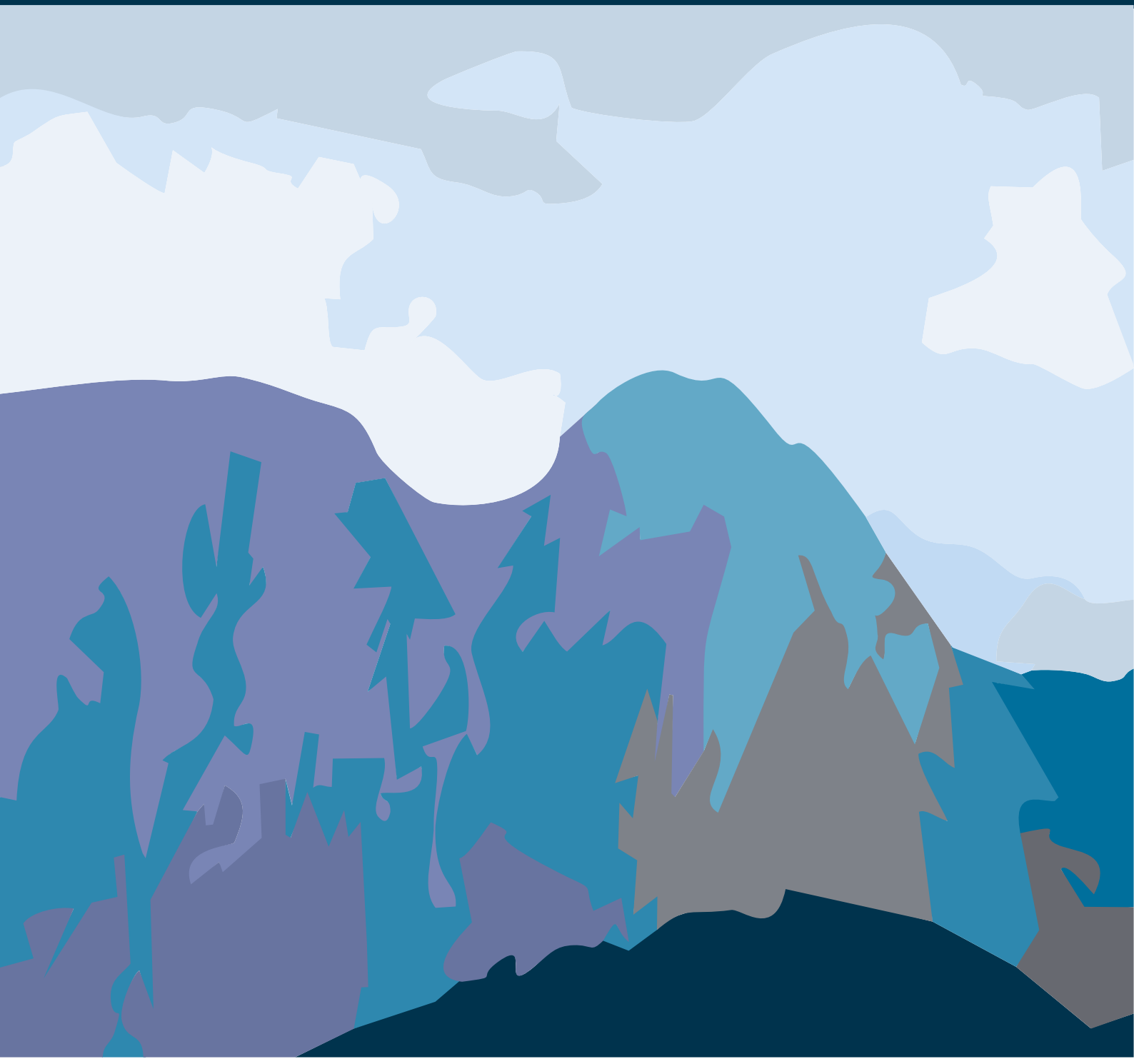
XLVII WINTER SCHOOL

FACULTY OF BIOCHEMISTRY, BIOPHYSICS AND BIOTECHNOLOGY
JAGIELLONIAN UNIVERSITY

MOLECULES, PATHWAYS, AND GAMES

8-12 FEBRUARY 2020, ZAKOPANE



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JAGIELLONIAN UNIVERSITY IN KRAKÓW

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We cordially invite you to participate in the Winter School of the Faculty of Biochemistry, Biophysics, and Biotechnology (FBBB) at the Jagiellonian University in Krakow. The honorary patronage of the Winter School was given by Prof. Zbigniew Madeja, Dean of the FBBB JU.

The School, which will take place from 8. (Saturday) to February 12, 2020 (Wednesday) in Zakopane, opens a series of events related to the celebration of the 50th anniversary of the establishment of the Institute of Molecular Biology of the Jagiellonian University. The acronym of the title of this year Winter School: Molecules, Pathways, and Games refers to initials of Prof. Marta Pasenkiewicz-Gierula, to whom the very first session of the School is dedicated.

Zakopane is a place that certainly favours scientific integration in the beautiful scenery of the Tatra mountains. Many interesting guests have already announced their participation in the School. Register, come to Zakopane, and enjoy one of the most exciting and inspiring scientific meeting organized by FBBB JU this year!

– dr hab. Krzysztof Murzyn

Prof. Marta Pasenkiewicz-Gierula

Urodziłam się w drugi dzień Świąt Bożego Narodzenia w 1949 roku w Krakowie. Bardzo miło wspominam czas spędzony w przedszkolu oraz w szkole podstawowej, za to o liceum wolę nie pamiętać. Pięcioletnie studia fizyki na Uniwersytecie Jagiellońskim odbyte w latach 1967-1972 były dla mnie wybawieniem z reżimu szkolnego i dobrym przygotowaniem do późniejszej pracy naukowej. Pracę magisterską z dziedziny fizyki cząstek elementarnych wykonałam w Zakładzie Fizyki Teoretycznej Instytutu Fizyki UJ w 1972 roku.

Zaraz po studiach w październiku 1972 roku zostałam zatrudniona na etacie naukowo-dydaktycznym w Zakładzie Biofizyki Instytutu Biologii Molekularnej Uniwersytetu Jagiellońskiego, kierowanym wówczas przez prof. dr hab. Stanisława Łukiewicza. Bardzo chciałam być biofizykiem, ale praca w Zakładzie Biofizyki była dla mnie wielkim wyzwaniem, gdyż moja wiedza biologiczna była wtedy znikoma; niestety, nadal taka jest, ale na trochę wyższym poziomie. W Zakładzie Biofizyki, zajmowałam kolejno stanowiska asystenta stażysty, asystenta, adiunkta, profesora nadzwyczajnego i profesora zwyczajnego. W latach 1979-1993 odbyłam 3 długoterminowe staże zagraniczne (w sumie 6 lat) – dwa w National Biomedical ESR Center, USA oraz w University of Tokyo i w Taisho Pharmaceutical Co., Japonia; odbyłam też szereg krótkoterminowych staży w USA, Japonii i Wielkiej Brytanii. Te staże zapoczątkowały współprace naukowe, które do dziś kontynuuję. Obecnie jestem kierownikiem zorganizowanego przez siebie w 2007 roku Zakładu Biofizyki Obliczeniowej i Bioinformatyki. W badaniach prowadzonych w Zakładzie używamy metod komputerowych, więc jedynym narzędziem w naszej pracy jest komputer – jest to możliwe dzięki młodemu (oprócz mnie), entuzjastycznemu zespołowi.

Moje zainteresowania badawcze zmieniały się wraz z kolejnymi stopniami naukowymi. Niemniej jednak, od początku zatrudnienia na Uniwersytecie Jagiellońskim pracuję nad zagadnieniami związanymi ze strukturą i dynamiką bioukładów. W okresie do uzyskania w 1979 roku stopnia doktora, zajmowałam się przede wszystkim strukturą centrów paramagnetycznych. W te zagadnienia wprowadził mnie i kierował moją pracą prof. Tadeusz Sarna (wtedy doktor). W tym okresie współpracowałam też z prof. Zygmuntem Wasylewskim (wtedy doktorem). Problematyką dynamiki centrów paramagnetycznych zainteresował mnie prof. Wojciech Froncisz (wtedy doktor habilitowany). Badania dynamiki kompleksów miedzi prowadziłam w National Biomedical ESR Center, USA we współpracy z profesorami J. Hyde'em, W. Fronciszem, W. Antholine'em, A. Jeśmanowiczem.

Z kolei prof. Akihiro Kusumi wprowadził mnie w tematykę błon lipidowych. Badania błonowe prowadziłam we współpracy z A. Kusumi oraz W. Karolem Subczyńskim. Wymienione badania zostały opisane w mojej rozprawie habilitacyjnej, na podstawie której uzyskałam stopień doktora habilitowanego w 1990 roku. Dzięki prof. A. Kusumi wyjechałam w 1991 roku na prawie 3-letni pobyt naukowy w Japonii, gdzie poznawałam metody modelowania molekularnego. Te metody stosuję obecnie w badaniach błon lipidowych. Komputerowe badania dynamiki błon, prowadzone wspólnie z moimi pierwszymi magistrantami

i doktorantami: Tomaszem Rogiem i Krzysztofem Murzynem, oraz z kolegami z Japonii umożliwiły mi uzyskanie tytułu naukowego profesora.

Według informacji z bazy danych Web-of-Science z dnia 31. 01. 2020 jestem współautorem 85 publikacji, cytowanych 3518 razy (3155 bez autocytowań), mój indeks h wynosi 35. Jestem też autorem lub współautorem dwóch rozdziałów w książkach wydanych przez Springer Verlag oraz trzech skryptów, a także szeregu prac metodycznych i przeglądowych.

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Program

Saturday 8.02

10:00	Departure from Kraków
12:30–15:30	*Registration and lunch*
15:30	Opening Ceremony
15:45–16:30	**Akihiro Kusumi–Opening lecture** Ultrafast single-molecule imaging revealed the compartmentalized architecture of the focal adhesion
16:35–17:00	**Martin Hof** Oxysterols and Truncated Oxidized Phospholipids in Model Membranes
17:00–17:30	*Coffee break*

Special Session
In honor of Prof. Marta Pasenkiewicz-Gierula
Chair: Zbigniew Madeja

17:30–17:50	**Karol Subczyński** Our friendship and collaboration began in Milwaukee
17:50–18:10	**Jerzy Ciarkowski** A short story of the long friendship
18:10–18:30	**Kazimierz Strzałka** Our Winter Schools – facts, legends, memories
18:30–18:50	**Halina Gabryś** On the origins of Biophysicists
20:00	*Welcome banquet*

Sunday 9.02

8:00–10:00	*Breakfast*
10:00–13:00	Free time
13:00–14:00	*Lunch*

Session 1
Molecules: Membrane structure and dynamics
Chair: Marta Pasenkiewicz-Gierula

14:00–14:25	**Wiesław Gruszecki** Carotenoids in membranes
14:30–14:55	**Anna Wiśniewska-Becker** Curcumin: a biomolecule of two faces
15:00–15:25	**Monika Rak** Polyprenyl-based lipoplexes potential for peptide modifications and bubble liposomes formation
15:30-16:00	*Coffee break*

Session 2
Molecules: Protein structure, dynamics, and function
Chair: Wiesław Gruszecki

16:00–16:25	**Gerald Kneller** Neutron spectroscopy of protein energy landscapes and dynamics
16:30–16:55	**Wladek Minor** Impact of reproducibility and artificial intelligence on structure based drug discovery
17:00–17:10	**Mariusz Madej** Oligopeptide acquisition by the main periodontopathogen
17:15–17:25	**Michał Gabruk** Insights into the oligomerization mechanism of LPOR with the use of (cryo)electron microscopy
17:30–19:00	Poster session 1
19:00	*Dinner*
20:30	*Wine Party*

Monday 10.02

8:00–10:00	*Breakfast*
10:00–13:00	Free time/Ski fun
13:00–14:00	*Lunch*

Session 3
Pathways: Signalling pathways and biochemical networks
Chair: Tomasz Róg

14:00–14:25	**Mateusz Kwitniewski** Chemerin: a multifunctional protein
14:30–14:55	**Andrzej Górecki** To dimerize or not to dimerize? Unusual strategies for transcriptional regulation by YY1
15:00–15:10	**Paweł Mystek** 3...2...1... G-protein αi subunits
15:15–15:25	**Gabriela Szczupaj** Structure elucidation of enkephalin analogs from NMR derived restraints
15:30–16:00	*Coffee break*

Session 4
Pathways: Modelling the molecular mechanisms of the biological processes
Chair: Maciej Bagiński

16:00–16:25	**Tomasz Róg** Role of lipids in entry mechanism of dopamine into d3 receptor
16:30–16:55	**Tomasz Borowski** Redox-active metalloenzymes–how computations supplement structural and biochemical studies
17:00–17:25	**Anna Wójcik-Augustyn** Insight into reaction mechanism of ATP Sulfurylase: theoretical studies
17:30–19:00	Poster session 2
19:00	*Dinner*
20:30	*Beer party*

Tuesday 11.02

8:00–10:00	*Breakfast*
10:00–13:00	Free time/Ski fun
13:00–14:00	*Lunch*

Session 5
Game of Life
Chair: Krzysztof Murzyn

14:00–14:25	**Maciej Bagiński** In-silico approach to combat telomerase: an important enzyme in cancer and senescence
14:30–14:55	**Jacek Międzobrodzki** How do bacteria communicate between themselves?
15:00–15:10	**Jakub Hryc** Computer models of poly-unsaturated monogalactolipid and digalactolipid bilayers
15:15–15:25	**Adrian Kania** Application of machine learning methods to biological classification of taxons
15:30–16:00	*Coffee break*

Session 6
Game over: pathology and disease
Chair: Jacek Międzobrodzki

16:00–16:25	**Martyna Elas** Uveal melanoma – the game has just begun
16:30–16:55	**Anna Pawlak** Is heptadienal a key player in observed photoreactivity of oxidised polyunsaturated fatty acids?
17:00–17:10	**Michał Sabat** Changes in metabolites concentrations leading to excessive fatigue in mitochondrial diseases: theoretical study with aid of skeletal muscle oxidative phosphorylation model
17:15–17:25	**Andrzej Kubiak** What is a link between L-glutamine depletion and prostate cancer cells biomechanics?
19:00	*Conference Dinner*

Wednesday 12.02

8:00–10:00	*Breakfast*
11:00	Departure to Kraków

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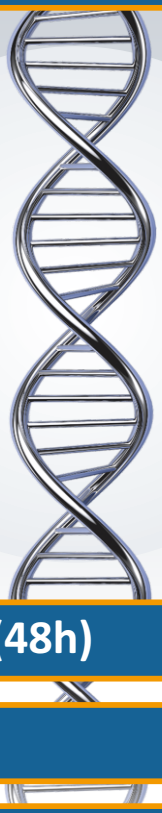

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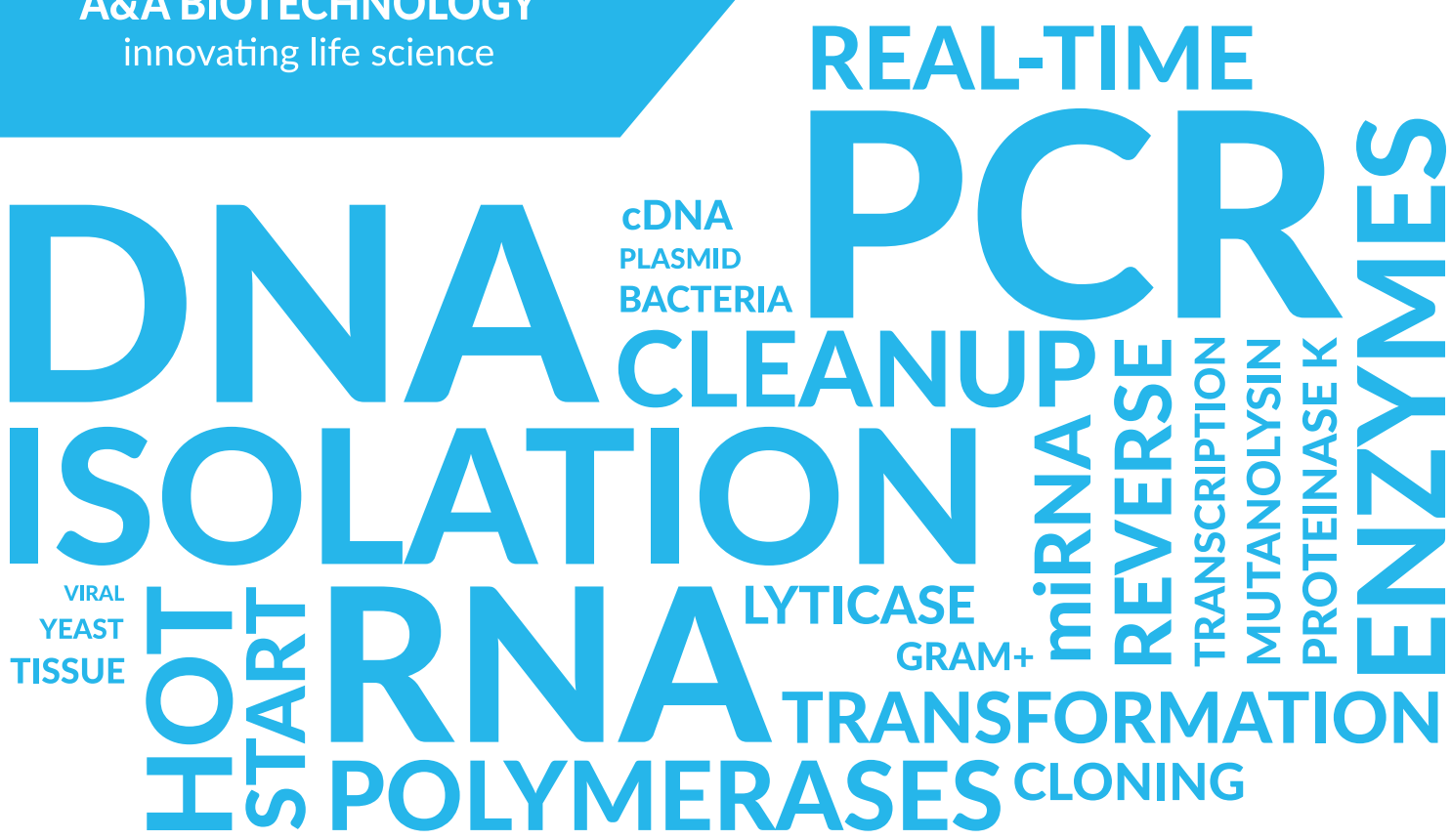

Special Session

S.1
A Short Story of the Long Friendship

J. Ciarkowski

Retiree from former *Faculty of Chemistry, University of Gdańsk, Wita Stwosza 63, 80-308 Gdańsk*

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A brief summary of the partnership between my group in Gdańsk and that of Prof. Marta Pasenkiewicz-Gierula will be given. The scientific collaboration, initiated by myself in 1997, soon grew up into personal friendship between Marta and me. The results of the cooperation will be outlined. While the formal collaboration, including common projects, expired by 2007, the friendship has been flourishing.

S.2
Our Winter Schools – Facts, Legends, Memories

K. Strzałka

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The history of our Winter Schools is inseparably connected with the establishment and functioning of the Institute of Molecular Biology and then the Faculty of Biochemistry, Biophysics and Biotechnology of the Jagiellonian University. Decades and generations have passed, the political system has changed, there have been several reforms of science and education, and our Winter Schools are still in progress and enjoy unflagging interest. The Schools themselves have also evolved and the present ones are very different in many ways from the first Schools of the Institute of Molecular Biology in the early 1970s. However, despite many changes and modifications, some things remain the same. Probably in these unchanging elements lies the secret of our Winter Schools, which despite the passage of years are still attractive and we like to participate in them.

S.3
Our Friendship and Collaboration Began in Milwaukee

W. K. Subczynski

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Dear Marta!

I find it interesting that our real friendship and collaboration began in Milwaukee, where we spent time together starting in March 1980. Aki Kusumi, who also was in Milwaukee at that time, helped us find common research interests in membrane biophysics. Our first paper published in *BBA – Biomembranes* in 1986, is our most cited paper. (~135 citations.) We were not always so successful: We published a paper in *JACS* in 1999 that I consider very good, but it has only one (self) citation. In the last few years, our collaboration has become more beneficial to us: In 2009, we received the NIH Fogarty grant, where I was the PI and you were the foreign PI. In 2017, we received the Harmonia grant and switched places—you were the PI and I was the foreign PI. These grants (the Harmonia grant is still active) help us to convene frequently, most often in Krakow, but also in Milwaukee.

I have a lot of happy, sometimes funny, memories from the times we spent together. In 1993, we were both in Japan, at Aki's invitation. You took me on a countryside excursion. We were walking through some forest and mountains when, suddenly, we realized we were being followed by a group of unfriendly monkeys. Luckily, we had some candies, which allowed us to escape these persecutors.

I hope our long collaboration will continue. We have many interesting plans, which easily will fill the next 10–20 years.

With best wishes,

Karol

Lectures

L.1

In-Silico Approach to Combat Telomerase: An Important Enzyme in Cancer and Senescence

M. Baginski[1], K. Serbakowska[1], U. Kalathiya[1], M. Padariya[1]

[1]*Department of Pharmaceutical Technology and Biochemistry, Faculty of Chemistry, Narutowicza St 11/12, 80-233 Gdansk, Poland*

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Telomerase is a reverse transcriptase enzyme involved in DNA synthesis at the end of linear chromosomes of eukaryotic organisms. Usually this enzyme is dormant but in most cancerous cells telomerase is reactivated in order to elongate part of DNA which is lost after each cellular division. This DNA elongation enables cancer cells to become immortal. Due to this fact telomerase became a new promising anticancer target. Discovery of this enzyme was also awarded by Nobel Prize in 2009. Despite extensive research, direct telomerase inhibitors are not yet introduced to clinics due to complexity of this enzyme and lack of knowledge about molecular details of its function. Molecular structures of this protein or its parts from simple organisms and human homology models are currently available. Using these models structure-based drug design efforts have been undertaken in many labs to find or design *de novo* potential inhibitors. Different *is silico* strategies have been applied and different chemical scaffolds have been explored. However, all these studies have their limitations and it has appeared that to combat telomerase in cancer cells is very challenging task. The presentation is an overview of all these newest discoveries including also our contribution to the field.

L.2
Redox-Active Metalloenzymes – How Computations Supplement Structural and Biochemical Studies

T. Borowski[1]

[1] *Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Niezapominajek 8, 30-239, Kraków, Poland*

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A large portion of biochemical reactions are connected with changes of reactants oxidation state, i.e. are redox processes, and as such are catalyzed by specialized enzymes, of which a very significant part are metalloenzymes. Their catalytic reaction mechanisms are typically multi-step processes often featuring short-living transient intermediates that are notoriously difficult to trap and characterize with experimental methods. As a consequence, studies on metalloenzymes require employing a wide panel of complementary research methods. This talk will showcase how computational chemistry tools (DFT, QM/MM, MD) complement experimental methods in our quest for understanding enzymatic reaction mechanisms at atomic level. Examples will be drawn from our own experience but also from literature.

L.3
Uveal Melanoma – The Game Has Just Begun

K. Jasińska-Konior[1], M. Szczygieł[1], A. Kozińska[1], A. Markiewicz[2], B. Romanowska-Dixon[2], M. Elas[1]

[1] *Department of Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Krakow, Poland*
[2] *Department of Ophthalmology and Ocular Oncology, Jagiellonian University Medical College, Kopernika 38, Kraków, Poland*

Metastasis is the main cause of the high mortality rate in uveal melanoma (UM). The primary tumor in the eye is successfully treated with high doses of radiation, however the available treatments for the metastasis are insufficient. Uveal melanoma cells are highly resistant to chemotherapy, and many other approaches, such as targeting kinase pathways or immunotherapy prove unsuccessful.

We describe UMCure2020 project [1], its goals and results obtained so far by the team of clinical centers, academic and business partners. Our role in the project is focused on participation in the virtual UM patient biobank and developing cellular and animal models of UM. Using the newly developed models, we study uveal melanoma cell radiosensitivity, both to photon and proton beam radiation, as well as features influencing cellular migration and adhesion, such as cellular elasticity and motility[2][3].

Failure of the traditional approaches to metastasis treatment in UM has become a starting point to dig deeper into the biology of UM. Understanding the liver tropism of UM cells, their dormancy mechanisms and interactions with liver environment may open new avenues for metastatic disease management and be the real game changers.

Acknowledgements:
UMCure2020 Horizon 2020 project no 667787

References:

[1] Rodrigues, M. et al. (2019) So Close, yet so Far: Discrepancies between Uveal and Other Melanomas. A Position Paper from UM Cure 2020. *Cancers* **7 (11)**: 1032.

[2] Jasińska-Konior, K. (2019) Increased elasticity of melanoma cells after low-LET proton beam due to actin cytoskeleton rearrangements. *Scientific Reports* **9 (1)**:7008.

[3] Jasińska-Konior, K. (2017) Proton beam irradiation inhibits the migration of melanoma cells. *PloS One* **10 (12)**: 1–16.

L.4
To Dimerize or Not to Dimerize? Unusual Strategies for Transcriptional Regulation by YY1.

A. Górecki

Department of Physical Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland

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The Yin Yang 1 (YY1) protein plays a key role in the control of cell growth and differentiation, and thus is important in a number of physiological and pathological processes in all vertebrates. It controls the expression of hundreds of genes, acting as an activator, repressor or initiator of transcription. Almost all the published results consider YY1 as a monomeric protein, in contrast to most transcription factors acting as dimers. Only two publications formulate the hypothesis of YY1 homodimerization [1], but none yet considers the impact of this process on the function of the transcription factor. Our latest studies show that the dimerization of human YY1 is effective and strongly depends on the concentration of zinc ions.

The C-terminal fragment of the YY1 protein consists of four C2H2 zinc fingers. This fragment is responsible for the interaction with DNA within the promoter regions. The N-terminal fragment is responsible for interacting with over 100 different proteins, thus regulating YY1's activity. Our data proved that the N-terminal fragment is intrinsically disordered [2], but can partially fold and form a dimer upon zinc presence. Conformational changes in the N-terminal region may additionally affect the regulation of transcription.

The effectiveness of the postulated dimerization process in controlling the expression of particular genes would depend on the occurrence of tandem sequences in promoter regions, their availability for YY1 dimers and finally on the presence of YY1 dimers. The presence of several sites recognized by YY1 is common for many genes, and the concentration of zinc ions, which affects YY1 dimerization, varies significantly during tumor transformation.

References:

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L.5
Carotenoids in Membranes

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Carotenoids belong to a group of natural pigments, ubiquitous in the biosphere. Physiological activity of carotenoids is tightly associated with their localization in biostuctures, including pigment-protein complexes and biomembranes. It has been established that localization and orientation of carotenoids in lipid membranes is determined by molecular interaction to lipids. Such a conclusion is based on the results of the experiments carried out with model systems consisting of carotenoid-containing lipid multi-bilayers. We will present new experimental approach to study carotenoids in membranes based on molecular spectroscopy and imaging of single unilamellar lipid vesicles. Application of fluorescence microscopy and Raman imaging enables to precisely determine localization and orientation of carotenoids in a single lipid bilayer membrane.

L.6
Oxysterols and Truncated Oxidized Phospholipids in Model Membranes

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Products of lipid and cholesterol oxidation are commonly, although in limited quantities, present in our bodies both under physiological and pathological conditions. While their recognition by proteins triggers many signaling pathways, their presence can also have severe effects on the physical properties of lipid membranes. In the lecture we will summarize our experimental and computational research on the behavior of truncated oxidized lipids and oxysterols in model lipid membranes. [1-6]

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L.7
Neutron Spectroscopy of Protein Energy Landscapes and Dynamics

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The intuitive concept of energy landscapes, which has been introduced to protein physics by Hans Frauenfelder [1], is interpreted from a neutron scattering perspective and connected to trajectory-based interpretations of neutron scattering spectra in the scenarios of quantum and classical mechanics [2,3]. It is shown that the energy landscape approach for complex systems leads to new qualitative and quantitative interpretations of quasielastic neutron scattering spectra in terms of "minimalistic models" and a first example is presented for the analysis of quasielastic neutron scattering spectra of free and inhibited human acetylcholine [4,5].

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L.8
Ultrafast Single-Molecule Imaging Revealed the Compartmentalized Architecture of the Focal Adhesion

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A world-fastest single fluorescent-molecule imaging-tracking station has been developed, which works at frame rates up to 30 kHz with single-molecule localization precisions down to 20 nm. Furthermore, this station allowed us to obtain PALM images of living cells (~30-nm localization precision for mEos3.2) every 0.5 s for a view-field of 10 x 10 μm, the fastest PALM ever performed with practical image sizes. Using this station, we found that virtually all phospholipids and transmembrane proteins (transferrin and EGF receptors) examined thus far underwent non-Brownian hop diffusion between actin-induced 110-nm-compartments in the plasma membrane (PM), once every ~10 and ~25 ms, respectively (T24 cells).

Surprisingly, membrane molecules readily enter and undergo hop diffusion in the focal adhesion (FA), a specialized PM domain responsible for the cellular attachment to and movement on the extracellular matrix. Ultrafast PALM of mEos3.2-conjugated paxillin (a cytoplasmic structural FA protein) simultaneously performed with single-molecule tracking of β3-integrin revealed that β3-integrin undergoes hop diffusion in the FA region that is often interrupted by temporary immobilizations at the FA-protein (paxillin) islands. These results support the FA archipelago model, in which FA-protein islands are sparsely distributed in the FA region compartmentalized by actin filaments, allowing the ready entrance and exiting of integrins through the channels between FA-protein islands for the integrin function at the FA-protein islands. This would allow for rapid formation and disintegration of FAs.

This talk is dedicated to Marta (Prof. Pasenkiewicz). I thank her for her friendship and long-term collaborations with me and congratulate her happy retirement.

L.9
Chemerin: A Multifunctional Protein

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Chemerin is a chemoattractant protein with adipokine properties. First identified as tazarotene- (retinoic acid-analog) induced gene 2 (TIG2) in tazarotene treated psoriatic skin, it has gained more attention over the past few years due to its multilevel impact on metabolism and immune responses. The pleiotropic actions of chemerin include chemotaxis of dendritic cells, macrophages and natural killers (NK) subsets, angiogenesis as well as regulation of adipogenesis and glucose metabolism. Although fat tissue and liver have been demonstrated as key sites of chemerin production, chemerin is also expressed at epithelial barriers, including skin epidermis. Our studies revealed that chemerin is structurally related to cathelicidins antimicrobial peptides and functions as an antibacterial agent in human epidermis. A chemerin-derived Val66-Pro85 peptide (p4) embodies the majority of chemerin's anti-microbial activity. Moreover, chemerin can contribute to the recruitment of immune cells subsets into inflamed skin. Expression of *TIG2* in epidermis is regulated by acute phase mediators, specific bacteria strains and cytokines associated with skin pathology like psoriasis. Together, these data suggest that chemerin can maintain skin homeostasis in several ways: as a chemoattractant ligand for different leukocytes such as dendritic cells and macrophages, and as an antimicrobial agent controlling growth of skin-associated microbes.

L.10
Why Do Bacteria Communicate Between Themselves?

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Microorganisms are not only the smallest, one cell-, but also phylogenetically the oldest organisms living on Earth. Their morphology, physiology and remarkable participation in many ecological processes makes these organisms well adapted to various environments, and they still gain new features to enabling the conquer of new ecological niches. Genetic adjustment of bacteria results in biochemical plasticity, communication between bacteria results in quorum sensing phenomenon, horizontal gene transfer enables effective defense against antibiotics, nucleic acids repair mechanisms enables colonization of odd environments. Taking above into account, bacteria are extremely differentiated and specialized organisms, not lacking the potential of quick change. The molecular mechanisms of bacterial adapting phenomena are investigated by advanced research techniques like polymerase chain reaction (PCR) techniques, DNA sequencing both Sanger and next generation sequencing (NGS) which results in single genes or gene cassette typing for bacteria qualification in biotechnology or discovery of clonal complexes for epidemiological investigation useful in diagnostics of infectious diseases.

L.11
Impact of Reproducibility and Artificial Intelligence on Structure Based Drug Discovery

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Structural Biology is the key component for structure- and fragment-based drug discovery. The scientific, technical, and computational progress of structural biology has generated an unprecedented number of structural models but has also uncovered some adverse outliers. Among hundreds of high quality models, a small number remind us that the process of structure elucidation is not fail-safe. The number of suboptimal structures is very low, but the ripple effect of suboptimal or sometimes even erroneous structures may invalidate many biomedical hypotheses when discovered. For example, the selection of buffer and the presence of tags used for protein purification can completely alter the results of experiments obtained by various techniques. The administrative regulations may improve the situation slightly, but a change of the attitude regarding data management systems is more critical. Biological data are highly interconnected, and effective data management systems must take into account the diversity of data and experimental methods and should also properly handle contradictory results when various techniques are used. Integrating these diverse data is not only a challenge for modern data management systems but is the most serious challenge facing all biomedical research. The significant progress in addressing reproducibility and artificial intelligence limitations may revolutionize drug discovery.

L.12
Is Heptadienal a Key Player in Observed Photoreactivity of Oxidised Polyunsaturated Fatty Acids?

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The mammalian retina contains a high level of lipids esterified with polyunsaturated fatty acids (PUFAs), including docosahexaenoic acid (22:6)[1], which are especially susceptible to oxidation. Even though reactive products of PUFAs oxidation could irreversibly modify key cellular constituents, ultimately leading to the onset of degenerative processes[2], the role of such products in phototoxicity of oxidised PUFAs has not been considered. However, it has been shown recently that lipids extracted from human and bovine retinas are photoreactive and upon irradiation with blue light may generate reactive oxygen species[3]. In this work we analysed photoreactivity of 2,4-heptadienal, which is a characteristic product of oxidation of ω-3 polyusaturated fatty acids.

The presence of 2,4-heptadienal in the oxidised bovine and human retinal lipids extracts was determined using Raman spectroscopy. Photoreactivity of 2,4-heptadienal was studied by EPR-oximetry and EPR-spin trapping. Photogeneration of singlet oxygen by heptadienal was measured using time-resolved phosphorescence at 1270nm.

Upon irradiation with blue light 2,4-heptadienal reveals moderate photoreactivity. It generates singlet oxygen with quantum yield approaching 0.1 when excited with 400 nm, which is slightly higher than that of human retinas lipid extract. The results suggest that 2,4-heptadienal could in part be responsible for the observed photoreactivity of oxidised retinal lipids.

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L.13
Role of Lipids in Entry Mechanism of Dopamine Into D3 Receptor

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Non-peptidic neurotransmitters are a group of polar and amphipathic compounds. Their receptors belong to two categories: large ion channels containing a binding cavity in the extra-membrane domain and G protein-coupled receptors (GPCR) containing a binding cavity in the transmembrane region. Interestingly, while polar neurotransmitters bind to ionotropic receptors predominantly, the amphipathic ones, which partition to the membrane-water interface, bind to GPCRs [1, 2]. This observation suggests that the binding of the amphipathic neurotransmitters to their target receptors may be mediated by the membrane.

Here, we used unbiased molecular dynamics (MD) simulations, random acceleration MD (RAMD) simulations, and free energy calculations to investigate the binding mechanism of dopamine to its target GPCR. Our results suggest that dopamine enters to its binding pocket via the membrane and support the membrane-mediated mechanism. First, using 1000 independent MD simulations, we show that membrane binding of dopamine precedes its interaction with the receptor. Only a small fraction (8%) of dopamine molecules associates with the receptor before the membrane. In 92% of the cases, dopamine first associates with the membrane-water interface. Moreover, we estimated a high free energy difference between the water phase and water-membrane interface, indicating that transfer of dopamine back to the water phase from the membrane-water interface is a slow process. Finally, we characterized a novel binding pathway that connects the membrane-water interface with the dopamine binding site traversing between the transmembrane α-helices 5 and 6 using RAMD simulations. Interestingly, this pathway does not feature any major free energy barriers allowing efficient dopamine entry into the binding pocket.

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L.14
Curcumin: A Biomolecule of Two Faces

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Curcumin, a natural yellow-orange dye used as a spice, food coloring and preservative, is believed to exhibit a broad range of therapeutic effects against different disorders. There are also data showing curcumin phototoxicity against bacteria, fungi and various mammalian cells. The mechanisms of curcumin different activities are not fully understood. Being insoluble in water curcumin accumulates in cell membranes where it may indirectly lead to the observed effects by altering the membrane environment.

We investigated the effects of curcumin on lipid order, phase transition and polarity in model liposome membranes using electron paramagnetic resonance (EPR) spin labeling technique. We also studied the photochemical activity of curcumin in liposomal membranes, by measuring the oxygen photo-consumption (by EPR) and singlet oxygen generation (directly, by detection of singlet oxygen ($^1O_2$) luminescence at 1270 nm) and indirectly (by detection of $^1O_2$ – produced cholesterol hydroperoxides using HPLC EC(Hg).

Curcumin rigidified the studied membranes at all positions along the lipid acyl chain. Polarity measurements revealed enhancement of water penetration by curcumin in the membrane center and in the polar headgroup region. Curcumin also strongly affected the DMPC phase transition abolishing it completely at the concentration of 10 mol%. The oxygen photoconsumption rate in liposomes containing curcumin was significantly higher than in liposomes irradiated without the dye. It was also shown that the main reactive oxygen species photogenerated by curcumin in liposome membranes was $^1O_2$.

The strong membrane structural effects of curcumin may have important consequences related to its protective properties. On one hand, increased lipid order should make membranes more resistant to penetration by different compounds such as oxidants, on the other hand, increased water penetration may mean more water soluble free radicals or transition metal ions which can cause lipid peroxidation. Observed photoactivity of curcumin in the membranes supports the latter conclusion. Therefore, although curcumin is recommended in diet as a valuable natural compound, caution should be recommended especially when skin is exposed to light.

Oral Presentations

O.1

Insights Into the Oligomerization Mechanism of LPOR With the Use of (Cryo)electron Microscopy

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LPOR, or light-dependent protochlorophyllide oxidoreductase, is an enzyme involved in the chlorophyll biosynthesis in oxygenic phototrophs. The plant isoforms of LPOR are known to form large, regular complexes with lipid membranes called prolamellar bodies, or PLB, however, the mechanism of the formation of PLB remains elusive.

In the present study, we have employed advanced electron microscopy techniques to study the oligomerization properties of LPOR isoforms. Our approach allowed us to characterize a few types of filamentous complexes that the enzyme can form. Moreover, we have identified the key factors that regulate the oligomerization process of LPOR, that is the plant lipids MGDG and PG. Additionally, the use of cryoelectron microscopy revealed that the filaments are composed of the multi-start helix of LPOR subunits wrapped around the lipid bilayer.

The results, combined with our spectroscopic measurements, allowed us to propose the mechanism of PLB formation and suggest a new role of LPOR in plastid development.

This work was supported by Bekker scholarship from Narodowa Agencja Wymiany Akademickiej NAWA (PPN/BEK/2018/100105) and START scholarship granted by Foundation for Polish Science (FNP) (024.2018).

O.2
Computer Models of Poly-Unsaturated Monogalactolipid and Digalactolipid Bilayers

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Monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) are the main lipids components of thylakoid membranes of chloroplasts. They play a crucial role in maintaining optimal efficiency of photosynthesis. The mesoscopic phase of aggregates spontaneously formed by MGDG molecules in water, depend strongly on the type of the acyl chains attached to the glycerol backbone of the molecule. MGDG with poly-unsaturated α-linolenoyl (di-18:3, *cis*) acyl chains forms predominantly nonlamellar phases. DGDG forms predominantly a lamellar phase.

Molecular dynamics (MD) simulations of fully hydrated di-18:3 MGDG and di-18:3 DGDG bilayers both in the lamellar phase were carried out at 295 K in the OPLS-AA force field for 300 ns and 1000 ns, respectively, using the GROMACS software package. Beforehand, the torsion term in the potential energy function for DGDG was corrected [1] as well as the atomic charges on the galactose moieties of MGDG and DGDG [2]. Time profiles of the potential energy and area per lipid indicated that the DGDG and MGDG bilayers reached thermal equilibrium during 600 ns and 100 ns, respectively, of the simulation times. In effect, 400-ns and 300-ns, respectively, trajectory fragments were used for analyses. Distributions of the MD generated conformations of Φ, Ψ and ω dihedral angles of the glycosidic linkage in the DGDG and MGDG molecules were compared to those in the crystal structures. The comparisons are favourable, which implies that the generated computer models of the DGDG and MGDG bilayers are trustful and can be used in the future research.

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O.3
Application of Machine Learning Methods to Biological Classification of Taxons

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Analysis of the relationship between taxons is often considered using phylogenetic trees. There are various approaches to construct them, including these based on distances and discrete characters [1]. In the first case, for nucleotide or amino acid sequences, assigned to different organisms, one begins with alignment and then a matrix of evolutionary distances (MED) is constructed. The phylogenetic tree is generated based on the MED. In the case of nucleotide sequences, the Kimura model, which is an extension of the Jucas - Cantor model, is usually used for MED generating. For amino acid sequences other methods are used [1].

In this work, the authors consider and compare different machine learning algorithms [2,3] to extract the relationship between taxons. The PCA algorithm or mountain grouping combined with chaos game is an innovative approach to this problem. Certain properties of amino acids (and thus proteins) were taken into consideration, especially those that differentiate subsequent individuals the most. More specifically, the PCA method selects the directions for the largest data diversity. Mountain grouping allows one to smooth the images obtained from the chaos game. Both the group of amino acid and nucleotide sequences were analyzed.

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O.4
What Is a Link Between L-Glutamine Depletion and Prostate Cancer Cells Biomechanics?

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One of features of cancer cells are changes in their metabolic activity [1], [2]. Thus many approaches are proposed to target cancer cell metabolism as a new anticancer therapy. One of them is targeting of glutamine transporters which is important due to the fact that glutamine is considered as major source of nitrogen for nucleotides synthesis [3]. Parallelly more attention is paid to mechanical cancer hallmarks. In general cancerous cell became more soft than their healthy counterparts[4]. Such changes in mechanics of cells and their surrounding affects mechanotransducive pathways leading to changes in cells behaviour including drug resistance[5], [6]. Thus the aim of our study was to determine how depletion of L-glutamine supplementation will affects prostate cancer mechanics. What is more we investigate how mechanical response of prostate cancer cells changes upon docetaxel treatment when L-glutamine is depleted.

While for prostate cancer cell cultured under standard conditions their mechanics is preserved (~6 kPa) over time (after 24, 48 and 72 h) depletion of L-glutamine leads to their significant stiffening with peak after 48 h (~10 kPa). Interestingly similar robust transient stiffening was observed when control cells were treated with 1 nM of docetaxel (~10 kPa). The mechanical response of prostate cancer cells to docetaxel was milder when cells were cultured with L-glutamine depletion, thus indicating affected mechanosensing of cells during this process.

Our results coupled together suggest that changes in L-glutamine supply affects not only cellular mechanics but also their response to anti-cancer action. Such findings provide important insight into way in which we understand mechanobiology of drug response and might be taken under consideration while looking for new therapeutic approaches.

AK acknowledge the support of InterDokMed project no. POWR.03.02.00-00-I013/16

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O.5
Oligopeptide Acquisition by the Main Periodontopathogen

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Porphyromonas gingivalis is the main pathogen involved in the development of chronic periodontitis. Due to its asaccharolytic character, this Gram-negative bacterium is unable to metabolise carbohydrates, but instead it acquires peptides provided by proteolytic enzymes called gingipains. Despite the fact that peptides are crucial for growth and survival of *P. gingivalis*, the mechanism of their uptake is completely unknown. This study presents the structural and functional characterisation of a RagAB complex transporting peptides through the outer membrane of *P. gingivalis*. Therefore, it sheds new light on the uptake of peptides by Gram-negative bacteria.

The crystal structure of RagAB was solved by molecular replacement. RagAB forms the hetero-tetrameric complex composed of two subunits of RagA and two subunits of RagB ($RagA_2B_2$). Examination of the RagAB dimer interface showed the electron density which can be modelled as a peptide of ~13 residues in length suggesting that both RagA and RagB form a peptide binding site. Single-particle cryo-electron microscopy revealed three dynamic states of RagAB: double closed, single closed and double open and show conformational changes of the plug domain upon substrate binding. Specificity of both complexes towards peptide length and amino acid composition was determined using mass spectrometry. The structure-function studies were carried out by comparing the growth of RagAB mutants in minimal medium with bovine serum albumin as a sole peptide source.

The results obtained in this study will contribute to better understanding of peptide transport by Gram-negative bacteria and may allow development of specific drugs attenuating virulence of *P. gingivalis*.

O.6
3...2...1... G-Protein αi Subunits

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Heterotrimeric G-proteins along with G-protein-coupled receptors (GPCRs) regulate many biochemical functions by transferring the signal from the extracellular space through the plasma membrane to the inside of the cell. We characterized lateral diffusion and G-protein subunit interactions in living cells using fluorescence recovery after photobleaching (FRAP) microscopy and fluorescence resonance energy transfer (FRET) detected by fluorescence lifetime imaging microscopy (FLIM), respectively. Despite the high similarity of the sequences, the studied subunits of the Gi family are characterized by their diverse location and signaling.

O.7

Polyprenyl-Based Lipoplexes Potential for Peptide Modifications and Bubble Liposomes Formation

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Lipofection is a widely used molecular biology technique and one of the most promising non-viral gene therapy strategies. However, one of the main drawbacks of using cationic lipids-based lipoplexes in DNA/RNA delivery is lack of targeting specificity.

We have addressed this issue using polyprenyl (PTAI - trimethylpolyprenylammonium iodides)-based lipofection. PTAI-based lipoplexes (complexes of lipids and DNA) were previously proved effective as DNA and RNA carriers *in vitro* as well as vaccine delivery vehicles *in vivo*. In order to test PTAI potential for targeted DNA delivery we have tested its's suitability for peptide modifications and bubble liposomes (BL) formation.

Our results show that lipoplexes formed with PTAI-11+DOPE+DC-cholesterol (1:1:1 molar ratio) using NanoAssemblr® device have small size (78,4±0,2 nm) and low polydispersity (PDI=0.138) ensuring passive targeting by enhanced permeability and retention (EPR) phenomenon in cancer. RGD-peptide modification does not alter the lipoplex size. PEG incorporation changes lipoplex positive charge (+23,1± 0,8mV) to neutral making them more appropriate for *in vivo* use. Interestingly, vehicles without DNA have small size but very high PDI. PTAI-11 is also good candidate for BL preparation using anionic liposomes consisting of DSPC+DSPG+mPEG-DSPE mixed with PTAI-11+DNA complexes. Resulting neutral liposomes entrap perfluoropropane and show higher efficiency of lipofection when combined with ultrasound (US) exposure *in vitro*. They also show higher efficiency with US *in vivo* in spleen, liver, stomach and intraperitoneal wall after intraperitoneal (IP) injection in mice.

Overall, our results show that PTAI are promising candidates for targeted gene delivery with peptide-modified lipoplexes as well as BL and US.

This work was financially supported by the National Science Centre, Poland within MINIATURA-2 grant no. 2018/02/X/NZ3/01566.

O.8

Changes in Metabolites Concentrations Leading to Excessive Fatigue in Mitochondrial Diseases. Theoretical Study With Aid of Skeletal Muscle Oxidative Phosphorylation Model.

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An excessive vulnerability to fatigue is common symptom in various mitochondrial diseases. Among proposed natural mechanisms of fatigue, the most important are increase of the muscle cytosol acidity and rise of the cytosolic phosphate ions concentration. Excessive fatigue could be described in the same way. Yet, proper connection between these changes in metabolites concentration and dysfunctions of bioenergetic enzymatic pathways are lacking. Moreover, the exact role of metabolite transporters in these pathways is also not defined. Effect of various combinations of work intensity and level of oxidative phosphorylation disruption on changes in metabolite concentrations was investigated through application of computer model. Results suggest increase of phosphate ions concentration as primary mechanism of fatigue in most of muscles. Relation between decrease of oxidative phosphorylation and work intensity shows linear dependence for mutations equally disturbing activity of each element of the pathway (which is equivalent to decrease of mitochondrial mass). In case of mutations which disrupts only one element of pathway fatigue threshold-exercise intensity dependence is exponential. Muscle phosphate levels were the most vulnerable for mutations of complex III and ATP synthase. However, mutations of ATP/ADP exchanger emerged as equally disruptive and able to significantly increase phosphate concentrations also in the rest state. Surprisingly decrease of phosphate transporter activity had nearly no effect on cytosolic phosphate and acidity. Together these results show that transporter proteins could be equally important as enzymes for proper work of bioenergetic system of the cell, and they should be duly included in the future structural biology investigations.

O.9
Structure Elucidation of Enkephalin Analogs From NMR Derived Restraints

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Enkephalins are natural penta-peptides discovered by Hughes and Kosterlitz in 1974. They are generated from proenkephalin via proteolytic cleavage. Preproenkephalin precursor is subject to complex cleavages and post-translational modifications resulting in enkephalins synthesis. Two different forms of enkephalins are known up-to-date. These forms are Leu-Enkephalins (Tyr-Gly-Gly-Phe-Leu) and Met-Enkephalins (Tyr-Gly-Gly-Phe-Met). These are endogenous penta-peptides which exhibit morphine-like properties. Different strategies have been used to improve the properties of the peptides to make them more amenable as therapeutics, such as cyclization or incorporation of unnatural amino acids within the peptide sequence.

The enkephalin series we studied was cyclized through aromatic rings. The structures of modified enkephalins were calculated with XPLOR basing on through-space restraints obtained from two-dimensional NMR spectra analysis. The elucidated structures of the modified enkephalins are part of structure-activity relationship studies. Our goal is to understand what structural features govern the opioid peptide selectivity and the activity towards a particular opioid receptor. The end goal of these studies is to help in designing new, better drugs.

O.10
Insight Into Reaction Mechanism of ATP Sulfurylase. Theoretical Studies.

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ATP sulfurylase (ATPS) is involved in the first step of the sulfate reduction process occurring in Sulfate Reducing Bacteria. It catalyzes the activation of ATP to adenosine 5'-phosphosulfate (APS). There are two hypothesis about mechanism of ATPS reaction. The first is SN-2 direct conversion of ATP to APS and pyrophosphate, where SO_4^{2-} attacks the α-phosphorus of the ATP phosphate group and reaction proceeds through pentavalent trigonal bi-pyramidal transition state [1,2,3]. The second one is two-step SN-1 mechanism, where ATP is cleaved to pyrophosphate and AMP anhydride, which subsequently reacts with sulfate leading to APS [4]. The ATPS mechanism was investigated with QM methods (DFT-D3/B3LYP). Seven models of ATPS active site were applied. One with magnesium cation was built based on the Molecular Dynamics simulation results performed for hexameric form of ATPS in complex with APS, Mg^{2+} and pyrophosphate (PPi). Six other models were constructed based on the crystal structure, which was solved for ATPS-APS-PPi complex (PDB: 1G8H) [2]. Interestingly, in this only crystal structure available for ATPS in complex with both of reaction products Mg^{2+} is not observed.

The presented results suggest that ATPS reaction proceeds by the SN-2 direct conversion of ATP to APS with the barrier of 12kcal/mol [5]. Interestingly, studies show that ATPS reaction proceeds without Mg^{2+}. The presence of magnesium cation affects the conformation of ATP, leading to high barriers for all tested mechanisms. Moreover, SN-1 two-step mechanism has been excluded due to high barrier of ATP cleavage and unstable AMP anhydride.

Acknowledgments:

The Swedish Nuclear Fuel and Waste Management Co (SKB) is acknowledged for financial support. and in part by PL-Grid Infrastructure. This research was supported in part by PLGrid Infrastructure.

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Sunday Poster Session

PS.1

Development of the N-Terminal-Derived Gα Protein Chimeras as Functional Probes to Study the Protein-Plasma Membrane Interaction

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Heterotrimeric G proteins, consisting of α, β, γ subunits, have a crucial role in signal transduction mediated by GPCR (G protein-coupled receptor) receptors. The interaction of Gα subunits with plasma membrane is not fully understood. Previous studies have shown that different Gα subunits are located in the particular plasma membrane surface [1,2]. The membrane binding of Gα subunits is mainly mediated by the covalently attached myristate and/or palmitate and the N-terminal positively charged α-helix. We would like to examine whether only the helix of Gα subunits is essential for the specific lipids-protein interaction or membrane-anchors are crucial for these interactions. Based on the hypothesis that N-terminal region of Gα subunits is capable of mimicking their interactions with membranes, protein chimeras containing the first thirty to forty N-terminal residues and Green Fluorescent Protein (GFP) have been constructed (Gαs, Gαi1, Gαi2 with GFP). All of N-terminal helixes of examining proteins have positive charged moieties, but the numbers of the residues vary between Gα subunits. In our studies chimera proteins have been produced *Escherichia coli* and purified by tag-based metal-ion affinity and ion exchange chromatography. Secondary structure and thermal stability of chimers were characterized by circular dichroism spectroscopy, differential scanning calorimetry and fluorescence spectroscopy of aromatic residues.

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PS.2
Role of CCL2/CCR2 Signaling in the Treatment of Ischemia

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The contribution of chemokines to the inflammatory response is a key factor in the pathogenesis of nervous system diseases including ischemic stroke. CCL2 is one of the major pro-inflammatory chemokines. This chemokine exhibits strong chemotactic properties and increases the migration and infiltration of monocytes and macrophages into the inflammatory site. That's why, the aim of study was to determinate the role of CCL2/CCR2 signaling in oxygen and glucose deprivation (OGD) model of ischemia.

The main model of research were organotypic hippocampal cultures (OHC), which were obtained from 6-7 days old pups, cultured by 7 days in standard conditions. One hour before OGD, modulator of CCR2 was added and then plates were removed into hypoxic chamber to induce ischemia model of stroke. 24 hours after damage, biochemical analyzes and measurements of mechanical properties of OHC subjected to OGD were performed.

Deprivation of oxygen was confirmed by significant increase in HIF-1α expression. What is more, increased mortality was detected in OHC what confirmed by significant increase in LDH release to cell culture medium. The main damage was localized in CA1 region of hippocampi. Compared to the control hippocampal slices, for OHC subjected to OGD, a significant decrease in stiffness was observed. Inhibition of CCL2 by use of its receptor (CCR2) antagonist - Irbesartan reduced mortality in OHC undergoing OGD.Those observation indicate that modulation of CCL2/CCR2 signaling might decrease neurons death caused by means of immune response.

Natalia Bryniarska acknowledges the support of InterDokMed project no. POWR.03.02.00-00-I013/16

PS.3

Violaxanthin De-Epoxidase Muteins as an Useful Tool in Molecular Mechanism of Violaxanthin De-Epoxidation Research

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Violaxanthin de-epoxidase (VDE) plays a key role in the light protecting process in plants. Under high light conditions, this enzyme converts violaxanthin to zeaxanthin via intermediate antheraxanthin. Lipids forming the inverted hexagonal phase, such as monogalactosyldiacylglycerol (MGDG), are required for VDE activity. Ascorbate, as a reduction agent, is also needed.

Five conservative amino acid residues were chosen and exchanged using site-directed mutagenesis, and expressed in *Escherichia coli*. The purity levels of each isolated type of VDE proteins were determined by the densitometric analysis of the electrophoresis gels. The violaxanthin de-epoxidation reactions were performed for 60 minutes in conditions optimal for the enzyme – at 25°C and pH 5.1 – in selected ascorbate concentrations. After the reactions, violaxanthin, antheraxanthin and zeaxanthin contents were determined using high-performance liquid chromatography (HPLC) with detection at 440 nm. The activities of VDE muteins were assessed by measuring the relative contents of violaxanthin compared to the wild-type VDE.

Some differences were detected, although data showed that all types of muteins can de-epoxidise violaxanthin at the same or lower level than wild-type protein. The explanation of these dissimilarities could be that chosen amino acid residues are directly involved in catalysis as well as influence on ascorbate, violaxanthin or MGDG binding by VDE.

PS.4

The As(v) Stress – How Does *Pheaodactylum tricornutum* Respond to Arsenic Stress?

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Phaeodactylum tricornutum is a diatom model used to conduct biochemical and molecular studies. It possesses an ability to grow in the presence of arsenic as well as to precipitate it in a form of a sediment.

The main aim of this work was to check an influence of different concentrations of As(V) (0.04 mg/L, 1 mg/L, 25 mg/L) on the level of expression of genes connected with the detoxification of As(V) (*ArsA* and *ArsB*), the arsenates' transport (*PIT*) and
P. tricornutum's response to oxidative stress (*SOD1, SOD2, KAT*). The RNA was isolated to evaluate the level of gene expression, using the RT-qPCR method. Growth kinetics was measured as a change in optical density at 600 nm (OD_{600}) and by directly cell counting.

Diatoms grew under all tested arsenic concentrations. The obtained results showed that As(V) influenced on the level of expression of all studied genes. However, expression of those genes depended not only on the type of analyzed genes but also on the concentration of arsenic. Furthermore, these studies revealed that the level of genes expression fluctuated in different colonies even if all other variables were kept constant.

PS.5

Effect of Low Oxygen Concentration On Sensing of Dna Double-Strand Breaks

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Reduced oxygen concentration influences numerous cellular processes. One of the consequences of hypoxia is disruption of DNA damage repair pathways, which can result in genetic instability. Cellular response to hypoxia is initiated by post-translational modifications (PTM) of HIF (hypoxia inducible factor) [1], followed by changes in PTMs and expression of DNA repair genes.

One of the first cellular responses to a double-strand DNA break is poly(ADP-ribosyl)ation of histones and proteins involved in DNA damage response, which plays important role in recruitment and activation of ATM kinase [2]. Within minutes after the formation of DNA damage, activated ATM phosphorylates histone H2AX at serine 139 (γH2AX). H2AX phosphorylation very quickly extends over long distances, as a signal for recruitment of repair proteins [3].

The aim of presented research was to investigate the effect of low oxygen concentration on formation of intranuclear foci containing factors involved in sensing of DNA double strand breaks. Individual DNA breaks were induced using a focused beam of blue light from an argon laser, in cells in cultures equilibrated with atmospheric or reduced oxygen concentration. Reducing concentration of oxygen in a cell culture on a microscope stage was achieved by limiting oxygen access to sample or by increasing the number of cells in a closed sample.

Poly(ADP-ribosyl)ation at the site of local DNA damage was decreased under conditions of reduced oxygen concentration. Low oxygen concentration resulted in partial loss of ability to phosphorylate histone H2AX and depletion of ATM kinase activation in response to DNA damage [4].

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PS.6

The Identification of *Enterococcus* spp. at the Species Level Using *rpoB* and *tuf* Genes Sequencing

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The *Enterococcus* genus consist of Gram-positive and facultative anaerobic cocci, colonizing humans and animals digestive tract. Enterococci belong to opportunistic pathogens that in specific conditions cause clinical infections, including urinary tract infections, endocarditis, and bacteraemia. The proper species identification will be useful for enterococcal infections targeted treatment. The aims of the presented research was to identify *Enterococcus* strains at the species level, by the PCR-based methods combined with DNA sequencing. The 16S rRNA gene sequencing is used in standard diagnostics, however, it does not allow precise identification of *Enterococcus* spp. due to the high similarity of the gene sequence for closely related species. In this study two methods of enterococci species identification were compared, the sequencing of the *rpoB* and *tuf* genes was used, and then the relationship between the tested strains was determined. Analysis of the *rpoB* and *tuf* genes sequences allowed the identification at the species level of 100% and 78% strains, respectively. The phylogenetic analysis of kinship indicated the differences in the evolution of the *rpoB* and *tuf* genes in particular species of *Enterococcus* spp.

PS.7
Nrf2/Keap1 Axis Determines Endothelial Cell Fate

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The gradual decline of cellular protein homeostasis (proteostasis) is a hallmark of ageing. Loss of redox balance is postulated to contribute to this process. Nrf2 is a stress-responsive transcription factor repressed by a redox-sensitive protein Keap1. Nrf2 has emerged as a guardian of cellular homeostasis. However, Nrf2 level and its activity may decrease with age. We aimed to elucidate the significance of Nrf2 in endothelial cell and aorta function with a focus on ageing and proteostasis.

We found that although Nrf2 is considered a key regulator of redox status, its depletion in primary human ECs does not lead to oxidative damage. It is achieved due to S-nitrosation of NOX4. Markedly increased protein S-nitrosation in Nrf2-deficient cells triggers premature senescence and disturbs proteostasis. Interestingly, those effects are not dependent on Nrf2 transcriptional activity, but on its ability to scavenge Keap1. We show that Keap1 serves as a critical component of the machinery responsible for S-nitrosation in ECs. Additionally, a complementary phenotype is observed in human ECs isolated from aged donors and in aortas from Nrf2 tKO mice. The ultrastructure and phenotype of Nrf2 tKO aortas are significantly altered, rendering them susceptible to aortic aneurysm formation.

In conclusion, we show that Keap1 governs protein S-nitrosation and aggregation in endothelial cells. This serves as an oxidative damage escaping mechanism relying on S-nitrosation of NOX4 and redirects ECs from death to senescence. Hence, our data unveil a novel non-canonical role of Nrf2/Keap1 axis in endothelial cell biology.

PS.8

Physico-Chemical Studies of Hydroxyapatites Changes in Biomaterials of Ceramic Type *In Vitro*

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The increasing number of injuries, fractures and bone tumours along with developments in regenerative medicine result in growing demand for bone substitute materials [1]. The aim of engineering of biomaterials is to produce non-toxic and biocompatible scaffold that would accelerate regeneration of the tissue [2]. Various microstructural and physico-chemical properties of biomaterials, such as surface chemistry, surface roughness, topography, mechanical features and interfacial free energy (hydrophobic/hydrophilic balance) are crucial for cell adhesion, proliferation and differentiation. These factors are also critically important to the overall utility of a particular material [3].

In current study the structural characteristics and physico-chemical properties of biomaterials consisting of chitosan, β-1,3-glucan and hydroxyapatite were determined with the use of X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM) and scanning electron microscopy (SEM). These techniques allowed to assess the changes of the hydroxyapatite caused by stem cells (ADSC – *Adipose tisuue-Derived Stem Cells* and BMDSC - *Bone Marrow-Derived Stem Cells)* cultured directly on the ceramic-based biomaterial. The bioactivity and biocompatibility of biomaterials were determined without the need for performing animal testing in accordance with the 3R (replacement, reduction, refinement) principle.

Conducted experiments showed that this ceramic-based composite possesses a large biomedical potential as a novel biomaterial for the regeneration of bone tissue.

Acknowledgment

Financial was provided by the Foundation for Polish Science within Reintegration grant no. POWROTY/2017-4/14.

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PS.9

Exploring the Heterogeneity and Function of Bone-Marrow Endothelial Cells

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All blood cells are derived from hematopoietic stem cells (HSCs). HSCs resides in bone marrow, where they require a specialized microenvironment referred to as niche.

It was shown that HSCs localize in direct proximity to endothelial cells (ECs) that are crucial component of HSCs niche. However, the BM ECs are heterogenous and it remains unclear which fraction of ECs supports proper function of HSCs and regenerates the vascular niche after injury. Therefore our aim is to prospectively identify different fractions of ECs and study their function in BM niche.

Using flow cytometry, ability to bind whole IgG fraction and expression of several surface antigens we identified three distinct subpopulations of BM ECs: IgG+Ly6C-, IgG+Ly6C+ and IgG-Ly6C+. Combining the cytometric analysis with single-cell RNA sequencing (sc-RNAseq) data, we showed that IgG+Ly6C- fraction possess phenotype of sinusoidal ECs, while IgG+Ly6C+ and IgG-Ly6C+ express arteriole markers. We observed that frequency of IgG+Ly6C+ population decreases in old mice. Additional functional studies showed that only IgG+Ly6C- ECs phagocytize dextran.

To further explore the heterogeneity of BM endothelial cells we perform analysis of single-cell RNA sequencing (sc-RNAseq) data. Analysis revealed *Ramp3* and *ApelinR1* as potential markers of Ly6C+IgG+ cells. Moreover, analysis of potential developmental trajectories indicated that this subpopulation may represent the ECs progenitors of BM vasculature. Identification of novel marker genes will allow further isolation of endothelial bone marrow cell populations.

Concluding, our analyzes of endothelial heterogeneity allows for the prospective identification of different endothelial cell fractions building bone marrow niche.

PS.10

Refinement of the OPLS-AA Force Field Parameters for Phospholipids. Theoretical Studies on Triacetin Molecule

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The triacetin molecule, which contains moiety present in glycerol backbone of phospholipids has never been considered as a model molecule. It is particularly interesting molecule, since there is large resources of the experimental data obtained for its condensed phase, such as density and enthalpy of vaporization.

In the presented studied, we focused on the OPLS-AA parameters such as Ryckeart-Bellman coefficients describing torsional potential and partial charge, which are responsible for reproduction of non-bonding interactions. In order to adjust appropriate parameters for torsion angles, the methods of Monte Carlo and linear programming were used. The first one, by introducing a temperature factor, prevents stuck in the local minimum. The disadvantage of this approach is the lack of repeatability and arbitrariness in the selection of initial parameters. However, it works in practice. The second is based on some results from linear algebra, and the entire estimating process is repeatable. The execution process is much shorter. The presented results show the refinement procedure, including QM and ab initio MD calculation, applied for parametrization of triacetin molecule and the effect of the new parameters on reproduction of known liquid state properties of these molecule.

Acknowledgments:

This research project is supported by grant No 2015/17/D /ST4/00555 (National Science Center) and in part by PL-Grid Infrastructure.

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PS.11

Autophagy-Related Additive Effect of Bergamottin and Simvastatin on the Viability of Glioblastoma Multiforme Cells

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Bergamottin is a furanocoumarin commonly found in grapefruits, pomelos and bergamot fruits. It is supposed to negatively affect the activity of numerous drugs via the interference with their intracellular metabolism. This effect has been ascribed to bergamottin-induced inhibition of the CYP3A4 isoform of cytochrome P450, which participates in the metabolism of ca. 50% of the currently used drugs. Recently, bergamottin has also been found to affect the growth of glioblastoma multiforme (GBM) cells via the inhibition of small G protein Rac1. The study aim was to estimate the suitability of combined Rac1 targeting for the GBM treatment. For this purpose, we subjected T98G and U87 cells to the combined Bergamottin/Simvastatin treatment. Simvastatin competitively inhibits 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, causing mevalonate deficit, the inhibition of cholesterol synthesis, and the blockade of Rac1 isoprenylation. In our hands, bergamottin (10-100 μM) and simvastatin (1-10 μM) decreased the proliferation of T98G and U87 cells in a dose-dependent manner. We also observed an limiting effect of these compounds on GBM cell migration and transmigration capacity. Our data show the additive effects of bergamottin and simvastatin on the viability of GBM cells. Rac1 inhibition results in disruption of the mTOR signaling pathway, its deactivation and an induction of mTOR-dependent autophagy. Therefore, the postulated effect of bergamottin and simvastatin on Rac1 activity can involve the disturbances mTOR signaling, which induce the “suicidal” autophagy of GBM cells.

This work was financially supported by Polish National Science Centre (UMO-2015/19/D/NZ3/00273 to D.R.).

PS.12
The *In Silico* Model of the Mixed Cancer Culture Growth

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The topic of cancer heterogeneity is recognized as a major problem in cancer treatment. One way to study heterogeneity involves creating a defined *in vitro* mixtures of different cell lines. Corresponding *in silico* models allow one to proceed a parallel study under highly controlled conditions. Our objective is to demonstrate such computational model of a mixed cancer culture. The program introduces two cancer cells lines ("B16" and "LLC") differing in respect of proliferation rate as well as the cell size (two size available) and the dynamics of growth. Consequently, the simulation was designed as to recognize only the steric relations of mixed culture with exclusion of any biological and environmental factors. Triple objectives had been envisaged in such experimental setting. 1. To create a tool enable to simulate the dynamics of cell mixture (which due to complexity of task is unfeasible in an analytical way). 2. To test mixed culture growth in different conditions up to boundaries anyhow not available for *in vitro* cultures. 3. To shed light on laboratory data of parallel conducted *in vitro* experiments. The variety of parameters was tested revealing both advantages and limitations of the model. The comparison of simulation results to experimental data suggests that observed *in vitro* accelerated growth of mixed cultures does not result from the additive effect of purely steric relations occurring on the level of each cell line separately but from the heterogeneity itself. In other words, mixed culture seems to create a new biological quality.

PS.13

Bacterial Isolates From *Callitriche cophocarpa* in Study on Arsenic Bioremediation of Waters

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The aim of this study was to investigate bacterial symbionts of *Callitriche cophocarpa* towards arsenic resistance and siderophores production in order to potential utility in water remediation.

Plant samples collected from the natural stands were incubated with 500 μM of As(III) ($NaAsO_2$) or As(V) ($NaHAsO_4x7H_2O$) in sterilized river water. From 7 days-old cultures 13 different As-resistant bacterial strains were isolated and genetically identified.

Obtained isolates were tested towards arsenic resistance and siderophores production. Resistance test was performed by culturing on LB agar plates enriched with As(III) of concentration 5, 10, 25, 35, 50 mM and As(V) also with two higher concentrations, i.e. 150 and 267 mM, for 24h at 22 $^\circ$C. To estimate siderophores production single colonies of isolates were inoculated on LB agar plates with As(III), As(V) of 500 μM for 5 days and then plates were covered with CAS-agar medium to perform colorimetric reaction. Plates were incubated for 24h, and siderophores production efficiency was calculated as a ratio of sum of colony diameter and width of yellow halo of the reaction area to colony diameter.

All isolates were resistant to all applied concentrations of As(V). As(III) was visibly more toxic – two isolates were not able to grow with As(III), then resistance decreased according to increasing concentration. Only one isolate presented resistance to 50 mM.

Seven isolates presented ability to produce siderophores. Isolates from As(III) treated plants were less efficient in siderophores production than obtained from cultures with As(V).

PS.14
Influence of DAL-PEG-DK5 on the Intracellular Persistence of *S. Aureus* in the Human Keratinocyte Cell Line

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Methicillin-resistant *Staphylococcus aureus* (MRSA) belongs to the relatively aerobic, gram positive cocci is one of the major opportunistic strains, which are common in nosocomial infections. In response to different environmental stimuli those particular strains have the ability to change expression of genes encoding virulence factors. The rapid emergence of multidrug-resistant pathogens has become a global issue, which prompted the search for new substances with antibacterial properties. Antimicrobial peptides (AMPs) belong to the group of compounds, which possess strong antimicrobial properties and are secreted by most of the organisms. As a host defense peptide they are promising potential therapeutic molecules because of their close binding to negatively charged bacterial cell membrane. This thesis presents the results of research conducted with usage of peptide conjugate DAL-PEG-DK5 composed of the synthetic enkephalin analogue DAL and lysine-rich derivative of temporin 1CEb, which is DK5. The aim of the research was to evaluate influence of DAL-PEG-DK5 on the intracellular persistence of *S. aureus* USA300 in the human keratinocyte cell line (HaCaT). The results clearly indicated that DAL-PEG-DK5 treatment reduced statistically significant intracellular level of USA300 inside human keratinocytes. Furthermore, the results demonstrate the mechanism of action of the tested compound, which in a very short time (0-10 min) passes through the bacteria by damaging their membranes. Understanding the mechanism of action enables in vivo testing and may also lead to designing of novel peptide-based compounds with similar structure.

PS.15

Epidermal Growth Factor (EGF) Promotes Cytoskeletal Rearrangements and Augments Invasive Potential via Cx43/ROS Dependent Manner Within Human Glioblastoma Multiforme Cells *In Vitro*

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Glioblastoma multiforme (GBM) is the fourth on a four-grade scale of advancement of the gliomas. Gliomas are tumors which have arised i.a. from glia in the brain. GBM is the most common type of cancer in humans. GBM therapy is mainly based on surgical resection of tumor and implementation of chemotherapy. However, because of the brain-blood barrier, the accessibility of therapeutical substances are limited. During GBM progression the invasive cells migrate through parenchyma to other regions of brain, sometimes fundamental for patient survival. High migration ability of GBM cells and formation of metastases is the main reason for malignant and deadly GBM character. Epidermal growth factor (EGF) is a molecule that evokes numerous intracellular signaling cascades by binding to its receptor (EGFR). EGF is secreted by both normal and tumor cells. It is exuded also in brain, especially during its continuum damage. Such abnormalities could occur during glioblastoma tumor growth or after tumor resection which is used as a primary method of GBM therapy. Interestingly, in glioblastoma tumors there is observed a very common mutation of EGFR which is called EGFR variant III, truncated form showing constitutive activity. Taking into account such issues, we hypothesized that GBM-induced brain tissue microdamage evokes EGF hypersecretion which stimulate invasiveness of GBM cells. In the research we used glioblastoma multiforme T98G cell line., We confirmed the significant role of EGF in GBM progression and its ability to augment its invasive properties via inducing cytoskeletal rearrangements (F-actin/vinculin) and mild ROS overproduction within T98G cells. This phenomenon was corelated with ROS-dependent increased motile activity, proliferative potential enhancement and overexpression of cytosolic level of malignancy-related marker - Cx43. Furthermore, observed intracellular changes were accompanied by cell metabolic profile (ATP/lactate production) modulation. In conclusion, we demonstrate that EGF is crucial modulator of GBM development/progression.

The present study was financially supported by the Student Research Grant Program at Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University (grant no. 4/SPB/2019)

PS.16
Detection of G-Quadruplexes and Heme Oxygenase 1 Interactions by Proximity Ligase Assay

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G-quadruplexes (G4) are stacked nucleic acid structures that form within guanine-rich DNA or RNA sequences. These structures appear to play an important role in various biological functions, such as DNA replication and transcription, telomere maintenance, or in RNA metabolism. Several helicases, namely Brip1, Blm, Wrn and Pif1 can unwind G-quadruplex structures *in vitro*. However, their role *in vivo* is unclear, since the visualisation of proteins that bind to the G4 structures is not easily demonstrated experimentally.

In the present study, to the best of our knowledge for the first time, we adapted proximity ligase assay (PLA) for the analysis of G4 partners. Initially, this technique was developed for the sensitive detection of protein interactions and modifications. As a positive control for PLA we visualized interactions between Ku70 and Ku80 proteins which form dimers located in the cell nucleus. Using PLA we showed in cells co-localization of G-quadruplexes with its known modifier, Brip1 helicase. Moreover, we have also demonstrated that heme oxygenase-1 protein is located in the vicinity of G4 suggesting its potential role in modification of G4 stuctures, both in cell lines and primary hematopoietic cells. All studied interactions with G4 were observed in the form of discrete fluorescent spots located in the cell nucleus that disappeared after DNase treatment, proving the specificity of the reaction.

Concluding, the versatility of PLA that allows for analysis of different cell types both by microscopy and by flow cytometry, makes this technique a powerful tool for the detection of G4-protein interactions in cells.

PS.17
What Is a Link Between L-Glutamine Depletion and Prostate Cancer Cells Biomechanics?

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One of features of cancer cells are changes in their metabolic activity [1], [2]. Thus many approaches are proposed to target cancer cell metabolism as a new anticancer therapy. One of them is targeting of glutamine transporters which is important due to the fact that glutamine is considered as major source of nitrogen for nucleotides synthesis [3]. Parallelly more attention is paid to mechanical cancer hallmarks. In general cancerous cell became more soft than their healthy counterparts[4]. Such changes in mechanics of cells and their surrounding affects mechanotransducive pathways leading to changes in cells behaviour including drug resistance[5], [6]. Thus the aim of our study was to determine how depletion of L-glutamine supplementation will affects prostate cancer mechanics. What is more we investigate how mechanical response of prostate cancer cells changes upon docetaxel treatment when L-glutamine is depleted.

While for prostate cancer cell cultured under standard conditions their mechanics is preserved (~6 kPa) over time (after 24, 48 and 72 h) depletion of L-glutamine leads to their significant stiffening with peak after 48 h (~10 kPa). Interestingly similar robust transient stiffening was observed when control cells were treated with 1 nM of docetaxel (~10 kPa). The mechanical response of prostate cancer cells to docetaxel was milder when cells were cultured with L-glutamine depletion, thus indicating affected mechanosensing of cells during this process.

Our results coupled together suggest that changes in L-glutamine supply affects not only cellular mechanics but also their response to anti-cancer action. Such findings provide important insight into way in which we understand mechanobiology of drug response and might be taken under consideration while looking for new therapeutic approaches.

AK acknowledge the support of InterDokMed project no. POWR.03.02.00-00-I013/16

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PS.18

Dead or Alive? Evaluation of Chlorophyll Fluorescence in Algae as Viability Test

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Diatom *Phaeodactylum tricornutum* and green alga *Chlamydomonas reinhardtii* are commonly used in molecular biology as model organisms. Their growth may be assessed by multiple simple techniques. In contrast, there are few methods allowing assessment of their viability. One of them could be cell counting based on chlorophyll autofluorescence with automatic cell counters.

To verify the above presumption, we applied the method of counting cells showing chlorophyll autofluorescence as a viability test in single-cell algal cultures. We compared that to the use of maximum quantum yield of photosystem II (Fv/Fm) and MTT assays, commonly used for assessing vitality. Cell death was induced by thallium chloride and sodium azide. Multiple measurements were started 30 min after addition of toxic agents and were performed every 4 hours for 12 hours.

The highest differences in viability were recorded for Fv/Fm measurements after treatment with sodium azide. In this case, the values of Fv/Fm decreased about tenfold compared to the untreated control. For thallium chloride, signal reduction was considerably smaller, however still significant. Results of MTT assay also showed larger changes for sodium azide-treated samples compared to thallium chloride-treated group.

Direct cell-counting method based on fluorescence did not show substantial changes in viability within the timeframe of the experiment. No differences were observed for organisms treated with thallium chloride nor for those treated with sodium azide.

Obtained results demonstrated that although the counting of autofluorescence exhibiting cells is convenient and useful for cell growth monitoring, it is not suitable for direct estimation of their viability.

Project supported by Rada Kół Naukowych UJ (Council of Science Clubs Jagiellonian University)

PS.19
The Dark Side of the Fungus

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Melanins are a group of black ubiquitous polymers observed in animal (as well as human), plant, fungal and bacterial cells, where they are present mostly as protective agents against various environmental factors. In fungi, they do not occur often and probably, they are mainly responsible for survival under harmful conditions.

Among fungal endophytes isolated from deciduous trees, it was noted that some of them produce dark pigment in its mycelium, especially on the bottom. The increase of the black color intensity with the age of the culture was observed in one of them. Regarding the fact that melanin has a dark color, it was decided to analyze if melanin is present in its mycelium. For this purpose, electron paramagnetic resonance (EPR) spectroscopy was used in the temperature of liquid nitrogen, forasmuch as melanin has unpaired electrons in the structure. The EPR spectrum of the mycelium revealed a very strong melanin signal. The melanin type detected in these analyses was identified as pheomelanin. Because the medium, in which the fungus had grown, also darkened, it was verified whether melanin is secreted to the environment. No melanin was detected in the culture medium even with zinc acetate addition to the sample to increase the signal intensity.

Due to the fact that melanin is not a pigment commonly found in fungal cells, and on the other hand revealing many unusual biological properties and functions, the biological significance of this pigment in adaptability of the studied fungus should be tested in the future.

PS.20
Specific Inhibition of DYRK1A Tyrosine Kinase for Disease Modulation

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High-resolution structures of proteins and protein-inhibitor complexes open possibilities for rational drug and inhibitor design. In particular, the structures of protein inhibitor complexes reveal the specific interactions in the binding pocket, comprising hydrogen binding patterns and other polar and hydrophobic interactions. Protein kinase inhibition is well established for therapeutic intervention in various disease models, with dozens of approved protein kinase inhibitor drugs against a limited number of targets. Currently, attention is shifting toward novelty in protein kinase targeting, novel inhibition mechanisms and greater diversity in disease areas. New targets are emerging from genomics and other studies, and this expands a variety of targets, targeting approaches and active compounds. We have established a collaborative project in drug discovery and design targeting proteins using X-ray structure, aided drug design, and synchrotron radiation facilities in Europe. Among kinases, DYRK1A is one of five members of the Dual-specificity tyrosine (Y) phosphorylation-Regulated Kinase (DYRK) family. The DYRK1A gene is located in the Down Syndrome critical region and regulates cellular processes related to proliferation and differentiation of neuronal progenitor cells during early development. This observation has refocused researchers on its role in neurodegenerative diseases accompanied by the recently proposed contributions of DYRK1A to diabetes. Here we report a set of scaffolds and their promising derivatives, not generally known for DYRK1A inhibition, demonstrating their effects in biochemical assays and also in cell cultures. These inhibitors effectively block the tau phosphorylation, crucial in related diseases and the obtained crystal structures support the rational design for state-of-the-art therapeutics.

PS.21

Responsiveness of Human Bronchial Epithelial Cells and Fibroblasts From Asthmatic and Non-Asthmatic Patients in EMTU Co-Cultures on the TGF-β_1

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Asthma-related airway wall remodeling is associated with i.a. damage of bronchial epithelium and subepithelial fibrosis. Functional interactions between human bronchial epithelial cells (HBECs) and fibroblasts (HBFs) are known as the epithelial-mesenchymal trophic unit (EMTU) and are necessary for a proper functionality of lung tissue. However, a high concentration of TGF-β_1 in the asthmatic bronchi drives the structural disintegrity of epithelium with epithelial-to-mesenchymal transitions (EMT) of HBECs and fibroblast-to-myofibroblast transitions (FMT) of HBFs. Previous reports indicate different intrinsic properties of HBECs and HBFs from asthmatic (AS) and non-asthmatic (NA) patients, which affect their EMT/FMT potential. Hovewer, little is known about the nature of interactions between these cells in the EMTU model. Here, we investigated the effects of TGF-β_1 on EMT markers of HBECs cultured in the air-liquid-interface (ALI) and effectiveness of FMT in HBFs co-cultured in the EMTU. Our results show that: i) AS co-cultures are more sensitive to TGF-β_1 than the NA ones; ii) HBFs increase the transepithelial electrical resistance (TEER) values in epithelium; iii) in response to TGF-β_1, HBFs AS efficiently than HBFs NA diminish the TEER values, reduce the ALI-related (*MUC5AC, DNAH9*) and EMT-related genes (*CDH2, ACTA2, SNAI1/2*) in HBECs AS than HBFs NA. Moreover, the FMT potential of HBFs AS measured by the expression of *ACTA2* is effectively diminished by NA than AS HBECs. These results suggest a protective effect of HBFs on the properties of TGF-β_1-treated HBECs and vice versa and show the EMTU model as the useful tool for the *in vitro* studies of asthmatic airway remodeling.

Supported by the grant from PNSC 2015/17/B/NZ3/02248 (MM). DW is the beneficiary of ETIUDA scholarship (2019/32/T/NZ3/00405); PNSC.

PS.22

Trichostatin A Attenuates the TGF-β_1-Induced Myofibroblastic Transition of Human Bronchial Fibroblasts From Asthmatics

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Fibroblast-to-myofibroblast transition (FMT) is the key process involved in the subepithelial fibrosis progression observed in remodeled airways in most patients with diagnosed asthma. In response to TGF-β_1, resident human bronchial fibroblasts (HBFs) activate the profibrotic TGFβ/Smad2/3 signaling pathway, acquire a highly contractile myofibroblast phenotype and secrete increased levels of extracellular matrix proteins. It is known that epigenetics is involved in the progression of asthma, but the role of histone deacetylases (HDACs) activity in the progression of subepithelial fibrosis and FMT remains unclear. In this study we investigate a molecular link between TGF-β_1-induced myofibroblastic shifts of HBFs and HDACs activity using trichostatin A (TSA) – a potent inhibitor of HDACs. HBFs isolated from asthmatic donors were cultured in serum-free conditions with TSA and TGF-β_1 for 1 hour (analyses of activation of Smad signaling), 24 hours (gene expression- qPCR) or 4 days (analyses of protein levels by in-cell ELISA, Western blot or immunofluorescence). Our results demonstrate the inhibitory effect of TSA on the percentage of myofibroblasts and levels of profibrotic genes (*ACTA2, TAGLN, FN, TNC*) and proteins (α-SMA, fibronectin) in TSA/TGF-β_1-treated populations of HBF. TSA has no inhibitory effect on the Smad2/3 phosphorylation and intracellular localization, but effectively inhibits the levels of HDAC2. These results suggest that HDACs inhibition is associated with the diminished efficiency of FMT in the TGF-β_1-treated populations of HBF and show the ability of TSA for the suppression of subepithelial fibrotic changes in the airway wall, but extensive studies are needed to clarify the therapeutic potential of TSA in asthma.

Supported by the grant from PNSC 2019/03/X/NZ3/01801 to M.P. and 2015/17/B/NZ3/02248 to M.M. DW is the beneficiary of ETIUDA scholarship (2019/32/T/NZ3/00405); PNSC.

PS.23

Post-PDT Antitumor Immune Response: A Game With a Lot of Players

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Recently, immune checkpoint immunotherapy has shown promising clinical results. However, there is still a large number of patients who respond poorly; therefore, more potent combinational therapies are required. In this context, immunotherapy based on the modulation of immune checkpoints like PD-1/PD-L1 in combination with photodynamic therapy (PDT) may be an innovative therapeutic strategy for more resistant cancers. Herein, we employed polarity-tunable bacteriochlorin-based photosensitizers that absorb near-infrared light to compare the PDT efficacy after protocols targeted to tumor vasculature, endothelial cells or tumor tissue. We performed the analysis of the molecular mechanisms of PDT crucial for the generation of antitumor immunity and indicated that PDT-induced cell death might affect the integrity of the host tissue and develop acute local inflammation. Using the multitarget approach, it was possible to evaluate whether modulation of inflammatory mediators determines the mode of cell death and the susceptibility to systemic PD-1/PD-L1. More importantly, we present that PDT in vivo contributed to an immunogenic environment in the tumor, which may enhance PD-1/PD-L1 checkpoint blockade therapy by stimulating a strong and long-term antitumor immune response. We demonstrated that optimized PDT regressed the growth of not only primary- but also distant tumors examined in bilateral syngeneic and pseudo-metastatic mouse models. Moreover, based on rechallenge experiments, we assessed the generation of immune memory able to prevent tumor relapse. Finally, we suggest that the combination of PDT with PD-1/PD-L1 axis inhibition designed in the current study offers a new strategy for treating metastatic cancers with primary tumors accessible by PDT.[1-2]

Acknowledgments: This work was supported by the NCN within the grant no 2016/22/E/NZ7/00420 and FNP within the START 071.2019 program.

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PS.24

Particular Outline of Mechanisms Regulating Doxorubicin Resistance Development Within Human Glioblastoma Multiforme Cells

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Despite the udnoubted progress in filed of numerous tumors therapies, glioblastoma multiforme (GBM) treatment is still an indisputable challenge for contemporary neurooncology. This state of affairs is a result of specific brain tissue anatomy implicating difficult distribution of applied drugs. What is more, frequent clinical picture of such tumor is related to drug-resistance development and malignancy enhancement during/after therapeutic cycles. Recent clinical trials indicate strong antineoplastic effect of anthracycline drug – doxorubicn (DOX) within mentioned disease entity and suggest its incorporation into GBM treatment strategies (NCT02758366, NCT01851733). However, due to a significant lack of informations about mechanisms involved in response of GBM cells to doxorubicin, we performed numerous experiments using microscopy techniques (fluorescence microscopy, TIRFM, TEM) and biochemical analysis (Western blot) in order to get a detailed picture of such phenomenon with particular attention to drug resistance development. On our hands, DOX-treated GBM cells showed notable enhancement of invasive phenotype rather than apoptosis activation. In particular, cellular response to doxorubicin was related to i.a migration activity stimulation, cytoskeleton architecture rearrangement and increase of invasive phenotype markers levels (Snail-1, Cx43, Vimentin). Such effect was accompanied by mitochondrial dynamics modulation and autophagy (but not cell death) induction. Interestingly, long term analyzes revealed prominent polyploidity formation that presumably comes from endomitosis (Ki67 positive cells; metaphase chromosomes presence). It is also worth emphasizing that after several days from DOX exposition, we observed GBM cell population renewal. Collectively, our experiments provided results that describe chosen mechanisms of cellular response to DOX during GBM therapy and indicate potential pro-invasive activity of such drug. Thus, we suggest that described phenomenon should be taken into consideration during application of doxorubicin in glioblastoma multiforme therapy.

The present study was financially supported by the Polish Ministry of Science and Higher Education (grant no. 0161/DIA/2019/4 to M.P) and in part by Research Grant Program at Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University (grant no. MNS/18/2019 to D.R)

PS.25
Modeling Cancer Growth With Evolutionary Game Theory

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Cancer - one of the most complicated condition studied in biomedical research, poses a great challenge not only for clinicians or experimentalists but also computational scientists. A full comprehension of the interplay between healthy and cancer cells with all its aspects is beyond our current knowledge [1]. Yet a system where two subpopulations of competing cells evolve towards fixation can be described as a game between two players. Recent studies highlight benefits from modeling cancer progression in a game-like, theoretical framework [2]. The prisoner's dilemma successfully captures the essential features of cancer growth and allows to test hypotheses and formulate claims in a quantitative manner. Ultimately no model is perfect - it is a matter of how well a given set of assumptions corresponds with the key aspects of the modelled system and how useful a given description is when it comes to gaining insight about the underlying mechanisms and generating new knowledge. The aim of our research was to re-assess the population dynamics under the presented model upon some changes in cancer representation that, in our opinion, reflects the biological process better. Using a hypothetical cancer cells population we verified three essential features of cancer growth within our model in silico . The Gompertzian growth curve - describing the basic pattern of tumor growth. The log-kill hypothesis as a model for the effect of cytotoxic chemotherapy on tumor size. Finally, the Norton–Simon hypothesis considering the rate of cancer cell death in response to treatment at the time of it.

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PS.26

Functional Links Between Metabolic Pathways and Mitochondrial Dynamics – Potential Implications for Polyploidy Formation Within Human Glioblastoma Multiforme Cells

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Glioblastoma multiforme (GBM) is the most common type of central nervous system (CNS) tumors that occur in children under 15 years of age. It represents the third most common cause of death in neoplasm patients under the age of 40. Despite the development of GBM treatment strategies, the average survival time of patients with GBM has not changed within the last 30 years and does not exceed 12-14 months from the time of diagnosis. Moreover, the mechanisms responsible for the development and progression of GBM still remain unclear, despite the numerous data on its pathogenesis. Recently, development of polyploidy accompanied by the rearrangements of mitochondrial architecture have been pinpointed as crucial for GBM drug-resistance and tumor recurrence. Therefore, we focused on the functional links between the metabolic profile and mitochondrial dynamics/architecture during the development of polyploidy in GBM populations. For this purpose, the effect of an array of metabolic inhibitors (of glycolysis, pyruvate transport, OXPHOS and mitochondria-dependent pyrimidine biosynthesis) and/or cytostatic drugs (doxorubicin, temozolomide, carmustine, Cis-Pt, 5-fluorouracil) on the formation of polyploidy (polyploid giant cells; PGCs) was verified. The obtained results indicate that mitochondrial fusion and PGCs formation strongly depends on OXPHOS-related electron transport chain (in particular, coenzyme Q: cytochrome c – oxidoreductase; Complex III) and mitochondria-dependent de novo pyrimidine biosynthesis. Furthermore, the observed effects were accompanied by the modulation of ATP/lactate production. Collectively, our data suggest the crucial role of mitochondria-dependent bioenergetic status for GBM polyploidy. This may have fatal consequences for GBM progression/recurrence.

The present study was financially supported by Research Grant Program at Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University (grant no. MNS 16/2019; N19/MNS/000018 to D.R)

PS.27

Investigation of Antioxidant and Structural Properties of Tauroursodeoxycholic Acid (TUDCA) in a Model Photoreceptor Membranes Made of Synthetic Lipids

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Tauroursodeoxycholic acid (TUDCA), the taurine conjugate of the ursodeoxycholic acid (UDCA), has been known and used from ancient times as a therapeutic compound in traditional Chinese medicine. TUDCA has recently been gaining significant interest as neuroprotective agent, including visual disorders. It has been shown that TUDCA efficiently protects photoreceptors against photodamage. However, the exact mechanism of its action has not been elucidated.

In this work we investigated antioxidant activity of TUDCA and its impact on structural properties of model photoreceptors membranes of different composition using electron paramagnetic resonance spectroscopy and spin labelling technique. The results show that TUDCA acts as an effective antioxidant against free radicals-induced lipid oxidation in model photoreceptor membranes. However, none of the bile acids studied, quenches singlet oxygen. TUDCA induces distinct changes in the polarity and fluidity in all investigated model membranes, especially in these containing low cholesterol concentration. Together our results suggest that cytoprotective activity of TUDCA in photoreceptors may be in part consequence of its sole physico-chemical properties.

Acknowledgements:

Faculty of Biochemistry, Biophysics and Biotechnology of Jagiellonian University, a partner of the Leading National Research Center (KNOW) supported by the Ministry of Science and Higher Education (Grant number 35p/11/2017, URL: https://app.dimensions.ai/details/grant/grant.7424462)

PS.28

The Bioinformatics Testing of the miRNA Molecule Refers to Functional Subdivision

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During RNA interference, mRNA and short molecules (e.g. miRNA) interact with each other. As a result, the protein complex with miRNA cleaves the target transcript and the corresponding protein is not synthesized [1]. There are many bioinformatic tools for predicting the targets and binding sites for miRISC (RISC within mounted miRNA is called miRISC (miRNA-RISC)). They are based on complementarity, phylogenetic conservativeness of both miRNA and mRNA, estimation of the free energy of miRNA/mRNA duplex, and evaluation of the accessibility of the transcript region [2]. However, these methods often provide different predictions for the target sites [3]. For these reasons some insights into the structure of the miRNA molecule should be considered. The miRNA molecule is often divided into two parts, namely: the so-called seed (approximately the first 7 nt) and the puzzle – the remaining part of the miRNA molecule.

This work focuses on verifying the internal miRNA structure on the basis of the EIIP parameter, estimating duplex binding energy (within 3'UTR, 5'UTR and CDS), including a confirmation of the role of the 3'UTR region using the tool miRanda, and non-random relations within the miRNAs set.

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PS.29
Identification and Characterization of Posttranslationally Modified Isoforms of BacSp222 Bacteriocin

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BacSp222 is a peptide bacteriocin secreted by the zoonotic strain *Staphylococcus pseudintermedius* 222. The peptide combines properties of bacteriocin and virulence factor as it has bactericidal as well as cytotoxic and immunomodulatory activities against eukaryotic cells. We have shown that BacSp222 is posttranslationally modified by butanedioic (succinyl) groups. Three isoforms of BacSp222 were found in the culture medium: unmodified isoform 1, isoform 2 containing one butanedioic group, and isoform 3 with two butanedioic groups.

This communication describes the results of studies concerning chemical identification and biosynthesis of isoforms, the influence of modification on molecule conformation and biological activity. The identity of isoforms was determined by mass spectrometry and Edman degradation technique. The studies carried out by HPLC have shown that all isoforms are produced simultaneously at each stage of bacterial culture growth, but the ratio between isoforms depends on time and availability of nutrients. The export of bacteriocin is very efficient because none of the isoform was detected in the bacterial lysate. The isoforms occur in free form in culture medium and are not associated with intracellular follicles or multi-molecular protein aggregates. Circular dichroism studies did not show significant differences in conformation between isoforms 1 and 2. On the other hand, succinylation significantly reduced bactericidal and cytotoxic activity.

Presented results suggest that described posttranslational modification to some degree protects producer cells against autotoxicity of secreted bacteriocin. However, further research is required concerning the mechanism of formation of the derivatives and the effect of succinylation on the immunogenic properties of BacSp222.

The study was supported by grant No 2018/31/B/NZ3/01226 (to P.M.) from National Science Centre, Poland.

PS.30

Towards the Embryonic Transfer in Mice and Generation of New Mouse Strains

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Transfer of murine embryos is a quintessential step for mouse breeding procedures such as rederivation of contaminated mouse strains, recovery of cryopreserved (vitrified) embryos, and generation of genetically modified mice. The latter starts with the modification of mouse zygotes. While the zygotes can be transferred both at the stage of the zygote to oviducts (early transfer) or after *in vitro* culture as blastocysts to the uterus, devitrified embryos can be transferred only to the uterus (late transfer). Therefore, we aimed to optimize both early and late embryo transfers.

We successfully obtained high numbers of mouse zygotes from female donor mice after the treatment with pregnant mare serum gonadotropin (PMSG), human chorionic gonadotropin (hCG) 48 hours later, and mating with males of the same strain. Then, zygotes were either transferred directly to oviducts at 0.5 day *post coitum* (*dpc*) or cultured until the blastocyst stage, when they were transferred to uterus horns with the surgical or non-surgical method at 3.5 *dpc*. We used pseudopregnant FVB/J mice mated with vasectomized males as recipients of embryo transfer.

Finally, in order to optimize the microinjection method, we injected mouse zygotes with a plasmid harboring GFP gene and further cultured them *in vitro*. Upon the microinjection, we obtained viable embryos, which developed to the compact morula stage and showed green fluorescence.

PS.31

Expect the Unexpected – the Significance of Type of Expressing Vector in Promoter Activation Studies

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All cells need a source of energy to maintain basic survival processes. Proliferating cells have additional energetic requirements to grow and divide. A major source of cellular energy and new cell mass is glucose. Fast-growing cells rely primarily on glucose fermentation even in oxygen-rich conditions. Proliferating cells produce lactate to regenerate NAD^+, however lactate generation may have secondary benefits for tumor cells. The resulting acidification of the extracellular microenvironment promotes survival, growth and invasive behavior of malignant cancer cells via upregulation of metalloproteinase activity.

Our recent studies indicate that after the inhibition of glycolysis, using 2-deoxyglucose
(2-DG), the mRNA level and activity of matrix metalloproteinase 2 (MMP-2) decrease.
MMP-2 is an enzyme involved in extracellular matrix degradation in various processes, such as embryonic development and wound healing. Increased MMPs activity is observed also during epithelial-mesenchymal transition. To examine the possibility that MMP-2 mRNA decrease results from its promoter activity regulation we prepared expression vector with the reporter gene under the control of MMP-2 promoter with various sequence fragments deletions. However, it was not possible to precisely localize the region regulating promoter activation in response to 2-DG. Using another expression vector we checked if observed changes in promoter activation are the result of the response of the vector itself to the presence of 2-DG, rather than the regulatory element within the promoter. The obtained results indicate that the choice of expression vector is not without significance. This study raises the problem of choosing the right model for experiments using expression vectors.

PS.32

Selected Environmental Factors in IAA Production by Plant Endosymbiotic Bacteria

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Pseudomonas putida, isolated from underground parts of *Tussilago farfara* growing on mercury contaminated soil, was shown to be able to produce indole-3-acetic acid (IAA) and thus identified as Plant Growth Promoting Bacteria.

The presented studies aimed to verify the ability of IAA production by isolated strain in growth medium with 0.01% of Hg and in medium without Hg. Additionally, the effect of bacterial growth phase and oxygen availability on IAA concentration were tested for 96 hours. The significance of oxygen on IAA production was tested in two variants: tightly closed 15 ml falcon tube with 5 ml medium (variant 1) and Erlenmeyer flask ventilated with sterile air (variant 2). The concentration of IAA was monitored by absorbance measurement of Salkowski reagent's colorful product at 540 nm.

Generally, the growth of bacteria seems to be independent of mercury presence in the medium in both tested variants. In variant 2 the rate of bacteria cell divisions was higher. It was also observed that mercury has no crucial impact on IAA production. No clear correlation between endogenous IAA concentration and bacterial growth phase was noticed, yet the hormone level changed cyclically during the culturing. It was found that in variant 2 maximal IAA concentration was higher than in variant 1. Furthermore, in variant 2 production of water soluble pigment was observed. While studying the effect of two different concentrations of exogenous IAA on level of endogenous IAA and rate of bacteria culturing, no inhibition of bacterial IAA synthesis or culture growth was observed.

Monday Poster Session

PM.1

Acclimatisation of *Thalassiosira pseudonana* Photosynthetic Membranes to Different Environmental Temperature

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The greenhouse effect results in warming the planet's surface and a higher ocean heat content. The changing water temperature may impact Earth's ecosystems. In the present study the effect of different environmental temperatures (from 12 to 23°C) on a model diatom species, *Thalassiosira pseudonana,* was tested under laboratory conditions. In this work we analysed the temperature effect on the fluidity and chemical composition of thylakoid membranes and selected physiological aspects of the investigated diatom species. We observed a slight decrease of the growth rate and chlorophyll concentration, but an increase of the protein concentration, in diatom cells cultured at lower temperatures. The analysis of the thylakoid membrane composition isolated from diatoms cultured under the conditions of low temperatures, indicated an increase of polyunsaturated and a decrease of saturated fatty acids, accompanied by a higher lipid:protein ratio. These changes resulted in the preservation of the thylakoid membrane fluidity in the polar and hydrophobic regions of the membranes and finally in maintaining the photosynthetic efficiency as determined by measurements of the optimum quantum yield (Fv/Fm). The obtained results show that regulation of the fatty acid and protein content represents one of the most important strategies of diatoms for the optimization of photosynthetic membrane under environmental temperature changes.

PM.2

Spectroscopic Assessment of Osteogenesis Process on Ceramic Biomaterials *In Vitro*

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Despite the exceptional healing capacity of bone, infection, tumor, trauma and surgery can cause remarkable bone loss requiring reconstructive techniques to restore its form and role [1]. Tissue engineered products (TEPs), including biomaterials containing either cells and/or growth factors, play an important role in bone regeneration by providing the necessary substrate for cell adhesion, proliferation, and differentiation. They exhibit optimal features such as macroporosity and 3D structure to modulate cell activity and function, ensuring osteogenesis and angiogenesis processes within it [2, 3].

In present study FT-IR and Raman spectroscopies were applied to characterise the composite ceramic-based biomaterial consisting of chitosan, β-1,3-glucan (curdlan) and hydroxyapatite. These techniques also allowed to evaluate the production of bone formation markers by two different stem cells type seeded on its surface: Adipose tissue-derived stem cells (ADSC) and Bone marrow-derived stem cells (BMDSC). Mapping and imaging analysis confirmed biocompatibility and bioactivity of the tested biomaterial without the need for performing animal testing in accordance with the 3R (replacement, reduction, refinement) principle. Moreover, this approach enables detection of structural changes of the organic and inorganic phase of prepared biocomposite *in vitro*.

Conducted research showed that designed biomaterial of ceramic type has a large biomedical potential in regenerative medicine due to its high biocompatibility. Additionally, it was proven that vibrational spectroscopic methods are an effective tool to study the structure of biological samples, reducing the cost of experiments and shorten the time of analysis.

Acknowledgment

Financial was provided by the Foundation for Polish Science within Reintegration grant no. POWROTY/2017-4/14.

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PM.3

Cellular Uptake of Lipoplexes Based on PTAI-11, DOPE and DC-cholesterol in DU145, B16F10 and XC Cell Lines With Different Susceptibility to PTAI-Based Lipofection

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Lipofection is a technique of introducing nucleic acids into eukaryotic cells with chemical carriers - lipids. This method brings great potential for use in research, industry and clinical therapies. PTAI (polyprenyltrimethylammonium iodides) are one of efficient DNA and RNA carriers but mechanism of their cell entry remains unknown.

The aim of the study is to identify endocytosis uptake pathways of PTAI-based lipopexes (complexes of DNA and lipids) in cells showing different susceptibility to PTAI-based lipofection: DU145 (human prostate cancer), B16F10 (mouse melanoma) and XC (Rous sarcoma) cell lines.

It has been proven that lipoplexes are internalized via endocytosis, but the exact mechanism of this process is not known. In order to identify the exact pathways, we have used endocytosis inhibitors. To reduce probability of false positive results, we examined the effect of inhibitors on cell viability and proliferation.

The results show that one of our most effective lipoplexes based on PTAI-11+DOPE+DC-cholesterol are uptaken in DU145 mainly by clathrin-mediated endocytosis, but also via caveolae-mediated pathway. Clathrin-mediated endocytosis and macropinocytosis are dominant for B16 cells, while for XC cells all three: clathrin- and caveolae-mediated mechanisms and micropinocytosis are relevant. Uptake mechanisms differ between cell lines in serum-free (SF) conditions. The results so far show an interesting difference also between SF and serum-supplemented (SS) conditions - tests carried out in the presence of serum showed no significant differences between different cell lines.

These findings bring new information about the mechanisms of PTAI-based lipoplexes uptake and may play an important role in the improvement of effectiveness of PTAI-based lipofection.

PM.4

New *Serratia marcescens* Strains With High Keratinases and Gelatinases Activity Obtained by Directed Evolution and Mutagenesis

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Serratia marcescens in commonly found Gram-negative bacteria with great potential for use in biotechnology as a producer of hydrolytic enzymes: keratinases and gelatinases. These extracellular enzymes can be easily harnessed for improving waste management generated by food industry. Feather generated as a byproduct of poultry industry is mostly composed of keratin, which is very difficult to utilize. Keratinases can potentially solve this problem as they are capable of breaking down this protein. Gelatin hydrolysates also have many uses, and can be obtained, by using gelatinases. However, only bacterial strains with highest activity of these enzymes are ensure commercial success of *Serratia*-based biopreparates.

Through selection and mutagenesis we identified strains of *S. marcescens* with significantly improved activity of lipases, keratinases and gelatinases in crude extracts. Additionally, we developed and improved methods for both quantitative and rapid qualitative analysis of aforementioned enzymes.

Research is funded by grant 6/SPB/2019.

PM.5

Balancing the Composition of Tuff-Based Fertilizer for Aquatic Plants Using *Spirodela polyrhiza* (L.) as a Model

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Duckweed (*Spirodela polyrhiza* L.) is a model aquatic plant. Its rapid growth and high nutrient accumulation raised an attention for industrial biomass production. Tuff is a soft and porous volcanic rock and can be considered as a source of minerals ensuring cheap and rapid growth of duckweed. However, phosphorus and iron bioavailability is dependent on pH. Thus, these microelements may be depleted in highly alkaline tuff-enriched cultures.

Potentiometric titration revealed that tuff-enriched medium exhibited limited buffering properties and allowed simple and stable pH tuning at the stage of medium composition. The effect of initial pH of artificial tuff-based media on the growth and photosynthetic activity of *S. polyrhiza* was investigated in laboratory scale experiment. In particular, pigment composition and activity of Photosystem II and NPQ were monitored through the course of experiment. Finally, we tested the effect of iron supplementation for optimization of tuff-based fertilizer. Change of initial pH had little impact on growth of *Spirodela polyrhiza* L. Both, iron and the inorganic phosphorus improved its growth, proving to be essential for composing the formula of tuff-based fertilizer.

PM.6
Blue Light Induced Activation of Phospholipase C in the Melanopsin Signaling Pathway in HEK293 Cell Line

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Melanopsin is a member of the G-protein coupled receptors family. It is involved in non-image-forming responses to light including circadian rhythm, regulation of sleep, pupil response and other. Although significant efforts research have been devoted to different cell subtypes and their behavioral responses to light activation, signaling cascade involving melanopsin photoactivation is still poorly characterized. In this study, we analyzed the effect of photoactivation of melanopsin and different types of phospholipase C in HEK293 cells in vitro. To determine the optimal condition of blue light exposure, survival of HEK293 cells expressing melanopsin was measured by MTT assay and image analysis of nuclear propidium iodide (PI) fluorescence at 0 hr and 24 hr after the cell irradiation. Real Time PCR measurements were carried out to investigate which type of phospholipase C is responsible for melanopsin activation, which was determined by mRNA level of FOS. The inhibition of PLC, induced by blue light irradiation, was analyzed by measurement of changes in concertation of intracellular calcium ions. Our result showed that only PLCβ1 and PLCβ4 subtypes were activated, by exposure of cells blue light, suggesting that only beta family PLC was involved in melanopsin signaling pathway. It was also observed, that treatment of the cell with PLC inhibitor -U73122, resulted in significant but not complete reduction of intracellular calcium level. Therefore the melanopsin signaling pathway is not limited to phospholipase C, but also another protein could be involved. Phospholipase C plays a significant role in blue light activated melanopsin signaling pathway.

Acknowledgements: Supported by a research grant SYMFONIA 2 2013/08/W/NZ3/00700 from the NSC.

PM.7

Uncovering the Phototoxic Potential of Ambient Particulate Matter – The Molecular Approach

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Smog (contraction of the words smoke and fog) is a type of intense air pollution, a phenomenon affecting an increasing number of people, which is almost impossible to break away from. EU limit values of coarse particulate matter (PM_{10}) were exceeded for over a 100 days in several sampling facilities throughout Małopolskie Voivodeship. According to the WHO 9 out of 10 people breathe air containing high levels of pollutants, levels exceeding WHO guideline limits. These fact provoked scientists all over the world to investigate the impact of suspended matter on human life and health. Most of those studies concentrate on the respiratory, nervous and circulatory system but recently interest has begun to arouse on the impact of particulate matter on skin condition including skin barrier disfunction and skin aging. Interestingly, not a single study has considered the potential role of light in the skin toxicity induced by particulate matter. It should be stressed that despite the low light intensity during winter months, the amount may be sufficient for triggering certain photochemical processes. To understand the role of light in $PM_{2.5}$-mediated skin toxicity we have incubated HaCaT cells with particulate matter samples and irradiate them using solar simulator that gives a unique opportunity to resemble conditions occurring in a life-like situations. We have performed an array of experiments to examine the effect of particulate matter on cell viability and oxidative damage. Our results suggest that photochemical process induced by light irradiation increase toxicity of $PM_{2.5}$ against human keratinocytes.

PM.8

Improvement of Extracellular Lipase Activity From *S. marcescens*, Obtained by Selection and Directed Evolution

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Serratia marcescens in ubiquitous Gram-negative bacteria was identified as capable for producing extracellular lipases. These enzymes can be used as an ingredient for improving washing-powder facilities, waste oils management or biofuel production.

Through selection and directed mutagenesis we identified strains of *S. marcescens* with significantly improved activity of lipases. UV mutagenesis were used for increasing genetic variation and further screening of suitable strains. Plate assays based on reactions with tween-80 and bromothymol blue allowed rapid identification of the most valuable clones. Crude enzymatic extract exhibited at least trifold increase in activity in comparison to non-modified strains. Thus, obtained strains are promising tool for obtaining commercially valuable biopreparates and might be used as one of the ways to produce biofuels.

Research is funded by grant 6/SPB/2019.

PM.9
Simply Method for Seed-Derived Callus Induction From *Nicotiana tabacum*

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Callus is an undifferentiated plant tissue. Similarly like stem cells in animals the callus can transform to other types of tissues, induction of suspension cultures or for generating artificial seeds (useful for commercially important species with naturally low seed production). Thus, callus culture is an important tool in plant production and biotechnology [1, 2]. Callus can be induced by hormonal treatment of explants (usually leaf fragments, nods, roots and shoots) [2]. For many species' explants it was shown that seeds and seedlings can also be used [3-5]. Such approach has many advantages: sterilization of seeds is much easier than other types of explants and callus induction is faster in comparison to other types of tissues. *Nicotiana tabacum* is an important plant used commercially and as a model plant. However, no method for seed-derived callus was developed *N. tabacum*. Here we present a novel and efficient method of induction of *N. tabacum* cv. Samsun seed-derived callus. Our results indicate that combination of BAP and 2,4-D exhibited highest potential for calli formation and was significantly better in comparison to two other methods. [2-3].

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PM.10
Molecular Effect of Mitochondrial–Related Mutation G171H in Cytochrome *b* in *Rhodobacter capsulatus* and Its Influence on ROS Production

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Cytochrome bc_1 plays an essential role in electron transport chain. It takes part in proton motive force formation. Cytochrome *b* subunit of bc_1 complex, being encoded by mitochondrial DNA, is more prone to mutations compared with other subunits encoded by nuclear DNA. Some of these mutations are associated with mitochondrial disorders in humans. In this work we examined the effects of mutation Y155H, which was found in cytochrome *b* in a Prader - Willi Syndrome patient. Using a purple bacterial model we introduced an analogous mutation G171H in *Rhodobacter capsulatus*. To assess the functionality of mutated bc_1 complex we performed biochemical analysis, electron paramagnetic resonance (EPR) experiments and flash–induced electron transfer measurements. The mutated enzyme shows reduced activity at higher pH values, however the K_M, defining the affinity of the enzyme to quinone, is the same for G171H and wild type protein (WT). Interestingly, the kinetics of reactive oxygen species (ROS) formation in G171H mutant significantly differs from WT. For the first few seconds of enzymatic reaction the mutant, unlike WT, displays reduced free radicals production. So far, this phenomenon has not been described in any other mutants of cytochrome bc_1. To explain this observation we perform detailed spectroscopic analysis of this mutant. It is expected to provide further insight into mechanism of ROS production.

PM.11

Application of Fourier Transform Infrared Microspectroscopy for Imaging of Fish Kidney Tissue

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Fourier Transform Infrared Spectroscopy (FT-IR) is emerging approach widely used for studying biological systems that allows qualitative and quantitative analysis of the most important cellular macromolecules, such as proteins, lipids, nucleic acids and carbohydrates. It enables detection not only the chemical structure of compounds, but also assessment of their conformation. FT-IR is, among others, a sensitive method to trace the conformation of proteins and their secondary structure, which makes it useful in monitoring and diagnostics of various diseases. FT-IR imaging of tissue is based on the rule that different chemical bonds within cells or tissue absorb different regions of the mid-infrared, that can be associated with the presence and composition of biomolecules (*e.g.* lipids, proteins, glycogen). Hence, these bands can be applied as biomarkers for the biochemical response of cells and tissues to different pathologies [1-3].

Considering the fact that the pathological changes are associated with changes in biochemical composition, which are specific for tissue, the chemical and structural comparative analysis of healthy and pathologically altered rainbow trout kidney tissue; the latter one was isolated from diseased fish suffering from infection caused by *Aeromonas*, was performed. To determine the effect of bacterial infection on morphology and changes in the profile of biomolecules in both tissues, FT-IR microspectroscopy has been applied. The experimental results can provide valuable insights to explain molecular mechanism involved in the pathogenesis of diseases caused by *Aeromonas* bacteria.

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PM.12

Photoreactive Properties of Melanin Pigments Derived From Keratinous Structures and Its Effect on Viability and Mechanical Properties of HaCaT Cells

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Melanin pigments are produced in a multistage process by specialized cells such as the melanocytes. In the skin, melanin once produced is quickly transferred to neighbouring keratinocytes. Melanin, in particular the brown-black eumelanin is perceived as an effective photoprotective agent against solar radiation due to its ability to absorb and dissipate light in the UV-vis range and scavenge reactive oxygen species. While eumelanin does it very efficiently, the yellow-reddish pheomelanin, on the other hand, seems to be less photoprotective and even photoreactive. This is due to the fact that physicochemical properties of the two types of melanin differ significantly. Recently, it was shown that pheomelanin can generate reactive oxygen species, in particular singlet oxygen much more efficiently than eumelanin . However, most of the studies done so far were made on synthetic models of melanin pigments thus the lack of natural melanins to carry out such experiments leaves many questions unsolved. In this study, we examine photoreactive properties of melanins isolated from hair obtained from donors of different skin phototypes. We demonstrate that melanin from lighter skin generate more singlet oxygen than those from dark skin individuals thus demonstrating photoreactive properties, instead of photoprotective effect. Moreover, the enhanced photoreactivity of melanins derived from Fitzpatrick type I/II skin lead to the modifications of cell cytoskeleton architecture.

Acknowledgements: This work was supported by National Science Center (NCN) grant Sonata-2015/19/D/ST4/01964.

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PM.13

Uncovering the Phototoxic Potential of Ambient Particulate Matter – The Biophysical Approach

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Poland has one of the most polluted air in the European Union. An astonishing 7 million deaths worldwide each year can be attributed to ambient air pollution according to the WHO report and only 10% of world population lives in a city that complies with WHO air quality guidelines. For this reason, numerous studies on the impact of air pollution on human life and health are being conducted, mostly regarding respiratory, circulatory and nervous system, and recently our natural barrier against pathogens, mechanical injuries and adverse effects of solar radiation – skin. Although photochemically active components like benzo[a]pyrene and heavy metals are found in particulate matter samples and their contact with skin cells is inevitable not a single study has taken this factor into account. It should be emphasized that despite a low intensity of sunlight during winter months, the amount might be sufficient for triggering certain photochemical processes. In this study we have characterized photoreactive properties of fine particulate matter ($PM_{2.5}$) collected in Krakow during different seasons of the year. In order to do so we have monitored photoinduced consumption of oxygen using several wavelengths from UV and visible region, trapped and identified free radicals generated by $PM_{2.5}$ samples and examined their kinetics. Furthermore, we have characterized the ability of $PM_{2.5}$ components to photogenerate singlet oxygen ($^1O_2$) – one of the most hazardous oxygen species. It is important to underline, that the oxidative stress caused by the excessive generation of free radicals may lead to skin ageing and are involved in the pathogenesis of skin diseases, such as skin tumours or psoriasis.

PM.14

The Effects of Traumatic Acid on Physiology of Microalgae *Phaeodactylum tricornutum* and *Chlamydomonas reinhardtii*

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Phaeodactylum tricornutum and *Chlamydomonas reinhardtii* are microalgae, highly promising in industrial biotechnology. Due to their high grow rates and biosynthetic capacity, their potential usefulness in the fields of biofuels and recombinant proteins production was suggested [1,2,3]. One of the challenges in their industrial usage is an accumulation of biomass, which may be solved by exogenous application of phytohormones [4,5]. Traumatic acid (TA) is a plant hormone, known for stimulating cell divisions in damaged tissues. TA, tested on microalgae *Chlorella vulgaris*, boosted cell culture density [6,7].

In this study, we tested effects of different TA concentrations on algal growth. Notably, effects on cell number, activity of Photosystem II and chlorophyll content have been investigated.

Research were financed by Complexity Garage, project GZ/01/2019.

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PM.15
Computer Simulation of Biochemical and Chemical Steps in Melanogenesis

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Melanin pigments are synthesized in a multi-stage process known as melanogenesis. They are produced and stored in cell-type-specific, membrane-bound compartments termed melanosomes, which are specialized intracellular organelles located in melanocytes. Most natural melanins are mixtures of eumelanin (black to brown) and pheomelanin (yellow to reddish-brown) in varying ratios. In was experimentally demonstrated that pheomelanin is produced as first, the synthesis of eumelanin starts later, but every granules of natural pigment contains both kinds of melanins [1,2]. The key regulatory enzyme in the synthesis of melanin is tyrosinase, which catalyses multiple steps in melanin biosynthesis, demonstrating the socalled monophenolase and diphenolase activities. In first part of the work, we discuss the full kinetic model of tyrosinase activity, examining the effect of mono- and diphenols concentrations on dopaquinone production [3]. In the second part, the kinetic switching model of eu- and pheomelanin productivity based on classic reaction scheme of melanogenesis known as modified Rapper-Mason scheme is discussed [1,2,3]. All computer simulations were performed for different concentrations of the basic reagents: mono- and diphenoles, enzymes and thiols. Simulations were performed using CellDesigner and COPASI systems. The following conclusions can be drawn from the simulation results: (1) existence of switching mechanism in production of eu- and pheomelanin was confirmed, (2) rate of pheomelanin production is strongly influenced by the concentration of thiols and the initial concentration of tyrosine, (3) the production of eumelanin really starts to dominate in melanin synthesis after the exhaustion of available thiols.

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PM.16

Anticancer Effect of the New Manganese Porphyrin MnTPPS and Sodium Ascorbate Against MCF-7 and AT-2 Cells In Vitro

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Porphyrin are known for their photodynamic effect which is used in phototherapy of tumors. Activated by electromagnetic radiation they can producethe reactive forms of oxygen which could damage cancer cells. It has been shown that generationof ROS is effective way to induce cancer cells death. I this research we used specially projected manganese porphyrin working independently of light and able to generate the ROS in reaction with cytosolic pool of sodium ascorbate. In our hands it lead to internal cell damage followed by cell death. The presence of 5 μM porphyrin in the system reduces the concentration at which ASC becomes toxic and lead to necrosis of cancer cells while normal cells remain relatively untouched. It was observed that MnTPPS - ASC system, cause destabilization of the actin cytoskeleton and damage of the cell membranes (0.5 mM ASC, 5 μM MnTPPS). Moreover, MnTPPs-ASC tandem leadd to changes in mitochondrial membrane potential causing disruption in work of this organellum. In summary: our results confirm the possibility of using the MnTPPS – ASC tandem in cancer treatment.

The present study was financially supported by Research Grant Program at Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University (grant no. MNS 16/2019; N19/MNS/000018 to D.R) and by Polish National Science Centre (UMO-2015/19/D/NZ3/00273 to D.R.).

PM.17

Chicken Chorioallantoic Membrane as a Model for Analyzing the Effect on Angiogenesis in Melanoma and Glioblastoma Multiforme Tumors

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Currently, cancer is recognized as the second most common cause of death in the world. In 2018, it was the cause of 9.6 million deaths in the world (WHO data). The complex and diverse nature of cancer is one of the reasons of using a wide variety research models, highlighting particular features.

Here, we describe our experience of using chorioallantoic membrane (CAM) of a fertilized chicken egg to study tumors of murine and human origin. CAM is a model enabling studying various biological processes, including normal and tumor angiogenesis. It also found application in research on cancer development and biology, tumor metastasis and cancer therapies, with particular emphasis on anti-vascular therapies [1,2].

In our research, we used human glioblastoma multiforme cells and several melanoma lines. Our results demonstrate that the tumor cells form tumors with visible vascularization a few days after implantation. In addition, melanoma cells do not lose their ability to produce pigment.

The formation of blood vessels in tumors and the retention of melanoma cell pigmentation, makes the CAM assay an interesting model in cancer field. Moreover, the CAM model can be used in various studies on cancer progression and cancer therapies.

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PM.18
The Biological Role of MCPIP3 in the Central Nervous System

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MCPIP3 is a member of the MCPIP family of proteins. These proteins are involved in maintaining the homeostasis of the immune system mainly through the degradation of inflammatory transcripts [1,2]. The biological function of MCPIP3 is not fully understood, however, its contribution to the regulation of vascular inflammation has been confirmed [3]. MCPIP3 has been also recognized as a key regulator of the interferon pathway in macrophages [4]. Some genome-wide association studies suggest its involvement in the pathogenesis of Alzheimer's disease [5]. In line with this premise, MCPIP3 is highly expressed in the brain, according to the Human Protein Atlas [6].

Using qPCR we have confirmed the expression of MCPIP3 in different mouse brain structures. To investigate the details of the regulation of the MCPIP3 level in the central nervous system we have employed the U251-MG (human astrocytoma) cell line. Stimulation of these cells with pro-inflammatory factors, such as IL-1β and PMA leads to the elevated expression of the gene encoding MCPIP3 (*ZC3H12C)*. This up-regulation was further confirmed by RNA FISH analysis performed on the MBE (mouse brain capillary endothelium) cells stimulated with IL-1β. We have also shown that MCPIP3 destabilizes its own transcript. Additionally, MCPIP1 and tristetraprolin (TTP) are involved in the MCPIP3 mRNA decay. Using bioinformatics analysis we have identified possible ARE motifs involved in the TTP binding. We have also studied the role of MCPIP3 in the regulation of proinflammatory mRNA's level. The direct interaction of MCPIP3 with some proinflammatory transcripts was further confirmed by RNA immunoprecipitation.

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PM.19
Bacteria in the Game of Life Against Reactive Oxygen Species During PDI

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Application of inorganic semiconductor as heterogeneous photocatalysts, especially TiO_2, in the environmental and biomedical processes has been extensively investigated. However, the antimicrobial application of semiconductor nanoparticles seems to be one of the most promising due to the increased antibiotic resistance and lack of effective treatment modality. Highly-active anatase TiO_2 nanoparticles have been synthesized and the surface modification by porphyrin derivatives was performed followed by acid post-treatment.The photocurrents measurements also confirmed the sensitization of titania particles by porphyrins in the range of visible light, which was with good accordance with theoretical study. Ground state electronic structures and spectra of free-base as well as zinc complexes of porphyrin were calculated with density functional level of theory (DFT). The antimicrobial performance of the prepared nanoparticles was evaluated against a model pathogenic bacterium (gram-negative *E. coli* and Gram-positive *S. aureus*). After a short-term incubation of bacteria with synthetized nanomaterials (1 g/L) and irradiation with blue-light (420±20 nm) at dose of 10 J/cm^2, 3-4 logs of *Escherichia coli* and 2-3 logs of *Staphylococcus aureu*s had been inactivated. Further decrease in bacteria viability was observed after PDI potentiation with H_2O_2 or KI addition and results in completed microorganisms eradication after use of lower material concentration (0.1 g/L). Moreover, the SEM examination of bacteria death after each mode of PDI showed that there were different mechanisms of cellular disruption in dependence of generated oxidative stress.

PM.20
Structure Elucidation of Enkephalin Analogs From NMR Derived Restraints

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Enkephalins are natural penta-peptides discovered by Hughes and Kosterlitz in 1974. They are generated from proenkephalin via proteolytic cleavage. Preproenkephalin precursor is subject to complex cleavages and post-translational modifications resulting in enkephalins synthesis. Two different forms of enkephalins are known up-to-date. These forms are Leu-Enkephalins (Tyr-Gly-Gly-Phe-Leu) and Met-Enkephalins (Tyr-Gly-Gly-Phe-Met). These are endogenous penta-peptides which exhibit morphine-like properties. Different strategies have been used to improve the properties of the peptides to make them more amenable as therapeutics, such as cyclization or incorporation of unnatural amino acids within the peptide sequence.

The enkephalin series we studied was cyclized through aromatic rings. The structures of modified enkephalins were calculated with XPLOR basing on through-space restraints obtained from two-dimensional NMR spectra analysis. The elucidated structures of the modified enkephalins are part of structure-activity relationship studies. Our goal is to understand what structural features govern the opioid peptide selectivity and the activity towards a particular opioid receptor. The end goal of these studies is to help in designing new, better drugs.

PM.21
Yeastie Boostie – Making Diatoms Make More Goodie

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Phaeodactylum tricornutum diatoms are known to produce pigments with anti-cancer and other beneficial properties. Among them, fucoxanthin and diadinoxanthin are the most well-studied ones. Finding new ways of increasing production yields of those pigments is an interesting and important area of applicative science.

In this study, we tested if the addition of spent yeast and hops sludge recovered from the beer-making process could increase the production of beneficial pigments by *P. tricornutum* cells. The control diatom cultures were incubated in standard f/2 medium. The spent beer-making sludge was obtained after the final fermentation of beer with US-05 yeasts. We tested the yields of fucoxanthin, diadinoxanthin and diatoxanthin pigments produced by diatoms' cells in control cultures and in cultures containing either 1 or 2 grams of freeze-dried spent sludge per 1 liter of diatom culture.

We observed that after one week of growth, the number of diatom cells in cultures with sludge additions was the same or higher than in control cultures. Furthermore, the amount of fucoxanthin produced per diatom cell was significantly higher in the cases of both sludge concentrations tested. Additionally, the amount of produced diadinoxanthin increased when cells were incubated with the lower concentration of yeast sludge added to the cell culture. In contrast to that, the amount of produced diatoxanthin was lower in both cases of yeast additions.

This study supports the notion that adding spent beer-fermentation sludge to *P. tricornutum* cultures can greatly increase the production of beneficial pigments in the diatoms' cells.

PM.22

Let Your Column Live a Long and Happy Life – Using Porous Graphitized Carbon for Ascorbic Acid Analysis

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Vitamin C (L-ascorbic acid) is essential for all living organisms. It plays a vital role as a cofactor for many enzymes (*e.g.* collagen biosynthesis, iron metabolism, neutralising excess hydrogen peroxide), an antioxidant, and an essential part of the immune system. This led to development of multiple chromatographic methods for quantification of the ascorbic acid and its oxidised form – the dehydroascorbic acid. Most of these methods use octadecyl silica as the stationary phase, coupled with an acidic mobile phase. The main drawback of this type of setup is the accelerated column aging due to the hydrolysis of the stationary phase.

We have addressed this problem by developing a novel chromatographic method using porous graphitized carbon as the stationary phase. Due to their chemistry, such stationary phases are chemically resistant and even highly acidic mobile phases may be employed. We propose a simple extraction procedure requiring between 100 and 600 mg of the sample followed by a 15 min chromatographic gradient run with spectrophotometric detection at 245 nm. Our method was tested on multiple plant samples yielding satisfactory sensitivity and repeatability.

The proposed method performs as well as previously described chromatographic methods, with additional robustness and longer column lifespan, which leads to reduced analysis costs per sample. We hope it can be applied not only in the laboratory setup but also in various industrial branches, *e.g.* the food or pharmaceutical industry.

PM.23

New Formyl Peptide Receptor 2 Agonist Exerts the Neuroprotective Effect on the LPS-induced Cell Death in Microglia Cells

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INTRODUCTION: Neuroinflammation is a complex multicellular process that plays an important role in a variety of neuropsychiatric disorders. Recently the role of Formyl Peptide Receptor-2 (FPR2) a seven transmembrane G protein-coupled receptor, as important target in brain processes modulation has been suggested. Especially the role of its agonists in the neuroprotection and resolution of inflammation is strongly underlined. In line, an increasing number of synthesized compounds targeting FPRs, especially FPR2 agonists, have been disclosed in patents.

AIM: The aim of this study was to determine, whether the new agonist with ureidopropanamide scaffold (CMC23) is able to modulate the cell death processes evoked by lipopolysaccharide (LPS) treatment in primary microglia cells.

METHODS: Primary microglia cultures were prepared from cortices of 1-2 days old rat offspring. Microglia cells were pre-treated with CMC23 (0,1-10 μM) and next exposed to nonspecific immune system activator – lipopolysaccharide (LPS, 100 ng/ml). After 24 h of incubation, cell death was determined by lactate dehydrogenase release (LDH test).

RESULTS: Pre-treatment of FPR2 receptor agonist – CMC23 (0,1-10 μM) dose-dependent suppressed the LPS-evoked LDH release after 24h of incubation.

CONCLUSION: We found that CMC23 exerts neuroprotective effect in LPS-activated microglia. Furthermore it may be postulated that FPR2 play a role as target for endogenous pro-resolving pathways following inflammatory activation.

Supported by the grant no. 2017/26/M/NZ7/01048, National Science Centre, Poland

PM.24

Combined Chemotherapy With Hyperthermia and Calcitriol in the Human and Mouse Cell Model of Pancreatic Cancer

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Combined therapies are currently being developed to support the use of chemotherapy drugs. An interesting option seems to be the combination of gemcitabine (popular chemotherapeutic against pancreatic cancer) with hyperthermia and calcitriol (active form of vitamin D_3). Gemcytabine is a strong and specific analogue of deoxycytidine and is affecting the DNA synthesis process [1]. Mild Hyperthermia (<42°C) is a type of targeted therapy. High temperature can modify the cell membrane and support drug delivery or lead to cell death [2]. Calcitriol, has a wide range of effects related to cell proliferation, differentiation and apoptosis, oncogenesis and regulation of angiogenesis [3].

The aim of the study was to investigate the combined effects of hyperthermia and the active form of vitamin D_3 with chemotherapy against mouse and human pancreatic cancer cells.

Human (PANC-1) and murine (Panc02) pancreatic cancer cells were heated to 41 ° C for 20 minutes and treated with gemcitabine (0.001 - 1000 μM) and calcitriol dose of 100 nM. Cells survival and proliferation was evaluated with counting cells using a hemocytometer and MTT test. Apoptosis and necrosis was examined by flow cytometry.

The results showed the use of three treatment components most strongly reduces the number of cancer cells. In conclusion, our results suggest that the combination of hyperthermia and calcitriol with gemcitabine can lead to better anti-cancer effects than using the drug alone.

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PM.25
Glycofullerenes in Pancreatic Cancer Research *In Vitro*

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Carbon nanomaterials have been gaining its popularity in the context of medical application for several years, therefore researchers are highly interested in exploring their biological properties. One of the most promising compounds for oncological purposes is a highly water-soluble glucosamine derivative of [60]fullerene (**GF**), for which first successful research results had been published [1]. Following thesis covers the influence of **GF** on pancreatic cancer cells PANC-1. This reaserch describes PANC-1 cells proliferation, migration and metabolic activity after incubation with different concentration of [60]fullerene derivative. The final results depict the influence of glycofullerene (**GF)** on various cancer cellular events and potential application of fullerene nanomaterials against carcinogenesis of PANC-1 cells.

Acknowledgments:

M Serda thanks National Science Center (Poland) for the support (grant number UMO-2016/23/D/NZ7/00912). M Krzykawska-Serda thanks National Science Center (Poland) for the support (grant number UMO-2018/29/B/NZ5/02954).

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PM.26

The TGF-β_1-Induced Predisposition of 3D *In Vitro* Cultured Asthma-Derived Human Bronchial Fibroblasts to Myofibroblasts Transition is Enhanced in Comparison to Hbfs Derived From non-Asthmatics

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In vitro cell cultures are widely used in science. Although their usefulness is invaluable, they have many limitations. In recent years, a growing branch of *in vitro* research has been studied in 3D systems which more closely reflect *in vivo* conditions. For several years we have been using an *in vitro* model of human bronchial fibroblasts (HBFs) obtained from bronchoscopy sections from patients with asthma (AS) and from patients in whom asthma was excluded (NA)[1]. Numerous experiments indicate inherent features of HBFs AS favouring their fibroblast-to-myofibroblast transition (FMT) in '2D' cultures[2]. As HBFs interact with extracellular matrix proteins in the connective tissue, we have recently decided to expand our '2D model' to include the *in vitro* cell cultures in 3D using commercially available collagen gels. Our preliminary studies showed that 1.5 mg/ml concentration of collagen is suitable for the HBFs growth. Next, we compared fibrosis-related genes in TGF-β_1-activated HBFs from the AS and NA groups in the '2D' and 3D model. HBFs AS maintained an increased expression of pro-fibrotic genes (ACTA2, FN, COL1A1, SERPINE1) vs HBFs NA in the 3D conditions similarly to the '2D' ones. It is probably related to the TGF-β/Smad2/3 profibrotic pathway intensification (observed increased nuclear translocation of p-Smad2/3 proteins). Moreover, the extracellular matrix contractility is higher in HBFs AS. In summary, our results indicated an enhanced potential of the FMT in HBF AS populations in the 3D cultures similarly to the '2D' conditions.

Supported by the Polish National Science Centre (PNSC) grant (2015/17/B/NZ3/02248) to MM. DW obtained financial resources from the ETIUDA scholarship (2019/32/T/NZ3/00405); PNSC.

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PM.27

Condensed Phase Properties of N-Pentadecane, as Emerging From the Application of Biomolecular Force Fields

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The OPLS-AA force field is successfully applied in Molecular Dynamics (MD) simulations of carbohydrates, proteins and nucleic acids [1]. While it provides satisfactory reproduction of experimental properties, for decane and shorter alkanes, it fails for the longer hydrocarbons and thus also for phospholipids common in membranes. There were several attempts to refine it, i.e. altering partial charges of H and C atoms in hydrocarbon chains to reproduce dipole moment (OPLS-QQ) of C-H bond and reparameterization of selected torsional potentials (OPLS-MP).

In the presented work we compared reproduction of various condensed phase properties by six sets of parameters applicable for simulations of phospholipid bilayers: the original OPLS-AA; OPLS-QQ with alternate point charges for C and H atoms of aliphatic chains ; OPLS-MP with selected torsion potentials refitted to reproduce the experimentally determined melting point (OPLS-MP) of n-pentadecane; and other force fields, including: CHARMM36 [2], Stockholm Lipids (Slipids) - originally developed for phospholipids [3], Berger Lipids - widely used and developed on the basis of OPLS -united atom (OPS-UA) and GROMOS set of parameters [4].

Acknowledgments:

This research project is supported by grant No 2015/17/D /ST4/00555 (National Science Center) and in part by PL-Grid Infrastructure.

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PM.28

Comparison of Phenotypic and Genetic Methods in Species Identification of Clinically Relevant Bacteria From *Streptococcus* and *Enterococcus* Genera

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Bacteria from *Streptococcus* and *Enterococcus* are clinically relevant opportunistic pathogens. In humans, diseases associated with streptococci occur chiefly in the respiratory tract, bloodstream or as skin infections. Enterococci are a common cause of bacteremia and infective endocarditis. Bacterial identification is difficult due to the high genetic and taxonomic similarities and several reclassifications within genus. The development of molecular biology techniques has made it possible to introduce a rapid and reliable diagnostics of the infections caused by these microorganisms.

The aim of the study was to identify *Streptococcus* and *Enterococcus* at the species level using phenotypic methods both with antibiotic susceptibility testing and genetic ones.

Methods used in routine microbiological diagnostics allowed the identification of 20 isolates of clinical origin, 15 of the genus *Streptococcus* and 5 *Enterococcus,* 3 non streptococci species have been additionally identified. For precise identification and determination of the most differentiating method, the Sanger sequencing of the *16S rRNA*, *sodA*, *tuf* and *rpoB* genes was used. The *rpoB* gene sequencing had the highest discriminatory power both for the streptococci and enterococci and allowed for species identification of 19 streptococci, all enterococci and 3 non streptococci species.

The obtained results indicate that gene sequence analysis is more reliable than phenotypic tests used in routine diagnostic, but a compilation of several methods and molecular markers have to be used to unambiguously confirm species identification results.

The investigations were created as a result of research project no. UMO-2016/21/N/NZ6/00981 (for M. K. S.) financed by the National Science Centre Poland.

PM.29

Analysis of Expression of Selected Genes in Response to Iron Depletion in Cyanobacterium *Synechocystis* sp. PCC 6803

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Cyanobacteria are small, green, usually unicellular bacteria, which have an ability to produce organic compounds in the process of oxygenic photosynthesis. Their photosynthetic complexes are localized in thylakoid membranes and are closely related to plants' photosynthetic machinery. The cyanobacterium *Synechocystis* sp. PCC 6803 became the first photosynthetic organism which genome was fully sequenced. Nowadays these organisms are one of the most important model objects in experimental biology.

The goal of our study was to examine how iron replete and iron deplete conditions affect expression of selected genes encoding proteins involved in iron metabolism and photosynthesis in *Synechocystis* PCC 6803. For this purpose we used wild type (WT) strain and a deletion mutant *hhoA*$^{-}$, lacking serine protease HhoA. Bacteria were grown in a medium containing normal levels of iron and a medium supplemented with deferoxamine B (DFB) which chelates Fe ions. Real-time quantitative PCR was used to analyze transcript levels of selected genes. Results will be presented and discussed with focus on cellular iron metabolism in cyanobacterial cells.

PM.30
Chemical and Photochemical Stability of Beta-Carotene Endoperoxides

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Carotenoids are natural pigments found in plants, bacteria, fungi, cyanobacteria and algae. They have excellent ability to quench singlet oxygen and triplet states of chlorophylls and function as natural photoprotectors. The quenching of singlet oxygen may occur physically, during which the energy is dissipated in the form of heat, or chemically, when the structure of the carotenoid changes. Such dual features are shown by β-carotene, which has anti-oxidative and pro-oxidative properties. All-trans β-carotene readily reacts with oxygen, yielding a series of mono- and diendoperoxides, which are likely to be the major cause for pro-oxidant activity of carotenoids [1, 2]. The aim of the project was to study the formation of endoperoxides of β-carotene and determine their stability and reactivity under various conditions. The endoperoxides of β-carotene were synthetised photochemically and purified by high performance liquid chromatography. 5,8-endoperoxide of β-carotene was used as a representative compound. Its stability and photostability was investigated in various solvents, in methanol, ethanol, toulen, acetone and chloroform. Also micellar medium were used and the endoperoxide was placed in several detergents: LDAO, β-OG and Triton X-100 in 20 mM Tris buffer. The effect of environment acidity was studied in buffers at different pH values. High performance liquid chromatography was used to monitor stages of synthesis and degradation of the endoperoxide.

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PM.31

Two Pathways of Energy Dissipated by Heat in Plant Photosystem

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Plant photosynthesis operates based on the cooperation of two photosystems, with the reaction centers characterized by light absorption at 680 nm (Photosystem II) and 700 nm (Photosystem I). Efficient photosynthesis is possible thanks to activity of antenna pigment-protein complexes absorbing photons and transferring excitation energy towards the reaction centers. The major light-harvesting pigment–protein complex of plants, LHCII, is the most abundant membrane protein, comprising more than half of the chlorophyll pool in the biosphere [1,2]. We show [3] that spontaneous formation of supramolecular structures by LHCII, the largest antenna of plants, gives rise to two energetically coupled states characterized by the fluorescence emission close to 680 nm (E680) and to 700 nm (E700). The results of the spectroscopic experiments reveal operation of the thermally-driven up-conversion of E700 to E680, both in isolated LHCII and in *Arabidopsis thaliana* leaves. This mechanism appears essential for excitation balance between two photosystems.

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