Annual Report **2004**

INTERNATIONAL INSTITUTE OF MOLECULAR
AND CELL BIOLOGY IN WARSAW

Director
Jacek Kuznicki

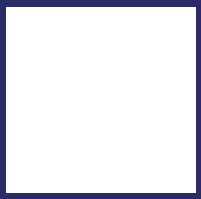
Deputy Director for scientific matters
Michal Witt

Deputy Director for administrative matters
Maria Kleska

Financial Manager
Hanna Iwaniukowicz

Chairman of the International Advisory Board
Angelo Azzi

Deputy Chairman of the International Advisory Board
Leszek Kaczmarek



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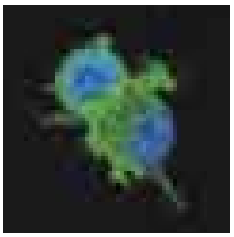
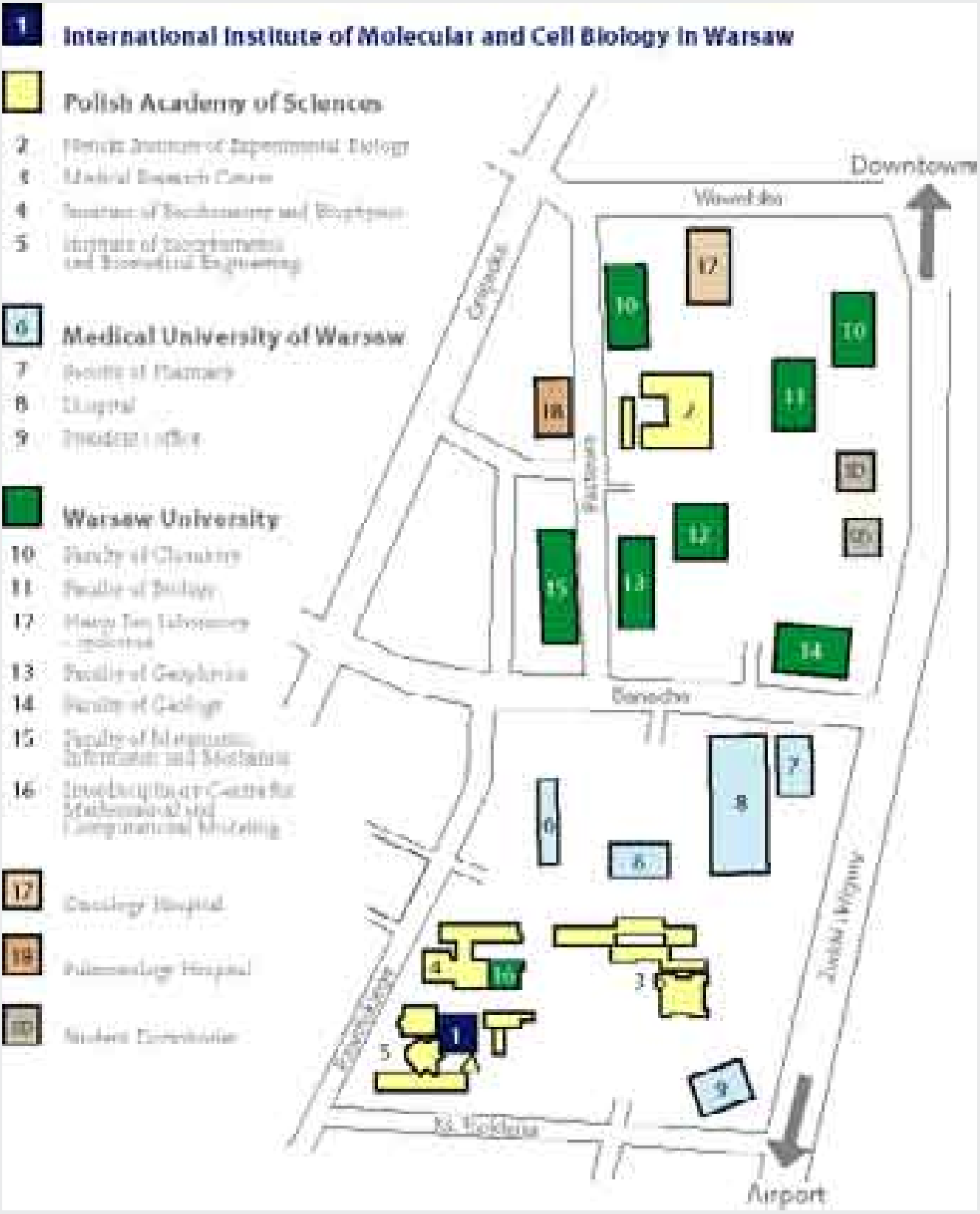
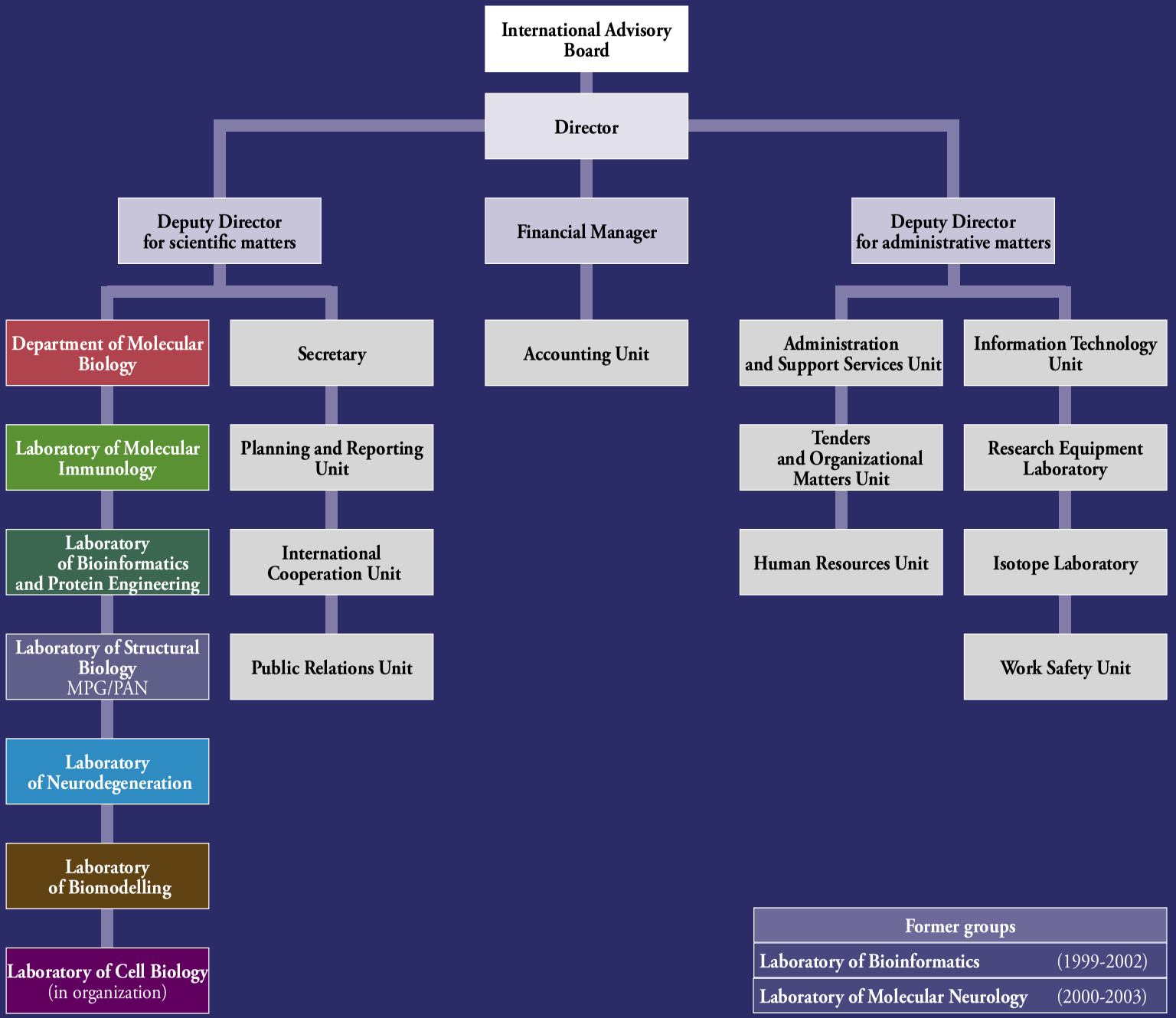


Map of the Ochota Campus



Structure of the International Institute of Molecular and Cell Biology in Warsaw



Former groups	
Laboratory of Bioinformatics	(1999-2002)
Laboratory of Molecular Neurology	(2000-2003)

Directors and Administration

Jacek Kuznicki
Director

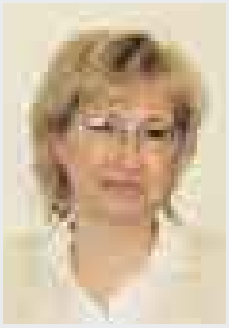
Beata Tkacz
Director's Assistant

Agnieszka Ziemka
Planning and Reporting Manager

Michal Witt
Deputy Director for scientific matters

Urszula Bialek-Wyrzykowska
Foreign Affairs Manager

Magdalena Glogowska
Foreign Affairs Manager



Maria Kleska
Deputy Director for administrative matters

Agnieszka Karbowska
Tenders Specialist

Krystyna Domanska
Human Resources Specialist

Hanna Iwaniukowicz
Financial Manager

Sylwia Adamiec
Accounting Specialist

Monika Nowicka
Payroll Specialist

Ewa Blazewicz
Secretarial Assistant

International Advisory Board of the International Institute of Molecular and Cell Biology in Warsaw

2002-2006 term

Chairman:
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Deputy Chairman:
Leszek Kaczmarek

Members:

Ken-ichi Arai

Director, Institute of Medical Science, University of Tokyo 4-6-1, Shiroganedai, Minato-ku, Tokyo 108, Japan

Angelo Azzi

Director, Institute of Biochemistry and Molecular Biology, University of Berne, Buhlstrasse 28, CH-3012 Berne, Switzerland

Alexey A. Bogdanov

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Rector Magnificus Utrecht University, Universiteitsweg 100, 3584 CG Utrecht, PO BOX 80125, 3508-TC, The Netherlands

Robert Huber

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Wieland Huttner

Scientific Member and Director, Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, D-01307 Dresden, Germany

Leszek Kaczmarek

Chairman, Division II Biological Sciences, Polish Academy of Sciences, PKiN, Pl. Defilad 1, 00-901 Warsaw, Poland; Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteur St, 02-093 Warsaw, Poland

Oleg A. Krishtal

Deputy Director, The Bogomoletz Institute of Physiology, Head of the Department of Cellular Membranology, Bogomoletz Institute of Physiology, Kiev, Ukraine

Andrzej B. Legocki

President, Polish Academy of Sciences, PKiN, Pl. Defilad 1, 00-901 Warsaw, Poland

Participants of the meeting of the International Advisory Board, June, 2004

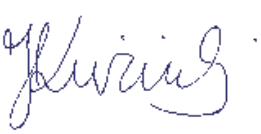
From left: Professors W. Huttner, M. Witt, R. Przewlocki, J. Mallet, A. Azzi, L. Kaczmarek, J. Kuznicki, M.Z. Ratajczak, S. Majewski, A.A. Bogdanov, M. Zylicz, M.J. Nalecz, W.H. Gispen

Important Dates in the Institute's History

Sept. 1991	The proposal to create the Institute was published in the UNESCO Bulletin of MCBN
June 1994	State Committee for Scientific Research (KBN) accepts the activities aimed at establishing the Institute
Oct. 1994	Presidium of Polish Academy of Sciences (PAN) votes to support the Institute
May 1995	An agreement between Poland and UNESCO to establish the Institute
June 1996	The Molecular and Cell Biology Department is created by PAN
June 1997	Polish Parliament passes a bill to found the Institute
May 1998	Prof. A. Azzi is nominated as the Director of IIMCB
Jan. 1999	The Institute commences its independent activities; Prof. J. Kuznicki appointed as Acting Director
July 1999	Dr. J. Dastych is appointed as Leader of the Laboratory of Molecular Immunology
Oct. 1999	Prof. M. Zylicz is appointed as Chair of the Department of Molecular Biology
April 2000	An agreement between the Max Planck Society (MPG) and the Polish Academy of Sciences (PAN) to launch a Joint MPG-PAN Junior Research Group
Nov. 2000	Dr. M. Bochtler is appointed as Leader of the Laboratory of Structural Biology (Joint MPG-PAN Junior Research Group), and Dr. M. Hetman as Leader of the Laboratory of Molecular Neurology
Dec. 2000	Dr. J. Rychlewski is appointed as Leader of the Laboratory of Bioinformatics
Jan. 2001	The MPG-PAN Junior Research Group commences its activities
June 2001	Prof. J. Kuznicki is elected by the International Advisory Board as Director of the Institute, begins to complete the Laboratory of Neurodegeneration. After consultation with UNESCO, the official nomination was signed by the President of PAN on February 1, 2002
Mar. 2002	Dr. J.M. Bujnicki is nominated as Acting Leader of the Laboratory of Bioinformatics and in June being appointed as Leader of the Laboratory of Bioinformatics
June 2002	Dr. S. Filipek is appointed as Leader of the Laboratory of Biomodelling
Nov. 2002	New members of the International Advisory Board nominated for 2002-2006 term
Jan. 2003	Status of the Centre of Excellence in Molecular Bio-Medicine is granted by the European Commission within 5th Framework Programme
June 2003	The 5th meeting of the International Advisory Board of IIMCB; First evaluation of two research groups
June 2004	The 6th meeting of the International Advisory Board of IIMCB

Directors' note

The next year, the next challenges, the next problems. Some of the laboratories have been dissolved, new ones have been created. It's sad to see old friends going, but on the other hand we stick to the strict rules of the Institute encouraging a constant flow of people. We believe that this is the right direction. We feel fortunate to recruit new brilliant people who start their activities here, although the recruitment process is never easy. Slowly but steadily the Institute is defining its scope towards, broadly speaking, protein research, with all the variations on this theme. This is broad enough to accommodate cell biology, and genetics, computer modelling, organic chemistry, biochemistry, biophysics and so on. We base our criteria on human resources and their qualities rather than strictly define the specializations we want. However, the new equipment purchased gives an added value to what we had before: definitely IIMCB has become one of the most modern Polish institutes with regard to the research equipment. As well as the quality of the faculty members, the technical basis we can offer is another component of critical mass reached after several years of fruitful activity of the Institute. We do run quite a number of international programs; one coordinated within 5FP has been successfully completed, within 6FP we have already three programs to go. The Institute's Center of Excellence is blooming with brilliant seminars of visitors and the hands-on experience of our students traveling abroad giving more international flavor to the everyday activities. Furthermore, to be more internationally communicative we have improved our web page where everyone can trace all the details of IIMCB's activities in real time. Please keep checking!



Description of the Institute's Activities

The Organization of Research at IIMCB

Generally the scope of research at the Institute is focused on basic biomedical problems with special emphasis on protein structure/function analysis. IIMCB's structure consists of six research groups: Department of Molecular Biology (Zylicz), Laboratory of Molecular Immunology (Dastych; activity discontinued on December 31, 2004), Laboratory of Bioinformatics and Protein Engineering (Bujnicki), Laboratory of Structural Biology Max Planck/PAN (Bochtler), Laboratory of Neurodegeneration (Kuznicki), Laboratory of Biomodelling (Filipek).

Among the major research topics are:

1. the role of molecular chaperones in cell transformation, especially Hsp90 and Hsp70 regulatory activity of p53 transcription; the characterization of novel human testes specific protein kinase (Zylicz's group)
2. novel technology for in vitro immunotoxicity testing ("cell-chip technology"); signaling pathways regulating the cytokine expression in mast cells (Dastych's group)
3. sequence-structure-function relationships of methyltransferases, nucleases and DNA repair proteins; development of tools and protocols for protein structure prediction; functional analysis of genes/proteins identified by bioinformatics (Bujnicki's group)
4. crystallographic structure-function studies of staphostatin-type inhibitors of cysteine peptidases, lysostaphin-type and other peptidoglycan hydrolases, enzymes of the ubiquitin-proteasome pathway (Bochtler's group)
5. gene/protein/cell-level studies of molecular mechanisms of neurodegenerative diseases pathogenesis; involvement of ubiquitination in pathomechanism of Alzheimer disease (Kuznicki's group)
6. modelling of rhodopsin oligomer/rhodopsin G protein (transducin) complex, of higher oligomers of rhodopsin molecules in native membranes and of presinilins PS1 and PS2 (Filipek's group)

Awards, Honors and Titles

- Membership of the Polish Academy of Sciences, Prof. J. Kuznicki, since Dec. 2004
- Professorship Award from Foundation for Polish Research (FNP) to Prof. J. Kuznicki, 2004-2006
- Habilitation degree to Dr. Slawomir Filipek
- Habilitation degree to Dr. Urszula Wojda
- Researchers from the Laboratory of Bioinformatics and Protein Engineering achieved very high scores in the 6th Community-Wide Experiment on the Critical Assessment of Techniques for Protein Structure Prediction (CASP-6). Two groups, „KOLINSKI-BUJNICKI" (a duumvirate of dr. Janusz Bujnicki and prof. Andrzej Kolinski from the University of Warsaw) and „GeneSilico" (a group of dr. Bujnicki's students) achieved the 2nd and 5th rank in the inofficial overall ranking (according to the fully automated assessment available at http://bioinformatics.buffalo.edu/casp6). According to expert assessors (http://predictioncenter.llnl.gov/casp6/meeting/presentations/talks.html), the KOLINSKI-BUJNICKI group was ranked on the 1st/2nd position (depending on the scoring system) in the New Folds category and the 3rd position in Comparative Modeling. The GeneSilico group was ranked as the 2nd in Fold-Recognition (Homology).
  This is a similar score to that achieved two years earlier in the previous edition of the experiment (CASP-5), where Bujnicki Janusz and GeneSilico achieved ranks 2nd and 5th in the inofficial overall ranking, respectively, with the 1st position in Comparative Modeling for Bujnicki-Janusz (http://csb.stanford.edu/levitt/CASP5_AutoAssessor/casp5_summary_v2.txt).

Education

has been effectuated throughout the doctoral program in partnership with other research and educational institutes of Ochota Campus (13 students), in collaboration with Utrecht University (eight students: five in Warsaw, two in Cracow, one

in Poznan), with the Postgraduate School of Molecular Medicine (three students), with the Foundation for Polish Science (two students); (see section „Educational Activities" p. 51).

The Media Visibility and Popularization of Science

In 2004 IIMCB researchers presented results of their research on Academic Internet Television Network and other TV programs. They gave numerous press interviews in leading Polish (Wprost, Polityka, Rzeczpospolita, Gazeta Wyborcza, Świat Nauki, Academia, Forum Akademickie) and foreign (The Scientist, German Research) newspapers and journals. Also our researchers were present in various radio broadcasts and Internet portals (www.onet.pl, www.esculap.pl, www.naukawpolsce.pl). Furthermore, they gave lectures at charity (for example Polish Children's Fund) and educational (UNESCO, EMBO Teacher Courses) organizations. IIMCB researchers attended the Warsaw Science Festival, which is the most popular and successful event bringing science to society in Poland. In it's laboratory at IIMCB Science Festival School (SFN) carried out 44 one-day workshops for secondary and high school students and four courses for Warsaw biology teachers. SFN organized also 21 lectures that attracted over 1000 listeners (see section „Popularization of Science" page 53).

Publishing NEWSKO

This electronic weekly bulletin gives Ochota Campus community current information on seminars, symposia, conferences, job opportunities and other essential events. NEWSKO which has been published at the Institute every Thursday for the last six years, integrates scientists, students and medical doctors at the Ochota Campus and plays a role as the communication platform for all Centres of Excellence at Ochota Campus.

Computer Network

The IIMCB computer network, managed by Rafal Flis and Andrzej Kociubinski, is implemented over a structured network of copper fifth category cable. Active elements of the network are: four optic fiber transceivers, seven 3Com/HP 24-port Ethernet 10/100 Mb/s switches and two HP 8-port Ethernet 10/100/1000 Mb/s switches. We are connected directly to several different Research Institutes in Ochota Campus through fiber optics. There are more than 100 workstations, notebooks, tablets and pads in the network protected by a local firewall operating under Windows NT/W2K/XP/CE, Linux, BSD, Solaris and Mac OS. We have twelve Institute servers (Intel based) used for e-mail, intranet, Internet, dns, dhcp, applications files, remote access, proxy, firewall, terminal services, multimedia and video streaming. These servers operate under Windows NT/W2K/2003, BSD and Linux.

Users can remotely access the local network through VPN from home or elsewhere. We have a connection to the Ochota Campus through the Gigabit Ethernet. Next year, we are planning to develop the connection to upgrade the local network to the Gigabit Ethernet bone. A plan for future purchases includes mass storage and multimedia for common usage of the Institute labs.

Visit of Prof. Frederico Mayor from Fundacion Cultura de Paz (Spain), June 2004

From left: Prof. J. Kuznicki, Prof L. Kuznicki, Prof F. Mayor, Prof. M. J. Nalecz, Prof. M. Witt

Activities of the Centre of Excellence in Molecular Bio-Medicine

The activities of the Centre of Excellence in Molecular Bio-Medicine focus on four objectives (I) improvement of research quality in biomedical sciences, (II) extension of the range and scope of education and training in the field, (III) promotion and popularisation of molecular medicine and human genetics as innovative and modern branches of basic and applicable research, and (IV) strengthening of the international position of IIMCB as a centre, where basic and applied research, as well as education and training, are carried out at the highest level. These objectives were taken into consideration while implementing the Centre's activities depicted in the workplan throughout all workpackages.

Workpackage 1

The second International Annual Symposium took place in the 18th month of the project. It consisted of an open research symposium and a closed meeting of International Advisory Board (IAB). Research symposium included lectures given by candidates for the new lab leader position at IIMCB (**Dr. E. Brown**, USA; **Dr. A. Majewska**, USA; **Dr. P. Stepien**, Poland and **Dr. S. Werten**, France) and of a summary session of doctoral fellows within the Utrecht University PhD program.

Workpackage 2

Within this workpackage, four major activities were planned. Within activity (3) **M. Olszewski** visited **Prof. E F. Knol**, at the Department of Dermatology and Alergology, University Medical Centre in Utrecht, The Netherlands and **M. Bucko-Justyna** visited **Prof. B. Burgering** at the Department of Physiological Chemistry and Centre for Biomedical Genetics, University Medical Centre in Utrecht, The Netherlands. **Prof. Edward F. Knol** visited IIMCB afterwards. Within activity (4) **Dr. Małgorzata Mossakowska**, co-ordinator of the Polish Centenarians Project, and **Dr. Katarzyna Broczek**, a geriatrician working for the project, visited **Prof. Claudio Franceschi**, affiliated with Italian National Research Centre on Ageing in Ancona and University of Bologna, Italy. They visited a number of research and medical centres specializing in the problems of elderly people. Then Prof. Franceschi came to IIMCB where he gave a lecture entitled "Genetics of longevity" and presented the principles of the 6th Framework Programme Project "Genetics of Healthy Ageing (GEHA)". Most importantly, Prof. Franceschi representing the Italian National Research Centre on Ageing in Ancona and Prof. Kuznicki representing IIMCB, agreed to formalize existing bilateral collaborations of scientists working in both institutions and thus to create an exchange program in the area of biomedical sciences.

Workpackage 3

Within this workpackage fourteen scientists from various European laboratories visited the Centre: **Prof. B. B. Burgering** (The Netherlands), **Prof. J. Damborsky** (Czech Republic), **Prof. H. Grosjean** (France), **Prof. R. Herrmann** (Germany), **Prof. D. Hibner** (France), **Prof. T. Hupp** (United Kingdom), **Prof. Jäättelä** (Denmark), **Prof. S. Klimasauskas** (Lithuania), **Prof. E. Knol** (The Netherlands), **Prof. M. R. Kreutz** (Germany), **Prof. G. Maravić** (Croatia), **Prof. G. Meiss** (Germany), **Prof. A. M. Pingoud** (France) and **Prof. J. Weiner III** (Germany). They all presented seminars for all scientists at the Centre and the Ochota Campus and led extensive scientific discussions with the researchers at the Centre. A number of scientific co-operations were initiated that are expected to result in common research projects and publications. These visits were particularly important for young scientists at the Centre who gained the opportunity to discuss their projects with experts in the field and to develop new ideas. **Dr. J. Dastych**, Head of the Laboratory of Molecular Immunology, visited the National Institute of Public Health and the Environment in Bilthoven, The Netherlands where he was hosted by Prof. H. Van Loveren.

Workpackage 4

Within this workpackage an intense exchange of students and post-docs between the Centre and established European laboratories took place. Two foreign guests worked at the Centre: **Prof. M. Kreutz** (Germany) and **D. Religa** (Sweden). Three Polish researchers from the Centre worked in various European laboratories: **J. Jolkowska** worked in the MRD Laboratory at the University Children Hospital in Tubingen, Ger-

many under the supervision of **Prof. P. Bader, L. Lipinski** worked in the Department of Physiological Chemistry and the Centre for Biomedical Genetics at the University Medical Centre in Utrecht, The Netherlands under the supervision of **Prof. B. Burgering**, and **E. Purta** worked at the Institute of Microbiology, ULB Brussels, Belgium under the supervision of **Prof. L. Droogmans**. Activities within this workpackage resulted in preparation of five scientific papers.

Workpackage 5

The conference **"Molecular Mechanisms of Neurodegeneration and Neuroprotection"**, a joint event organized by Prof. Jacek Kuznicki from the Centre and Prof. Bozena Kaminska from the Nencki Institute of Experimental Biology, took place in 2003 and was described in the First Annual Periodic Report.

Workpackage 6

The workshop **"Molecular basis of Alzheimer's disease"** was organized in cooperation with Polish Academy of Sciences Medical Research Centre. One of the main goals of the workshop was to present a current state of research on the biochemical and genetic basis of Alzheimer's disease and related neurodegenerative conditions. The other topic of the workshop was the ethical and philosophical aspect of molecular diagnosis of Alzheimer's disease and the philosophical foundation of modern genetics. Invited speakers: **Prof. Ulrich Finckh** (Germany), **Prof. Rob Layfield** (United Kingdom), **Prof. Marc Cruts** (Belgium), **Prof. Fred van Leeuwen** (The Netherlands), **Prof. Paweł P. Liberski** (Poland), and **Prof. Paweł Łuków** (Poland) presented lectures covering various aspects of Alzheimer's disease, frontotemporal dementia, prion disease and Parkinson's disease. Additionally seven lectures presented by Polish scientists gave an insight into research performed in Polish institutions.

Molecular Medicine Lecture Series: "Pneumology, Hematology and Psychiatry" included lectures by: **Prof. B. Andresen** (Denmark), **Prof. P. Bader** (Germany), **Prof. P. F. Pignatti** (Italy) and **Prof. C. Wijmenga** (The Netherlands).

Lecture Series "Enzyme structure and mechanism" hosted **Prof. T. Clausen** (Austria).

Workpackage 7

The second of the three Integrated Courses of the Postgraduate School of Molecular Medicine (SMM) planned within this workpackage: **"Advances in Molecular Medicine: Molecular therapy in clinical practice"** was a joint event organized by the Centre, SMM and the Wielkopolska Cancer Centre in Poznan. This event included lectures by invited guests: **Prof. H. Gaspar** (United Kingdom), **Prof. S. Gay** (Switzerland), **Prof. Dirk Schadendorf** (Germany), **Prof. D. Trono** (Switzerland), and **Prof. M. Wiznerowicz** (Switzerland). The course included lectures, laboratory workshops, and clinical presentations.

Workpackage 8

One of the main objectives of the Centre of Excellence project is a promotion of the International Institute of Molecular and Cell Biology as a leading research centre in molecular biomedicine, both domestically and internationally, and the popularization of science in the society. To meet the first objective, a Public Relation Unit is being organized and two IIMCB fellows **M. Głogowska** and **A. Ziemka** have taken postgraduate training in PR. The Centre is very active in bringing science to society; it organized five events within 8th Warsaw Science Festival and supported numerous activities of the Science Festival School. Other promotional activities included the workshop "Bridges in Life Sciences" and Prime Minister Marek Belka's visit to IIMCB. Additionally, two promotional publications were prepared and published in 2004: Annual Report 2003 and a promotional leaflet on IIMCB activities.

Scientific Meetings and Lectures

- 4th Integrated Course – Advances in Molecular Medicine: *Molecular therapy in clinical practice*, 29.03.-02.04.2004, Poznan, organized by: Postgraduate School of Molecular Medicine (SMM), Department of Cancer Immunology, Oncology Chair, University of Medical Sciences in Poznan, Wielkopolska Cancer Center in Poznan and IIMCB within the Centre of Excellence in Molecular Bio-Medicine project
- Seminar *Management tools for excellent science* 30.03.2004, organized by Polish Academy of Sciences, Max Planck Society and IIMCB
- Seminar *A novel platform for cell-specific molecular analysis,* 21.04.2004 co-organized by IIMCB, TK Biotech (Poland) and Arcturus (USA)
- Seminar of participants of KBN ordered research grant: *Genetic and Environmental Longevity Factors in a Group of Polish Centenarians,* 7-8.05.2004 (approx. 50 participants from 20 Polish institutes) organized by IIMCB
- Internal review session of the Institute, Mierki, Poland, 28.05.2004, organized by IIMCB
- *SMM Spring School Lecture Course on Human Genetics,* 3-4.06.2004, Warsaw, co-organized by IIMCB and SMM
- International Annual Symposium, 25.06.2004, Warsaw, Poland, IIMCB within the Centre of Excellence in Molecular Bio-Medicine project
- Workshop *Molecular basis of Alzheimer's disease,* 2-3.07.2004, Warsaw, Poland, organized by IIMCB within the Centre of Excellence in Molecular Bio-Medicine project and Medical Research Center PAN
- Workshop *Bridges in Life Sciences*, 06.10.2004, Warsaw, Poland; organized by IIMCB in cooperation with National Institutes of Health and Cedar Sinai Medical Center, USA
- Annual Scientific Report Session, 18-19.10.2004, Warsaw, organized by SMM, Medical University of Warsaw and IIMCB
- 6th Winter School *From gene to protein, from structure to function and dysfunction*, 29.11-3.12.2004, Warsaw, organized by SMM and IIMCB

Lectures by invited guests

- Edward Brown, (Department of Radiation Oncology, Massachusetts General Hospital, USA) *Can antitumor therapy be improved with multiphoton laser scanning microscopy?* 25.06.2004
- Anna Majewska, (Center for Learning and Memory, Massachusetts Institute of Technology, USA) *Imaging of synaptic structure in the visual cortex in vivo,* 25.06.2004
- Piotr Stepien, (Department of Genetics, University of Warsaw, Poland) *Human mitochondria: much more than just energy production,* 25.06.2004
- Maria Barcikowska (Medical Research Center, Warsaw, Poland) *Frontotemporal dementia: disease or syndrome?* 02.07.2004
- Pawel P. Liberski (Medical University of Lodz, Poland) *Molecular diagnosis of a prion disease,* 03.07.2004
- Pawel Lukow (Institute of Philosophy Warsaw University, Poland) *Informed consent and genetic information,* 03.07.2004
- A. Kowalska (Institute of Human Genetics, Poznan, Poland) *AD related mutations in Polish population,* 03.07.2004
- Peter Hedera (Vanderbilt University, USA) *Ethical principles and pitfalls of genetic testing for dementia,* 03.07.2004
- Zbigniew Zalewski (Jagellonian University, Krakow, Poland) *Philosophical aspects of genetic testing,* 03.07.2004
- Magdalena Gacia, (Medical Research Center, Warsaw, Poland) *PSEN1 promoter polymorphisms in Polish AD cohort,* 03.07.2004
- Jaroslaw Marszalek (Interdisciplinary Faculty of Biotechnology UG/AMG, Poland) *Specialized mitochondrial Hsp 70 system involved in Fe-S clusters biogenesis – functional interactions and evolution,* 09.12.2004
- Krzysztof Drabikowski, (Friedrich Miescher Institute, Basel, Switzerland) *Sumoylation in C. elegans, a global approach,* 23.12.2004
- V. Renugopalakrishnan (Harvard Medical School, Boston, USA) *Bionanotechnological applications of proteins – a bottom up approach,* 13.09.2004

Lectures within Centre of Excellence in Molecular Bio-Medicine project

- Peter Bader (University Children's Hospital Tübingen, Germany) *Chimerism and Minimal Residual Disease in Children transplanted for ALL,* 10.03.2004
- Brage S. Andresen (Unit for Molecular Medicine,Inst. of Human Genetics, Aarhus University, Denmark) *Missplicing at the root of phenotypic variation in human disease - Examples from the Acyl-CoA dehydrogenase genes,* 17.03.2004
- Boudewijn Burgering (Department of Physiological Chemistry University Medical Center Utrecht, The Netherlands) *Forkhead transcription factors and the control of cellular oxidative stress,* 02.04.2004
- Cisca Wijmenga (University Medical Center Utrecht, Complex Genetics Group Dept.of Biomedical Genetics, The Netherlands) *The hunt for celiac disease genes: a genomics approach,* 26.05.2004
- Saulius Klimasauskas (HHMI International Research Scholar, Institute of Biotechnology, Vilnius, Lithuania) *Mechanisms of DNA cytosine methylation,* 22.06.2004
- Sebastiaan Werten (Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France) *Structural biology of transcription cofactors,* 25.06.2004
- Ulrich Finckh (University Hospital Eppendorf, Institute of Human Genetics, Germany), *Genetic studies on Alzheimer's disease: current status and future prospects,* 02.07.2004
- Robert Layfield (University of Nottingham Medical School, Great Britain), *The role of ubiquitin-mediated proteolysis in neurodegenerative disorders,* 02.07.2004
- Fred van Leeuwen (Netherlands Institute for Brain Research, Amsterdam, The Netherlands), *Molecular misreading as a contributor to the neuropathogenesis of Alzheimer's disease,* 02.07.2004
- Marc Cruts (Department of Molecular Genetics VIB8 University of Antwerp, Belgium), *Mutation detection and characterization in neurodegenerative conditions,* 03.07.2004
- Tim Clausen (Research Institute of Molecular Pathology, Austria) *Structure and function of the DegS stress sensor,* 23.08.2004
- Henri Grosjean (Laboratoire d'Enzymologie et Biochimie Structurales, CNRS, Gif-sur-Yvette, France) *tRNA modification in the three Domains of Life,* 23.08.2004
- Gordana Maravic (Department of Biochemistry and Molecular Biology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia) *rRNA methylation and antibiotic resistance,* 27.08.2004
- Pier Franco Pignatti (Instituto di Biologia e Genetica, Universita di Verona, Italy) *Molecular biomedicine and the unraveling of complex phenotypes: the example of cardiovascular disease genetics and pharmacogenetics,* 6.09.2004
- January Weiner III (Division of Bioinformatics, The Westfalian Wilhelms University of Muenster, Germany) *Circular permutations and the domain-wise evolution of proteins,* 10.09.2004
- Jiri Damborsky (EMBO/HHMI Scientist Josef Loschmidt Professor of Chemistry National Centre for Biomolecular Research Masaryk University, Brno, Czech Republic) *Computer-assisted protein engineering of enzymes,* 24.09.2004
- Claudio Franceschi (University of Bologna, Department of Immunology INRCA, Ancona, Italy), *Genetics of longevity,* 01.10.2004
- Ted Hupp (University of Edinburgh, Cancer Research Centre, UK) *Control of gene expression by the tumour suppressor p53,* 10.11.2004
- Michael R. Kreutz (Department of Neurochemistry and Molecular Biology Leibnitz Institute for Neurobiology in Magdeburg, Germany) *The enigmas of synapto-dendritic Ca^{2+}-signaling,* 18.11.2004
- Alfred M. Pingoud (Institute of Biochemistry, Justus-Liebig-Universitaet, Giessen, Germany) *Structure, function and evolution of homing endonucleases,* 26.11.2004
- Gregor Meiss (Institute of Biochemistry, Justus-Liebig-Universitaet, Giessen, Germany) *Regulation and enzymology of apoptotic nucleases,* 26.11.2004
- Krzysztof Skowronek & Sebastian Pawlak, (Laboratory of Bioinformatics and Protein Engineering, IIMCB) *Prediction-validation approach for preliminary structural characterization of restriction endonucleases: case studies,* 26.11.2004
- Marcin Feder, (Laboratory of Bioinformatics and Protein Engineering, IIMCB) *A new, unusual family of PD-(D/E)XK nucleases,* 26.11.2004
- Marja Jäättelä (Institute of Cancer Biology of the Danish Cancer Society, Denmark) *Lysosomal control of programmed cell death,* 06.12.2004
- Edward F. Knol (University Medical Center, Department of Dermatology and Allergology, Utrecht, The Netherlands) *Local and systemic factors in atopic dermatitis: relation atopy and other allergic diseases,* 08.12.2004
- Rolf Herrmann (Institut fur Medizinische Physik und Biophysik Charite – Iniversitatsmedizin, Berlin, Germany) *Signal transduction from GPCRs to G proteins,* 10.12.2004

Lectures by IIMCB researchers

- Slawomir Filipek (Laboratory of Biomodelling, IIMCB) *Rhodopsin and G-protein-coupled receptors - activation, oligomerization and complexation,* 13.01.2004
- Jacek Kuznicki (Laboratory of Neurodegeneration, IIMCB) *Ubiquitination complexes, presenilins, and Alzheimer disease - lab projects,* 20.01.2004
- Maciej Zylicz (Department of Molecular Biology, IIMCB) *Molecular chaperones involvement in cell transformation,* 27.01.2004
- Michal Gajda (Laboratory of Bioinformatics and Protein Engineering, IIMCB) *Design of new protein fold with Rosetta,* 3.02.2004

- Sergej Odintsov (Laboratory of Structural Biology Max Planck/PAN, IIMCB) *Recombinant immunotoxins: protein engineering for cancer therapy,* 10.02.2004
- Lech Trzeciak (Departament of Molecular Biology, IIMCB) *Tssk3 - the Testis-Specific Protein Kinase 3-how far did we get?,* 24.02.2004
- Gang Zhao (post-doc from China, guest of Laboratory of Neurodegeneration, IIMCB) *LHRH-PE40 and Calmyrin Story,* 02.03.2004
- Michal Kurowski, (Laboratory of Bioinformatics and Protein Engineering, IIMCB) *Sequence and structure – based protein families classification. How similar is similar?,* 09.03.2004
- Krzysztof Skowronek (Laboratory of Bioinformatics and Protein Engineering, IIMCB) *The art of producing protein in Escherichia coli: a selection of tricks,* 16.03.2004
- Sebastian Pawlak (Laboratory of Bioinformatics and Protein Engineering, IIMCB) *Diversity of omes?,* 23.03.2004
- Andrzej Lewandowicz (Laboratory of Neurodegeneration, IIMCB) *The new transition state analogues of purine nucleoside phosphorylase - design and properties* / The work summary from Albert Einstein College of Medicine, New York, USA, 31.03.2004
- Urszula Wojda (Laboratory of Neurodegeneration, IIMCB) *Gene therapy: concepts, tools, problems,* 06.04.2004
- Aleksandra Szybinska, (Laboratory of Neurodegeneration, IIMCB) *Nanog, a pluripotency sustaining factor,* 20.04.2004
- Joanna Boros (Department of Molecular Biology, IIMCB) *Mcm1p-Fkh2p-Ndd1p complex – the story unwinds,* 27.04.2004
- Henryk Korza (Laboratory of Structural Biology Max Planck/PAN, IIMCB) *Polio – the history?,* 18.05.2004
- Izabela Sabala (Laboratory of Structural Biology Max Planck/PAN, IIMCB) *Peptidoglycan hydrolases: LytM & Co.,* 28.05.2004
- Michal Kurowski (Laboratory of Bioinformatics and Protein Engineering, IIMCB) *Bioinformatic methods for the analysis of human DNA repair enzymes,* 28.05.2004
- Jan Kosinski (Laboratory of Bioinformatics and Protein Engineering, IIMCB) *Molecular phylogenetics of DNA:m5c methyltransferases,* 28.05.2004
- Krystiana Krzysko (Laboratory of Biomodelling, IIMCB) *G protein activation by G-protein-coupled receptor dimers,* 28.05.2004
- Violetta Adamczewska (Laboratory of Immunology, IIMCB) *Mercuric ions activate calcineurin and upregulate NFAT- dependent IL-4 promoter activity in mast cells,* 28.05.2004
- Dominika Trzaska (Laboratory of Immunology, IIMCB) *Role of interleukin-4 3'UTR in the regulation of mRNA stability,* 28.05.2004
- Maciej Olszewski (Laboratory of Immunology, IIMCB) *Intracellular trafficking and storage of TNF-alpha in mast cell granules,* 28.05.2004
- Magda Gacia (Laboratory of Neurodegeneration, IIMCB) *Molecular characterization of Polish Alzheimer's disease population,* 28.05.2004
- Marta Bucko-Justyna (Department of Molecular Biology, IIMCB) *TSSK3 protein kinase and its interacting protein RUSC2 : new players in signal transduction?,* 28.05.2004
- Aleksandra Helwak (Department of Molecular Biology, IIMCB) *Interaction between molecular chaperones from the HSP 70 family and p53 tumor suppressor,* 28.05.2004
- Lukasz Bojarski (Laboratory of Neurodegeneration, IIMCB) *Cell membrane Presenilin 1 - seven or eight transmembrane domains?,* 28.05.2004
- Grzegorz Kudla (Department of Molecular Biology, IIMCB) *Evolution of Hsp70 and its role in p53 folding,* 28.05.2004
- Lukasz Bojarski (Laboratory of Neurodegeneration, IIMCB) *Beta-catenin - on the crossroads of skin tumors and Alzheimer's disease,* 01.06.2004
- Magdalena Gacia, (Laboratory of Neurodegeneration, IIMCB) *Would you like regular coffee or decaf? – a short review on caffeine properties,* 22.06.2004
- Urszula Wojda (Laboratory of Neurodegeneration, IIMCB) *Calcium binding proteins in Alzheimer's disease,* 02.07.2004
- Slawomir Filipek (Laboratory of Biomodelling, IIMCB) *Possible involvement of brain CacyBP/SIP & Sgt1 proteins in ubiquitination,* 02.07.2004
- Matthias Bochtler (Laboratory of Structural Biology Max Planck/PAN, IIMCB) *Standard mechanism protease inhibitors vs. staphopain B-staphostatin B complex,* 28.10.2004
- Maciej Olszewski (Laboratory of Immunology, IIMCB) *The sorting hat or how trafficking of TNF-alpha to mast cell granules is dependent on glycosylation,* 04.11.2004
- Adam Sobczak (Laboratory of Neurodegeneration, IIMCB) *Role of the c-Jun transcription factor in axonal regeneration,* 25.11.2004
- Grzegorz Kudla (Department of Molecular Biology, IIMCB) *Evolution and function of nucleotide usage in the mammalian Hsp70 family genes,* 16.12.2004

Grants

International

6th Framework Programme

- "Co-ordinated Internet-linked networks for promoting innovation, exchanging knowledge and encouraging good practice to enhance bioscience education in European schools"; J. Bryk (SFN) (at the final stage of the contract negotiations)
- "Genetic Testing in Europe - Network for test development harmonization, validation and standardization of services", (LSHB-CT-2004-512148), 30,000 EUR, 2005-2009; M. Witt
- "Abnormal proteins in the pathogenesis of neurodegenerative disorders" (LSHM-CT-2003-503330); 161,200 EUR; 2004-2006, and supplementary grant from KBN 457,000 PLN; J. Kuznicki
- "Mechanisms of transgene integration and expression in crop plant plastids: underpinning a technology for improving human health" (LSHG-CT-2003-503238); 164,160 EUR; 2004-2007, and supplementary grant from KBN 477,000 PLN; J.M. Bujnicki

5th Framework Programme

- "Continuing Education for European Biology Teachers" (QLG7-CT-2002-00573), Subcontract; 23,430 EUR, 2004; J. Bryk (SFN)
- "Centre of Excellence in Molecular Bio-Medicine" (QLK6-CT-2002-90363); 350,000 EUR and supplementary grant from KBN 996,000 PLN and coordination grant from KBN 30 000 PLN 2003-2005; J. Kuznicki
- "Exploiting the HSP70 chaperone machine for novel therapeutic strategies in human diseases and for the engineering of productive cellular biomolecular factories" (QLK3-CT-2000-00720); 64,776 EUR and supplementary grant from KBN 197,000 PLN, 2003-2004; M. Zylicz
- Novel non-antibiotic treatment of staphylococcal diseases (QLK2-CT-2002- 01250); 238,382 EUR and supplementary grant from KBN 776,000 PLN, 2002-2005; M. Bochtler

Other International Funds

- Grant NIH "Kinetoplastid SL RNA biogenesis", 20,000 USD annually, 2004-2008; J.M. Bujnicki
- Utrecht University fellowships for five PhD students (M. Witt's lab, IIMCB and Institute of Human Genetics, PAS, Poznan; M. Zylicz's lab, IIMCB; A. Lipkowski's lab, Center for Experimental and Clinical Medicine, PAN, Warsaw; L. Kaczmarek's lab, Nencki Institute, PAN, Warsaw; R. Przewlocki's lab, Institute of Pharmacology, PAN, Cracow); 10,000 EUR annually from 2004 to 2007
- Grant NIH "Discovering new human DNA repair genes by bioinformatics" (WSU03043), 44,040 USD, 2003- 2004; J.M. Bujnicki
- EMBO Young Investigator Program (Project No. 741) 26,000 USD and 50,000 PLN annually from 2002 to 2005; J.M. Bujnicki
- The Max Planck Society (MPG) – the Polish Academy of Sciences (PAN) MPI-CBG Junior Research Group Program; 240,000 DM annually from 2001 to 2006; M. Bochtler
- Utrecht University fellowships for three doctoral students (in M. Zylicz's and J. Dastych's labs at IIMCB, and one in R. Przewlocki's lab Cracow); 55,000 Hfl annually from 2000 to 2004
- HHMI mini-grant to foster collaboration "Bioinformatics-guided engineering of DNA methyltransferases", 15,000 USD; 2003-2004; J.M. Bujnicki with S. Klimasauskas (Vilnius, Lithuania)
- HHMI mini-grant to foster collaboration "Molecular causes underlying the partial folding of a microtubule-associated protein domain", 15,000 USD; 2003-2004; J.M. Bujnicki with J. Otlewski (Wroclaw, Poland)

Polish

Research Grants from the State Committee for Scientific Research

- KBN-Polonium "Etude comparitive d'ARN-methyltransferases de differents organismes: un modele pour l'evolution des systemes enzimatiques de modification des acides nucleiques", 2004-2005; J.M. Bujnicki
- Polish-German project (KBN-DAAD) "Protein-nucleic acid and protein-protein interactions in biomedically important enzymes involved in nucleic acid metabolism (DNA repair and degradation)", 2004-2005; J.M. Bujnicki
- Polish-Czech project "Protein engineering of dehalogenating biocatalysts", 2004-2005; J.M. Bujnicki)
- "Combating bacterial resistance against MLSb antibiotics by design of a novel type of inhibitors against Erm methyltransferases". (KBN-0611/P05/2004/27); 93,000 PLN; 2004-2006; M. Feder
- "Differences in action of stress-induced and constitutively synthesized Hsp70" (KBN-0408/P04/2004/27); 550,200 PLN; 2004-2007; M. Zylicz
- "Modelling of G Protein-Coupled Receptor and their interactions with drugs in case of opioid receptors" (KBN-0624/P05/2003/25); 120,000 PLN; 2003-2006; S. Filipek
- "Identification of specificity determinants of restriction endonucleases by bioinformatics and mutagenesis" (KBN-0344/P04/2003/24); 300,000 PLN; 2003-2006; J.M. Bujnicki
- "Application of bioinformatic tools to characterization of enzymes involved in DNA repair" (KBN-0503/P05/-2003/24); 30,800 PLN; 2003-2004; M. Kurowski
- "Hsp90 in Cancerogenesis" (KBN-0203/P04/2002/22); 462,000 PLN; 2002-2005; A. Wawrzynow
- "Calcium binding proteins interaction with presenilin 1 (PS1) in lymphocytes of Alzheimer's disease patients and healthy controls" (KBN-0436/P04/2001/20); 325,000 PLN; 2001-2004; J. Kuznicki

Ordered Grants from the State Committee for Scientific Research

- Ordered Grant from the State Committee for Scientific Research (KBN-K089/P04/2004) "New bioinformatic tools for proteomics and structural genomics"; 1,850,000 PLN; 2004-2007; J.M. Bujnicki
- Bilateral Polish-German Ordered Research Grant (KBN-K064/P05/2003) "The transduction of neuronal Ca^{2+}-signals via EF-hand Calcium-Binding Proteins Caldendrin and Calmyrin in Alzheimer's disease and psychotic disorders"; 955,400 PLN; 2003-2006; (Director: U. Wojda with cooperation with Dr. M.R. Kreutz, Department of Neurochemistry, Molecular Biology, Leibniz Institute for Neurobiology, Magdeburg, Germany)
- "Genetic and Environmental Longevity Factors in a Group of Polish Centenarians" (KBN-022/P05/1999); 1,500,000 PLN; 2001-2005; (Director: J. Kuznicki); 22 groups in Poland
- "Addiction: Neurobiological Basis, Mechanisms, Methods of Prophylaxis and Treatment" (KBN-033/P05/2000); 3,400,000 PLN; 2001-2005; (Director: R. Przewlocki)

Research Grants from the State Committee for Scientific Research co-ordinated by other institutions

- "Novel Vaccines against Campylobacter Jejuni" (KBN-6/P06K/04321); 250,000 PLN; 2001-2004; (coordinator: E.K. Jagusztyn-Krynicka, co-operator: J.M. Bujnicki), co-ordinated at the Biology Department of the University of Warsaw

Other Research Grants

- Research Grant from Foundation for Polish Science (SP 10/04) „Beta-catenin metabolism in health and disease"; 240,000 PLN; 2004-2006; (J. Kuznicki)

International Cooperation

With The Max Planck Society

Based on the agreement between the Max Planck Society (MPG) and the Polish Academy of Sciences (PAN), Dr. Matthias Bochtler has run a protein crystallography group in the IIMCB for more than four years. The equipment and running costs for the lab, including personnel, are provided by the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden (MPI-CBG). IIMCB covers local operational costs, maintenance and provides administrative support. The lab, which employs over ten people, has been active in the structural biology of peptidases, proteases and protein degradation. The group has been first to publish the structures of several new peptidase clans, and, in studies on the staphopain-staphostatin system, has discovered a novel cysteine peptidase inhibitor mechanism. Recently, the group has focused more on eukaryotic protein degradation. Close contacts been MPG and PAN/IIMCB were strengthened by the recruitment of Dr. Marta Miaczynska of Max Planck Institute of Molecular Cell Biology and Genetics in Dresden for a lab leader position at IIMCB: the Laboratory of Cell Biology led by her will start its activities in February 2005.

On 30 March a seminar "Management tools for excellent science" took place at IIMCB; this seminar coorganized by PAN, MPG and IIMCB gave an opportunity for Dr. Barbara Bludau (MPG Secretary General) and other MPG representatives' to visit IIMCB.

With the Utrecht University

Research collaboration program with Utrecht University was initiated by Prof. Willem Gispen, Rector of the Utrecht University. The main goal of this program was to foster Polish – Dutch exchange of scientific information and strengthening the research cooperation through bilateral visits of staff members and their students. Furthermore, eight Polish doctoral students received four-year fellowships to work in Poland on their doctoral thesis. In the spring of 2005 Marta Bucko, Maciej Olszewski and Katarzyna Starowicz will defend their thesis in Utrecht.

With Italian National Research Centre on Ageing (INRCA), Ancona

After few years of successful cooperation, existing collaborations of scientists between Italian National Research Centre on Ageing in Ancona and IIMCB were formalized. Prof. Claudio Franceschi representing INRCA and Prof. Jacek Kuznicki representing IIMCB have signed the agreement on creation of an exchange program in the area of biomedical sciences.

Foreign Visits to IIMCB:

1. 6-9.02.2004: Ms NG Teng Hwee and Mr Boon Swan Foo, Agency for Science, Technology and Research, Singapore
2. 17.02.2004: Prof Fotis Kafatos, European Molecular Biology Laboratory, Heidelberg, Germany
3. 23.03.2004 : Information trip for German Science Editors, Robert Bosch Stiftung delegation
4. 23.07.2004: Prof. Dame Julia Higgins (Foreign Secretary and Vice President) and Dr. Bernie Jones (Head of Science Policy Section), Royal Society of London
5. 29.07.2004: Ms Mona Ip (Second Secretary for Trade Matters), Canadian Embassy
6. 6.10.2004: delegation from National Institutes of Health and Cedar Sinai Medical Center, USA
7. 7.10.2004: delegation of German Parliamentarians
8. 18.10.2004: Prof. Bernard Pau (Scientific Director) and Patrick Gaudray (Deputy Director), Centre National De La Recherche Scientifique, France

IIMCB International Representation:

1. 19-24.04.2004: "Towards a New Partnership in Science and Technology", Polish Science Forum in Tokyo; Prof. Jacek Kuznicki
2. 21-23.05.2004: Networking workshop for UNESCO Life Sciences Research Centers in Eastern and Central Europe, Paris; Prof. Jacek Kuznicki, Prof. Michal Witt, Prof. Alicja Zylicz, Prof. Maciej Zylicz, Dr. Janusz Bujnicki, Dr. Jaroslaw Dastych, Magda Glogowska MSc
3. 30.06.2004: "Brain drain, brain gain: new challenges"; conference supported by the European Commission, Paris; Prof. Jacek Kuznicki
4. 14-18.11.2004: Conference on Biotechnology, Scientific Centre in Vienna/PAN; Prof. Jacek Kuznicki.

Infrastructure and Working Environment

The infrastructure of the Institute is fully adapted to the safety and biosafety regulations for chemistry and molecular biology laboratories. All laboratories have been equipped with modern apparatus in accordance with the highest international standards. In addition to the regular equipment in each wet laboratory, there are pieces of apparatus shared by all researchers. There are centrifuges and ultracentrifuges, sets of FPLC and HPLC systems, chromatography system ACTA, a real-time thermocycler, fluorescence microscopes, phosphoimagers, incubators and shakers for bacterial cultures, electroporators for transfections and transformations, freezers (-70°C). There are

One of our laboratories

also five cell culture labs fully equipped with incubators, laminar-flow hoods, and microscopes, three coldrooms, and two sets of water deionizing units. The isotope laboratory has been recently equipped (including a new scintillation counter) compliant with the Polish and EU law regulations. The Laboratory of Structural Biology, fully financed by the Max Planck Society, Max Planck Institute of Molecular Cell Biology and Genetics in Dresden, is furnished with the most modern research equipment including a high brilliance rotating anode generator (RU-H3RHB from MSC), Max-Flux confocal optical mirrors, a MAR345 low noise X-ray detector and a cryosystem.

Due to its uniqueness, this equipment serves members of the entire scientific community interested in protein crystallography analysis. Among new pieces of equipment recently acquired at IIMCB are: surface plasmon resonance measuring system Biacore 3000, confocal microscope, flow cytometer, CD spectropolarimeter, microplate reader.

The building is equipped with ventilation, air conditioning, smoke alarms and fire escapes according to current regulations. Offices and lecture halls are separated from the laboratory space. Three lecture halls allow for intensive seminar programs, without any restrictions concerning time schedules. The practical courses are organized in a separate laboratory that is an important element for comfort and work safety. Being part of a large building complex, IIMCB has access to the following: six lecture halls (from 20 to 300 people), an exhibition hall, a hotel, a cafeteria, as well as other facilities belonging to the neighboring research institutes of the Ochota Campus. The IIMCB facilities, as well as the whole campus complex, are fully accessible for the disabled. Medical, social and legal services are accessible to the entire staff on-site. A security guard system operates on the entire campus around the clock.

Surface plasmon resonance measuring system Biacore 3000

Department of Molecular Biology

staff

Head:
Maciej Zylicz, PhD, Professor

Associate Head:
Alicja Zylicz, PhD, Professor

Research Assistant:
Lech Trzeciak, MD, PhD (until 30th April 2004)
Marcin Klejman PhD

PhD students:
Joanna Boros, MSc; Marta Bucko-Justyna, MSc; Malgorzata Gutkowska, MSc; Aleksandra Helwak, MSc; Grzegorz Kudla, MSc; Leszek Lipinski, MSc; Dawid Walerych, MSc; Bartosz Wawrzynow, MSc

Secretary:
Grazyna Orleanska, MSc

Technician:
Wanda Gocal

Maciej Zylicz, PhD

Degrees

Professor, 1992

DSc.Habil. in molecular biology, Institute of Biochemistry and Biophysics, PAN, Warsaw, Poland 1986

PhD in biochemistry, Medical University of Gdansk, Poland 1979

MSc in physics and biology, University of Gdansk, 1977

Post-doctoral Training

1993-1994 Visiting Professor, University of Utah, Medical Center, Institute of Oncology, Salt Lake City, UT, USA

1982-1984 University of Utah, Department of Cellular, Viral and Molecular Biology, Salt Lake City, UT, USA

1979-1981 University of Gdansk, Department of Biochemistry, Gdansk

Professional Employment

since 1999 Head of Department of Molecular Biology, IIMCB

1994-1999 Head of Department of Molecular and Cellular Biology, Faculty of Biotechnology, University of Gdansk

1991-1994 Head of Department of Molecular Biology, University of Gdansk

1990-1993 Vice President, University of Gdansk

1988-1991 Associate Professor, Department of Molecular Biology, University of Gdansk

1981-1988 Assistant Professor, Department of Biochemistry, University of Gdansk

Other Professional Activities

2000-2004 Chair of Biology, Earth Sciences and Environmental Protection Commission of State Committee for Scientific Research

Membership in Scientific Societies, Organizations and Panels

- Member of EMBO
- Member of EMBO Council
- Member of Advisory Editorial Board of EMBO Journal and EMBO Reports and IUBM Life
- Polish delegate to EMBC (2001-2004)
- Polish delegate to Life Science Committee of ESF
- Member of the Selection Committee for EMBO YIP (2001-2003)
- Member of the Selection Committee for the special DFG programs
- Member of Polish Academy of Sciences
- Member of American Society of Biochemistry and Molecular Biology
- Member of Academia Europaea
- Member of the State Committee for Scientific Research (1997-2004)
- Member of Polish Academy of Arts and Sciences

Honors, Prizes, Awards

1. Prime Minister Award for Scientific Achievements, 2002
2. "L. Marchlewski" Award of Biochemistry and Biophysics Committee PAN, 2001
3. "Heweliusz" Prize for the Scientific Achievements, awarded by the President of Gdansk, 1993
4. Award of Foundation for Polish Science (FNP), 1999
5. Award of the Polish Biochemical Society for the best biochemistry work performed in Polish laboratories, 1996
6. Award of Ministry of Education, 1994
7. Award of Polish Academy of Sciences, 1990
8. Individual Award of Polish Academy of Sciences for Scientific Achievements, 1986

Doctorates

Liberek K, Skowyra D, Osipiuk J, Banecki B, Wojtkowiak D, Jakobkiewicz J, Puzewicz J, Barski P, King F.

DSc Performed in Department

Liberek K, Marszalek J, Konieczny I, Wawrzynow A, Banecki B.

Professor Titles Received:

Liberek K, Marszalek J, Konieczny I, Wawrzynow A.

Publications

77 publications in primary scientific journals including: two papers published in Cell, six in EMBO J., six in PNAS and 25 in J. Biol. Chem. These papers were cited more then 4,500 times with an average citation per paper of 60.

Selected publications since 2001

- Mycko PM, Cwiklinska H, Szymanski B, Kudla G, Kilianek L, Odyniec A, Brosnan CF, Selmaj KW (2004) Inducible heat shock protein 70 promotes myelin autoantigen presentation by HLA Class II. *J. Immun.*172: 202-213
- Kudla G, Helwak A, Lipinski L (2004) Gene conversion and GC-content evolution in mammalian Hsp70. *Molecular Biology and Evolution,* 21: 1438-1444
- Jassem J, Jassem E, Jakobkiewicz-Banecka J, Rzyman W, Badzio A, Dziadziuszko R, Kobierska-Gulinda G, Szymanowska A, Skrzypski M, Zylicz M (2004) P53 and K-ras mutations are frequent events in microscopically negative surgical margins from patients with non-small cell lung carcinoma. *Cancer,* 100: 1951-1960
- Dworakowska D, Jassem E, Jassem J, Peters B, Dziadziuszko R, Zylicz M, Jakobkiewicz-Banecka J, Kobierska-Gulida G, Szymanowska A, Skokowski J, Roessner A, Schneider-Stock R (2004) MDM2 gene amplification: a new independent factor of adverse prognosis in non-small cell lung cancer (NSCLC). *Lung Cancer* 43: 285-295
- Muller L, Schaupp A, Walerych D, Wegele H, Buchner J (2004) Hsp90 regulates the activity of wild type p53 under physiological and elevated temperatures. *J. Biol.Chem.,* 279: 48846-48854
- Walerych D, Kudla G, Gutkowska M, Wawrzynow B, Muller L, King FW, Helwak A, Boros J, Zylicz A, Zylicz M (2004) Hsp90 Chaperones Wild-type p53 Tumor Suppressor Protein. *J. Biol. Chem.,* 279: 48836-48845

- Jassem E, Niklinski J, Rosell R, Niklinska W, Jakobkiewicz J, Monzo M, Chyczewski L, Kobierska G, Skokowski J, Zylicz M, Jassem J (2001) Types and localisation of p53 gene mutations. A report on 332 non-small cell lung cancer patients. *Lung Cancer,* 34: 47-51
- Zylicz M, Wawrzynow A (2001) Insights into the function of Hsp70 chaperones. *IUBMB,* 51: 283-287
- King FW, Wawrzynow A, Hohfeld J, Zylicz M (2001) Co-chaperones Bag-1, Hop and Hsp40 regulate Hsc70 and Hsp90 interactions with wild-type or mutant p53. *EMBO J.* 20: 6297-6305
- Zylicz M, King FW, Wawrzynow A (2001) Hsp70 interactions with the p53 tumour suppressor protein. *EMBO J.* 20: 4634-4638
- Genevaux P, Wawrzynow A, Zylicz M, Georgopoulos C, Kelley WL (2001) DjlA is a Third DnaK Co-chaperone of Escherichia coli, and Dj1A-mediated Induction of Colanic Acid Capsule Requires Dj1A-DnaK Interaction. *J. Biol. Chem.* 276: 7906-7912
- Banecki B, Wawrzynow A, Puzewicz J, Georgopoulos C, Zylicz M (2001) Structure–function analysis of the zinc-binding region of the ClpX molecular chaperone. *J. Biol. Chem.* 276: 18843-18848
- Kaczanowski R, Trzeciak L, Kucharczyk K (2001) Multi-temperature single-stranded conformation polymorphism. *Electrophoresis* 22: 3539-3545.

Description of Current Research

The scientific objective of our department is focused mainly on the role of molecular chaperones in cell transformation.

The p53 tumor suppressor protein is a transcription factor (Figure 1) which regulates cellular response to stress, abnormal cell proliferation and DNA damage. More than 50% of human cancers are shown to have mutations within the p53 gene. In some cases mutations in the p53 gene are an early event leading to cell transformation, for example p53 mutations are frequent events in microscopically negative surgical margins from patients with no-small cell lung carcinoma. Moreover the inactivation of p53 function which leads to cell transformation occurs also during cytoplasmic sequestration of wild type p53 protein or overexpesion of MDM2, an E3 ligase required for ubiqiutination and subsequent degradation of p53. MDM2 gene amplification is a new independent factor of adverse prognosis in non-small cell lung cancer.

It has been known for years that mutant p53 tumor suppressor protein coimmunoprecipitates with members of the Hsp70 and Hsp90 families. Such interactions lead to the formation of a p53 multi-chaperone complex that is responsible for the stabilization and sequestration of p53 to the cytoplasm. Hsp90 inhibitors can partially disrupt these interactions, which results in the degradation of mutant p53. With the use of highly purified proteins, we identified intermediate reactions that lead to the assembly of molecular chaperone complex with wild-type or mutant p53 protein. The presence of Hsp90 in a complex with wild-type p53 inhibits binding of Hsp40 and Hsc70 to p53. The conformational mutant of p53, which possesses low affinity towards Hsp90, can form a stable multichaperone complex in which Hsp90 is bound to mutant p53 indirectly (mut p53-Hsp40-Hsc70-Hop-Hsp90). Several independent methods, such as plasmon resonance, immunoprecipitation, ELISA and cross-linking were used to demonstrate that Hsp90 directly, in the absence of any other co-chaperones, is associated with wild-type p53 but not with mutant p53 protein.

Wild-type p53 is a structurally unstable protein, which undergoes conformational changes at elevated temperatures. We proposed that during heat shock, cytoplasmic p53 possessing the wild-type sequence could temporarily adopt a mutant conformation, subsequently initiating the formation of a multichaperone complex that could partially stabilize wild-type p53. Results from a recently published paper by Wang and Chen (2003) support our hypothesis. Binding molecular chaperones to p53 inhibited the ability of MDM2 to promote p53 ubiquitination and degradation, resulting in the stabilization of both p53 and MDM2. The evidence for Hsp90 binding to mutant p53 is conclusive, whereas the exact nature of cellular interactions between Hsp90 and genotypically wild-type p53 possessing either wild-type or mutant conformation still remains to be elucidated.

In this year's report, we demonstrate that the chaperone activity of Hsp90 is required for wild-type p53 transcriptional activity. Specific Hsp90 inhibitors, geldanamycin and radicicol inhibit p53-dependent transcriptional activity by dissociation of p53 from its target DNA - promoter sequence sites. Results from a reconstituted in vitro system clearly show that Hsp90 positively regulates p53 DNA binding to a specific promoter sequence, moreover this Hsp90 activity is ATP dependent (Figure 2). At the same time presence of ATP can dissociate Hsp90-p53 complex. These results suggest that the influence of Hsp90 on p53 DNA binding cannot be explained by the passive protection of wild-type p53 conformation, caused by static association with Hsp90. There are at least two possibilities that could explain the mechanism for Hsp90's positive

regulation of p53 DNA binding to the promoter sequence at 37°C. First, Hsp90 inhibits p53 aggregation or catalyses the disaggregation of p53 protein at elevated temperatures. Such a mechanism was previously discovered for chaperones belonging to prokaryotic and eukaryotic Hsp70 families. Second, the Hsp90 association with wild-type p53 could induce the partial unfolding of p53. Following the dissociation of this Hsp90-p53 complex in the presence of ATP, p53 could spontaneously refold back into a wild-type conformation with high affinity to the p21 promoter sequence. A similar mechanism of molecular chaperone action was proposed in the case of Hsp100 involved in protein folding and proteolysis. Recent data from the Ted Hupp laboratory suggests that Hsp90 in the presence of MDM2 could indeed partially unfold p53.

Elevated temperature or overproduction of Hsp70 (Figure 3 and unpublished results) is shifting the equilibrium of p53 towards "non-active" conformation (Figure 4). Probably similar "non-active" conformation could be reached in the case of p53 – structural mutant. We propose that partial unfolding of p53 could be catalyzed by Hsp90, and after Hsp90 dissociation, the subsequent spontaneous refolding of p53 allows it to reach its native conformation. This Hsp90 chaperone activity, triggered by ATP hydrolysis, will not only increase p53 DNA binding to the promoter sequence but also allow MDM2 – dependent degradation. In addition, the retention of p53 in wild-type conformation by transient Hsp90 interactions would also inhibit the formation of a multichaperone-p53 complex, which prevents p53 from MDM2-dependent degradation and import to the nucleus. At stress conditions, depending on the ratio between free Hsp90 and Hsp70 chaperones, p53 will reach native or non-active conformation. Additionally post-translational modification of p53 and regulatory loops, p53-dependent transcription of Hsp70 and MDM2, will influence this dynamic homeostasis (Figure 4).

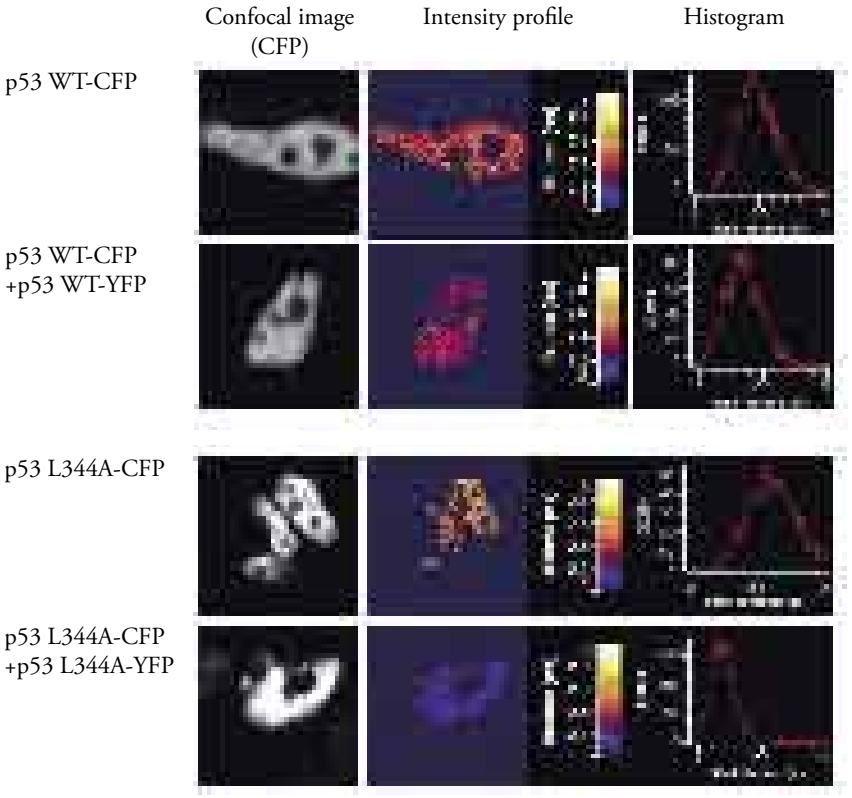


Figure 1 Formation of p53 heterodimers in HeLa cells.

FRET measurements using the time-gated fluorescence lifetime imaging method.

Cells were transfected either with CFP-p53 fusion proteins alone or co-transfected with CFP and YFP-p53 vectors. The L344A is a mutation in the tetramerization domain of p53 abolishing p53 tetramerization (Joanna Boros, unpublished results).

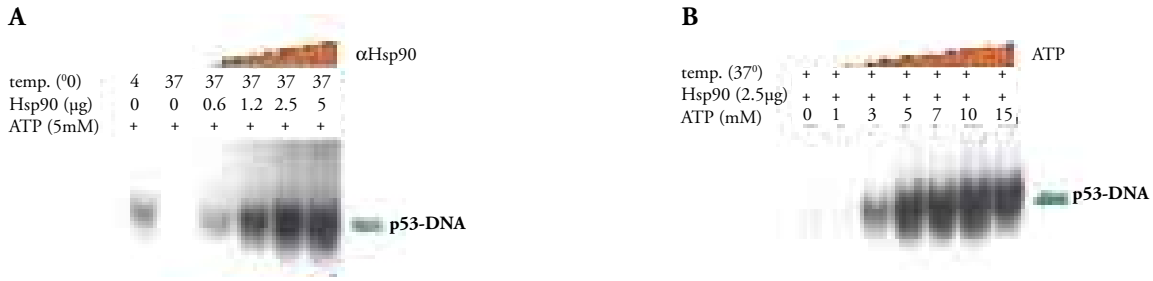


Figure 2 Hsp90α, in ATP-dependent reaction, chaperones p53 in binding to the p21 promoter sequence (Dawid Walerych et al., 2004).

The DNA binding activity of p53 was quantified by EMSA (gel-shift) assay.

A. p53 after pre-incubation for 1 h at 37°C is not able to bind to p21 promoter sequence unless Hsp90 is present during pre-incubation time.

B. this reaction is ATP-dependent.

Figure 3 Colocalization of Hsp70 with aggregated p53 in HeLa cells.

Cells were co-transfected with Hsp70 and p53 ts mutant. Presence of Hsp70 (read) and p53 (green) were detected by immuno-staining with appropriate antibody. Overproduction of Hsp70 causes aggregation of p53 at permissive temperature which leads to inactivation of transcriptional activity of p53 (Grzegorz Kudla, unpublished results).

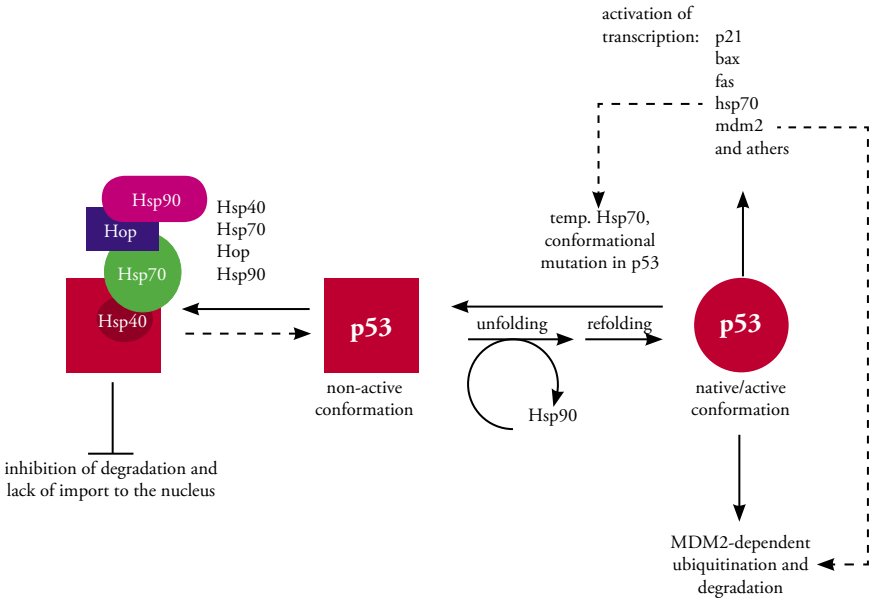
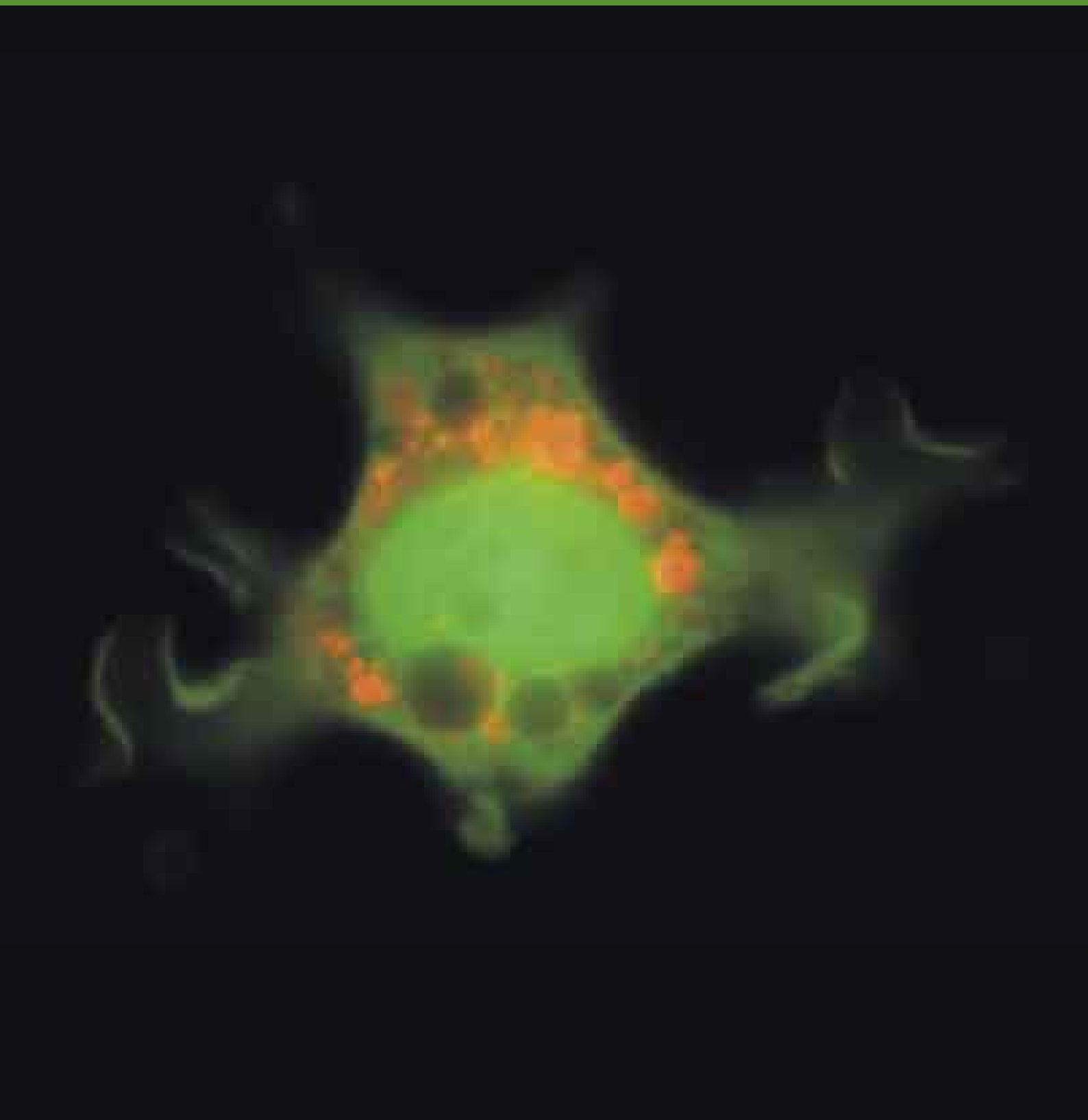


Figure 4

Hypothetical model for the role of molecular chaperones in maintaining the level of p53 in cells.

Laboratory of Molecular Immunology



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Undergraduate student:
Patrycja Zembek

Technician:
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Jaroslaw Dastych, PhD

Degrees

DSc.Habil. Medical University of Lodz, Poland, 2003

PhD in medical biology, Medical Academy, Lodz, Poland, 1991

MSc in molecular biology, University of Lodz, Poland, 1983

Post-doctoral Training

1992-1995 Laboratory of Allergic Diseases, National Institutes of Health, Bethesda, MD, USA

Professional Employment

1999-2004 Head, Laboratory of Molecular Immunology, IIMCB

1995-1998 Acting Head, Allergy Research Section, Department of Biogenic Amines Polish Academy of Sciences (PAN), Lodz, Poland

1992-1995 Senior Researcher, Allergy Research Section, Department of Biogenic Amines PAN

1985-1992 Assistant, Allergy Research Section, Department of Biogenic Amines PAN

1983-1985 Technician, Allergy Research Section, Department of Biogenic Amines PAN

Other Professional Activities

1998-1999 Visiting Scientist, Laboratory of Allergic Diseases, National Institutes of Health, Bethesda, MD, USA

Membership in Scientific Societies, Organizations and Panels:

- Polish Society for Experimental and Clinical Immunology
- American Academy of Asthma Allergy and Clinical Immunology

Honours, Prizes, Awards

- Fulbright Scholarship, 1989
- Fogarty International Fellowship, 1992-1995

Selected Publications since 1999

- *Ringerike T, Ulleras E, Volker R, Verlaan B, Eikeset A, Trzaska D, Adamczewska V, Olszewski M, Walczak-Drzewiecka A, Arkusz J, Van Loveren H, Nilsson G, Lovik M, Dastych J, Vandebriel R.J (2005) Detection of immunotoxicity using T-cell based cytokine reporter cell lines ("Cell Chip"). *Toxicology* 206(2): 257-272
- *Ulleras E, Trzaska D, Arkusz J, Ringerike T, Adamczewska V, Olszewski M, Wyczolkowska J, Walczak-Drzewiecka A, Al-Nedawi K, Nilsson G, Bialek-Wyrzykowska U, Stepnik M, Loveren HV, Vandebriel RJ, Lovik M, Rydzynski K, Dastych J (2005) Development of the "Cell Chip": a new in vitro alternative technique for immunotoxicity testing. *Toxicology* 206(6): 245-256
- *Walczak-Drzewiecka A, Wyczolkowska J, Dastych J (2003) Environmentally relevant metal and transition metal ions enhance FcεRI mediated mast cell activation. *Environ. Health Perspect.* 111, 5: 708-713
- *Taylor M, Dastych J, Sehgal D, Sundstrom M, Nilsson G, Akin C, Mage RG, Metcalfe DD (2001) The kit activating mutation D816V enhances stem cell factor-dependent chemotaxis. *Blood 98:* 1195-1199
- Dastych J, Wyczolkowska J, Metcalfe DD (2001) Characterization of α 5-integrin-dependent mast cell adhesion following FcεRI aggregation. *Int. Arch. Allergy Immunol.* 125: 152-159
- Wyczolkowska J, Weyer A, Dastych J (2000) Inhibitory effect of wheat germ agglutinin on mouse mast cell adhesion to fibronectin. *Int. Arch. Allergy Immunol.* 122: 216-223
- *Fukui M, Whittlesey K, Metcalfe DD, Dastych J (2000) Human mast cells express the hyaluronic-acid-binding isoform of CD44 and adhere to hyaluronic acid. *Clin. Immunol.* 94: 173-178
- Dastych J, Walczak-Drzewiecka A, Wyczolkowska J, Metcalfe DD (1999) Murine mast cells exposed to mercuric chloride release granule associated N-acetyl-β-D-hexosaminidase and secrete IL-4 and TNF-α. J. *Allergy Clin. Immunol.* 103: 1108-1114.

*Papers marked with an asterisk have the IIMCB affiliation of the authors

Description of Current Research

The research of the Laboratory of Molecular Immunology in 2004 focused on two aspects of cytokine expression in mast cells. We investigated the signaling events responsible for xenobiotic-mediated IL-4 expression and the intracellular trafficking of TNF-α protein.

Mercuric ions mediated IL-4 expression

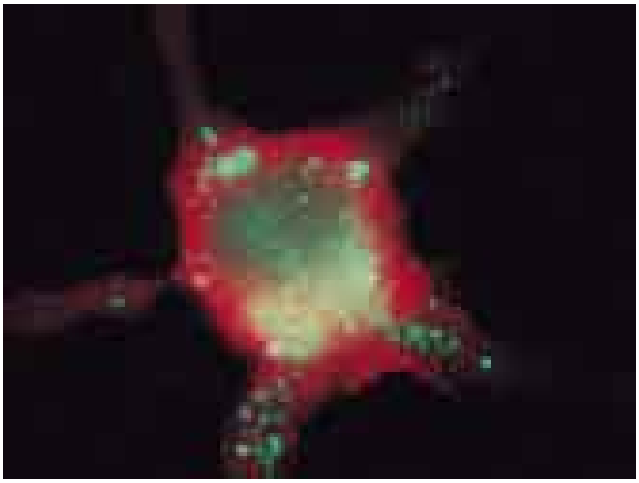
Cytokines including IL-4 are critical regulators that orchestrate the immune response by interconnecting dispersed elements of immune system into one functional entity. Cellular stress itself is frequently associated with changes in cytokine expression. Different damaging factors may induce expression of immunomodulatory cytokine genes resulting in immunomodulation. We observed that several metal and transition metal ions induced and enhanced allergen-mediated IL-4 expression. All these effects of metal and transition metal ions on mast cells were observed in concentrations, which might be relevant for the environmental exposure in air pollution.

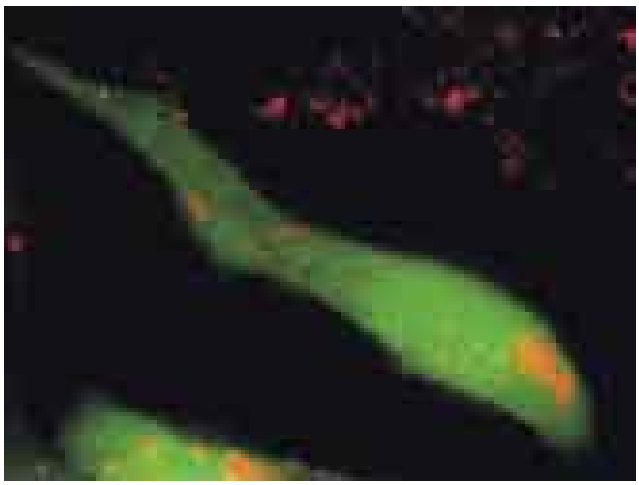
We investigated the role of calcineurin in signal transduction mechanisms regulating IL-4 expression in mast cells exposed to mercuric ions. We have gained new evidence for the critical role of this enzyme in upregulation of IL-4 by mercuric ions. In a series of experiments we investigated the effect of mercuric ions on IL-4 promoter activity in mast cells. $HgCl_2$ upregulated IL-4 promoter activity in mast cells in a process, which required NFAT binding site and was sensitive to calcineurin (CaN) inhibitors: Cyclosporin A (CsA) and FK506. Furthermore Hg^{2+} activated transcription driven by an artificial NFAT-dependent promoter containing three NFAT sites and increased cacineurin activity in vitro. These observations are consistent with the hypothesis that Hg^{2+} ions increase the activity of calcineurin that in turn upregulates NFAT, which binds to a specific DNA motif present in IL-4 promoter resulting in IL-4 expression. Thus, Hg^{2+} ions are able to activate a calcineurin/NFAT signaling pathway in immune cells. We have previously observed that another signaling pathway, namely JNK/c-Jun, is involved in mercuric-mediated IL-4 expression. Thus mercuric ions are capable of inducing the two signaling pathways in immune cells that control expression of several immunomodulatory cytokines, and this could be an important molecular mechanism mediating immunotoxic activities of mercuric compounds observed in vivo.

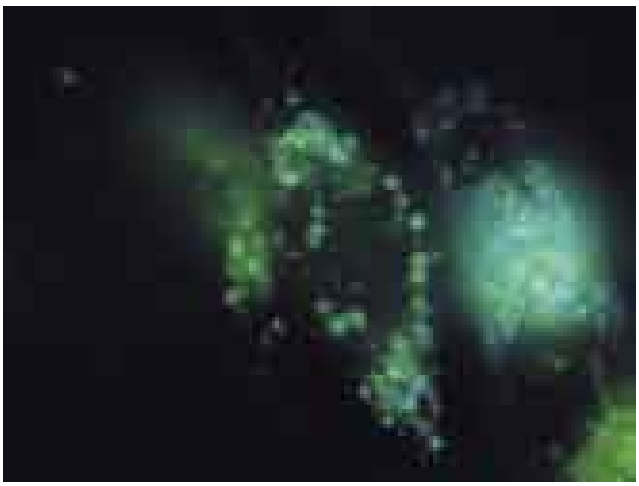
Intracellular trafficking of TNF-α protein

An important post-transcriptional mechanism engaged in TNF-α expression in mast cells is the intracellular trafficking of TNF-α protein leading to the storage of this cytokine in mast cell granules. This unique feature of mast cell TNF-α

allows for a very fast release of this cytokine upon mast cell contact with bacteria or parasite at the infection site. We have employed transient expression of TNF-α-EGFP fusion protein in mast cells to study the mechanisms underlying the storage of cytokine in cytoplasmic granules. To determine what pathway is utilized to direct TNF-α to cytoplasmic granules and what amino acid motifs are responsible for the sorting process we constructed a fusion protein covering the full sequence of TNF-α, N-terminally fused to EGFP. The transfection of mast cells with DNA constructs coding for the TNF-α-EGFP fusion protein resulted in the apparent granular pattern of fluorescence, which co-localized with several granule markers. These observations suggest that TNF-α-EGFP fusion protein was efficiently sorted to secretory granules. This sorting process was inhibited by both brefeldin A and monensin. Considering the relationship between lysosomes and secretory granules and following TNF-α sequence analysis it was determined whether TNF-α is sorted through a mannose-6-phosphate receptor dependent pathway. It was observed that ammonium chloride and tunicamycin blocked TNF-α -EGFP fusion protein delivery to secretory granules. In situ mutagenesis experiments have confirmed the necessity of N-linked glycosylation for the efficient sorting of TNF-α into rodent mast cell granules. Thus we have evidence that TNF-α travels from ER to mast cell granules via brefeldin A and monensin sensitive route and utilizing the cation-dependent MPR pathway







From top to bottom:

RBL-2H3 rat mast cells were transfected with pEGFP-F plasmid encoding for farnesylated variant of EGFP and following overnight incubation counterstained with DAPI. Cells were imaged on Leica DM IRE2 microscope equipped with TCS SP2 AOBS confocal scanhead at IIMCB.

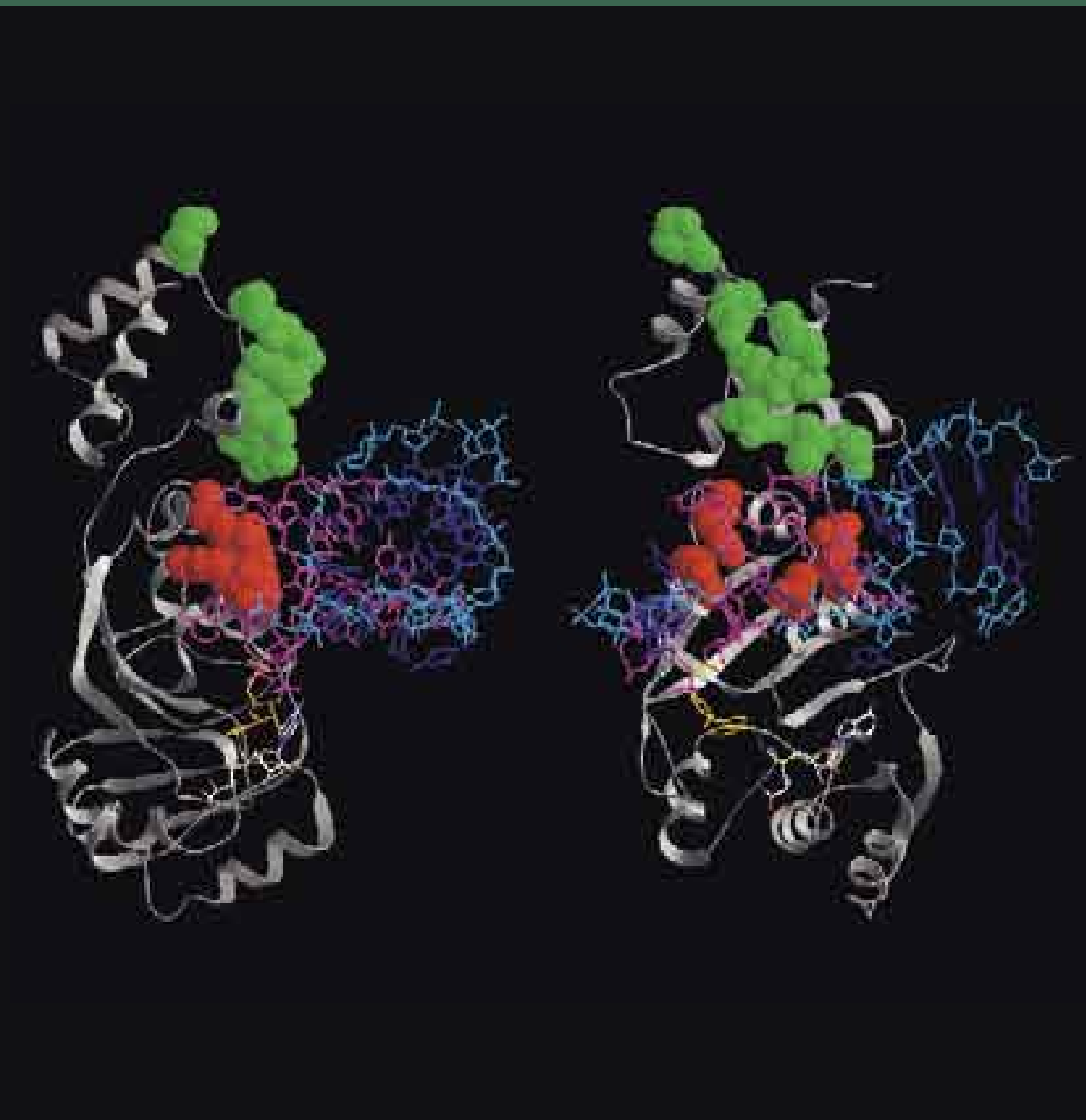
RBL-2H3 cells were incubated with Texas Red-Dextran conjugate overnight and the following day transfected with pEGFP-N1 plasmid. Cells were imaged on Olympus IX70 inverted microscope equipped with FView-II cooled CCD camera.

RBL-2H3 cell, transfected with cytokine-GFP fusion, fixed and immunofluorescently stained against RMCP-2 (TRITC).

RBL-2H3 cells were cotransfected with plasmids encoding for fusion proteins TNF-ECFP and TNF-EYFP. Following transfection cells were imaged on Olympus IX70 inverted microscope equipped with FView-II cooled CCD camera. Secretory granules are visualized and white colour reveals areas where ECFP and EYFP fluorescence overlaps.

Author: Maciej Olszewski, MSc

Laboratory of Bioinformatics and Protein Engineering



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Michal Rajkowski, BSc

Janusz Bujnicki, PhD

Degrees

2005 DSc.Habil Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw

2001 PhD in bioinformatics; University of Warsaw, Faculty of Biology

1998 MSc in microbiology; University of Warsaw, Faculty of Biology

Professional Experience

since 2002 Head of the Laboratory of Bioinformatics and Protein Engineering IIMCB

2001-2002 Group Leader, Molecular Evolution Research Group, Laboratory of Bioinformatics, IIMCB

2001 Visiting Scientist, Computational Biology Branch, National Center for Biotechnology Information, NLM, NIH, Bethesda, MD, USA (with Dr. E.V. Koonin)

1999-2000 Research Scientist, Bioinformatics Laboratory, IIMCB (with Dr. L. Rychlewski)

1998-2000 Senior Research Assistant, Molecular Biology Research Program, Henry Ford Health System, 1 Ford Place 5D, Detroit, MI, USA (with Dr. L.C. Lutter)

Awards

2002 EMBO/Howard Hughes Medical Institute Young Investigator Program award 2003, Fellowship for Young Scientists of the Foundation for Polish Science

2002 Award of the Polish Society of Genetics (the best Polish genetics-related publication in the year 2001: Trends Biochem Sci. 2001 Jan; 26(1): 9-11)

2001 Award of the Polish Biochemical Society (the best Polish publication on nucleic acid biochemistry in the year 2000: FASEB J. 2000 Nov; 14(14): 2365-2368)

Papers published by the Bujnicki group in 2004

- Armengaud J, Urbonavicius J, Fernandez B, Chaussinand G, Bujnicki JM, Grosjean H (2004) N2-methylation of guanosine at position 10 in tRNA is catalyzed by a THUMP domain containing, AdoMet-dependent methyltransferase, conserved in Archaea and Eukaryota. *J. Biol. Chem.* 279: 37142-37152
- Bujnicki JM, Feder M, Ayres CL, Redman KL (2004) Sequence-structure-function studies of tRNA:m5C methyltransferase Trm4p and its relationship to DNA:m5C and RNA:m5U methyltransferases. *Nucleic Acids Res.* 30; 32(8):2453-2463
- Bujnicki JM, Oudjama Y,Roovers M, Owczarek S,Caillet J, Droogmans L (2004) Identification of a bifunctional enzyme MnmC involved in the biosynthesis of a hypermodified uridine in the wobble position of tRNA. *RNA* Aug;10(8):1236-42
- Koudan EV, Bujnicki JM, Gromova ES (2004) Homology modeling of the CG-specific DNA methyltransferase SssI and its complexes with DNA and AdoHcy. *Biomol. Struct. Dyn.* Dec;22(3):339-45
- Maravic G, Bujnicki JM, Flögel M (2004) Mutational analysis of basic residues in the N-terminus of the rRNA:m6A methyltransferase ErmC'. *Folia Microbiol.* 49(1): 3-7
- Nes WD, Jayasimha P, Zhou W, Kanagasabai R, Jin C, Jaradat TT, Shaw RW, Bujnicki JM (2004) Sterol methyltransferase: functional analysis of highly conserved residues by site-directed mutagenesis. *Biochemistry* 20;43(2):569-576
- Paziewska A, Wyrwicz L.S, Bujnicki J.M, Bomsztyk K, Ostrowski J (2004) Cooperative binding of the hnRNP K three KH domains to mRNA targets *FEBS Lett.* 577: 134-140
- Potluri S, Khan AA, Kuzminykh A, Bujnicki JM, Friedman AM, Bailey-Kellogg C (2004) Geometric analysis of cross-linkability for protein fold discrimination. *Pac. Symp. Biocomput.* 447-458
- Roovers M, Wouters J, Bujnicki JM, Tricot C, Stalon V, Grosjean H, Droogmans L (2004) A primordial RNA modification enzyme: the case of tRNA:m1A methyltransferase. *Nucleic Acids Res.* 22; 32(2):465-476
- Saravanan M, Bujnicki JM, Cymerman I.A, Rao D.N, Nagaraja V (2004) Type II restriction endonuclease R.KpnI is a member of the HNH nuclease superfamily. *Nucleic Acids Res.* Vol. 32, No. 20:6129–6135
- Sasin J, Bujnicki J.M (2004) COLORADO3D, a web server for the visual analysis of protein structures. *Nucleic Acids Res.* 32: 586-589
- Schafer P, Scholz SR, Gimadutdinow O, Cymerman IA, Bujnicki JM, Ruiz-Carrillo A, Pingoud A, Meiss G (2004) Structural and functional characterization of mitochondrial EndoG, a sugar non-specific nuclease which plays an important role during apoptosis. *J Mol Biol.* 23; 338(2):217-28
- Ye X, O'Neil P.K, Foster A. N, Gajda M.J, Kosinski J, Kurowski M.A, Bujnicki JM, Friedman A.M, Bailey-Kellogg C (2004) Probabilistic cross-link analysis and experiment planning for high-throughput elucidation of protein structure. *Protein Sci.* 13: 3298-3312.
- Book: "Practical Bioinformatics". Vol. 15 in Nucleic Acids and Molecular Biology series; Editor: Bujnicki JM, Springer-Verlag 2004; ISBN: 3-540-20613-2

Current Research

The research of the Laboratory of Bioinformatics and Protein Engineering is focused on bioinformatics, biochemistry, and evolution of protein-nucleic acid interactions. Of particular interest are the sequence-structure-function relationships in enzymes that catalyze covalent modifications or cleavage of nucleic acids, such as methyltransferases, nucleases and proteins involved in DNA repair. We are also involved in the development and applications of software tools for the analysis of data concerning protein sequence-structure-function relationships. Our preferred modus operandi is to integrate various types of experimental data (generated in-house or by collaborators) and theoretical predictions to infer the structure and function of proteins.

From the theoretical end, our current focus is on the development of tools and protocols for purely theoretical prediction of protein structures (see for instance our protein structure prediction "meta server" at (http://genesilico.pl/meta/) as well as for determination of protein structures based on heterogeneous, low-resolution, noisy and ambivalent experimental data. We are also involved in genome-scale phylogenetic analyses, with the focus on identification of genes/proteins, which belong to particular families. Structural and evolutionary predictions obtained from bioinformatics analyses are then combined to infer the protein function.

From the experimental end, the goal is to characterize the function of new genes/proteins identified by bioinformatics and to use the theoretical prediction to guide protein engineering, using rational and random approaches, as well as the combination of both. The ultimate goal is to design proteins with new properties, in particular enzymes with new desired functions, which have not been observed in nature.

Projects

The development of new software tools for structural genomics and proteomics – in collaboration with Prof. Andrzej Kolinski (University of Warsaw) and Dr. Matthias Botchler (IIMCB); funded by KBN, also in collaboration with Prof. David Baker (University of Washington, Seattle, WA, USA, funded by NIH).

The development of software tools for the inference of phylogenies using protein structures and sequences; funded by EMBO & HHMI.The discovery of novel human DNA repair enzymes using bioinformatics and their biochemical characterization – in collaboration with Prof. Ashok Bhagwat (University of Michigan, Detroit, MI, USA); funded by NIH.

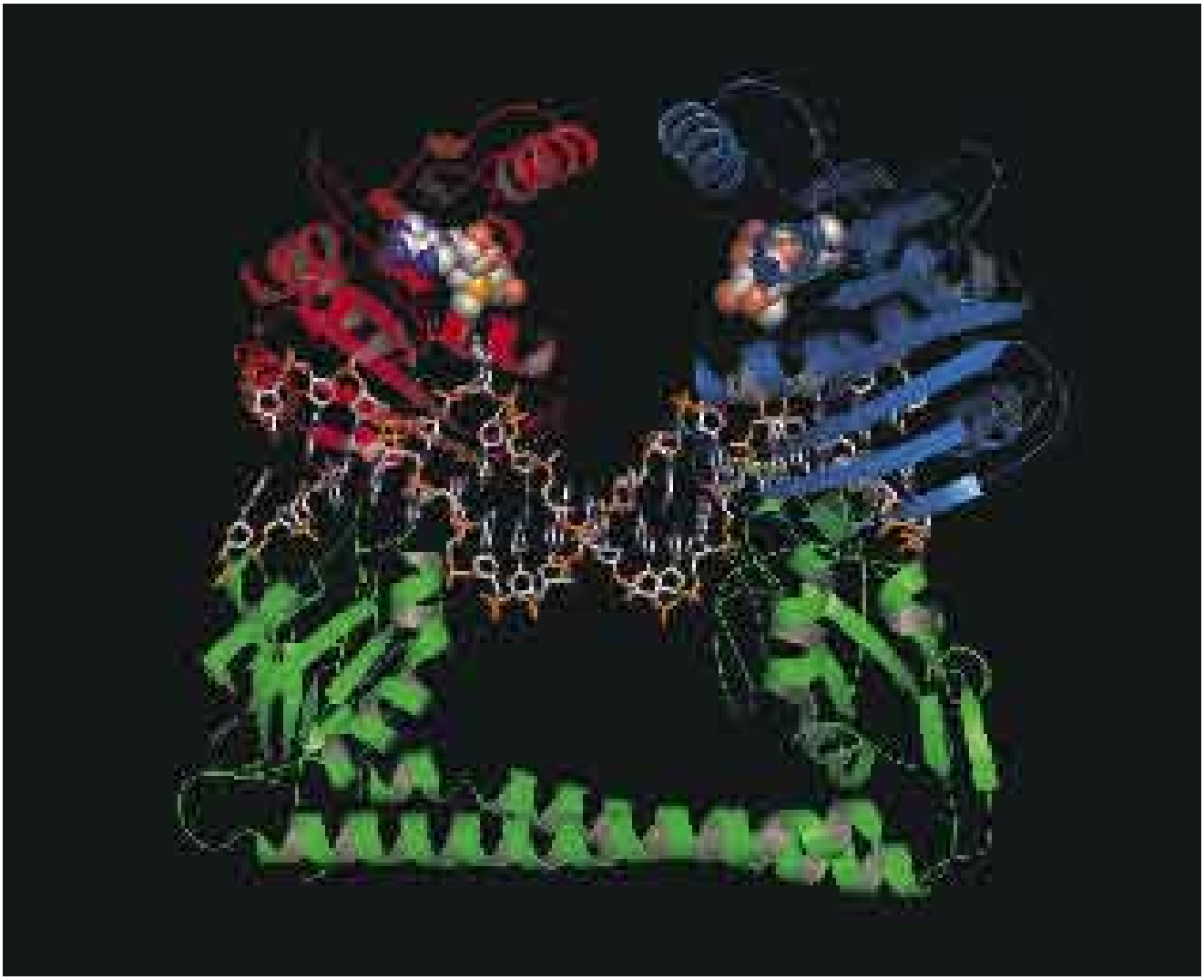
The characterization of protein-DNA recognition in restriction enzymes; in-house research funded by KBN, also in collaboration with Prof. Alfred Pingoud (Justus-Liebig Universitaet, Giessen, Germany, collaboration funded by DAAD).

The evolution of structure-function relationships in RNA MTases (funded by EMBO & HHMI). Key collaborators: Dr. Bruno Lapeyre (CNRS, Montpellier, France), Dr. Henri Grosjean (CNRS, Gif-sur-Yvette, France), Dr. Louis Droogmans (University of Bruxelles, Belgium), Dr. Gordana Maravic (University of Zagreb)

The bioinformatics-guided engineering of DNA methyltransferases with new properties – in collaboration with Dr. Monika Radlinska (Warsaw University); funded by EMBO & HHMI; also in collaboration with Dr. Saulius Klimasauskas (Institute of Biotechnology, Vilnius, Lithuania, collaboration funded by HHMI).

The bioinformatics-guided engineering of protein stability – in collaboration with Prof. Jacek Otlewski (Wroclaw University; collaboration funded by HHMI).

The classification and evolution of S-adenosylmethionine-dependent methyltransferases – in collaboration with Drs. Eugene Koonin and L. Aravind (NCBI, NIH, Bethesda, MD USA).



Model of a type I restriction-modification enzyme, complex of HsdS and HsdM subunits with S-adenosyl-L-methionine and substrate DNA (J.M.Bujnicki and A.Obarska, unpublished data). Graphics - courtesy of J.Kosinski.

Laboratory of Structural Biology MPG/PAN

[Joint Max Planck Society/Polish Academy of Science (MPG/PAN) Junior Research Group]

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The equipment and running costs for the lab, including personnel, are provided by the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden (MPI-CBG).

Matthias Bochtler, PhD

Degrees

PhD in biochemistry, Technische Universitaet München, Germany, 1999

MSc in experimental physics, Ludwig Maximilians-Universitaet München, Germany, 1995

Research Training

1999-2000 the Max Planck Institut für Biochemie, Martinsried, Germany

1996-1999 Research Assistant, MPI für Biochemie, Martinsried, Germany

1995-1996 internship, the Department of Medical Microbiology, University of Regensburg, Germany

1992-1993 guest student, Cambridge University, United Kingdom

1990-1992 studies in physics, Munich University, Germany

Honors, Prizes, Awards

EMBO/HHMI Young Investigator award, 2004

Crystal award, Germany, 2000

Crystal award, Germany, 1998

Scholarship from Deutsche Studienstiftung and the Bavarian State, 1990-1992

Selected Publications since 2003

- Groll M, Bochtler M, Brandtstetter H, Clausen T, Huber R (2005) Molecular machines for protein degradation. *Chembiochem.* 6(2):222-56
- Bochtler M, Odinstov SG, Marcyjaniak M, Sabala I (2004) Similar active sites in lysostaphins and D-Ala-D-Ala metallopeptitades. *Protein Science* 13: 854-861
- Marcyjaniak M, Odintsov SG, Sabala I, Bochtler M (2004) Peptidoglycan amidase MepA is a LAS metallopeptidase. *J. Biol. Chem.* 279(42): 43982-43989
- Filipek R, Szczepanowski R, Sabat A, Potempa J, Bochtler M (2004) Prostaphopain B Structure: A Comparison of Proregion-Mediated and Staphostatin-Mediated Protease Inhibition. *Biochemistry* 43(44): 14306-14315
- Odinstov SG, Sabala I, Marcyjaniak M, Bochtler M (2004) Latent LytM at 1.3A resolution. *J. Mol. Biol.* 335: 775-785
- Dubin G, Krajewski M, Popowicz G, Stec-Niemczyk J, Bochtler M, Potempa J, Dubin A, Holak TA. (2003) A novell class of cysteine protease inhibitors: solution structure of staphostain A form Staphylococus aureus. *Biochemistry* 42: 13449-13456
- Filipek R, Rzychon M, Oleksy A, Gruca M, Dubin A, Potempa j, Bochtler M (2003) The Staphostatin-staphopain complex: a forward binding inhibitor in complex with its target cysteine protease. *J. Biol. Chem.* 278: 40959-40966
- Rzychon M, Filipek R, Sabat A, Kosowska K, Dubin A, Potempa J, Bochtler M. (2003) Staphostantins resemble lipocalins, not cystatins in fold. *Protein Sci.* 12: 2252-2256
- Song HK, Bochtler M, Azim MK, Hartmann C, Huber R, Ramachandran R. (2003) Isolation and characterization of the prokaryotic proteasome homolog Hs1VU (C1pQy) from *Thermotoga maritima* and the crystal structure of Hs1V. *Biophys. Chem.* 100: 437-452

Description of Current Research

The MPG-PAN Junior Research Group is interested in structure-function studies of peptidases, proteases and proteins involved in protein degradation. We are currently focusing on three major projects.

Staphostatin-type inhibitors of cysteine peptidases

Staphostatins are the endogenous, highly specific inhibitors of staphopains, the major secreted cysteine proteases from Staphylococcus aureus. We have shown that (i) staphostatins are cysteine protease inhibitors with a lipocalin-like fold, (ii) they act as competitive, active site-directed inhibitors that span the active site clefts of their target proteases in the same orientation as substrates, (iii) their binding modes resemble the "ion-molecule" binding modes that have been proposed for the Michaelis complex of papain-type enzymes and their substrates. These findings imply that staphostatins represent a structurally and mechanistically novel family of cysteine peptidase inhibitors, with some clear similarities, but also pronounced differences, to standard mechanism serine protease inhibitors. To compare the mechanisms of staphostatin B mediated and proregion mediated inhibition of staphopain B, we have solved the structure of prostaphopain B. From this work, we conclude that the staphopain B proregion uses a variant of the usual "backwards"-binding mode that protects proregions from proteolytic cleavage by the mature parts of papain-like enzymes.

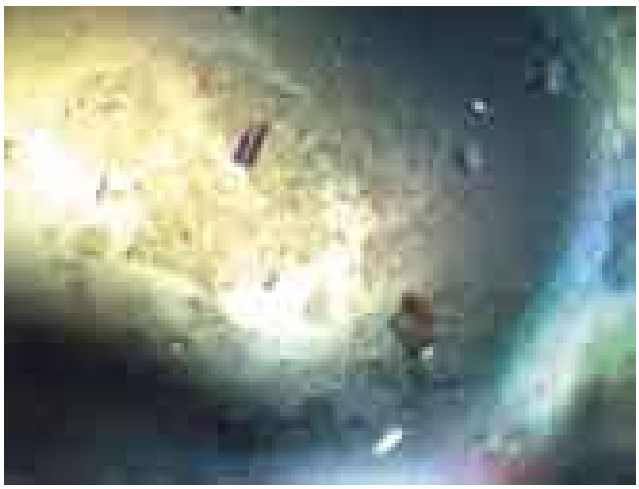
Lysostaphin-type and other peptidoglycan hydrolases

LAS enzymes are metallopeptidases that have been grouped together as a result of our work and that are unrelated to "standard" metallopeptidases such as gelatinases or collagenases. The first step towards the definition of this new group of metallopeptidases was our determination of the crystal structure of LytM a model protein for the pharmacologically interesting peptidase lysostaphin. The LytM fold turned out to be unprecedented in peptidases and proteases, and gave rise to the definition of a new clan of peptidases (MO). In spite of the overall uniqueness of the structure, we found striking similarities between the active sites of LytM, D-Ala-D-Ala-peptidases and the N-domain of sonic hedgehog, that suggested the definition of a new group of metallopeptidases, the LAS enzymes. Using consensus motifs from the structural work, we then predicted and subsequently confirmed that another peptidase family of unknown fold and mechanism, the MepA-like enzymes, are LAS enzymes. This work defines a metallopeptidase with over 300 member sequences in the database, and it provides an example for the successful exploitation of structural information for biological predictions.

Enzymes of the ubiquitin-proteasome pathway

The ubiquitin-proteasome pathway is essential for the degradation of cytosolic and nuclear proteins in eukaryotes. Protein ubiquitination requires the sequential activity of three enzy-

mes: a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and a ubiquitin-ligase (E3). The ubiquitin-transfer machinery is hierarchically organized: for every ubiquitin-activating enzyme, there are several ubiquitin-conjugating enzymes, and most ubiquitin-conjugating enzymes can in turn interact with multiple ubiquitin-ligases. Despite the central role of ubiquitin-activating enzyme in this cascade, a crystal structure of this enzyme is not available. The enzyme is thought to consist of an adenylation domain, a catalytic cysteine domain, a four-helix bundle, and, possibly, a ubiquitin-like domain. During the last year, we have managed to crystallize a fragment of ubiquitin-activating enzyme and to solve its structure. In addition, a review on large proteolytic enzymes has been published.



From top to bottom:

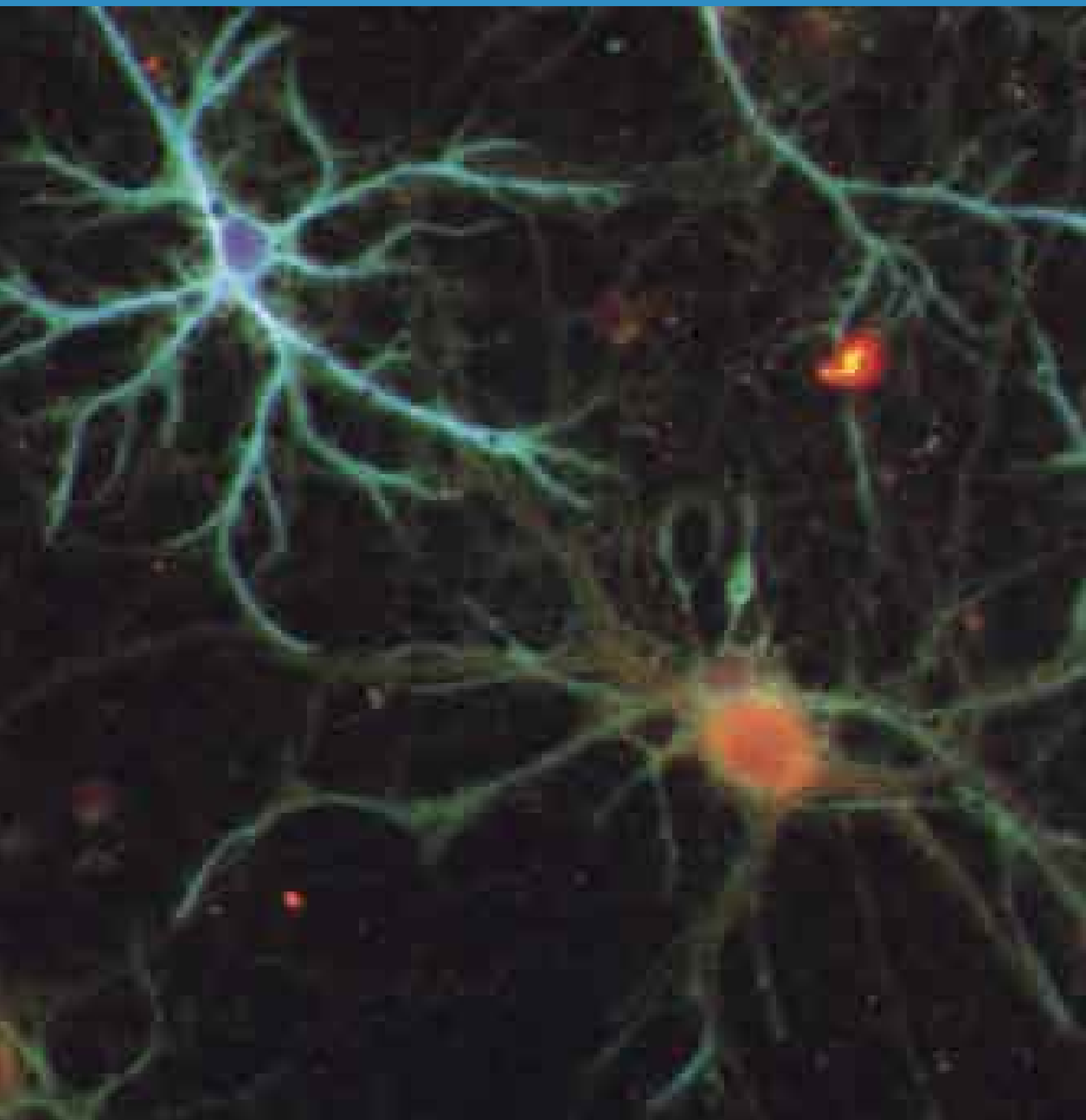
Wild type staphopain B-staphostatin B complex crystals

Crystals of a ubiquitin-activating enzyme fragment (second catalytic cysteine half domain) in the H3 crystal form

Crystals of a ubiquitin-activating enzyme fragment in the H32 crystal form, derivatized with tantalum bromide

Diffraction pattern of a crystal of a ubiquitin-activating enzyme fragment

Laboratory of Neurodegeneration



staff

Head:
Jacek Kuznicki, PhD, Professor

Associate Professors:
Urszula Wojda, PhD, DSc.Habil.
Cezary Zekanowski, PhD, DSc.Habil.

Post-doctoral fellows:
Andrzej Lewandowicz, MD, PhD (started May 2004)
Monika Mysiak, PhD (started January 2005)
Marta Wisniewska, PhD (started October 2004)
Gang Zhao, PhD (ended September 2004)

PhD students:
Magdalena Blazejczyk, MSc; Lukasz Bojarski, Wojciech Michowski, MSc; Adam Sobczak, MSc; Aleksandra Szybinska, MSc

Centenarian Program:
Malgorzata Mossakowska, PhD (co-ordinator); Katarzyna Broczek, MD, PhD (geriatrician); Malgorzata Kupisz-Urbanska, MD

Linked with the Laboratory of Calcium Binding Proteins at the Nencki Institute of Experimental Biology
Head: Dr. Anna Filipek, DSc.Habil.

Collaboration with Prof. Dr. hab. Maria Barcikowska, MD, PhD, Professor and Head Department of Neurodegenerative Disorders, Medical Research Center, Polish Academy of Sciences and Department of Neurology, MSWiA Hospital, Warszawa

Jacek Kuznicki, PhD

Degrees

Professor, 1993

DSc.Habil. Nencki Institute of Experimental Biology PAN, Warsaw, Poland, 1987

PhD in biochemistry, Nencki Institute of Experimental Biology PAN, Warsaw, 1980

MSc in biochemistry, Warsaw University, 1976

Post-doctoral Training

1981-1984 Visiting Fellow, Laboratory of Cell Biology headed by E.D. Korn, National Institutes of Health, Bethesda, MD, USA

Professional Employment

since 2002 Director and Head of the Laboratory of Neurodegeneration, IIMCB

2000-2001 Director, Centre of Excellence for Studies on Mechanisms of Neurodegeneration Phare Sci-Tech II located at the Nencki Institute of Experimental Biology PAN, Warsaw

1999-2001 Acting Director, IIMCB; Organizer and Director of Centenarian Program

1996-2002 Head of Laboratory of Calcium Binding Proteins, the Nencki Institute of Experimental Biology PAN, Warsaw

1992-1995 Visiting Professor at the National Institute of Mental Health, Laboratory of Clinical Science, Bethesda, MD, USA

1991-1992 Deputy Director (Scientific Director), Nencki Institute of Experimental Biology PAN, Warsaw

1986-1992 Associate Professor and Head of Laboratory of Calcium Binding Proteins, Nencki Institute of Experimental Biology PAN, Warsaw

1984-1985 Research Associate, Nencki Institute of Experimental Biology PAN, Warsaw

1981-1984 Visiting Fellow, NIH, Laboratory of Cell Biology, Bethesda, MD, USA

1980-1981 Post-doctoral Fellow, Nencki Institute of Experimental Biology PAN, Warsaw

1976-1980 PhD Student, Nencki Institute of Experimental Biology PAN, Warsaw

Membership in Scientific Societies, Organizations and Panels

- Member of the Polish Academy of Science - since December 2004
- Member of American Society for Biochemistry and Molecular Biology, since 2003
- Head of Advisory Board of the Science School Festival, since 2002
- Member of the Biochemical Society (England), since 1995
- Member of the Polish Neuroscience Society, since 1991
- Member of the Polish Society for the Advancement of Science and Arts, since 1991
- Vice-president of the Polish Biotechnology Committee, 1996-1999 and 2000-2002
- Member of the Polish Biotechnology Committee, 1990-2002
- Co-Editor of Advances in Biochemistry (published in Polish), 1989-1992
- Member of the Polish Biochemical Society, since 1977
- General Secretary of the Polish Biochemical Society, 1989-1991

Honors, Prizes, Awards

- Professorship Award of Foundation for Polish Research (FNP), 2004-2006
- Prime Minister Award for the scientific achievements, 2003
- Award from Division of Biological Sciences PAN for the work on calcium binding proteins, 2001
- Polish Anatomical Society Award for the article on calcium binding proteins published in "Advances in Cell Biology", 1987
- Skarzynski Award of Polish Biochemical Society for the best review article in Advances in Biochemistry, 1986
- Parnas Award of Polish Biochemical Society for the publishing of the best paper in biochemical research, 1977
- Mozolowski Award, Polish Biochemical Society for outstanding Polish young biochemists, 1977
- Magna cum laude, University of Warsaw, 1976

Selected Publications

- *Bhattacharya S, Lee Y.-T, Michowski W, Filipek A, Kuznicki J, Chazin W (2005) The modular structure of SIP facilitates its role in stabilizing multi-protein assemblies. *(submitted)*
- *Blazejczyk M, Wojda U, Sobczak A, Spilker C, Bernstein H-G, Gundelfinger ED, Kreutz MR, Kuznicki J (2005) Binding characteristics and cellular expression profiles question a major role of presenilin2/calmyrin interaction in Alzheimer's disease. *(submitted)*
- *Bernstein H-G, Blazejczyk M, Rudka T, Gundelfinger ED, Dobrowolny H, Bogerts B, Kreutz MR, Kuznicki J, Wojda U (2005) The Alzheimer disease-related calcium-binding protein calmyrin is prominently expressed in human forebrain with an altered distribution in Alzheimer's as compared to normal aging brains. *Neuropathol and App Neurobiol (in press)*
- *Puzianowska-Kuznicka M, Kuznicki J (2005) Genetic alterations in accelerated ageing syndromes. Do they play a role in natural ageing? *Int J Biochem Cell Biol* 37:947-960
- *Zekanowski C, Religa D, Safranow K, Maruszak A, Dziedziejko V, Styczynska M, Gacia M, Golan M, Peplonska B, Chlubek D, Kuznicki J, Barcikowska M (2004) The -22c/t polymorphism in presenilin 1 gene is not connected with late-onset and early-onset familial Alzheimer's disease in Poland. *J Neural Transm. Oct 12; [Epub ahead of print]*
- *Lee Y-T, Jacob J, Michowski W, Nowotny M, Kuznicki J, Chazin WJ (2004) Human Sgt1 binds HSP90 through the CS domain and not the TPR domain, *J Biol Chem* 279, 16511-16517
- *C Zekanowski, D Religa, C Graff, S Filipek, J Kuznicki (2004) Genetic aspects of Alzheimer's disease. *Acta Neurobiol Exp* (Wars), 64, 19-31
- *Nowotny M, Spiechowicz M, Jastrzebska B, Filipek A, Kitagawa K, Kuznicki J (2003) Calcium-regulated interaction of Sgt1 with S100A6 (calcyclin) and other S100 proteins. *J Biol Chem* 278, 26923-26928

- *Zekanowski C, Styczynska M, Peplonska B, Gabryelewicz T, Religa D, Ilkowski J, Kijanowska-Haladyna B, Kotapka-Minc S, Mikkelsen S, Pfeffer A, Barczak A, Luczywek E, Wasiak B, Chodakowska-Zebrowska M, Gustaw K, Laczkowska J, Sobow T, Kuznicki J, Barcikowska M. (2003) Mutations in presenilin 1, presenilin 2 and amyloid precursor protein genes in patients with early-onset Alzheimer's disease in Poland. *Experimental Neurol* 184, 991-996
- *Zekanowski C, Peplonska B, Styczynska M, Gustaw K, Kuznicki J, Barcikowska M (2003) Mutation screening of the MAPT and the STH genes in Polish patients with clinically diagnosed frontotemporal dementia (FTD). *Dement Geriatr Cogn Disord 16,* 126-131
- *Peplonska B, Zekanowski C, Religa D, Czyzewski K, Styczynska M, Pfeffer A, Gabryelewicz T, Golebiowski M, Luczywek E, Wasiak B, Barczak A, Chodakowska M, Barcikowska M, Kuznicki J (2003) Strong association between Saitohin gene polymorphism and tau haplotype in the Polish population. *Neurosci Lett* 348 163-166
- *Wojda A, Wolnik-Brzozowska D, Lubka M, Mossakowska M, Witt M (2003) The 102-year old woman with translocation (7;12) and infertility in anamnesis *J Appl Genet* 44, 425-427
- *Palczewska M, Groves P, Batta G, Heise B, Kuznicki J (2003) Calretinin and calbindin D28k have different domain organizations. *Protein Sci* 12, 180-184
- *Filipek A, Jastrzebska B, Nowotny M, Kuznicki J, (2002) CacyBP/SIP, a Calcyclin and Siah-1-interacting Protein, Binds EF-hand Proteins of the S100 Family. *J Biol Chem* 277, 28848-28852
- *Filipek A, Jastrzębska B, Nowotny M, Kwiatkowska K, Hetman M, Surmacz L, Wyroba E, Kuznicki J (2002) Ca^{2+} -dependent Translocation of the Calcyclin-Binding Protein in Neurons and Neuroblastoma NB-2a cells. *J Biol Chem* 277, 21103-21109
- *Billing-Marczak K, Buzanska L, Winsky L, Nowotny M, Rudka T, Isaacs K, Belin MF, Kuznicki J (2002) AP2-Like cis element is required for calretinin gene promoter activity in cells of neuronal phenotype differentiated from multipotent human cell line DEV. *Biochim Biophys Acta* 1577, 412-420
- *Palczewska M, Groves P, Ambrus A, Kaleta A, Kövér KE, Batta G, Kuznicki J (2001) Structural and biochemical characterization of neuronal calretinin domain I-II (residues 1-100); comparison to homologous calbindin D28k domain I-II (residues 1-93). *Eur J Biochem* 268, 6229-6237
- Nowotny M, Bhattacharya S, Filipek A, Krezel AM, Chazin WJ, Kuznicki J (2000) Characterization of the interaction of calcyclin (S100A6) and calcyclin-binding protein. *J Biol Chem* 275, 31178-31182
- Jastrzębska B, Filipek A, Nowicka D, Kaczmarek L, Kuznicki J (2000) Calcyclin (S100A6) binding protein (CacyBP) is highly expressed in brain neurons. *J Histochem Cytochem* 48, 1195-1202
- Lesniak W, Jezierska A, Kuznicki J (2000) Upstream stimulatory factor is involved in the regulation of the human calcyclin (S100A6) gene. *Biochim Biophys Acta* 1517, 73-81
- Palczewska M, Groves P, Kuznicki J (1999) Use of Pichia pastoris for expression, purification and characterization of rat calretinin „EF-hand" domains. *Protein Expres Purif* 17, 465-464
- Billing-Marczak K, Przybyszewska M, Kuznicki J (1999) Measurements of $[Ca^{2+}]$ using fura-2 in glioma C6 cells expressing calretinin with GFP as a marker of transfection: no Ca^{2+}-buffering provided by calretinin. *Biochem Biophys Acta* 1449, 169-177
- Filipek A, Kuznicki J (1998) Molecular cloning and expression of a mouse brain cDNA encoding a novel protein target of calcyclin. *J Neurochem* 70, 1793-1798
- Groves P, Finn BE, Kuznicki J, Forsen S (1998) A model for target protein binding to calcium-activated S100 dimers. *FEBS Lett* 421, 175-179

*Papers marked with an asterisk have the IIMCB affiliation of the authors

Current Projects

We study at genetic, protein and cellular levels the molecular mechanisms involved in the pathogenesis of neurodegenerative diseases such as Alzheimer disease (AD). We are also interested in the involvement of ubiquitination in the pathologies of these diseases.

To determine the spectrum of mutations in a group of Polish patients with clinically diagnosed familial AD and patients with mild cognitive impairment (MCI) and family history of AD, we performed a screening for mutations in the presenilin 1 (PSEN1), presenilin 2 (PSEN2) and amyloid precursor protein (APP) genes. Five previously recognized pathogenic mutations in PSEN1 gene (H163R, M139V, F177L) and APP gene (T714A, V715A), and five novel putative mutations in PSEN1 gene (P117R, I213F, L226F, and L424H) and PSEN2 gene (Q228L) were identified. The novel mutations are absent in a group of 225 control subjects and 115 sporadic AD patients. The frequency of mutations was 19%, which is consistent with other studies using the same definition of a patient with familial AD. The patients with no mutations detected were older than the patients with mutation identified. Also a frequency of APOE4 allele was higher in this group. It could be concluded that screening for mutations in the three genes can be included in a broad diagnostic program directed

at patients with a positive family history or age of onset generally before 55 years. Attempts are currently undertaken to characterize novel mutations using in vitro cell system and in silico analysis. Mutations in the presenilin 1 (PSEN1) gene are highly penetrant. The only common exception is a mutation E318G, reported initially as a pathogenic one, and connected with a variable age of onset. Then the E318G mutation has been found in a number of healthy control individuals, as well as in early- and late-onset sporadic AD patients, and in familial AD patients. The results suggest that the E318G mutation could be regarded as one of: a rare polymorphism, a neutral mutation, or a mutation with incomplete penetrance leading to the disease in particular cases or populations. To determine whether E318G mutation is related causally to AD in the Polish population E318G mutation frequency was assessed in a total of 659 subjects. When the mutation frequencies were compared to healthy controls, no significant differences between the groups were found. Our results indicate that E318G mutation is not related causally to AD in the Polish population, either as a risk factor or a disease causing mutation. The studied groups of familial AD patients, and unrelated sporadic AD cases (late-onset AD) were diagnosed in the Department of Neurodegenerative Disorders of Medical Research Center of the Polish Academy of Sciences in Warsaw, headed by Prof. M. Barcikowska, MD).

Analysis of Ca^{2+}-binding protein calmyrin and its interaction with presenilin 2

One of the mechanisms that seems to be involved in the early stages of AD is perturbed Ca^{2+}-signaling, but little is known on the role of Ca^{2+}-binding proteins in neurodegeneration mechanisms. One of the recently described Ca^{2+}-binding proteins from the EF-hand protein family is mirystoylated calmyrin. Calmyrin's involvement in AD pathogenesis was suggested based on its ability to interact with presenilin 2, shown using yeast-two-hybrid system. Calmyrin can interact also with other cellular targets including the polo-like kinases SNK and FNK in the human brain. To check the possibility that calmyrin-dependent signaling is disturbed in familial AD pathogenesis we determined calmyrin co-localization with PS2 in human healthy versus AD brain, as well as comparing by affinity chromatography calmyrin interaction with PS2 from normal and familial AD lymphocytes with described mutation in PS2. We also analyzed calmyrin and presenilin 2 distribution in rat brains and searched for other calcium-dependent protein targets of calmyrin. Using anti-calmyrin antibodies we analyzed the localization of calmyrin in a normal human and AD brains and compared it with the known distribution of PS2. We used sections of brains of healthy middle aged persons (two males and two females), of 10 neuropathologically confirmed patients with AD (five males, five females, mean age = 69.1 years), and of an age-matching group without dementia (seven neurologically normal subjects, three males, four females, mean age = 72.7 years). Calmyrin immunoreactivity in the normal healthy human brain was found to be unevenly distributed with prominent immunostaining in pyramidal neurons and interneurons. No apparent differences were visible between the staining of brain sections from younger and older non-demented patients. In AD brain a substantial loss of calmyrin-immunopositive neurons was observed in all regions. Immunoreactive neurons, however, displayed stronger staining that was especially concentrated in perinuclear regions. Calmyrin-immunosignals associated also with diffuse and senile plaques, hallmarks of AD. Neuronal localization of calmyrin in the human brain and altered distribution in the AD brains are in agreement with the hypothesis that calmyrin could be involved in the AD pathogenesis. However, binding characteristics and cellular expression profiles of calmyrin and presenilin 2 question a major role of their interaction in Alzheimer's disease.

Subcellular localization of calmyrin was further studied by transfection of primary rat neurons with calmyrin-EGFP construct showing calmyrin presence in the cell body as well as in the nucleus. The above-mentioned studies on calmyrin were performed in collaboration with Dr. Hans-Gert Bernstein from the Department of Psychiatry in the Otto-von-Guericke-University in Magdeburg and Dr. Michael R. Kreutz from Department of Neurochemistry, Molecular Biology, Leibniz Institute for Neurobiology in Magdeburg. All brains were obtained from pathologists after medical examination in accordance to the ethics and rules outlined by German law and the local ethics commission of the University of Magdeburg.

Polish Centenarians Program

This multidisciplinary program entitled "Environmental and Genetic Factors of Polish Centenarians' Longevity" was ordered by KBN and is being directed by J. Kuznicki. The scientific aim of the program is to collect information concerning the environmental determinants of healthy aging in Polish centenarians and to provide material to study the several aspects of longevity including the search for genetic determinants. The organization of medical examination, blood analysis and database are being co-ordinated by Dr. M. Mossakowska. Medical visits and medical examinations are being made by geriatricians: K. Broczek (the Warsaw group), K. Wieczorowska-Tobis (the Poznan group), A. Klich-Raczka and J. Zyczkowska (the Cracow group). The program consists of 7 original projects, in which 22 research groups from different laboratories in Poland are taking part:

– The health status evaluation of Polish centenarians, including the cardiac system, Dr. T. Grodzicki

– The neurological and neuropsychological status of Polish centenarians with particular estimation of dementia risk factors, extra-pyramidal function and postural stability, Dr. A. Pfeffer

– The psychological aspects of functioning in Polish centenarians, Dr. E. Szelag

– The evaluation of neuroendocrine system, mineral balance and osseous system, Dr. B. Baranowska

– The immune system of Polish centenarians including the function of CD8+CD28 – sub-population of T lymphocytes, Dr. E. Sikora

– The evaluation of the antioxidant status in Polish centenarians, Dr. B. Klapcinska

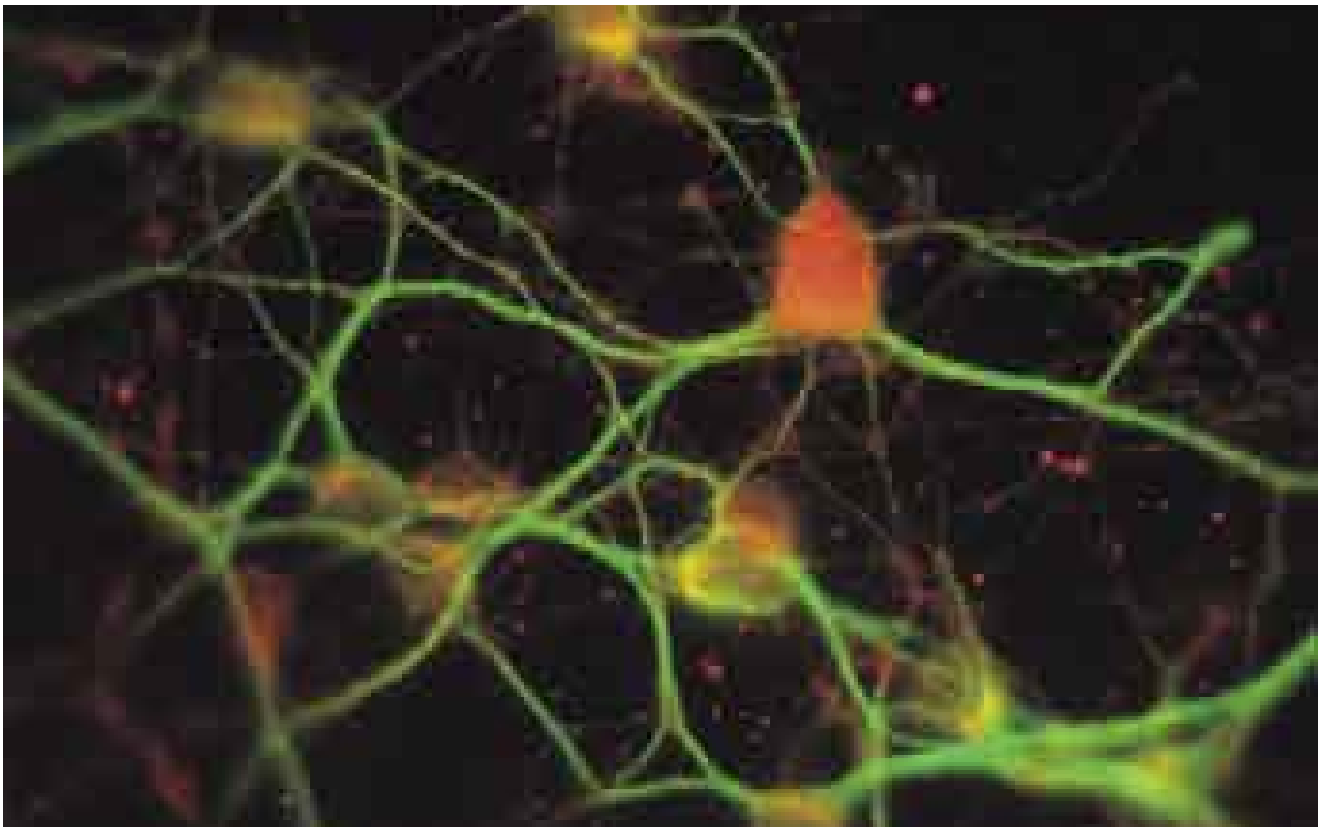
– The establishment of DNA, RNA and immortalized lymphocytes bank. Study on chromosomal aberrations and polymorphism of genes connected with aging, Dr. M. Witt.

Since the beginning of the project about than 350 centenarians have been visited and genetic material has been collected from many of them and from 82 people of 65 years old. The DNA, RNA and immortalized lymphocytes bank consists of samples taken from both centenarians and healthy subjects as well as from Alzheimer disease patients. The bank contains: gDNA – 300 samples, cDNA – 148, immortalized cell lines – 153. The material from the bank is being used also by research groups in Poland, which are not directly involved in the program.

The social aim of the program is to draw the public's attention to the aging population, the living conditions of old people and to attract young Polish medical doctors into gerontology. The pilot of this program began at the IIMCB in autumn 1998.

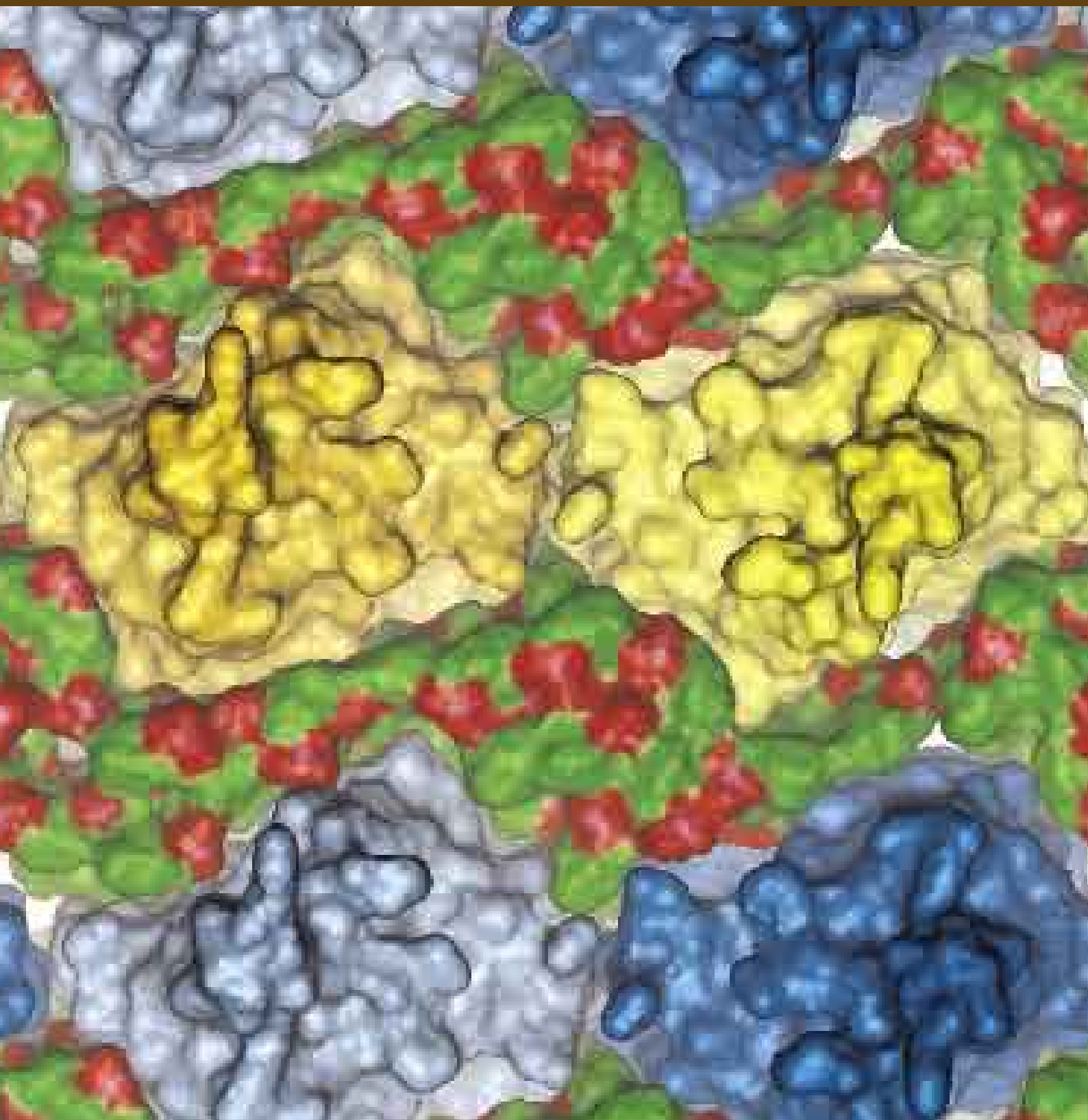
Major projects and funding

- APOPIS "Abnormal proteins in the pathogenesis of neurodegenerative disorders", (EU Integrated Project in VI FP)
- Studies of proteins involved in b-catenin ubiquitination (SIP, Sgt1) (grant from USA – FIRCA, KBN grant)
- The search for a functional bio-marker of familial Alzheimer disease – identification of the proteins that change affinity as a result of presenilin mutations (Polish-German and KBN grants)
- Molecular characterization (genotypes at PSEN1, PSEN2, APP, MAPT, and STH genes) of Polish Patients with Familial Alzheimer's Disease, Sporadic Alzheimer's Disease Patients, and Frontotemporal-Dementia (KBN grant)
- Polish Centenarians Programme „Studies on Environmental and Genetic Aspects of Longevity" (KBN grant) *(http://www.iimcb.gov.pl/centenarians/centenarians.htm)*



Immunostaining of granullar rat neurons: MAP2 - marker of neurons in green, overexpression of calmyrin in red. Author: Magdalena Blazejczyk, MSc

Laboratory of Biomodelling



staff

Head:
Slawomir Filipek, PhD, DSc

PhD students:
Anna Modzelewska, MSc;
Krystiana Krzysko, MSc;
Michał Kolinski, MSc

Undergraduate students:
Magdalena Kolczewska, Ewelina Siadkowska

Slawomir Filipek, PhD, DSc

Degrees

DSc.Habil. in medicinal chemistry, Warsaw University, Faculty of Chemistry, 2004

PhD in theoretical chemistry, Warsaw University, Faculty of Chemistry, 1993

MSc in quantum chemistry, Warsaw University, Faculty of Chemistry, 1985

Post-doctoral Training

2001, 2002 Visiting scientist, Department of Ophthalmology, University of Washington, Seattle, WA, USA

Professional Employment

since 2002 Head of the Laboratory of Biomodelling, IIMCB

since 1993 Assistant Professor, Warsaw University, Faculty of Chemistry

1985-1992 Assistant, Warsaw University, Faculty of Chemistry

Honors, Prizes, Awards

2000-2002 Scientific awards-stipends of Rector of Warsaw University

Publications

30 publications since 2000 in primary scientific journals

Publications 2003-2004

- *Park PS, Filipek S, Wells JW, Palczewski K (2004) Oligomerization of G protein-coupled receptors: past, present, and future. *Biochemistry* 43:15643-15656
- *Filipek S, Krzysko KA, Fotiadis D, Liang Y, Saperstein DA, Engel A, Palczewski K (2004) A concept for G protein activation by G protein-coupled receptor dimers: the transducin/rhodopsin interface. *Photochem. Photobiol. Sci.* 3: 628-638
- *Suda K, Filipek S, Palczewski K, Engel A, Fotiadis D (2004) Blue native gel electrophoresis and chemical cross-linking of rhodopsin dimmers and oligomers in native disc membranes. *Mol. Membr. Biol.* 21: 435-446
- *Fotiadis D, Liang Y, Filipek S, Saperstein D.A, Engel A, Palczewski K (2004) The G protein-coupled receptor rhodopsin in the native membrane. *FEBS Letters* 564: 281-288
- *Zekanowski C, Religa D, Graff C, Filipek S, Kuznicki J (2004) Genetic aspects of Alzheimer's disease. *Acta Neurobiol. Exp.* 64: 19-31
- *Imanishi Y, Yang L, Sokal I, Filipek S, Palczewski K, Baehr W (2004) Diversity of guanylate cyclase-activating proteins (GCAPs) in teleost fish: characterization of three novel GCAPs (GCAP4, GCAP5, GCAP7) from zebrafish (Danio rerio) and prediction of eight GCAPs (GCAP1-8) in pufferfish (Fugu rubripes). *J. Mol. Evol.* 59: 204-217
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- *Zhu L, Jang GF, Jastrzebska B, Filipek S, Pearce-Kelling SE, Aguirre GD, Stenkamp RE, Acland GM, Palczewski K (2004) A naturally occurring mutation of the opsin gene (T4R) in dogs affects glycosylation and stability of the G protein-coupled receptor. *J. Biol. Chem.* 279:53828-53839
- *Liang Y, Fotiadis D, Maeda T, Maeda A, Modzelewska A, Filipek S, Saperstein DA, Engel A, Palczewski K (2004) Rhodopsin signaling and organization in heterozygote rhodopsin knockout mice. *J. Biol. Chem.* 279:48189-48196
- *Fotiadis D, Liang Y, Filipek S, Saperstein DA, Engel A, Palczewski K (2003) Rhodopsin dimers in native disc membranes. *Nature* 421: 127-128
- *Liang Y, Fotiadis D, Filipek S, Saperstein DA, Palczewski K, Engel A (2003) Organization of the G Protein-coupled Receptors Rhodopsin and Opsin in Native Membranes *J. Biol. Chem.* 278: 21655-21662
- *Schadel SA, Heck M, Maretzki D, Filipek S, Teller DC, Palczewski K, Hoffman KP, (2003) Ligand Channeling within a G-protein-coupled Receptor: The Entry and Exit of Retinals in Native Opsin. *J. Biol. Chem.* 278: 24896-24903
- *Mirzadegan T, Benko G, Filipek S, Palczewski K (2003) Sequence Analyses of G-Protein-Coupled Receptors: Similarities to Rhodopsin. *Biochemistry-US* 42: 2759-2767
- *Maeda T, van Hooser J.P, Driessen C.A.G.G, Filipek S, Janssen J.J.M, Palczewski K (2003) Evaluation of the role of the retinal G protein-coupled receptor (RGR) in the vertebrate retina in vivo. *J. Neurochem.* 85: 944-956
- *Noorwez Sm, Kuksa V, Imanishi Y, Zhu L, Filipek S, Palczewski K, Kaushal S (2003) Pharmacological Chaperone-mediated in Vivo Folding and Stabilization of the P23H-opsin Mutant Associated with Autosomal Dominant Retinitis Pigmentosa. *J. Biol. Chem.* 278: 14442-14450
- *Filipek S, Teller DC, Palczewski K, Stenkamp R (2003) The Crystallographic Model of Rhodopsin and Its Use in Studies of Other G Protein-Coupled Receptors. *Annu. Rev. Biophys. Biomol. Struct.* 32: 375-397
- Filipek S, Stenkamp RE, Teller DC, Palczewski K (2003) G Protein-Coupled Receptor Rhodopsin: A Prospectus, *Annu. Rev. Physiol.* 65: 851-879
- *Fritze O, Filipek S, Kuksa V, Palczewski K, Hofmann KP, Ernst OP (2003) Role of the Conserved NPXXY(X)5,6F Motif in the Rhodopsin Ground State and During Activation. *Proc. Natl. Acad. Sci. USA* 100: 2290-2295

*Papers marked with an asterisk have the IIMCB affiliation of the authors

Current research and projects

We have modeled the structure of a big complex containing rhodopsin oligomer (six molecules) and trimeric rhodopsin G protein (transducin) (Fig. 1). The model revealed that dissociation of oligomer into single monomers of rhodopsin was not necessary prior to complex formation with transducin. Furthermore, a positive cooperation effect in the binding of G protein by activated rhodopsin was discovered (Fig. 2). Monomeric rhodopsin is still required in existing models of activation of rhodopsin and passing a signal to transducin. However, these models don't take into account existence of rafts of proteins and phospholipids.

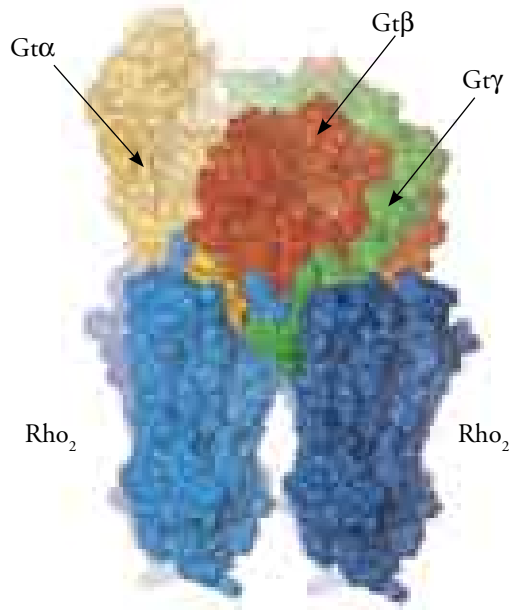


Fig. 1. Complex of transducin (Gtαβγ) and rhodopsin tetramer ($2Rho_2$).

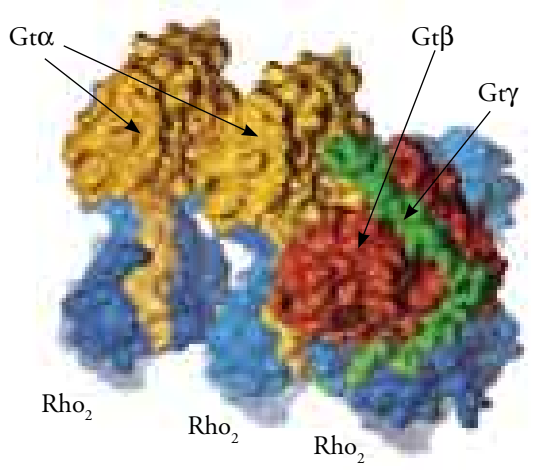


Fig. 2. Complex of two transducin molecules (Gtα after dissociation out of Gtβγ, and the Gtαβγ) and three rhodopsin dimers. View from the cytoplasm.

In heterozygous rhodopsin +/- mice as compared with age-matched WT mice, the length of rod outer segments (ROS) was shorter by 30-40%, and the average diameter of ROS was reduced by ~20%. Together, the reduction of the volume of ROS was ~60% in rhodopsin +/- mice (cooperation with Department of Ophthalmology, University of Washington, Seattle, WA, USA). Atomic force microscopy of WT and rhodopsin +/- disc membranes revealed, in both cases, rhodopsin organized in paracrystalline and raft-like structures (cooperation with M.E. Müller Institute for Microscopy, Biozentrum, University of Basel, Switzerland). From this data it was concluded that the differences in physiological responses measured in WT and rhodopsin +/- mice are due to structural changes of the whole ROS, and not due to a lower density of rhodopsin in discs.

We plan to continue investigating the formation of dimers and higher oligomers of rhodopsin molecules in native membranes of ROS (Fig. 3). This membrane contains unsaturated lipids (docosahexaenoyl chains) in high concentrations which greatly influence kinetics of signal transduction in vision process. Our next aim is investigating the processes of deactivation of rhodopsin by building models of complexes of rhodopsin kinase and arrestin with oligomeric rhodopsin. Such models can serve as templates for other G protein-coupled receptors (GPCRs) and we investigate processes that the opioid receptors (mu, delta and kappa) are involved in: dimerization, activation, passing the signal to G protein and finally deactivation and internalization.

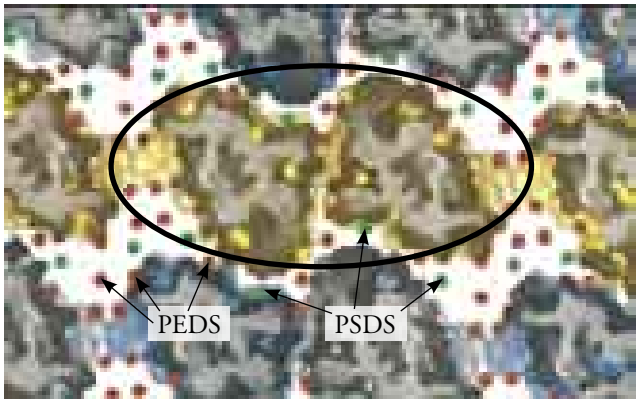


Fig. 3. The model of rhodopsin oligomer in the membrane. View from cytoplasmic side while cytoplasmic loops were removed. Single rhodopsin dimer marked by ellipse. Positions of phosphorus atoms of phospholipids marked by spheres: red - PEDS, green - PSDS.

We also modeled the structures of presenilins (PS1 and PS2), the proteins forming γ-secretase complex which is responsible for overproduction of β-amyloid, forming plaques in the brain during Alzheimer Disease (cooperation with Laboratory of Neurodegeneration at IIMCB and Department of Neurodegenerative Disorders at Medical Research Centre, Warsaw). Many mutations of presenilins were found in areas that may serve as binding regions to other membrane components of γ-secretase complex (Aph-1, nicastrin and Pen-2). Bearing in mind difficulties in the structural characterization of membrane proteins, we plan to construct a molecular model of membrane part of γ-secretase complex including substrate amyloid precursor peptide (APP). Then it would be possible to predict which mutations are pathological and possibly to design new drugs directed to disrupt interactions of γ-secretase complex with APP.

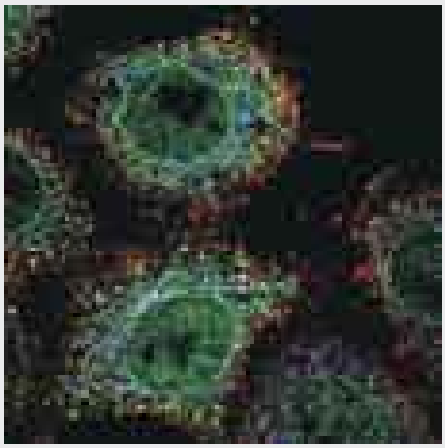
Laboratory of Cell Biology

(since April 2005)

staff

Head:
Marta Miaczynska, PhD

PhD students:
Marta Brewinska, MSc
Anna Zarebska, MSc



Marta Miaczynska, PhD

Degrees

1997 PhD in genetics, University of Vienna, Austria

1993 MSc in molecular biology, Jagiellonian University in Cracow, Poland

1991 BSc in biological sciences, University of Wolverhampton, UK

Research Training

2001-2005 senior postdoctoral fellow in the Max Planck Institute for Molecular Cell Biology and Genetics (MPI-CBG) in Dresden, Germany

1997-2000 postdoctoral training at the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany

1993-1996 PhD studies in the Institute of Microbiology and Genetics, University of Vienna, Austria

1990-1991 exchange student at the University of Wolverhampton, Wolverhampton, UK

Fellowships and awards

1999-2000 Long Term Postdoctoral Fellowship of the Human Frontier Science Program Organization (HFSPO)

1998-1999 Erwin Schrödinger Postdoctoral Fellowship from the Austrian Science Fund (FWF)

1993-1996 Bertha von Suttner PhD Scholarship from the Austrian Ministry of Science 1990-1991 Studentship of the European Community Tempus Scheme

Research

The laboratory will study the interface between the processes of endocytic transport and signal transduction. In particular we will investigate the role of endosomal compartments in signalling, focusing on the function of APPL proteins which act as signal transducers by shuttling between the endosomes and the nucleus.

Educational Activities

Utrecht University Doctoral Program

The Utrecht University doctoral program is based on an agreement between the Polish Network for Cell and Molecular Biology UNESCO/PAN and the Utrecht University (The Netherlands). This is a part of the research collaboration program initiated by Prof. Wilem Gispen to facilitate the exchange of scientific information and ideas among Polish and Dutch scientists and graduate students and allow for short-term research visits of the staff members and their students from Poland to Utrecht and vice versa. The doctoral program itself offers three four-year doctoral positions. The doctoral thesis will be defended in front of the dissertation committee of the Medical Faculty of Utrecht University. As a result of the publicly advertised competition three students were accepted: M. Bucko (M. Zylicz's lab, IIMCB), M. Olszewski (J. Dastych's lab, IIMCB) and K. Starowicz (R. Przewlocki's lab, Institute of Pharmacology, PAS, Cracow). Because of the success of this part of the program, the next recruitment has been announced and performed. Five positions have been filled for the period of 2003-2007: J. Boros (M. Zylicz's lab, IIMCB: Formation of p53 dimers and tetramers in vivo), M. Geremek (M. Witt's lab, IIMCB and Institute of Human Genetics, PAN, Poznan: Genetic analysis of primary ciliary dyskinesia/Kartagener Syndrome [PCD/KS]), M. Lukowiak (A. Lipkowski's lab, Center for Experimental and Clinical Medicine, PAN, Warsaw: Pharmacology of opioid peptides. The application of polymers as carriers of the opioid peptides), P. Michaluk (L. Kaczmarek's lab, Nencki Institute, PAN, Warsaw: Role of MMP-9 in neuronal plasticity), M. Piechota (R. Przewlocki's lab, Institute of Pharmacology, PAN, Cracow: Role of the melanocortin system in chronic inflammatory pain in comparison to neuropathic pain). In the mid-term all PhD students presented the progress of their research to Prof. Gispen within a comprehensive review session. IIMCB coordinates the entire program on a Polish site.

Postgraduate School of Molecular Medicine (SMM) (www.iimcb.gov.pl/smm/index.html)

Medical Universities in Warsaw, Poznan, Szczecin, Gdansk as well as the International Institute of Molecular and Cell Biology, the Nencki Institute and the Foundation for Experimental and Clinical Oncology have jointly founded the Postgraduate School of Molecular Medicine.

The main goal of the School is to offer a new post-graduate doctorate program in the field of molecular medicine, which is addressed to medical, biology and pharmacy students in Poland. SMM is formally affiliated with the Medical University of Warsaw, which is responsible for the administration of the school. According to its by-laws, the School is managed by the Director and the Scientific Council elected by the founding institutions. At present the Director's position is held by Prof. L. Konarska from the Pharmacy Department, Medical University of Warsaw. SMM admits students (up to ten per year) for the four-year doctoral program. The candidates are requested to present a scientific program of their doctoral research, the scientific merit of which is carefully evaluated by the Recruitment Committee of SMM as well as independent judges in Poland and abroad. Seven groups of students were accepted during the period of 1998-2004. Successful candidates accomplish their scientific program, under supervision of their mentors, in different laboratories throughout Poland. The members of SMM Scientific Council annually evaluate student progress. The mentor program offered to the students includes theoretical (lectures, seminars) and practical courses (laboratory sessions) on select topics of modern molecular biology and medicine. Each SMM student is awarded a stipend (full or supplemental). Furthermore, SMM helps students to participate in short-term scientific training in leading Polish and foreign laboratories. In parallel to funds generated by founding institutions, SMM activities are supported by subsidies from the Polish Ministry of Health, Ministry of Research and Informatization, Kronenberg Foundation, UNESCO-ROSTE, European Commission within the 5th Framework Programme (Centre of Excellence in Molecular Bio-Medicine of IIMCB), CNRS (France). Additional financial support comes from the French government supporting the costs of participation of outstanding French scientists in mentoring and organizational activities of SMM as well as short-term scholarships for the training of SMM students in laboratories in France.

In 2004 the following courses were organized:

- 4th Integrated Course - Advances in Molecular Medicine: "Molecular therapy in clinical practice", 29.03.-02.04.2004, Poznan, organized by: Postgraduate School of Molecular Medicine (SMM), Department of Cancer Immunology, Oncology Chair, University of Medical Sciences in Poznan, Wielkopolska Cancer Center in Poznan and IIMCB within the "Centre of Excellence in Molecular Bio-Medicine" project. The course was organized by Prof. Andrzej Mackiewicz and Dariusz Kowalczyk, PhD hab. Over fifty physicians, researchers and students, including seventeen students from Postgraduate School of Molecular Medicine (SMM) attended the meeting and medical sessions. The course program provided an intense research and hands-on experience to SMM students in the field of molecular and gene therapy approach in clinical practice. Students attended state-of-the-art research seminars, had clinical presentations and completed laboratory practice. The lectures presented ranged from innovative vectors used for the introduction of foreign DNA into stem cells and cancer cells followed to treatment of genetic and metabolic diseases using gene therapy.
- SMM Spring School Lecture Course on Human Genetics, 3-4.06.2004, Warsaw, organized by SMM and IIMCB. This annual obligatory course for all first-year students was organized by Prof. Michal Witt. The lectures were given by fourteen eminent Polish scientists from the major clinical and research institutions in Poland. The course was open for the public. PhD students of Utrecht University doctoral program participated in the event.
- Annual Scientific Report Session, 18-19.10.2004, Warsaw, organized by SMM, Medical University of Warsaw and IIMCB. Invited lecture "Glial cells – partners of neurons" was delivered by Prof. Helmut Kettenmann. The French-Polish agreement on creation of European Research Group "From basic oncology to cancer biotherapy" was signed by Prof. Bernard Pau representing the French National Center for Scientific Research (CNRS) and Prof. Andrzej Trzebski representing Polish Academy of Sciences (PAN).
- SMM students presented the results of their scientific activity during the 2003/04 academic year. Sessions were attended and reviewed by a committee consisting of the members of the SMM Scientific Council and foreign collaborators. The Committee evaluated scientific merit and progress of scientific work performed by each student.
- 6thWinter School "From gene to protein, from structure to function and dysfunction", 29.11-3.12.2004, Warsaw. This additional obligatory annual course was organized by Prof. Liliana Konarska. The lectures were given by twenty-six outstanding scientists and academic teachers from the most famous clinical and research institutions in Poland. Students preparing a doctoral thesis within the international doctorate program of Utrecht University and IIMCB participated in the event.

Popularization of Science

The aim of the Science Festival School (SFN) is to reduce the gap between science and society in Poland by conducting educational activities popularizing the theme of biology - open lectures, workshops for students and all interested people, courses for biology teachers, and exhibitions. All activities are focused on improving biology education and awareness of biology in society. The co-founders of the Science Festival School are four biological institutes: International Institute of Molecular and Cell Biology (IIMCB), Nencki Institute of Experimental Biology PAN (IBD), Institute of Biochemistry and Biophysics PAN (IBB), Warsaw Agricultural University (WAU) and Warsaw Festival of Science. IIMCB hosts SFN' laboratory, office and administration.

Prof. J. Kuznicki is the President of the SFN Scientific Board which consists of: Prof. J. Duszynski (IBD), Prof. M. Fikus (Warsaw Festival of Science), Prof. W. Ostoja-Zagórski (IBB) and Prof. H. Wedrychowicz (WAU).

The Institutes finance the School's activities and sponsors supply chemicals and equipment. In the year 2004, sponsors were: Fermentas, MP Biomedicals, Scie-Plas, Symbios and Prona Agarose. Important media partners are Gazeta Wyborcza, Polska Agencja Prasowa.

The SFN cooperates with many partners in science-popularizing initiatives: Akademia Szkoły z Klasą, DANA Foundation (with International Brain's Week), Radio Bis (with Piknik Naukowy), Nowa Era publisher, UE Centre of Excellence at IBD (BRAINS), Warsaw Center of Excellence for Teachers and students and professors from the Institutes, Warsaw University and Agricultural University and 21. Warsaw Scouting Group. International partners are: National Center for Biotechnology Education at the University of Reading UK, European Molecular Biology Organization with Science and Society Programme, Network of Youth Excellence, UNESCO.

Main activities in 2004:

- **Workshops for young participants.** During the whole academic year workshops of 4-5 hours duration for students from secondary and high-schools were organized. Participants used laboratory equipment, techniques and conducted real-life experiments covering topics such as examining DNA by PCR methods, bacterial transformation, gene cloning, protein fingerprinting or molecular diagnosis. The practical experiments were supported by lectures presenting the theoretical basis of molecular biology, genetics and its techniques. Amongst others, SFN hosted groups of Warsaw Technical University students and finalists of the Polish Biology Olympiad who advanced to the international final. Besides that, lessons for primary school children were organized to perform in an easy and attractive way the basics of molecular biology and genetics. Total amount of participants: over 1000.
- **Open lectures.** SFN presents theoretical issues of modern biology by organizing open lectures given by top Polish scientists. Lectures about genomics, evolution, genetic diseases biotechnology, immunology are given every two weeks. On average, the lectures attract about 70 listeners. In cooperation with UE Centre of Excellence at IBD (BRAINS) one-day conference for teachers was organized, where invited speakers presented topics from neurobiology and biochemistry to psychology.
- **Courses for biology teachers.** A programme to train biology teachers was started in response to a big interest amongst schools. Participants spending a few days on such a course not only have the chance to learn how to use modern laboratory equipment and molecular techniques, but also how to perform some "kitchen biology experiments " that can be easily implemented in the classroom. SFN started offering the courses regularly in its laboratory and decided to spread the idea countrywide. Through the support of Gazeta Wyborcza and Akademia Szkoły z Klasą, SFN organized 5 weekend meetings at Teachers Excellence Centers throughout the country. In cooperation with the publisher of Nowa Era, one-day workshops on kitchen laboratory methods were organized in the 10 biggest cities. Finally SFN was the main organizer of a workshop for biology teachers coordinated by European Molecular Biology Organization. This event was one of nine in Europe financed by the European Union as

a part of the project "Continuing Education for European Biology Teachers". A total of 74 teachers from all over the country (21 from Warsaw) and 3 from the Ukraine participated in a four-day workshop, 17-20 June 2004. The second edition of this workshop was organized in November 2004 provided for teachers from small cities and villages, financed by UNESCO Polish Committee (31 participants). Total amount of participants over the year 2004: 470 people.

Programme of „Molecular Biology at the Beginning of the 21st Century" workshop

17 –20 June 2004

Lectures and practical courses:

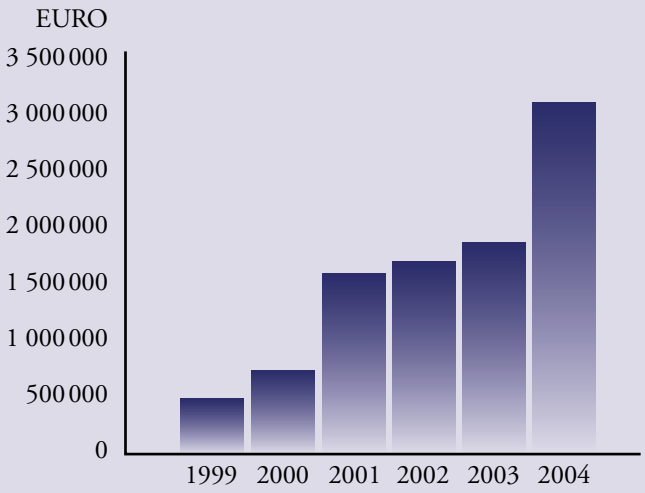
- "Genomic" Dr. Paweł Golik, Department of Genetics Warsaw University
- "About tumors" Prof. Janusz Siedlecki, Oncology Institute in Warsaw
- "Genetics in medicine" Prof. Tadeusz Mazurczak, Institute of Mother & Child in Warsaw
- "XLAB presentation" Dr. Eva-Maria Neher, Experimental Laboratory for Young People
- Laboratory techniques in modern biology – workshop part I ("Find a mutation in CFTR gene" – molecular diagnosis; "Colourful bacteria – transformation of bacteria by pGLO and pBluescript plasmids"; "Protein fingerprinting"
- Computer classes – workshop part II (Biology sources in the Internet; How to use BioEdit program; Watching proteins – Rasmol program)
- "Ask a scientist" – a mini-conference with Prof. E. Bartnik and Prof. P. Stępień, Department of Genetics Warsaw University
- Are molecular biology experiments in school possible? – workshop part III ("Easy electrophoresis"; "Isolation of DNA from onion"; "Fruit enzymes")
- Interactions in groups– workshop part IV (How science works; How science works; Phylogenetics
- Biotechnology" Prof. Piotr Stępień, Department of Genetics WU
- **Science Festival in Warsaw** (14-18 September 2004). SFS took part in The VIII Festival of Science with three 2-day workshops: Examine your own DNA, Enzymes around us and On tracks of proteins.
- **Science Picnic** (22 May 2004). SFS participated in Radio BIS VIII Science Picnic in cooperation with KNBM. Green bacteria exhibition, isolation of DNA from onion, modeling DNA were presented.

Near future

Despite continuation of regular activity, SFN concentrates on providing external funding for additional initiatives.

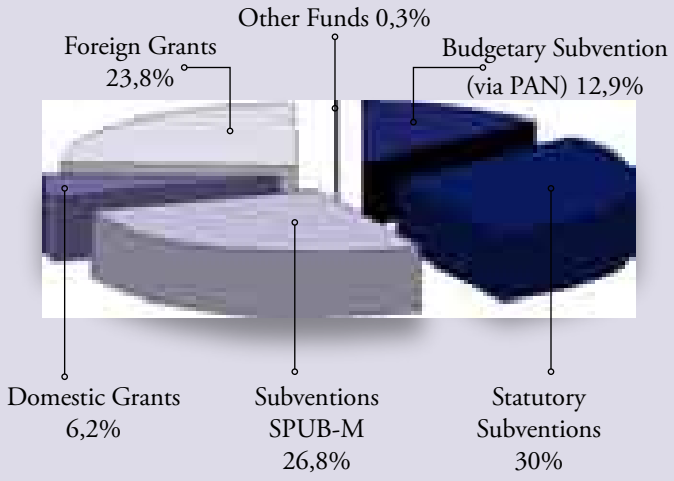
- In November 2004 Polish National Commission for UNESCO accepted the project "Science of Modern Biology – Exploratory Resources for Biology Teachers and Students" in the year 2005. This project aims to develop various resources to supplement existing biology curricula and help teachers engage young people in current issues of modern biology. In particular, SFN will develop materials and practical experimental kits to be used in classrooms and produce a comprehensive database of educational graphics, diagrams and animations.
- SFN applied for the EU 6th Frame Program along with such respected organizations as EMBO and National Centre for Biotechnology Education at the University of Reading, UK to establish a broad platform for exchanging ideas, protocols and disseminations of the work of similar organizations in Europe. Visit www.eurovolvox.org for further information.

Diversity of Funding IIMCB `2004



Sources	amounts in PLN	amounts in EURO*
Budgetary Subventions	6 351 738	1 557 180
Domestic Grants – KBN/MNiI	564 300	138 343
Foreign Grants – EU, Max-Planck Institute, EMBO, NIH	2 170 309	532 069
Other Funds	29 542	7 242
Investments Subventions	3 418 420	838 053
Total	12 534 308	3 072 888

** 1 EURO = 4,0790 PLN at 31st Dec `2004*



Expenses of IIMCB

PROFIT & LOSS STATEMENT	amounts in PLN
NET REVENUE ON SALES AND EQUIVALENTS:	9 778 074
Net revenue on sales of products	5 347 326
Change of work in progress	4 430 748
OPERATIONAL ACTIVITY COSTS	9 889 507
Depreciation	377 940
Materials and energy	3 792 754
Services	902 151
Fees and taxes	336 560
Salaries and wages	2 752 056
Social and health insurance	558 144
Other operational expenses	1 169 902
PROFIT / LOSS ON SALES	-111 433
OTHER OPERATIONAL INCOME	95 489
Subventions	89 835
Other operational income	5 654
OTHER OPERATIONAL EXPENSES	3 206
Other operational expenses	3 206
PROFIT / LOSS ON OPERATIONAL ACTIVITY	-19 150
FINANCIAL INCOME	179 444
Interests; in this:	123 418
Others	56 025
FINANCIAL EXPENSES	94 828
Interests; in this:	219
Others	94 609
PROFIT / LOSS ON BUSINESS ACTIVITY	65 466
EXTRAORDINARY ITEMS	0
GROSS PROFIT / LOSS	65 466
INCOME TAX	0
NET PROFIT / LOSS	65 466

Staff at IIMCB

(as of April 2005)

Name	Function	Employer
Jacek Kuznicki	Director	IIMCB
Michal Witt	Deputy Director for scientific matters	IIMCB (1/2)
Maria Kleska	Deputy Director for administrative matters	IIMCB
Hanna Iwaniukowicz	Financial Manager	IIMCB
Sylwia Adamiec	Accounting Specialist	IIMCB
Monika Nowicka	Payroll Specialist	IIMCB
Beata Tkacz	Director's Assistant	IIMCB
Dorota Urbanowska	Director's Assistant	IIMCB (maternity leave)
Agnieszka Karbowska	Tenders Specialist	IIMCB
Urszula Bialek-Wyrzykowska	Foreign Affair Manager	CEMBM/IIMCB (1/2)
Magda Glogowska	Foreign Affair Manager	CEMBM
Agnieszka Ziemka	Planning and Reporting Manager	IIMCB
Krystyna Domanska	Human Resources Specialist	IIMCB (1/2)
Ewa Blazewicz	Secretarial Assistant	IIMCB
Rafal Flis	Network Manager	IIMCB
Andrzej Kociubinski	Network Manager's Assistant	IIMCB (1/2)
Andrzej Sliwowski	Network Manager	IIMCB (sick leave)
Robert Banasiak	Electrician & Technical Support	IIMCB

Department of Molecular Biology

Name	Function	Employer
Maciej Zylicz	Head	IIMCB
Alicja Zylicz	Assistant Head	IIMCB
Lech Trzeciak	Research Assistant	IIMCB (until April, 2004)
Marcin Klejman	Research Assistant	IIMCB
Marta Bucko-Justyna	PhD Student	Utrecht fellowship
Grzegorz Kudla	PhD Student	SMM
Aleksandra Helwak	PhD Student	IBB
Leszek Lipinski	PhD Student	IBB
Malgorzata Gutkowska	PhD Student	UW/FNP
Joanna Boros	PhD Student	Utrecht fellowship
Dawid Walerych	PhD Student	SMM
Bartosz Wawrzynow	MSc Student	Volunteer
Grazyna Orleanska	Secretary	IIMCB
Wanda Gocal	Technician	IIMCB

Laboratory of Molecular Immunology

Name	Function	Employer
Jaroslaw Dastych	Head	IIMCB
Violetta Adamczewska	PhD Student	IIMCB
Dominika Trzaska	PhD Student	IIMCB
Maciej Olszewski	PhD Student	Utrecht fellowship
Patrycja Zembek	MSc Student	Volunteer

Laboratory of Bioinformatics and Protein Engineering

Name	Function	Employer
Janusz M. Bujnicki	Head	IIMCB
Krzysztof Skowronek	Research Coordinator	EU
Michal Boniecki	PhD Student	IIMCB
Ryszard Matlak	Research Assistant	IIMCB
Agnieszka Chmiel	PhD Student	IIMCB
Iwona Cymerman	PhD Student	SMM
Michal Gajda	PhD Student	IIMCB
Marcin Feder	PhD Student	SMM
Michal Kurowski	PhD Student	IIMCB
Sebastian Pawlak	PhD Student	IIMCB
Elżbieta Purta	PhD Student	IIMCB
Joanna Sasin	PhD Student	IIMCB
Paulina Sroczynska-Obuchowicz	PhD Student	IIMCB
Karolina Tkaczuk	PhD Student	IIMCB
Jan Kosinski	MSc Student	Volunteer
Agnieszka Obarska	MSc Student	Volunteer
Stanislaw Dunin-Horkawicz	MSc Student	Volunteer
Grzegorz Papaj	MSc Student	IIMCB
Marcin Pawlowski	MSc Student	Volunteer
Malgorzata Durawa	PhD Student	IIMCB
Michał Wrzesinski	Office Manager	IIMCB
Michal Rajkowski	Computer Administrator	EU
Maciej Stopa	Computer Administrator	EU

Laboratory of Structural Biology MPG/PAN

Name	Function	Employer
Matthias Bochtler	Head	Max Planck
Izabela Sabala	Post-doctoral Fellow	EU/Max Planck
Honorata Czapinska	Post-doctoral Fellow	FNP Fellowship
Grzegorz Chojnowski	PhD Student	Max Planck/KBN grant
Renata Filipek	PhD Student	Max Planck (DFG)
Henryk Korza	PhD Student	Max Planck
Magdalena Lipka	PhD Student	Max Planck
Małgorzata Marcyjaniak	PhD Student	EU/Max Planck
Sergey Odintsov	PhD Student	Max Planck/Nencki Fellow
Monika Sokolowska	PhD Student	Max Planck
Roman Szczepanowski	PhD Student	EU/Max Planck
Magdalena Kaus	PhD Student	Max Planck/IIMCB

Laboratory of Biomodelling

Slawomir Filipek	Head	IIMCB
Anna Modzelewska	PhD Student	IIMCB/UW
Krystiana Krzysko	PhD Student	IIMCB
Michal Kolinski	PhD Student	SMM

Laboratory of Cell Biology

Name	Function	Employer
Marta Miaczynska	Head	IIMCB (since April 2005)
Marta Brewinska	PhD Student	IIMCB (since Feb. 2005)
Anna Zarebska	PhD Student	IIMCB (since Feb. 2005)

Laboratory of Neurodegeneration

Name	Function	Employer
Jacek Kuznicki	Head	IIMCB
Urszula Wojda	Associate Professor	IIMCB
Cezary Zekanowski	Associate Professor	IIMCB (½)
Andrzej Lewandowicz	Post-doctoral Fellow	IIMCB/EU (since May, 2004)
Monika Mysiak	Post-doctoral Fellow	IIMCB (since Jan, 2005)
Marta Wisniewska	Post-doctoral Fellow	IIMCB (since Oct, 2004)
Gang Zhao	Post-doctoral Fellow (until Sept, 2004)	Polish Gov. Fellowship
Magdalena Blazejczyk	PhD Student	IIMCB/Nencki Fellow
Lukasz Bojarski	PhD Student	IIMCB/Nencki Fellow
Adam Sobczak	PhD Student	IIMCB/Nencki Fellow
Wojciech Michowski	PhD Student	Nencki Fellow
Aleksandra Szybinska	PhD Student	IIMCB
Malgorzata Mossakowska	Centenarians Project - coordinator	IIMCB
Katarzyna Broczek	Centenarians Project	KBN grant
Malgorzata Kupisz-Urbanska	Centenarians Project	KBN grant

School of the Science Festival

Name	Function	Employer
Jaroslaw Bryk	Head until February 2004, now consultant	IIMCB/Nencki/IBB
Joanna Lilpop	Head since March 2004	IIMCB/SMM
Anna Lorenc	Consultant	SMM
Wojciech Kuban	Teacher	IBB
Takao Ishikawa	Teacher	UW
Sebastian Pawlak	Teacher	IIMCB
Urszula Brykczyńska	Teacher	UW
Aleksandra Kwiatkowska	Teacher	UW
Berenika Pokorska	Teacher	UW
Agata Rogowska	Teacher	UW
Anna Zarebska	Teacher	IIMCB
Maciej Kotlinski	Teacher	UW
Aleksandra Paczkowska	Teacher	UW
Wojciech Grajkowski	Teacher	Nencki
Grzegorz Papaj	Teacher	UW
Marta Badurek	Volunteer	Nencki/IBB
Jakub Urbanski	Volunteer	
Malgorzata Minta	Volunteer	UW
Pawel Mazur	Volunteer	UW

Coworkers of Prof. R. Przewlocki, Institute of Pharmacology PAN, Cracow

Name	Function	Employer
Barbara Ziolkowska	Research Assistant (since March 2002)	KBN grant
Katarzyna Starowicz	PhD Student	Utrecht Univ. Fellowship
Malgorzata Piechota	PhD Student	Utrecht Univ. Fellowship