THE CZECH ACADEMY OF SCIENCES

INSTITUTE OF BIOTECHNOLOGY

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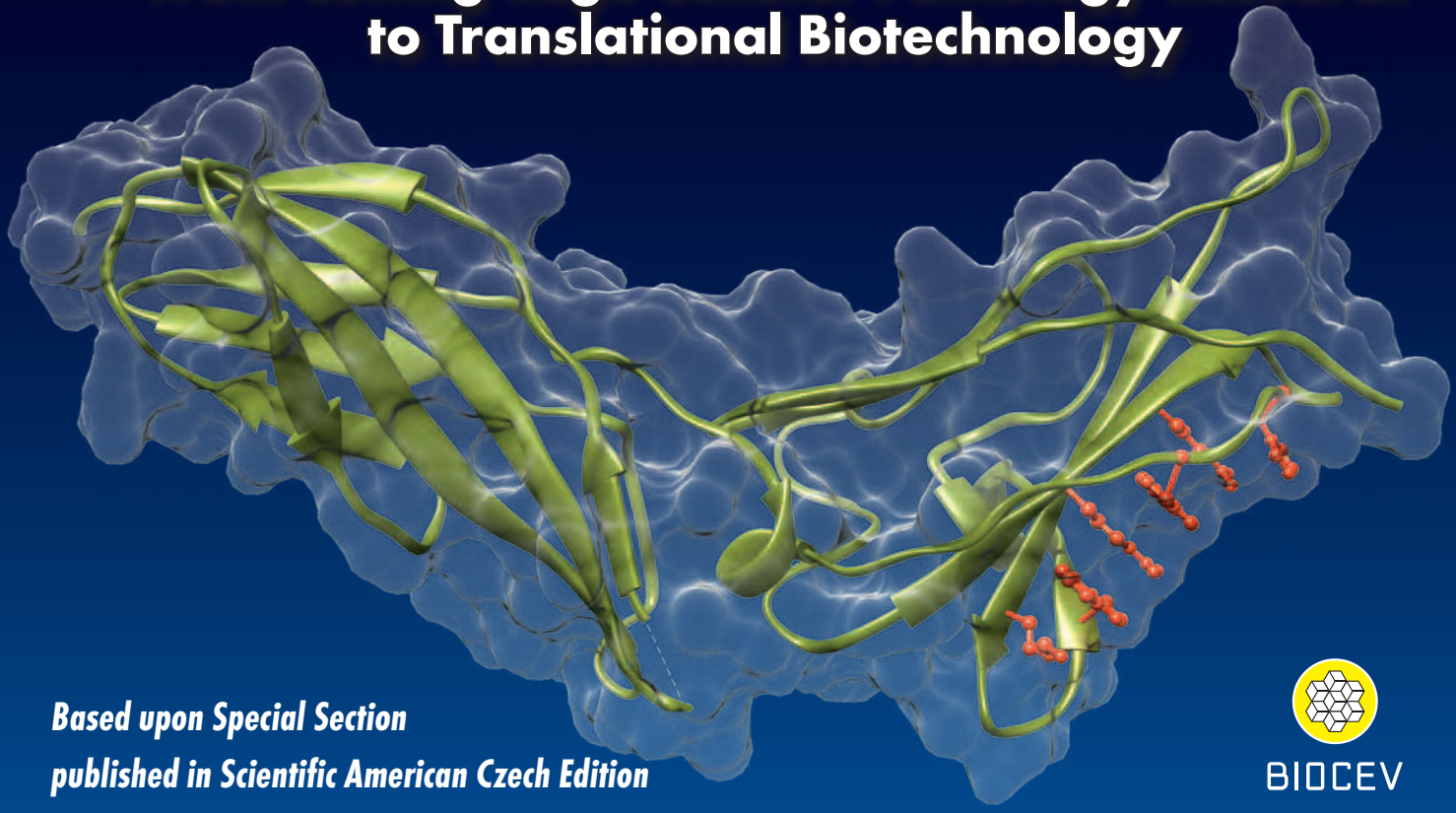
JOURNEY THROUGH LIFE

From Cutting-Edge Cellular Pathology Research to Translational Biotechnology



Based upon Special Section published in Scientific American Czech Edition

BIOCEV

For a Healthier Life

The Institute of Biotechnology of the Czech Academy of Sciences, v. v. i. (IBT) is a very young institution; it was established in 2008. During its brief existence it has already achieved a number of remarkable results. It focuses on basic research in molecular biological sciences at the highest level, with prospective transfer of biotechnological methods and molecular tools to diagnose and treat pathological condition of the cell into human and veterinary medicine, or into other key areas of human activity.

Let's Join Forces

A key place in the development of the Institute is held by its involvement in project BIOCEV, 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 (Faculty of Science and First Faculty of Medicine), whose objective is to establish and maintain scientific centers of excellence in biotechnology and biomedicine.

Funding was provided by the European Regional Development Fund through the Research and Development for Innovation Operational Program.

The Institute participated in preparation of the project, the implementation part of which was completed at the end of 2015.

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

In the New Place toward New Goals

The Institute moved to a new building in the BIOCEV Center in Vestec in January 2016 and is involved in two of the five research programs of the Center. We want to take full advantage of this opportunity and produce highest-quality scientific results, which will be transferred into clinical practice.

The new seat of IBT

In Partnership with Universities

The Institute contributes to education of students from the Czech Republic and abroad at all levels of university studies. The students learn experimental techniques and methodologies of scientific research while working in the laboratories under the guidance of experienced researchers and preparing their bachelor's, master's or doctor's thesis. Leading researchers lecture at a number of Czech and foreign universities. The IBT study programs are prepared in cooperation with the following Czech universities: Charles University, University of Chemistry and Technology Prague, Czech Technical University, Czech Agricultural University, the University of South Bohemia in České Budějovice. Approximately 15 undergraduate and 30 graduate students are currently working at IBT.

The IBT PhD program is part of the Postgraduate Studies in Biomedicine, associating Charles University and many institutes of the Czech Academy of Sciences. The PhD program also takes advantage of the curricula offered by the University of Chemistry and Technology. The individual study courses are organized by the respective sectoral councils. The studies must be completed with a state examination and dissertation defense at the respective university.

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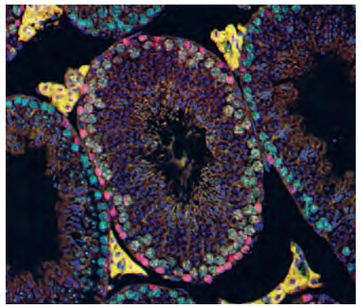
Research Program 1: Development of Therapeutic and Diagnostic Procedures



Histology of mouse testes.

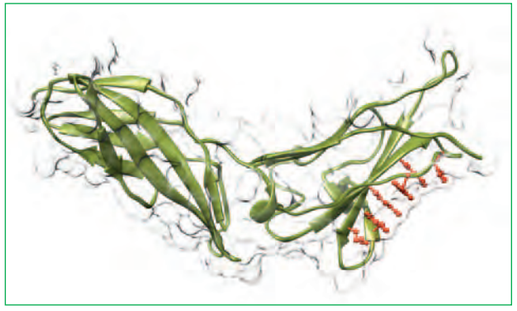
The unifying elements of research program 1 are the study of pathological conditions in the cell, identification of causes of these conditions, changes in expression profiling of selected genes, detection of changes in the localization and modification of selected proteins, and identification of other molecules related to induction of the pathology.

The objective of the program is to develop new procedures for disease prevention and to prepare new methods for disease monitoring and diagnostics and tools for molecular therapy of associated pathologies.

Research Program 2: Structural Biology and Protein Engineering

The aim of program 2 is to investigate novel biotechnologically, diagnostically, and medicinally relevant biomolecules, proteins and nucleic acids that can be constructed using methods of molecular biology and protein engineering.

Understanding the structures of the studied molecules and their mutual interactions will help to modify their biological activities, making them useful in diagnostics or as drugs or advanced materials.



The structure of the interferon gamma receptor 2

The Institute Is Responsible for Two Core Facilities in the BIOCEV Centre

The two facilities are the Center for Molecular Structure, and Quantitative and Digital PCR.

The Center of Molecular Structure provides a comprehensive approach to the study of the spatial structure, function and biophysical properties of biological molecules.

Quantitative and digital PCR specializes in providing real-time PCR (RT-qPCR) services and courses.

Assoc. Prof. Jana Pěknicová, Ph.D.
Director of IBT CAS, v. v. i.

Plasticity of Tumor Cells and Treatment of Tumors

The working group under the leadership of Jiří Neužil (Laboratory of Molecular Therapy) together with their Czech colleagues and an international team published, in the prestigious journal *Cell Metabolism* (IF 17.565), an article demonstrating that cancer cells without mitochondrial DNA (mtDNA) show tumor growth delay and that re-emergence of tumors is associated with acquisition of mtDNA from the host cells, resulting in partial restoration of the mitochondrial function.

The results indicate that the horizontal transfer of mtDNA from the host cells to tumor cells with compromised respiratory functions leads to restoration of breathing and of tumor growth. As well, these results suggest the pathophysiological processes to overcome mtDNA damage, and show a high plasticity of the malignant cells.

It was shown that vitamin E derivatives targeted to mitochondria kill tumor cells. Some of these substances prepared and tested by the group are protected by national and international patents. One such substance is in clinical testing as a potential therapeutic for the treatment of selected tumors.

Better Drugs to Treat Psoriasis

The team of Petr Malý (Laboratory of Ligand Engineering) has been involved in preparation of new types of small binding proteins called recombinant ligands. If we prepare a small binding protein so that it binds to the cell surface receptor and elicits an inhibitory or stimulatory reaction, we can use this protein very efficiently in the development of new-generation therapeutics. The advantage of recombinant ligands lies in their easy preparation from bacterial cultures, high stability, structural resistance, and relatively simple possibility of modification.

The prepared REX ligands are protected by a Czech patent, which is currently being extended to an international patent.

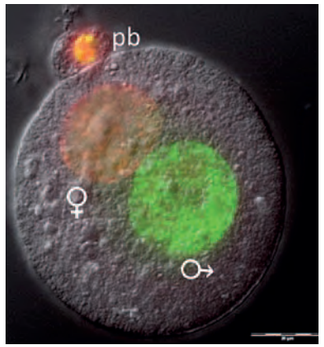
Towards New Life

Head:
Kateřina Hortová, Ph.D.

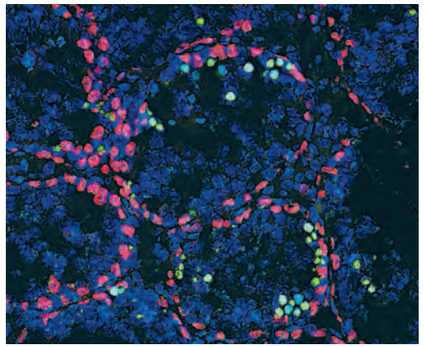
Fertilization is a highly specialized interaction between gametes that culminates in the formation of a zygote and development of a new individual. The sperm-egg interactions in mammals consist of series of specialized and regulated events that initially involve egg-induced activation of the sperm and ultimately result in reciprocal sperm-induced activation of the egg. Sperm motility, metabolism, capacitation and acrosome reaction are modulated by factors associated with the egg, its non-cellular or cellular components and fluids, present in both male and female reproductive tracts. Sperm proteins and the glycoprotein envelope of the egg (zona pellucida) take also part in this highly specific interactions between gametes.

Let Men Be Fertile!

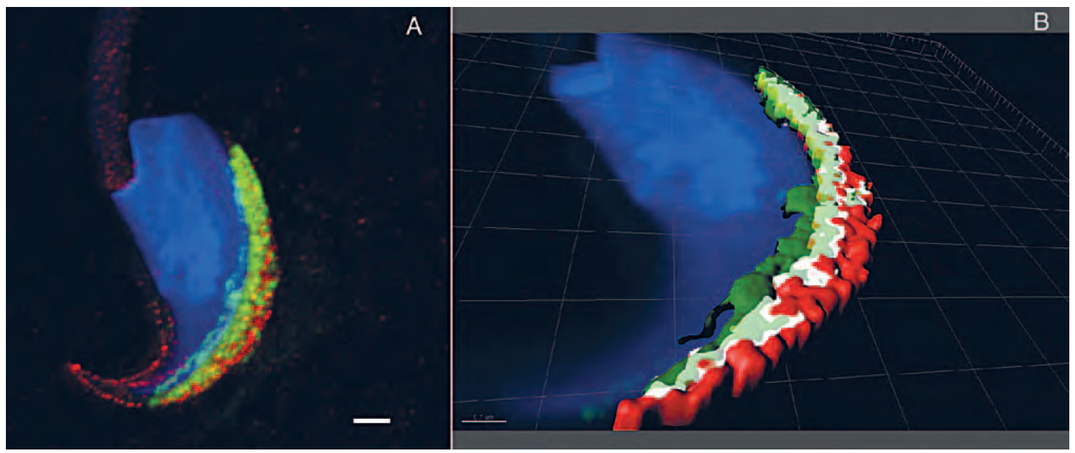
The Laboratory of Reproductive Biology focuses on studying and characterization of molecular mechanism of fertilization including sperm maturation (capacitation, acrosome reaction, composition of seminal fluid), sperm–egg interaction during fertilization as well as development of specific tools (monoclonal antibodies) for detection of male infertility. At the same time, the group is involved in characterization of reproductive parameters modified due to Diabetes mellitus and selected environmental factors. This research area includes, beside others, identification of epigenetic mechanisms participating in changes in gametogenesis connected to gene expression. The outcome of this research led already to several prototypes and commercial kits production, serving as tools for identification of sperm parameters in Centres of Assisted Reproduction.



Mouse embryo: Male and female pronuclei stained with antibody against H4K12ac histone modification (*green*) and 5mC DNA methylation (*red*) show transfer of specific epigenetic marks coming exclusively from sperm (*green*), and take an active part in the early embryogenesis (Vieweg and Dvorakova-Hortova K. et al. *Clinical Epigenetics*, 2015).

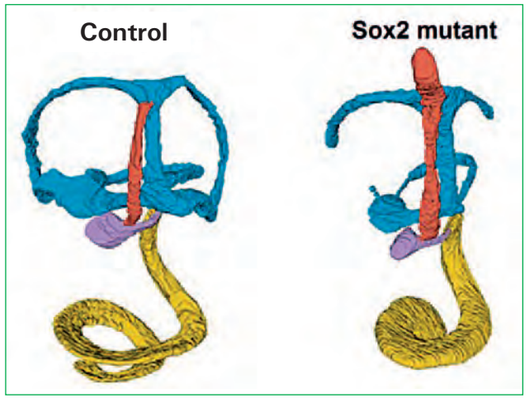


Mouse testis: Evaluation of apoptosis (*green*) by TUNEL assay after the exposure of individuals to environmental factors (Elzeinova et al., *Exp Toxicol Pathol.*, 2013).



Detection of proteins CD46 (*green*) and β1 integrin (*red*) in mouse sperm head by super-resolution STED microscopy (A), Co-localization lines of selected proteins (*white*) analyzed by Imaris software (B). The scale bar indicates 0.7 micrometers. Nucleus (*blue*). (Frolikova et al., *Sci Rep.*, 2016)

To Enjoy the Future World of Sounds



Comparison of inner ear development with a partial deletion of transcription factor Sox2 using a transgenic mouse model (Dvorakova M et al., 6:38253, *Scientific Reports* 2016).

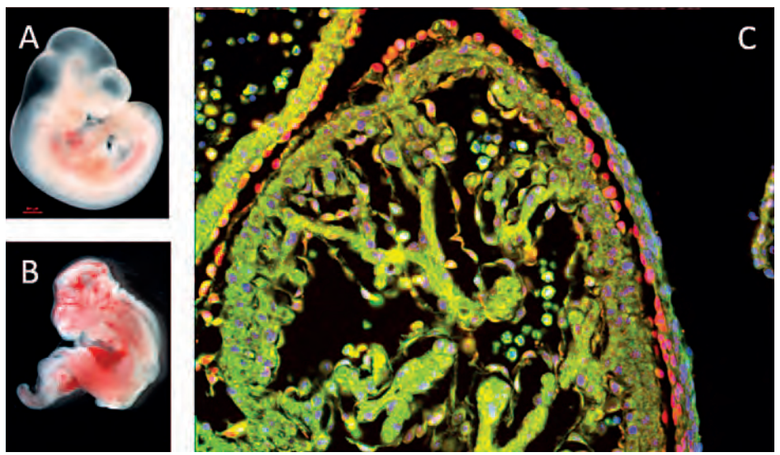
In one of our projects we focus on the analyses of transcriptional regulation in neurosensory embryonic development. We aim to identify genes and signaling pathways that are necessary for the development of specific cell types of the inner ear. This is a critical step for our understanding of pathophysiological processes in hearing disorders associated with the death of hair cells, supporting cells and neurons, and with loss of neuronal contacts. Approximately 71 million Europeans suffer hearing impairment or loss.

Head:
Gabriela Pavlínková, Ph.D.

We use mouse transgenic mutants to analyze interactions and cooperation of transcription factors, ISLET1, SOX2, and bHLH neurosensory specification factors in the development of neural and sensory lineages in the inner ear.

Reducing the Risk of Congenital Defects

Diabetes mellitus during pregnancy can have a negative impact on the fetus development. Insufficiently managed hyperglycemia of mother during the first trimester increases the risk of congenital defects and spontaneous abortions. Neural tube defects, caudal regression and cardiovascular defects are the most frequent congenital defects. Clinical tests have shown that the most frequent birth defects in diabetic embryopathy are cardiovascular defects, particularly atrioventricular defects, hypoplastic left heart and transpositions of large vessels. In addition, the risk of cardiovascular diseases and diabetes is increased in children and adults that had been exposed to unfavorable conditions during embryonic development, a process called fetal programming. Laboratory of Molecular Pathogenetics focuses on the identification of genetic factors that may, in combination with maternal diabetes environment, influence embryonic and fetal development and thus increase the risk of cardiovascular defects and functional changes of the heart. We have used gene expression profiling methods and an animal model to identify changes in signal pathways important for the morphogenesis and maturation of the fetal heart that are related to the crucial step in heart development – septation. We have demonstrated then a mutation of transcription factor called hypoxia-inducible factor 1 alpha results in an increased risk for cardiovascular defects in diabetic embryopathy. We have shown that the combination of gene mutation and teratogenic diabetic environment alteres signaling pathways, increases the risk of diabetic embryopathy, and induces changes in the process of fetal programming.



Changes in embryonic development exposed to diabetic environment. (*A*) A normal development and (*B*) a diabetic embryopathy. (*C*) Imunohistochemical staining of expressed genes that induce changes in the morphogenetic process during heart development.

To Intervene Accurately and Efficiently

Head:
Prof. Jiří Neužil, Ph.D.

Cancer pathologies are a growing problem in industrial countries in spite of the unprecedented effort of many scientists around the world. This very frustrating perspective is caused by an extraordinary plasticity of tumor cells, i.e. their capability to escape the treatment, overcome adverse situations and utilize alternative sources of energy. It is not becoming clear that it is unrealistic to cure cancer with drugs that target a single signaling pathway or one gene. It is therefore necessary to find a common intervention 'point' that could not be mutated in cancer cells and that would be 'universal' (and selective) for a number of tumor types. Mitochondrial respiration appears to be such an 'Achilles' heel' of cancer.

New Small Molecules

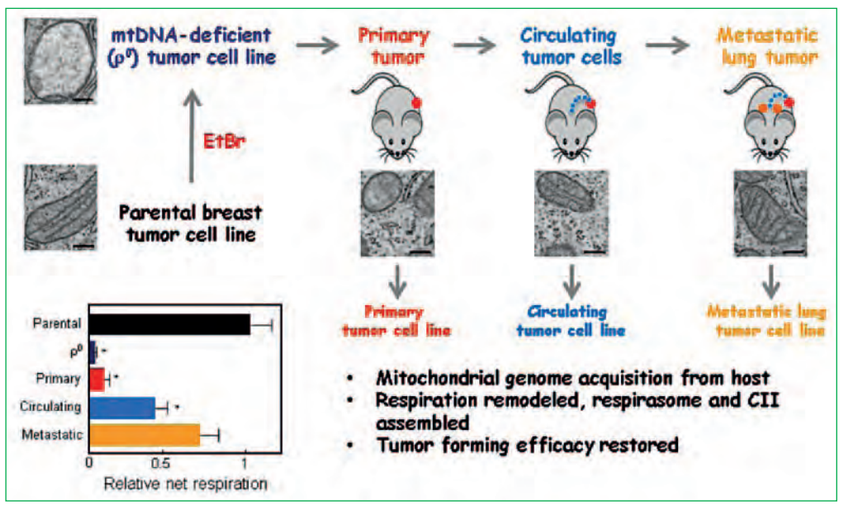
Our group focuses on a new approach to cancer therapy, when we focus on new small molecules that we design and that affect mitochondrial respiration. We refer to these substances as „mitocans" (from „mitochondria" and „cancer"). These agents are very efficient (and, at the same time, selective for tumor cells) also against highly recalcitrant tumors, such as pancreatic cancer, high-Her2-high breast cancer or triple-negative breast tumors. One of the substances we designed (that are protected by international patents) is now entering clinical tests.

Verified by Experiments

Our research on horizontal transfer of mitochondria in experimental animals further corroborates the notion that mitochondrial respiration is an appropriate molecular target for treatment of neoplastic pathologies. We discovered that cancer cells with strongly damaged mitochondrial DNA (mtDNA), when grafted into experimental mouse, form tumors only after restoring their mitochondrial respiration. This process involves trafficking of mitochondria with DNA from the surrounding, stromal cells. As the cancer cells cannot form tumors before restoring respiration, it is obvious that respiration is essential for formation and progression of tumors. It is consistent with the above mentioned proposition that respiration is a plausible target for treatment of tumor diseases. We are currently studying the molecular mechanisms and regulation of horizontal intercellular transfer of mitochondria and utilization of this novel phenomenon for development of new approaches to cancer therapy.

MORE TO EXPLORE:

Tan A et al. (2015) *Cell Metab* 21, 81–94.



Obtaining mitochondria by tumors without mitochondrial DNA. Cancer cells that lack mitochondria obtain them from the host organism – in this case from the mouse – and they can grow and form metastases only restoring mitochondrial respiration.

Treating Tumors!

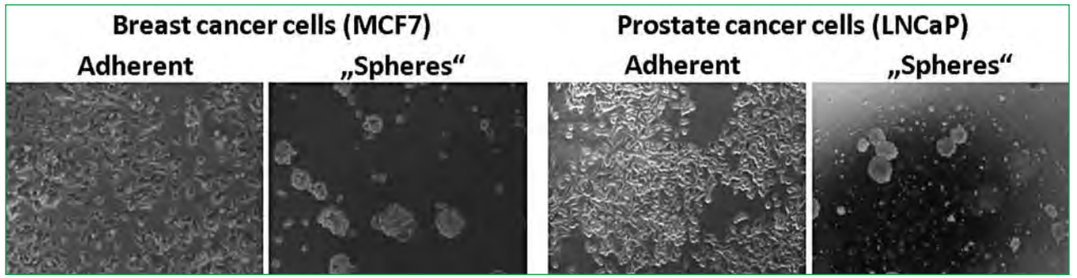
Cancer is the second most frequent cause of death in developed countries. A timely and accurate diagnosis and appropriate therapy are crucial for a successful treatment. Despite enormous progress in tumor diagnosis and treatment it is evident that a many patients do not respond adequately to the therapy or they do respond but the tumor gradually develops resistance and the treatment becomes ineffective. Tumor resistance is one of the fundamental problems of cancer therapy as most patients do not die from the primary tumor but from the secondary tumors that show resistance to the used anti-cancer drugs. One of the possible causes of tumor resistance may be a special type of tumor cells called tumor-initiating cells, or also cancer stem cells. The cells show the characteristics of stem cells, and substantial resistance to anticancer drugs.

Head:
Jaroslav Truksa, Ph.D.

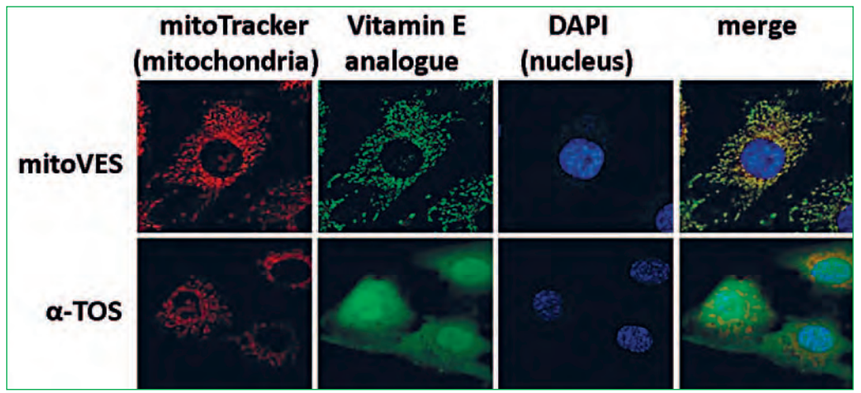
Let's Use All Weapons

Laboratory of Tumor Resistance focuses on biology and characterizing of tumor-initiating cells. We mostly use the model of so-called „spheres" in our laboratory, when the cancer cells are grown in a special medium and grow as small „microtumors," i.e. three-dimensional structures that exhibit an increased number of tumor-initiating cells, and thus permit to study their various characteristics. Our research goals include characterization of molecules that are involved in drug resistance – such as expression and regulation of the so-called ABC-transport proteins, and also finding characteristic features of those cells that can be used in diagnosis and prognosis of tumor diseases.

The group also focuses on developing and testing new anticancer drugs that are targeted into mitochondria such as mitochondrially targeted vitamin E succinate (MitoVES), and also monitoring of transfer of mitochondria among cells, both in collaboration with the Laboratory of Molecular Therapy. Further research topic of the laboratory represents iron metabolism and its alterations in the tumor-initiating cells and possible use of iron metabolism-related genes in diagnosis or treatment of cancer.



Tumor cells of breast cancer (MCF7) and prostate cancer (LNCaP) in culture, grown as adherent cells and as „spheres"



Evidence of mitochondrial localization of mitochondrially targeted derivative of vitamin E (MitoVES) against the original substance (α-TOS) which does not exhibit mitochondrial distribution. Source: Truksa et al. *ARS*, 2015.

From Healthy Cells to Healthy Organisms

Head:
Prof. Mikael Kubista, Ph.D.

Laboratory of Gene Expression is Europe's leading academic laboratory specialized in high-throughput gene expression profiling and single-cell analysis using quantitative PCR in real time (qPCR). We are working on several basic research projects in the field of developmental biology and stem cells, and also on applied projects in the fields of cancer and neurological research. We also develop methods and applications for nucleic acid analysis and are also interested in the area of standardization. The laboratory is equipped with unique devices for single cell collection from suspension – CellCelector (ALS, Germany), and for high-throughput analysis of gene expression – Biomark (Fluidigm, U.S.).

Frogs as a Model of Development

We use *Xenopus laevis* as a model organism in our developmental biology projects to explore the following phenomena:

- the role of nitric oxide signaling in early development
- the function of nitric oxide in regeneration and wound healing
- localization of maternal mRNAs, miRNAs and proteins in the egg and their distribution among blastomeres in the early development.

We collaborate with University of Notre Dame, USA; and Whitehead Institute, MIT, USA.

Two-days-old embryo of Xenopus laevis as a model organism for developmental biology

For Restoring the Nerve Cells

In the field of neural regeneration we use the mouse and rat models to study:

- gene expression changes in glial cells during brain development and after ischemic brain damage
- gene expression changes in glial cells during aging
- gene expression changes in glial cells during aging in the mouse model of Alzheimer disease
- astrocytic subpopulation identified by single-cell gene expression analysis
- identification of new markers for astrocytes.

We collaborate with the Institute of Experimental Medicine, Czech Academy of Sciences.

To Cure Cancer More Efficiently

In the field of cancer research:

- we evaluate a panel of markers for the profiling and characterization of circulating tumor cells to guide treatment.
- we investigate functional and molecular markers of DNA repair and miRNAs in tumor and healthy tissue from a group of patients with colorectal carcinoma.

Collaborating institutions: AdnaGen; TATAA Biocenter; First Faculty of Medicine, Charles University; Institute of Experimental Medicine, Czech Academy of Sciences.

Picking individual cells in prostate cancer with the help of CellCelector

Molecules of Life

Head:
Assoc. Prof. Bohdan Schneider, Ph.D.

For many people, the word „molecule" represents only a dim memory of high school chemistry. Although molecules form the material world around us, and actually also our physical selves, we rarely ponder how the individual molecules give rise to something so incredibly complex, such as the human body. We can view biomolecules as nanoscopic biological systems or machines that can arrange themselves into larger organized entities and thus create the living matter. However, the living matter is not a static object. It is changing constantly due to the activity of the biomolecules that are clustering in mutual interactions, transforming themselves chemically, producing new molecules and decomposing the old ones, including themselves. It is essential in all these processes that the molecules can correctly recognize each other.

What Makes Biomolecules So Interesting for Research?

Despite the tremendous progress in the field of molecular biology made in the last few decades, we are still at the threshold of a detailed understanding of how biomolecular processes work. Research and development in this field has the potential to bring entirely new materials, pharmaceuticals, and other applications. In our lab, we study two types of biomolecules, proteins and DNAs. With proteins, we use methods of so-called directed evolution to search for proteins that will bind with high specificity to so-called cytokines, molecules of the innate immune system. Such proteins have potential diagnostic or therapeutic applications. Regarding DNA, we are interested in its basic structural characteristics, which are important for understanding and possibly influencing the genetic processes. Since both proteins and DNAs developed in aqueous environment during evolution, we are also interested to learn as much as possible about their interactions with water and dissolved ions.

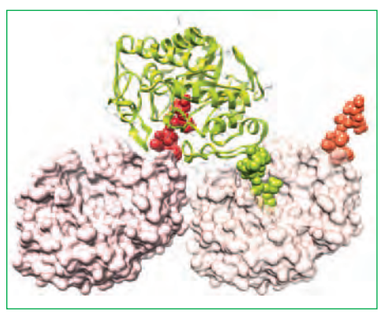
In our lab, we succeeded in solving the previously unknown structure of interferon gamma receptor 2. Main chain of the protein is shown in *green*, the *white* area indicates the surface of the protein molecule. Side chains of the amino acid motif that stabilizes the receptor molecule are highlighted in *red*. The structure has been deposited in the Protein Data Bank under the code 5eh1.

Investment that Pays off

To keep up with the world in our fields of research, we must apply the most advanced experimental and computational approaches. In this respect, it is a great advantage that our laboratory is a part of a newly built BIOCEV center, equipped with top-notch devices and technologies. Since today's science is international, it is also necessary to collaborate with leading domestic and foreign institutions in our field.

From Basic Research to Practical Application

The study of biomolecules is in many ways a fascinating intellectual activity. However, we as scientists are well aware that satisfying our desire for knowledge is not the main motivation for supporting science from the societal perspective. Instead, this motivation arises from the practical outcomes of science. Therefore, in our laboratory we are studying biomolecular systems where one can suppose that a detailed knowledge about them, their properties and possible modifications would lead to the development of new diagnostic, therapeutic or biotechnological preparations. Currently, we devote most of our attention to designing and further exploring molecules that would block the biological activities of cytokines, already mentioned above, which, if disregulated, can cause serious autoimmune diseases such as psoriasis and Crohn's disease.



Old yellow enzyme is an enigmatic class of flavoproteins which can mediate their activity by defined and reversible aggregation. Figure shows sterical blocking of the active site of XdpB, one of Old yellow enzymes, PDB netry 5epd.

Tailor-made Proteins

Head:
Petr Malý, Ph.D.

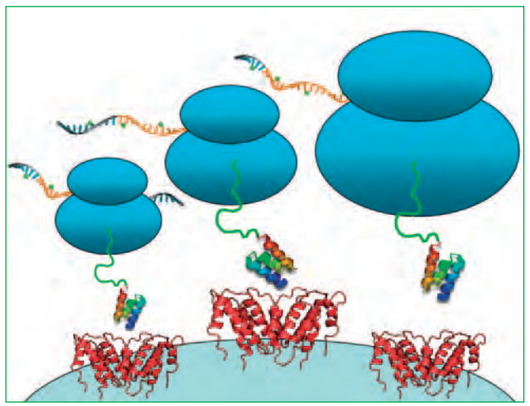
Proteins form the basic structural and metabolic components of every living organism and affect its state in health or disease. Protein engineering deals with structure-function studies of particular proteins and this includes targeted modifications of protein molecules leading to an improvement of their properties such as higher specificity of intermolecular interactions, an increased binding affinity to the chosen molecular target, or a generated blocking function. The Laboratory of Ligand Engineering is focused not only on the "molecular design", represented by in-silico modeling and by molecular modifications of proteins or their particular domains using gene-fusion approaches, but also on the generation and characterization of novel binding proteins selected from high-complex collections of ligand molecules, so-called combinatorial protein libraries.

For Better Diagnostics

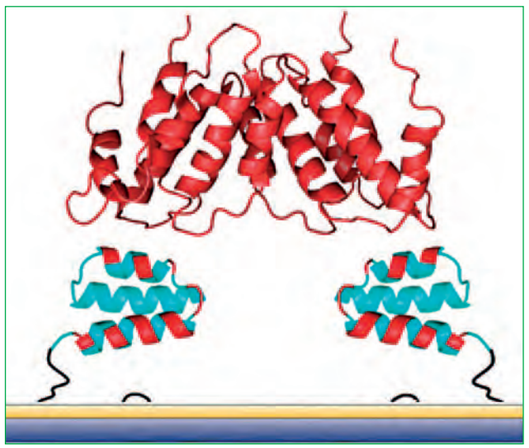
Recently we have established a model of three-helix bundle of albumin-binding domain (ABD) of streptococcal protein G as a master scaffold for the generation of high-complex combinatorial library of a theoretical complexity of up to 100 trillion variants. Protein of interest serves as a molecular target for the selection of high-affinity binding proteins that are further characterized for their specificity, biochemical properties as well as biophysical parameters such as thermal stability, binding kinetics or the ability to form multi-mers. During several past years, the established ABD model has been used for the selection and characterization of high-affinity protein variants binding human interferon gamma, but also for the generation of binders targeting human prostate cancer serum oncomarkers important for more-complex in vitro diagnostics.

NO to Autoimmune Diseases!

Inhibitory binding proteins targeted to human IL-23 cytokine (called ILP binders) and IL-23 receptor (REX binders) are of a special value as they block the binding of the cytokine to cell-surface receptors, leading to the suppression of signaling cascade into T lymphocyte nucleus and a subsequent secretion of several pro-inflammatory modulators playing a critical role in the development of several autoimmune diseases. These ligands are patented as promising alternatives to commonly used antibodies and might be useful for the development of topical anti-psoriatic drugs.



Based on a genetic information carried by the particular messenger RNA, ribosome complexes produce the corresponding protein variant binding to the immobilized protein target. The bound ribosome complexes are dissociated and the released genetic information carried by the RNA is, after the reverse transcription to DNA, identified by sequencing.



The generated binding proteins can be immobilized to the surface of a microfluidic chip and serve as high-affinity capture proteins for serum diagnostics of tested individuals.

Biological Functions Lie in Three-dimensional Organization of Atoms of Bio-molecules and in Their Interactions

Head:
Jan Dohnálek, Ph.D.

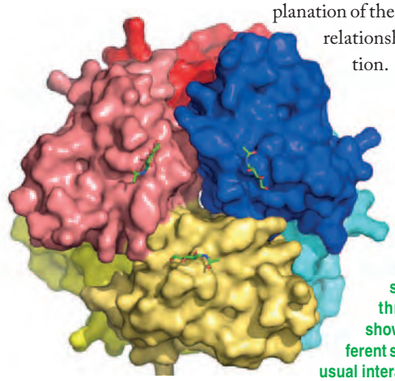
The tools of single crystal X-ray diffraction help us describe structure of biological molecules at atomic detail and explain principles of function of proteins. In the Laboratory of structure and function of biomolecules we apply crystallography mainly in studies of previously unknown enzymes and receptors. We analyze our targets at atomic detail and elucidate properties of proteins with practical application. The results are applicable in drug development (inhibition of retroviruses, fungal diseases, cancer), in immunology (immune system receptors) and in processing of food and hazardous substances (enzymatic treatment and protection of food, degradation of warfare agents).

Innate Defense against Diseases

Protein interactions play a crucial role in the human immune system. Upon contact a cell of the innate immune system has to recognize the normal cell of the organism from a virus-infected or cancer cell. Interactions between the cell surface receptors and their ligands are determinant in this process. If receptors of a natural killer cell send signals to their mother cell to destroy the enemy, a complex of proteins is secreted causing a rapid degradation of the contacted cell. In collaboration with the Faculty of Science of the Charles University and with the Institute of Microbiology CAS we determined the structure and the related properties of several C-type lectin-like ligands and receptors of natural killer cells: mouse C-lrb, mouse NKR-P1A, and human LLT1. These studies are aimed at determination of the interaction details and then utilization of the new knowledge for human health.

Against other human pathogens

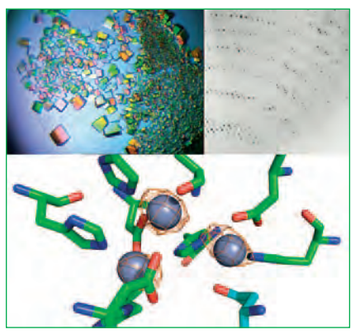
Enzymes secure a dramatically increased efficiency of the necessary chemical reactions using the exact arrangement of the key chemical groups. Plants utilize nucleases of type I for non-specific cleavage of nucleic acids in tissue development and apoptotic functions. In collaboration with the Institute of Plant Molecular Biology, Biology Centre CAS and with the University of Chemistry and Technology Prague we determined the structure of the tomato multifunctional nuclease 1 with anticancer effects and explained its stability and principles of specificity. Similar enzymes are used also by human pathogens such as Legionella and Leishmania. We want to contribute to the fight against some major diseases by explanation of their structure-function relationship and role in infection.



The crystal structure of the extracellular part of the LLT1 ligand of human natural kill cell receptor. Molecular surface of a complex of three protein dimers is shown, each dimer is in different shades of one color. The usual interaction interfaces of the dimers are oriented into the hexamer interior and glycosylation chains of this protein are exposed to the exterior of the hexamer. The first unit of N-acetylglucosamine is represented by sticks.

Importance of research infrastructures and international collaboration

Our laboratory significantly contributed to the establishment of the Centre of Molecular Structure BIOCEV, now part of an Instruct center. It collaborates with the European infrastructure Instruct, the University of Oxford and the University of York. Our studies also include new enzymes for biotechnologies in collaboration with Novozymes A/S in Copenhagen.



Above left: Protein crystals (flavin oxidase) in polarized white light. Above right: A single crystal X-ray diffraction pattern of an enzyme. Below: Exploitation of anomalous scattering in crystallographic analysis enables exact identification of zinc ions in the active center of the plant multifunctional nuclease TBN1; spheres indicate positions of the zinc ions, the surrounding amino acid residues are represented by sticks and the net represents a contour of electron density corresponding to the signal of the anomalous scattering of zinc at a given wavelength of X-rays and identifies the element and its coordinates.

Metalohydrolases as Targets for Tumor Diagnosis and Therapy

Head:
Cyril Bařinka, Ph.D.

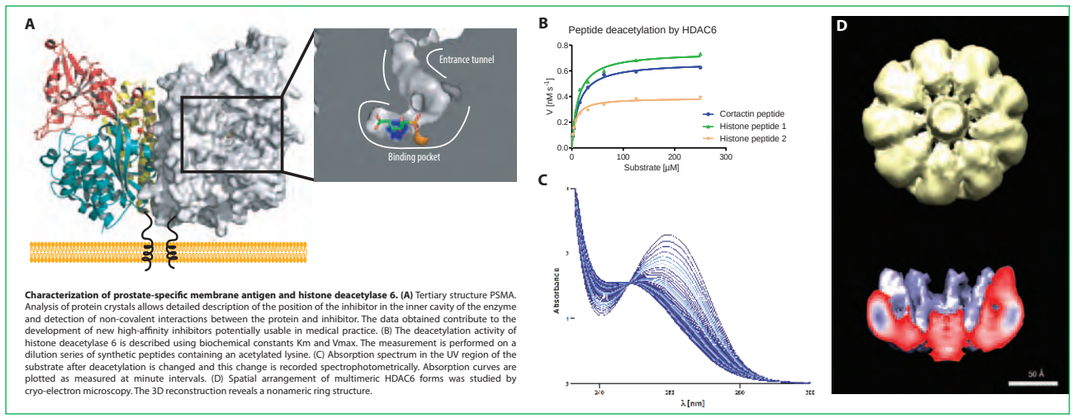
Our laboratory studies metalohydrolases, especially the family of histone deacetylases and M28 proteases (e.g., prostate-specific membrane antigen – PSMA). Mutations or aberrant expression of these enzymes are associated with neoplastic malignancies as well as a number of neurological disorders, including e.g. Alzheimer's disease, schizophrenia, and amyotrophic lateral sclerosis. Our research activities are aimed at two main objectives: (i) to understand the role of the studied metalohydrolases in physiological and pathological processes, and (ii) to develop reagents that specifically recognize target proteins and can be used for diagnostic and/or therapeutic purposes.

A Wide Range of Experimental Approaches

To achieve the first objective, we use a broad spectrum of experimental approaches involving the production of recombinant proteins in diverse heterologous expression systems (bacterial, yeast, insect, and mammalian cell cultures), their purification and subsequent biochemical, biophysical and structural characterization. We use modern methods of molecular biology to modify the structure of naturally occurring proteins and monitor the impact of the mutation on their function. Enzyme activity and substrate specificity are studied by high-performance liquid chromatography, or fluorescent techniques using peptide microarrays. Biophysical characterization includes, e.g., differential scanning calorimetry, circular dichroism or surface plasmon resonance. The primary tool for studying the three-dimensional structure of the studied proteins and their interactions with newly developed reagents include then electron microscopy and X-ray crystallography.

Recognize and Cure

We use the knowledge garnered through our basic-research oriented studies to develop tools (i.e. small molecules or macromolecules) that can be used as research reagents or in clinical practice. Such translational research is performed in collaboration with research centers and industrial partners in the Czech Republic and abroad. In collaboration with our foreign colleagues, we for example solved three-dimensional structures of several dozen PSMA complexes with small-molecule inhibitors and our data laid foundations for the structure-assisted development of the next generation compounds. Together with the Technical University of Munich we have recently filed a European patent application describing the development of high-affinity macromolecules targeting PSMA that can be used for prostate cancer imaging. In conclusion, in our projects we aim at crossing the boundaries of the basic research and translate our findings into practical biomedical applications.



Characterization of prostate-specific membrane antigen and histone deacetylase 6. (A) Tertiary structure PSMA. Analysis of protein crystals allows detailed description of the position of the inhibitor in the inner cavity of the enzyme and detection of non-covalent interactions between the protein and inhibitor. The data obtained contribute to the development of new high-affinity inhibitors potentially usable in medical practice. (B) The deacetylation activity of histone deacetylase 6 is described using biochemical constants Km and Vmax. The measurement is performed on a dilution series of synthetic peptides containing an acetylated lysine. (C) Absorption spectrum in the UV region of the substrate after deacetylation is changed and this change is recorded spectrophotometrically. Absorption curves are plotted as measured at minute intervals. (D) Spatial arrangement of multimeric HDAC6 forms was studied by cryo-electron microscopy. The 3D reconstruction reveals a nonameric ring structure.

Providing Cell's Shape and Movement

Head:
Zdeněk Lánský, Ph.D.

Structural proteins that form the basis of cytoskeletal networks provide rigidity to the cellular content and are able to generate mechanical forces. This role of the cytoskeleton is essential for a wide variety of key cellular processes, such as, cell division, cell motility or morphogenesis (Civelekoglu-Scholey & Scholey 2010; Abu Shah & Keren 2013). Mechanical forces are generated in two basic ways: by cytoskeletal filaments that grow and shrink, and by proteins that bind to the cytoskeletal filaments and are able to move these filaments, such as, for example, molecular motors. How do these individual elements of the cytoskeleton cooperate to generate coherent behavior of the cytoskeletal networks and ensure the correct course of the cellular processes is not fully understood.

We Understand Increasingly Complex Systems

To answer these questions we use a bottom-up research strategy, increasing the level of complexity of the investigated systems. We isolate individual cytoskeletal proteins, the individual elements of the cytoskeletal complexes, which we combine in vitro and investigate their self-assembly. We explore such reconstituted system down to single molecule level using advanced biophysical methods. We employ optical microscopy enabling the visualization of single molecules (Joo et al. 2008), and optical tweezers enabling manipulation of single molecules and the measurement of mechanical forces (Moffitt et al. 2008). We combine our experimental approach with mathematical modeling, which provides quantitative understanding of the molecular mechanisms underlying the examined cytoskeletal system.

The Principle of Motion Discovered

Recently, using this approach, we showed that the relative movement of cytoskeletal filaments can be driven by a force analogous to pressure that drives the piston in a gas-filled cylinder (Lansky et al. 2015). We found that cytoskeletal proteins, which are confined in the space between cytoskeletal filaments, in which they move by diffusion (Braun et al. 2011), generate pressure similarly to gas particles enclosed in a cylinder. We quantified this „protein pressure" and demonstrated that it is high enough to move cytoskeletal filaments relative to each other and could thus be relevant for the rearrangement of the cytoskeleton during the cell cycle. Our approach thus enables a detailed understanding of the interplay of the individual components that generate mechanical forces inside the cell, gradually gaining a comprehensive understanding of the cellular processes driven by cytoskeletal networks.

MORE TO EXPLORE:

https://www.biocev.eu/en/programme/structural-biology-and-protein-engineering/structural-proteins-and-their-complexes/

References:

Abu Shah E, Keren K, Current Opinion in Cell Biology 2013, 25 (5), 550–557

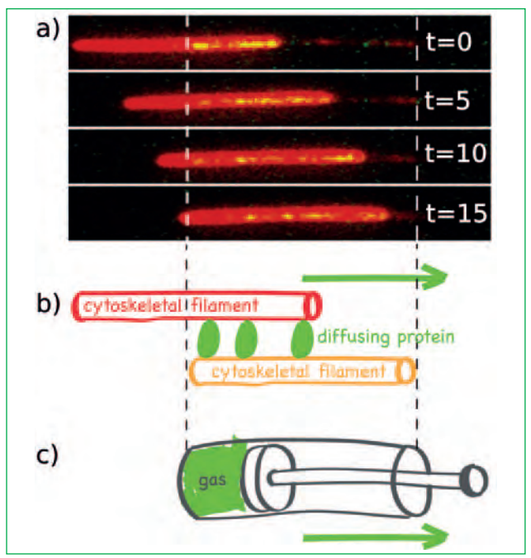
Civelekoglu-Scholey G, Scholey JM, Cellular and molecular life sciences 2010, 67 (13), 2231-2250

Braun M et al., Nature Cell Biology 2011, 13 (10), 1259–1264

Joo C et al., Annual review of biochemistry 2008, 77, 51–76

Lansky Z et al., Cell 2015, 160 (6), 1159–1168

Moffitt et al., Annual review of biochemistry 2008, 77, 205–228



The relative movement of cytoskeletal filaments propelled by the pressure of diffusing proteins. **a) Micrographs of two cytoskeletal filaments at time t = 0 to15 minutes. One filament (*dim red*) is firmly attached to the substrate and its ends are indicated by white dashed lines. The second filament (*bright red*) moves along the attached filament. The force driving this movement is generated by cytoskeletal proteins ASE1 (*green*), which are diffusing in the space between the filaments. b) Schematic illustration of the experiment presented in panel a). c) Macroscopic analogy of the experiment in panel a), diffusing particles of gas generate pressure in the closed space of the cylinder. This pressure manifests itself as the movement of the piston.**

Centre of Molecular Structure

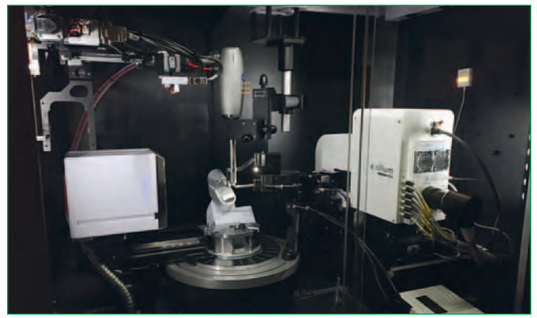
Head:
Frédéric Vellieux, Ph.D.

The Centre of Molecular Structure (CMS) encompasses several laboratories providing a complex approach to studies of three-dimensional structure, function and biophysical properties of biological molecules. Together with core facilities in CEITEC, CMS is a part of Czech Infrastructure for Integrative Structural Biology (CIISB), national affiliated centre of INSTRUCT (European Integrated Structural Biology Infrastructure).

CMS provides expertise, measurements and assistance in the following areas:

- crystalisation of biological molecules
- single crystal x-ray diffraction
- crystal structure solution
- microcalorimetric techniques for monitoring of interactions and stability (ITC, DSC)
- determination of parameters of molecular interactions (SPR)
- interaction analysis of biomolecules by microscale thermophoresis technique (MST)
- determination of protein primary sequence on microgram scale and of arrangement of disulphide bridges (FTMS)
- characterisation of post-translational modifications
- tertiary and quaternary structure mapping.

Bruker Daltonics 15T-Solarix XR FT-ICR ultra-high resolution mass spectrometer at the CMS, with electrospray and MALDI ion sources, coupled to an Agilent Technologies 1200 HPLC system.



Bruker D8 Venture diffractometer with a high-flux liquid Gallium MetalJet D2 X-ray source, Photon II detector and Kappa goniometer. This is also equipped with an ISX motorized stage for in-situ X-ray diffraction experiments, enabling screening of diffraction properties in crystallization trays.

Quantitative and Digital PCR

Head:
Lucie Langerová

The IBT qPCR Core Facility is one of Europe's leading academic service providers specialized in high-throughput gene expression analysis using real-time quantitative PCR (qPCR). It is the best equipped and experienced laboratory for nucleic acid analysis in the Czech Republic offering services on the unique and powerful microfluidic high-throughput BioMark™ System from Fluidigm. It uses integrated fluidic circuits known as dynamic arrays for gene expression analysis, genotyping analysis and digital array technology for absolute quantification of PCR targets and copy number variation analysis. 9,216 reactions can be processed in a single run.

Our aim is to make state-of-the-art qPCR technologies and know-how related to nucleic acids analysis available to academic researchers on attractive conditions. We contribute to clients' workflow from extraction of samples, to qPCR, data analysis and any downstream applications. We have separate laboratories for extraction, pre-PCR, PCR, and post-PCR to eliminate any risk for contaminations. We also offer academic researchers the possibility to perform their own experiments at our facility. We also support the Prague TATAA Biocenter in real-time PCR courses.

Core facility Genecore and its equipment for nucleic acid analysis including BioMark, loading stations, qPCR instruments and robotic liquid handling

Service Technology Laboratory

Head:
Lukáš Werner, Ph.D.

The Service Technology Laboratory (STL) is a section within Core Facility department of the Institute of Biotechnology in BIOCEV. Our laboratory was founded as a member of preclinical Test Sites in the CAS and we are primarily oriented to medicinal chemistry and preclinical development. Our experience comprises preparation of various Test Items including process optimization and initial scale-up. Good Laboratory Practice (GLP) certification of prepared compounds is an option for GLP part of preclinical development. Synthetic work is offered either on contract basis or as a part of academic collaboration. STL laboratory can also assist with formulation development as well as with chemical and physical stability studies including sub visible particles analysis. Our laboratory is frequently involved in both study design and evaluation. If required, we can also assist with composing of patent applications and documentation for the State Institute for Drug Control. STL is also very much opened for academic collaboration in the area of synthetic organic chemistry and SAR studies.