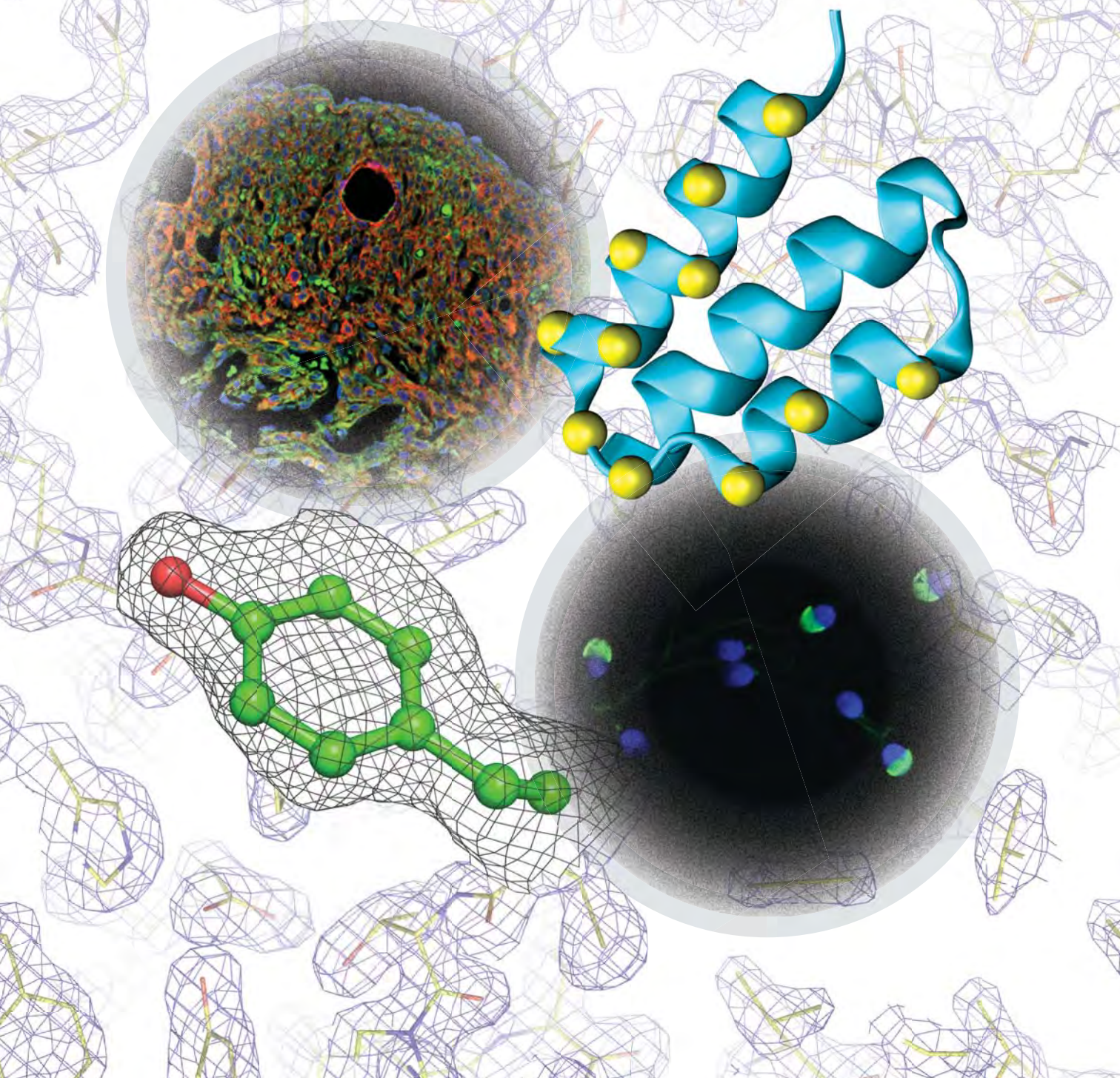
Institute of Biotechnology

Academy of Sciences of the Czech Republic

2008 – 2012



Contents

Introduction

Dear Reader,

You are opening the first Scientific Report of the Institute of Biotechnology of the Academy of Sciences of the Czech Republic, v. v. i., which summarizes its activities during the five years of its existence. It is therefore a young institution, founded in 2008, with Peter Šebo appointed as its first director.

The activities of the Institute of Biotechnology are focused on excellent basic research in molecular biology with prospective transfer of biotechnological methods and tools to human and veterinary medicine or other important areas of human activity.

The beginnings of the Institute were not easy; most groups were newly established and the Institute had limited financial possibilities to assist their establishment and development. Another limitation was the allotted space. Despite these problems, by honest and intensive scientific work our researchers have shown that they are able to publish a number of good publications in international journals with a solid impact factor every year. The Institute demonstrated not only viability but continuous growth, which is documented in this Scientific Report.

The Institute started with eight laboratories in 2012. The development of the laboratories and specification of their activities resulted in separation of the Laboratory of Ligand Engineering (Head P. Šebo) into the existing Laboratory of Ligand Engineering (Head P. Malý) and a new group, Laboratory of Biological Recognition (Head B. Schneider). At the end of 2012, a new Laboratory of Tumour Resistance (Head J. Truksa) was established, originating from the parental Laboratory of Molecular Therapy (Head J. Neužil).

The Institute activities are also oriented toward transfer of scientific knowledge into practice. In the project "Centre of Molecular Methods for Monitoring Diffuse Pollution of the Environment" (with participation of several biotech companies) we created many prototypes for commercial production (monoclonal antibodies against selected pollutants for construction of kits for their detection in the environment and monitoring of environmental pollution (Laboratory of Reproductive Biology).
Research at the Laboratory of Molecular Therapy has been sponsored by private donors, which is a remarkable achievement within the Academy. These funds enable faster transfer of results (anti-cancer agents) to preclinical and clinical trials.
The Laboratory of Ligand Engineering filed a patent application (Reg. No. PV_2012-829) presenting a new generation of protein binders usable for development of novel drugs as an alternative to conventional drugs based on neutralizing monoclonal antibodies.
The Laboratory of Biological Recognition filed several applications for prototypes (software) to study the structure of proteins. Research in other groups is also directed towards practical applications, mainly to diagnostics.

Our research groups are relatively successful in obtaining grants, even though termination of the important programmes of the Ministry of Education in 2011 ("Research Centres 1M", NRP II) was not without consequences to our budget. However, researchers now receive approximately the same amount of grant funding as the institutional funding.

Our Institute maintains collaboration with other Institutes of the Academy and Universities, both in grant applications and publication activity. Our study programmes (Bachelor, Master, PhD) are performed in cooperation with the Universities. Six of our scientists lecture at Universities and work in expert committees and scientific councils of faculties.

International cooperation is one of the key factors for work of all our groups and is promoted at all levels at the Institute. Our groups participate in joint projects, bilateral agreements, or simply during solution of a particular scientific problem. We received eminent scientists from abroad and enjoyed their interesting lectures. As well, our institute is open to foreign PhD students. Every year, our researchers take part in regular meetings (Symposium “Immunology and Biology of Reproduction, with International Participation” (Žďár nad Sázavou) and “Discussions in Structural Biology” (Nové Hrady)).

In collaboration with the TATA Biocenter Prague, the Laboratory of Gene Expression develops completely new technologies, jointly organizing courses of analysis of gene expression by qRT-PCR (using unique instrument Biomark). Another sophisticated instrument, VEVO 770, serves for non-invasive imaging and quantitative evaluation of models of various diseases, thus providing the Laboratory of Molecular Therapy a unique position in Central and Eastern Europe.

The international journal ‘Reproductive Biology and Endocrinology’ (RB & E) is issued under the auspices of our Institute. RB & E represents a global platform for reproductive and developmental biologists, reproductive endocrinologists, and many others. For more information see: http://www.rbej.com.

The good standing of our Institute has been confirmed by honours awarded to our researchers. Cyril Bařinka obtained the prestigious five-year J.E. Purkynje Fellowship and EMBO grant in 2010. Jaroslav Truksa was awarded by the Kellner Foundation (2012). In 2009, Jana Pěknicová received a medal from the International Coordination Committee for Immunology of Reproduction (ICCIR) acknowledging her work in the ICCIR and Development of Reproductive Immunology. Several researchers were acknowledged for the best publication in their field of study or for the best presentation at symposia.

Scientists from our Institute are also active in popularizing scientific results, by giving lectures and participating in the “Week of Science” and ”Open-Door Days” organized by the Academy, as well as by appearances on TV (TV24: Planet Science: Infertility - Disease of 21st Millennium (2009), CT2: DIAGNOSIS - Male infertility (2011) and in printed media (journals Tyden, Reflex, Instinkt, Scientific American, Vesmir, etc.).

Another significant activity of the Institute, playing a crucial role in its further development, is involvement in the project BIOCEV within the framework of the Research and Development for Innovation Programme of the Ministry of Education of the Czech Republic (www.biocev.eu). The Institute of Biotechnology, along with five institutes of the Academy of Sciences (Institute of Molecular Genetics, 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 the First Medical Faculty) participated in the preparation and implementation the project. The Institute is involved in two of the five BIOCEV programmes - WP 3 (Structural Biology and Protein Engineering) and WP 5 (Development of Therapeutic and Diagnostic Procedures).
Research and development of novel biotechnologies by a young emerging institute with high scientific potential, in collaboration with long-established and experienced research institutions, could bring great benefits to society, particularly concerning the socio-economical impacts on various areas of population health.

I am convinced that the Institute of Biotechnology has all the necessary prerequisites for further development, fostering acquisition of ground-breaking knowledge and results, and consequently publications in top scientific journals. As such, the Institute represents an invaluable platform for transfer of highly innovative scientific findings to their practical application.

Prague, February 25, 2013

Jana Pěknicová
Director

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Cell Pathology and Molecular Therapy

Laboratory of Molecular Therapy

Jiří Neužil

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RESEARCH TOPICS

Jiri Neuzil is the Head of the Laboratory of Molecular Therapy (LMT). In collaboration with his Australian group, the laboratory focuses on the design and development of novel anti-cancer agents, in particular vitamin E analogues, that are efficient and selective for malignant cells. The researchers are especially interested in the molecular mechanism of apoptosis caused by such agents, which includes pathways of mitochondrial destabilisation. They have defined and characterized the group of 'mitocans', small molecules relaying their anti-cancer activity via mitochondria. These agents are particularly intriguing from the translational point of view, since it is now clear that tumours are extremely heterogeneous in their mutations, even within different regions of the same tumour. Therefore, mitochondria may present an invariant target that can be utilized for cancer therapy.

A particular focus of the group is on mitocans from the group of vitamin E analogues, represented by the redox-silent compound α-tocopheryl succinate (α-TOS). This agent exerts high apoptogenic activity towards a variety of cancer cell lines while being non-toxic to normal cells. This *in vitro* pro-apoptotic activity is matched by its anti-cancer activity in mouse models of tumours, which the group has shown for colorectal, breast and mesothelioma tumours. This finding endows α-TOS with substantial translational spin and/or makes it a lead compound for the design of more efficient agents.

In the attempt to characterize the pathway(s) regulating apoptotic signalling in cancer cells triggered by α-TOS, the researchers discovered a new target of anti-cancer drugs, the mitochondrial complex II (CII). Interestingly, they found that α-TOS (and similar compounds) interacts with the proximal and distal ubiquinone (UbQ)-binding site of CII, thereby replacing UbQ in CII. CII, by means of its succinate dehydrogenase (SDH) activity, converts succinate to fumarate as a component of the tricarboxylic acid (TCA) cycle. Electrons released from this reaction are normally intercepted by UbQ and donated to CIII of the electron transport chain (ETC), part of oxidative phosphorylation. In case UbQ is replaced by α-TOS or similar compounds, the electrons recombine with molecular oxygen and cause generation of superoxide, triggering the apoptosis signalling cascade. Since genes coding for the subunit of CII mutate only exceptionally, the complex presents a very intriguing target for anti-cancer drugs.

In the attempt to increase the apoptogenic activity of α-TOS, the agent was modified by tagging with the cationic triphenylphosphonium (TPP) group, which causes the accumulation of the agent (mitochondrially targeted vitamin E succinate, MitoVES) at the interface of the mitochondrial inner membrane and the mitochondrial matrix, enhancing the apoptogenic activity of the parental compound by 1–2 orders of magnitude. Importantly, this increase in toxicity towards cancer cells did not compromise the selectivity of the agent for malignant cells. The approach of the group to cancer therapy by tagging anti-cancer agents with lipophilic cations epitomised by TPP presents a novel paradigm of efficient cancer therapy. The group is further pursuing this line of research to synthesize novel, more efficient anti-cancer agents targeting mitochondria. In particular, they plan to initiate translational research, whereby delivering their novel agents to the clinic. If successful, this may change the landscape of current and future cancer therapeutic modalities potentially leading to efficient cancer treatment.

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Sandra Lettlová / Undergraduate Student

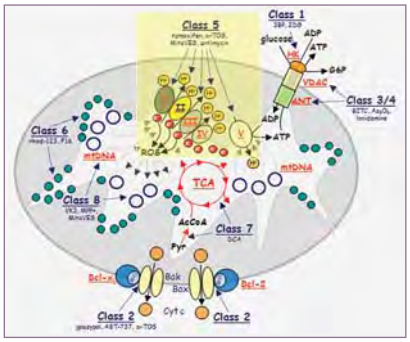

Fig 1: The classification of mitocans. Mitocans, small molecules with anti-cancer activity that act upon mitochondria, are classified into several classes. Class 1 mitocans comprises agents targeting hexokinase (HK), Class 2 compounds acting on Bcl-2 family proteins (BH3 mimetics and similar compounds), Class 3 and 4 compouds with redox-inhibitory function and acting on the voltage-dependent anion channel (VDAC) and adenine nucleotide translocator (ANT) channel proteins, Class 5 agents targeting the electron transport chain (ETC), Class 6 lipophilic compounds targeting the inner membrane, Class 7 agents targeting the tricarboxylic acid (TCA) cycle, and Class 8 compounds acting on mitochondrial DNA (mtDNA). Highlighted are the Class 5 mitocans and CII, which comprise α-tocopheryl succinate (α-TOS) and mitochodnrially targeted vitamin E succinate (MitoVES).

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Dong, L. F., Jameson, V. J. A., Tilly, D., Cerny, J., Mahdavian, E., Marin-Hernandez, A., Hernandez-Esquivel, L., Rodriguez-Enriquez, S., Stursa, J., Witting, P. K., Stantic, B., Rohlena, J., Truksa, J., Kluckova, K., Dyason, J. C., Ledvina, M., Salvatore, B. A., Moreno-Sanchez, R., Coster, M., Ralph, J., Smith, A. J., Neuzil, J. Mitochondrial targeting of vitamin E succinate enhances its pro-apoptotic and anti-cancer activity via mitochondrial complex II. *Journal of Biological Chemistry*, 286: 3717-3728, 2011. ISSN 0021-9258.

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GRANT SUPPORTS

Jiří Neužil

The Grant Agency of the Academy of Sciences of the Czech Republic:
IAA500520602 Cell death pathways in mesothelioma cells exposed to pharmacological and immunological inducers of apoptosis: genomic and proteomic approach. 2006-2008.

Grant Agency of the Czech Republic:
GA305/07/1008 Role of mitochondria in cardiomyocyte apoptosis during myocardial infarction: from cells to the whole animal. 2007-2009.

The Grant Agency of the Academy of Sciences of the Czech Republic:
IAA500520702 A novel model of apoptosis initiation by "mitocans". 2007-2009.

Academy of Sciences of the Czech Republic:
KAN200520703 The use of ultrasound in nanomedicine. 2007-2011.

Grant Agency of the Czech Republic:
GA204/08/0811 Mechanism of ROS generation and mitochondrial permeabilization. 2008-2010.

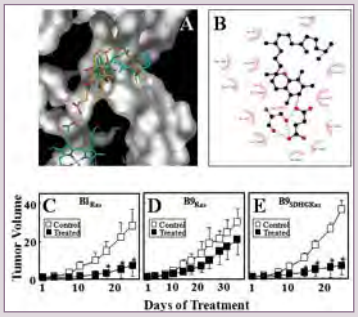

Fig 2: Model of the interaction of α-TOS with CII and its effect on complex II (CII)-functional and dysfunctional tumours. Molecular modeling indicates the position of α-TOS in relation to UbQ and heme between the succinate dehydrogenase C (SDHC) and SDHD subunits of CII (A), and its interaction with the Ser68 (shown here as Ser42 as used in the study by Sun et al 2005), which binds ubiqhinone (UbQ) in the proximal quinone (Q_P) site. Strong hydrogen bonds of the oxo groups of the succinyl moiety of α-TOS with the SDHC's Ser68 are indicated (B). Parental B1, SDHC-deficient B9 and SDHC-reconstituted B9$_{SDHC}$ cells were transformed with H-Ras and grafted into nude mice to form tumours. The carcinomas were treated with α-TOS, indicating a good response of the CII-functional and very little response of the CII-compromised tumours (C-E).

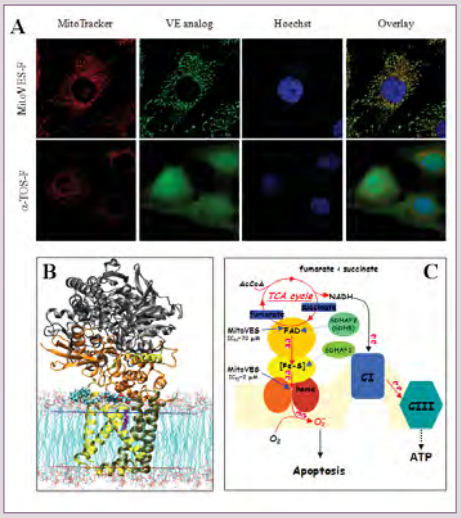

Fig 3: Mitochondrial localisation of MitoVES, its interaction with CII and the molecular mechanism of its effect on CII. A. Mouse breast cancer NeuTL cells were incubated with MitoTracker Red and fluorescently labelled MitoVES or α-TOS and inspected by confocal microscopy. Hoechst 33342 was used to visualise the nuclei. B. Molecular modelling indicated the position of MitoVES at the interface of the MIM and matrix components of CII. C. MitoVES inhibits both SDH and succinate quinine reductase (SQR) activity of CII, the former with IC_{50} ~ 70 μM, the latter with IC_{50} ~ 2 μM. Due to this scenario, the conversion of succinate to fumarate occurs in the presence of MitoVES, albeit at a lower rate, generating electrons that are forced to transverse to the membrane components of CII. In the situation when UbQ is displaced from the Q_P site in CII, electrons lack their natural acceptor and recombine with molecular oxygen to give rise to superoxide. This then triggers a series of reactions that result in the induction of the apoptotic cascade, resulting in the demise of the cell.

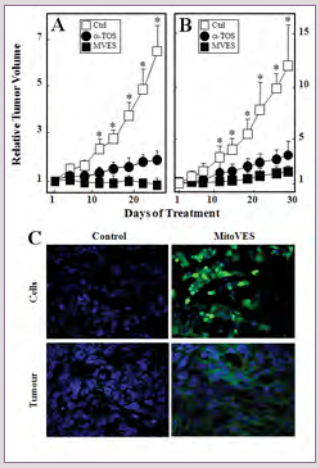

Fig 4: MitoVES efficiently suppresses tumours and induces pseudohypoxia in cancer cells and in tumours. FVB/N c-neu transgenic mice with spontaneous breast carcinomas (A) and nude mice with xenografts derived from colorectal HCT116 cells (B) were treated with α-TOS and with MitoVES at 10-fold lower doses than that of the former. Ultrasound imaging documents a superior activity of MitoVES over its untargeted counterpart. C. HCT116 colon cancer cells were stably transfected with the ODD-GFP gene-containing plasmid. The cells were exposed to 5 μM MitoVES for 12 h and green colour, indicative of the accumulation of the chimeric ODD-GFP protein observed using confocal microscopy (upper images). Sections from HCT116ODD-GFP-derived tumours treated with MitoVES were inspected by confocal microscopy, revealing green fluorescence. The in vitro and in vivo experiments document that the mechanism by which MitoVES induces apoptosis is similar in both settings.

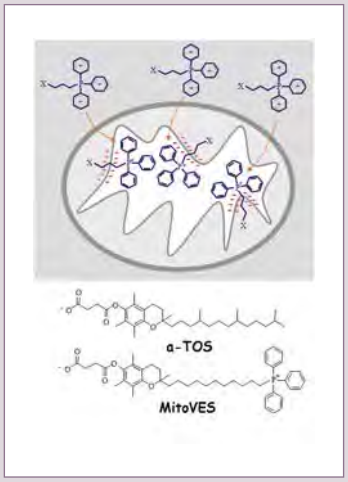

Fig 5: Principle of targeting to mitochondria. Hydrophobic compounds are modified by tagging with the positively charged triphenylphosphonium (TPP). At the neutral and acidic pH, the charge is delocalized on the flanking phenyl groups, therefore the agent can freely move across membranes. Once in the matrix, the negative potential at the matrix phase of the mitochondrial inner membrane (MIM) causes docking the compound across the interface of the MIM and the matrix, considerably increasing its concentration in mitochondria. Below are shown structures of α-tocopheryl succinate and its mitochondrially targeted homologue with some 20-50-fold high toxicity towards cancer cells.

Grant Agency of the Czech Republic:
GAP301/10/1937 Mechanisms of efficient killing of cancer stem cells. 2010-2013.

Karel Vališ
The Grant Agency of the Academy of Sciences of the Czech Republic:
KJB500970904 Molecular mechanism of apoptosis induced by alfa-tocopheryl succinate in cancer cells. 2009-2010.

Jakub Rohlena
Grant Agency of the Czech Republic:
GAP301/12/1851 Induction of apoptosis in cancer cells by mitochondria-specific compounds; the role of respiratory complex II. 2012-2014.

Jaroslav Truksa
Grant Agency of the Czech Republic:
GAP305/12/1708 Modulation of mitochondrial functions in cancer cells by the mitochondrially targeted vitamin E analogues. 2012-2014.

Laboratory of Gene Expression

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RESEARCH TOPICS

The Laboratory of Gene Expression is a Europe's leading academic laboratory specialized in high-throughput gene expression profiling and single-cell analysis using real-time quantitative PCR (qPCR). We participate in several basic research projects in the field of developmental biology and stem cells, several applied projects of clinical and medical relevance, in particular in cancer and neurological research, and we are involved in development of methods and applications including standardization.

Our developmental research uses the model organism *Xenopus laevis*. *Xenopus* has many advantages such as outer fertilization and outer embryonic development, inducible ovulation, large size of oocytes, large number of ovulated oocytes, transparent embryos and convenient conditions for breeding. Measuring temporal profiles of key developmental biomolecules (microRNAs, mRNAs and proteins) in individual blastomeres in the earliest developmental stages (1 to 64 cells) we can monitor the formation of gradients that underlie differentiation and eventually lead to the formation of body axis. We developed qPCR tomography to measure gradients even within cells and revealed that several mRNAs are polarized already in the original oocyte. The *Xenopus* oocyte has two differentially coloured hemispheres called animal and vegetal. The oocyte was cryo-sliced into 30-µm thin slices along the animal-vegetal axis, mRNA was isolated, cDNA prepared and quantified with qPCR. We observed three groups of maternal mRNAs, with different polarizations in the oocyte. One group of mRNAs was most abundant in the animal hemisphere. The other two groups were more abundant in the vegetal hemisphere, with the mRNAs in the third group being exclusively found in the most distal part of the vegetal hemisphere. The gradients remain throughout the initial cell divisions.

We study the role of glial cells during development and repair. Glial cells are implicated in neurodevelopment, synapse formation, neuroprotection, regenerative mechanisms after CNS injury, and the maintenance of extracellular ionic and transmitter homeostasis. Astrocytes is the main glial cell type in the brain and is involved in the maintenance of the blood-brain barrier, regulation of water and ionic homeostasis the metabolism of amino acid neurotransmitters, as well as providing energy and nutrient support to neurons. Using single cell expression profiling we found several distinct astrocytic populations to be present in the mouse cortex that seem to correlate to the extent of cell swelling during oxygen-glucose deprivation. Using the inhibitors we studied the contribution of Cl- and K+ channels, co-transporters and excitatory amino acid transporters to oxygen-glucose deprivation induced changes of the astrocytic volume. We found two main astrocytic subpopulation that differ in expression of inwardly rectifying K^+ channels (Kir4.1), K(2P) channels (TREK-1 and TWIK-1), and Cl^- channels (ClC2). Using mouse model, collecting astrocytes at 10, 20, 30 and 50 days after birth and 3, 7 and 14 days after focal cerebral ischemia for profiling we found transcriptional fully mature astrocytes express mainly Eaat1, Glul, Aqp4 and Kcnj10. After a brain injury the situation rapidly changes: the astrocytes are reactive and express Eaat1, Glul, Aqp1, Aqp9, Snap25, hyperpolarization-activated cation channels Hcn1, Hcn2, Hcn3, glutamate receptors Grin2a, Gria2, Gria3, Grm5 and potassium channels Kcna3.

We have developed SOP for expression profiling of cancer cells extracted from circulation (blood) and from primary tumours. A multivariate model for the analysis of expression results has been developed and is evaluated for use in therapy. The presence of the tumour cells in circulation is a very poor prognostic factor for patients, indicating metastasis, and characterization of the tumour cells by means of expression profiling can have significant value as a therapy indicator. By expression profiling we can identify

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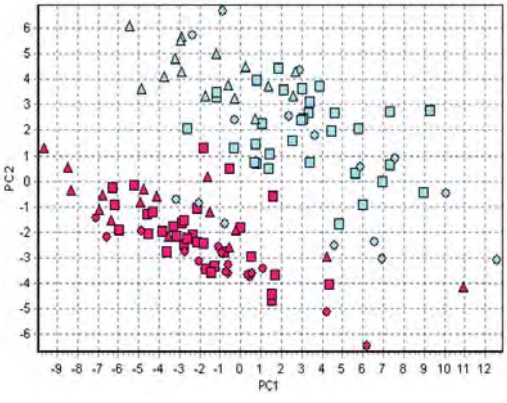

Fig 1: Principal component analysis of single blastomeres. The animal blastomeres (in red) have distinct localization then the vegetal blastomeres (in blue). In total 4x8 cell stage (triangles), 4x 16 cell stage (squares) and 1x 32 cell stage (circles) of Xenopus leavis embryos were analysed.

Laboratory of Gene Expression

Fig 2: Model organism Xenopus leavis.

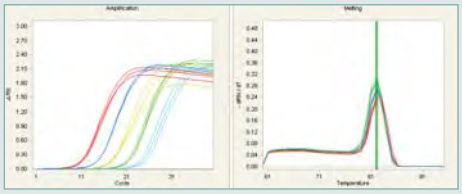

Fig 3: Standard curve of YWHAZ gene and melting curve analysis.

cancer stem-like cells, which are more aggressive because of their ability to differentiate and their higher resistance to drugs. Currently, in collaboration with AdnaGen and TATAA Biocenter we are evaluating a panel of 24 markers for the profiling and characterization of circulating tumour cells that will be used to guide treatment.

The laboratory is also part of the European project SPIDIA (www.spidia.eu) developing methods for optimization of the pre-analytical processes of molecular diagnostics. Our contributions consist in qPCR analysis of mRNA in blood and tissue samples. Several studies report that degradation and deregulation processes take place during the pre-analytical procedures when the sample is prepared for analysis, and the measured profiles are influenced by the handling of the samples rather than by the underlying biology. Currently, no reliable biomarkers are available to identify the changes that occur during the pre-analytical process. We have monitored and validated a set of biomarker candidates to assess the pre-analytical variation of mRNA levels in the blood samples collected in EDTA and PAXgene tubes that would reflect degradation/deregulation.

Our laboratory also develops working cooperations with other institutes, for example Institute of Experimental Medicine. In our mutual project, we investigate functional and molecular markers of DNA repair in tumor and healthy tissues from a group of patients with sporadic forms of colorectal carcinom. An important aspect of the project is to clarify the abnormal epigenetic profile (methylation) of tumour cells in the concomitance with their genetic constitution, which will improve our understanding of the different sporadic colorectal carcinom phenotypes.

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Slyskova, J., Korenkova, V., Collins, A. R., Prochazka, P., Vodickova, L., Svec, J., Lipska, L., Levy, M., Schneiderova, M., Liska, V., Holubec, L., Kumar, R., Soucek, P., Naccarati, A., Vodicka, P. Functional, Genetic, and Epigenetic Aspects of Base and Nucleotide Excision Repair in Colorectal Carcinoma. *Clinical Cancer Research* 2012 Oct 19. ISSN 1078-0432.

GRANT SUPPORTS

Mikael Kubista
The Grant Agency of the Academy of Sciences of the Czech Republic:
IAA500520809 Mechanisms of embryonic stem cells early differentiation. 2008-2010.

Grant Agency of the Czech Republic:
GA301/09/1752 Formation of spatiotemporal molecular gradients in early development of Xenopus laevis. 2009-2011.
The Grant Agency of the Academy of Sciences of the Czech Republic:
IAA500970904 MicroRNA expression during early development of Xenopus laevis and mRNA degradation. 2009-2011.

Grant Agency of the Ministry of Health of the Czech Republic:
NS9976 Gene expression profiling in cancer circulating cells (CTCs) in breast carcinoma patients - a tool for early metastasis detection and therapy individualisation. 2009-2011.

Ministry of Education, Youth and Sports:
7E09019 Standardisation and improvement of generic pre-analytical tools and procedures for in vitro diagnostics. 2009-2012.

Grant Agency of the Czech Republic:
GAP303/10/1338 Cell volume regulation in glial cells during brain ischemia/reperfusion. 2010-2012.

Ministry of Education, Youth and Sports:
ME10052 Cellular Expression Signatures in Idiopathic Pulmonary Fibrosis. Ministry of Education, Youth and Sports. 2010-2012.

Ministry of Education, Youth and Sports:
CZ.1.07/2.3.00/30.0045, Biotechnological expert in structural biology and gene expression, OP Education for Competitiveness, 2012 – 2015.

Vlasta Korenková
Grant Agency of the Czech Republic:
GAP304/12/1585 Molecular DNA repair characteristics in colorectal tumor tissue. 2012-2015.

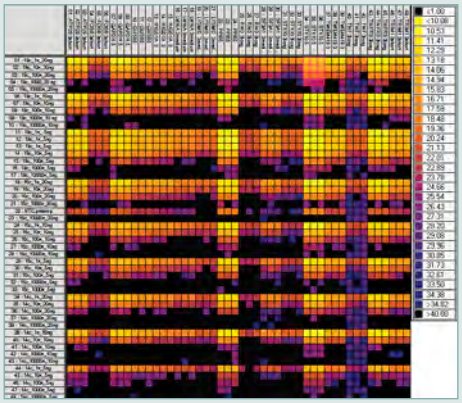

Fig 4: Gene expression results may be viewed as a heat map showing 2,304 reactions per run.

Laboratory of Reproductive Biology

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RESEARCH TOPICS

Fertilization is a highly specialized interaction between gametes that culminates in the formation of a zygote and development of a new individual of the species. The sperm-egg interactions in mammals consist of a series of specialized and regulated events that initially involve egg-induced activation of the spermatozoon and ultimately result in the sperm-induced activation of the egg. Sperm motility, metabolism, capacitation and acrosome reaction are modulated by factors associated with the egg, its acellular or cellular investments, or fluids bathing both the male and female reproductive tracts. Sperm and zona pellucida proteins participate in the highly specialized interaction between the gametes.

Our group has focused on studying the molecular mechanism of fertilization since 1990. We have investigated the role of cell surface and acrosomal proteins of mammalian spermatozoa during capacitation, acrosome reaction and binding of the sperm to the glycoprotein network of the oocyte's zona pellucida. Other studied aspects included the unique regulation of protein tyrosine phosphorylation of mammalian sperm proteins during capacitation, identification and localization of these proteins and activators in mammalian spermatozoa and their connection with the cytoskeleton, and the motility of sperm. Another interesting biomodel for studying the fertilization strategy is the fish model (chondrostean and teleostean fish).

Recent studies indicate that more than 15 % of human population suffer from infertility-related problems. Thorough elucidation of the mechanisms of fertilization is thus crucial for all centres of assisted reproduction, where 2 % of children are now born, and the numbers are expected to further increase. Our laboratory deals with the molecular mechanisms of fertilization, namely with the nature of sperm proteins playing a role in fertilization. During the last 10 years we discovered a number of new proteins that are fundamental for the sperm-oocyte binding and we developed methods and molecular tools (monoclonal antibodies and kits) for their study. Many of them are also used in the centres of assisted reproduction and have been successfully commercialized.

The decreasing quality of human environment has a negative influence on animals, including humans. Our project „The Centre of Molecular Methods for Monitoring the Diffuse Pollution of the Environment" was focused on the new systems for monitoring the diffuse environmental pollution. The diffuse pollution means long-lasting contamination of the environment with very low concentrations of pollutants, residues of a variety of anthropogenic activities, together with a clearly proved negative influence on the health and reproduction of mammals. The central focus was mainly on substances with endocrine activity, such as hormonal contraceptives, certain medications including antibiotics, and substances used in detergents and paints. The monitoring system is based on the determined impact of selected pollutants on both mammalian organisms *in vivo* (reproductive organs, gametes, expression of selected genes and reproduction) and cells *in vitro* (toxicity, viability and function). In cooperation with commercial companies, several kits for the detection of these substances have been developed. The direct effect of selected substances on the estrogen receptors will be elucidated in the testes and sperm.

Recent data suggest that the changes in gene expression and deleterious effects of the environmental pollutants on the male reproductive function are directed via epigenetic changes rather than direct genetic modifications. Identification of the epigenetic mechanisms involved in the changes of gametogenesis-related gene expression will lead to better understanding of male reproduction and prevention of increasing infertility.

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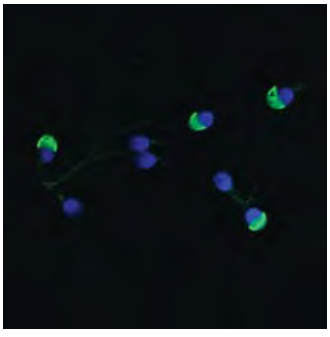

Fig 1: Immunofluorescence staining of the acrosome in the human sperm obtained with monoclonal antibody Hs-14.

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Ded, L., Dostalova, P., Dorosh, A., Dvorakova-Hortova, K., Peknicova, J. Effect of estrogens on boar sperm capacitation in vitro. *Reproductive Biology and Endocrinology*, 8:87, 2010. doi: 10.1186/1477-7827-8-87. ISSN 1477-7827.

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GRANT SUPPORTS

Jana Pěknicová

Grant Agency of the Czech Republic:
GA524/06/0817 Ultrastructure, Energetic and Competition in Spermatozoa: A Comparative Study Using Two Model Species of Chondrostean and Teleostean Fishes. 2006-2008.

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NR8932 Differential expression of Pregnancy Associated Plasma Protein - A (PAPP-A) and Insulin-like growth factor-binding protein 4 (IGFBP4) in follicular fluid and blood of women in correlation with ovarian hyperstimulation syndrome (OHSS). 2006-2008.

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Eureka project: OE 211 ELISA kits for detection of environmental quality. 2006-2008.

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Ministry of Education, Youth and Sports:
Center: 1M06011 The Centre of Molecular Methods for Monitoring the Diffuse Pollution of the Environment. 2006-2011.

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GD523/08/H064 Biotechnologies of mammalian gametes. 2008-2011.

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NS10009 Expression of genes and proteins playing a role in the human reproduction - damage biomarkers. 2009-2011.

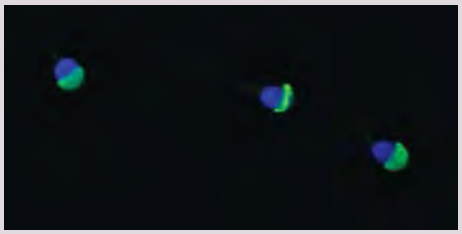

Fig 2: Immunofluorescence staining of the acrosome in the boar sperm obtained with monoclonal antibody Hs-8.

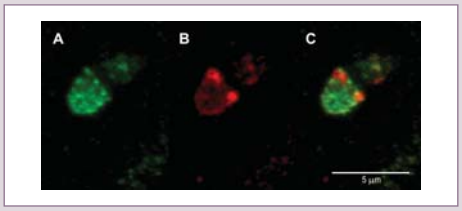

Fig 3: Double staining of acrosomal and cytoskeletal proteins in boar sperm head. Immunofluorescence with monoclonal antibodies Hs-14 against acrosome proteins (green) - (A), TU-12 against beta-tubulin (red) – (B) and co-localization with both antibodies (C).

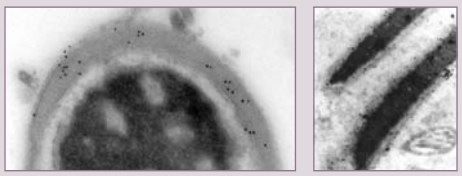

Fig 4: Boar sperm acrosomal proteins in electron microscopy. Black dots in the sperm head (acrosome).

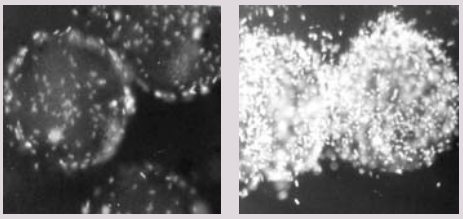

Fig 5: Sperm – egg binding. Monoclonal antibody against binding proteins blocks sperm attact to oocyte (left side). Control without antibody (right side).

Grant Agency of the Czech Republic:
GA523/09/1793 Effect of endocrine disruptors on reproductive parameters and expression of selected genes in mouse gonads. 2009-2012.

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P503/12/1834 Identification of epigenetic biomarkers of male germ cell disorders linked to adverse environmental factors. 2012 – 2015.

Jonáková Věra
Grant Agency of the Czech Republic:
GA303/06/0895 Characterization of proteins secreted by the male reproductive tract and their role in individual steps of the reproduction process. 2006-2008.

Maňásková-Postlerová Pavla
Grant Agency of the Czech Republic:
GA303/09/1285 Study of the sperm membrane proteins with zona pellucida binding activity. 2009-2011.

Dostálová Pavla
Grant Agency of the Charles University:
GAUK 151-43-253149. Identification and characterization of estrogenic receptors in testes and sperm. 2012-2014.

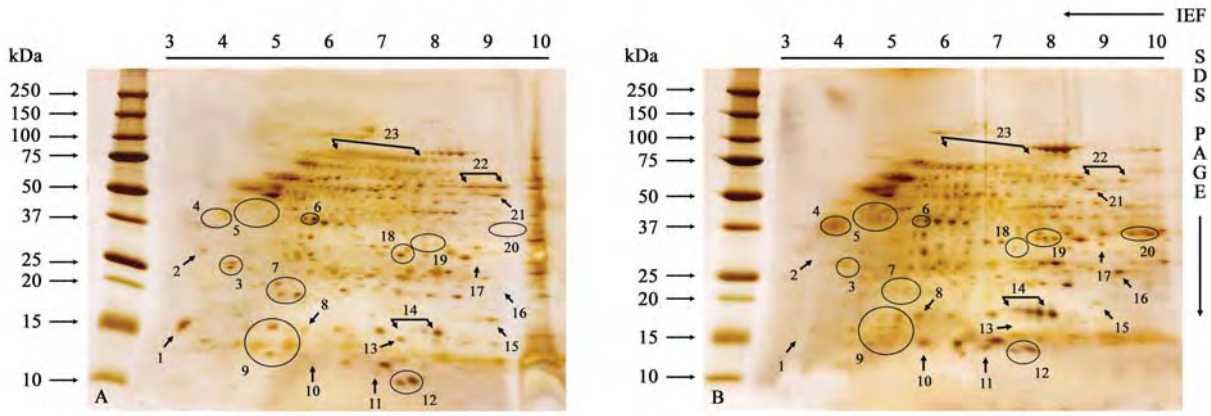

Fig 6: 2D protein profile of ejaculated (A) and capacitated (B) sperm isolated from the sperm surface. Corresponding arrows indicate qualitative and quantitative differences between profiles of ejaculated and capacitated sperm.

Laboratory of Molecular Pathogenetics

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RESEARCH TOPICS

Our research program is focused on the molecular mechanisms in pathologies associated with diabetes mellitus. We use animal models and gene expression profiling to identify molecular targets for the development of preventive and diagnostic strategies.

The overarching goal of our research has been to identify key molecular players in cardiovascular pathologies caused by the exposure to diabetes. We have analysed changes induced by intrauterine diabetic environment in the developing embryonic heart. Diabetes-exposed embryos showed an increased incidence of cardiac malformations by 28 % compared to embryos from non-diabetic pregnancies. Using the mouse as an experimental system and global gene expression profiling, we have identified target genes which can serve as an indicator for specific abnormalities in the heart development and function, and genes contributing to developmental heart defects in diabetic pregnancies and heart dysfunctions in the adult.

We demonstrated that exposure to maternal diabetes resulted in dysregulation of the hypoxia-inducible factor 1 (HIF-1) pathway in the developing embryo. For the first time, we linked HIF-1-regulated pathways and the development of congenital malformations in diabetes-exposed embryos. We showed that the environmental (maternal diabetes) and genetic (*Hif1a* mutation) factors reduce HIF-1 activity in embryos and result in cardiovascular malformations. Results of this research were selected for the platform presentation at the 49th Annual Meeting of the Teratology Society, Puerto Rico, 2009; and the 52nd Annual Meeting of the Teratology Society, USA, 2012.

Additionally, we have analysed the role of HIF-1 pathways in response to the conditions of hypoxia in the adult heart. We have performed gene expression profiling of *Hif1a* partially deficient adult mutant mice. In contrast to non-mutant mice, sustained hypoxia activates the transcriptional responses of the majority of analysed genes in the *Hif1a* mutant left ventricle (LV). Our results show that gene expression was differently regulated in the right and left heart ventricles and that it was significantly affected by hypoxia, gender, and *Hif1a* partial deficiency.

Subsequently, we were interested in the early molecular and physiological changes induced by diabetes and a possible role of HIF-1 pathways in cardiac responses to diabetes in the adult heart. We investigated the cardiac responses to diabetic conditions, including changes in LV echocardiographic parameters, tissue remodelling, and transcriptional profile modulations in the adult heart. Diabetes was associated with significant increase in LV systolic diameter in the *Hif1a* heterozygous mutant group only. To explore the tissue-specific changes induced by diabetes, we analysed the expression of 13 selected genes in the LV of the heart. The expression levels of mRNA of *Vegfa* were significantly affected by genotype in the LV of the diabetic heart. Since our RT-qPCR analysis demonstrated a significant effect of genotype on the *Vegfa* mRNA expression, we analysed the cardiac expression of VEGF-A, a key HIF-1 target gene product. We showed that diabetes decreased VEGF-A protein levels in the coronary vessels of *Hif1a* mutant hearts compared to controls. For the first time, we showed that HIF-1 pathways are involved in the early manifestation of pathological changes induced by diabetic environment in the heart. Furthermore, impaired HIF-1 regulation accelerated the progress of pathological changes in the diabetic heart. Our observations are in line with increasing evidence that the HIF-1-regulated system is compromised in the diabetic heart.

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Zuzana Hampejsová, BSc / Master Student
Lada Škvorová / Technician

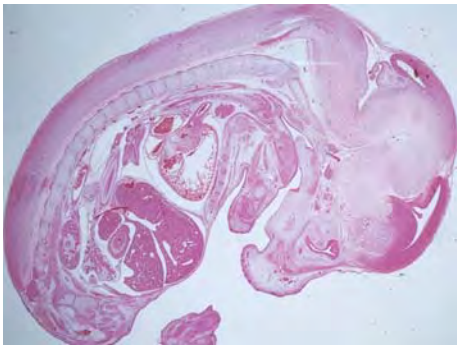

Fig 1: Longitudinal section of E14.5 embryos, stained with hematoxylin and eosin.

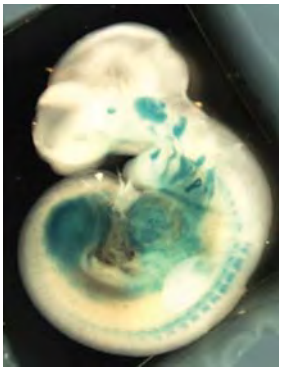

Fig 2: Whole mount: LacZ staining of Isl1-Cre transgene in E10.5 embryos.

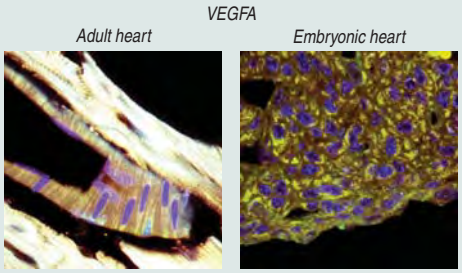

Fig 3: Confocal imaging of transverse sections of adult and E14.5 embryonic heart stained with anti-VEGFA antibody (green), Hoechst 33342 (blue) as a nuclear counterstain.

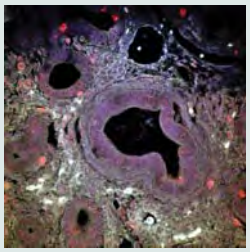

Fig 4: Confocal imaging of cellular proliferation at E14.5 embryos with anti- phospho-histone H3 antibody to detect mitotic cells (red).

Fig 5: Heat map: Global gene expression profile of control (2) and diabetes-exposed E10.5 embryos (5) analyzed by microarrays. Each colored vertical line represents the expression signal for one gene and each column represents individual embryo. Pavlinkova et al. BMC Genomics 2009, 10:274.

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Salbaum, J.M., Kruger, C., Zhang, X., Delahaye, N.A., Pavlinkova, G., Burk, D.H., Kappen, C. Altered gene expression and spongiotrophoblast differentiation in placenta from a mouse model of diabetes in pregnancy. *Diabetologia*, 54: 1909-1920, 2011. ISSN 0012-186X.

GRANT SUPPORTS

Gabriela Pavlínková

IRG Reintegration Grant:
224760, Molecular Mechanisms in Diabetic Embryopathy, Marie Curie Actions – Seventh Research Framework Programme People. 2008-2012.

Grant Agency of the Czech Republic:
GA301/09/0117, Molecular Mechanisms in Diabetic Embryopathy. 2009-2013.

Ministry of Education, Youth and Sports:
CZ.1.07/2.3.00/30.0020, Biotechnological expert, OP Education for Competitiveness. 2012 – 2015.

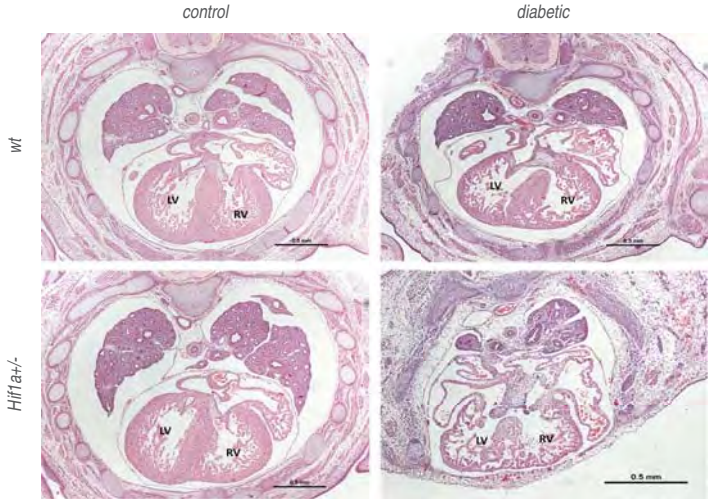

Fig 6: Morphological changes in embryos exposed to maternal diabetes. Transverse sections of E14.5 thorax of Wt and Hif1a$^{+/-}$ demonstrated an increased rate of cardiovascular defects in diabetes-exposed embryos, including ventricular septal defects and thin myocardial walls. RV, right ventricle; LV, left ventricle.

Laboratory of Immunopathology and Immunotherapy

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RESEARCH TOPICS

Our research is focused on the study of development of immunopathologies associated either with insufficient function of the immune system (immunodeficiencies) or with too strong activity targeting the body's own structures and cells (autoimmune diseases). The object of our study is to identify the molecules or cell populations playing a role in the development of these diseases, with the purpose to develop therapeutics specifically modulating the pathological activity of autoreactive cells or triggering reparative processes in the tissues.

One of our particular goals is immunomodulation of the pathological immune response in systemic lupus erythematosus (SLE), which represents a prototypic autoimmune disease associated with production of pathological autoantibodies against self DNA (anti-dsDNA).

Curvent therapy is non-specific and affects also important natural physiological functions of the immune system. Protective immunomodulatory effects of modified de-lipidated bacterial lipopolysaccharide (dLPS) have been shown in patients with severe sepsis, which may implicate its possible application in autoimmune diseases.

Our research is aimed at specific inhibition of pathological functions of autoreactive B cells in the mouse model of SLE (and *in vitro* in cultured peripheral blood mononuclear cells of patients with SLE).

The inhibitory effect is based on the usage of dLPS covalently bound to a specific peptide that mimics ds-DNA as the main autoantigen in SLE. This peptide is composed of 10 amino acids and it was shown that its central DWEYS motif is specifically recognized by anti-dsDNA autoantibodies, and therefore it is called DNA mimetope.

The advantage of our approach is usage of dLPS and DNA mimetope just within one and the same molecular complex. The expected imunotherapeutic effect might cause modulation and/or full elimination of the pathological autoimmune reaction, for instance due to induction of anergy of B lymphocytes carrying immunoreceptors specific for the DNA mimetope and reduction of serum titres of autoantibodies.

Another major topic of our research is more associated with direct clinical application in the field of autoimmune diseases. Anti-TNF therapy represents a powerful therapeutic tool currently used in clinical rheumatology to cure patients with the refractory form of systemic rheumatic diseases such as rheumatoid arthritis or juvenile idiopathic arthritis. However, up to 10 % of patients may display resistance to this therapy and moreover the reaction cannot be anticipated using the standard clinical approach.

We identified a unique population of peripheral plasma cells characterized as $CD19^{+}CD20^{-}CD27^{high}CD38^{+}CD138^{+}$. Subsequently, significant differences in the frequencies of these plasma cells between patients responding and resistant to anti-TNF therapy have been demonstrated. All responders had at least 13-fold reduced frequencies of these cells on day 0 in comparison to non-responders and this was also associated with significant improvement of the clinical parameters.

Thus, these specific plasma cells represent biological markers predicting the outcome of anti-TNF therapy and their evaluation might serve as an effective and inexpensive marker usable in clinical practice.

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Zuzana Kerdíková MSc / PhD Student

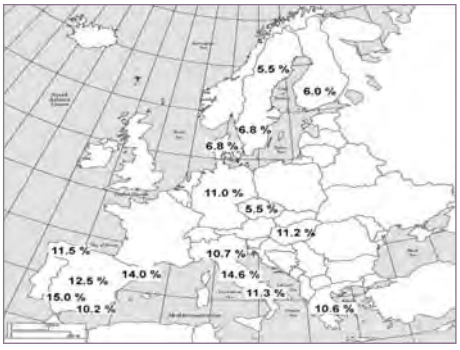

Fig 1: The frequency of PD3.1 A allele in Europe displays geographical gradient.
Development of autoimmune diseases such as systemic lupus erythematosus (SLE) is partly determined by the presence of mutations in genes. One of example could be the gene for programmed cell death 1 (PDCD1) and mutation which causes replacement of guanine (G) by adenine (A) (called PD1.3 G/A polymorphism).

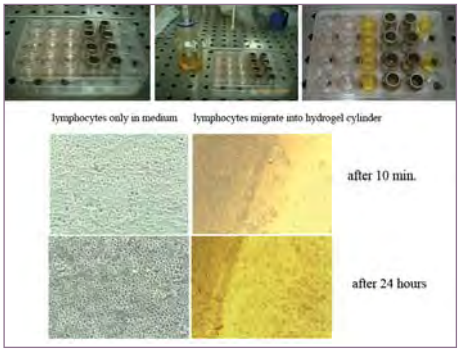

Fig 2: Migration of human leukocytes into polymeric hydrogel
Autoimmune diseases such as rheumatoid arthritis is associated with destruction of inflamed joint.

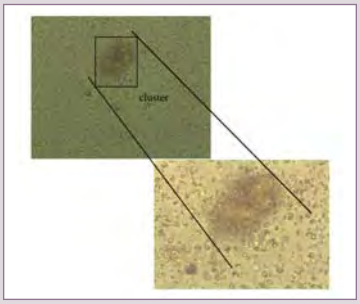

Fig 3: Human leukocytes form follicular-like structures in polymeric hydrogel
It is known that majority of cells of the immune system gain their functional competence within follicular structures known as germinal centers which are in the body located in the spleen, liver or lymh nodes. It would be a great advantage for immunology research to have an experimental system which can mimic the process ongoing in these centers in vitro.

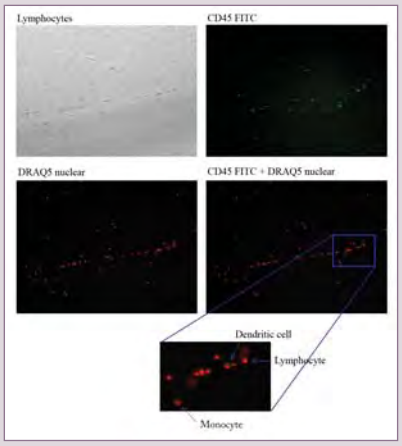

Fig 4: Human leukocytes entering polymeric hydrogel
Human leukocyte population is composed of many cell types that are known to participace in immune response to antigenic challenge and form lymphoid follicles within lymph nodes. Polymeric hydrogel might represent suitable environment where such follicles could be generated in vitro.

We are now planning to verify our findings in a larger cohort of patients and to standardize the detection method of the observed plasma cell population for laboratories in the Czech Republic. Another output is providing clinicians with a very fast, informative and inexpensive test in order to include into anti-TNF therapy only those patients who will respond and to improve clinical care by its individualization.

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GRANT SUPPORTS

Šárka Růžičková

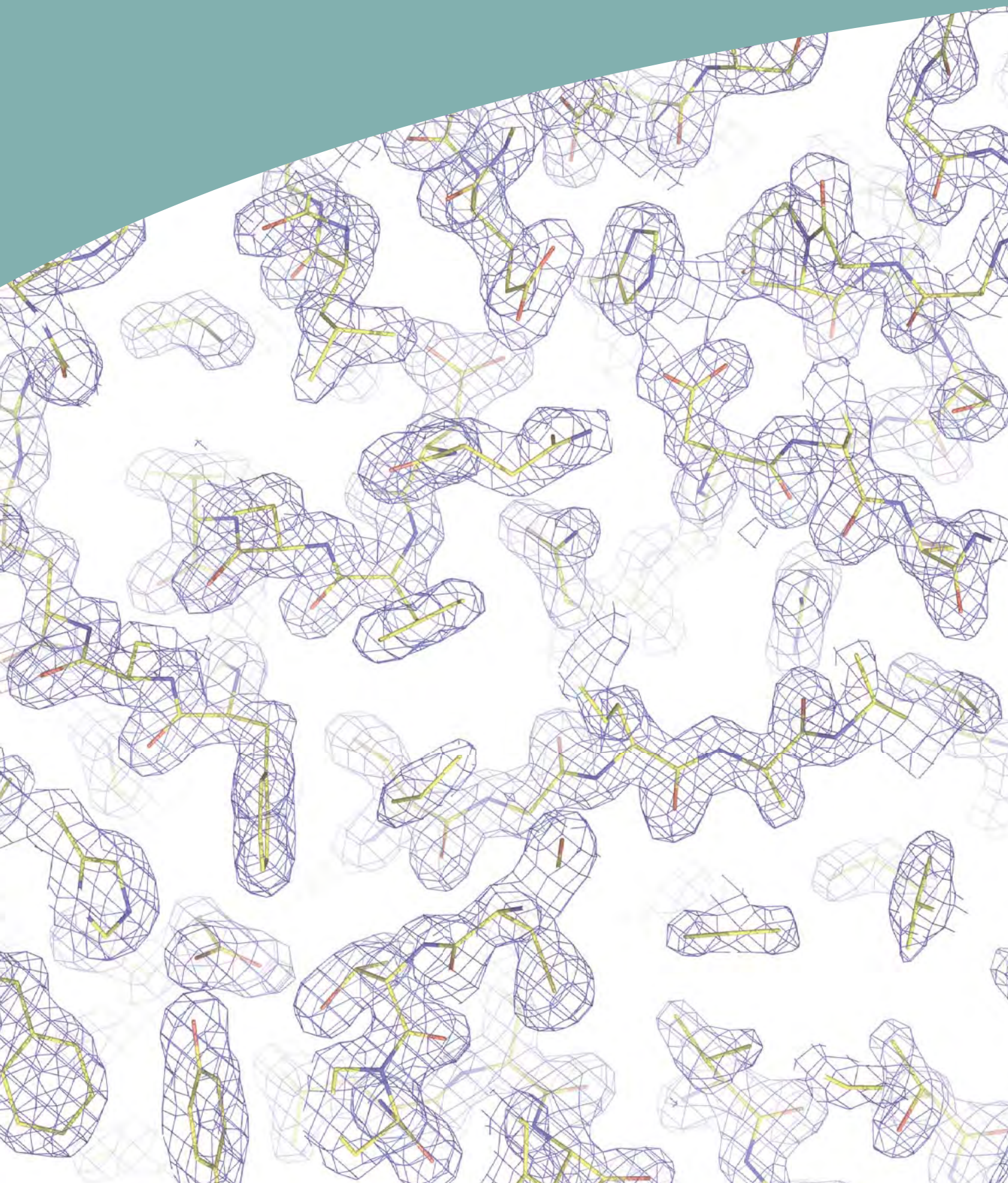
Grant Agency of the Czech Republic:
GA310/06/0477 The role of NK and NKT cells in the development and pathogenesis of rheumatoid arthritis. 2006-2008.

Ministry of Health of the Czech Republic:
NT11414-5 Disturbances of differentiation of B-lymphocytes in patients with defects of humoral imunity. 2010-2014.

Roche s. r.o.:
12-393 Identification of population of plasma cells as a marker predicting response to anti-TNF therapy. 2012 – 2013.

Bristol-MyersSquibb spol. s r.o.:
61169 Workshops and mini-courses organization focused on usage of scientific, methodological and clinical approaches for detection of a unique population of plasma cells as predictor marker for outcome of anti-TNF therapy. 2012 – 2013.

Structural Biology and Protein Engineering



Laboratory of Biomolecular Recognition

Bohdan Schneider

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http://www.structbio.eu/BS

RESEARCH TOPICS

Recognition between biological macromolecules (proteins and nucleic acids) is an important step in all biological processes. We study interactions between these molecules with the goal to understand the mechanisms of their mutual recognition by combining computer approaches of bioinformatics and molecular modelling with biophysical and crystallographic experimental studies to characterize the interactions at the submolecular level.

Bioinformatic database studies concentrate on systematic comparison of structures of the analysed molecules and understanding of their conformational behaviour. We rely on expert use of publicly available database resources, principally the Protein Data Bank (PDB, http://www.rcsb.org/) and GenBank (http://www.ncbi.nlm.nih.gov/genbank/) and also some other more specialized databases, e.g. from a wide spectrum of services available at the European Bioinformatics Institute. For the energy-based molecular modelling, we use public servers and services available to academic users.

Biophysical measurements combine spectrophotometric and thermodynamic techniques and detailed structural information of the studied molecules is provided by crystallographic analysis.

In all our studies, we concentrate on those aspects of the interactions that lead to specific recognition of molecules with potential diagnostic, medical or biotechnological use.

Studied systems and processes

- Bioinformatic analysis and computer study of the structure, dynamics, and solvation of nucleic acids and proteins and interactions in their complexes
  - o structural aspects of the protein/DNA interface classified by unique bioinformatic tools
- Analysis and engineering of protein-protein and protein-DNA interactions, namely
  - o increasing the affinity of receptor of human interferon γ towards human interferon γ by rational design
  - o analysis of interactions between bacterial transposases and single-stranded DNA

Methods

- Bioinformatics
  - o analysis of data from databases available in the public domain as the Protein Data Bank
  - o molecular modelling and computer simulations of biomacromolecules
    - we apply techniques of empirical potential, such as molecular dynamic and *in silico* mutation as well as quantum mechanics
- Biophysical methods of characterization of biomolecules and their interactions
  - o thermodynamic techniques such as surface, microcalorimetry plasmon resonance (SPR)
  - o spectroscopy (circular dichroism) and dispersion techniques (DLS)
- Crystallography
- Proteins for most of our studies are also expressed and purified in the laboratory.

Equipment used in the lab

Facilities for expression and purification of prokaryotic & eukaryotic proteins including a room for eukaryotic tissue cultures

Crystallization room operated at 18 °C, equipped with a crystallization robot

Diffractometer Rigaku equipped with rotating anode, image plate detector and cryo-protection

MEMBERS

of the Laboratory of Biomolecular Recognition

Bohdan Schneider, Dr / Head of Laboratory

Lada Biedermannová, PhD / Research Scientist – maternity leave

Jiří Černý, PhD / Research Scientist

Pavel Mikulecký, MSc / PhD Student

Jaroslav Nunvář, MSc / PhD Student

Iva Zusková, MSc / PhD Student

Jiří Zahradník, MSc / Diploma Student

Jan Dohnálek, PhD / Research Scientist (partial affiliation)

Ivan Slabý, Dr / Research Scientist (partial affiliation)

Jiří Vondrášek, Dr / Research Scientist (partial affiliation)

Karel Pufler, Msc / Technician

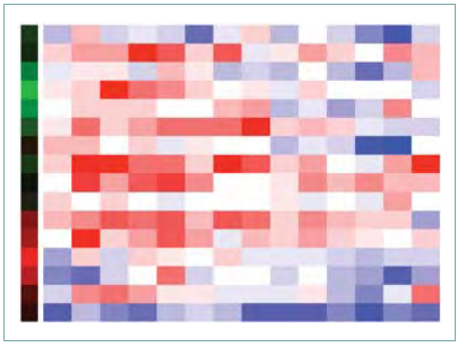

Fig 1: Interactions between proteins and DNA described by bioinformatic tools. Displayed are correlations between peptide conformers, so called peptide blocks (plotted on the vertical axis) and dinucleotide conformers (horizontal axis) that form direct polar contacts. Figure displays relative abundance of protein-DNA contacts formed between a particular peptide and DNA conformer. Red indicates statistical over- and blue under-representation. The distributions are plotted for structures of protein/DNA complexes determined with crystallographic resolution between 1.90 and 2.80 Å [Schneider, Černý, Svozil, Gelly & de Brevern: in preparation (2013)].

SPR measurements on the campus and in collaboration with Prof Homola, Institute of Photonics & Electronics
Measurements of dispersity of the studied systems by dynamic light scattering (DLS) in the laboratory.
The computer cluster (80 CPUs in total) with the necessary molecular modelling software.

SELECTED PAPERS

Neidle, S., Schneider, B., Berman, H. M. Fundamentals of DNA and RNA structure. In Gu, J., Bourne, Philip E. (ed.). *Structural Bioinformatic Fundamentals of DNA and RNA structure 3.* USA: Wiley-Blackwell, 41-76, 2009. ISBN 978-0-470-18105-8.

Joseph, A.P., Agarwal, G., Mahajan,, Gelly, J.Ch., Swapna, L., Offmann, B., Cadet, F., Bornot, A., Tyagi, M., Valadié, H., Schneider, B., Etchebest, C., Srinivasan, N., de Brevern, A.G. A short survey on protein block *Biophysical reviews*, 2: 137-147, 2010. ISSN 1867-2450.

Kratochvilova, I., Todorciuc, T., Kral, K., Němec, H., Buncek, M., Sebera, J., Zalis, S., Vokacova, Z., Sychrovsky, V., Bednarova, L., Mojzes, P., Schneider, B. Charge transport in DNA oligonucleotides with various base-pairing pattern *Journal of Physical Chemistry B*, 114: 5196–5205, 2010. ISSN 1520-6106.

Benda, L., Schneider, B., Sychrovsky, V. Calculating the Response of NMR Shielding Tensor .sigma.(31P) and 2J(31P,13C) Coupling Constants in Nucleic Acid Phosphate to Coordination of the Mg2+Cation. *Journal of Physical Chemistry A*, 115: 2385-2395, 2011. ISSN 1089-5639.

GRANT SUPPORTS

Bohdan Schneider

Ministry of Education, Youth and Sports:
1) MEB021032 Complex structural analysis of interaction at the protein – nucleic acid interface by unique bioinformatic descriptors. 2010-2011.

Grant Agency of the Czech Republic:
2) GAP305/10/2184 Structure-function relationships underlying protein-protein interactions. 2010-2014.

Grant Agency of the Czech Republic:
3) GAP305/12/1801 Molecular mechanisms of association of a novel type of transposase with repetitive palindromic elements. 2012-2014.

Jiří Vondrášek

Grant Agency of the Czech Republic:
GAP302/10/0427 Production and characterization of biologically active recombinant human ameloblastin -a hard tissue regeneration and differentiation inducing protein. 2010-2012.

Lada Biedermannová

Grant Agency of the Czech Republic:
GAP205/12/P729 Effect of protein hydration shell on the stability of protein complexes. 2012-2014.

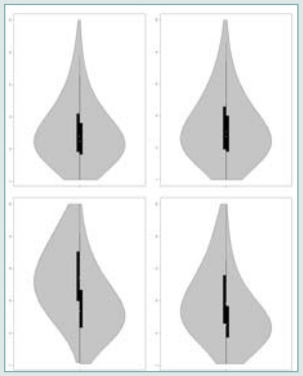

Fig 2: Distributions of crystallographic B-factors (that measure vibrations of atoms) at and outside the protein/DNA interface. Each "violin plot" shows two distributions of B-factors, outside the interface on the left and at the interface on the right. Two violin plots on the top show B-factors for protein atoms and symmetrical shape of the plots shows that B-factors of protein atoms are virtually the same at and outside the interface. Two bottom violin plots show B-factors for DNA atoms and their highly asymmetric shape shows larger vibrations outside the interface (large B-factors) than at the interface (low values of B-factors). Distributions are plotted for structures of protein/DNA complexes determined with crystallographic resolution between 1.90 and 2.80 Å. [Schneider, Černý, Svozil, Gelly & de Brevern: in preparation (2012)].

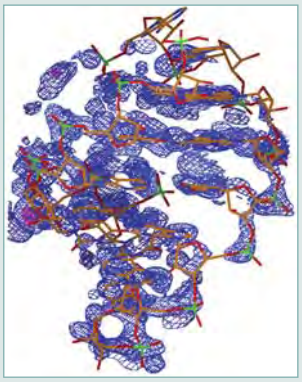

Fig 3: A view of the automated fit of an RNA double helix structure into the experimentally phased electron density of the structure by Ennifar & Dumas, unpublished crystal structure. Pavelčík & Schneider: Acta Crystallographica D 64:620 (2008).

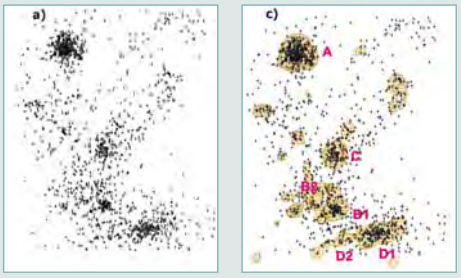

Fig 4: Technique of "Fourier averaging" developed to cluster distributions of points in Cartesian or parametric (here torsion) space. Figure a) shows the original distribution of experimental points, and figure c) calculated pseudo electron density of the point distribution in gold with assigned centers of the high-density clusters labeled A, B1, B2, C, D1, and D2. Svozil, Kalina, Omelka & Schneider: Nucleic Acids Research 36:3690 (2008) and Schneider, Morávek & Berman Nucleic Acids Research 32:1666 (2004).

Laboratory of Ligand Engineering

Petr Malý

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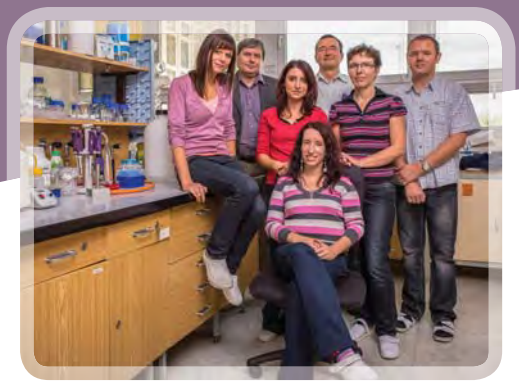

RESEARCH TOPICS

Artificial binding proteins derived from small protein scaffolds represent a valuable alternative to commonly used antibodies. Novel binders with engineered affinity, high specificity or designed inhibitory function attract attention as key components for the development of novel biosensing devices, *in vivo* diagnostics and next-generation therapeutics. Small, robust, and soluble proteins with high thermal and hydrodynamic stability and without disulphide bonds are amenable to rational improvement and can be easily modified by gene-fusion approaches.

In our laboratory, which operates since 2008, we focus on engineering novel binders raised against human cytokines, their receptors or tumour markers. 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 libraries (Fig. 1). Using randomization of 11 pre-determined amino acid residues we engineered a library of a theoretical complexity 10^{14} protein variants which was successfully used, in combination with ribosome display selection, for the generation of unique binders of human interferon gamma (hIFNγ) with Kd in the nanomolar range (Ahmad et al. 2012) (Fig. 2). High-affinity binders of hIFNγ were then used as key components for the construction of a novel SPR biosensor with the improved sensitivity for detection of hIFNγ in diluted blood plasma (Šípová et al. 2012)(Fig. 3).

The assembled combinatorial library has been used in another project leading to the generation of inhibitory ABD variants targeting human IL-23 or its receptor (IL-23R), key elements of the Th17-mediated pro-inflammatory pathway. In this running project we have already identified a collection of ABD variants (REX binders) with nanomolar Kd value for the binding of recombinant hIL-23R or IL-23R-Fc chimera. These so-called REX binders inhibited binding of p19 protein (α subunit of IL-23) to the IL-23 receptor in several arrangements of direct competition ELISA (Fig. 4). We further demonstrate that REX variants bind to human cell lines K-562 and THP-1 and this binding correlates with IL-23R cell-surface expression. As binding of REX proteins to THP-1 cells can be substantially diminished by a high dose of the p19 protein, we conclude that we identified novel IL-23R-binding inhibitors that might be useful in designing novel anti-inflammatory tools. This is of special value as the molecular structure of the IL-23/IL-23R complex has not been described yet and, therefore, designing efficient inhibitors of IL-23 function with a promising therapeutic potential remains cumbersome. Currently, we have been working on the characterization of IL-23 binding ABD variants (ILP binders) and investigation of a possible immunomodulatory function of the most promising REX and ILP candidates.

In another part of our research, we closely collaborate with several Czech biotech companies in the project AFFIBINDER supported by the Ministry of Industry and Trade of the Czech Republic. Within this project, we develop a novel fluorescence-based screening method for large-scale identification of high-affinity binders raised against selected human oncomarkers. As a part of this project we develop novel high-affinity binders of human KLK2 and PSP94 prostate cancer biomarkers.

MEMBERS

of the Laboratory of Ligand Engineering

Petr Malý, PhD / Head of Laboratory
Milan Kuchař, PhD / Research Fellow
Hana Petroková, PhD / Research Fellow
Peter Šebo, PhD / Research Fellow (part time)
Lucie Vaňková, MSc / PhD Student
Lucie Marečková, MSc / Research Assistant
Petra Kadlčáková / Technician

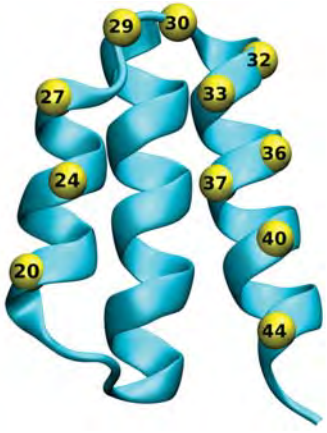

Fig 1: Albumin Binding Domain (ABD) of Streptococcal protein G was used as a scaffold. 11 pre-determined positions of a total 46 amino acids were randomized to generate a combinatorial library of 10^{16} codon variants.

SELECTED PAPERS

Krejcirikova, V., Pachl, P., Fabry, M., Maly, P., Rezacova, P., Brynda, J. Structure of the mouse galectin-4 N-terminal carbohydrate-recognition domain reveals the mechanism of oligosaccharide recognition. *Acta Crystallographica Section D-Biological Crystallography*, 67: Pt3, 204-211, 2011. ISSN 0907-4449.

Bibova, I., Linhartova, I., Stanek, O., Rusnakova, V., Kubista, M., Suchanek, M., Vasakova, M., Sebo, P. Detection of immune cell response to M. tuberculosis-specific antigens by quantitative polymerase chain reaction. *Diagnostic Microbiology and Infectious Disease*, 72: 68-78, 2012. ISSN 0732-8893.

Ahmad, J.N., Li, J., Biedermannova, L., Kuchar, M., Sipova, H., Semeradtova, A., Cerny, J., Petrokova, H., Mikulecky, P., Polinek, J., Staněk, O., Vondrasek, J., Homola, J., Maly, J., Osicka, R., Sebo, P., Maly, P. Novel high-affinity binders of human interferon gamma derived from albumin-binding domain of protein G. *Proteins: structure, function and bioinformatics*, 80: 774-789, 2012. ISSN: 0887-3585.

Sipova, H., Sevcu, V., Kuchar, M., Ahmad, J.N., Mikulecky, P., Osicka, R., Maly, P., Homola, J. Surface plasmon resonance biosensor based on engineered proteins for direct detection of interferon-gamma in diluted blood plasma. *Sensors and Actuators B*, 174: 306-311, 2012. ISSN: 0925-4005.

GRANT SUPPORTS

Peter Šebo

Academy of Sciences of the Czech Republic:
KAN200520702 Nanoimmunosensors for detection of cytokines. 2007-2011.

Petr Malý

Grant Agency of the Czech Republic:
GAP303/10/1849 Immunomodulatory ligands suppressing function of human IL-23. 2010-2013.

Ministry of Industry and Trade of the Czech Republic:
FR-TI4/667 Novel binding biomolecule development for tumor in vitro diagnostics. 2012-2014.

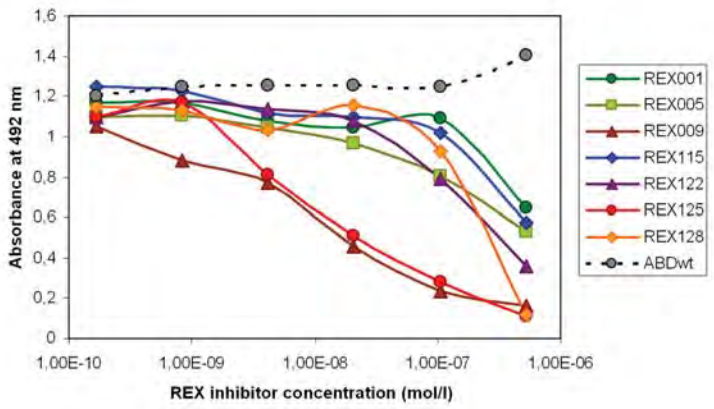

Fig 4: Detection of inhibitory binders of human IL-23R by a competition ELISA. Selected REX variants compete with a recombinant p19 protein (alpha subunit of IL-23) for binding to the immobilized IL-23 receptor produced in E. coli SHuffle strain. REX inhibitors were serially diluted in PBS + 0,05% Tween +1% BSA solution containing 20 nM p19 protein. Increasing concentration of REX proteins in the solution with a constant concentration of p19 leads to the suppresion of p19/IL-23R binding, in a striking contrast to the unmutated ABD wild-type control. Binding of p19 to the immobilized receptor was detected by an anti-human IL-23 polyclonal antibody.

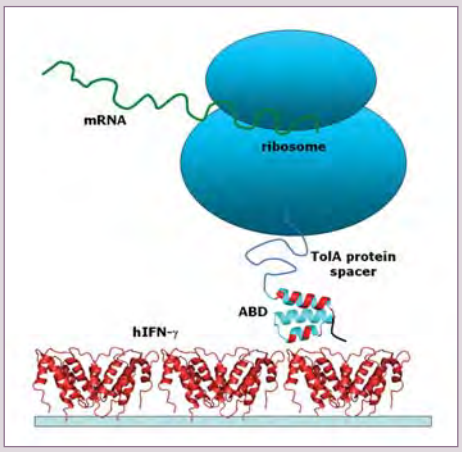

Fig 2: The principle of ribosome display selection. The genotype (mRNA) is linked to the phenotype (peptide) within the same ternary complex of a stalled ribosome, to which both the stop-codon-less mRNA and the nascent and folded polypeptide remain bound, the latter protruding from the ribosome channel and being capable to bind immobilized targets. Upon washing-out of unbound ribosome complexes, the mRNA is extracted, reverse-transcribed, amplified by PCR, transcribed into mRNA and used in a new selection cycle, to enrich for genes encoding binders of the target. An increase of a washing stringency in each selection cycle leads to the selection of high-affinity binders. The figure shows the screening of human interferon gamma binders.

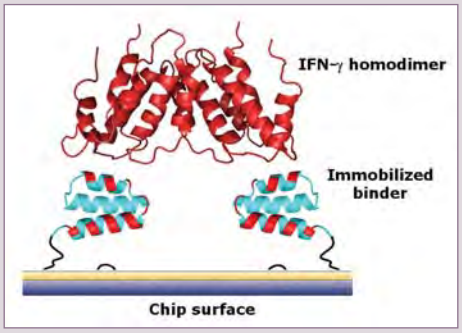

Fig 3: Design of a biosensor for the human interferon gamma detection. A small high-affinity binder recognizing the human cytokine is immobilized to the chip surface. If the cytokine is present in the analyzed sample it bounds to the immobilized binder and is, subsequently, detected by an optoelectronic manner (Surface Plasmon Resonance)..

Laboratory of Structural Biology

Cyril Bařinka

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RESEARCH TOPICS

The Laboratory of Structural Biology (LSB) was established in March 2010. We use methods of molecular, cell and structural biology to elucidate the structure-function relationship of several diagnostic/therapeutic targets. Ongoing projects include:

1. Identification and development of novel reagents, tools and techniques targeting human glutamate carboxypeptidase II (GCPII) and its paralogues/orthologues.

GCPII is a membrane-bound zinc metallopeptidase implicated in several physiological processes. Within the nervous system, GCPII exerts its peptidase activity by hydrolysing N-acetyl-aspartyl-glutamate and the jejunal form of the enzyme acts as folate hydrolase, thus participating in the absorption of dietary folates (Fig. 1). GCPII-specific inhibitors have been reported to be neuroprotective in multiple preclinical models of neurodegeneration. Additionally, given the GCPII over-expression in prostate carcinoma and within the neovasculature of solid tumours, the protein serves as an attractive target for imaging and therapy of a variety of cancers.

Our efforts are focused on addressing questions pertaining to the fundamental (patho) physiological roles of GCPII in healthy tissues as well as in cancer and neurodegeneration. We solved the first high-resolution X-ray structure of human GCPII and these data, together with more than 40 reported structures of GCPII-inhibitor complexes, serve as a basis for our extensive programme aimed at the structure-assisted design of novel inhibitory compounds targeting GCPII (Fig. 2). In addition to projects involving small-molecule GCPII inhibitors we are actively pursuing the development of biologics (Anticalin-based scaffolds) as novel agents for prostate cancer imaging. In our basic-oriented research we aim at unravelling putative non-enzymatic role(s) of human GCPII (receptor, chaperone) and defining functions of GCPII orthologues in several model organisms, including *A. thaliana* and *C. elegans* (Fig. 3).

2. Structure-function studies of histone deacetylases (HDACs)

Lysine acetylation is a major post-translational modification that plays a key role in many physiological processes and is believed to have a broad spectrum of modulatory functions within the cell. Acetylation levels of cellular targets are regulated by opposite activities of histone acyltransferease and histone deacetylases (HDACs). Eighteen human HDACs identified so far can be divided into four major classes and our laboratory is specifically interested in class IIb (HDAC6, HDAC10) and class IV (HDAC11) proteins. To address basic questions related to the structure-function relationship of these hydrolases we employ a panel of molecular biology, biochemistry and enzymology techniques targeting the individual HDACs as well as their cognate substrates.

In addition to our own research, we actively participate in several collaborative projects that take advantage of a portfolio of techniques established in our lab. These techniques include heterologous protein expression in several systems including *E. coli*, *K. lactis*, insect S2 and Hi5 cells, and mammalian HEK293 cells, enzymatic and physicochemical characterization of purified proteins and X-ray structure determination, including robotic screening of crystallization conditions.

MEMBERS

of the Laboratory of Structural Biology

Cyril Bařinka, PhD / Head of Laboratory
Jiří Pavlíček, PhD / Postdoc
Zora Nováková, PhD / Postdoc
Glenda P. Alquicer Barrera, PhD / Postdoc
Dana Borovská, MSc / Research Assistant
Veronika Jetenská, MSc / Research Assistant
Petra Pospěchová, MSc / Research Assistant
Ľubica Škultétyová, MSc / PhD Student
Jakub Ptáček, MSc / PhD Student
Petr Daniel, Bc / Diploma Student

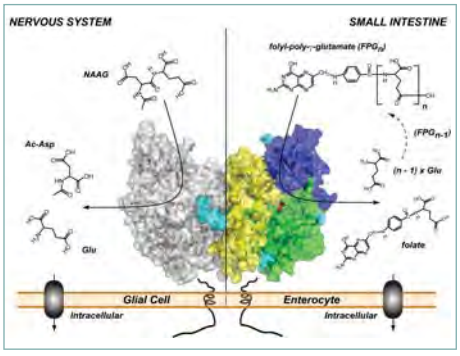


Fig 1: Homodimer of human GCPII (crystal structure) tethered to the biological membrane. One monomer shown in semitransparent surface representation with individual domains of the extracellular part colored green (protease domain), blue (apical domain), and yellow (C-terminal); the second monomer is colored gray. N-linked sugar moieties are colored cyan, and the active-site Zn2+ ions are shown as red spheres.

SELECTED PAPERS

Zhang, A.X., Murelli, R.P., Barinka, C., Michel, J., Cocleaza, A., Jorgensen, W.L., Lubkowski, J., Spiegel, D.A. A remote arene-binding site on prostate specific membrane antigen revealed by antibody-recruiting small molecules. *Journal of the American Chemical Society*, 132: 12711-12716, 2010. ISSN 0002-7863.

Plechanovova, A., Byun, Y., Alquicer, G., Skultetyova, L., Mlcochova, P., Nemcova, A., Kim, H.-J., Navratil, M., Mease, R., Lubkowski, J., Pomper, M., Konvalinka, J., Rulisek, L., Barinka, C. Novel substrate-based inhibitors of human glutamate carboxypeptidase II with enhanced lipophilicity. *Journal of Medicinal Chemistry*, 54: 7535-7546, 2011. ISSN 0022-2623.

Alquicer, G., Sedlak, D., Byun, Y., Pavlicek, J., Stathis, M., Rojas, C., Slusher, B., Pomper, M.G., Bartunek, P., Barinka, C. Development of a high-throughput fluorescence polarization assay to identify novel ligands of glutamate carboxypeptidase II. *Journal of Biomolecular Screening*, 17:1030-1040, 2012. ISSN 1087-0571.

Pavlicek, J., Ptacek, J., Barinka, C. Glutamate carboxypeptidase II: an overview of structural studies and their importance for structure-based drug design and deciphering the reaction mechanism of the enzyme. *Current Medical Chemistry*, 19:1300-1309, 2012. ISSN 0929-8673.

Barinka, C., Rojas, C., Slusher, B., Pomper, M. Glutamate carboxypeptidase II in diagnosis and treatment of neurologic disorders and prostate cancer. *Current Medical Chemistry*, 19:856-870, 2012. ISSN 0929-8673.

GRANT SUPPORTS

Cyril Bařinka

Ministry of Education, Youth and Sports:
ME10031 Structure-based design of ligands and inhibitors targeting glutamate carboxypeptidase II and its homologs. 2010 – 2012.

EMBO:
1978, Installation Grant. 2010 – 2015.

Academy of Sciences of the Czech Republic:
Fellowship Jana Evangelisty Purkyně. 2010 – 2014.

IRG Reintegration Grant:
249220, Design and development of novel reagents, tools, and techniques targeting human glutamate carboxypeptidases II and III, Marie Curie Actions - Seventh Research Framework Programme People. 2010 – 2014.

EMBO:
ASTF 56 – 2012, Short Term Fellowship. 2012.

Ministry of Education, Youth and Sports:
CZ.1.07/2.3.00/30.0045, Biotechnological expert in structural biology and gene expression, OP Education for Competitiveness. 2012 – 2015.

Grant Agency of the Czech Republic:
GAP301/12/1513 Novel biologics for cancer imaging. 2012 – 2016.

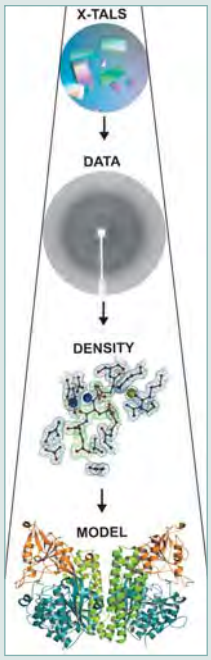


Fig 2: A workflow of the X-ray structure determination starting with the production of diffraction quality crystals (A), data collection at a synchrotron facility (B), structure solving and refinement (C) and model building and analysis (D).

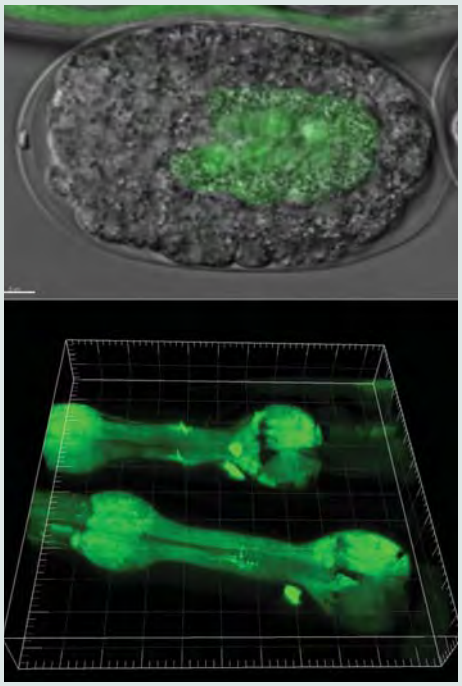
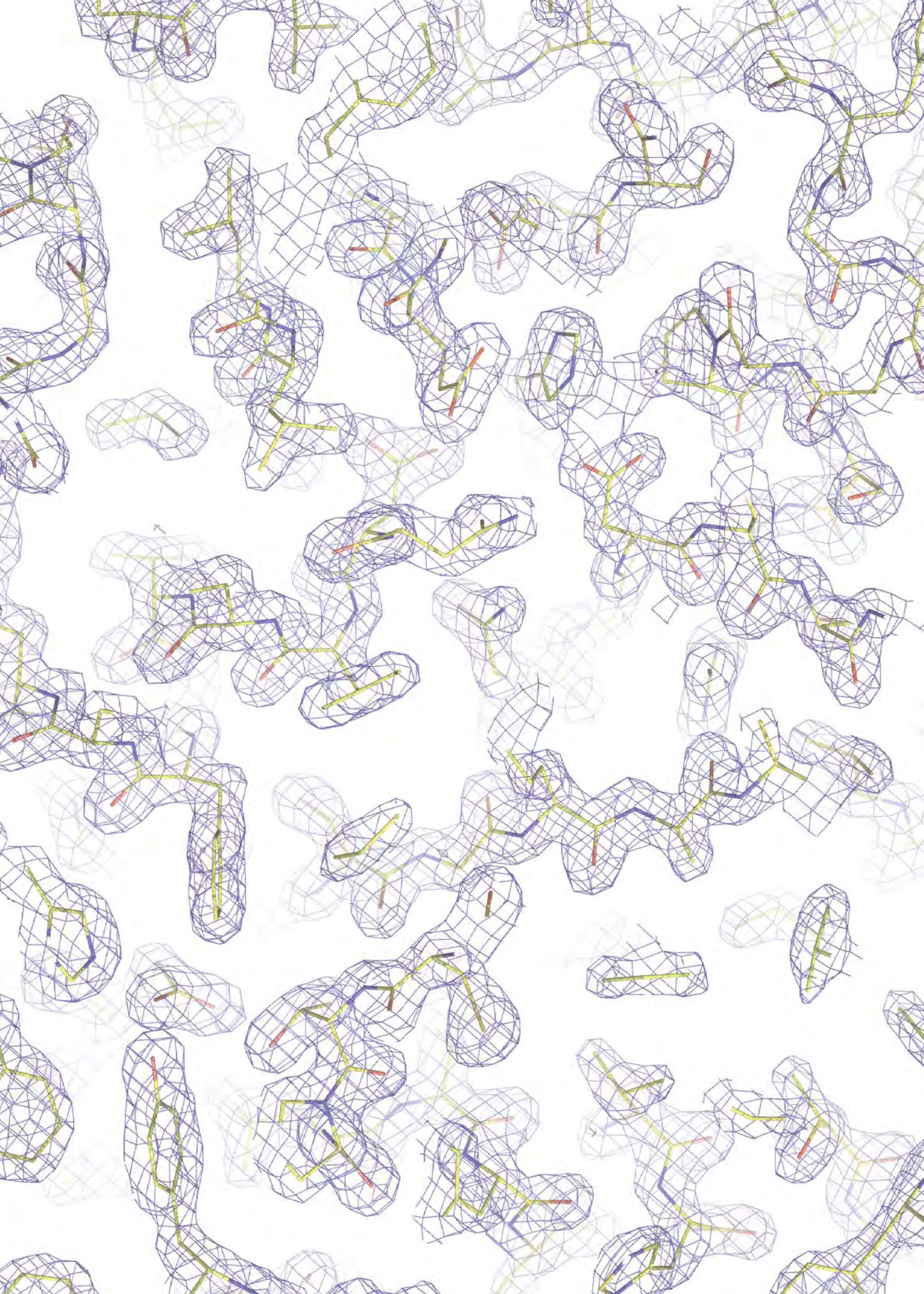


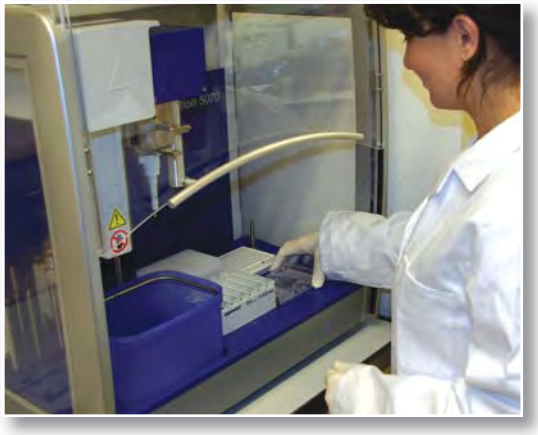
Fig 3: The expression of green fluorescence protein (GFP) controlled by the R57.1a promoter in C.elegans embryo (upper panel) and a subset of head neurons (3D reconstruction; lower panel).



qPCR Core Facility

The BTU qPCR Core Facility was founded in 2009 and has grown to become 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 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. 9,216 reactions can be processed in a single run. The laboratory has also conventional qPCR instruments including the LightCycler 480 Real-Time PCR System and LightCycler 96 Real-Time PCR System from Roche, CFX96 and CFX384 from Bio-Rad, StepOne Real-Time PCR System and 7500 Fast Real-Time PCR System from Life Technologies, Eco Real-Time PCR System from Illumina and the Rotor Gene 6000 from Qiagen. The latter is particularly suited for high resolution melt analysis. Sample preparation and processing is automated using the QIACube form Qiagene. Assay plates are assembly with the robotic liquid handling workstation Eppendorf epMotion P 5073. Sample quality is assessed using the NanoDrop ND-1000 Spectrophotometer, Qubit 2.0 Fluorometer from Life Technologies, Experion Automated Electrophoresis Station from Bio-Rad the and 2100 Bioanalyzer from Agilent Technologies.

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 Vevo 770

Visual Sonics' Vevo 770 – the high-resolution in vivo micro imaging system devised specifically for non-invasive small animal research – is in operation since 2007, and as of 2010 has been moved to the newly-reconstructed pavillion CH. It offers spatial resolution down to 30 microns, and because of its non-invasive nature allows longitudinal studies. It provides researchers with a simple method for efficient viewing of extremely small physiological structures and for imaging of living tissues and blood flow with near-microscopic resolution. Vevo 770 has been developed specifically for mice, but is readily adaptable to other small animal models.

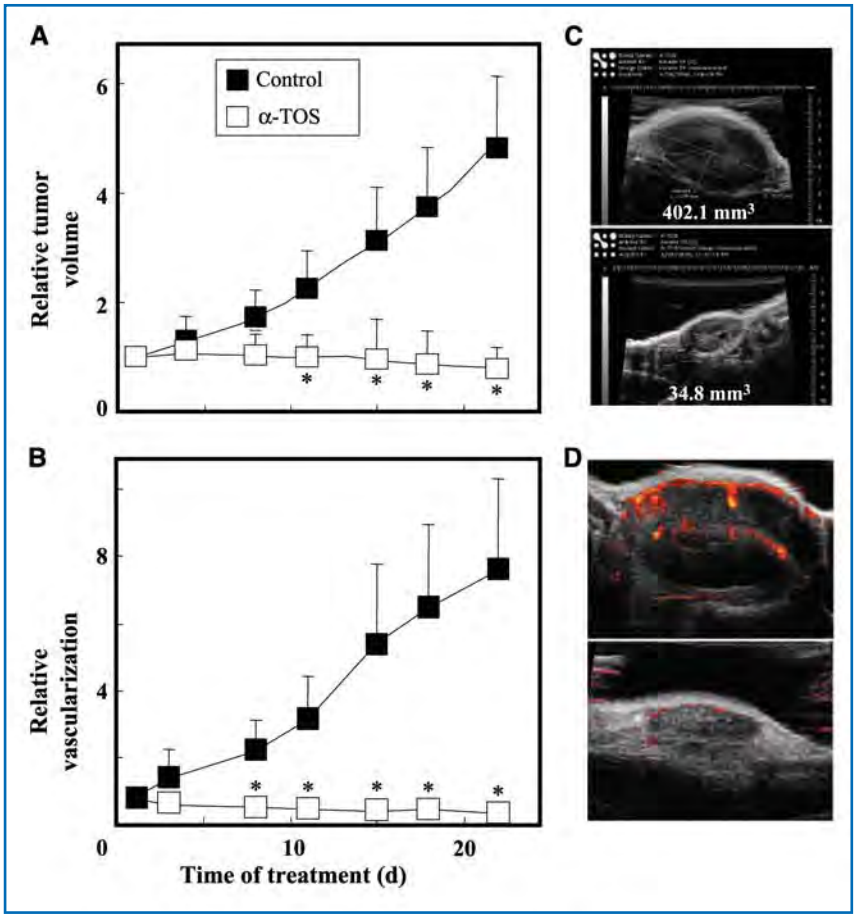


Fig: Transgenic FVB/N c-neu mice with spontaneous breast tumors were treated by alpha-tocopheryl succinate. Kinetics of tumor growth (A) and vascularization (B) were quantified by the Vevo 770 instrument. Shown are typical images of control and treated tumours (panel C) and their vascularization (yellow and red spots – panel D).

Financial and Administration Services

Head, Deputy Director: Jan Škoda

Administrative services (back row, from left):
Jan Škoda, Klára Knížková, Ilona Dita, Lukáš Veselý,
Monika Kopřivová

Glass washing services (front row, from left):
Hana Boháčková, Zdeňka Kšír*ová*

Financial services are partially supplied by the Financial Department of the Institute of Molecular Genetics of the AS CR, v. v. i.

Publications

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Laboratory of Molecular Pathogenetics

2009

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Laboratory of Immunopathology and Immunotherapy

2008

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Laboratory of Biomolecular Recognition

2008

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Laboratory of Ligand Engineering

2011

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Laboratory of Structural Biology

2010

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Seminar Speakers

2008

Axel Spahr *(University of Ulm, Germany)*
Peter Sutovsky *(University of Missouri – Columbia, USA)*
Anders Stählberg *(Gothenburg University, Sweden)*
Anna Coll *(University of Girona, Switzerland)*
Asgeir Brevikj *(Norwegian Institute of Public Health, Norway)*
Alexander G. de Brevern *(Université Paris – Diderot, France)*
David Stuart *(Oxford University, United Kingdom)*
Petter Lyngstaadas *(University of Oslo, Norway)*
Etienne Joly *(INSERM Toulouse, France)*

2009

Jacques Samarut *(Institute of Functional Genome, Lyon, France)*
Marino Zerial *(Max Planck Institute for Cell Biology and Genetics, Dresden, Germany)*
Graham Warren *(Max F. Perutz Laboratory, Vienna, Austria)*
Pavel Tomančák *(May Planck Institute for Cell Biology and Genetics, Dresden, Germany)*
Michael W. Pfaffl *(Central Institute for Nutrition and Food Science, Technical University of Munich, Germany)*
Heinrich H. D. Meyer *(Central Institute for Nutrition and Food Science, Technical University of Munich, Germany)*
Andreas Weinhäusel *(Department Health & Environment, Molecular Medicine, AIT Austrian Institute of Technology, Austria)*
Richard Brereton *(Centre for Chemometrics, University of Bristol, UK)*
Alexander Weis *(AdnaGen AG, Germany)*
Sigfried Hauch *(AdnaGen AG, Germany)*
Vladimír Beneš *(EMBL Heidelberg, Germany)*
Heimo Müller *(Institute of Pathology, Medical University Graz, Austria)*

2010

Jesús del Maro *(Centro de Investigaciones Biológicas C.I.B., Spain)*
Mario Zoratti *(University of Padova, Italy)*

2011

Alexander G. de Brevern *(Université Paris – Diderot, Paris, France)*
Jean - Christophe Gelly *(Université Paris – Diderot, Paris, France)*

2012

Satish Kumar Gupta *(National Institute of Immunology, New Delhi, India)*
Michael Chandler *(CNRS – Centre national de la recherche scientifique , Toulouse, France)*

Conferences

2008

XIV. Symposium of Czech Reproductive Immunologist with International Participation, 29th May – 1st June 2008, Zdar nad Sazavou
Laboratory of Reproductive Biology

2009

XV. Symposium of Czech Reproductive Immunologist with International Participation, 29th – 31th May 2009, Zdar nad Sazavou
Laboratory of Reproductive Biology

Mitochondria, Apoptosis and Cancer, EMBO Workshop, 1st – 3rd October 2009, Prague
Laboratory of Molecular Therapy

VII. Discussions in Structural Molecular Biology, 12th – 14th March 2009, Nove Hrady
Laboratory of Biomolecular Recognition

2010

XVI. Symposium of Czech Reproductive Immunologist with International Participation, 28th – 30th May 2010, Zdar nad Sazavou
Laboratory of Reproductive Biology

VIII. Discussions in Structural Molecular Biology, 18th - 20th March 2010, Nove Hrady
Laboratory of Biomolecular Recognition

2011

XVII. Symposium of Czech Reproductive Immunologist with International Participation, 26th – 29th May 2011, Zdar nad Sazavou
Laboratory of Reproductive Biology

Mitochondria, Apoptosis and Cancer, EMBO Workshop, 27th – 29th October 2011, Singapore
Laboratory of Molecular Therapy

IX. Discussions in Structural Molecular Biology, 24th – 26th March 2011
Laboratory of Biomolecular Recognition

Develompents in Real-time PCR. From Preanalytics to Molecular Diagnostics. Symposium. June 13th – 17th, Prague
Laboratory of Gene Expression

2012

XVIII. Symposium of Czech Reproductive Immunologist with Internationat Participation, 25th – 26th May 2012 Zdar nad Sazavou
Laboratory of Reproductive Biology

Annual Meeting of the Society for Free Radical Research, 28th November – 1st December 2012, Brisbane, Qld, Australia
Laboratory of Molecular Therapy

X. Discussions in Structural Molecular Biology, 22th – 24th March 2012, Nove Hrady
Laboratory of Biomolecular Recognition

Attune Acoustic Focusing Cytometer Workshop – 25th September 2012, Prague
Laboratory of Immunopathology and Immunotherapy

Courses

2008 – 2011

Regularly every year: qPCR TATAA courses: Hands-on qPCR; Sample preparation and quality control; Experimental design & data analysis
Laboratory of Gene Expression

2012

qPCR TATAA courses: Hands-on qPCR; Sample preparation and quality control; Experimental design & data analysis; qPCR for microRNA analysis
Laboratory of Gene Expression

Attune Acoustic Focusing Cytometer Workshop, 25 th September 2012
Laboratory of Immunopathology and Immunotherapy

Training Center for the Czech Republic

Fully automated cell separator **Robosep (Stem Cell Technologies)** for immunomagnetic isolation of various cell types is available as a service in the laboratory since 2008. From the same year multiplex analyzer **BioPlex 200 (BioRad)** operating on the principle of flow cytometry and Luminex technology was installed in the laboratory and is offered also a service to customers.
Laboratory of Immunopathology and Immunotherapy

Conferences of the Institute of Biotechnology AS CR, v. v. i.

The First Conference of the Institute of Biotechnology AS CR, v. v. i.

The first Conference of the Institute of Biotechnology AS CR, v. v. i., was held on October 12–13, 2010. This event took place in a conference centre of the Academy of Sciences of the Czech Republic, the Liblice Castle. All laboratories of the Institute presented their research goals and results. As a guest, the participants welcomed Professor Prem Ponka from McGill University, Montreal, Canada.

The Second Conference of the Institute of Biotechnology AS CR, v. v. i.

The second institutional conference took place on November 14–15, 2011. This event was also held in the Liblice Castle, similarly as previous year. The focus of this conference was on the presentation of young scientists, including PhD students.

Project BIOCEV

The title of project BIOCEV represents the acronym of 'Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University in Vestec'. BIOCEV is a joint project of six institutes of the Academy of Sciences of the Czech Republic (Institute of Molecular Genetics AS CR, v. v. i., Institute of Biotechnology AS CR, v. v. i., Institute of Microbiology AS CR, v. v. i., Institute of Physiology AS CR, v. v. i., Institute of Experimental Medicine AS CR, v. v. i., and Institute of Macromolecular Chemistry AS CR, v. v. i.) and two faculties of Charles University in Prague (Faculty of Science and First Faculty of Medicine). The goal of project BIOCEV is to establish a European Centre of Excellence in biomedicine and biotechnology. The Institute of Biotechnology as one of the Project partners will relocate to the newly built Centre of Excellence, BIOCEV, in 2015. The IBT researchers will participate in BIOCEV research programmes, each of them dealing with a number of separate research projects.

Membership in CzechBio association

The Institute of Biotechnology was one of the founding members of the CzechBio association (association of Czech biotech companies). The association was established in December 2008 with the aim to become a national biotech and biomedicine platform in the Czech Republic and to strengthen cooperation between the academic and the private sector. From its original 21 founding members the association has grown steadily – it now includes 31 private companies, four research institutes and one university. In time, CzechBio has become a respected organization representing the industry interests towards both the national government and foreign organizations.

Where We Are

Location of the Institute of Biotechnology AS CR, v. v. i.

The Institute of Biotechnology AS CR, v. v. i., is located on the Prague – Krč campus of Biomedical Academy Institutes. The campus is easily reachable by public transport or by car.

Address of the Institute of Biotechnology AS CR, v. v. i.

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Impressum

Published by
© Institute of Biotechology AS CR, v. v. i.

Editors
Jana Pěknicová, Ilona Dita

Layout & Design
Lucie Gabrielová

Pictures & Credits
Martin Vítek / Heads of the Research Groups

Printed by
H.R.G. spol. s r. o.
February 2013

Special thanks to Šárka Takáčová for the English revision.

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