

**NATIONAL CENTRE FOR SCIENTIFIC RESEARCH
"DEMOKRITOS"**

INSTITUTE OF BIOLOGY

**2011
ANNUAL REPORT**

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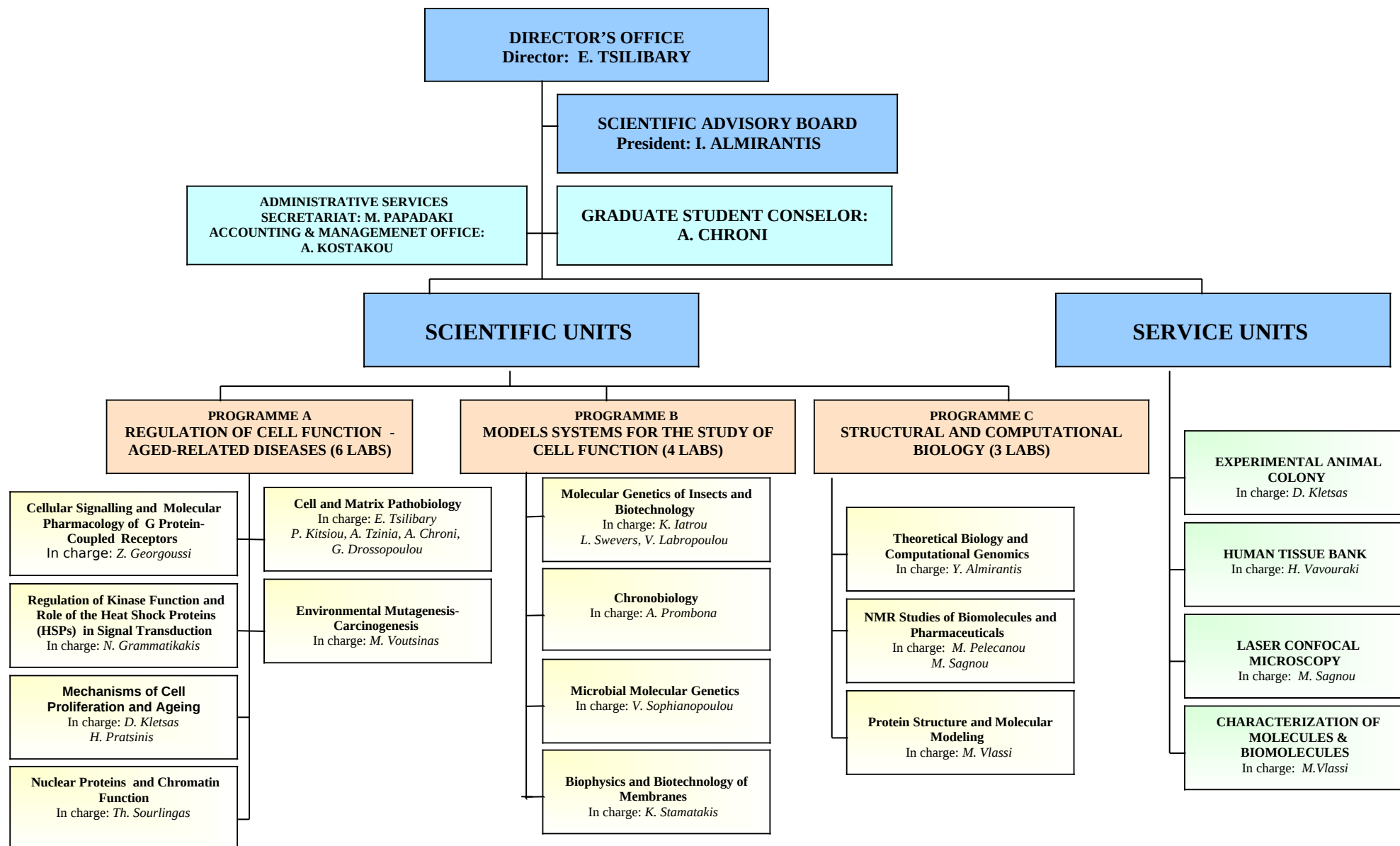
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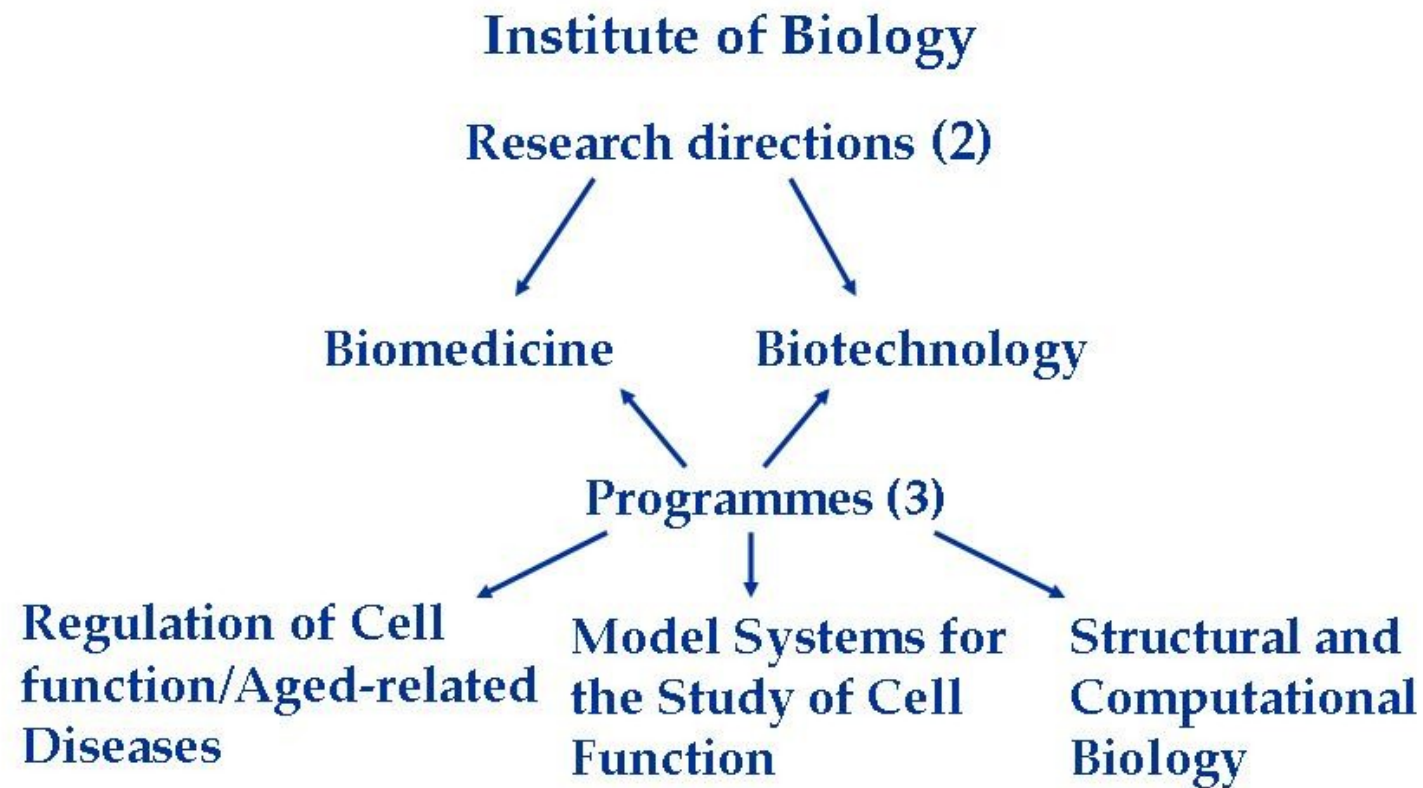
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ORGANISATION CHART





PERSONNEL



DIRECTOR

Tsilibary Effie

MD, Cell Biologist

ACTING DIRECTOR

Vlassi Metaxia

Physicist-Chrystallographer

SCIENTIFIC STAFF

Research Directors

Almirantis Yannis
Georgoussi Zafiroula-Iro
Iatrou Kostas

Chemist
Biochemist
Professor of Biochemistry and Molecular
Biology
Biologist
Pharmacist
Biologist
Biologist
MD, Cell Biologist
Physicist-Chrystallographer

Kletsas Dimitris
Pelecanou Maria
Sophianopoulou Vassiliki
Swevers Luc
Tsilibary Effie
Vlassi Metaxia

Senior Researchers

Chroni Aggelika
Grammatikakis Nikolaos
Konstantopoulou Maria
Labropoulou Vassiliki
Prombona Anastasia
Sourlingas Thomae
Stamatakis Konstantinos
Tzinia Athina
Voutsinas Gerassimos

Biologist
Cell Biologist
Biologist
Biochemist
Biologist
Biologist
Biologist
Biochemist
Biologist

Researchers

Kitsiou Paraskevi
Vavouraki Helen

Biologist
Radiopharmacist

Lecturers

Drossopoulou Garifallia
Pratsinis Haris
Sagnou Marina

Biologist
Chemist
Biologist/ Chemist

Technical Specialists

Panagiotopoulou Angeliki
Stefanou Dimitra

Biochemist
Agronomist

RESEARCH TECHNICIANS

Avgeris Socrates
Doulgeridis George
Kakkos Stilianos
Kopanelis Dimitris (Retired)
Kotsopoulou Eleni
Pantazi-Mazomenou Anastassia
Zafiroopoulos Ioannis

ADMINISTRATIVE STAFF

Girlemis Dimitrios	Technician for electronic equipment & maintenance of equipment
Kostakou Athanassia	Accountant
Papadaki Margarita	Secretary
Pragastis Apostolos	Administrator

EMERITUS & COLLABORATING SCIENTISTS

Emeritus Scientists

Ignatiadou Lydia (Dr. Hydrobiologist) - *Emeritus*
Leventis Minas (DDS) - *Collaborating Scientist*
Papageorgiou George (Dr. Biophysicist) - *Emeritus*
Papageorgiou Spyros (Dr. Physicist) - *Emeritus*
Sekeris Kalliope (Dr. Biochemist) - *Emeritus*
Sideris Eleftherios (Dr. Geneticist) - *Emeritus*
Stathakos Dimitrios (Dr. Biochemist) - *Emeritus*
Tsimilli - Michael Meropi (Dr. Biologist) - *Collaborating Scientist*

LLaboratory

Iatrou K.
Vavouraki H.
Stamatakis K.
Almirantis I.
Sourlingas Th.
Sophianopoulou V.
Kletsas D.
Stamatakis K.

POSTDOCTORAL FELLOWS

Fellow

Benaki Demetra (NCSR "Demokritos")
Fragouli Apostolia (NCSR "Demokritos")
Koussis Konstantinos (Programme)
Kythraioti Georgia (Programme)
Lambidonis Antonios (IKY)
Mavrogonatou Eleni (Programme)
Nikolaou Christoforos (NCSR "Demokritos")
Tsitoura Panagiota (Programme)
Vamvakas Sotirios - Spiridon (NCSR "Demokritos")

Supervisor

Pelekanou M.
Tsilibary E.
Iatrou K.
Iatrou K.
Voutsinas G.
Kletsas D.
Almirantis I.
Iatrou K.
Kletsas K.

GRADUATE STUDENTS

Student

Aliberti Sofia (NCSR "Demokritos")
 Apostolou - Karabelis Konstantinos (NCSR "Demokritos")
 Argyri Letta (Programme)
 Armatas Andreas (NCSR "Demokritos")
 Athanassopoulos Alexandros (NCSR "Demokritos")
 Daniil George (NCSR "Demokritos")
 Dafnis Ioannis (Programme)
 Dimozi Anastasia (NCSR "Demokritos")
 Galeou Aggeliki (NCSR "Demokritos", *MSc*)
 Georganta Irene (Programme)
 Ioannidis Konstantinos (NCSR "Demokritos")
 Kapodistria Katerina (NCSR "Demokritos")
 Karkoulis Panagiotis (NCSR "Demokritos")
 Kolliopoulou Anna (NCSR "Demokritos")
 Konstantinou Vasos (Programme)
 Koutmos Theodore (NCSR "Demokritos")
 Kostomiri Mirto (NCSR "Demokritos")
 Leontiadis Leonidas (NCSR "Demokritos")
 Magkrioti Christiana (Programme)
 Papadopoulou Adamadia (Programme)
 Papakonstantinou Maria (NCSR "Demokritos")
 Repouskou Anastasia (NCSR "Demokritos")
 Salpea Paraskevi (NCSR "Demokritos")
 Tsoதாக Nikos (NCSR "Demokritos")
 Vaggelatos Ioannis (NCSR "Demokritos")
 Xidous Marios (NCSR "Demokritos")

Supervisor

Grammatikakis N.
 Almirantis I.
 Chroni A.
 Kletsas D.
 Sophianopoulou V.
 Chroni A.
 Chroni A. -*PhD obtained*
 Kletsas D.
 Prombona A. - *MSc obtained*
 Georgoussi Z.
 Iatrou K.
 Kitsiou P.
 Voutsinas G. -*PhD obtained*
 Iatrou K.
 Kletsas D. -*PhD obtained*
 Tsilibary E.
 Pelecanou M.
 Georgoussi Z.
 Iatrou K./V. Labropoulou -*PhD obtained*
 Kletsas D.
 Georgoussi Z.
 Prombona A. -*PhD obtained*
 Sourlingas Th. -*PhD obtained*
 Tsilibary E.
 Sophianopoulou V.
 Sourlingas Th.

GRADUATE RESEARCH ASSOCIATES

Fellow

Raptis Konstantinos (MD)
 Raptopoulos Dimitris (Dr. Biologist)
 Sellis Diamadis (*MSc*)

Supervisor

Vavouraki H.
 Iatrou K.
 Vlassi M.

COLLABORATING GRADUATE STUDENTS

Student (University)

Delimitsou Aggeliki (Univ. of Athens, *MSc*)
 Fotopoulos Vassilis (Univ. of Ioannina)
 Geranios Pavlos (Univ. of Athens, *MSc*)
 Konstantatou Evmorphia (Univ. of Athens)
 Konstantonis Dimitris (Univ. of Athens)
 Lagopati Nefeli (Athens Polytechnic School)
 Lefaki Maria (Univ. of Athens, *MSc*)
 Liakou Maria (Univ. of Athens, *MSc*)
 Polichronopoulos Dimitris (Univ. of Athens, *MSc*)
 Peristeri Eleutheria (Univ. of Athens, *MSc*)
 Rapti Maria - Aikaterini (Univ. of Athens, *MSc*)
 Tsiagas Ioannis (Univ. of Athens, *MSc*)
 Verouti Sofia (Univ. of Athens)

Supervisor

Voutsinas G.
 Tzinia A.
 Sophianopoulou V.
 Voutsinas G.
 Kletsas D.
 Tsilibary E./Tzinia A.
 Kletsas D. - *MSc obtained*
 Kletsas D.
 Almyrantis I. - *MSc obtained*
 Voutsinas G.
 Kletsas D. - *MSc obtained*
 Almyrantis I. - *MSc obtained*
 Tsilibary E. .

UNDERGRADUATE STUDENTS AND OTHER IN TRAINING

Student (University)

Supervisor

Balis Christos (Univ. of Athens)	Chroni A.
Bassoyianni Angeliki - Stephania (Univ. of Athens)	Voutsinas G.
De Wilde Ruben (Univ. of Gent, Belgium)	Swevers L.
Diakatou Markella (Univ. of Athens)	Voutsinas G.
Florou-Servou Amalia (Univ. of Athens)	Georgoussi Z.
Golfinopoulou Christina (Univ. of Athens)	Chroni A.
Kyritsis Konstantinos (Univ. of Thessaloki)	Chroni A.
Kritharidou Anna (Univ. of Patras)	Georgoussi A.
Nikou Theodora (Agricultural Univ. of Athens)	Stamatakis K.
Pantazopoulou Vassiliki (Univ. of Athens)	Sophianopoulou V. - <i>undergraduate dissertation completed</i>
	Konstantopoulou M.
Panteleri Rafaela (Univ. of Thessaloniki)	Georgoussi Z.
Sachinidis Athanassios (Univ. of Thessaloniki)	
Siouti Eleni (Univ. of Athens)	Kletsas D.
Vrana Evi - Stavroula (Univ. of Athens)	Georgoussi Z.

INTRODUCTION



The Institute of Biology (IB) is one of eight institutes of National Center for Scientific Research (NCSR) Demokritos. The Center has the unique feature of combining scientists with different expertise who conduct basic research and collaborate. The thrust is optimal research and technology progress in the thematic areas covered by research interests of researchers from the different institutes.

The IB with 23 faculty members, has recently obtained significant new and upgraded equipment through competitive funding for infrastructure for the total amount of over 500.000 €; Two major service laboratories, the animal colony and the human tissue bank have been certified by international standards (ISO 9000/2001); these laboratories are gradually upgraded by adding new equipment, offices and/or office space, and space for experimentation.

The IB has significant independent and collaborating activities aiming at internationally competitive research and the achievement of excellence. One of the features of the IB is multidisciplinary biomedical and biotechnological research, evident from the nature of the three programs and the published research papers as well. Interdisciplinary research related to life sciences and the environment is a unique and characteristic advantage (an “earmark”) of NCSR Demokritos and the IB as well. Presently, the conducted interdisciplinary research focuses on the environment, the development of innovative molecules and biomolecules for diagnostic and therapeutic use, nanomaterials for medical and imaging purposes, targeted drug delivery, etc. These activities form a dynamic research cluster which includes many IB researchers. Moreover, the main independent IB activities focus on the one hand on biomedical research with different biochemical, cellular, molecular, pharmaceutical, proteomic and other approaches; on the other hand, biotechnological research is performed related to the environment, using similar approaches. Finally, structural, bio-informatical and theoretical approaches make up one more research direction of the IB.

Retired, emeritus researchers proved to be very active once again contributing publications, seminars, participation to IB research projects etc, thus being a significant part of IB productivity.

In 2011, the PhD candidates, awardees of the “Akogionoglou Award” (in honor of the internationally recognized scientist who was a researcher and Head of the IB ((1969-1975, 1979-1982), was: It should be noted that all submitted applications were extremely competitive, an indication of research of outstanding quality which is performed at the IB.

Apart for the hopeful news for nascent researchers-scientists, the IB was faced with the extremely saddening event, to bid farewell to two young PhD candidates, Anastasia Dimozi and Andreas Armatas, who passed away due to an accident on 15/1/2012. In their honor and memory, the Center inaugurated in 2012 awards and two studentships, on in each of their names. It is hoped that these awards and studentships will reflect the promise of quality research which was demonstrated by the two unjustly lost young people.

A victory was conquered following merging of different research institutes, which was proposed in the Fall 2011 and put to action in March 2012, that of maintaining the IB intact, with a new identity, as “**Institute of Biosciences and Applications**” (IB-A), in the new structure of the Center, which is now comprised five Research Institutes.

I wish to thank the members of the IB scientific advisory committee (Dr. I. Almirantis, Dr. A. Chroni, Dr. M. Pelecanou, Dr. C. Stamatakis, and Dr. M. Vlassi) and the Educational Committee (Dr. A. Chroni, Dr. M. Sagnou) who supported the task of upgrading the IB. I am also thankful to all researchers who participated in various committees, as well as the Vice-Director of the IB, Dr. Vlassi, who substantially contributed to a smooth operation of the IB and helped with my numerous administrative tasks, especially under extremely stressful conditions (inside and outside the Center), during discussions for merging of different biologically-oriented institutes.

Despite the various obstacles and difficulties (particularly intense this year due to the financial crisis), the support and confidence constantly provided by the majority of researchers is a main source of optimism and confidence in successfully achieving the aim of upgrading the IB towards excellence. It is my belief that based on objective indexes such as: funding, number of publications, citations, etc, the IB progressively becomes recognized as an internationally competitive institute (“RAND” report, 2011). I have trust in the research potential of IB researchers who prove on a daily basis their contribution and keep trying harder and getting better, and I extend the best wishes to all members for best success in their continued efforts. With a positive attitude, persistence in the aim of excellence and un-relented effort, the unprecedented financial crisis which we experience during the last three years, can be reverted to a series of opportunities for all; more so for young scientists who represent hope for an immediately better future and promising perspectives.

Finally, I wish to heartfully thank Ms. Athanasia Kostakou, the IB accountant, and Ms. Margarita Papadaki, the IB secretary.



Effie C. Tsilibary, MD, PhD

Head, Institute of Biology
April 2012

PROGRAMME A:
REGULATION OF CELL FUNCTION
AGED-RELATED DISEASES

Research

Group:

Cellular Signalling and Molecular Pharmacology of G Protein-Coupled Receptors

Research Staff

Iro Georgoussi, Research Director

Leonidas Leontiadis, Graduate Student

Eirini- Maria Georganta, Graduate Student

Maria- Pagona Papakonstantinou, Graduate Student

Evi-Stavroula Vrana, Undergraduate Student

Anna Kritharidou, Undergraduate Student

Thanassis Sachinidis, Training Student

Amalia Florou-Zervou, Training Student

Research Interests

The research interests of our group are focused on the **elucidation of the molecular mechanisms governing the G protein-coupled receptors (GPCRs) signalling**. We use as a model system the opioid receptors because of their involvement in pain perception and in mechanisms related to tolerance and dependence upon chronic drug administration.

More specifically our objectives aim to identify:

- ◆ novel opioid-or other GPCR interacting partners and signaling pathways in order to define novel pharmacological factors
- ◆ transcription factors and genes whose action is altered upon opioid receptor activation,
- ◆ pharmacologically novel ligands that bind to the μ , δ and κ -opioid receptors as potential new compounds- analgesics- to alleviate chronic pain.

2011 Findings

New signalling pathways mediated upon activation of μ -, δ - and κ - opioid receptors

In an attempt to investigate the signaling pathways mediated upon activation of the **μ , δ and κ** -opioid receptors (μ -OR, δ -OR, κ -OR) and understand the mode of action of Regulators of G protein Signaling (RGS proteins) in opioid signalling we investigated whether selectivity of coupling between the μ -OR, δ -OR and κ -OR with members of RGS proteins exist. We thus determined **RGS2** protein as a novel interacting protein. We identified that RGS2 interacts differentially with the three opioid receptor subtypes and demonstrated the ability of this protein to modulate opioid receptor signalling.

We also demonstrated that **spinophilin**, a neural scaffolding protein enriched in dendritic spines interacts specifically with the C-terminal domains of the δ - and μ -opioid receptors and G proteins. Binding of spinophilin modifies cAMP levels and MAPK phosphorylation mediated by both opioid receptors, thus establishing spinophilin as a new interacting partner participating in a multimeric signaling complex that displays a differential regulatory role in opioid receptor function.

Activation of transcription factors upon δ -opioid receptor stimulation and their role in neurite outgrowth

In an attempt to elucidate the molecular mechanisms involved in the phosphorylation of transcription factors after opioid administration, we demonstrated that the δ -opioid receptor (δ -OR) serves as a platform for the formation of a multi-component signalling complex, "signalosome" consisting of p-STAT5B- c-Src kinase and specific subunits of G proteins. This "signalosome" is implicated in cell survival and plays a critical role in neurite length and outgrowth in Neuro2A cells upon δ -opioid receptor activation with specific agonists (Fig. 1).

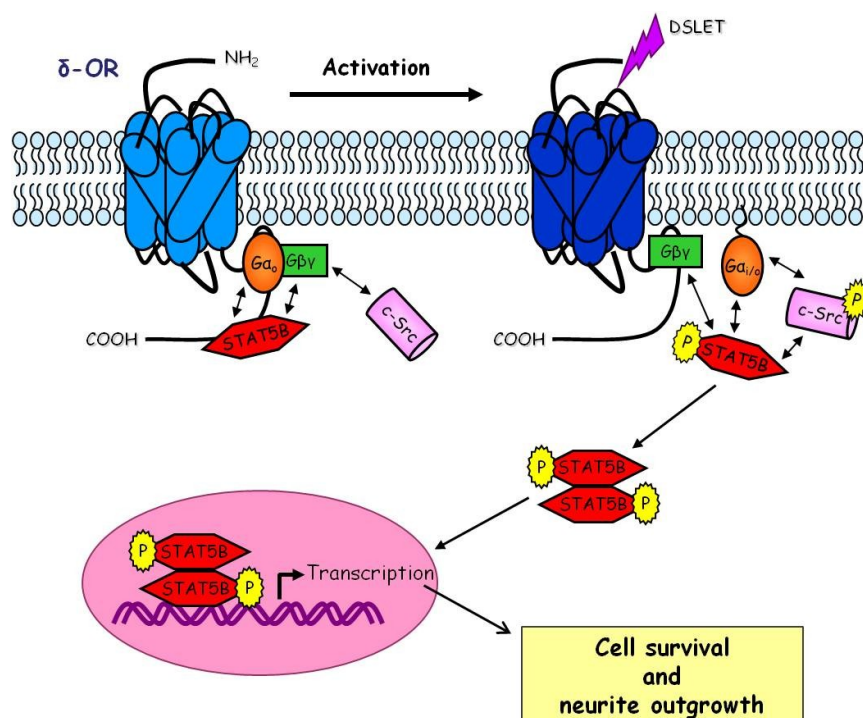


Figure 1

Putative signaling pathway of the δ -opioid receptor-STAT5B-G protein complex, implicated in STAT5B phosphorylation leading to cell survival and neurite outgrowth

Pharmacological evaluation of new selective compounds targeting the different G protein coupled receptors / development of high throughput screens

Under the 6th Framework we participate in the EU consortium “NORMOLIFE” (LSHC-CT2006-037733). Our group characterized new pharmacological ligands (synthesized by other members of the consortium) that bind to the μ -, δ - or κ -opioid receptors, expressing mixed agonistic and antagonistic properties. These analogs display also potent *in vivo* analgesic effects as assessed using brain-region specific primary network co-cultures and microelectrode neurochip technology prior to animal studies.

Molecular mechanisms mediated by the olfactory receptors of the mosquito

***Anopheles gambiae*:** In collaboration with the group of Insect Molecular Genetics and Biotechnology of the Institute of Biology and under the 7th FP framework with the EU consortium “ENAROMaTIC” coordinated by Prof. K. Iatrou (IBE). Our efforts focused on the elucidation of the signalling mechanisms regulating the *Anopheles gambiae* mosquito olfactory receptors. We found that the mosquito odorant receptors OR1 and OR2, exhibit a reverse orientation as compared to the classical GPCRs. We also performed studies on the binding parameters of these receptors using tritiated ligands.

Publications

Vandormael B., Fourla D, Gramowski A., Weiss D., Georgoussi Z. and Tourwé D. (2011) Design and Synthesis of Dmt1-[dermorphin] tetrapeptide as μ/δ opioid receptor agonists. *J. Med.Chem.*, 54, 7848-7859.

Pasquinucci, L., Parenti, C., Turnaturi, R., Aricò, G., Marrazzo A., Prezzavento O., Ronsisvalle G., Georgoussi Z., Fourla D-D., Scoto G-M., and Ronsisvalle G.m (2012) The benzomorphan-based LP1 ligand is a suitable MOR/DOR agonist for chronic pain treatment *Life Sciences*, 90, 66-70.

Georgoussi, Z., Georganta, E-M., Milligan, G. (2012). The other side of opioid receptor signalling: Regulation by protein-protein interaction. *Curr. Drug Targets*, Vol. 13, 80-102.

Articles in Press

Fourla D., Papakonstantinou, M.-P., Vrana E-M. and Georgoussi Z. Selective interactions of spinophilin with the C-terminal domains of the δ - and μ -opioid receptors and G proteins differentially modify opioid receptor signalling (IF 4.243)

Articles in Books and Conference Proceedings

Leontiadis, L. J., Papakonstantinou M -P. and Georgoussi Z. (2010) Regulator of G protein signaling 4: a novel regulator of μ - and δ -opioid receptor signaling. *Review of Clinical Pharmacology and Farmakokinetikes International Edition*, 24 (2), 168-170,

Georganta, E-M., Agalou, A., Georgoussi, Z. STAT5B forms dynamic complexes with the δ -opioid receptor and selective G protein subunits. *Review of Clinical Pharmacology and Farmakokinetikes International Edition*, 24 (2), 91-94

Georgoussi, Z (2010) «Seven Transmembrane Receptors and G proteins: Health, pathogenesis and development of new drugs » Summer School N.C.S.R. "DEMOKRITOS" p.117-118

Presentations at Scientific Conferences

Georgoussi Z. (2011) "The other side of opioid receptor signaling: regulation by protein-protein interaction" 23^d Biennial Meeting of the ISN-ESN International Society for Neurochemistry, August 28- September 2, 2011 Athens, Greece (invited speaker).

M.-P. Papakonstantinou , L.J. Leontiadis, M. Sarris, F. Nikolos and Z. Georgoussi. RGS2 and RGS4 proteins act as novel modulators of kappa and delta opioid receptors signaling. European Opioid Conference, April 13-15, 2011 Krakow, Poland (Poster)

M.-P. Papakonstantinou and Z. Georgoussi. Kappa opioid receptor signalling is differentially modulated by the Regulators of G protein Signaling RGS4 and RGS2 proteins. 23rd ISN-ESN Biennial Meeting of the International Society of Neurochemistry and the European Society of Neurochemistry, August 28- September 1, 2011 , Athens Greece

E-M. Georganta, and Z. Georgoussi. δ -opioid receptor-induced neurite outgrowth is mediated by G proteins and the STAT5B transcription factor European Opioid Conference, April 13-15, 2011 Krakow, Poland

E-M. Georganta, and Z. Georgoussi. The δ -opioid receptor mediated neurite outgrowth involves G proteins and the Signal Transducer and Activator of Transcription 5B (STAT5B) ISN-ESN Biennial Meeting of the International Society of Neurochemistry and the European Society of Neurochemistry, August 28- September 1, 2011 Athens, Greece

Tsitoura, P., Swevers, L., Georgoussi -Z., and Iatrou, K. A functional expression platform for neuronal GPCRs and ligand-gated ion channels of mammalian and invertebrate origin, ISN-ESN Biennial Meeting of the International Society of Neurochemistry and the European Society of Neurochemistry, August 28- September 1, 2011 Athens, Greece

Makarona, E Georganta, E-M., Georgoussi Z. and Tsamis C. Zinc Oxide Nanotextured Substrates for the Development of Neuronal Circuits. Eurosensors, Athens, 2011

Lorella Pasquinucci, Rita Turnaturi, Carmela Parenti, Giuseppina Aricò, Giovanna Maria Scoto, Zafiroula Georgoussi, Danai-Dionysia Fourla and Giuseppe Ronsisvalle, Medicinal Chemistry 2011, Catania 28-30 June 2011

P. Tsitoura, L. Swevers, Z. Georgoussi, K. Iatrou (2011). Silkworm functional expression platforms for neuronal GPCRs and ligand-gated ion channels. Second Second International Symposium on "*Bombyx mori* Functional Genomics and Modern Silkworm", October 22-23, 2011, Chongqing, China

M.-P. Papakonstantinou ,L.J. Leontiadis, F. Nikolos and Z. Georgoussi. RGS2 and RGS4 proteins: novel modulators of kappa and delta opioid receptor signaling. 10th Pharmacology Symposium, 12th March, 2011, Athens, Greece

M.-P. Papakonstantinou and Z. Georgoussi. RGS2 and RGS4 proteins are novel modulators of kappa opioid receptor signalling. 25th Hellenic Society for Neuroscience Meeting, November 24-26, 2011, Patras, Greece. (Poster)

M.-P. Papakonstantinou and Z. Georgoussi. RGS2 and RGS4 proteins: novel interacting partners of kappa opioid receptor. 62nd Conference of the Hellenic Society of Biochemistry and Molecular Biology, December 9 -11, 2011, Athens, Greece. (Poster)

E-M. Georganta and Z. Georgoussi. δ -opioid receptor-induced neurite outgrowth is mediated by G proteins and the STAT5B transcription factor. 10th Pharmacology Workshop, 12 March 2011, Athens, Greece (oral presentation).

E-M. Georganta and Z. Georgoussi. δ -opioid receptor activation leads to neurite outgrowth and neuronal survival via a STAT5B-G α i/o pathway. 62nd Greek Conference of Biochemistry and Molecular Biology, 9-11 December 2011, Eugenides Foundation, Athens, Greece (oral presentation).

Educational Activities

Z. Georgoussi:

Supervision of the Ph.D theses of the graduate students:

E. Georganta L. Leontiadis (University of Athens) and M. Papakonstantinou (University of Patras)

Member of the advisory committee in the Department of Biology of the University of Athens for the Ph.D candidates E. Georganta and L. Leontiadis

Member of the advisory committee in the Department of Pharmacy of the University of Patras for the Ph.D candidate M. Papakonstantinou.

Member of the Recruitment Committee for postdoctoral fellowships in the I.B.A.

Teaching in the Postgraduate Graduate Program on the "Molecular Basis of Human Diseases" in the University of Athens, Sector of Biochemistry and Molecular Biology in the Department of Biology

Graduate Program, "Molecular basis of Human Disease" of the University of Athens Department of Biochemistry and Molecular Biology, University of Athens, with title: G proteins in health and disease"

Participation in the Educational Exchange Program of the University of Catania, Sicily, Italy

Participation in the Postgraduate Course of the Polish Academy of Sciences, Warsaw, Poland

Research Seminars held by I. Georganta and M. Papakonstantinou under the postgraduate program of the IBA

Research Seminars held by L. Leontiadis and I. Georganta in the University of Athens, Department of Biochemistry & Molecular Biology and the Department of Animal Physiology respectively, Faculty of Biology, Athens

Other Scientific Activities

Z. Georgoussi:

Co-founder together with Prof. K. Iatrou and Dr L. Swevers of the spin-off company GENEXPA

Reviewer of Articles for the Scientific Journals, Molecular Pharmacology, Journal of Neurochemistry, Journal of Pharmacology and Experimental Therapeutics, Cellular Signaling, Neuropharmacology, Neuropharmacology Journal of Biotechnology, Journal of Neuroscience, BioMed Cell Biology

Reviewer of grant proposals of the General Secretary for Research of Cyprus (RPF), Cyprus, General Secretary of Research and Technology, National Scholarship Foundation (IKY)

Training in *Cell Imaging* in the National Institute on Drug Abuse (NIDA), Baltimore, Maryland USA in Dr Shipenberg's lab (29 June - 4 August 2011)

Member of the WISE (Working group of Women in Science) of FEBS

Member of "Periktioni" Network representing the N.C.S.R. "Demokritos" for the Greek Women Scientists

Member of the Scientific Organizing Committee of the 23d International Neurochemistry conference (August 2011)

Member of the International Research Network for palliative care "*Normolife*", related with the design, development and assessment of new analgesic compounds

Member of the Organizing Committee of the 62nd Panhellenic Conference of Biochemistry and Molecular Biology, December 2011, Athens

Speaking Invitations

23d International Neurochemistry Meeting and Biennial Meeting of the ISN-ESN International Society for Neurochemistry, Session: Cytoskeletal Interactions in the Regulation of GPCR signaling, on "*The other side of opioid receptor signaling: regulation by protein-protein interaction*" August 28- September 2, 2011 Athens, Greece, (Z. Georgoussi)

Other Distinctions and Awards

Honorable distinction with financial Award of the Greek Society for of Pharmacology (2011) for the work entitled «*A signalosome formed between the δ -opioid receptor, STAT5B and G protein subunits is implicated in neuronal survival and neurite outgrowth*» by M. Georganta, A. Agalou, Z. Georgoussi (I. Georganta).

Other Activities for the Institute of Biology

Z. Georgoussi:

Member of the evaluation committee for postdoctoral researchers

Responsible for functioning of ultracentrifuges Beckman L8 & Optima max, speedvac concentrator, spectrometer

Impact factor (for 3 publications): 11,912

Citations for 2011 (without self citations): 21

Citations for 2007-2011 (without self citations): 105

h-factor: 14

Laboratory Equipment and Common Usage Equipment (IB)

- Tissue culture: incubators laminar flow, microcentrifuges, inverted microscope
- Biochemistry: isolation of recombinant proteins, affinity chromatography, gel filtration
- Molecular biology: DNA, RNA and protein electrophoresis, microcentrifuges, DNA sequencer (IBA), ultracentrifuges Beckman L8 & Optima, Scintillation counter, spectrometer (IB), sonicator, spectrometer, speedvac concentrator, scintillation counter (IB)
- Molecular Pharmacology: BRANDEL harvester for multiple filtration, dowex columns for adenylyl cyclase assays and IP3 formation
- Cell Biology : FACS, confocal microscope, luminometer, fluorescence automatic cell sorter (IBA), fluorescence microscope, confocal microscope (IB), microplate reader for fluorescence and luminescence (for GFP, fluorescein, β -galactosidase, luciferase) (IB)

Current external funding

Research Program entitled *NORMOLIFE -Development of new therapeutic substances and strategies for treatment of pain patients with advanced stages of cancer*, funded by the European Union coordinated by: Prof. A. Lipkowski, with responsible Scientist from Greece: Z. Georgoussi

Duration: 12/2006-12/2009

Total funding of the program (*consortium*): 2.039.925 €

Total funding (of the lab): 541.331 €

Funding of the lab for the year 2011: 26.000 €.

Research Program entitled *ENAROMaTIC- European Network for Advanced Research on Olfaction for Malaria Transmitting Insect Control*, funded by the European Union as responsible Scientist and program Coordinator: Prof. K. Iatrou

Duration: 12/2008-12/2012

Total Funding of the program (*consortium*): 2.500.000 €

Total funding (lab): 50.000

Total funding (lab) for 2011: 12.000 €

Pending proposals:

Bilateral joint proposal cooperation between Hungary-Greece (ESPA 2007-13) entitled «*Selective opioid-neurotensin mosaic peptide analogs targeting heterodimerization between respective receptors*» Prof. G. Toth, Hungarian Academy of Science, GSRT, September 25, 2009 (Coordinating scientist: Z. Georgoussi)

Research Group:

Regulation of Kinase Function and Role of the Heat Shock Proteins (HSPs) in Signal Transduction

Research Staff

Nikos Grammatikakis, Senior Researcher

Sofia Aliberti, Graduate Student

Research Interests

A) Cell Signaling

- Mechanisms of mammalian kinase regulation during normal differentiation and disease
- Chemotherapeutical inhibition of oncogenic kinase activity

B) Cellular Responses to Stress and Nutrition

- Regulation of Chaperone Protein Activity
- Identification of Signaling Mediators (including kinases and transcriptional factors) which are modulated by the Chaperone Machinery in response to Stress and Dietary Factors

C) Cell Cycle Regulation

- The Chaperone Machinery as an effector of cellular Stress in cell cycle progression

D) Novel Molecular Chaperones

- Characterization and study of a group of novel Molecular Chaperones identified in our lab and their potential role as mediators of the assembly and activity of ErbB2, Raf, Akt, Cdk4 and I-kappaB kinases (IKK) in cell proliferation and cell cycle progression. Our study extends to learning how the activity of these novel signal modulators is regulated by Growth conditions and Stress (Radiation and chemotherapeutic drugs).

2011 Findings

The first part of our work in 2004 was carried in our lab in the US (Harvard University/BIDMC). Upon transferring to the Institute of Biology at the NCSR Demokritos we encountered a major hardship and delay in establishing a working environment, especially in regards to finding a proper laboratory space. Nevertheless, we have been able to get around this problem and establishing new collaborations with investigators from the Signaling field continue our experimental work in other labs in the area. The results of the work we have carried out both in the US and in Greece during the past year can be summarized as follows:

In general lines we have continued testing experimentally the model we had proposed 9 years ago, namely that “the activity of a select group of kinases might -in addition to the classic mechanisms- be possibly conformationally modulated by Cdc37 and the chaperone machinery”. To this end, our recent data indicate that p50Cdc37 is the same molecule suspected since the 1980s to, together with Hsp90, bind to and possibly regulate Src (reviewed in: Brugge J., 1986: Interaction of the Rous sarcoma virus protein pp60src with the cellular proteins pp50 and pp90. *Curr Top Microbiol Immunol* 123:1-22). As our current data also show, p50Cdc37 is a crucial regulatory factor for a group of G1-specific kinases which is functionally dependent on interactions with the Chaperone Machinery (HSPs). This group of kinases includes Raf and Src, ErbB2, Akt, the I-kappaB kinases (IKKs), MLK3, the heme-regulated eIF-2alpha kinase (HRI) and, among the cyclin-dependent-kinases, Cdk4, Cdk6 and Cdk9. Further, as our overall data show, the entire assembly and coordinate regulation of at least three crucial signaling kinase modules (Raf>Mek>Erk, IKK>IkappaB>NFkappaB and Cdk4>Cdk4/CyclinD>pRb) might be mediated by the chaperone machinery under physiological conditions. In the above process, the ATPase/folding activity of the participating Hsp90 and Hsp70 plays a central role. Finally, we have reported a novel tripartite connection, that of the MAPK kinase module and of the 14-3-3 family of adaptors with the Heat Shock Factor-1 (HSF-1) and formulated a hypothesis of how the nucleocytoplasmic localization, and as result the transcriptional activity of the later might be affected by upstream signaling.

Articles in Press or to be submitted

Sofia Aliberti, et al. «THE ROLE OF THE HSP90 SIGNALING SYSTEM IN MEDIATING ERBB2 SIGNALING».

This is a collaborative project with colleagues from abroad. The paper has been submitted.

Georgia Messaritou, Sofia Aliberti, et al. « MOLECULAR INTERACTIONS AND FUNCTION OF 14-3-3 (MONOMERS AND DIMERS)»

This is a collaborative project with colleagues from the Fleming Institute. The paper is under preparation.

El Hamidieh Avraam, et al. «Cell Surface CDC37 Participates in Extracellular HSP90 Mediated Cancer Cell Invasion »

This is a collaborative project with colleagues from the Institute Pasteur. The paper has been accepted for publication.

Citations 2011 (without self- citations): 56

Total Citations 2008-2011 (without self- citations): 382

h-factor: 18

Research Group: Mechanisms of Cell Proliferation and Ageing

Research Staff

Dimitris Kletsas, Research Director

Haris Pratsinis, Lecturer

Dimitrios Stathakos, Emeritus Scientist

Sotirios-Spyridon Vamvakas, Postdoctoral Fellow

Eleni Mavrogonatou, Postdoctoral Fellow

Vasos Konstantinou, Graduate Student- *Phd obtained in 2011*

Adamantia Papadopoulou, Graduate Student

Anastasia Dimozi*, Graduate Student

Andreas Armatas*, Graduate Student

Maria - Aikaterini Rapti, Collaborating Graduate Student (*MSc*) - *MSc obtained in 2011*

Maria Lefaki, Graduate Student (*MSc*) - *MSc obtained in 2011*

Dimitris Konstantonis, Graduate Student

Eleni Liakou, Graduate Student (*MSc*)

Eleni Siouti, Undergraduate Student - *Undergraduate dissertation completed in 2010*

Research Interests

The Laboratory is focusing on tissue repair during development and ageing with an emphasis on the role of growth factors, and especially that of TGF- β . The action of growth factors on cell proliferation and extracellular matrix production, as well as the responsible signaling pathways are investigated. Alternative mechanisms of cell proliferation and differentiation, such as autocrine regulation, cell-matrix interactions, exogenous stresses and the effect of mechanical forces are also studied.

Main goal of the Laboratory is the investigation of the mechanisms of ageing and longevity. The structural and functional characteristics of the senescent cell - as a result of successive duplications or of exogenous stresses - in comparison to that of the young or the cancer cell are investigated. Especially, we are interested on the role of the senescent - somatic and stem - cell in the process of ageing and the development of age-related diseases, including cancer. In this direction, we study the interaction between the senescent stromal fibroblasts and adjacent cancer cells. Emphasis is given in tissues, such as the intervertebral disc, the degeneration of which provokes severe dysfunctions during ageing.

Aim of our studies is the elucidation of the mechanisms underlying the regulation of tissue homeostasis, especially during ageing, and furthermore the contribution, through research networks, in the development of cell replacement therapies. Finally, we study natural products and new synthetic compounds with putative anti-cancer, anti-ageing/anti-oxidant and wound healing action, as well as their mode of action.

2011 Findings

In our laboratory the role of growth factors in tissue repair is being studied with a special emphasis in the action of TGF- β . Having in mind the different repair strategies between fetuses and adults, and our previous data demonstrating that TGF- β inhibits the proliferation of human skin fibroblasts of fetal origin but stimulates adult ones, we have shown that TGF- β activity is regulated by the presence of extracellular matrix components, and especially hyaluronic acid.

Main goal of the laboratory is the study of the functional features of senescent cells, as well as, their role in the development of age-related diseases including cancer. We have observed that various anti-tumour treatments, such as ionizing radiation or some chemotherapeutic drugs, induce premature senescence of the stromal cells, that in turn favour the growth of cancer cells.

In addition, we continued our research on the cellular physiology of intervertebral disc degeneration, and especially the cellular response to various stresses, such as hyperosmolality, oxidative stress, and cyclic mechanical stretching. It was observed that these stresses stimulate intracellular signalling pathways leading to cell proliferation regulation and premature senescence.

During our study of the relation between stress and ageing, we compared skin fibroblasts from patients undergoing glucocorticoid treatment for extended time-periods with normal ones and we observed no significant differences in their physiology in vitro.

A further goal of our work is the use of cell therapies in age-related degenerative disorders, by employing either autologous somatic cells or mesenchymal stem cells. We have shown that senescent cells possess a diminished differentiation capacity compared to young ones (Figure 1). Furthermore, by studying artificial skin substitutes available in the market we observed that the factors they secrete inhibit the fibrotic responses of human skin fibroblasts (normal and chronic wound-derived).

In the framework of the EU-funded AgroCos project a library of 480 compounds – isolated from plant extracts – was studied and a considerable number of them was found to possess antioxidant activity and the capacity to protect human skin fibroblasts from the cytotoxic effects of UV-radiation.

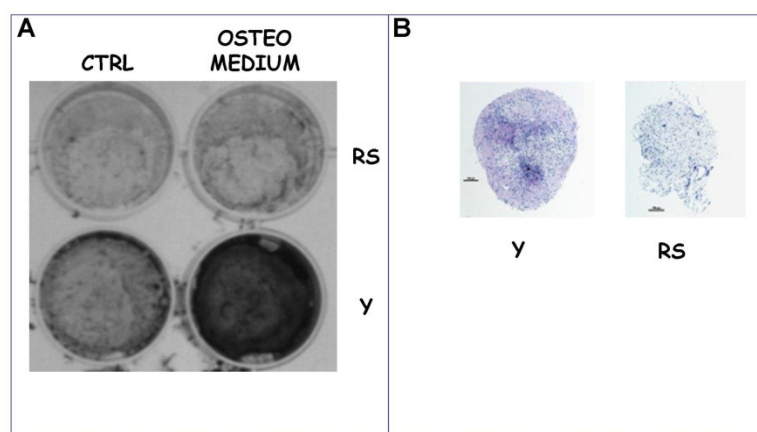


Figure 1. Effect of cellular ageing on differentiation capacity. A. Osteoblastic differentiation in the presence of a special culture medium (osteo-medium) of young (Y) and senescent (RS) fibroblasts from periodontal ligament (von Kossa staining),

B. Chondrocytic differentiation of young (Y) and senescent (RS) mesenchymal stem cells in three-dimensional cultures (proteoglycan expression).

Publications

Pratsinis, H., Dimozi, A., Pilichos, K., Tsagarakis, S., Yiacooumettis, A., Kletsas, D. (2011). Previous chronic exogenous glucocorticoid administration in vivo does not affect functional characteristics and cellular lifespan of human skin fibroblasts in vitro. *Exp. Dermatol.* 20, 529-531.

Magoulas, G.E., Bariamis, S.E., Athanassopoulos, C.M., Haskopoulos, A., Dedes, P.G., Krokidis, M.G., Karamanos, N.K., Kletsas, D., Papaioannou, D., Maroulis, G. (2011). Syntheses, antiproliferative activity and theoretical characterization of acitretin-type retinoids with changes in the lipophilic part. *Eur. J. Med. Chem.* 46, 721-737.

Grivas, T.B., Vasiliadis, E.S., Kaspiris, A., Khaldi, L., Kletsas, D. (2011). Expression of matrix metalloproteinase-1 (MMP-1) in Wistar rat's intervertebral disc after experimentally induced scoliotic deformity. *Scoliosis* 6,9.

Papaioannou, K.A., Markopoulou, C.E., Gioni, V., Mamalis, A.A., Vayouraki, H.N., Kletsas, D., Vrotsos, I.A. (2011). Attachment and proliferation of human osteoblast-like cells on guided bone regeneration (GBR) membranes in the absence or presence of nicotine: an in vitro study. *Int. J. Oral. Maxillofac. Implants* 26, 509-519.

Liu, Q., Humpe, A., Kletsas, D., Warnke, F., Becker, S.T., Douglas, T., Sivananthan, S., Warnke, P.H. (2011). Proliferation assessment of primary human mesenchymal stem cells on collagen membranes for guided bone regeneration. *Int. J. Oral. Maxillofac. Implants* 26, 1004-1010.

Papadopoulou, A., Kletsas, D. (2011). Human lung fibroblasts prematurely senescent after exposure to ionizing radiation enhance the growth of malignant lung epithelial cells in vitro and in vivo. *Int. J. Oncol.* 39, 989-999.

Articles in Press

Mavrogonatou, E., Kletsas, D. (2012). Differential response of nucleus pulposus intervertebral disc cells to high salt, sorbitol, and urea. *J. Cell. Physiol.* 227, 1179-1187 (IF: 3,986)

Makropoulou, M., Aligiannis, N., Gonou-Zagou, Z., Pratsinis, H., Skaltsounis, A.L., Fokialakis, N. (2012). Antioxidant and cytotoxic activity of the wild edible mushroom *Gomphus clavatus*. *J. Med. Food* 15, 216-221. (IF: 1,461)

Fokialakis, N., Alexi, X., Aligiannis, N., Siriani, D., Meligova, A.K., Pratsinis, H., Mitakou, S., Alexis, M.N. (2012). Ester and carbamate ester derivatives of Biochanin A: Synthesis and in vitro evaluation of estrogenic and antiproliferative activities. *Bioorg. Med. Chem.* 20: 2962-2970. (IF: 2,978)

Zampeli, D., Pratsinis, H., Eliades, T., Eliades, G., Kletsas, D., Papagiannoulis, L. (2012). In vitro estrogenicity of dental resin sealants. *Pediatr. Dent.* (in press) (IF: 1,831)

Pratsinis, H., Constantinou, V., Pavlakis, K., Sapkas, G., Kletsas, D. (2012). Exogenous and autocrine growth factors stimulate human intervertebral disc cell proliferation via the ERK and Akt pathways. *J. Orthop. Res.* 30, 958-964. (IF: 2,976)

Anastasiadi, M., Pratsinis, H., Kletsas, D., Skaltsounis, A.L., Haroutounian, S.A. (2012). Grape stem extracts: Polyphenolic content and assessment of their in vitro antioxidant properties. *LWT-Food Sci. Technol.* (in press). (IF: 2,292)

Wuertz, K., Vo, N., Kletsas, D., Boos, N. (2012). Inflammatory and catabolic signalling in intervertebral discs: The roles of NF- κ B and MAP Kinases. *Eur. Cell Mater.* 23, 103-20. (IF: 9,65)

Articles in Books and Conference Proceedings

D. Kletsas "Cellular senescence and cancer development: antagonistic and synergistic relations" in "Cancer Biomarkers" (A.G. Gerogakilas, ed.) Science Publishers, 2012

Presentations at Scientific Conferences

D. Kletsas (2011) Cellular senescence: molecular mechanisms and means of prevention. 3rd International Workshop on Wound Technology. January 16-17, Paris, France (invited speaker).

D. Kletsas (2011) Cellular senescence: mechanism, role on tissue homeostasis and implications in cell replacement therapies. 8th European Congress "Healthy and Active Ageing for all Europeans" International Association of Gerontology and Geriatrics. April 14-17, Bologna Italy (invited speaker).

E. Mavrogonatou, D. Kletsas (2011). High osmolality reduces the proliferation rate of nucleus pulposus intervertebral disc cells, negatively regulates their response to exogenous growth factors and activates an enhanced DNA repair mechanism. 38th Annual Meeting of the International Society for the Study of the Lumbar Spine (ISSLS), June 14-18, 2011, Gothenburg, Sweden.

H. Pratsinis, S. Iconomopoulos, C. Neidlinger-Wilke, D. Kletsas (2011). Effects of cyclic strain on the activation of signaling pathways and the proliferative behaviour of intervertebral disc cells in vitro. 38th Annual Meeting of the International Society for the Study of the Lumbar Spine (ISSLS), June 14-18, 2011, Gothenburg, Sweden.

H. Pratsinis, V. Constantinou, K. Pavlakis, G. Sapkas, D. Kletsas (2011). Proliferative response of human intervertebral disc cells to exogenous and autocrine growth factors: Involvement of pivotal signaling pathways. 38th Annual Meeting of the International

Society for the Study of the Lumbar Spine (ISSLS), June 14-18, 2011, Gothenburg, Sweden.

D. Kletsas (2011) Cellular senescence: molecular mechanisms and implications in tissue homeostasis. 3rd FEBS Advanced Lecture Course "Matrix Pathobiology, Signaling and Molecular Targets", September 2-7, 2011, Island of Spetses, Greece (invited speaker).

H. Pratsinis, S. Chrissouli, V. Velissariou, A. Anastasiou, D. Kletsas (2011). Growth Factors and Signaling Pathways Involved in the Amniotic Fluid-Induced Proliferation of Human Skin Fibroblasts. 3rd FEBS Advanced Lecture Course "Matrix Pathobiology, Signaling and Molecular Targets", September 2-7, 2011, Island of Spetses, Greece.

C. Gialeli, D. Kletsas, G.N. Tzanakakis, N.K. Karamanos (2011). Targeting epidermal growth factor receptor in colon cancer. 3rd FEBS Advanced Lecture Course "Matrix Pathobiology, Signaling and Molecular Targets", September 2-7, 2011, Island of Spetses, Greece.

C.J. Malavaki, A. Roussidis, D. Kletsas, T. Tsegenidis, A.D. Theocharis, G.N. Tzanakakis, N.K. Karamanos (2011). Invasion of breast cancer cells is related to the PDGF-R mediated expression of heparin sulphate proteoglycans. 3rd FEBS Advanced Lecture Course "Matrix Pathobiology, Signaling and Molecular Targets", September 2-7, 2011, Island of Spetses, Greece.

A. A. Armatas, N.K. Karamanos, D. Kletsas (2011). Hyaluronan as regulator of the differential response of fetal and adult dermal fibroblasts to TGF- β . 3rd FEBS Advanced Lecture Course "Matrix Pathobiology, Signaling and Molecular Targets", September 2-7, 2011, Island of Spetses, Greece.

Papadopoulou, A. Scorilas, D. Kletsas (2011) Expression of kallikrein genes in senescent human fibroblasts. 4th International Symposium on Kallikreins and Kallikrein-Related Peptidases. 2-4 September, 2011, Rhodes, Greece.

D. Kletsas (2011) DNA damage, cellular senescence and role in tissue homeostasis. EMBO Workshop "Chromosome: Structure, damage & repair". 25-28 September, Cape Soumio, Greece (invited speaker).

H. Pratsinis, A. Dimozi, A. Papadopoulou, M. Lefaki, K. Pilichos, S. Tsagarakis, AM. Yiacoimettis, D. Kletsas (2011). Stress, cell senescence and tissue homeostasis. 5th Hellenic Conference for Wound Healing and Ulcers, 17-18 March 2011, Athens.

E. Mavrogonatou, A. Dimozi, H. Pratsinis, D. Kletsas (2011). The role of stresses in intervertebral disc-cell proliferation. 5th Hellenic Spine Conference, 27-30 October 2011, Pafos, Cyprus.

V. Constantinou, K. Soultanis, K. Pavlakis, H. Pratsinis, G. Sapkas, D. Kletsas (2011). Stimulation of intervertebral disc-cell proliferation by autocrine and exogenous growth factors through the MEK/ERK and PI3K/Akt signaling pathways. 5th Hellenic Spine Conference, 27-30 October 2011, Pafos, Cyprus.

D. Kletsas (2011) Cellular senescence. 67th Hellenic Orthopaedic Conference. 12-16 October, Athens (invited speaker).

E. Mavrogonatou, D. Kletsas (2011). Nucleus pulposus disc cells' immediate osmo-regulatory response is triggered by hypertonicity and not by increased ionic concentration. 62nd National Conference of Biochemistry and Molecular Biology, 9-11 December 2011, Athens.

R. Tenta, M. Xanthopoulou, M. Skyrianou, H. Pratsinis, D. Kletsas, T. Nomikos (2011). Antiproliferative action of pumpkin seed extracts on PC-3 prostate cancer cells. 62nd National Conference of Biochemistry and Molecular Biology, 9-11 December 2011, Athens.

R. Tenta, M. Xanthopoulou, M. Skyrianou, H. Pratsinis, D. Kletsas, S. Antonopoulou, E. Fragopoulou (2011). Antiproliferative effects of red and white wine extracts in PC-3 prostate cancer cells. 62nd National Conference of Biochemistry and Molecular Biology, 9-11 December 2011, Athens.

A.A. Armatas, N.K. Karamanos, D. Kletsas (2011). Hyaluronan as a regulator of proliferative response of fetal dermal fibroblasts to TGF- β . 62nd National Conference of Biochemistry and Molecular Biology, 9-11 December 2011, Athens.

H. Pratsinis, A. Armatas, A. Dimozi, D. Kletsas (2011). Paracrine effects of young human skin fibroblasts on senescent cells: Implications for cell-based therapeutic approaches. 62nd National Conference of Biochemistry and Molecular Biology, 9-11 December 2011, Athens.

A. Dimozi, D. Kletsas (2011). Oxidative stress inhibits proliferation and provokes premature senescence in intervertebral disc cells. 62nd National Conference of Biochemistry and Molecular Biology, 9-11 December 2011, Athens

D. Kletsas (2011) Molecular mechanisms of cellular senescence in the intervertebral disc. 17th Seminar on Spinal Biomechanics and Biotechnology. 2-3 December 2011, Athens (invited speaker).

Educational Activities

"Mechanisms of cell senescence and their role in tissue homeostasis", NCSR "Demokritos" Summer School, 1 hour, 30 students. (D. Kletsas)

Supervision of the PhD theses of Adamantia Papadopoulou, Anastasia Dimozi and Andreas Armatas. (D. Kletsas) "Cell senescence and tissue homeostasis" Post-graduate Master's Degree in Biochemistry, Chemistry Department of the University of Athens, 2 hours, 15 students. (D. Kletsas)

"Cell senescence and tissue homeostasis" Post-graduate Master's Degree in Physiology, Medical School of the University of Athens, 2 hours, 30 students. (D. Kletsas)

"Cell senescence and carcinogenesis" Lecture in the framework of the class entitled "Oncogenes and growth factors in cancer biology" Medical School of the University of Athens, 2 hours, 20 students. (D. Kletsas)

"Cell Culture-Tissue Culture", Post-graduate Master's Degree "Applications of Biology in Medicine", Department of Biology of the University of Athens, 6 hours, 20 students. (D. Kletsas, H. Pratsinis and E. Mavrogonatou)

Member of three examination committees for Ph.D. & M.Sc. theses in the University of Athens (Department of Chemistry and Dental School) and University of Patras. (D. Kletsas)

V. Constantinou concluded his Ph.D. thesis entitled "The role of growth factors on the proliferation of intervertebral disc cells, via the MEK/ERK and the PI3K/Akt pathways, during disc degeneration" in the Medical School of the University of Athens, which was unanimously accepted and awarded the degree "Excellent". (Scientific Supervisor D. Kletsas).

M.A. Rapti concluded her M.Sc. thesis entitled "Investigation on the effect of anticancer drugs on stromal cells" in the framework of the Master's Degree Programme of the Biology Department of the University of Athens; the thesis was unanimously accepted and awarded the degree "Excellent". (Scientific Supervisor D. Kletsas).

M. Lefaki concluded her M.Sc. thesis entitled "Interactions of stromal and cancer cells" in the framework of the Master's Degree Programme of the Biology Department of the University of Athens; the thesis was unanimously accepted and awarded the degree "Excellent". (Scientific Supervisor D. Kletsas).

Other Scientific Activities

Member (Secretary General) of the Administrative Board of the Hellenic Society for Biochemistry and Molecular Biology (D. Kletsas). Member (Secretary) of the Research Club for Connective Tissue and Matrix Biology of the Hellenic Society for Biochemistry and Molecular Biology (D. Kletsas). Member of the Administrative Board of the European Tissue Repair Society (D. Kletsas). Secretary of the Biology Section (Europe) of the International Association of Gerontology and Geriatrics (IAGG) (D. Kletsas)

Editorial board member of the scientific journals “Biogerontology”, “European Spine Journal”, “PLOS One”, “Fibrogenesis and Tissue Repair”, “Open Longevity Science”, “Open Spine Journal” και “Journal of Dental Biomechanics”. (D. Kletsas)

Member of the Organizing Committee of the 3rd FEBS Advanced Lecture Course “Matrix Pathobiology, Signaling and Molecular Targets”, September 2-7, 2011, Island of Spetses, Greece (D. Kletsas)

Member of the Scientific Committee of the 21st European Tissue Repair Society (ETRS) Meeting, October 5-7, 2011, Amsterdam (D. Kletsas)

Review of grant proposals to the AO Foundation (D. Kletsas)

Reviewing of manuscripts submitted to European Spine Journal, PLoS ONE, Journal of Cellular Physiology, Molecular Cancer Therapeutics, Biogerontology, Journal of Investigative Dermatology, Molecular Carcinogenesis, Mechanisms of Ageing and Development, Cell Biochemistry & Function, Osteoarthritis and Cartilage, Dental Materials, American Journal of Orthodontics and Dentofacial Orthopedics, Journal of Photobiology, Archives of Dermatological Research, Journal of Dental Biomechanics (D. Kletsas) and Experimental Dermatology, Journal of Molecular Biochemistry (H. Pratsinis).

Other Distinctions and Awards

E. Mavrogonatou, D. Kletsas. High osmolality reduces the proliferation rate of nucleus pulposus intervertebral disc cells, negatively regulates their response to exogenous growth factors and activates an enhanced DNA repair mechanism. ISSLS Gothenburg, Sweden, 14-18 June 2011 – Winner of the 2011 ISSLS Poster Prize.

Ioannovitch prize from the Organizing and Scientific Committee of the 5th Hellenic Conference for Wound Healing and Ulcers, (2011) to the communication entitled “Stress, cell senescence and tissue homeostasis” by H. Pratsinis, A. Dimozi, A. Papadopoulou, M. Lefaki, K. Pilichos, S. Tsagarakis, AM. Yiacoymettis, and D. Kletsas.

“Vicky Zachariou” prize for the best oral presentation in the 5th Hellenic Spine Conference (2011) to the communication entitled “Stimulation of intervertebral disc-cell proliferation by autocrine and exogenous growth factors through the MEK/ERK and PI3K/Akt signaling pathways” by V. Constantinou, K. Soultanis, K. Pavlakis, H. Pratsinis, G. Sappas, and D. Kletsas.

D. Kletsas (2011) Cellular senescence: Mechanisms and role in tissue homeostasis. Lecture in the framework of the seminars organized by the Department of Chemistry of the University of Athens, March 15, 2011, Athens (invited speaker).

D. Kletsas (2011) Cellular senescence: Mechanisms and role in tissue homeostasis. April 11, 2011, University of Leipzig (invited speaker).

D. Kletsas (2011) Regulation of cell proliferation and senescence in the intervertebral disc. May 16, 2011, University of Ulm (invited speaker).

D. Kletsas (2011) Mechanisms of Tissue Homeostasis: The Role of Cellular Senescence. May 26, 2011, University of Bern (invited speaker).

D. Kletsas (2011) Promotion of biotechnological research in Greece and in Europe. 1st International Forum Biosciences and Cancer. November 4 2011, Athens (invited speaker).

Other Activities for the Institute of Biology

D. Kletsas:

Scientific Supervisor of the Experimental Animal Colony

Supervisor of the Fluorescence Activated Cell Sorting Facility

Member of the Finances Committee of NCSR “Demokritos”

Member of the Committee on Services-Providing Laboratories of NCSR “Demokritos”

Member of the Hygiene and Security Committee of NCSR “Demokritos”

H. Pratsinis:

Member of the advisory committee of the post-graduate Ph.D. fellows A. Dimozi and A. Athanasopoulou

Responsibility (jointly with Dr. A. Prombona) for the conducted tours and public relations of the Institute of Biology (starting from November 2011).

Impact Factors:

D. Kletsas (for 5 publications): 13,285

H. Pratsinis (for 1 publication): 4,159

Citations 2011 (without self-citations):

D. Kletsas: 435

H. Pratsinis: 118

Total Citations 2007-2011 (without self-citations):

D. Kletsas: 2060

H. Pratsinis: 476

h-factor:

D. Kletsas: 25

H. Pratsinis: 16

Laboratory equipment

The lab has the necessary equipment for gene and protein expression analyses, as well as for cell biology studies. In particular, a fully equipped cell culture room exists. Dr. D. Kletsas is the supervisor of the aforementioned equipment. In addition, some institutional facilities exist in the lab (under the same supervisor), such as a flow cytometer FACSCalibur (Becton-Dickinson), a Coulter Counter cell counter and a UV-visible light microscope Axioplan (Zeiss) bearing a CCD camera and a software for image capturing and processing.

Current External Funding

Project entitled *Disc-degeneration linked pathologies: novel biomarkers and diagnostics for targeting treatment and repair (GENODISC)*, funded by EE with Coordinator Dr. J. Urban (Greek Coordinator: D. Kletsas)

Duration: 2008-2010

Total programme funding: 2.997.144€

Funding of the lab for 2011: 55.375 €.

Project entitled *From Biodiversity to Chemodiversity: Novel Plant Produced Compounds with Agrochemical and Cosmetic Interest (AgroCos)*, funded by EU with Coordinator Prof. A.-L. Skaltsounis, University of Athens - (Coordinator for NCSR "D": D. Kletsas)

Duration: 2010-2014

Total programme funding: 2.903.633 €

Funding of the lab for 2011: 0€.

Project entitled *Investigation of the effect of neonatal fibroblasts on the pro-inflammatory phenotype of senescent cells*, funded by Organogenesis Inc., Massachusetts, USA with Coordinator Dr. D. Kletsas

Duration: 2010-2011

Total programme funding: 50.000 \$

Funding of the lab for 2011: 14.895 \$

Program entitled *Contribution of the intracellular cross-talk of ER α / β with EGF-R and IGF-R in the development and progress of breast cancer: Functional cell characteristics, expression of bioactive molecules and EMT induction*, funded by GSRT (THALES project) with Prof. N.K. Karamanos (Department of Chemistry, University of Patras) as the scientific supervisor - (NCSR "D" supervisor: Dr. D. Kletsas)

Duration: 2010-2014

Total funding : 600.000 €

Funding of the lab for 2011: 0€.

Beginning from 2011, according to the decision of the 445th Meeting of the Board of NCSR "Demokritos" (29-11-2010), a Specialized Services Unit entitled "Development of Cell Assay Systems and Assays of Bioactive Products" has started to operate under the scientific supervision of Dr. D. Kletsas and staffed by H. Pratsinis, E. Mavrogonatou, A. Papadopoulou and M. Lefaki. The activities of the Unit include the isolation of primary cell cultures from somatic and mesenchymal stem cells and the development of the appropriate cell culture systems for assaying synthetic or natural bioactive products

regarding their wound healing, anti-ageing, and anti-cancer behavior. During 2011 cytotoxicity and MMP-expression studies were commissioned by the company KORRES S.A. – NATURAL PRODUCTS.

* **Note:** All lab members would like to express their great sorrow for the tragic loss of our beloved colleagues, Ph.D students, Anastasia Dimozi and Andreas Armatas in a car accident in the 15th of January, 2012. Natasa and Andreas participated in most of the lab's activities, had presented their scientific results in national and international conferences and had published their work in international scientific journals and books. Their ethics, diligence and capabilities indicated a luminous future. They will always stay alive in our memory.

Research Group: Nuclear Proteins and Chromatin Function

Research Staff

Thomais Sourlingas, Senior Researcher

Kalliope Sekeri, Emeritus Researcher

Marios Xidous, Graduate Student

Paraskevi Salpea, Graduate Student – *PhD obtained in 2011*

Research Interests

Our research is focused on the functional role of the histone subtypes and their epigenetic post translational modifications, as well as the effects that histone deacetylase inhibitors have on gene expression and cellular function in various biological processes.

1. Ageing-Apoptosis: Investigation of the relationship of the expression profile of the histone subtypes of the histone H1 family (somatic subtypes and H1) and epigenetic histone modifications (phosphorylation, acetylation and methylation) during ageing and apoptosis in cell strains and lines. We are also studying the changes that these epigenetic modifications bring about in the expression profiles of age-dependent genes in human peripheral blood leucocytes.
2. Mammalian Biological Clock: The role of histone acetylation and methylation in the regulation of the mammalian biological clock.
3. Psychiatric Disorders: How changes in histone epigenetic modifications and in the expression of the H1 DNA linker histone subtypes affect chromatin remodelling (reorganization) events in human peripheral blood leucocytes from individuals with psychiatric disorders.

2011 Findings

1. We examined the effects that two histone deacetylase inhibitors (HDACs), trichostatin A (TSA) and nicotinamide, have on clock function. Real-time PCR showed that TSA increases the mRNA levels of the early response clock gene *per1* and that nicotinamide inhibits this TSA-induced effect. ChIP assays showed that treatment with TSA increases histone acetylation levels in the glucocorticoid response element region and in the region of the transcription start site of this gene. These TSA-induced effects on the acetylation levels are repressed if nicotinamide is applied concomitantly with TSA. Moreover, nicotinamide blocks the acute response of *per1* induced by dexamethasone. This response is necessary for the synchronization of circadian rhythm in cell cultures. ChIP experiments showed that this inhibition of the acute response is associated with a decrease in the lysine 4 trimethylation levels of histone H3 (H3K4me3) in the promoter region of this gene. We conclude that the decrease in H3K4me3 levels in the *per1* promoter is the repressive mechanism by which nicotinamide inhibits the *per1* acute response induced by dexamethasone. These results reveal the existence of a novel, as yet to be reported, mechanism with which clock function is regulated.
This study is being carried out by M. Xydous within the framework of his doctoral thesis. The general aims of this project are being carried out within the framework of a research collaboration with the laboratory of “Chronobiology” (Group Leader, Dr. Anastasia Prombona) of the Institute of Biology N.C.S.R. “D”.
2. We studied the histone acetylation and methylation levels in the promoter regions of the *H1°* and the *dfna5* genes, which are differentiation- and age-related genes. The results showed that:
 - In *dfna5* the H3K4me3 levels are higher in neonatal (cord blood) samples as compared to those of young donors, a finding which is in agreement with this gene's expression level.
 - Histone acetylation in the *H1°* gene region in lymphocytes were found to be higher in samples from elderly donors versus those from young donors. These results are in agreement with the expression levels of this gene.Within the framework of this study, we also analyzed epigenetic changes genomewide as a function of age.

- Using next generation sequencing technology, we found regions with age-dependent alterations in DNA methylation.
- Concomitant to the above, methylation of H3K27, a histone modification associated with transcriptional repression, was also studied genomewide in monocytes of neonatal (cord blood) as compared to monocytes from adult donors. From this line of work, we found that the genomic loci that showed increased methylation levels of H3K27 were colocalized with those that showed the greatest differences in DNA methylation as a function of age.

With this series of experiments Paraskevi Salpea completed her PhD thesis work, which she presented in December, 2011.

The second part of this thesis work was accomplished in the laboratory of Dr. Bruce Howard, Head of the Laboratory of Molecular Growth Regulation, of the National Institute of Child Health and Human Development; National Institutes of Health (NIH) within the framework of a research collaboration between the two labs.

Articles in Books and Conferences Proceedings

Sourlingas, T. G. The functionally discrete biological roles of the H1 linker histone subtypes. In "Histones: Class, Structure and Function" (Chang-Hui Shen, ed.), chapter 1, pp. 1-34, Nova Science Publishers, Inc. 2011.

Educational Activities

Summer School (IB/NCSR "D") "Histone Variants and Post Translational Modifications: Fundamental Factors in Chromatin Remodeling Events during Aging and Apoptosis" (1 hour).

Seminar: "Cell Cycle: Checkpoints and Consequences for Normal Cellular Function" within the framework of the course "Cell Cultures-Tissue Cultures" of the Graduate Masters' Program: Applications of Biology in Medicine, Dept. of Biology, University of Athens (6 hours, 20 students).

Supervision of the doctoral thesis work of Marios Xydous, biologist, recipient of a 4-year scholarship from NCSR "D". Title of thesis work: "The effect of histone acetylation and methylation levels in the regulation of the mammalian biological clock". This thesis work has been completed. Part of this work has been submitted for publication in *BBA: Gene Regulatory Mechanisms* which has been accepted and will be published in 2012.

Supervision of the doctoral thesis work of **Paraskevi Salpea**, biologist, recipient of a 4-year scholarship from NCSR "D". Title of thesis work: "Study of acetylation and methylation in the reorganization of chromatin during aging". **This thesis work has been completed and was presented** in the Dept. of Biological Chemistry, Medical School, University of Athens in **December, 2011**. A manuscript was submitted to *Nucleic Acids Research* which was accepted and will be published in 2012.

Member of the Internal Advisory Committee for the doctoral thesis work of M. Xydous, P. Salpea and A. Galeos.

Other activities in the Institute of Biology

Member of the IB Committee for the receipt of new materials and services and for the examination and characterization of materials unsuitable for use and to be destroyed that have been acquired by funds from the Demokritos budget.

Other scientific activities

Scientific collaborations:

- With the laboratory of Bruce Howard, Head of the Laboratory of Molecular Growth Regulation of the National Institute of Child Health and Human Development; National Institutes of Health (NIH). Within the framework of this collaboration, P. Salpea, biologist, who completed her doctoral thesis work in our lab, also worked in the lab of Dr. Howard on a project that was part of her thesis work.
- With Dr. A. Prombona (Laboratory of Chronobiology), Biology Institute, NCSR "D". This research collaboration involves the study of the effects of histone

modifications on genes that regulate the mammalian biological clock (circadian rhythm) and potential consequences to cellular function and carcinogenesis.

- With the Dept. of Biological Chemistry of the Medical School, University of Athens (Associate Prof. P. Moutsatsou). The collaboration involves "The study of the induction of apoptosis by ursolic acid in the MCF-7 breast cancer cell line.

Citations 2011 (without self-citations): 18

Total Citations 2007-2011 (without self-citations): 78

h-factor: 6

Laboratory Equipment and Common Usage Equipment (IB) (Allocated to T. Sourlingas)

- Cell cultures: Laminar flow, cell culture incubator (IB), cell culture incubator, light microscope, phase contrast microscope, 2 CO₂ tanks, humidity (liquid) sterilizer incubator (IB), dry sterilizer incubator (IB), nitrogen tank for storage of cell cultures, nitrogen tank for storage of cell cultures (IB), tabletop refrigerated centrifuge with three heads (IB), 2 glass apparatus for filtering cell culture material.
- Protein analysis, etc.: floor standing refrigerated centrifuge Sorvall with three heads (IB), water circulator, 2 powersupplies, 2 electrophoresis apparatus, 2 protein transfer apparatus (Westerns), shaker/waterbath, 2 scales (accuracy and standard), pH-meter (IB), 2 stirrer/hotplates, 2 vortex, eppendorf tube shaker, refractometer (densitometer for solutions), H₂O distillation apparatus.
- Standard refrigerator for reagents, small refrigerator for food.

IB Common Usage Equipment Outside of the Lab that T. Sourlingas is responsible for

β-counter

Deep freezer (-80°C)

-40°C freezer

Research Group: Cell & Matrix Biochemistry/Pathobiology

Research Staff

Fotini-Effie Tsilibary, Research Director

Athina Tzinia, Senior Researcher

Angelika Chroni, Senior Researcher

Paraskevi Kitsiou, Researcher

Garyfallia Drossopoulou, Lecturer

Apostolia Fragouli, Postdoctoral Fellow

Nikos Tsotakos, Graduate Student

Katerina Kapodistria, Graduate Student

Theodore Koutmos, Graduate Student

Ioannis Daphnis, Graduate Student - *PhD obtained in 2011*

Georgios Daniil, Graduate Student

Letta Argyri, Graduate Student

Nefeli Lagopati, Collaborating Graduate Student

Sofia Verouti, Collaborating Graduate Student

Vassilis Photopoulos, Collaborating Graduate Student

Christina Golfinopoulou, Undergraduate Student

Christos Balis, Undergraduate Student

Konstantinos Kyritsis, Training Student

Eleni Kotsopoulou, Research Technician

Research Interests

1. Study of kidney function: **A)** Regulation of gene expression and epigenetic mechanisms in glomerular podocytes in physiological and diabetic conditions, **B)** Renoprotective role of Vitamin D₃ on glomerular podocytes. **C)** Nephron signalling in pancreatic β -cells: Cross talk between nephron signalling and insulin survival signalling. **D)** anticancer properties of UV-activated TiO₂ nanoparticles
2. Neurodegenerative Disorders: **A)** *In vivo* effects of collagenase B (MMP-9) enhancing neuronal plasticity and cognitive functions in the brain of transgenic mice (TgMMP9); **B)** the protective role of MMP9 overexpressed in the brain of 5XFAD mice, a model of Alzheimer's disease (TgMMP9 x Tg5XFAD), **C)** Elucidation of the role of apoE4, a major risk factor for Alzheimer's disease, in the pathogenesis of the disease. **D)** Effect of Oleuropein in the formation of Alzheimer's amyloid plaques
3. Arthropathies: Expression of MMP-9 in septic and aseptic Arthritis
4. Biological activities of TiO₂ innovative nanoparticles for diagnostic and therapeutic use.
5. Atherosclerotic disease: Molecular mechanisms of atherosclerosis - Structure function relationship of proteins involved in lipoprotein metabolism pathways.

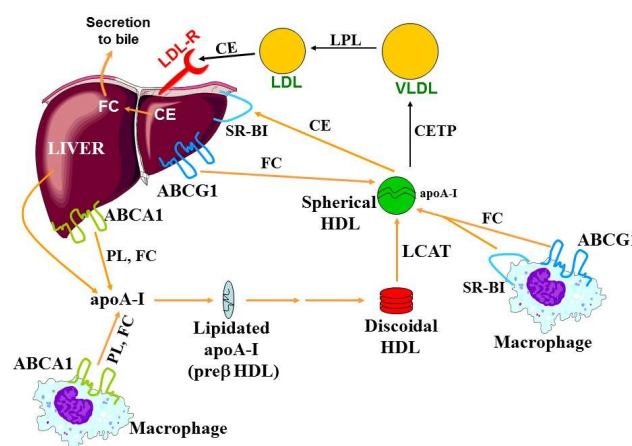
2011 Findings

Research related to human apolipoproteins structure and function

Study of structure-function relationship of apolipoprotein A-I (apoA-I), which is the major protein component of high-density lipoprotein (HDL). Elucidation of the role of apoA-I in the biogenesis and atheroprotective properties of HDL, as well as in plasma cholesterol and triglycerides homeostasis.

Apo A-I must leave the circulation and pass the endothelium to exert its atheroprotective actions in the arterial wall. We demonstrated that the carboxyl-terminal domain of apoA-I is mandatory for the transendothelial transport of lipid-free apoA-I but not of prelipidated apoA-I particles. Our findings indicate that stringent structure-function relationships underline the specificity of transendothelial apoA-I transport.

In another study, we showed that changes in specific amino acid residues of apoA-I not only affect HDL biogenesis, but also result in disturbance of plasma total cholesterol and triglycerides homeostasis and may lead to severe hypertriglyceridemia.



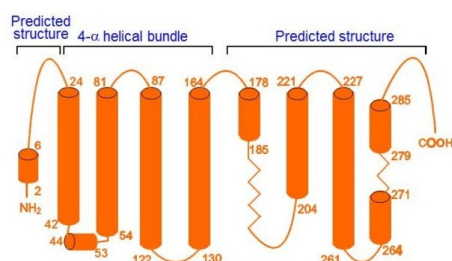
Pathway of HDL metabolism

Study of structure-function relationship of apolipoprotein E (apoE). Elucidation of the role of apoE in the pathogenesis of atherosclerosis and Alzheimer's disease.

ApoE has three common isoforms in humans, apoE2, apoE3 and apoE4. We examined whether three type III hyperlipoproteinemia predisposing apoE3 variants, namely R136S, R145C and K146E affect the biophysical properties of the protein. Our findings suggest that single amino acid changes in the functionally important region 136–150 of apoE3 can affect the molecule's stability and conformation in solution and may underlie functional consequences. However, the magnitude and the non-concerted nature of these changes, make it unlikely that they constitute a distinct unifying mechanism leading to type III hyperlipoproteinemia pathogenesis.

ApoE is the only medium-size apolipoprotein that is expressed in brain and forms HDL-type lipoproteins. ApoE4 isoform is the major genetic risk factor for Alzheimer's disease. We studied the functions of truncated apoE4 forms *in vitro* and *in vivo* in order to identify the domains of apoE4 required for the biogenesis of apoE-containing HDL. Overall, the *in-vivo* and *in-vitro* data are consistent and showed that apoE4-185 is the shortest truncated form that supports formation of discoidal apoE4-containing HDL particles.

To facilitate our studies on the role of apoE4 in the pathogenesis of cardiovascular disease and neurodegenerative diseases, we developed a simple expression and purification procedure for the production of recombinant human apoE4. Our approach results in rapid, high-yield production of structurally and functionally competent apoE4 as evidenced by secondary structure measurements, thermal and chemical melting profiles and the kinetic profile of solubilization of phospholipid vesicles. A variety of apoE4 single amino acid mutants, as well as wild-type and mutant apoE3 forms, have been also expressed with this system, all resulting in comparable levels and purity of protein. The structural and functional characterization of these proteins is underway.



Secondary structure of human apolipoprotein E

The neuroprotective role of MMP-9

For the *in vivo* study of MMP9 we have generated transgenic mice overexpressing MMP9 in the brain (TgMMP9). These animals were crossed with animal models for AD (5XFAD) to produce double transgenic animals (TgMMP9/Tg5XFAD). Double transgenic animals exhibit

improved cognitive abilities and increased secretion of the neuroprotective soluble APP fragment (sAPP α), compared to Tg5XFAD animals.

Studies of signaling pathways involved in pancreatic β -cell survival.

Our findings demonstrated that incubation of pancreatic β TC-6 cells with anti-nephrin antibodies, induced nephrin clustering, association of PI3K with nephrin, and localization of the complex at the plasma membrane. The increased interaction of nephrin with PI3K induced the phosphorylation/activation of Akt; this effect was inhibited by wortmannin and LY294002, indicating that Akt activation was PI3K-dependent. Activation of nephrin resulted in increased Bad phosphorylation/inactivation; hence nephrin-induced PI3K/Akt activation trigger anti-apoptotic signalling.

Anticancer activities of photo-activated TiO₂ nanoparticles

Differently treated, activated TiO₂ nanoparticles were assessed for specifically inducing cell apoptosis and death in breast tumor cells with low (MCF-7) and high (MDA-MB-468) invasive properties. Among the different particles studies, the sol-gel samples were most effective, compared to Degussa P25 or Sigma Aldrich samples.

Renoprotective role of vitamin D₃

Our findings demonstrated that in the presence of 1,25-dihydroxyvitamin D₃ (calcitriol) and its analogue paricalcitol, cell-surface associated nephrin which was suppressed by high glucose was restored to normal levels, whereas the glucose-induced severe down-regulation of podocalyxin expression was partially restored. Podocalyxin and nephrin re-activation of expression is in part mediated by vitamin D receptor, VDR, which could act on transcription factor(s) regulating nephrin and podocalyxin expression, such as WT1. Hence vitamin D₃ and paricalcitol, which has fewer side effects and could be clinically valuable, play a significant role in maintaining the expression of important, specialized components of podocytes and thus protect against loss of the permselective glomerular filtration barrier.

Publications

Ohnsorg P. M., Rohrer L., Perisa D., Kateifides A., Chroni A., Kardassis D., Zannis V. and von Eckardstein A. (2011) The carboxy-terminus of apolipoprotein A-I (ApoA-I) is necessary for the transport of lipid-free ApoA-I but not pre-lipidated ApoA-I particles through aortic endothelial cells. *J. Biol. Chem.*, 286, 7744-7754.

Daniil G., Phedonos A. A. P., Holleboom A. G., Motazacker M. M., Argyri L., Kuivenhoven J. A. and Chroni A. (2011) Characterization of antioxidant/anti-inflammatory properties and apoA-I-containing subpopulations of HDL from family subjects with monogenic low HDL disorders. *Clin. Chim. Acta*, 412, 1213-1220.

Kateifides A. K., Gorshkova I. N., Duka A., Chroni A., Kardassis K. and Zannis V.I (2011) Alteration of negatively charged residues in the 89 to 99 domain of α poA-I affects lipid homeostasis and the maturation of HDL. *J. Lipid Res.*, 52, 1363-1372.

Vezerides A., Chroni A. and Zannis V. I. Domains of apoE4 required for the biogenesis of apoE-containing HDL. *Ann. Med.*, 43, 302-311 (2011).

Besler C., Heinrich K., Rohrer L., Doerries C., Riwanto M., Shih D. M., Chroni A., Yonekawa K., Stein S., Schaefer N., Mueller M., Akhmedov A., Daniil G., Manes C., Templin C., Wyss C., Maier W., Tanner F. C., Matter C. M., Corti R., Furlong C., Lusis A. J., von Eckardstein A., Fogelman A. M., Lüscher T. F., Landmesser U. Mechanisms underlying adverse effects of HDL on eNOS-activating pathways in patients with coronary artery disease. (2011) *J. Clin. Invest.*, 121, 2693-2708.

Argyri L., Skamnaki V., Stratikos E. and Chroni A. A simple approach for human recombinant apolipoprotein E4 expression and purification. *Prot. Express. Purif.*, 79, 251-257 (2011).

Georgiadou D., Chroni A., Vezerides A., Zannis V. I. and Stratikos E. Apolipoprotein E3 mutants linked with development of Type III Hyperlipoproteinemia alter the protein's thermodynamic properties.(2011) *PLoS ONE*, 6(11), e27037.

[Enhanced neuronal plasticity and elevated endogenous sAPP \$\alpha\$ levels in mice over-](#)

[expressing MMP9](#). Fragkouli A, Papatheodoropoulos C, Georgopoulos S, Stamatakis A, Stylianopoulou F, Tsilibary EC, Tzinia AK. J Neurochem. 2011 Dec 22. doi: 10.1111/j.1471-4159.2011.07637.x. [Epub ahead of print]

[Expression levels of matrix metalloproteinase \(MMP\)-9 and its specific inhibitor TIMP-1, in septic and aseptic arthritis of the knee](#). Fotopoulos VC, Tzinia A, Tzurbakis M, Kalfakakou V, Levidiotou-Stefanou S, Georgoulis A. Knee Surg Sports Traumatol Arthrosc. 2011 Sep 24. [Epub ahead of print]

[Matrix metalloproteinase-9 participates in NGF-induced \$\alpha\$ -secretase cleavage of amyloid- \$\beta\$ protein precursor in PC12 cells](#). Fragkouli A, Tzinia AK, Charalampopoulos I, Gravanis A, Tsilibary EC. J Alzheimers Dis. 2011;24(4):705-19.

Articles in Press

Dafnis I., Tzinia A., Tsilibary E. C., Zannis V. I. and Chroni A. An apolipoprotein E4 fragment is involved in neuroinflammatory response. (*Neuroscience Accepted*)

Articles in Books and Conference Proceedings

Georgiadou D., Vezeridis A., Chroni A., Zannis V. I. and Stratikos E. Apolipoprotein E3 mutants linked with development of Type III Hyperlipoproteinemia alter the protein's thermodynamic properties. *The FEBS Journal*, 278 supplement 1, p. 284, P15.9 (2011).

Daniil G., Phedonos A. A. P., Holleboom A. G., Motazacker M. M., Argyri L., Kuivenhoven J. A. and Chroni A. Characterization of antioxidant/anti-inflammatory properties and apoA-I-containing subpopulations of HDL from family subjects with monogenic low HDL disorders. *The FEBS Journal*, 278, supplement 1, p451, YSF.20 (2011).

Georgiadou D., Vezeridis A., Chroni A., Zannis V. I. and Stratikos E. Apolipoprotein E3 mutants linked with development of Type III Hyperlipoproteinemia alter the protein's thermodynamic properties. *Eur. Biophys. J.*, 40 (Suppl 1) p. S139 (2011).

Dafnis I., Tzinia A., Tsilibary E. C., Zannis V. I. and Chroni A. An apolipoprotein E4 fragment is involved in neuroinflammatory response. *J. Neurochem.*, 118, Supplement 1, p204 (2011).

Tsilibary EC, Drossopoulou G, Tsotakos N and Koutmos T. Phenotypic Changes of Podocytes in Diabetic Renal Disease. In "European Journal of Clinical Investigation" vol 41 (Suppl. 1), pp. 84-84, 2011.

Tzinia A, Fragkouli A, Tsilibary EC: MMP-9 in Alzheimer's disease (oral presentation, A. Tzinia, invited speaker, COST BM1001, Brain Extracellular Matrix in Health and Disease, Loano (Italy), May 22-24, 2011

Presentations at Scientific Conferences

Georgiadou D., Vezeridis A., Chroni A., Zannis V. I. and Stratikos E. Apolipoprotein E3 mutants linked with development of Type III Hyperlipoproteinemia alter the protein's thermodynamic properties. *36th FEBS Congress*, 25-30 June, 2011, Torino, Italy

Daniil G., Phedonos A. A. P., Holleboom A. G., Motazacker M. M., Argyri L., Kuivenhoven J. A. and Chroni A. Characterization of antioxidant/anti-inflammatory properties and apoA-I-containing subpopulations of HDL from family subjects with monogenic low HDL disorders. *36th FEBS Congress*, 25-30 June, 2011, Torino, Italy

Georgiadou D., Vezeridis A., Chroni A., Zannis V. I. and Stratikos E. Apolipoprotein E3 mutants linked with development of Type III Hyperlipoproteinemia alter the protein's thermodynamic properties. *8th European Biophysics Congress*, 23-27 August 2011, Budapest, Hungary

Dafnis I., Tzinia A., Tsilibary E. C., Zannis V. I. and Chroni A. An apolipoprotein E4 fragment is involved in neuroinflammatory response. *23rd Biennial Meeting of International Society for Neurochemistry and European Society for Neurochemistry*, 28 August- 1 September 2011, Athens, Greece

Verouti SN., Drossopoulou G., Fragopoulou E., Demopoulos AC., Iatrou C., Tsilibary EC. VDR-activators upregulate the expression of podocalyxin and nephrin in Human Glomerular Epithelial Cells (HGEC)/Podocytes cultured in high glucose levels (2011). *62nd*

Conference of the Hellenic Society for Biochemistry and Molecular Biology (HSBMB). Eugenides Foundation, December 9-11, 2011, Athens, Greece.

Georgiadou D., Chroni A., Vezerides A., Zannis V. I. and Stratikos E. Biophysical analysis of apolipoprotein E3 mutants linked with development of Type III Hyperlipoproteinemia. *14th Meeting of Hellenic Society of Lipidology, Atherosclerosis and Vascular Disease*, 13-15 October 2011, Athens, Greece.

Argyri L., Skamnaki V., Stratikos E. and Chroni A. A simple approach for human recombinant apolipoprotein E4 expression and purification. *4th Meeting of Research Working Groups of Hellenic Atherosclerosis Society*, 2-3 december 2011, Athens, Greece.

Georgiadou D., Chroni A., Vezerides A., Zannis V. I. and Stratikos E. Biophysical analysis of apolipoprotein E3 mutants linked with development of Type III Hyperlipoproteinemia. *62nd Conference of the Hellenic Society for Biochemistry and Molecular Biology*, 9-11 December 2011, Athens, Greece.

K. Kapodistria, P. Politis, E. Tsilibary, A. Charonis, E. Kotsopoulou, P. Kitsiou (2011). Nephrin, a transmembrane protein of glomerular epithelial cells, is involved in pancreatic beta-cell survival signalling. *62nd Annual Meeting of Hellenic Society of Biochemistry & Molecular Biology*, (9-11 December 2011, Athens)

Fotopoulos VC, Tzinia A, Tzurbakis M, Kalfakakou V, Levidiotou-Stefanou S, Georgoulis. Matrix metalloproteinases 9 (MMP-9) and tissue inhibitors of metalloproteinases 1 (TIMP-1), can potentially contribute to differential diagnosis of infectious arthritis of the knee. *4th Panhellenic Conference of Greek Society for Arthroscopy, knee surgery and sports injuries* 22-25 June (2011).

Other Scientific Activities

EC Tsilibary,: i) Member (Representative from Greece) of COST Action BM1001/ECM Net: Brain Extracellular Matrix in Health and Disease (2011-2014); ii) Memembr of COST Action BM0702- "EUROKUP" (Urine Kidney Proteomics); Scientific Editor for PLoSOne; iv) evaluator of abstracts for the 48th Annual Meeting of the European Renal Association -European Dialysis Transplant Association (ERA-EDTA); v) Evaluator of research proposals of ANR (Agence Nationale de Recherche, FR; vi) Co-ordinator of session on "Biology & Biochemistry of Diseases-I", 62nd Annual Meeting EEBMB, Decemebr 9, 2011; vii) invited speaker, 45th Annual Meeting of the European Society for Clinical Investigation, April 13-16, 2011, Herakleion, Crete (workshop 13: Molecular Apects of Renal Disease); viii) invited speaker, Association of Emeritus Scientists of NCSR Demokritos: " Propective use of stem cells in chronic and incurable diseases: How close is medical practice to therapeutic applications of cellular therapy?"; ix) evaluator of MSc assays for post-doctoral programme: " Molecular and Applied Physiology" (1. Review on Alzheimer's disease; 2. Tumor-suppressive protein p53 and mutations occurring in breast cancer); x) reviewer for scientific journals; PLoSOne, Photodiagnosis and Photodynamic Therapy, Microfluidics, Cells/Tissues/Organs, Kidney International, Am. J. Physiology Renal, Brain Research, BBA, J. Biol. Chemistry, Environmental Science & Technology, etc.

A. Tzinia: invited speaker BM1001, Brain Extracellular Matrix in Health and Disease, Loano (Italy), May 22-24, 2011. Invited speaker: "MMP9 in Alzheimer's Disease.

A. Chroni: i) Lead Guest Editor, Special Issue on "Lipids and lipoproteins in atherosclerosis", Journal of Lipids; ii) Organization of the 1st Scientific Meeting of COST Action BM0904 "HDL: From Biological Understanding to Clinical Exploitation", 28-29 January 2011, Athens; iii) Reviewer for scientific journals Atherosclerosis, Annals of Rheumatic Diseases, Clinica Chimica Acta, Biochimica et Biophysica Acta- Molecular and Cell Biology of Lipids, Hellenic Journal of Atherosclerosis; iv) Member of the Core Group of COST Action BM0904. Participation in Core Group meeting in Milan, Italy, 23 September 2011; v) Kardassis D., Choni A. and Sidiropoulos P. Proinflammatory HDL as a biomarker for cardiovascular disease in patients with chronic inflammatory diseases. *Atheroma*, 15(2), pp. 1-2, 2011.

P. Kitsiou: Reviewer for scientific journals *Current Diabetes Reviews*, *Current HIV Research*, *PLoS ONE*, *Recent Patents on Endocrine, Metabolic & Immune Drug Discovery*

G. Drossopoulou: Reviewer for scientific journals: *PLoS ONE*, *Journal of Nephrology*.

Other Distinctions and Awards

Fotopoulos VC, Tzinia A, Tzurbakis M, Kalfakakou V, Levidiotou-Stefanou S, Georgoulis. matrix metalloproteinases 9 (MMP-9) and tissue inhibitors of metalloproteinases 1 (TIMP-1), can potentially contribute to differential diagnosis of infectious arthritis of the knee. 4th Panhellenic Conference of Greek Society for Arthroscopy, knee surgery and sports injuries 22-25 June (2011). (1st prize)

Educational Activities

Ioannis Dafnis presented his PhD thesis entitled "Structure-function relationship of apolipoprotein E in brain" at the Department of Chemistry, University of Athens. Supervisor: Dr. A. Chroni. The PhD degree was awarded with Honors.

- 1) EC Tsilibary: i) Three-hour lecture for post-doctoral course "Pathobiochemistry", Department of Biology, National & Kapodistrian University of Athens: ("Molecular mechanisms and therapeutic approaches of diabetes mellitus", 22.03.2011); ii) Three-hour lecture for post-doctoral course "Molecular and Applied Physiology", Medical School, National & Kapodistrian University of Athens: ("Cell apoptosis in diseases: a beneficial or damaging process?", 15.11.2011)
- 2) A. Chroni, i) Member of the Institute of Biology Advisory Committee of PhD students N. Tsotakos, I. Vaggelatos, G. Daniil and M. Kostomoiri, ii) "Lipids and apolipoproteins: From cardiovascular disease to Alzheimer's disease". Guest lecturer in graduate course "Clinical Chemistry II", Clinical Chemistry Graduate Program, Department of Chemistry, University of Athens. May 26 2011 (2h -5 students), iii) "Lipoprotein metabolism pathways and atherosclerosis. The association between atherosclerosis and Alzheimer's disease." Guest lecturer in graduate course "Human Biochemistry", Biochemistry Graduate Program, Department of Chemistry, University of Athens. 2 June 2011 (3 hours- 10 students). iv) Presentation: "Structure-function relationship of human apolipoproteins: role of apolipoprotein A-I in atherosclerosis and of apolipoprotein E in Alzheimer's disease". 1st Summer School on Proteins "Proteins: from gene to structure and beyond...", Biology Department, University of Athens, 1-2 July 2011 (1 hour).

Other Activities for the Institute of Biology

A. Chroni:

1. Person in charge for education issues in IB. Representative of IB in the Education Committee of NCSR Demokritos
2. Member of the Scientific Advisory Committee of IB.
3. Person in charge for the operation of FPLC

EC Tsilibary:

1. Head, Institute of Biology, Member of the Executive Committee, NCSR Demokritos
2. Coordinator of the committee for external affairs, NCSR Demokritos
3. Coordinator of the committee for the organization of the workshop "NCSR Demokritos- 50 years\'

Impact Factors (for 10 publications): 48,816

Citations 2011 (without self-citations): E C Tsilibary: 78, A Tzinia: 23, P. Kitsiou: 17, A. Chroni: 68, G. Drossopoulou: 53. Total: 239

Total Citations 2007-2011 (without self-citations): ΕΦΚ Τσιλιμπάρη: 264, A. Tzinia: 82, P. Kitsiou: 59, A. Chroni: 304, G. Drossopoulou: 265. Total: 974

h-factor: EC Tsilibary: 29 (30), A. Tzinia: 9, P. Kitsiou: 6, A. Chroni: 13, G. Drossopoulou: 9

Laboratory Equipment and Common Usage Equipment (IB)

FPLC (common equipment)

Refrigerated shaking incubator for cells (equipment purchased with grant funds, A. Chroni)

Tabletop refrigerated microcentrifuge Heraeus (equipment purchased with grant funds, A. Chroni)

Waterbath (equipment purchased with grant funds, A. Chroni)
 2 Magnetic stirrers (equipment purchased with grant funds, A. Chroni)
 Eppendorf microcentrifuge
 Water-bath Heto
 Ultrasound-Sonics (equipment purchased with grant funds, E. Tsilibary)
 PCR apparatus
 2 Digital balances (one was purchased with grant funds, A. Chroni)
 3 Freezers -20°C (one was purchased with grant funds, A. Chroni)
 3 Refrigerators
 2 CO₂ incubators (equipment purchased with grant funds, E. Tsilibary)
 2 Laminar Flow (one was purchased with grant funds, E. Tsilibary)
 Table top centrifuge (equipment purchased with grant funds, E. Tsilibary)
 Microwave oven
 pH meter
 Incubator
 5 Gel electrophoresis apparatuses (two were purchased with grant funds, A. Chroni)
 5 Power supplies (two were purchased with grant funds, A. Chroni)
 4 Vortex (one was purchased with grant funds, A. Chroni)
 3 Shaking platforms (one was purchased with grant funds, A. Chroni)

Current External Funding

Program entitled *Functional genomics of inborn errors and therapeutic interventions in high density lipoprotein (HDL) metabolism* funded by the European Union with the Principal Investigator for NCSR "Demokritos": A. Chroni,

Duration: 2007-2009

Total funding (lab): 294.000€

Funding of the lab for 2011: 20.000€.

Program entitled *Study of functional interactions between HDL and cholesterol transporter ABCG1 - Role in inflammation and atherosclerosis* funded by the Hellenic Society of Lipidology, Atherosclerosis and Vascular Disease with the Principal Investigator for NCSR "Demokritos": A. Chroni,

Duration: 2010-2011

Total funding (lab): 8.000€

Funding of the lab for 2011: 4.000€.

Program entitled *Targeted strategies for new treatment approaches for cardiovascular and inflammatory diseases based on the protective functions of high density lipoprotein (HDL)* funded by GSRT with the Principal Investigator for NCSR "Demokritos": A. Chroni,

Duration: 18/2/2011-17/2/2012

Total funding (lab): 100.400€

Funding of the lab for 2011: 38.640€.

Program entitled *Apolipoprotein E mutations and hereditary Lipoprotein Glomerulopathy: mechanism of pathogenesis and diagnostic value* funded by the Hellenic Atherosclerosis Society with the Principal Investigator for NCSR "Demokritos": A. Chroni,

Duration: 2011-2012

Total funding (lab): 5.000€

Funding of the lab for 2011: 2.500€.

Abbot Hellas (Donation for Research and Development)

Title: "Renoprotective role of vitamin D", Principal Investigators for NCSR: Dr. E. Tsilibary, Dr. G. Drossopoulou

Total funding: 30.000 €

2011 funding: 6.800 €

COST program ECMNet-BM1001: "*Brain Extracellular Matrix in Health and Disease*", funding from the European Science Foundation - COST Action; E. Tsilibary, member of the Management Committee and (representative from Greece.

Duration: 15/12/2010 - 14/12/2014

1000 € for participation as representative from Greece, Loano, IT, May 22-24

COST program ECMNet-BM1001: *"Brain Extracellular Matrix in Health and Disease"*, funding from the European Science Foundation - COST Action; A.Tzinia, representative from Greece.

Duration: 15/12/2010 – 14/12/2014

Dr A. Tzinia, as 2nd representative from Greece, was funded with 850 € for her participation at the annual meeting in Loano, IT, May 22-24

European Science Foundation - COST Action BM0904

Title: *"HDL - From Biological Understanding to Clinical Exploitation."*

Member of the Management Committee A. Chroni, 8/6/2010 – 7/6/2014

Note: Submitted-pending applications

EC Tsilibary:

1. Application for "Program of Excellence" (Participants: A. Tzinia, A. Chroni, P. Kitsiou, G. Drossopoulou): "Common pathogenetic mechanisms and pathways of the matrix-related diseases of Diabetes and Alzheimer's disease resulting in apoptotic cell death" (DIABET-AL) (EC Tsilibary: Principal Investigator) - **Approved for funding (360000 €), 10/2012-10/2015**
2. REGPOT (participants: A. Chroni, P. Kitsiou): Center of Excellence for Multidisciplinary Approaches to identify Targets and Tools interfering with Disease: CEMADis (EC Tsilibary: Principal Investigator)
3. Application for "Collaboration-2" "Pancreatic beta cells functionality and regeneration: the role of liraglutide" (G. Chrousos, Principal Investigator; AS Charonis": co-PI; EC Tsilibary, P. Kitsiou, participants;); **Approved for funding (total budget 1335000€; Team of EC Tsilibary: 270000),12/2012-12/2015)**
4. Application for "Collaboration-2": "Pre-clinical evaluation of novel, synthetic neurosteroids for interfering with neurodegenerative diseases" (Microneurotrophins) (EC Tsilibary, participant)
5. Application for "Collaboration-2": "CleanN-cement (EC Tsilibary, participant)

A. Tzinia:

Alzheimer's Association Grand 2012 : title of proposal 'Neuroprotective role of MMP9 in animal models of Alzheimer disease' (Principal Investigator A. Tzinia, co-Investigator E Tsilibary)

Program duration: 3 years

Budget: 240.000 E

A. Chroni:

Two research grant proposals (Synergasia 2011 and Aristeia) have been submitted to General Secretariat for Research and Technology of Greece.

Research Group: Environmental Mutagenesis -Carcinogenesis

Research Staff

Gerassimos Voutsinas, Senior Researcher

Antonis Lampidonis, Postdoctoral Fellow (IKY Fellowship)

Panagiotis Karkoulis, Graduate Student - *PhD obtained in 2011*

Eumorphia Konstantatou, Collaborating Graduate Student

Eleutheria Peristeri, Collaborating Graduate Student (*MSc*)

Angeliki Delimitsou, Collaborating Graduate Student (*MSc*)

Michaela Diakatou, Undergraduate Student

Angeliki-Stephania Bassoyianni, Undergraduate Student

Sokratis Avgeris, Research Technician

Research Interests

1. Identification and validation of drug targets for cancer therapy
2. Development and evaluation of biomarkers for diagnosis, prognosis and response to treatment in human diseases
3. Development of genetic testing protocols for molecular diagnosis of human genetic diseases

2011 Findings

1. Thymidylate synthase inhibition induces p53-dependent and p53-independent apoptotic responses in human urinary bladder cancer cells

PURPOSE: In search for more effective clinical protocols, the antimetabolite drug 5-fluorouracil (5-FU) has been successfully included in new regimens of bladder cancer combination chemotherapy. In the present study, we have investigated the effects of 5-FU treatment on apoptosis induction in wild-type and mutant p53 urinary bladder cancer cells. **METHODS:** We have used MTT-based assays, FACS analysis, Western blotting and semi-quantitative RT-PCR in RT4 and RT112 (grade I, wild-type p53), as well as in T24 (grade III, mutant p53) and TCCSUP (grade IV, mutant p53) human urinary bladder cancer cell lines. **RESULTS:** In the urothelial bladder cancer cell lines RT4 and T24, 5-FU-induced TS inhibition proved to be associated with cell type-dependent (a) sensitivity to the drug, (b) Caspase-mediated apoptosis, (c) p53 stabilization and activation, as well as Rb phosphorylation and E2F1 expression and (d) transcriptional regulation of p53 target genes and their cognate proteins, while an E2F-dependent transcriptional network did not seem to be critically engaged in such type of responses. **CONCLUSIONS:** We have shown that in the wild-type p53 context of RT4 cells, 5-FU-triggered apoptosis was prominently efficient and mainly regulated by p53-dependent mechanisms, whereas the mutant p53 environment of T24 cells was able to provide notable levels of resistance to apoptosis, basically ascribed to E2F-independent, and still unidentified, pathways. Nevertheless, the differential vulnerability of RT4 and T24 cells to 5-FU administration could also be associated with cell-type-specific transcriptional expression patterns of certain genes critically involved in 5-FU metabolism.

2. The resurgence of Hormone-Sensitive Lipase (HSL) in mammalian lipolysis

The ability to store energy in the form of energy-dense triacylglycerol and to mobilize these stores rapidly during periods of low carbohydrate availability or throughout the strong metabolic demand is a highly conserved process, absolutely essential for survival. In the industrialized world the regulation of this pathway is viewed as an important therapeutic target for disease prevention. Adipose tissue lipolysis is a catabolic process leading to the breakdown of triacylglycerols stored in fat cells, and release of fatty acids and glycerol. Mobilization of adipose tissue fat is mediated by the MGL, HSL and ATGL, similarly functioning enzymes. ATGL initiates lipolysis followed by the actions of HSL on diacylglycerol, and MGL on monoacylglycerol. HSL is regulated by reversible phosphorylation on five critical residues. Phosphorylation alone, however, is not enough to activate HSL. Probably, conformational alterations and a translocation from the cytoplasm to lipid droplets are also involved. In accordance, Perilipin functions as a master regulator of lipolysis, protecting or exposing the triacylglycerol core of a lipid droplet to lipases. The prototype processes of hormonal lipolytic control are the β -adrenergic stimulation and suppression by insulin, both of which affect cytoplasmic cyclic

AMP levels. Lipolysis in adipocytes is an important process in the management of body energy reserves. Its deregulation may contribute to the symptoms of type 2 diabetes mellitus and other pathological situations. We, herein, discuss the metabolic regulation and function of lipases mediating mammalian lipolysis with a focus on HSL, quoting newly identified members of the lipolytic proteome.

Publications

Stravopodis, D.J., P.K. Karkoulis, E.G. Konstantakou, S. Melachroinou, A. Thanasopoulou, G. Aravantinos, L.H. Margaritis, E. Anastasiadou and G.E. Voutsinas (2011) Thymidilate synthase inhibition induces p53-dependent and p53-independent apoptotic responses in human urinary bladder cancer cell lines, *J Cancer Res Clin Oncol* 137, 359-374.

Lampidonis, A.D., E. Rogdakis, G.E. Voutsinas and D.J. Stravopodis (2011) The resurgence of Hormone-Sensitive Lipase (HSL) in mammalian lipolysis, *Gene* 477, 1-11.

Presentations at Scientific Conferences

Karkoulis, P.K., D.J. Stravopodis, A.D. Velentzas and G.E. Voutsinas (2011) 17-DMAG induces functional impairment of heat shock protein 90-assisted signaling pathways in human urinary bladder cancer cells, 62nd Congress of the Hellenic Society of Biochemistry and Molecular Biology, 9-11 December 2011, Athens, Greece.

Voutsinas G. (2011) Molecular basis of Tuberous Sclerosis - novel therapeutic approaches, 37th Panhellenic Medical Conference, 20 May 2011, Athens, Greece.

Voutsinas G. (2011) Molecular basis and therapeutic approaches of Tuberous Sclerosis, 11th Panhellenic Conference of Child Neurology, 10-11 December 2011, Athens, Greece.

Educational Activities

P. Karkoulis successfully defended his Ph.D. thesis entitled «*Study of signal transduction mechanisms under the effect of chemotherapeutic agents in human cancer*», in July 2011, at the Department of Biology of NKUA.

Lecture (2 hours) and practical laboratory exercise (3 hours): "Cytotoxicity study on conventional and targeted chemotherapeutic drugs" included in the course for "Cell and tissue cultures", in the frame of the Post-Graduate Specialization Diploma "Biological Applications in Medicine" of the Departments of Biology and Medicine of the National Kapodistrian University of Athens (NKUA), Athens.

Lectures (2 x 3 hours): "Pharmacological targeting of Hsp90" and "Molecular diagnosis of genetic diseases" included in the course for "Molecular Biology - Systemic and in silico approaches", in the frame of the Post-Graduate Specialization Diploma "Biological Applications in Medicine" of the Departments of Biology and Medicine of the National Kapodistrian University of Athens (NKUA), Athens.

Course: "Introduction to Molecular Biology", including 16 hours practical laboratory exercises, in the American College of Greece (Deree College), Aghia Paraskevi Attikis, Greece.

Lecture (1 hour) entitled "Tuberous Sclerosis: Molecular basis and therapeutic approaches", patient update at a meeting organized by the Greek Association for Tuberous Sclerosis, 12 November 2011, Ilissia Hotel, Athens, Greece.

Other Scientific Activities

Participation in Greek and International scientific bodies and organizations:

1. Reviewer for Chemotherapy Journal, International Journal of Cancer, Journal of Molecular Biochemistry, Molecules and PLoS ONE.
2. Reviewer for the Union for International Cancer Control (International Union Against Cancer).
3. Press conference of Novartis for a novel therapeutic approach of Tuberous Sclerosis, 15 November 2011, Athens, Greece.
4. Greek Alliance for Rare Diseases (PESPA) (Treasurer).
5. Greek Alliance for Rare Diseases (Member of the Scientific Committee).

6. Tuberous Sclerosis Association of Greece (EEOS) (Member of the Scientific Committee).
7. Meeting of the Council of Alliances of Eurordis, 12 May 2011, Amsterdam, Netherlands.
8. Eurordis Membership Meeting 2011, 13-14 May 2011, Amsterdam, Netherlands.
9. Novartis TSC Patient Advocacy Advisory Meeting, 15-16 May 2011, Amsterdam, Netherlands.
10. Final Report (January 2011) from the Greek Conference in the frame of the Europlan Project of EU-Eurordis, organized by PESPA, 26-27 November 2010, Athens, Greece.
11. International TSC Research Conference 2011, 22-25 September 2011, Belfast, Northern Ireland.
12. Member of the Organizing Committee of the 5th Conference of PESPA, "Autoimmune diseases: Today and tomorrow", 25-26 November 2011, Athens, Greece.
13. Member of the Organizing Committee of the 62nd Conference of HSBMB, 9-11 December 2011, Athens, Greece.

Other Activities for the Institute of Biology

Head of the Laboratory for "Molecular Diagnosis of Genetic Diseases" (E1609), rendering genetic testing services for Tuberous Sclerosis and Neurofibromatosis.

In charge for the operation of ABI Prism 310 Genetic Analyzer (Applied Biosystems), Mx3000P QPCR system (Stratagene), Image Analysis System (Vilber Lourmat), LAS-4000 Luminescent Image Analyzer (Fuji-Film) and FLA-7000 Fluorescent Image Analyzing System (Fuji-Film) of the Institute of Biosciences and Applications, NCSR "Demokritos".

Member of 1 internal advisory committee of a scholar working on his thesis (P. Karkoulis).

Impact factors (for 2 publications): 4,982

Number of citations for 2011 (without self-citations): 86

Number of citations 2007-2011 (without self-citations): 303

h-factor: 11

Laboratory Equipment and Common Usage Equipment (IB)

- Old equipment charged to G. Voutsinas or equipment acquired through research program funding: Laminar flow cabinets for cell cultures, CO₂ incubator, refrigerated centrifuge, bench-top microcentrifuge, waterbath, refrigerator, freezers, deep-freezer, agarose and polyacrylamide gel electrophoresis apparatuses, computer, laser printer.
- Common equipment of the Institute of Biosciences and Applications in the responsibility of G. Voutsinas: Thermal cycler, ABI Prism 310 Genetic Analyzer (Applied Biosystems), QPCR Mx3000P system (Stratagene), Image Analysis System (Vilber Lourmat), LAS-4000 Luminescent Image Analyzer (Fuji-Film), FLA-7000 Fluorescent Image Analyzing System (Fuji-Film).

Current External Funding

Research project entitled *Hsp90 inhibition as a new tool against human urinary bladder cancer*, financed by the Miltiades Empeirikos Public Welfare Foundation. Project Coordinator: D.J. Stravopodis (Dept of Biology, University of Athens)

Duration: 1/7/2010 – 31/6/2011

Total funding (laboratory): 6.000 €

Laboratory funding for 2011: 3.000 €.

Research project entitled *Molecular diagnosis of Neurofibromatosis type 1*, financed by the American College of Greece. Project Coordinator: G.E. Voutsinas

Duration: 1/11/2010 – 31/10/2012

Total funding (laboratory): 10.000 €

Laboratory funding for 2011: 3.000 €.

Note: Three (3) research proposals have been approved for funding:

1. «Non-ionizing electromagnetic radiations: biologic effects», Research proposal for funding submitted to the Ministry of Education, Life-long Learning and Religion.
2. «Contribution of intracellular communication of ER α / β with EGR-R and IGF-R in the development and progression of breast cancer: functional properties of cells, expression of bio-active molecules and induction of EMT», Research proposal for funding submitted to the Ministry of Education, Life-long Learning and Religion.
3. «Sequencing and genome characterization of lactic acid bacteria *Streptococcus macedonicus*, *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *lactis* and *Lactobacillus acidipiscis*. Physiological, evolutionary and technological extensions», Research proposal for funding submitted to the Ministry of Education, Life-long Learning and Religion.

PROGRAMME B:
***MODEL SYSTEMS FOR THE
STUDY OF CELL FUNCTION***

Research

Group:

Molecular Genetics of Insects and Biotechnology

Research Staff

Kostas Iatrou, Research Director

Luc Swevers, Senior Researcher

Vassiliki Labropoulou, Senior Researcher

Lydia Ignatiadou, Emeritus Scientist

Konstantinos Koussis, Postdoctoral Fellow

Panagiota Tsitoura, Postdoctoral Fellow

Georgia Kythraioti, Postdoctoral Fellow

Konstantinos Ioannidis, Graduate Student

Christiana Magrioti, Graduate Student - *PhD obtained in 2011*

Ruben De Wilde, Visiting Graduate Student

Dimitris Raptopoulos, Special Collaborator

Dimitra Stefanou, Technical Specialist

Dimitris Kopanelis, Research Technician

Research Interests

- 1. Regulatory mechanisms controlling insect physiological functions.** (a) Oogenesis in lepidopteran insects. (b) Mechanisms of immunosuppression in lepidopteran insects following parasitization by hymenopteran endoparasitoids. (c) Mechanisms controlling olfactory function in the malaria mosquito vector *Anopheles gambiae*.
- 2. Molecular biology and genetic manipulation of insect nuclear polyhedrosis viruses.** (a) Recombinant viruses as vectors for insect genetic transformation. (b) Modified viruses as vectors for human gene therapy and cellular immunization applications.
- 3. Functional genomics.** (a) Systems for production of proteins of economic importance in lepidopteran insect and mammalian cell lines. (b) High throughput screening systems for detection of bioactive substances (activators and inhibitors of pharmacological targets) in chemical libraries and collections of natural products (plants and microorganisms).

2011 Findings

Analysis of small RNA pathways in the silkworm, *Bombyx mori*.

The contribution of the basic machinery of the three pathways of small RNAs to the process of dsRNA-mediated gene silencing was determined by "RNAi-of-the-RNAi" experiments. The results show contribution of components of the siRNA but also of the piRNA pathway in the silencing process. The expression levels of the basic factors of the siRNA pathway were also measured during metamorphosis of the silkworm and differences in expression were observed among different strains, tissues and developmental stages.

Parasitization of lepidopteran insects - the interference of CcBV Ank proteins with the transcription of immune response genes in the host

Six members of the ankyrin-repeat protein family (Ank) of *Cotesia congregata* Bracovirus (CcBV), were examined in vitro for their ability to interfere with the host's induced innate immune responses. Five of the examined Anks suppressed transcriptional activity controlled by Relish1 of *Bombyx mori*, a Rel/NFkB transcription factor of the Imd pathway, to various degrees while only one member of the Ank family, Ank2, displayed transcription inhibitory activity for the Toll immune pathway. When overexpressed in insect cells, Ank2 was shown to be ubiquitinated, a finding suggesting that the protein may be degraded by the proteasome or participate in other, ubiquitin-controlled signal transduction pathways. The same protein was also found to associate with the microtubules in structures resembling mitotic spindles. In conclusion, besides their role as inhibitors of innate immunity-related transcriptional activity, viral Ank proteins seem to fulfil additional but as yet undetermined roles.

Ligands of olfactory proteins as repellents

We continued our screening studies for identification of natural molecules with repellent action against the mosquito vector of the malaria parasite, *Anopheles gambiae*. Molecules isolated from essential oils obtained from a Greek plant collection were examined for binding to odorant binding proteins and bioactivity in behavioural experiments involving live mosquito populations. The most active repellent compounds were also evaluated in natural field tests in Nigeria for repellent action against *A. gambiae* and *Culex sp.* and were shown to possess be equally well repellent activities similar to, if not superior than the well known mosquito repellent DEET. For mosquito odorant receptors, which are ligand-gated heteromeric cation channels, we developed a highly sensitive functional, bioluminescence assay capable of measuring receptor activation upon ligand stimulation. All relevant measurements upon receptor activation confirmed that recombinant odorant receptors expressed in insect cells are functionally active. EC50 values were determined for some of the receptor ligands and essential oils extracts were also examined for their capability to activate odorant receptors. Finally our studies focused on point mutations for odorant receptor OR7 (Orco), an essential receptor subunit, which heteromerizes with other receptor subunits to form the functional cationic channels.

Engineering of baculovirus vectors for transduction of mammalian cells and insect transformation

Further studies were carried out that investigate the functional role of baculoviruses deficient for the gene *lef-8* that encodes a component of the viral RNA polymerase. Experiments indicate that rescue of the deficient virus is not possible by co-transfection with expression constructs for Lef-8 protein. Instead, it was observed that successful rescue requires the re-introduction of the *lef-8* gene into the deficient virus, either under the control of its own promoter or that of a heterologous promoter.

Screening systems for substances with molting-accelerating activity and endocrine disruptors

A functional test was developed for detection of compounds that interact with the ecdysone receptor (EcR) of crustaceans. The method is based on expression of chimeric proteins consisting of the fusion of the ligand-binding domain of EcR with the DNA-binding or activation domain of the Gal4 transactivator.

Screening systems for substances with serotonergic activity.

To discover lead compounds with serotonergic activity, specific functional screening platforms were developed, in collaboration with the laboratory of Molecular Pharmacology of the IB-A (Dr. Z. Georgoussi), which are based on insect cell lines over-expressing the human serotonergic receptors 5HT-3A (K/Ca ion channel) and 5HT-4A (G protein-coupled receptor). With assays that measure the release of intracellular calcium and cAMP in the presence of serotonin and known partial agonists and antagonists, the functionality of the two systems was confirmed. The available platforms are currently used for the screening of plant extracts with the aim of identifying lead compounds for new pharmaceuticals that activate or inhibit the function of the specific serotonergic targets.

Publications

Terenius, O., Papanicolaou, A., Garbutt, J.S., Eleftherianos, I., Huvenne, H., Sriramana, K., Albrechtsen, M., An, C., Aymeric, J.-L., Barthel, A., Bebas, P., Bitra, K., Bravo, A., Chevalier, F., Collinge, D.P., Crava, C.M., de Maagd, R.A., Duvic, B., Erlandson, M., Faye, I., Felföldi, G., Fujiwara, H., Futahashi, R., Gandhe, A.S., Gatehouse, H.S., Gatehouse, L.N., Giebertowicz, J., Gómez, I., Grimmelikhuijzen, C.J., Groot, A.T., Hauser, F., Heckel, D.G., Hegedus, D.D., Hrycaj, S., Huang, L., Hull, J., Iatrou, K., Iga, M., Kanost, M.R., Kotwica, J., Li, C., Li, J., Liu, J., Lundmark, M., Matsumoto, S., Meyering-Vos, M., Millichap, P.J., Monteiro, A., Mrinal, N., Niimi, T., Nowara, D., Ohnishi, A., Oostra, V., Ozaki, K., Papakonstantinou, M., Popadic, A., Rajam, M.V., Saenko, S., Simpson, R.M., Soberón, M., Strand, M.R., Tomita, S., Toprak, U., Wang, P., Wee, C.W., Whyard, S., Zhang, W., Nagaraju, J., French-Constant, R.H., Herrero, S., Gordon, K., Swevers, L., and Smagghe, G. (2011). RNA interference in Lepidoptera: an overview of successful and unsuccessful studies and implications for experimental design. *J. Insect Physiol.* 57, 231-245.

Mommaerts, V., Hagenaars, A., Meyer, J., De Coen, W., Swevers, L., Mosallanejad, H., and Smagghe, G. (2011). Impact of a perfluorinated organic compound PFOS on the terrestrial pollinator *Bombus terrestris* (Insecta, Hymenoptera). *Ecotoxicology* 20, 447-456.

Zervoudi, E., Papakyriakou, A., Georgiadou, D., Evnouchidou, I., Gajda, A., Poreba, M., Salvesen, G.S., Drag, M., Hattori, A., Swevers, L., Vourloumis, D., and Stratikos, E. (2011). Probing the S1 specificity pocket of the aminopeptidases that generate antigenic peptides. *Biochemical J.* 435, 411-420.

Verhaegen, Y., Parmentier, K., Swevers, L., Renders, E., Rougé, P., Soin, T., De Coen, W., Cooreman, K., and Smagghe, G. (2011). The heterodimeric ecdysteroid receptor complex in the brown shrimp *Crangon crangon*: EcR and RXR isoform characteristics and sensitivity towards the marine pollutant tributyltin. *Gen. Comp. Endocr.* 172, 158-169.

Swevers, L., Liu, J., Huvenne, H., and Smagghe, G. (2011). Search for limiting factors in the RNAi pathway in silkworm tissues and Bm5 cells: the RNA-binding proteins R2D2 and Translin. *PLoS ONE* 6:e20250.

Magkrioti, C., Iatrou, K. and Labropoulou, V. (2011). Differential inhibition of BmRelish1-dependent transcription in lepidopteran cells by bracovirus ankyrin-repeat proteins. *Insect Biochem Mol Biol.* 41:993-1002.

Tsitsanou, KE, , Thireou, T., Drakou, CE, Koussis, K, Keramioti, MV, Leonidas, DD, Eliopoulos, E, Iatrou, K, Zographos, SE (2011) *Anopheles gambiae* odorant binding protein crystal complex with the synthetic repellent DEET: implications for structure-based design of novel mosquito repellents. *Cell Mol Life Sci* DOI 10.1007/s00018-011-0745- z.

Ferreira, J.G., Andersen, J.H., Borja, A., Bricker, S.B., Camp, J., Cardoso da Silva, M., Garses, E., Heiskanen, A.S., Humborg, C., Ignatiades, L., Lancelot, C., Menesquen, A., Tett, P., Hoepffner, N. and Claussen, U., (2011). Overview of eutrophication indicators to assess environmental status within the European Marine Strategy Framework Directive. [*Estuarine, Coastal Shelf Science*, 93:117-131.](#)

Articles in Press

De Geyter, E., Swevers, L., Soin, T., Geelen, D., and Smagghe, G. (2012). Saponins do not affect the ecdysteroid receptor complex but cause membrane permeation in insect culture cell lines. *J. Insect Physiol.* 58, 18-23 (IF = 2.031).

Ignatiades L. (2012). Mixotrophic and heterotrophic dinoflagellates in the eutrophic coastal waters of the Aegean Sea (eastern Mediterranean Sea). *Botanica Marina* (in Press). (IF: 1.623)

Metaxatos A. and L. Ignatiades, (2012). Clearance rate in the venerid bivalve *Callista chione* (L) in response to endemic algal species and bacteria: effects of cell biovolume and body size. *Marine Freshwater Behav Physiol* (in Press). (IF: 0.701)

Articles in Books and Conference Proceedings

Swevers, L., and Smagghe, G. (2012). Use of RNAi for control of insect crop pests. In: "Arthropod-Plant Interactions, Novel Insights and Approaches for IPM", G. Smagghe & I. Diaz (Eds.). Springer-Verlag, Dordrecht. In Press.

Presentations at Scientific Conferences

L. Swevers, Liu, J., Huvenne, H., and Smagghe, G. (2011). The RNAi response in lepidopteran insects: can it be exploited for pest control? Annual Scientific Conference "Horticulture – Science, Quality, Diversity and Harmony", May 26th-28th, Iași, Roumania.

L. Swevers, (2011). The RNAi response in insects and its potential for pest control. 2011 In Vitro Biology Meeting, June 4th-8th, Raleigh, North Carolina, USA.

J. Liu, Swevers, L., Huvenne, H., and Smagghe, G. (2011). Expression, transfection, co-localization, purification, and dsRNA inhibition and degradation of a dsRNase in the silkworm *Bombyx mori*. 3rd International Symposium on Insect Physiology, Biochemistry and Molecular Biology (IPBMB), July 2nd - 6th, Shanghai, China.

R. De Wilde, Swevers, L., Verhaegen, Y., Rougé, P., Cooreman, K. and Smagghe, G. (2011). *Crangon crangon* ecdysone receptor and retinoid-X-receptor complex: dimerization influenced by ligand binding? VIIth International Conference On Arthropods: Chemical, Physiological, Biotechnological And Environmental Aspects, Stefan Kopeć Memorial Conference, 18th-23rd September, Białka Tatrzańska, Poland.

- L. Swevers, Kolliopoulou, A., Liu, J., Huvenne, H., and Smagghe, G. (2011). Small RNA pathways in the silkworm-derived Bm5 cell line. Sixth International Symposium on Molecular Insect Science, 2nd-5th October, Amsterdam, The Netherlands.
- H. Huvenne, Swevers, L., De Vos, W., and Smagghe, G. (2011). Gaining insight in the dsRNA uptake mechanism in insect cell lines. Sixth International Symposium on Molecular Insect Science, 2nd-5th October, Amsterdam, The Netherlands.
- K. Iatrou (2011). Malaria mosquito vector control beyond insecticides: plant essential oils as sources of bioactive compounds with mosquito repellent properties. Annual Symposium of the Faculty of Horticulture, University of Agricultural and Veterinary Medicine "Ion Ionescu De La Brad, Iasi, Romania, 26-28 May, 2011.
- P. Tsitoura, K. Koussis and K. Iatrou (2011). Functional expression of *Anopheles gambiae* odorant receptors in lepidopteran insect cells. Sixth International Symposium on "Molecular Insect Science", October 2-5, 2011, Amsterdam, The Netherlands.
- P. Tsitoura, L. Swevers, Z. Georgoussi and K. Iatrou (2011). A functional expression platform for neuronal GPCRs and ligand-gated ion channels of mammalian and invertebrate origin. ISN-ESN-2011, 23rd Biennial Meeting, International Society for Neurochemistry – European Society for Neurochemistry, August 28- September 1, 2011, Athens, Greece.
- P. Tsitoura, K. Koussis and K. Iatrou (2011). Expression of mosquito odorant receptors in lepidopteran insect cells EMBO/FEBS Lecture Course on Channels and Transporters, International School of Biophysics, Erice, Italy, 11-17 May 2011.
- K. Koussis, P. Tsitoura, M. Konstantopoulou, T. Kröber, M. Bourquin, F.R. Dani, O. Marinotti, M.F. Walter, P.M. Guerin, K. Iatrou (2011). *Anopheles gambiae* odorant binding protein, odorant receptor and behavioral screens for identification of new mosquito repellents of natural origin. Fifth International Meeting on "Molecular and Population Biology of Mosquitoes and Other Disease Vectors", 24-30 July 2011, Kolymbari, Crete, Greece.
- K. Koussis, P. Tsitoura, M. Konstantopoulou, T. Kröber, F.R. Dani, P. Pelosi, P.M. Guerin, K. Iatrou (2011). Odorant-binding proteins as frontier probes for compounds effecting mosquito olfactory responses. 21st European Chemoreception Research Organization (ECRO) Congress, Manchester Conference Centre, UK, 7-10 September, 2011.
- P. Tsitoura, L. Swevers, Z. Georgoussi, K. Iatrou (2011). Silkworm functional expression platforms for neuronal GPCRs and ligand-gated ion channels. Second Second International Symposium on "*Bombyx mori* Functional Genomics and Modern Silkworm", October 22-23, 2011, Chongqing, China.
- K. Koussis, T. Kröber, M. Bourquin, M. Konstantopoulou, F.R. Dani, P. Pelosi, P.M. Guerin and K. Iatrou (2011). *Anopheles gambiae* odorant binding proteins provide a fast track for discovery of mosquito repellents from plant extracts. Sixth International Symposium on Molecular Insect Science, 2-5 October 2011, NH Grand Krasnapolsky, Amsterdam, The Netherlands.
- C. Magkrioti, K. Iatrou, V. Labropoulou (2011). Ankyrin-repeat proteins of the polydnavirus ccbv differentially inhibit the imd and toll pathways in lepidopteran cells. Sixth International Symposium on Molecular Insect Science, 2-5 October 2011, NH Grand Krasnapolsky, Amsterdam, The Netherlands.
- K. Koussis, M. Konstantopoulou, T. Kröber, P.M. Guerin and K. Iatrou (2011). Odorant binding protein-based screens for discovery of natural compounds effecting mosquito olfactory responses. Structure- & Computer-Aided Design Workshop: Bioactive Molecules & Materials, National Hellenic Research Foundation, Institute of Organic & Pharmaceutical Chemistry, Athens, Greece, 7 – 11 November, 2011.
- K. Iatrou (2011). Malaria vector control beyond insecticides: rational approaches for the identification of olfaction-based behavior modifiers for Anopheline mosquitoes. Symposium on "Insect Olfaction & Taste: Identifying, Clarifying and Speaking about the Key Issues", 2011 ESA Annual Meeting, November 13-16, 2011, Reno, Nevada, USA.
- K.E. Tsitsanou, T. Thireou, C.E. Drakou, K. Koussis, M.V. Keramioti, D.D. Leonidas, E. Eliopoulos, K. Iatrou, S.E. Zographos. (2011). New molecular targets for the structure-

based design of novel mosquito repellents. Fifth International Meeting on "Molecular and Population Biology of Mosquitoes and Other Disease Vectors", 24-30 July 2011, Kolymbari, Crete, Greece.

K.E. Tsitsanou, T. Thireou, C.E. Drakou, K. Koussis, E. Eliopoulos, K. Iatrou, S.E. Zographos (2011). AgamOBP1 crystal complex with DEET: a new molecular target for the design of novel mosquito repellents. XX1st Congress of the European Chemoreception Research Organization (ECRO), September 7-10, 2011, Manchester, UK, Abstracts, p.123.

K.E. Tsitsanou, C.E. Drakou, A. Thireou, E. Eliopoulos, K. Iatrou and S.E. Zographos (2011). AgamOBP1 is a molecular target for the development of novel insect repellents. 5th Conference of the Hellenic Crystallographic Association, University of Thessaly, 24-25 September 2010, Larisa, Greece. Poster presentation ("*Nikos Oikonomakos*" Award for the best poster presentation).

Other Scientific Activities

Invited Speakers

- Section Horticulture Technologies, Annual Scientific Conference "Horticulture – Science, Quality, Diversity and Harmony", May 26th-28th, Iași, Roumania (L. Swevers)
- Plenary Symposium, 2011 In Vitro Biology Meeting, June 4th-8th, Raleigh, North Carolina, USA (L. Swevers)
- RNAi Symposium, Sixth International Symposium on Molecular Insect Science, 2nd-5th October, Amsterdam, The Netherlands (L. Swevers)
- Plenary speaker, Annual Symposium of the Faculty of Horticulture, University of Agricultural and Veterinary Medicine "Ion Ionescu De La Brad, Iasi, Romania, 26-28 May, 2011 (K.Iatrou).
- 21st European Chemoreception Research Organization (ECRO) Congress, Manchester Conference Centre, UK, 7-10 September, 2011 (K. Iatrou).
- Second International Symposium on "Bombyx mori Functional Genomics and Modern Silk Road", October 21-23, 2011, Chongqing, China (K. Iatrou).
- Symposium on "Insect Olfaction & Taste: Identifying, Clarifying and Speaking about the Key Issues", 2011 Annual Meeting of the Entomological Society of America, November 13-16, 2011, Reno, NV, USA (K. Iatrou).

Member, Steering Committee, International Lepidopteran Genome Project Consortium (K. Iatrou).

Editor of Scientific Journal: "The Journal of Insect Science" (K. Iatrou)

Member of the Editorial Board of the Scientific Journals: "Sericologia", "Insect Biochemistry and Molecular Biology", "Archives of Insect Biochemistry and Physiology", "Open Biotechnology Journal", "Journal of Biomedicine and Biotechnology", and "Current Biotechnology" (K. Iatrou)

Member of the Editorial Board of the Scientific Journal: "Archives of Insect Biochemistry and Molecular Biology" (L. Swevers)

Member of the Editorial Board of the Scientific Journal: "Mediterranean Marine Science Journal" (L. Ignatiadou)

Member of Meeting Organizing Committee: The Second International Symposium on "Silkworm Functional Genomics and Modern Silkroad", Southwest University, Chongqing, China, October 21-23, 2011 (organization by the Department of International Cooperation, Ministry of Science and Technology, PRC; the Chongqing Science and Technology Commission, Chongqing, China; and the State Key Laboratory of Silkworm Genome Biology, Southwest University, China). (K. Iatrou)

Organizer, 2nd Joint Symposium of EU/FP7 Funded Projects for Malaria Vector Biology and Control", Orthodox Academy of Crete, Kolymbari, Crete, Greece - 24th July 2011 (K. Iatrou)

Reviewer of Articles for the Scientific Journals "Insect Biochemistry and Molecular Biology", "Insect Molecular Biology", "Journal of Insect Science", "Sericologia", "Journal of Insect Physiology", "Journal of Biomedicine and Biotechnology", "Molecular Biology Reports", "Parasites & Vectors", "Comparative and Functional Genomics", "Journal of Virological Methods", "Current Biotechnology", "Molecular Microbiology", "Journal of Molecular Biology", "Pakistan Journal of Scientific & Industrial Research", "Iranian Journal of Applied Animal Science", "International Journal of Biological Sciences" (K. Iatrou)

Reviewer of Articles for the Scientific Journals "Central European Journal of Biology", "Plant Protection Science", "Archives of Insect Biochemistry and Molecular Biology", "Insect Biochemistry and Molecular Biology" (2 x), "PLOS ONE" (4 x), "African Journal of Biotechnology", "Journal of Insect Physiology, Ecotoxicology", "Bulletin of Entomological Research", "Insect Molecular Biology" (L. Swevers).

Reviewer of Articles for the Scientific Journals "Marine Ecology", "Mediterranean Marine Science Journal", "Environmental Science and Pollution Research" (L. Ignatiadou)

Member of the Committee for Recruitment of Two External Collaborators for the Scientific Program "RASTAS-SPEAR: RAdiation-Shapes Thermal protection investigAtionS for high-Speed EArth Re-entry" (Institute of Material Science, NCSR "Demokritos") (L. Swevers).

Creation of the laboratory for the Provision of Specialized Scientific and Technological Services and Products with the title "Distribution of Biotechnological Products and Services" (K. Iatrou).

Educational Activities

C. Magkrioti presented her Ph.D. thesis with title "Interactions of proteins from the endosymbiont virus of the parasitoid hymenopteran *Cotesia congregata* with proteins of the immune response of Lepidoptera" during December 2011 at the Department of Biology of the National University of Athens (supervisors K. Iatrou and V. Labropoulou).

One-hour lecture entitled "Targeting the olfactory functions of mosquitoes for the drastic reduction in the spreading of infectious diseases" at the Summer School of NCSR "Demokritos" (K. Iatrou).

One-hour lecture entitled "The RNAi mechanism in insects and its potential for pest control" at the Summer School of NCSR "Demokritos" (L. Swevers).

Two-hour lecture with title "Expression of target proteins in insect cells for discovery of bio-active lead molecules" at the 1st Summer School of the Department of Biology, National University of Athens (K. Iatrou).

Other Activities for the Institute of Biology

Supervision of Ph.D. thesis of Theodoros Georgomanolis, IB graduate student (University of Athens) (K. Iatrou, L. Swevers).

Supervision of Ph.D. thesis of Konstantinos Ioannidis, IB graduate student (University of Athens) (K. Iatrou, L. Swevers).

Supervision of Ph.D. thesis of Christiana Magkrioti, IB graduate student (University of Athens) (K. Iatrou, V. Labropoulou).

Supervision of Ph.D. thesis of Anna Kolliopoulou, IB graduate student (University of Athens) (L. Swevers).

Member of the Internal Committee for supervision of graduate students with scholarship from NCSR "Demokritos" at the IB: Christiana Magkrioti, Konstantinos Ioannidis, Maria Papakonstandinou (L. Swevers).

Responsible for the functioning of the following instruments: Fluostar Microplate Fluorometer, HPLC Hewlett Packard, microplate luminometer TECAN InfiniTE M-200 (L. Swevers).

Member of the Internal Committee for supervision of graduate students with scholarship from NCSR "Demokritos" at the IB: Christiana Magkrioti, Konstantinos Ioannidis (V. Labropoulou).

Responsible for tours and public relations of the Institute of Biology, NCSR "Demokritos" from September 2010 (V. Labropoulou).

Impact Factors (for 8 publications): 31,05

Citations 2011 (without self- citations): 233

Iatrou K.: 117

Swevers L.: 60

Labropoulou V.: 49

Ignatiadou L.: 78

Total Citations 2007-2011 (without self- citations): 909

Iatrou K.: 412

Swevers L.: 237

Labropoulou V.: 222

Ignatiadou L.: 309

h-factor:

25 (K. Iatrou)

16 (L. Swevers)

9 (V. Labropoulou)

17 (L. Ignatiadou)

Laboratory Equipment and Common Usage Equipment (IB)

- Cell culture: incubators, bio-reactors, laminar flow, inverted fluorescence microscope, microcentrifuges with cooling.
- Incubators for rearing of insects (silkworm) in controlled environment.
- Production of proteins: affinity chromatography, purification of antibodies
- Biochemistry and molecular biology: DNA, RNA and protein electrophoresis, microcentrifuges, DNA sequencer (IB), ultracentrifuges (IB), Nuclear Magnetic Resonance (NMR) spectrometer (IB), sonicator (IB), micro-spectrometer, speedvac concentrator (IB), scintillation counter (IB), HPLC.
- Cell Biology: automatic cell sorter (IB), fluorescence microscope, confocal microscope (IB), high-throughput screening for bio-active substances and protein interactions: microplate reader for fluorescence and luminescence (for GFP, rhodamin, fluorescein, β -galactosidase, luciferase and other photo-proteins) and studies of protein interactions by fluorescence resonance energy transfer (FRET) (IB).

Current External Funding

Program FWO-Vlaanderen F 6/12 (Belgium) with title *Key mechanisms of systemic RNA interference (RNAi) in insects* with coordinating scientist in Greece Dr. Luc Swevers (Coordinator: G. Smagghe, (Belgium)

Duration: 1/2009-12/2012

Total funding: 1.170.200€

Total lab funding: 0€.

PENED 2005 project entitled *Mechanisms of immunosuppression in lepidopteran insects following parasitization by hymenopteran endoparasitoids: the role of the interactions between proteins produced by hymenopteran endosymbiotic polydnaviruses and hemocyte proteins of the lepidopteran hosts*, funded by GSRT (Coordinator K. Iatrou).

Duration: 3/2006-3/2009

Total funding (lab): 57.600 €

Funding of the lab for 2011: 0 €.

Project entitled *ENAROMaTIC - European Network for Advanced Research on Olfaction for Malaria Transmitting Insect Control*, funded by the European Union with Scientific Coordinator K. Iatrou.

Duration: 12/2008-12/2012

Total funding (consortium): 2.500.000 €

Total funding (lab): 563.000 €

Funding of the lab for 2011: 219.217 €

Co-funding of the lab from GSRT for 2010: 879,61€

Note: The following proposals have been also submitted and are funded (#1) or are under evaluation (#2-#5):

1. Scientific Program “Thalis”, Strengthening of inter-scientific and inter-institutional research and innovation with possibility of attraction of high quality scientists from abroad through conduction of basic and applied research of excellence: “Genomic and functional approach to understand the resistance of insects and mites against insecticides and development of applications for its management” Scientific Coordinator: J. Vontas, University of Crete. Coordinating Scientist for research team at NCSR “Demokritos” (K. Iatrou, L. Swevers, V. Labropoulou, M. Konstantopoulou): K. Iatrou. **Approved - 90.000€** funding for the research team during the whole duration of the program.
2. Bilateral S & T Cooperation (Greece-Slovakia). “New Approaches for Insect Transformation”. Coordinator: L. Swevers. Requested lab funding: 15.000€.
3. Operational Program “Training and Life-Long Learning”, Action “Excellence”: “Comparison of the RNAi response between coleopteran and lepidopteran insects”. Coordinator: L. Swevers. Requested lab funding: 325.200€.
4. Operational Program “Training and Life-Long Learning”, Action “Excellence”: “Olfaction-based behaviour modifiers of plant origin for protection against mosquito-born infectious diseases – the malaria paradigm”. Coordinator: K. Iatrou. Requested lab funding: 456.000€.
5. ERC Synergy Grant 2012: “Induction of RNAi in Insects by Non-pathogenic Virus Inhibition”. Third Principal Investigator: Luc Swevers. Requested lab funding: 2.088.000.

Research Group: *Pending (Collaboration with the laboratory of Molecular Genetics of Insects and Biotechnology - In charge: Dr. K. Iatrou)*

Research Staff

Maria Konstantopoulou, Senior Researcher

Kelly Martinou, Collaborating Scientist

Anatsassia Pantazi – Mazomenou, Research Technician

Rafaela Panteleri, Undergraduate Student

Research Interests

- Chemical ecology: isolation and identification of biologically active compounds, relating to insect chemical communication and plant – insect interactions (pheromones, volatile compounds of plant origin etc.) that may be used in integrated pest management programs.
- Isolation and identification of secondary metabolites (mainly of plant origin) acting on insect physiology and/or behavior (behavior modifying agents - infochemicals). Laboratory and field evaluation of bioactivity of the isolated metabolites; study of their mode of action.
- Development of specialized chemical attractants for insects and technologies for their application in pest population control. Biodegradable materials endowed with controlled release rate and UV protection properties.
- Biochemistry of insect olfactory receptors with emphasis on the localization and isolation of protein receptors for semiochemicals.
- Endosymbiotic bacteria of insects: Isolation and studies on their mutualistic relations with host insects.
- Microorganisms and Biotechnology: Isolation of naturally occurring microorganisms and their biologically active secondary metabolites (toxins) aiming to their incorporation in insect population management.

2011 Findings

In the context of the EC funded project ENAROMaTIC (collaboration with the Laboratory of "Insect Molecular Genetics and Biotechnology", Prof. K. Iatrou), the collection of endemic botanical species was continued and our current collection comprises of over 300 species belonging to 68 plant families. Steam distillation in a modified Clevenger apparatus is employed to extract essential oils. The crude extracts are tested with the high throughput screening assay for OBPs ligand identification. Extracts exhibiting positive response are fractionated by means of solid phase extraction using increasing polarity solvents. Fractions are evaluated for biological activity. Gas chromatography is employed to identify differences in eluates between crude extracts and their fractions. Tentative identification of biologically active compounds is performed using Mass Spectroscopy. Four compounds positively identified have shown biological activity both in laboratory and in small scale field experiments.

In the framework of the project: "Development of ecological and innovative technologies for the population management of the pine processionary moth, *Thaumetopoea pityocampa*, in urban and suburban environments" experiments were conducted for the micro-encapsulation of the insect's pheromone in various biodegradable polymers. The rate of pheromone desorption (release rate) out of the polymers under evaluation under laboratory, artificial ageing and field conditions were recorded. Evaluation of commercially available containers and applicators for the chosen bio-polymers was conducted aiming to the ease of use and effective application of the Mating Disruption Method. Final report of results and technology transfer was submitted to the concerned company.

In the framework of the project: "Research and development of innovative tools for palm pest management, *Rhynhophorous ferrugineus* (Coleoptera: Curculionidae)" experiments were conducted to study the effect of entomopathogenic nematodes, entomopathogenic fungi, the effectiveness of the currently used kairomonal mixture and the possibility of its improvement, and the effectiveness of various types of traps currently in use.

Final report of results and technology transfer was submitted to the concerned company.

Publications

Hegazi, E., Konstantopoulou, M., Herz, A., Khafagi, W., Agamy, E., Showiel, S., Atwa, A., Gehan M., El-Aziz, A., Safaa, M., Abdel-Rahman, S. (2011). Seasonality in the occurrence of two lepidopterous olive pests in Egypt. *Insect Science* 18: 565-574.

Hegazi, E., Khafagi, W., Herz, A., Konstantopoulou, M., Hassan, S., Agamy, E., Abd El-Aziz, G., Ali, S., Showeil, S. (2011). Dispersal and field progeny production of *Trichogramma* species released in olive orchard in Egypt. *Biocontrol* DOI 10.1007/s10526-011-9420-4.

Articles in Press

Hegazi, E.M., Konstantopoulou, M.A., Khafagi, W.E., Herz, A., Raptopoulos, D.G., Agamy, E., Abd El-Aziz, G., Ali, S. E., Abdel-Rahman, S. (2012). Oviposition behavior of *Palpita unionalis* in different olive varieties. *Phytoparasitica* (In press). IF: 0.527.

Presentations at Scientific Conferences

K. Koussis, P. Tsitoura, M. Konstantopoulou, T. Kröber, M. Bourquin, F.R. Dani, O. Marinotti, M.F. Walter, P.M. Guerin, K. Iatrou (2011). *Anopheles gambiae* odorant binding protein, odorant receptor and behavioral screens for identification of new mosquito repellents of natural origin. Fifth International Meeting on "Molecular and Population Biology of Mosquitoes and Other Disease Vectors", 24-30 July 2011, Kolymbari, Crete, Greece.

K. Koussis, P. Tsitoura, M. Konstantopoulou, T. Kröber, F.R. Dani, P. Pelosi, P.M. Guerin, K. Iatrou (2011). Odorant-binding proteins as frontier probes for compounds effecting mosquito olfactory responses. 21st European Chemoreception Research Organization (ECRO) Congress, Manchester Conference Centre, UK, 7-10 September, 2011.

K. Koussis, T. Kröber, M. Bourquin, M. Konstantopoulou, F.R. Dani, P. Pelosi, P.M. Guerin and K. Iatrou (2011). *Anopheles gambiae* odorant binding proteins provide a fast track for discovery of mosquito repellents from plant extracts. Sixth International Symposium on Molecular Insect Science, 2-5 October 2011, NH Grand Krasnapolsky, Amsterdam, The Netherlands.

K. Koussis, M. Konstantopoulou, T. Kröber, P.M. Guerin and K. Iatrou (2011). Odorant binding protein-based screens for discovery of natural compounds effecting mosquito olfactory responses. Structure- & Computer- Aided Design Workshop: Bioactive Molecules & Materials, National Hellenic Research Foundation, Institute of Organic & Pharmaceutical Chemistry, Athens, Greece, 7 - 11 November, 2011.

E. Lioulia, C. Koutsonikou, R. Panteleri, D. Mademtzoglou, M. Konstantopoulou and P. Mavragani-Tsipidou. Evaluation of the insecticidal and genotoxic activity of the essential oils of the plants *Azadirachta indica*, *Pimpinella anisum* and *Carum carvi*. Proceedings of the 33rd Scientific Conference of Hellenic Society for Biological Sciences, Edessa, May 19-21, 2011.

A. Michaelakis, P. Milonas, D. Papachristos, D. Kontodimas, F. Karamaouna, K. Koutrouli, C. Pontikakos, D. Raptopoulos, N. Babilis and M. Konstantopoulou (2011). Monitoring *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae) populations and their management by employing mating disruption. 14th Panhellenic Meeting of Hellenic Entomological Society, ES, 11 - 14 October, Naples, Greece. Abstract: 155-157.

A. Martinou, P. Milonas, D. Raptopoulos, N. Babilis and M. Konstantopoulou (2011). Toxicity of TETRASTOP® on the black bean aphid *Aphis fabae* Scopoli. 14th Panhellenic Meeting of Hellenic Entomological Society, ES, 11 - 14 October, Naples, Greece. Abstract: 339-341.

Other Scientific Activities

Member of the supervising committee for Diploma of undergraduate student in the Department of Genetics, Development and Molecular Biology, School of Biology, Aristotle University of Thessaloniki (Assoc. Prof. P. Tsipidou-Mavragani).

Reviewer of the following international scientific journals: Chemosphere, Journal of Agricultural and Food chemistry, Entomologia Experimentalis et Applicata, Bulletin of Insectology, Journal of Applied Entomology, Crop Protection, Insect Science and Journal of Pest Science.

Member of the editorial board of the scientific journal "*Tunisien Journal of Plant Protection*" specialist for the Chemical Ecology issues.

Member of the Cultural and Environmental Committee of the Board of NCSR "Demokritos".

Substitute member of the committee for the public bidding contest for the "Canteen housed in the installations of NCSR "Demokritos".

Other Activities for the Institute of Biology

Responsible for radioprotection of the radioactive source Co-60, with activity 5470 Ci (March 2004-).

Impact Factors (for 2 publications): 3,320

Citations 2011 (without self-citations): 24

Total Citations 2007- 2011 (without self-citations): 111

h-factor: 8

Laboratory Equipment

The laboratory is adequately equipped for analysis and identification of chemical compounds: gas chromatograph (GC), high pressure liquid chromatography (HPLC) fitted with an up to four solvent mixing system and supported by the appropriate software for recording and processing results. In addition various distillation devices, chromatography columns, a UV apparatus, an ultrasound apparatus and a solid phase microextraction (SPME) system are available.

Insect rearing units (including CT room).

Wind tunnel for studies of insect behavior under the effect of various chemical *stimuli*.

Stereoscope equipped with a camera, Incubators, laminar flow hood.

Protein purification and isolation: protein electrophoresis, western blot, centrifuge.

Current External Funding

Participation in the EU Research project entitled *ENAROMaTIC - European Network for Advanced Research on Olfaction for Malaria Transmitting Insect Control* (Coordinator: Prof. K. Iatrou).

Duration: 12/2008-12/2012

Total project funds: 2.500.000 €

Total funds for the Coordinator's laboratory: 563.000 €

Funds (2011) for the laboratory derive through the total Coordinator's funds

Participation in the action "Innovation Vouchers for Small and Medium Enterprises", Secretariat for Research and Technology (GSRT) of the Ministry for Education Lifelong Learning and Religion: "Development of ecological and innovative technologies for the population management of pine processionary moth, *Thaumetopoea pityocampa*, in urban and suburban environments" (Coordinator Dr. M. Konstantopoulou).

Duration: 4/2011-8/2011

Total funding (lab): 7.000 €

Funding of the lab for 2011: 0 €

Participation in the action "Innovation Vouchers for Small and Medium Enterprises", Secretariat for Research and Technology (GSRT) of the Ministry for Education Lifelong Learning and Religion: "Research and development of innovative tools for palm pest management, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae)" (Coordinator Dr. M. Konstantopoulou).

Duration: 6/2011-10/2011

Total funding (lab): 7.000 €

Funding of the lab for 2011: 0 €

Note 1: Research program "Thales" - Enhancing interdisciplinary and multidisciplinary research and innovation with the potential to attract high level researchers from abroad by conducting basic and applied research excellence. Ministry for Education Lifelong Learning and Religion: "Genomic and functional analysis for insect and acari resistance to insecticides" Coordinator: J. Vontas, University of Crete. Research partner from Institute of Biology, NCSR "D": Prof. K. Iatrou, in collaboration with Luc Swevers, Vasiliki Labropoulou and M. Konstantopoulou.

A financing of 90,000 Euros has been approved and will be made available for the group during the period for the project.

Research Group: Chronobiology

Research Staff

Anastassia Prombona, Senior Researcher

Anastasia Repouskou, Graduate Student - PhD obtained in 2011

Aggeliki Galeou, Graduate Student (MSc) - MSc obtained in 2011

Research Interests

Investigation of the biological clock function in plants

Study of rhythmically expressed genes in *Phaseolus vulgaris*. Regulation of genes involved in the synchronization of the biological clock by input light signals and photoperiodism. Role of rhythmically expressed genes in the central oscillator function. Interaction of clock proteins.

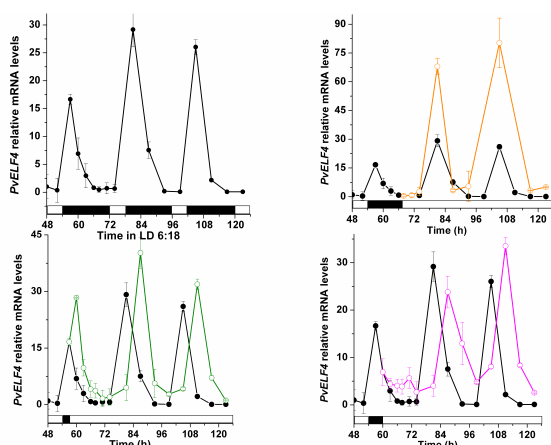
Involvement of the biological clock function in carcinogenesis

Regulation of cell cycle and cell proliferation by the biological clock in mouse fibroblasts and cancer cell lines. Study of the effects of modulated acetylation levels on the biological clock function and the cell cycle. Elucidation of the role of the circadian time in proliferation of cancer cells during the application of drugs (chronotherapy).

2011 Findings

Investigation of the biological clock function in plants

In order to study the resynchronization of the *Phaseolus vulgaris* clock by light signals at night, experiments of A. Galeou for her MSc thesis aimed at exploring the light responsiveness of evening specific genes at different phases of their rhythmic expression. Thus, we examined the phase-specific light responsiveness and the resulting rhythmic expression of the evening-peaking *PvTOC1* and *PvELF4* genes. The study of the oscillatory pattern of both revealed that they are symphasic, not only under different photoperiods but also after artificial dawn at night. In addition, they both exhibit responsiveness to light application at darkness, only at the peak phase of the rhythm, which occurs a few hours after lights-off. Unexpectedly, at phases different from peak, *PvTOC1* and *PvELF4* are not light responsive, but can, with the exception of phases attained towards the end of the night, be resynchronized. The mechanism, by which these differential responses occur, is under investigation. Experiments with leaf movements signify the involvement of evening-specific - and not morning-specific elements - of the clock in this physiological process (unpublished results).



ELF4 of *Phaseolus vulgaris* is rhythmically expressed under photoperiod of LD 6:18 (6h light:18h dark). Artificial dawn at the beginning of the night (3h after dusk: green line, 6h after dusk: pink line) results in phase change (resynchronization) of the oscillation. In contrast, artificial dawn at the end of the night (4h before dawn: orange line), does not have any effect on the phase, that is identical to the photoperiodic rhythm (black line). Day is depicted by white and night by black boxes on the x-axis.

function in carcinogenesis

In the last year of her PhD work (defense: January 2011) A. Repouskou explored the hypothesis whether the accumulation and stability of c-MYC onco-protein are regulated by the circadian clock. For this purpose she examined the endogenous protein levels in clock synchronized N2A (mouse neuroblastoma) cell cultures at 4-hour intervals as well as the degradation and synthesis rate of the protein at different circadian times. The results documented the regulation of the c-MYC protein by the circadian clock at various levels

Involvement of the biological clock

that results in rhythmic levels in the accumulation of the oncoprotein (unpublished results).

The present activity is performed in collaboration with Dr. T. Sourlingas (Head of Laboratory of Nuclear Proteins and Chromatin Function).

Articles in Press

Galeou A., Prombona A. (2012). Light at night resynchronizes the evening-phased rhythms of *TOC1* and *ELF4* in *Phaseolus vulgaris*. *Plant Science* 184 (2012) 141- 147, i.f: 2,481

Educational Activities

A. Repouskou defended her PhD thesis with title: «Circadian clock and histone acetylation: their interaction with the cell cycle in *in vitro* murine cell cultures» (January 2011, Department of Biology, National and Kapodistrian University of Athens) (Scientific supervisor: Dr. A Prombona).

A. Galeou presented her Master thesis with title: «Identification of regulatory elements of a diurnally expressed gene of *Phaseolus vulgaris*» (May 2011, Department Science of Plant Production, Agricultural University of Athens) (Scientific supervisor: Dr. A. Prombona).

Teaching in Lecture Series with Title: “Applications of Biological Sciences in Medicine: Systemic and *in silico* approaches” (Dr. A. Prombona, 3 hour-lecture in the summer semester), Department of Biology, National and Kapodistrian University of Athens

Other Activities for the Institute of Biology

Information guidance of visiting school classes about the activities of the Institute (A. Prombona)

Citations 2011 (without self-citations): 4

Total Citations 2007- 2011 (without self-citations): 39

h-factor: 5

Common Usage IB Equipment in the Laboratory (In charge: A. Prombona)

Thermal Cycler 2 blocks (Biorad)

Thermal Cycler (MJ Reasearch)

Electroporator (BTX, ECM 399)

Hybridization Oven (Stuart Scientific)

Spectrophotometer (Hitachi)

French Press (Aminco)

Incubator 37°C (Gallenkamp)

Note: The submitted proposal «Histone acetyltransferase Tip60 is a potential modulator of clock-controlled genes implicated in liver metabolism and obesity» with postdoc researcher Dr. A. Repouskou has been supported with 150000 euro from the Program: Support of Postdoctoral Researchers, ESPA 2007-2013 in collaboration of the Laboratory of Chronobiology (Head: Dr. A. Prombona) and the Department Genes and Behavior (Head: Prof. G. Eichele) at the Max-Planck Institute for Biophysical Chemistry (Karl Friedrich Bonhoeffer Institute), Göttingen (Germany).
Budget for 2011: 0 euro

Research Group:

Microbial Molecular Genetics

Research Staff

Vassiliki Sophianopoulou, Research Director

Eleftherios Sideris, Emeritus Scientist

Ioannis Vaggelatos, Graduate Student

Alexandros Athanassopoulos, Graduate Student

Pavlos Geranios, Collaborating Graduate Student

Vassiliki Pantazopoulou, Undergraduate Student – *Undergraduate dissertation completed in 2011*

Interests

Our group is primarily interested in several aspects concerning i) the expression, function, cell biology and evolution of transport proteins and ii) membrane organization. Our model organism of choice is the non-pathogenic ascomycetes *Aspergillus nidulans*, a classic model genetic system since the 1950's. The two last decades several *A. nidulans* transporters specific for amino acid transport have been cloned and studied in respect to their transcriptional, post-translational and cellular control of expression.

A. Transporters of medical, pharmacological and agricultural importance

Recognition and transport of amino acid-neurotransmitters (proline, glutamate), through cellular membranes via specific transmembrane transporters

Activities:

a) identification and regulation of the expression of genes encoding amino acid transporters b) isolation and characterization of factors that regulate directly or indirectly the activity of amino acid transporters ie *trans*-acting molecular determinants involved in topogenesis/recycling/endocytosis of amino acid transport systems (eisosomal proteins, CKI kinases, aldolases) and c) studies on structure-function relationships of amino acid transporters.

Intermediate and Long-term objectives: better understanding of the molecular basis of neurodegenerative diseases, possible identification of new pharmaceutical targets and future development of highly-targeted drugs.

B. Fungal membrane organization

Activities:

a) identification and molecular, cellular characterization of eisosomal and eisosome-associated proteins involved in lateral compartmentation of fungal plasma membrane b) functional characterization of eisosome and eisosome-associated proteins c) structure-function analysis of eisosome and eisosome-associated proteins

Intermediate and Long-term objectives: knowing the role of specific fungal proteins, including those in contact with the cells of the host, is one of the strategies to develop very specific anti-mycotic drugs with little or no side effects on human patients, which is not the case with most of the first-line anti-mycotic drugs.

The principal current interest of the lab is to use *A. nidulans* as a model system for:

- Genetically and biochemically dissecting the **structure-function relationships** underlying **amino acid transporter** topogenesis, function and specificity
- Identifying the pathways and molecular mechanisms involved in the **membrane trafficking and endocytosis** of specific transporters in response to various physiological, developmental and genetic signals
- Studying the role of **eisosomal proteins** in fungal membrane organization in respect to membrane trafficking and endocytosis of specific transporters, cell cycle progression and animal/human infections

2011 Findings

A. Transporters of medical, pharmacological and agricultural importance

The CkiA casein kinase I is essential in *A. nidulans* and is localised to the cytoplasm and the nucleus. We have investigated the effect of three characterized point mutations and of depletion of the CkiA activity, on the activity, localization and integrity of AgtA (dicarboxylic amino acid specific transporter) and to a lesser detail on the activity and localization of PrnB (specific proline transporter). Both point mutations and CkiA depletion result in mislocalisation of AgtA to the vacuole followed by proteolysis. Experimental and genomic data indicate that both the signalling of amino acid transporter traffic and the functions of the casein kinases I have diverged considerably between the Saccharomycotina and the Pezizomycotina (A. Apostolaki et al., 2012, *Mol. Microbiol.* 84(3):530-49).

B. Fungal membrane organization

In an attempt to find possible substrates of the CkiA (casein kinase I) of *A. nidulans* -alleles of which result in disruption of A

amino acid transporter targeting to plasma membrane- we have studied *A. nidulans* eisosomal proteins stated to be involved in lateral compartmentation of fungal plasma membranes. (see I. Vangelatos et al., 2010). The eisosomal proteins are universally and quite strictly conserved in ascomycetes, however this evolutionary conservation is in contradiction with an elusive functional significance. Our comparative analysis of the eisosomes of *Aspergillus nidulans* and *Saccharomyces cerevisiae* reveal striking differences in the assembly and developmental fate of these structures between these two model organisms (see C. Scazzocchio et al., 2011). Moreover, we have investigated the subcellular localization of non mobile eisosomes at different developmental stages of *A. nidulans* sexual cycle (ascospore development and germination) and their role in cell cycle progression. Our results showed no association between eisosomal proteins and sexual cycle progression and *no direct relation between* PilA eisosome-like structures and clathrin-mediated endocytosis. On the other hand our results suggest correlation between PilA foci and sphingolipid biosynthesis (A. Athanasopoulos and V. Sophianopoulou in preparation; see Fig. 1).

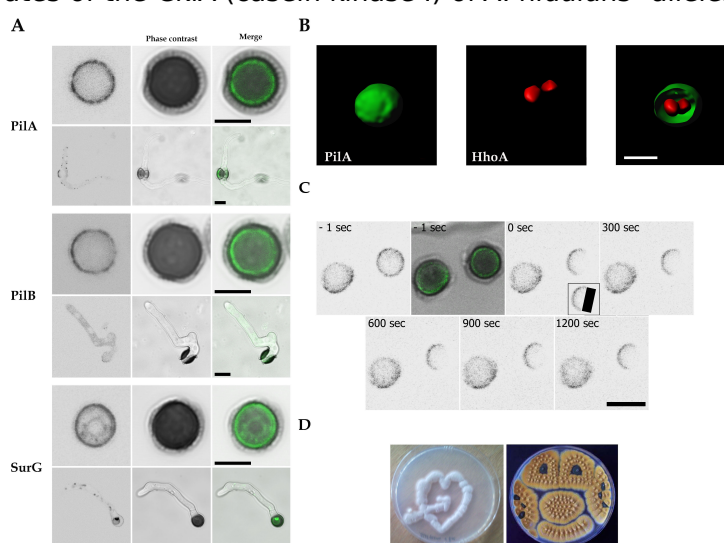


Figure 1 Cellular distribution of eisosomal proteins: (A) Polar sections of confocal images of ungerminated ascospores and young mycelia of *Aspergillus nidulans* strains expressing the eisosomal proteins PilA-GFP, PilB-GFP and SurG-GFP. (B) 3-D view of PilA and HhoA (histone 1) after isosurface extraction of confocal z-stack 0.5µm thick sections, using the 3-D visualization software Imaris 7. 2.3. Interior view of ascospore showing both nuclei of a binucleate ascospore. Green and red correspond to PilA and HhoA respectively. (C) Time-lapse experiments in ungerminated ascospores expressing PilA-GFP, showing extremely low mobility of eisosomal PilA after photobleaching. (D) Solid culture of an *Aspergillus nidulans* strain being "in love" and "happy".

Publications

Scazzocchio, C., Vangelatos, I., and Sophianopoulou, V. (2011). Eisosomes and membrane compartments in the ascomycetes: a view from *Aspergillus nidulans*. Invited Addendum Article: **Commun. Integr. Biol.** Vol. 4(1): 64-68. <http://www.ncbi.nlm.nih.gov/pubmed/21509182>

Articles in Press

Apostolaki, A., Harispe, L., Calcagno-Pizzarelli, AM., Vangelatos, I., Sophianopoulou, V., Arst Jr, A., Peñalva, MA., Sotiris, A., and Scazzocchio, C. (2012). The activity of an *Aspergillus nidulans* essential casein kinase I is required for delivery of amino acid

transporters to the plasma membrane. **Mol. Microbiol.** 84(3):530-49 <http://www.ncbi.nlm.nih.gov/pubmed/22489878> (IF: 5).

Articles in Books and Conference Proceedings

A. Apostolaki, L. Harispe, A.M. Calcagno-Pizzarelli, I. Vangelatos, V. Sophianopoulou, H.N.Jr. Arst, M.A. Penalva, S. Amillis, C. Scazzocchio 2011. 62^o Meeting of the Hellenic Society of Biochemistry and Molecular Biology: CkiA activity is required for amino acid transporter delivery to the plasma membrane in *Aspergillus nidulans*. (Poster in Intracellular trafficking session, page 44).

V. Sophianopoulou: Summer School NCSR «Demokritos» July 2011. Life Sciences: Molecular organization of protein microdomains in fungal plasma membrane and their role in fungal infections. Abstract book pages 142-143.

3. 2nd Annual International Symposia of Mycology (ISM-2012). Invitation to deliver an oral presentation at session ISM 1-3: Fungal Genetics and Genomics. Guangzhou, China (V. Sophianopoulou).

Presentations at Scientific Conferences

1. V. Sophianopoulou 2011. Model Microorganisms in Basic Research: Molecular organization of plasma membrane microdomains in fungi and their implication in fundamental cellular processes and pathogenicity. Abstract of the 4th Congress of the Hellenic Scientific Society “Mikrobiokosmos”, pages 18-19 (invited speaker: V. Sophianopoulou).

2. A. Athanasopoulos, H. Boleti, V. Sophianopoulou 2011. Abstract of the 62^o: Meeting of the Hellenic Society of Biochemistry and Molecular Biology: Molecular organization of plasma membrane in fungi: subcellular distribution of eisosomal proteins in the ascomycetes *Aspergillus nidulans*. (Oral presentation: A. Athanasopoulos, in session VII-Cell-cell communication, signaling and intracellular trafficking page 22).

Other Scientific activities

Editorial Advisor Board Member of “The Open Mycology Journal” (Bentham Science Publishers) (V. Sophianopoulou).

Referee for Molecular Membrane Biology, Comparative Biochemistry and Physiology and the Journal of Molecular Biochemistry (V. Sophianopoulou).

Invited speaker at the the 4th Congress of the Hellenic Scientific Society “Mikrobiokosmos” (V. Sophianopoulou).

Member of the Advisory Committee for the PhD thesis of I. Vangelatos, University of Ioannina (V. Sophianopoulou).

Member of the Advisory Committee for the PhD thesis of A. Athanasopoulos, National and Kapodistrian University of Athens (V. Sophianopoulou).

Member of the Advisory Committee for the MA thesis of P. Geranios, National and Kapodistrian University of Athens (V. Sophianopoulou).

Member of the Evaluation Committee for the PhD thesis of Christos Gournas, Department of Biology, National and Kapodistrian University of Athens (V. Sophianopoulou).

Evaluator of IKY for MA Grants (V. Sophianopoulou).

Akokiounoglou award for PhD thesis (I. Vangelatos)

Educational Activities

Four-hour lectures on “Microbial Biotechnology-Model Systems of Molecular Microbiology” a Post-Graduate Course at University of Athens Department of Biology (V. Sophianopoulou).

Coordinator of the Post-Graduate Course on “Chromatin structure and regulation of gene expression” at IB (V. Sophianopoulou).

Presentation with title: “Study of the role of fungal eisosomal membrane microdomains in animal infections” at the summer school of NCSR “Demokritos” Abstract book 123-125 (V. Sophianopoulou).

“Ideodromies after 4”: New ideas and proposals of IB researchers: Presentation with title: Investigation of the biological role of eisosomal proteins in basal cellular processes: results, perspectives and considerations. February 2011 (V. Sophianopoulou).

Bibliographic seminar: Reassessment of the role of plasma membrane domains in the regulation of vesicular traffic in yeast (3/3/2011) (A. Athanasopoulos).

Other Activities for the Institute of Biology

V. Sophianopoulou:

- Member of the Scientific Consulting Board at IB (resignation 06/2010)
- President of the Education Committee at IB (until 07/2010)
- Member of the Education Committee at NCRS Demokritos (until 07/2010)
- Supervision of the PhD. thesis of the graduate student I. Vangelatos at the IB (University of Ioannina)
- Supervision of the PhD thesis of the graduate student A. Athanasopoulos at the IB (University of Athens)
- Supervision of the Master thesis of the graduate student P. Geranios at the IB (University of Athens)
- Supervision of Diploma Thesis of the undergraduate student V. Pantazopoulou at the IB (University of Athens)
- Member of the Internal Committee for supervision of graduate students with scholarship from NCSR “Demokritos” at the IB: N. Sdralia
- Member of the Examination Committee for the recruitment of new graduate students at the IB (Biology)
- Deputy member of the committee responsible for receiving the IB materials from the regular budget of 2011 (V. Sophianopoulou)
- Deputy member of the examination committee for PhD scholarships in IB (V. Sophianopoulou)

Impact Factor (for 1 publication):.5

Citations 2011 (without self-citations): 29

Total Citations 2006-2010 (without self-citations): 165

h-factor: 12

Laboratory Equipment

Power supply Consort E432 και E861, Gel Drier Heto GD1, Hybridization oven Stuart S1 20H, apparatus of Pulsed-Field Gel electrophoresis Rotaphor Type V computerized (Biometra), horizontal gel electrophoresis instrumentation (Consort και Pharmacia), vertical gel electrophoresis instrumentation (Boehringer-Ingelheim, Bioproducts BV 101), protein transfer instrumentation (Boehringer-Ingelheim, Bioproducts BB 100), Heat block Heto Chill Master Comfort CB 8-30E and miniplus 3 Gilson, UV Transilluminator UVP, INC TM-20, Analytical balance ADA71/L, BioDoc-It™ Imaging System UVP, Gradient PCR Takara TP600, Microflow Advanced Bio safety Cabinet ClassII, Refrigerated Centrifuge Kubota 7780 (V. Sophianopoulou).

Current External Funding

EU programme (FP7-PEOPLE-2009-RG Marie Curie Actions—European Re-integration Grants -ERG) entitled *Eisosomal proteins in Aspergillus nidulans: regulators of endocytosis, cell wall synthesis, membrane sub-domain organization and cell cycle*. (Responsible Scientist: V. Sophianopoulou)

Duration: 2011-2013

Total funding (lab): 45.000€

Funding of the lab for 2010: 0 €.

Early termination of the programme December 2011

Project entitled *Structure-function relations of bacteria transporters and their eukaryotic homologues* funded by GSRT (“Thalis” as “Central Research Group”). Coordinator: S. Frilingos, University of Ioannina (Coordinator of Central Research Group: V. Sophianopoulou).

Duration: 2011- 2013

Total funding ~520.000 €

Total funding of the lab: ~78.000 €.

Funding of the lab for 2010: 0 €.

Project entitled *Development of genetic and genomic tools with Minos transposon and their applications in model organisms*, funded by GSRT (“Thalis” as as “Member of a Central Research Group”). Coordinator: Ch. Savakis, Biomedical Sciences Research Center Al. Fleming (Coordinating IB scientist: V. Sophianopoulou).

Duration: 2011- 2013

Total funding ~535.000 €

Total funding of the lab: ~20.000 €.

Funding of the lab for 2010: 0 €.

Note: The following proposals have been submitted and are under evaluation

1. Joint Research and Technology projects 2011 – 2013: GREECE – CZECHIA (GSRT). Title: Study of the role of fungal eisosomal membrane microdomains in animal infection (Coordinator: V. Sophianopolou).
2. «Aristeia» (GSRT-ESPA): Title: Molecular organization of plasma membrane in fungi and how is implicated in fundamental cellular processes and pathogenicity (Coordinator: V. Sophianopoulou).

Research Group: Biophysics and Biotechnology of Membranes

Research Staff

Kostas Stamatakis, Senior Researcher

George Papageorgiou, Emeritus Scientist

Meropi Tsimilli – Michael, Collaborating Scientist

Theodora Nikou, Summer Student

Research Interests

We investigate the time-dependent changes (induction) of chlorophyll a (Chl a) fluorescence in model cyanobacteria and higher plants, with emphasis on the role of carotenoids as photon collectors. In contrast to chlorophylls and phycobilins, the light harvesting mechanism of carotenoids and the supply of electronic excitation from them to the Chls a of the reaction centers of photosystems I and II (PSI, PSII) has not been described in satisfactory detail. Our research focuses on the role of carotenoids in the balanced excitation of the reaction centers of PSI and PSII, so that they turn over at the same rate and the quantum yield of photosynthesis becomes maximized.

Time-dependent changes of Chl a fluorescence (induction) in model cyanobacteria and its technological applications.

Studies on the photosynthetic Hydrogen production.

2011 Findings

The oxidoreduction state of intersystem electron carriers (plastoquinones, Cyt b6f) is the chemical signal that initiates the state transitions mechanism in cyanobacteria, which regulates the amount of electronic excitation (EE) delivered from peripheral antenna complexes to the core complexes of Photosystems (PS) I and II (PSI_{CC}, PSII_{CC}). In cyanobacteria the peripheral antenna is the extrinsic phycobilisome (PBS) which can couple to both intramembranous core complexes. The chlorophyll (Chl) a fluorescence induction (FI) kinetics of cyanobacteria, recorded by exciting the PBS (orange light) comprises a fast OPS transient and a slower SMT transient, with the SM rise corresponding to a state 2 to state 1 transition. When *Synechococcus* sp PCC 7942 cells are suspended in hyperosmotic medium, the SM amplitude is lower, compared to hypo-osmotic cell suspensions, while the OPS transient remains unchanged. Such cells are locked in state 2 and are characterized by a diminished PSII share of the PBS excitation and an enhanced PS I share [K. Stamatakis, M. Tsimilli-Michael, G. C. Papageorgiou *Biochim. Biophys. Acta* 1767 (2007) 766–772]. Here, we compare cells adapted to blue actinic light (direct Chl a excitation and exclusion of the PBS) with cells adapted to orange light (PBS-sensitized Chl a excitation) with respect to the hyperosmotic suppression of state 1 fluorescence.

Publications

GC Papageorgiou, Govindjee (2011) Photosystem II fluorescence. Scaling from the past. *Photochem Photobiol B: Biology* 104, 258–270). (IF 2,253)

Articles in Press

R Kana, O Komarek, E Kotabova, G.C.Papageorgiou, Govindjee and O Prasil (2012, in press) Slow S to M rise in cyanobacteria is due to a state 2 to state 1 transition. *Biochim. Biophys. Acta-Bioenergetics* (IF 3,688)

Articles in Books and Conferences Proceedings

Papageorgiou GC (2011) Fluorescence emission from the photosynthetic apparatus. Chapter 18 *in*: Eaton-Rye J and Tripathy B (eds) *Photosynthesis: Plastid Biology, Energy Conversion and Carbon Assimilation..* Springer, Netherlands (in press, 2011).

Papageorgiou GC (2011) Contributions of Govindjee, 1955–1969. Chapter 31 *in*: Eaton-Rye J and Tripathy B (eds) *Photosynthesis: Plastid Biology, Energy Conversion and Carbon Assimilation..* Springer, Netherlands (in press, 2011).

Papageorgiou GC (2011) Foreword (invited by the editors). In Itoh S, Mohanty P, Guruprasad KN (Eds) Photosynthesis – Overviews on Recent Progress and Future Perspectives. I. K. International Publishing House Pvt. Ltd., New Delhi, India

Presentations at Scientific Conferences

K. Stamatakis, M. Tsimilli-Michael, GC Papageorgiou (2011). Hyper-osmotic effects on the distribution of electronic excitation between the photosystem II and photosystem I core complexes. Molecular Bioenergetics of Cyanobacteria: From Cell to Community, April 10-15, 2011, Sant Feliu de Guixols, Spain.

D. Benaki, K. Stamatakis, E. Mikros, M. Pelecanou (2011). Curcumin and β - Amyloid Peptide: A promising interaction. Biologically Active Peptides XII, April 27 – 29, 2011, Praha, Czech Republic.

R Kaňa, O Komárek, E Kotabová, GC Papageorgiou, Govindjee, O Prášil (2011) Is the slow S to M fluorescence rise in cyanobacteria due to a state transition? Gordon Research Conference on Photosynthesis, Davidson, NC, USA, June 11-12, 2011

Stamatakis K., Tsimilli-Michael M., Papageorgiou G. C. (2011) On the question of the light-harvesting role of β -carotene in cyanobacteria 12th Congress Hellenic Botanical Society 29/9-2/10 Rethimno Crete.

Other Scientific Activities

Editorial Board Member:

GC Papageorgiou: Associate Editor & Editorial Board Member of Photosynthetica. To Photosynthetica (Springer)

Scientific manuscript reviewer:

K. Stamatakis: Photosynthetica, Photochem Photobiol B: Biology

GC Papageorgiou: Biochim Biophys. Acta (Bioenergetics), Photosynth. Research, Photosynthetica, Frontiers in Photosynthesis.

Other Activities for the Institute of Biology

Member of Scientific Advisory Board of IB

Citations 2011 (without self-citations): 24

Total citations 2007-2011 (without self-citations): 69

h-factor: 7

Laboratory Equipment and Common Usage Equipment (IB) (Allocated to K. Stamatakis)

- Orbital shaker incubator Gallenkamp Model INR-400 (London, UK)
- Sorvall RMC-14 Refrigerated Microcentrifuge (Sorvall Dupont USA)
- Dual – Beam Spectrophotometers,
 - (i) Hitachi-557 dual wavelength absorption spectrophotometer (Hitachi, Tokyo, Japan)
 - (ii) Jasco double beam spectrophotometer UVIDEK 610 (JASCO Japan spectroscopic Co. LTD)
- Fluorometers:
 - (i) Handy-PEA, Hansatech, King's Lynn, Norfolk, UK)
 - (ii) PAM; Heinz Walz, Effeltrich, Germany,
- Oxygen meters: Clark-type oxygen electrode (DW1; Oxygraph, Hansatech, King's Lynn, U.K.)
- Hitachi F-2500 spectrofluorometer (IB) (Hitachi High Technologies Corporation, Japan)

Current External Funding

Participation to the programme IRAKLEITOS II entitled *Green fruit's photosynthesis: correlation with the peculiarities of the internal microenvironment and their photoprotective and metabolic demands*, funded by the Greek Ministry of Education, Lifelong Learning and Religious Affairs (Coordinator: Ass. Professor Y. Petropoulou, Univ. of Patras)

Duration: 2009-2012

Total programme funding: 45.000€

Funding for the lab for 2011: 0€

Note:

A proposal entitled *Photosynthesis: a green source for green energy* (GSRT) has been submitted and is under evaluation.

PROGRAMME C:
STRUCTURAL AND
COMPUTATIONAL BIOLOGY

Research Group: Theoretical Biology and Computational Genomics

Research Staff

Yannis Almirantis, Research Director

Spyros Papageorgiou, Emeritus Scientist

Christoforos Nikolaou, Postdoctoral Fellow

Konstantinos Apostolou – Karabelis, Graduate Student

Yannis Tsiagas, Collaborating Graduate Student (*MSc*) – *MSc obtained in 2011*

Dimitris Polichronopoulos, Collaborating Graduate Student (*MSc*) – *MSc obtained in 2011*

Research Interests

Probabilistic and statistical aspects in genome organization - Non-randomness at several length scales.

- Deviations from randomness at the level of nucleotide n-tuplets. Patterns related to the functionality of genomic regions and to the global genome structure.
- Deviations from randomness at the “middle” length scale, expressed as clustering of similar nucleotides. Use of such approaches for the distinction of coding and non-coding segments.
- Long range correlations and Zipf laws in the genome structure. Power laws in the distribution of exons and of other genomic functional localizations.
- DNA sequences seen as genomic text – Linguistic features in the genome: redundancy – multiple coding – asymmetry etc.
- “Conservation laws” at the genome structure. The case of “Chargaff’s 2nd parity rule”. The use of deviations from this law in the study of genomic dynamics and evolution.
- Evolution at the genomic level. Formulation of minimal evolutionary scenarios compatible with the observed probabilistic features of genomes. Interpretation of the above mentioned probabilistic features either by selectionist or mutationist causality.

Pattern formation in biological systems - Self-organization and evolution.

- Early development – Left-right asymmetries – Mechanisms of activation of Hox genes during limb development.
- Reaction-diffusion systems – Spontaneous symmetry breaking and pattern-formation in systems with feedbacks.
- Prebiotic and early evolution as a complex self-organization procedure.

2011 Findings

Statistical methods, including block-entropy-based approaches in the study of long-range features of genomic sequences seen as symbol-series, either considering the full alphabet of the four nucleotides or the binary purine / pyrimidine character set, have already been used. Recently, we studied the scaling properties of block entropy $H(n)$ in entire chromosomes, with the symbol alphabet inscribing the functional roles “protein-coding” and “non-coding”, using 1s and 0s respectively.

Now, we study the chromosomal distribution of copies of Transposable Elements (TEs) from several repeat families, in genomes of model organisms from distant taxa, using: **(i)** the scaling properties of block entropy (with 1 and 0 denoting nucleotides belonging or not to the studied repeat type), and **(ii)** the standard “box-counting” method; searching for fractality and for the determination of the corresponding self-similarity dimension.

In previous studies, it has been shown that the sizes of the spacers separating TEs of the same repeat family follow power-law-like distributions in several cases of eukaryotic organisms. Our recent findings using block-entropy and box-counting verify the appearance not only of power-law distributions but also of fractality in many instances. A clear difference has been found between compact genomes (e.g., *Arabidopsis thaliana*, *Drosophila melanogaster*) where fractality is well-pronounced and frequent, and lengthy genomes, where well-shaped fractality is met only sporadically.

The findings of this study seem to converge with other results for an active role of TEs in the emergence of a specific spatial pattern in chromatin folding, which recently has been found to form a so-called “fractal globule”.

Publications

S. Papageorgiou. Physical forces may cause Hox gene collinearity in the primary and secondary axes of the developing vertebrates. *Development Growth & Differentiation* (2011) 53, 1. (Impact Factor: 2.28)

Articles in Press

A.Klimopoulos, D.Sellis, Y.Almirantis. Widespread occurrence of power-law distributions in inter-repeat distances shaped by genome dynamics. *Gene* (2012) DOI: 10.1016/j.gene.2012.02.005(Impact Factor: 2.27)

S. Papageorgiou. Comparison of models for the collinearity of Hox Genes in the developmental axes of vertebrates. *Current Genomics* (2012) (accepted for publication).

Presentations at Scientific Conferences

D. Polychronopoulos, G. Giannakopoulos, C. Nikolaou, G. Paliouras, Y.Almirantis Analyzing the DNA composition of ultraconserved sequences with N-gram Graphs. 6th Conference of the Hellenic Society for Computational Biology and Bioinformatics. University of Patras 7-9/10/2011.

Educational Activities

Giannis Tsiagkas presented his master thesis prepared in the framework of the MSc "BIOINFORMATICS", Faculty of Biology, University of Athens. Thesis Title: "Study of the genome organization and evolution through the investigation of the genomic distribution of CpG Islands" April 4th, 2011. Supervisor: Y.Almirantis.

Dimitris Polychronopoulos presented his master thesis prepared in the framework of the MSc "BIOINFORMATICS", Faculty of Biology, University of Athens. Thesis Title: "Study of the distribution of Conserved Not Expressed Elements in several genomes. Relations with genomic functionality and evolution" April 4th, 2011. Supervisor: Y.Almirantis.

- Teaching (16H) of the course "An Introduction to Computational Genomics" in the framework of the "Post Graduate Specialisation Diploma" in Bioinformatics, Biology Department, U.O.A.

- Teaching (12H) of the course "An Introduction to Computational Genomics" in the framework of the Post Graduate Specialisation Diploma in Clinical Biochemistry and Molecular Diagnostics, Biology Department, U.O.A.

Other Activities for the Institute of Biology

President of the Scientific Advisory Board of the Institute (Yannis Almirantis)

Citations 2011 (without self- citations): 11

Total Citations 2007-2011 (without self- citations): 104

h-factor: 11

Research Group: NMR Studies of Biomolecules and Pharmaceuticals

Research Staff

Maria Pelekanou, Research Director

Marina Sagnou, Lecturer

Demetra Benaki, Postdoctoral Fellow

Angeliki Panagiotopoulou, Technical Specialist

Myrta Kostomoiri, Graduate Student (MSc)

Research Interests

Studies of the **structure, interactions**, and **structure/activity** relations of compounds of pharmacological interest for the diagnosis and/or therapy of various diseases.

Our efforts are focused on two major groups of molecules:

1. Bioactive organic compounds designed for targeted pharmacological action either on their own or after suitable labeling/complexation

In an established and ongoing collaboration with IRRP, our group is engaged in the development of novel complexes of rhenium and technetium, for the diagnosis and/or therapy of prevailing diseases like cancer and Alzheimer's disease. The project involves organic synthesis and/or chemical modification of pharmacophoric molecules that will render the complexes tissue specific, investigation of the coordination properties of rhenium and technetium, studies of the structural characteristics, dynamic properties, interactions of the synthesized complexes, as well as their *in vitro* and *in vivo* biological evaluation in the appropriate model systems. Furthermore, our team is involved in the development and biological evaluation of dual-targeted anticancer complexes of platinum, the design and synthesis of novel radiotracers (¹¹¹In-125 or Tc-99m) for imaging of drug resistant epilepsy, as well as in the investigation of new derivatives of curcumin as multi-functional agents for multi-targeted treatment of common ailments.

2. Polypeptides

The activity includes studies on the conformation and interactions of bioactive peptides with NMR and CD with special focus on the β -amyloid peptide (β -AP) of Alzheimer's disease. A thorough examination of the structural transition of β -AP(1-40) to amyloid fibrils - the principal component of amyloid plaques that characterize the disease - and its interactions with compounds, like thioflavin T, oleuropein, curcumin, that may intervene in the toxic aggregation process, has been conducted. In addition, in collaboration with IRRP, the structure-activity relationship of peptides of the neuroprotective family of Humanin is studied along with the development of specially designed labeled derivatives to be used in the investigation of the mechanism of action of this peptide family.

2011 Findings

Aiming at the development of new radiodiagnostic agents for Alzheimer's disease (AD), a number of rhenium/technetium-99m complexes with benzothiazole and curcumin as pharmacophoric moieties were synthesized in collaboration with IRRP. The complexes were fully characterized and evaluated *in vitro* in brain sections from AD patients and it was shown that they retain the affinity of the pharmacophore for amyloid plaques. In the frame of funding from the John S. Latsis Public Benefit Foundation, biodistribution experiments in normal mice and transgenic models of AD revealed an equally encouraging *in vivo* profile, prompting for further investigation.

In 2011 the structural characterization with NMR of novel rhenium dextran complexes was completed within the framework of an IAEA program aiming at the development of suitable

radiopharmaceuticals for the detection of the sentinel lymph node, the primary site of tumor cell metastasis. In addition, the coordination of the tricarbonyl $M(\text{CO})_3^+$ ($M = \text{Re}$, $^{99\text{m}}\text{Tc}$) core with new bidentate OO ligands (maltoze, ligand kojic acid) and monodentate phosphines was investigated. The biological evaluation of ruthenium, palladium, and platinum complexes of the anticancer agent 2-(4'-aminophenyl)benzothiazole in MCF7 and MDA231 cell lines was also advanced.

Within the framework of the FP7 program EURIPIDES, elacridar, the clinically used P-glycoprotein (Pgp) inhibitor, was chemically modified to allow the incorporation of iodine in its structure. The iodinated derivative displayed comparable to elacridar Pgp inhibition in vitro. Furthermore, after its successful radiolabeling with I-125, biodistribution results and dynamic imaging studies of the radioiodinated analogue in healthy mice demonstrated its potential as a SPECT tracer in the delineation of the role of Pgps in pharmacoresistance.

In the field of peptides, the study of derivatives of the neuroprotective colivelin especially designed and synthesized to serve as probes in the investigation of its mode of action was advanced. The structure of colivelin derivatives was studied with NMR and labeling of one with $^{99\text{m}}\text{Tc}$ was effected for biodistribution studies in mice. In the same area, the study with NMR, CD and fluorescence spectroscopy of the interaction of curcumin with the β -amyloid peptide of Alzheimer's disease (β -AP) is to be submitted for publication as part of our search for potential inhibitors of the aggregation of β -AP to toxic oligomeric/polymeric forms.

[Papagiannopoulou, D., Tsoukalas, C., Makris, G., Raptopoulou, C. P., Psycharis, V., Leondiadis, L., Gdniazdowska, E., Koźmiński, P., Fuks, L., Pelecanou, M., Pirmettis, I., Papadopoulos, M. S.](#) (2011). Histidine derivatives as tridentate chelators for the *fac*- $[M(\text{CO})_3]$ ($M = \text{Re}$, $^{99\text{m}}\text{Tc}$, ^{188}Re) core: Synthesis, structural characterization, radiochemistry and stability. [Inorganica Chimica Acta](#) 378, 333-337.

[Tsotakos, T., Tsoukalas, C., Patsis, G., Panagiotopoulou, A., Nikolić, N., Janković, D., Djokić, D., Raptopoulou, C. P., Terzis, A., Papagiannopoulou, D., Pelecanou, M., Papadopoulos, M., Pirmettis, I.](#) (2011). Benzimidazole derivatives as NSO ligands for the *fac*- $[M(\text{CO})_3]^+$ ($M = \text{Re}$, $^{99\text{m}}\text{Tc}$). [Inorganica Chimica Acta](#) 377, 62-68.

Kyprianidou, P., Tsoukalas, C., Chiotellis, A., Papagiannopoulou, D., Raptopoulou, C. P., Terzis, A., Pelecanou, M., Papadopoulos, M., Pirmettis, I. (2011). First example of well-characterized Re and $^{99\text{m}}\text{Tc}$ tricarbonyl complexes of ciprofloxacin and norfloxacin in the development of infection-specific imaging agents. [Inorganica Chimica Acta](#), 370, 236-242.

[Chiotellis, A., Tsoukalas, C., Pelecanou, M., Pirmettis, I., Papadopoulos, M.](#) (2011). [New \$^{99\text{m}}\text{Tc}\(\text{CO}\)_3\(\text{NNO}\)\$ complexes in the development of 5-HT_{1A} receptor imaging agents.](#) [Radiochimica Acta](#), 99, 307-315.

[Fernandez Núñez, E.G., Linkowski, B., Teodoro, R., Pereira Wiecek, D., da Silva, N. G., Papadopoulos, M., Pelecanou, M., Pirmettis, I., de Oliveira Filho, R. S., Duatti, A., Pasqualini, R.](#) (2011). [Parameters optimization defined by statistical analysis for cysteine-dextran radiolabeling with technetium tricarbonyl core.](#) [Applied Radiation and Isotopes](#), 69, 663-669.

[Sagnou, M., Benaki, D., Triantis, C., Tsotakos, T., Psycharis, V., Raptopoulou, C.P., Pirmettis, I., Papadopoulos, M., Pelecanou, M.](#) (2011). [Curcumin as the OO bidentate ligand in "2 + 1" complexes with the \$\[M\(\text{CO}\)_3\]^+\$ \(\$M = \text{Re}\$, \$^{99\text{m}}\text{Tc}\$ \) tricarbonyl core for radiodiagnostic applications.](#) [Inorganic Chemistry](#), 50 (4,) 1295-1303.

Articles in Press

Liolios, C. C., Zikos, C., Fragogeorgi, E., Benaki, D., Pelecanou, M., Pirmettis, I., Ioannidis, N., Sanakis, Y., Raptopoulou, C., Terzis, A., Boschetti, F., Papadopoulos, M., Sivolapenko, G., Varvarigou, A. (2012). A new bombesin-copper complex based on a bifunctional cyclam derivative. *Eur. J. Inorg. Chem.*, *in press* (IF 2.909)

Benaki, D., Zikos, C., Karachaliou, C. E., Tsitsilonis, O., Leondiadis, L., Kalbacher, H., Voelter, W., Papadopoulos, M., Pirmettis, I., Pelecanou, M., Livaniou, E. (2012). Alpha Thymosin-derivative complexes with $^{185/187}\text{Re}$ and $^{99\text{m}}\text{Tc}$: Structural analysis and initial biological evaluation. *Chemical Biology & Drug Design*, *in press* (IF 2.527)

Pirmettis, I., Arano, Y., Tsotakos, T., Okada, K., Yamaguchi, A., Uehara, T., Morais, M., Correia, J. D. G., Santos, I., Martins, M., Pereira, S., Kyprianidou, P., Triantis, C., Pelecanou, M., Papadopoulos, M. (2012). New $^{99\text{m}}\text{Tc}(\text{CO})_3$ mannosylated dextrans bearing S-derivatized cysteine chelator for sentinel lymph node detection. *Molecular Pharmaceutics*, *in press* (IF 5.4)

Makris, G., Karagiorgou, O., Papagiannopoulou, D., Panagiotopoulou, A., Raptopoulou, C. P., Terzis, A., Psycharis, V., Pelecanou, M., Pirmettis, I., Papadopoulos, M. S. (2012). Rhenium(I) and technetium(I) tricarbonyl complexes with $[\text{N}^{\text{S}}\text{O}]$ -type chelators: Synthesis, structural characterization, and radiochemistry. *Eur. J. Inorg. Chem.*, *in press* (IF 2.909)

Giglio, J., Fernández, S., Jentschel, C., Pietzsch, H.-J., Papadopoulos, M., Pelecanou, M., Pirmettis, I., Paolino, A., Rey, A. (2012). Design and development of $^{99\text{m}}\text{Tc}$ "4+1" labelled dextrane-mannose derivatives as potential radiopharmaceuticals for sentinel lymph node detection. *Nuclear Medicine and Biology*, *in press* (IF 2.6)

Sagnou, M., Mitsopoulou, K. P., Koliopoulos, G., Pelecanou, M., Couladouros, E., Michaelakis, A. (2012). Evaluation of naturally occurring curcuminoids and related compounds against larvae of *Culex pipiens*. *Acta Tropica*, *in press* (IF 2.5)

Articles in Books and Conference Proceedings

Kostomoiri, M., Zikos, C., Benaki, D., Slaninova, J., Pirmettis, I., Papadopoulos, M., Pelecanou, M., Livaniou, E. Design and synthesis of specific colivelin derivatives for *in vitro* and *in vivo* studies. In "Biologically Active Peptides; XIIth Conference" (J. Slaninova), vol. 12, Symposium Series, Prague, Czech Republic, 2011; *in press*

Karachaliou, C.-E., Zikos, C., Benaki, D., Pelecanou, M., Tsitsilonis, O., Papadopoulos, M., Pirmettis, I., Livaniou, E. Synthesis, characterization and $^{99\text{m}}\text{Tc}$ -radiolabelling of specific derivatives of alpha thymosin peptides for *in vitro* and *in vivo* studies. In "Biologically Active Peptides; XIIth Conference" (J. Slaninova), vol. 12, Symposium Series, Prague, Czech Republic, 2011; *in press*

Benaki, D., Stamatakis, K., Mikros, E., Pelecanou, M. Spectroscopic investigation of the interaction of curcumin with β -amyloid peptide (1-40) with Fluorescence, CD, and NMR. In "Biologically Active Peptides; XIIth Conference" (J. Slaninova), vol. 12, Symposium Series, Prague, Czech Republic, 2011; *in press*

Presentations at Scientific Conferences

M. Kostomoiri, C. Zikos, D. Benaki, J. Slaninova, I. Pirmettis, M. Papadopoulos, M. Pelecanou, E. Livaniou (2011). Design and synthesis of specific colivelin derivatives for *in vitro* and *in vivo* studies. Biologically Active Peptides XII, April 27-29, Prague, Czech Republic.

C.-E. Karachaliou, C. Zikos, D. Benaki, Pelecanou, M., O. Tsitsilonis, M. Papadopoulos, I. Pirmettis, E. Livaniou (2011). Synthesis, characterization and $^{99\text{m}}\text{Tc}$ -radiolabelling of specific derivatives of alpha thymosin peptides for *in vitro* and *in vivo* studies. Biologically Active Peptides XII, April 27-29, Prague, Czech Republic.

D. Benaki, K. Stamatakis, E. Mikros, M. Pelecanou. Spectroscopic investigation of the interaction of curcumin with β -amyloid peptide (1-40) with Fluorescence, CD, and NMR. Biologically Active Peptides (2011). XII, April 27-29, Prague, Czech Republic.

A. Panagiotopoulou, C. Tsoukalas, C. Raptopoulou, V. Psycharis, I. Pirmettis, M. Papadopoulos, M. Pelecanou (2011). Synthesis and characterization of new *fac*- $[\text{Re}(\text{OO})(\text{P})(\text{CO})_3]$ and $\text{Re}(\text{OO})(\text{P})_2(\text{CO})_2$ complexes. 19th International Symposium on Radiopharmaceutical Sciences, 29 August – 2 September, Amsterdam.

C. Triantis, A. Lazopoulos, T. Tsotakos, C. Tsoukalas, A. Drakopoulos, C. Raptopoulou, V. Psycharis, M. Pelecanou, I. Pirmettis, M. Papadopoulos (2011). Synthesis and characterization of new *fac*-[M(NO)(P)(CO)₃] and M(NO)(P)₂(CO)₂ complexes (M=Re, ^{99m}Tc) 19th International Symposium on Radiopharmaceutical Sciences, 29 August - 2 September, Amsterdam.

A. Lazopoulos, C. Triantis, C. Tsoukalas, T. Tsotakos, C. Raptopoulou, V. Psycharis, M. Pelecanou, I. Pirmettis, M. Papadopoulos (2011). A convenient route leading to mixed neutral *fac*-[M(NO)(P)(CO)₃] and M(NO)(P)₂(CO)₂ (M = Re, ^{99m}Tc) complexes coupled to amine pharmacophores 19th International Symposium on Radiopharmaceutical Sciences, 29 August - 2 September, Amsterdam.

V. Vassiliadis, C. Triantis, T. Tsotakos, D. Papagiannopoulou, M. Pelecanou, C. Raptopoulou, V. Psiharis, I. Pirmettis, M. Papadopoulos (2011). Synthesis and characterization of novel *fac*-[M(NO/NS)(P)(CO)₃] and [M(NO/NS)(P)₂(CO)₂] complexes (M = Re, ^{99m}Tc)", 19th International Symposium on Radiopharmaceutical Sciences, 29 August - 2 September, Amsterdam. *J. Lab. Comp. Radiopharm.*, 54, P-263, S352.

B. Mavroidi, C. Methenitis, M. Sagnou, A. Philippopoulos, M. Pelecanou, S. Theocharis (2011) Complexes of Pd(II) and Pt(II) with benzothiazole derivatives as anticancer agents with combined activity 4th European Conference on Chemistry for Life Sciences, August 31 - September 3, Budapest, Hungary

G. Makris, M. Pelecanou, I. Iakovou, T. Christoforidis, I. Pirmettis, M. S. Papadopoulos, D. Papagiannopoulou (2011). In vitro and in vivo studies of novel *fac*-[^{99m}Tc(NSO)(CO)₃] complexes bearing bifunctional chelators, for application in targeted radiopharmaceuticals. Annual Congress of The European Association of Nuclear Medicine, October 2011, Birmingham, UK, *Eur. J. Nucl. Med. Mol Imaging* PW060, 2011-S-1711-EANM

G. Makris, C. P. Raptopoulou, A. Terzis, M. Pelecanou, I. Pirmettis, M.S.Papadopoulos, D.Papagiannopoulou, (2011) Rhenium(I) and technetium(I) tricarbonyl complexes with tridentate NSO and NSN bifunctional agents: Synthesis, structural characterization, radiochemistry in vitro and in-vivo stability", 11th Sigma-Aldrich Young chemists Symposium, October 17-19, Pesaro, Italy, FC6.

G. Makris, M. Pelecanou, I. Pirmettis, M.S. Papadopoulos, D. Papagiannopoulou (2011). In vitro and in vivo studies of two novel organometallic technetium-99m complexes bearing bifunctional chelators, for application in targeted radiopharmaceuticals, 12th Conference of Medicinal Chemistry, April 12-15, Patras, P18, p.84

V. Vassiliadis, C. Triantis, T. Tsotakos, D. Papagiannopoulou, M. Pelecanou, C. Raptopoulou, V. Psyharis, I. Pirmettis and M. Papadopoulos (2011). Synthesis and characterization of novel *fac*-[M(NO/NS)(P)(CO)₃] and [M(NO/NS)(P)₂(CO)₂] complexes (M = Re, ^{99m}Tc)" 12th Conference of Medicinal Chemistry, April 12-15, Patras, P02 p.68

A. Panagiotopoulou, C. Tsoukalas, C. Raptopoulou, V. Psycharis, I. Pirmettis, M. Papadopoulos, M. Pelecanou (2011). New "2+1" mixed ligand rhenium complexes of the general formula *fac*-[Re(OO)(L)(CO)₃]" 12th Conference of Medicinal Chemistry, April 12-15, Patras

G. Makris, M. Pelecanou, I. Pirmettis, M. S. Papadopoulos, D. Papagiannopoulou (2001). Novel [Re/^{99m}Tc(L)(CO)₃] complexes with NSO and NSN tridentate bifunctional chelators. Synthesis, Characterization and in-vitro stability. 12th Conference in Advanced Medicinal Chemistry Thessaloniki

V. Vassiliadis, C. Triantis, T. Tsotakos, M. Pelecanou, C. Raptopoulou, V. Psyharis, I. Pirmettis, M. Papadopoulos and D. Papagiannopoulou (2011). The 2+1 Approach for the developemtnof novel [^{99m}Tc(NS)(P)(CO)₃]-type radiopharmceuticals: Synthesis, structural studies and radiochemistry», 12th Conference in Advanced Medicinal Chemistry, Thessaloniki

G. Makris, M. Pelecanou, I. Pirmettis, M. Papadopoulos, D. Papagiannopoulou (2011). In vitro and in vivo studies of two novel organometallic technetium-99m complexes bearing bifunctional chelators for application in targeted radiopharmaceuticals 15th Panhellenic Pharmaceutical Congress, May 13-15, Athens, PS005.

A. Panagiotopoulou, C. Tsoukalas, K. Raptopoulou, V. Psycharis, I. Pirmettis, M. Papadopoulos, M. Pelecanou (2011). Synthesis and characterization of new mixed complexes *fac*-[Re(OO)(L)(CO)₃]. 15th Panhellenic Pharmaceutical Conference, May 13 - 15, Athens

C. Triantis, C. Tsoukalas, T. Tsotakos, K. Raptopoulou, A. Terzis, M. Pelecanou, I. Pirmettis, M. Papadopoulos (2011). Novel mixed Tc-99m complexes with acetylacetone and phosphine. 15th Panhellenic Pharmaceutical Conference, May 13 - 15, Athens

C. Triantis, A. Lazopoulos, T. Tsotakos, C. Tsoukalas, A. Drakopoulos, K. Raptopoulou, V. Psycharis, M. Pelecanou, I. Pirmettis, M. Papadopoulos (2011). Synthesis and characterization of novel mixed complexes *fac*-[M(NO)(P)(CO)]₃ KAI M(NO)(P)₂(CO)₂ (M = Re, ^{99m}Tc). 15th Panhellenic Pharmaceutical Conference, May 13 - 15, Athens

A. Lazopoulos, C. Triantis, C. Tsoukalas, T. Tsotakos, K. Raptopoulou, V. Psycharis, M. Pelecanou, I. Pirmettis, M. Papadopoulos (2011). Targeted mixed complexes *fac*-[M(NO)(P)(CO)]₃ and M(NO)(P)₂(CO)₂ (M = Re, ^{99m}Tc). 15th Panhellenic Pharmaceutical Conference, May 13 - 15, Athens

Educational Activities

"Applications of NMR in Medicine" in the framework of the graduate course "Introduction to Research Methodology", School of Medicine, Univ. of Athens, December 2011 (3 hours, 30 students), M. Pelecanou

"A little curry every day keeps the doctor away" Lecture in the Summer School 2011 of NCSR "Demokritos", (1 h; 80 students), M. Sagnou

Other Scientific Activities

Member of the Local Organizing Committee of the International Conference Euromar 2013 that will be held in Crete in July 2013 (M. Pelecanou)

Reviewer for the scientific journals: Inorganic Chemistry, Journal of Medicinal Chemistry (M. Pelecanou)

Presentation of the results of the study "Development of a diagnostic agent for Alzheimer's disease" funded by John S. Latsis Public Benefit Foundation (Scientific Research Projects 2010) in a ceremony, held on May 5th, 2011. During the event a short movie was screened, produced by the Foundation and directed by S. Birsim, on the activities of all research teams funded in 2010.

The daily newspaper ETHNOS published on May 6th, 2011 an article on the activity of our team in the field of Alzheimer's disease

<http://www.ethnos.gr/article.asp?catid=22733&subid=2&pubid=63015229>

Other Activities for the Institute of Biology

M. Pelecanou:

- Member of the Scientific Advisory Board of the Institute of Biology
- Responsible for the operation of the Circular Spectropolarimeter (CD) with the support of the specialized technical scientist Dr. A. Panagiotopoulou
- Co-responsible for the NMR Lab of the NCSR "D", (Internal Project 949, Protocol Number. A.C.305/Subj. 18).
- Safety Responsible for the Institute of Biology

M. Sagnou:

- Responsible for the operation of the Confocal microscope
- Deputy for educational affairs
- Responsible for graduate student seminars

D. Benaki:

- Responsible for the NCSR “D” Blood Bank

Impact Factors (for 6 publications): 12,686

Citations 2011 (without self-citations):

M. Pelecanou: 66

M. Sagnou: 23

Total Citations 2007-2011 (without self-citations):

M. Pelecanou: 212

M. Sagnou: 55

h-factor:

M. Pelecanou: 15

M. Sagnou: 5

Laboratory Equipment

Circular Spectropolarimeter (CD). The instrument, JASCO J-720 equipped with a Peltier temperature controller, is placed in IB and belongs to the Crystallography Center. Responsible for IB: Dr. M. Vlassi and M. Pelecanou. Dr. A. Panagiotopoulou (Technical Specialist) is responsible for the maintenance and service rendering for NCSR “D” users as well as of other research and academic institutions.

NMR Spectrometers 250 and 500 MHz, Bruker. The spectrometers are placed in the building “Sholi” and they belong to three institutes, i.e. Biology, Physical Chemistry, and RRP. Responsible for IB: M. Pelecanou. The Technical Specialist Dr. A. Panagiotopoulou is responsible for the maintenance and service rendering for NCSR “D” users as well as of other research and academic institutions.

HPLC (old, from Dr. S. Loucas Lab); recent service by the electronic's engineer D. Gkirklemis

High precision balance of six decimals (from Dr. S. Loucas Lab)

UV for TLC, rotary evaporator, glassware oven

Current External Funding

Program entitled *European Research initiative to develop Imaging Probes for early In-vivo Diagnosis and Evaluation of response to therapeutic Substances*, funded by EU with Dr Varvarigou (IRRP, NCSR “Demokritos”) as scientific coordinator of the Hellenic team; IB collaborator: Dr. M. Sagnou.

Duration: 2008-12

Total Funding for Greece: 318.000 €

Total funding (lab): 16.000 €

Funding of the lab for 2011: 13.000 €

Program title *Development of Alzheimer's disease diagnostic agent*, funded by TT Hellenic Post Bank, TTBank with Scientific Coordinator Dr. M. Pelecanou

Duration: 2007 - 2010

Total funding (lab): 20.000 €

Funding of the lab for 2011: 5.000 €.

Note 1 : The following have been approved and the funding will start in 2012

1. Program Title: *Development of Novel Natural Product-based Imaging Probes for Early Diagnosis and Therapeutic Application in Multi-Drug Resistant Tumors*, funded by EU (FP7-ERA.Net RUS), Scientific responsible and coordinator Dr. M. Sagnou (total amount 400.800€)
2. Program Title: Development and screening of novel beta amyloid peptide inhibitors for Alzheimer's disease, funded by GSRT ("Collaboration" ESPA 2007-2013) with coordinating institution the Goulandris Museum of Physical History and scientific responsible for the IB Dr M. Pelecanou; amount for the lab 100.000 €
3. Program Title: *Biosynthesis and genetic identification of cyclic peptide with potential therapeutic properties against Alzheimer's disease: inhibitors of protein aggregation*" (Coordinator: Dr. E. Gonos, Laboratory of Molecular and Cellular Aging, Institute of Biological Research and Biotechnology, National Hellenic Research Foundation, Athens; Scientific coordinator for the NCSR "Demokritos" team Dr. Pelecanou; funding for the lab 29.000 €
4. Program Title: *Directed Evolution of Small-Molecule Therapeutics Against Neurodegenerative Diseases*, funded by GSRT ("Aristeia" 2011); Scientific coordinator Dr. G. Skretas; Scientific coordinator for the IB, NRCR "D" Dr. Pelecanou.
The lab budget has not yet been finalized.

Note 2 : Submitted, under evaluation:

1. *Development of novel targeted diagnostic rhenium and technetium tools with dual tumor imaging potential.*
Empirikion Foundation
November 2010
Scientific coordinator: M. Pelecanou
Total Budget: 15.000 €
2. *Integrated Drug Delivery and Activation Device for Localized and Effective Cancer and Wound Infusion Therapy (THERAFUSE).*
In the frame of "Collaboration 2011- Synergy of Productivity and Research Entities focused on scientific and technological domains-fields ".NSRF 2007-2013, GSRT
Coordinator: Constelex Association
Total Budget: 815.000 €

Research Group: Protein Structure and Molecular Modeling

Research Staff

Metaxia Vlassi, Research Director

Diamadis Sellis, Graduate Associate

Research Interests

Our current research activities focus on

1) Structural studies of proteins with emphasis on sequence repeat-containing proteins aiming to elucidate sequence-structure-function relationships and the structural determinants of sequence repeat-mediated protein interactions. The approach followed includes mainly structural bioinformatics techniques such as *in silico* 3D-modeling (homology/comparative modeling & threading), docking, molecular dynamics simulations etc.

2) Studying the dynamics of protein structure by means of *in silico* molecular dynamics (MD) simulations and development of related computing tools.

3) 3D-modeling of enzymes of mainly medical interest and of their interactions with potential inhibitors aiming at unraveling their inhibition mechanism, towards the development of new pharmaceuticals.

2011 Findings

1) Arginine/Serine (RS) Repeats

We have previously used molecular dynamics (MD) simulations to study the conformational preferences of peptides containing short repeats of consecutive RS dipeptides corresponding to the RS-domain of Lamin-B Receptor (LBR).

In 2011 we performed MD simulations on a conserved LBR peptide (R2'), overlapping with its RS repeats, both in isolation and in complex with the kinase, SRPK1, which modulates the RS-mediated protein interactions. A new version of our Gromita GUI (see below) was used for this purpose. In total, our MD studies shed light on the conformational preferences of the RS repeats before and after their phosphorylation and showed, for the first time, that SRPK1 uses a common recognition mechanism for its RS-containing substrates, irrespective of their RS-repeat length. This work is accepted for publication (Sellis *et al.*, *in Press*)

2) The Gromita Software.

In a previous work, we have developed the computer program, Gromita (<http://bio.demokritos.gr/gromita>), which is a Graphical User Interface (GUI) to version 4 (4.0.x) of the MD program, Gromacs. The related article (Sellis *et al. Bioinformatics and Biology Insights* 2009) was highly visible (3059 views within 58 weeks after publication).

In 2011, we developed an updated new Gromita version (v3), which is compatible with the latest Gromacs-4 versions and includes new features like the *genion* utility, which replaces solvent molecules by monatomic ions and allows neutralization of the solvent for MD simulations with explicit solvent treatment. Construction of a new web site for this Gromita version is under development.

3) Molecular Modeling of Enzyme/inhibitors Interactions

Using molecular docking techniques, in combination with MD simulations, we have previously shown that a series of nucleoside analogues with adequate tumor-inhibitory effects could effectively dock into the active site of human poly(A)-specific ribonuclease (PARN) (see annual report 2009 and Balatsos *et al. Biochemistry* 2009), which can, therefore, be used as a target for the design of new anticancer agents.

Our new *in silico* molecular docking experiments on a novel series of synthetic nucleosides into the active site of PARN suggested that the high inhibitory activity of an uracil-based nucleoside analogue (named U1) can be attributed to its slow-binding and slow-release from

the active site of the enzyme. Our prediction was verified experimentally (using kinetic analysis) by our collaborators. In total, our studies showed that such compounds (uracil-based) can be therefore used as basis for the development of new, more specific and more potent PARN inhibitors. This work is accepted for publication (Balatsos *et al.*, *in Press*).

Articles In Press

Sellis D, Drosou V, Vlachakis D, Voukkalis N, Giannakouros T, Vlassi M. (2012) Phosphorylation of the arginine/serine repeats of lamin B receptor by SRPK1-insights from molecular dynamics simulations. *Biochim Biophys Acta (General Subjects)* 1820(1):44-55. *Epub 2011 Oct 26*. IF₂₀₁₁ = 4.663

Balatsos N, Vlachakis D, Chatzigeorgiou V, Manta S, Komiotis D, Vlassi M, Stathopoulos C (2012) Kinetic and in silico analysis of the slow-binding inhibition of human poly(A)-specific ribonuclease (PARN) by novel nucleoside analogues. *Biochimie*. 94(1):214-21. *Epub 2011 Oct 24*. IF₂₀₁₂ = 3.787

Presentations at Scientific Conferences

D. Sellis, V. Drosou, D. Vlachakis, N. Voukkalis, T. Giannakouros, M. Vlassi* (2011) Phosphorylation of the Arginine/Serine Repeats of Lamin B Receptor by SRPK1 - Insights from Molecular Dynamics Simulations. 62nd Congress of the Hellenic Society for Biochemistry and Molecular Biology(HSBMB), Dec 9-11, 2011, Athens. Pp. 79 (* *oral presentation*)

D. Sellis, T. Giannakouros, M. Vlassi* (2011) Using Molecular Dynamics simulations to study the conformation of Arg/Ser repeat-containing peptides. Proceedings of the 6th Conference of the Hellenic Society for Computational Biology and Bioinformatics (HSCBB), Oct 7-9, 2011, Patras. Pp. 12 (**oral presentation*)

Other Scientific Activities

- Referee for *Nucleic Acid Research* (1 article)
- Member of the national network BE/OPT-XFEL (Network to Optimize use of the European X-FEL by the Greek Research Community)
- Member of the national network “INSTRUCT” (“INSTRUCT: An Integrated Structural Biology Infrastructure for Europe”).
- Member of the network “Center for Crystallographic Studies of Macromolecules”

Educational Activities

Lectures on “Principles of X-Ray Crystallography: Applications in Structural Biology” in the framework of the post-graduate program (towards a Masters degree) entitled “Clinical Biochemistry – Molecular Diagnosis” (Dr. M. Vlassi, Dept. of Biology, National & Kapodistrian University. of Athens)

Other Activities for the Institute of Biology

Vice Director of IB (since Nov. 2010)

Member of the Scientific Advisory Board of IB (since Oct. 2010)

Surrogate Member of the Scientific Advisory Committee of NCSR “D”

President of the Committee for computing and network support of IB

Member of the committee for consumables delivering at IB

Member of the committee for instrumentation delivering at IB in the framework of EPAN

Responsible scientist for the use of common instrumentation (i.e. shaking incubator etc)

Member of the Committee for examining the level of knowledge of the Greek language of EU citizens

Citations 2011 (without self- citations): 27

Total Citations 2007-2011 (without self- citations): 146

h-factor: 11

Laboratory Equipment

Personnal Computers (>5) purchased mostly through research programmes

Note: Grant applications approved in 2011

- (i) A grant application entitled “*The new Biology of intrinsically disordered proteins: A targeted, multidisciplinary analysis of IDP structure, function and properties in real time and true cellular conditions*” (coordinator: S. Georgatos, UoI) was approved in Oct. 2011
- (ii) A grant application entitled “*Mitochondrial dysfunction in neurodegenerative disorders*” (coordinator: A. Plaitakis, UoC) was approved in Oct 2011.

S E R V I C E U N I T S

- *HUMAN TISSUE BANK*
- *EXPERIMENTAL ANIMAL COLONY*
- *LASER CONFOCAL MICROSCOPY*
- *CHARACTERIZATION OF
PROTEINS AND BIOACTIVE
MOLECULES*

HUMAN TISSUE BANK

Research Staff

Helen Vavouraki, Researcher

Minas Leventis, Collaborating Scientist

Konstantinos Raptis, Graduate Research Associate

Stilianos Kakkos, Research Technician

Lab Description - Research Interests

Human Tissue Bank, one of the first legislated laboratories of NCSR "DEMOKRITOS", collects tissues of human origin, it process them and produces grafts to be used in reconstructive surgery. Its operation follows the European Directives 23/2004, 17/2006 and 86/2006 as well as the recommendations of IAEA.

Member of the European Association of Tissue Banks, it is the unique in Greece applying its "knowhow" in the processing of a great variety of human tissues. During the almost 40 years of continuous functioning it has delivered more than 42000 tissue preparations without any problem relevant to their quality. All procedures taking place in the Bank are fully computerized and accredited according to ISO 9001/2008. We are constantly care for the quality control issues, and our compliance with the Greek and European stds

The grafts preparations are delivered to hospitals, health clinics and laboratories, all over the country.

The scientific research interests of the Bank are focused mainly in the study of the activity of the produced grafts , in the optimization of the production methods, the introduction of new techniques, the process of new tissues and the development of new grafts. There are therefore, collaborations with university medical and other departments in order to promote Public Health .

2011 Graft production - Findings

For our collaboration with the private sector which promotes and delivers our grafts to the hospitals, we processed tissues from 103 donors from different hospitals, producing 265 tissue preparations in total, to be used in the Orthopedic Surgery.

We have delivered also various other tissue preparations for scientific collaboration purposes, to hospitals, University labs, e.t.c.

Publications

Markopoulou CE, Dereka XE, Vavouraki HN, Pepelassi EE, Mamalis AA, Karoussis IK, Vrotsos IA. (2011) "Effect of rhTGF- β 1 combined with bone grafts on human periodontal cell differentiation." "Growth Factors: 29(1):14-20.

Papaioannou, K., Markopoulou, C., Gioni V., Mamalis A. Vavouraki H., et al. (2011) "Attachment and Proliferation of Human Osteoblast-Like Cells on Guided Bone Regeneration (GBR) Membranes in the Absence or Presence of Nicotine: An In Vitro Study". International Journal of Oral and Maxillofacial Implants (IntJOMIv 26 (3): 509-519.

Articles in Press

Pantou A.L, Markopoulou C.E., Dereka X.E., Vavouraki H., Mamalis A., Vrotsos I.A. "The effect of Platelet-Rich Plasma (PRP) combined with a bone allograft on human Periodontal Ligament (PDL) cells". Cell and Tissue Banking, (in press) Epub 2010 Dec 1.(I.F 2010:1,157)

Other Activities for the Institute of Biology

Scientific Responsible of Human Tissue Bank.

Quality Manager of Human Tissue Bank (ISO 9001/2008)

Training visits of University students in the Bank.

Other Scientific Activities

Reviewer in the scientific Journal Platelets

Member of European Committee for the establishment of unique nomenclature of human tissues and cells

Member of European auditors-net of human tissue and cells banks

Expert of National Transplant Organization and Ministry of Health in the field of Human Tissue Banking

Impact Factors (for 2 publications): 4,772

Citations 2011 (without self- citations): 15

Total Citations 2007-2011 (without self- citations): 43

h-factor: 5

Laboratory Equipment

Deep freezers, refrigerators for the preservation of tissues and their derivatives

Operating Room equipment

Customized surgical saw

Cutting mill

Lyophilizer

Laminar Flow

Packaging equipment

Sterility tests equipment

General laboratory equipment

Note:

A Project under the name “ Research, development and delivery of tissue grafts-tissue repair products” has been suggested and approved by the Directory Board of NCSR “D”

EXPERIMENTAL ANIMAL COLONY

Research Staff

Dimitris Kletsas, Research Director

Ioannis Zafiropoulos, Research Technician

George Doulgeridis, Research Technician

Description

The animal facility maintains and reproduces inbred strains of experimental animals. The following strains are currently available:

- Mice, strain SWR SWISS ALBINO
- Rats, strains WISTAR ALBINO and ETB
- Rabbits, strain NZW ALBINO
- Mice, strain SCID

During 2011 the Animal Facility provided the following animals:

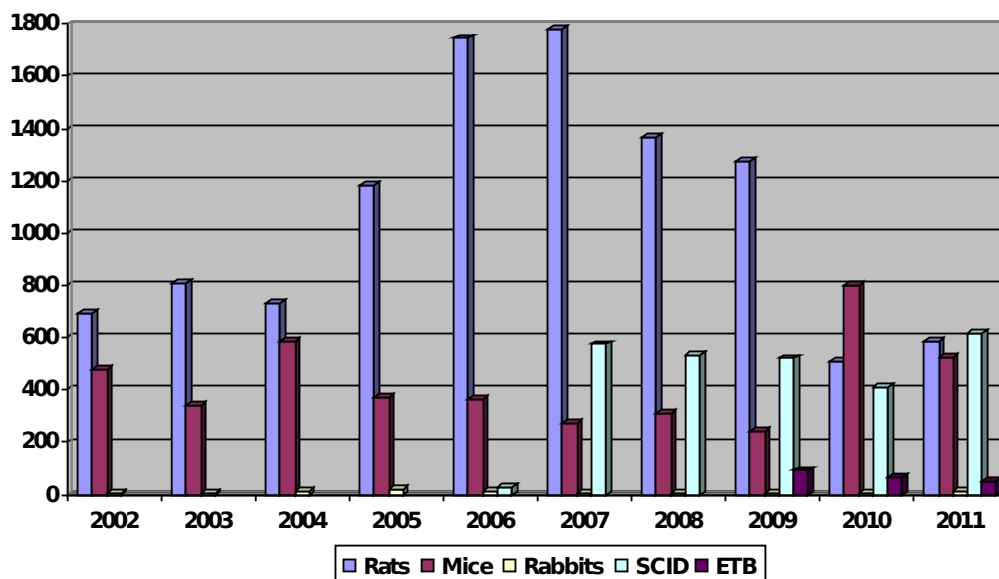
Users	Rats WISTAR	Rats ETB	Mice	Rabbits NZW	Mice SCID
Institute of Biology	0	0	91	2	62
Institute of Radioisotopes & Radiodiagnostics	29	0	415	7	557
External Users	555	50	13	0	0
External Users (maintenance)	0	0	6	0	0
Total	584	50	525	9	619

The number and species of animals produced are dictated by the needs of research programs within the Institutes of NCSR "Demokritos", mainly the Institutes of Biology and Radioisotopes-Radiodiagnostic Products. In addition, animals are provided outside the Centre in research laboratories, Universities, pharmaceutical companies, etc.

The colonies of mice, rats and SCID mice have been renewed. New colonies of transgenic animals have also been developed.

The building has (and is currently being) upgraded, a new quarantine room has been created, new instruments have been purchased, the quality of animals has been tested locally by a veterinarian and by a certified laboratory abroad, in order to conform with the ISO 9001:2008 certification.

DISPOSAL OF LABORATORY ANIMALS 2002-2011



LASER CONFOCAL MICROSCOPY

Research Staff
Marina Sagnou, Lecturer

Description

The current Unit activities include:

- a) The study of cellular, molecular and biochemical phenomena on cells and tissues using confocal microscopy imaging techniques
- b) The use of confocal microscopy as a tool to explore the surface area and penetration potential of novel and known material
- c) The application of immunohistochemistry, face-contrast, Nomarsky etc techniques on both fixed and living cells.

2011 Findings

During the year 2011, there seemed to be a rather increased demand for the the study of cellular, molecular and biochemical phenomena using confocal microscopy imaging techniques by both the Local Institute of Biology researchers, and those from the University of Athens, The Agricultural University, the Technical University as well as some Hospital Units.

Furthermore, it was this year's achievement, to initiate the exploration of the surface area and penetration potential of novel and known material, as a new ground of application for this technique, by both NCSR "D" researchers and external industry collaborators.

CHARACTERIZATION OF PROTEINS & BIOACTIVE MOLECULES

Research Staff

Metaxia Vlassi, Research Director

Maria Pelecanou, Research Director

Aggeliki Panagiotopoulou, Technical Specialist

Description

The service unit for Characterization of Molecules and Biomolecules (CMB) has been established in 2003 and comprises two pre-existing laboratories: 1) the Centre for Crystallographic Studies of Macromolecules (CCM) and 2) the Nuclear Magnetic Resonance laboratory (NMR).

- CCM consists of a) a state-of-the-art X-ray system for diffraction experiments on macromolecules and b) a Circular Dichroism (CD) spectropolarimeter. CCM has been financed by the General Secretariat for Research and Technology (GSRT, EPET II program) as a network of related to molecular structure research groups from "Demokritos" and from other Research/Academic Institutions of Greece. CCM operates at NCSR "Demokritos" since fall 1998.
- The NMR laboratory consists of a) a 250 MHz NMR spectrometer and b) a 500 MHz ADVANCE DRX NMR spectrometer. The latter has been funded in the framework of a GSRT program entitled: 'Up-grading the infrastructure of NCSR "Demokritos" and is shared by the Institutes of Physical Chemistry, Biology and Radioisotopes & Radiodiagnostic Products.

2011 Findings

Both laboratories of the CMB service unit mainly support related to molecular structure research activities of the Physical Chemistry, Biology and Radioisotopes & Radiodiagnostic Products Institutes of NCSR "Demokritos", thus contributing to the research and development program of the Centre. In addition, the unit also serves external users mainly from other Research and Academic Institutions.

EDUCATIONAL ACTIVITIES

EDUCATION

The Institute of Biology continues its Graduate Course Programme, which has been successfully carried out for the past 40 years. This Programme includes:

- a. Training of young scientists at the postdoctoral level
- b. Pre-graduate and graduate thesis work
- c. Courses at the graduate level
- d. Lecture Contributions to the Summer School of the NCSR "Demokritos"

During the year 2011, 9 scientists were trained at the postdoctoral level at our Institute. Furthermore, 31 graduate students worked toward the completion of their doctoral thesis research work under the supervision of scientists of the Institute and on projects which were given to them by their respective supervisors.

During the year 2011, 6 of our graduate students finished their thesis work and became PhDs and 5 students completed their Master thesis.

Moreover, 8 students from Greek Universities are carrying out their pre-graduate project thesis work at the Institute. Additionally, during 2011, one graduate student from abroad visited IB and 4 students did practical job training in laboratories at the Biology Institute.

In addition to the above, scientists of the Biology Institute carried out the following series of courses and seminars within the framework of the Graduate School Programme of the Greek Universities:

- *Lecture entitled "Cytotoxicity study on conventional and targeted chemotherapeutic drugs" (seminar and practical laboratory exercise) " included in the course for "Cell and tissue cultures", in the framework of the Post-Graduate Specialization Diploma "Biological Applications in Medicine" (Dr. G. Voutsinas, Department of Biology and Medical School, University of Athens)*
- *Lecture entitled "Pharmacological targeting of Hsp90" included in the course for "Molecular Biology - Systemic and in silico approaches", in the framework of the Post-Graduate Specialization Diploma "Biological Applications in Medicine" (Dr. G. Voutsinas, Department of Biology and Medical School, University of Athens)*
- *Lecture entitled "Molecular diagnosis of genetic diseases" included in the course for "Molecular Biology - Systemic and in silico approaches", in the framework of the Post-Graduate Specialization Diploma "Biological Applications in Medicine" (Dr. G. Voutsinas, Department of Biology and Medical School, University of Athens)*
- *Lecture entitled "7TM Receptors and G proteins in health and disease" in the framework of the postgraduate course "Biochemistry" (Dr. Z. Georgoussi, Department of Biology, University of Athens)*
- *Teaching in the framework of the postgraduate course "Molecular Base of Human Diseases" (Dr. Z. Georgoussi, Department of Biology, University of Athens)*
- *Lecture entitled "Cell senescence and tissue homeostasis" in the framework of the postgraduate course "Biochemistry" (Dr. D. Kletsas, Department of Chemistry, University of Athens)*
- *Lecture entitled "Cell senescence and tissue homeostasis" in the framework of the postgraduate course "Physiology" (Dr. D. Kletsas, Medical School, University of Athens)*
- *Lecture entitled "Cell senescence and Carcinogenesis" in the framework of the postgraduate course "Oncogenes and growth factors in cancer biology" (Dr. D. Kletsas, Medical School, University of Athens)*
- *Teaching in the framework of the postgraduate programme "Application of Biology in Medicine", the course "Cell cultures - Tissue cultures" (Dr. D. Kletsas, Dr. H. Pratsinis and Dr. E. Mavrogonatou, Department of Biology, University of Athens)*

- Lecture entitled “Cell Cycle: Checkpoints and Consequences for Physiological Cell Function” in the framework of the postgraduate course: “Applications of Biology to Medicine” (**Dr. Th. Sourlingas**, Department of Biology, University of Athens).
- Lecture entitled “Molecular mechanisms and therapeutic approaches of diabetes mellitus” in the framework of the course “Pathobiochemistry” (**Dr. E. Tsilibary**, Department of Biology, University of Athens)
- Lecture entitled “Cell apoptosis in diseases: a beneficial or damaging process?” in the framework of the course “Molecular and applied physiology”, (**Dr. E. Tsilibary**, Medical School, University of Athens)
- Lecture entitled “Lipids and apolipoproteins: From cardiovascular disease to Alzheimer’s disease” in the framework of the graduate course “Clinical Chemistry II” (**Dr. A. Chroni**, Department of Chemistry, University of Athens)
- Lecture entitled “Lipoprotein metabolism pathways and atherosclerosis. The association between atherosclerosis and Alzheimer’s disease” in the framework of the postgraduate course “Human Biochemistry” (**Dr. A. Chroni**, Department of Chemistry, University of Athens)
- Lecture entitled “Molecular Biology: Systemic and in silico Approaches” in the framework of the postgraduate course “Applications of the Biological Sciences in Medicine” (**Dr. A. Prombona**, Department of Biology, University of Athens)
- Lectures entitled “Eukaryotic microorganism as model systems for functional expression and characterization of transmembrane transporters of higher organisms” in the framework of the postgraduate course “Microbial Biotechnology – Model Systems of Molecular Microbiology” (**Dr. V. Sophianopoulou**, Department of Biology, University of Athens)
- Teaching in the framework of the postgraduate program “Bioinformatics”, the course “Introduction to Computational Biology” (**Dr. I. Almyrantis**, Department of Biology, University of Athens)
- Teaching in the framework of the postgraduate program “Clinical Biochemistry and Molecular Diagnostics”, the course “Introduction to Computational Biology” (**Dr. I. Almyrantis**, Department of Biology, University of Athens)
- Lecture on “Principles of X-Ray Crystallography: Applications in Structural Biology” in the framework of the post-graduate program (towards a Masters degree) entitled “Clinical Biochemistry – Molecular Diagnosis” (**Dr. M. Vlassi**, Dept. of Biology, National & Kapodistrian University of Athens)
- Teaching in the framework of the graduate course “Introduction to Research Methodology”, the course “Applications of NMR in Medicine” (**Dr. M. Pelecanou**, Medical School, University of Athens)

During July 2011, the Summer School of NCSR “Demokritos” was held and had included talks from the researchers of the Institute of Biology and of invited speakers coming from other Greek Institutions and abroad. The seminars of Biology related to the Summer School are presented analytically in the following pages.

Within the framework of the Graduate School Programme, are also organized, on a regular basis, bibliographical seminars and seminars presenting progress in current research work. These seminars are presented by all the graduate students of the Institute and supplemented by scientific seminars presented by other researchers of the Institute as well as invited guest speakers from other Greek or foreign Educational and/or Scientific Research Institutes. The seminars accomplished the past year (2011) are presented analytically in the following pages.

Finally, the educational endeavours of the Biology Institute also include those accomplished by **Dr. V. Labropoulou** (until 6/2011) and **Dr. H. Pratsinis** and **Dr. A.**

Prombona (from 7/2011), who give informative seminars to High School, University and Military School students.

COMPLETION/AWARD OF DOCTORAL THESES IN 2011

GRADUATE STUDENT	TITLE OF DOCTORAL THESIS	ADVISOR (in Institute of Biology)	UNIVERSITY
Ioannis Dafnis	Structure-function relationship of apolipoprotein E in brain	A. Chroni	Department of Chemistry University of Athens
Panagiotis Karkoulis	Study of signal transduction mechanisms under the effect of chemotherapeutic agents in human cancer	G. Voutsinas	Department of Biology University of Athens
Vasos Konstantinou	The role of growth factors on the proliferation of intervertebral disc cells, via the MEK/ERK and the PI3K/Akt pathways, during disc degeneration	D. Kletsas	Medical School University of Athens
Christiana Magrioti	Interactions of proteins from the endosymbiont virus of the parasitoid hymenopteran <i>Cotesia congregata</i> with proteins of the immune response of Lepidoptera	K. Iatrou & V. Labropoulou	Department of Biology University of Athens
Anastasia Repouskou	Circadian clock and histone acetylation: their interaction with the cell cycle in <i>in vitro</i> murine cell cultures	A. Prombona	Department of Biology University of Athens
Paraskevi Salpea	Study of acetylation and methylation in the reorganization of chromatin during aging	Th. Sourlingas	Medical School University of Athens

**LECTURE CONTRIBUTIONS TO
THE 2011 SUMMER SCHOOL
OF THE NCSR "DEMOKRITOS"**
(July 2011)

DATE	SPEAKER	TITLE
11/7/11	Dr. L. Swevers Institute of Biology, NCSR "Demokritos"	The RNAi mechanism in insects and its potential for pest control
11/7/11	Dr. K. Iatrou Institute of Biology, NCSR "Demokritos"	Targeting the olfactory functions of mosquitoes for the drastic reduction in the spreading of infectious diseases
13/7/11	Dr. Th. Sourlingas Institute of Biology, NCSR "Demokritos"	Histone Variants and Post Translational Modifications: Fundamental Factors in Chromatin Remodeling Events during Aging and Apoptosis
13/7/11	Dr. E. Tsilibary Institute of Biology, NCSR "Demokritos"	Mechanisms of apoptosis in age-related diseases
13/7/11	Dr. D. Kletsas Institute of Biology, NCSR "Demokritos"	Mechanisms of cell senescence and their role in tissue homeostasis
14/7/11	Dr. V. Sophianopoulou Institute of Biology, NCSR "Demokritos"	Study of the role of fungal eisosomal membrane microdomains in animal infections
14/7/11	Dr. M. Sagnou Institute of Biology, NCSR "Demokritos"	A little bit of curry once a day keeps the doctor away

SEMINAR PROGRAMME 2011
INSTITUTE OF BIOLOGY

DATE	SPEAKER	TITLE
27/1/11	Dr. E. Reboutsika BSRC "Al. Fleming"	Stemness settles SoxB genes down to work
2/2/11	Dr. V. Sophianopoulou Institute of Biology, NCSR "Demokritos" ("The Road of Ideas" Lecture Series of the IB)	Investigation of the biological role of eisosomal proteins in basal cellular processes: results, perspectives and considerations
3/2/11	A. Galeou Institute of Biology, NCSR "Demokritos"	Circadian control of carbon availability for Arabidopsis plant growth at night
3/2/11	P. Karkoulis Institute of Biology, NCSR "Demokritos"	Internalization of NK cells into tumor cells requires ezrin and leads to programmed cell-in- cell death
10/2/11	K. Apostolou - Karambelis Institute of Biology, NCSR "Demokritos"	A New Method for Assessing the Effect of Replication on DNA Base Composition Asymmetry
17/2/11	Th. Koutmos Institute of Biology, NCSR "Demokritos"	Notch Activation Differentially Regulates Renal Progenitors' Proliferation and Differentiation Toward the Podocyte Lineage in Glomerular Disorders
17/2/11	K. Kapodistria Institute of Biology, NCSR "Demokritos"	Nephrin is expressed on the surface of insulin vesicles and facilitates glucose-stimulated insulin
23/2/11	Dr. Z. Georgoussi Institute of Biology, NCSR "Demokritos" ("The Road of Ideas" Lecture Series of the IB)	The other side of opioid receptor signalling: regulation by protein-protein interactions-New developments and future perspectives
24/2/11	A. Papadopoulou Institute of Biology, NCSR "Demokritos"	Ionizing radiation-induced long-term expression of senescence markers in mice is independent of p53 and immune status
24/2/11	A. Dimozi Institute of Biology, NCSR "Demokritos"	Oxidative stress-induced ATM activation
3/3/11	A. Charonis National Foundation of Biomedical Research, Academy of Athens	The importance of proteomics for diagnosis of chronic kidney disease
4/3/11	A. Athanassopoulos Institute of Biology, NCSR "Demokritos"	Reassessment of the role of plasma membrane domains in the regulation of vesicular traffic in yeast
17/3/11	G. Daniil Institute of Biology, NCSR "Demokritos"	Interleukin-10 Facilitates Both Cholesterol Uptake and Efflux in Macrophages
17/3/11	M. Kostomiroi Institute of Biology, NCSR "Demokritos"	A Humanin Derivative Reduces Amyloid Beta Accumulation and Ameliorates Memory Deficit in Triple Transgenic Mice
24/3/11	A. Kolliopoulou Institute of Biology, NCSR "Demokritos"	Cell-specific RNA interference by peptide- inhibited-peptidase-activated siRNAs
24/3/11	A. Armatas Institute of Biology, NCSR "Demokritos"	The Matricellular Protein CCN1/CYR61 Induces Fibroblast Senescence and Restricts Fibrosis in Cutaneous Wound Healing
29/4/11	Dr. V. Ntziachristos Institute for Biological and Medical Imaging, Helmholtz Zentrum München."	Illuminating biological discovery with advanced imaging methods

8/6/11	Dr. B. Howard Laboratory of Molecular Growth Regulation National Institute of Child Health and Human Development National Institutes of Health (NIH)	Postnatal development- and age-related changes in DNA methylation patterns in the human genome
4/11/11	Assistant Prof. G. Spyroulias Dpt. of Pharmacy, Univ. of Patras"	Structural Biology through Spectroscopy of Biomolecular NMR
2/12/11	Prof. G. Paxinos Neuroscience Research Australia Barker St and Hospital Rd, Randwick, Sydney, Australia	Brain, behavior and evolution
8/12/11	K. Ioannidis Institute of Biology, NCSR "Demokritos"	Lepidopteran nuclear polyhedrosis viruses: Molecular characterization and biotechnological applications
15/12/11	S. Verouti Institute of Biology, NCSR "Demokritos"	VDR-activators up-regulate the expression of podocalyxin and nephrin on Human Glomerular Epithelial Cells (HGEC)/ podocytes cultured on high glucose.
20/12/11	Dr. M. Kalamvoki Cover Viral Oncology Labs University of Chicago, USA	Immediate early events that co-opt with the HSV-1 genome activation. Cycling around the CLOCK

COLLECTIVE DATA

FINANCIAL REPORT 2011

INTERNAL FUNDING FROM THE SPECIAL ACCOUNT DEPARTMENT AND FUNDING FROM THE GSRT

	PROGRAMME (COORDINATOR: E. TSILIBARY, Head of IB).
<u>INCOME</u>	464
CARRIED OVER FROM 2010	56.101,20
FUNDING FROM NCSR "D"	60.000,00
MATCHING FUNDS	
INCOME FROM SERVICES	
DONATIONS FROM COMPANIES	
TRANSFER FROM OTHER RESOURCES	4.244,48
TOTAL INCOME	120.345,68

PROGRAMME	
1334	1475
-	
55.460,27	953,7
26.000,00	
17.000,00	1.200,7
12.460,27	2.154,4

<u>EXPENSES</u>	
EQUIPEMENT	9.914,90
SUPPLIES	22.842,17
SALARIES	600,00
TRAVELS	3.210,29
OTHER EXPENSES	15.927,70
COMMITTED	611,68
TRANSFER FROM OTHER RESOURCES	36.627,88
<u>TOTAL EXPENSES</u>	89.734,62

0,00	0,0
3.229,97	0,0
38.535,88	0,0
0,00	0,0
1.254,63	0,0
211,25	0,0
71,37	0,0
43.303,10	0,0

**EXTERNAL FUNDING FROM THE PROGRAMMES OF THE
INSTITUTE (Programmes that are coordinated by the Head of IB
are included)**

SOURCE OF FUNDING (number of programmes)	FUNDING (in EUROS)			
	Programme A	Programme B	Programme C	INSTITUTE
European Union (5)	101.375	220.097	13.000	334.472
General Secretariat for Research & Technology (1)	38.640	-	-	38.640
Empirikion Foundation (1)	3.000	-	-	3.000
Hellenic Society of Lipidology, Atherosclerosis and Vascular Disease w (2)	6.500	-	-	6.500
Hellenic Post Bank, TTBank (1)	-	-	5.000	5.000
American College of Greece (1)	7.000	-	-	7.000
Organogenesis Inc. (1)	11.280	-	-	11.280
Abbot Hellas (1)	6.800	-	-	6.800
TOTAL	174.595	220.097	18.000	412.692

COLLECTIVE DATA ON PRODUCTIVITY OF SCIENTIFIC PROGRAMMES

	P R O G R A M M E			INSTITUTE
	A	B	C	
Researchers	11	7	4	23*
Technical Specialist	-	1	1	2
Emeritus & Collaborating Scientists	2	4	1	8**
Postdoctoral Fellows	4	3	2	9
Graduate Students	17	7	2	26
Collaborating Graduate Students	10	1	2	13
Graduate Research Associates	-	1	1	3!!!
Undergraduate Students	10	4	-	14
Research Technicians	2	1	-	6@
Administrative Staff	-	-	-	4
Total Personnel	55	29	13	108
Publications in Peer-Reviewed Journals	21	12	7	42[#]
Publications (Average) in Peer-Reviewed Journals per Scientist	1.909	1.714	2.333	1.826
Cumulative Impact Factor in Peer-Reviewed Journals (number of publications)	83.042 (21)	36.623 (12)	14.966 (7)	139.403[#] (42)
Average Impact Factor in Peer-Reviewed Journals	3.954	3.051	2.138	3.319
Cumulative Impact factor per Scientist	7.549	5.231	3.741	6.061
Proceedings to Conferences	8	3	3	14
Proceedings (Average) per Scientist	0.727	0.428	0.75	0.608
Total Publications	29	15	10	56[#]
Publications (Average) per Scientist	2.636	2.142	2.5	2.434
Citations	973	314	137	1439*
International Patents	-	-	-	-
Greek Patents	-	-	-	-
Presentations to International Conferences	25	19	10	52[£]
Presentations (Average) per Scientist to International Conferences	2.272	2.714	2.5	2.260
Presentations to Greek Conferences	25	9	13	47
Presentations (Average) per Scientist to Greek Conferences	2.272	1.285	3.25	2.043
Total Presentations to Conferences	50	28	23	99[£]
Presentations (Average) per Scientist to Conferences	4.545	4	5.75	4.304

* 1 Scientist of Human Tissue Bank is included

** 1 Collaborating Scientist of Human Tissue Bank is included

!!! 1 Collaborating Graduate Associate of Human Tissue Bank is included

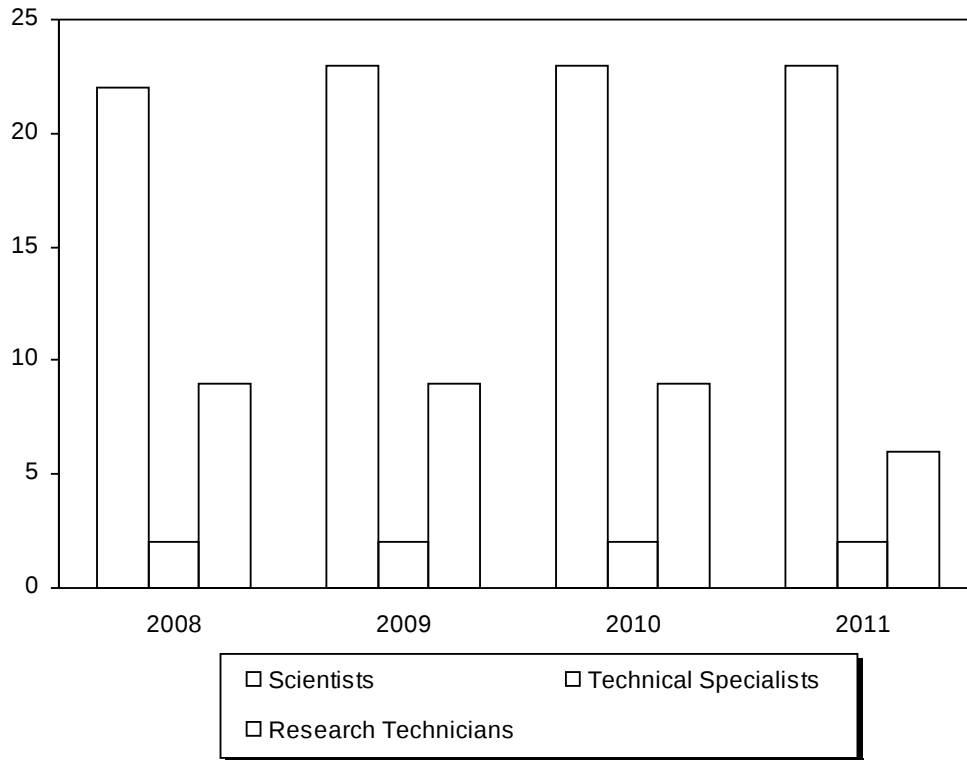
@ 2 Research Technicians who are occupied in Experimental Animal Colony and 1 Research Technician who is occupied in Human Tissue Bank are included

2 publications of Human Tissue Bank are included

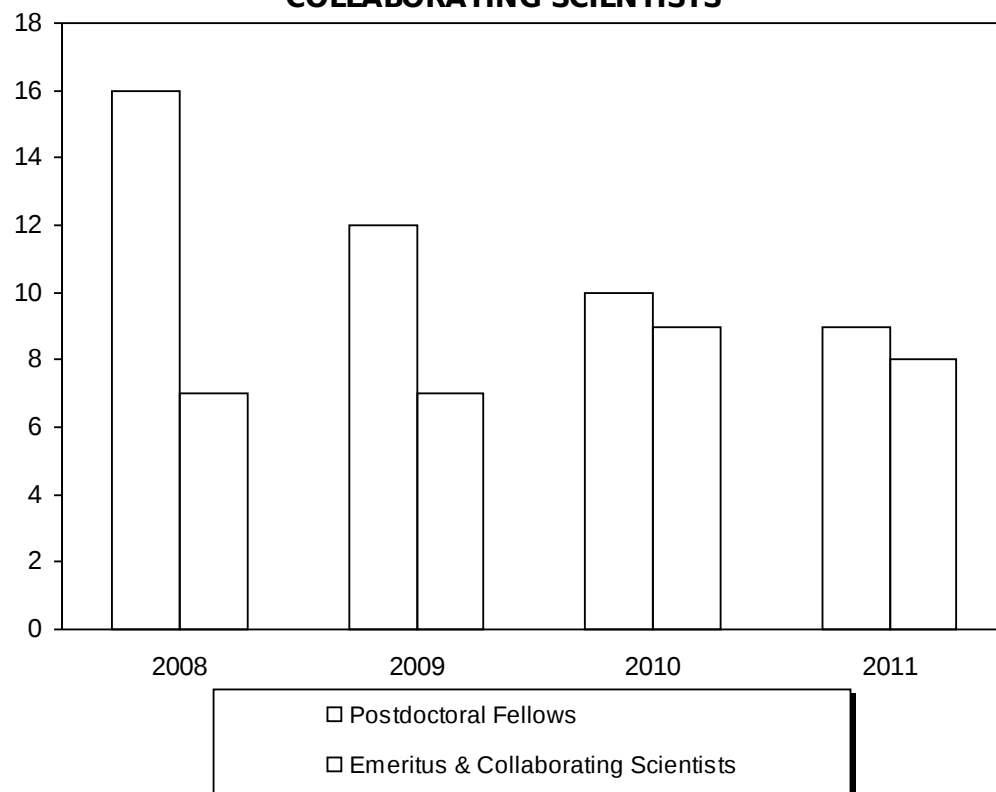
£ 2 common presentations of A and B programme are included

CHANGES OF IB STAFF DURING 2008-2011

TENURED EMPLOYEES



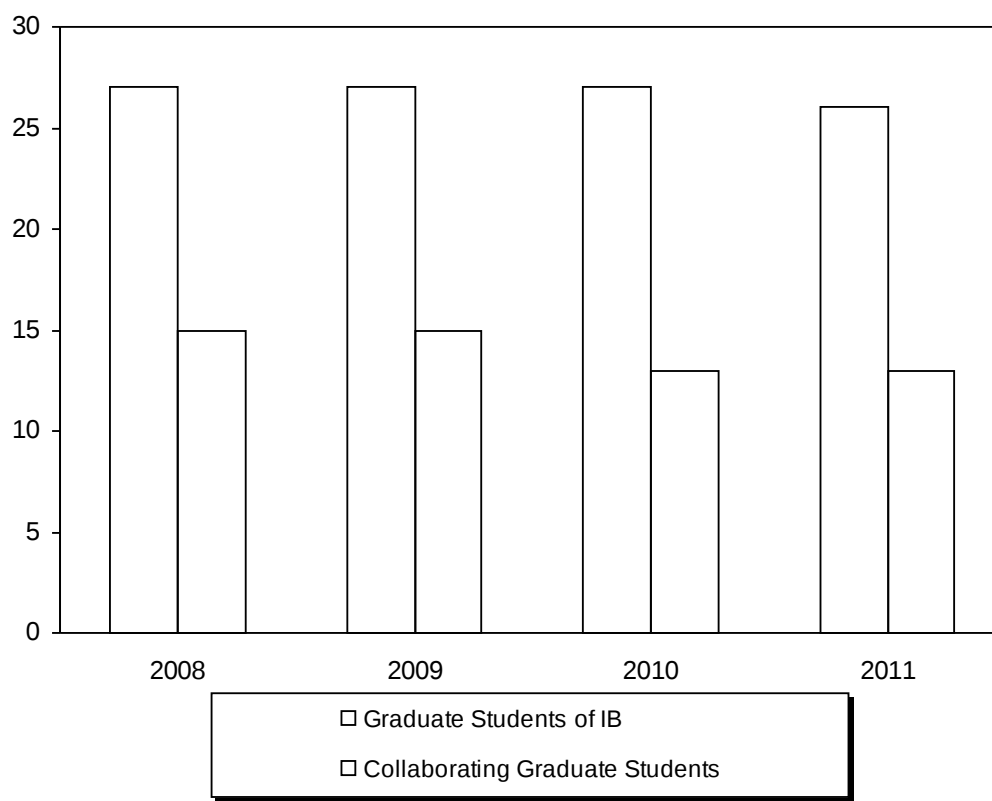
POSTDOCTORAL FELLOWS and EMERITUS & COLLABORATING SCIENTISTS



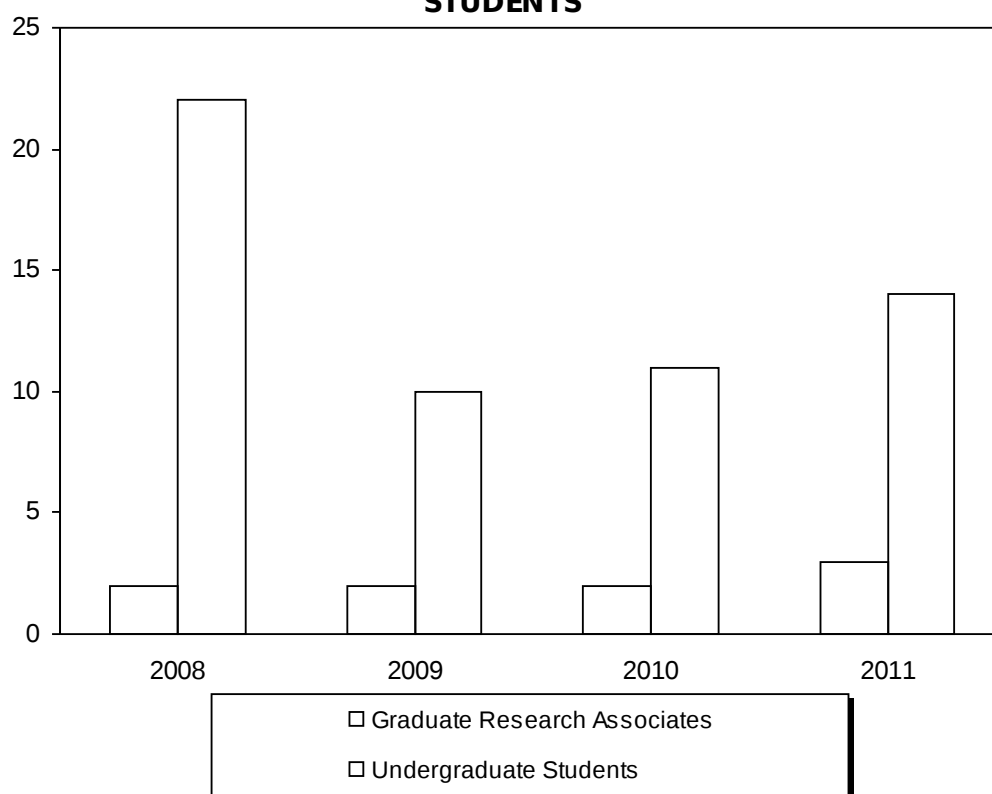
CHANGES OF IB STAFF

DURING 2008-2011

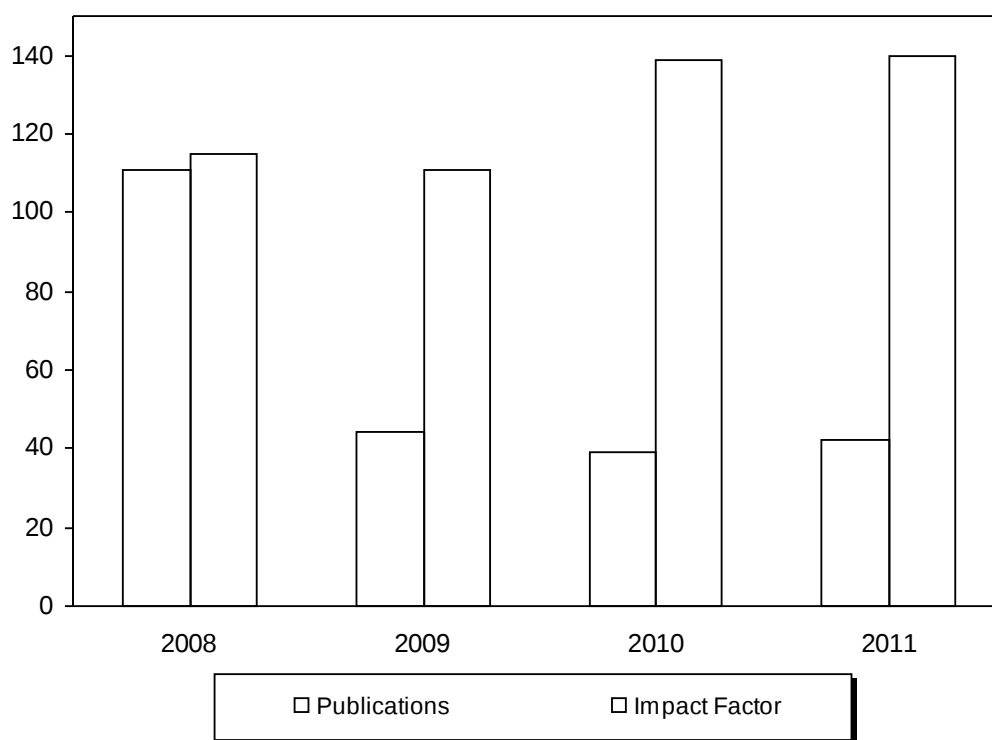
GRADUATE STUDENTS



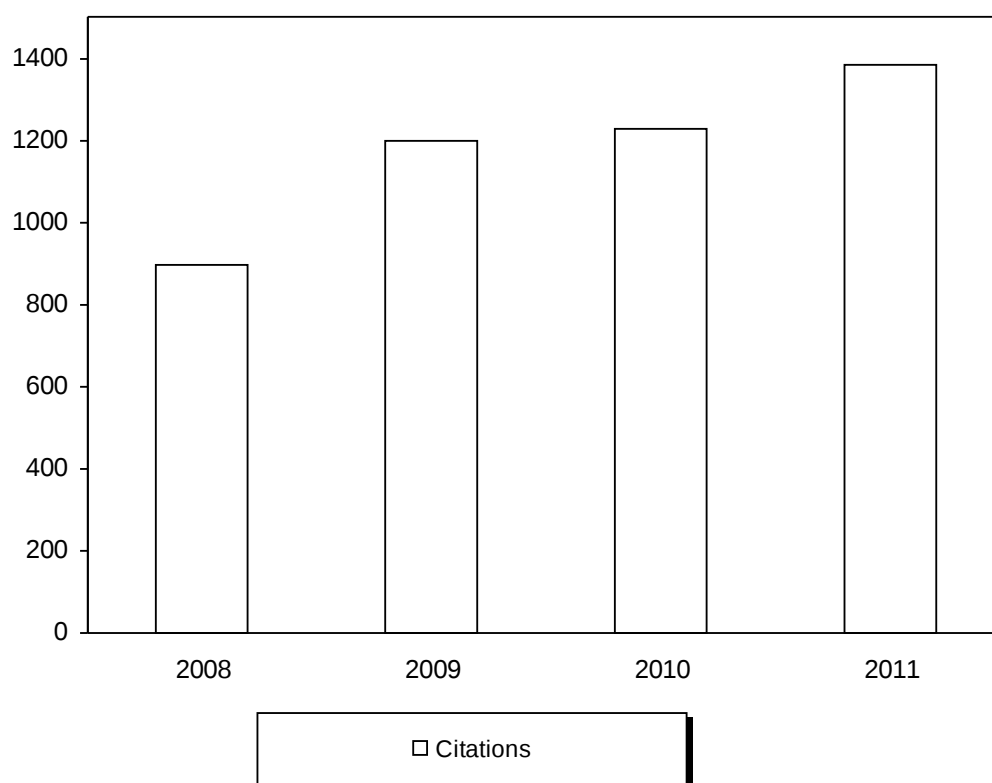
GRADUATE RESEARCH ASSOCIATES AND UNDERGRADUATE STUDENTS



PUBLICATIONS IN PEER-REVIEWED JOURNALS AND CUMULATIVE IMPACT FACTOR DURING 2008-2011



CITATIONS OF THE INSTITUTE 2008-2011



CUMULATIVE EXTERNAL FUNDING OF THE INSTITUTE DURING 2008-2011 (EURO)

