БЪЛГАРСКА АКАДЕМИЯ на НАУКИТЕ
1869

BULGARIAN ACADEMY OF SCIENCES

INSTITUTE OF BIOPHYSICS AND BIOMEDICAL ENGINEERING

SCIENTIFIC SESSION „Biomedicine and Quality of Life – Youth in Science“ 26 – 27 June 2017

BOOK OF ABSTRACTS

Program Committee:

- **Chair:** Corr. Memb. Prof. Andon Kossev, DSc
- **Members:**
    - Corr. Memb. Prof. Krassimir Atanassov, DSc DSc
    - Corr. Memb. Prof. Ilza Pajeva, DSc
    - Prof. Albena Momchilova, DSc
    - Prof. Ivaylo Christov, DSc
    - Prof. Rositsa Raikova, DSc
    - Prof. Stefka Taneva, DSc
    - Prof. Antoaneta Popova, PhD
    - Assoc. Prof. Natalia Krusteva, PhD
    - Assoc. Prof. Tania Pencheva, PhD

Organizing Committee:

- **Chair:** Assoc. Prof. Tania Pencheva, PhD
- **Members:**
    - Assoc. Prof. Tonya Andreeva, PhD
    - Assoc. Prof. Sashka Krumova, PhD
    - Assist. Prof. Kamelia Hristova, PhD
    - Assist. Prof. Vassia Atanassova, PhD
    - Assist. Prof. Peter Vassilev, PhD
    - Severina Semkova, PhD

With the kind support provided by:

СЪЮЗ НА УЧЕНИТЕ В БЪЛГАРИЯ

ELTA '90

More than Technology

the innovative for biology & medicine

MED future

Table of Contents

Session 1

„Photosynthesis“

Photosynthetic Performance, Lipid Composition and Production of Antioxidants in *Solanum licopersicum* as Affected by High Light at Low Temperature

Milena Gerganova[1], Livia Spisher[2], Felix Kessler[2], Maya Velitchkova[1]

[1]*Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences*
E-mail: milena@bio21.bas.bg

[2]*Laboratory of Plant Physiology, Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland*

Abstract: Plant growth and yields depend on the effectiveness of photosynthesis. Long-term climate change driven by extreme temperatures and high light may have a deleterious impact on plant physiology and in turn negative effects on crop yields. For optimal functioning, the photosynthetic machinery in chloroplasts continuously adapts to changing environmental conditions. Concomitant with lipid phase changes and organization of pigment-protein complexes, under environmental stress the defense systems are activated and production of antioxidant compounds occurred allowing the plants to cope and acclimate to unfavorable conditions. In present study, photosynthetic performance and alterations of membrane lipids, prenylquinones and carotenoids were evaluated in tomato plants (*Solanum licopersicum*) after development for two and six days at 12/10 ºC and optimal or high light intensity. The content and unsaturation degree of MGDG and DGDG, the main lipids of thylakoid membranes were analyzed by means of non-target lipidomics and compared with results from control plants. At low temperatures and high light intensity, the most striking changes were observed for the prenylquinones and the degree of saturation of fatty acids. The data suggest that increased concentration of α-tocopherol is important for protection against low temperature stress and for proper function of photosynthetic apparatus.

Keywords: Photosynthesis, Prenylquinones, Fatty acids, Carotenoids.

Acknowledgements
This work is supported by Bulgarian-Swiss Research Program under Project IZEBZO-143169/1 and Project № 136/12.05.2016 of the Program for Career Development of Young Scientists, BAS.

Regulatory Effect of Salicylic Acid on the Photosynthetic Activity of Rice Plants under Physiological Conditions

Ekaterina Yotsova, Anelia Dobrikova, Martin Stefanov, Emilia Apostolova

Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences
E-mails: katya13@bio21.bas.bg, ekaterina_yotsova@abv.bg

Abstract: The aim of this study was to investigate the regulatory role of exogenous application of salicylic acid (SA) on the photosynthetic apparatus of rice plants under physiological conditions. Pulse Amplitude Modulated (PAM) and low-temperature chlorophyll fluorescence measurements, P700 redox state measurements, photochemical activities of both photosystems (PSII-mediated electron transport and PSI-mediated electron transport) and the kinetic parameters of the oxygen-evolving reaction were used to assess the effect of different concentrations of SA (10, 50, 100 μM) on the photosynthetic apparatus of rice seedlings. The pigment contents and some growth parameters were also determined. Data showed that exogenous SA causes: (i) an influence on the energy transfer between pigment-protein complexes of the thylakoid membranes only at the highest studied concentration; (ii) a modification of the Mn clusters of the oxygen-evolving complex; (iii) an influence on the interaction between the secondary quinine acceptor (Q_B) and the plastoquinone; (iv) changes in the oxidation-reduction properties of P700. Observed effects of SA on the rice photosynthetic activity strongly depended on its concentration, as only the application of 10 μM SA had a stimulating effect on the functional activity of the rice photosynthetic apparatus under physiological conditions.

Keywords: Salicylic acid, Photochemical activity, Pigments, P700, Chlorophyll fluorescence.

Acknowledgements
This study is supported by Bulgarian Academy of Sciences and the Project № DFNP-137/12.05.2016 of the Program for Career Development of Young Scientists, BAS. The authors thank the company "Ekoprim", Bulgaria for providing the seeds.

Thermoluminescense Study of Photosystem II Activity and Fatty Acid Composition of Chloroplast Membranes from Leaves of *Petasites hybridus* Plants

Vesela Yordanova[1*], Albena Ivanova[2], Albena Momchilova[1], Svetlana Momchilova[3], Liliana Maslenkova[1]

[1]*Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences*
E-mail: v.v.yordanova.bul@abv.bg

[2]*Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences*

[3]*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences*

**Corresponding author*

Abstract: Representatives of genus *Petasites* are shown to be potential sources of high levels of bioactive compounds, especially sesquiterpene esters, with very promising aspects of therapeutic utility. Currently there are no comprehensive studies on the effects of abiotic and phenological factors on photosynthetic activity and metabolic profile of biologically active components of Bulgarian populations of *Petasites*. The aim of the present study is an investigation of structural – functional characteristics of photosynthetic apparatus in *Petasites hybridus* (common butterbur) in changing environmental conditions. For this purpose thermoluminescence (TL) emission from intact leaves and isolated chloroplast membranes and their fatty acid composition were examined. Plant material was collected from populations growing at various locations in Bulgaria. The observed differences in TL curve intensity and the relative contributions of the main B- and AG (afterglow) bands to the overall TL emission without essential changes in emission temperatures and the cycling of photosystem II charge pairs suggest some adaptive changes in the number of PSII centers and assimilatory potential in the leaves in dependence on the growth conditions. The comparison of fatty acid composition of total lipophilic extracts revealed differences between plants from investigated regions and strikingly, a substantial quantity of unsaturated very-long fatty acids was observed.

Keywords: *Petasites hybridus*, Photosynthetic activity, Fatty acid composition.

Acknowledgements: *This work was supported by Project № DFNP-131/12.05.2016 of the Program for Career Development of Young Scientists, BAS.*

Photoinhibition of Photosystems in Thylakoid Membranes of *Arabidopsis thaliana*, Wild Type and Mutant lut2 at Low Temperature

Konstantin Dobrev, Daniela Stanoeva, Maya Velichkova, Antoaneta Popova

Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences
E-mail: kostja2@yahoo.co.uk

Abstract: Carotenoids are responsible for performing important functions in photosynthetic process: as accessory pigments in light absorption and in dissipation of excessive absorbed light energy, deactivating stress-induced reactive oxygen species and triplet chlorophyll. The role of lack of the carotenoid lutein, in high light induced alterations in structural organization and functional activity of the main pigment-protein complexes was evaluated using isolated thylakoid membranes of *Arabidopsis thaliana*, wt and mutant lut2, deficient in lutein. Alterations in photochemical activity of photosystem I and photosystem II were determined by a Clark-type electrode in the presence of exogenous electron donors and acceptors. Activity of oxygen-evolving complex and of the grana and stroma situated photosystem II complexes was evaluated by determination of flash oxygen yields and initial oxygen burst under continuous illumination without addition of electron acceptor. High light induced alterations in energy transfer and interaction between the main pigment protein complexes was determined by analyzing low-temperature (77K) fluorescence emission and excitation spectra. Pulse Amplitude Modulated (PAM) fluorescence was applied for evaluation of maximal quantum efficiency of PSII (Fv/Fm).

Results obtained are discussed in respect to the role of lutein for the organization and sensitivity of photosynthetic apparatus towards high light intensity treatment.

Keywords: Photosystem II, Photoinhibition, Lutein, *Arabidopsis thaliana*.

Acknowledgements
This work was supported by the Project DFNP-134/12.05.2016 of the Program for Career Development of Young Scientists, BAS.

Dependence of Photoinhibition Extent on Acclimation of Tomato Plants to Different Light and Temperature Conditions

Aygyun Faik, Gabriela Petrova, Milena Gerganova, Maya Velitchkova

Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences
E-mail: aygyun@bio21.bas.bg

Abstract: Among all environmental factors, light intensity is one of the most important for photosynthetic organisms. Despite it is a limiting growth factor, exposure of plants to excessive light can cause damages of the photosynthetic apparatus and photoinhibition occurs. In nature, plants have evolved different strategies to manage the relative excitation of photosystem II (PSII) and photosystem I (PSI). In the present study photoinhibition of thylakoid membranes isolated from tomato plants (*Solanum lycopersicum* cv. M82) acclimated to different light intensity and temperature conditions for 6 days was investigated. The extent of high light-induced damage of both photosystems was estimated on the basis of photochemical activity of photosystems in presence of artificial electron acceptor and donors. In order to unravel the alternations in energy distribution and energy interaction between pigment-protein complexes 77K fluorescence emission and excitation spectra were analysed. More sensitive towards high light treatment (1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$) in respect to PSII photochemical activity exhibit thylakoid membranes isolated from high temperature-treated plants contrary to the high temperature and high light-treated plants which demonstrate the highest resistance. A relative increase of energy transfer from PSII to PSI during photoinhibitory treatment is more expressed in thylakoid membranes isolated from plants acclimated to high temperature.

Keywords: Photosystem II, Photosystem I, Photoinhibition, 77K fluorescence.

Acknowledgements
This work is supported by Bulgarian-Swiss Research Program under Project IZEBZO-143169/1.

Light-induced Changes in Fluorescence Characteristics of *Paulownia* Grown in Soils with Different Salinity

Martin Stefanov[1], Ekaterina Yotsova[1], Yuliana Markovska[2], Emilia Apostolova[1]

[1]*Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences*
E-mail: martin@bio21.bas.bg

[2]*Faculty of Biology, Sofia University "St. Kliment Ohridski"*

Abstract: The aim of this investigation was to assess the effects of the light stress on the photosystem II photochemistry and energy transfer between pigment-protein complexes of two lines of *Paulownia* (*Paulownia tomentosa x fortunei* and *Paulownia elongata x elongata*) grown in soils with different salinity. Pulse amplitude modulated (PAM) chlorophyll fluorescence, low temperature (77K) chlorophyll fluorescence and absorption spectroscopy were used to estimate the impacts of high light intensity on photosynthetic apparatus. The light-induced changes in pigment composition were accompanied with to an inhibition of the primary photochemistry of photosystem II (Fv/Fm) and a decrease of the ratio of photochemical to nonphotochemical processes (Fv/F_0). Data revealed that light stress increases energy transfer from photosystem II to photosystem I and influences on the energy transfer from chlorophyll *b* to chlorophyll *a*. The experimental results also showed that energy redistribution between both photosystems was unaffected by soil salinity while this process is influenced by light stress in both lines *Paulownia*. In summary, data in the investigation revealed some of the reasons for the light sensitivity and salt tolerance of the studied *Paulownia* lines.

Keywords: *Paulownia*, Absorption spectra, Chlorophyll fluorescence spectra, Light stress, Salinity.

Acknowledgements
This work was supported by the Bulgarian Academy of Sciences and Project № DFNP-138/12.05.2016 of the Program for Career Development of Young Scientists, BAS.

Relations between Photosynthetic Performance and Polyphenolics Productivity of *Artemisia alba* Turra *in vitro* Tissue Cultures

Nia Petrova[1], Petya Koleva[2], Violeta Velikova[3], Tsonko Tsonev[3], Tonya Andreeva[1], Stefka Taneva[1], Sashka Krumova[1], Kalina Danova[2]

[1]*Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences*
E-mails: zlatkova.nia@gmail.com, t_andreeva@abv.bg, sgtaneva@gmail.com, sashka@bio21.bas.bg

[2]*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences*
E-mails: danova@abv.bg, petya_k0leva@abv.bg

[3]*Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences*
E-mails: violet@bio21.bas.bg, tsonev@gmail.com

Abstract: Establishing of optimal growth conditions and secondary metabolites production *in vitro* is vital for the biotechnological development of medicinal plants. In previous work the interrelations between treatments with plant growth regulators *in vitro* and terpenoid levels were studied for the fragrant shrub *Artemisa alba*. In the present work the interrelations between the supplementation of plant growth regulators (benzyladenine, BA and indole-3-butyric acid, IBA), the structural and functional characteristics of the photosynthetic (thylakoid) membranes and the productivity of antioxidant polyphenolic compounds in the medicinal plant *Artemisia alba* Turra is investigated. We assayed colorimetrically the total phenolic and flavonoid levels of total leaf extracts, the structural characteristics of isolated thylakoid membranes from the aerial parts by circular dichroism and atomic force microscopy and their functionality by pulse amplitude fluorescence modulated imaging.

Our results reveal a complex non-linear relationship between BA and IBA supplementation, the polyphenolic levels and the architecture and functionality of the photosynthetic thylakoid membranes. The cultures treated only with IBA structurally and functionally resembled the untreated control and had lower polyphenolics content, while the features of those treated with BA or combination of both hormones strongly depended on the concentration of the supplemented plant growth regulators.

Keywords: *Artemisa alba,* Benzyladenine, Indole-3-butyric, Polyphenolic compounds, Thylakoid membrane.

Acknowledgements
This project is supported by the Bilateral Agreement Program between the Bulgarian and Czech Academies of Sciences.

Session 2

„Cellular and Molecular Mechanisms in Pathology“

Anti-angiogenic Properties of a New Anti-tumor Agent Erufosine

Irina Georgieva[1], Martin Berger[2], Albena Momchilova[1], Rumiana Tzoneva[1]

[1]*Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences*
E-mails: georgieva.irina5@gmail.com, tzoneva@bio21.bas.bg

[2]*German Cancer Research Center, Heidelberg, Germany*
E-mail: m.berger@dkfz.de

Abstract: Angiogenesis is extremely important for tumor growth and metastasis. Therefore, many tumors enhance the proliferation of endothelial cells in order to provide the cancer with the necessary supplements. Thus the suppression of tumor growth by inhibition of angiogenesis is a new approach in anti-tumor therapy [1]. Erufosine is a membrane-acting anti-tumor lipid with high sensitivity to tumor cells, most probably due to the difference in membrane fluidity of cancer and normal cells. While the apoptotic effect of erufosine on different cancer cells is well studied, little is known about its anti-angiogenic properties. Therefore, our work was concentrated on the effect of erufosine on endothelial cells. For that purpose *in vitro* angiogenic model was established consisting of proliferating and resting endothelial cells cultured on coatings of adhesive proteins as fibrinogen, fibronectin and collagen [2]. To analyze the action of erufosine on cell survival, monolayer permeability and actin cytoskeleton reorganization on proliferating and resting Human Umbilical Vein Endothelial Cells (HUVECs) we used MTT test, FITC-labeled dextran permeability assay and actin staining technique. Protein coatings increased the survival rate of HUVECs treated with erufosine: IC50 of erufosine for HUVECs seeded on protein coated surfaces was higher compared to the non-coated surfaces. HUVEC monolayer permeability was altered by the action of erufosine. Resting HUVECs showed higher resistance to erufosine than proliferating HUVECs. The present study affirms erufosine as a multi-modal anti-tumor agent with complex and directed action both against the survival processes of cancer cells and against tumor angiogenesis.

Keywords: Erufosine, Tumor angiogenesis, Anti-angiogenic therapy.

Acknowledgements
This work was supported by projects DFNI B02/5 and DFNI B02/23.

References

1. Chometon G., F. Cappuccini, A. Raducanu, M. Aumailley, V. Jendrossek (2014). The Membrane-targeted Alkylphosphocholine Erufosine Interferes with Survival Signals from the Extracellular Matrix, Anti-cancer Agents in Medicinal Chemistry, 14, 578-591.
2. Zerp S., S. Vink, G. A. Ruiter, P. Koolwijk, E. Peters, A. H. van der Luit, D. de Jong, M. Budde, H. Bartelink, W. J. van Blitterswijk, M. Verheij (2008). Alkylphospholipids Inhibit Capillary-like Endothelial Tube Formation *in vitro:* Antiangiogenic Properties of a New Class of Antitumor Agents, Anti-cancer Drugs, 19, 65-75.

Effects of Newly Synthesized Derivatives of Caffeine-8-thioglycolic Acid on Isolated Rat Synaptosomes and Human Neuroblastoma Cell Line SH-SY5Y

Alexandra Kasabova-Angelova[1], Magdalena Kondeva-Burdina[1], Javor Mitkov[2], Maya Georgieva[2], Virginia Tzankova[2], Alexander Zlatkov[2]

[1]*Laboratory "Drug Metabolism and Drug Toxicity", Department "Pharmacology, Pharmacotherapy and Toxicology", Faculty of Pharmacy, Medical University, Sofia*
E-mail: alexandrakasabova89@gmail.com

[2]*Department "Pharmaceutical Chemistry", Faculty of Pharmacy, Medical University, Sofia*

Abstract: Oxidative stress is implicated in the pathogenesis of many diseases, such as neurodegenerative, cancer and others. The increased production of reactive oxygen species (ROS) lead to damage of number of biomolecules: lipids, proteins, DNA, etc.

Caffeine is the most commonly used psychostimulant, which revealed protective effects in conditions of chronic liver and neurodegenerative diseases [1-3].

The aim of the study was to evaluate the effects of newly synthetized derivatives of caffeine-8-thioglycolic acid (in concentration 100 μM) on isolated rat synaptosomes and cell line SH-SY5Y. On human neuroblastoma cell line SH-SY5Y, most of the derivatives didn't show statistically significant neurotoxic effects, compared to the control (non-treated cells). Only JTA-2Ox, JTA-11, JTA-12 and JTA-13 decreased cell viability, measured by MTT-test.

On isolated rat brain synaptosomes, administered alone, all the compounds revealed statistically significant neurotoxic effects, compared to the control (non-treated synaptosomes). JTA-1, JTA-2 and JTA-3 showed lowest neurotoxic effects and were investigated in a model of 6-hydroxydopamine-induced oxidative stress. Under the conditions of 6-hydroxydopamine-induced oxidative stress, the compounds JTA-1 and JTA-2, with proven antihypoxic effects, show a statistically significant neuroprotective effects on isolated rat synaptosomes, by preserving the synaptosomal viability and the level of reduced glutathione. The possible mechanism of neuroprotection might be due to the potential competition between the 6-hydroxydopamine and JTA-1 and JTA-2 for binding to the dopamine receptor.

Keywords: Neuroprotection, Caffeine, Synaptosomes, SH-SY5Y cell line.

Acknowledgements
This work was supported by Project №570/21.01.2016 of Council of Medical Science, Medical University, Sofia.

References

1. Bravi F., C. Bosetti, A. Tavani, S. Gallus, C. La Vecchia (2013). Coffee Reduces Risk for Hepatocellular Carcinoma: An Updated Meta-analysis, Clinical Gastroenterology and Hepatology, 11(11), 1413-1421.e1.
2. Cardina R., M. Piciocchia, D. Martinesa, L. Scribanoa, M. Petraccob, F. Farinati (2013). Effects of Coffee Consumption in Chronic Hepatitis C: A Randomized Controlled Trial, Digestive and Liver Disease, 45, 499-504.
3. Kolahdouzan M., M. J. Hamadeh (2017). The Neuroprotective Effects of Caffeine in Neurodegenerative Diseases, CNS Neuroscience & Therapeutics, 23(4), 272-290.

Implication of Interaction between Tumor-supressor Protein p53 and HMGB1 in Oncogenesis Process

Maria Schröder, Jordana Todorova, Iva Ugrinova

Roumen Tsanev Institute of Molecular Biology, Bulgarian Academy of Sciences
E-mails: marias82@abv.bg, jordanabg@yahoo.com, ugryiva@gmail.com

Abstract: Tumor development and response to therapy both depend on the balance between apoptosis ("programmed cell death") and autophagy ("programmed cell survival"). The High mobility group box 1 protein HMGB1 and the tumor suppressor protein p53 are essential partners in cellular fate determination in terms of apoptosis and autophagy regulation. It is shown, that they form a complex, which regulates the balance between tumor cell death and survival. Any change in the delicate p53 – HMGB1 relationship would lead to misbalance in cell death pathways and might result in increased levels of autophagy and cell survival, respectively to drug resistance. The main goal of our investigation was to elucidate, whether the post-translational modifications /acetylation/ and the acidic C-tail of HMGB1 influence the interaction of HMGB1 with p53 in human lung carcinoma cells A549. Furthermore, we explore the localization of p53 in cells treated with exogenous HMGB1.

We used protein-protein binding assay to test the affinity of differently modified HMGB1 proteins and immunofluorescent microscopy to monitor p53 localization in the cells.

Based on the experimental results we can draw the conclusion that the stimulation with HMGB1 results in translocation of cytoplasmic p53 in the nucleus. In addition, the post-translational acetylation and the acidic C-tail of HMGB1 affect its interaction with p53 and might be developed further as a putative therapeutic target in cancer treatment.

Keywords: HMGB1, p53, Autophagy, Apoptosis.

Acknowledgements
This work was supported by Grant DN01/10 16.12.2016 of the National Science Fund of Bulgaria.

The Motility of Non-small Cell Lung Cancer Cells is Stimulated by HMGB1

Maria Petrova[1], **Ivan Iliev**[2], **Iva Ugrinova**[1]

[1]*Roumen Tsanev Institute of Molecular Biology, Bulgarian Academy of Sciences*
E-mails: mhristova84@abv.bg, ugryiva@gmail.com

[2]*Institute of Experimental Morphology, Pathology and Anthropology with Museum*
Bulgarian Academy of Sciences
E-mail: taparsky@abv.bg

Abstract: Non-small cell lung cancer is the most common form of lung cancer. The causes of this disease are still poorly understood. HMGB1/RAGE is identified as a ligand-receptor pair that plays an important role in tumorigenesis. HMGB1 and RAGE levels are higher in most human tumors and HMGB1 overexpression is associated with tumor progression. We investigated the effect of HMGB1 protein and its truncated form, lacking the C terminus, on RAGE expression and cell motility of lung cancer cell lines – A549 (p53 positive) and H1299 (p53 negative). The results demonstrate that the motility of both cell lines was stimulated only by the full length HMGB1. The C-tail removal resulted in the formation of a stable ligand/receptor complex and substantial increase of exogenous RAGE half-life but did not provoke downstream cellular events. Our results suggest that HMGB1/RAGE signaling should be considered as an essential process for the development of non-small cell lung cancers with great invasive potential. The truncated form plays the role of a blocking molecule that "locks" the receptor and inactivates it. This makes the tailless molecule a promising therapeutic agent that competes for the biologically active HMGB1 ligand and prevents the downstream signaling through RAGE.

Keywords: HMGB1, RAGE, Cell motility, Tumorigenesis, Non small cell lung cancer.

Acknowledgements
This work was supported by grant DN01/10 16.12.2016 of the National Science Fund of Bulgaria.

Radiotherapy of Malignant Neoplasms Using Brachytherapy

Georgi Varbanov, Radostin Mihaylov, Denitsa Simeonova

Medical University "Prof. Dr. Paraskev Stoyanov", Varna
E-mail: vargeorg@gmail.com

Abstract: Brachytherapy is a type of intercellular radiotherapy in which the source of radioactive energy is placed near the organ or directly in it. The ionizing energy destroys the structure of DNA and thus limits cell division. The exact placement of the ionizing material protects the surrounding tissues from exposure to radiation and allows the administration of higher doses of radiation, compared to teletherapy. Brachytherapy is used for treatment of cancer of the prostate, vagina, cervix, breast, skin, esophagus, etc. Depending on the place where it is administrated, brachytherapy can be classified as interstitial, intracavitary, interlaminar, intravascular. Depending on the dose of ionizing rays, brachytherapy is divided into several categories: HDR, LDR, PDR, permanent and electronic. A recent analysis of information from more than 100,000 men with prostate cancer, using Surveillance, Epidemiology, and End Results (SEER)-Medicare database, shows that external-beam radiation therapy causes more long-term side effects and is more expensive than brachytherapy. The researchers found that little more than 7% of men who received external-beam radiation therapy experienced side effects, such as narrowing of the urethra and bleeding in the bladder, compared to about 3% of men who had brachytherapy.

Keywords: Brachytherapy, Radiotherapy, Neoplasms.

Acknowledgements
This work is an extended version of a results presented at Student's Session "Biophysics – Clinical Applications: Principles, Concepts and Methods" at the Department of Physics and Biophysics at the Medical University of Varna. We are grateful to our supervisor, Assist. Prof. Yordanka Eneva and the Head of the Department of Physics and Biophysics – Assoc. Prof. Stefan Krustev, D.Sc.

Calorimetric Markers for Monitoring Non-transplanted and Subjected to Autologous Stem-cells Transplantation Patients with Multiple Myeloma

Avgustina Danailova[1], Sashka Krumova[1], Svetla Todinova[1], Lidia Garcheva[2], Stefka Taneva[1]

[1]*Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Science*
E-mails: avgustina_danailova@abv.bg, sashka@bio21.bas.bg, todinova@abv.bg, sgtaneva@gmail.com

[2]*National Specialized Hospital for Active Treating of Haematological Diseases, Sofia*
E-mail: lgartcheva@gmail.com

Abstract: Multiple myeloma (MM) is a neoplastic disease most often associated with secretion of abnormal monoclonal immunoglobulins (paraproteins or M proteins) in the blood stream. Our previous large scale studies on the MM serum proteome demonstrated that differential scanning calorimetry (DSC) can well be applied to characterize and stratify MM cases with diagnostic and monitoring purposes [1-3].

In this work we explore the changes in the dynamics of the calorimetric features of MM sera derived from patients that were subjected to autologous stem-cells transplantation (ASCT) as well as from non-transplanted ones during treatment and compare them with the immunological marker (M protein concentration) routinely used in the clinical practice. Our data show that as expected patients that underwent ASCT in general had lower M protein level compared to the non-transplanted cases. Irrespective of the application of ASCT or not the shape similarity parameter and the weighted average center of the calorimetric profiles were correlated with the M protein level and thus with the clinical status of the patients showing that these parameters are suitable for patients monitoring during treatment.

Keywords: Multiple myeloma, Differential scanning calorimetry, Monoclonal immunoglobulins, Autologous stem-cells transplantation.

Acknowledgements
This work was supported Project № DFNP-140/12.05.2016 of the Program for Career Development of Young Scientists, BAS.

References

1. Todinova S., S. Krumova, L. Gartcheva, C. Robeerst, S. G. Taneva (2011). Microcalorimetry of Blood Serum Proteome: A Modified Interaction Network in the Multiple Myeloma Case, Anal Chem, 83, 7992-7998.
2. Todinova S., S. Krumova, R. Radoeva, L. Gartcheva, S. G. Taneva (2014). Calorimetric Markers of Bence Jones and Nonsecretory Multiple Myeloma Serum Proteome, Anal Chem, 86, 12355-12361.
3. Krumova S., S. Todinova, A. Danailova, V. Petkova, K. Dimitrova, L. Gartcheva, S. G. Taneva (2015). Calorimetric Features of IgM Gammopathies. Implication for Patient's Diagnosis and Monitoring, Thermochim Acta, 615, 23-29.

Determination of the Indirect Effect of Ethanol on the Reduction of Embryonic and Placental Weight through a Model of Mediation

Teodora Taseva[1], **Nelly Olova**[2]

[1]*Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences*
E-mail: tasevatk@bio21.bas.bg

[2]*The Babraham Institute, Cambridge, UK*

Abstract: The intrauterine growth restriction is a key feature of the fetal alcohol syndrome. One of the major factors controlling embryonic and placental growth is the imprinted *Igf2/H19* locus [2]. Its normal imprinted expression is determined by the CpG methylation levels of four differentially methylated regions (DMRs). In the present study, we hypothesized that 5.8 g/kg ethanol applied during preimplantation stage of pregnancy, can indirectly induce growth retardation in the middle of gestation through a change in the CpG methylation of the *Ifg2/H19* DMRs. To test this, we used a simple mediation model as described by Andrew Hayes [1], using the package *PROCESS* and IBM SPSS Statistics 21. Multiple regression analysis and bootstrapping algorithms were applied to assess each component of the proposed mediation model. The results from the analysis confirm a mediatory role for the change in CpG methylation levels of placental *Igf2/H19* locus in the interaction between ethanol and placental weight (PM = 0.357, CI = –34.83 to –0.38). The change in CpG methylation of the embryonic *Igf2/H19* locus was not classified as a mediator, and thus the ethanol directly affected embryo weight (PM = 0.528, CI = –6.24 to 0.084). Our results help reveal the mechanisms underlying ethanol teratogenicity, which so far remained elusive.

Keywords: Ethanol, Fetal alcohol syndrome, Imprinted genes, Mouse embryo, Placenta, Mediation model.

Acknowledgements
This work was supported by grant №BG051PO001-3.3.06-0025, financed by the European Social Fund and Operational Programme Human Resources Development (2007-2013) and co-financed by Bulgarian Ministry of Education and Science.

References

1. Preacher K., A. Hayes (2004). SPSS and SAS Procedures for Estimating Indirect Effects in Simple Mediation Models, Behav Res Methods Instrum Comput, 36(4), 717-731.
2. Reik W., K. Davies, W. Dean, G. Kelsey, M. Constância (2001). Imprinted Genes and the Coordination of Fetal and Postnatal Growth in Mammals, Novartis Found Symp, 237, 19-31.

Session 3

„Bioinformatics and Mathematical Modelling“

Introduction to Wikipedia for Researchers in Biomedicine and Quality of Life: Findings from an Ongoing Project

Vassia Atanassova, Olympia Roeva

Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences
E-mails: vassia.atanassova@gmail.com, olympia@biomed.bas.bg

Abstract: Under the Program for career development of young scientists of the Bulgarian Academy of Sceince, the project "Expanding Wikipedia's scientific-educational corpus with bio-inspired computational paradigms in the field of bioinformatics" was approved in 2016. The aim of the project is to highlight the current research of different approaches to the theory of calculations inspired by nature and collective intelligence, along with their applications in a variety of fields, in particular bioinformatics. In view of the established need, we have also set the ambitious goal of promoting such a dynamic and interdisciplinary area in Bulgaria to a wide group of users, students, lecturers and researchers while emphasizing the achievements of Bulgarian scientists and research teams. To realize the transfer of knowledge on the widest scale, we planned to use the open platform of the online Wikipedia encyclopedia in its Bulgarian version [1].

As a result of the project work in certain cases, some of the terms in Bulgarian language have been established and validated. In view of the existing criticisms of Wikipedia's open platform, it was important that we approach the field of bioinformatics professionally with the necessary competencies and access to scientific literature and scientific databases that as researchers at the Bulgarian Academy of Sciences we have the privilege of using.

With this report, we aim to present the results of the project shortly before its completion and to popularize among young scholars and PhD students who participate in the scientific session "Biomedicine and Quality of Life" the idea that they can also contribute to building a quality public scientific and educational resource within their fields of research competence, using the platform of Wikipedia [2].

Keywords: Bioinformatics, Education, Science, Transfer of knowledge, Wikipedia.

Acknowledgements
This report is supported under the Project № DFNP-124/12.05.2016 of the Program for Career Development of Young Scientists, BAS.

References

1. https://bg.wikipedia.org
2. Marinova M., V. Atanassova (2010). Innovations in Higher Education: Experience with Wikipedia, Proc. of 8th Conference "Management of innovations", 1-2 July 2010, Varna, Bulgaria 100-105. Available at http://wikimedia.bg/downloads/research/Marinova-2010.pdf (access date May 30, 2017).

A Note on Possible Use of Majorization in Problems from Bioinformatics

Peter Vassilev, Todor Stoyanov

Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences
E-mails: peter.vassilev@gmail.com, tstoyanov72@gmail.com

Abstract: In the present paper we outline some of the basic theoretical aspects of the theory of majorization [1]. We make a short review of the uses and capabilities of Lorenz curves to rank different alternatives related both to quality and quantity. Later on we focus our attention on situations where majorization can improve the performance of different algorithms, e.g., related to partitioning problems [2]. Further, we investigate results obtained by InterCriteria Analysis [3] and ways to combine them with majorization techniques to obtain better evaluation.

Keywords: Majorization, Schur convexity (Concavity), InterCriteria analysis, Bioinformatics.

Acknowledgements
The first author is grateful for the support provided by the National Science Fund of Bulgaria under Grant No. DFNI-I-02-5 "InterCriteria Analysis: A New Approach to Decision Making".

References

1. Arnold B. C., A. W. Marshall, I. Olkin (2011). Inequalities: Theory of Majorization and Its Applications, Springer, New York.
2. Parida L. (2007). Pattern Discovery in Bioinformatics: Theory & Algorithms, CRC Press.
3. Atanassov K., V. Atanassova, G. Gluhchev (2015). InterCriteria Analysis: Ideas and Problems, Notes on Intuitionistic Fuzzy Sets, 21(1), 81-88.

InterCriteria Decision Making Approach to Behterev's Disease Analysis

Bistra Zaharieva[1], Lyubka Doukovska[1], Simeon Ribagin[2], Irina Radeva[1]

[1]*Institute of Information and Communication Technologies, Bulgarian Academy of Sciences*
E-mails: bissi_5@yahoo.com, doukovska@iit.bas.bg, iradeva@iit.bas.bg

[2]*Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences*
E-mail: sim_ribagin@mail.bg

Abstract: Behterev's disease is a chronic inflammatory disease, affecting the spine and the sacroiliac joints [3]. This disease causes disability and has hard consequence both for the patients and for society in general. In this paper, we make the consequent step in a series of research, aimed at proposing the application of the novel approach of InterCriteria Analysis (ICA) [1] to medical data, aimed at the discovery of correlations between important healthy indicators [2], based on available medical data. The aim is improvement of the quality of life of such patients through specific authentic methodology of kinesitherapy and ergotherapy. What is researched is the health status of the patients suffering from Rheumatoid spondylitis in relation to their quality of life. The main concept of the ICA approach is to estimate the objects on the basis of several criteria. In the current study, we apply the ICA analysis over the results of a medicine and physiotherapeutic treatment and kinesitherapeutical program. The results obtained from the analysis show strong correlation between some of the indicators. The present paper proves the application of the original InterCriteria decision making approach, which eases the analysis of the relations between the criteria, giving better clinical quality.

Keywords: InterCriteria decision making approach, Behterev's disease, Rheumatoid spondylitis, Ankylosing spondylitis.

Acknowledgements
The authors are thankful for the support provided by the National Science Fund of Bulgaria under Grant No. DFNI-I-02-5 "InterCriteria Analysis: A New Approach to Decision Making".

References

1. Atanassov K., D. Mavrov, V. Atanassova (2014). InterCriteria Decision Making: A New Approach for Multicriteria Decision Making, Based on Index Matrices and intUitionistic Fuzzy Sets, Issues in Intuitionistic Fuzzy Sets and Generalized Nets, 11, 1-8.
2. McHorney C., J. Ware, J. Lu, C. Sherbourne (1994). The MOS 36-Item Short-form Health Survey (SF-36), III. Tests of Data Quality, Scaling Assumptions and Reliability across Diverse Patient Groups, Med Care, 32(4), 40-66.
3. Rudwaleit M., A. Metter, J. Listing, J. Sieper, J. Braun (2006). Inflammatory Back Pain in Ankylosing Spondylitis: A Reassessment of the Clinical History for Application as Classification and Diagnostic Criteria, Arthritis Rheum, 54(2), 569-578.

ICrAData – Software for InterCriteria Analysis

Nikolay Ikonomov[1], Peter Vassilev[2], Olympia Roeva[2]

[1]*Institute of Mathematics and Informatics, Bulgarian Academy of Sciences*
E-mail: nikonomov@math.bas.bg

[2]*Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences*
E-mails: peter.vassilev@gmail.com, olympia@biomed.bas.bg

Abstract: In this paper, we consider the InterCriteria Analysis (ICrA) [3], which is based on the apparatuses of Index Matrices [1] and Intuitionistic Fuzzy Sets [2]. We demonstrate the application of ICrA using the software ICrAData. ICrAData implements five different algorithms for InterCriteria relations calculation, namely μ-biased, Balanced, ν-biased, Unbiased and Weighted. The results from ICrA are displayed in two panels – table and graphic view, and can also be exported in various formats: matrices, vectors, and graphics. In table view the column data can be sorted in ascending or descending order. The graphic view has options for sizing the intuitionistic fuzzy triangle, showing a grid and assigning different colors for the points. Moreover, a selected point in the graphic is outlined in the table view.

All functionalities of the ICrAData software are illustrated by examples.

Keywords: InterCriteria Analysis, Software.

Acknowledgements
The authors are thankful for the support provided by the Bulgarian National Science Fund under Grant Ref. No. DFNI-I-02-5 "InterCriteria Analysis: A New Approach to Decision Making".

References

1. Atanassov K. (2014). Index Matrices: Towards an Augmented Matrix Calculus, Studies in Computational Intelligence, 573.
2. Atanassov K. (2012). On Intuitionistic Fuzzy Sets Theory, Springer, Berlin.
3. Atanassov K., D. Mavrov, V. Atanassova (2014). InterCriteria Decision Making: A New Approach for Multicriteria Decision Making, Based on Index Matrices and Intuitionistic Fuzzy Sets, Issues in Intuitionistic Fuzzy Sets and Generalized Nets, 11, 1-8.

Generalized Net Model of Proximal Humeral Fractures Diagnosing

Simeon Ribagin[1], **Bistra Zaharieva**[2], **Tania Pencheva**[1]

[1]*Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences*
E-mails: sim_ribagin@mail.bg, tania.pencheva@biomed.bas.bg

[2]*Institute of Information and Communication Technologies, Bulgarian Academy of Sciences*
E-mail: bissi_5@yahoo.com

Abstract: Proximal humeral fractures are extremely common injuries and they occur primarily in older patients. They represent 5.7% of diagnosed fractures [3] of and are the third most common fracture pattern seen in elderly [2]. Fractures of the proximal humerus usually occur after a low energy fall. Due to the variety of factors which influence the classification and the diagnosis of these fractures, early detection is the key factor for an appropriate and successful treatment. Accordingly, in the present study we present a successful example of Generalized Nets (GN) [1] application in traumatology and propose a novel approach to timely detection and diagnosing of proximal humeral fractures. The developed GN-model provides a framework that can be used by primary care practitioners to guide diagnostic processes for patient suspected to have fracture of the proximal humerus. Moreover, when running the developed model with real patients' data, the obtained results may be analyzed by the recently proposed approach of intercriteria analysis, which will permit discovery of new relations as well as improvement of the model accuracy.

Keywords: Proximal humeral fractures, Generalized nets, Diagnosing.

Acknowledgements
Work presented here is partially supported by the Project № DFNP-142/12.05.2016 of the Program for Career Development of Young Scientists, BAS and National Science Fund of Bulgaria under Grant No. DFNI-I-02-5 "InterCriteria Analysis: A New Approach to Decision Making".

References

1. Atanassov K. (1991). Generalized Nets, Singapore, World Scientific.
2. Horak J., B. E. Nilsson (1975). Epidemiology of Fracture of the Upper End of the Humerus, Clin Orthop Relat Res, 112, 250-253.
3. Roux A., L. Decroocq, S. El Batti, N. Bonnevialle, G. Moineau, C. Trojani, P. Boileau, F. de Peretti (2012). Epidemiology of Proximal Humerus Fractures Managed in a Trauma Center, Orthopaedics & Traumatology: Surgery & Research, 98, 715-719.

Generalized Net of Cluster Analysis Using CLIQUE: Clustering in QUEst

Veselina Bureva, Stanislav Popov, Velichka Traneva, Stoyan Tranev

University "Prof. Dr. Assen Zlatarov", Burgas
E-mail: vbureva@btu.bg

Abstract: The current research studies one of the techniques for subspace grid-based cluster analysis. The main steps in the process of detecting groups of objects with similar behavior are: dividing the data space into a finite number of cells, forming a grid-based structure, detecting groups of similar objects, and defining the clusters. There are different approaches to grid-based cluster analysis. More common algorithms are STING: Statistical Information Grid Approach, WaveCluster and CLIQUE. STING explores statistical information stored in grid cells, WaveCluster clusters objects using a wavelet transform method and CLIQUE represents a grid- and density-based approach for clustering in a high-dimensional data space. A more thorough study on CLIQUE is done in the current work [1]. It automatically identifies the subspaces of the multidimensional data space and allows better clustering than the one in the original space.

The CLIQUE algorithm cluster analysis process is modeled using the Generalized nets (GNs) apparatus. The theory of Generalized nets was introduced by Prof. Atanassov and is presented in [3, 4]. GNs are defined in a way that is principally different from the ways of defining the other types of Petri nets. Generalized nets are used for describing and modelling real processes as well as to simulate and control them. They can help us to determine an improvement to the real process. The created generalized net presents the steps of the CLIQUE cluster analysis process. It is part of a series of models describing data mining processes [2].

Keywords: Cluster analysis, Generalized net, Subspace grid-based cluster analysis.

Acknowledgements
The authors are grateful for the support provided by the National Science Fund of Bulgaria under grant DN 02/10 New Instruments for Knowledge Discovery from Data and Their Modelling.

References

1. Agrawal R., J. Gehrke, D. Gunopulos, P. Raghavan (1998). Automatic Subspace Clustering of High Dimensional Data for Data Mining Applications, Proc. of the 1998 ACM SIGMOD International Conference on Management of Data, Seattle, USA, June 01-04, 94-105.
2. Atanassov K. (2016). Generalized Nets as a Tool for the Modelling of Data Mining Processes, Innovative Issues in Intelligent Systems, Vol. 623 of the Series Studies in Computational Intelligence, 161-215.
3. Atanassov K. (1991). Generalized Nets, World Scientific, Singapore.
4. Atanassov K. (2007). On Generalized Nets Theory, Prof. M. Drinov Academic Publishing House, Sofia.

Session 4

„Natural Products“

Natural Flavonoids from *Silybum marianum* L. (Milk Thistle): *in vitro* and *in silico* Studies of Pharmacokinetic Properties and Toxicity

Antonia Diukendjieva, Merilin Al Sharif, Petko Alov, Tania Pencheva, Ivanka Tsakovska, Ilza Pajeva

Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences
E-mails: antonia.diukendjieva@biomed.bas.bg, merilin.al@biomed.bas.bg, petko@biophys.bas.bg, tania.pencheva@biomed.bas.bg, ITsakovska@biomed.bas.bg, pajeva@biomed.bas.bg

Abstract: In the recent years natural products re-emereged as a rich source of compounds for drug discovery [1]. However, most of their pharmacokinetic properties, mechanisms of action and potential toxic effects are not well defined.

In the present study we explored important ADME/Tox properties and biochemical interactions of flavonolignans that are the main components of milk thistle (*Silybum marianum* L.) [2]. Predictions by knowledge-based expert systems were performed based on which three potential toxic effects (chromosome damage *in vitro*, estrogen receptor alpha modulation and skin sensitization) and two metabolic transformations (glucuronidation and oxidative *O*-demethylation) were outlined as most plausible among the studied compounds. Molecular modelling studies were further performed and stereospecific interactions of silybin A and silybin B (the main components of *Silybum marianum*) with the ligand-binding domain of the human estrogen receptor alpha were explored. For evaluation of gastrointestinal absorption after oral administration the parallel artificial membrane permeability assay (PAMPA) [3] and an in house QSAR model [4] for prediction of PAMPA permeability were used. According to the results obtained from both methods most of the studied flavonolignans may be considered as highly permeable in the gastrointestinal tract, which is a good prerequisite for sufficient bioavailability.

Keywords: *Silybum marianum L.*, Flavonolignans, ADME/tox.

Acknowledgements
The financial support by the Project № DFNP-141/12.05.2016 of the Program for Career Development of Young Scientists, BAS and the National Science Fund of Bulgaria project DCOST 01/11-2016 is gratefully acknowledged.

References

1. Harvey A. L., R. Edrada-Ebel, R. J. Quinn (2015). The Re-emergence of Natural Products for Drug Discovery in the Genomics Era, Nature Reviews, Drug Discovery, 14, 111-129.
2. Gazák R., D. Walterová, V. Kren (2007). Silybin and Silymarin-new and Emerging Applications in Medicine, Current Medicinal Chemistry, 14, 315-338.
3. Kansy M., F. Senner, K. Gubernator (1998). Physicochemical High Throughput Screening: Parallel Artificial Membrane Permeation Assay in the Description of Passive Absorption Processes, Journal of Medicinal Chemistry, 41, 1007-1010.
4. Diukendjieva A., L. Marinov, P. Alov, I. Tsakovska, I. Pajeva (2015). *In silico* Modelling to Predict Transcellular Permeability of Bioactive Compounds, Series G. Medicine, Pharmacy and Dental Medicine, Union of Scientists in Bulgaria, 18, 187-190.

Flavonoid Glycosides Extracted from *Inula* are Raft Making Molecules

Ralitsa Veleva[1,2*], Tanya Topouzova-Hristova[1], Aneliya Kostadinova[2], Veselina Moskova-Doumanova[1], Albena Momchilova[2], Galya Staneva[2]

[1]*Faculty of Biology, Sofia University "St. Kliment Ohridski"*
E-mail: a._k.c@abv.bg

[2]*Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences*

**Corresponding author*

Abstract: In present-day pharmacology there have been an extending number of investigations on medicinal plants. There are some scientifically proven health-related beneficial activities of extracts of the herbs of the genus *Inula.* We used extracts from *Inula oculus-christi* L., enriched with flavonoid glycosides (FGs) to study the cell morphology changes. We have observed that the extracts impaired cell periphery and disrupted intercellular contacts [1]. The mechanisms of this activity need further investigations and we first studied the lipid organization in model membranes before examining the native membranes. We found that FGs from *Inula* decrease the fluidity of Sphingomyelin (SM) and Phosphatidylcholine (PC) membranes. The phase transition temperature of SM increases with several degrees and the phase transition cooperativity decreases as a function of lipid/FGs ratio. The presence of Cholesterol (Ch) changes the observed trend and FGs are able to fluidize SM/Ch (1/1) membranes. This SM/Ch ratio corresponds to the ratio of these two lipids in the specialized cellular domains called *rafts*. One could suggest that FGs extracted from *Inula* make the cellular membrane *rafts* more fluid. By using ternary PC/SM/Ch mixtures, modeling *rafts* surrounded by liquid-disordered environment, we showed that FGs from *Inula* are rather *raft* making molecules by increasing the fraction of *rafts* compared to the control conditions. Further investigations are planned to reveal a link between membrane organization, cell functioning and human health benefits.

Keywords: *Inula,* Model membranes, *Raft* domains, Extracts.

Acknowledgements
The present work was supported by the Science Fund – Sofia University "St. Kliment Ohridski" under Grant 80-10-2/12.04.2017.

References

1. Veleva R., V. Moskova-Doumanova, M. Todorova, A. Trendafilova, J. Doumanov, T. Topouzova-Hristova (2017). Cytotoxicity of Flavonoid Glycosides, Flavonoid Aglycones and Phenolic Acids from *Inula oculus-christi* L. on Mammalian Cell Lines, Journal of Bioscience and Biotechnology, in press.

Optimized Structure-based Methodology for Studying PPARγ Partial Agonists

Merilin Al Sharif, Antonia Diukendjieva, Petko Alov, Ivanka Tsakovska, Ilza Pajeva

Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences
E-mails: merilin.al@biomed.bas.bg, antonia.diukendjieva@biomed.bas.bg, petko@biophys.bas.bg, ITsakovska@biomed.bas.bg, pajeva@biomed.bas.bg

Abstract: The peroxisome proliferator-activated receptor (PPAR) γ is a master regulator of the lipid and glucose metabolism, thus being a valuable drug target [1]. Since its full activation is related to adverse effects, researchers focus on the discovery of novel compounds with a partial agonists' protein-ligand interaction pattern [2].

The aim of this study was to optimise a docking algorithm for molecular modelling of PPARγ partial agonists.

Therefore, a dataset with structures and activities of PPARγ partial agonists was constructed. A comparative study of different scoring functions' performance was conducted by redocking the partial agonists' structures from selected PPARγ protein-ligand complexes in the PDB [3]. The docking protocols' performance regarding pose reproducibility and interpretability in the context of the collected activity data was estimated.

As a result, an optimised docking protocol was developed to successfully correlate the docking scores of the studied compounds with their experimentally derived activity values and to provide the best matching degree with their original binding modes.

Overall, these results could be useful for designing molecular modelling studies of novel PPARγ partial agonists by selection of reliable docking poses to predict their binding mode and for ranking them in respect to their agonistic activity using the calculated docking scores.

Keywords: PPARγ, Partial agonists, Docking, Optimisation.

Acknowledgements
The study is supported by the project grant from the National Science Fund of Bulgaria under Grant DM 01/1/2016.

References

1. Azhar S. (2010). Peroxisome Proliferator-activated Receptors, Metabolic Syndrome and Cardiovascular Disease, Future Cardiology, 6(5), 657-691.
2. Chigurupati S., S. A. Dhanaraj, P. Balakumar (2015). A Step Ahead of PPARγ Full Agonists to PPARγ Partial Agonists: Therapeutic Perspectives in the Management of Diabetic Insulin Resistance, European Journal of Pharmacology, 755, 50-57.
3. PDB, Protein Data Bank, http://www.rcsb.org/ (access date May 09, 2017).

Structure and Stability of Hemocyanin and Peptides from Garden Snail *Cornu aspersum*

Radostina Velikova, Lyudmila Velkova, Aleksandar Dolashki, Pavlina Dolashka

Institute for Organic Chemistry with Center for Phytochemistry, Bulgarian Academy of Sciences
E-mail: radostina.1988@abv.bg

Abstract: The hemolymph of molluscan snails is a complex mixture of biochemically and pharmacologically-active components such as peptides and proteins. The structures stability, temperature and pH-dependent denaturation of one of isolated peptide was determined by CD. Loss of α-helical structure occurred in the range 50-85 °C depending on pH. The peptide was not stable towards thermally-induced unfolding.

The native molecule of CaH, isolated from hemolymph of garden snail *C. aspersa*, is constructed from three different subunits – βc-CaH, α_N-CaH and α_D-CaH (Mw ~ 400 kDa), and each of them containing eight functional units (Mw ~ 50kDa).

The structural and conformational stability of the CaH, βc-CaH subunit and the βc-CaH-g functional unit (Fu) were analyzed by CD in wide pH-T range (T °C 20÷85 °C and pH 2÷12). To provide details on the stability of the protein, Fu βc-CaH-g was compared with the native molecule of CaH and the subunit βc-CaH via pH–T diagrams, typical phase portraits for stability and denaturation reversibility. Increasing the temperature from 25 °C to 60 °C, the reversible denaturation of the molecule of protein also increases, opening a reversibility window within the range of pH 5.5-8.0 for subunit βc-CaH and the range of pH 5.0-9.0 for functional unit βc-CaH-g and as a result of which the real thermodynamic parameters (Δ Cp, $\Delta H°_{exp}$, and $\Delta G°_{exp}$) were determined.

The obtained results lead to the conclusion that the carbohydrate structure influences the stability of the native molecule CaH and βc-CaH subunit, but does not affect the stability of one isolated functional unit.

Keywords: Photosystem βc-CaH subunit (SU), βc-CaH-g functional unit (Fu), Circular dichroism (CD), Stability, Temperature and pH-dependent denaturation.

Acknowledgements
This work was supported by a research grant № DN 01/14 from 19.12.2016 of the National Science Fund of Bulgaria.

Antioxidant Screening of Peptides Isolated from the Hemolymph of Garden Snail *Cornu aspersum*

Yulian Voynikov[1,2], Radoslav Abrashev[3], Nedelina Kostadinova[3], Ekaterina Krumova[3], Aleksandar Dolashki[1], Lyudmila Velkova[1], Pavlina Dolashka[1]

[1]*Institute of Organic Chemistry with Centrum of Phytochemistry, Bulgarian Academy of Sciences*
E-mail: yulian_voynikov@yahoo.com

[2]*Faculty of Pharmacy, Medical University, Sofia*

[3]*Stephan Angeloff Institute for Microbiology, Bulgarian Academy of Sciences*

Abstract: Antioxidative peptides have been utilized by various species to combat pathogenic microorganisms and stress. In the present study, the antioxidant potential of protein fractions obtained from the hemoplymph of the garden snail *Cornu aspersum* was evaluated. Proteins were separated from the isolated moluskan hemolymph into three fractions with masses of < 10 kDa, 10-30 kDa and > 30 kDa. Two complementary test methods were employed for the preliminary antioxidative screening, involving measurement of the radical scavenging activity on 1,1-Diphenyl-2-picrylhydrazyl free radical (DPPH), and the inhibition of *nitro blue tetrazolium* (NBT) reduction by photochemically generated O_2^-. Caffeic acid was used as a positive control. On the DPPH assay all fractions were able to reduce the stable free radical DPPH to the yellow-colored 1,1-Diphenyl-2-picrylhydrazyl, with the peptide fraction below 10 kDa exhibiting better free radical scavenging activity than those with higher molecular weight. All tested fraction revealed comparable inhibition on the reduction of NBT by photochemically generated superoxide anion radicals, but the effect was less pronounced than that of caffeic acid.

Keywords: *Cornu aspersum*, Peptide, Protein, DPPH, NBT.

Acknowledgement
This work was supported by a research grant № DN 01/14 from 19.12.2016 of the National Science Fund of Bulgaria.

Antibacterial Activity of Peptides from Snail *Cornu aspersum*

Alexandra Nissimova, Aleksander Dolashki, Lyudmila Velkova, Pavlina Dolashka

Institute for Organic Chemistry, Center for Phytochemistry, Bulgarian Academy of Sciences
E-mail: anissimova@yahoo.com

Abstract: The emergence of multi-resistant bacteria requires the development of antibiotics with new mechanisms of action. Antimicrobial peptides are a unique and diverse group of molecules that have a great potential for use in new antimicrobial drugs, as many of them have a pronounced cytotoxicity to a number of multi-drug resistant bacteria. It is known that *Cornu aspersum* snail mucus contains a number of biologically active components. Ten biologically active peptides from *Cornu aspersum* snails contained in a fraction below 10 kDa were isolated by high pressure reverse phase chromatography (HPLC) on a Nucleosil column C18.

The molecular masses of these peptides between 1 and 5 kDa were determined by mass spectrometry analysis (MALDI-TOF). The amino acid sequences of the peptides were determined by their MS/MS spectra which indicate that they are tryptophan, tyrosyl and proline reach peptides. We also analyzed a glycopeptide with a mass of 4113 Da, whose amino acid sequence was determined by MS/MS analysis – KARYCGAEVTAN.

Some of these peptides show antibacterial activity against bacterial strains *E. coli, C. perfringens, P. acnes* and *S. aureus*.

Keywords: Antimicrobial peptides, *Cornu aspersum*, Mass spectrometry, *E. coli, C. perfringens, P. acnes, S. aureus*.

Acknowledgements
This work was supported by a research grant № DN 01/14 from 19.12.2016 of the National Science Fund of Bulgaria.

Investigation of Extracts Derived from the Plant *Geum urbanum* L. as Drug Candidates with Antimicrobial and Cytotoxic Activity

Lyudmila Dimitrova[1], Maya M. Zaharieva[1], Nedelina Kostadinova[2], Milena Popova[3], Iva Tsvetkova[1], Spiro M. Konstantinov[4], Vassya Bankova[3], Hristo Najdenski[1]

[1]*The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences*
Department of Infectious Microbiology
E-mail: lus22@abv.bg

[2]*The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences*
Section of Mycology

[3]*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences*
Laboratory "Chemistry of Natural Products"
E-mail: bankova@orgchm.bas.bg

[4]*Medical University Sofia, Faculty of Pharmacy*
Department of Pharmacology, Pharmacotherapy and Toxicology
E-mail: konstantinov.spiromihaylov@gmail.com

Abstract: The medicinal plant *Geum urbanum* L. has been used in the Bulgarian folk medicine for the treatment of inflammatory and infectious diseases of the gastro-intestinal tract, but the data about its chemical composition and biological activities are still scanty. The aim of our study was to evaluate the antimicrobial, cytotoxic and radical-scavenging potential of extracts obtained from *Geum urbanum* L. on pathogenic bacterial strains, normal transformed and malignant cell lines. The mode of action of the biologically active extracts was examined regarding: (i) bactericidal effect; (ii) influence on bacterial motility and quorum sensing; (iii) apoptosis induction in sensitive cancer cell lines.

Methanol, petroleum ether, ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH) extracts were obtained from the aerial and underground parts of *G. urbanum* L. Minimal inhibitory and bactericidal concentrations (MIC/MBC) were determined by serial dilution method. Bacterial growth rate was determined by time kill assay. Swarming motility and inhibitory potential on the synthesis of pyocianin were studied on *P. aeruginosa* (PA01). Cytotoxicity and apoptosis induction were investigated by colorimetric tests for dehydrogenase and caspase-3 activity. IC_{50} values were calculated with GraphPadPrizm software.

All extracts exhibited antibacterial activity against *Staphylococcus aureus, Staphylococcus epidermidis* and *Bacillus cereus*. The antimicrobial and radical-scavenging properties were most pronounced in EtOAc extracts. The IC_{50} values for tumor cell lines corresponded to MIC and MBC depending on the extract type. EtOAc and *n*-BuOH extracts inhibited the swarming motility and synthesis of pyocianin by PA01.

Taken together, our results indicate that *Geum urbanum* L. is a perspective new source of bioactive compounds with possible potential for treatment of certain skin infections. The established corresponding cytotoxicity supposes activity against bladder cancer with favorable antibacterial effect.

Keywords: *Geum urbanum*, Antibacterial activity, Quorum sensing, Cytotoxicity.

Acknowledgements
This study was supported by DFNP-70/27.04.2016 of the Program for Career Development of Young Scientists, BAS and the Alexander von Humboldt Foundation within Alumni Program "Equipment subsidies" for Maya Zaharieva.

Session 5

„Biomaterials and Model Systems“

Polyelectrolyte Multilayer Coatings for Surface Biofunctionalization

Svetozar Stoichev[1], Tonya Andreeva[1], Stefka G. Taneva[1], Rumen Krastev[2]

[1]*Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences*
E-mail: svetlio85@abv.bg

[2]*The Natural and Medical Sciences Institute at the University of Tübingen (NMI), Germany*

Abstract: The interest in the construction of nanomaterials for biofunctionalization of surfaces for different applications is constantly growing in the recent years. This interest stems from the need to create materials with specific physicochemical, optical and electrical properties that meet the requirements of the biological tissues.

The present investigation is focused on the optimization of the physicochemical and mechanical properties of polyelectrolyte multilayers by addition of Hofmeister anions and on development of novel hybrid polyelectrolyte films by incorporation of ultrathin graphene oxide (GO) layers. The polymeric matrix was built up by natural biocompatible and biodegradable polyelectrolytes – hyaluronic acid (HA) and chitosan (Chi). GO is known as the thinnest and most robust material with unique electrical, thermal and mechanical features.

We demonstrated that the physicochemical properties of HA/Chi multilayers can be successfully modified both by incorporation of GO-layers and by addition of Hofmeister anions [1, 2]. Changing the counteranion from chaotropic to kosmotropic results in an increase of the hydration, thickness, micro- and macro-roughness, and hydrophilicity of the films, whereas the stiffness and anti-thrombogenicity are enhanced in a reverse manner. Film stiffness could be varied over two orders of magnitude by adjusting the type of the counteranion. We also proved that the introduction of kosmotropic anions improves the films biocompatibility contributing to a decrease of the albumin adsorption and complete anti-thrombogenicity.

Keywords: Polyelectrolyte multilayers, Graphene oxide, Nanomaterials, Hybrid films, Hofmeister effect.

Acknowledgements
This work is supported by Bulgarian Academy of Sciences, Project № DFNP-139/12.05.2016 of the Program for Career Development of Young Scientists, BAS.

References

1. Andreeva T. D., A. K. Danailova, P. Terziyska, S. B. Krumova, S. G. Taneva, R. Krastev (2016). The Hofmeister Anion Effect on Morphology of Polyelectrolyte Multilayers for Biofunctionalization of Cardiovascular Stents, Bulg Chem Commun, 48(A), 23-28.
2. Andreeva T. D., H. Hartmann, S. G. Taneva, R. Krastev (2016). Regulation of the Growth, Morphology, Mechanical Properties and Biocompatibility of Natural Polysaccharide-based Multilayers by Hofmeister Anions, J Mater Chem B, 4(44), 7092-7100.

Electrical Stimuli Responsive Biomaterials in Tissue Engineering and Anti-tumor Therapy

Tihomira Stoyanova, Albena Momchilova, Rumiana Tzoneva

Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences
E-mail: tihomira_stoyanova@abv.bg

Abstract: Purpose: Biomaterials that respond to electrical stimuli have gained tremendous research interest in the area of tissue engineering and tumor treatment [1]. During the recent years, there has been growing research on the cell response to the applied electric field which can reflect to biological processes important for tissue regeneration or tumor restriction. The goal of the current presentation is to gain a rational design of electrical stimuli responsive biomaterials which could govern the cell behavior in such a way that can influence cell adhesion, protein adsorption and angiogenesis. We present three groups of stimuli responsive biomaterials: novel electroconductive cryogels, electrospun nanofibres and gelatin-based 3D scaffolds for tissue engineering or tumor treatment.

Findings: Novel fabricated electrically conducting 2-hydroxyethylcellulose/polyaniline (HEC/PANI) nanocomposite cryogels showed excellent cell survival rate and the application of low electrical filed to the attached to the materials endothelial cells triggered changes in cell orientation – they became oriented in parallel to the electrical field [1]. The applied alternative current (AC) to the fibroblasts adhered to the poly(etherimide) (PEI) electrospun fiber materials affected their adhesion and adsorption of fibronectin [2]. The applied electrical filed to gelatin-based hydrogels with elasticity comparable to human tissues influenced cell attachment, protein synthesis, Vascular Endothelial Growth Factor (VEGF) and membrane metalloproteinases (MMP's) production [3].

Conclusion: The presented data revealed the electrical stimuli responsive biomaterials as a rational solution for creating scaffolds for tissue regeneration or tumor treatment and restriction.

Acknowledgements
The work was supported by DNTS 01/003, DFNI-B 02/5 and DFNI-B 02/23 projects.

References

1. Petrov P., P. Mokreva, I. Kostov, V. Uzunova, R. Tzoneva (2016). Novel Electrically Conducting 2-hydroxyethylcellulose/polyaniline Nanocomposite Cryogels: Synthesis and Application in Tissue Engineering, Carbohydrate Polymers, 140, 349-355.
2. Pehlivanova V., V. Krasteva, B. Seifert, K. Lützow, I. Tsoneva, T. Becker (2013). The Role of Alternating Current Electric Field for Cell Adhesion on 2D and 3D Biomimetic Scaffolds Based on Polymer Materials and Adhesive Proteins, Journal of Materials Research, 28, 2180-2186.
3. Tzoneva R., V. Uzunova, S. Apostolova, A. Krüger-Genge, A. Neffe, F. Jung, A. Lendlein (2016). Angiogenic Potential of Endothelial and Tumor Cells Seeded on Gelatin-based Hydrogels in Response to Electrical Stimulations, Clinical Hemorheology and Microcirculation, 64, 941-949.

In vitro and *in vivo* Visualization of Internalization and Localization of Theranostic Nanoparticles in Tumors after Electroporation

Severina Semkova[1], Biliana Nikolova[1], Shuhei Murayama,[2] Elena Stoyanova[3], Iana Tsoneva[1], Zhivko Zhelev[1,4], Ichio Aoki[2], Rumiana Bakalova[2,5]

[1]*Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences*
E-mail: severina.yordanova@gmail.com

[2]*Department of Molecular Imaging and Theranostics, National Institute of Radiological Sciences, National Institute for Quantum and Radiological Science and Technology, Chiba, Japan*
E-mail: murayama@nirs.go.jp

[3]*Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences*
E-mail: elena.n.st@gmail.com

[4]*Medical Faculty, Trakia University, Stara Zagora*
E-mail: zh_zhelev@yahoo.com

[5]*Medical Faculty, Sofia University "St. Kliment Ohridski"*
E-mail: ra_bakalova@yahoo.com

Abstract: One of the recent topics of biomedical research is related to combine opportunities for diagnostic and therapy within a multifunctional nanomaterial ("theranostic nanoparticles") [1]. Promising in this area are two actively investigated science directions – contemporary chemotherapy and nanoparticle-based drug delivery systems. Electro-assisted delivery and internalization is a new approach in this direction [2].

The aims of the study was to investigate the theranostic potential of multimodal nanoparticles, loaded with different contrast and/or drug agents, the visualization of internalization and localization in cancer cells (*in vitro*) and in solid tumors (*in vivo*).

For experiments were used: Colon 26 cancer cells line; mice models; characterization of nanoparticles by DLS analysis and spin-ultrafiltration; electrotreatment; MTS – proliferation test, *in vitro* and *in vivo* fluorescent imaging techniques.

We found that electroporation facilitated penetration, localization and retention of nanoparticles in colon cancer cells both in vitro and in vivo. Improvement of existing and development of new combinations of electrochemotherapy and nanosized long-circulating drug delivery systems have the potential for future therapeutic strategy in personalized treatment of solid tumors.

Keywords: Theranostic, Multimodal Nanoparticles, Drug Delivery, Electroporation, Electrochemotherapy, Fluorescent Imaging.

Acknowledgements
The study was supported by the Project № DFNP-133/12.05.2016 of the Program for Career Development of Young Scientists, BAS.

References

1. Kunjachan S., J. Ehling, K. Storm, T. Lammers (2015). Noninvasive Imaging of Nanomedicines and Nanotheranostics: Principles, Progress, and Prospects, Chem Rev, 115, 10907-10937.
2. Nikolova B., S. Atanasova, Tsv. Mudrov, I. Tsoneva, Zh. Zhelev, R. Bakalova, I. Aoki (2015). Image-guided Electro-assisted Drug Delivery: Comparison between Two Types of Electrodes, International Journal of Bioautomation, 19(2), 259-266.

Polycaprolactone/poly (Ethylene Oxide) Cryogels as Novel Drug Carriers in Anti-tumor Therapy

Veselina Uzunova[1], Petar Petrov[2], Martin Berger[3], Albena Momchilova[1], Rumiana Tzoneva[1]

[1]*Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences*
E-mails: vesi.uzunova@abv.bg, tzoneva@bio21.bas.bg

[2]*Institute of Polymers, Bulgarian Academy of Sciences*
E-mail: ppetrov@polymer.bas.bg

[3]*German Cancer Research Center, Heidelberg, Germany*
E-mail: m.berger@dkfz.de

Abstract: Purpose: Macro-porous hydrogels, synthesized below the freezing point of the solvent (cryogels), have recently gained significant interest in the biomaterial field due to their unique forming properties The Novel polycaprolactone (PCL)/poly (ethylene oxide) PEO cryogels combine useful properties for drug carriers as mechanical stability, prolonged degradation (PCL) and improved release characteristics (PEO). The goal of the present study was to test PCL/PEO cryogels as potential drug carries of new anti-tumor agent erufosine (EPC_3) and Doxorubicin (Dox).

Procedures: The scaffolds of cryogels were prepared via the combination of cryogenic treatment and photochemical crosslinking [1]. The cytotoxicity of cryogels on Graffi tumor cell line and fibroblasts was tested by MTT method. A spectrophotometric technique was used to determine the rate of drug release from scaffolds. Light microscopy was used to detect the cytotoxic effect of EPC_3-loaded scaffolds on cancer cells.

Findings: The cryogels did not show cytotoxicity on any cell types (80-100% viability). The spectrophotometric measurement showed that the composition PCL/PEO (2:1, wt. %) of cryogels was optimal for Doxorubicin release from the scaffolds. The maximal drug release was found in the first two hours and was kept constant for next 8 days. The microscopic observations on breast cancer cells in the presence of EPC_3-loaded scaffolds (PCL/PEO/EPC3 = 2:1:1) showed complete cell degradation after 4 h of incubation duo to the high release of EPC_3 from the scaffold.

Conclusion: The EPC_3- and Dox-loaded scaffolds prepared from the newly synthesized cryogels shows promising release characteristics suitable for their use as drug carries in anti-tumor therapy.

Keywords: Erufosine, Doxorubicin, PCL/PEO biodegradable cryogels, Cytotoxicity, Drug release.

Acknowledgements
This work was supported by Project № DFNP-128/12.05.2016 of the Program for Career Development of Young Scientists, BAS and Grants DFNI B02/5 and DFNI B02/23 of the National Science Fund of Bulgaria.

References

1. Petrov P., P. Mokreva, I. Kostov, V. Uzunova, R. Tzoneva (2016). Novel Electrically Conducting 2-hydroxyethylcellulose/polyaniline Nanocomposite Cryogels: Synthesis and Application in Tissue Engineering, Carbohydr Polym, Elsevier, 140, 349-355.

Influence of Resveratrol on Domain Organization and Membrane Fluidity

Rusina Hazarosova*, Albena Momchilova, Galya Staneva

Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences
E-mail: r_hazarosova@abv.bg

**Corresponding author*

Abstract: Resveratrol (RV) is an antioxidant that has attracted the scientists' attention in the last decade. We are interested in studying particular domains in the membrane, called rafts, enriched in sphingolipids, cholesterol and specialized proteins. They are implicated in a plethora of cellular processes. Both DPH fluorescent anisotropy and DPH-Tempo quenching method were used to monitor RV effect on the fluidity and the raft formation. Lipid mixtures of phosphatidylcholine (PC)/sphingomyelin (SM)/cholesterol (Ch) were used to mimic the cellular rafts. Two types of phosphatidylcholine were chosen to probe the degree of fatty acid unsaturation at *sn-2* position of phosphatidylcholine: palmitoyl-docosahexaenoyl phosphatidylcholine and palmitoyl-oleoyl phosphatidylcholine. We found that RV exhibits complex behavior depending on the SM/Ch ratio, degree of fatty acid unsaturation as well as RV/lipid ratio. RV is able to increase the raft fraction in monounsaturated PC ternary mixture at 2/1 SM/Ch ratio and to decrease it at 1/1 SM/Ch. In polyunsaturated PC ternary mixtures, the general trend of RV is to inhibit raft formation. Lipid/RV ratio is essential for the observed phenomena. Since RV changes physicochemical features of lipid bilayer in different ways it might be suggested that one of the roles of polyphenols is to tune the lipid order, fluidity and organization in the membranes.

Keywords: Resveratrol, Lipid raft, Docosahexaenoic acid, Oleic acid, Lipid ordering, TEMPO, DPH.

Acknowledgments
This work was financially supported by research project DFNI B02/23 of the National Science Fund of Bulgaria.

A Study of Functional Activity of the Retina after Irradiation with Visible Light and Ionizing Radiation from ^{60}Co

Pavlin Tsonev[1,2], Maya Tanovska[2], Ulia Vinogradova[3], Lilia Vladimirova-Mihaleva[2], Evgenii Krasavin[3], Mihail Ostrovskii[3]

[1]*Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences*
E-mail: vladimirova@phys.uni-sofia.bg

[2]*Sofia University "St. Kliment Ohridski", Faculty of Physics*

[3]*Laboratory of Radiation Biology*
International Intergovernmental Organization Joint Institute for Nuclear Research (JINR)
Dubna, Russia

Abstract: In this work we studied the functional activity of the mouse retina after a significant period after irradiation with visible light and ionizing radiation. The study has been carried out by using electroretinography.

It has been established that the recovery time for the physiological activity of the retina depends on the duration of the stimulation by visible light. In this case the recovery of the retina has been observed in one hour after the influence of the visible light. This recovery has no permanent character.

The study of the functional activity of the retina in 8-9 months after the impact of the ionizing radiation has showed, that the adult mouse retina obeys a high radiation resistance. Previously, in the Laboratory of Radiation Biology of JINR Dubna, has been estimated that the influence of accelerated protons and γ-quants in a doses of 14 Gy does not lead to reduction of the a- and b-waves in the retinogram of the mouse eye retina [1-3].

The study of the dynamics of the changes in the physiological activity of the mouse eye retina in 8-9 months after total irradiation with protons and γ-quants with a dose of 5 Gy has showed that the irradiation excites a rectification of magnitudes of the a- and b-waves in the retinogram.

The magnitudes in the retinogram of the mouse eye retina after irradiation do not restore until control values.

Keywords: Retina, Ionizing radiation, Electroretinography.

References

1. Tronov V. A., U. V. Vinogradova, M. U. Loginova, V. A. Poplinskaya, M. A. Ostrovskii (2012). Radio-resistance Mechanisms of Terminally Differentiated Mature Retinal Cells, Cytology, 54(3), 261-269 (in Russian).
2. Tronov V. A., U. V. Vinogradova, K. N. Lyahova, V. A. Poplinskaya, M. A. Ostrovskii (2014). Damage and Functional Retinal Repair of Mice after Exposure to Genotoxin Agents, Radiation Biology, Radioecology, 54(4), 385-392 (in Russian).
3. Vinogradova U. V. (2015). Study of Damage and Processes of Recovery of Mouse Retina after Irradiation with Accelerated Protons and the Action of Methylnitrosourea, Autoreferate of PhD Thessis, Moskow, 3-21 (in Russian).

Dried Blood Spots as Alternative, Archival Material for Detection of Viral Agents (Measles, Mumps, Rubella)

Stefka Krumova, Svetla Angelova, Adelina Pavlova, Silviya Voleva, Elitsa Golkocheva-Markova, Petia Genova-Kalou, Adelina Pavlova, Irina Georgieva

Department of Virology, National Centre of Infectious and Parasitic Diseases (NCIPD), Sofia
E-mail: stefka.krumova@gmail.com

Abstract: In recent years there appears a variety of new and innovative applications of the dried blood spots (DBS). The areas of their range of application are medicine, neonatology, virology, microbiology, toxicology pharmacokinetics and others [1, 2].

The study **aimed** at optimizing and introducing DBS analysis as an alternative approach for the detection of specific viral markers of vaccine preventable diseases – measles, mumps and rubella in Bulgaria.

Material and methods: In the first half of 2017, a total 32 blood samples (16 sera samples and 16 DBS) from children (n = 16) between 1 and 13 years of age were collected as part of measles/rubella surveillance in Bulgaria from 10 districts in the country. Serological methods (indirect ELISA assay) for detection of specific measles, mumps and rubella IgG/IgM antibodies were used.

Results: The marker (ELISA IgM) for acute measles infection in 2 patients (2 serum samples and 2 DBS) was detected. Positive IgG results for all tested viral agents in 13/16 (81.25%) sera and in 12/16 (75%) DBS were found. In 3/16 children protective IgG antibodies not found because they were under the age of first dose MMR vaccine.

Conclusion: These initial data is shown that DBS are suitable as an alternative approach (non-invasive, inexpensive, not requiring cold chain for transport and storage) for laboratory diagnosis and surveillance in virology.

Keywords: DBS, Measles, Mumps, Rubella, ELISA.

Acknowledgements
This study was conducted with the assistance of project № DM 03/1, 12.12.16 "Dried blood spots (DBS) as alternative, archive material for detection of viral agents (measles, mumps, rubella, hepatitis B virus) in Bulgarian population", "Competition for Financial Support for Project of Junior Researchers – 2016", National Science Fund of Bulgaria.

References

1. Hannon W., B. Therell (2014). Overview of the History and Applications of Dried Blood Spot Samples. In: Dried Blood Spots: Applications and Techniques, Li W., M. S. Lee (Eds), Wiley, 3-15.
2. Demirev P. (2013). Dried Blood Spots: Analysis and Applications, Anal Chem, 85(2), 779-789.

Session 6

„Neurophysiology, Movement and Sport Physiology“

The Effect of Co-activation of Antagonist Muscles on Motor Cortex Excitability: A Transcranial Magnetic Stimulation Study

Kapka Mancheva[1], **Diana I. Stephanova**[1], **Werner Wolf**[2], **Andon Kossev**[1]

[1]*Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences*
E-mails: kapka_mancheva@abv.bg, dsteph@bio.bas.bg, kossev@bio.bas.bg

[2]*Institut für Infomationstechnik, Universität der Bundeswehr München, Neubiberg, Germany*
E-mail: Werner.Wolf@UniBw.de

Abstract: The effect of unilateral tonic muscle activity with and without co-activation of the antagonists on motor cortex excitability has been studied in seven right handed healthy volunteers. Contralateral motor evoked potentials (MEPs) were recorded from the first dorsal interosseous muscles of right hands in response to transcranial magnetic stimulation (TMS) during relax, isometric index finger abduction and antagonistic co-activation. The intracortical facilitation (ICF), short- and long-latency intracortical inhibition (SICI and LICI) were investigated by paired-pulse TMS.

The unilateral tonic activation of the right hand facilitated MEPs in response to single-pulse TMS. The increase of MEP amplitudes was significantly greater during isometric index finger abduction compared to co-activation of antagonist muscles. During paired-pulse TMS with short interstimulus intervals, the SICI (interstimulus interval of 3 ms) was not influenced by the unilateral tonic activity while ICF (interstimulus interval of 13 ms) was suppressed. During paired-pulse TMS with longer interstimulus interval (100 ms) the LICI was not influenced during isometric index finger abduction while during antagonistic co-activation the LICI was significantly less pronounced.

The decreased LICI is assumed to reflect mechanisms underlying the co-activation of antagonists.

Keywords: Transcranial magnetic stimulation, Motor evoked potential, Intracortical facilitation, Short-latency intracortical inhibition, Long-latency intracortical inhibition, Co-activation.

Acknowledgements
The study was supported by Project № DFNP-125/12.05.2016 of the Program for Career Development of Young Scientists, BAS.

A Complex Semi-automated Method for Kinetic and Two-dimensional Kinematic Motion Analysis

Katerina Kirilova, Plamen Gatev

Institute of Neurobiology, Bulgarian Academy of Sciences
E-mails: kat_kirilova@abv.bg, pgatev@yahoo.com

Abstract: Functional reach [1] (FR) – maximally reaching forward with the arms while standing is a well-known dynamic balance test, but different movement strategies were reported [2]. This study was motivated by the need of affordable and robust approaches for their evaluation. We introduce a complex semi-automatic method based on 2D-motion capture for measuring distances, joint angles and velocities in the sagittal plane during FR combined with pedobarographic measurements and problem-oriented algorithms for their evaluation.

Passive markers were placed on anatomical landmarks. Subjects performed FR on a pedobarographic platform (Tekscan), recording Center-of-Pressure (COP) excursions. Kinematic data were synchronously video-recorded (Canon Powershot 230HS, no significant lens distortions, tested beforehand). One trial lasted 30s, sampling rate: 30 fps. For better synchronization video-trimming by sound was applied (Solveig). Specialized free software for marker and markerless automatic tracking (Viana.NET, Kinovea) was applied. Planar pole calibration was done by previously measured inter-marker distance. COP excursions in the sagittal plane were measured by Tekscan software. All data were further processed by problem-oriented algorithms (MatLab). We successfully evaluated the kinematic measures: FR, mean forward and return hand velocities; and their corresponding COP measures. The method is prospective for concerted kinetic and 2D kinematic analysis of dynamic standing balance.

Keywords: Functional reach, Posture, 2D-Motion analysis, Center-of-Pressure.

Acknowledgements
This study was partially funded by grant TK 02/60 with the National Science Fund of Bulgaria.

References

1. Duncan P., D. Weiner, J. Chandler, S. Studenski (1990). Functional Reach: A New Clinical Measure of Balance, Journal of Gerontology, 45(6), M192-M197.
2. Liao C., S. Lin (2008). Effects of Different Movement Strategies on Forward Reach Distance, Gait and Posture, 28(1), 16-23.

Influence of Unconscious Semantic Priming for a Wide Focus on a Decision-visuomotor Task in Young and Older Non-professional Drivers

Milena Staneva, Valentina Grigorova

Institute of Neurobiology, Bulgarian Academy of Science
E-mails: mil_ilieva@abv.bg, valgrig04@yahoo.com

Abstract: The study was aimed at estimating of subliminal semantic priming effect for wide attention focus on a decision-visuomotor task performed in traffic scenarios similar to those using in non-professional drivers` license tests. The traffic task was presented in two conditions: 1) immediately after priming and: 2) after primed the same way adaptation of reactive saccades. Thus, we estimated whether priming was shared between the two visuomotor tasks. We also assessed an aging effect on decision-visuomotor task performance. The priming was based on modified scrambled sentence task of Bargh et al. [1]. Ten of twenty traffic scenarios were displayed across the whole screen of the computer monitor and ten were displayed within the central 25% of the screen area.

30 young (Y, aged 20-30 years) and 30 older (O, aged 50-63 years) subjects took part in the study. They were divided equally into three groups according to the purposes of the examination: 1) after priming – Y and O; 2) after a saccade adaptation – YS and OS; 3) Controls – YC and OC.

The estimation was based on an analysis of subjects' response times (RT) of the first vehicle touch determined as the delay between picture appearance and the onset of subject' touch.

The outcomes revealed that RTs were significantly lower: of young control group than those of the older control group and; when small than large scenarios were presented. It was also established that RTs of both Y and O groups were larger than RTs of respective YC and OC groups, i.e. RT increased after priming. On the contrary, RT decreased after saccade adaptation in both age groups, but mostly in OS group. These results suggest: subliminal semantic priming instead positive has rather negative effect on decision-visuomotor task performance due probably shared attention resources between the priming and the task; an aging effect on decision-visuomotor task performance.

Keywords: Spatial selective attention, Reaction time, Saccade adaptation.

References

1. Bargh J. A., M. Chen, L. Burrows (1996). Automaticity of Social Behavior: Direct Effects of Trait Construct and Stereotype-activation on Action, Journal Personality and Social Psychology, 71, 230-244.

Effect of Proactive Mode of Processing on Event-related Oscillatory Brain Responses in Children

Plamenka Nanova, Vasil Kolev, Juliana Yordanova

Institute of Neurobiology, Bulgarian Academy of Sciences
E-mail: pnanova@yahoo.com

Abstract: Purpose: Proactive cognition is characterized by the formation and active maintenance of internal task representation, which may affect the processing of incoming stimuli. The aim was to compare proactive and retroactive modes of processing on oscillatory brain responses in children. It was hypothesized that both the temporal synchronization of event-related oscillations as reflected by phase-locking to stimulus and local synchronization as reflected by oscillation magnitude, would be affected [1, 3, 4].

Procedure: Thirty six children aged 7-10 years were studied. Auditory event-related potentials (ERPs) were recorded in four conditions, in which two cognitive factors (working memory activation and motor task relevance) were either present or not. Children were divided into two groups according to their proactive or retroactive mode of processing. Phase-locking and single-trial magnitude of ERPs were analyzed in delta (0.5-4 Hz), theta (4-7 Hz), slow (7-10 Hz), and fast (10-14 Hz) alpha frequency bands [2].

Findings: Proactive mode of processing was characterized by decreased phase locking of delta and slow alpha oscillations, and reduced magnitude of theta oscillations. These effects did not depend on the processing condition indicating a trait effect.

Conclusions: The results demonstrate that proactive processing in children reduces the temporal and local synchronization of oscillatory neural responses to incoming stimuli.

Keywords: Time-frequency ERP components, Children, Cognition, Auditory modality.

Acknowledgement
Work was supported by the Grant DFNI B-01/24 of the National Science Fund of Bulgaria.

References

1. Kolev V., T. Demiralp, J. Yordanova, A. Ademoglu, Ü. Isoglu-Alkaç (1997). Time-frequency Analysis Reveals Multiple Functional Components during Oddball P300, NeuroReport, 8(8), 2061-2065.
2. Nanova P., V. Kolev, J. Yordanova (2011). Developmental Gender Differences in the Synchronization of Auditory Event-related Oscillations, Clinical Neurophysiology, 122(5), 907-915.
3. Yordanova J., V. Kolev (1998). Event-related Alpha Oscillations Are Functionally Associated with P300 during Information Processing, NeuroReport, 9(14), 3159-3164.
4. Yordanova J., V. Kolev (2009). Event-related Brain Oscillations: Developmental Effects on Power and Synchronization, Journal of Psychophysiology, 23(4), 174-182.

Determining Energy Expenditure from Heart Rate Monitoring during Training Sessions at Bulgarian Wrestlers

Nikolay Zaekov[1], Lubomir Petrov[1], Krassimir Rankov[1], Milena Nikolova[1], Rasho Makaveev[2], Nikola Nikolov[2]

[1]*National Sports Academy "Vassil Levski", Department of Physiology and Biochemistry, Sofia*
E-mail: nzaekov@abv.bg

[2]*National Sports Academy "Vassil Levski", Department of Wrestling and Judo, Sofia*

Abstract: A linear relationship between heart rate (HR) and oxygen consumption during physical activity has been established. Most equations for assessing the energy expenditure by HR monitoring during physical activity are based on this linear dependence.

Hiilloskorpi et al. [1] analyze three equation models and suggest two equations for each model – for low and high activity. Usually the boundary between the two activities is in the range of 80 to 100 beats per minute [3].

Aim of the study was to determine physical activity`s part of daily energy expenditure by heart rate monitoring and using data to control athletes' nutritional intake.

Group of 10 male and 4 female wrestlers (age = 21 ± 1.8) at national level, who train 15 hours per week served as subjects in this investigation.

The 72-hour HR recording was performed with a TEMEO cardiotelemetric system (made in Bulgaria) [2]. The energy expenditure during physical activity is determined by Method 1 of Hiilloskorpi et al. [1].

The determined daily energy expenditure is compared to the theoretically calculated. The deference is less than 100 kcal, but if subjects change the intensity, volume or duration of the workouts or increase their number, the difference will be much more evident.

Keywords: Energy expenditure, Heart rate monitoring, Physical activity.

Acknowledgements
We thank our colleagues and investigated persons for their assistance.

References

1. Hiilloskorpi H. K., M. E. Pasanen, M. G. Fogelholm, R. M. Laukkanen, A. T. Mänttäri (2003). Use of Heart Rate to Predict Energy Expenditure from Low to High Activity Levels, Int J Sports Med, 24(5), 332-338.
2. Mateev H., I. Simova, T. Katova, N. Dimitrov, I. Christov (2011). TEMEO – A Novel Mobile Heart Rhythm Telemonitoring System, Computing in Cardiology, Hangzhou, China, 833-836.
3. Spurr G. B., A. M. Prentice, P. R. Murgatroyd, G. R. Goldberg, J. C. Reina, N. T. Christman (1988). Energy Expenditure from Minute-by-minute Heart-rate Recording: Comparison with Indirect Calorimetry, American Journal of Clinical Nutrition, 48, 552-559.

Manually Defined Continuous Positive Airway Pressure versus Mathematically Calculated for Obstructive Sleep Apnea

Krassimir Rankov, Nikolay Zaekov, Milena Nikolova

National Sports Academy "Vassil Levski", Sofia
E-mail: krankov@gmail.com

Abstract: The determination of therapeutic pressure in patients with Obstructive sleep apnea (OSA) is crucial. Manual adjustment of Continuous positive airway pressure (CPAP) under polysomnography (PSG) is considered the better practice. Recently, different formulas were presented for predicting CPAP using body mass index, neck circumference and apnea/hypopnea index.

The aim of the study was to assess the correlation between the values of pressure calculated by two formulas as well as manual obtained pressure values.

Forty patients (33 male, 7 female) with suspected OSA were examined by PSG. Manual titration was applied using standard protocol. For predicting CPAP were used two formulas [1, 2]:

$$\text{Pressure} = 0.16\times\text{BMI} + 0.13\times\text{NC} + 0.04\times\text{AHI} - 5.12 \quad (1)$$

$$\text{Pressure} = 0.193\times\text{BMI} + 0.077\times\text{NC} + 0.02\times\text{AHI} - 0.611 \quad (2)$$

For men the determined pressure was higher when using the Eq. (1) whereas with the Eq. (2) the pressure was lower than predicted. For women the differences were larger.

The pressures calculated with the Eq. (1) and the Eq. (2) for both genders were lower and higher, respectively, than those experimentally obtained. From a practical point of view, when it is not possible to determine pressure experimentally, the Eq. (2) should be preferred because higher pressure prescription is better for OSA therapy.

Keywords: Obstructive sleep apnea, Continuous positive airway pressure, Titration, Prediction.

Acknowledgements
We thank our colleagues and investigated persons for their assistance.

References

1. Rowley J. A., G. S. Tarbichi, M. S. Badr (2005). The Use of a Predicted CPAP Equation Improves CPAP Titration Success, Sleep Breath, 9(1), 26-32.
2. Sériès F. (2000). Accuracy of an Unattended Home CPAP Titration in the Treatment of Obstructive Sleep Apnea, Am J Respir Crit Care Med, 162(1), 94-97.