner R, Durand T, Rossmeisl M, Abumrad NA, Kopecky J, Kuda O (2019); Lipokine 5-PAHSA is Regulated by Adipose Triglyceride Lipase and Primes Adipocytes for de novo Lipogenesis in Mice; DIABETES; doi.org/10.2337/db19-0494



Orbitrap Fusion coupled with Nano-LC Dionex Ultimate 3000 (Thermo Scientific)

TSQ Quantiva coupled with LC Dionex Ultimate 3000 (Thermo Scientific)

Pegasus® 4D GCxGC-TOFMS (Leco Corporation) coupled with MultiPurpose Sampler MPS (Gerstel)

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SAARSTEDT


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BIOCEV

BIOCEV: has started to assert its position as a first class science and research centre and meets all prerequisites to continue to play this role in the future.

BIOCEV: brings together enthusiastic teams of well-trained scientists from six institutes of the Czech Academy of Sciences and two faculties of Charles University, all working together under one roof.

BIOCEV: has been actively developing five research programmes