

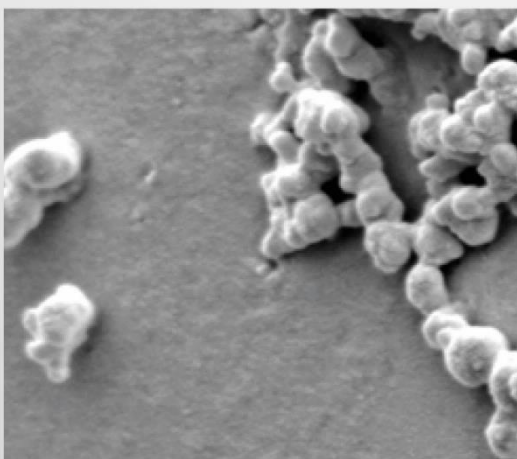
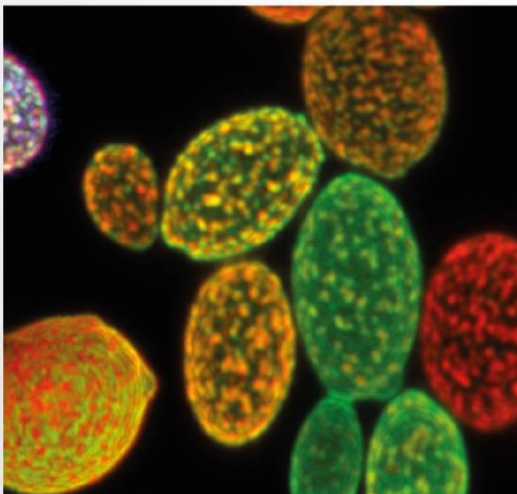
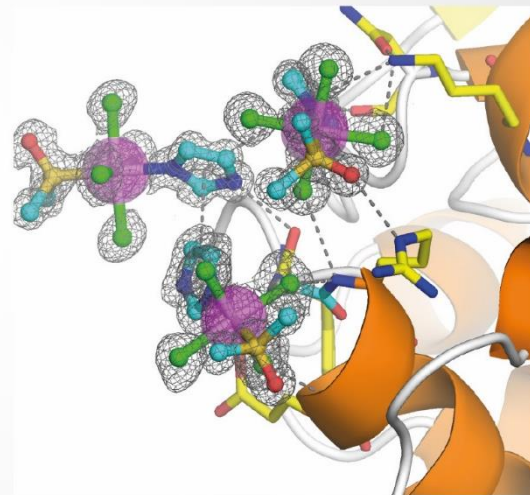
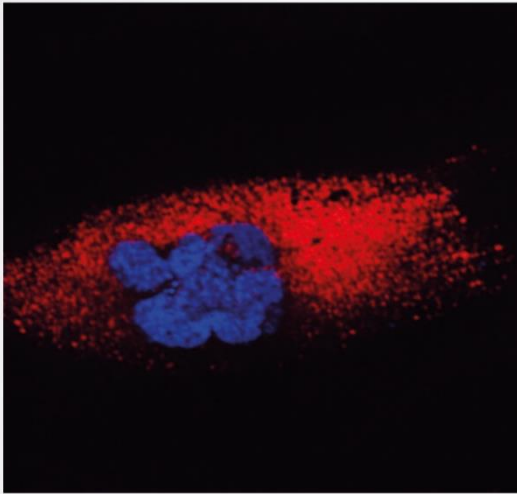


DEMOKRITOS
NATIONAL CENTRE FOR SCIENTIFIC RESEARCH



INSTITUTE
OF BIOSCIENCES
& APPLICATIONS

ANNUAL REPORT
2020



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

INSTITUTE OF BIOSCIENCES & APPLICATIONS

ANNUAL REPORT 2020

AGIA PARASKEVI

OCTOBER 2021

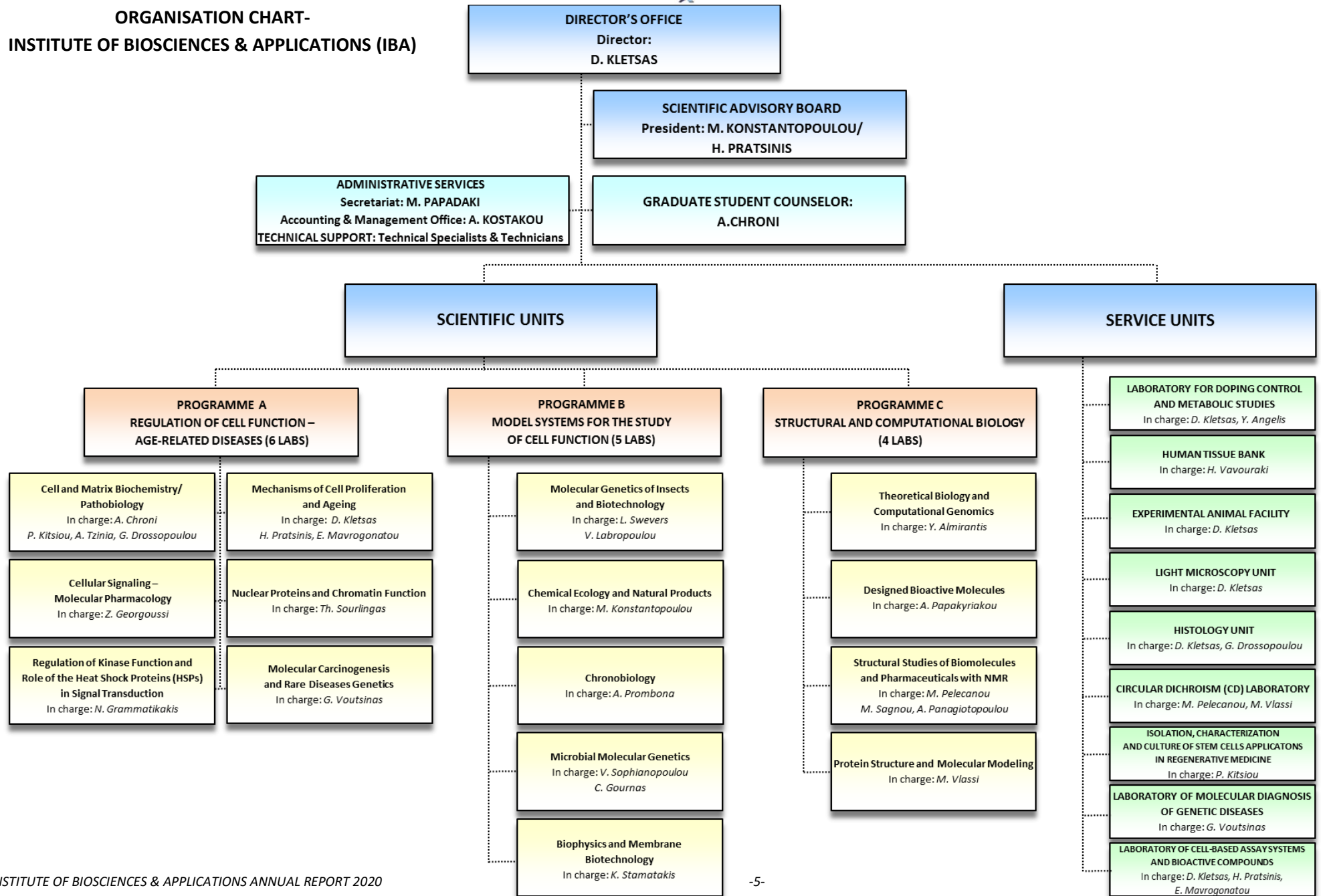
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**ORGANISATION CHART-
INSTITUTE OF BIOSCIENCES & APPLICATIONS (IBA)**



IBA PERSONNEL

DIRECTOR

Kletsas Dimitris

Dr. Biologist

SCIENTIFIC STAFF

Research Directors

Almirantis Yannis

Dr. Theoretical Biologist

Chroni Angeliki

Dr. Chemist

Georgoussi Zafiroula-Iro

Dr. Biochemist

Kletsas Dimitris

Dr. Biologist

Pelecanou Maria

Dr. Pharmacist

Sophianopoulou Vassiliki

Dr. Molecular Biologist, Microbiologist

Stamatakis Konstantinos

Dr. Biologist

Swevers Luc

Dr. Biologist

Vlassi Metaxia

Dr. Physicist-Chrysallographer

Voutsinas Gerassimos

Dr. Biologist

Senior Researchers

Grammatikakis Nikolaos

Dr. Molecular Biologist

Drossopoulou Garyfalia

Dr. Biologist

Kitsiou Paraskevi

Dr. Biologist

Konstantopoulou Maria

Dr. Biologist

Labropoulou Vassiliki

Dr. Biochemist

Papakyriakou Athanasios

Dr. Chemist

Pratsinis Harris

Dr. Chemist

Prombona Anastasia

Dr. Molecular Plant Biologist

Sourlingas Thomae

Dr. Biologist

Tzinia Athina

Dr. Biochemist

Vavouraki Helen

Dr. Pharmacist

Researchers

Gournas Christos

Dr. Biologist

Mavrogonatou Eleni

Dr. Biologist

Sagnou Marina

Dr. Biologist/ Chemist

Functional Scientific Personnel

Panagiotopoulou Angeliki

Dr. Biochemist

TECHNICAL SPECIALISTS

Angelis Yiannis

Dr. Chemist

Athanasopoulos Alexandros

Dr. Biologist

Avgeris Socratis

Technologist (MSc)

Fragkaki Argyro

Dr. Chemist

Kioukia Nassia

Dr. Pharmacist

Kiوسي Polyxeni

Dr. Chemist

Stefanou Dimitra

Agronomist

ADMINISTRATIVE & TECHNICAL SUPPORT

Kostakou Athanasia	Accountant
Chlapana Foteini	Research Technician
Doulgeridis George	Laboratory Animal Caretaker
Filippidou Maria	Administrative Officer
Lionis Ioannis (MSc)	Electronics Engineer
Loui Stella	Technologist
Papadaki Margarita (MSc)	Administrative Officer
Pavlaki Maria	Supporting Personnel
Tzouvara Vassiliki	Administrative Officer
Vlahos Panayiotis (MSc)	Network Administrator
Vlahou Maria	Administrative Officer
Zafeiropoulos Giannis	Laboratory Animal Technician

EMERITUS & COLLABORATING SCIENTISTS

Scientists

Iatrou Kostas (Dr. Biochemist & Molecular Biologist) – *Emeritus*
 Ignatiades Lydia (Dr. Hydrobiologist) – *Collaborating*
 Papageorgiou George (Dr. Biophysicist) – *Collaborating*
 Papageorgiou Spyros (Dr. Physicist) – *Collaborating*
 Sekeri Kalliope (Dr. Biochemist) – *Collaborating*
 Tsimilli-Michael Merope – *Collaborating*

IBA Researcher

Georgoussi Z.
 Georgoussi Z.
 Stamatakis K.
 Almirantis Y.
 Sourlingas Th.
 Stamatakis K.

POSTDOCTORAL FELLOWS

Fellow

Christoforidis Christoforos
 Dafnis Ioannis
 Feng Min
 Fytrou Anastasia
 Galeou Angeliki
 Gkolfinopoulou Christina
 Halevas Eleftherios
 Kaminari Archontia
 Karanikou Maria
 Kolliopoulou Anna
 Kontogiannatos Dimitrios
 Koutsoumpeli Eleni
 Kythreoti Georgia
 Matiadis Dimitris
 Mavroidi Barbara
 Mpakali Anastasia
 Papadopoulou Adamantia

IBA Researcher

Kletsas D.
 Chroni A.
 Swevers L.
 Konstantopoulou M.
 Prombona A.
 Chroni A.
 Sagnou M.
 Tzinia A.
 Chroni A.
 Swevers L.
 Swevers L.
 Konstantopoulou M.
 Iatrou K.
 Pelecanou M.
 Pelecanou M.
 Papakyriakou A.
 Kletsas D.

GRADUATE RESEARCH ASSOCIATES

Research Associate

Betsi Petri-Christina (MSc)
 Bondi Labrina
 Dasenaki Ioanna (MSc)
 Dedemadi Anastasia-Georgia (MSc)
 Goula Olga
 Klamarias Lykourgos (Veterinarian)
 Kosmidis Eleutherios
 Ninios Ioannis (PhD)
 Panagopoulou Lydia (MSc)
 Patra Christina
 Raptopoulos Dimitris (PhD)
 Sakellariou Panagiotis (PhD)
 Santorinios Dimitris
 Smyrniotis Stefanos
 Theofanidi Eleni
 Valanti Effie

IBA Researcher

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 Voutsinas G.
 Konstantopoulou M.
 Sourlingas Th.
 Kletsas D.
 Kletsas D.
 Vavouraki H.
 Vavouraki H.
 Vavouraki H.
 Vavouraki H.
 Konstantopoulou M.
 Kletsas D.
 Voutsinas G.
 Voutsinas G.
 Drossopoulou G.
 Chroni A.

PHD CANDIDATES AND MSc STUDENTS

PhD candidate (University)

Adamopoulou Maria (Univ. of Copenhagen, Denmark)
 Angelopoulou Maria (NKUA)
 Athanasoulis Alexandros (NKUA)
 Biratsi Ada (NKUA)
 Broussos Panayiotis (AUA)
 Chiniadis Lykourgos (AUA)
 Dedemadi Anastasia-Georgia (NKUA)
 Farmaki Danae (NKUA)
 Fotopoulou Asimina (Univ. of Patras)
 Giakoumidaki-Vogiatzi Aikaterini (AUA)
 Kalampalidis Alexandros (NKUA)
 Karoussiotis Christos (NKUA)
 Kouroumalis Anastasios (NKUA) – *PhD obtained*
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 Lesgidou Nastazia (DUT)
 Manikas Neoklis (AUA)
 Megarioti Amalia (NKUA)
 Mountaki Christina (NKUA)
 Pallaki Paschalina (NKUA)
 Papagiannis Achilleas (AUTH)
 Sofokleous Valentinos (NKUA)
 Symeonof Alexandra (NKUA)
 Tsimelis Efstathios (Univ. of Patras)
 Vayenos Dimitris (NTUA)

Supervisor in IBA

Kletsas D.
 Kletsas D.
 Papakyriakou A.
 Sophianopoulou V.
 Stamatakis K.
 Papakyriakou A.
 Chroni A.
 Prombona A.
 Kletsas D.
 Stamatakis K.
 Papakyriakou A.
 Georgoussi Z.
 Kletsas D.
 Kletsas D.
 Vlassi M.
 Konstantopoulou M.
 Gournas C.
 Chroni A.
 Georgoussi Z.
 Chroni A.
 Sagnou M.
 Georgoussi Z.
 Kletsas D.
 Stamatakis K.

MSc student (University)

Almpani Charalampia (NKUA)
 Dimozi Maria (NKUA)

Supervisor in IBA

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 Kletsas D.

Kaplani Eleni (NKUA)	Kletsas D.
Kelemen Marina – Gemma (Univ. of Patras)	Kletsas D.
Kypraiou Anastasia (Univ. of Patras)	Kletsas D.
Micha Asimina	Chroni A.
Nigianni Georgia (Univ. of Patras)	Sagnou M.
Ntogka Christina (NKUA) – <i>MSc obtained</i>	Kletsas D.
Ren Feifei (South China Agricultural University)	Swevers L.
Valianatos Alexandros (NKUA)	Sophianopoulou V.

UNDERGRADUATE & OTHER TRAINING STUDENTS

Student (University)	Supervisor in IBA
Byrne Vania – Nicole (NKUA)	Mavrogonatou E.
Delli Anna (NKUA) – <i>Dissertation completed</i>	Voutsinas G.
Gaitanos Spiros (NKUA)	Sophianopoulou V.
Gosda Vassiliki (Metropolitan College) – <i>Dissertation completed</i>	Voutsinas G.
Ifanti Georgia-Despina (NKUA)	Gournas C.
Maraidonis Ioannis-Nektarios (NKUA)	Labroboulou V.
Mazurek Yuzef-Alexander (UWA)	Swevers L.
Menoudaki-Evangelou Athanasia (Metropolitan College) – <i>Dissertation completed</i>	Voutsinas G.
Myridakis Antonis (NKUA) – <i>Dissertation completed</i>	Georgoussi Z.
Papanagnou Dimitris (Univ. of Crete)	Georgoussi Z.
Paralikas Nikos (NKUA)	Voutsinas G.
Poizatzi Aglaia (NKUA)	Georgoussi Z.
Ragusa Chiara (Univ. of Catania, ERASMUS)	Georgoussi Z.
Skoutelas Petros (Univ. of Patras)	Sophianopoulou V.
Stathourou Maria (NKUA)	Georgoussi Z.
Stefanatou Chrysanthi (AUA)	Prombona A.
Syropoulou Vassiliki (Univ. of Ioannina)	Chroni A.
Triantafyllopoulou Maria (Metropolitan College) – <i>Dissertation completed</i>	Voutsinas G.
Zakopoulou Tatiana (NKUA)	Sophianopoulou V.
Zuccala Sara (Univ. of Catania, ERASMUS)	Georgoussi Z.

INTRODUCTION

The Institute of Biosciences and Applications (IBA) is one of the five Institutes of the National Centre for Scientific Research (NCSR) “Demokritos”. The Centre, among the best in Greece and in Europe, is characterized by multidisciplinary and the unique coexistence of different fields of science, as well as the collaboration among scientists of different disciplines, towards the promotion of science and innovation. Within this framework, the IBA focuses its research and developmental activities in various areas of life sciences and especially in the study of cellular function, with an emphasis on age-related diseases, on biotechnology and the environment, as well as on structural and computational biology. In addition, the Institute houses a considerable number of laboratories, some of them unique in Greece, providing advanced technological research services to the Public and the Private Sector.

Within 2020, despite the covid-19 pandemic, there was a significant upgrading of IBA’s research and developmental work. For this achievement I want to congratulate the 25 researchers and the total 138 members of the Institute. I want to thank the Vice Director G. Voutsinas and the members of the previous Scientific Committee of the IBA M. Konstantopoulou (President), A. Chroni, Z. Georgoussi, H. Pratsinis, L. Swevers (Members), those of the new Committee H. Pratsinis (President) M. Konstantopoulou, V. Labropoulou, A. Tzinia, G. Voutsinas (Members), and A. Kostakou (Representative of the administrative and technical personnel) for the orderly function of the Institute, as well as the Responsible of the Educational Committee A. Chroni and the Deputy M. Sagnou for the upgrading of the education within IBA.

The implementation of important programs for the function and upgrading of the infrastructure of the IBA continued within 2020. A special reference is needed to those colleagues who worked, in collaboration with all the members of the Institute (researchers, technical and administrative staff), for the implementation of these projects, namely SANITURA (Drs. V. Labropoulou and H. Pratsinis), OPENSREEN-GR (Drs. K. Iatrou, A. Chroni, Z. Georgoussi, and H. Pratsinis), BIOIMAGING-GR (Drs. V. Labropoulou, H. Pratsinis, and M. Sagnou) and INSPIRED (Drs. A. Chroni, A. Panagiotopoulou, M. Pelecanou and M. Vlassi). The successful implementation of these projects, as well as of all the competitive grants the members of the IBA have attracted, increased significantly the number of young researchers, renewed the infrastructures and supported considerably the scientific and developmental effort of the Institute.

The upgrading of the Laboratory for Doping Control and Metabolic Studies continued with the renewal of the analytical infrastructure, while a Memorandum of Understanding has been signed between the Deputy Ministers of Development and Investments and of Sports for the financial support of the Laboratory, including the recruitment of new scientists. I want to congratulate Y. Angelis (Scientific Director) and A. Fragkaki (Quality Manager), as well as all the members of the Laboratory for their efforts.

Unfortunately, within 2020 we experienced the loss of three distinguished researchers that played an important role in the development of the Institute, those of V. Mazomenos, G. Papageorgiou and L. Stasinopoulou, as well as of the previous IBA student Th. Tartas. We will remember them with respect and affection.

Our Institute has a long tradition in postgraduate education since the 1960s. During 2020, education within IBA has been greatly upgraded. The training of a significant number of post-doctorate fellows, post-graduate students, and diploma and pre-graduate students continued, while the majority of the faculty members participated in post-graduate courses of a number of Universities in Greece. The Interinstitutional Post-Graduate Educational Program (IPEP) entitled “Applied Biochemistry: Clinical Chemistry, Biotechnology and Evaluation of Pharmaceutical Products” between IBA and the Department of Chemistry of the University of Patras has continued, while the participation of the IBA in the IPEP “Athens International School for Neurosciences” has been

renewed. In addition, in collaboration with the European Learning Laboratory for the Life Sciences (ELLS) of the European Molecular Biology Laboratory (EMBL), a training course for secondary school biology teachers was organized with great success. Finally, the researchers of the IBA participated in the Summer School of NCSR “Demokritos”, as well as in a number of activities for the dissemination of science to the general public. Congratulations to all colleagues participating in these activities.

This year, Mrs. M. Papadaki, who served as IBA Secretary for 18 years, left the Institute. I want to thank her for her efforts during all these years and wish her every success in her new career. Finally, I want to thank the IBA Accountant Mrs. A. Kostakou for her significant contribution to the orderly function of the Institute.

Dimitris Kletsas, PhD
IBA Director
October 2021

PROGRAMME A

"REGULATION OF CELL FUNCTION – AGE-RELATED DISEASES"

Research Group: Cellular Signaling–Molecular Pharmacology

Research Staff

Zafiroula-Iro Georgoussi, Research Director

Paschalina Pallaki, Graduate Student
Christos Karoussiotis, Graduate Student
Alexandra Symeonof, Graduate Student
Antonis Myridakis, Undergraduate Student-*BSc obtained in 2020*
Dimitris Papanagnou, Chemist, Trainee
Aglaia Poizantzi, Graduate MSc student
Maria Stathourou, Graduate MSc student
Sara Zuccala, Undergraduate Student, Erasmus
Chiara Ragusa, Undergraduate Student, Erasmus

Kostas Iatrou, Research Director (Emeritus)
Lydia Ignatiades, Research Director (Retired)
Georgia Kythreoti, Postdoctoral Fellow

Research Interests

The research activities of the laboratory of Cellular Signaling and Molecular Pharmacology are focused on the elucidation of the regulatory mechanisms governing G protein-coupled receptors (GPCRs) signaling pathways. Emphasis is given on the mechanisms acting beyond the G protein paradigm that are implicated in various physiological and pathological conditions.

As a model system we use the three opioid receptor subtypes (δ , μ and κ) which are involved in neurotransmission, neurogenesis and neuronal plasticity and are implicated in tolerance and dependence upon prolonged drug administration, pain, and emotional behaviors such as anxiety, depression and stress.

More specifically our objectives are focused on three interconnected thematic areas, which include:

- The elucidation of novel signaling pathways and analysis of genes, transcription factors and proteins - whose function is altered upon opioid administration and are implicated in neuronal differentiation and outgrowth.
- The elucidation of the molecular mechanism of autophagy in neuronal plasticity under stress conditions in animal models.
- The pharmacological characterization of new bioactive compounds for opioid or other GPCRs using high throughput cell-based screening assays in order to identify “smart drugs” to alleviate pain or other diseases of the central nervous system.

2020 Findings

Alternative signal transduction pathways induced upon opioid receptor activation

Autophagy: an unexplored mechanism of the opioid receptor function: Given that the dynorphin/ κ -opioid receptor (κ -OR) system plays key role in modulating anxiety and stress related behaviors and that κ -OR antagonists are considered as important pharmacological targets for depressive disorders and anxiety-like behaviors we identified that specific κ -OR analogs induce the autophagic machinery. Our studies propose that a new signaling pathway involving Gai/o proteins, ERK1,2 and p-CREB regulate the transcription of *becn1* autophagic gene upon κ -OR activation with the selective κ -OR agonist U50,488H. Moreover, we indicate that U50,488H administration in mice induces autophagy exclusively in hippocampus and decreases the levels of the synaptic proteins spinophilin, PSD-95 and SNAP-25 (Figure 1). Behavioral studies in mice [force swim test, (FST)] upregulates autophagy in hippocampus an effect that it is blocked upon administration of the κ -OR specific antagonist norBNI. In parallel the levels synaptosomal proteins upon FST are decreased and this

effect is blocked by norBNI administration. These results demonstrate that under stress conditions the dynorphin release results in κ -OR-induction of autophagy in hippocampus leading to synaptic alterations.

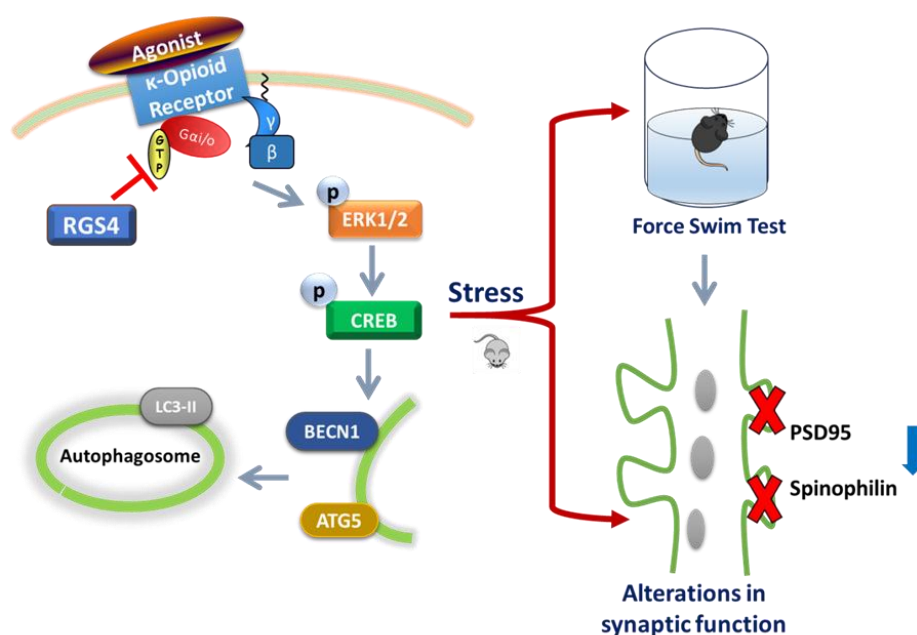


Figure 1: Schematic representation of a putative signaling pathway through which activation of the κ -opioid receptor with selective agonists induces the autophagic machinery and leads to synaptic alterations and in hippocampal neurons under stress conditions.

The role of RGS4 protein: Previous observations from our lab have shown that RGS4 negatively regulates opioid receptor signaling and that RGS4 is implicated in δ -opioid-receptor (δ -OR) dependent neuronal differentiation and neurite outgrowth. This negative regulatory mechanism is exerted by a “non-canonical” signaling pathway regulating STAT5B-inducible gene expression (Georganta et al., 2010, 2013; Pallaki et al., 2017). In this regard, we found using primary neuronal cultures from RGS4^{-/-} mice that the levels of specific autophagic markers are dramatically reduced with a concomitant increase of p-Akt, p-JNK and p-ERK1, 2 kinases. These observations demonstrate that RGS4 is plausible implicated in autophagy regulation.

In collaboration with Prof. J. Selent, Pompeu Fabra University, Barcelona, Spain under the auspices of the COST-action-GLISTEN (*GPCR Ligand Interactions, Structures and Transmembrane Signaling*) using molecular dynamics and site directed mutagenesis we have identified the structural determinants and the critical amino-acids responsible for the interaction of δ -OR with RGS4. The high degree of conservation among opioid receptors points to a conserved interaction mode between helix VIII of opioid receptors and the N-terminal region of RGS4 and allowed as to propose a structural model of a ternary complex including the δ -OR, $G_{i\alpha}$ and RGS4 protein complex.

Pharmacological characterization of bioactive compounds in cell-based throughput platforms

In the context of our participation in the EU consortium “*NORMOLIFE NETWORK*” (collaboration with Drs L. Pasquinucci, G. Ronsisvalle & R. Turnaturi, University of Catania), our group characterized using specific cell-based assays the pharmacological profile of new bioactive compounds. These compounds exert functional selectivity towards a specific opioid receptor pathway (*biased agonism*), thus providing new dimensions for the use of δ -opioid receptor analogs for pain management.

Development of neuronal networks on graphene microelectrode nanostructures

In collaboration with Dr P. Dimitrakis of the INN Institute of NCSR “D” efforts were made to develop neuronal cells on graphene nanoribbon interconnects. These studies aim in the design of biosensors that could allow to investigate the cellular (electrical) responses and their plausible variations upon electrical or chemical stimulation.

Publications

Pasquinucci L., Parenti C., Ruiz-Cantero M. C., Georgoussi Z., Pallaki P., Cobos E.J., Amata E., Marrazzo A., Prezzavento O., Arena E., Dichiara M., Salerno L., Turnaturi R. (2020) “*Novel N-Substituted Benzomorphan-Based Compounds: From MOR-Agonist/DOR-Antagonist to Biased/Unbiased MOR Agonists*”. *ACS Med. Chem. Lett.* 28;11(5):678-685. doi: 10.1021/acsmchemlett. 9b00549 (IF: 3.737).

Karoussiotis C., Marti-Solano M., Stepniewski, MT., Symeonof, A., Selent J. and Georgoussi, Z. (2019) “*A highly conserved δ -opioid receptor region essential for RGS4 recruitment*” *FEBS J* 287(4):736-748 doi.org/10.1111 /febs.15033 (IF: 4.739)

Articles in Press

Georgoussi Z., Karoussiotis C., Sotiriou A., Papavranoussi-Daponte D., Polissidis A.V., Nikolettou V. (2020). “*The role of κ -Opioid Receptor-Induced Autophagy in Synaptic Alterations*”. *FASEB Journal* 34, S1, <https://doi.org/10.1096/fasebj.2020.34.s1.02897> (IF:4.411)

Other Publications

Georgoussi Z., Karoussiotis C., Sotiriou A., Papavranoussi-Daponte D., Polissidis A.V., Nikolettou V. (2020). “*The role of κ -Opioid Receptor-Induced Autophagy in Synaptic Alterations*”. *FASEB Journal* 34, S1, <https://doi.org/10.1096/fasebj.2020.34.s1.02897>

Presentations at Scientific Conferences

Georgoussi Z., Karoussiotis C., Sotiriou A., Papavranoussi-Daponte D., Polissidis A.V., Nikolettou V. (2020). “*The role of κ -Opioid Receptor-Induced Autophagy in Synaptic Alterations*”. *Experimental Biology 2020, San Diego CA, USA, April, 2020*

Georgoussi Z. (2020) Conference of the Hellenic Society of Algology “*Cellular Signaling of opioid receptors: Targeting new opioids*”, Athens 24 October, 2020

Educational Activities

- Member of the organizing committee for the establishment of the International Masters course in Neurosciences entitled “*Athens International Master’s Programme for Neurosciences*” together with the Kapodistrian University of Athens, the NHRF, BRFAA, the Institutes Pasteur and the Fleming Institute.
- Co-corganizer of the course “*Cellular and Molecular Neurosciences*” of the Athens International Master’s Programme in Neurosciences.
- Supervisor of the PhD dissertations of the Graduate students: Paschalina Pallaki, Christos Karoussiotis, and Alexandra Symeonof.
- Supervisor of the BSc theses of the Undergraduate students: Antonis Myridakis, Department of Biology, Kapodistrian University of Athens.
- Supervision of the MSc students A. Poizatzi and M. Stathourou, Athens International Master’s Program in Neurosciences, Kapodistrian University
- Supervisor of the Undergraduate students Sara Zuccala and Chiara Ragusa from the University of Catania, Department of Pharmacology, Erasmus program.
- Member of the Advisory Committee in the Department of Biology of the Kapodistrian University in Athens for the PhD students P. Pallaki, S. Koutoglou C. Karoussiotis and A. Symeonof.

- Teaching in the Interdepartmental Master Course program of the Kapodistrian University of Athens, Department of Biochemistry and Molecular Biology “*Molecular Basis of Human Disease*” entitled “*G-Protein Coupled Receptors in Health and Disease*”, (6 hours, 30 students).
- Teaching in the Athens “*International Master's Programme in Neurosciences*” National Kapodistrian University, Department of Biology, entitled “*Signaling of Neurotransmitter Receptors*” and “*Neuropeptides*” (18 hrs, 18 students).
- Teaching in the Bilateral Masters Course of the IBA, NCSR “D” in collaboration with the faculty of Chemistry, University of Patras, on “*Applied Biochemistry, Medicinal Chemistry, Biotechnology, Pharmaceutical products*”, entitled: “*Cellular Signaling of Transmembrane Receptors-Molecular Pharmacology*” (4 hours, 8 students).
- IBA-Summer Scientific Laboratories for High School students on “*Protein Expression Methodologies in Different Cell Systems*” (hands on, group of 3 students).
- Antonis Myridakis defended his Diploma Thesis in the Faculty of Biology of the Kapodistrian University of Athens entitled “*Opioid receptors: Novel Signaling Pathways*” (Scientific Advisor: Z. Georgoussi).

Invitations

Z. Georgoussi: Invited speaker of the Hellenic Society of Algology, talk entitled “Cellular Signaling of Opioid receptors: Targeting novel opioids”, Athens 24 October 2020

Other Scientific Activities

Z. Georgoussi:

Other Scientific and Administrative activities

- National Representative, Intergovernmental Framework for European Cooperation in Science and Technology, COST-action- CA18133 entitled “*European Research Network on Signal Transduction (ERNEST)*”.
- Substitute National Representative, Intergovernmental Framework for European Cooperation in Science and Technology, EU–COST Action CA18240 entitled “*ADHEsion GPCR Network: Research and Implementation Set the path for future Exploration*” (*Adher N' Rise*).
- Member of the Administrative and Educational board of the «Athens International Master's Programme in Neurosciences».
- Member of the evaluation committee for the MSc Students of the «Athens International Master's Programme in Neurosciences».
- Member of the Research Consortium “*Normolife Network*” responsible for the identification of novel opioid compounds to alleviate pain.
- Member of the evaluation Committee for the recruitment of the Associate Professorship position, Department of Biochemistry, University of Thessaly.

Reviewing of funding and evaluation applications

- National Research Development and Innovation Office (NKFIH) of Hungary
- Cyprus Research Promotion Foundation (RPF)
- Operational Programme Competitiveness, Entrepreneurship and Innovation (EPAnEK)
- Hellenic General Secretary for Research and Technology (GSRT)
- State Scholarship Foundation (IKY)
- The Hellenic Foundation for Research and Innovation (H.F.R.I.)

Reviewer in scientific Journals

- Molecular Pharmacology, Journal of Neurochemistry, Journal of Pharmacology and Experimental Therapeutics, Cellular Signaling, Neuropharmacology, Neuropharmacology Journal of Neuroscience, BioMed Cell Biology, BioMed Research International, Journal of Biological Chemistry, Letters in Drug Design & Discovery, Current Drug Discovery Technologies,

CNS Neuroscience & Therapeutics, Neurochemistry, Nature Neuroscience, Frontiers in Neurosciences

Other Activities in the Institute of Biosciences & Applications and NCSR “Demokritos”

- Elected Member of the Scientific Council of the Institute of Biosciences and Applications (IBA), (07/2018-07/2020)
- Member of the working group of the National Roadmap Research Infrastructure “OPENSREEN-GR: *An Open – Access Research Infrastructure of Target-Based Screening Technologies and Chemical Biology for Human and Animal Health, Agriculture and the Environment*” coordinated by the IBA.
- Responsible scientist for the ultracentrifuges Beckman Coulter OPTIMA-MAX και L8-80M and the Speed Vac, Savant of the IBA, NCSR “D”

Impact factor (for 2 publications): 8.129

Citations for 2018 (without self-citations): 39

Citations for 2014-2018 (without self-citations): 181

h-factor: 19 (Scopus), 20 (Google Scholar)

Current external funding

Programme EU–COST Action CA18133 με τίτλο *European Research Network on Signal Transduction (ERNEST)* funded by the EU–COST Action with Dr. Z. Georgoussi as Member and National Representative of the Management Committee

Duration: 04/2019- 03/2023

Total Funding: 164000 €

Funding for 2020: 0

Programme EU–COST Action CA18240 entitled *“ADHEsion GPCR Network: Research and Implementation Set the path for future Exploration” (Adher N’ Rise)* as member and Substitute National Representative of the Management Committee

Duration: 11/2019- 11/2023

Total Funding: 120000 €

Funding for 2020: 0

Kostas Iatrou: Insect Physiology and Ethology and Applications

Research Interests

- Control of olfactory functions in the malaria mosquito vector *Anopheles gambiae*
- Oogenesis in lepidopteran insects as a model of terminal differentiation induced by ecdysteroids
- Genetically modified nuclear polyhedrosis viruses as tools for genetic transformation of insect and mammalian cells
- Production of recombinant proteins in insect cell cultures
- Development of cell-based assays for fast screening of natural and synthetic collections of small molecules and discovery of novel regulators of cellular functions
- Assessment of statistical ecological models for the impact of the climatic change on the biodiversity of sea phytoplanktonic systems based on analysis of data of the MedOBIS database generated by L. Ignatiades concerning the Eastern Aegean Sea (L. Ignatiades)

2020 Findings

Insect odorant receptors (ORs) are heteromeric ion channels consisting of a common subunit, ORco, and one of multiple variable subunits, ORx, to which odorants acting as specific receptor agonists or antagonists bind. In cultured lepidopteran cells expressing only ORco, functional homomeric cation channels are formed that are activated by specific ORco agonists. We have recently shown that certain mosquito repellents block odor-specific responses of multiple *Anopheles gambiae* ORs and that the inhibition is caused by their binding ORco. In cell cultures expressing ORco alone, these repellents inhibit the function of the homomeric channel. Based on these findings, we have predicted that small natural or synthetic molecules that bind to the ORco subunit and block the *ex vivo* function of the homomeric channel, might also inhibit the function of multiple heteromeric odorant receptors and thus cause anosmia to mosquitoes.

The previous functional characterization of 100 natural volatile compounds using our insect cell-based system for expression of Orco of the mosquito malaria vector *A. gambiae* resulted in the identification of 15 hits acting as ORco antagonists acting as "anosmia"-causing agents for the "tiger" mosquito, *Aedes albopictus*, vector of multiple, highly infectious agents. The classification of the antagonists as orthosteric or allosteric, in terms of their capacity to bind to a known ORco agonist site, allowed the design of compound blends for induction of greater degrees of "anosmia" on the target mosquitoes with some of them displaying bioactivities comparable to that of the widely used repellent DEET.

This year, we attempted to determine specific characteristics of the bioactive compounds through the design of a pharmacophore that predicts the binding of orthosteric antagonists (and agonists) on a specific binding pocket of ORco as well as determine additional structural determinants of bioactive binders, orthosteric and allosteric (collaboration with Drs. Ada Thireou, Agricultural University of Athens and Thanos Papakyriakou, IBA). A mutagenesis project involving three specific amino acids of the orthosteric binding site has been undertaken and the analysis of the consequences of the mutations on the functionality of the homomeric ORco is in progress.

Finally, a considerable portion of our activities this year has been dedicated to the design and generation of a new series of vectors allowing *ex vivo* expression of N-terminally tagged ORco channels in insect cells. The new vectors should allow the stable synthesis, isolation and purification of large quantities of native ORco channels by tag affinity chromatography and tag release following treatment with a specific protease. The effort to confirm the functionality of the new ORco expression systems is in progress with the aim of using them as tools for determining the atomic structure of ORco in its apo-form and as a complex with its antagonists by X-ray crystallography or cryo-electron microscopy (collaboration with Dr. Spyros Zographos, National Hellenic Research Foundation, NHRF).

Publications

Kythreoti, G, Sdralia, N, Tsitoura, P, Papachristos, P, Michaelakis, AP, Karras, V, Ruel, DM, Yakir, E, Bohbot, JD, Schulz, S and Iatrou, K (2020). Volatile allosteric antagonists of mosquito odorant receptors inhibit human-host attraction. *J Biol Chem* 2020 Dec 9; jbc.RA120.016557. doi: 10.1074/jbc.RA120.016557. Online ahead of print. (IF 4.238)

Ignatiades, L (2020). Taxonomic diversity, size-functional diversity, and species dominance interrelations in phytoplankton communities: a critical analysis of data interpretation. *Mar. Biodivers.* 50, 58 (2020). <https://doi.org/10.1007/s12526-020-01086-4>. (IF 1.487)

Other scientific activities

Evaluation Committees

Evaluation of research grant application for the panel "CE20 - Biologie des animaux, des organismes photosynthétiques et des microorganismes" for the National Research Foundation of France (ANR).

Evaluation of members of the Department of Biology and Human Biology, University of Haifa, Israel for promotion to the rank of Associate Professor.

Participation in editorial boards of scientific journals

Member, Editorial Boards for "Sericologia", "Insect Biochemistry and Molecular Biology", "Archives of Insect Biochemistry and Physiology", "BioMed Research International" and "Insects".
Section editor, "Mediterranean Marine Science Journal" (L. Ignatiades)

Reviews of scientific manuscripts

Manuscript reviewer for the scientific journals "Insect Biochemistry and Molecular Biology", "BioMed Research International", "Current Research in Biotechnology", "Acta Tropica".

Other scientific activities not covered by previous questions

National Representative and Management Committee Member for EU-COST Action CA18133 entitled "European Research Network on Signal Transduction (ERNEST)"

Total Impact Factor for peer-reviewed publications in 2020

5.725 (Iatrou K + Ignatiades L)

Bibliographic citations in 2020 (without self-citations)

Iatrou K: 216 (Scopus)

Ignatiades L: 67 (Scopus)

Total bibliographic citations for 2016-2020 (without self-citations)

Iatrou K: 961 (Scopus)

Ignatiades L: 512 (Scopus)

h-factor:

Iatrou K: 36 (Scopus), 45 (Google scholar)

Ignatiades L: 21 (Scopus), 28 (Google Scholar)

External funding

Research program entitled με τίτλο *Identification of new insect olfactory and taste enhancers of natural or synthetic origin* (funded by Inscent, Inc., USA)

Program duration: 2017-2021

Total funding (*for the duration of the program*): 40.000€

Participating research groups: K. Iatrou, NCSR "D"

Principal Investigator: K. Iatrou

Funding of the NCSR "D" research group for the year 2020: 0€

Research program entitled *3D-ORco: The structure of ORco, the odorant receptor co-receptor of mosquitoes* (funded by ELIDEK)

Program duration: 2020-2023

Total funding (*for the duration of the program*): 180.000€

Participating research groups: S. Zographos, NHRF, K. Iatrou, NCSR "D"

Principal Investigator: S. Zographos

Funding of the NCSR "D" research group for the year 2020: 18.000€ (from HNRF Special Accounts)

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

Research Staff

Nikos Grammatikakis, Senior Researcher

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)

Research Group: Mechanisms of Cell Proliferation and Ageing

Research Staff

Dimitris Kletsas, Research Director

Harris Pratsinis, Senior Researcher

Eleni Mavrogatou, Researcher

Adamantia Papadopoulou, Postdoctoral Fellow

Christophoros Christophoridis, Postdoctoral Fellow

Anastasios Kouroumalis, Graduate Student – *PhD obtained in 2020*

Asimina Fotopoulou, Graduate Student

Efstathios Tsimelis, Graduate Student

Eleni Liakou, Graduate Student

Maria Angelopoulou, Graduate Student

Maria Adamopoulou, Graduate Student

Christina Ntogka, Collaborating Graduate Student (MSc) – *MSc obtained in 2020*

Charalambia Almpani, Collaborating Graduate Student (MSc) – *MSc obtained in 2020*

Marina-Gemma Kelemeni, Collaborating Graduate Student (MSc)

Anastasia Kypraiou, Collaborating Graduate Student (MSc)

Eleni Kaplani, Collaborating Graduate Student (MSc)

Maria Dimozi, Collaborating Graduate Student (MSc)

Vania-Nicole Byrne, Collaborating Undergraduate Student (BSc) – *BSc obtained in 2020*

Research Interests

The Laboratory is focusing on the investigation of the mechanisms of ageing and longevity. Cellular senescence - as a result of successive duplications *in vitro* - and premature senescence - as a response of the cells to exogenous stresses - are investigated. The structural and functional characteristics of the senescent cell are studied. Especially, we are interested in 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 senescent stromal fibroblasts and adjacent cancer cells. Emphasis is given on tissues, such as the intervertebral disc, the degeneration of which provokes severe dysfunctions during ageing.

In addition, tissue repair during development and ageing is studied, with an emphasis on the role of growth factors, and especially that of TGF- β . The mechanisms regulating cell proliferation and extracellular matrix production, as well as the responsible intracellular signaling pathways are investigated. In parallel, alternative regulatory mechanisms of cell proliferation and differentiation, such as autocrine regulation, cell-matrix interactions, cell responses to exogenous stresses (e.g. ionizing and UV radiation) or the effect of mechanical forces are studied.

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

2020 Findings

Main goal of the Laboratory is the study of the mechanisms implicated in cellular senescence, of the senescent phenotype and of the role of senescent cells in tissue homeostasis and in the manifestation of age-related diseases. We showed that decorin, a proteoglycan which when overexpressed in the breast stroma inhibits the development of adjacent tumors, is down-regulated in breast stromal fibroblasts rendered prematurely senescent after exposure to ionizing radiation. The phenomenon is mainly attributed to the action of autocrine growth factors (e.g. of FGF and

VEGF) and to the impairment of autophagy. In addition, secreted factors from various breast cancer cells of different molecular profile further down-regulate decorin expression, suggesting a co-operative action of cancer and senescent cells in the promotion of tumorigenesis [Figure 1A]. Decorin down-regulation in senescent breast stromal fibroblasts fills in their tumor promoting phenotype based on to the creation of a favorable microenvironment and reinforces the hypothesis that senescent cells - *via* their secretome - may lead to the remodeling of tissue architecture, contributing to the manifestation of age-related diseases [Figure 1B].

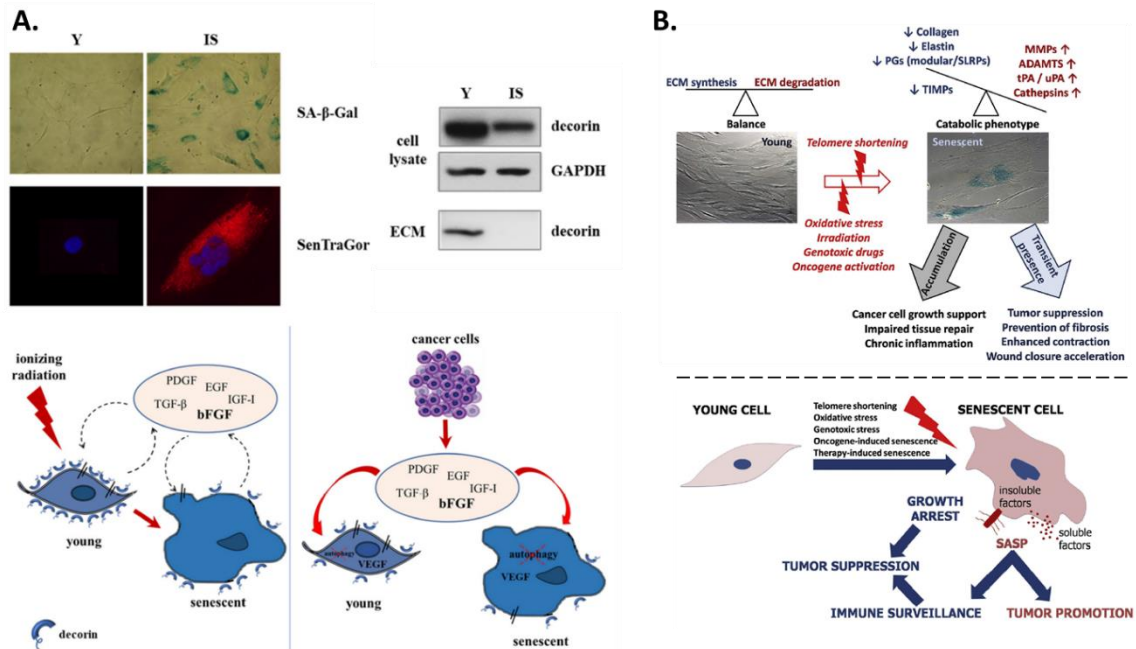


Figure 1: A. Ionizing radiation-induced prematurely senescent breast stromal fibroblasts are characterized by decreased expression and deposition to the extracellular matrix of the proteoglycan decorin, phenomenon mainly attributed to the action of autocrine growth factors (e.g. of FGF and VEGF) and to the impairment of autophagy and is reinforced by the interaction with breast cancer cells [Y: young cells, IS: ionizing radiation-induced senescent cells, Mavrogonatou et al., *Cancers (Basel)*. 2021 Apr 20;13(8):1987]. B. Senescent cells' accumulation may - due to their secretome - lead to tissue architecture remodeling, contributing to the promotion of age-related pathologies, including cancer [Mavrogonatou et al, *Matrix Biol.* 2019 75-76:27-42; Mavrogonatou et al., *Semin Cancer Biol.* 2020 62:182-191].

In vitro cultures of senescent cells are characterized by significant morphological alterations, including their bigger size and enlargement. On the other hand, in previous studies of our Laboratory, we showed that senescent fibroblasts retain their spindle-like shape in the tissue *in vivo*. After restoration of senescent fibroblasts' typical fibroblastic morphology by culturing them on special photolithographically micropatterned substrates, we demonstrated that cells preserve their functional traits. Our findings support the use of classical culture conditions to study the mechanisms of senescence.

In parallel, research regarding the effect of UVB radiation on the viability and premature senescence of dermal fibroblasts is carried on.

Low back pain constitutes one of the most important chronic age-related diseases, in the manifestation of which intervertebral disc degeneration is a critical parameter. Given the absence of vascularization in the intervertebral disc, we attempted to elucidate whether improvement of the tissue physiology could be achieved using pharmacological approaches. In this direction, we administered zoledronic acid in rabbits and assessed the kinetics of its accumulation in the disc by LC/MS techniques. Our findings pinpointed to its transient accumulation in the periphery of the disc

(in the annulus fibrosus) and its absence from the center (the nucleus pulposus). The estimated concentrations do not seem to affect disc cells' physiology.

Our Laboratory has shown in several previous studies that mechanical forces lead periodontal ligament fibroblasts to osteoblastic differentiation. We demonstrated that TNF- α , which is overexpressed under conditions of inflammation, decreases the ability of cells to differentiate through an oxidative stress- and premature senescence-independent mechanism.

Finally, we continued our research on the investigation of natural products and novel synthetic compounds with anti-cancer, anti-oxidant and cosmetic applications.

Publications

Mavrogonatou E, Pratsinis H, Kletsas D. (2020). The role of senescence in cancer development. *Semin Cancer Biol.* 62, 182-191. (IF: 15.707)

Kanioura A, Petrou P, Kletsas D, Tserepi A, Chatzichristidi M, Gogolides E, Kakabakos S. (2020). Three-dimensional (3D) hierarchical oxygen plasma micro/nanostructured polymeric substrates for selective enrichment of cancer cells from mixtures with normal ones. *Colloids Surf B Biointerfaces.* 187, 110675. (IF: 5.268)

Papadopoulou A, Kanioura A, Petrou PS, Argitis P, Kakabakos SE, Kletsas D. (2020). Reacquisition of a spindle cell shape does not lead to the restoration of a youthful state in senescent human skin fibroblasts. *Biogerontology.* 21, 695-708. (IF: 4.207)

Zilelidou EA, Milina V, Paramithiotis S, Zoumpopoulou G, Poimenidou SV, Mavrogonatou E, Kletsas D, Papadimitriou K, Tsakalidou E, Skandamis PN. (2020). Differential modulation of *Listeria monocytogenes* fitness, *in vitro* virulence, and transcription of virulence-associated genes in response to the presence of different microorganisms. *Appl Environ Microbiol.* 86, e01165-20. (IF: 4.792)

Feretis T, Katselis C, Papanikolaou IG, Apostolou K, Tsikalakis S, Toutouzas KG, Theodoropoulos G, Trigka EA, Saetta AA, Alexakis N, Konstandoulakis M, Tsarea K, Karamperi M, Kletsas D, Patsouris E, Manouras A, Zografos GC, Papalois A. (2020). ATSC transplantation contributes to liver regeneration following paracetamol-induced acute liver injury through differentiation into hepatic-like cells. *Am J Stem Cells.* 9, 36-56. (IF: 3.333)

Papadopoulou A, Cantele A, Koletsi D, Eliades T, Kletsas D. (2020). Short- and long-term treatment with TNF- α inhibits the induction of osteoblastic differentiation in cyclic tensile-stretched periodontal ligament fibroblasts. *Eur J Orthod.* 42, 396-406. (IF: 3.075)

Manou D, Bouris P, Kletsas D, Götte M, Greve B, Moustakas A, Karamanos NK, Theocharis AD. (2020). Serglycin activates pro-tumorigenic signaling and controls glioblastoma cell stemness, differentiation and invasive potential. *Matrix Biol Plus.* 6-7, 100033. (IF: -)

Kanioura, A., Constantoudis, V., Petrou, P., Kletsas, D., Tserepi, A., Gogolides, E., Chatzichristidi, M., Kakabakos, S. (2020). Oxygen plasma micro-nanostructured PMMA plates and microfluidics for increased adhesion and proliferation of cancer versus normal cells: The role of surface roughness and disorder. *Micro and Nano Engineering.* 8, 100060. (IF: -)

Zoumpopoulou, G., Papadimitriou, K., Alexandraki, V., Mavrogonatou, E., Alexopoulou, K., Anastasiou, R., Georgalaki, M., Kletsas, D., Tsakalidou, E., Giaouris, E. (2020). The microbiota of Kalathaki and Melichloro Greek artisanal cheeses comprises functional lactic acid bacteria. *LWT Food Science and Technology.* 130, 109570. (IF: 4.006)

Panagiotaki KN, Spyrou K, Zachariadis M, Pratsinis H, Kouloumpis A, Boutsika LG, Enotiadis A, Gournis D, Giannelis EP, Sideratou Z. (2020). Non-porous phosphonated ionic silica nanospheres as nanocarriers for efficient intracellular delivery of doxorubicin. *Materials Today Communications.* 23, 100787. (IF: 2.678)

Konstantinou, D., Mavrogonatou, E., Zervou, S.K., Giannogonas, P., Gkelis, S. (2020). Bioprospecting sponge-associated marine cyanobacteria to produce bioactive compounds. *Toxins (Basel)*. 12, 73. (IF: 4.546)

Articles in Press

Papastathopoulos A, Lougiakis N, Kostakis IK, Marakos P, Pouli N, Pratsinis H, Kletsas D. (2021). New bioactive 5-arylcarboximidamidopyrazolo[3,4-c]pyridines: Synthesis, cytotoxic activity, mechanistic investigation and structure-activity relationships. *Eur J Med Chem*. 218, 113387. (IF: 5.572)

Mavrogonatou E, Papadopoulou A, Fotopoulou A, Tsimelis S, Bassiony H, Yiacooumettis AM, Panagiotou PN, Pratsinis H, Kletsas D. (2021). Down-regulation of the proteoglycan decorin fills in the tumor-promoting phenotype of ionizing radiation-induced senescent human breast stromal fibroblasts. *Cancers (Basel)*. 13, 1987. (IF: 6.126)

Mavrogonatou E, Kouroumalis A, Papadopoulou A, Pratsinis H, Kletsas D. (2021). Cell-based therapies for the regeneration of the intervertebral disc: promises and challenges. *Acta Orthopaedica Et Traumatologica Hellenica*. 72, 21-29. (IF: -).

Veroutis D, Kouroumalis A, Lagopati N, Polyzou A, Chamilos C, Papadodima S, Evangelou K, Gorgoulis VG, Kletsas D. (2021). Evaluation of senescent cells in intervertebral discs by lipofuscin staining. *Mech Ageing Dev*. 199, 111564. (IF: 5.432)

Sklirou AD, Angelopoulou MT, Argyropoulou A, Chaita E, Boka VI, Cheimonidi C, Niforou K, Mavrogonatou E, Pratsinis H, Kalpoutzakis E, Aligiannis N, Kletsas D, Trougakos IP, Skaltsounis AL. (2021). Phytochemical study and in Vitro screening focusing on the anti-aging features of various plants of the Greek flora. *Antioxidants (Basel)*. 10, 1206. (IF: 6.312)

Other Scientific Activities

Participation in Greek and international scientific bodies and organizations

President of the Administrative Board of the Hellenic Society for Biochemistry and Molecular Biology (D. Kletsas)

Member (Secretary General) of the Research Club for Connective Tissue and Matrix Biology of the Hellenic Society for Biochemistry and Molecular Biology (D. Kletsas)

Member of the FEBS Fellowships Committee (D. Kletsas)

Member of the Sectorial Scientific Council for Bio-Medical Sciences of the National Council for Research and Innovation (D. Kletsas)

National Representative in the Committee “Health, demographic change and wellbeing” of Horizon 2020 (D. Kletsas)

Substitute Member of the National Committee on the welfare of animals used for scientific purposes (D. Kletsas)

Member of the Regional Research and Innovation Council of Attica Region (ΠΣΕΚ-Α) (D. Kletsas)

Member of the Ethics Committee of BSRC “Alexander Fleming” (D. Kletsas)

Member of the Committee for the selection of Director of Biomedical Research of BSRC “Alexander Fleming” (D. Kletsas)

Member of the Evaluation Committee for GRAMMER European Spine Journal Award (D. Kletsas)

Participation in editorial boards of scientific journals

Editorial board member of the Journals “Ageing Research Reviews”, “Biogerontology”, “Mechanisms of Ageing and Development”, “Experimental Gerontology”, “European Spine Journal”, “PLOS ONE” και “Journal of Orthopaedic Research Spine”, “Antioxidants” (D. Kletsas)

Editorial board member of the Journal “Cosmetics” (H. Pratsinis)

Participation in committees for the reviewing of research proposals

Ligue Suisse contre le cancer (D. Kletsas)

Reviewing of manuscripts in scientific journals

European Spine Journal (4), Tissue engineering, Frontiers in Oncology, Rejuvenation Research, Mechanisms of Ageing and Development (5), Biogerontology (4), Oxodative Medicine and Cellular Longevity, Matrix Biology, Wound Repair and Regeneration (2), Experimental Gerontology (2), Journal of Investigative Dermatology (2), Oncogene, Matrix Biology Plus (5), Ageing Research Reviews, FEBS Journal, Annals of the New York Academy of Sciences, Theranostics, Cells, Scientific Reports, Cell & Tissue Research, Journal of Biological Research, Aging Cell, BBA - Molecular Basis of Disease, Plos One, EMBO Journal (D. Kletsas)

Arabian Journal of Chemistry (2), Bioorganic and Medicinal Chemistry Letters, Cosmetics (3), Cytotechnology, Drug Design Development and Therapy, Genes & Diseases, International Journal of Molecular Sciences (2), Marine Drugs, Molecules (2), Nanomaterials, PLoS ONE, Research Square (H. Pratsinis)

Aging, PLoS ONE, International Journal of Environmental Research and Public Health, International Journal of Molecular Sciences (2), Biomedicine & Pharmacotherapy (9), American Journal of Orthodontics & Dentofacial Orthopedics (9), Molecules (2), Cancers (5), Biomolecules, Pharmaceuticals, Life, Frontiers in Microbiology, Clinical Interventions in Aging, BioMed Research International, Electronic Journal of Biotechnology (E. Mavrogonatou)

Educational Activities

Member of the special interinstitutional committee and lecturer in the Joint Post-Graduate Programme (JPGP) in “Applied Biochemistry: Clinical Chemistry, Biotechnology, Evaluation of Pharmaceutical Products” of the Department of Chemistry of the University of Patras and of the Institute of Biosciences and Applications of NCSR “Demokritos” (D. Kletsas)

Responsible for the Organization of the Lab Seminar for the training of Secondary Education Biology Teachers in collaboration with the European Learning Laboratory for the Life Sciences (ELLS) of the European Molecular Biology Laboratory (EMBL) (11-13/9/2020) (D. Kletsas)

Supervision of the PhD theses of Asimina Fotopoulou, Efstathios Tsimelis, Eleni Liakou, Maria Angelopoulou and Maria Adamopoulou (D. Kletsas)

Supervision of the theses for the acquisition of a Master’s degree of Christina Ntogka, Marina-Gemma Kelemen, Eleni Kaplani, Anastasis Kypraiou and Maria Dimozi (D. Kletsas)

Supervision of the thesis for the acquisition of a Master’s degree of Charalampia Almpani (H. Pratsinis)

Supervision of the thesis for the acquisition of a Bachelor’s degree of Vania-Nicole Byrne (E. Mavrogonatou)

“Cell senescence and tissue homeostasis”, NCSR “Demokritos” Summer School, 1 hour, 100 students (D. Kletsas)

“*In vitro* studies of natural and synthetic bioactive products”, NCSR “Demokritos” Summer School, 1 hour, 100 students (H. Pratsinis)

“Cell senescence and carcinogenesis”, Lecture in the framework of the class entitled “Thoracic Oncology”, Medical School of the University of Athens, 1 hour, 50 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 tissue homeostasis”, Post-graduate Master’s Degree in Cosmetology, Department of Pharmacy of the University of Athens, 10 students (D. Kletsas)

“Cell senescence: Molecular mechanisms and role in tissue homeostasis”, Harokopio University, 2 hours, 15 students (D. Kletsas)

Post-graduate Master’s Degree “Applied Biomechanics and Biomaterials in Orthopaedics”, 1 hour, 20 students (D. Kletsas)

“Cell proliferation and tissue homeostasis. Growth factors: Structure, receptors and signal transduction. Cell senescence and tissue homeostasis. Methodologies for the study of cell proliferation”, 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)

“Cell systems in the research of carcinogenesis”, Post-graduate Master’s Degree “Neoplastic Disease in Humans: Diagnosis, Modern Treatment and Research”, Medical School of the University of Athens, 2 hours, 20 students (H. Pratsinis)

Participation in the Inter-Institutional Joint Post-Graduate Programme (JPGP) in “Applied Biochemistry: Clinical Chemistry, Biotechnology, Evaluation of Pharmaceutical Products” between the Institute of Biosciences and Applications and the University of Patras, 6 hours, 8 students (D. Kletsas, H. Pratsinis and E. Mavrogonatou)

Participation in the Lab Seminar for the training of Secondary Education Biology Teachers in collaboration with the European Learning Laboratory for the Life Sciences (ELLS) of the European Molecular Biology Laboratory (EMBL) (11-13/9/2020) (D. Kletsas, H. Pratsinis and E. Mavrogonatou)

Member of examination committees for PhD, MSc and BSc theses

Anastasios Kouroumalis concluded his PhD thesis entitled “Study of the effect of the extracellular environment on the gene expression and function of intervertebral disc cells”. The thesis was unanimously accepted and awarded the degree “Excellent” (Scientific Supervisor D. Kletsas)

Christina Ntogka concluded her MSc thesis entitled “Study of the senescence of intervertebral disc cells” in the framework of the Master’s Degree Programme of the Biology Department of the University of Athens “Applications of Biology in Medicine”. The thesis was unanimously accepted and awarded the degree “Excellent” (Scientific Supervisor D. Kletsas)

Charalampia Almpiani concluded her MSc thesis entitled “Study of the action of thymosin peptides on human dermal fibroblasts for the regulation of cellular functions related to healing” in the framework of the Master’s Degree Programme of the Pharmacy Department of the University of Athens “Cosmetology-Dermatopharmacology”. The thesis was unanimously accepted and awarded the degree “Excellent” (Scientific Supervisor H. Pratsinis)

Vania-Nicole Byrne concluded her BSc thesis entitled “Calcium homeostasis in intervertebral disc cells” for the acquisition of a Bachelor’s degree in the Biology Department of the University of Athens. The thesis was unanimously accepted and awarded the degree “Excellent” (Scientific Supervisor E. Mavrogonatou)

Other Activities in IBA and in NCSR “Demokritos”

D. Kletsas:

Director of IBA & Member of the Administrative Board of NCSR “Demokritos”

Scientific and Administrative Responsible of the Experimental Animal Facility

Scientific and Administrative Responsible of the Light Microscopy Unit

Responsible for the function of the Fluorescence Activated Cell Sorting Facility

H. Pratsinis:

President of the Scientific Board of IBA

Responsible for the conducted tours in the Institute of Biosciences and Applications of NCSR “Demokritos”

E. Mavrogonatou:

Member of the Committee for the receipt of deliverables for the project E-12388

Member of the Committee for the Evaluation of offers for the supply of laboratory consumables of the project EE12421

Substitute member of the Committee for the selection of external collaborators for the implementation of the project E-12315

Impact Factors

D. Kletsas (for 7 out of 9 publications): 40.388

H. Pratsinis (for 2 publications): 18.385

E. Mavrogonatou (for 4 publications): 29.051

Citations 2020 (without self-citations)

D. Kletsas: 751

H. Pratsinis: 249

E. Mavrogonatou: 172

Total Citations 2015-2020 (without self-citations)

D. Kletsas: 3932

H. Pratsinis: 1110

E. Mavrogonatou: 531

h-factor

D. Kletsas: 46 (Scopus), 52 (Google Scholar)

H. Pratsinis: 31 (Scopus), 34 (Google Scholar)

E. Mavrogonatou: 18 (Scopus), 20 (Google Scholar)

Current External Funding

Project entitled “Study of the accumulation of anti-cancer compounds in intervertebral disc tissues and their effect on cellular aging (MIS 5047829)”, co-financed by Greece and the European Union (European Social Fund-ESF) through the Operational Programme “Human Resources Development, Education and Lifelong Learning 2014-2020”

Scientific Supervisor: Dr. D. Kletsas

Duration: 2020-2021

Total programme funding (for the entire duration of the programme): 41.541,50 €

Funding of the lab for 2020: 20.000 €

Project entitled “Development of innovative cosmeceuticals based on the greek flora (CosmAGE)” (T2EAK-02583, MIS 5070022), co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE

Scientific Supervisor: Dr. D. Kletsas

Duration: 2020-2023

Total programme funding (for the entire duration of the programme): 229.999,99 €

Funding of the lab for 2020: 92.000 €

Current External IBA Funding

Project entitled “SANITURA (TARGET IDENTIFICATION AND DEVELOPMENT OF NOVEL APPROACHES FOR HEALTH AND ENVIRONMENTAL APPLICATIONS)”, funded by the General Secretariat for

Research and Innovation (Action for the Strategic Development on the Research and Technological Sectors, Operational Programme “Competitiveness, Entrepreneurship and Innovation”, NSRF 2014-2020)

Scientific Supervisor: Dr. D. Kletsas

Duration: 2017-2020

Total programme funding (for the entire duration of the programme): 740.000,00 €

Funding for 2020: 222.000,00 €

Project entitled “OPENSREEN-GR: A Greek Research Infrastructure for visualizing and monitoring fundamental biological processes, funded by the General Secretariat for Research and Innovation (Action “Reinforcement of the Research and Innovation Infrastructure”, Operational Programme “Competitiveness, Entrepreneurship and Innovation”, NSRF 2014-2020)

Scientific Supervisor: Dr. D. Kletsas

Duration: 2017-2020

Total programme funding (for the entire duration of the programme): 899.600,00 €

Funding for 2020: 537.000,00 €

Project entitled “BIOIMAGING-GR: A Greek Research Infrastructure for Visualizing and Monitoring Fundamental Biological Processes”, funded by the General Secretariat for Research and Innovation (Action “Reinforcement of the Research and Innovation Infrastructure”, Operational Programme “Competitiveness, Entrepreneurship and Innovation”, NSRF 2014-2020)

Scientific Supervisor in IBA: Dr. D. Kletsas

Duration: 2017-2020

Total programme funding (for the entire duration of the programme): 211.250,00 €

Funding for 2020: 60.000,00 €.

Project entitled “Ensuring long-term sustainability of excellence in chemical biology within Europe and beyond (EU-OPENSREEN-DRIVE)”, funded by the European Union (Action “Reinforcement of the Research and Innovation Infrastructure”, Operational Programme “Competitiveness, Entrepreneurship and Innovation”, NSRF 2014-2020)

Scientific Supervisor in IBA: Dr. D. Kletsas

Duration: 2019-2023

Total programme funding (for the entire duration of the programme): 16.875,00 €

Funding for 2020: -

Project entitled “The Routes of Bee”, funded by the General Secretariat for Research and Innovation (Flagship national initiative for creating research networks in the value chains of "Olive", "Vineyard", "Honey" and "Livestock", NSRF 2014-2020)

Scientific Supervisor in IBA: Dr. D. Kletsas

Duration: 2017-2020

Total programme funding (for the entire duration of the programme): 39.200,00 €

Funding for 2020: -

Project entitled “Laboratory for Doping Control”, funded by the Ministry of Culture and Sports

Scientific Supervisor: Dr. D. Kletsas

Duration: 2020-2023

Total programme funding (for the entire duration of the programme): 1.200.000,00 €

Funding for 2020: 480.000,00 €

Research Group: Nuclear Proteins and Chromatin Function

Research Staff

Thomae Sourlingas, Senior Researcher

Kalliope Sekeri, Research Collaborator, Retired A' Grade

Anastasia-Georgia Dedemadi, Collaborating Graduate Student (MSc)

Research Interests

Basic factors that affect chromatin remodeling and thus gene expression and cellular function are the histone subtype constitution of nucleosomes and histone post translational epigenetic modifications. Changes in the subtype constitution and in histone modifications can activate or inactivate genes that are involved in biological processes and pathological states. Based on the aforementioned, the lab's present research interests are focused on:

1. Cancer: We are studying changes in the histone subtype constitution and in histone post translational modifications in age-related cancers, as well as in other cancer cell types. Observed differences in histone subtype levels or differences in epigenetic modifications of these cancer cell types with respect to normal controls may possibly be used as biomarkers for the specific cancer cell type or as targets for future intervention. Within this framework, the efficiency of histone deacetylase inhibitors (HDACIs) as proapoptotic anticancer agents will also be studied.
2. Aging/Senescence: We are studying the relationship amongst the expression profiles of the histone subtypes and their epigenetic modifications during aging and the role that histone epigenetic modifications play in gene-expression changes of age-related genes.
3. Psychotic Disorders: We are investigating how changes in the chromatin constitution and the expression profiles of the histone H1 subtypes and histone post translational epigenetic modifications are associated with observed chromatin remodeling events (conformational changes) in chromatin of human peripheral blood leucocytes from individuals with psychiatric disorders.
4. Chromatin and the Mammalian Biological Clock: We are investigating the role of chromatin conformational changes that are brought about by changes in the levels of histones and their post translational epigenetic modifications in the regulation of the mammalian biological clock in cell cultures (in collaboration with Dr. Prombona, head of the Chronobiology Lab).

2020 Findings

Cancer and Histones

Histone deacetylase inhibitors (HDACIs) are used as tools for the investigation of epigenetic regulation of chromatin and they can be used alone, or in combination with other anticancer agents, in therapeutic regimens. For these reasons, we studied three histone H3 epigenetic modifications (acetylation, trimethylation and dimethylation) and three histone H1 subtypes (H1.0, H1.3 and H1.5) in the absence and presence of the HDACI, sodium butyrate, in three leukemic cell lines (K562, NB4, Molt4) as compared to normal lymphocytes. Of importance are our findings showing that in the presence of sodium butyrate histone H3 acetylation and trimethylation, but not H3 dimethylation, increase significantly. Of great interest was the finding that while the levels of the H1.0 subtype increase in the presence of sodium butyrate, for the first time we see another effect, which has not been previously reported. We observe a statistically significant decrease in H1.3. The levels of the H1.5 subtype, associated with closed heterochromatin, do not change. H1.0 has been well-studied, but not H1.3. Thus these results are of special significance. It is also

important that these 2 subtypes (H1.0 and H1.3) that were found to be affected by this inhibitor are related to chromatin regulatory functions, while H1.5, which was found not to be affected, is associated with closed inactive, heterochromatin. Histone H3 dimethylation which was also found to remain unaffected in the presence of this drug, is also associated with closed heterochromatin. It is noted that the increase of H1.0 with the simultaneous decrease of H1.3 in the presence of butyrate is observed only in the leukemic cell lines. Sodium butyrate does not affect the protein levels of physiological lymphocytes. This was also associated with butyrate-induced mortality rates. In lymphocytes we do not observe an increase in mortality, in contrast to NB4 and Molt4 cells where we have a statistically significant decrease in viability in the presence of this HDAC inhibitor. These results suggest that these histone epigenetic factors may be used as biomarkers of the efficacy of HDACIs in therapeutic regimens.

Other scientific activities

Reviewer of scientific publications: Biochemistry and Cell Biology, Apoptosis, Leukemia Research, Life Sciences, Acta BB Sinica, J Pharmaceutical Sci Exp Pharmacology

Scientific collaborations:

- With Dr. A. Prombona (Laboratory of Chronobiology), Institute of Biosciences and Applications, 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 the potential consequences to cellular function and carcinogenesis.
- With the Neurobiology Research Institute of the Th. Th. Cozzika Foundation (Dr. Margarita Chrysanthou-Piterou). Within the framework of this program we are studying changes in the expression levels of histone genes in leucocytes of patients with bipolar disorder and schizophrenia.
- With the Professor I. Angelopoulos, University of Athens, Medical School, 1st Department of Psychiatry, Eginition Hospital. We are studying changes in the expression levels of histone genes in leucocytes of patients with bipolar disorder and schizophrenia.

Educational Activities

Within IBA

“DNA organization, histones and chromatin function” within the framework of the course “Molecular and Cell Biology – Molecular Biotechnology” of the Bi-Institutional Graduate Masters’ Program, “Applied Biochemistry, Clinical Chemistry, Biotechnology, Evaluation of Pharmaceutical Products”, Institute of Biosciences and Applications and University of Patras (2 hours, 8 students)

Outsine IBA

“Cell Cycle: Checkpoints and Consequences for Normal Cellular Function when Cell Cycle Progression Dysfunctions” within the framework of the course “Ageing and Age-Related Diseases” of the Graduate Masters’ Program: Applications of Biology in Medicine, Dept. of Biology and Medical School of the University of Athens (3 hours, 21 students)

Other activities in the Institute of Biosciences and Applications

Responsible for the organization of research seminars of the graduate students of IBA

Member of the IBA 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

Citations for 2020 (without self-citations): 17

Total citations for 2015-2019 (without self-citations): 149

h-factor:

Scopus: 11

Google Scholar: 13

Research Group: Cell & Matrix Biochemistry/Pathobiology

Research Staff

Angeliki Chroni, Research Director

Athina Tzinia, Senior Researcher

Paraskevi Kitsiou, Senior Researcher

Garyfalia Drossopoulou, Senior Researcher

Archontia Kaminari, Postdoctoral Researcher

Ioannis Dafnis, Postdoctoral Researcher

Christina Gkolfinopoulou, Postdoctoral Researcher

Maria Karanikou, Postdoctoral Researcher

Christina Mountaki, PhD student

Achillea Papagiannis, PhD student

Anastasia Georgia Dedemadi, PhD student

Asimina Micha, MSc student

Effie Valanti, Collaborating scientist

Eleni Theofanidi, Collaborating scientist

Vasiliki Syropoulou, Undergraduate student

Research Interests

1. *Study of molecular mechanisms of neurodegenerative conditions and diseases of the Central Nervous System*
 - A. Analysis of the structure-function relationship of apoE4, the major risk factor for Alzheimer's disease, and its role in the mechanisms of disease pathogenesis.
 - B. Study of cell protection and survival mechanisms in age-related diseases like Alzheimer's disease and type II Diabetes.
2. *Molecular mechanisms of dyslipidemias and atherosclerosis*
 - A. Elucidation of the mechanisms underlying disorders of high density lipoprotein (HDL) metabolism that result in changes of HDL atheroprotective properties. Specific (dys)functions and compositional changes of HDL could be targeted as biomarkers for the assessment of cardiovascular risk and/or effects exerted by HDL modifying therapies.
 - B. Analysis of structure-function relationship of apoA-I and apoE and their role in atherosclerosis.
3. *Diabetes Mellitus and Diabetic Kidney Disease*
 - A. Study of pancreatic β -cell survival mechanisms in diabetic conditions: i) Cross talk between nephrin and survival signaling pathways in pancreatic insulin producing beta cells ii) Effect of liraglutide (a human GLP-1 analogue) on nephrin signaling and islet β -cell survival in db/db lepr^{-/-} type 2 diabetic mice
 - B. Kidney function: Regulation of gene expression and epigenetic mechanisms in glomerular podocytes.
 - i) Mode of liraglutide action in prevention of podocyte and β -cell apoptosis and on the process of regeneration of β -cells from mesenchymal stem cells (MSCs). Renoprotective role of VitaminD3 on glomerular kidney function, using animal models of Type2 Diabetes.
 - ii) Analysis of the insulin-mediated survival pathway in isolated rat glomeruli and animal models of Type2 Diabetes. Role in prevention and/ or treatment of diabetic nephropathy. Interactions with signaling pathways that regulate podocyte survival.

2020 Findings

1. Study of molecular mechanisms of neurodegenerative conditions and diseases of the Central Nervous System

Alzheimer's disease (AD) is the most common neurodegenerative disease affecting more than 40 million people worldwide without any effective therapy up to date. Existing therapeutic approaches focus primarily on A β peptides, while others explore different neuroprotective strategies such as the identification and use of natural products, and new synthetic biomolecules involved in signaling associated with the pathophysiology of neuronal degeneration in AD. Towards this direction, our research interests focused on the study of molecules such as phenoxazines and MARK4 Kinase regulators as therapeutic agents against AD. Specifically in the context of a collaboration with Dr. B. Charlie-(Aristotle University of Thessaloniki), 12 substances were tested for their toxicity in neuroblastoma cell lines (model of nerve cell study) by the MTT method and for their action in regulating the activity of key proteins such as AKT kinase and GSK3b in primary hippocampal neurons by western analysis. Four of the above compounds were selected for further characterization.

In addition, it was investigated whether the supplemental diet with raisins, that contains antioxidative phenolic and other components, can prevent the accumulation of A β and neuroinflammatory responses in the brain of mice models of Alzheimer's disease (5xFAD). Animals fed a diet supplemented with raisins for 3 months displayed reduced levels of A β and TNF α in the brain as compared with animals fed a normal diet or diet supplemented with glucose/fructose.

2. Study of the molecular mechanisms of dyslipidemia and atherosclerosis

We evaluated the effect of A164S mutation, the only apoA-I mutation associated with increased cardiovascular risk and mortality in general population despite the presence of normal HDL-cholesterol levels, on the properties of apoA-I and HDL. Our analyses showed that this apoA-I mutation leads to the formation of dysfunctional HDL particles with impaired endothelial protective capacity, that may underlie the increased cardiovascular risk and mortality observed in carriers of apoA-I[A164S] mutant (Gkolfinopoulou et al. Cell Mol Life Sci. doi: 10.1007/s00018-020-03583-y). Furthermore, we evaluated the effect of V19L mutation, the only apoA-I mutation associated with increased HDL-cholesterol levels and decreased CAD risk, on the conformational integrity and atheroprotective properties of apoA-I and HDL (Gkolfinopoulou et al. Biochim. Biophys. Acta Mol. Cell. Biol. Lipids, doi: 10.1016/j.bbailip.2019.158593).

3. Diabetes Mellitus and Diabetic Kidney Disease

A) In mouse pancreatic β -cells (β TC-6), nephrin signaling promotes cell survival by inhibiting apoptosis. Our results demonstrated that exposure of β TC6 cells to increased glucose concentration led to downregulation of nephrin signaling without affecting nephrin expression. Impairment of nephrin signaling was attributed to increased internalization of nephrin which was accompanied by increased PKC α expression. Taken together the above results suggest that PKC-dependent nephrin internalization may serve as an ignition spark for impaired beta-cell signaling in long-standing hyperglycemia.

B) Kidney function: Regulation of gene expression and epigenetic mechanisms in glomerular podocytes.

Mode of liraglutide action in prevention of podocyte and β -cell apoptosis and on the process of regeneration of β -cells from mesenchymal stem cells (MSCs). Renoprotective role of Vitamin D3 on glomerular kidney function, using animal models of Type 2 Diabetes.

We investigated the role of secreted condition media (CM) from human Amniotic Fluid Mesenchymal Stem/Stromal Cells (CM-AF-MSC) in isolated rat glomeruli cultured in the presence of normal (5mM) and diabetic (25mM) glucose levels. Expression levels of nephrin and podocalyxin were decreased in the presence of diabetic glucose levels. This effect was rescued by CM-AF-MSC treatment. Additionally, the PI3K-pAkt-mediated survival pathway was affected under diabetic

conditions. However, CM-AF-MSC treatment significantly decreased the high-glucose-mediated apoptosis presented in isolated glomeruli.

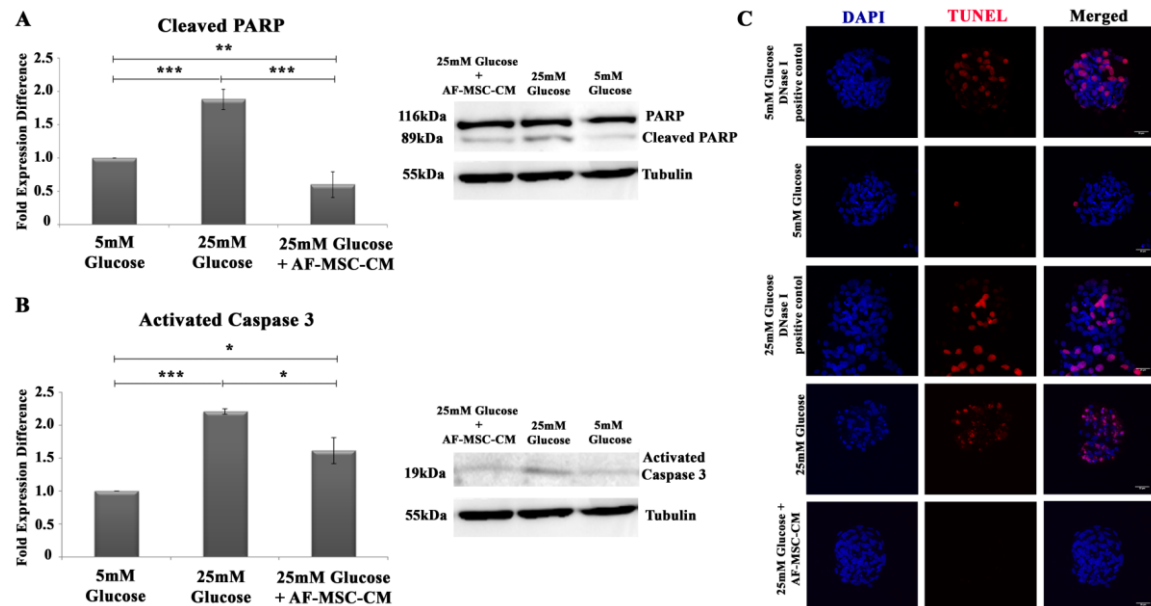


Figure: High-glucose mediated alterations of protein and mRNA levels of cleaved PARP and activated caspase proteins were restored to normal levels after CM-AF-MSC treatment.

Publications

Kavetsou E., Katopodi A., Argyri L., Chainoglou E., Pontiki E., Hadjipavlou-Litina D., Chroni A. and Detsi A. Novel 3-aryl-5-substituted-coumarin analogues: Synthesis and bioactivity profile. *Drug Dev. Res.* 81, 456-469 (2020). (IF 4.360)

Gkolfinopoulou C., Bourtsala A. and Chroni A. Structural and functional basis for increased HDL-cholesterol levels due to the naturally occurring V19L mutation in human apolipoprotein A-I. *Biochim. Biophys. Acta Mol. Cell. Biol. Lipids* 1865, 158593 (2020). (IF 4.698)

Chroni A., Rallidis L., Vassou D., Gkolfinopoulou C., Papakosta P., Zervou M.I., Goulielmos G.N., Kiouri E., Pappa D., Eliopoulos E. and Kardassis D. Identification and characterization of a rare variant in apolipoprotein A-IV, p.(V336M), and evaluation of HDL functionality in a Greek cohort with extreme HDL cholesterol levels. *Arch Biochem. Biophys.* 696:108655 (2020). (IF 4.013)

O. Trohatou, A. Mantsiou, M. Makridakis, A. Vlahou, A. Charonis, M. Roubelakis and Drossopoulou G. (2020). Secreted conditioned media from human amniotic fluid mesenchymal stem/stromal cells ameliorate podocyte injury in isolated diabetic rat glomeruli. *AJP-Renal Physiol.* doi: 10.1111 (2020) (IF 3.144)

Articles in Press

Gkolfinopoulou C., Soukou F., Dafnis I., Kellici T. F., Sanoudou D., Mavromoustakos T., Stratikos E. and Chroni A. Structure-function analysis of naturally occurring apolipoprotein A-I L144R, A164S and L178P mutants provides insight on their role on HDL levels and cardiovascular risk. *Cell Mol Life Sci.* doi: 10.1007/s00018-020-03583-y. (2021). (IF 9.261)

Lagopati, N., Kotsinas, A., Veroutis, D., Evangelou, K., Papaspyropoulos, A., Arfanis, M., Falaras, P., Kitsiou, P.V., Pateras, I., Bergonzini, A., Frisan, T., Kyriazis, S., Tsoukleris, D.S., Tsilibary, E.-P.C., Gazouli, M., Pavlatou, E.A., Gorgoulis, V.G. (2021). Biological Effect of Silver-modified Nanostructured Titanium Dioxide in Cancer. *Cancer genomics & proteomics* 18 (3), 425-439. (IF 3.280)

Book Chapters or Proceedings

Gkolfinopoulou C, Soukou F., Stratikos E. and Chroni A. Insights from structure-function analysis of naturally occurring apolipoprotein A-I mutants L144R, A164S and L178P on their role on HDL levels and cardiovascular risk. *Atherosclerosis*, 315: e64 (2020).

Presentations at Scientific Conferences

Gkolfinopoulou C, Soukou F., Stratikos E. and Chroni A. Insights from structure-function analysis of naturally occurring apolipoprotein A-I mutants L144R, A164S and L178P on their role on HDL levels and cardiovascular risk. 88th European Atherosclerosis Society Congress, 4-7 October 2020, virtual congress

Chroni A., Rallidis L., Vassou D., Gkolfinopoulou C., Papakosta P., Zervou M.I., Goulielmos G.N., Kiouri E, Pappa D., Eliopoulos E. and Kardassis D. Structural and functional characterization of a rare variant in apolipoprotein A-IV, p.(V336M), and evaluation of HDL functionality in a Greek cohort with extreme HDL-cholesterol levels. 9th Congress of the Hellenic Atherosclerosis Society, 3-5 December 2020

Gkolfinopoulou C., Soukou F., Dafnis I., Sanoudou D. and Chroni A. Understanding the association of the naturally occurring A164S mutation in apolipoprotein A-I with increased cardiovascular risk despite the presence of normal HDL-cholesterol levels. 9th Congress of the Hellenic Atherosclerosis Society, 3-5 December 2020

Other Scientific Activities

Participation in Greek and international scientific bodies and organizations

A. Chroni:

Vice president of the Coordinating Committee of the Working Group "Study of Pathophysiology of Atherosclerosis" of the Hellenic Atherosclerosis Society

Deputy member of the General Assembly of the Hellenic Foundation for Research and Innovation

G. Drossopoulou:

Member of the committee "Kidneys and Diabetes Melitus" of the Hellenic Society of Nephrology

Participation in research panels of research proposals

A. Chroni:

Evaluator of proposals submitted under the Call "Establishment of (potential) Business Teams of the European Innovation Partnership for productivity and sustainability of agriculture" (NSRF 2014-2020)

Review of Scientific publications

A. Chroni: *Bioscience Reports*, *Frontiers in Molecular Biosciences*, *Journal of Inflammation Research*, *Scientific Reports*, *International Journal of Molecular Sciences*, *Life Sciences*, *British Journal of Pharmacology*, *BBA-Molecular and Cell Biology of Lipids*, *Molecular Genetics and Genomic Medicine*, *ACS Chemical Neuroscience*, *Frontiers in Neurology*

A. Tzinia: *PLoS ONE*, *Cellular Physiology and Biochemistry*, *Neural Regeneration Research*

P. Kitsiou: *Current Diabetes Reviews*, *PLoS ONE*, *Recent Patents on Endocrine, Metabolic & Immune Drug Discovery*

G. Drossopoulou: *Current Diabetes Reviews*, *Journal of Nephrology*, *PLoS ONE*, *Nutrients*, *International Journal of Environmental Research and Public Health*, *American Journal of Nephrology*

Other scientific activities

A. Chroni: “HDL-cholesterol: Reappraisal of its clinical value”, Webinar series on “Mechanisms of atherosclerosis - Recent data” organized by the Working Group “Study of Pathophysiology of Atherosclerosis” of the Hellenic Atherosclerosis Society, 30 March 2020

Scientific distinctions and awards

2nd Award for Basic Research: Chroni A., Rallidis L., Vassou D., Gkolfinopoulou C., Papakosta P., Zervou M.I., Goulielmos G.N., Kiouri E, Pappa D., Eliopoulos E. and Kardassis D. Structural and functional characterization of a rare variant in apolipoprotein A-IV, p.(V336M), and evaluation of HDL functionality in a Greek cohort with extreme HDL-cholesterol levels. 9th Congress of the Hellenic Atherosclerosis Society, 3-5 December 2020

A. Chroni, research protocol award from the Hellenic Society of Lipidology, Atherosclerosis and Vascular Disease

Educational Activities

A. Chroni:

Member of the Inter-Departmental Committee in the joined MSc Programme “Applied Biochemistry: Clinical Chemistry, Biotechnology, Evaluation of Pharmaceutical Products” of the Department of Chemistry of the University of Patras and of the Institute of Biosciences and Applications of NCSR “Demokritos”.

Faculty in the joined MSc Programme “Applied Biochemistry: Clinical Chemistry, Biotechnology, Evaluation of Pharmaceutical Products” of the Department of Chemistry of the University of Patras and of the Institute of Biosciences and Applications of NCSR “Demokritos”.

- Title of lecture: “Cardiovascular disease and current therapeutic approaches” (1hr – 8 students, Course: “Molecular and Cellular Biology – Molecular biotechnology”)
- Title of lecture: “Methods for analysis and characterization of proteins, lipids and hydrocarbons” (2 hrs - 8 students, Course: “Biochemical Analysis- Clinical Biochemistry”)

Guest lecturer in graduate course “Clinical Chemistry”, “Clinical Chemistry-Molecular Diagnostics” Graduate Program, Faculties of Biology, Chemistry and Nursing, University of Athens

- Title of lecture: “Alzheimer’s disease: a) diagnosis (clinical, laboratory, imaging), b) risk factors (genetic factors, age, sex, cardiovascular factors, traumatic injuries, depression, medications, etc.), c) latest data on the mechanisms of occurrence and progression of the disease and on treatment” (2 hours – 25 students)

Chair of the PhD Advisory Committee of C. Mountaki and A. G. Dedemadi at the Department of Chemistry, University of Athens

MSc supervisor for A. Micha

Member of PhD Advisory Committee of E. Valanti at the School of Medicine, University of Athens and A. Papagianni at the School of Medicine, Aristotle University of Thessaloniki

Presentation entitled «Postgraduate studies in the Institute of Biosciences and Applications” at the 55th Summer School of NCSR “Demokritos”, July 2020, (20 min, 450 students)

G. Drossopoulou:

Guest Lecturer in the postgraduate specialisation programme (MSc) “Applied Biochemistry: Clinical Chemistry, Biotechnology, Evaluation of Pharmaceutical Products”, Department of Chemistry, University of Patras and Institute of Biosciences and Applications (IBA), NCSR “Demokritos” (4 hours – 7 students). Lectures on “Biochemical Analysis – Clinical Biochemistry”

Guest lecturer in MSc postgraduate program “Molecular and Applied Physiology”, Medical School, University of Athens “Regulation of Apoptosis in disease progression: Is it desirable or must be avoided?” October 2020 (3 hours lecture - 23 students)

Guest lecturer in MSc postgraduate course Clinical Biochemistry – Molecular Diagnostics, Unit: Physiology Chapters, Department of Biology, National and Kapodistrian University of Athens, “Metabolic Syndromes – Diabetes Mellitus and its complications” (3 hours – 32 students)

Other Activities at the Institute of Biosciences and Applications and NCSR “Demokritos”

A. Chroni:

Person in charge for education issues in the Institute of Biosciences and Applications (IBA)

Representative of IBA in the Education Committee of NCSR “Demokritos”

Member of Special Committee for the utilization of research findings, NCSR "Demokritos"

G. Drossopoulou:

Person in charge for the Histology Unit of the Institute of Biosciences and Applications (IBA)

Impact factors

A. Chroni: 13.071

G. Drossopoulou: 3.144

Citations 2020 (without self-citations)

A. Chroni: 130 (Scopus)

A. Tzinia: 71 (Scopus), 101(Google Scholar)

P. Kitsiou: 35 (Scopus)

G. Drossopoulou: 58 (Scopus)

Total Citations 2015-2020 (without self-citations)

A. Chroni: 864 (Scopus)

A. Tzinia: 435 (Scopus), 570 (Google Scholar)

P. Kitsiou: 175 (Scopus)

G. Drossopoulou: 466 (Scopus)

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A. Chroni: 23 (Scopus); 27 (Google Scholar)

A. Tzinia: 18 (Scopus); 21 (Google Scholar)

P. Kitsiou: 11 (Scopus, Google Scholar)

G. Drossopoulou: 14 (Scopus)

Current External Funding

Project entitled “High Density Lipoprotein (HDL) Structure, Function and Dysfunction: Prospects for New Diagnostic and Therapeutic Approaches for Cardiovascular Disease”, funded under the call “Supporting researchers with an emphasis on young researchers” of the Ministry of Education and Religious Affairs of Greece/ NSRF 2014-2020, with Dr. A. Chroni as Principal Investigator.

Duration: 2018-2020

Total funding (lab): 40.600€

Funding of the lab for 2020: 3.654 €

Project entitled “Complementary neuroprotective action of currant as a natural dietary supplement. Highlighting the mechanisms of action of currant using animal models of neurodegenerative diseases”, funded under the call “RESEARCH-CREATE-INNOVATE” of the Ministry of Economy and Development of Greece/ NSRF 2014-2020 with Dr. A. Chroni as Principal Investigator for NCSR Demokritos.

Duration: 2018-2022

Total funding (lab): 188.000 €

Funding of the lab for 2020: 60.000€

Project entitled “New therapies aiming to improve the atheroprotective and immunomodulatory properties of HDL for the treatment of autoimmune and cardiovascular diseases”, funded under the call “RESEARCH-CREATE-INNOVATE” of the Ministry of Economy and Development of Greece/ NSRF 2014-2020 with Dr. A. Chroni as Coordinator of the project.

Duration: 2020-2022

Total funding (lab): 250.000 €

Funding of the lab for 2020: 20.000€

Project entitled “The National Research Infrastructures on Integrated Structural Biology, Drug, Screening, Efforts and Drug Target Functional Characterization-INSPIRED”, funded under the call “Reinforcement of the Research and Innovation Infrastructure” of the Ministry of Economy and Development of Greece/ NSRF 2014-2020 with Dr. G. Nounessis as Principal Investigator for NCSR Demokritos and Dr. A. Chroni as team member.

Duration: 2018-2021

Total funding (lab): 16.000 €

Funding of the lab for 2020: 6.000€

Project entitled “Podocyte and β -cell survival in diabetes mellitus: The role of Liraglutide” Industrial Postdoc Researcher Program funded by Stavros Niarchos Foundation/NCSR “Demokritos” and NOVO NORDISK HELLAS

Principal Investigator: G. Drossopoulou

Duration: 2017-2020

Funding of the lab for 2018: 27.650€

Project entitled “Nephroprotective role of VitaminD3”, funded by AENORASIS SA

Principal Investigator: G. Drossopoulou

Duration: 2017-2021

Funding of the lab for 2018: 13.825€

Research Group: Molecular Carcinogenesis and Rare Disease Genetics

Research Staff

Gerassimos Voutsinas, Research Director (Researcher A')

Stefanos Smyrniotis, Post-graduate research collaborator

Labrina Bondi, Graduate student

Stamatia Katarachia, Collaborating Graduate Student

Vassiliki Gosda, Undergraduate student

Athanasia Menoudaki-Evangelou, Undergraduate student

Maria Triantafyllopoulou, Undergraduate student

Anna Deli, Undergraduate student

Nikolaos Paralikas, Undergraduate student

Dimitris Santorinios, Graduate research collaborator

Socratis 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. Pathogenesis and molecular analysis of human genetic diseases

2020 Findings

Malignancy grade-dependent mapping of metabolic landscapes in human urothelial bladder cancer: Identification of novel, diagnostic, and druggable biomarkers

Background: Urothelial bladder cancer (UBC) is one of the cancers with the highest mortality rate and prevalence worldwide; however, the clinical management of the disease remains challenging. Metabolomics has emerged as a powerful tool with beneficial applications in cancer biology and thus can provide new insights on the underlying mechanisms of UBC progression and/or reveal novel diagnostic and therapeutic schemes. Methods: A collection of four human UBC cell lines that critically reflect the different malignancy grades of UBC was employed; RT4 (grade I), RT112 (grade II), T24 (grade III), and TCCSUP (grade IV). They were examined using Nuclear Magnetic Resonance, Mass Spectrometry, and advanced statistical approaches, with the goal of creating new metabolic profiles that are mechanistically associated with UBC progression toward metastasis. Results: Distinct metabolic profiles were observed for each cell line group, with T24 (grade III) cells exhibiting the most abundant metabolite contents. AMP and creatine phosphate were highly increased in the T24 cell line compared to the RT4 (grade I) cell line, indicating the major energetic transformation to which UBC cells are being subjected during metastasis. Thymosin β 4 and β 10 were also profiled with grade-specific patterns of expression, strongly suggesting the importance of actin-cytoskeleton dynamics for UBC advancement to metastatic and drug-tolerant forms. Conclusions: The present study unveils a novel and putatively druggable metabolic signature that holds strong promise for early diagnosis and the successful chemotherapy of UBC disease.

Publications

Iliou A., A. Panagiotakis, A.F. Giannopoulou, D. Benaki, M. Kosmopoulou, A.D. Velentzas, O.E. Tsitsilonis, I.S. Papassideri, G.E. Voutsinas, E.G. Konstantakou, E. Gikas, E. Mikros and D.J. Stravopodis (2020) Malignancy Grade-Dependent Mapping of Metabolic Landscapes in Human Urothelial Bladder Cancer: Identification of Novel, Diagnostic, and Druggable Biomarkers, *International Journal of Molecular Sciences* 21: 1892. (IF: 4.556)

Articles in Press

Giannopoulou A.F, A.D. Velentzas, A.K. Anagnostopoulos, A. Agalou, N.C. Papandreou, D.G. Koumoundourou, E.G. Konstantakou, V.I. Pantazopoulou, A. Delis, M.T. Michailidi, D. Valakos, P. Syntichaki, V.A. Iconomidou, O.E. Tsitsilonis, I.S. Papassideri, G.E. Voutsinas, D. Thanos, D. Beis, E. Anastasiadou, G.Th. Tsangaris and D.J. Stravopodis (2021) Molecular Seeds Controlling Metastasis are Sown Early during Mutant BRAF-dependent Cutaneous Melanomagenesis, *Cancers* 2021, 13, 2024. <https://doi.org/10.3390/cancers13092024>. (IF: 6.639)

Other Scientific Activities

Head of the Laboratory for “Molecular Diagnosis of Genetic Diseases” (E1609), rendering genetic testing services for Tuberous Sclerosis Complex and Neurofibromatosis type I.

Reviewer of research articles for: *Scientific Reports*, *Cells*, *Cancer Management and Research*, *Journal of Human Genetics* (2 articles), *OncoTargets and Therapy* (2 articles), *Molecules*, *Cancers* (2 articles), *SAGE Open Medicine*.

Educational Activities

Stefanos Smyrniotis has presented his Dissertation entitled “Role of mitochondrial unfolded protein response in cancer development” (September 2020), at the Department of Biological Applications & Technology of the University of Ioannina, Greece.

Vassiliki Gosda has presented her Dissertation entitled “Mitochondrial unfolded protein response following inhibition of LONP1 protease in cancer cells” (June 2020), at the Department of Biomedical Sciences of Metropolitan College of Greece.

Athanasia Menoudaki-Evangelou has presented her Dissertation entitled “Mitochondrial unfolded protein response following inhibition of mitochondrial translation in cancer cells” (June 2020), at the Department of Biomedical Sciences of Metropolitan College of Greece.

Maria Triantafyllopoulou has presented her Dissertation entitled “Mitochondrial unfolded protein response following exposure to high levels of reactive oxygen species in cancer cells” (June 2020), at the Department of Biomedical Sciences of Metropolitan College of Greece.

Practical laboratory internship of Anna Deli, Nikolaos Paralikas, and Dimitris Santorinios has been successfully completed.

Lecture entitled “Molecular mechanisms of carcinogenesis and pharmacological inhibition of selected cellular targets for therapy”, Common Post-Graduate Program “Applied Biochemistry” of the Dept of Chemistry, University of Patras, and the Institute of BioSciences and Applications, NCSR “Demokritos”, Thursday 30 April 2020 (number of attendants: 8 students, teaching hours: 2).

Lab training and lectures of Secondary Education Biology Teachers at the ELLSconnect Learning LAB 2020, co-organized by IBA and EMBL, September 2020 (number of attendants: 4 teachers, teaching hours: 30).

Teaching of the undergraduate course: “Introduction to Molecular Biology” (4 semesters), American College of Greece (Deree College), Agia Paraskevi Attikis, January - December 2020 (number of attendants: 80 students, teaching hours: 184).

Lecture entitled "Molecular mechanisms of carcinogenesis and pharmaceutical inhibition of selected cellular targets for therapy", Post-Graduate Program "Development of New Drugs: Research, Circulation, and Access", Department of Medicine, National and Kapodistrian University of Athens, February 22, 2020, Athens (number of attendants: 30 students, teaching hours: 3).

Lecture entitled “Molecular mechanisms of carcinogenesis and pharmaceutical inhibition of selected cellular targets for therapy”, Post-Graduate Program “Applications of Biology in Medicine”,

Departments of Biology and Medicine, National and Kapodistrian University of Athens, April 7, 2020, Athens (number of attendants: 20 individuals, teaching hours: 3).

Other Activities at the Institute of Biosciences and Applications and NCSR “Demokritos”

In charge for the operation of 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”.

Deputy Director of IBA (1-1-20 until 15-11-20 and 16-11-20 until 31-12-20).

Member of the Research Council of the Institute (ESI) (January 1, 2020 – December 31, 2020).

Member of the Research Committee of NCSR “Demokritos” (January 1, 2020 – December 31, 2020).

Alternate member of the Appeals Committee of NCSR “Demokritos” (January 1, 2020 – December 31, 2020).

Member of the Bid Evaluation Committee for hiring a Biopathologist-Microbiologist for NCSR “Demokritos” (December 2020).

Member of 7 Reviewer Committees for hiring scientific collaborators at IBA (June 3, June 29, October 1, October 12, October 19, October 21, December 21, 2020).

Impact factors (for 1 publication): 4.556

Number of citations for 2020 (without self-citations): 107

Number of citations 2016-2020 (without self-citations): 392

h-factor: 19

PROGRAMME B

"MODEL SYSTEMS FOR THE STUDY OF CELL FUNCTION"

Research Group: Molecular Genetics of Insects and Biotechnology

Research Staff

Luc Swevers, Research Director

Vasiliki Labropoulou, Senior Researcher

Dimitrios Kontogiannatos, Postdoctoral Fellow

Min Feng, Postdoctoral Fellow

Feifei Ren, Collaborating Graduate Student

Ioannis Nektarios Maraidonis, Diploma Student

Aleksander Józef Mazurek, Diploma Student

Dimitra Stefanou, Technical Specialist

Research Interests

- (1) Analysis of small RNA (miRNA, siRNA, piRNA) pathways in Lepidoptera. Development of methods for improvement of RNAi efficiency in lepidopteran insects. Development and improvement of methods for screening of molecules, mainly antimicrobial peptides (AMPs), with antiviral properties.
- (2) Analysis of the immune response against RNA virus infections in lepidopteran insects: small RNAs and “cytokines”.
- (3) Development of methods for insect pest control: development of nuclear polyhedrosis viruses as transformation vectors, production of recombinant RNA viruses and “viral-like particles” (VLPs), exploration of the use of transposable elements for insect transformation, environmental RNAi, insect growth regulators.
- (4) Functional genomics: development of high-throughput screening systems for the detection of biologically active compounds: ecdysone agonists and moulting-accelerating compounds, functional expression of metabolic enzymes that are involved in insecticide resistance.

2020 Findings

Virus-like particles (VLPs) for efficient delivery of dsRNA to insect pests. The baculovirus expression vector system (BEVS) was used to successfully produce VLPs based on the capsid shell protein (CSP) of cytoplasmic polyhedrosis virus. Interestingly, CSP fusion proteins with GFP were also found to assemble in VLPs that formed high-order aggregates. CSP-GFP-based VLPs were efficiently taken up by Bm5 cells and midgut tissue from the silkworm that was cultured *ex vivo*. In addition, the capacity of the BEVS to produce dsRNA hairpins was evaluated. Co-expression of the dsRNA-binding protein B2 or knockdown/knockout of *dicer-2* in the insect cell lines increased dsRNA production by the BEVS. However, the increase in dsRNA was independent of the presence of the dsRNA hairpin in the recombinant baculoviruses, indicating that dsRNA production by very late baculovirus promoters may not be an efficient process. Other experiments indicated the exceptional stability of dsRNA molecules in cellular extracts and it remains to be investigated whether exogenously applied (in vitro synthesized) dsRNA can become incorporated during VLPs in baculovirus-infected cells (HFRI program).

Identification of hemocyte populations of the silkworm and the impact of baculovirus infection.

Following single-cell RNA sequencing, 20 cell clusters with their marker genes were identified in silkworm hemocytes. The sequencing data were subsequently correlated with the cell types and showed that the infection caused great changes in their prevalence, more specifically an increase in pro-hemocytes was observed (collaboration with South China Agricultural University, Guangzhou, China).

Metabolome analysis of baculovirus infection in hemolymph of silkworm. The analysis revealed the importance of amino-acid metabolism during baculovirus infection. However, no significant correlations between metabolite abundances in hemolymph and immune gene expression were

observed. In contrast to other virus infections, glucolysis and glutaminolysis do not play an important role during baculovirus infection (collaboration with South China Agricultural University, Guangzhou, China).

Insect antimicrobial peptides and viral infections. Experiments of blocking of infection of insect cells by the virus AcMNPV-YFP showed a decrease in fluorescence of 27% by Cecropin A, 50% by IBP2 and 33% by B2 protein. The C-terminal domain of Spätzle was cloned, a cytokine that is capable to activate the Toll receptor. Stably transformed cell lines were generated that produce the above-mentioned molecules and will be used in assays to evaluate the activation of NF κ B and the transcription of AMP genes.

Original publications

Swevers, L., Feng, M., Ren, F., and Sun, J. (2020). Antiviral defense against Cypovirus 1 (*Reoviridae*) infection in the silkworm, *Bombyx mori*. Arch. Insect Biochem. Physiol. 103, e21616. (IF: 1.536)

Wang, L., Swevers, L., Van Meulebroek, L., Meeus, I., Vanhaecke, L., and Smagghe, G. (2020). Metabolomic analysis of Cricket paralysis virus infection in *Drosophila* S2 cells reveals divergent effects on central carbon metabolism as compared with silkworm Bm5 cells. Viruses 12, 393. (IF: 3.816)

Kolliopoulou, A., Kontogiannatos, D., and Swevers, L. (2020). The use of engineered plant viruses in a trans-kingdom silencing strategy against their insect vectors. Front. Plant Sci. 11, 917. (IF: 4.407)

Feng, M., Fei, S., Xia, J., Labropoulou, V., Swevers, L., and Sun, J. (2020). Antimicrobial peptides as potential antiviral factors in insect antiviral immune response. Front. Immunol. 11, 2030. (IF: 5.085)

Wei P., Demaeht P., De Schutter K., Linda Grigoraki L., Labropoulou V., Riga M., Vontas J., Ralf Nauen R., Dermauw W., Van Leeuwen T. (2020). "Overexpression of an alternative allele of carboxyl/choline esterase 4 (CCE04) of *Tetranychus urticae* is associated with high levels of resistance to the keto-enol acaricide spiroticlofen". Pest Manag Sci 76: 1142-1153. (IF: 3.750)

Original publications that are accepted for 2020

Swevers, L., Denecke, S., Vogelsang, K., Geibel, S., and Vontas, J. (2021). Can the mammalian organoid technology be applied to the insect gut? Pest Manag. Sci. 77, 55-63. (IF: 3.750)

Vlogiannitis, S., Mavridis, K., Dermauw, W., Snoeck, S., Katsavou, E., Morou, E., Harizanis, P., Swevers, L., Hemingway, J., Feyereisen, R., Van Leeuwen, T., and Vontas, J. (2021). Reduced proinsecticide activation by cytochrome P450 confers coumaphos resistance in the major bee parasite *Varroa destructor*. Proc. Natl. Acad. Sci. USA. 118(6):e2020380118. (IF: 9.412)

Feng, M., Kolliopoulou, A., Zhou, Y., Fei, S., Xia, J., Swevers, L., and Sun, J. (2021). The piRNA response to BmNPV infection in the silkworm fat body and midgut. Insect Science 28, 662–679. (IF: 2.791)

Ren, F., Swevers, L., Lu, Q., Zhao, Y., Yan, J., Li, H., and Sun, J. (2021). Effect of mutations in Capsid Shell Protein (CSP) on the assembly of BmCPV virus-like particles (VLPs). J. Gen. Virol. 102, 001542. (IF: 3.376)

Feng, M., Xia, J., Fei, S., Peng, R., Wang, X., Zhou, Y., Wang, P, Swevers, L., and Sun, J. (2021) Identification of Silkworm Hemocyte Subsets and Analysis of Their Response to Baculovirus Infection Based on Single-Cell RNA Sequencing. Front. Immunol. 12, 645359. (IF: 5.085)

Feng, M., Fei, S., Xia, J., Zhang, M., Wu, H., Swevers, L., and Sun, J. (2021). Global Metabolic Profiling of Baculovirus Infection in Silkworm Hemolymph Shows the Importance of Amino-Acid Metabolism. Viruses 13, 841. (IF: 3.816)

Publications in books and conference proceedings

Kontogiannatos, D., Kolliopoulou, A., and Swevers, L. (2021). The “Trojan Horse” approach for successful RNA interference in insects. In: “*RNAi for Plant Improvement and Protection*”, B. Mezzetti, J. Sweet, L. Burgos (Eds.), pp 25-39. CAB International 2021

Editing of publications of scientific books

“Novel Insights into Insect Viral Immunity”. Research Topic - *Frontiers Immunology*. Editors: Liang Jiang, Xiao-Qiang Yu, Luc Swevers

Presentations in International Conferences

Kontogiannatos, D., Kolliopoulou, A., Zhao, Y., Ren, F., Lu, Q., Sun, J., and Swevers, L. (2020). Evaluation of production of Virus-Like Particles’ components by the Baculovirus Expression Vector System for efficient dsRNA delivery in insects. 4th iPlanta Conference “Contribution of RNAi to sustainable agriculture, food safety and security”, 26-28 February, Athens, Greece.

De Schutter, K., Reyna, Z.M., Christaens, O., Taning, C.N.T., Swevers, L., Dubruel, P., Van Damme, E.J.M., and Smagghe, G. (2020). DsRNA-delivery systems: elucidation of uptake in plant and insect cells. 4th iPlanta Conference “Contribution of RNAi to sustainable agriculture, food safety and security”, 26-28 February, Athens, Greece.

Christiaens, O., Reyna, Z.M., Tardajos, M.G., Dash, M., De Schutter, K., Swevers, L., Van Damme, E., Dubruel, P., and Smagghe, G. (2020). The development of dsRNA carriers to improve RNAi efficacy in recalcitrant insect species. 4th iPlanta Conference “Contribution of RNAi to sustainable agriculture, food safety and security”, 26-28 February, Athens, Greece.

Feng, M., Xia, J., Fei, S., Zhou, Y., Wang, X., Wang, P., Swevers, L., and Sun, J. (2020). Identification of silkworm hemocyte subsets and analysis of their response to BmNPV infection based on single-cell sequencing. The 3rd International Symposium on Sericultural Sciences and the 21st Century Silk Road, 21-22 October, Chongqing, China.

Kontogiannatos, D., Kolliopoulou, A., Ren, F., Feng, M., Sun, J., and Swevers, L. (2020). Production of RNA Hairpins and Viral-like Particles by the Baculovirus Expression Vector System. *Entomology 2020*. ESA's Virtual Annual Meeting, November 11-25, 2020.

Presentations in Greek Conferences

Kontogiannatos, D., Koutrotsios, G., Barbarigou, C.-G., Ksekalaki, S., Pratsinis, H., Kletsas, D., Swevers, L., Kourti, A., Tarantilis, P., and Zervakis, G.I. (2020). Biotechnological approach for the culture of the entomopathogenic fungus *Cordyceps militaris*: production of mycelium biomass and initial evaluation of the activity of cordysepin. 12th Pan-Hellinic Conference “Biosciences & Unified Health”, 27/11/2020 - 29/11/2020, Athens.

Kolliopoulou, A., Mazurek, A.J., Kontogiannatos, D., and Swevers, L. (2020). Silencing of the *Dicer-2* gene in lepidopteran cells using RNAi and CRISPR/Cas9. 12th Pan-Hellinic Conference “Biosciences & Unified Health”, 27/11/2020 - 29/11/2020, Athens.

Other scientific activities

Organization of scientific conferences or participation in organization committees of conferences

L. Swevers: Organization of the 4th iPLANTA Conference, “Contribution of RNAi to sustainable agriculture, food safety and security”, Cost Action EU (Cost Action CA15223 ‘Modifying plants to produce interfering RNA’), Athens, Greece (February 2020).

V. Labropoulou: Member of the organization committee of the 4th iPLANTA Conference.

Participation in editorial boards of scientific journals

L. Swevers: Member of the editorial board of the scientific journals: “Archives of Insect Biochemistry and Physiology” and “Journal of Insect Science”.

L. Swevers: Guest associate editor in Comparative Immunology (Frontiers in Immunology).

Participation in committees for evaluation of research projects

L. Swevers: Member of the committee of experts of the Funds of Scientific Research FWO-Vlaanderen (Belgium) “Bio2” (Functional Biology) for the evaluation of research proposals (PhD and postdoc scholarships, national research programs).

L. Swevers: Expert for the funding agency “Agence Nationale de la Recherche (ANR)” (one proposal) (France) (L. Swevers).

Reviews for research journals:

L. Swevers: Reviewer of scientific articles for the journals “Archives of Insect Biochemistry and Physiology” (2x), “Biochimica et Biophysica Acta – General Subjects”, “BMC Microbiology”, “Environmental Pollution”, “Frontiers in Physiology” (2x), “General and Comparative Endocrinology”, “Heliyon”, “Insect Science” (4x), “Journal of Pesticide Science”, “Journal of Insect Physiology” (6x), “Journal of Invertebrate Pathology”, “Journal of Pest Science” (2x), “Molecular Genetics and Genomics”, “Molecular Immunology” (2x), “Pest Management Science” (3x), “Pesticide Biochemistry and Physiology”, “Phytoparasitica”, “PLoS ONE”, “Scientific Reports”, “Science of the Total Environment”, “Transgenic Research”, “Virus Research” (2x).

Educational activities

Inside IBA

L. Swevers:

Participation in the Bi-Institutional Program of Graduate Studies of “Applied Biochemistry: Clinical Chemistry, Biotechnology, Validation of Pharmaceutical Drugs” (collaboration with the Chemistry Department of the University of Patras)

- Molecular Pharmacology – Immunology: lecture with title: “RNAi as an antiviral defense system in insects” (1 hour, 8 students)
- Molecular and Cellular Biology – Molecular Biotechnology: lecture with title “The Baculovirus Expression System” (1 hour, 8 students)

V. Labropoulou:

- Supervision of the diploma thesis of Ioannis Nektarios Maraidonis.
- Participation in the Bi-Institutional Program of Graduate Studies of “Applied Biochemistry: Clinical Chemistry, Biotechnology, Validation of Pharmaceutical Drugs” (collaboration with the Chemistry Department of the University of Patras). Lecture with title “Innate immunity in insects” (2 hours, 8 students).
- Laboratory seminar for teachers of secondary education (IBA – EMBL). Instructor Laboratory workshop. Theoretical principles of laboratory methods (Western blot analysis, PCR). 11-13/09/2020.

Outside IBA

L. Swevers:

- Member of the evaluation committee of PhD thesis (External Examiner): Elise Vogel: “Optimizing RNA interference for insect pest control”. Zoological Institute, KU Leuven, Belgium (July 2020).
- Member of the evaluation committee of PhD thesis (External Examiner): Lina Mingels: “Role of small RNAs in antiviral immunity and systemic RNA interference of insects”. Zoological Institute, KU Leuven, Belgium (December 2020).
- One hourly lecture with title “CRISPR/Cas and the rise of genome editing techniques” at the Summer School of NCSR “Demokritos”.

Other activities at IBA and NCSR “D”

L Swevers:

- Member of the scientific board of the institute (until June 2020).
- Responsible for the following instruments: Fluostar Microplate Fluorometer, HPLC Hewlett Packard, microplate reader for luminescence and fluorescence TECAN InfiniTE M-200.

V. Labropoulou:

- Member of the scientific board of IBA (from 13 /07/2020)
- Team member of the Light Microscopy unit of IBA
- Committee member for selection of external collaborators in projects of INN and IP&T

Total impact factor for publications during 2020

L Swevers: 14.844 (for 4 publications)

V. Labropoulou: 8.835 (for 2 publications)

Total citations for 2020 (without self-citations)

L Swevers: 370

V. Labropoulou: 21

Total citations for 2015-2020 (without self-citations)

L Swevers: 1568

V. Labropoulou: 217

h-factor:

L Swevers: Scopus: 32, Google Scholar: 37

V. Labropoulou: Scopus: 12, Google Scholar: 15

Current External Funding

Project entitled “Characterization of extracellular RNA-signals and their role in antiviral immunity in insects”

Organization: FWO-Vlaanderen (Belgium)

Coordinator: J. Vanden Broeck; responsible for the Greek participation: L. Swevers

Duration: 1/2019-12/2022

Total funding: 700.800 €

Total funding for the laboratory: 0 €

Total funding for the laboratory during 2020: 0 €

Program entitled “Modifying plants to produce interfering RNA (iPlanta)”

Organization: EU COST (COST Action CA15223)

Coordinator: B. Mezzetti

National representative: L. Swevers

Duration: 10/2016-03/2021

Total funding for the period 05/20 until 03/21: 95.220,00 €

Total funding for the laboratory: 0 €

Total funding for the laboratory during 2020: 0 €

Graduate Student Overseas Study Program, South China Agricultural University (Guangzhou, People’s Republic of China)

Duration: 12/2019-11/2020

Scholarship for Feifei Ren: 18.000 €

Program entitled “Viral-like particles for increased RNAi delivery in insects” (VLP-RNAi, #785).

Organization: Hellenic Foundation for Research and Innovation (H.F.R.I.) under the “First Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of

high-cost research equipment grant”.

Coordinator: L. Swevers

Duration: 02/2020-05/2022

Total funding for the laboratory: 152.000 €

Total funding for the laboratory during 2020: 66.650 €

Research Group: Chemical Ecology and Natural Products

Maria Konstantopoulou, Senior Researcher

Dimitris Raptopoulos, Graduate Research Associate (PhD)

Eleni Koutsoumpeli, Postdoctoral Fellow

Anastasia Fytrou, Postdoctoral Fellow

Petri-Christina Betsi, Graduate Research Associate (MSc)

Ioanna Dasenanki, Graduate Research Associate (MSc)

Neoklis Manikas, PhD candidate

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. Chemical synthesis of semiochemicals (infochemicals).

Development of specialized dispensers for semiochemicals acting as attractants/repellants for insects and technologies for their application in pest control. Biodegradable materials endowed with controlled- release rate and UV protection properties.

Development of biocides of biological origin (Biological Control Agents. BCAs) and methods/technologies of application, aiming to incorporating them in integrated management methods for pest of agricultural and urban environment but also for pests of public health concern such as mosquitoes.

Biochemistry of insect olfactory receptors with emphasis on the localization and isolation of protein receptors for semiochemicals.

Microorganisms and Biotechnology: Isolation of naturally occurring microorganisms and their biologically active secondary metabolites (toxins) aiming to their incorporation in insect population management.

2020 Findings

Within the framework of the programs OLEFINE (OLEaginous yeast platforms for FINE chemicals) and PHERA (Pheromones for Row crop Applications) HORIZON 2020: i) biotechnological methods using yeasts in order to produce insect sex pheromones and ii) the verification that yeast-produced pheromones are homologous to the chemically produced ones were continued. Also the study of the electrophysiological response (electro-antenography EAG and electro-antenography system connected to the gas chromatograph GC-EAD) of the antennae of male insects to pheromone blends produced by yeast fermentation was carried out. In addition, bioassays on male insects in a wind tunnel were performed and monitored using a camera recording system. The analysis of their behavior is done through a specialized software (behavioral tracking). To control the activity of biophomones, field experiments were carried out in three regions in Greece (northern and central) and also in Cyprus, and their homology to chemically synthesized pheromones was verified. In particular, we performed monitoring experiments and trap-shut-down experiments using a flowable polymeric matrix in which the sex pheromone was formulated (Figure 1). We continued the research on the optimization of systems for the slow release of semiochemicals through their inclusion in non-toxic, biodegradable and environmentally friendly polymers and biopolymers endowed with special characteristics aiming at protecting the labile semiochemicals from ultraviolet

radiation. We measured the release rate of biopesticides formulated in the flowable polymer (aged naturally under field conditions) using Solid Phase Micro Extraction (SPME) (Figure 1).

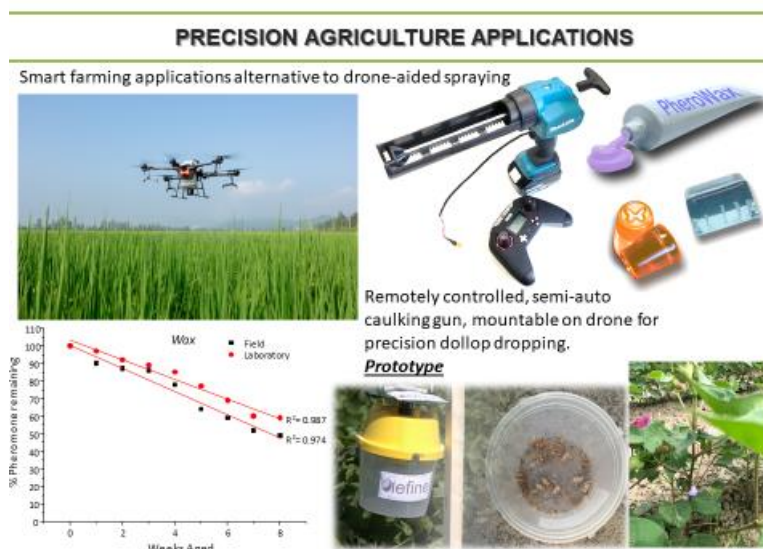


Figure 1

We maintain the insect colonies of crop pests of great economic importance that were established during the previous years, namely *Helicoverpa armigera*, *Ostrinia nubilalis*, *Lobesia botrana*, *Tuta absoluta* and *Plodia interpunctella*. They serve in electrophysiological and behavioral experiments but also for tissue cropping for the isolation of enzymes involved in pheromone synthesis. Recently, a colony of the Diamondback moth *Plutella xylostella*, a serious pest of cruciferous plants has been established with the aim of isolating enzymes involved in pheromone synthesis, in the framework of OLEFINE.

The effort for identification of infochemicals and other bioactive metabolites of natural origin (Biological Control Agents, BCAs) for their use as "smart" insecticides was continued. We tested the effect of secondary metabolites of *Pistacia lentiscus* and the entomopathogenic strain SMU-21 of *Mucor hiemalis* on the viability of *Lobesia botrana* larvae. In addition, we studied the bioactivity of the essential oils of aromatic plants and determined the activity of chemical compounds using electrophysiological and viability tests on adult and larvae of *L. botrana* respectively (in collaboration with the Technical University of Cyprus). We proceeded to chemical analysis of the plant fractions by gas chromatography-mass spectrometry (GC-MS) for the determination of bioactive substances.

We continued the study of the cytostatic / cytotoxic action as well as the antioxidant action of plant extracts and their fractions from the collection of extracts, which has been generated and is maintained in the laboratory, in collaboration with the Laboratory of Cell Proliferation and Aging of IBA.

Publications

Holkenbrink C., Ding B.J., Wang H.L., Dam M.I., Petkevicius K., Kildegaard, Leonie Wenning K.R., Sinkwitz Ch., Lorántfy B., Koutsoumpeli E., França L., Pires M., Bernardi C., Urrutia W., Mafra-Neto A., Ferreira B.S., Raptopoulos D., Konstantopoulou M., Löfstedt Ch., Borodina I. 2020. Production of moth pheromones for pest control by yeast fermentation. *Metabolic Engineering* 62: 312-321. (IF: 9.783)

Articles in Press

Petkevicius K., Koutsoumpeli E., Betsi P. Chr., Ding B. J., Kildegaard K. R., Jensen Hilbert, Mezo N., Mazziotta A., Gabrielsson A., Sinkwitz Chr., Lorantfy B., Holkenbrink C., Löfstedt Chr., Raptopoulos D., Konstantopoulou M., Borodina I. Biotechnological production of the European corn borer sex pheromone in the yeast *Yarrowia lipolytica*. *Biotechnology Journal*. (IF: 4.677)

Presentations at Scientific Conferences

Koutsoumpeli E., Raptopoulos D., Konstantopoulou M. 2020. Biologically produced pheromones: Electrophysiological and behavioural responses of *Helicoverpa armigera*. XXVI International Congress of Entomology, Helsinki, Finland.

Holkenbrink C., Ding B.J., Wang H.L., Dam M.I., Petkevicius K., Kildegaard, Leonie Wenning K.R., Sinkwitz Ch., Lorántfy B., Koutsoumpeli E., França L., Pires M., Bernardi C., Urrutia W., Mafra-Neto A., Ferreira B.S., Raptopoulos D., Konstantopoulou M., Löfstedt Ch., Borodina I. 2020. Production of moth pheromones for pest control by yeast fermentation. Annual virtual meeting of AIChE. Abstract 602019.

Other Scientific Activities

Union of Greek Researcher representative in National Council for Research and Innovation (NCRI)

Member of the Attica Region Research and Innovation Council (PSEK) 2017-2021.

Researcher and Specialist Functional Scientist representative in NCSR "Demokritos" Board of Directors

Member of the international claim committee for the XII European Entomological Congress 2022 held in Crete.

Member of organizing committee of XII European Entomological Congress 2022 held in Crete

President of the Union of Greek Researcher

Reviewer of research proposals for General Secretary Research and Technology

Reviewer and member of the committee of proposals for the Research, Innovation and Excellence Call of the University of Thessaly PhD candidates' scholarships

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, Journal of Pest Science, Bulletin of Entomological Research, Pest Management Science, Journal of Chromatography B, PLoS-One, Journal of Chemistry, Journal of Medicinal Plant Research, Research in Veterinary science, Journal of essential oil bearing plants, Insects

Topic Editor of Open Access Journal Insects

Special Issue Editor of Special Issue of Agronomy: "Medicinal and Aromatic Plants (MAPs): The Connection between Cultivation Practices and Biological Properties"

Educational Activities

Participation in the Interinstitutional Program of Postgraduate Studies: «Applied Biochemistry Clinical Chemistry and Biotechnology, evaluation of pharmaceutical plants» between of IBE and Department of Chemistry of University of Patras.

Other activities for the Institute of Biosciences & Applications

President of the Scientific Advisory Board (ESI) of the Institute (06/2019-06/2020) and member (07/2020- now).

Participation as full member in the project “Administration of the research infrastructure and materialization of programs of identification of bioactive compounds in NCSR “D” of the Action {OPENSOURCE-GR: Research infrastructure of open access for targeted screening technologies and detection of bioactive molecules to protect Health, Livestock, Agriculture and the Environment”.

Participation as full member in the project of project team project: SANITURA (Determination of objectives and development of innovative approaches for applications in health and the environment), funded by GSRT (Strategy Development Action Research and Technology Organizations, Operational Program "Competitiveness, Entrepreneurship and Innovation ", NSRF 2014- 2020.

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

Impact Factors (for 1 publication): 9.783

Citations 2020(without self-citations): 67

Total Citations 2016- 2020 (without self-citations): 287

h-factor: 14 (Scopus), 17 (Google Scholar)

Current External Funding

Project entitled *Oleaginous yeast platforms for Fine chemicals* (Horizon 2020, Call: H2020-NMBP-2016-2017/H2020-NMBP-BIO-2017, GRANT AGREEMENT 760798) funded by EU, and Scientific responsible Dr. M. Konstantopoulou.

Duration: 1/1/2018 – 31/12/2021

Total program funding: 441.311 €

Laboratory Funding for 2020: 124.233,2 €

Project entitled *Bio Based Industries Joint Undertaking, PHERA “Pheromones for Row crop Applications”* (Horizon 2020, Subcontractor) funded by EU, and Scientific responsible Dr. M. Konstantopoulou.

Duration: 1/1/2020 – 31/12/2023

Total program funding: 45.000 €

Laboratory Funding for 2020: 45.000 €

Research Group: Chronobiology

Research Staff

Anastasia Prombona, Senior Researcher

Angeliki Galeou, Postdoctoral Fellow

Danae Farmaki, PhD student

Chrysanthi Stefanatou, Diploma student

Research Interests

The research interests of our laboratory are concentrated on the study of the circadian oscillator function in *Phaseolus vulgaris* and in mammalian cell culture systems.

The circadian clock of plants

This project focuses on the study of rhythmically expressed genes in *P. vulgaris* primary leaves and their synchronization under different photoperiods and the application of light at night. In addition, we investigate the regulation of clock gene expression and the molecular mechanisms of the oscillator function by implementation of an in vitro protoplast system from *P. vulgaris* leaves. Additionally, the study of the interaction of the molecular oscillator with the plant defence during *Pseudomonas* infection is investigated.

The circadian clock of mammals-correlation with pathological conditions

This project concentrates on the study of the role of the biological circadian clock in carcinogenesis. More specifically, we investigate the regulation by the circadian clock of the expression of the oncogene *c-MYC* and the interaction of the oncoprotein with components of the circadian clock. In addition, we investigate the effect of bioactive and pharmaceutical compounds on the clock function and the oncogenesis. The regulation of gene expression related to chromatin remodeling at the promoters of clock genes under physiological and pathological conditions is also investigated.

2020 Findings

The circadian clock of P. vulgaris

We have investigated the interplay of the circadian clock with the pathogen triggered immunity (PTI) of *P. vulgaris* following infection with *Pseudomonas syringae* (Psp) as a compatible interaction system during the initial stages of the infection process. In particular, we studied the oxidative defence a. by measuring the oxidative burst caused by the elicitor peptide flagellin 22 (flg22) and b. by infecting leaves with Psp bacteria and analyzing the in planta growth and the gene expression changes of peroxidases and respiratory burst oxidase homologs (RBOHs). In order to uncover any possible role of the circadian clock in the defence process, all experiments were performed repeatedly at different times of the day. Our results reveal that the oxidative defence is light and clock dependent. Ongoing experiments explore the role of central clock elements in the observed rhythmic processes. This work is implemented by Dr. A. Galeou with the participation of C. Stefanatou (unpublished results).

Interaction of the circadian clock with pathological conditions

This project aims to identify among the clock protein modulators those that improve the function of the circadian clock and result in recession of malignant properties such as proliferation and metastasis dynamics of human cancer cell lines. In addition to the phenotypic characteristics study in the presence and absence of clock modulators, gene expression analysis of clock, cell cycle and stemness genes is performed. Ongoing experiments indicate that the clock function is differentiated among cell lines of variant origin (metastatic, primary tumor) for the same cancer type. This work constitutes part of the PhD work of D. Farmaki.

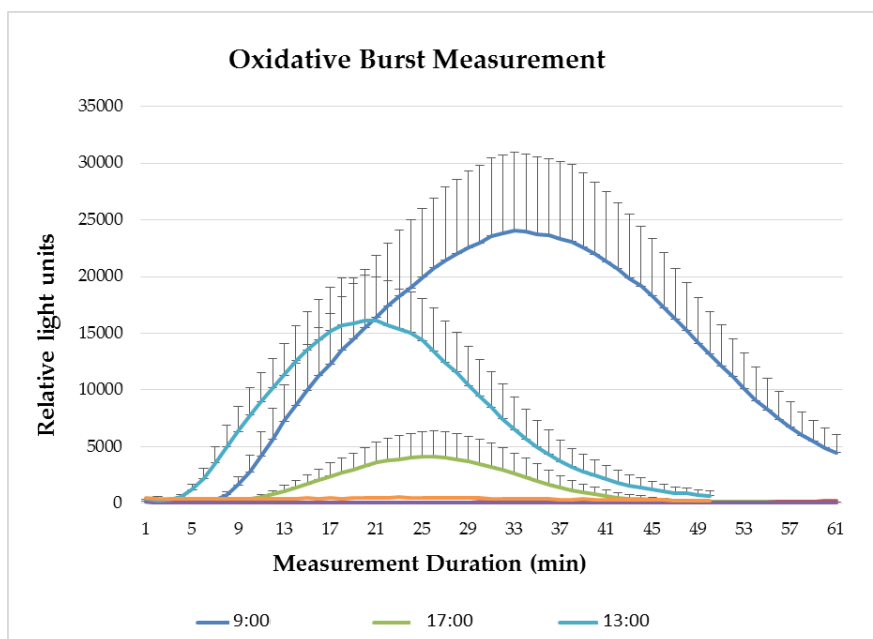


Figure 1: The oxidative burst of bean leaves discs measured at different times of the day. Plants were grown for 12-15 days under photoperiod with equal day-night length and transferred to continuous light. Leaf discs of 5 mm diameter were cut at 3 different times of the subjective day (at 9:00, 13:00 and 17:00 hour). Twenty four hours later, the oxidative burst, that is the production of free radicals (reactive oxygen species, ROS) and hydrogen hyperoxide, was measured as the response to flg22, a peptide of the bacterial elicitor flagellin. The produced ROS react with Luminol by oxidizing it and photons are produced. The Luminometer measurements were performed every 1 min for the duration of 1 hour. In a 96-well plate 6 control samples (without flg22) and 18 samples with flg22, were measured. Error bars represent the standard deviation of the repeated samples.

Publications 2020

Galeou, A., Prombona, A. (2020) Daily rhythmic leaf movements and expression of circadian clock genes are differentially synchronized by light in *Phaseolus vulgaris*. *Plant Gene* 23, 100245. (IF: 1.67).

Education

Supervision of the PhD candidate D. Farmaki

Supervision of the Diploma student C. Stefanatou

Other activities in IBA

Person in charge for the collection and safe removal of chemical waste

Impact Factor for the 2020 publications: 1.67

Citations for the year 2020 (without self citations): 37

Citations for the years 2015-2020 (without self citations): 126

h-index: 9

Current External Funding

Project entitled “The circadian clock in the defence and the phytoprotection of *Phaseolus vulgaris* during infection by *Pseudomonas*, Operational Programme “Human Resources Development, Education and Lifelong Learning” in the context of the project “Reinforcement of Postdoctoral Researchers - 2nd Cycle” (MIS-5033021), implemented by the State Scholarships Foundation (IKY).
Scientist in charge: Dr. A. Galeou

Duration of the Programme: 24 months
Total funding (for the 24 months): 26.400 €
Funding for the year 2020: 6.600 €
Funding of the Laboratory for the year 2020: 6.600 €

Research Group: Microbial Molecular Genetics laboratory

Research Staff

Vicky Sophianopoulou, Research Director

Christos Gournas, Researcher

Ada Biratsi, Post-Graduate Fellow (PhD candidate)

Amalia Megarioti, Post-Graduate Fellow (PhD candidate)

Alexandros Valianatos, Post-Graduate Student (Master Thesis)

Tatiana Zakopoulou, Undergraduate Student (Diploma Thesis)

Spiros Gaitanos, Undergraduate Student (Diploma Thesis)

Petros Skoutelas, Undergraduate Student (Practical work)

Georgia-Despina Ifanti, Undergraduate Student (Practical work)

Research Interests

Our group is primarily interested in the function, regulation and organization of the fungal plasma membrane (PM). The plasma membrane, as the boundary between the cell and its environment, is a platform for numerous functions. A major challenge is to understand how the PM coordinates these diverse processes while maintaining its barrier function, and the mechanisms underlay them. The general concept of our work is to understand how specific compartments/domains of the plasma membrane regulate their structure and function to promote the activity and /or trafficking of nutrient transporters, persistence of fungal cells, critical stress responses and how these phenomena are related to fungal pathogenicity (*I. Vangelatos et al., 2010; C. Scazzocchio et al., 2011; A. Athanasopoulos et al., 2013, 2015; Gournas et al, 2018; Athanasopoulos et al., 2019*).

Our models of choice are two of the most genetically tractable organisms: The non-pathogenic ascomycetes *Aspergillus nidulans* and the budding yeast *Saccharomyces cerevisiae*, fungi that have been extensively exploited for the discovery of a plethora of mechanisms underlying biological processes and for which state of the art genetic tools are available.

Our group has contributed to the understanding of the above processes, mainly in two subjects:

A) Regulation of expression, structure-function relationships, specificity, trafficking and evolution of amino acid transporter proteins

B) The organization of the lateral compartmentalization of the fungal plasma membrane

Long-term objectives: identification of new pharmaceutical targets and future development of highly-targeted antifungals/vaccines.

In addition, our group is interested in elucidate the molecular mechanisms underlie detoxification of toxic amino acid analogues. L-Azetidine-2-Carboxylic Acid (AZC) is a toxic analogue of proline, produced by the flowering plant *Convallaria majalis* (valley lily), certain species of the genus *Polygonatum* and beets (*Beta vulgaris*). In nature, AZC has a protective role for the plants that produce it, and at the same time is a toxic substance for a multitude of organisms, including various bacteria, fungi and even mammals. Our studies targeted to understanding the molecular mechanism(s) underlie detoxification and catabolism of AZC in soil fungi.

Medium and long-term objectives: design new and specialized antifungal substances to protect crops of nutritional and economic significance.

2020 Findings

In respect to the study of the organization and physiological role of eisosomes:

1) It was shown that Lsp1 is required for the optimal expansion of MCCs in Quiescent yeasts. Moreover, expansion of eisosomes occurs only in respiratory-active quiescent yeasts, and is defective in strains carrying deletions in genes of the respiratory chain. Strains lacking MCCs show defective utilization of ethanol as carbon source, indicating that eisosomes are required for

respiration. This phenotype is due to problematic mitochondrial function, since strains lacking MCCs show defective mitochondrial membrane potential and dramatically reduced long-term survival in the stationary phase, while their population is enriched in senescent and dead cells.

2) It was shown that Iron and copper, elements related to respiration, the function of mitochondria and oxidative stress, do not affect the number of eisosomal spots or the expression of PilA in *A. nidulans*. In addition, strains lacking the core eisosomal genes show no hypersensitivity to copper or iron.

3) The effect of several transcription factors on the expression of Pil1 and Lsp1 was studied. Results showed that the number of MCCs increases upon glucose depletion, but mainly in the simultaneous presence of a non-fermentable carbon source. Preliminary evidence suggests that this regulation is independent of the general Carbon-catabole Repressor Mig1.

4) It was shown that the number of PilA spots in the hyphae of *A. nidulans* increases upon nitrogen starvation, up to 10-fold upon glucose starvation, and to a lesser extent in the presence of poor carbon sources glycerol, fructose or ethanol. On the contrary, no effect was observed at the expression levels of SurG, PilB and Nce102 core eisosomal proteins under the same conditions.

5) It was shown that in dormant conidiospores of *A. nidulans*, the sorting of SurG and AnNce102 to the vacuole is performed via clathrin-dependent endocytosis which seems to not require ubiquitin or the AP2-adaptor of clathrin. On the contrary, in the growing hyphae the sorting to the vacuole seems to occur directly from the Golgi, without passage from the plasma membrane.

Publications in peer-reviewed journals

Esch, B.M., Limar, S., Bogdanowski, A., Gournas, C., More, T., Sundag, C., Walter, S., Heinisch, J.J., Ejsing, C.S., André, B., et al. (2020). Uptake of exogenous serine is important to maintain sphingolipid homeostasis in *Saccharomyces cerevisiae*. *PLoS Genet.* 16, e1008745 (IF: 5.45)

Manuscripts accepted for publication in 2021

A. Biratsi, A. Athanasopoulos, V. N. Kouvelis, C. Gournas, and V. Sophianopoulou. (2021) A highly conserved mechanism for the detoxification and assimilation of the toxic phytoproduct L-azetidine-2-carboxylic acid in *Aspergillus nidulans*. *Scientific Reports* (IF: 4.38)

Other Scientific activities

Participation in Greek and international meetings and federations:

- Head of the Microbial Molecular Genetics lab of IBA (2004-today) (V. Sophianopoulou).
- Member of the Evaluation Committee for the recruitment of Collaborating Researchers at IBA within the Industrial Research Fellowship Program of Stavros Niarchos at NCSR “Demokritos” (V. Sophianopoulou).
- Member of the editorial board of *Scientific Reports* (Published by Springer Nature) (V. Sophianopoulou)
- Member of the Advisory Committee of the department of Biology, NKUA, for the PhD thesis of A. Biratsi (V. Sophianopoulou).
- Members of the Advisory Committee of the department of Biology, NKUA, for the PhD thesis of A. Megarioti (V. Sophianopoulou, C. Gournas).
- Member of the Co-ordination Committee of the Post-graduate Education Course (MSc) on “Applied Biochemistry: Clinical Chemistry, Biotechnology, Evaluation of Pharmaceutical Products” at the Department of Chemistry, University of Patras (2018-2020) (V. Sophianopoulou).
- Member of the Interdepartmental Committee of the Post-graduate Education Course (MSc) on “Applied Biochemistry: Clinical Chemistry, Biotechnology, Evaluation of Pharmaceutical

Products” at the Department of Chemistry, University of Patras and Institute of Biosciences and Applications NCSR “D” (2018-2020) (V. Sophianopoulou).

- Member of the Evaluation Committee for the recruitment of post-graduate students within the Post-graduate Education Course (MSc) on “Applied Biochemistry: Clinical Chemistry, Biotechnology, Evaluation of Pharmaceutical Products” at the Department of Chemistry, University of Patras (2018-2020) (V. Sophianopoulou).
- Responsible for the practical training of students from the University of Patras, within the program “Competitiveness, Entrepreneurship and Innovation” 1/08/20-30/09/20 (V. Sophianopoulou).
- Responsible for the practical training of students from the University of Patras, within the program “Competitiveness, Entrepreneurship and Innovation”. 17/09/20-17/11/20 (C. Gournas).
- Member of the Advisory Committee for the Master’s thesis of A. Vallianatos, Biology Department, University of Athens (V. Sophianopoulou).
- Member of the Advisory Committee for the Dipoma thesis of S. Gaitanos, Biology Department, University of Athens (V. Sophianopoulou).
- Member of the Advisory Committee for the Dipoma thesis of T. Zakopoulou, Chemistry Department, University of Athens (V. Sophianopoulou and C. Gournas).
- Member of the Examination Committee for the Doctoral thesis of Georgia Papadaki, entitled “Mechanisms of subcellular membrane trafficking”. Department of Biology, NKUA (C. Gournas).
- PC member of the 11th Hellenic Conference on Artificial Intelligence (AI) (SETN 2020) Workshop titled “AI in Natural Sciences and Technology”, September 2 & 4 - Athens (V. Sophianopoulou, A. Athanasopoulos).
- Participation at the Teacher Training seminar, co-organised by the IBA and the European Learning Laboratory for the Life Sciences (ELLS) of the European Molecular Biology Laboratory (EMBL) (V. Sophianopoulou and C. Gournas).
- Deputy member of the Electoral Body for the tenure of Assistant Professor, University of West Attica, School of Health and Welfare Sciences, Department / Institute of Biomedical Sciences (V. Sophianopoulou).
- Deputy member of the Electoral Body for the tenure of Assistant Professor, of the School of Plant Sciences, Agricultural University of Athens, with the subject "Microbiology - Biotechnology of Microorganisms" (V. Sophianopoulou).

Referee of peer-review articles published in International Journals
Biomolecules, MDPI (V. Sophianopoulou)

FEMS letters, Oxford Academic; Biology of the Cell, Wiley (C. Gournas)

Other lectures or presentations of scientific content (except research conferences)

Teacher Training seminar, co-organised by the IB-A and the European Learning Laboratory for the Life Sciences (ELLS) of the European Molecular Biology Laboratory (EMBL), 11/9-13/9 (V. Sophianopoulou and C. Gournas)

Distinctions and Awards

- Invitation to join the Editorial Board of International Journal of Molecular Sciences, as Topic Editor (V. Sophianopoulou).
- Our article “On the Evolution of Specificity in Members of the Yeast Amino Acid Transporter Family as Parts of Specific Metabolic Pathways [Link: http://www.mdpi.com/1422-0067/19/5/1398](http://www.mdpi.com/1422-0067/19/5/1398) on 13/11/20 has been viewed more than 1910 times and downloaded 1422 times.

Training activities

Post-graduate Education Courses on:

- “Applied Biochemistry: Clinical Chemistry, Biotechnology, Evaluation of Pharmaceutical Products” at the Department of Chemistry, University of Patras (7 students, V. Sophianopoulou 3 hours, C. Gournas 2 hours).
- “Molecular Biology: Systemic and *in silico* Approaches”, a Graduate Mandatory Course of the Interdisciplinary (Faculty of Biology and Medical School, National and Kapodistrian University of Athens. Two - Years Graduate Program “Applications of Biology in Medicine” (20 students, 4 hours V. Sophianopoulou)

Other activities at the IBA and the NCSR “D”

Lecture at the 55th Summer School of NCSR “D” entitled “Investigation the compartmentalization of the plasma membrane, aiming to develop a new generation of antifungal drugs” (V Sophianopoulou).

Total Impact Factor for original publications in 2020

5. 45 (1 publication)

Bibliographic references for 2020 (without self-citations)

V. Sophianopoulou: 100

C. Gournas: 124

Total citations 2016-2020 (without self-citations)

V. Sophianopoulou: 341

C. Gournas: 406

h-factor

V. Sophianopoulou: 20

C. Gournas: 13

Current external funding

Grant entitled “Plasma membrane organization in quiescence”, by the Fondation Santé - Biomedical Research Grants

Scientific coordinator: C. Gournas

Duration: 02/2019 - 10/2021

Total funding for the laboratory: 50.000 €

Funding of the laboratory during 2020: 18.649,79 €

Grant entitled “Study of the role of eisosomal proteins in the quiescent state of fungi”, funded by the ministry of finance and development (Support in researchers, emphasizing on new Researchers, 2nd cycle).

Scientific coordinator: C. Gournas; Academic advisor: V. Sophianopoulou

Duration: 04/2020-01/2022

Total funding for the laboratory: 45.500 €

Funding of the laboratory during: 24.029,34 €

Research Group: Biophysics and Membrane Biotechnology

Research Staff

Kostas Stamatakis, Research Director

George C. Papageorgiou, Collaborating Scientist† (3-11-20)

Merope Tsimilli-Michael, Collaborating Scientist

Dimitris Vayenos, PhD candidate

Panayiotis Broussos, PhD candidate

Aikaterini Giakoumidaki-Vogiatzi, PhD candidate

Research Interests

Photosynthetic cyanobacteria are preferable candidates for the sequestration of large quantities CO₂ from the atmosphere because they can grow in extreme or/and specified environmental conditions (temperature, pressure, salinity, pH, chemical composition) and are capable of binding CO₂ to produce high energy chemical compounds using sunlight. The rapidly growing cyanobacteria constitute a very promising and CO₂ emission-free source for biofuels production which can substitute for other carbon-dependent natural sources of energy. The production and accumulation of sucrose in cyanobacteria is connected to their adaptation to extreme environmental conditions. Our research addresses the following themes:

(a) We investigate the production of hydrogen (H₂) by cyanobacteria through the process of anaerobic "dark fermentation" of the sucrose they accumulated under salinity stress.

(b) Terpenes are the largest group of secondary metabolites and are used by industry (production of drugs, cosmetics, food technology). Study of the production of terpenes from genetically modified strains of the cyanobacterium *Synechocystis* sp PCC 6813 (S6813) capable of producing terpenes. They are synthesized from acetyl-coenzyme-A (acetyl-CoA) or from glycolysis intermediates.

(c) We study, also, the time dependent changes of chlorophyll α fluorescence (F_{Chla}), or fluorescence induction, in cyanobacteria, giving emphasis to the distribution of the electronic excitation to the reaction centers of photosystem I (PSI) and photosystem II (PSII), as an indicator of protective mechanism against the destructive effects of the reactive oxygen species (ROS) that are produced during photosynthesis.

(d) We study the photosynthetic apparatus of the haptophyte *Phaeocystis antarctica* and of a novel Ross Sea dinoflagellate (RSD) that hosts *P. antarctica* chloroplasts as kleptoplasts. Both algae are dominant in the phytoplankton of Ross Sea in Antarctica. Our research led to the discovery of the kleptoplasty.

(e) We have developed a new method for the evaluation and assessment of antibacterial properties of materials and surfaces by means of time-dependent changes of F_{Chla} (Patent No. OBI 20140100263/02.05.2014). Specifically, the method relates F₀, the initial value of F_{Chla} kinetic trace (OJIP), upon a transition from darkness to continuous light of cyanobacteria. Since cyanobacteria are gram negative, we can use them as a guide for antibacterial assay.

2020 Findings

Sucrose, a compatible osmolyte in cyanobacteria, functions both as an energy reserve and as osmoprotectant. Sugars are the most common substrates used by microorganisms to produce hydrogen (H₂) by means of anaerobic dark fermentation. Cells of the unicellular, non-nitrogen fixing, freshwater cyanobacterium *Synechococcus elongatus* PCC7942 accumulate sucrose under salt stress. In the present work, we used this cyanobacterium and a genetically engineered strain of it (known as PAMCOD) to investigate the optimal conditions for (a) photosynthetic activity, (b) cell proliferation and (c) sucrose accumulation, which are necessary for H₂ production *via* anaerobic

dark fermentation of the accumulated sucrose. PAMCOD (Deshnium et al. in *Plant Mol Biol* 29:897–902, 1995) contains the gene *codA* that codes for choline oxidase, the enzyme which converts choline to the zwitterion glycine betaine. Glycine betaine is a compatible osmolyte which increases the salt tolerance of *Synechococcus elongatus* PCC7942. Furthermore, glycine betaine maintains cell proliferation under salt stress and results in increased sucrose accumulation. In the present study, we examine the environmental factors, such as the NaCl concentration, the culture medium pH, and the carbon dioxide content of the air bubbled through it. At optimal conditions, sucrose accumulated in the cyanobacteria cells up to 13.5 mol per mole Chl α . Overall, genetically engineered *Synechococcus elongatus* PCC7942 produces sucrose in sufficient quantities such that it may be a viable alternative (a) to sucrose synthesis, and (b) to H₂ formation via anaerobic dark fermentation.

Viscose fabrics were modified with alginate and doped with copper ions, to investigate the effect of copper doping on their antibacterial and UV blocking properties. The modified fabrics were characterized using FTIR, UV spectroscopy, SEM and chromatometric analysis and their copper uptake properties were investigated. The antibacterial effect of the treatments was evaluated before and after repeated washings (up to 50 cycles). The resulting composite materials showed excellent antibacterial properties with up to 100 % reduction of bacteria, even from very low copper content, while alginate/copper treatment increased the UV radiation protection index by 261 %, offering a composite fabric with improved performance.

Oxidized multi-walled carbon nanotubes (oxCNTs) were functionalized by a simple non-covalent modification procedure using quaternized hyperbranched poly(ethyleneimine) derivatives (QPEIs), with various quaternization degrees. Structural characterization of these hybrids using a variety of techniques, revealed the successful and homogenous anchoring of QPEIs on the oxCNTs' surface. Moreover, these hybrids efficiently dispersed in aqueous media, forming dispersions with excellent aqueous stability for over 12 months. Their cytotoxicity effect was investigated on two types of gram(-) bacteria, an autotrophic (cyanobacterium *Synechococcus* sp. PCC 7942) and a heterotrophic (bacterium *Escherichia coli*). An enhanced, dose-dependent antibacterial and anti-cyanobacterial activity against both tested organisms was observed, increasing with the quaternization degree. Remarkably, in the photosynthetic bacteria it was shown that the hybrid materials affect their photosynthetic apparatus by selective inhibition of the Photosystem-I electron transport activity. Cytotoxicity studies on a human prostate carcinoma DU145 cell line and 3T3 mouse fibroblasts revealed that all hybrids exhibit high cytocompatibility in the concentration range, in which they also exhibit both high antibacterial and anti-cyanobacterial activity. Thus, QPEI-functionalized oxCNTs can be very attractive candidates as antibacterial and anti-cyanobacterial agents that can be used for potential applications in the disinfection industry, as well as for the control of harmful cyanobacterial blooms.

Publications

Heliopoulos S. N., Kouzilos N. G., Papageorgiou S. K., Stamatakis K., Katsaros F. K. (2020) Viscose fabric functionalized with Copper and Copper Alginate treatment toward Antibacterial and UV blocking Properties. *Fibers and Polymers* 21: 1238-1250 (IF 1.797)

Vayenos D., Romanos Em. G., Papageorgiou G. C., Stamatakis K. (2020) The freshwater cyanobacterium *Synechococcus* sp. PCC7942 under salt stress: A cell factory for sucrose and hydrogen production. *Photosynth. Research* 146: 235-245 (IF 3,216)

Heliopoulos S. N., Kythreoti G., Lyra K.M., Panagiotaki N.K., Papavasiliou A., Sakellis E., Papageorgiou S., Kouloumpis A, Gournis D., Katsaros K.F., Stamatakis K., Sideratou Z. (2020) Cytotoxicity effects of water-soluble multi-walled carbon nanotubes decorated with quaternized hyperbranched poly(ethyleneimine) derivatives on autotrophic and heterotrophic Gram-Negative bacteria. *Pharmaceuticals* 2020, 13: 293 (IF 4.616)

Kefallinou, D., Ellinas, K., Speliotis Th., Stamatakis, K., Gogolides, E., Tserepi. A. (2020) Optimization of antibacterial properties of “hybrid” metal-sputtered superhydrophobic coatings, 10, 25; doi:10.3390/coatings10010025Surfaces (IF 2.436).

Abstracts in Greek Conferences

Broussos, P-I., Romanos, G.E. Stamatakis, K (2020). Study of Hydrogen Production by Cyanobacteria. 1st Panhellenic Scientific Meeting of Plant Physiologists February 7-8, 2020, Athens, Greece

Giakoumidaki-Voyiatzi A., Melis A., Stamatakis K. (2020). Study of Photosynthesis of cyanobacteria capable of producing terpenes. 1st Panhellenic Scientific Meeting of Plant Physiologists February 7-8, 2020, Athens, Greece

Stamatakis K., Broussos, P-I., Giakoumidaki-Voyiatzi A., Papageorgiou G.C. (2020) Studies of photosynthesis and its applications. 1st Panhellenic Scientific Meeting of Plant Physiologists February 7-8, 2020, Athens, Greece

Member in Scientific committees

Member of the International Organizing Committee of the 11th International Meeting “Photosynthesis and Hydrogen Energy Research for Sustainability”.

Impact factor (3 publications): 9.629

Citations in 2020 (without self-citations): 69 (Scopus), 92 (Google Scholar)

Total Citations in 2015 - 2020 (without self-citations): 312 (Scopus), 346 (Google Scholar)

h-factor: 13 (Scopus), 16 (Google Scholar)

Current External Funding

Partnership and Cooperation Agreement (NSRF) 2014-2020, European Regional Development Fund (ERDF) Development of New Innovative Energy Technologies of Low Carbon Footprint for the Enhancement of Excellence in the Region of Western Macedonia – SUB-PROJECT 01_uowm,. MIS code 5047197 of The Operational Program “Competitiveness, Entrepreneurship & Innovation” (EPAnEK) co-financed by Greece and the European Union.

Principal Investigator Prof. Amanatidou Elisavet.

Funding: 10000 €

PROGRAMME C

“STRUCTURAL AND COMPUTATIONAL BIOLOGY”

Research Group: Theoretical Biology and Computational Genomics

Research Staff

Yannis Almirantis, Research Director

Spyros Papageorgiou, Collaborating Scientist

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 and at the “middle” length scale: ‘word’ use, clustering of similar nucleotides etc. Patterns related to the functionality of genomic regions and to the global genome structure. Distinction of coding and non-coding segments.
- Long range correlations and Zipf laws in the genome structure. Power laws in the distribution of exons, transposable elements, CpG-islands, CNEs and of other genomic elements.
- 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.

Foundations of Science – Epistemology of the limits

- Modern science and traditional knowledge – Convergence and interactions.
- Convergence and interactions between the sciences and humanities.
- Causality and extended causality. From Aristotle to C.G.Jung – Meaningfulness and non-causal correlation.
- Epistemic consequences of alternative medicine – Foundations of homeopathy.

2020 Findings

We revisit the topic of human genome guanine-cytosine content under neutral evolution. For this study, the de novo mutation data within human is used to estimate mutational rate instead of using base substitution data between related species. We then define a new measure of mutation bias which separate the de novo mutation counts from the background guanine-cytosine content itself, making comparison between different datasets easier. We derive a new formula for calculating limiting guanine-cytosine content by separating CpG-involved mutational events as an independent variable. Using the formula when CpG-involved mutations are considered, the guanine-cytosine content drops less severely in the limit of neutral dynamics. We provide evidence, under certain assumptions, that an isochores-like structure might remain as a limiting configuration of the neutral mutational dynamics.

Publications

Papageorgiou S. (2020). Hox gene collinearity may be related to noether theory on symmetry and its linked conserved quantity. *J (MDPI)* 3(2), 151-161.

Other Scientific activities

Reviewer of scientific papers

BMC Bioinformatics, Bioinformatics, GENE, BMS-CBIO, Journal of Theoretical Biology, OBM Integrative and Complementary Medicine, Royal Society Open Science.

Publication in arXiv

Li W., Almirantis Y., Provata A. (2020). Revisiting the Neutral Dynamics Derived Limiting Guanine-Cytosine Content Using the Human De Novo Point Mutation Data. arXiv preprint arXiv:2008.01781, - arxiv.org

Teaching

- Teaching (15H) 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 (3H) 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.

Impact Factor (for 1 publication): -

Citations 2020 (without self- citations): 77

Total Citations 2015-2020 (without self-citations): 286

h-factor: 15 (Scopus)

Research Group: Designed Bioactive Molecules Laboratory

Research Staff

Athanasios Papakyriakou, Senior Researcher

Anastasia Mpakali, Postdoctoral Fellow

Lykourgos Chiniadis, PhD Student

Alexandros Athanasoulis, PhD Student

Alexandros Kalampalidis, PhD Student

Research Interests

Design and synthesis of inhibitors for the M1 family of zinc aminopeptidases and structure-based discovery of allosteric inhibitors of the insulin-regulated aminopeptidase (IRAP) in collaboration with the group of Dr. Efstratios Stratikos.

Biochemical study of LMTK3 kinase activity using small molecule inhibitors in conjunction with computational study of their interaction and biophysical study of the interaction between LMTK3 inhibitors and β -tubulin (Collaboration with Prof. George Giamas, University of Sussex, UK)

Design of bioactive compounds based on the structures of β -adrenoreceptors (Collaboration with Prof. Barbara Richichi, University of Florence, Italy).

Computational study of the stabilization mechanism of PIN1 isomerase via phosphorylation by JNK kinases (Collaboration with Prof. Salvatore Papa, University of Leeds, UK).

Crystallographic study of Ru(II)-based complexes using model proteins and molecular dynamics studies of acetylcholine receptors in complex with toxins (Collaboration with Dr. Petros Giastas, Agricultural University of Athens, Greece).

2020 Findings

The design and synthesis of zinc aminopeptidase inhibitors based on the β -amino- α -hydroxy acid scaffold of bestatin has revealed several nanomolar inhibitors which can be optimized as selective inhibitors for ERAP2.

The mechanism of PIN1 stabilization via phosphorylation by JNK has been elucidated (Lepore A. et al. *Hepatology* 2021) and the low affinity of an LMTK3 kinase for α -/ β -tubulin dimers has been established using biophysical and computational methods (Cilibrasi C, et al. *Mol Cancer* 2021).

Small molecular weight inhibitors of IRAP with significant selectivity against the homologous ERAP1/ERAP2 enzymes have been discovered through structure-based virtual screening. One compound has been shown to inhibit the processing of the natural substrate oxytocin in the absence of measurable inhibition of small substrates (Temponeras I. et al. *Pharmaceuticals* 2021).

The crystallographic study of the antimetastatic Ru(II) compound NAMI-A in complex with hen egg white lysozyme has produced 4 crystal structures at near-atomic resolution, which contribute to the investigation of the protein ruthenation selectivity and mechanism of action via the time-dependent hydrolysis of the Ru(II) ligands (Chiniadis L. et al. *Inorganic Chemistry* 2021).

The preparation and publication of 2 manuscripts for the design, synthesis and biochemical evaluation of novel RANKL trimerization inhibitors (Rinotas V. et al. *J. Med. Chem.* 2020), and for the design, synthesis and biochemical evaluation of sphingosine kinase SK1 inhibitors (Papakyriakou A. et al. *ACS Med. Chem. Lett.* 2020) have been completed.

Publications

Ditsiou A, Cilibrasi C, Simigdala N, Papakyriakou A, [...] Prodromou C, Klinakis A, Stebbing J, Giamas G. (2020) The structure-function relationship of oncogenic LMTK3. *Science Advances* 6(46):eabc3099. Doi: 10.1126/sciadv.abc3099. (IF: 13.1)

Rinotas V, Papakyriakou A, Violitzi F, [...] Couladouros E, Kontopidis G, Eliopoulos E, Douni E. (2020) Discovery of Small-Molecule Inhibitors of Receptor Activator of Nuclear Factor- κ B Ligand with a Superior Therapeutic Index. *Journal of Medicinal Chemistry* 63(20):12043-12059. (IF: 6.2)

Papakyriakou A, Cencetti F, Puliti E, Morelli L, Tricomi J, Bruni P, Compostella F, Richichi B. (2020) Glycans Meet Sphingolipids: Structure-Based Design of Glycan Containing Analogues of a Sphingosine Kinase Inhibitor. *ACS Medicinal Chemistry Letters* 11(5):913-920. (IF: 4.0)

Mavridis G., Arya R., Domnick A., Zoidakis J., M. [...] Georgiadis D, Tampé R., Papakyriakou A., Stern L.J., and Stratikos E. (2020) A systematic re-examination of processing of MHC-I-bound antigenic peptide precursors by ER aminopeptidase 1. *Journal of Biological Chemistry*, 295(21):7193-7210. (IF: 4.2)

Chiniadis L., Bratsos I., Bethanis K., Karpusas M., Giastas P and Papakyriakou A. (2020) High-resolution crystal structures of a “half sandwich”-type Ru(II) coordination compound bound to hen egg-white lysozyme and proteinase K. *Journal of Biological Inorganic Chemistry*, 25(4):635-645. (IF: 3.2)

Articles in Press

Lepore A, Choy PM, Lee NC, Carella MA, [...] Papakyriakou A, Giamas G, Bubici C, Papa S. (2021) Phosphorylation and stabilization of PIN1 by JNK promote intrahepatic cholangiocarcinoma growth. *Hepatology* May 28. Doi: 10.1002/hep.31983. (IF: 14.7)

Cilibrasi C, Ditsiou A, Papakyriakou A, Mavridis G, Eravci M, Stebbing J, Gagliano T, Giamas G. (2021) LMTK3 inhibition affects microtubule stability. *Molecular Cancer* 20(1):53. Doi: 10.1186/s12943-021-01345-3. (IF: 15.3)

Kalampalidis, A., Peppas, A., Schnakenburg, G., Papakyriakou, A., Tsoupras, A., Zabetakis, I., Philippopoulos, A.I. (2021) Antithrombotic and antiplatelet activity of an organometallic rhodium(I) complex incorporating a substituted thieno-[2,3-d]-pyrimidine ligand: Synthesis, structural characterization, and molecular docking calculations. *Applied Organometallic Chemistry*, 35 (6), art. No. e6210, doi: 10.1002/aoc.6210. (IF: 3.1)

Presentations at Scientific Conferences

A. Temponeras, L. Chiniadis, A. Papakyriakou and E. Stratikos, “Discovery of selective inhibitor leads by targeting an allosteric site in insulin-regulated aminopeptidase”, Poster at the 6th International Electronic Conference on Medicinal Chemistry, 1–30 Nov. 2020

Other Scientific Activities

Member of the Evaluation Panel of the Hellenic Foundation for Research & Innovation (ELIDEK)

Journal referee in *Molecules*, *International Journal of Molecular Sciences*, *Pharmaceuticals*, *Marine Drugs* (MDPI)

Treasurer of the Hellenic Crystallographic Association (HeCrA)

Other lectures or scientific presentations

A. Papakyriakou, “Structure-based discovery of bioactive molecules and computational study of protein dynamics” 4th February 2020, School of Medicine, University of Patras, Greece.

Teaching activities

Interdepartmental Program for Postgraduate Studies of IBE and the Department of Chemistry, University of Patras; Molecular Pharmacology, 2nd Semester 2020 (8 hours).

Other Activities at the NSCR “Demokritos”

Member of the Scientific Advisory Committee of “Lefkippos” Technology Park in NCSR.

Total Impact Factor for publications in 2020: 30.7

Citations for 2020 (excluding self- citations): 200 (Scopus)

Total Citations 2015-2020 (excluding self- citations): 780 (Scopus)

h-factor: 23 (Google scholar), 20 (Scopus)

Current External Funding

Research grant *ARIA “Atomic Resolution Insight into the Antigen processing machinery”*, funded by from the Hellenic Foundation for Research & Innovation and GSRT (Coordinator: A. Papakyriakou)

Duration: 11/10/2018 – 10/10/2021

Total Program Funding: 180.000€

Funding of the lab for 2020: 60.000€

Research Group: Structural Studies of Biomolecules and Pharmaceuticals with NMR

Research Staff

Maria Pelecanou, Research Director

Marina Sagnou, Researcher

Angeliki Panagiotopoulou, Functional Scientist B

Barbara Mavroidi, Postdoctoral Fellow (MSc, PhD)

Eleftherios Halevas, Postdoctoral Fellow (MSc, PhD)

Dimitris Matiadis, Postdoctoral Fellow (PhD)

Valentinos Sofokleous, PhD candidate

Georgia Nigianni, Postgraduate student

Research interests

Development (design, synthesis, characterization, structural analysis, biological evaluation) of new compounds or pharmacologically active derivatives for the diagnosis and/or therapy of diseases, as well as for biotechnological and nanotechnological applications. Our main fields of research are Alzheimer's disease and cancer, and our tools are **NMR** and **CD spectroscopies** for structural studies, combined with methodologies of **organic synthesis, complexation/labeling, encapsulation, and biological evaluation** of the new compounds/agents in appropriate systems. Biological evaluation includes interaction studies with biomolecules, biochemical, microscopic and cellular assays in cancer and primary cell lines, as well as *in vivo* biodistribution in experimental animals in collaboration with INRASTES.

Currently, our research activity focuses on:

- pharmacophoric molecules/bioactive natural products (such as 2-(4'-aminophenyl)-benzothiazole, curcumin, isatin, quercetin, chrysin, crocin), derivatives of these compounds with improved pharmacological properties, as well as their metal complexes (with copper, palladium, platinum, gallium, zinc, silver) for synergistic activity from the metal and the pharmacophoric ligand. The compounds are studied either free or encapsulated in nanostructures (nanoparticles, liposomes) for increased bioavailability and targeted release.
- complexes of rhenium and radioactive technetium-99m with appropriate ligands designed for targeted action for diagnosis with SPECT and/or therapy of widespread diseases, in collaboration with INRASTES. Within the same framework, gallium complexes for targeted PET diagnosis of tumours.
- inhibitors of the aggregation process of the β -amyloid peptide ($A\beta$) of Alzheimer's disease to cytotoxic oligomeric and polymeric forms. The effect of potential inhibitors (natural products, synthetic organic molecules, neuroprotective peptides, biosynthetic cyclic peptides), as well as targeted liposomes, on the aggregation process of $A\beta$ is studied with CD, but also through the reduction of the $A\beta$ toxicity (rescue effect) in primary neuronal cells and neuronal cancer cell lines.

2020 Findings

In 2020 the activity of the group in the utilization of natural products for the development of novel bioactive compounds continued, with the fruitful and productive contribution of the post-doctoral fellows D. Matiadis (organic synthesis), E. Halevas (complexation, nanoencapsulation) and B. Mavroidi (chemical analysis, biological evaluation). Representative examples include:

- synthesis of a series of pyrazoline derivatives from monocarbonyl curcumin analogues and of their silver complexes with significant antifungal ($< 0.25 \mu\text{g/mL}$) and antimicrobial activity
- evaluation of selected pyrazoline derivatives from monocarbonyl curcumin analogues for DNA interaction

- synthesis and crystallographic analysis of novel vanadium-curcumin and gallium-chrysin complexes and their encapsulation in cationic liposomal nanoparticles and magnetic nanoparticles for anticancer action
- synthesis and crystallographic characterization of novel copper-curcumin, copper-quercetin, copper-chrysin and zinc-chrysin complexes and evaluation of their antioxidant, genotoxic, and cytotoxic activity
- synthesis and biological evaluation of novel dendrimeric nanoparticles with encapsulated artemisinin

During 2020, following the Greek patent (OBI#1009516) and the approved international application under the Patent Cooperation Treaty (#PCT/EP2019/057235) regarding our findings on the cyclopentadienyl-bearing benzothiazole and benzimidazole complexes with radioactive ^{99m}Tc and its non-radioactive Re counterpart, which present remarkable blood-brain barrier (BBB) penetration and ideal properties for diagnosis (^{99m}Tc) and therapy (Re) of Alzheimer's disease (AD), we entered the national PCT phase in Europe, USA, China, and India. In the same field of AD, the evaluation of derivatives of the natural indole isatin as neuroprotective agents with CD, cellular assays, and electronic microscopy was published, while mechanistic investigations are being conducted in primary neuronal cells from transgenic 5xFAD mice. Furthermore, the evaluation with CD and thioflavin T assay of phenylbenzothiazole decorated liposomes with BBB penetration potential as inhibitors of the A β aggregation process was also published. In the field of anticancer applications, the synthesis and evaluation of curcuminoids as photosensitisers in prostate cancer photodynamic therapy was published in collaboration with NTUA. At the stage of manuscript preparation is the synthesis and evaluation of ^{99m}Tc and Re complexes bearing phenylbenzothiazole derivatives against breast cancer.

Taking advantage of the IB-A infrastructure, our activity is also expanding to new areas through collaborations, with characteristic examples the:

- confocal microscopy study of selective staining of systemic amyloidosis tissue for diagnostic applications (Prof. I. Andreadou, School of Pharmacy, and Assoc. Professor E. Kastiris, School of Medicine, NUA)
- CD study of the aggregation process of light chain polypeptides responsible for Amyloid Light-chain (AL) amyloidosis, (Prof. I. Andreadou, School of Pharmacy, NUA)
- application of metabolomics in the study of the effect of pesticides on native and cultivated plants of the Greek flora (Assistant Prof. K Aliferis, AUA)
- development of quantitative NMR methods (q-NMR) for application in purity assessment of organic compounds, antibiotics, hormones (Chemical Metrology Lab, General Chemical State Laboratory)
- confocal microscopy study of novel porphyrin and cyclodextrin nanomaterials for photodynamic therapy of tumours (Dr. K. Yannakopoulou, INN)

Publications

Kazantzis, K. T., Koutsonikoli, K., Mavroidi, B., Zachariadis, M., Alexiou, P., Pelecanou, M., Politopoulos, K., Alexandratou, E., Sagnou, M. (2020). Curcumin derivatives as photosensitizers in photodynamic therapy: photophysical properties and in vitro studies with prostate cancer cells. *Photochem. Photobiol. Sci.* 19, 193-206 (IF: 2.831)

Sagnou, M., Novikov, F. N., Ivanova, E. S., Alexiou, P., Stroylov, V. S., Titov, I. Y., Tatarskiy, V. V., Vagida, M. S., Pelecanou, M., Shtil, A. A., & Chilov, G. G. (2020). Novel curcumin derivatives as P-glycoprotein inhibitors: Molecular modeling, synthesis and sensitization of multidrug resistant cells to doxorubicin. *Eur J Med Chem*, 198, 112331 (IF: 5.572)

Matiadis, D., Sagnou, M. Pyrazoline Hybrids as Promising Anticancer Agents: An Up-to-Date Overview. *Int. J. Mol. Sci.* 2020, 21, 5507 (IF: 5.923)

Halevas, E., Mavroidi, B., Antonoglou, O., Hatzidimitriou, A., Sagnou, M., Pantazaki, A. A., Litsardakis, G., Pelecanou, M. (2020). Structurally characterized gallium-chrysin complexes with anticancer potential. *Dalton Trans.* 49, 2734-2746 (IF: 4.174)

Matiadis, D., Mavroidi, B., Panagiotopoulou, A., Methenitis, C., Pelecanou, M. Sagnou, M. (2020). (E)-(1-(4-Ethoxycarbonylphenyl)-5-(3,4-dimethoxyphenyl)-3-(3,4-dimethoxystyryl)-2-pyrazoline: Synthesis, Characterization, DNA-Interaction, and Evaluation of Activity Against Drug-Resistant Cell Lines. *Molbank*, 1, M1114 (IF: 0.54)

Salvanou, E. A, Stellas, D., Tsoukalas, C., Mavroidi, B., Paravatou-Petsotas, M., Kalogeropoulos, N., Xanthopoulos, S., Denat, F., Laurent, G., Bazzi, R., Roux, S., Bouziotis, P. (2020). A Proof-of-Concept Study on the Therapeutic Potential of Au Nanoparticles Radiolabeled with the Alpha-Emitter Actinium-225. *Pharmaceutics*. 12, E188. (IF: 6.07)

Halevas, E., Pekou, A., Papi, R., Mavroidi, B., Hatzidimitriou, A. G., Zahariou, G., Sanakis, Y., Litsardakis, D., Sagnou, M., Pelecanou, M., Pantazaki, A. A. (2020). Synthesis, physicochemical characterization and biological properties of two novel Cu(II) complexes based on natural products curcumin and quercetin. *J. Inorg. Biochem.* 208, 111083 (IF: 3.94)

Sagnou, M., Mavroidi, B., Kaminari, A., Boukos, N., Pelecanou, M. (2020). Novel Isatin Thiosemicarbazone Derivatives as Potent Inhibitors of β -Amyloid Peptide Aggregation and Toxicity. *ACS Chem. Neurosci.* 11, 2266-2276 (IF: 4.486)

Halevas, E., Mavroidi, B., Nday, C.M., Tang, J., Smith, G.C., Boukos, N., Litsardakis, G., Pelecanou, M., Salifoglou, A. (2020). Modified magnetic core-shell mesoporous silica nano-formulations with encapsulated quercetin exhibit anti-amyloid and antioxidant activity. *J. Inorg. Biochem.* 213, 111271 (IF: 3.94)

Mourtas, S., Mavroidi, B., Marazioti, A., Kannavou, M., Sagnou, M., Pelecanou, M., Antimisiaris, S.G. (2020). Liposomes Decorated with 2-(4'-Aminophenyl)benzothiazole Effectively Inhibit A β 1-42 Fibril Formation and Exhibit in Vitro Brain-Targeting Potential. *Biomacromolecules*, 21, 4685-4698 (IF: 6.46)

Liolios, C., Shegani, A., Roupa, I., Kiritsis, Christos., Makarem, A., Paravatou-Petsotas, M., Pelecanou, M., Bouziotis, P., Papadopoulos, M., Kopka, K., Pirmettis, I. (2020). Synthesis, characterization and evaluation of ⁶⁸Ga labelled monomeric and dimeric quinazoline derivatives of the HBED-CC chelator targeting the epidermal growth factor receptor. *Bioorg. Chem.* 100, 103855 (IF: 4.831)

Matiadis D., Tsironis D., Stefanou V., Boussias S., Panagiotopoulou A., McKee V., Igglessi-Markopoulou O., Markopoulos J. (2020). Synthesis, biological evaluation and structure-activity relationship of novel 5-arylidene tetramic acid derivatives with antibacterial activity against methicillin-resistant *Staphylococcus aureus*. *Bioorg. Med. Chem. Lett.* 30(10):127107 (IF: 2.82)

Kollatos, N., Mitsos, C., Manta, S., Tzioumaki, N., Giannakas, C., Alexouli, T., Panagiotopoulou, A., Schols, D., Andrei, G., Komiotis, D. (2020) Design, Synthesis, and Biological Evaluation of Novel C5-Modified Pyrimidine Ribofuranonucleosides as Potential Antitumor or/and Antiviral Agents. *Med Chem.* 16(3), 368-384 (IF: 2.53)

Kollatos, N., Mitsos, C., Karetso, R., Antonaki, S.D., Panagiotopoulou, A., Tzioumaki, N., Manta, S., Schols, D., Komiotis, D. (2020). Design, Synthesis, and Biological Evaluation of Novel C5-Modified Pyrimidine Acyclic Nucleosides. *J Pharm Drug Res.* 3(1): 257-267 (IF: -)

Ntatsi, G., Aliferis, K.A, Panagiotopoulou, A., Roupheal, Y., Savvas, D. (2020) ¹H NMR metabolic profiling dataset of spiny chicory (*Cichorium spinosum* L.) exposed to abiotic stresses. *Data in Brief* 30,105622, 1-4 (IF: 1.13)

Articles in Press

Halevas, E., Mitrakas, A., Mavroidi, B., Athanasiou, D., Gkika, P., Antoniou, K., Samaras, G., Lialiaris, E., Hatzidimitriou, A., Pantazaki, A., Koukourakis, M., Sagnou, M., Pelecanou, M., Lialiaris, T. (2021). Structurally characterized copper-chrysin complexes display genotoxic and cytotoxic activity in human cells. *Inorganica Chim. Acta*, 515, 120062 (IF: 2.44)

Matiadis, D., Karagiaouri, M., Mavroidi, B., Nowak, K.E., Katsipis, G., Pelecanou, M., Pantazaki, A., Sagnou, M. (2021). Synthesis and antimicrobial evaluation of a pyrazoline-pyridine silver(I) complex: DNA-interaction and anti-biofilm activity. *BioMetals*, 34, 67-85 (IF: 2.949)

Halevas, E., Hatzidimitriou, A., Mavroidi, B., Sagnou, M., Pelecanou, M., Matiadis, D. (2021). Synthesis and structural characterization of (E)-4-[(2-hydroxy-3-methoxybenzylidene)amino]butanoic acid and its novel Cu(II) complex. *MolBank*, 1, 1179, 1-24. (IF: 0.54)

Halevas, E., Mavroidi, B., Pelecanou, M., Hatzidimitriou, A.G. (2021). Structurally characterized zinc complexes of flavonoids chrysin and quercetin with antioxidant potential. *Inorganica Chim. Acta*, 523, 120407 (IF: 2.44)

Halevas, E., Arvanitidou, M., Mavroidi, B., Hatzidimitriou, A.G., Politopoulos, K., Alexandratou, E., Pelecanou, M., Sagnou, M. (2021) A novel curcumin gallium complex as photosensitizer in photodynamic therapy: Synthesis, structural and physicochemical characterization, photophysical properties and *in vitro* studies against breast cancer cells. *J. Mol. Struct.* 1240, 130485 (IF: 2.011)

Halevas, E., Mavroidi, B., Kokotidou, Ch., Mitraki, A., Pelecanou, M., Sagnou, M., (2021) Advanced bis-MPA hyperbranched dendritic nanocarriers of artemisinin with anticancer potential, *J Nanopart Res*, accepted (IF: 2.132)

Matiadis, D., Stefanou, V., Tsironis, D., Panagiotopoulou, A., Igglesi-Markopoulou, O., Markopoulos, J. (2021). Synthesis and preliminary biological evaluation of antibacterial and antifungal 5-arylidene tetramic acid-cadmium(II) complexes. *Arch. Pharm.* 1-11, 2100305 (IF: 3.751)

Papasavva, A., Shegani, A., Kiritsis, C., Roupa, I., Ischyropoulou, M., Makrypidi, K., Pilatis, I., Loudos, G., Pelecanou, M., Papadopoulos, M., Pirmettis, I. (2021). Comparative study of a series of 99mTc(CO) 3 mannosylated dextran derivatives for sentinel lymph node detection. *Molecules*. 26 (16), 4797 (IF: 4.411)

Shegani, A., Ischyropoulou, M., Roupa, I., Kiritsis, C., Makrypidi, K., Papasavva, A., Raptopoulou, C., Psycharis, V., Hennkens, H. H., Pelecanou, M., Papadopoulos, M., Pirmettis, I. (2021). Synthesis and evaluation of new mixed "2 + 1" Re, 99mTc and 186Re tricarbonyl dithiocarbamate complexes with different monodentate ligands. *Bioorg. Med. Chem.* 47, 116373 (IF: 3.073)

Panagiotakis, S., Mavroidi, B., Athanasopoulos, A., Charalambidis, G., Coutsolelos, A. G., Paravatou-Petsotas, M., Pelecanou, M., Mavridis, I. M., Yannakopoulou, K. (2022). Unsymmetrical, monocarboxyalkyl meso-arylporphyrins in the photokilling of breast cancer cells using permethyl- β -cyclodextrin as sequesterant and cell uptake modulator. *Carbohydr. Polym.* 275, 118666 (IF: 9.381)

Granted patents applications in 2020

In 09/06/20 our international patent application within the Patent Cooperation Treaty (PCT/EP2019/057235, 22/03/19) entitled "*Tricarbonyl complexes of transition metals with benzo-heterocyclic derivatives of the cyclopentadienyl anion*" (inventors M. Pelecanou, M. Sagnou, B. Mavroidi (IB-A), M. Papadopoulos, I. Pirmettis, A. Shegani (INRASTES), regarding the remarkable BBB penetration potential of the tricarbonyl complexes of transition metals with benzo-heterocyclic derivatives of the cyclopentadienyl anion for application in diagnosis and therapy of the CNS diseases, was approved with all claims judged as **novel**, **patentable**, and of **industrial applicability**.

Entering the PCT national phase, the application was submitted for evaluation to the Patent Offices of Europe, USA, India, and China.

Other Research Activities

Review of scientific publications

Molecular Pharmaceutics (ACS), Radiation Research (Allen Press) (M. Pelecanou)

Molecules, Marine Drugs, Pharmaceuticals, Nutrients, International Journal of Molecular Science, Cancers, Inorganics, Catalyst, Foods – MDPI, Archiv der Pharmazie (Wiley) και Letters in Drug Design & Discovery – Bentham (M. Sagnou)

Journal of Biomolecular Structure and Dynamics (Taylor & Francis), Polyhedron (Elsevier) (A. Panagiotopoulou)

Educational Activities

Within IBA:

Participation in the NCSR “Demokritos” Summer School 2020: Presentation of the liquid sample NMR Spectroscopy (Principles and Applications of NMR spectroscopy) (A. Panagiotopoulou)

Lectures in the framework of the Interdisciplinary Postgraduate Program of the Faculty of Chemistry, University of Patras and the IB-A, NCSR “Demokritos” titled “Applied Biochemistry: Clinical Chemistry, Biotechnology, Evaluation of Pharmaceuticals”. Special options – drug development (semester 1); Molecular Pharmacology (DNA structure, DNA targeting drugs, RNA structure, RNA targeting drugs, molecular biology and genetic engineering) 8 students (M. Sagnou)

Member of the three-member committee:

Anna Pekou: Postgraduate student, Postgraduate Studies in Chemistry Program - Option “Biochemistry”, AUTH, Sep 2019-Feb 2020. Title: “Study of the biological activity of novel polyphenol-copper complexes” (M. Sagnou)

Karagiaouri Maria: Postgraduate student, Postgraduate Studies in Synthetic Chemistry, Biochemistry and Applications” Program - Option “Biochemistry”, AUTH, Sep 2019-Feb 2020. Title: “Studies of the effect of a silver complex with DNA metabolism enzymes and bacterial biofilm. Potential antimicrobial and antioxidant activity of propolis enriched polymers” (M. Sagnou)

Other Activities for the Institute of Biosciences & Applications

M. Pelecanou:

- Co-responsible (with M. Vlassi) for the operation of the circular dichroism spectrometer (CD) at IB-A (E-11811, Provincial Council No. 515, 12/6/2013)
- Co-responsible (with Lean. Leondiadis) for the operation of the NMR Laboratory of NCSR “Demokritos” (Internal Project E10949, Provincial Council No. 305 / Issue 18, 1/3/2002)

M. Sagnou:

- Member of the BIOIMAGING-GR monitoring group within the framework of the Infrastructure Roadmap together with H. Pratsinis, V. Lambropoulou
- Deputy education responsible

A. Panagiotopoulou

- Responsible for the Blood Bank of NCSR “Demokritos”
- Functional support of the CD spectropolarimeter and the 250 MHz and 500 MHz NMR spectrometers.

Total Impact Factor for 2020 publications

M. Pelecanou: 36.774

M. Sagnou: 28.385

A. Panagiotopoulou: 6.232

Citations 2020 (without self-citations):

M. Pelecanou: 97 (Scopus)

M. Sagnou: 65 (Scopus)

A. Panagiotopoulou: 24 (Scopus)

Total Citations 2015-2020 (without self-citations):

M. Pelecanou: 476 (Scopus)

M. Sagnou: 321 (Scopus)

A. Panagiotopoulou: 52 (Scopus)

h-factor:

M. Pelecanou: 21 (Scopus)

M. Sagnou: 15 (Scopus)

A. Panagiotopoulou: 6 (Scopus)

Current External Funding

Project entitled “Development of a radiodiagnostic agent for breast cancer”, funded by Stavros Niarchos Foundation and Biokosmos SA., within the framework of the Industrial Scholarship Program NCSR “Demokritos” - Stavros Niarchos Foundation

Duration: 3 years 2017-2020

Collaborating teams: M. Papadopoulos and I Pirmettis (INRASTES, NCSR “Demokritos”), Ch. Tsoukalas (Biokosmos SA)

Scientific Responsible: M. Pelecanou

Industrial Scholarship Fellow: B. Mavroidi

Total Program Funding: 65.459,88 €

Funding of the Lab for 2020: 10,909.98 €

Project entitled “Nature-loaded Targeted Nanoparticles for Prostate Cancer Therapy”, funded by Stavros Niarchos Foundation and Biohellenika S.A, within the framework of the Industrial Scholarship Program NCSR “Demokritos” - Stavros Niarchos Foundation

Duration: 3 years 2018-2021

Scientific Responsible: M. Sagnou

Industrial Scholarship Fellow: E. Halevas

Total Program Funding: 65.459,88 €

Funding of the Lab for 2020: 13,253.82 €

Project entitled “Greek Medicinal Plants for Photoprotection”, funded by Stavros Niarchos Foundation and PharmaGnose S.A. within the framework of the Industrial Scholarship Program NCSR “Demokritos” - Stavros Niarchos Foundation

Duration: 3 years 2020 -2023

Collaborating teams: Dr. A. Argiropoulou, PharmaGnose S.A

Scientific Responsible: M. Pelecanou

Industrial Scholarship Fellow: B. Mavroidi

Total Program Funding: 65.459,88 €

Funding of the Lab for 2020: 2.343,84 €

Project entitled “Innovative Radiopharmaceuticals with Fluoride-18: Research, Development and Introduction to the Greek Market for Diagnosis / Progression of Major Diseases with PET”, funded

by EREVNO-DIMIOURGO-KENOTOMO with I. Pirmettis (INRASTES, NCSR "D") as scientific coordinator. Responsible for IB-A: M. Pelecanou

Duration: 3 years 2017-2020

Collaborating teams: NCSR "Demokritos", Biokosmos SA

Total Program Funding: 998.075€

Funding of the Lab for 2020: 100.000 €

Project entitled "Development of a novel iatrotechnological product for effective wound healing (LIPOALCOPLAST)", funded by EREVNO-DIMIOURGO-KENOTOMO (2nd round) with G. Koliakos (Biohellenica SA) as scientific coordinator and in collaboration with POWERPHARM SA. Responsible for IB-A: M. Sagnou

Duration: 3 years

Total Program Funding: 713.727,16 €

Funding of the Lab for 2020: approved for funding, administrative clearance pending

Project entitled "Synthesis and characterization of magnetic dendrimeric nanocarriers with encapsulated flavonoid metal complexes for targeted anticancer activity" in the framework of "Supporting Researchers with Emphasis on Young Researchers - Round B" of the Operational Program "Human Resource Development, Education and Lifelong Learning" (ESPA 2014-2020) (EDULL 103).

Duration: 15 months

Young Researchers: Dr E. Halevas, Dr. B. Mavroidi

Scientific Coordinator: M. Pelecanou

Total Program Funding: 50.050 €

Project entitled "Monocarbonyl analogs of Curcumin (MAC) as larvae against the mosquito *Culex pipiens*" in the framework of "Supporting Researchers with Emphasis on Young Researchers - Round B" of the Operational Program "Human Resource Development, Education and Lifelong Learning" (ESPA 2014-2020) (EDULL 103).

Duration: 15 months

Young Researcher: Dr. D. Matiadis

Scientific Coordinator: Dr A. Michaelakis, Benaki Phytopathological Institute

Scientific responsible for IBA: M. Sagnou

Research Group: Protein Structure and Molecular Modeling

Research Staff

Metaxia Vlassi, Research Director

Nastazia Lesgidou, PhD student

Research Interests

- Protein folding
- Sequence/structure relationships of amino-acid repeats / Role in protein-protein interactions
- Molecular dynamics of proteins
- Molecular dynamics simulations & development of related tools
- Kinases
- Protein interactions
- Intrinsically disordered proteins
- Structure & dynamics of enzymes as potential therapeutic targets

The approach we follow includes *in silico* techniques such as homology/comparative 3D-modeling and molecular dynamics simulations.

Findings 2020

- With the aim to elucidate sequence/structure/function relationships of proteins linked to diseases (kinases in particular), in 2020:

1) We continued our *in silico* structural studies on the tyrosine kinase Tyk2, which plays a pivotal role in signal transduction and is related to various diseases. More precisely, in 2020 we built on our previous molecular dynamics (MD) studies on the wild type Tyk2 protein and its P1104A variant that confers protection against autoimmune diseases (see also Lesgidou *et al*, *Bioinformatics* 2018). The new MD simulations were performed in timescales of several microseconds and applied on forms of both proteins corresponding to pre-phosphorylation steps of the catalytic cycle of the kinase. Due to lack of known crystal structures for such Tyk2 forms, we first constructed appropriate 3D-models as initial structures for the MD simulations. The aim of these studies is to elucidate the structural consequences of this particular Tyk2 aminoacid substitution towards an in-depth understanding of the protective mechanism linked to the P1104A variant. The large-scale MD simulations were performed in the National HPC facility-ARIS @GRNET under project ID: *KIN_IMMUNMD_II* (pr008030) approved in 2019 (computational resources granted: 1.500.000,0 core-hours). The obtained long MD trajectories were analyzed using local resources and specialized MD analysis procedures. Our MD results so far, support the idea that the P1104 to Ala substitution promotes inactive Tyk2 conformations (Figure). Further analyses of the above MD trajectories, are in progress.

In addition, and in order to build on the above studies, a new proposal for extra computational time on ARIS@GRNET, was prepared in 2020 (to be submitted in 2021).

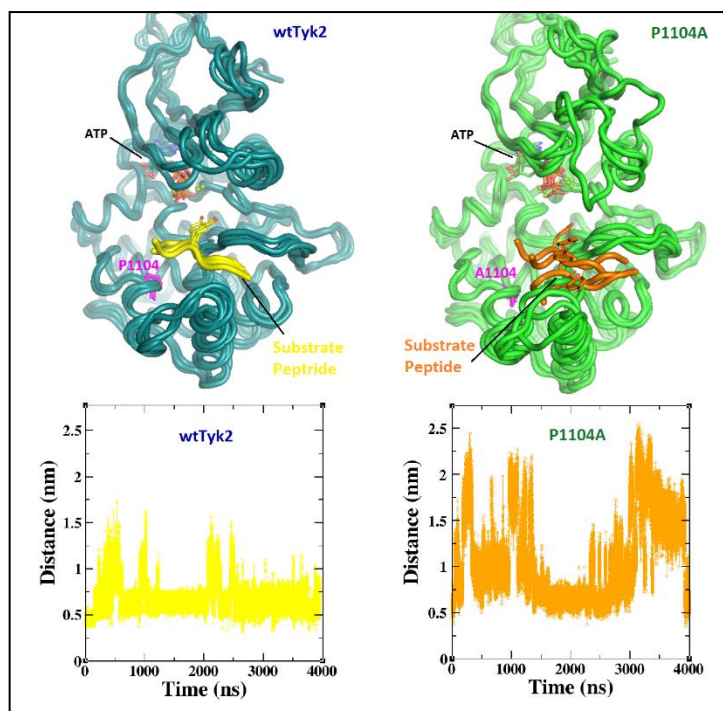


Figure. Results from large-scale MD simulations (4 μ s, each) of Tyk2 and P1104A variant, in the presence of potential substrate-peptide. (Upper panel): Ribbon representations of representative conformations of wtTyk2 (left) and P1104A (right) for the time range of 1 to 4 μ s of the corresponding MD trajectories. (Lower panel): Monitoring of the distance between the peptide and the corresponding catalytic site. The large-scale MD simulations were performed on the National supercomputer ARIS @GRNET (pr008030). Our MD results suggest that the conformations adopted by the P1104A variant are incompatible with substrate binding. These results further support the idea that this particular aminoacid substitution stabilizes inactive conformations of Tyk2, as suggested by our previous MD studies (Lesgidou et al *Bioinformatics* 2018)

2) We extended the *in silico* studies on the SRPK1 kinase, which we have also studied in the past (see Sellis et al *BBA-General Subjects* 2012; Vlassi et al *Data in Brief* 2019)). More precisely, in 2020, we performed multiple MD simulations of SRPK1 in complex with peptides from a human protein linked to carcinogenesis. 3D-models of SRPK1 binary complexes that we constructed previously, were used as initial structures. Interaction of these two proteins was observed biochemically by our collaborators, T. Giannakouros group (Aristotle University of Thessaloniki). The aim of these studies is to elucidate the structural details of this particular protein interaction of SRPK1.

- Finally, in 2020, potential end-users continued to express interest in new versions of the GROMITA-GUI we have previously developed in the lab (<http://gromita.bio.demokritos.gr>).

Other Scientific Activities

- M.V.: Reviewer for a journal of the American Chemical Society (ACS Omega) (one article)
- M.V.: Member of the European Network INSTRUCT-ERIC (INSTRUCT: An integrated Structural Biology Infrastructure for Europe)
- M.V.: Member of the related National Research Infrastructure project: “INSTRUCT-EL, an initiative of Greek Researchers related to Structural Biology” The related RI “**INSPIRED: The National RIs on Integrated Structural Biology, Drug Screening efforts and Drug-target functional characterization**” is funded by the GSRT (funding approved: June2018. Role of MV: member of the group of Scientists of the NCSR “Demokritos” node and responsible for the Computational activities of the node).
- Member of the group of Scientists of the NCSR “Demokritos” node of the **ELIXIR-GR** National Infrastructure.

Educational Activities

- ✓ Supervision of the PhD Thesis of N. Lesgidou (Democritus University of Thrace, Dept of Molecular Biology & Genetics) entitled: “Structural and dynamics studies of proteins related to diseases using molecular dynamics simulations” (started: February 2019)
- ✓ Lecture entitled “Principles of X-Ray Crystallography: Applications in Structural Biology” in the framework of the post-graduate program: “Clinical Biochemistry – Molecular Diagnosis” (Depts. of Biology/Chemistry/Nursing, National & Kapodistrian University of Athens).
- ✓ Lecture entitled: “Protein Structure – Experimental & Theoretic approaches” in the framework of the post-graduate program: “Applied Biochemistry: Clinical Chemistry, Biotechnology, Pharmaceutical Products evaluation” co-organized by IB_A of NCSR “Demokritos” and the University of Patras”.
- ✓ Lecture entitled: “Protein Structure – Experimental & Theoretic approaches” in the framework of the post-graduate program: “Bioinformatics-Computational Biology” (Dept. of Biology, Kapodistrian University of Athens)

Other Activities at IBA & NCSR “D”

In charge (with Dr. M. Pelecanou) of the Circular Dichroism (CD) service unit of IBA

Member of various committees of NCSR “D”

Citations 2020 (excluding self-citations): 39

Total citations 2016-2020 (excluding self-citations): 129

h-factor: 14

Current External Funding

“INSPIRED: The National Research Infrastructures on Integrated Structural Biology, Drug Screening Efforts and Drug Target Functional Characterization”, implemented under the Action “Reinforcement of the Research and Innovation Infrastructure”, funded by the Operational Programme “Competitiveness, Entrepreneurship and Innovation” (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund) (Role of M. Vlassi: member of the group of Scientists of the NCSR “Demokritos” node and responsible for the Computational activities of the node) (Coordination: NHRF).

Duration: Sept 2018 – Sept 2021

Budget for the computational activities of the NCSR “D” node: 36.000€.

Budget 2020: 18.600,0 €

Project entitled *“MD simulations of a kinase linked to autoimmune diseases. II* (project ID:KIN_IMMUNMD_II) in the framework of the 8th Call for Production Projects Accessing the supercomputer system ARIS @ GRNET (P.I.: M. Vlassi.).

Duration Dec 2019-Dec 2020

Total resources: 1.500.000,0 core-hours

ELIXIR-GR.

Role of M. Vlassi: member of the *group* of Scientists of the NCSR “Demokritos” node.

CENTRAL PROJECTS

IBA

Beyond the hitherto described activities of each Lab, IBA's personnel were engaged in a series of central projects, funded by the supervising authority, i.e. the General Secretariat for Research and Innovation (GSRI).

SANITURA

Almost all IBA's researchers (with the exception of those hired recently) participated in the project TARGET IDENTIFICATION AND DEVELOPMENT OF NOVEL APPROACHES FOR HEALTH AND ENVIRONMENTAL APPLICATIONS (acronym: SANITURA, MIS 5002514), belonging to the Action for the Strategic Development on the Research and Technological Sectors, funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund). Coordinator of the project is the Director of IBA. Aim of the project is the support of IBA research activities falling into the National Research and Innovation Strategies (RIS), such as the delineation of disease mechanisms, the identification of biomarkers and novel treatment targets, the development of preclinical disease models, the discovery of bioactive molecules and natural products for pharmaceutical and cosmetic applications, and the design of technologies for suppressing the environmental footprint of the agro-food sector. The total budget for the project is 740,000 €, 222,000 € of which were distributed during 2020.

In this period SANITURA supported with salaries seven (7) external collaborators, who delivered approximately 42 person-months, while IBA's researchers provided approximately 26 person-months. The tenders for the acquisition of equipment and consumables were completed successfully this year, as the project is approaching its finalization. During 2020, SANITURA supported eleven (11) publications in international peer-reviewed journals.

OPENSREEN-GR



IBA coordinates the project AN OPEN-ACCESS RESEARCH INFRASTRUCTURE OF CHEMICAL BIOLOGY AND TARGET-BASED SCREENING TECHNOLOGIES FOR HUMAN AND ANIMAL HEALTH, AGRICULTURE AND THE ENVIRONMENT (acronym: OPENSREEN-GR, MIS 5002691), which is implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund). Coordinator of the project is the Director of IBA. OPENSREEN-GR aims at the consolidation of a national infrastructure for medium to large-scale molecular target-based screening of existing libraries of synthetic small molecules, but also natural products. This infrastructure will provide the necessary equipment, as well as, trained personnel for implementing a vast array of bioactivity assessment methodologies. Beyond the identification of novel bioactive compounds, OPENSREEN-GR can proceed to their characterization, through studies of their biological effects and of the underlying molecular mechanisms, making use of a variety of model systems both *in vitro* and *in vivo*. These studies can also be combined with bioinformatics tools (*in silico*) for modeling the interactions of the bioactive compounds with their cellular targets, as well as, for analyzing structure-activity relationships (SAR). Apart from IBA, six other research and academic institutions are members of OPENSREEN-GR: Aristotle University of Thessaloniki, Democritus University of Thrace, University of Ioannina, National Hellenic Research Foundation, Biomedical Research Foundation of the Academy of Athens, and Agricultural University of Athens.

The total budget for IBA within the project is 899,600 €, a significant part of which (537,100 €) was distributed during 2020. Five IBA's researchers participated during this year in OPENSREEN-GR, providing approximately 7.5 research person-months. Moreover, nine external collaborators were recruited, delivering approximately 52 research person-months. During 2020 the acquisition of a state-of-the-art microplate analyzer, capable for reading absorbance, fluorescence, and

chemiluminescence, equipped with dispenser, gas and temperature controllers, bright field imaging optics, and plate stacker (Figure 1) took place. Moreover, an international tender for obtaining a Luminex analyzer, an automated liquid handling system (robotic workstation) and eleven more pieces of equipment was nearly finalized (being at the stage of contract signing). OPENSREEN-GR supported three publications by IBA researchers in international peer-reviewed journals within 2020.

Moreover, the national infrastructure OPENSREEN-GR is connected to the corresponding European Research Infrastructure Consortium (ERIC) EU-OPENSREEN, through the participation in the European project EU-OPENSREEN-DRIVE, which is currently in progress.

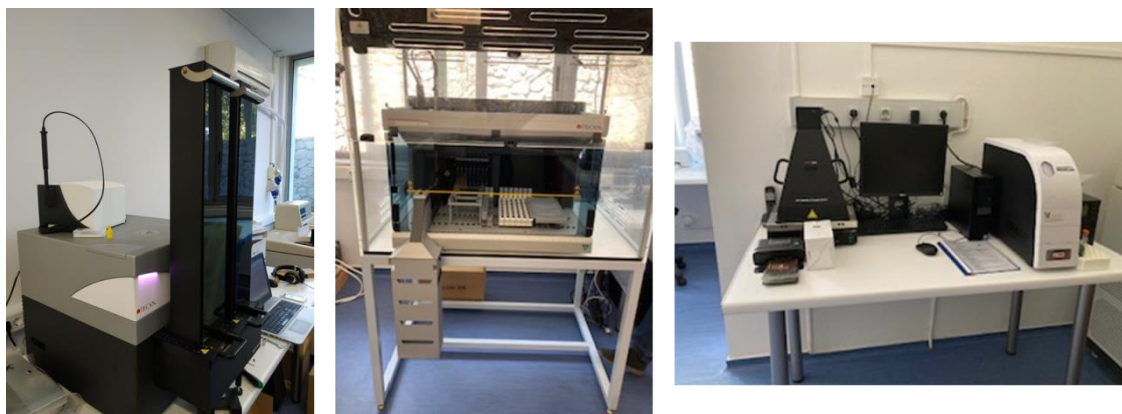


Figure 1: Part of the laboratory equipment of the Research Infrastructure OPENSREEN-GR in IBA

BIOIMAGING-GR



IBA is one of the eleven partners of the project A GREEK RESEARCH INFRASTRUCTURE FOR VISUALIZING AND MONITORING FUNDAMENTAL BIOLOGICAL PROCESSES (acronym: BIOIMAGING-GR, MIS 5002755), which is implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund), and is coordinated by the Foundation for Research and Technology-Hellas (FORTH). Regarding IBA's part of the project, it aims at the optimal function of the Imaging Unit, consisting mainly in an inverted confocal / multiphoton microscope and two UV / visible microscopes (one upright and one inverted). Hence, this Unit is expected to contribute in the development of research collaborations for the *in vitro* identification and imaging of biomolecules, facilitating the study and delineation of biological mechanisms, cellular functions, biochemical pathways, as well as, the development of novel small molecules capable for selective interactions with the above molecular and cellular systems. Head of the IBA's part of the project is the Director of the Institute, while the scientific team comprises 14 more IBA researchers and an external collaborator, the Imaging Unit operator. The total budget for IBA within the project is 211,250 €, 60,000 € of which were distributed during 2020. This funding supported the salary of the Imaging Unit operator for 12 person-months, while the rest team members provided approximately 4.5 person-months. Moreover, BIOIMAGING-GR supported three publications by IBA researchers in international peer-reviewed journals within 2020.

INSPIRED

IBA is also member of the project THE NATIONAL RESEARCH INFRASTRUCTURES ON INTEGRATED STRUCTURAL BIOLOGY, DRUG SCREENING EFFORTS AND DRUG TARGET FUNCTIONAL CHARACTERIZATION (acronym: INSPIRED, MIS 5002550), which is implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund), and is coordinated by the National Hellenic Research Foundation (NHRF). INSPIRED utilizes integrated approaches of Structural Biology and includes platforms for protein samples' preparation, their biophysical characterization, and the identification of their three-dimensional structures by means of X-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy and *in silico* approaches. Main aim of INSPIRED is to ensure a functional management scheme for the existing national Structural Biology infrastructures, as well as, for the data generated from the corresponding approaches for the benefit of the scientific, technological and economic development of the country. INSPIRED comprises 14 partners (6 Research Institutes and 8 Universities), while NCSR "Demokritos" is involved through the relevant activities and infrastructures of three Institutes (INN, IBA, and INRASTES) with a total budget of 220,000 €. IBA researchers with the relevant expertise are involved in research activities including protein sample purification / isolation, circular dichroism (CD) studies, NMR and *in silico* structural studies. The total budget for IBA activities includes 48,000 € for salaries of two research collaborators (30 person-months), as well as, 15,000 € for NMR spectrometers' maintenance. During 2020, an external collaborator was funded by the project for 12 person-months. INSPIRED supported one publication by IBA researchers in an international peer-reviewed journal within 2020.



THE ROUTES OF BEE

IBA participates with a team of 10 researchers, headed by the Director of the Institute, in the flagship national initiative entitled CREATION OF A NATIONAL RESEARCH NETWORK IN THE VALUE CHAIN OF "HONEY" (acronym: THE ROUTES OF BEE), established as part of the 'Creation of national research networks in the value chains of "Olive", "Vineyard", "Honey" and "Livestock"', funded by the national section of GSRT's Programme of Public Investment and coordinated by the Aristotle University of Thessaloniki. Within the framework of the characterization and promotion of the Greek Honey varieties, IBA researchers are implementing studies of the intracellular antioxidant activity, as well as, of the atheroprotective and possible anti-cancer properties of selected honey samples of Greek origin, with a total budget amounting to 39,200 €.

SERVICE UNITS

- ✓ **LABORATORY FOR DOPING CONTROL AND METABOLIC STUDIES**
- ✓ **HUMAN TISSUE BANK**
- ✓ **EXPERIMENTAL ANIMAL FACILITY**
- ✓ **LASER CONFOCAL MICROSCOPY**
- ✓ **HISTOLOGY UNIT**
- ✓ **CIRCULAR DICHROISM (CD) LABORATORY**
- ✓ **ISOLATION, CHARACTERIZATION AND CULTURE OF STEM CELLS APPLICATIONS IN REGENERATIVE MEDICINE**
- ✓ **LABORATORY OF MOLECULAR DIAGNOSIS OF GENETIC DISEASES**
- ✓ **LABORATORY OF CELL-BASED ASSAY SYSTEMS AND BIOACTIVE COMPOUNDS**

LABORATORY FOR DOPING CONTROL AND METABOLIC STUDIES

Personnel

Dimitris Kletsas, Research Director, Institute Director, Administrative Director

Yiannis Angelis, Dr. Chemist, Scientific Director

Nasia Kioukia, Dr. Pharmacist

Polyxeni Kiousi, Dr. Chemist

Argyro Fragkaki, Dr. Chemist, Quality Manager

Stella Loui, Research Technician

Foteini Chlapana, Research Technician

Maria Vlahou, Administrative Officer

Maria Fillipidou, Administrative Officer

Vassiliki Tzouvara, Administrative Officer

Maria Pavlaki, Supporting Personnel



Non-regular employees

Panagiotis Sakellariou, Dr. Biologist

Olga Goula, Chemist

Christoforos Christoforidis, Dr. Chemist

Efstathios Tsimelis, Medical Laboratories Technologist (MSc)

Lab description – Scientific interests

Since 2019, according to the Law 4589/29-1-2019 and to the common Ministers' Decision 84397 (FEK 3201/21-8-2019) the Doping Control Laboratory of Athens (DCLA) was transferred to the Institute of Biosciences and Applications. The Laboratory was founded in 1986 and is located in the Olympic Athletic Center of Athens (OAKA) complex, in a dedicated three floor building, built for the doping control testing of the 2004 Olympic Games.

DCLA was accredited by the International Olympic Committee (IOC) in 1995 and later on (2000) by ESYD, the Greek accreditation body, according to the requirements of the International Standard ISO/IEC 17025:2005. DCLA is a WADA accredited laboratory since 2003, according to the International Standard of Laboratories. Furthermore, DCLA is accredited by the Association of Official Racing Chemists (AORC) in the field of anti-doping testing in horses.

In 1997, the DCLA performed the doping control of the World Championships in Athletics. After that, DCLA performed the doping control analyses of the athletes' samples during the 28th Summer Olympic Games in Athens. Also, DCLA has undertaken the analysis of doping control sample during the Mediterranean Games of Mersin (Turkey) in 2013.

DCLA has analyzed athlete's samples for a number of different NADOS and IFs such as the Greek Anti-doping Council (ESKAN), Cyprus Anti-Doping Authority (Cyprus NADO) (CYADA), Tunisian Anti Doping Agency (Tunisian NADO), Israel NADO, Turkish Anti-doping Committee, UEFA, FIBA, National Anti-Doping Commission of Albania (Albanian NADO), Federation Internationale de Volleyball (FIVB), Union Cycliste Internationale (UCI), Portugal NADO (ADoP), Anti-doping Centre of Bulgaria (Bulgarian NADO), National Anti-doping Organisation of Malta (NADO Malta).

In the field of equine samples analyses, DCLA regularly analyses samples from the Jockey Club of Greece and the Hellenic Equestrian Federation. DCLA is equipped with a high-quality infrastructure including, among others, GC (2), GC/MS (5), GC/C/IRMS, GC/TOF/MS, LC/TOF/MS, GC/HRMS (3), etc.

Members of the scientific staff are under constant training and education and attend international meetings and symposia, such as the annual Manfred Donike Workshop and the USADA Symposium. In addition, scientific staff has participated as international experts in the 20th Winter Olympic Games in Torino (Italy) in 2006, the 30th Summer Olympic Games in London (Great Britain) in 2012,

the 22nd Winter Olympic Games in Sochi (Russia) and the 31st Summer Olympic Games, in Rio de Janeiro (Brazil).

DCLA, beyond sample analyses (within 2018 more than 3.000 samples have been analyzed), has undertaken a number of research programmes in the anti-doping research field, either partially funded by WADA or exclusively funded by OAKA. All projects have been conducted with the collaboration of external bodies, like governmental, university and WADA laboratories, in order to ensure optimum scientific performance and results. The results of these projects have been published in peer-reviewed journals and presented in international anti-doping congresses.

WADA inspection within 2018 led to a number of proposals to the Ministry as a prerequisite for the accreditation of the Laboratory. These led to the transfer of DCLA and its personnel to the IBA/NCSR “Demokritos”.

NCSR “Demokritos” has assured:

- a. the amount of 2.000.000 euros for purchasing state-of-the art analytical instruments and
- b. the amount of 1.280.000 euros for a period of three years as to cover functional expenses of DCLA, the recruitment of new personnel and the upgrading of its infrastructure.

The immediate goals of DCLA are the recruitment of new personnel and the upgrading of the research infrastructure, the reacquisition of the accreditation by WADA, the coverage of the necessities to support doping control for the Greek athletic community, as well as for international partners, mainly in the area of Southeast Mediterranean, the recovery of activities in equine doping control and the upgrading of research activity in the fields of chemical analysis and metabolic studies.

The formal transfer of the Laboratory to NCSR “Demokritos” has been finalized within 2020, as the Olympic Athletic Centre of Athens “Spyros Louis” and NCSR “Demokritos” signed a protocol for the transfer of the whole infrastructure and the use of the building housing the Laboratory. Furthermore, a memorandum of collaboration has been signed between the Deputy Minister of Development and Investments and the Deputy Minister of Sports for the financial support of the Laboratory with 480.000 euros annually for three years. With this, 6 new scientists have been recruited, raising the total number of scientists to 10, compared to 4 within 2019. In addition, within 2020 the procedures for the purchase of two LC/HRMS instruments, as well as of consumables, that cost 1.336.000 and 60.000 euros, respectively, have been started. These processes have been finalized within 2021.

During 2020 the Laboratory worked on the revocation of the absence of conformity and the reacquisition of the accreditation by WADA and despite the problems arisen due to the COVID-19 pandemic, key steps have been made towards this direction. For example, in 2021 the accreditation of the Laboratory from the Association of Official Racing Chemists for equine doping control was renewed.

Publications

Fragkaki A.G., Petropoulou G., Athanasiadou I., Kiouisi P., Kioukia-Fougia N., Archontaki H., Bakeas E., Angelis Y.S. (2020). Determination of anabolic androgenic steroids as imidazole carbamate derivatives in human urine using liquid chromatography–tandem mass spectrometry. *Journal of Separation science*, 43, 2154-2161 (IF: 2.878).

Sakellariou P., Kiouisi P., Fragkaki A.G., Lyris E., Petrou M., Georgakopoulos C. Angelis Y.S. (2020) Alternative markers for Methylnoretestosterone misuse in human urine. *Drug Testing and Analysis*, doi.org/10.1002/dta.2887. (IF: 2.903).

Sakellariou, P., Angelis, Y.S. (2020). Current methods of direct and indirect blood doping detection. *Pharmakeftiki Journal* 32, III, 2020 111-132. (IF:-)

HUMAN TISSUE BANK

Personnel

Helen Vavouraki, PhD, Senior Researcher- Scientific Responsible

Yiannis Ninios, PhD, Research Collaborator

Lydia Panagopoulou, MSc, Research Collaborator

Eleutherios Kosmidis, MSc, Scientific Collaborator

Christina Patra, Research Technician

Laboratory Description – Research Interests

Development activity

The main objective of Human Tissue Bank (HTB) is the collection of various human tissues, their processing and the production of grafts to be used in Regenerative Medicine and Reconstructive Surgery.

It is one of the first legislated laboratories of NCSR “DEMOKRITOS”, recently licensed as a Tissue and Cell Bank by the Ministry of Health (Greek Government Gazette 768, 26.02.2021) after thorough preparation and submission of all necessary documents.

Member of the European Association of Tissue Banks, it is the only bank of its kind in Greece, applying its “knowhow” in the processing of a great variety of human tissues. It has gradually developed the necessary expertise in each procedure (procurement, tissue processing, irradiation sterilization and disposal of tissue grafts) involved in the Tissue Banking. All the above activities comply with the European Directives 23/2004, 17/2006 and 86/2006 concerning human tissues and cells, as they were implemented in Greece by the 26/2008 Law, as well as, to the recommendations of IAEA.

In over 45 years of continuous operation, HTB has delivered more than 53.000 tissue grafts without any reported quality-related problems. All procedures taking place in the Bank are fully computerized and accredited according to ISO 9001/2015. It is a great concern for us to constantly be updated in quality control topics and to ensure our compliance with the Greek and European Standards.

The produced grafts are delivered to various hospitals, health clinics, medical and dental laboratories, all over the country.



Presentation of Human Tissue Bank's activities in scientific congresses

Research activity

The Tissue Bank's research interests are mainly focused on the study of the activity of the produced grafts, the optimization of the production methods, the processing of new types of tissues, the development of new techniques and new graft products. Funded, moreover, by a NSRF Research Project, we are developing a new, specially designed bone graft to accompany a patented surgical tool, used in Neurosurgery.

Our research activities are therefore based on collaborations with universities and healthcare institutions, in order to promote Public Health by launching improved products, publishing original papers and participating in doctorate degrees (Ph.D. theses)

2020 Findings

Despite all problems entrained by the SARS-CoV-2 pandemic, HTB managed to collect adequate tissues from collaborating hospitals. More precisely, we have processed 225 femur heads from living donors and we have produced and delivered 848 bone grafts to be used in Dentistry and Orthopedic Surgery. Meanwhile, we have processed 9 cranium grafts from Neurosurgery Depts.

Scientific collaboration with hospitals, University labs, etc., has resulted in the processing of new types of tissues, the application of new optimized techniques and the production of improved and customized products e.g., for child facial Surgery.

Participation in Congresses/Fairs

HAOMS 2020 AESTHETICS, organized by the Hellenic Association of Oral and Maxillofacial Surgery, Athens, January 17-18.

Virtual MEDICA, Germany-all over the world, November 16-19: TALOS-BIO.

Other Scientific Activities

Reviewer in the scientific Journals: Cell and Tissue Banking (Springer)

Member of Scientific Committee and Reviewer of the journal ACTA ORTHOPAEDICA ET TRAUMATOLOGICA HELLENICA (peer-review Journal in English)

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

Research Proposals Assessor

Other Activities at the NCSR "DEMOKRITOS"

Member of the Health and Safety Committee of NCSR "DEMOKRITOS"

Educational activities

Joint MSc Program "Applied Biochemistry: Clinical Chemistry, Biotechnology, Evaluation of Pharmaceutical Products", April 2020

Course: Tissue Banking-Development, Production and Quality Control of Tissue grafts

55th Summer School, 13-17 July 2020

Lecture: Tissue Grafts – New orientations

Current External Financing

NSRF Project 2014 – 2020:

Project title: TALOS-BIO - Development of a new, specially designed bone graft to accompany the EPO patented TALOS, used in Neurosurgery.

Duration: June 2018 – October 2021

Research Team: Y. Ninios, L. Panagopoulou, E. Kosmidis

Research Supervisor: H. Vavouraki

Overall funding: 188.800 €

Funding for 2020: 49.747,61 €

Citations 2020 (without self- citations): 5

Total Citations 2016-2020 (without self- citations): 31

h-factor: 9

EXPERIMENTAL ANIMAL FACILITY

Personnel

Dimitris Kletsas, Research Director, Scientific and Administrative Responsible

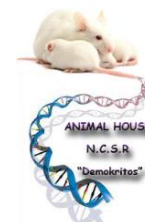
Giannis Zafeiropoulos, Laboratory Animal Technician

George Doulgeridis, Laboratory Animal Caretaker

Lykourgos Klamarias, Veterinarian

Laboratory Description

The Animal House of the Institute of Biosciences and Applications of the National Centre for Scientific Research “Demokritos” is one of the first organized and specialized Laboratories for the breeding and disposal of small laboratory animals in Greece. Currently, the aim of the Animal House is the supply of small laboratory animals to be used in experimentation in basic science, in the development of therapeutic regimes and new technologies, as well as in educational purposes. Today, the Animal House functions in compliance with the national and international standards and covers the most recent demands for research conducted on animal models.



The function of Committee for the Evaluation of Research Protocols and the Committee for the Wellbeing of Laboratory Animals, the continuously educated personnel, the up-to-date infrastructure, the accreditation of the Quality Management System according to ISO 9001:2015, the program for the control and assurance of the animals’ health, as well as the organization of educational seminars, guarantee the quality of the services provided. Finally, The Scientific and Administrative Head of the Animal House is an alternate member of the National Committee for the well-being of Laboratory Animals.

The Animal House has three facilities accredited according to the ΠΔ 56/2013 (Α.Π. 310181/24-06-2019)

- Facility for Animal Reproduction: EL25BIObr019
- Facility for Animal Disposal: EL25BIOsup020
- Facility for Animal Experimentation: EL25BIOexp039

Animal strains

The animal facility during 2020 maintained and reproduced the following strains:

1. Mice, strain CFW SWISS ALBINO
2. Mice, strain NOD SCID (immunocompromised)
3. Mice, strain SKH1 (hairless)
4. Mice, strain C57Bl/6 (wild type and transgenic AD 5xFAD and TgMMP-9)
5. Rats, strain WISTAR ALBINO
6. Rabbits, strain New Zealand ALBINO
7. Colonies of aged mice and rats are also available

During 2020, the Animal Facility provided the following animals:

Users	Rats WISTAR	Mice SKH1	Mice CFW	Mice C57Bl/6J	Rabbits NZW	Mice SCID	Total
IBA	45	10	0	35	10	9	109
INRASTES	76	0	303	0	2	298	679
External users	86	0	67	3	4	140	300
Total	207	10	370	38	16	447	1088

The operation of the Animal House, according to ΠΔ 56/2013, is supported by the following Committees:

Committee for the Evaluation of the Research Protocols - (Α.Π. 310295-24/06/2019):

Members: D. Kletsas, L. Klamarias, D. Mastellos, a representative from the the Veterinary Division of the Attica Prefecture.

Substituting Members: A. Tzinia, E. Livaniou, P. Sarris.

Committee for the Wellbeing of Laboratory Animals - (Α.Π. 310181-24/06/2019):

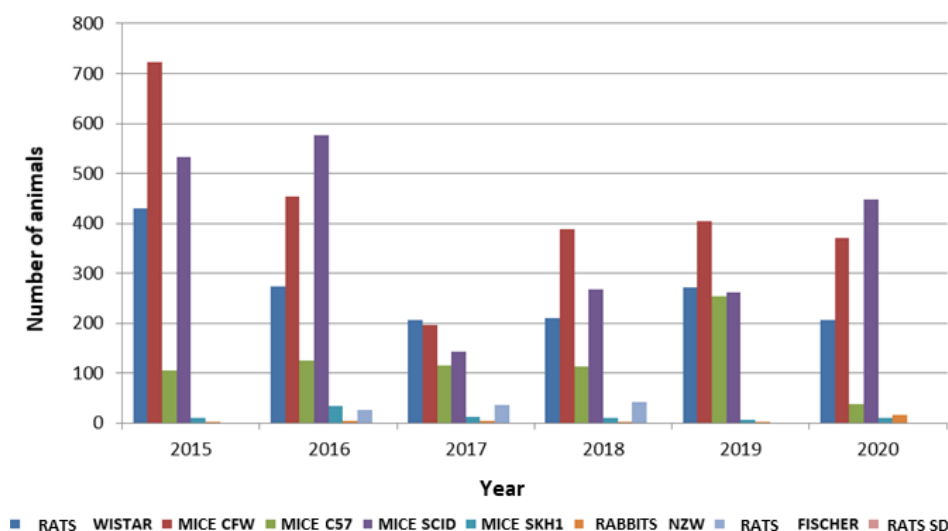
Members: D. Kletsas, L. Klamarias, E. Livaniou, G. Zafeiropoulos, G. Doulgeridis.

The administrative and scientific responsible of the Experimental Animal Facility is a substituting member of the National Committee for the wellbeing of laboratory animals.

During 2020

- The Animal House has upgraded the accreditation of the Quality Management System to ELOT EN ISO 9001:2015.
- Three new protocols for animal experimentation have been approved.
- The Safety Regulation and the Regulation of Medical Inspection have been renewed.
- The Director of the Animal House has been appointed as Alternate Member of the National Committee for the Wellbeing of the Laboratory Animals.
- A new system of telemetry for the surveillance of the environment of the Animal house has been established.
- A new colony for the reproduction of New Zealand rabbits has been established.
- The personnel of the Animal House followed several educational seminars.
- The Colony of Aged Animals has been enriched with new species.
- During 2020 at least 13 papers from NCSR “Demokritos” researchers have been published in peer-reviewed journals where the support from the Animal House has been acknowledged.

DISPOSAL OF LABORATORY ANIMALS 2015-2019



LIGHT MICROSCOPY UNIT

Personnel

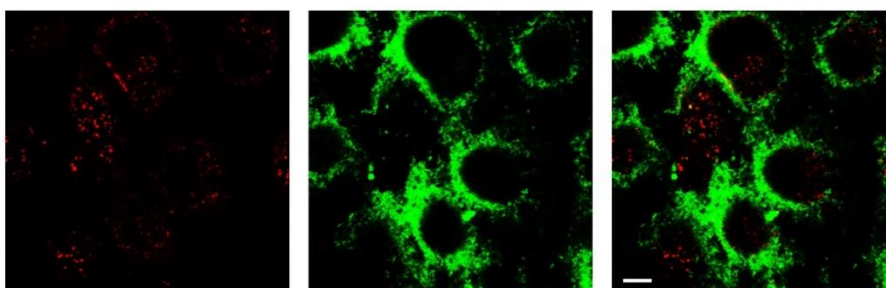
Dimitris Kletsas, Research Director, Institute Director, Scientific and Administrative Responsible

Vassiliki Labropoulou, Senior Researcher

Harris Pratsinis, Senior Researcher

Marina Sagnou, Researcher

Alexandros Athanasopoulos, Research assistant



Laboratory Description – Progress during 2020

The light microscopy unit of the Institute of Biosciences and Applications (IBA) of NCSR “Demokritos” has been operating since July 2016 with a new generation, modern equipment, serving the imaging needs of both the research laboratories of the Institute and other Institutes of NCSR “Demokritos”, as well as external research centers and Universities such as the NKUA, the NTUA, the Agricultural University of Athens as well as some Hospital Units. The light microscopy unit is equipped with a Nikon E600 Confocal Microscope and a state-of-the-art Leica TCS SP8 multiphoton microscope, with a fully automated galvanometric stage. The system is accompanied by a climate chamber for the strict control of all environmental variables (humidity, temperature, CO₂, O₂, N₂).

The light microscopy unit offers a wide range of application of optical microscopy:

- Multi-Channel Fluorescence Microscopy, covering UV, Visible and Infrared Spectrum
- Multi-color 3D Imaging
- Live Cell Imaging
- 2-Photon Microscopy
- Second Harmonic imaging
- Förster Resonance Energy Transfer (FRET) to monitor molecular interactions in live or fixed specimens
- Fluorescence Recovery After Photobleaching (FRAP)
- Cell/tissue Colocalization analysis
- Calcium imaging
- Differential Interference Contrast (DIC) microscopy (known as Nomarski microscopy)
- Image processing and analysis (with specialized software programs such as ImageJ / Fiji and Imaris (Bitplane))

Leica TCS SP8 MP



During 2020 the light microscopy unit recorded 300 operating hours, many of which have been collaborations with external research centers and universities. The unit collaborated with research teams from the IBA and INN as well as with various third-party academic institutions in protocols dealing with:

- The study of new chemical compounds aiming at the discovery of new active or imaging agents
- The study of the induction of genotoxic lesions and cellular senescence
- The expression of specific proteins associated with mechanisms involved in age-associated diseases such as dyslipidemia, diabetes mellitus and Alzheimer's disease
- Functional characterization of opioid receptors
- Imaging and cellular identification of new dendrimers, liposomal preparations and nanostructures for the transport of bioactive substances
- Imaging of biofilms in the nasal mucosa of patients with chronic rhinosinusitis and correlation with their clinical characteristics

The activities of light microscopy unit support the scientific work of the various research teams, contributing to scientific publications, doctoral theses and the development of collaborations among the users of the Unit. Moreover, the Unit actively supports the various NCSR “Demokritos” educational programs participating in presentations, demonstrations and workshops, for all educational grades.

At the same time, IBA participates in the “Greek Research Infrastructure for the Visualization and Monitoring of Fundamental Processes in Biology and Medicine (BIOIMAGING-GR)”, which is funded by GSRI in which, besides the Institute, 10 additional Research centers and Universities participate.

HISTOLOGY UNIT

Personnel

Dimitris Kletsas, Research Director, Institute Director, Administrative Responsible
Garyfalia Drossopoulou, Senior Researcher, Scientific responsible

Laboratory Description – Research Interests

The Histology Unit (HU) of the Institute of Biosciences and Applications (IBA) was established in 2019 and is housed in an especially formed site, in the basement of the central building of the Institute.

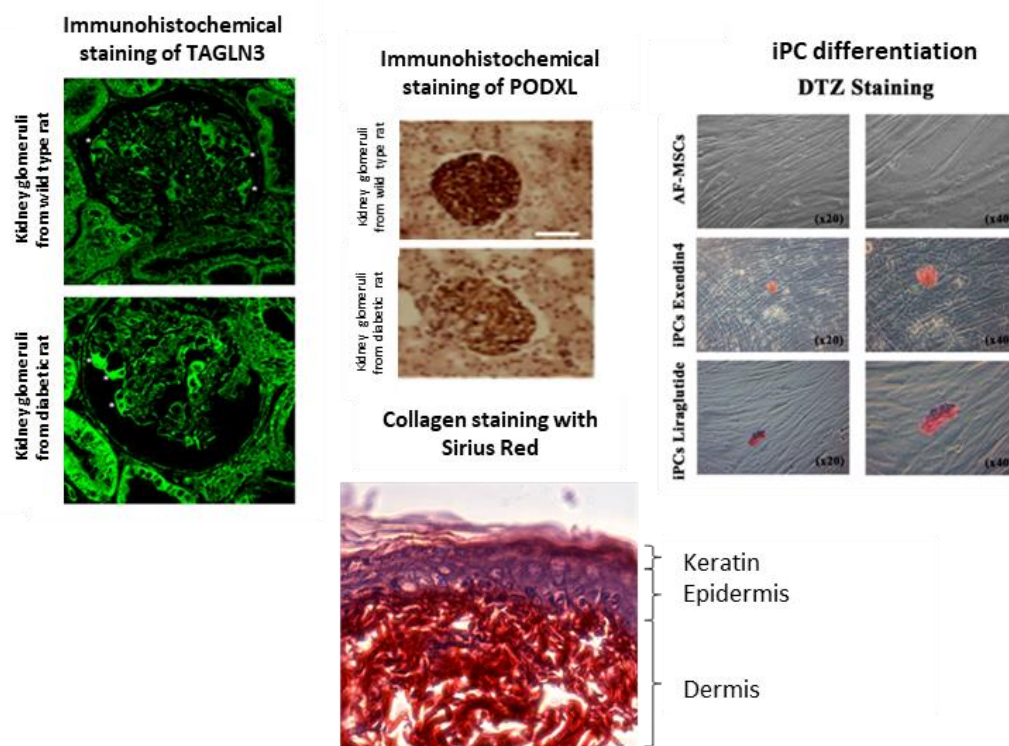
The HU supports the scientific work of several research groups and focuses on the investigation and phenotypic analysis at the tissue level of human and animal model specimens that are related to several pathologies, many of which derive from the Experimental Animal Facility of IBA.

The HU is fully equipped with hoods, tissue processing and embedding equipment, microtome, a cryostat and a vibratome, as well as all small necessary equipment.

Users can perform tissue processing, embedding, sectioning and staining with a series of stains. IBA researchers can also be trained to use selected equipment and then be able to acquire their own tissue sections. The unit can support protocol set up and application.

During 2020 IBA HU, participated in research protocols regarding the following:

- Mode of action of TAGLN3 in the survival of glomerular podocytes.
- Role of secreted conditioned media from human amniotic fluid mesenchymal stem/stromal cells in ameliorating the establishment and development of Diabetic Nephropathy.
- Differentiation of insulin producing cells (IPCs) by mesenchymal stem cells.
- Intervertebral disc degeneration
- Skin ageing
- Cancer development in experimental animal models



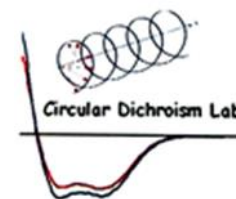
CIRCULAR DICHROISM (CD) LABORATORY

Personnel

Metaxia Vlassi, Research Director

Maria Pelecanou, Research Director

Angeliki Panagiotopoulou, Functional Research Scientist B'



User Committee

Dr. Metaxia Vlassi

Dr. Maria Pelecanou

Dr. Angeliki Chroni

Dr. Georgios Nounesis (INRASTES)

Dr. Efstratios Stratikos (INRASTES)

Laboratory Description - Object

The equipment of the Circular Dichroism Laboratory (CD) includes a JASCO-715 spectropolarimeter equipped with Peltier system for temperature control. This CD infrastructure was acquired in 1998 within the framework of the "Center for Crystallographic Studies of Macromolecules (CCM)", which was financed through a grant (EPET) from the General Secretariat for Research and Technology as a network of three Institutes of NCSR "Demokritos" (the former Biology, Physical Chemistry, Radioisotopes & Radiodiagnostics Products) and other Greek academic institutions. It is located at room Y-35 of the Institute of Biosciences & Applications and is operating under the supervision of IB-A scientists. In 2013 (13/06/2013), the CD Lab was included in the category of Specialized Services of NCSR "D", whereas in 2018, after re-categorization, the service was classified in the category of Specialized Services with Research Activity (decision of the Board of Directors 28-03-2018, Protocol # 010/2018-299 4/4/2018).

Circular Dichroism (CD) is a well-established spectroscopic technique based on the differential absorption of circularly polarized light from optically active molecules and it is widely used for

- Conformational analysis of biological macromolecules (e.g. proteins)
- Investigation of protein-protein interactions as well as interaction/complexation of proteins with various ligands, metals, stabilizers, inhibitors, drugs, etc. and interactions/complexation of nucleic acids
- Investigation of the influence of denaturants, solvents and ligands on the conformation and stability of macromolecules
- Monitoring conformational changes under different conditions/thermal stability studies
- Comparative structure and stability studies of proteins and their mutants

The CD method is highly accurate, sensitive, non-destructive to the sample and gives reliable and reproducible results.

Achievements - Progress in 2020

The CD unit has been widely used since 1998 by research teams of NCSR "D" and other academic / research organizations from all over Greece, for structural analyzes and studies of interactions of biological macromolecules. It should be noted that the CD spectrophotometer is one of the few in Greece and the only one in Athens that provides specialized scientific services to external users. The CD unit has contributed so far to scientific publications and doctoral dissertations as well as to the development of collaborations both within NCSR "D" and with other research institutes. The Scientists involved also contributed to the training of new users (students, researchers) as well as to addressing technical and scientific problems related to CD applications.

In 2020, as in previous years, the CD Lab has supported research projects of at least 12 groups of the 3 participating NCSR "D" Institutes and other Greek academic institutions such as the National and Kapodistrian University of Athens (Pharmaceutical Dept, Chemistry Dept), the National Technical University of Athens (Chemical Engineering Dept), the University of Thessaloniki (Chemistry Dept) and the NHRF (Institute of Biology, Pharmaceutical Chemistry and Biotechnology). Income from the provision of services is solely used to cover the operation and repair needs of the spectrophotometer.

The CD Laboratory participates in the Project: "INSPIRED: The National Research Infrastructures on Integrated Structural Biology, Drug Screening Efforts and Drug Target Functional Characterization" (coordinator: NHRF)", implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund) (funding approved in June 2018).

ISOLATION, CHARACTERIZATION AND CULTURE OF STEM CELLS APPLICATIONS IN REGENERATIVE MEDICINE

Personnel

Paraskevi Kitsiou, Senior Researcher (Admin)

Athina Tzinia, Senior Researcher

Garyfallia Drossopoulou, Senior Researcher

Laboratory Description – Research Objectives

The laboratory of Cell & Matrix Pathobiology focuses on isolation, culture and characterization of stem cells from different tissues of various organisms aiming at their use in therapeutic applications in the frame of regenerative Medicine.

The laboratory successfully isolates and characterizes human umbilical cord, human umbilical cord blood (UCB) stem cells and stem cells from other sources (skin and fat tissue). The mesenchymal stem cells isolated from fat tissue are used by orthopedists for the confrontation of damage of articulations (such as knee and hip) In addition, the laboratory has been expanded in isolation and culture of stem cells emanating from olfactory mucous, in collaboration with Dr A. Charonis and Dr P.

Politis {Research, Biomedical Research Foundation Academy of Athens (BRFAA), as well as: Dr Pedro Escada, Dr José Pratas-Vital (Hospital de Egas Moniz, Centro Hospitalar de Lisboa, Ocidental, Lisbon, Portugal), Dr. Ch. Gogo (EKPA, Attikon Hospital, B' Neurosurgical Clinic). The olfactory mucous is of great importance because it contains nervous tissue which regenerates very rapidly due to its high content of stem cells (OECs).

Progress achieved

During 2020, the laboratory focus on the isolation and culture of mesenchymal stem cells isolated from mouse fat tissue. Characterization of the above-mentioned cells was achieved by the use of flow cytometry.

Funding

The research activities of the laboratory of Cell & Matrix Pathobiology are funded by the company Biophylaxis, S.A.

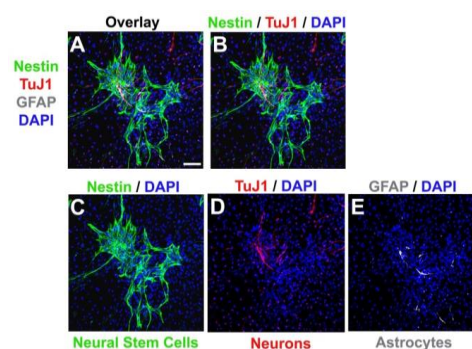


Figure 1: Culture of OECs results to enriched population of neural stem cells (NSC), as it appears from the use of appropriate indicators. These cells maintain the ability of proliferation and renewal.

LABORATORY OF MOLECULAR DIAGNOSIS OF GENETIC DISEASES

Personnel

Gerassimos Voutsinas, Research Director

Socratis Avgeris, Research Technician

Laboratory Description - Objective

The Laboratory of Molecular Diagnosis of Genetic Diseases was founded in 2011 and specializes in genetic testing of Tuberous Sclerosis Complex (TSC) and Neurofibromatosis types 1 and 2 (NF1 and NF2). The protocols used in the lab derive from previous research and have been developed in the frame of programs funded by the General Secretariat for Research and Technology (GSRT) and the American College of Greece (Deree).

Establishment of genetic testing for TSC by mutation detection in TSC1 and TSC2 genes, and for NF1 and NF2, in NF1 and NF2 genes, allows confirmation of clinical diagnosis in patients whether these fulfill or not the agreed criteria. At the same time, it may contribute decisively to differential diagnosis, clearly distinguishing TSC or NF from diseases with a similar phenotype. It is also possible to confirm or exclude the presence of disease in patient relatives, who do not exhibit a relevant phenotype, as well as to separate hereditary from *de novo* cases. Genetic counseling sessions are mandatory before and after testing.

It is worth mentioning that the above genetic analyses are complex and time-consuming, as in fact each family usually presents a distinct mutation, since the above genes are characterized by the absence of hot spots for mutations. Finally, in order to give emphasis on the necessity for family genetic testing, followed by genetic counseling sessions, it should be noted that, while both of these neurodermatological syndromes are autosomal dominant with 100% penetration, they may lead to so different phenotypes that - within the same family - one patient may have symptoms difficult to detect, while another one may express a particularly severe phenotype.

LABORATORY OF CELL-BASED ASSAY SYSTEMS AND BIOACTIVE COMPOUNDS

Personnel

Dimitris Kletsas, Research Director

Harris Pratsinis, Senior Researcher

Eleni Mavrogonatou, Researcher

Adamantia Papadopoulou, Postdoctoral Fellow

Anastasios Kouroumalis, Postdoctoral Fellow

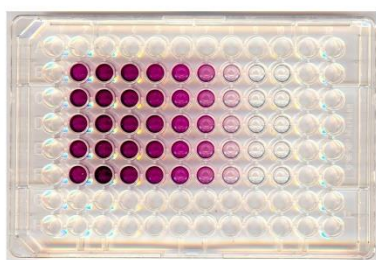
Laboratory Description

The activities of the Laboratory comprise the isolation of primary somatic or mesenchymal stem cells, and the development of the appropriate cell assay systems for the evaluation of synthetic or natural bioactive products regarding their wound healing, anti-ageing, and anti-cancer properties.

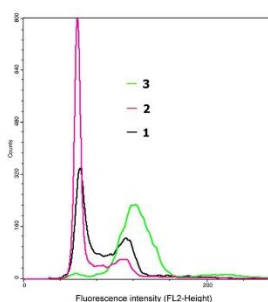
2020 Findings

During 2020 the service lab performed *in vitro* studies on the effect of acidity on senescent human cells, as well as *in vivo* experiments on the effect of alkaline water on the health of rats, in the framework of the signed contract with the company AHB Group S.A. In addition, the service lab conducted cytotoxicity tests of several materials for LAVIPHARM S.A.

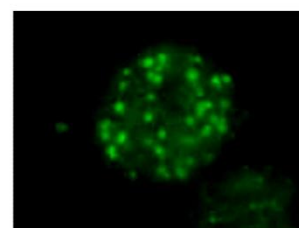
CYTOTOXICITY ASSESSMENT



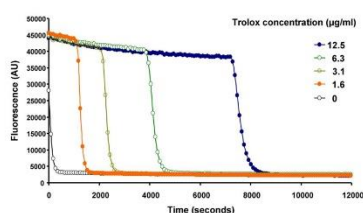
CELL-CYCLE ANALYSIS



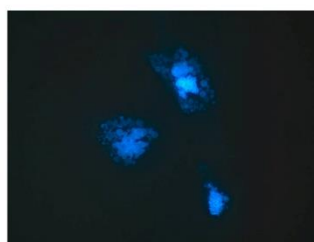
DNA DAMAGE ANALYSIS



ANTIOXIDANT ACTIVITY ASSAY



STUDY OF APOPTOSIS



ORGANOTYPIC CELL CULTURES



EDUCATIONAL ACTIVITIES

EDUCATION

The Institute of Biosciences and Applications has continued the successful implementation of a variety of educational programs and activities in 2020, which include:

- a) the training of postdoctoral researchers
- b) the training of undergraduate students and the supervision of postgraduate (PhD, MSc and diploma) students to successfully complete their project,
- c) the organization of postgraduate level courses and seminars,
- d) the participation of IBA researchers as lectures in the Annual Summer School of NCSR “Demokritos” for undergraduate and graduate students,
- e) the organization of a “hands-on” Summer Camp for Lyceum students, and
- f) the participation in dissemination and science communication activities to promote science, inform the public on research activities in IBA and promote research careers to young people.

It should be mentioned that due to the exceptional conditions and restrictions imposed due to the COVID-19 pandemic all educational programs and activities were accordingly modified to comply with the applied health protocols and rules.

During 2020, **17** postdoctoral researchers were trained at IBA, while, **24** PhD students and **10** MSc students carried out their research projects at the Institute under the supervision of IBA researchers.

In 2020, **1** graduate student concluded his PhD thesis and **5** students completed their Master’s theses successfully fulfilling their course requirements.

Moreover, **10** undergraduate students from Greek Universities have been performing their final year project at IBA, **5** of which completed their project successfully. Additionally, **10** undergraduate students undertook research practice in IBA laboratories.

Additionally, IBA researchers participated as lectures in various courses within the framework of Postgraduate Programmes of several Greek Universities:

- Lecture entitled “*Molecular mechanisms of carcinogenesis and pharmaceutical inhibition of selected therapeutic cellular targets*” in the framework of the Post-Graduate Specialization Diploma “*Drug development: Research, commercialization and accessibility*” (**Dr. G. Voutsinas**, Medical School, University of Athens)
- Lecture entitled “*Molecular mechanisms of carcinogenesis and pharmaceutical inhibition of selected therapeutic cellular targets*” in the framework of the Post-Graduate Specialization Diploma “*Applications of Biology in Medicine*” (**Dr. G. Voutsinas**, Department of Biology and Medical School, University of Athens)
- Lecture entitled “*G protein- coupled receptors in health and disease and drug development*” in the framework of the postgraduate course “*Molecular Basis of disease*” (**Dr. Z. Georgoussi**, Department of Biology, University of Athens)
- Lecture entitled “*G protein- coupled receptors in health and disease and drug development*” in the framework of the postgraduate course “*Molecular and Applied Physiology*” (**Dr. Z. Georgoussi**, Medical School, University of Athens)
- Course lecturing entitled “*Signaling of Neurotransmitter Receptors*” in the framework of the postgraduate course “*Athens International Master’s Programme in Neurosciences*” (**Dr. Z. Georgoussi**, Department of Biology, University of Athens)
- Lecture entitled “*Cell senescence and carcinogenesis*” in the framework of the class entitled “*Thoracic oncology*”, (**Dr. D. Kletsas**, Medical School, 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 tissue homeostasis” in the framework of the postgraduate course “Cosmetology” (**Dr. D. Kletsas**, Department of Pharmacology, University of Athens)
- Lecture entitled “Cell senescence: Molecular mechanisms and role in tissue homeostasis” (**Dr. D. Kletsas**, Harokopio University)
- Teaching in the Post-graduate Master’s Degree “*Applications of Biology in Medicine*” with lectures entitled “*Cell proliferation and tissue homeostasis. Growth factors: Structure, receptors and signal transduction. Cell senescence and tissue homeostasis. Methodologies for the study of cell proliferation*” and laboratory visits (**Dr. D. Kletsas**, **Dr. H. Pratsinis** and **Dr. E. Mavrogonatou**, Department of Biology, University of Athens)
- Lecture entitled “*Cell systems in the research of carcinogenesis*” in the framework the Postgraduate Master’s Degree “*Neoplastic Disease in Humans: Diagnosis, Modern Treatment and Research*” (**Dr. H. Pratsinis**, Medical School, University of Athens)
- Lecture entitled “*Cell Cycle: Checkpoints and Consequences for Normal Cellular Function when Cell Cycle Progress Dysfunctions*” in the framework of the course “*Cell cultures – Tissue cultures*” within the postgraduate programme “*Application of Biology in Medicine*” (**Dr. Th. Sourlingas**, Department of Biology & Medical School, University of Athens)
- Lecture entitled “*Alzheimer’s Disease: a) diagnosis (clinical, laboratory, imaging), b) risk factors (genetics, age, sex, cardiovascular, trauma, depression, drugs etc), c) recent data for the mechanisms of disease initiation and progress and therapeutic strategies*” in the framework the Postgraduate Master’s Degree “*Clinical Biochemistry-Molecular diagnosis*” (**Dr. A. Chroni**, Department of Chemistry, University of Athens)
- Lecture entitled “*Regulation of Apoptosis in disease progression: Is it desirable or must be avoided?*” in the framework of the graduate course “*Molecular and Applied Physiology*” (**Dr. G. Drossopoulou**, Medical School, University of Athens)
- Lecture entitled “*Metabolic Syndromes – Diabetes Mellitus and its complications*” in the framework of the graduate course “*Clinical Biochemistry – Molecular Diagnostics*” (**Dr. G. Drossopoulou**, Department of Biology, University of Athens)
- Lecture entitled “*Organization and Function of fungal plasma membrane,*” in the framework of the Inter-Institutional Joint Post-Graduate Programme “*Molecular and Cellular Biology and Biotechnology*” (**Dr. V. Sophianopoulou**, Medical School & Dept. 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)
- Lectures on “*Principles of X-Ray Crystallography: Applications in Structural Biology*” within the course “*Current biochemical and biophysical analytical methods*” in the framework of the postgraduate program (towards a Masters degree) entitled “*Clinical Biochemistry – Molecular Diagnosis*” (**Dr. M. Vlasi**, Department of Biology, Chemistry & Nursing, University of Athens)
- Lecture entitled “*Protein Structure – Experimental & Theoretic approaches*” in the framework of the postgraduate program “*Bioinformatics-Computational Biology*” (**Dr. M. Vlasi**, Department of Biology, University of Athens)

Moreover, in July 2020, within the framework of the 55th NCSR “Demokritos” Summer School, IBA researchers delivered a series of lectures on various frontier subjects of biological interest (see list below).

In September 2020, 11-13/09/2020, the collaboration of IBA with the European Learning Laboratory for the Life Sciences ELLS) which is a member of the European Molecular Biology Laboratory (EMBL) resulted in the successful organization and implementation of a Laboratory Workshop specifically addressed to Biology Secondary School teachers. The workshop aimed to support the life-long

learning process for High-school Biology Teachers giving them the opportunity to update their knowledge and experience both in theoretical aspects of frontier biochemistry and molecular biology issues as well as practical demonstrations of laboratory techniques such as SDS-PAGE electrophoresis, PCR, DNA protocols, western blotting etc (**D. Kletsas, H. Pratsinis, E. Mavrogonatou, B. Labropoulou, L. Swevers, A. Prombona, V. Sophianopoulou, Ch. Gournas, G. Voutsinas, Z. Georgousi**)



In 2020 the joined **MSc Programme “Applied Biochemistry: Clinical Chemistry, Biotechnology, Evaluation of Pharmaceutical Products”** (FEK 3923/2018) was successfully organized by the Department of Chemistry of the University of Patras and the Institute of Biosciences and Applications of NCSR "Demokritos". **8** postgraduate students were enrolled in this programme. The students attended courses at the University of Patras and IBA. Currently, they perform their MSc thesis' projects. IBA researchers taught in all four courses of the MSc programme:

- Biochemical Analysis – Clinical Biochemistry
 - Lecture entitled “*Functional testing of organs and endocrine glands*” (**Dr. G. Drossopoulou**)
 - Lecture entitled “*Chemical ecology: Applications of bioactive secondary metabolites of natural origin*” (**Dr. M. Konstantopoulou**)
 - Lecture entitled “*Methods for the analysis and study of proteins, lipids and carbohydrates*” (**Dr. A. Chroni**)
- Advanced Biochemistry
 - Lecture entitled “*Protein Structure – Experimental & Theoretic approaches*” (**Dr. M. Vlassi**)
 - Lecture entitled “*Signaling pathways of G protein- coupled receptors in health and disease and drug development*” (**Dr. Z. Georgousi**)
 - Lecture entitled “*Transcription factors (CREB, CREM, NF-kB, AP-1, STAT), identification methods for transcription factors activation*” (**Dr. G. Drossopoulou**)
 - Lecture entitled “*Ca²⁺ signaling*” (**Dr. E. Mavrogonatou**)
 - Lecture entitled “*Signaling pathways of main cytokines and developmental factors IL-1, TNF- α , TGF- β (SMAD proteins), PDGF, EGF, FGF*” (**Dr. D. Kletsas**)

- Lecture entitled *“Extracellular matrix interaction with cells”* (**Dr. H. Pratsinis**)
- Lecture entitled *“Lateral compartmentalization and function of plasma membrane: eisosomes and transmembrane transporters”* (**Dr. V. Sophianopoulou & Dr. C. Gournas**)
- Molecular Pharmacology – Immunology
 - Lecture entitled *“Opioid analgesics [morphine, morphine derivatives, analgesic receptor theory, agonists and antagonists, encephalines and endorphins, receptor mechanisms (μ , κ , δ and σ receptors)”* (**Dr. Z. Georgoussi**)
 - Lecture entitled *“Pharmaceutical activity on the receptors (the role of the receptor, neurotransmitters and hormones, receptors, signal transduction, receptor structural rearrangements, agonists and antagonists design, representative agonists, reverse antagonists, desensitization and sensitization, tolerance and addiction, cellular receptors, types and subtypes of receptors)”* (**Dr. Z. Georgoussi & Dr. Papakyriakou**)
 - Lecture entitled *“Pharmaceutical activity on enzymes (binding interactions, competitive and non-competitive inhibitors, allosteric inhibitors, the catalytic role of enzymes, enzyme regulation, isoenzymes, pharmaceutical applications of inhibitors (antimicrobial, antiviral and human enzyme inhibitors)”* (**Dr. Papakyriakou**)
 - Lecture entitled *“Structure and function of nucleic acids (DNA structure, anti-DNA drugs, RNA, anti-RNA drugs, drugs derived from nucleic acid structural motifs, molecular biology and genetic engineering)”* (**Dr. M Sagnou**)
 - Lecture entitled *“The adrenergic neuronal system (the adrenergic system, adrenergic receptors, adrenergic transducers, catecholamine biosynthesis and metabolism, neurotransmission, drug-targets, adrenergic binding area, structure-activity-relationships, agonists, antagonists of adrenergic receptors, other drugs affecting adrenergic transduction”* (**Dr. A. Papakyriakou**)
 - Lecture entitled *“The RNAi as a defence mechanism against insect viruses”* (**Dr. L. Swevers & Dr. B. Labropoulou**)
- Molecular and Cellular Biology – Molecular Biotechnology
 - Lecture entitled *“Development, Production and testing of tissue grafts”* (**Dr. E. Vavouraki**)
 - Lectures entitled *“Recombination and hybridization”, “Which part of eukaryotic genome is transcribed and translated”, “DNA mapping”, “Structure and function of genes”, “Sequence vectors, expression vectors”, “Molecular carcinogenesis”, “Rare diseases”* (**Dr. G. Voutsinas**)
 - Lectures entitled *“Strain selection, genetic recombination”, “Vectors - DNA (plasmids, cosmids, phages)”, “Cloning vectors”, “Methods of transfection and recombination of genetic information”* (**Dr. C. Gournas**)
 - Lecture entitled *“Diabetes – Diabetic nephropathy: Current therapeutic approaches”* (**Dr. G. Drossopoulou**)
 - Lecture entitled *“Cellular senescence as an anti-aging therapeutic target”* (**Dr. D. Kletsas**)
 - Lecture entitled *“Flow cytometry”* (**Dr. E. Mavrogonatou**)
 - Lecture entitled *“Methods for the evaluation of cytotoxicity-cytostatic activity”* (**Dr. H. Pratsinis**)
 - Lecture entitled *“DNA organisation, histones and chromatin function”* (**Dr. Th. Sourlingas**)
 - Lecture entitled *“Viral DNA replication, prokaryotic and eukaryotic cell”, “Synthetic DNA, cDNA, gene libraries”* (**Dr. V. Sophianopoulou**)
 - Lecture entitled *“Bacteriophage expression system: generation of recombinant viral vectors, via homologous recombination and delocalization”* (**Dr L. Swevers & Dr. B. Labropoulou**)
 - Lecture entitled *“Cardiovascular diseases and current therapeutic approaches”* (**Dr. A. Chroni**)

Furthermore, in 2020 the IBA researcher **Dr. Z. Georgoussi** participated for another year in the joined MSc Programme **“Athens International Master’s Programme in Neurosciences”** (ΦΕΚ 3802/2018).

COMPLETION/AWARD OF DOCTORAL THESES IN 2020

GRADUATE STUDENT	TITLE OF DOCTORAL THESIS	IBA SUPERVISOR	UNIVERSITY
Anastasios Kouroumalis	<i>Study of the effect of the external environment on the gene expression and cellular function of intervertebral disc cells</i>	D. Kletsas	School of Medicine, University of Athens

COMPLETION OF MASTER THESES IN 2020

POSTGRADUATE STUDENT	TITLE OF MSc THESIS	IBA SUPERVISOR	UNIVERSITY
Christina Ntogka	<i>Study of intervertebral disc cells' senescence</i>	D. Kletsas	Dept. Biology, University of Athens
Charalambia Almpani	<i>Study of thymosin peptides' action in human skin fibroblasts for the regulation of cellular functions related to wound healing</i>	H. Pratsinis	Dept. Pharmacy, University of Athens
Pekou Anna	<i>Biological evaluation of novel polyphenolic copper complexes</i>	M. Sagnou	Dept. Chemistry, University of Thessaloniki
Karagkiaouri Maria	<i>Studies on the interaction of a silver complex with DNA metabolism enzymes and bacterial biofilm. Potentail antimicrobial and antioxidant activity of propolis enriched polymers.</i>	M. Sagnou	Dept. Chemistry, University of Thessaloniki
Nigianni Georgia	<i>Synthesis of moncarbonyl curcumin derivatives and their biological evaluation</i>	M. Sagnou	Dept. Chemistry, University of Patras

COMPLETION OF FINAL YEAR UNDERGRADUATE PROJECTS 2020

UNDERGRADUATE STUDENT	FINAL YEAR PROJECT TITLE	IBA SUPERVISOR	UNIVERSITY
Antonis Miridakis	<i>Opioid Receptors: New signaling pathways</i>	Z. Georgoussi	Dept. Biology, University of Athens
Vania-Nicol Byrne	<i>Calcium homeostasis in intervertebral disc cells</i>	E. Mavrogonatou	Dept. Biology, University of Athens
Vassiliki Gosda	<i>Mitochondrial response of protein non-folding upon pharmacological inhibition of LonP1 mitochondrial protease in cancer cells</i>	G. Voutsinas	Metropolitan College of Greece
Athanasia Menoudaki-Evangelou	<i>Mitochondrial response of protein non-folding upon inhibition of mitochondrial translation in cancer cells</i>	G. Voutsinas	Metropolitan College of Greece
Maria Triantafyllopoulou	<i>Mitochondrial response of protein non-folding in the presence of increased levels of intracellular reactive oxygen species in cancer cells</i>	G. Voutsinas	Metropolitan College of Greece

IBA LECTURES' CONTRIBUTION TO THE 2020 SUMMER SCHOOL

DATE	SPEAKER	TITLE
13/7/2020	Dr. D. Kletsas	The multiple roles of cell senescence in tissue homeostasis
13/7/2020	Dr. L. Swevers	A novel CRISPR/Cas technology and gene editing
13/7/2020	Drs V. Sophianopoulou & C. Gournas	Exploring plasma membrane compartmentalization to develop a new generation of antifungal drugs
13/7/2020	Dr. H. Pratsinis	<i>In vitro</i> studies of natural and synthetic bioactive products
13/7/2020	Dr. T. Papakyriakou	Development of novel inhibitors based on computational and structural methods
13/7/2020	Drs E. Vavouraki & I. Ninios	Tissue grafts – new directions
13/7/2020	Dr. A. Chroni	Postgraduate research at IBA

COLLECTIVE DATA

SUMMARIZED DATA ON THE PRODUCTIVITY OF SCIENTIFIC PROGRAMMES

	PROGRAMME			INSTITUTE
	A	B	C	
Researchers & functional scientific personnel	11	7	6	25 ^a
Technical Specialists	2	1	-	7 ^b
Collaborating & Emeritus Scientists	3	2	1	6
Postdoctoral Fellows	7	6	4	17
PhD candidates	12	7	5	24
MSc students	7	2	1	10
Graduate Research Associates	6	3	-	16 ^c
Undergraduate and other training students	14	7	-	21
Administrative and Technical Support	-	-	-	12
Total Personnel	62	35	17	138
Publications in Peer-Reviewed Journals	24	14	22	63^d
Cumulative Impact Factor in Peer-Reviewed Journals	122.45	54.097	71.391	253.718
Proceedings in Conferences	2	1	-	3
Total Publications	26	15	16	66
Citations	1912	564	302	2912^e
International Patents	-	-	1	1
Greek Patents	-	-	-	1
Presentations in International Conferences	2	7	-	9
Presentations in International Conferences	5	5	-	10
Total Number of Presentations in Conferences	7	12	-	19

^a One Scientist of the Human Tissue Bank included

^b 4 Technical Specialists of the Laboratory for Doping Control and Metabolic Studies included

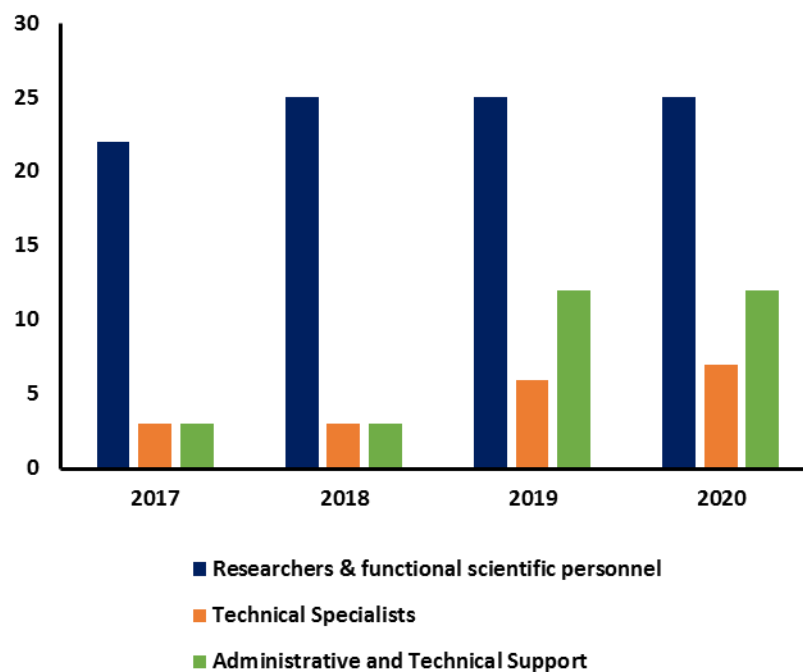
^c 4 Graduate Research Associates of the Human Tissue Bank, 1 of the Experimental Animal Facility and 2 of the Laboratory for Doping Control and Metabolic Studies included

^d 3 publications of the Laboratory for Doping Control and Metabolic Studies included

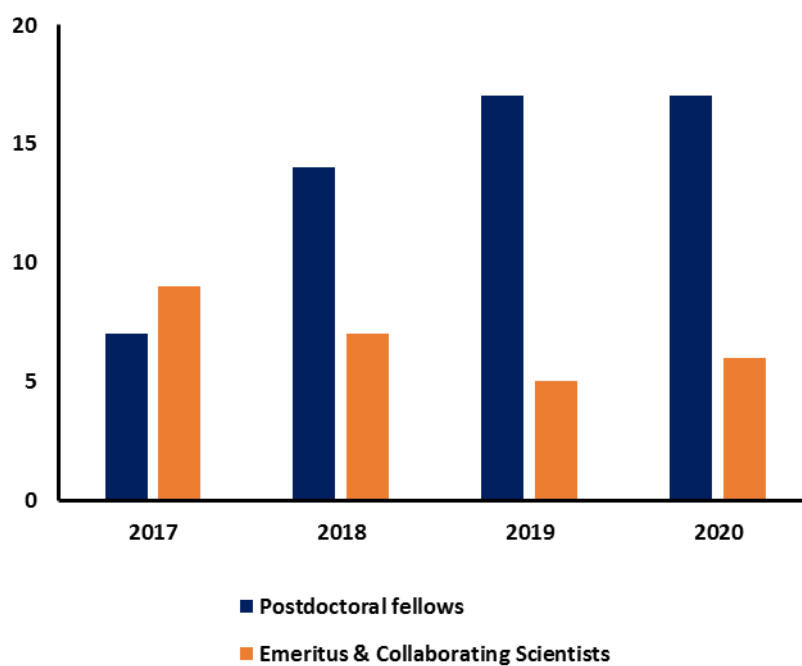
^e Citations of the Human Tissue Bank and of the Laboratory for Doping Control and Metabolic Studies included

CHANGES IN IBA STAFF DURING 2017-2020

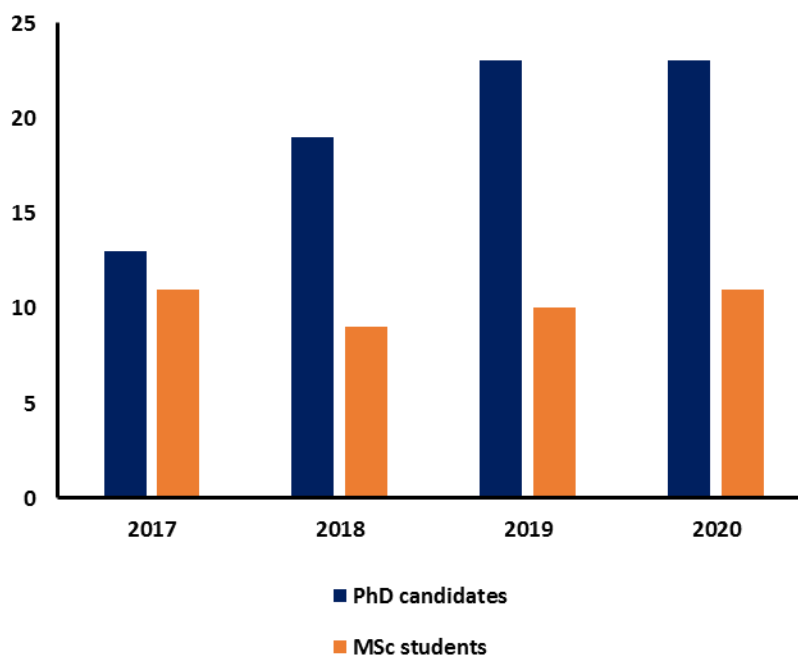
Tenured Staff



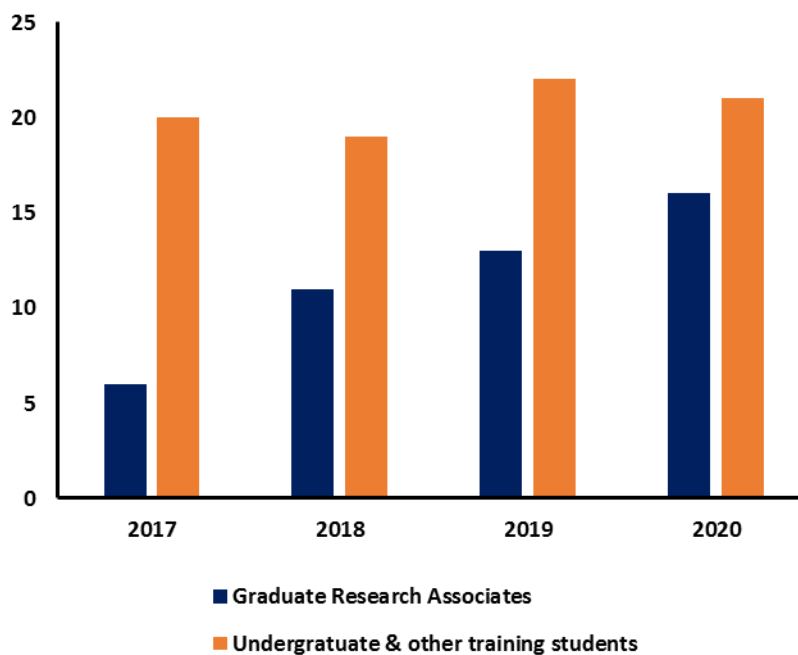
Postdoctoral fellows/ Emeritus & Collaborating Scientists



PhD candidates / MSc students

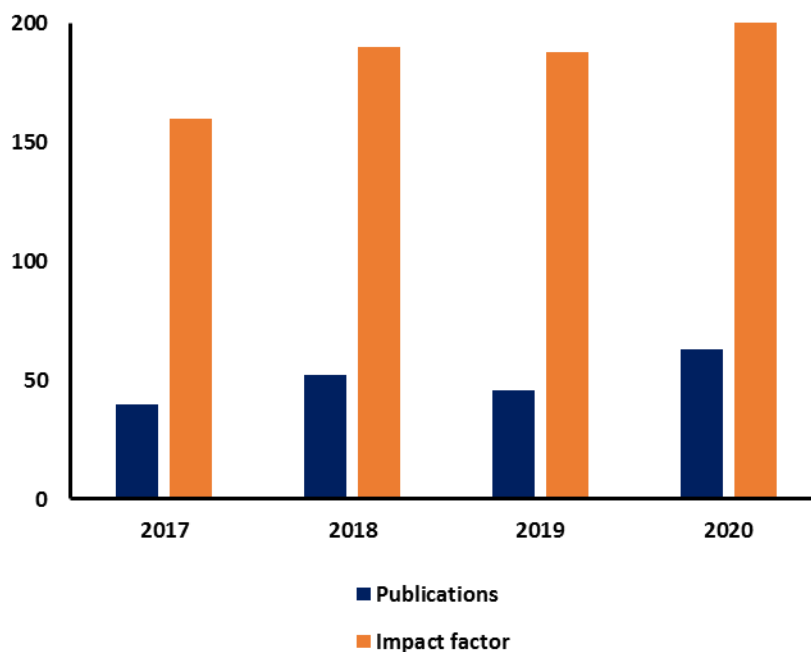


Undergraduate & other training students

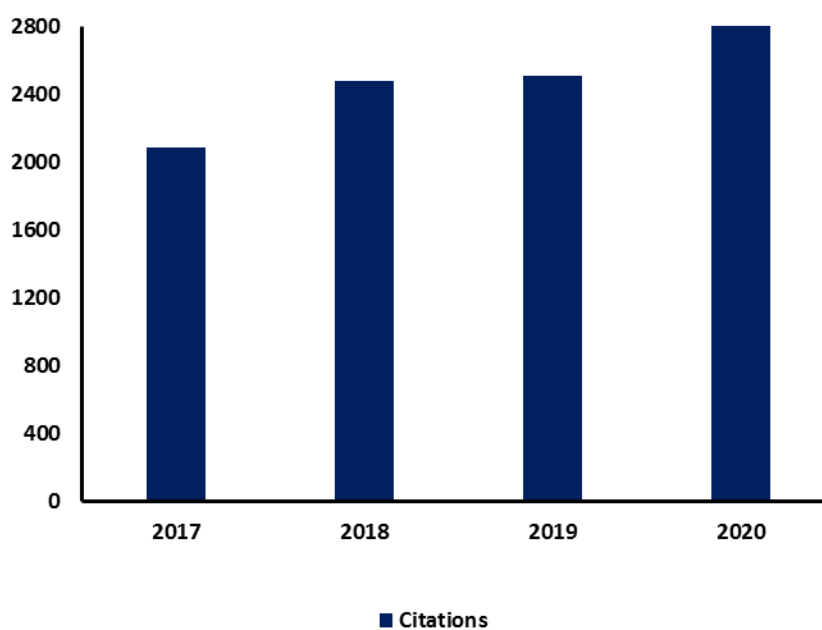


QUALITATIVE AND QUANTITATIVE DATA ON IBA PUBLICATIONS DURING 2017 – 2020

Publications in peer-reviewed Journals / Cumulative impact factor



Citations



EXTERNAL IBA FUNDING DURING 2017 – 2020

