

Questionnaire

Summary of the main activities of a scientific organization of the Slovak Academy of Sciences

Period: January 1, 2003 - December 31, 2006

I. Formal information on the assessed Organisation:

1. Legal name and address

Institute of Animal Biochemistry and Genetics
900 28 Ivanka pri Dunaji, Slovak Republic

2. Executive body of the Organisation and its composition

Directoriat	name	age	years in the position
director	RNDr. Ivan Hapala, CSc.	52	5
deputy director	hDoc. RNDr. Ľubor Košťál, CSc.	50	5
scientific secretary	Doc. RNDr. Miloslav Greksák, CSc.	70	5

3. Head of the Scientific Board

Doc. RNDr. Peter Šmigáň, DrSc.

4. Basic information about the research personnel

- i. Number of employees with a university degree (PhD students excluded) engaged in research and development and their full time equivalent work capacity (FTE) in 2003, 2004, 2005, 2006 and average number during the assessment period

ii. Organisation units/departments and their FTE employees with the university degree engaged in research and development

Research staff	2003		2004		2005		2006		average	
	No.	FTE	No.	FTE	No.	FTE	No.	FTE	No.	FTE
organisation in whole	23	20.25	26	22.05	30	23.95	30	25.25	27.25	22.875
Department of Bioenergetics	8	7.35	9	7.95	12	9.5	12	10.55	10.25	8.8375
Department of Immunogenetics	4	4	4	4	4	4	5	3.85	4.25	3.9625
Department of Endocrinology and Ethology	6	4.55	8	5.5	9	5.45	7	5.5	7.5	5.25
Department of Gravitational Physiology	5	4.35	5	4.6	5	5	6	5.35	5.25	4.825

5. Basic information on the funding

- i. Total salary budget¹ of the Organisation allocated from the institutional resources of the Slovak Academy of Sciences (SAS) in 2003, 2004, 2005, 2006, and average amount for the assessment period

Salary budget	2003	2004	2005	2006	average
total salary budget (millions of SKK)	9.303	9.445	10.040	10.275	9.766

6. URL of the Organisation's web site

<http://www.ubgz.sav.sk>

http://www.sav.sk/index.php?lang=en&charset=&doc=org-ins&institute_no=37

II. General information on the research and development activity of the Organisation:

1. Mission Statement of the Organisation as presented in its Foundation Charter

The mission of the Institute of Animal Biochemistry and Genetics, as defined in the Charter, is based on activities in four areas:

- Advancing knowledge in biosciences and agricultural sciences by performing high-quality basic research on genetic, biochemical and physiological processes in animals and microorganisms and transfer of this knowledge to agriculture, health care and industry.

¹ Sum of the brutto salaries without the fund contributions.

- Designing and implementing educational and research training programmes for Ph.D. students in biochemistry and animal physiology and participation in undergraduate education at partner universities.
- Providing consulting services and scientific expertise to administrative and decisive bodies, as well as dissemination of scientific knowledge in the society.
- Strengthening the position of science at international and national level improving thus the cultural and social awareness of the society

2. Summary of R&D activity pursued by the Organisation during the assessed period, from both national and international aspects and its incorporation in the European Research Area (max. 10 pages)

Membrane research experiences an exciting period in recent years. New ideas on membrane structure and its dynamics, as well as the newly acknowledged role of membrane-associated processes on the functioning of the cells and organisms make membrane studies more attractive than ever. Of particular importance is the increasing awareness of the role of membranes in the aetiology of some of the most severe diseases (e.g. neurological and cardiovascular diseases, cancer, diabetes, etc.). This is why membranes and their functions in normal and pathological conditions have been in the centre of our research interests during last four years. The broad spectrum of membrane-related problems is reflected also in the research program of our Institute. We are studying membrane phenomena at various levels of complexity (Fig. 1), such as: a) molecular interactions at membrane-like interfaces, b) membrane bioenergetics in methanoarchaea, c) formation and maintenance of membranes in eukaryotic cells, d) roles of membranes in cell-cell recognition, e) membrane receptors in neural signalling and control of behaviour. The results obtained during years 2003-2005 are summarized for each research area.

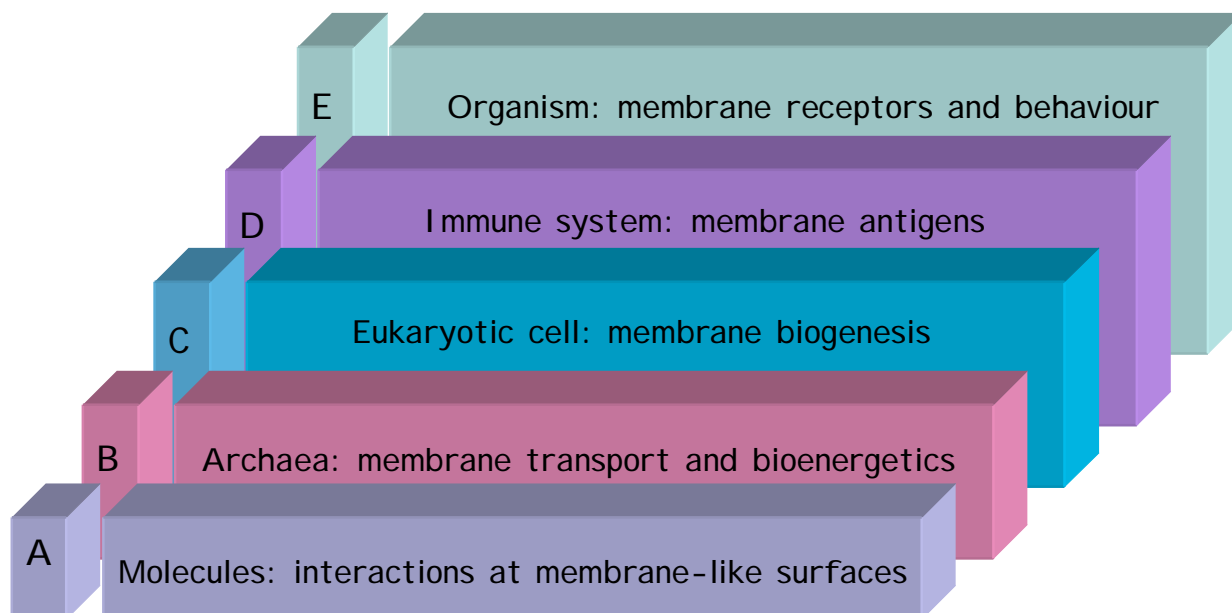


Fig. 1 Membrane functions studied at different levels of biological complexity at the Institute of Animal Biochemistry and Genetics

A. Interactions at the molecular level and development of novel biosensors

Biosensor research represents the boundary between biology, chemistry, nanotechnology, and engineering. Many proteins used in construction of biosensors are naturally associated with biological membranes. However, such proteins lose their conformation and activity if removed from membranes. We are therefore studying novel materials adhering in membrane-like layers to metal surfaces. Proteins can attach to the rough surface of these layers and maintain the active conformation. Conventional methods to tailor the surface properties of biosensors are based on self-assembled monolayers (SAMs) of thiol compounds. The disadvantage of SAMs is the absence of 3-D nature of the surface. To overcome this shortcoming, we combined the SAM concept with the unique properties of highly-branched nanostructures – dendrimers, and explored the way of constructing a high-performance biosensor interface.

Amino-terminated poly(amidoamine) dendrimers and hexadecanethiol (HDT) on a gold surface were examined as a prototypical biosensor matrix. Dendrimer terminated with amino groups was used to anchor receptors – enzymes, antibodies or aptamers, while the alkanethiol chains served as stabilizing components. In the first phase, studies of the alkanethiol-dendrimer assembly, effect of HDT/G1 dendrimer molar ratio, and imaging of the resulting charge distribution by the scanning electrochemical microscope were performed ([Svobodová et al. 2004](#)). The optimal assembly was used to design the electrochemical biosensor with glucose oxidase as the sensing element. Resulting glucose biosensor showed good physical and chemical parameters and excellent detection limit of 50 $\mu\text{mol/l}$ glucose.

Growing environmental concerns increase the need for monitoring of pollutants in agricultural crops and food products. We therefore started to design a bi-enzyme amperometric biosensor for the detection of organophosphate and carbamate pesticides, based on a direct assay. These pesticides inhibit acetylcholinesterase (AChE), a key enzyme in neuronal synaptic transmission. AChE hydrolyses acetylcholine to acetate and choline. Choline is subsequently oxidized by the second enzyme, choline oxidase (ChO), to betaine with the concomitant generation of H_2O_2 . H_2O_2 is then detected amperometrically. In our biosensor design, AChE-ChO bi-enzyme complex was attached to the alkanethiol-dendrimer matrix formed by the fourth dendrimer generation. Resulting biosensor was characterized by good stability and high sensitivity to acetylcholine. In the presence of organophosphate (dichlorvos) and carbamate (carbofuran) pesticides, and a carbamate drug (eserine), AChE activity was substantially decreased ([Šnejdárková et al. 2004](#)). The detection limits of 0.003, 0.04, and 0.1 nmol/l for dichlorvos, carbofuran and eserine, respectively, are suitable for the detection within the EU limits for drinking water. These results indicated that the biosensor assay designed in our laboratory can be a useful tool for screening pesticides in food samples.

Immunosensors represent another group of biosensors of a great interest. Affinity interaction of antibodies and antigens was studied by quartz crystal microbalance (QCM) technique in a flow mode. The QCM can detect changes in the frequency of crystal oscillations due to the changes in mass on the surface of the crystal. The frequency changes are not restricted to mass changes only, but also to the viscoelastic properties of the interface. In this type of biosensor, anti-human-IgG was anchored to alkanethiol-dendrimer matrix. Oriented immobilization of anti-human-IgG was achieved by use of Protein A interacting specifically with F_c portion of immunoglobulin. Comparison of biosensors constructed with and without Protein A revealed that the presence of Protein A increased the amount of bound antibodies 1.4-fold. On the other hand, the same detection limits of 7 nmol/l human IgG were observed for both types of biosensors. One of the important characteristics of this immunosensor was its ability to regenerate in 0.1M glycine-HCl pH 2.3 ([Svobodová et al. 2006](#)).

The advantage of alkanethiol-dendrimer architecture in comparison to SAMs is the higher local concentration of the functional terminal groups close to the sensor surface. This results in higher density of receptor molecules and improved biosensor sensitivity. The most

important characteristic of biosensors that distinguishes them from other analytical methods is the direct and immediate detection of the substances of interest or catalytic products. Therefore it is not surprising that biosensors play a key role in medical and clinical analysis, or in environmental and industrial monitoring.

B. Molecular basis of bioenergetic coupling on methanoarchaeal membrane

Methanoarchaea are phylogenetically very ancient organisms. The only energy pathway that satisfies their cellular energy demands is methanogenesis. Despite an extensive investigation of methane formation in methanoarchaea during the last 3 decades, mechanism of energy coupling on methanoarchaeal membranes and molecular mechanisms of these processes are still far from being completely understood. Recent studies have revealed that the bioenergetics of methanogens exhibits some unique features. First, methanogenesis is coupled to the generation of $\Delta\mu_{\text{H}^+}$ and $\Delta\mu_{\text{Na}^+}$. Second, both these gradients are directly utilised for ATP synthesis. The coexistence of these gradients in methanogens raised the question about their physiological role. Recently, we have obtained biochemical evidence indicating that both electrochemical gradients (H^+ and Na^+) in the cells of *Methanothermobacter thermautotrophicus* are directly involved in ATP synthesis via two different, H^+ and Na^+ dependent, ATP synthases. Surprisingly, only genes coding A_1A_0 ATPase/synthase subunits have been identified in the complete genome sequence of *M. thermautotrophicus*. Therefore, a systematic genetic approach to solving the problem of bioenergetic mechanisms in methanoarchaea has been employed in our laboratory since 1997. The project exploring mutants resistant to therapeutics and antimicrobial substances relevant as energy transformation inhibitors has provided a considerable amount of important results. A neomycin-resistant mutant of *M. thermautotrophicus* with a defect in Na^+ -dependent energetics was isolated and partly characterised in our laboratory ([Smigan et al. 1997](#)). It has been suggested that binding of the ATPase catalytic moiety to the membrane spanning part of at least one ATPase (probably the Na^+ -dependent one) is modified in this mutant. In an attempt to clarify this problem we have biochemically characterised this mutant. The results provided evidence that the mutant cells have an unimpaired H^+ -translocating ATPase (synthase), which is able to synthesise ATP even under alkaline conditions when Na^+ -dependent ATP synthesis is profoundly modified. In addition, links of $\Delta\mu_{\text{H}^+}$ and $\Delta\mu_{\text{Na}^+}$ coupled reactions, Na^+/H^+ antiport and membrane potential, have been also investigated. Lastly, SDS-PAGE analysis shows that the impairment of the Na^+ -dependent energetic system in the studied mutant strain is accompanied by changes in the spectrum of membrane-associated proteins. It seems that either pleiotropic or multiple mutations are present in the neomycin-resistant mutant. ([Majernik et al. 2003](#)).

To elucidate the discrepancy between biochemical and genetic findings and to achieve better understanding of the complex co-operation of $\Delta\mu_{\text{H}^+}$ and $\Delta\mu_{\text{Na}^+}$ bioenergetic subsystems in *M. thermautotrophicus*, we isolated and characterized a mutant resistant to the protonophorous uncoupler TCS capable to dissipate $\Delta\mu_{\text{H}^+}$ ([Cubonova et al. 2004](#)).

The mutant strain exhibited increased methane formation and elevated ATPase activity under non-growing conditions. ATP synthesis driven by methanogenic electron transport as well as by potassium diffusion potential in the presence of either H^+ or Na^+ ions was markedly reduced in the mutant strain. An abundant membrane-associated protein complex with molecular mass approximately 670 kDa was detected in the mutant strain after native PAGE. The results indicate that TCS resistance is associated with mutation(s) affecting uncoupler binding protein(s) and/or modulating the activity of unidentified ATPase. The uncoupler resistance in the mutant strain was not accompanied by changes in the level of subunits A,B and c of the A_1A_0 ATP synthase.

The Na^+/H^+ antiporter in methanoarchaea is a unique system able to balance the energies of $\Delta\mu_{\text{H}^+}$ and $\Delta\mu_{\text{Na}^+}$ and so it plays a key role in the cooperation of two electrochemical gradients. Potentially, a mutant with a lesion in Na^+/H^+ antiporter activity could reveal important information concerning the functions and coexistence of the two primary cycles located on the membrane of methanogenic Archaea. Very recently we have

isolated and biochemically characterized a mutant of *M. thermautotrophicus* resistant to amiloride - a specific Na^+/H^+ antiporter inhibitor ([Surin et al. 2007](#)). Our results have shown that mutant cells have a lesion in Na^+/H^+ antiporter activity accompanied by an increase in methane and ATP formation. ATP synthesis driven by potassium diffusion potential was profoundly inhibited in wild type cells by the presence of uncoupler TCS and sodium ions whereas approximately 50% inhibition was observed in mutant cells under the same conditions. Biochemical measurements were confirmed by MALDI TOF/TOF analysis that revealed increased levels of enzymes participating in methanogenesis in the amiloride resistant mutant.

The results of our research represent a progressive development in the studies of the complex problem of bioenergetic coupling in methanoarchaea. In addition, the results clearly show that our pioneering approach of combining biochemical, genetic and molecular biology methods in the study of energy transformation can be successfully applied also in this ancient phylogenetic group.

C. Membrane biogenesis: the study of lipid homeostasis in yeast

With respect to membrane structure and biogenesis, yeast *Saccharomyces cerevisiae* behaves as a typical eukaryotic organism and is thus often used as a model to study membrane-associated processes. The choice of this organism is justified also by the availability of a wide spectrum of specific powerful experimental tools, including the methods for genomic and proteomic analysis. In addition, fungal infections (mycoses) gain on significance due to a recent rise of immunocompromised subjects in human population (AIDS, cancer patients). All clinically relevant antifungal therapeutics target membrane biogenesis or membrane function. In this respect, *S. cerevisiae* is an extremely useful model to study molecular mechanisms of antimycotic mechanisms.

In the period 2003-2006, our research activities were focused on homeostasis of membrane phospholipids and neutral lipids. We were interested in the role of yeast lipid transfer proteins and lipid turnover in maintaining proper lipid composition of cellular membranes. The study how antifungal drugs exert their action via interference with ergosterol biogenesis or function is important for understanding the emerging resistance of fungal pathogens to clinically relevant antimycotics. Relatively recent topic of our research is the use of *S. cerevisiae* as a model for human neurological disorder, Nieman Pick type C.

Family of yeast phosphatidylinositol transfer proteins (PITPs) is implicated in regulation of many aspects of lipid biogenesis and turnover (reviewed in [Griac et al. 2006](#) and Griac, manuscript accepted). In the field of lipid transfer proteins we have analyzed the physiological role of the family of yeast PITPs in regulation of membrane phospholipid composition ([Schnabl et al. 2003](#)). We have shown that phosphatidylcholine (PC) transfer activity of the major yeast PITP, Sec14p, is not essential for its main function to maintain membrane lipid composition compatible with exocytosis ([Tahotna et al. 2007](#)). In addition, using PITPs as an experimental tool, we have provided evidence for the model describing the mechanism of phospholipid biogenesis regulation at the transcriptional level ([Holič et al. 2004](#)).

Lipid turnover generates important signalling molecules. It is one of the important mechanisms how the cell responds to the changing environment. In collaboration with Duquesne University, Pittsburgh (USA) we have revealed the role of two unknown yeast enzymes, homologous to mammalian and bacterial glycerophosphodiester phosphodiesterases. The first one, product of the yeast genome open reading frame (ORF) YPL110c, is a glycerophosphodiester phosphodiesterase involved in turnover of PC degradation products ([Fischer et al. 2005](#)). The other one, product of the ORF YPL206c, is phosphatidylglycerol specific phospholipase C involved in the regulation of cardiolipin branch of phospholipid biosynthesis (manuscript submitted).

Mutations in the NPC1 gene were identified as the major cause of severe neurological disorder Nieman Pick type C that is manifested by increased storage of cholesteryl esters in neuronal tissue. *S. cerevisiae* NCR1 gene shares significant homology with the human gene

encoding NPC1 protein and the yeast protein is able to substitute for defective human protein in NPC1 fibroblasts. We have found that yeast cells with deletion of *NCR1* gene show increased uptake and esterification of external cholesterol. In addition, localization studies with tagged Ncr1p indicate a dynamic subcellular distribution similar to the localization of NPC1 protein in human cells (manuscript in preparation).

Fungal squalene epoxidase (encoded by the *ERG1* gene in *S. cerevisiae*) is the target of widely used antimycotic terbinafine. In collaboration with the University of Graz (Austria) we performed mutational mapping of this rate-limiting enzyme in ergosterol biosynthetic pathway (Klobučníková et al. 2003, Leber et al. 2003). A computer model based on functional homologies to a bacterial hydroxylase was designed. The relevance of this model was confirmed by localization of mutations conferring hypersensitivity to terbinafine to putative FAD- and nucleotide-binding domains. Amino acid changes rendering yeast cells resistant to terbinafine clustered in a specific region on protein surface where the drug-binding site might be located (Ruckenstuhl et al., 2007).

Polyene antimycotics amphotericin B (AmB) and nystatin are widely used for treatment of systemic and topical mycoses. To understand the mechanisms of resistance to these two antifungals we isolated mutants showing selective resistance to either nystatin or AmB. Genetic and phenotypic characteristics of these two mutants revealed that their resistance is based on remarkably different mechanisms. Specific single-point mutation in the *ERG3* gene (encoding C5-sterol desaturase) and the resulting change in plasma membrane ergosterol content have been identified as the single cause of nystatin resistance. The AmB-resistant mutant shows unaltered lipid spectrum but changes in cell wall structure (Hapala et al., 2005).

Membranes are frequently attacked by reactive oxygen species (ROS) produced by extracellular or intracellular sources. The damage may be prevented by the activity of natural substances with antioxidative properties. As a part of a long-term international collaboration we have shown that plant extracts (Rooibos tea) significantly reduces the damage on cellular membranes induced in diabetes-like conditions (Uličná et al., 2006).

Our research is internationally highly accepted and its results were published in high-ranking scientific journals. Invitations to present the results at international meetings and to prepare reviews on the subjects are other important signs of international acceptance. Our approach confirmed the usefulness of yeast as a model in the studies of eukaryotic lipid homeostasis. We believe that understanding the functions of yeast PITPs will help to reveal the role of lipid transfer proteins in mammals where their defects cause serious neurodegenerative and malabsorption diseases. Similarities in subcellular localization and in phenotypes of NPC1 human fibroblasts and yeast cells without Ncr1 protein render the study of a yeast model of human Nieman Pick type C disease also highly promising. Moreover, the first structural model of the enzyme squalene epoxidase as well as the proof of specific mechanisms of the antifungal activity of two structurally similar polyene antimycotics open further possibilities in the search for improved efficiency of antifungal therapy.

D. Immunogenetics: functions of bovine cell membrane antigens in cell-to-cell communication

During the last four years the research program of the Institute in field of bovine cell membrane proteins has been concentrated on the study of the immunogenetic markers and their function in the bovine immune system. A set of new monoclonal antibodies (mAbs) recognising the cell surface antigens of bovine cells has been prepared using the standard hybridoma technique based on the xenoinmunization of BALB/c mice with bovine blood cells or bull spermatozoa. Using this procedure, three types of monoclonal antibodies with different cell specificity have been obtained. Four mAbs were analysed in more detail and their antigen specificity has been identified by serological, biochemical and histochemical methods.

MAB IVA-26 gave a positive reaction with the whole population of bovine lymphocytes, thrombocytes and a subpopulation of granulocytes. No reaction was detected on erythrocytes. Immunoperoxidase staining of tissue sections with mAb IVA-26 has shown strong staining in

the lymph node (cortical region), kidney (glomerules) and small intestinal epithelium (crypts). The IVA-26 clearly distinguished the medullar and the cortical zone of the bovine and pig thymus. IVA-26 immunoprecipitated proteins with molecular weight of 45 kDa (α chain) 14 kDa (β_2m) and 58 kDa ($\alpha+\beta_2m$) from lysates of detergent-solubilized, surface sulphobiotinylated bovine lymphocytes. This molecular weight correspond to the MHC class I molecule present on the majority of white blood cells having an important role in the presentation of intracellular antigens to cytotoxic T cells. The identity of IVA-26 was proved in immunoprecipitation assay where IVA-26 and MEM-147 (the standard reagent of human MHC class I molecule) precipitated the same protein (epitope) on bovine leukocytes ([Antalíková et al. 2004](#)).

IVA-41, another monoclonal antibody analysed for its specificity, recognises the polymorphic V antigen of the cattle erythrocyte cell membrane (blood group antigen). The antibody IVA-41 was tested in haemolytic assay on the panel of red blood cells from 2160 animals typed for 80 blood group antigens. The antibody showed excellent correlations ($r = 0.99$; $P < 0.0001$) with the reactions of standard polyclonal reagents recognising V antigen of cattle FV blood group system. Haemolytic as well as immunochemical analysis with monoclonal antibody IVA-41 revealed that V antigen of bovine red blood cells is the membrane bound, papain and pronase sensitive, trypsin and chymotrypsin resistant N-glycosylated sialoglycoprotein with molecular weight of 64, 56 and 50 kDa under nonreducing and 23 kDa under reducing conditions. Tissue tests showed the most intensive reaction in splenic cords of spleen red pulp, probably due to high concentration of erythrocytes in this tissue. In contrary to human blood groups as ABO or Lutheran system antigens, which are widely distributed on body tissues and should be therefore considered as histo-blood group antigens, it seems that the expression of bovine blood group V antigen is restricted to erythrocyte membrane. This work was the first attempt to analyse the chemical structure and tissue distribution of bovine red blood cell antigens by monoclonal antibody.

Monoclonal antibody IVA-285 has been prepared against surface immunoglobulin of bovine B lymphocytes reacting specifically with the light chain of bovine immunoglobulin. IVA-285 was derived clone producing antibody preferentially binding to the bovine B cells. IVA-285 detects bovine immunoglobulin level in all tested of body fluids of cattle. The apparent molecular weight of the band detected by IVA-285 was within the range 24-27 kDa corresponding to the molecular weight previously reported for the light chain of bovine Ig. The immunoperoxidase staining of cryostat tissue sections with mAb IVA-285 has shown a strong reaction with immunoglobulin-producing plasma cells and with immunoglobulin deposits throughout the cattle lymphoid and non-lymphoid tissues ([Antalíková et al. 2006](#)).

MCP/CD46 molecule of human cells is a widely distributed C3b/C4b binding regulatory glycoprotein of the complement system that has an important role in regulation of immune reactions of organism. In addition, CD46 is involved in immune protection of spermatozoa and in sperm binding to the zona pellucida of the oocytes. IVA-520 has been selected from the group of mAbs recognising the antigens common to leukocytes and spermatozoa. IVA-520 identified the molecule on bovine cells with similar properties (molecular weight, cell distribution) as human MCP molecule. IVA520-reacting molecule functionally behaves as the MCP molecule, showing cofactor activity for the factor I-mediated cleavage of bovine C3 complement. The main difference between the human and bovine MCP is the strong expression of MCP on bovine erythrocytes and its absence on human erythrocytes ([Antalíková et al. 2007](#)).

Monoclonal antibodies produced in our laboratory extend the palette of monoclonal antibodies for detection of the antigens in farm animals. The monoclonal antibody IVA-520 is the first antibody prepared against the bovine CD46 molecule and could be used for functional study of this molecule in bovine immune system. IVA-285 detecting the light chain of bovine immunoglobulin could be used as veterinary immunodiagnostic reagent for estimation of total level of bovine immunoglobulin and IVA-41 could be applied for detection of V antigen in cattle paternity control. The antibodies produced by hybridoma IVA-285 and IVA-520 are on the basis of agreement distributed by internationally established distributor EXBIO Praha, Czech Republic.

E. Signal molecules and their membrane receptors in the control of avian physiology and behaviour

Our research activities in the field of avian physiology are built up on the long tradition of the study of the neuroendocrine mechanisms of the development and growth in birds, mainly poultry. Our research was focused on the role of signal transduction mediated by steroid and thyroid hormones, melatonin and leptin via their action on membrane and nuclear receptors. More recent element in our research interest is the study of the role of neurotransmitters (mainly dopamine) and their membrane receptors in the control of avian behaviour and identification of brain structures involved in these processes.

Precocial species of birds are relatively mature and mobile from the moment of hatching, while newly hatched young of altricial species are relatively immobile, have closed eyes, lack down, and must be cared for by the adults. There are marked differences in physiology of precocial and altricial birds during early postnatal development. We published the first study of plasma biochemical parameters in a free-living altricial species, European starling, during the entire developmental period in a nest. An increase has been observed for all selected markers of intermediary metabolism. A decrease in lipid metabolism just before fledging may reflect an increased physical activity and changes in nutrition ([Juráni et al. 2004](#)). On the other side we studied the changes of plasma biochemical constituents during the first 100 days of life in the precocial domestic chickens. These were subjected to varying levels of food restriction which is routinely used in the broiler (meat type) parent stock production. The results of this study showed an interesting paradox. While the increased levels of corticosterone suggest that food restriction is stressful for birds, the increased enzymatic activities of *ad libitum* fed birds indicate muscle damage and suggest negative consequences of this treatment for health and welfare ([Rajman et al. 2006](#)).

In field of avian cosmic physiology the study of postnatal development of Japanese quail was aimed at determination of the hypodynamy effects on growth and development of body parts, organs, muscles and bones, as well as gonadal development and reproductive functions of male and female Japanese quail. We have also studied the variation in biochemical parameters and plasma levels of adrenal, thyroid and ovarian hormones. Obtained results indicate that Japanese quail chicks are capable to adapt, grow and develop under conditions of simulated microgravity, although the most of the examined quantitative parameters were significantly decreased in comparison with control. However, many biochemical parameters in plasma were not affected. Although the development of reproductive organs and their functions in Japanese quail reared under hypodynamy was delayed in comparison to the age-matched control, the test birds laid eggs and produced semen with a minimal delay ([Škrobánek et al. 2003, 2004, 2005](#)).

The discovery of hormone leptin is connected to its role in food intake and energy balance control. We studied the effect of *in ovo* leptin treatment on the activity of endocrine axes. Quail treated with leptin hatched earlier and had a higher body weight across the whole postnatal period than the saline-treated controls. These effects were accompanied by changes of endocrine and metabolic parameters suggesting that leptin acts as a general signal of low energy status to neuroendocrine systems in birds and it improves utilization of nutrients ([Lamošová et al. 2003](#)). Our other results showing substantial effect of leptin on food intake and feeding behaviour of quails ([Máčajová et al. 2004](#)) confirm this hypothesis.

Feather pecking, a behavioural disorder occurring in laying hens, is both a welfare and economic problem. The EU ban on the conventional battery cages from 2012 and an increased risk of feather pecking in alternative housing systems stimulated the interest of many research teams in this problem ([Sedlačková et al. 2003](#)). Our approach is unique by the use of neuroscience methods. We have formulated the hypothesis on the involvement of dopaminergic system in control of this behaviour. It was based on several arguments, including the fact that repeated treatment with dopaminergic agonists (such as apomorphine) induces an augmented pecking response accompanied by an alteration of the D1 and D2 dopamine receptor densities in birds (pigeons; [Acerbo et al. 2005](#)). There are differences in response of chicken lines selected for the high and low feather pecking propensity (HFP, LFP) to apomorphine. The HFP chicks displayed a more enhanced behavioural response to

acute apomorphine treatment than LFP birds. However, there were no differences in overall densities of dopamine receptors in the brain, as estimated by the homogenate binding technique, between both lines ([van Hierden et al. 2005](#)). Further study using quantitative ligand autoradiography, enabling higher anatomical resolution, proved up-regulation of both D1 and D2 dopamine receptor subtypes in several forebrain regions of HFP birds. The increase in the basal ganglia suggests a possible association of feather pecking with the dopaminergic reward system while up-regulated densities in areas of the “telencephalic feeding circuit” suggest a link to feeding behaviour ([Košťál et al. 2006](#)).

Reproductive behaviour plays an important role in natural mating systems where broiler breeder males compete for females. We investigated the effect of male-male competition compared with a noncompetitive situation on sexual behaviour, fertility, sperm quality, and morphological traits. Mating frequency consistently increased towards the end of the light phase. Significantly higher mating activity occurred in groups without competition. Heavier males and males with smaller combs had higher frequency of unsuccessful matings. Frequency of forced mating was not affected by the level of competition, but was more likely used by heavier males. Overall lack of association between mating behavior, morphometrical traits, sperm quality, and fertility suggest a multifactorial nature of the fertility of broiler breeder males. Fertility is also a relative parameter that depends upon the reproductive quality of the other male competitors within the group ([Bilčík et al. 2005](#), [Bilčík and Estevez, 2005](#)).

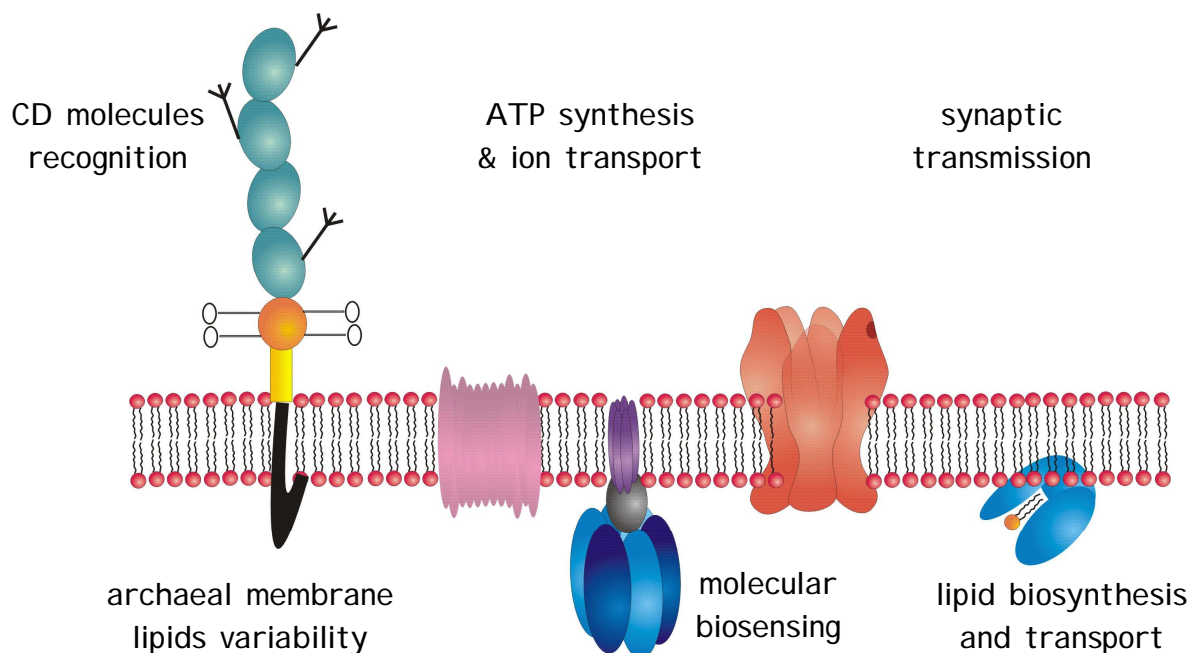
In order to measure physiological responses to various stimuli in freely moving birds we have adopted the radiotelemetric system used for laboratory rodents ([Savory and Kostal 1997](#)). Using this system we have monitored heart rate (HR), blood pressure (BP), body temperature (BT), and EEG in parent stock of meat type chickens over several 24-h periods. We found higher HR, BP, and BT in *ad libitum* group in comparison to restricted fed one. HR and BT were lower at night in all birds but with the decreases greater in the restricted fed group. Blood pressure tended to be higher at night ([Savory et al. 2006](#)). Using combination of behavioural observations with radiotelemetry we have tested the hypothesis whether stereotyped oral behaviour of broiler breeder hens subjected to routine quantitative food restriction is associated with de-arousal. The putative physiological indices of arousal, HR, BP, BT, and EEG in delta and theta frequency bands were highest during (stereotyped) feeder directed behaviour. On the other hand total time spent by all oral behaviours was related positively to an index of de-arousal (slow wave EEG) and negatively to an index of arousal (HR). These results provide apparently conflicting evidence of both arousal and de-arousal being associated with stereotypies ([Savory and Kostal 2006](#)).

During the past four years we have studied problems of growth, production, behaviour and welfare in poultry. We have employed and combined physiological, neurobiological and behavioural approach. Our results provided us solid basis for better understanding of these problems from a complex perspective.

To summarize, the focus of the Institute of Animal Biochemistry and Genetics on membranes and membrane-related processes has a traditional and innovative aspect. On one hand, it reflects the continuity of many research programs with the activities of our Institute in previous years. Promotion of research in areas in which we are nationally and internationally established is a logical and legitimate approach. However, it must be accompanied by conceptual and methodological innovations. We believe that we succeeded during the evaluated period both in terms of improved quality and extension of research in our traditional topics as well as in terms of introducing novel and promising directions of research. The novelty of our concept is to look at the membrane in its whole diversity (Fig. 2). That creates a common ground for all of the institute's laboratories to cooperate closely. One of the most important achievements characteristic for past four years was introduction of several novel experimental techniques (QCM analysis of intermolecular interactions, MALDI TOF/TOF analysis of proteins, HPLC and GC-MS analysis of lipids, *in situ* mapping of gene expression in brain) either directly at our institute or in cooperation with our partners. We may expect that the full benefit of these newly introduced progressive methods will be demonstrated in coming years. Research activities of our institute during the assessed period

yielded several principal results that significantly contributed to improved understanding of the roles of membranes in normal and pathological physiology. Some of those results have direct potential for practical application in agriculture, health care and technology. In general, we consider the last four years of our institute's activity as an important period in building the modern experimental base to study membranes and membrane-associated processes in their whole complexity.

Diversity of membrane functions



Dynamics of membrane components

Fig. 2 Different aspects of membrane-related processes studied at the Institute.

3. Concept of R&D activity of the Organisation for the next four years (max. 5 pages)

- i. Present state of knowledge and status of ongoing research related to the subject of the Concept, from both international and national perspective
- ii. Organisation's role or significance in the overall research effort within the field of the Concept on both the national and international scales
- iii. Objectives of the Concept
- iv. Proposed strategies and methods to be applied, and time schedule

In accordance with the long-term scientific mission of our Institute, the basic research focused on biological membranes and membrane-associated processes under normal and pathological conditions will be in the centre of our interest during the next four years. Membranes are not merely passive barriers. They are involved in complex reactions and are essential for central biological processes such as energy conservation or intracellular and cell-to-cell communication. Therefore, it is an advantage to study membranes and their functions at different levels of complexity, from formation of membranes and molecular interactions in the membrane, to the immunology and neural control of animal behaviour. For the years 2007-2010, the research in individual directions will be in part the extension and completion of the successful research directions from past four years, however, new problems and approaches will be incorporated into the research plans as well.

A. Interactions at the molecular level and development of novel biosensors

The most fundamental view at communication between molecules is the study of interactions at nanostructural level. Exploring this type of interactions has an enormous potential in biosensor application.

i) Raising concerns over human health issues highlight the need of simple, rapid and cost-effective analytical methods for monitoring contaminants in environment and agricultural products. Biosensors represent suitable and promising screening tool in this respect. A crucial stage in biosensing is the integration of biological recognition element and signal transducer utilising physical change accompanying the reaction. Self-assembled monolayers (SAMs) attached to the transducer act as a coupling layer between specific receptor and transducer. The communication between functional molecules, receptors, e.g. aptamers, antibodies or enzymes and transducer surface is a subject of extensive research. In many aspects, the phenomena at their interfaces mimic the situation in biomembranes. Electron transfer is of fundamental interest for construction of electrochemical sensors. The mass changes linked to protein-lipid, antigen-antibody, DNA-protein interactions are detected by quartz crystal microbalance biosensors. Such devices play important role in the environmental and food analysis, and in pharmaceutical, clinical and forensic applications.

ii) Major attention in biosensor research is currently paid to the design of the organized molecular assemblies forming functional interfaces between artificial support and biological fluids. Our concept of combining the advantage of SAMs with the special properties of nanostructures (dendrimers) to prepare a highly sensitive sensing matrix on gold sensor surface represents a significant improvement in this area. This novel architecture results in higher density of attached receptor macromolecules and in improvement of biosensor sensitivity. Our research is truly multidisciplinary and supported by collaboration and joint projects with the Comenius University, Slovakia; Technical University of Budapest, Hungary; Institute of Photonics and Electronics AS, Czech Republic.

iii) In the next four years we will conduct fundamental studies to gain the knowledge necessary for a predictable design and optimization of next generation of bioelectronic detection devices. Our research will draw on three advanced technologies: information processing, nanotechnology and biotechnology with the goal to develop advanced biosensors. The main objectives will include:

- development of affinity biosensors utilizing monoclonal antibody or DNA aptamers for detection of immunoglobulins, prions, etc. in nanomolar quantities, and
- development of enzyme-based biosensor for pesticide detection in environment.

iv) We will fulfil these aims by combining the unique features of biomolecular recognition with the appropriate physico-chemical transducer based on the electrochemical (enzyme sensor) and mechanical (affinity sensor) principle. During the first phase (year 1-2) we will optimize the procedures for immobilization of aptamers, antibodies, and enzymes and determine the changes of the thickness during the preparation of the individual sensor layers using laser ellipsometry. Year 3 will comprise inhibition studies of the enzyme acetylcholine esterase (important in synaptic transmission) as a suitable receptor in environmental biosensor, pesticide analysis in real samples and comparison of biosensor-derived results with HPLC. In year 4 we will focus on the optimization of sensor stability and regeneration.

B. Molecular basis of bioenergetic coupling on methanoarchaeal membrane

Methanoarchaea are descendant of the evolutionary oldest microorganisms. They feature chemically and structurally inimitable membranes capable to sense and communicate with surrounding environment exploring simple inorganic atoms and molecules.

i) The methanogenic archaea are indispensable in an anaerobic decomposition of organic matter and they are essential commensals in ruminants and humans. Methanogens produce vast amounts of methane globally every year. While methane emissions form potent greenhouse gas in the atmosphere, retained bio-methane may represent a real energy alternative for 21st century. Obviously, such situation sparks scientific interest in methanogenesis. Study of this process revealed unique ways how energy is transformed and stored in these organisms. In particular, methane formation is coupled to the generation of H⁺ and Na⁺ electrochemical gradients, which are used for ATP synthesis. Additionally, energy of the gradients can be balanced by Na⁺/H⁺ antiport, the anticipated key element in the cooperation between Na⁺- and H⁺-driven reactions. However, molecular mechanisms and enzymes participating in these processes have not yet been satisfactorily elucidated. This lack of knowledge evokes many of challenging questions about the two-sided nature of ATP synthesis and about role of Na⁺ in methanoarchaea.

ii) The specialized anaerobic laboratory established in our institute is unique not only in Slovakia but in the whole Central European area. A decade of applying genetic approach in our laboratory opened a new way to study energy conservation in methanoarchaea. This approach was accepted by international scientific community. We will continue in the study of some bioenergetic aspects of membrane-bound processes in methanogens, utilizing isolated mutants resistant to therapeutics and antimicrobial agents, with deficiencies in the bioenergetic machinery. Such work will provide novel information on the structure, functions and organization of archaeal membranes, of Na⁺/H⁺ antiporter, and of ATP synthase. Through our intellectual, personal and technical expertise we have particularly strong collaborative links to research institutes nationally and worldwide, especially with the University of York, UK, and the Ohio State University, USA, exploring exciting physiological aspects of thermophilic methanoarchaea.

iii) Our research will concentrate on elucidation of a molecular basis of some membrane-associated processes, especially communication link(s) between methane generation and ATP synthesis. Using specific mutant strains, we will be directly focused on

- coupling of methanogenesis to H⁺ or Na⁺ gradient-driven ATP synthesis
- genetic basis of resistance against therapeutics and antimicrobial agents
- genetic influence on membrane composition and lipid/protein interaction
- pilot bioenergetic study of methanogenic commensals in gastrointestinal tract of humans and ruminants

iv) Isolated mutants represent a powerful tool for comparative physiological and biochemical analysis. Particular, synthesis of ATP, methane and Na⁺/H⁺ antiport will be studied (year 1-2). In addition, proteomic approach using MALDI TOF/TOF will be used to identify differences in protein spectrum. Further, changes in ether lipids and squalene will be analyzed by HPLC and gas chromatography and lipid/protein interaction will be analyzed by quartz crystal microbalance (year 2-3). Finally, real-time PCR will be used for a transcriptomic analysis of an expression of bioenergetically interesting genes (transporters, ATPase, hydrogenases and putative Na⁺/H⁺ antiporter) to obtain a comprehensive characterisation of bioenergetic lesions in mutants and clarify some regulatory aspects of these processes (year 3-4).

C. Membrane biogenesis - the study of lipid homeostasis in yeast

Yeast is often used as a model of a eukaryotic cell. In many aspects, including the complexity of intracellular membranes and signalling it is a fairly typical eukaryotic organism.

i) Eukaryotic membrane contains many hundreds of different lipids. These lipids are non-randomly and very specifically distributed among the cell membranes, reflecting their specific roles in cell physiology and the status of the cell. Lipid biosynthetic pathways are relatively well described, including the roles of enzymes catalyzing individual biosynthetic steps.

In general, much less is known how the cells transport lipids from the environment or from the site of lipid synthesis to the target membranes and how they remodel their membranes. A serious problem with not fully understood mechanism is the oxidative damage to the membranes. Another clinically relevant problem is increased resistance of pathological fungi to the antimicrobial drugs. Defects in lipid transport, membrane remodelling, and the oxidative damage are behind the etiology of many serious human diseases. The study of these processes will have direct impact on the understanding of the molecular basis of numerous human diseases.

ii) Although the studies on the transport of phospholipids and sterols between subcellular membranes can be considered as the mainstream in membrane biogenesis research, our results represent a significant contribution in this area. Other aspects of our research, such as mechanisms of monitoring membrane lipid composition or lipid turnover, its physiological significance and regulation, represent novel directions with significant impact. Internationally, our laboratory collaborates with world leading lipidology laboratories: Technical University Graz and Karl Franzens University Graz, Austria; Duquesne University, USA; Indiana University, USA. We plan to strengthen the international cooperation by collaborative projects and by visits of scientists, exchange of ideas, materials, and methods. Importantly, we have been invited to participate in the EU FP7 project proposal – “Lipid enzymes, development of enzymes for lipid modification and activation”.

iii) Our research will be focused on the mechanisms how a eukaryotic model organism, yeast *Saccharomyces cerevisiae*, monitors its membrane lipid composition and how this membrane lipid composition is regulated in response to the changing environmental and intracellular conditions. Following problems will be in the centre of our research efforts:

- the role of lipid transfer proteins in the physiology of eukaryotic cell
- the role of phospholipid turnover in the regulation of lipid biosynthetic pathways
- mechanisms of neutral lipid homeostasis, including intracellular transport of sterols
- mechanisms of action of antifungal drugs and the study of mechanisms of increased resistance to clinically relevant antimycotics
- protective effects of natural antioxidants during membrane damage

iv) Methods of genetics, biochemistry, analytical chemistry, and molecular biology will be used to achieve the scientific objectives. In our laboratory we worked until now with individual genes or proteins, or with the small groups of related genes. The next logical step is the analysis on the whole genome and proteome level. Isolation of mutants or synthetic lethal interactions using gene deletion library will be performed first (year 1-2), followed by biochemical and genetic characterization using state-of-the-art analytical tools (years 2-3). Integrative approach into the overall cell physiology will be the last part of this coming research period.

D. Immunogenetics: functions of bovine cell membrane CD antigens in cell-to-cell communication

Evolution of multicellular organisms would not be possible without the recognition of different cell types and tissues. Immune system can fulfil this role due to its specific ability to communicate through different types of membrane receptor/antigen interactions.

i) Identification of CD molecules (antigens) incorporated in the cell membrane of immune competent and other cells is very important for understanding the structure and function of the immune system. Disorders in the expression or function of these molecules could lead to primary immune deficiency as Leukocyte Adherence Deficiency occurring in man and some animal species. In the last decade a set of monoclonal antibodies (mAbs) recognizing the CD antigens of farm animals has been prepared. While more than 300 such molecules have been identified in man, only 25 leukocyte molecules showing the similarity with human counterpart were accepted as CD antigens of cattle so far.

ii) Our research interest covers the study of the structure and the function of the immune system of farm animals. Particularly, our focus on distribution and characterization of bovine cell membrane CD molecules is unique in Slovakia and Czech Republic. Recently, several projects have been used for study on the production of mouse mAbs of cattle. A set of

mouse produced monoclonal antibodies (mAbs) recognizing the bovine CD9, CD11/18, CD41/61, CD45R, CD46 and CD62L has been prepared. Furthermore, some functional aspects of CD antigens in bovine immune system as the expression of adhesion molecules (CD18, CD62L) on the leukocytes of the Bovine Leukocyte Adherence Deficiency heterozygous cows and leukemic cows were studied. Our laboratory was one of the 15 participants of the international workshop for assignment the antigens and establishing the nomenclature of bovine CD antigens. We also intend to participate in the next workshop held under the auspices of International Veterinary Immunology Committee.

iii) Our main goals for next four years are:

- preparation of novel mAbs for identification of cell membrane CD molecules of cattle leukocytes and spermatozoa
- study of mutual relationships among some cell membrane CD antigens of mammary gland of dairy cows
- analysis of membrane antigens of bull spermatozoa and assigning physiological function of these antigens in bovine reproduction

iv) We will focus on an analysis of mAb recognizing the bovine homologue of human complement regulation molecule CD46. The structure of CD46 and its functional properties in complement regulation and its function in a reproduction process will be determined (year 1-2). MABs will be prepared by standard hybridoma technique and each antibody will be analysed by cytometrical, histochemical and biochemical methods (year 3-4). We plan to cooperate with the Institute of Molecular Genetics CAS in Prague, to perform the isolation and analysis of the chemical structure of sperm proteins. The best characterized mAbs, which are commercially attractive, will be offered for sale via EXBIO Praha company.

E. Signal molecules and their membrane receptors in the control of avian physiology and behaviour

At the most complex level, membrane processes participate in hormonal and neuronal signalling inevitable for the functioning of the whole organism. Understanding these processes is essential for understanding normal and abnormal physiology, as well as behaviour.

i) Avian physiology and neuroscience are important parts of both basic and applied science. Applied avian physiology is represented mainly by poultry science, serving for further development of poultry industry. Recently the focus in agriculture is moving from the highly efficient and profitable production to the production of 'healthy' products. Therefore poultry welfare, a particular issue within the poultry science, is getting an increasing attention from public as well as EU policymakers. Specific branch of applied avian physiology is cosmic physiology, studying the effects of environmental alterations related to space missions on avian organism. However, avian physiology and neuroscience generate also the fundamental basic science hypotheses and models. Spectacular advances in the study of the neurobiology of song learning for example have established birdsong learning as perhaps the major model system of learning. Similarly, the long-held dogma that no neurons are formed in adulthood was disproved also in songbirds.

ii) Our concept is to use a combined physiological and ethological approach focused on the study of hormones, neurotransmitters and their receptors in birds. International acknowledgement of our results in the field of poultry behaviour and welfare is illustrated by numerous collaborations with prestigious laboratories including the Scottish Agricultural College, UK, Swedish University of Agricultural Sciences, Sweden, or University of Maryland, USA, which resulted in the joint research projects and publications. Although birdsong learning and avian neurogenesis are the new topics at the department, even during this short time we have succeeded with grant applications at both national and international levels (FIRCA, in collaboration with the Duke University, USA). In addition, the problems of cosmic biology using avian model, Japanese quail, have been studied for nearly four decades. International acknowledgements of the results are represented by the collaboration with partners mainly in Russia and by the participation at the flight experiments aboard the orbital station.

iii) Using the models of welfare related behaviours in poultry and vocal communication in songbirds and parrots the main objectives for the forthcoming years are to study:

- neuro-endocrine control mechanisms of the normal and abnormal behaviour in birds
- the brain pathways and the selected signal molecules and their receptors in control of avian behaviour
- the molecular mechanisms of basal ganglia regeneration in birds

In field of avian cosmic physiology the main aim is to study:

- the postnatal development of Japanese quail at higher gravity (1.2-1.5 g)

iv) In order to fulfil these objectives, we will employ the methods of behavioural analysis, radiotelemetry, physiological methods (RIA, *in vitro* and *in vivo* techniques), receptor imaging techniques (quantitative ligand autoradiography, *in situ* hybridization, immunohistochemistry) and molecular techniques for the identification of behaviourally driven gene activation. During the first phase (year 1-2) we will identify the brain regions and pathways involved in control of poultry abnormal behaviour with an emphasis on dopaminergic mechanisms, study the effects of song nuclei damage on song production, examine the mechanisms of basal ganglia regeneration following lesions in songbirds and parrots, and estimate the effects of altered gravity on physiological functions in Japanese quail. In the second phase (year 3-4) we will study the neuroendocrine mechanisms controlling the abnormal behaviour in selected brain areas, study their function by pharmacological manipulation of their membrane receptors, investigate the cell types in the regenerated brain areas, their connectivity and synaptic transmission, and establish the relationship between abnormal conditions in birds and their biochemical and physiological characteristics.

Five model systems implemented at the Institute of Animal Biochemistry and Genetics enable a multilevel approach to study the complexity of membrane functions in living organisms. Those models, ranging from the basic molecular interactions at the artificial membrane-like surfaces to the membrane processes involved in control of animal physiology and behaviour, create a basis for the multidisciplinary analysis of membrane-associated processes. Proposed research activities reflect the advancing intra- and inter-institutional interactions. These bring an added value of complementing individual research groups in their expertise and experimental techniques. The major aim of the proposed concept is to advance the knowledge in membrane biosciences. In addition, some research activities proposed in this concept have a direct potential to influence human and animal health. Among them are: development of biosensors to monitor environmentally sensitive substances; the role of methanogens in the gastrointestinal tract of humans and animals; mechanism of action of clinically relevant antifungal drugs and protective effects of natural antioxidants on membranes; preparation of novel antigens to detect diseases in farm animals; the study of neuro-endocrine control mechanisms of the normal and abnormal behaviour in poultry. We believe that although we have set very ambitious goals, they are backed by qualified and motivated reaserch team.

III. Partial indicators of the main activities:

1. Research output

- i. **List of the selected publications documenting the most important results of basic research. Total number of publications in the whole assessed period should not exceed the average number of the research employees**

- [1] ACERBO, M.J. – VÝBOH, P. – KOŠŤÁL, Ľ. – KUBÍKOVÁ, Ľ. – DELIUS, J.D. Repeated apomorphine administration alterates dopamine D1 and D2 recep-tor densities in pigeon basal telencephalon. In *Experimental Brain Research*. Vol. 160 (2005), p. 533-537. (2.118- IF2005)

- [2] ANTALÍKOVÁ, J. - SIMON, M. - HOROVSKÁ, Ľ. - VALENTOVIČOVÁ, J. Monoclonal antibody produced against bovine MHC class I antigens. In *Folia Biologica*. Vol. 50 (2004), p. 29-31. (0.719 – IF2005)
- [3] ANTALÍKOVÁ, J. - SIMON, M. - JANKOVIČOVÁ, J. - HOROVSKÁ, Ľ. - DUŠINSKÝ, R. - HLUCHÝ, S. Monoclonal antibody to the light chain of bovine immunoglobulin. In *Hybridoma*. Vol. 25 (2006), p. 309-312 (0,404 – IF2005)
- [4] BILČÍK, B. – ESTEVEZ, I. Impact of male-male competition and morphological traits on mating strategies and reproductive success in broiler breeders. In *Applied Animal Behaviour Science*. Vol. 92 (2005), p. 307-323. (1.441- IF2005)
- [5] COLIN, P.G. - MAJERNÍK, A.I. – CHONG, J.P.J. – BOLT, E.L. A novel nuclease-ATPase (Nar71) from archaea is part of a proposed thermophilic DNA repair system. In *Nucleic Acids Research*. Vol. 32, no. 21 (2004), p. 6176-6186. (7.552-IF2005)
- [6] ČUBOŇOVÁ, Ľ. - SANDMAN, K. - HALLAM, S.J. – DELONG, E.F. – REEVE, J.N. Histones in Crenarchaea In *Journal of Bacteriology*. Vol. 187 (2005), p. 5482-5485. (4.167 - IF2005)
- [7] ČUBOŇOVÁ, Ľ. – ŠURIN, S. – MAJERNÍK, A. – ŠMIGÁŇ, P. Isolation and characterization of an uncoupler-resistant mutant of *Methanothermobacter thermoautotrophicus*. In *FEMS Microbiology Letters*. Vol. 233 (2004), p. 23-28. (2.057 – IF2005)
- [8] FISHER, E. – ALMAGUER, C. – HOLIČ, R. – GRIAČ, P. – PATTON-VOGT, J. Glycerophosphocholine-dependent growth requires Gde1p (YPL110c) and Git1p in *Saccharomyces cerevisiae*. In *Journal of Biological Chemistry*. Vol. 280, no. 43 (2005), p. 36110-36117. (5.854- IF2005)
- [9] GEORG, J. - SCHOMACHER, L. - CHONG, J.P.:J. - MAJERNÍK, A:I. - RAABE, M. - URLAUB, H. - MÜLLER, S. - CIIRDAEVA, E. - KRAMER, W. - FRITZ, H-J. The *Methanothermobacter thermoautotrophicus* ExoIII homologue Mth212 is a DNA uridine endonuclease. In *Nucleic Acids Research*. Vol. 34, no. 18 (2006), p. 5325-5336. (7,552 – IF2005)
- [10] GRIAČ, P. - HOLIČ, R. - TAHOTNÁ, D. Phosphatidylinositol-transfer protein and its homologues in yeast. In *Biochemical Society Transactions*. Vol. 34 (2006), p. 377-380. (3,099 – IF2005)
- [11] HAPALA, I. - KLOBUČNÍKOVÁ, V. – MAZÁŇOVÁ, K. – KOHÚT, P. Two mutants selectively resistant to polyenes reveal distinct mechanism of antifungal activity by nystatin and amphotericin B. In *Biochemical Society Transactions*. Vol. 33 (2005), p. 1206-1209. (3,099 – IF2005)
- [12] HIERDEN van, Y.M. – KOOLHAAS, J.M – KOŠŤÁL, Ľ. – VÝBOH, P. – SEDLAČKOVÁ, M. – RAJMAN, M. – JURÁNI, M. – KORTE, S.M. Chicks from a high and low feather pecking line of laying hens differ in apomorphine sensitivity. In *Physiology & Behavior*. Vol. 84, Iss. 3 (2005), p. 471-477. (2.183- IF2005)
- [13] HOLIČ, R. – ZÁGORŠEK, M. – GRIAČ, P. Regulation of phospholipid biosynthesis by phosphatidylinositol transfer protein Sec14p and its homologues. In *European Journal of Biochemistry*. Vol. 271 (2004), p. 4401-4408. (3.164 – IF2005)
- [14] JURÁNI, M. – VÝBOH, P. – ZEMAN, M. – LAMOŠOVÁ, D. – KOŠŤÁL, Ľ. – BLAŽÍČEK, P. Post-hatching dynamics of plasma biochemistry in free-living European starlings (*Sturnus vulgaris*). In *Comparative Biochemistry & Physiology, Part A*. Vol. 138 (2004), p. 89-95. (1.351 – IF2005)
- [15] KLOBUČNÍKOVÁ, V. – KOHÚT, P. – LEBER, R. – FUCHSBICHLER, S. – SCHWEIGHOFER, N. – TURNOWSKY, F. – HAPALA, I.: Terbinafine resistance in a pleiotropic yeast mutant is caused by a single point mutation in the ERG1 gene. In: *Biochemical and Biophysical Research Communication*, 2003, vol. 309, p. 666-671. (3.000 – IF2005)
- [16] LAMOŠOVÁ, D. - MÁČAJOVÁ, M. - ZEMAN, M. - MÓZEŠ, Š. - JEŽOVÁ, D.: Effect of in ovo leptin administration on the development of Japanese quail. In: *Physiological Research*, 2003, vol.52, p. 201-209. (1.806 – IF2005)

- [17] LEBER, R. - FUCHSBICHLER, S. – KLOBUČNÍKOVÁ, V. – SCHWEIGHOFER, N. – PITTERS, E. – WOHLFARTER, K. – LEDERER, M. – LANDL, K. – RUCKENSTUHL, Ch. – HAPALA, I. – TURNOWSKY, F.: Molecular mechanism of terbinafine resistance in *Saccharomyces cerevisiae*. In: *Antimicrobial Agents and Chemotherapy*, 2003, vol. 47, p. 3890-3900. (4.215 – IF2005)
- [18] MAJERNÍK, A. - ČUBOŇOVÁ, L. - POLÁK, P. - ŠMIGÁŇ, P. - GREKSÁK, M.: Biochemical analysis of neomycin-resistance in the methanoarchaeon *Methanothermobacter thermautotrophicus* and some implications for energetic processes in this strain. In: *Anaerobe*, 2003, vol. 9, p. 31-38. (0.776 – IF2005)
- [19] MAJERNÍK, I. A. – LUNDGREN, M. – McDERMOTT, P. – BERNANDER, R. - CHONG, J.P.J. DNA content and nucleoid distribution in *Methanothermobacter thermautotrophicus*. In *Journal of Bacteriology*. Vol. 187, no. 5 (2005), p. 1856-1858. (4.167- IF2005)
- [20] RAJMAN, M. - JURÁNI, M. - LAMOŠOVÁ, D.- MÁČAJOVÁ, M. - SEDLAČKOVÁ, M. - KOŠŤÁL, L. - JEŽOVÁ, D. - VÝBOH, P. The effects of feed restriction on plasma biochemistry in growing meat type chickens (*Gallus gallus*). In *Comparative Biochemistry and Physiology, Part A* Vol.145 (2006), p. 363-371. (1,351 – IF2005)
- [21] SAVORY, C.J. - KOŠŤÁL, L. Is expression of some behaviours associated with dearousal in restricted-fed chickens? In *Physiology & Behaviour*. Vol. 88 (2006), p. 473-479. (2,183 – IF2005)
- [22] SCHNABL, M. – OSKOLKOVA, O.V. – HOLIČ, R. – BREŽNÁ, B. – PICHLER, H. – ZÁGORŠEK, M. – KOHLWEIN, S.D. – PALTAUF, F. – DAUM, G. – GRIAC, P.: Subcellular localization of yeast Sec14 homologues and their involvement in regulation of phospholipid turnover. In: *European Journal of Biochemistry*, 2003, vol. 270, p. 3133-3145. (3.164 – IF2005)
- [23] SVOBODOVÁ, L.- ŠNEJDÁRKOVÁ, M.- POLOHOVÁ, V.- GRMAN, I.- RYBÁR, P.- HIANIK, T. QCM Immunosensor Based on Polyamidoamine Dendrimers. In *Electroanalysis*. Vol. 18, no. 19-20 (2006), p.1943-1949. (2,189 - IF2005)
- [24] ŠKROBÁNEK, P. – BARANOVSKÁ, M. – JURÁNI, M. – ŠÁRNIKOVÁ, B. Influence of simulated microgravity on leg bone growth and mineral deposition in developing Japanese quail chicks. In *Acta Veterinaria Brno*. Vol. 74 (2005), p. 475-481. (0.449-IF2005)
- [25] ŠNEJDÁRKOVÁ, M. – SVOBODOVÁ, L. – EVTUGYN, G. – BUDNIKOV, H. – KARYAKIN, A. – NIKOLELIS, DP – HIANIK, T. Acetylcholinesterase sensors based on gold electrodes modified with dendrimer and polyaniline. A comparative research. In *Analytica Chimica Acta*. Vol. 514 (2004), p. 79-88. (2.760 – IF2005)
- [26] ŠNEJDÁRKOVÁ, M. – SVOBODOVÁ, L. – NIKOLELIS, D. P. – WANG, J. – HIANIK, T.: Acetylcholine biosensor based on dendrimer layers for pesticides detection. In: *Electroanalysis*, 2003, vol. 15, no. 14, p. 1185-1191. (2.189 – IF2005)
- [27] ULIČNÁ, O. - VANČOVÁ, O. - BOŽEK, P. - ČÁRSKY, J. - ŠEBEKOVÁ, K. - BOOR, P. - NAKANO, M. - GREKSÁK, M. Rooibos Tea (*Aspalathus linearis*) partially prevents oxidative stress in streptozotocin-induced diabetic rats. In *Physiological Research*. Vol. 55, no. 2 (2006), p. 157-164. (1,806 – IF2005)
- [28] VALACHOVIČ, M. - BAREITHER, B.M. - SHAH ALAM BHUIYAN, M. - ECKSTEIN, J. - BARBUCH, R. - BALDERES, D. - WILCOX, L. - STURLEY, S.L. - DICKSON, R.C. – BARD, M. Cumulative Mutations Affecting Sterol Biosynthesis in the Yeast *Saccharomyces Cerevisiae* Result in Synthetic Lethality that is Suppressed by Alterations in Sphingolipid Profiles. In *Genetics*. Vol. 173, no. 4 (2006), p. 1893-1908. (4,289 – IF2005)
- [29] VANČEK, M. - VIDOVÁ, M. - MAJERNÍK, A. - ŠMIGÁŇ, P. Methanogenesis is Ca²⁺ dependent in *Methanothermobacter thermautotrophicus* strain ΔH. In *FEMS Microbiology Letters*. Vol. 258 (2006), p. 269-273. (2,057 – IF2005)

ii. List of monographs/books published abroad

iii. List of monographs/books published in Slovakia

iv. List of other scientific outputs specifically important for the Organisation

v. Table of research outputs

Table **Research outputs** shows research outputs in number of specified entries; these entries are then divided by FTE employees with a university degree (from Tab. Research staff) for all Organisation at the respective year; finally these entries are divided by the total salary budget (from Tab. Salary budget).

Research outputs	2003			2004			2005			2006			total			
	number	No. / FTE	No. / salary budget	number	No. / FTE	No. / salary budget	number	No. / FTE	No. / salary budget	number	No. / FTE	No. / salary budget	number	averaged number per year	av. No. / FTE	av. No. / salary budget
chapters in monographs, books published abroad	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.0	0.00	0.00
chapters in monographs, books published in Slovakia	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.0	0.00	0.00
CC publications	11	0.54	1.18	23	1.04	2.44	13	0.54	1.29	12	0.48	1.17	59	14.8	0.64	1.51
scientific publications indexed by other databases (specify)	1	0.05	0.11	1	0.05	0.11	0	0.00	0.00	0	0.00	0.00	2	0.5	0.02	0.05
scientific publications in other journals	2	0.10	0.21	0	0.00	0.00	1	0.04	0.10	0	0.00	0.00	3	0.8	0.03	0.08
publications in proc. of international scientific conferences	29	1.43	3.12	39	1.77	4.13	41	1.71	4.08	54	2.14	5.26	163	40.8	1.78	4.17
publications in proc. of nat. scientific conferences	9	0.44	0.97	6	0.27	0.64	13	0.54	1.29	7	0.28	0.68	35	8.8	0.38	0.90
active participations at international conferences	30	1.48	3.22	37	1.68	3.92	39	1.63	3.88	54	2.14	5.26	160	40.0	1.75	4.10
active participations at national conferences	9	0.44	0.97	6	0.27	0.64	15	0.63	1.49	8	0.32	0.78	38	9.5	0.42	0.97

vi. Renormalized publications²

Renormalized publications = number of CC publications in the given year times authorship's portion of the Organisation times the journal impact factor in 2005 divided by the median impact factor in the research field

Renormalised publications	2003			2004			2005			2006		
	number	No. / FTE	No. / salary budget	number	No. / FTE	No. / salary budget	number	No. / FTE	No. / salary budget	number	No. / FTE	No. / salary budget
Renormalized publications	5.06	0.25	0.54	14.6	0.66	1.54	8.24	0.34	0.82	6.93	0.27	0.67

vii. Standard manuscript page count³

Standard manuscript page count	2003			2004			2005			2006		
	number	No. / FTE	No. / salary budget	number	No. / FTE	No. / salary budget	number	No. / FTE	No. / salary budget	number	No. / FTE	No. / salary budget
page count	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0

viii. List of patents and patent applications

ix. Supplementary information and/or comments on the scientific output of the Organisation

One of our long-term goals is to increase the quality of our research. This is reflected also in our “publication policy” – to focus more on quality (represented by reputation and impact factor of publishing journals) than on mere quantity of

² This information is required only from the Organisations of the Section 2 of the Slovak Academy of Sciences.

³ This information is required only from the Organisations of the Section 3 of the Slovak Academy of Sciences.

published papers. 59 publications published in CC journals in the years 2003-2006 represent a moderate increase in the quantity of our production compared to 52 papers published in previous evaluation period. Much higher improvement can be seen with respect to the quality of published papers judged as Impact Factor (IF) of corresponding journals. Compared to previous evaluation period, the average IF improved significantly from IF_{av}=1.293 (1998-2002) to IF_{av}=1.930 (2003-2006). This significant progress was apparent for all four years in this evaluation period (IF₂₀₀₃: 1.685; IF₂₀₀₄: 1.710; IF₂₀₀₅: 2,226; IF₂₀₀₆: 2,259). This positive change in quality of output is a consequence of following factors:

- We succeeded in publishing our results in outstanding journals such as Nucleic Acids Research. (IF 7.552), Journal of Biological Chemistry (5.854), Apoptosis (4.540), Antimicrobial Agents and Chemotherapy (4.379), Genetics (4,289), Journal of Bacteriology (4.146), Biochimica et Biophysica Acta (3.510), European Journal of Biochemistry (3.164), Biochemical Society Transactions (3.099) or Biochemical and Biophysical Research Communications (3.000).
- A significant proportion of papers (39%) have been published in journals of very good quality (IF > 2.0) and 51% with better-than-average quality (IF>1.5).
- Since we are publishing in journals with subjects ranging from veterinary sciences to molecular biology, a ratio of actual IF to median IF for corresponding subject category may be more indicative about the quality of papers. Also this criterion shows that we are publishing in good quality journals: the average ratio IF/median IF ranged from 1.045 to 1.342 for all years of the evaluation period and the ratio was higher than one for more than 50 % of our publications.

Publishing in high-quality journals is not a simple task. The better the journal is, the more stringent tends to be the review process. Attempts to publish in first-class journals may result in a delay in publication rate, as we experienced this year. During first 6 weeks of 2007, 6 more papers have been published with another 4 papers waiting as “accepted for publication” in the pipeline. The positive message from these papers published or accepted too late to be included in the evaluation is that we succeeded to maintain high standard of publishing journals as the average IF for already published papers reached excellent value of 2.60.

2. Responses to the scientific output

Table **Citations** shows specified responses to the scientific outputs; these entries are then divided by the FTE employees with a university degree (from Tab. Research staff) for all Organisation at the respective year; finally these entries are divided by the total salary budget (from Tab. Salary budget).

Citations	2002			2003			2004			2005			total			
	number	No. / FTE	No. / salary budget	number	No. / FTE	No. / salary budget	number	No. / FTE	No. / salary budget	number	No. / FTE	No. / salary budget	number	averaged number per year	av. No. / FTE	av. No. / salary budget
Web of Science	132	6.5	14.2	116	5.3	12.3	164	6.8	16.3	215	8.5	20.9	627	156.8	6.9	64.2
SCOPUS	4	0.2	0.4	12	0.5	1.3	10	0.4	1.0	17	0.7	1.7	43	10.8	0.5	4.4
(specify Database 1)	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0	0.0
in monographs, conf. proceedings and other publications abroad	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0	0.0
in monographs, conf. proceedings and other publications in Slovakia	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0	0.0

i. List of 10 top-cited publications and number of their citations in the assessment period

[1] REINER, A- PERKEL, DJ- BRUCE, LL- BUTLER, AB- CSILLAG, A- KUENZEL, W- MEDINA, L- PAXINOS, G-SHIMIZU, T- STRIEDTER, G- WILD, M- BALL, GF- DURAND, S- GUTURKUN, O- LEE, DW- MELLO, CV-POWERS, A- WHITE, SA- HOUGH, G- KUBIKOVA, L_- SMULDERS, TV- WADA, K- DUGAS-FORD, J- HUSBAND, S- YAMAMOTO, K- YU, J- SIANG, C- JARVIS, ED Revised nomenclature for avian telencephalon and some related brainstem nuclei. Journal of Comparative Neurology-V473 (2004) -PP 377-414 (WoS cit. 72)

- [2] SCHROEDER,F.- JEFFERSON,J.R.- KIER,A.B.- KNITTEL,J.- SCALLEN,T.J.- WOOD,W.G.- HAPALA,I. Membrane cholesterol dynamics: cholesterol domains and kinetic pools. *Proceedings of the Society for Experimental Biology and Medicine*, Vol. 196, (1991), pp.235-252. (WoS cit. 32)
- [3] SREENIVAS, A. - PATTON-VOGT, J.L,- BRUNO, V. – GRIAC, P. – HENRY, S.A. A role for phospholipase D (P1d1p) in growth, secretion and regulation of membrane lipid synthesis in yeast. *Journal of Biological Chemistry*. Vol. 273, (1998) p.16635-16638. (WoS cit. 21)
- [4] PATTONVOGT, J.L. - GRIAC, P. - SREENIVAS, A, - BRUNO, V. – DOWD, S. – SWEDE, M.J. - HENRY, S.A. Role of the yeast phosphatidylinositol-phosphatidylcholine transfer protein (sec14p) in phosphatidylcholine turnover and INO1 regulation. *Journal of Biological Chemistry*. Vol.272 (1997), p. 20873-20883). (WoS cit.20)
- [5] MAJERNIK, A., GOTTSCHALK., G., DANIEL, R. Screening of Environmental DNA Libraries for the presence of genes conferring Na⁺(Li⁺)/H⁺ antiporter activity on *Escherichia coli*: characterization of the recovered genes and the corresponding gene products. *Journal of Bacteriology*. Vol.183 (2001) p. 6645-6653. (WoS cit. 19)
- [6] BILCIK, B. - KEELING, L.J. Changes in feather condition in relation to feather pecking and aggressive behaviour in laying hens. *British Poultry Science*. Vol. 40 (1999) p. 444-451. (WoS cit. 14)
- [7] ZEMAN, M. – GWINNER, E. – HERICHOVA, I. - -LAMOSOVA. D, - KOSTAL, L. Perinatal development of circadian melatonin production in domestic chicks. *Journal of Pineal Research*. 1999 Vol. 26 (1999) p. 28-34. (WoS cit. 14)
- [8] HERICHOVA, I - ZEMAN, M. - MACKOVA, M - GRIAC, P. Rhythms of the pineal N-acetyltransferase mRNA and melatonin concentrations during embryonic and post-embryonic development in chicken. *Neuroscience Letters*. Vol. 298 (2001) p.123 (WoS cit. 14)
- [9] BILCIK, B. – KEELING, L.J. Relationship between feather pecking and ground pecking in laying hens and the effect of group size. *Applied Animal Behaviour Science*.Vol.68 (2000) p.55-66. (WoS cit.12)
- [10] HIANIK, T.-SNEJDARKOVA, M. - SOKOLIKOVA, L. - MESZAR, E. - KRIVANEK, R. - TVAROZEK, V. - NOVOTNY, I. - WANG, J. Immunosensors based on supported lipid membranes, protein films and liposomes modified by antibodies, *Sensors and Actuators B-Chemical*. Vol.57 (1999) p. 201-212. (WoS cit.12)

[11] GRIAC, P, - SWEDE, M.J. – HENRY, - S.A The role of phosphatidylcholine biosynthesis in the regulation of the INO1 gene of yeast. *Journal of Biological Chemistry* Vol. 271 (1996), p.25692-25698 (WoS cit.12)

ii. List of top-cited authors from the Organisation (at most 10 % of the research employees) and their number of citations in the assessment period

[1] RNDr. Peter Griač, CSc. (WoS cit. 91)

[2] Mgr. Ľubica Kubíková, PhD. (WoS cit.88)

[3] RNDr. Ivan Hapala, CSc. (WoS cit. 87)

Prof. RNDr. Michal Zeman, DrSc. (part time contract, 115 WoS citations of papers published as a part of the scientific program of the Institute)

iii. Supplementary information and/or comments on responses to the scientific output of the Organisation

Due to problems with retrieval of citations not indexed in international databases, presented data on responses to publications are limited to Web of Science and Scopus databases.

The evaluation of the citation record of our Institute should consider the fact that a major part of our scientific production is in agriculture-related areas where citation rate is far lower compared to highly cited subjects like molecular biology and medical research. Moreover, the citation rate of many very interesting results in field of avian behaviour or biochemistry of archaea is undervalued because of the limited number of laboratories working in the field. With respect to this, the citation record of our Institute in the evaluation period is respectable. Compared to previous evaluation period, the average number of WoS citations increased significantly from 96 citations/year (1998-2001) to 157 citations/year (2002-2005). The number of citations per researcher per year rose from 6.5 in 2002 to 8.5 in 2005 (the average value for the whole period: 6.85). One of the positive aspects here is the fact that this increase can be attributed to citations of young researchers. Increasing number of publications in high-impact journals indicates that the positive trend in the citation rate could be retained also in the coming years.

3. Research status of the Organisation in the international and national context

- **International/European position of the Organisation**

- i. **List of the most important research activities documenting international importance of the research performed by the Organisation, incl. major projects (details of projects should be supplied under Indicator 4). Collective membership in the international research organisations, in particular within the European Research Area**

[1] ***Evaluation/validation of novel biosensors in real environmental and food samples***, QLK3 – 2000 – 01311, 5FP project

[2] ***Measuring and monitoring farm animal welfare***, COST Action 846

[3] ***Behaviour and its underlying physiological mechanisms related to reproduction and welfare problems in parent stock of meat type chickens***, U.S.-Slovak Science and Technology Program 021/2001

[4] ***Neutral lipids of the yeast *Saccharomyces cerevisiae****, FWF 15141 (Austria)

[5] ***Physiological effect of rooibos tea *Aspalathus linearis* and other natural antioxidants in some biochemical and physiological processes in animals and humans***. collaboration with Institute for Medical Science of Ageing, Nagakute, Japan

[6] ***Preparation of robust transforming system in cells *Methanothermobacter thermoautotrophicus****. Collaboration with University of York, Great Britain.

- ii. **List of international conferences (co-) organised by the Organisation**

[1] ***COST Action 846: Measuring and monitoring farm animal welfare, Final Meeting***, 23.-24. 3. 2006, Bratislava

[2] ***22nd International Conference on Yeast Genetics & Molecular Biology***, 7.-12. 8. 2005, Bratislava,

[3] ***31st Ethological Conference***, 15.-17.4. 2004, Poľana

[4] ***32nd Ethological Conference***, 27. -30. 4. 2005, Nečtiny, Czech Republic

[5] ***33rd Ethological Conference***, 19.- 22. 4. 2006, Jihlava, Czech Republic

- iii. **List of international journals edited/published by the Organisation**

-

iv. List of edited proceedings from international scientific conferences and other proceedings

[1] 31st Ethological Conference, 15.-17. 4. 2004, Poľana, Program and Abstracts

- **National position of the Organisation**

- i. **List of selected most important national projects (Centres of Excellence, National Reference Laboratories, Agency for the Promotion of Research and Development (APVV/APVT), National Research Programmes, Scientific Grant Agency of the Slovak Academy of Sciences and the Ministry of Education (VEGA), and others)**

- [1] ***Membrane-associated processes and their role in normal and pathological physiology of farm animals and their symbiotic microorganisms***, APVT-51-016502, doc. RNDr. Peter Šmigáň DrSc., 09/2002 - 12/2005
- [2] ***Yeast as a model for pathological defects of lipid homeostasis in eukaryotic cells***, APVT-51-029504, Mgr. Martin Valachovič PhD., 01/2005 -12/2007
- [3] ***Molecular genetic principles of membrane bound processes in normal and pathological animal physiology***, APVT-51-024904, doc. RNDr. Peter Šmigáň DrSc., 09/2005 - 12/2007
- [4] ***BIOMEMBRANES: cross-sectional educational program for graduate students and young scientists in life sciences***. ESF (European Social Fund) project # 13120200072. RNDr. Ivan Hapala, CSc., 02/2006 – 04/2008.
- [5] ***Interaction of proteins with supramolecular compounds and their application in biosensor construction on the basis of membranes and thin films***. VEGA 2/1017/21Ing. Maja Šnejdárková, CSc., 2001 – 2003.
- [6] ***Melatonin and leptin participation in control of poultry development and growth and analysis of factors regulating melatonin synthesis***. VEGA 2/1015/23, Ing. Dalma Lamošová, CSc., 2001 – 2003.
- [7] ***Adaptation mechanisms to disturbed membrane lipid biogenesis in yeast *Saccharomyces cerevisiae****. VEGA 2/1016/23, RNDr. Peter Griač, CSc., 2001-2003.
- [8] ***Measuring poultry welfare using behavioural and physiological methods***. VEGA 2/2080/22, RNDr. Ľubor Košťál, CSc., 2002 – 2004.
- [9] ***Postembryonic development of Japanese quail in the conditions of hypodynamy***. VEGA 2/3046/23, Ing. Peter Škrobánek, CSc., 2003 – 2005.

- [10] ***The use of monoclonal antibodies for analysis of immune functions and morphological structures of the cattle organs.*** VEGA 2/3045/03, Ing. Michal Simon, DrSc., 2003 – 2005.
- [11] ***Biochemical-genetic approach to the study of molecular mechanisms of energy transformation in methanoarchaea *Methanothermobacter thermoautotrophicus*.*** VEGA 2/3047/23, Doc. RNDr. Peter Šmigáň, DrSc., 2003 – 2005.
- [12] ***Electron transfer in biosensors and topography of the bioactive surfaces.*** VEGA 2/4131/24, Ing. Maja Šnejdárková, CSc., 2004 – 2006.
- [13] ***Mechanisms of membrane lipid homeostasis in the yeast *Saccharomyces cerevisiae*.*** VEGA 2/4130/24, RNDr. Peter Griač, CSc., 2004 – 2006.
- [14] ***Behavioural physiology of poultry related to production traits and welfare.*** VEGA 2/5127/25, RNDr. Ľubor Košťál, CSc. (RNDr. Boris Bilčík, PhD. since 2006), 2005 – 2007.
- [15] ***Production and application of monoclonal antibodies in biochemical, histochemical and functional analysis of some CD molecules (antigens) of bovine cell membranes.*** VEGA 2/6023/26, Ing. Michal Simon, DrSc., 2006 – 2008.
- [16] ***Study of energy transformation in methanoarchaea *Methanothermobacter thermoautotrophicus*: Isolation and characterization of amiloride, NO_3^- , DCCD and bafilomycin A_1 -resistant mutants.*** VEGA 2/6925/26, Doc. RNDr. Peter Šmigáň, DrSc., 2006 – 2008.
- [17] ***Development of reproductive ability in Japanese quail exposed to simulated microgravity.*** VEGA 2/6024/26, Ing. Peter Škrobánek, CSc., 2006 – 2008.

ii. List of national scientific conferences (co)-organised by the Organisation

- [1] 3rd Ivanka Days of Young Biologists, 17. 6. 2004, Ivanka pri Dunaji
- [2] 4th Ivanka Days of Young Biologists, 14. 6. 2006, Ivanka pri Dunaji

iii. List of national journals published by the Organisation

-

iv. List of edited proceedings of national scientific conferences/events

-

- **International/European position of the individual researchers**

- i. **List of invited/keynote presentations at international conferences, documented by an invitation letter or programme**

- [1] Greksák M.: *Aspalathus linearis* – hepatoprotective effect and modulation of oxidative stress during streptozotocine induced diabetes. In: XXIIIth Xenobiochemic symposium, 16.-19.05 2005, Valtice, Czech Republic.
- [2] Griáč P.: Phosphatidylinositol transfer protein and its homologues in yeast. In Biochemical Society Focused Meeting, Non-vesicular intracellular traffic, 15.-16.12. 2005, London, UK.
- [3] Klobučníková V., Mazáňová K., Kohút P., Hapala I.: Mechanisms of resistance to polyene macrolides in yeast: two mutants displaying different sensitivity to nystatin and amphotericin B. In Central European Symposium on Antibiotic Resistance (CESAR) 2006, The High Tatras, June 18-21 2006.
- [4] Košťál L.: Avian brain behaviour and the measurement of animal welfare. Avian Brain Conference, 23.-25. 8. 2005, Budapest, Hungary
- [5] Košťál L.: Neurobiology, psychopharmacology and welfare measurement in farm animals, 32nd Ethological Conference 27.-30.4. 2005, Nečtiny, Czech Republic
- [6] Košťál, L.: Abnormal behaviour in farm animals: definitions, classification and mechanisms. AGRIBEA seminar: Normal and abnormal behaviour. Paris, INRA, 15.12. 2006.
- [7] Šnejdárková M.: Dendrimers in biosensor technology. In Book of abstracts. International Conference on Advanced Materials, Micro- and Nanotechnology in 3rd calls of 6th FP projects“ (CAMIN 05 Smolenice).
- [8] Zeman M.: Hormonal control of avian behaviour. In 32nd Ethological Conference 27.-30.4. 2005, Nečtiny, Czech Republic

- ii. **List of employees who served as members of the organising and/or programme committees for international conferences**

- [1] I. Hapala - Steering Committee of the Yeast Lipid Conferences (elected member, 2002-date)
- [2] I. Hapala – Member of the Program Committee, 32th Annual Conference on Yeast, Smolenice, 2004; 34th Annual Conference on Yeast, Smolenice, 2006.
- [3] I. Hapala, P. Griáč, Members of the Organizing Committee, 22nd International Conference on Yeast Genetics & Molecular Biology, Bratislava, 7.- 12. 8. 2005

- [4] Ľ. Košťál, Head of the Organizing Committee; P. Výboh, M. Sedlačková, M. Rajman – Members of the Organizing Committee, 31st Ethological Conference, Poľana, 15.-17. 4. 2004
- [5] Ľ. Košťál, Member of the Scientific Steering Committee, Avian Brain Conference, 23.-25. 8. 2005, Budapest, Hungary
- [6] Ľ. Košťál, B. Bilčík, Organizing Committee, COST Action 846: Measuring and monitoring farm animal welfare, Final Meeting, Bratislava, 23.-24. 3. 2006
- [7] M. Rajman, Member of the Organizing Committee, 32nd Ethological Conference, Nečtiny, Czech Republic, 27. -30. 4. 2005
- [8] M. Rajman, Member of the Organizing Committee, 33rd Ethological Conference, Jihlava, Czech Republic, 19.- 22. 4. 2006
- [9] M. Zeman, Member of Scientific Board of the 5th Workshop: Fundamental Physiology and Perinatal Development in Poultry, Berlin, Germany, 5.-7. 9. 2003
- [10] M. Zeman, Member of the Organizing Committee, Vth Slovak Seminar on Animal Physiology, Nitra, 2.- 3. 6. 2003
- [11] M. Greksák, Member of the Programme Committee, XX. Biochemical Meeting, Piešťany, September 12.-16. 2006

iii. List of employees who served as members of important international scientific bodies (e.g. boards, committees, editorial boards of scientific journals)

- [1] Antalíková J. - Member of the International Reviewers Panel, Medical Science Monitor (USA)
- [2] Košťál Ľ. - Member of the International Advisory Board, Acta Veterinaria, Brno (ČR)
- [3] Simon M. - Member of the Editorial Board, Animal Science Papers and Reports, Jastrzebiec (Poland)
- [4] Zeman M. - Associate Editor, Neuroendocrinology Letter, Stockholm, (Sweden)
- [5] Zeman M. – Member of the Editorial Board, Biológia (SR)
- [6] Greksák M. – Member of the Editorial Board, General Physiology and Biophysics (SR)

iv. List of international scientific awards and distinctions

employees awarded by travel grants from international bodies:

- [1] Čuboňová Ľ., 2003, EMBO travel grant; EMBO course „Cellular and Molecular Biology of Membranes“, Corsica, France
- [2] Svobodová Ľ., 2003, Travel grant; The scanning electrochemical microscopy course, University of Texas, June 2003, Austin, Texas, USA

- [3] Hronská L., 2004, FEBS Short Term Fellowship, 3 months, Division of Biochemistry, Department of Medicine, University of Fribourg, Switzerland
- [4] Polohová V., 2004, FEBS (Federation of European Biochemical Societies) Youth Travel Fund grant; The Design and Building of Quartz Crystal Microbalance Biosensors course, the Hacettepe University, June 2004, Ankara, Turkey
- [5] Valachovič M., 2004, Travel grant from the Indiana University - Purdue University of Indianapolis; 95th American Oil Chemists' Society Annual Meeting & Expo. Cincinnati, USA
- [6] Holič R., 2005, FEBS travel grant; FEBS Advanced Course "Lipid-protein interaction in signalling and membrane traffic", Chieti, Italy
- [7] Griač P., 2005, Travel grant from the local organizers; Academy Colloquium, Lipid Moving Centre Stage, Amsterdam, the Netherlands
- [8] Griač P., 2005, The Biochemical Society, UK, Invited Speaker Travel Grant; Conference Nonvesicular intracellular traffic, London, UK
- [9] Hronská L., 2005, The Biochemical Society, UK, travel grant; Conference Nonvesicular intracellular traffic, London, UK
- [10] Rajman M., 2005, IBRO CEERC Summer school, 11.- 22. 7., Debrecen, Hungary
- [11] Rajman M., 2005, Travel grant from the local organizers; Avian Brain Conference, 24. – 26. 8. 2005, Budapest, Hungary
- [12] Svobodová L., 2005, Travel grant; ESF Research Conference on "Biological Surfaces and Interfaces", June 2005 San Feliu de Guixols (Spain)
- [13] Kubíková L., 2006, Short term fellowship funded by the FIRCA and Duke University; Duke University, Durham, USA
- [14] Kubíková L., 2006, IBRO CEERC travel grant; Federation of European Neuroscience Societies Forum 2007, Vienna, Austria, 8.-12. 7. 2006
- [15] Majerník A., 2006, The Royal Society, UK, short term fellowship; Department of Biology, University of York, York, United Kingdom
- [16] Polohová V., 2006, IUPAP (International Union of Pure and Applied Physics) Women in Physics Travel Grant Program, 11th International Conference on Electroanalysis, ESEAC 2006: June 2006, Bordeaux, France
- [17] Rajman M., 2006, IBRO travel grant; International IBRO Workshop, Regulatory mechanisms of synaptic transmission in the nervous system, Budapest, 26 - 28. 1. 2006
- [18] Šimočková M., 2006, Travel grant from the local organizers; 24th Small Meeting on Yeast Transport and Energetics, Czech Republic

- [19] Poloncová K., 2006, Travel grant from the local organizers, Conference: New Concepts in Lipidology, Noordwijkerhout, the Netherlands
- [20] Šimočková M., 2006, FEBS travel grant; Conference: New Concepts in Lipidology, Noordwijkerhout, the Netherlands
- [21] Valachovič M., 2006, FEBS travel grant; Conference: New Concepts in Lipidology, Noordwijkerhout, the Netherlands
- [22] Valachovič M., 2006, Program Officers Training Program sponsored by Ministry of Education, Slovak Republic; National Institutes of Health-Fogarty International Center, Bethesda, USA

- **National position of the individual researchers**

- i. **List of invited/keynote presentations at national conferences documented by an invitation letter or programme**

- [1] Hapala I.: Mechanisms of the antimycotics resistance in yeast: Analysis of pleiotropic mutant resistant to terbafine. 2nd Drobnica Memorial, Senec, 12-14. 11. 2003

- ii. **List of employees who served as members of organising and programme committees of national conferences**

-

- iii. **List of employees serving in important national scientific bodies (e.g. boards, committees, editorial boards of scientific journals)**

- iv. **List of national awards and distinctions**

- [1] Šnejdárková M., 2003, Prize of the Slovak Academy of Sciences for the collaboration with the universities; for the long term collaboration with the Faculty of Mathematics, Physics and Informatics of the Comenius University in Bratislava (with Prof. RNDr. T. Hianik, DrSc.) in field of the supramolecular structures utilization for the development of the chemosensitive interfaces in biosensors.
- [2] Valachovič M., 2003, 1st prize, Young Researchs Award, 50th Anniversary of the Slovak Academy of Sciences.
- [3] Svobodová L., 2003, 1st prize and the Award of the President of the Slovak Academy of Sciences, 50th Anniversary of the Slovak Academy of Sciences; Student Science

Conference, Bratislava, Chemical Section - for the contribution SVOBODOVÁ L., ŠNEJDÁRKOVÁ M.: Dendrimeric layers – unique nanomaterials as chemically sensitive interfaces.

- [4] Čuboňová Ľ., 2003, 3rd prize and the Award of the President of the Slovak Academy of Sciences, 50th Anniversary of the Slovak Academy of Sciences; Student Science Conference, Bratislava, Biological Section - for the contribution ČUBOŇOVÁ Ľ., ŠMIGÁŇ P.: An attempt to explain resistance of the methanoarchaea *Methanobacterium thermoautotrophicum* to the uncoupler of the oxidative phosphorylation: Study of the membrane bound processes.
- [5] Čuboňová Ľ., 2003, Honourable Mention, 2nd Memorial of Prof. Drobnica, Senec, Slovakia
- [6] Valachovič M., 2004, Postdoctoral scholarship from the Stefan Schwarz Foundation of the Slovak Academy of Sciences.
- [7] Svobodová L., 2005, Postdoctoral scholarship from the Stefan Schwarz Foundation of the Slovak Academy of Sciences.
- [8] Greksák M., 2006: Honorary Member of the Slovak Society for Biochemistry and Molecular Biology.

Supplementary information and/or comments documenting international and national status of the Organisation

Institute of Animal Biochemistry and Genetics is the only Institute in Slovakia that is systematically involved in the study of dynamics of membrane processes at various complexity levels, from membrane-like molecular assemblies to higher organisms. Most of the research topics studied by our research teams can be considered as distinct nationally as well as internationally. These “hallmark topics” of our Institute include:

- dendrimer-based enzyme biosensors
- Na⁺ and H⁺ gradients in the bioenergetics of methanoarchaea
- proteins involved in lipid homeostasis in yeast
- mechanisms of antimycotic resistance
- bovine CD antigens and their role in bovine physiology and pathophysiology
- neuroendocrine control of normal and abnormal behaviour in birds
- adaptive mechanisms to hypogravitation and hypergravitation in Japanese quail

Each of the Institute's research groups has contributed to the advancement of science also with respect to the development of specific methodological and analytical tools. To mention just two examples, establishment of anaerobic laboratory for the strictest anaerobic conditions and the pioneering use of mutants in the study of the bioenergetic machinery in methanoarchaea helped to identify two faces of bioenergetic processes in methanoarchaea - sodium and proton energetics. Similarly, complex behavioural studies in birds using in situ gene expression and receptor mapping in the brain together with remote measurement of physiological parameters and behavioural patterns or song learning have also brought a wealth of information that unravelled many open questions in the complex issue of the neuroendocrine control of behaviour in birds.

Very encouraging with respect to the future of our Institute was the national and international acknowledgement of good results of our young researchers. The positive messages expressed by several received honours, successful applications for grant and stipends in Slovakia are confirmed internationally by high success rate in obtaining grant support abroad (short-term fellowships, travel grants, etc.)

Numerous national and international collaborations also demonstrate the appreciation of the quality of our research. The Institute of Animal Biochemistry and Genetics has set up four joint research laboratories with Slovak universities based on formal agreements. In addition, our Institute is member of several consortia aimed at improvement of the research infrastructure in Slovakia. Most of research groups have intense contacts and collaborations with leading international laboratories, several of them lasting for more than 15 years. Our scientists are being invited as speakers to international conferences and to universities and research institutes. Many of them are active in the organisation of national and international conferences. This all proves that the Institute of Animal Biochemistry and Genetics has a solid position in the landscape of national and international science.

4. Project structure, research grants and other funding resources

- **International projects and funding**
 - i. **List of major projects within the European Research Area – 5th and 6th Framework Programme of the EU, European Science Foundation, NATO, COST, INTAS, CERN, etc. (here and in items below please specify: type of project, title, grant number, duration, funding, responsible person in the Organisation and his/her status in the project, e.g. coordinator, principal investigator, investigator)**

[1] European Science Foundation – COST project.

Measuring and monitoring farm animal welfare. COST Action 846, *duration:* 2000 – 2006 (Slovakia since 2001), RNDr. Ľubor Košťál, CSc., RNDr. Boris Bilčík, PhD. (Members of the Management Committee for Slovakia),.

Funding:

97 000 SK

[2] FP5 Project

Evaluation/validation of novel biosensors in real environmental and food samples. QLK3 – 2000 – 01311. *duration:* 1.9.2000 – 31.8.2003, – Ing. Maja Šnejdárková, CSc. – collaborator

Funding:

no direct transfer; supplies & travel costs

ii. List of other international projects incl. funding

[1] U.S. – Slovak Science and Technology Program project (partner University of Maryland, USA)

Behaviour and its underlying physiological mechanisms related to reproduction and welfare problems in parent stock of meat type chickens. ID 021/2001 *duration:* 2002 – 2006, RNDr. Ľubor Košťál, CSc. (co-investigator),

Funding:

503 555 SK

[2] International collaboration with Institute for Medical Science of Ageing, Nagakute, Japan.

Physiological effect of rooibos tea *Aspalathus linearis* and other natural antioxidants in some biochemical and physiological processes in animals and humans. *duration:* 2003 – 2005, Doc. RNDr. Miloslav Greksák, CSc. (coinvestigator).

Funding (from Institute for Medical Science of Ageing, Nagakute, Japan):

220 000 SK

[3] Fogarty International Research Collaboration Award, USA.

Molecular mechanisms of basal ganglia regeneration in songbirds. 2006 – 2009, Mgr. Ľubica Kubíková, PhD. (co-investigator), FIRCA R03TW007615-01.

Funding (NIH, USA):

600 000 SK (+ 100 000 SK from Slovak Academy of Sciences MVTs)

iii. List of other important projects and collaborations without direct funding

[1] International collaboration with University of York, Great Britain.

Preparation of robust transforming system in cells *Methanothermobacter thermoautotrophicus*. *duration:* 2001 – ongoing, Doc. RNDr. Peter Šmigáň, DrSc. (co-investigator).

[2] International collaboration with Graz University of Technology, Austria.

Neutral lipids of the yeast *Saccharomyces cerevisiae*. Cooperation as a part of project FWF 15141 (principal investigator prof. G. Daum). *duration:* 2005 – 2007, RNDr. Ivan Hapala, CSc. (co-investigator).

- **National projects and funding**

- i. **List of projects supported by the Agency for the Promotion of Research and Development (APVV/APVT), National Research Programmes, and their funding**

[1] **Membrane-associated processes and their role in normal and pathological physiology of farm animals and their symbiotic microorganisms.** APVT-20-016502. *Duration:* 2002-2005, Doc. RNDr. Peter Šmigáň, DrSc. (principal investigator).

Funding:

4 657 000 SK

[2] **Yeast as a model for pathological defects of lipid homeostasis in eukaryotic cells.** APVT-51-029504. *Duration:* 2005-2007, Mgr. Martin Valachovič, PhD. (principal investigator).

Funding:

1 000 000 SK

[3] **Molecular genetic principles of membrane bound processes in normal and pathological animal physiology.** APVT-51-024904. *Duration:* 2005-2007, Doc. RNDr. Peter Šmigáň, DrSc. (principal investigator).

Funding:

3 378 000 SK

[4] **Biosensors for fast diagnostics of prion diseases.** APVV-20-P01705. *Duration:* 2005 – 2007, Ing. Maja Šnejdárková, CSc. (co-investigator),

Funding:

338 000 SK

[5] European Social Fund project.

BIOMEMBRANES: cross-sectional educational program for graduate students and young scientists in life sciences. ESF project # 13120200072. Duration: 2006 – 2008, RNDr. Ivan Hapala, CSc. (coordinator),
440 000 SK

- ii. **Number of projects supported by the Scientific Grant Agency of the Slovak Academy of Sciences and the Ministry of Education (VEGA) for each year, and their funding**

VEGA	2003	2004	2005	2006
number	9	7	8	8
funding (millions of SKK)	0.790	0.866	0.849	1.040

• **Summary of funding from external resources**

External resources	2003	2004	2005	2006	total	average
external resources (millions of SKK)	2.604	2.899	3.814	5.290	14.607	3.652
external resources transferred to cooperating research organisations (millions of SKK)	0.000	0.000	0.000	0.000	0.000	0.000
ratio between external resources and total salary budget	0.280	0.307	0.380	0.515	1.482	0.370
overall expenditures (millions of SKK)	18.880	20.351	22.747	23.959	85.937	21.484

Supplementary information and/or comments on research projects and funding resources

With respect to funding from external sources, years 2003-2006 have been characterized by extension of our focus from smaller project supported by the VEGA to large projects funded by State Agency for Research and Development (APVV). The direct consequence of this shift of focus was a major increase in funding resources (2.4-fold increase from 2003 to 2006). Of particular significance were two major APVV projects focusing on biomembrane research that included about 75 % of the research

capacity of the Institute. These two projects provided a basis for the unification of the research program of the Institute.

Another positive aspect in funding over past four years was the successful penetration of the generation of young scientist generation into the funding scheme. Currently we have three projects (1 VEGA project, 1 APVV project, 1 NIH-supported joint project) with principal investigators younger than 35 years.

5. Organisation of PhD studies, other pedagogical activities

i. List of accredited programmes of doctoral studies (as stipulated in the previously effective legislation as well as in the recently amended Act on the Universities)

[1] Biochemistry

- 14-10-9 (old legislation –131/1997 Ordinance)
- 4.1.22 (new legislation - 131/2002 Act) contract with Comenius University, Faculty of Science and Slovak Technical University, Faculty of Chemical and Food Processing Technology

[2] Animal Physiology

- 15-17-9 (old legislation –131/1997 Ordinance)
- 4.2.10 (new legislation - 131/2002 Act) contract with Comenius University, Faculty of Science

ii. Summary table on doctoral studies (number of internal/external PhD students; number of students who completed their study by a successful thesis defence; number of PhD students who quitted the programme)

PhD study	31.12.2003			31.12.2004			31.12.2005			31.12.2006		
number of potential PhD supervisors												
PhD students	number	defended thesis	students quitted	number	defended thesis	students quitted	number	defended thesis	students quitted	number	defended thesis	students quitted
internal	11	-	3	10	5	1	10	1	4	10	2	3
external												
supervised at external institution by the research employees of the assessed organisation												

iii. Postdoctoral positions supported by

a) external funding (specify the source)

- [1] Ľ. Kubíková (2001-.2005), Duke University, Durham, North Carolina, USA. Funding source: NIH
- [2] M. Valachovič (2001 - 2004), Department of Biology, Indiana University - Purdue University Indianapolis, Indianapolis, USA. Funding source: NIH
- [3] B. Bilčík (2001-2005), University of Maryland, College Park, Maryland, USA. Funding source: USDA
- [4] A.I. Majernik (2003-2005), Centre for Extremophile Research, Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY, U.K.. Funding source: local grant
- [5] Ľ.Čuboňová (2004-2006), Department of Microbiology, Ohio State University, Columbus, OH 43210, USA. Funding source: local grant

b) internal funding - the Slovak Academy of Sciences Supporting Fund of Stefan Schwarz

- [1] Valachovič M., 2004-2008
- [2] Svobodová L., 2005-2009

iv. Summary table on pedagogical activities in undergraduate programmes for each year

Teaching	2003	2004	2005	2006
lectures (hours/year)	36	32	56	63
practicum courses (hours/year)	636	296	288	500
supervised diploma works (in total)	4	5	3	4
members in PhD committees (in total)	6	6	7	6
members in DrSc. committees (in total)	3	3	4	3
members in university/faculty councils (in total)				
members in habilitation/inauguration committees (in total)	2	3	1	2

v. List of published university textbooks

vi. Number of published academic course books

vii. List of joint research laboratories/facilities with the universities

The Institute of Animal Biochemistry and Genetics established 4 joint research laboratories with university faculties based on formal contract and another 2 based on informal agreements.

Joint research laboratories based on formal contract:

- [1] Faculty of Physics, Mathematics and Informatics, Comenius University Bratislava (project collaboration with Department of nuclear physics and biophysics)
- [2] Faculty of Science, Comenius University Bratislava (project collaboration with Department of Biochemistry, Dept. of Animal Physiology and Ethology, Dept. of Microbiology and Virology, Dept. of Genetics)
- [3] Faculty of Chemical and Food Processing Technology, Slovak Technical University, Bratislava (project collaboration with Institute of Biochemistry, Nutrition and Health Protection, Institute of Biotechnology and Food Processing)
- [4] University of Veterinary Medicine Košice (project collaboration with Department of Normal Anatomy, Histology and Physiology).

Joint research laboratories based on informal agreement:

- [5] Faculty of Agrobiological Sciences, Slovak Agricultural University, Department of Veterinary Disciplines
- [6] Faculty of Medicine, Comenius University, Bratislava, 3rd Department of Internal Medicine, Laboratory of Pharmacobiochemistry

viii. Supplementary information and/or comments on doctoral studies and pedagogical activities

Based on the agreements with the universities, Institute of Animal Biochemistry and Genetics is actively involved in the education at the Comenius University and Slovak Technical University. As a part of this collaboration, our employees are giving lecture courses, seminars and laboratory courses, as well as supervising the diploma theses of students that perform their experimental work at our Institute.

PhD education has been one of the priorities of our Institute also in the past evaluation period. We have been able to keep high standards of our PhD programs in Biochemistry and in Animal Physiology with the average number of 10 PhD students participating in the programs each year. In the years 2003-2004, 13 new students entered our programs and 8 finished the PhD studies by successful defense of the thesis (with 4 students still preparing for the defense after formal finishing the PhD course). The dropout rate was very low with only 1 student terminating untimely. The quality of the PhD theses has been highly appreciated by the Defence Committees. During past four years, a position at the Institute was offered to four most gifted students.

One of major accomplishments in PhD program during the evaluation period is the launch of a new educational program “**BIOMEMBRANES: cross-sectional educational program for graduate students and young scientists in life sciences**” supported by a grant of the European Social Fund. This program is in a sense the pioneering act as it provides a complex interdisciplinary education in biomembrane-related research in biochemistry, molecular biology, biophysics and physiology for PhD students from universities and research institutes in the region of Bratislava. The program is a joint activity of three institutes of Slovak Academy of Sciences (Institute of Animal Biochemistry and Genetics, Institute of Molecular Physiology and Genetics, Institute of Virology). It is open to all PhD students and young scientists interested in biomembranes. Each institute offers the theoretical lecture courses and practical laboratory courses, each in the specific areas of its special expertise. The specific

“added value” of the program is the opportunity to have a hands-on experience with the sophisticated unique equipment and methods available at participating institutes. The details about the program can be found on www.ubgz.sav.sk/biomembrany/

Another activity that should be mentioned here is organization of a minisymposium “Ivanka days of young biologists”. We started to organise this minisymposium 7 years ago with the aim to give PhD students the possibility to present their results in short talks and in discussion. During the evaluation period, two minisymposia have been organised (in 2004 and 2006). The minisymposia are organized as competitions. Presentations in three categories (biochemistry and molecular biology; cell biology and microbiology, physiology and general biology) are evaluated by a jury and the best presentation in each category is awarded. Very good quality of presentations has been typical for all minisymposia so far and the jury could conclude every year that it is a pleasure to discuss science with so many talented and enthusiastic young people. More information about the minisymposia can be found on www.ubgz.sav.sk/stvrte_ivanske_dni

6. Direct output to the society

(applications of results, popularisation and outreach activities)

i. List of the most important results of applied research projects

ii. List of the most important studies commissioned for the decision-making authorities, the government and NGOs, international and foreign organisations

iii. List of the most important popularisation activities

Lectures:

- [1] Košťál Ľ.: “What do animals signal with their behaviour about the housing conditions”
Lecture for students of the Agricultural College in Ivanka pri Dunaji. European Science Week, 6.11.2003
- [2] Greksák M.: “Methanohogens in the physiology of digestion in animals and human”
Lecture for students of the Agricultural College in Ivanka pri Dunaji, European Science Week, 6.11.2003
- [3] Hapala I.: Newspaper debate for the daily Pravda about “Dolly the sheep, which had three mothers” in Pravda’s Weekly supplement, 1. 3. 2003, p. XI

- [4] Hapala I.: "Research structure of the Institute of Animal Biochemistry and Genetics"
Lecture for students of the Agricultural College in Ivanka pri Dunaji, European Science Week, 6.11. 2003
- [5] Juráni M.: Lecture: "Mutations in Agapornis Fisher" 1st international congress of breeders „EXOTA“, Olomouc 2003, CR
- [6] Juráni, M.: Agapornis – breeding and genetics of mutations. Lecture at the fiftieth anniversary of the "Slovak union of exotic bird breeders. Slovak Union of Breeders, Bratislava, 26.10.2006.
- [7] Majerník A.: Coexistence between human and microorganisms.
20.11.2006 – Lecture for High-school students, Grosslingova Str. Bratislava
24.11.2006 – Lecture for High-school students, Grosslingova Str. Bratislava
- [8] Greksák M.: Rooibos tee – a delicious beverage capable prevention against modern civilization diseases based on so called "oxygen stress". Presentation for the public at the village Ivanka pri Dunaji, European Science Week (21.11.2006.)

Papers:

- [9] Šnejdárková M., Svobodová L., Hianík T.: Nanomaterials in biosensors. Highly branched molecules. Quark, 6, 16-17. (2003)
- [10] Košťál, L.: Bird and animal brains are similar. Newspaper article in the daily SME 17.2.2005
- [11] Škrobánek, P.: The space quail from Ivanka. Newspaper article in "Ivanské novinky", October 2005, p. 10-12
- [12] Hapala I.: Eurofonds: will the expectations meet the reality? Forum Scientiae et Sapientiae, 2006, vol XIII, No.3, p. 26-27.
- [13] Newspaper article in the Novohradské noviny; 36/2006, p. 33; Mihály František: Great discussion with the biochemist Doc. RNDr. Miloslav GREKSÁK, CSc.
- [14] Visiting the breeder RNDr. Marián Juráni. Article in the journal „EXOTA - Journal for breeders“, No. 3 (2006), p. 14-16.

Other:

- [15] Zeman M.: Discussion about "The time change and biological rhythms" in the programme "Afternoon with the radio" (Slovenský rozhlas, 25. 10. 2003).
- [16] Hapala I.: Discussion in the programme "Metamorphosis" (Slovenský rozhlas, 14. 2. 2003).
- [17] Hapala I.: Guest in the TV programme TV morning (TV channel Markíza, 23.1.2003).

- [18] Svobodová L.: Discussion about biosensors in the programme “Enter – broadcast about science, technology and informatics” (Slovenský rozhlas, 5.6.2003).
- [19] Hapala I.: Interview for radio-broadcast Okey on the space physiology research project „Prepelica“ on the occasion of the anniversary of SPUTNIK II launch to the orbit.
- [20] Svobodová, L.: Application of biosensors. TV broadcast, programme “Science and technology” (TV channel STV2, 11.10.2005).

iv. List of patents issued abroad, incl. revenues

v. List of the patents issued in Slovakia, incl. revenues

vi. List of licences sold abroad, incl. revenues

vii. List of licences sold in Slovakia, incl. revenues

viii. List of contracts with industrial partners, incl. revenues

[1] Monoclonal antibodies against human antigens are widely used for scientific and diagnostic purposes. Most of these mAbs are commercially available. However, mAbs specific for antigens of domestic animals are much less common and only a small portion (about 7%) of human mAbs are cross-reactive and could be used for testing in domestic animals. The mAbs produced in our Institute enlarge the palette of monoclonal antibodies for detection of the cell surface antigens of cattle. Some of the mAbs could be used for immunodiagnostic purposes as IVA 281 suitable for measuring the immunoglobulin concentration or/and for the study of cattle immune system. mAbs with potential commercial value that were produced in our institute were distributed by the company EXBIO Praha, Czech Republic. As a part of the agreement, the mAbs IVA50 (CD9), IVA 35 (CD18), IVA 30 (CD41/61), IVA 103 (CD45R), IVA 94 (CD62 L), IVA 285 (Ig light chain) are offered in the product catalogue of EXBIO Praha www.exbio.cz (veterinary reagents). The financial compensation for our monoclonals is based on a sort of „barter trade“ system: EXBIO company has been supplying our Institute with monoclonal antibodies offered in their catalogue in the total value of 110.000,- SK during the past four years.

ix. List of research projects with industrial partners, incl. revenues

x. Summary of outreach activities

Outreach activities	2003	2004	2005	2006	total
studies for the decision sphere, government and NGOs, international and foreign organisations					0
articles in press media/internet popularising results of science, in particular those achieved by the Organization	1	0	2	3	6
appearances in telecommunication media popularising results of science, in particular those achieved by the Organization	6	1	1	0	8
public popularisation lectures	4	0	0	4	8

xi. Supplementary information and/or comments on applications and popularisation activities

Presentation of the results of our research programs and a general promotion of science in the public is one of important aims of our Institute. In addition to numerous lectures, popularization papers and interviews listed above, three activities should be presented in more detail.

In year 2003 several significant research achievements of the Institute were presented in “The 15th anniversary of Slovak Academy of Sciences” exhibition, which took place in the Slovak National Museum in Bratislava. Our Institute was represented by research activities in the field of the space research and gravitational physiology. Special attention was given to the project “Prepelica” (the Japanese quail) of the Štefanik mission. Our aims and results in the field of space research were explained on a panel presentation while authentic space Incubator used to transport quail eggs from the Earth to the space station MIR was an attractive and interactive part of the exhibition. Our presentation received favourable reactions from the public and media (weekly supplement Science of the daily Sme, 2.7. 2003). The exhibition continued in 2004 and toured Slovakia with a great success.

To improve the communication of science to general public and to increase the awareness of people about the role of science in the society, Institute of Animal

Biochemistry and Genetics has been actively participating in the European Science Week in past three years 2004-2006. Institute is open to visitors one day during this week, we provide a guided tour through the laboratories with demonstrations of some aspects of our experimental work and our staff is available and ready to answer any questions related to science. These "Open days" are also the opportunity to invite high-school students and university students to present them the research program of our Institute and the possibilities they have to work and study here. Popular science lectures that represent an integral part of our European Science Week activities are listed in previous paragraphs.

To attract gifted student for work at our institute means to come out of our laboratories and to approach the students before they decide about their future. As a part of this endeavour, two young scientists (A. Majerník and M. Valachovič) started a lecture series at high schools in Bratislava with the aim to publicize results of a scientific research and to present some aspects of our research to students. Lectures run since November 2006 till May 2007 with the following topics: Coexistence between man and microorganisms (A. Majerník, November 2006), Biology and chemistry in every day life (M. Valachovič), Life in extreme conditions (A. Majerník), Yeast. The Trojan horse (M. Valachovič), What can be found in our guts? (A. Majerník).

7. Background and management. Staffing policy and implementation of findings from previous assessments

i. Summary table of personnel

Personnel	2003	2004	2005	2006
all personnel	43	43	47	47
research employees from Tab. Research staff	23	26	30	30
FTE from Tab. Research staff	20.25	22.05	23.95	25.25
averaged age of research employees with university degree	47.7	46.2	44.4	42.8

ii. Professional qualification structure

Number of	2003	2004	2005	2006
DrSc.	3	3	3	3
PhD / CSc.	16	19	19	19
Prof.	-	1	1	1
Doc./Assoc. Prof.	3	2	2	3

iii. Status and development of research infrastructure incl. experimental, computing and technical base (description of the present infrastructure, premises, and material and technical resources. Infrastructure, instrumentation and major technical equipment necessary for the achievement of the objectives specified in the research Concept)

Experimental research represents the principal activity of the Institute of Animal Biochemistry and Genetics. To be competitive at the international level, modern experimental research requires state-of-the art instrumental infrastructure. As in many scientific institutes in Slovakia, building and renewal of research infrastructure has been neglected for years. The absence of modern instrumentation hinders us often from performing experiments which are needed for the full completion of our research projects. In many cases we were dependent on cooperation with other institutes and universities in Slovakia or abroad.

This situation began to improve during last few years. Thanks to increased funding, our laboratories have been equipped with small instrumentation and appliances. In addition we were able to obtain much needed modern instrumentation. Project "Biotechnology centre BITCET" supported the purchase of computer-controlled bioreactor Applicon, which has been adopted for use at the strict anaerobic conditions. Joined support from the Presidium of SAS and project BITCET enabled the purchase of the Agilent HPLC apparatus equipped with diode array detector and ESA CoronaCAD detector. Scientists from our institute could use other major instruments purchased through the project BITCET. Unfortunately, the location of these appliances at the institutes of SAS in Bratislava prevents from a more effective use of these so much needed experimental tools. International collaborations and grants supported purchase of less expensive but very important instrumentation: PCR machine, radiotelemetric system to monitor physiological parameters of free moving birds, portable centrifuge, and

electrophoresis apparatuses to name few. Institutional money from SAS and from the State research programs to buy scientific equipment is very limited and only few larger instruments were purchased recently.

The Institute's infrastructure includes other indispensable instruments that are frequently outdated and kept in the working condition only by an extreme effort from the servicing companies and the scientists themselves. These instruments include: ultracentrifuge, UV-VIS spectrophotometer, spectrofluorimeter, anaerobic box, scintillation counter, fluorescence microscope, gas chromatograph, large-volume centrifuges, luminometer, yeast tetrad dissection apparatus, incubators and particularly a large low-speed high-diameter centrifuge built for studies of the effects of increased gravitation on experimental animals. This centrifuge has been upgraded and rebuilt in last two years in a joint effort of our Institute with the Institute of Experimental Endocrinology SAS and Institute of Measurement SAS. After this upgrade, the centrifuge can operate in several computer-controlled modes at gravity ranging from 1G to 6G and blood samples can be withdrawn during the operation from rats. The centrifuge offers a worldwide unique possibility to study in rats the short-term and medium-term (hours to days) effects of hypergravity on various parameters ranging from hormonal status to gene expression.

Current computer infrastructure is rather outdated and problematic. Local area network is based on coaxial cabling with the speed of 10 Mbs, with one central file and print server and slow radio connection to Computing centre SAS in Bratislava. In the connection with moving the institute to the main academic campus in Bratislava, we plan to build new LAN with structured wiring with the speed 100/1000Mbs and direct connection to campus backbone.

To fulfil all objectives of the research Concept, the research infrastructure of our institute needs to be completed and modernized. In the course of the next 4-5 years we plan to purchase some major essential instruments. The most needed upgrades include equipment for:

- imaging techniques (fluorescence microscope, fluorescence-associated cell sorter, cryocut)
- analytical techniques (gas chromatograph with mass spectroscopy detector, completion of HPLC apparatus with fraction collector and microdialysis accessory)
- physiology research (inhalation anesthesia for birds and small laboratory animals, radiotelemetric system)
- general biochemistry and microbiology (ultracentrifuge and large-volume refrigerated centrifuge, anaerobic box, analytical balances)

- biophysical research (quartz microbalance QCM, apparatus for electrochemical measurements Autolab)

We plan to purchase the above mentioned instruments through the combination of resources: State research programs (APVV), Institutional funds, EU framework programs, European Structural Funds, and International collaborative grants. We are aware that only combination of resources and participation in international programs will allow us to modernize the Institute's research infrastructure to carry out the research at the internationally competitive level.

iv. Status and development of bibliographic resources, activities of the Organisation's library and/or information centre

Library employees:	1 full time librarian
Library equipment:	PC with internet connection and printer
Library collection:	5199 book units
Special collections:	285 book units
Acquisitions (2003-2006):	113
Cost of book/journal acquisitions (2003–2006):	123 000.- SKK
Subscribed periodicals:	2003 - 25 titles
	2004 - 25 titles
	2005 - 22 titles
	2006 - 23 titles
Library loans and services (2003 - 2006)	
Loans of books and journals:	1504
Interlibrary loans:	381
Bibliographic searches:	30

v. Describe how the results and suggestions of the previous assessment were taken into account

In the previous evaluation of the period 1999-2002, our Institute has been ranked in the highest grade "A". The Evaluation Committee stated that the results of our Institute have met international standards, the research program is balanced and the orientation on membrane-related processes opens new research possibilities.

The evaluation and recommendations and our response for individual areas are summarized below:

1. Scientific program and output:

Previous evaluation: Focus on membrane-related processes for the major part of research projects was evaluated as a positive step in the integration of research program. Improvement in quantitative parameters (increase in number of CC-indexed publications to 52) as well as in qualitative parameters (significant increase in the quality of publications reflected in the average IF of 1.293; increase of the average number of SCI-indexed citations to 4.69 per scientist per year) were noted. The only recommendation has been to publish scientific monographs in our traditional research areas (avian physiology, methanogens).

Response of the Institute: In the period 2003-2006 we achieved further improvement of quantitative and particularly qualitative characteristics of research output. The increase in the total number of CC-indexed publications (from 52 to 59) was accompanied by the significant improvement in the quality of papers (increase of journal average IF by 50% from 1.29 to 1.93) as well as in the number of citations (average number of WOS citations per scientist per year increased by almost 50 % from 4.69 to 6.85). Monograph summarizing the results in the area of avian physiology (obtained as a part of the space flight program of our Institute) is already in press and another monograph on biomembrane structure and functions is in preparation as a part of the PhD educational program Biomembranes.

2. Personal management, education, infrastructure:

Previous evaluation: Personal management has been evaluated positively with respect to the age and professional structure. Committee highly appreciated the quality of PhD program at the Institute as the best in the group of agricultural research institutes. Critical comments were addressed to relatively poorer research infrastructure (aged equipment, limited availability of new scientific literature) and the Commission recommended to integrate more women into the scientific management, e.g. in Scientific Board of the Institute.

Response of the Institute: The personal infrastructure has been changed in a positive way since we were able to employ the most talented graduates from our PhD

program. This policy resulted in a significant improvement of the average age of research scientists from 47.6 to 42.8 years. The return of 4 young scientists back from long-term stays in the UK and USA was another very positive aspect. These young scientists have been fully integrated into the management of science at our Institute: three of them are currently principal investigators of research projects and three (2 of them females) were elected to the Scientific Board. We succeeded in maintaining the high standards of our PhD program and extended our educational activities to universities and other institutes. Our Institute is coordinating a major European Social Fund project "Biomembranes" aimed at specialized professional education of PhD students and young scientists in the Bratislava region. The relative problematic situation with equipment improved as well: during past four years, all research groups significantly re-equipped their laboratories with smaller appliances and the Institute purchased two larger instruments (HPLC with two special detectors and a fermentor for special anaerobic cultivation of microorganisms). In addition, the Institute is participating in a large state program "Biotechnology Center BITCET" aimed at improvement of the infrastructure at university departments and scientific institutes with biotechnology-related programs. As participants of this project we have access to state-of-the-art equipment as gene sequencing and gene synthesis instruments, FPLC, LC-MS, plasma resonance instrument, etc.

3. Integration into the national and international research programs, funding:

Previous evaluation: The Evaluation Committee positively appraised the broad scientific cooperation with prominent scientific institutions all over the world. The funding from national sources was evaluated as appropriate, however, the Committee recommended to pay more attention to EU projects and generally to diversify more the sources for project funding.

Response of the Institute: The Institute has maintained all active collaborations from previous period and extended it in some new promising directions. With respect to international funding, we shifted the policy from a large number of short-term travel grants to larger projects (COST, FIRCA, Slovak-US Science and Technology Program) with higher funding supporting experimental work and infrastructure of our Institute. Despite our efforts we were not so successful in obtaining support from EU projects. In addition to our participation in one COST project, we were involved in the three applications for FP6 projects. These applications have not passed the final stage of the evaluation procedure.

With respect to national funding, past four years were characterized by a major shift from financing via smaller grants from VEGA grant agency to larger projects funded by Agency for Science and Research (APVV). During past four years we were principal investigators of 3 APVV projects and co-investigators in another one while the number of VEGA-supported projects has been maintained on the same level. As a result, total support from national sources almost tripled over past four years.

vi. Supplementary information and/or comments on management, research infrastructure, and trends in personnel development

In the period 2003-2006, the Institute did not undergo any major changes in the organization and management. Elections in 2006 confirmed the previous director as the only candidate. The management of the Institute (deputy director, scientific secretary, chair of the Scientific board) remained in the positions. Significant changes took place in the Scientific Board where 3 new elected members are below the age of 40 years. This change indicates that the young generation is prepared to take more responsibility for the performance of the Institute.

Generally, personal development was characterized by positive as well as negative changes during the past four years. The major negative change was the administrative reduction of the number of permanent positions from 47 to 44 that was justified by the Presidium by high number of researchers on long-term stays abroad. The situation was rather difficult when these young researchers started to come back from their stays. On one hand, this return of four promising young researchers during two years is definitely a positive change. On the other hand, it caused serious problems with covering their salaries and this positive development was associated with a significant reduction of the income of the whole staff. Despite these problems with salaries, we were able to offer positions at the Institute to three most talented PhD graduates during this evaluation period.

The major task with respect to the management of the Institute and the status of the infrastructure for the next 2-3 years will be the relocation of the Institute to the Academy Campus in Bratislava. The preparatory phase started already during the evaluated period. Although this process is and will be complicated, nevertheless, it is inevitable for the future of the Institute from several aspects. It will solve the complicated situation with the ownership of the properties in Ivanka. We believe that relocation will contribute to the better accessibility of the Institute for students (graduate, PhD) and in this way increase the number of motivated young scientist studying and working at the Institute. It will also

contribute to the better accessibility of the devices located at the collaborating institutions in Bratislava for our researchers. Although we were the members of several consortia aimed at improvement of the research infrastructure, commuting makes the routine use of devices available through them complicated. Central Campus will also provide better chances for informal contacts so necessary for potential collaborations with other academic institutions. And finally, relocation will also solve our current problems with the quality of the internet access. We are aware of the potential impact on the personal and organizational structure of the Institute. The process of relocation will definitely affect the scientific output within the following period of evaluation. However, we believe that the final result of this process will mean improvement of the infrastructure and that it will refresh the research potential of the Institute.

Other information relevant to the assessment

-