Characteristics of main research directions investigated at the institute and the achievements 2010−2014

Institute	Institute of Biotechnology of the CAS, v. v. i.

On January 1st 2008, the Academy of Sciences of the Czech Republic (AS CR) established a new public research institution, the Institute of Biotechnology AS CR, v. v. i., at its biomedical research campus in Prague-Krč. The Institute experienced some initial challenges, since several of its groups were established the time of creation of the institute, others were in their initial phases of existence. The new laboratories were started by young researchers who returned from successful international research stages. Dr. C. Barinka returned from the National Cancer Institute, MD, USA, and established the Laboratory of Structural Biology, Dr. Sindelka returned from the Whitehead Institute, Boston, and established an independent research sub-group in the Laboratory of Gene Expression. One of the groups, Laboratory of Tumour Resistance, headed by Dr. J. Truksa, was created by splitting from the parent group (Laboratory of Molecular Therapy – Prof. J. Neuzil, Head). Another group, Laboratory of Structure and Function of Biomolecules, headed by J. Dohnalek, migrated from the Institute of Macromolecular Chemistry of the Academy of Sciences. Establishment of other new groups is planned (Structural Bioinformatics and Structural Proteins and Their Complexes).

The activities of the Institute of Biotechnology are focused on excellent basic research in biomedical molecular biology with prospective transfer of biotechnological methods and tools to human and veterinary medicine or other important areas of human activity.
The scientific activities of the Institute cover the broad area from cell pathology and human diseases (Research team 1) to protein engineering and structural biology (Research team 2).

The projects of groups within Team 1 represent top basic research focusing on elucidation of molecular mechanisms of serious diseases with a significant impact on the health of the population. This involves a complex approach to the research of human diseases, starting from the molecular-biological background at the level of genes related to a particular disease, moving to biological, biochemical and proteomic studies of proteins participating directly or indirectly in the origin or process of the disease, identification of biomarkers for diagnostic purposes, application of model organisms and systems for revealing and understanding of new functions of genes and their products, as well as regulation of pathways in which they participate.

Team 2 focuses on research excellence related to the study of biotechnologically and medically important biomolecules, proteins and nucleic acids. The aim is to understand the structures and intermolecular interactions of biomolecular systems and, in a broader sense, their structure-function relationships, in order to be able to increase their desired effect developing them into medically or biotechnologically useful products. The studied molecular systems are produced by advanced techniques of molecular biology and protein engineering, their structure, stability and activity determined by the state-of-the-art structural and biophysical experimental and computational methods.

In the period 2010-2014, IBT scientists published 149 publications in peer-reviewed journals, six chapters in scientific books, filed two patents, and commercialized 22 applied results. One laboratory (Laboratory of Immunopathology and Immunotherapy) was discontinued at the end of 2014 due to the instability and lack of performance. The practical outputs of the activities of the Institute represented by patents, licenses and technologies are commercialised in cooperation with private investors.

1. Research objectives of the Team 1 "Development of Therapeutic and Diagnostic Procedures".

Team 1 comprised six individual sub-groups, all focusing on understanding the molecular and cellular bases of a variety of pathological states. They have been engaged in cutting-edge basic research aimed at providing better understanding of molecular mechanisms of highly socio-economically challenging pathologies with considerable impact on the population health. The focus is on a complex approach to the research of human diseases, starting with molecular aspects at the level of genetic regulation of selected pathologies, to biological, biochemical and proteomic studies that are relevant both to the genesis and progress of the pathologies, and identification of relevant diagnostic biomarkers. Model organisms and systems are used to uncover novel functions of genes, their products and the regulatory aspects. An important output of ground-breaking features will be identification of novel and efficient diagnostic and therapeutic approaches.

1.1. Scientific profile and the main specific results of the Team 1

In the period 2010-2014, Team 1 published 113 publications in peer-reviewed journals, five chapters in scientific books, filed one patent, and 12 applied results. The team efforts are primarily focused on top-quality basic research with potential application results in diagnosis and treatment of diseases. The scientific profile and main results of the individual groups under Team 1 are presented below.

1.1.1. Laboratory of Reproductive Biology

The laboratory focuses on studying molecular mechanisms of reproduction and the nature of specific sperm proteins playing a role during sperm maturation, such as capacitation, acrosome reaction and sperm-egg fertilisation. During the last 10 years, its members discovered a number of new proteins that are fundamental for the sperm-egg binding and developed methods and molecular tools (monoclonal antibodies and kits) for their study. Many of them are also used in Centres of Assisted Reproduction and have been successfully commercialised (12 applied results). *In vitro* experiments demonstrated the role of oestrogens and oestrogen receptors during capacitation in mammalian spermatozoa and also the potential negative impact of oestrogens on the sperm reproductive fitness in the female reproductive tract (Ded et al., 2010). The laboratory tested a number of environmental pollutants and determined their negative effect on mammalian reproduction (Elzeinova et al. 2012, Zatecka et al., 2014a, 2014b, Brieño-Enríquez et al., 2015).

1.1.2. Laboratory of Molecular Therapy

The laboratory is interested in better understanding of cancer biology, more specifically, focusing on the role of mitochondria in tumour formation and progression and, as well, as a target for efficient anti-cancer therapy. The laboratory has two main objectives: 1. Mitochondria as a target for novel mitocans, i.e. anti-cancer agents modified to associate with mitochondria; and 2. Transfer of mitochondria between stromal and tumour cells and its role in tumour initiation and progression.

For several years, the group has been studying novel anti-cancer agents that are efficient against tumours and that have been modified by tagging with a delocalised cationic group (triphenylphosphonium, TPP+). This modification causes their preferential association with mitochondria. This approach has been applied to vitamin E succinate, a selective anti-cancer agent that was described by the group a while ago. Thus, mitochondrially targeted vitamin E succinate (MitoVES) has been documented to kill cancer cells of different origin some 20-50-fold more efficiently than found for its non-targeted counterpart, and to very efficiently suppress tumorigenic angiogenesis. Complex biochemical, cell biological as well as molecular modelling studies confirmed mitochondrial complex II as the preferred target for MitoVES. The group then focused on complex I-targeting anti-cancer agents, starting with the widely used therapeutics against breast cancer, tamoxifen. They synthesised mitochondrially targeted tamoxifen (MitoTAM) by tagging the parental compound with TPP+, and found that MitoTAM

was much more efficient in killing Her2^{high} breast cancer cells than the parental compound. Further and very unexpectedly, MitoTAM killed Her2^{high} cell more efficiently than Her2^{low} cells, exactly opposite to tamoxifen. This was remarkable in particular for effects on Her2^{high} tumours in a mouse model, where several mice became tumour-free, and the tumour did not reappear within 5-6 months post treatment cessation. This indicated complete eradication of Her2^{high} breast carcinomas. The group recently started studies on another anti-cancer agent targeting complex I that suppresses the fatal pancreatic carcinomas and that seems very intriguing. These studies are ongoing. With regard to the novel anti-cancer agents targeting mitochondria, the group has been coining the term 'mitocans', which encompasses a wide group of small molecules with anti-cancer activity acting via mitochondria, and classified mitocans into several groups based on their molecular mechanism of action. Key results were published in a number of papers with some of the outstanding ones being Tan et al., accepted 2014, Tomaset al. 2014, Stapelberg et al. 2014).

1.1.3. Laboratory of Gene Expression

The laboratory is a Europe's leading academic group specialised in high-throughput gene expression profiling and single-cell analysis using real-time quantitative PCR (qPCR). They have several basic research projects in the field of developmental biology and stem cells as well as applied projects within cancer and neurological research. They also develop methods for quality control and assessment of nucleic acid analyses, and are active in the area of standardisation (Bustin et al., 2013, Huggettt et al., 2013).

The long-term interest of the group in developmental biology lies in early development of *Xenopus laevis* and localization of maternal mRNAs along the main developmental axes. They performed detailed analysis of maternal mRNAs along the animal-vegetal axis using the qPCR tomography technique they developed some years ago. They also studied distribution of miRNAs and found them too to be asymmetrically distributed within the oocyte (Flachsova, et al., 2013).

The laboratory was a member of EU-funded consortium called SPIDIA (2009-2012), where the goal was to develop standard operating procedures for the pre-analytical process in molecular diagnostics. The work resulted in several publications and is also the base of nine new ISO guidelines that are currently being produced. Dr. Kubista, the Head of the laboratory, is a member of the international working group drafting these new guidelines (Bustin et al., 2009, the paper has over 3,000 citations).

The laboratory is considered the leader in the field of quality control and assessment in molecular diagnostics, and has been contributing to various guidelines and methodology papers. Since 2009, based on their unique equipment, the laboratory has been offering core facility services to academic and occasional industrial customers. In 2014, this facility was integrated as an official core facility of the BIOCEV. It provides complete service from experimental design and assay development to quantification and data analysis, and is operated by a core facility manager.

1.1.4. Laboratory of Molecular Pathogenetics

The main focus of the Laboratory is to identify the molecular mechanisms in pathological changes during embryonic development. In the last five years, they have developed and progressed in two major projects. The first project determines the molecular causes of pathological changes associated with diabetic exposure. For the first time, the members of the group linked HIF-1-regulated pathways and the development of congenital malformations in diabetes-exposed embryos and provided compelling evidence that impairment of HIF-1α-controlled hypoxia-response pathways may play a functionally causative role in diabetic embryopathy. In the other important project in the field of diabetic embryopathy, they showed that diabetes mellitus during pregnancy alters transcriptional profiles in the placenta, affecting cells of embryonic and maternal origin. Their collaborative research of tran-scriptome changes associated with diabetic embryopathy has been summarised in a book. To further investigate the role of HIF-1 pathways in cardiovascular pathologies and in combination with diabetic exposure, they analysed the molecular changes in the adult heart using a mouse model. Their

results show that the dysfunction of HIF1 pathways considerably affects transcriptional regulation in the heart. (Bohuslavova et al., 2010).
The second major project is focused on establishment of the functional role of transcriptional factor ISLET1 in neurosensory development. Within this project, the laboratory established a fruitful collaboration with Prof. Syka's group at the Institute of Experimental Medicine AS CR and Prof Bernd Fritzsch's group from the USA. Using transgenic mice and Cre-loxP system, they have analysed the role of specification transcription factors (Sox2, Atoh1, and Neurod1) in neurosensory system of the inner ear (Bohuslavova et al., 2013).

1.1.5. Laboratory of Tumour Resistance

The laboratory was established in February 2013, and its activity can thus be monitored for two years only. Previous work was conducted at the Institute of Biotechnology within the Molecular Therapy Group, from which this laboratory branched off. The laboratory focuses on the biology of tumour-initiating cells, their iron metabolism and regulation and expression of ABC transporters, important mediators of cancer resistance. During the monitored period of 2010-2014, it achieved three important outcomes, focusing on mtDNA transfer, the role of MiR-126 in the regulation of mitochondrial functions, and the regulation and role of the IDO1 enzyme in the biology of tumour-initiating cells (Tan et al. accepted 2014, Tomasetti al. 2014, Stapelberg et al. 2014).

1.1.6. Laboratory of Immunopathology and Immunotherapy

The laboratory was involved in research and development of new diagnostic and therapeutic tools available at the molecular or cellular level in disorders of humoral immunity in humans.
The Laboratory, due to the instability and insuffiient performance, was discontinued at the end of 2014.

Collectively, the above statements concerning the performance over the last five years and planned future research (see below) of five current laboratories of Team 1 clearly document their excellent scientific quality and ability to remain leaders in their research areas. Importantly, the presented research includes highly sophisticated and break-through aspects of basic research, innovative and world top methodological approaches as well as translational research with patents and the ensuing transfer of the basic research results into practice. Several laboratory Heads are world-leading scientists in their respective research field.

2. Research objectives of Team 2 "Structural Biology and Protein Engineering"

The main objective has been the study of biotechnologically and medically important biomolecules, proteins and nucleic acids. The aim of the team is to understand structures and intermolecular interactions of biomolecular systems and, in a broader sense, their structure-function relationships, in order to be able to increase their desired effect, so that they can be developed into medically or biotechnologically useful products. The studied molecular systems are produced by advanced techniques of molecular biology and protein engineering, and their structure, stability and activity determined by state-of-the-art structural and biophysical experimental and computational methods.

2.1. The main scientific results of Team 2

In the period 2010-2014, Team 2 published 36 publications in peer-reviewed journals, two chapters in a scientific book, and filed one patent and 10 applied results. The primary research efforts have been directed into four main areas that demonstrate the team's achievements but, more importantly, the future potential of the team to play an internationally recognized role in basic as well as oriented biomedical research. The main result areas are as follows.

2.1.1. Structure-assisted drug design

This research is aimed at the development of small molecules targeting human glutamate carboxypeptidase II (GCPII) that could be used as imaging or therapeutic modalities. The international recognition of the team's contribution to this field was underscored by the invited reviews on structural (Pavlicek et al., 2012) and biological (Barinka et al., 2012) aspects of GCPII.

2.1.2. Protein engineering

Combining techniques of *in vitro* protein evolution and computer modelling for targeted modulation of protein properties such as binding affinity to medically important proteins (Mikulecky et al. 2013, Kuchar et al. 2014) (Malý et al. Czech patent No.304514, 2014, and published international patent application WO 2014/079399 A1, 2014).

2.1.3. Structural bioinformatics

Bioinformatics is used as a tool to better understand biomolecular structures, for large-scale comparison of structures, description of general structural behaviour of nucleic acids and proteins, and for discovery of structural motifs (Schneider et al., 2014a, 2014b).

2.1.4. Structure-function studies of proteins

This area represents an important line of the team's research. These studies are based on employment of modern methods of structural and biophysical molecular analysis including X-ray crystallography (Pavlicek et al., 2012, Skalova et al., 2015).

2.2. Evolution and current state of Team 2

The evaluation period 2010-2014 can be characterised as the time of the Team consolidation. the major tasks within this period were to establish, develop, stabilise, and sustain the Team's research activities. The goal has been to design and build a strong, internationally recognised research group with synergy between individual research laboratories of the Team, whose expertise would span from bioinformatics to expression and characterisation, structure determination, and finally testing the biological activity of the studied molecular systems.
The composition of the Team reflects the composition of one of the BIOCEV Research Programmes, Programme 3 "Structural Biology and Protein Engineering". The heart of the Team was founded in 2008 as a research laboratory with broad expertise stretching from bioinformatics to crystallography and protein engineering. This rather 'unstructured' group has evolved into two currently functional laboratoriess, "Laboratory of Ligand Engineering", (with P. Malý as its Head) and "Laboratory of Biomolecular Recognition" (with B. Schneider as the Head) and a planned bioinformatic laboratory "Structural Bioinformatics of Proteins".
The team had been substantially strengthened when C. Bařinka joined the IBT in 2009 and he is now the Head of the "Laboratory of Structural Biology". While the group "Laboratory of Structure and Function of Biomolecules" of J. Dohnalek has always been planned as a part of IBT, principally as the cornerstone of the BIOCEV Research Programme 3, the group was transferred from the Institute of Macromolecular Chemistry in July 2013.
To summarise, the structure-oriented biomedical and biotechnological research of the IBT is secured by four well-established research laboratories within Team 2, and by a perspective of opening two new, dynamic research groups.

References
Team 1:

Bohuslavova R, Kolar F, Kuthanova L, Neckar J, Tichopad A, Pavlinkova G.: Gene expression profiling of sex differences in HIF1-dependent adaptive cardiac responses to chronic hypoxia. J Appl Physiol, 109(4):1195-202, 2010.

Bohuslavova R, Skvorova L, Sedmera D, Semenza GL, Pavlinkova G. Increased susceptibility of HIF-1α heterozygous-null mice to cardiovascular malformations associated with maternal diabetes. J Mol Cell Cardiol. 60:129-41, 2013.

Brieño-Enríquez M. A., García-López. J., Cárdenas D. B., Guibert S., Cleroux E., Děd L., Hourcade JdD., Pěknicová J., Weber M., del Mazo, J.: Exposure to endocrine disruptor induces transgenerational epigenetic deregulation of MicroRNAs in primordial germ cells. PLoS ONE, accepted

Bustin S. A., Benes V., Garson J.A.,...Kubista M., Mueller R., Nolan T.,. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clinicla Chemistry 55:611-622, 2009

Bustin, S. A,….Kubista, M,….Vandesompele, J.: The need for transparency and good practices in the qPCR literature. Nature Methods, 10: 1063–1067, 2013.

Ded L., Dostalova P., Dorosh A., Dvorakova-Hortova K., Peknicova J.: Effect of estrogens on boar sperm capacitation in vitro. 13:8, Reprod Biol Endocrinol. 8:87, 2010.

Elzeinova, F., Pěknicova, J., Ded, L., Kubatova A., Margaryan H., Dorosh A., Makovicky P., Rajmon, R.: Adverse effect of tetracycline and doxycycline on testicular tissue and sperm parameters in CD1 outbred mice. Exp Toxicol Pathol. 65: 911-917, 2013

Flachsova, M., Sindelka, R., Kubista, M.: Single blastomere expression profiling of Xenopus laevis embryos of 8 to 32-cells reveals developmental asymmetry. Scientific Reports, 3: 2278, 2013.

Huggett, J. F., Foy, C. A., Benes, V., Emslie, K., Garson, J. A., Haynes, R., Hellemans, J., Kubista, M., Mueller, R. D., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J., Bustin, S. A. The digital MIQE guidelines: Minimum Information for Publication of Quantitative Digital PCR Experiments. Clinical Chemistry, 59(6): 892-902, 2013.

Tan AS, Baty JW, Dong LF, Bezawork-Geleta A, Endaya B, Goodwin J, Bajzikova M, Kovarova J, Peterka M, Yan B, Pesdar EA, Sobol M, Filimonenko A, Stuart S, Vondrusova M, Kluckova K, Sachaphibulkij K, Rohlena J, Hozak P, Truksa J, Eccles D, Haupt LM, Griffiths LR, Neuzil J, Berridge MV. Mitochondrial genome acquisition restores respiratory function and tumorigenic potential of cancer cells without mitochondrial DNA. Cell Metab. 21:81-94, 2015. accepted 2014

Stapelberg M, Zobalova R, Nguyen MN, Walker T, Stantic M, Goodwin J, Pasdar EA, Thai T, Prokopova K, Yan B, Hall S, de Pennington N, Thomas SR, Grant G, Stursa J, Bajzikova M, Meedeniya AC, Truksa J, Ralph SJ, Ansorge O, Dong LF, Neuzil J. Indoleamine-2,3-dioxygenase elevated in tumor-initiating cells is suppressed by mitocans. Free Radic Biol Med. 67: 41-50, 2014.

Tomasetti M, Nocchi L, Staffolani S, Manzella N, Amati M, Goodwin J, Kluckova K, Nguyen M, Strafella E, Bajzikova M, Peterka M, Lettlova S, Truksa J, Lee W, Dong LF, Santarelli L, Neuzil J. MicroRNA-126 suppresses mesothelioma malignancy by targeting IRS1 and interfering with the mitochondrial function. Antioxid Redox Signal. 21: 2109-25, 2014.

Zatecka E., Ded L., Elzeinova F., Kubatova A., Dorosh A., Margaryan H.,Dostalova P., Korenkova V., Hoskova K., Peknicova J.: Effect of zearalenone on reproductive parameters and expression of selected testicular genes in mice. Reprod. Toxicology 45: 20-30, 2014a.

Zatecka E., Castillo J., Elzeinova E., Kubatova A., Ded L., Peknicova J., Oliva R.:The effect of tetrabromobisphenol A on protamine content and DNA 1 integrity in mouse sperm. Andrology 2(6): 910-917, 2014b.

Team 2:

Barinka C., Rojas C., Slusher B., Pomper M.: Glutamate Carboxypeptidase Ii in Diagnosis and Treatment of Neurologic Disorders and Prostate Cancer. Curr. Med. Chem. 19: 856-870, 2012.

Cerny J., Biedermannova L., Mikulecky P., Zahradnik J., Charnavets T., Sebo P., Schneider B.: Redesigning protein cavities as a strategy for increasing affinity in protein-protein interaction. Interferon-γ receptor 1 as a model. Biomed Res. Int., 2015: in press, accepted December 2014.

Kuchar M., Vankova L., Petrokova M., Cerny J. Osicka R., Pelak O., Sipova H., Schneider B., Homola J., Sebo P., Kalina T, Maly P. Human Interleukin-23 Receptor Antagonists Derived from an Albumin-Binding Domain Scaffold Inhibit Il-23-Dependent Ex Vivo Expansion of Il-17-Producing T-Cells. Proteins 82: 975-989, 2014.

P Mikulecky P., Cerny J., Biedermannova L., Petrokova H., Kuchar M., Vondrasek J., Maly P, Sebo P., Schneider B.: Increasing Affinity of Interferon-Gamma Receptor 1 to Interferon-Gamma by Computer-Aided Design. Biomed Res. Int. 2013: Artn 752514, 2013.

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. Curr. Med. Chem. 19: 1300-1309, 2012.

Schneider B., Cerny J., Svozil D., Cech P., Gelly J.C., de Brevern A.G.: Bioinformatic Analysis of the Protein/DNA Interface. Nucleic Acids Res. 42: 3381-3394, 2014.

Schneider B., Gelly J.C., de Brevern A.G., Cerny J.: Local Dynamics of Proteins and DNA Evaluated from Crystallographic B Factors. Acta Cryst. D 70: 2413-2419, 2014.

Skalova T., Blaha J., Harlos K., Duskova J., Koval T., Stransky J., Hasek J, Vanek O., Dohnalek J.: Four Crystal Structures of Human Llt1, a Ligand of Human Nkr-P1, in Varied Glycosylation and Oligomerization States. Acta Cryst. D 71: 578-591(accepted Dec 2014), 2015.

Other research-related activities – BIOCEV project

One of the main reasons why IBT was founded in 2008 was to concentrate a critical mass of research potential in terms of people and equipment able to produce excellent science at the interface between structural biology, biotechnology and medicine in the BIOCEV centre funded by the European Regional Fund.

The Institute of Biotechnology actively supports implementation of the project BIOCEV, G. Pavlinkova is a member of the Council of BIOCEV and B. Schneider is the Head of the BIOCEV Research Project 3 and a member of the Scientific Council of BIOCEV, J. Dohnalek a member of the Scientific Council of BIOCEV and coordinator of the BIOCEV core facilities.

Team 1 is involved in the BIOCEV project by participating in its research programme and by establishment of the qPCR and dPCR core facility (V. Korenkova, Head), which split off the Laboratory of Gene Expression (M. Kubista, Head).

Members of the Team 2 have participated in planning of the BIOCEV centre. Specifically, the Head of “Laboratory of Structure and Function of Biomolecules”, J. Dohnálek, provided significant input into the design, planning and realization of core facilities of BIOCEV. He and the members of his team designed the crystallisation and diffraction central facility, structural biology laboratories, and secured obtaining crystallography-related equipment. The Head of “Laboratory of Biomolecular Recognition”, B. Schneider, designed biophysical facilities and has been acting as the Head of the BIOCEV Research Project 3 since 2009.

Important activities have been pursued in European research infrastructures INSTRUCT and ELIXIR. The Czech Infrastructure for Integrative Structural Biology (CIISB), which has been jointly formed by the CEITEC and BIOCEV centres, is connected to the European infrastructure for integrative structural biology INSTRUCT; J. Dohnalek plays the role of the INSTRUCT spokesperson for the Czech Republic. The team also actively participates in ELIXIR.cz linked to the European bioinformatic infrastructure ELIXIR; J. Cerny is a member of the Executive Council and B. Schneider of the Board of ELIXIR.cz. Both infrastructures will be instrumental in the future development of the team activities.

Significant awards to employees
The very high standing of our Institute has been emphasised by honours awarded to our researchers. C. Barinka obtained the prestigious five-year J. E. Purkyne Fellowship and EMBO installation grant in 2010. J. Truksa was awarded by the grand from the Kellner Foundation (2012). Several researchers were acknowledged for the best publication in their field of study or for the best presentation at symposia.

Application of the results
The Institute's 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 a number of prototypes for commercial production and for 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 pre-clinical and clinical trials. The Laboratory of Ligand Engineering filed a patent application presenting a new generation of protein binders applicable to development of novel drugs as an alternative to conventional drugs based on neutralising 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.

Academic and international cooperation
Our Institute maintains collaboration with other Institutes of the Academy of Sciences of the Czech Republic and Universities, both in grant applications and publication activity. Our study programmes (Bachelor, Master, PhD) are performed in cooperation with Czech universities. Six of our scientists lecture at Czech and foreign universities and work in expert committees and scientific councils of several university Faculties.

International cooperation is one of the key factors of research of all our groups and is promoted at all levels in the Institute. Our groups participate in joint projects, bilateral agreements, or simply in solution of a particular scientific problem. We hosted eminent scientists from abroad, who delivered excellent lectures. We are open to foreign PhD students, and several are currently carrying out their research activities in our institute.

Both Teams every year organise national and international meetings. J. Peknicova (Team 1), is the main organiser of an annual Symposium of Immunology and Biology of Reproduction with International Participation in Trest (in 2014 was the 20th Anniversary Symposium). The Symposium enables communication between workers in research and in practice (reproductive medicine, centers of assisted reproduction and others). B. Schneider (Team 2) is the main organiser of an annual major national meeting, Discussions in Structural Molecular Biology.

Members of Team 2 play an important role in the Czech and international structural biology community. J. Dohnalek is the President, and B. Schneider Treasurer of the Czech Society for Structural Biology (http://structbio.org), B. Schneider is also a member of the Commission on Biomolecules of the International Union of Crystallography.

Journal publishing
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.

Scientists from our Institute are also active in popularising scientific results, by giving lectures and participating in the "Week of Science" and "Open-Door Days" organised by the Academy, as well as by appearances on TV and in printed media.

Research Report of the team in the period 2010–2014

Institute	Institute of Biotechnology CAS, v. v. i.

Scientific team	Development of Diagnostic and Therapeutic Procedures

2.1. Research objectives of the Team "Development of Therapeutic and Diagnostic Procedures"

The team comprises six individual sub-groups, all focusing on understanding the molecular and cellular bases of a variety of pathological states. They concern cutting-edge basic research aimed at providing better understanding of the molecular mechanism of highly socio-economically challenging pathologies with considerable impact on the population health. The focus is on a complex approach to the research of human diseases, starting with molecular aspects at the level of genetic regulation of selected pathologies, to biological, biochemical and proteomic studies that are relevant both to the genesis and progress of the pathologies, and identification of relevant diagnostic biomarkers. Model organisms and systems will also be used to uncover novel functions of genes, their products, and the regulatory aspects. An important output of ground-breaking features will be identification of novel and efficient therapeutic approaches.

The common denominator of all these projects is the pathological state of the cell, more precisely, uncovering of the molecular mechanisms underlying this state by profiling of selected genes, detection of changes in the localization and modification of relevant proteins, and identification of additional molecules that are involved in the initiation and progression of the pathological state. Further, the focus will be on preparation of the rationale for prevention and/or treatment of these states as well as the design of diagnostic methodologies for monitoring the pathologies. This approach is comparable with those of contemporary top biomedical research institutions and is likely to deliver breakthrough results published in leading journals and intellectual property leading to commercialization of results.

2.2. Scientific profile and the main specific results of the team

In the period 2010-2014, the team published 113 publications in peer-reviewed journals, five chapters in scientific books, filed one patent, and obtained twelve applied results. The team efforts are primarily focused on high-quality basic research with potential application of the results in the diagnosis and treatment of diseases. The scientific profile and the main results of individual groups are presented here:

2.2.1. Laboratory of Reproductive Biology

The laboratory has been focusing on studying the molecular mechanism of reproduction and the nature of specific sperm proteins playing a role during sperm maturation such as capacitation, acrosome reaction and sperm-egg fertilization. During last 10 years we discovered a number of new proteins that are fundamental for the sperm-egg 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. We focused on two major areas of research: 1. The role of oestrogens and

oestrogenic receptors during capacitation in mammalian spermatozoa; and 2. The effect of environmental pollutants on reproduction.

In vitro experiments have demonstrated the role of oestrogens and oestrogen receptors during capacitation in mammalian spermatozoa. This effect is mediated mainly by hyperphosphorylation of sperm proteins and premature calcium influx. These processes lead to a decreased ability of sperm to undergo acrosome reaction. The role of oestrogens was also confirmed by *in vivo* experiments. Together with Dr. K. Dvorakova-Hortova, Charles University, Faculty of Science, Prague, we found that exposure to 17β-estradiol leads to premature capacitation of mouse sperm in the epididymis with a potential negative impact on the sperm reproductive fitness in the female reproductive tract (Ded et al., 2010).

The process of sperm ubiqutination is another important step of sperm maturation. The ubiquitin-activating enzyme (UBA1) was detected in the sperm acrosome and a specific inhibitor (Pyr41) of UBA1 that alters sperm capacitation and sperm-egg coat penetration and inhibits porcine fertilization was characterized. In collaboration with prof. P. Sutovsky, Columbia University, USA, we found that ubiquitination of the “fertilization” protein spermadhesin (AQN1) and acrosin inhibitor (SPINK2) may play an important role in the pathway controlling the capacitation-associated changes in sperm protein phosphorylation. Our findings support the idea that the ubiquitin-proteasome system plays an essential role in fertilization. (Yi Y.J., et al., 2012).

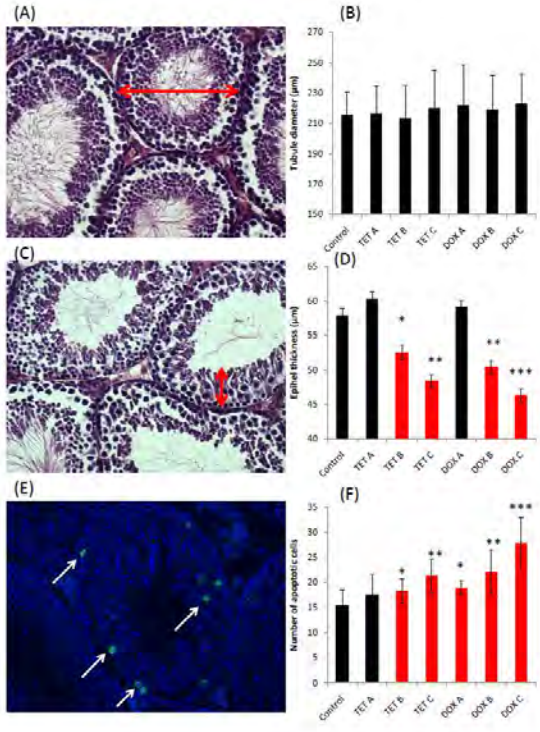


Fig.1. Analysis of the testicular tissue of mice after treatment with antibiotics during adolescence.
(A) Histological picture of the control animals - ongoing intensive sperm production. Arrows indicate the diameter of the channels. (B) Data of channel diameter measurements in the control and experimental animals. (C) Histology of the testis in animals treated with antibiotics. Loss of sperm production, changes in the channels. (D) Data of channel diameter measurements in the control and experimental animals. (E) The process of cell death - cells marked in green, cell nuclei are indicated in blue. (F) Graphical evaluation of the number of dead cells in histological sections of the testis in the control and experimental animals (Elzeinova et al., 2013).

We have also tested a number of environmental pollutants with endocrine activity such as endocrine disruptors (tetrabromobisphenol A, zearalenone, vinclozolin and the others) as well as other substances (antibiotics, oestrogens). In collaboration with Dr. R. Oliva and Dr J. Castillo, University of Barcelona, Spain we found that these substances affect the hormonal system, reproductive organs, spermatogenesis, sperm motility, expression of selected genes, expression of protamines in the sperm nucleus and their ratio, and induce epigenetic factors. We proved existence of a paradoxical effect of certain endocrine disruptors (bisphenol A and zearalenone), in which lower concentration of the substance has a greater negative effect on reproductive parameters than the higher dose. This finding is important for assessing the impact of these substances, which are present as pollutants in the environment. Furthermore, we proved that pollutants could induce trans-generational epigenetic deregulation of microRNA

expression, influencing their key role in germ cell differentiation, without changing the DNA (Zatecka et al., 2014a, 2014b). In cooperation with commercial companies, twelve prototypes for the detection of these substances have been developed.

2.2.2. Laboratory of Molecular Therapy

We have been interested in better understanding of cancer biology, more specifically, focusing on the role of mitochondria in tumour formation and progression and, as well, as a target for efficient anti-cancer therapy. We have two major areas of research: 1. Mitochondria as a target for novel mitocans, i.e. anti-cancer agents modified to associate with mitochondria; and 2. Transfer of mitochondria between stromal and tumour cells and its role in tumour initiation and progression.

Our group has been studying for several years novel anti-cancer agents that are efficient against tumours and that have been modified by tagging with a delocalised cationic group (tri-phenylphosphonium, TPP+). This modification causes their preferential association with mitochondria. This approach has been applied to vitamin E succinate, a selective anti-cancer agent that we described a while ago. Thus, mitochondrially targeted vitamin E succinate (MitoVES) has been documented to kill cancer cells of different origin some 20-50-fold more efficiently than found for its non-targeted counterpart, and to very efficiently suppress tumorigenic angiogenesis. Complex biochemical, cell biological as well as molecular modelling studies confirmed mitochondrial complex II as the preferred target for MitoVES. We then focused on complex I-targeting anti-cancer agents, starting with the widely used therapeutics against breast cancer, tamoxifen. We synthesised mitochondrially targeted tamoxifen (MitoTAM) by tagging the parental compound with TPP+, and found that MitoTAM was much more efficient in killing $Her2^{high}$ breast cancer cells than the parental compound. Further and very unexpectedly, MitoTAM killed $Her2^{high}$ cell more efficiently than $Her2^{low}$ cells, exactly opposite to tamoxifen. This was remarkable in particular for effects on $Her2^{high}$ tumours in a mouse model, where several mice became tumour-free, and the tumour did not come back within 5-6 months post treatment cessation. This indicated complete eradication of $Her2^{high}$ breast carcinomas. We have recently started studies on another anti-cancer agent targeting complex I that suppresses the fatal pancreatic carcinomas and that seems very intriguing. These studies are ongoing. With regards of our novel anti-cancer agents targeting mitochondria, we have been coining the term ‘mitocans’, which encompasses a wide group of small molecules with anti-cancer activity acting via mitochondria, and we have classified mitocans into several groups based on their molecular mechanism of action. We also, for the first time, showed that tagging mitochondria-acting anti-cancer agents with a delocalised cationic group (TPP+) makes them superior in anti-cancer efficacy and, further, alters their biological activity.

Some two years ago we started a project in collaboration with Prof. Michael Berridge from Wellington, New Zealand, on mitochondrial transfer from stroma to tumours. We found that cancer cells with mitochondria without DNA (ρ^0 cells) form syngeneic tumours when grafted into mice with a 3-4 week delay compared to the parental cells. Analysis of malignant cells from these tumours resulted in the discovery of horizontal transfer of mitochondria from stroma to tumour cells, since the isolated cells, which were originally deprived of their mitochondrial DNA (mtDNA) now contained mtDNA with the polymorphism of the host. These cells were, again, capable of respiration, which linked the mitochondrial function and the capacity of cancer cells to form tumours (Tan et al., 2015). We believe that this is the first documentation of mtDNA acquisition by way of mitochondrial horizontal transfer *in vivo*, and that these results are not only relevant to cancer but are addressing some of the very fundamental questions of cell biology as well as evolution.

2.2.3. Laboratory of Gene Expression

Our laboratory is a Europe's leading academic group specialized in high-throughput gene expression profiling and single cell analysis using real-time quantitative PCR (qPCR). We have several basic research projects in the field of developmental biology and stem cells as well as applied projects within cancer and neurological research. We also develop methods for quality control and assessment of nucleic acid analyses, and we are active in the area of standardization (Bustin et al., 2013).

Our long-term interest in developmental biology lies in early development of *Xenopus laevis* and localization of maternal mRNAs along the main developmental axes. We performed detailed analysis of maternal mRNAs along the animal-vegetal axis using the qPCR tomography technique we developed some years ago. We also studied distribution of miRNAs and found them too to be asymmetrically distributed within the oocyte (Huggettt et al., 2013). This is the first finding ever that microRNAs can be oriented within cells. Further, we studied the distribution of mRNAs among dividing cells of embryos from 8- to 32-cell stage showing the asymmetric distribution in the oocyte propagates (Flachsova et al., 2013). In 2013, Dr. Sindelka returned from a 4-year post-doctoral stay at the Whitehead Institute, Boston, and established an independent research sub-group studying the role of nitric oxide during early development, wound healing and regeneration using the *Xenopus* model.

Several members of our group collaborate with Dr. Anderova's laboratory at the Institute of Experimental Medicine, studying neural regeneration using mouse and rat models. Our contribution is single-cell expression profiling in glial cells cells (Rusnakova et al., 2013, Honsa et al., 2014).

In the cancer field, we have been collaborating with Dr. Vodicka's group at the Institute of Experimental Medicine of the Academy of Sciences of the Czech Republic (Slyskova et al., 2012). We have also collaborated with First and Third Medical Faculties of Charles University on a circulating tumour cells project. In collaboration with Dr Sabine Kasimir Bauer in Essen and Dr Katarina Kolostova at the Medical Faculty, Charles University, we characterized circulating tumour cells by expression profiling using a panel of 48-96 markers. We were successful in identifying signatures indicating treatment responses.

Our laboratory was a member of EU-funded consortium called SPIDIA (2009-2012), where the goal was to develop standard operating procedures for the pre-analytical process in molecular diagnostics. The work resulted in several publications and is also the base of nine new ISO guidelines that are currently being produced. Dr. Kubista, the head of our laboratory, is a member of the international working group drafting these new guidelines (Bustin et al., 2013).

Our laboratory is considered the leader in the field of quality control and assessment in molecular diagnostics, and we have been contributing to various guidelines and methodology papers (Huggettt et al., 2013). Since 2009, based on our unique equipment, our laboratory has been offering core facility services to academic and occasional industrial customers. In 2014, our facility was integrated as an official core facility of the BIOCEV. It provides complete service from experimental design and assay development to quantification and data analysis, and is operated by a core facility manager.

2.2.4. Laboratory of Molecular Pathogenetics

The main focus of our research is to identify molecular mechanisms in pathological changes during embryonic development. In the last five years, we have developed and progressed in two major projects. The first project determines the molecular causes of pathological changes associated with diabetic exposure. In collaboration with Prof. G. Semenza, Johns Hopkins University School of Medicine, USA, we linked HIF-1-regulated pathways and the development of congenital malformations in diabetes-exposed embryos and provided compelling evidence that impairment of HIF-1α-controlled hypoxia-response pathways may play a functionally

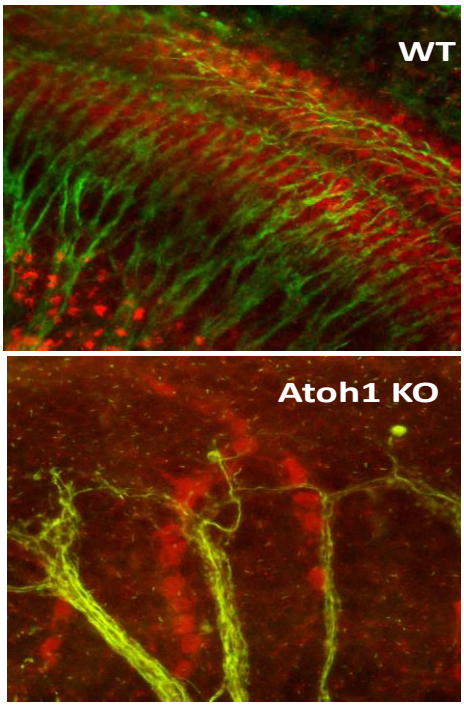


Fig.2. Differences in the innervation between the wildtype (WT) and mutant (*Atoh1*KO) cochlea.

causative role in diabetic embryopathy (Bohuslavova et al., 2013). In the other important project in the field of diabetic embryopathy, we showed that diabetes mellitus during pregnancy alters transcriptional profiles in the placenta, affecting cells of embryonic and maternal origin (Salbaum et al., 2011). Our collaborative research of transcriptome changes associated with diabetic embryopathy has been summarize in a book volume of *Comprehensive Toxicology*. To further investigate the role of HIF-1 pathways in cardiovascular pathologies and in combination with diabetic exposure, we analyzed molecular changes in the adult heart using a mouse model (Bohuslavova et al., 2014). Our results show that the dysfunction of HIF1 pathways considerably affects transcriptional regulation in the adult heart Our data also reveal significant differences between males and females in cardiac adaptive responses to hypoxia and indicate the necessity of optimizing diagnostic and therapeutic procedures in clinical practice with respect to gender. Together with Prof. F. Kolar‘s group, Institute of Physiology AS CR, we also showed that HIF-1α regulates early cardiac responses to diabetes in the adult heart and that HIF-1α deregulation may influence the increased risk for diabetic cardiomyopathy. Our results are in line with increasing evidence that the HIF-1-regulated system is compromised in the diabetic heart (Bohuslavova et al., 2010).

The second major project is focused on establishment of the functional role of the transcriptional factor ISLET1 in neurosensory development. Within this project we established a fruitful collaboration with Prof. Syka’s group at the Institute of Experimental Medicine AS CR and Prof. Bernd Fritzsch’s group from the USA. Using transgenic mice and Cre-loxP system, we have analyzed the role of specification transcription factors (Sox2, Atoh1, and Neurod1) in neurosensory system of the inner ear (Fig.2).

2.2.5. Laboratory of Tumour Resistance

Our laboratory was established in February 2013, and its activity can thus be monitored for two years only. Previous work was conducted at the Institute of Biotechnology within the Molecular Therapy Group, from which our laboratory branched off.

Our group is focusing on the biology of the tumour-initiating cells and the function and biology of mitochondria including utilization of mitochondria as targets for cancer therapy. We are also interested in iron metabolism and in the expression and function of ABC transporter proteins in the biology of tumour-initiating cells. The main aim of our research is to describe and identify novel targets of cancer cells, especially of the tumour-initiating cells, which could be utilized to remove, re-program or specifically induce apoptosis in these cells. During the monitored period of 2010-2014, we achieved three important outcomes, focusing on mtDNA transfer, the role of MiR-126 in the regulation of mitochondrial functions, and the regulation and role of the IDO1 enzyme in the biology of tumour-initiating cells.

Our first project documents the important role of mitochondria in carcinogenesis. Cancer cells without mitochondrial DNA (mtDNA) form tumours in mice with a considerable lag. However, cancer cells derived from these tumours and their metastases show reversal to the normal mitochondrial function. Their mtDNA is of the host origin (from the host mouse), and this clearly documents that the so-called horizontal mitochondrial transfer has occurred, providing cancer cells with functional mitochondria. Our role in this research was to identify and validate SNP polymorphisms that documents mtDNA transfer from host cells to cancer cells.

Our second project helped identify the important role of MiR-126 regulation in cell growth and invasive capacity of mesothelioma cancer. High expression of the small non-coding RNA MiR-126 dramatically inhibits the propensity of mesothelioma tumours to grow, involving considerable changes in mitochondrial function elicited by RS1 down-regulation. We support the idea that MiR-126 is one of the important regulators of mesothelioma carcinogenesis that could be clinically used as a biomarker. Our role was to validate the proposed scheme in which MiR-126 down-regulates IRS1 through its 3‘UTR using the luciferase reporter assay.

The third piece of work shows that mitochondrially targeted vitamin E analogues are able to down-regulate the levels of the IDO1 protein in tumour-initiating cells. These cells are thought to survive the therapy and later on give rise to tumour relapse. Our data show that cultures of tumour-initiating cells *in vitro* show higher protein levels and activity of IDO1, possibly resulting in inhibition of the immune response. Since the mitochondrially targeted vitamin E analogue is able to suppress IDO1 levels, it possibly has high translational potential. We have utilized our expertise in assessing the expression of several important regulatory genes via qPCR.

2.2.6. Laboratory of immunopathology and immunotherapy

The laboratory was involved in research and development of new diagnostic and therapeutic tools available at the molecular or cellular level in disorders of humoral immunity in humans. The most important object of interest is direct modulation of autoimmune condition using a modified recombinant autoantigens and study of unconventional memory B cells that play a key role in the development of a variety of immune disorders (Palenicek et al., 2011, Adamcova et al., 2013).

References:

Adamcova M., Ruzickova S., Simko F.: Multiplexed immunoassays for simultaneous quantification of cardiovascular biomarkers in the model of Hg-nitro-L-arginine methylester (L-

Bohuslavova R, Kolar F, Kuthanova L, Neckar J, Tichopad A, Pavlinkova G.: Gene expression profiling of sex differences in HIF1-dependent adaptive cardiac responses to chronic hypoxia. J Appl Physiol, 109(4):1195-202, 2010.

Bohuslavova R, Skvorova L, Sedmera D, Semenza GL, Pavlinkova G. Increased susceptibility of HIF-1α heterozygous-null mice to cardiovascular malformations associated with maternal diabetes. J Mol Cell Cardiol. 60:129-41, 2013.

Bohuslavova R., Kolar F., Sedmera D., Skvorova L., Papousek F., Neckar J., Pavlinkova G. Partial deficiency of HIF-1α stimulates pathological cardiac changes in streptozotocin-induced diabetic mice. BMC Endocr Disord. 14:11-25, 2014.

Brieño-Enríquez M. A., García-López. J., Cárdenas D. B., Guibert S., Cleroux E., Děd L., Hourcade JdD., Pěknicová J., Weber M., del Mazo, J.: Exposure to endocrine disruptor induces transgenerational epigenetic deregulation of MicroRNAs in primordial germ cells. PLoS ONE, accepted

Bustin S. A., Benes V., Garson J.A.,...Kubista M., Mueller R., Nolan T.,. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clinical Chemistry 55:611-622, 2009

Bustin, S. A,….Kubista, M,….Vandesompele, J.: The need for transparency and good practices in the qPCR literature. Nature Methods, 10: 1063–1067, 2013.

Ded L., Dostalova P., Dorosh A., Dvorakova-Hortova K., Peknicova J.: Effect of estrogens on boar sperm capacitation in vitro. 13:8, Reprod Biol Endocrinol. 8:87, 2010.

Elzeinova, F., Pěknicova, J., Ded, L., Kubatova A., Margaryan H., Dorosh A., Makovicky P., Rajmon, R.: Adverse effect of tetracycline and doxycycline on testicular tissue and sperm parameters in CD1 outbred mice. Exp Toxicol Pathol. 65: 911-917, 2013

Flachsova, M., Sindelka, R., Kubista, M.: Single blastomere expression profiling of Xenopus laevis embryos of 8 to 32-cells reveals developmental asymmetry. Scientific Reports, 3: 2278, 2013.

Honsa P., Pivonkova H., Harantova L., Butenko O., Kriska J., Dzamba D., Rusnakova V., Valihrach, L., Kubista, M., Anderova, M. Increased Expression of Hyperpolarization - Activated Cyclic Nucleotide-Gated (HCN) Channels in Reactive Astrocytes Following Ischemia. GLIA, 2014.

Huggett J. F., Foy C. A., Benes, V., Emslie K., Garson J. A., Haynes R., Hellemans J., Kubista M., Mueller R. D., Nolan T., Pfaffl M. W., Shipley G. L., Vandesompele J., Bustin S. A. The digital MIQE guidelines: Minimum Information for Publication of Quantitative Digital PCR Experiments. Clinical Chemistry, 59(6): 892-902, 2013.

Palenicek T., Rambosek L., Ruzickova S., Vaculin S., Hoschl C.: The influence of a subanasthetic dose of ketamine on circulating pro-inflamantory cytokines and serotonin in brain. Psychological Medicine, 41(8): 1787-1789, 2011.

Rusnakova V., Honsa P., Dzamba D., Stahlberg A., Kubista M., Anderova M. Heterogeneity of Astrocytes: From Development to Injury – Single Cell Gene Expression. PLoS ONE 8(8): e69734, 2013.

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, 18:5878-5887, 2012.

Stapelberg M, Zobalova R, Nguyen MN, Walker T, Stantic M, Goodwin J, Pasdar EA, Thai T, Prokopova K, Yan B, Hall S, de Pennington N, Thomas SR, Grant G, Stursa J, Bajzikova M, Meedeniya AC, Truksa J, Ralph SJ, Ansorge O, Dong LF, Neuzil J. Indoleamine-2,3-dioxygenase elevated in tumor-initiating cells is suppressed by mitocans. Free Radic Biol Med. 67: 41-50, 2014.

Tan AS, Baty JW, Dong LF, Bezawork-Geleta A, Endaya B, Goodwin J, Bajzikova M, Kovarova J, Peterka M, Yan B, Pesdar EA, Sobol M, Filimonenko A, Stuart S, Vondrusova Kluckova K, Sachaphibulkij K, Rohlena J, Hozak P, Truksa J, Eccles D, Haupt LM, Griffiths LR, Neuzil J, Berridge MV. Mitochondrial genome acquisition restores respiratory function and tumorigenic potential of cancer cells without mitochondrial DNA. Cell Metab. 21:81-94, 2015. accepted 2014

Tomasetti M, Nocchi L, Staffolani S, Manzella N, Amati M, Goodwin J, Kluckova K, Nguyen M, Strafella E, Bajzikova M, Peterka M, Lettlova S, Truksa J, Lee W, Dong LF, Santarelli L, Neuzil J. MicroRNA-126 suppresses mesothelioma malignancy by targeting IRS1 and interfering with the mitochondrial function. Antioxid Redox Signal. 21: 2109-25, 2014.

Yi Y J., Zimmerman S. W., Manandhar G., Odhiambo J. F., Kennedy C., Jonáková V., Maňásková-Postlerová P., Sutovsky M., Park C.S., Sutovsky P.: Ubiquitin-activating enzyme (UBA1) is required for sperm capacitation, acrosomal exocytosis and sperm-egg coat penetration during porcine fertilization. International J. of Andrology 35(2), 196-210 (2012)

Zatecka E., Ded L., Elzeinova F., Kubatova A., Dorosh A., Margaryan H.,Dostalova P., Korenkova V., Hoskova K., Peknicova J.: Effect of zearalenone on reproductive parameters and expression of selected testicular genes in mice. Reprod. Toxicology 45: 20-30, 2014a.

Zatecka E., Castillo J., Elzeinova E., Kubatova A., Ded L., Peknicova J., Oliva R.:The effect of tetrabromobisphenol A on protamine content and DNA 1 integrity in mouse sperm. Andrology 2(6): 910-917, 2014b.

3. Research Plan of the team for 2015–2019

Maximum length of 3 pages.

The future plans of the five laboratories of Team I are highly innovative and are bound to deliver ground-breaking results touching on the very basics of cell and molecular biology, clearly greatly enriching our basic scientific knowledge, also branching to development and establishment of diagnostic a therapeutic approaches relevant to some of the most vexing pathologies of industrial countries with overwhelming socio-economic impact.

3.1. Laboratory of Reproductive Biology

Our future research will build on previous results and with the new methodological approaches the molecular mechanisms of selected processes in reproduction will be specified. Our research will be directed towards the following five areas.

The effect of environmental pollutant on reproductive parameter will be studied and the influence of endocrine disruptor in diabetic environment on male reproduction will be investigated (collaboration with Dr. G. Pavlinkova (Laboratory of Molecular Pathogenetics, IBT) and prof. M. Macek, 2nd Faculty of Medicine, Motol University Hospital).

Identification of epigenetic biomarkers of male germ cell disorders linked to adverse environmental factors will be studied. A complex assessment of the effect of tetrabromobisphenol A on testicular physiology with regard to the specific effects on different testicular cell types (Sertoli cells) during different developmental periods will be used.

Lifestyle and environmental factors affecting sperm epigenome and embryonic development with focus on assessing the potential correlation between identified epigenetic fertility factors related to smoking with IVF/ICSI (intracytoplasmic sperm injection) will be determined. The number of oocytes, and embryos, the percentage of fertilized oocytes (fertility rate) and pregnancy outcomes will be registered. Biostatistical comparison of murine sperm epigenome with human sperm epigenome will be performed in order to determine specific genomic sequences susceptible to cigarette smoke/hormonal mediated defects.

The influence of estrogens and xenoestrogens on sperm estrogen receptors and their impact on mammalian reproduction will be studied, focusing on the detection and localization of estrogen receptors (ERs) during sperm development: sorting of spermatic cells in different developmental stages; monitoring of splicing variants of ERs by PCR; isolation of subcellular protein fractions from spermatogenic cells and sperm; testing of selected antibodies against ERs with different specificity.

Protein network dynamics during sperm capacitation, acrosome reaction (AR) and sperm-egg interaction will be monitored by investigating of molecular mechanisms responsible for dynamics of Izumo1 and CD46 during AR and sperm-egg interaction, followed up by characterization tetraspanin protein (CD81, CD46, CD9) dynamics and monitoring the dynamics of proteins (Izumo1, Integrins, ADAMs) during sperm-egg interaction and fusion.

3.2. Laboratory of Molecular Therapy

Our focus for the next few years is to continue in our ongoing projects and develop them into novel areas of research with emphasis on highly innovative aspects of our studies. One arm of our studies will be pursuing mitochondria as a translational relevant target for novel anti-cancer agents, the other will be the mechanism and functional aspects of mitochondrial transfer between tumour stroma and cancer cells.

The first large project, i.e. targeting mitochondria as an efficient way to cure cancer, is based on our previous experience of over 10 years in this area of research. Currently, we are close to finalising a project, in which we modified tamoxifen, a frequently used drug against breast cancer by its tagging with the TPP+ group to target cancer cell mitochondria. We will conduct the final experiments that will allow submission of this work into a top journal. Recently, we filed a patent application protecting MitoTAM and similar compounds as very efficient drugs against Her2-high breast cancer. At this stage, we are planning a proper pre-clinical trial that should result in phase I/phase II clinical trial in 2016/2017. We have a dedicated investor who financially supports our translational work.

The second major project concerns horizontal mitochondrial transfer. This project is of paramount importance for better understanding of basic cell biology and evolution, as well as for developing novel anti-cancer therapeutic approaches. We have observed that cancer cells without mtDNA (ρ^0 cells) form syngeneic tumours in mice with a considerable delay compared to parental cells. A question arises, what happens in (ρ^0 cells during the 3-4 weeks post-grafting and before a palpable tumour appears and starts progressing. We will study the molecular events after grafting ρ^0 cells into syngeneic mice. We will identify the changes that the originally ρ^0 cell has to undergo following acquisition of mtDNA in terms of mitochondrial function recovery and tumour initiation and progression. We will perform experiments to delineate the precise molecular events in transfer of mitochondria from the host to (ρ^0 cancer cells, including in vivo settings, using a transgenic mouse with red fluorescent mitochondria with grafted (ρ^0 cells with green fluorescent nuclei.

3.3. Laboratory of Gene Expression

Our main goal for the next five years is to continue developing our expertise in single-cell and subcellular profiling with next-generation sequencing and proteomics technologies, and we will apply these to our projects in developmental biology and basic cancer and neurological research.

Recently, we initiated collaboration with Prof. Norman Dovichi at Univ. of Notre Dame, USA, on quantitative deep proteome analysis, and we have started whole transcriptome profiling and profiling of non-coding RNA using next-generation sequencing of single cells. Collecting single cells from 2, 4 and 8-cell stages of *Xenopus* development, our goal is to create the first complete spatio-temporal map of key biomolecules and cell fate determinants of a developing vertebrate embryo. This will become a blueprint for future studies of the body plan formation.

In the field of neural regeneration, we will study expressional changes in individual glial cells during postnatal brain development induced by ischemic brain injury during aging and in an Alzheimer mouse model. The goal is to identify astrocytic sub-populations and follow re-activation and differentiation at the single-cell level. Within our cancer research, we will study heterogeneity of tumours and circulating tumour cell populations by single-cell expression profiling. Last year, we purchased a unique instrument called CellCollector from ALS, Germany, for single-cell selection for downstream analysis. In collaboration with ALS, we will develop work-flow expression profiling, which will allow us to tremendously increase the throughput, reduce the time of analysis (which is critical in single-cell profiling), and also to correlate mRNA and non-coding RNA profiles to immunological markers, such as surface proteins. This will make our single-cell work-flow the most powerful in the world and will open new avenues for basic as well as applied research.

We also give high priority to teaching and training of PhD students and post-doctoral fellows. We have strategic collaboration with the TATAA Biocenters and organize hands-on training courses for Czech as well as foreign students in different aspects of molecular analyses from

sample preparation, quality assessment, qPCR &NGS profiling, and data analysis. Our laboratory is mainly responsible for advanced courses including single-cell profiling, digital PCR and next-generation sequencing.

3.4. Laboratory of Molecular Pathogenetics

We plan to continue our research by focusing on two basic topics. First, we will investigate the effects of diabetic environment on the developing heart. We will build on our global transcriptome analyses of the embryonic heart and extend our research to the epigenome of diabetes-exposed heart. For this purpose, we have recently submitted a grant proposal to investigate developmental programing mechanisms in the diabetes-exposed heart. Together with Professor Görlach's (Technische Universität München, Germany), Benes's (EMBL Heidelberg), and Kolar's group (Institute of Physiology AS CR), we will analyse the genetic and epigenetic factors in diabetic embryopathy and developmental programming.

Second, we will continue our research on neurosensory development. Together with Professor Bernd Fritzsch (University of Iowa, USA) and Professor Syka (Institute of Experimental Medicine, AS CR), we plan to submit three manuscripts on Islet1 function in the inner ear development. Our break-through finding regarding the collaboration of Islet1 and Sox2 transcription factors in embryonal development should result in a publication in a journal with high impact factor. Two talented PhD students are involved in this research and they should graduate in the next two and three years, respectively.

Additionally, we have established a new line of research and received funding in collaboration with the Laboratory of Reproductive Biology (our Institute) and Professor Milan Macek, Second Faculty of Medicine, Motol University Hospital, investigating the negative effects of diabetes mellitus on male fertility.

3.5. Laboratory of Tumour Resistance

In the upcoming years, we will focus on detailed description of genes that we identified as genes that are differentially expressed in tumour-initiating cells (TICs) and may be important for their biology. These genes are related to iron metabolism as well as ABC transporters, whose expression is altered in TICs. The physiological role of these genes will be studied by inducible overexpression and gene knockout using TALEN or CRISPR approach. The effect of these manipulations on cellular proliferation, invasiveness and ability to generate and maintain tumour-initiating cells *in vitro* and *in vivo* will be studied.

Further, we will conduct our studies on mitochondrial regulation and function. We are especially focusing on the regulation of mitochondrial function and biogenesis by miRNA, and we have identified several interesting candidate miRNAs whose expression is altered in cells with compromised mitochondrial function and which are also differentially expressed in tumour-initiating cells. The effect of these miRNAs will be studied by constitutive and inducible over-expression and determination of its impact on mitochondrial structure, function and on the composition and stability of the mitochondrial respiratory complexes and supercomplexes.

We also plan, in cooperation with the Molecular Therapy Group, to continue our participation in studies that document the transfer of mtDNA between stromal cells of the host origin and cancer cells devoid of mtDNA. These studies will shed light on these basic molecular processes and will delineate and document the importance of mitochondrial OXPHOS in initiation and progression of cancer in mouse syngeneic models that are currently used in the laboratory.

3.6. Laboratory of Immunopathology and Immunotherapy

The Attestation Committee of the Institute and the Council of the Institute, due to the instability and low long-term productivity of the group, proposed to terminate the activities of the group. The group ended in December 31, 2014.

Collectively, the above statements concerning the performance over the last five years plus planned research of five laboratories of the team clearly document their cutting-edge quality and the promise that they will become leaders in their areas of research.

Importantly, this research includes highly sophisticated and break-through aspects of basic research, innovative and world top methodological approaches as well as translational research with patents and the ensuing transfer of results of basic research into practice. Several laboratory Heads are world-leading scientists in their respective research field.

Research Report of the team in the period 2010–2014

Institute	Institute of Biotechnology of the CAS, v. v. i.

Scientific team	Structural Biology and Protein Engineering

2.1. Research objectives of the Team "Structural Biology and Protein Engineering"

The main objective is to study biotechnologically and medically important biomolecules, proteins and nucleic acids. Our aim is to understand the structures and intermolecular interactions of biomolecular systems and, in a broader sense, their structure-function relationships, in order to be able to increase their desired effect, so that they can be developed into medically or biotechnologically useful products. The studied molecular systems are produced by advanced techniques of molecular biology and protein engineering; their structure, stability, and activity determined by the state-of-the-art structural and biophysical experimental and computational methods.

2.2. The main scientific results of the Team 2

Our primary research efforts have been directed into four main areas that demonstrate our achievements, but more importantly the future potential of the team to play an internationally recognized role in basic as well as oriented biomedical research. These main result areas are:

2.2.1. Structure-assisted drug design. Aimed at the development of small-molecules targeting human glutamate carboxypeptidase II (GCPII) that could be used as imaging or therapeutic modalities. The international recognition of our contribution to these studies was underscored by the invited reviews on structural (Pavlicek, Ptacek *et al*. 2012) and biological (Barinka, Rojas *et al*. 2012) aspects of GCPII.

2.2.2. Protein engineering. Combining techniques of *in vitro* protein evolution and of computer modeling for targeted modulation of protein properties such as binding affinity to medically important proteins (Mikulecky, Cerny *et al.* 2013) (Kuchar, Vankova *et al.* 2014) (Malý et al., Czech patent No.304514, 2014, and published international patent application WO 2014/079399 A1, 2014).

2.2.3. Structural bioinformatics. Bioinformatics as a tool to better understand biomolecular structures, large scale comparison of structures, description of general structural behavior of nucleic acids and proteins, discovery of structural motifs (Schneider, Cerny *et al.* 2014) (Schneider, Gelly *et al.* 2014).

2.2.4. Structure-function studies of proteins represent an important line of our research. These studies are based on employment of modern methods of structural and biophysical molecular analysis including x-ray crystallography (Navratil, Ptacek *et al*. 2014) (Skalova, Blaha *et al.* 2015).

2.3. Evolution and the current state of the Team 2

The evaluation period 2010-2014 can be characterized as the time of team instituting; our major tasks within that period were to establish, develop, stabilize, and sustain our research activities. The goal has been to design and build a strong, internationally recognized research group with synergy between individual Research Laboratories of

the team whose expertise would span from bioinformatics to expression and characterization, to structure determination, and finally to testing of biological activity of the studied molecular systems.

One of the main reasons why IBT was founded in 2008 was to concentrate a critical mass of research potential in terms of people and equipment able to produce excellent science at the interface between structural biology, biotechnology, and medicine in the BIOCEV center funded by the European Regional Fund. Therefore, composition of the team at IBT reflects the composition of one of the BIOCEV Research Programs, Program 3 “Structural Biology and Protein Engineering” (two research labs of Program 3 are from the Institute of Microbiology AS CR).

The heart of the team was founded in 2008 as a research laboratory with broad expertise stretched from bioinformatics to crystallography and to protein engineering. This rather unstructured group has evolved into two currently functional labs, “Laboratory of Ligand Engineering”, P. Malý, Head (the average FTE 4.4 during years 2010-2014), and “Laboratory of Biomolecular Recognition”, B. Schneider, Head (the average FTE 4.7), and a planned bioinformatic lab, “Structural Bioinformatics” J. Černý, Head.

The team has been substantially strengthened when C. Bařinka joined IBT in 2010 as Head of the “Laboratory of Structural Biology” (the average FTE 5.4). He received prestigious personal grants from the EMBO (Installation grant) and the Czech Academy of Sciences (Fellowship of J. E. Purkyne), which further underscore the national and international recognition of his research accomplishments.

While “Laboratory of Structure and Function of Biomolecules” of J. Dohnálek has always been planned as a part of IBT, principally as the cornerstone of the BIOCEV Research Program 3, it formally joined IBT in July 2013 (average FTE 1.6), and most of the research results of this group are reported under the Institute of Macro-molecular Chemistry. A second new research group, “Structural Proteins and Their Complexes” (Z. Lánský, Head), has been opened in January 2015 and our report mentions plans of this and bioinformatic research groups for the period 2015-2019.

To summarize, the structure oriented biomedical and biotechnological research of IBT is secured by four well-established research labs with research personnel (including PhD students) at a level of about 5 FTE each, and by a perspective of two to be opened dynamic research groups.

2.4. Other research-related activities

Members of the team have participated very actively in planning and construction of the BIOCEV center. Specifically, the Head of “Laboratory of Structure and Function of Biomolecules”, J. Dohnálek provided significant input in the design, planning and realization of the core facilities of BIOCEV. He and the members of his team designed the crystallization and diffraction central facility and secured obtaining the related equipment. The Head of “Laboratory of Biomolecular Recognition”, B. Schneider, designed biophysical facilities that have been the first BIOCEV central facilities to be operational. He has also played a role of the Head of the BIOCEV Research Project 3 since 2009. We have to note that these activities allowed us to influence the BIOCEV design to some extent but also have represented a substantial workload influencing our scientific output.

Important activities have been pursued in European research infrastructures INSTRUCT and ELIXIR. The Czech Infrastructure for Integrative Structural Biology (CIISB), which has been jointly formed by the CEITEC and BIOCEV centers, is connected to the European infrastructure for integrative structural biology INSTRUCT; J. Dohnálek plays

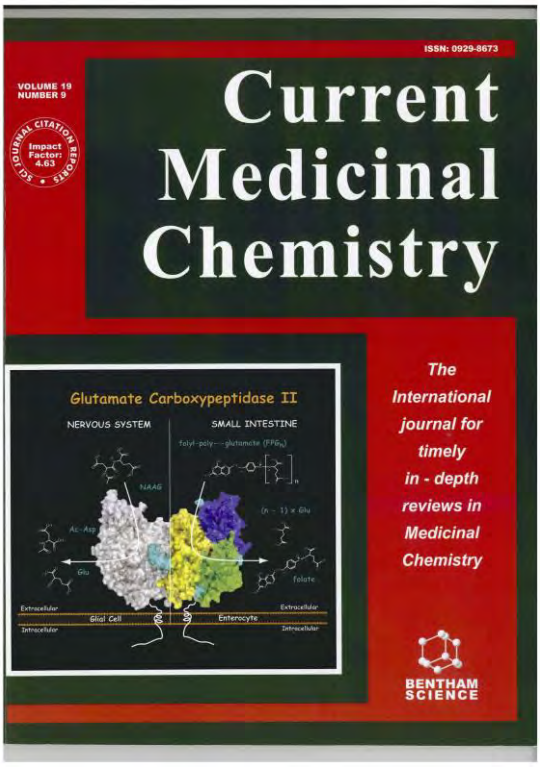
the role of an Instruct spokesperson for the Czech Republic and he and B. Schneider are members of the Executive committee of CIISB. The team also actively participates in ELIXIR.cz linked to the European bioinformatic infrastructure ELIXIR; J. Černý is a member of the Executive Council and B. Schneider of the Board of ELIXIR.cz. Both infrastructures will be instrumental in the future development of the team activities.

2.5. Overview of the research activities of the Team 2

Research activities of the team encompass four major areas: 1. Structure-assisted drug design, 2. Protein engineering, 3. Structural bioinformatics, and 4. Structure-function studies of proteins.

2.5.1. Structure-assisted drug design

The projects aimed at “traditional” structure-assisted drug development were directed to GCPII-specific small-molecules. We were able to contribute significantly to the understanding of the structural features of human GCPII with relation to the inhibitor design by the structural, biochemical, and biological characterization of different chemical classes of GCPII-specific probes. Our results were reported in several manuscripts and additional findings are in various stages of completion. Moreover, our expertise in this field formed a basis for a consultancy agreement with a French pharma company (Institute de Recherche Pierre Fabre and Dr. Barinka).



Cover page of Current Medicinal Chemistry featuring a crystal structure of human GCPII (Pavlicek, Ptacek *et al.* 2012).

In collaboration with Spiegel lab we identified a previously unreported arene-binding site on GCPII that drastically enhances inhibitor binding to GCPII and can be used for the design of novel ultra-high affinity compounds (Zhang, Murelli *et al.* 2010). We designed, synthesized and analyzed a series of GCPII inhibitors with enhanced lipophilicity that were aimed at the targeting of GCPII residing within the neuraxis (Plechanovova, Byun *et al.* 2011). We developed a novel assay based on several libraries totaling over 50,000 small-molecule compounds using their fluorescence polarization screening (Alquicer, Sedlak *et al.* 2012) and solved X-ray structures of a series of urea-based inhibitors, where the terminal glutamate moiety was replaced with more lipophilic functionalities that could be used for the rational design of novel glutamate-free GCPII inhibitors (Pavlicek, Ptacek *et al.* 2014).

In addition to the aforementioned published studies, we undertook several other projects describing interactions between GCPII and small molecules of different chemical classes and these projects are now very close to completion with a minimum of experimental work remaining.

2.5.2. Protein Engineering

Research oriented to targeted modulation of protein function *e. g*. to increasing protein affinity to the desired target, requires the integration of a host of methodological approaches, namely computer modeling, structural bioinformatics, biophysical characterization of proteins, measurement of their interactions, but mainly skills of protein expression and purification and special techniques of molecular biology such as phage or ribosome display. During the last five years, the team has implemented all these techniques and applied them successfully to various targets thus opening an avenue to future applications.

Historically, the first target of our effort to find high affinity binders or to increase affinity of the existing ones, was interferon-γ. The task was approached by two complementary ways, by *in vitro* protein evolution by ribosome display, and by *in silico* mutation analysis and molecular dynamics simulations.

The first approach, which was based on the ribosome display selection, used the three-helix bundle of the albumin-binding domain (ABD) of streptococcal protein G as a master scaffold for generation of a high-complex combinatorial DNA library with a theoretical complexity of trillions of ABD variants. This library was used as proof-of-concept for selection of unique ABD-derived binders of human interferon-γ with sub-to-low nanomolar range of binding affinity (Ahmad, Li *et al.* 2012). The usefulness of the availability of such high-affinity IFNγ binders for improved human diagnostics was then demonstrated in the following work where two of these binders (ABD020 and ABD275) were used as strong capture binding components, immobilized on the chip of a surface plasmon resonance (SPR) biosensor, for human IFNγ detection. It has been demonstrated that concentrations of IFNγ as low as 0.2 nM can be detected in both buffer and albumin-depleted 2% human plasma using the reported SPR biosensor (Sipova, Sevcu *et al.* 2012).

An alternative, computer-aided approach to finding high-affinity binder to interferon-γ searched for mutations in interferon-γ natural ligand, its receptor 1. We developed two generally applicable *in silico* driven protocols. In the first strategy, we mutated the amino acid residues at the interferon-γ/receptor interface (Mikulecky, Cerny *et al.* 2013). The other approach was more unorthodox, looking for mutations in cavities in the receptor molecule that would stiffen it and increase its binding (Cerny, Mikulecky *et al*. 2015). We designed approximately 30 receptor variants; they were all expressed in *E. coli* and their affinities to interferon-γ measured by SPR. Of these ~30 variants, our straightforward *in silico* protocol succeeded in finding two receptor variants with the affinity increased about five-fold and seven-fold compared to the wild-type receptor (besides several other variants with smaller affinity increases).

The proven concept of the ABD library was then successfully used in the following project, which led to the generation of high-affinity antagonists of IL-23 receptor, a key member of IL-23-mediated cell signaling and Th17-driven pro-inflammatory axis. A collection of recombinant human IL-23 receptor binding proteins were generated and characterized, and several variants were identified as efficient IL-23R blockers, suppressing IL-23 cytokine binding to the IL-23R receptor (Kuchar, Vankova *et al.* 2014). These IL-23R antagonists (REX binders) might therefore be useful in designing novel anti-inflammatory biologicals (Malý et al., Czech patent No.304514, 2014, and published international patent application WO 2014/079399 A1, 2014). Due to the low-molecular weight of ABD-derived binders (5 kD), they can be further modified to serve as a non-immunoglobulin alternative for trans-dermal delivery systems important for development of novel topical drugs for treatment of psoriasis. The model of ABD-derived library was also used for generation of other inhibitory binders targeted to human IL-23 cytokine

(Vaňková et al., in preparation), playing a substantial role in mediating several autoimmune diseases.

The research project aimed at the development of high-affinity binders that could serve to detect oncomarkers. In these projects, we developed three collections of binders raised against novel important prostate cancer oncomarkers, binders of human prostate secretory protein 94 (PAP binders), to human kallikrein-2 (KLA binders) and human kallikrein-11 (HIP binders). These binders could serve as novel components for improved and more complex prostate cancer diagnosis (Marečková et al. submitted). In these projects, we collaborated with the Czech companies EXBIO Praha and Protean.

Our model of ABD combinatorial library and the established ribosome selection system was further used for generation of Shiga toxin specific binders that can be displayed on the surface of genetically modified lactic acid bacteria as an efficient *in vivo* detoxication bacterial cleaner of Stx1B toxin, produced by enterohemorrhagic *E. coli* and *Shigella dysenteriae*, thus preventing from hemolytic uremic syndrome and acute renal failure (Zadravec *et al*., in preparation). The project was undertaken in collaboration with Dr. A. Berlec, Jozef Stefan Institute, Ljubljana, and Prof. B. Štrukelj, University of Ljubljana, Slovenia.

Interleukin-23 (IL-23) is a heterodimeric cytokine of covalently bound p19 and p40 subunits that interact with their cognate IL-23/ IL-12β1 receptors thus promoting intracellular signaling. We developed unique IL-23 receptor blocking proteins with *ex vivo* immunosuppressive function (Kuchar, Vankova *et al*. 2014).

In parallel to the projects aimed at interactions between GCPII and small molecules (see 2.5.3), we commenced the development of GCPII-specific macromolecular ligands that could be used for prostate cancer imaging and therapy. The development of these ligands was done in collaboration with prof. Skerra (TUM, Munich, Germany) and was based on phage display of Anticalins, 20 kDa non-immunoglobulin scaffolds. Selected Anticalin binders with picomolar affinities offer a viable alternative to antibody-based GCPII-specific ligands for biomedical applications. These findings provide the basis for a European patent application that is being jointly filed with TUM.

We produced four new monoclonal antibodies specifically recognizing GCPII. Their detailed characterization revealed that two of them outperform mAbs used in clinical testing. Given these findings we plan using protein engineering to modify our mAbs (humanization, Fab expression) with the hope of translation into clinic. Finally, in collaboration with Prof. Konvalinka group (IOCB, Prague, Czech Republic) we carried out a detailed comparison of GCPII-specific mAbs “the most widely used” in the field (Tykvart, Navratil *et al.* 2014).

2.5.3. Structural bioinformatics

Structural bioinformatics and the related computer modeling techniques, especially analyzing protein structures, are in a sense integral to other research approaches, and are therefore discussed in the relevant section of this report. Here we mention mainly activities in studying DNA-related structural bioinformatics.

Our unique ability to classify DNA conformers (Cech, Kukal *et al.* 2013) and initiation of collaboration with the French group of Dr. de Brevern (Université Paris-7) opened an avenue for a rigorous structural description of protein/DNA interfaces (Schneider, Cerny *et al.* 2014). We used the concept of structural alphabets to classify dinucleotide (Svozil, Kalina *et al.* 2008) and pentapeptide (Joseph, Agarwal *et al.* 2010) building blocks in over a thousand crystal structures of protein-DNA complexes. Assembling the mutually interacting dinucleotide and pentapeptide building blocks into “interaction matrices” revealed the preferred interaction motifs at the protein-DNA interface and allowed structural comparison at and outside the interface. The analyzed data demonstrated important differences between complexes of various types of proteins such as transcription factors and nucleases, distinct interaction patterns for the DNA minor groove relative to the major groove and phosphate, and importance of water-mediated contacts.

The dynamics of protein and nucleic acid structures is as important as their average static picture. The carefully curated dataset used in the previous study of the protein-DNA interactions (Schneider, Gelly *et al.* 2014) was used to find out how the crystal-derived B-factors represent the dynamic behavior of atoms of these complexes. Expectedly, the solvent-accessible amino acids have B-factors larger than residues at the biomolecular interfaces, while the core-forming amino acids are the most restricted in their movement. A unique feature of the latter group is that their side chain and backbone atoms are restricted in their movements to the same extent; in all other amino acid groups the side chains are more floppy than the backbone. Surprising are low values of B-factors of the water molecules bridging proteins with DNA. The above mentioned features are pronounced clearly only in structures with high crystallographic resolution; analysis of B-factors in structures with low resolution could lead to incorrect conclusions.

Our expertise in analysis of DNA and RNA conformational space led to several fruitful collaborations on the properties of NMR spectra of nucleic acids (Benda, Schneider *et al.* 2011) and to studies of DNA electric conductivity (Kratochvilova, Todorciuc *et al.* 2010) (Kratochvilova, Vala *et al.* 2013).

Specific bacterial DNA sequence segments, so-called repetitive extragenic palindromes (REP), turned our interest into a less studied phenomenon of structured or folded single stranded DNA. These DNA sequences are related to a putative transposase (with proven nuclease activity) and we performed analysis of phylogenetic distribution of particular REP classes in genomic sequences of tens of bacterial strains belonging to the *Pseudomonas fluorescens* and *Stenotrophomonas sp*. species (Nunvar, Licha *et al.* 2013) and to this end also analyzed the genome of *S. maltophilia* (Nunvar, Elhottova *et al.* 2014). The results show that REP elements constitute intriguing dynamic components of bacterial genomes, and indicate that REP diversification and proliferation are ongoing processes. High numbers of REPs have apparently been retained during the entire evolutionary time since the establishment of these two bacterial lineages, probably because of their so far not understood beneficial effect on host long-term fitness.

So far unpublished research of spectroscopic and thermodynamic solution properties of REP-related oligonucleotides from six bacterial species (Charnavets, Necasova *et al*. 2015, submitted to Bioplymers) identified their unexpectedly complex conformational

behavior. Intriguingly, for several REPs, the circular dichroism spectra suggest guanine tetraplexes as potential alternative or additional structural elements to the expected stem loop hairpins.

In collaboration, we also surveyed the possibility to study nuclease activity of the REP-related transposases by surface plasmon resonance (Bockova, Springer *et al.* 2015).

2.5.4. Structure-function studies of proteins

The effort to understand relationships between the structure and function of biomolecules is the general underpinning of our research. The related studies focus on the targets relevant for treatment of human diseases or applicable in biotechnologies. The natural methodological tools are x-ray crystallography, computer modeling, bioinformatics, and biophysical techniques characterizing the state and interactions of the molecules.

Expertise in single crystal biological X-ray crystallography lies in *de novo* phasing of unknown structures with the use of crystallographic experimental phasing techniques (including MIR, SIR, MAD, and SAD) and their combination with molecular replacement MR (Koval, Lipovova *et al.* 2013). We also significantly contributed to the knowledge of eukaryotic zinc-dependent nucleases by our discovery of several principal properties of a plant nuclease TBN1 (Koval, Lipovova *et al.* 2013). The results of the last two and other crystallographic projects (*e.g*. (Stepankova, Duskova *et al.* 2013)) have been published and are reported in the report of Institute of Macromolecular Chemistry.

We contributed to the explanation of the C-type lectin like natural killer cell receptors and their protein ligands by determining the structure of the mouse ligand Clr-g and of human receptor ligand LLT1 (Skalova, Blaha *et al.* 2015). This study brought insights into the interaction principles of these immune system proteins. We contributed to functional characterization of a new protein factor HelD of *Bacillus subtilis* RNA polymerase (Wiedermannova, Sudzinova *et al.* 2014). HelD enhances RNA polymerase activity in an ATP-dependent manner, most likely by recycling RNAp from the template DNA.

A collaboration between the Laboratory of Ligand Engineering at the IBT and the crystallographic group at the Institute of Molecular Genetics AS CR in Prague resulted in the description of high-resolution crystal structure of the N-terminal CRD domain of the mouse Galectin-4 in complex with lactose. The lactose binding affinity was characterized by fluorescent measurements and two lactose-binding sites were identified (Krejcirikova, Pachl *et al.* 2011).

Several projects aimed at deeper understanding of the role(s) of GCPII and its orthologs and paralogs in physiological processes. We structurally and biochemically characterized the folate-hydrolyzing activity of GCPII and its natural H475Y variant. This study offered a detailed description of GCPII involvement in the folate metabolism (Navratil, Ptacek *et al.* 2014). A more biotechnologically oriented project resulted in successful production of *in vivo* biotinylated GCPII. This construct was extensively used by us and several collaborating labs in a variety of experimental settings (Tykvart, Sacha *et al.* 2012). To better understand the physiological role of GCPII in non-mammalian species, we work on structural, enzymatic and biological characterization of GCPII orthologs from *K. lactis*, *S. cerevisiae*, *C. elegans*, *A. thaliana*, *D. rerio*, *X. laevis*, *S. mansoni*, and *F. hepatica*. We reported a detailed characterization of human NAALADase L, a paralog of GCPII, and showed that its primary physiological function is associated with the final stages of protein/peptide digestion and absorption in the human digestive system (Tykvart, Barinka *et al.* 2015). Our expertise in to GCPII studies was

witnessed by the invitation to write reviews on the structural (Pavlicek, Ptacek *et al.* 2012) and biological (Barinka, Rojas *et al.* 2012) aspects of the enzyme.

Projects aimed at the characterization of histone deacetylases (HDACs) represent a new research direction initiated in 2011. We invested heavily into the search for a robust expression system for the facile production of various HDACs and their variants, and successfully implemented a Gateway-based high-throughput pipeline for screening suitable combinations of expression hosts and affinity tags which is, in addition to our HDAC-related projects, used by several collaborating labs for expression of various recombinant proteins.

Aiming at solving the X-ray structure of human HDAC6, we produced the full-length protein and a number of its variants and subjected them to extensive screening of crystallization conditions. We were able to obtain diffracting crystals for one of the constructs at crystallographic resolution of 5.5 Å, but this data is insufficient for structure solving. We continue in this direction using different variants of orthologous HDACs as well as more constructs of human HDAC6. In addition to HDAC6, we expressed and purified three additional human HDACs (-8, -10, and -11). Our longer-term goal is to express all human HDACs (11 total) and characterize their substrate specificity using microarrays and proteome-based libraries of acetylated peptides.

Using acetylome microarrays together with a combinatorial library of acetylated tripeptides we analyzed the substrate specificity of HDAC6 and its isolated HDAC domains (collaboration with prof. Schutkowski, Halle, Germany). The identified substrate hits are now being verified using purified peptides in solution.

References

JN Ahmad, J Li, L Biedermannova, M Kuchar, H Sipova, A Semeradtova, J Cerny, H Petrokova, P Mikulecky, J Polinek, O Stanek, J Vondrasek, J Homola, J Maly, R Osicka, P Sebo & P Maly (2012). Novel High-Affinity Binders of Human Interferon Gamma Derived from Albumin-Binding Domain of Protein G. *Proteins* **80**: 774-789.

G Alquicer, D Sedlak, Y Byun, J Pavlicek, M Stathis, C Rojas, B Slusher, MG Pomper, P Bartunek & C Barinka (2012). Development of a High-Throughput Fluorescence Polarization Assay to Identify Ligands of Glutamate Carboxypeptidase Ii. *J. Biomol. Screen.* **17**: 1030-1040.

C Barinka, C Rojas, B Slusher & M Pomper (2012). Glutamate Carboxypeptidase Ii in Diagnosis and Treatment of Neurologic Disorders and Prostate Cancer. *Curr. Med. Chem.* **19**: 856-870.

L Benda, B Schneider & V Sychrovsky (2011). 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. *J Phys Chem A* **115**: 2385-2395.

M Bockova, T Springer, I Necasova, J Nunvar, B Schneider & J Homola (2015). Monitoring RAYT Activity by Surface Plasmon Resonance Biosensor. *Anal Bioanal Chem*, in press, 10.1007/s00216-015-8491-y.

P Cech, J Kukal, J Cerny, B Schneider & D Svozil (2013). Automatic Workflow for the Classification of Local DNA Conformations. *BMC Bioinform.* **14**: 205.

AP Joseph, G Agarwal, S Mahajan, J-C Gelly, LS Swapna, B Offmann, F Cadet, A Bornot, M Tyagi, H Valadié, B Schneider, C Etchebest, N Srinivasan & AG de Brevern (2010). A Short Survey on Protein Blocks. *Biophys. Rev.* **2**: 137-145.

J Cerny, L Biedermannova, P Mikulecky, J Zahradnik, T Charnavets, P Sebo & B Schneider (2015). Redesigning protein cavities as a strategy for increasing affinity in protein-protein interaction. Interferon-γ receptor 1 as a model. *Biomed Res. Int.*, **2015**: in press, accepted December 2014. doi: 10.1155/2015/716945.

T Koval, P Lipovova, T Podzimek, J Matousek, J Duskova, T Skalova, A Stepankova, J Hasek & J Dohnalek (2013). Plant Multifunctional Nuclease Tbn1 with Unexpected Phospholipase Activity: Structural Study and Reaction-Mechanism Analysis. *Acta Cryst. D* **69**: 213-226.

I Kratochvilova, T Todorciuc, K Kral, H Nemec, M Buncek, J Sebera, S Zalis, Z Vokacova, V Sychrovsky, L Bednarova, P Mojzes & B Schneider (2010). Charge Transport in DNA Oligonucleotides with Various Base-Pairing Patterns. *J Phys Chem*. **B114**: 5196-5205.

I Kratochvilova, M Vala, M Weiter, M Sperova, B Schneider, O Pav, J Sebera, I Rosenberg & V Sychrovsky (2013). Charge Transfer through DNA/DNA Duplexes and DNA/RNA Hybrids: Complex Theoretical and Experimental Studies. *Biophys. Chem*. **180-181**: 127-134.

V Krejcirikova, P Pachl, M Fabry, P Maly, P Rezacova & J Brynda (2011). Structure of the Mouse Galectin-4 N-Terminal Carbohydrate-Recognition Domain Reveals the Mechanism of Oligosaccharide Recognition. *Acta Cryst. D* **67**: 204-211.

M Kuchar, L Vankova, H Petrokova, J Cerny, R Osicka, O Pelak, H Sipova, B Schneider, J Homola, P Sebo, T Kalina & P Maly (2014). Human Interleukin-23 Receptor Antagonists Derived from an Albumin-Binding Domain Scaffold Inhibit IL-23-Dependent Ex Vivo Expansion of IL-17-Producing T-Cells. *Proteins* **82**: 975-989.

P Mikulecky, J Cerny, L Biedermannova, H Petrokova, M Kuchar, J Vondrasek, P Maly, P Sebo & B Schneider (2013). Increasing Affinity of Interferon-Gamma Receptor 1 to Interferon-Gamma by Computer-Aided Design. *Biomed Res. Int.* **2013**: Artn 752514.

M Navratil, J Ptacek, P Sacha, J Starkova, J Lubkowski, C Barinka & J Konvalinka (2014). Structural and Biochemical Characterization of the Folyl-Poly-Gamma-L-Glutamate Hydrolyzing Activity of Human Glutamate Carboxypeptidase Ii. *FEBS J.* **281**: 3228-3242.

J Nunvar, D Elhottova, A Chronakova, B Schneider & I Licha (2014). Draft Genome Sequence of *Stenotrophomonas Maltophilia* Strain 5ba-I-2, a Soil Isolate and a Member of a Phylogenetically Basal Lineage. *Genome Announc.* **2**.

J Nunvar, I Licha & B Schneider (2013). Evolution of REP Diversity: A Comparative Study. *BMC Genomics* **14**: 385.

J Pavlicek, J Ptacek & C Barinka (2012). Glutamate Carboxypeptidase Ii: An Overview of Structural Studies and Their Importance for Structure-Based Drug Design and Deciphering the Reaction Mechanism of the Enzyme. *Curr. Med. Chem.* **19**: 1300-1309.

J Pavlicek, J Ptacek, J Cerny, Y Byun, L Skultetyova, MG Pomper, J Lubkowski & C Barinka (2014). Structural Characterization of P1 '-Diversified Urea-Based Inhibitors of Glutamate Carboxypeptidase Ii. *Bioorg. Med. Chem. Lett.* **24**: 2340-2345.

A Plechanovova, Y Byun, G Alquicer, L Skultetyova, P Mlcochova, A Nemcova, HJ Kim, M Navratil, R Mease, J Lubkowski, M Pomper, J Konvalinka, L Rulisek & C Barinka (2011). Novel Substrate-Based Inhibitors of Human Glutamate Carboxypeptidase II with Enhanced Lipophilicity. *J. Med. Chem.* **54**: 7535-7546.

B Schneider, J Cerny, D Svozil, P Cech, JC Gelly & AG de Brevern (2014). Bioinformatic Analysis of the Protein/DNA Interface. *Nucleic Acids Res.* **42**: 3381-3394.

B Schneider, JC Gelly, AG de Brevern & J Cerny (2014). Local Dynamics of Proteins and DNA Evaluated from Crystallographic B Factors. *Acta Cryst. D* **70**: 2413-2419.

H Sipova, V Sevcu, M Kuchar, JN Ahmad, P Mikulecky, R Osicka, P Maly & J Homola (2012). Surface Plasmon Resonance Biosensor Based on Engineered Proteins for Direct Detection of Interferon-Gamma in Diluted Blood Plasma. *Sensor. Actuat. B-Chem.* **174**: 306-311.

T Skalova, J Blaha, K Harlos, J Duskova, T Koval, J Stransky, J Hasek, O Vanek & J Dohnalek (2015). Four Crystal Structures of Human Llt1, a Ligand of Human Nkr-P1, in Varied Glycosylation and Oligomerization States. *Acta Cryst. D* **71**: 578-591(accepted Dec 2014).

A Stepankova, J Duskova, T Skalova, J Hasek, T Koval, LH Ostergaard & J Dohnalek (2013). Organophosphorus Acid Anhydrolase from Alteromonas Macleodii: Structural Study and Functional Relationship to Prolidases. *Acta Crystallogr F* **69**: 346-354.

D Svozil, J Kalina, M Omelka & B Schneider (2008). DNA Conformations and Their Sequence Preferences. *Nucleic Acids Res.* **36**: 3690-3706.

J Tykvart, C Barinka, M Svoboda, V Navratil, R Soucek, M Hubalek, M Hradilek, P Sacha, J Lubkowski & J Konvalinka (2015). Structural and Biochemical Characterization of a Novel Aminopeptidase from Human Intestine. *J. Biol. Chem.*, 10.1074/jbc.M114.628149.

J Tykvart, V Navratil, F Sedlak, E Corey, M Colombatti, G Fracasso, F Koukolik, C Barinka, P Sacha & J Konvalinka (2014). Comparative Analysis of Monoclonal Antibodies against Prostate-Specific Membrane Antigen (Psma). *Prostate* **74**: 1674-1690.

J Tykvart, P Sacha, C Barinka, T Knedlik, J Starkova, J Lubkowski & J Konvalinka (2012). Efficient and Versatile One-Step Affinity Purification of *in vivo* Biotinylated Proteins: Expression, Characterization and Structure Analysis of Recombinant Human Glutamate Carboxypeptidase Ii. *Protein Expres. Purif.* **82**: 106-115.

J Wiedermannova, P Sudzinova, T Koval, A Rabatinova, H Sanderova, O Ramaniuk, S Rittich, J Dohnalek, Z Fu, P Halada, P Lewis & L Krasny (2014). Characterization of Held, an Interacting Partner of RNA Polymerase from Bacillus Subtilis. *Nucleic Acids Res.* **42**: 5151-5163.

AX Zhang, RP Murelli, C Barinka, J Michel, A Cocleaza, WL Jorgensen, J Lubkowski & DA Spiegel (2010). A Remote Arene-Binding Site on Prostate Specific Membrane Antigen Revealed by Antibody-Recruiting Small Molecules. *J. Am. Chem. Soc.* **132**: 12711-12716.