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#ISFMS2022

the 4th ISFMS—Biochemistry, Molecular Biology and Druggability of Proteins

University of Florence
Florence, Italy
06 – 09 September 2022

Conference Chairs
Prof. Dr. Claudiu T. Supuran
Prof. Dr. Paola Gratteri
Prof. Dr. Silvia Selleri
Prof. Dr. Clemente Capasso

Conference Committee
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Organised by

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4th ISFMS—Biochemistry, Molecular Biology and Druggability of Proteins
06 – 09 September 2022, Florence, Italy

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Tuesday 06 September 2022: 14:30 – 19:00

Wednesday 07 September 2022: 09:00 - 13:30 / 15:00 - 18:45

Thursday 08 September 2022: 09:00 - 13:30/ 15:00 – 18:45/

Conference Dinner: 20:30

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Day 1 - Tuesday, 6 September 2022, 14:30 - 19:00 (CEST)

14:30 - 15:30	Registration Desk Open (Check-in)
15:30 - 15:45	Greetings from Prof. Debora Berti, Prorector for research, University of Florence
15:45 - 16:00	Welcome and chairing from Prof. Claudiu T. Supuran, chair of the conference
16:00 - 17:00	**Keynote talk : Shoukat Dedhar** *"Targeting the Tumour Microenvironment"*
17:15 - 19:00	Welcome cocktail

Day 2 - Wednesday, 7 September 2022, 09:00 - 18:45 (CEST)

	Session I. Chaired by Dr. Clemente Capasso
09:00 - 10:00	***Keynote Talk :*** **W. Alexander Donald** *" Multiplexed screening of thousands of natural products for protein binding in native mass spectrometry"*
10:00 - 10:30	**Daniel Lietha** *"Structure and force activation of adhesion signalling"*
	10:30 - 11:00 – Coffee Break
	Session I. Chaired by Dr. Clemente Capasso
11:00 - 11:30	**Catherine Michaux** *"Revealing intrinsic disorder and aggregation properties of the DPF3 zinc finger proteins"*
11:30 - 12:00	**Felix Nagel** *"Biophysical and Structural Insights into a Pancreatitis Associated Kazal Inhibitor"*
12:00 - 12:30	**Mietha Van der Walt** *"Application of reverse pharmacophore mapping to search for protein targets of istradefylline – a drug repurposing perspective"*
12:30 - 13:00	**Spyridon Gourdoupis** *"FEN1 as a target for confining oncogenesis"*
13:00 - 13:30	**Filippo Favretto** *"Functional and Structural Characterization of two Cyclophilins from the parasite Toxoplasma gondii"*
	13:30 - 15:00 – Lunch
	Session II. Chaired by Prof. Dr. Paola Gratteri and Prof. Dr. Claudiu T. Supuran
15:00 - 16:00	***Keynote Talk*** **: Giuseppina De Simone** *"Biochemical and structural studies on human carbonic anhydrase IX: a promising therapeutic target for cancer treatment"*
16:00 - 16:30	**Anne Susemihl** *"BCL11B CCHC Zinc Finger Domain and Its Potential for Cancer Therapy"*
16:30 - 17:00	**Aida Serra** *"Industry by-product EVs (BP-EVs) as a novel compound delivery platform for protein target within the Central Nervous System"*
	17:00 - 17:30 – Coffee Break
17:30 - 18:00	**Elena Krachmarova** *"Effect of SARS-CoV-2 ORF6 protein on DNA replication in eukaryotic cells"*
18:00 - 18:45	***Poster Session 1***

Day 3 – Thursday 8 September 2022, 09:00 - 18:45 (CEST)

	Session III. Chaired by Prof. Dr. Silvia Selleri and Dr. Clemente Capasso
09:00 - 10:00	**Keynote Talk : Binghe Wang** *"Examining the Therapeutic Effects of Carbon Monoxide: Seeing both the Forest and the Trees in Understanding Its Hemoprotein Targets"*
10:00 - 10:30	**Jiri Cerven** *"Targetting Topoisomerase IIa: back to the drawing board via Z-DNA"*
	10:30 – 11:00 – Coffee break
11:00 - 12:00	**Keynote Talk : Marianna Patrauchan** *"Molecular pillars of calcium signaling in a bacterial pathogen: Pseudomonas aeruginosa"*
12:00 - 12:30	**Patrycja Gralewska** *"ATRi and CHK1i significantly increased chromosomal abnormalities caused by olaparib in HGSOC BRCAMUT and BRCAWT ovarian cancer cell lines"*
12:30 - 13:00	**Katarzyna Walczewska-Szewc** *"How can interaction with prolyl oligopeptidase possibly trigger α-synuclein aggregation?"*
13:00 - 13:30	**Marco Pedretti** *"Functional characterization of cystathionine gamma lyase from Pseudomonas aeruginosa"*
	13:30 – 15:00 – Lunch
	Session IV. Chaired by Prof. Dr. Giuseppina De Simone
15:00 - 16:00	**Keynote Talk : Jean-Yves Winum** *" Innovative approaches targeting carbonic anhydrases: an update "*
16:00 - 16:30	**Gyula Batta** *" DMSO Induced Unfolding of the Antifungal Disulfide Protein PAF: a combined NMR and DSC study "*
16:30 - 17:00	**Leandar Litov** *" Human interferon gamma is a potential inhibitor of SARS-Cov-2 Orf6 activity "*
	17:00 – 17:30 – Coffee break
	Session IV. Chaired by Prof. Dr. Giuseppina De Simone
17:30 - 18:00	**Massimiliano Gasparrini** *"Human extracellular NAMPT and NAPRT mediate inflammatory response through TLR4-binding: molecular insights into the mechanism of interaction"*
18:00 - 18:45	***Poster Session 2***
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Day 4 – Friday 9 September 2022, 09:00 - 16:15 (CEST)

	Session V. Chaired by Prof. Dr. Jean-Yves WINUM
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10:00 - 10:15	**Arianna Giacomini** *"Inhibition of Carbonic Anhydrase IX induces mitochondrial oxidative stress and apoptosis in multiple myeloma"*
10:15 - 10:45	**Carsten Zeilinger** *"Strategies to display susceptibility of targets for compounds"*
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	Session V. Chaired by Prof. Dr. Jean-Yves Winum
11:15 - 12:15	**Keynote Talk : Lukasz Jaremko** *"Biological motions, gene regulation and drugs "*
12:15 - 12:45	**Barbara Jachimska** *"Fibrinogen corona on the dendrimer nanoparticles?"*
12:45 - 13:15	**Olga Alekseeva** *"Variation in viral tropism belongs to IDPR positions in structural proteins of enterovirus"*
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	Session IV. Chaired by Prof. Dr. Claudiu T. Supuran, Prof. Dr. Paola Gratteri, Prof. Dr. Silvia Selleri and Dr. Clemente Capasso
14:45 - 15:45	**Keynote Talk : Vladimir Uversky** *"Protein structure-function continuum: On the roles of intrinsic disorder in protein functionality"*
	15:45 – 16:15 Awards Ceremony & Closing Remarks

Certificate of Attendance

Participants of the event will be able to download an electronic Certificate of Attendance by accessing their dashboards on Sciforum.net once the event is concluded. The certificates will be found under "My Certificates" category.

Welcome from the chairs

Dear Colleagues,

We would like to invite you to The 4th ISFMS: Biochemistry, Molecular Biology and Druggability of Proteins, which will be held in the wonderful city of Florence, Italy, in the period September 6–9, 2022.

The conference will be hosted at the University of Florence, in the biomedical campus nearby the main city hospital, which is easily accessible from the airport, train station or city center. Apart the high quality science which will be presented during the event, our fantastic location will afford the possibility to visit the city in which Leonardo da Vinci, Michelangelo and Galileo Galilei (to mention just a few personalities) lived and created some of their unique artistic and scientific masterpieces. The friendly and peaceful atmosphere of our city will surely favor nice scientific interactions and the possibility to establish interesting contacts with scientists from all over the world in a collaborative manner.

We look forward to seeing you in Florence!

Prof. Dr. Claudiu T. Supuran **Conference Chair**
University of Florence, Italy

Prof. Dr. Paola Gratteri
Conference Chair
University of Florence, Italy

Prof. Dr. Silvia Selleri
Conference Chair
University of Florence, Italy

Dr. Clemente Capasso
Conference Chair
National Research Council, Italy

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Sciforum is an event planning platform that supports open science by offering the opportunity to host and participate in academic conferences. It provides an environment for scholarly exchange, discussion of topics of current interest, building of networks and establishing collaborations.

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4th ISFMS: Biochemistry, Molecular Biology and Druggability of Proteins will be held at University of Florence, Florence, Italy, on 06 – 09 September 2022.

This event will bring together leading researchers at the cutting edge of peptide and protein science, from around the world and across the broad field of peptides and proteins, to share the results of their recent studies. Meeting participants will have the opportunity to present posters and short talks on their work and discuss their research in a stimulating and collegial environment.

Conference Venue

University of Florence
Viale G. Morgagni, Piazza S.Marco, 4 - 50121 Florence, Italy

Registration Desk

The desk for registration, information and distribution of documents will be open from 14:30 on 06 September 2022.

Use of Masks in University of Florence

Masks should be worn in the venue facilities during the talks and when moving across common areas. You may take them off during the coffee or lunch breaks, and when going outside the building. Thank you for your collaboration!

Disclaimer

Delegates will receive a name-badge at the Information Desk, upon registration. The badge must be worn prominently in order to gain access to the congress area during all scientific and social events. Admission will be refused to anyone not in possession of an appropriate badge.

Insurance

The organizers do not accept liability for personal accident, loss, or damage to private property incurred as a result of participation in the *4th ISFMS: Biochemistry, Molecular Biology and Druggability of Proteins*. Delegates are advised to arrange appropriate insurance to cover travel, cancellation costs, medical, and theft or damage of belongings.

Florence and Italy

Florence is a city in central Italy and the capital of the Tuscany region. During the Middle Ages, Florence was a centre of European trade and finance, and one of the wealthiest cities of that era.

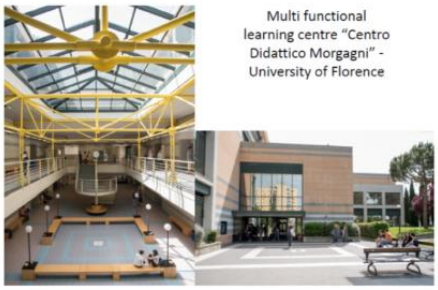
The city attracts millions of tourists each year, and UNESCO declared the Historic Center of Florence a World Heritage Site in 1982. The city is noted for its culture, Renaissance art and architecture and monuments. The city also boasts numerous museums and art galleries, such as the Uffizi Gallery and the Palazzo Pitti, and still influences the fields of art, culture and politics to this day. Due to Florence's artistic and architectural heritage, Forbes has ranked it as one of the most beautiful cities in the world.

For more information visit http://www.firenzeturismo.it/en/.

University of Florence

The University of Florence is an Italian public research university located in Florence, Italy. It comprises 12 schools and has about 60,000 students enrolled. The first university in Florence was the Studium Generale, which was established by the Florentine Republic in 1321. The Studium was recognized by Pope Clement VI in 1349 and authorized to grant regular degrees. The Pope also established that the first Italian faculty of theology would be in Florence. The Studium became an imperial university in 1364 but was moved to Pisa in 1473 when Lorenzo the Magnificent gained control of Florence. Charles VIII moved it back from 1497 to 1515, but it was moved to Pisa again when the Medici family returned to power.

The modern university dates from 1859, when a group of disparate higher-studies institutions grouped together in the Istituto di Studi Pratici e di Perfezionamento, which a year later was recognized as a full-fledged university by the government of newly unified Italy. In 1923, the Istituto was officially denominated as University by the Italian Parliament.



Multi functional learning centre "Centro Didattico Morgagni" - University of Florence

The Grand Hall of the Chancellor's Office of the University of Florence

How to Reach the Venue

Florence may be reached directly by plane. The airport has connections with many of the main transport hubs in Europe (Rome, Milan, Paris, Frankfurt, Munich, Zurich) and a tram service connects the airport to the city centre. By train, it is a 90-minute trip from Rome or Milan where the largest airports in Italy are located. Pisa airport is just one hour from Florence by train and there are many flights into this city too.

The biomedical campus is 15 min by tram (T1) from the main train station which is in the city centre. Florence is a relatively small city (around 300 000 residents) and the public transportation (trams, trains, buses) is efficient and inexpensive.

Venue: Biomedical campus, University of Florence

Address: Viale G. Morgagni, Piazza S.Marco, 4 - 50121 Florence, Italy

Keynote speakers

Prof. Dr. Shoukat Dedhar
Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, Canada

Prof. Dr. Binghe Wang
Department of Chemistry, and Center for Diagnostics and Therapeutic, Georgia State University, Atlanta, GA, USA

Prof. Dr. Marianna A. Patrauchan
Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK, USA

Prof. Dr. William Donald
Dipartimento di Farmacia e Biotecnologie, Università di Bologna, Italy

Prof. Dr. Jean-Yves Winum
IBMM - Pôle Chimie Balard Recherche, University of Montpellier, Montpellier, France

Prof. Dr. Vladimir N. Uversky
Department of Molecular Medicine, Morsani College of Medicine, University of South Florida, Tampa, USA

Prof. Dr. Giuseppina De Simone
Biostructure and Bioimaging Institute-CNR, Naples, Italy

Prof. Dr. Daniel P. Flaherty
Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, USA

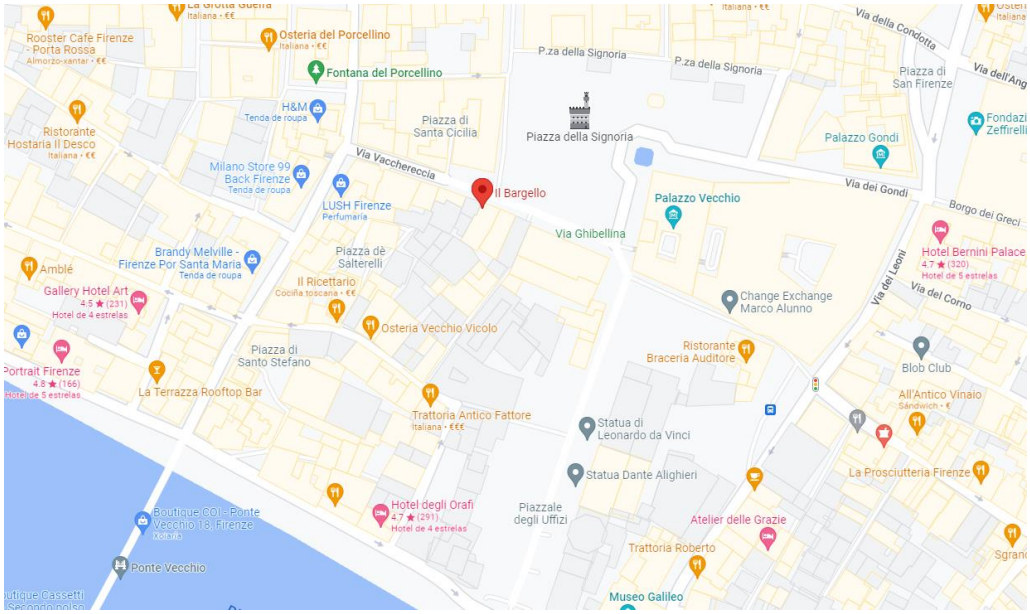
Dr. Łukasz Jaremko
Molecular Diagnostics and Drug Discovery (MD3) Group, Red Sea Research Center (RSRC), King Abdullah, University of Science and Technology (KAUST), Kingdom of Saudi Arabia

Social Events

Conference Dinner

Thursday 08 September, 20:30
Price: 50€ *Tickets must be purchased in advance.*

The Conference Social Dinner will take place on 8 September at 20:30h at Ristorante IL Bargello. Address: Piazza della Signoria 4/red 50122 Firenze FL



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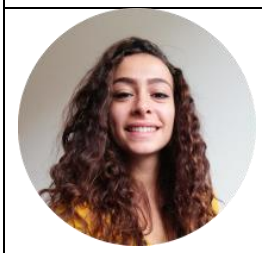
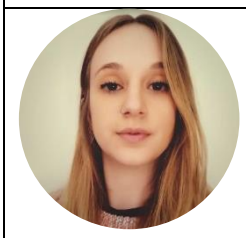
	**Sara Ottolini** Email: sara.ottolini@mdpi.com
	**Carmen Ruiz** Email: carmen.ruiz@mdpi.com

Targeting the Tumor Microenvironment

Shoukat Dedhar

BC Cancer Research Institute, Vancouver, BC, Canada

The “druggability” of proteins depends on extensive understanding of not only the structure of the protein, but also its role in cellular physiology and mechanism of action.

Our research interests are in understanding the role of the tumor microenvironment (TME) in the progression of cancer. The TME is a complex and highly heterogeneous component of tumors, consisting of blood vessels, fibroblasts and extracellular matrix, and different types of immune cells that include neutrophils, macrophages and T and B cells.

A key component of the TME of solid tumors is a poorly developed vasculature leading to a decrease in the oxygen tension (hypoxia), in various parts of the tumor. Tumor cells within the hypoxic niche of solid tumors thus need to adapt in order to survive. Hypoxia triggers the stabilization of the transcription factors, HIF-1/2, which are normally degraded within the cytoplasm under ambient oxygen tension (20%). HIF -1 regulates the expression of a large number of genes, of which genes that regulate cellular metabolism and pH are critical for the metabolic “re-wiring” of tumor cells to promote survival.

Some of the strongest HIF-1 responsive genes encode Carbonic Anhydrases IX and XII proteins, both of which are cell surface integral plasma membrane proteins, which play a critical role in regulating the acidic pH generated due to the inhibition of oxidative phosphorylation and a switch to glycolysis due to a build-up of lactic acid. CAIX/XII catalyze the conversion of extracellular carbon dioxide to bicarbonate, which couples with cell surface bicarbonate transporters to internalize the bicarbonate and neutralize the acidic pH. A consequence of CAIX/XII activity is the generation of extracellular protons, which contribute to an increasingly acidic extracellular pH, which, besides promoting cell migration and invasion, is also highly immunosuppressive , and is detrimental for immunotherapeutic approaches.

Multiplexed Screening of Thousands of Natural Products for Protein-Ligand Binding in Native Mass Spectrometry

William A. Donald

School of Chemistry, University of New South Wales, Sydney, Australia

The structural diversity of natural products offers unique opportunities for drug discovery, but challenges associated with their isolation and screening can hinder the identification of drug-like molecules from complex natural product extracts. In this talk, I will report a multistage, high-resolution native mass spectrometry approach to rapidly identify natural products that bind to therapeutically relevant protein targets. By directly screening crude natural product extracts containing thousands of drug-like small molecules using a single, rapid measurement, we could identify novel natural product ligands of human drug targets without fractionation. This method should significantly increase the efficiency of target-based natural product drug discovery workflows.

References:

JL Bennett, GTH Nguyen, WA Donald, Protein–Small Molecule Interactions in Native Mass Spectrometry, Chem. Rev., 2022, 122, 7327-7385.
GTH Nguyen, JL Bennett, S Liu, SE Hancock, DL Winter, DJ Glove, WA Donald, Multiplexed Screening of Thousands of Natural Products for Protein–Ligand Binding in Native Mass Spectrometry, J. Am. Chem. Soc., 2021, 143, 21379-21387.
GTH Nguyen, TN Tran, MN Podgorski, SG Bell, CT Supuran, WA Donald, Nanoscale ion emitters in native mass spectrometry for measuring ligand–protein binding affinities, ACS Cent. Sci., 2019, 5, 308-318.

Structure and force activation of adhesion signalling

Daniel Lietha

Margarita Salas Center for Biological Research, Spanish National Research Council (CIB-CSIC)

The Focal Adhesion (FA) complex plays a key role in mesenchymal cell motility and during tumour progression provides a means for cancer cells to invade neighbouring tissues and initiate metastasis. FAs display a layered architecture and initially assemble under low force on the cytoplasmic side of the plasma membrane, but are then force activated by contractile actomyosin fibres attached to the complex to trigger FA signals. Focal Adhesion kinase (FAK) is a key signalling component in FAs that forms the first layer closest to the membrane in FAs. It is thought to be the key force sensor that converts the mechanical force in FAs into a biochemical signal. Both, the molecular architecture and mechanism of force activation of FA signalling is currently unknown.

Using high resolution cryo-electron microscopy we obtained structural information at atomic resolution of the first layer in FAs comprised by FAK oligomers bound to the lipid membrane. This reveals large conformational changes of FAK upon membrane binding that trigger the assembly of FAK oligomers. This oligomeric membrane bound state represents a primed, but not yet activated state of FAK and we propose that forces in FAs will trigger activation of FAK by opening the structure and exposing the active site. To test this hypothesis we employed single molecule atomic force microscopy, which enabled us to demonstrate that FAK can be mechanoactivated in the low pico newton range - the force range occurring natively in FAs – well below forces that would result in domain unfolding.

In conclusion, our study for the first time provides details on the structural arrangement of the first layer in FAs formed by FAK oligomers and how mechanical force in FAs triggers biochemical signals to promote cell motility and tumour invasion.

Revealing intrinsic disorder and aggregation properties of the DPF3 zinc finger proteins

Julien Mignon[1], Denis Mottet[2], Vladimir Uversky[3], Eric A Perpète[1], Catherine Michaux[1]

[1] University of Namur
[2] University of Liège
[3] University of South Florida

Double PHD fingers 3 (DPF3) is a zinc finger protein, found in the BRG1-associated factors (BAF) chromatin remodelling complex, and is involved in the regulation of gene expression. Two DPF3 isoforms have been identified, respectively named DPF3b and DPF3a. Very few structural data are available for these isoforms, and their specific functionality remains unclear.

According to disorder predictors, both isoforms present disordered structures. While DPF3b shows scores of a moderately disordered protein, DPF3a is a highly disordered protein with scores comparable to those of α-synuclein or tau, two very well-known intrinsically disordered proteins.

The characterization of DPF3's disordered nature is therefore fully pertinent for a better understanding of their functionality. Furthermore, the identification of specific aggregation properties can also reveal new DPF3 functions, making it a new drug targetable amyloidogenic protein. By combining predictive disorder algorithms, as well as spectroscopic, microscopy, and scattering techniques, we report the intrinsically disordered character and prone-to-aggregate in vitro behaviour of DPF3 proteins.

Biophysical and Structural Insights into a Pancreatitis-Associated Kazal Inhibitor

Felix Nagel[1], Anne Susemihl[1], Norman Geist[1], Gottfried Julius Palm[2], Michael Lammers[2], Mihaela Delcea[1]

[1] Biophysical Chemistry, Institute of Biochemistry, University of Greifswald, Greifswald, Germany
[2] Synthetic and Structural Biochemistry, Institute of Biochemistry, University of Greifswald, Greifswald, Germany

The serine protease inhibitor Kazal type 1 (SPINK1) contains an N34S mutation present in 2% of the population. The mutation was found two decades ago and is strongly associated with chronic pancreatitis. However, the mechanism of action of this important variant has remained elusive. We recombinantly expressed and refolded SPINK1 and human cationic trypsin, carried out extensive biophysical characterization and analyzed the affinity, kinetics, thermodynamics and structure of the SPINK1-trypsin complex. SPINK1 and the N34S variant are functionally and structurally indistinguishable, proving that the N34S variant is a “silent bystander” and is not a cause of chronic pancreatitis. Binding of SPINK1, however, causes a change in rotamer conformation of the catalytic His63 in trypsin, which probably contributes to the proteolytic stability of the inhibitor. Through mutagenesis studies, we identified key residues within the SPINK1 binding loop. Most interestingly, a T40P variant increases the half life of the enzyme-inhibitor complex by over 10-fold and is found in many animal models including mice, naked moles, pigs and cows but not in humans. However, binding of human SPINK1 to trypsin variants of these species is not affected by the T40P mutation, indicating that the inhibitor and enzyme of the same organism form one highly specific, integrated unit. Our structure–function analysis of SPINK1–trypsin interactions enabled us to answer a medical question, and we obtained further evidence for a “silent bystander” model of the N34S mutation. Additionally, we provide a structural and biophysical framework for designing more potent and specific trypsin inhibitors in the future.

Application of reverse pharmacophore mapping to search for protein targets of istradefylline – a drug repurposing perspective

Mietha Magdalena Van der Walt, Arnold Petrus Smith

Human Metabolomics, Faculty of Natural and Agricultural Sciences, Potchefstroom Campus, North-West University, Private Bag x6001, Box 269, Potchefstroom 2531, South Africa

Drug repurposing (DR) is gaining popularity as a low-risk and low-cost drug development strategy compared to traditional drug discovery. Preapproved (e.g., FDA-approved) drugs are utilized to identify novel clinical applications. The choice of drug target is vital since not all druggable proteins are necessarily drug targets. Identifying protein targets based on reverse pharmacophore mapping is gaining attention, especially after the development of free-accessible web servers, e.g., PharmMapper. Here, we investigate whether computational tools (e.g., PharmMapper) predicting protein targets of a preapproved drug have the capability to identify viable drug target(s), which may lead to identifying alternative clinical applications for a low-risk and low-cost DR strategy. The A2A adenosine receptor (AR) antagonist istradefylline is an FDA-approved drug for treating Parkinson's Disease-related motor symptoms. Additionally, literature reports antidepressant-like activity of istradefylline via A2A AR modulation in rodent models of depression. However, the exact mechanism is debatable and unclear. We tested our DR strategy by assessing istradefylline's DR likeliness towards depression. PharmMapper was utilized to predict protein targets for istradefylline. The retrieved predicted protein targets were analyzed and interpreted via computational target identification platforms to reveal potential drug target(s), drug-target-pathway(s) (DTPs), and diseases of interest for istradefylline. The identified protein targets theoretically suggested istradefylline as a Parkinson's Disease treatment option, for which it is clinically FDA-approved. DR of istradefylline as an antidepressant is plausible, based on the retrieved DTPs, suggesting mechanisms of action similar to MAOIs and SSRIs. However, the TCA drug class was eliminated, agreeing with a previous metabolomics study where istradefylline and imipramine didn't share similar metabolic profiles. Concluding, reverse pharmacophore mapping is a low-risk and low-cost assessment tool to identify potential protein targets of preapproved drugs – it is recommended for a bird's-eye view for DR.

FEN1 as a target for confining oncogenesis

Spyridon Gourdoupis, Lukasz Jaremko

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Flap Endonuclease 1 (FEN1) is a structure specific endo/exo-nuclease that maintains DNA integrity by participating in DNA replication and repair1. FEN1 is a characteristic biomarker to all cancers2, its overexpression has been correlated with chemotherapy resistance3 and it has been established as a therapeutic target for human cancers4. All proposed inhibitors lack specificity since they target the active site which is conserved among all endonucleases. Here we demonstrate an NMR-based approach for drug screening by targeting the disordered flexible arms that bind DNA bypassing the conserved active site potentially leading to more specific inhibitors.

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Functional and Structural Characterization of two Cyclophilins from the parasite *Toxoplasma gondii*

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Cyclosporin A (CsA) is an immunosuppressant drug, which exerts its action by binding with nanomolar affinity to cyclophilins (Cyps), a family of proteins belonging to the class of peptidyl-prolyl-cis-trans isomerases (PPIases). Different studies demonstrated that CsA has also a significant anti-microbial activity against many parasites, including Toxoplasma gondii, the causative agent of toxoplasmosis. Therefore, parasite Cyps have received increasing attention for the development of alternative therapeutic strategies against parasites mediated diseases.

Herein, we describe the characterization at functional and structural level of two novel members of the Cyp family from T. gondii, named TgCyp18.4 and TgCyp23, that were recombinantly prepared in E. coli strains. TgCyp23 displays the highest sequence identity with human CypA (hCypA), the archetypical member of the Cyp family, but it possesses an N-terminal extension of 40 amino acids. TgCyp23 is a highly active general PPIase (kcat/Km ≈ 3.8 *106 M-1s-1) and binds CsA with high affinity (Kd ≈ 82 nM, IC50 ≈ 0.6 nM), similarly to hCypA (Kd ≈ 14 nM, IC50 ≈ 1 nM). On the other hand, TgCyp18.4 has several amino acid substitutions in the active site and CsA binding region that cause a significant decrease in both its PPIase activity (kcat/Km ≈ 1.0 *104 M-1s-1) and affinity for CsA (Kd ≈ 9.3 µM, IC50 ≈ 288 nM). Using site directed mutagenesis to replace a key Trp residue, crucial for CsA binding, we obtained a new TgCyp18.4 protein with ~54-fold greater affinity for CsA. Interestingly, we also demonstrated that both TgCyp23 and TgCyp18.4 can act in vitro as molecular chaperones, independently of their PPIase activity. These findings, combined with crystallographic data, suggest that TgCyp18.4 and TgCyp23 might have distinct functions in T. gondii, supporting anti-parasite interventions based on selective targeting of cyclophilins.

BCL11B CCHC Zinc Finger Domain and Its Potential for Cancer Therapy

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The transcription factor B Cell Lymphoma/Leukemia 11B (BCL11B) exerts a bi-directional role in cancer. Its oncogenic potential becomes relevant in the context of T cell acute lymphoblastic leukemia (T-ALL) with BCL11B being over-expressed in 10 % of these cases. Additionally, BCL11B knockouts in culture lead to T cells acquiring natural killer cell properties, further solidifying BCL11B's role as emerging cancer target. A prerequisite for BCL11B functionality is its dimerization, which is mediated by an atypical CCHC zinc finger motif within the N-terminal region of the protein. Understanding the nature of BCL11B oligomerization and its zinc binding properties may pave the way to use BCL11B in targeted cancer therapy. However, zinc fingers are known to be extremely difficult to target and are generally considered nearly undruggable. To approach this problem, we recombinantly expressed and purified the fluorescently-tagged N-terminal BCL11B domain, and developed a FRET assay for high throughput compound library screening and real-time tracking of BCL11B oligomerization. We showed that zinc binding is essential for oligomerization of the N-terminal domain. Therefore, zinc binding properties of the CCHC zinc finger domain were determined using UV-Vis spectroscopy and isothermal titration calorimetry. Additionally, high affinity monoclonal antibodies against the N-terminal BCL11B domain were generated. Single-chain variable fragments or CDR-loop derived peptides of these antibodies are promising candidates for targeted inhibition of BCL11B and may serve as potential tools for T cell reprogramming.

Industry by-product EVs (BP-EVs) as a novel compound delivery platform for protein target within the Central Nervous System

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Extracellular vesicles (EVs) are a promising tool to assist on cell and tissue specific compound delivery and represent a potential way to drastically improve the efficiency of therapeutic compounds and to reduce the toxicity of multiple therapeutic agents. However, the identification of reliable and safe sources of EVs to be useful as nanocarriers and the achievement of further biochemical characterization of available EVs nanocarriers are still undergoing and foremost pending tasks. We have recently uncovered the existence of a novel source of EVs with excellent nanocarrier-oriented properties and safe profiles. These vesicles, obtained from food industry by-products, which, in turn, contribute to the progress of circular economy, are tremendously efficient in the time to reach the central nervous system through oral administration. Here, we will depict the capacity of these vesicles to trespass the blood-brain barrier and reach brain tissues in vivo by 'in vivo fluorescent imaging'. We will also provide shotgun proteome-wide analyses focused on one hand to contribute to the identification of target molecules in the membrane of BP-EVs to further analyze the specific target capacities of these vesicles, while on the other hand, these might help to potentially identify endogenous cargoes. Finally, we will indicate the preliminary results already generated that account for the capacity of BP-EVs to accommodate specific compound molecules as nanovectors.

Effect of SARS-CoV-2 ORF6 protein on DNA replication in eukaryotic cells

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SARS-CoV-2 is a novel coronavirus responsible for causing the coronavirus disease 2019 (COVID-19) pandemic. Most of the research on SARS-CoV-2 has been focused on viral entry and replication. However, to date, the functions of the individual SARS-CoV-2 viral proteins, particularly how they affect human cells, remain largely unknown. SARS-CoV-2 viral genome encodes for 16 non-structural, four structural and nine accessory proteins. Although the accessory proteins do not play critical roles in viral replication, some of them are key factors in immune evasion. Among all accessory proteins, ORF6 has been shown as the most toxic one. It has been demonstrated that ORF6 prevents nucleocytoplasmic RNA transport via interactions with host proteins; however, the mechanism of its cell toxicity still remains unidentified.

In this study, we analysed the toxic effect of ORF6 on WISH and PC3 human cell lines. We found that the replication rates of cells overexpressing ORF6 were reduced, and they accumulated phosho-H2AX indicating replication stress. ORF6-overexpressing cells accumulated co-transcriptional R-loops, likely due to impaired RNA export. As R-loops block the replication and trigger DNA damage response; these results implicate ORF6 as a threat to genome integrity during S-phase of the cell cycle.

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Examining the Therapeutic Effects of Carbon Monoxide: Seeing both the Forest and the Trees in Understanding Its Hemoprotein Targets

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Carbon monoxide (CO) is an endogenous signaling molecule primarily produced via heme degradation by heme oxygenase (HO)-1 and -2 with HO-1 being inducible and HO-2 being constitutive. Despite is public perception of being a poisonous gas, CO has been shown in animal models to have therapeutic effects in the treatment of a variety of inflammation-related problems such as colitis, ischemia-reperfusion injury, gastric injury, liver injury, and acute kidney injury, among many others. In terms of its mechanism(s) of action, CO is known to bind to a large number of hemoproteins with at least 25 identified molecular targets. This presentation aims to examine some critical issues in developing CO as a therapeutic agent with a special focus on analyzing the various hemoprotein targets. Such analyses take into consideration of target organs, delivery forms, binding affinity of protein targets with CO, and binding competitions. The information to be presented will be important for the feasibility assessment of using CO as a therapeutic agent.

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Targetting Topoisomerase IIa: back to the drawing board via Z-DNA

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Topoisomerase IIa (Topo II) is a key member of the extensive enzyme family recognizing and altering DNA supercoiling. Topo II resolves and maintains the topological integrity of genomic states during DNA replication and mitosis, transcription, and recombination. As a consequence, a great number of anticancer therapies have been devised to target various steps of the human TOP2A gene product [1]. In 1993, one of our labs reported that Drosophila Topo II exhibits a 100-fold greater affinity for Z-DNA than for B-DNA and furthermore, that GTP irreversibly inactivates the enzyme while increasing its affinity for Z-DNA 5-10 fold [2]. Using comprehensive bioinformatics analyses we have now identified Z-alpha domains and GTP binding sites in Topo II (see also Abstract Slychko et al.). Topo II–Z-DNA–GTP complexes have been modeled by molecular docking techniques, and the results correspond well with our in silico identified Z-alpha domain and GTP-binding sites. We anticipate that these results and their experimental verification will lead to new concepts for targeting Topo II in anticancer drug discovery. [1] Vann, KR, Oviatt, AA, Osheroff, N (2021). Topoisomerase II poisons: converting essential enzymes into molecular scissors. Biochemistry 60: 1630-1641. [2] Arndt-Jovin, DJ, Udvardy A, Garner MM, Ritter S, Jovin TM (1993). Z-DNA binding and inhibition by GTP of Drosophila topoisomerase II. Biochemistry 32: 4862-4872.

ATRi and CHK1i significantly increased chromosomal abnormalities caused by olaparib in HGSOC $BRCA^{MUT}$ and $BRCA^{WT}$ ovarian cancer cell lines

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Olaparib was the first FDA approved PARP inhibitor preventing the repair of DNA damage, which creates synthetic lethality in BRCAMUT high-grade serous ovarian cancers (HGSOCs). However, it is often insufficient as monotherapy, for both BRCAMUT and BRCAWT ovarian cancers. DNA damage response (DDR) includes activation of ataxia telangiectasia and Rad-3 related protein (ATR) and checkpoint kinase 1 (CHK1) and may be crucial in response to PARP inhibitor (PARPi) treatment. The objective of the study was to increase the effectiveness of PARPi by combining this drug with ATR (AZD6738, ATRi) or CHK1 (MK8776, CHK1i) kinase inhibitors. Homologous recombination deficient – PEO-1 (BRCA2MUT) and proficient – OV-90 (p53MUT) and SKOV-3 (p53 null), ovarian cancer cell lines were used. Metaphase spread, cell cycle distribution, cleaved caspase-3 and γH2AX expression by western blot and immunofluorescence staining were assessed. Our data showed that expression levels of γH2AX and cleaved caspase-3 were elevated upon combination treatments in comparison with PARPi monotherapy, however to the comparable level as with ATRi or CHK1i monotherapy. Interestingly for us, the level of chromosomal abnormalities was higher after combination treatment, followed by no significant changes in the distribution of the G2/M phase of the cell cycle in SKOV-3 and PEO-1 compared to PARPi monotherapy. These data confirmed that both PARPi:ATRi and PARPi:CHK1i combinations caused inappropriate entry into mitosis leading to increased DNA damage, chromosomal aberrations and genome instability. However, in the case of OV-90 cells, where the high number of DNA damage exceeded the cell's ability to repair them, activation of the DDR pathway led to programmed cell death without entering mitosis. This research was funded by the National Science Centre, Poland, project grant number: Sonata Bis 2019/34/E/NZ7/00056.

How can interaction with prolyl oligopeptidase possibly trigger α-synuclein aggregation?

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The formation of protein aggregates is considered one of the leading causes of neuronal malfunction and subsequent brain damage in many neurodegenerative diseases. In Parkinson's disease, the proteins involved in pathogenic aggregates are α-synucleins. The causes of aggregate formation are still unclear, but we already know that specific processes can accelerate or delay aggregation. One such process is the interaction with prolyl oligopeptidase. It has been shown experimentally that synuclein aggregation can be reduced by inhibiting prolyl oligopeptidase (PREP). Interestingly, this effect cannot simply be related to the enzyme's catalytic function inhibition since different PREP inhibitors interfere with synuclein aggregation differently, not necessarily correlated to their inhibition potency. Our previous numerical studies provided an opportunity to observe differences in the dynamics of the enzyme inhibited by different compounds and allowed us to identify regions of proteins potentially involved in the interaction between PREP and α-synuclein. Here we use molecular dynamics modelling to study specific interactions between disordered synuclein and PREP. Tracing the course of such an interaction is the first step toward explaining why a direct interaction between synuclein and PREP triggers the aggregation process.

Functional characterization of cystathionine gamma-lyase from *Pseudomonas aeruginosa*

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Cystathionine gamma-lyase (CGL) is a pyridoxal-5'-phosphate (PLP)-dependent enzyme that is involved in the metabolism of L-cysteine by catalyzing the hydrolysis of L-cystathionine to L-cysteine. Moreover, CGL catalyzes several hydrogen sulfide (H2S)-generating reactions, including the hydrolysis of L-cysteine and L-homocysteine to pyruvate and butyrate, respectively, as well as the condensation of both the substrates to L-cystathionine. H2S has recently emerged as an important gaseous signaling molecule that plays a crucial role in promoting resistance to clinically relevant antibiotics in bacterial pathogens, including Pseudomonas aeruginosa (Pa) and Staphylococcus aureus (Sa) [1]. In the search for novel druggable targets, herein, we performed a thorough characterization of CGL from Pa (PaCGL). We overexpressed the protein in E. coli and analyzed its molecular and kinetic properties. Native PaCGL is a tetramer composed of four identical subunits, each containing a PLP molecule. PaCGL efficiently produces L-cysteine via α, γ-elimination of L-cystathionine as demonstrated by the kinetic analysis in combination with the identification of reaction products by RP-HPLC. Of note, PaCGL can also efficiently produce H2S using L-cysteine or L-homocysteine as substrates. Together with the information obtained from the crystal structure (PDB: 7BA4), our data support the strong involvement of CGL in sulfur metabolism of Pa (both cysteine biosynthesis and H2S generation), therefore making it an attractive target for pathogen inhibitor design.

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Innovative Approaches Targeting Carbonic Anhydrases: An Update

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The zinc metalloenzymes carbonic anhydrases (CAs, EC 4.2.1.1) are considered as drug targets for several pathologies, and different inhibitors found clinical applications as diuretics, antiglaucoma agents, anticonvulsants, and anticancer agents/diagnostic tools. Their main drawback is related to the lack of isoform selectivity leading to serious side effects for all pathologies in which they are employed. Even if the potency of carbonic anhydrase inhibitors has been greatly improved in the last years, with inhibitors reaching inhibition constants in the femtomolar range, the selectivity issue remains important.
In this presentation, two recent approaches to targeting carbonic anhydrases, initiated and developed in our laboratory, will be discussed. These new strategies dealing or with multivalent approach, or with boron-based small molecule inhibitors, may open new opportunities in the drug design of innovative isoform-selective carbonic anhydrase inhibitors with biomedical applications.

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DMSO-Induced Unfolding of the Antifungal Disulfide Protein PAF: A Combined NMR and DSC Study

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PAF and related antifungal proteins are known to have highly stable beta-barrel folds around room temperature due to the presence of 3-4 disulfide bonds. However, unfolded states persist and contribute to the thermal equilibrium, and low-populated states might influence the biological effects. Such sporadic states were detected earlier in our CEST-NMR experiments. As an alternative, to explore equilibria during chemical unfolding, we studied PAF and its inactive variant, PAFD19S, using NMR spectroscopy and differential scanning calorimetry (DSC). Dimethyl-sulfoxide (DMSO), a common solvent in pharmaceutical studies, was applied for denaturation. The unfolding was completely reversible, as was shown by adding excess H2O. Folded structures disappeared in DMSO/H2O solvent mixture above ca. 80 v/v % DMSO concentration. The NOE-based NMR structures of the two proteins were determined in 50 v/v % DMSO/H2O solution. Interestingly, the NMR visible 3D structures of PAFD19Sand PAF are rather similar in the denaturing mixtures, in contrast to comparisons of their native folds in water. The study of the NMR relaxation dynamics proved that the termini of the two proteins retained increased mobility, in agreement with previous NMR results in the absence of a denaturing agent. DSC experiments—a combination of heat and chemical unfolding—showed that PAF is more stable than the inactive PAFD19S variant.

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Human interferon gamma is a potential inhibitor of SARS-Cov-2 Orf6 activity

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The SARS-CoV-2 virus expresses 4 structural, 16 non-structural and 9 accessory proteins. The latter are not necessary for in vivo replication but are responsible for the virus pathogenicity and suppression of innate immune response. One of the most important SARS-CoV-2 accessory proteins is Orf6. This is a 61 amino acid residue long protein that antagonizes with type-I interferon signaling, a key component for antiviral response of the host cell, by targeting the Rae1-Nup98 complex. The highly acidic Orf6 C-terminal part appears to be responsible for the interaction with the cellular Rae1-Nup98 complex, which participates in the mRNA nuclear export. It was shown that the SARS-CoV-2 Orf6 is the most toxic virus protein and contributes significantly to COVID-19 lung pathology and disease outcome. This necessitates the search for possible inhibitors of this protein.

Interferon gamma (hIFNg) is a crucial immunomodulating cytokine, consisting of an alpha-helical globule and two highly flexible C-terminal tails. With its basic sequence, this particular domain of IFNg is a perfect binding partner candidate for neutralizing the acidic SARS-CoV-2 Orf6 C-terminus.

Here, we report first results from a molecular modelling study on the possible inhibition of the SARS-CoV-2 Orf6 by either a full-length hIFNg homodimer, or a few peptides, encompassing the C-terminal domain of the cytokine. Our in silico experiments demonstrate that the two molecules interact with high affinity due to the strong electrostatic attraction between them. Upon binding of either hIFNg C-terminal peptides or the full-length cytokine molecule, the C-terminal domain of the SARS-CoV-2 Orf6 is effectively neutralized, which would potentially impede its binding to the Rae1-Nup98 complex.

Human extracellular NAMPT and NAPRT mediate the inflammatory response through TLR4-binding: molecular insights into the mechanism of interaction

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The enzymes nicotinamide phosphoribosyltransferase (NAMPT) and nicotinate phosphoribosyltransferase (NAPRT) catalyze key reactions in intracellular NAD biosynthesis, starting from nicotinamide and nicotinic acid, respectively. In addition to their essential role in the maintenance of steady-state NAD levels, an interesting feature of both enzymes is that they are secreted into the extracellular milieu, where they behave as cytokines with pro-inflammatory activity, even if the mechanism of their secretion and their physiological function in the extracellular space are still uncertain1. Our previous data collected in human macrophages demonstrated that extracellular NAMPT and NAPRT trigger the NF-kB pathway, stimulating the secretion of inflammatory cytokines through the activation of Toll-like receptor 4 (TLR4)2. Their physical interaction with the receptor was further confirmed by surface plasmon resonance studies using the recombinant proteins2,3. With the aim to investigate the molecular binding mechanism, we first found that, despite a high degree of structural conservation, the bacterial orthologs of human NAMPT and NAPRT were not able to elicit an inflammatory response2. Based on this, by combining bioinformatics, functional, and structural studies, we identified some surface-exposed regions that are particularly rich in positively charged residues and unique to the human enzymes, which could be responsible for TLR4 binding2,4. By performing multiple sequence alignments of the two enzymes from different species, we explored the evolutionary transition of NAMPT and NAPRT from simple catalytically active proteins to signaling enzymes. We found that the acquisition of the candidate determinants for TLR4 binding emerged in higher eukaryotes and has been perfected in mammals.

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Bacterial Carbonic Anhydrase Inhibitors for the Treatment of Drug Resistant Bacteria: A Classical Drug Scaffold Gets a New Purpose

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There is a critical need to identify new strategies to combat the ever-growing problem of antibiotic resistant bacterial pathogens. Among the most problematic pathogens in terms of resistance threats are the Gram-positive Enterococcus faecium and E. faecalis as well as the Gram-negative Neisseria gonorrhoeae. Our team has identified that inhibition of the bacterial carbonic anhydrases from both pathogens represent viable antimicrobial therapeutic targets. To this end we have designed and synthesized novel inhibitors of these carbonic anhydrases that display potent efficacy against both VRE and N. gonorrhoeae strains. Our studies have also demonstrated that analogs adopt an alternative binding pose not traditionally observed for carbonic anhydrase inhibitors. Moreover, the analogs possess favorable pharmacokinetic properties and display efficacy in vivo against VRE and N. gonorrhoeae. Taken together these results confirm that targeting bacterial carbonic anhydrases may be a suitable strategy for treating VRE and N. gonorrhoeae infections.

Inhibition of Carbonic Anhydrase IX induces mitochondrial oxidative stress and apoptosis in multiple myeloma

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Multiple myeloma (MM) arises from the clonal proliferation of malignant plasma cells in the bone marrow (BM). Since BM niches are characterized by low oxygen levels, MM cells have to adapt their metabolism to the hypoxic BM milieu. This generates high quantities of toxic metabolic acids that induce the expression of proteins involved in the prevention of H+ ions accumulation and intracellular pH maintenance. In this context, carbonic anhydrase IX (CA IX), a hypoxia-induced cell-surface enzyme, sustains cancer cell survival under hypoxic and acidosis conditions by regulating intracellular pH. Indeed, CA IX overexpression has been reported in several types of solid cancer and correlates with poor prognosis. However, no data are available about the impact of CA IX in MM. Our preliminary data demonstrate that MM cells express high levels of CA IX under both normoxic and hypoxic conditions, suggesting that inhibition of CA IX activity may affect MM survival.

Here, we investigated the anti-MM activity of two CA IX inhibitors, the clinical grade sulphonamide SLC-0111 (Phase Ib/II clinical trials) and its analog FC-531. In vitro, both CA IX inhibitors significantly reduced KMS-11 MM cell growth, FC-531 being more potent compared to SLC-0111. Interestingly, CA IX inhibition strongly induced mitochondrial ROS production, mitochondrial membrane depolarization and apoptotic cell death. Accordingly, Western blot analysis revealed a significative induction of ROS-mediated DNA damage and caspase activation, as demonstrated by increased levels of gH2AX and cleaved PARP and caspase 3. Finally, CA IX inhibition significantly reduced the growth of KMS-11 tumor xenografts in NOD/SCID mice. Importantly, similar results were obtained with Bortezomib-resistant KMS-11 cells, suggesting that CA IX inhibition may represent a valid therapeutic approach for both naïve and proteosome inhibitor-refractory MM patients.

Altogether these findings demonstrate the therapeutic potential of CA IX inhibitors for the treatment of MM.

Strategies to display susceptibility of targets for compounds

Carsten Zeilinger

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Target-oriented trawling of compound libraries can help to identify novel substances that inactivate the protein function or increase the affinity for the natural ligand or clients or help to restore proteins activity corrupted by environmental or mutagenic influences. With protein microarrays a highly miniaturized assay system was used to display protein-ligand or protein-protein interaction using fluorescently labeled ligand to visualize binding and displacement events. The application and its novel range demonstrated by protein ligand/protein interaction using purified target proteins such as heat shock proteins, transcription factor, receptor or used for flux measurements through ion channels. Purified full-length Hsp90 or Hsp70 transferred onto nitrocellulose microarrays binds fluorescently labeled ATP whereas in presence of well-known high affine anti Hp90 inhibitor such radicicol displace ATP resulting in a low fluorescence signal. This assay format enabled later simultaneous screening of natural product libraries, predictive selected compound libraries to identify novel selective anti Hsp90 or Hsp70 inhibitors against Hsp from pathogenic cells. In addition IC50 values compared with alternate methods such as ITC, thermophoresis or AFM. Furthermore amino acid exchanges in the ATP pocket gave positions with generate an increase or loss of ATP binding. These positions were used to test the genome database for candidate which are novel compound producer. Exploitation of protein-protein interaction with other client proteins such as Hsf1, TP53 and other gave different affinities for Hsp90 and used for testing of novel inhibitors.It was also possible to spot HeLa cells or liposomes with purified Cx26 onto the microarray under preservation of its transport activity for Lucifer Yellow through connexin 26 hemichannels. Cx26 was activated by temperature while the mutated channel or cold temperature or high Ca2+ does not activate the channel for Lucifer Yellow uptake. The uptake assay was transferred to preselected compounds which may restore loss of function mutations.

Biological motions, gene regulation and drugs

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The structural studies delivering the static architectures of biomolecules became well established in the last four decades, with a variety of atomic resolution methods, including X-ray crystallography, solution and solid-state NMR, and cryogenic electron microscopy. The evidence of numerous studies on both fundamental and applied biochemical and medical phenomena indicated that the static structural information is not sufficient. More often the missing link to describe the biological function is the molecular mobility, the dynamics. Here I would like to introduce the concept of biological motions which is more general than dynamics and likely better to describe the biomolecular processes of human health and disease. First, I will focus on the biophysical techniques monitoring and detecting the biological motions of biomolecules. Then I will show how the biological motions can link the molecular details with the biological function, like helping us to understand the molecular bases of the cancer-driving mutations. The protein function cannot be often easily linked with the static molecular architecture, this becomes even more pronounced in the case of IDPR, so in intrinsically disordered proteins and protein regions. Here on the example of epigenetic writers and nucleosomes, I will demonstrate the pivotal role of biological motions and the link between modifiability and mobility of the flexible histone tails. The final part of my talk will explain how the protein dynamics can facilitate target identification for the drug-discovery campaign and help in optimizing the lead compounds. In short, I argue that soon modern drug design will be assisted by detailed studies of the biological motions of targeted biomolecules and ligands.

Fibrinogen corona on the dendrimer nanoparticles

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Nanoparticles play an essential role in the dynamic development of nanomedicine. The formation of the nanoparticle–protein complex is a critical element in predicting the in vivo pathway of nanoparticles and, consequently, their biodistribution and toxicity. Serum fibrinogen has a decisive influence on the opsonization process of carriers used in DDS. The interaction of fibrinogen with dendrimer molecules was monitored in multiple directions using advanced analytical methods and molecular simulations. Measurements of electrophoretic mobility and circular dichroism (CD) were used to determine the stability and secondary structure of the protein. It was found that the Zeta potential of the molecules is closely correlated with the Zeta potential values of fibrinogen and the degree of corona coverage. The fibrinogen corona's stability on the dendrimers' surface was monitored by measuring the adsorption kinetics under dynamic conditions by QCM-D and SPR spectroscopy. Fibrinogen molecules efficiently adsorb to negatively and positively charged dendrimer molecules over a wide pH range. However, significant changes in the secondary structure of the protein are observed depending on the types of functional groups that make up the outer layer of the dendrimer structure. The orientation of a protein determines both its flexibility and the availability of optimal protein binding sites and functions. The protein orientation and degree of changes in protein structures and their partial dysfunction depend on interaction with the carrier. CD spectra showed a reduction in the α-helix and increased the β-sheet content for the conjugate system compared to the native protein. Appropriate selection of the topography and surface composition and detailed information about the spatial structure of proteins allow controlling both the orientation and properties of adsorbed proteins on the nanoparticle surface. The changes in the conformation of the fibrinogen molecule were confirmed using molecular dynamics simulations.

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Variation in viral tropism belongs to IDPR positions in structural proteins of enterovirus

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Oncolytic viruses belong to a new class of antitumor therapy for the treatment of aggressive malignant neoplasms. Viral tropism is determined by virion internalization efficiency, depending on the presence of specific receptors and cofactors on the cell surface. Oncolytic Echovirus 1 (strain Farouk, E1-F) uses neonatal Fc receptor (FCGRT) for entry into cells. This receptor acts as a pocket factor for virus uncoating, and there are no Enterovirus B strains capable of effective cell infection with no expression of this protein. Using the bioselection method, we receive a derivative strain from canonical E1-F on HEK293T cells with the knockout of the FCGRT gene. This strain can effectively replicate on HEK293T ΔFCGRT cells. Over the course of the genome sequence analysis, we detect a significant number of non-synonymous substitutions in a structural segment of new strain polypeptide. The amino acid sequence analysis of structural proteins interacting with FCGRT indicates changes in intrinsically disordered protein region (IDPR) positions. The emergence of these regions may lead to a change in the new strain tropism to the already known cellular factors. The assessment of the tropism of the new strain to tumor cell lines shows a significant change in comparison with the original strain. In vitro experiments demonstrate that the oncolytic potential is preserved. The further study of IDPR positions in structural proteins can shed some light on the process of virus host change.

1-1 Safety studies of the long-acting insulin analogs in the scope of the biological properties of metabolites formed after the exposure of the drug to the blood plasma environment

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Insulin analogs designed to exhibit an extended profile of action due to the addition of basic amino acids at the terminus of protein's B-chain undergo rapid degradation after subcutaneous administration. What is worth noticing is that only a few derivatives of analogs are obtained in body fluids as a result of interaction with degradative enzymes naturally present in the body. These compounds differ from the parent molecules by the lack of one or more terminal basic amino acids.

The project, the results of which are presented on the poster, was aimed to verify whether the abovementioned chemical modifications of the primary structures of insulin analogs lead to alterations in the biological properties of the administrated drugs. It was also important to verify whether the metabolites of insulin analogs are safe for diabetic patients.

The poster contains a summary of the experiments examining the biological activity of insulin analogs and the observations of processes occurring at the cellular level after activation of the cell membrane receptors by these drugs. The main task was to explain to what extent the observed properties of derivatives differ from the characteristics of the parent molecules. The results obtained were also compared to the known properties of human insulin, used here as the reference.

1-2 Live cell imaging of DPP3 and SH2D3C protein interaction using bimolecular fluorescence complementation

Ana Tomašić Paić, Lucija Horvat, Mihaela Matovina

Ruđer Bošković Institute

The identification of protein–protein interactions (PPIs) plays a pivotal role in understanding the functional relationships between proteins. Complex maps of proteins provide potential clues to the cellular function of still-uncharacterized proteins. In order to directly visualize protein interactions in living mammalian cells, we use a bimolecular fluorescence complementation (BiFC) method. The BiFC assay is crucial for determining the subcellular locations of protein interactions, as well as providing insight into the biological functions of newly discovered protein complexes. Based on the reconstitution of a fluorescent protein in vivo, we confirm the interaction of two proteins in mouse embryo fibroblasts (NIH3T3) and in HeLa cells: SH2 domain containing protein 3C (SH2D3C) and dipeptidyl peptidase 3 (DPP3). DPP3 is a ubiquitous, intracellular Zn-dependent protease, a cytosolic enzyme known to cleave dipeptides from the amino-termini of 3–10 aa long peptides in vitro. In addition to its enzymatic activity, DPP3 is involved in the regulation of the Nrf2/Keap1 signalling pathway. It is able to block the ubiquitination of Nrf2 by competing to interact with Keap1. SH2D3C acts as an adapter protein and has proposed roles in cell migration and adhesion, tissue organization, and the regulation of the immune response. SH2D3C protein has internally disordered regions (IDRs) and six isoforms. All isoforms have the C-terminal domain that shows homology with Cdc25/Ras-GEF domain, while all except the shortest isoform 3 have the N-terminal SH2 domain. SH2D3C has been identified as a prey in the SILAC-MS screen of dipeptidyl peptidase 3 (DPP3) interactome, and the interaction has been confirmed by Co-IP on overexpressed proteins in TREX HEK293T cells. The preliminary co-localization experiments together with BiFC analysis showed that DPP3 and SH2D3C proteins co-localize in the cytosol and in the membrane ruffles, possibly indicating that the interaction has a role in cell migration.

1-3 A PDE2-PKA dependent regulation of NCLX is mediating neuronal survival and cognitive function

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Impaired phosphodiesterase (PDE) function and mitochondrial Ca2+ ([Ca2+]m) signaling lead to ischemic damage and dysfunction in learning and memory. We monitored [Ca2+]m transients in hippocampal neurons evoked by caffeine and depolarization. We found that [Ca2+]m efflux is apparent in WT but diminished in neurons deficient in the mitochondrial Na+/Ca2+-exchanger (NCLX). Depolarization-induced Ca2+ transients alone failed to evoke strong [Ca2+]m efflux in WT neurons. As caffeine is an inhibitor of PDE2, it can affect [Ca2+]m by inhibiting mitochondrial PDE2. We show that pretreatment with the selective PDE2 inhibitor Bay-60-7550 effectively rescued [Ca2+]m efflux triggered by neuronal depolarization, as caffeine. Furthermore, inhibition of PDE2 acts by diminishing the Ca2+ dependent change of mitochondrial cAMP thus promoting NCLX phosphorylation at its regulatory PKA S258-residue. Selective PDE2 inhibition also enhanced [Ca2+]m efflux triggered by neuromodulators Vasopressin and Norepinephrine. We found that protection of neurons against excitotoxic insults, conferred by PDE2 inhibition in WT neurons, is strongly diminished in NCLX-KO neurons, thus is NCLX-dependent. Finally, administration of Bay-60-7550 enhanced learning and memory in object recognition test in WT but not in NCLX-KO mice. Our results identify a link between PDE2 and [Ca2+]m signaling. [Ca2+]m dyshomeostasis is a prominent feature of multiple disorders, thus the link between NCLX and PDE2 may provide new therapeutic targets.

1-4 ZnR/GPR39 Modulates Saliva Composition via Regulation of Na^+/K^+ ATPase activity

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Introduction: Zn2+ is selectively concentrated in the secretory granules of the salivary glands. Interestingly, symptoms of zinc deficiency include a loss of taste sensation and reduced saliva secretion (dry mouth). How zinc regulates these functions is not well understood. In the salivary duct cell line, HSY, extracellular Zn2+ activates a Zn2+ -sensing receptor, ZnR/GPR39. This receptor is a G-protein coupled receptor that triggers intracellular Ca2+ release and the subsequent regulation of signaling that is associated with epithelial functions. However, the link between ZnR/GPR39 and salivary function is not well understood.

Aim: We hypothesize that ZnR/GPR39 controls salivary gland function via the activation of Ca2+ signaling and the subsequent regulation of epithelial solute transport by the Na+/K+ ATPase pump. This transporter is present on the basolateral plasma membrane of salivary epithelial cells and regulates intracellular solute concentration.

Results: We found that Zn2+ enhances ion transport activity that is reduced in the presence of the inhibitor of the pump, ouabain. Similarly, the silencing of Na+/K+ ATPase using siRNA constructs abolished the enhanced ion transporter activity. Moreover, we monitored the Zn2+-dependent increase in K+ transport activity in acute slices of salivary parotid glands that were reduced by ouabain. In contrast, in ZnR/GPR39 knockout tissues, this activity was abolished, indicating that Zn2+ via ZnR/GPR39 regulates Na+/K+ ATPase activity. Finally, we found that salivary ion composition was disrupted in ZnR/GPR39 knockout mice, with the increased concentration of Na+ and K+ in the saliva.

Conclusion: Our results suggest that, in salivary gland cells, ZnR/GPR39 activates Na+/K+ ATPase activity, regulating ion gradient and saliva composition.

1-5 Proteomic Characterization of Feline Obesity

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Obesity has become a global problem and a huge healthcare concern of the 21st century, reducing the quality and length of life of humans and animals by increasing morbidity and mortality. As in humans, feline overweight and obesity have become a rising problem with a prevalence ranging between 19 and 52 %.

A total of 6 healthy lean cats and 12 naturally occurring overweight and obese cats, of which 6 were metabolically healthy and 6 were metabolically unhealthy, were included in the study. Serums were subjected to protein extraction, digestion and isobaric labeling via tandem mass tags (TMT). LC-MS/MS data were analysed to identify and quantify the detected proteins. Statistical and bioinformatic analyses were performed using R and Cytoscape.

Among the group of cats, 11 proteins were detected as significantly differentially expressed. Apolipoprotein C-III and factor H increase in feline obesity. Apo C is proposed as a new risk factor for obesity in humans. Factor H, the regulatory protein in the activation of complement system, has been suggested as a link between obesity and metabolic disorders. Moreover, several studies of human and animal obesity have shown that obesity is associated with the reduced inhibition of coagulation, antithrombin (AT III), causing a hypercoagulable state. The obtained data strongly suggest a link between feline obesity and the synthesis, secretion, and deacylation of ghrelin, an orexigenic hormone that stimulates food intake in a dose-dependent manner.

Numerous similarities between cats and humans are postulated, as diabetes mellitus in cats strongly resembles human type 2 diabetes and dyslipidaemia also occurs with feline obesity. The present study extends the knowledge on feline obesity and on the development of its related disorders, highlights similarities compared with human obesity, and may help in revealing new biomarkers for early diagnosis and obesity control using cats as a model.

1-6 Deregulated levels of RUVBL1 induce transcription-dependent replication stress

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The frequent occurrence of transcription and DNA replication in cells results in many encounters, and thus conflicts, between the transcription and replication machineries. These conflicts constitute a major intrinsic source of genome instability, which is a hallmark of cancer cells. The INO80 family members RUVBL2 and RUVBL1 (Tip48 and Tip49) are overexpressed in cancers from many different tissue types. Here, we report that both the excess and the deficit of the chromatin regulator RUVBL1 impede DNA replication as a consequence of altered transcription. Surprisingly, cells that either overexpressed or were silenced for RUVBL1 had slower replication fork rates and accumulated phosphorylated H2AX, dependent on active transcription. However, the mechanisms of transcription-dependent replication stress were different when RUVBL1 was overexpressed and when depleted. RUVBL1 overexpression led to increased c-Myc-dependent pause release of RNAPII, as evidenced by higher overall transcription, much stronger Ser2 phosphorylation of Rpb1- C-terminal domain, and enhanced colocalization of Rpb1 and c-Myc. RUVBL1 deficiency resulted in increased ubiquitination of Rpb1 and reduced mobility of an RNAP subunit, suggesting accumulation of stalled RNAPIIs on chromatin. Overall, our data show that by modulating the state of RNAPII complexes, RUVBL1 deregulation induces replication-transcription interference and compromises genome integrity during S-phase.

1-7 Acridines as antifungal agents – yeast topoisomerase II targeting

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Derivatives of acridine and acridone are characterized by a wide range of biological activity and unique properties. Used in industry as pigments and drugs: for giardiasis, accelerating wound healing, anti-cancer and antimicrobial. Such a wide biological activity of these derivatives is due to the flat structure and the conjugated double bonds, thanks to which they interact with DNA-dependent enzymes or tyrosine kinase receptors. Despite the fact that acridine derivatives have antifungal activity, these compounds are mainly tested for antitumor and antibacterial activity. This is probably related to the problems with transport through the fungal cell wall. On the other hand bacterial or human topoisomerases targeting by acridine derivatives is also effectively evaluated in contrast to fungal enzyme inhibition. Although topoisomerases are ubiquitous enzymes, evolutionarily conserved, mammalian and fungal enzymes show structural differences and in sensitivity to inhibitors. In an effort to develop biologically active antifungals that are able to target yeast topoisomerase II activity several C-1311 as well as C-1305 derivatives were synthesized. Starting compounds exhibited anticancer activity correlated with inhibitory effect on human topo II although no antifungal activity was observed. To overcome transport inefficiency the lead structures were modified by change in lipophilicity and by combining with amino acids, which increases their bioavailability. Some new derivatives are fungicidal and severely affect C. albicans hyphal growth in different inducing media as well as biofilm formation. Those compounds exhibit antifungal activity even against C. albicans fluconazole resistant strains with MIC value ≥ 8 µg mL-1. Our results also indicate that selected synthetized compounds with antifungal activity target yeast topoisomerase II activity. Taking into account all the results obtained for modifying acridine structures the search for antifungal drug candidates targeting yeast topoisomerases among those derivatives is undoubtedly worth continuing and can help overcome the phenomenon of drug resistance.

1-8 Identification and characterisation of the first mollusc core 1 β1,3-galactosyltransferase

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Glycoprotein-N-acetylgalactosamine β1,3-galactosyltransferase (T-synthase, EC 2.4.1.122) catalyses the transfer of the monosaccharide galactose from UDP-Gal to GalNAc-Ser/Thr, synthesising the core 1 O-glycan structure (T-antigen) of mucin-type O-glycans. Although such structures have been described in snails, so far, no core 1 β1,3-galactosyltransferase has been determined there. In this study, we identified, cloned, expressed and characterised the glycoprotein-N-acetylgalactosamine β1,3-galactosyltransferase from the freshwater snail Biomphalaria glabrata, which is the first cloned T-synthase of mollusc origin.

The sequence of the enzyme was identified by a BlastP search of the NCBI Biomphalaria glabrata database using the human T-synthase sequence (NP_064541.1) as a template. The gene codes for a 388 amino acid long type II transmembrane protein with two putative N-glycosylation sites, a short cytoplasmic domain at the N-terminus, a transmembrane part and a catalytic domain at the C-terminus. The coding sequence was synthesised and expressed in Sf9 insect cells. The human enzyme requires a chaperon called Cosmc to be folded properly; nevertheless, no ortholog of Cosmc has been found in any of the known invertebrate genomes. The expression product of the putative enzyme displays core 1 β1,3-galactosyltransferase activity using pNP-α-GalNAc as a substrate. This shows that it does not require any specific factor to have high enzymatic activity and that insect cells represent an adequate expression system. The enzyme shows high sequence homology (49.40% with Homo sapiens, 49.14% with Caenorhabditis elegans, 53.69% with Drosophila melanogaster) and similar biochemical parameters compared to previously characterised T-synthases from other species.

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1-9 Variation in viral tropism belongs to IDPR positions in structural proteins of enterovirus

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Oncolytic viruses belong to a new class of antitumor therapy for the treatment of aggressive malignant neoplasms. Viral tropism is determined by virion internalization efficiency, depending on the presence of specific receptors and cofactors on the cell surface. Oncolytic Echovirus 1 (strain Farouk, E1-F) uses neonatal Fc receptor (FCGRT) for entry into cells. This receptor acts as a pocket factor for virus uncoating, and there are no Enterovirus B strains capable of effective cell infection with no expression of this protein. Using the bioselection method, we receive a derivative strain from canonical E1-F on HEK293T cells with the knockout of the FCGRT gene. This strain can effectively replicate on HEK293T ΔFCGRT cells. Over the course of the genome sequence analysis, we detect a significant number of non-synonymous substitutions in a structural segment of new strain polypeptide. The amino acid sequence analysis of structural proteins interacting with FCGRT indicates changes in intrinsically disordered protein region (IDPR) positions. The emergence of these regions may lead to a change in the new strain tropism to the already known cellular factors. The assessment of the tropism of the new strain to tumor cell lines shows a significant change in comparison with the original strain. In vitro experiments demonstrate that the oncolytic potential is preserved. The further study of IDPR positions in structural proteins can shed some light on the process of virus host change.

1-10 Molecular detection of *Staphylococcus aureus* fibronectin-binding protein A from bovine mastitis: a potential biomarker in lateral flow assay

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Bovine mastitis constitutes a serious issue in the dairy industry. The so-called golden standard for the diagnosis of mastitis is based on the determination of the somatic cell count, which, although it allows a rapid diagnosis of infection, does not recognize an etiological factor. Presently, there is no fast, bacterial species-recognizing test in the diagnostic market.

Therefore, the aim of this study was to detect fibronectin-binding protein A (FnBPA) of Staphylococcus aureus (SA) isolated from milk samples with diagnosed mastitis to be used as a biomarker in an immunodiagnostic assay.

Three representative clinical strains were grown onto Columbia blood agar for further analysis, including DNA isolation and protein isolation. Bacterial DNA was subjected to amplification by the PCR method with specific primers for the detection of the fnbA gene encoding FnBPA. In turn, a mixture of bacterial surface proteins was subjected to SDS-PAGE and a band corresponding to the size of FnBPA was detected in immunoblotting with both SA-positive serum samples. This study was supported by the National Centre for Research and Development (grant no. LIDER/9/0029/L-10/18/NCBR/2019).

The detection of the products of amplification visualized by ethidium bromide confirmed the presence of the fnbA gene in all studied strains. In turn, the investigation of FnBPA among isolated proteins showed corresponding bands both for polyacrylamide gel and membranes marked with antibodies from serum isolated from cows infected with S. aureus. The bands were not detected in the presence of serum isolated from healthy animals (SA-negative). The received results indicate the conservativity, specificity, and immunoreactivity of FnBPA, and thus it can be considered as a good marker in an innovative lateral flow assay for the rapid diagnosis of mastitis caused by S. aureus, which is one of the most common etiological factors in bovine mastitis.

1-11 Differential impact of lipid raft depletion on platelet-derived growth factor (PDGF)-induced ERK1/2 MAP-kinase, SRC and AKT signaling

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It has become clear that lipid rafts functions as signaling hotspots connecting cell surface receptors to intracellular signaling pathways. However, the exact involvement of lipid rafts in receptor tyrosine kinase signaling is still poorly understood. In this study, we have analyzed platelet-derived growth factor (PDGF) receptor b (PDGFR-b) signaling in two different cell lines depleted of cholesterol and as a consequence, disruption of lipid rafts. Cholesterol depletion of BJ-hTERT fibroblasts using methyl-b-cyclodextrin (MbCD) did not affect PDGFR-b activation as measured by its tyrosine phosphorylation. However, we did observe a small reduction in AKT phosphorylation and a more robust decrease of ERK1/2 activation. In contrast, in the osteosarcoma cell line U2OS, we noticed a deficient receptor activation. Interestingly, in U2OS cells, the ERK1/2 pathway was unaffected, but instead AKT and SRC signaling was reduced. These results suggest that cell type specific wiring of signaling pathways can lead to differential sensitivity to cholesterol depletion. Furthermore, MbCD treatment had a much more pronounced morphological effect on U2OS compared to BJ-hTERT cells. This is consistent with a previous report claiming that cancer cells are more sensitive to cholesterol depletion than normal cells. Our data supports the possibility that cholesterol lowering drugs may impede tumor growth.

Poster 1-12

1-12 1α,25-dihydroxyvitamin D3 and tacalcitol suppress the activation of STAT3 and anchorage-independent growth in T98G glioblastoma cells

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Glioblastoma multiforme (GBM) is the most malignant and lethal primary brain tumour in adults. The therapeutic options are limited making the prognosis of patients poor. Abundant evidence has shown that 1α,25-dihydroxyvitamin D3, the biologically active form of vitamin D3, has antitumoral effects in many types of cancer cells in vivo and in vitro, including GBM. The clinical use of 1α,25-dihydroxyvitamin D3 in cancer is prevented by side effects such as hypercalcemia and hypercalciuria. Therefore, there is a need of 1α,25-dihydroxyvitamin D3 analogues with reduced hypercalcemic and hypercalciuric side effects. In the work presented, effects of 1α,25-dihydroxyvitamin D3 and the vitamin D3 analog tacalcitol on downstream signaling and anchorage-independent growth in T98G glioblastoma cells were investigated. Treatment with 1α,25-dihydroxyvitamin D3 or tacalcitol decreased the level of phosphorylated p70S6 kinase, known to regulate cell growth by inducing protein synthesis. Tacalcitol slightly increased the phosphorylation of PLCg, which modulates several cellular and physiological functions necessary for tumorigenesis. STAT3 is a critical mediator of tumor progression and the result demonstrated that 1α,25-dihydroxyvitamin D3 and tacalcitol suppressed the activation of STAT3. The suppressive effect of STAT3 was also stronger for tacalcitol than for 1α,25-dihydroxyvitamin D3. The results from the anchorage-independent growth assay in soft agar, an assay for detecting malignant transformation of cells, showed that both 1α,25-dihydroxyvitamin D3 and tacalcitol inhibited anchorage-independent growth. In conclusion, the results indicate that 1α,25-dihydroxyvitamin D3 and vitamin D analogues have the potential to inhibit tumorigenicity in GBM, making these compounds a possible therapeutic alternative for GBM treatment.

1-13 Extensive bioinformatics analyses reveal phylogenetically conserved Topoisomerase II sites with novel putative functions

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Topoisomerases are key enzymes that catalyze changes in the topological state of DNA allowing replication, transcription, recombination, and chromosome segregation. They are ubiquitous in all domains of life, from Bacteria and Archaea to Eukarya. In medicine, mainly Topoisomerase II serves as an effective molecular target in several cancer therapies [1]. Therefore we decided to carry out comprehensive bioinformatic analyses of all to date available protein sequences of Topoisomerase II obtained from several thousands of organisms covering the whole tree of life. We found that there are several previously unidentified extremely conserved protein regions and further inspected their potential function in silico. Finally, we identified that some of these regions have putative GTP/GDP binding functions which may serve to rationalize the reported [2] effects of such small molecules (as well as Z-DNA) on Topoisomerase II activities (see also Abstract Červeň et al.). Experimental verification will provide novel avenues for modulating Topoisomerase II function in normal and cancer cells. [1] Vann, KR, Oviatt, AA, Osheroff, N (2021). Topoisomerase II poisons: converting essential enzymes into molecular scissors. Biochemistry 60: 1630-1641. [2] Arndt-Jovin, DJ, Udvardy A, Garner MM, Ritter S, Jovin TM (1993). Z-DNA binding and inhibition by GTP of Drosophila topoisomerase II. Biochemistry 32: 4862-4872.

Poster 1-14

1-14 The role of *Galleria mellonella* cationic protein 8 in insect immunity

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Antimicrobial peptides are the main tools of insects' immune response. The type of synthesized peptides and their levels vary depending on the pathogen attacking the host organism and on the fitness of the infected host. Galleria mellonella cationic protein 8 (GmCP8) is a 9.2 kDa polypeptide detected in the hemolymph of Galleria mellonella larvae. So far, this protein has been shown to act as an opsonin and an inhibitor of some serine proteases. We have isolated GmCP8 from G. mellonella hemolymph and here we report its antimicrobial properties against Gram-positive and Gram-negative bacteria and against fungi. This activity was associated with changes observed on the surface of microorganisms, and with their membrane permeabilization. Additionally, the GmCP8 protein inhibited the activity of metalloproteinase thermolysin. Thermolysin-like metalloproteinases are secreted by many pathogens to digest host proteins, including these of immune properties. We also analyzed the level of expression of genes encoding the GmCP8 protein in the gut and in the fat body of G. mellonella after oral infection with insect entomopathogen Pseudomonas entomophila. Our results show that GmCP8 protein is involved in multiple aspects of insect immunity, given its important role in humoral defence mechanisms.

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Pseudomonas entomophila was a kind gift from Dr. Frideric Boccard (France), whose group isolated this strain in Guadeloupe. The work was financed by National Science Centre, Poland, project number 2020/37/B/NZ6/00167.

1-15 Genetic diversity of peptidylarginine deiminase and the pathogenicity of *Porphyromonas gingivalis*

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Periodontitis (PD) is one of the most common and widespread chronic inflammatory disease. Crucial contributors to the disease are considered bacterial dysbiosis and secreted bacterial virulence factors. One such factor is peptidylarginine deiminase (PPAD), produced by human oral opportunistic pathogen, Porphyromonas gingivalis (Pg). PPAD catalyzes post-translational modification of proteins, which may alter their biological functions. Recent evidence of the critical role of PPAD in gingival cells infection and citrullination encourages studies of the PPAD gene sequence and function in the context of bacterial pathogenicity.

Bioinformatic analysis of the PPAD was conducted in gene sequences deposited in NCBI database (n=63). All clinical strains were verified using NCBI Gene and BioSample (PD, n=60; Ctrl, n=3). The analysis of missense mutations, polymorphic and synonymous variants was conducted using the BLAST program against the W83 Pg reference strain (AF100668.1).

Out of 105 nucleotide substitutions identified, 52 that caused a change in the structure of the encoded protein were divided into 15 conservative and 26 non-conservative mutations. The analysis of the frequency of the individual substitutions allowed the identification of missense mutations (25%) and polymorphic variants (>25%). Moreover, the multi-nucleotide substitutions at eight codons were found. In addition, seven missense mutations, one polymorphic variant (36. 51%) and seven synonymous variants were located in close proximity to the PPAD active center. The polymorphic variant was an A->G transition, resulting in the substitution of asparagine with aspartic acid, nearby position Asn 297 of the PPAD.

The presence of missense mutations and polymorphic variants close to the active center of PPAD in the majority of PD-derived Pg strains (in opposite to controls) indicate a significant heterogeneity of PPAD and may have an effect on Pg pathogenicity. This thesis needs verification in further in vitro studies.

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1-16 Albumin as a platform for designing biologically active carriers

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Due to the specificity of their structure, protein systems are adapted to carry various types of ligands. The structure of many proteins potentially allows two kinds of immobilization of a therapeutic agent, either on the outer surface of the protein or within the protein structure. The BSA structure will define two main active centers, the so-called Sudlow I and II. The conducted studies concern the determination of the effectiveness of BSA as a potential carrier of 5-fluorouracil (5-FU). 5-Fluorouracil is a broad-spectrum anticancer drug against solid tumors. The research was carried out on many levels, determining the number of physicochemical properties of the system using complementary measurement techniques. Critical conditions for complex formation were determined, enabling both the optimization of the system and the achievement of significant correlations between the drug form and the effective localization of the active substance in the structure of the protein molecule. The presence of two amino groups in the 5FU structure contributes to the deprotonation of the molecule at high pH values (pH> 8). This was confirmed by UV-vis and 1H NMR spectroscopic studies in a wide range of pH. The experimental research was supplemented by simulations using molecular dynamics (MD). The 5FU ligand and AN1/AN3 tautomers were randomly docked in the six BSA subdomains. The most effective complex formation was found with the AN3 form.

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1-17 Insecticide-mediated allosteric changes in mosquito voltage-gated sodium channels.

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Mosquitoes are the primary vectors of severe diseases such as malaria, yellow fever, dengue, West Nile fever, or Zika, transmitted in more than 100 countries. They represent 17% of the global infectious diseases, causing almost one million deaths each year. Prevention of vector-borne disease transmissions is largely implemented by the use of insecticides. However, mosquitoes have developed resistance to the most commonly used insecticides. This poses a serious threat, especially at a time of global warming which may affect the extent of vector-borne disease outbreaks. To find new chemicals that could reduce disease transmission, a thorough understanding of molecular mechanisms of insecticide mode of action is required.

One of the major molecular targets of insecticide action is voltage-gated sodium channels (VGSCs). These transmembrane proteins initiate and propagate action potentials in response to membrane depolarization in nerve and muscle. The structure of the α-subunit of VGSC consists of a single polypeptide chain that folds into four domains built up from six transmembrane helices each. Helices S1-S4 constitute the voltage-sensing-domain with helix S4 acting as a voltage sensor, while helices S5 and S6 contribute to the ion-conducting pore. Here, we present a detailed analysis of selected insecticide interactions with Anopheles gambiae mosquito and human (type hNav1.7) VGSCs. Using molecular dynamics computational approach, we investigate the volume of lateral fenestrations present in VGSCs that could enable ligand access to the central cavity of a channel. We delineate a mechanism of allosteric conformational changes in VGSCs induced by insecticide binding. We also propose a new, photoswitchable acyl sulfonamide compound with an azobenzene functional group that could enable light-modulated VGSC conductance in the insect central nervous system.

Our modeling is the first step in the development of a new class of selective, light-controlled insecticides and may facilitate research in insect biology.

1-18 C-reactive protein inhibitors: from cardiovascular disease to malignant processes

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Background

Monomeric C-reactive protein (mCRP) is a critical molecule that perpetuates the aggregation of blood cells linked to acute coronary disease and can play role in carcinogenesis.

Aims

To perform a literature data up-date regarding the possible prognostic and therapeutic role of anti-mCRP substances.

Methods

Over 9,500 papers indexed in Medline were focused on the prognostic or therapeutic role of mCRP in cancer. After using specific criteria for inclusion and exclusion, over 100 original research-type studies were selected and use to understand the mechanistic role of mCRP in inflammation and cancer.

Results

mCRP plays pro-coagulant role and influence the inflammation-based indexes. Except the CRP to albumin ratio, it was also found to be associated with the systemic immune-inflammation/systemic inflammation response indexes, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, aggregate index of systemic inflammation, and monocyte-to-lymphocyte ratio. As all of these indexes are known as prognostic parameters for patients with carcinomas, mCRP inhibitors could be future potential anti-cancer agents used successfully in oncotherapy. The literature data are still scarce, and more complex experimental studies are necessary for a better understanding of the process.

Conclusions

The inhibition of mCRP might have benefits for patients with pro-coagulant disorders but the clinical implications in oncology is far to be understood. This study was partially funded by the CNCS – UEFISCDI, project number PN-III-P4-ID-PCE2020-1540.

1-19 The Influence of DNA damage on glycosylases crosstalk via charge transfer. Electronic Properties of (4*S*/R) spiroiminodihydantoin: a Theoretical Approach.

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The seed of life is concealed in the base sequence in DNA, which has been continuously verified by repair proteins. Genetic information is permanently exposed to external and endocellular factors, which can lead to different lesion formations. Its stability depends on the protein efficiency of recognition and repair systems. Many types of glycosylases can recognize and remove lesioned nucleobases. Therefore, the stability of genetic information depends on how rapidly they recognize DNA damage. Since the end of the twentieth century, the theory states that charge transfer is involved in protein cross-talking through ds-DNA. The glycosylases contained clusters [4Fe-4S]2+, e.g., Endo III and MutY have been frequently investigated. For these reasons, a comparative analysis of short ds-oligo containing (4S/R) spiroiminodihydantoin and 7,8-dihydro-8-oxo-2'-deoxyguanosine is proposed. Moreover, the electronic properties of the discussed ds-oligo in the context of non-equilibrated and equilibrated solvent modes were taken into theoretical consideration. All energetic calculations were performed at the M062x/D95**//M062x/6-31++G** level of theory. The initial geometry has been optimized via the ONIOM methodology. The lowest adiabatic ionization potential (AIP) was assigned for ds-DNA containing a (4S) spiroiminodihydantoin. Surprisingly, the highest adiabatic electron affinity (AEA) was also observed for 4S diastereomer. A lower vertical ionization potential and higher vertical electron affinity were denoted for (4S) spiroiminodihydantoin in a non-equilibrated solvent state. The above supported faster 4S diastereomer recognition by glycosylases than the opposite one, i.e., 4R under the electron transfer mechanism.

1-20 Choline transporter-like protein 1 (CTL1/SLC44A1) as a novel molecular target therapy for hepatocellular carcinoma

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Hepatocellular carcinoma, the most common type of primary liver cancer, has a high incidence and mortality rate, and it is desirable to develop new therapeutic agents to overcome unmet medical needs. Choline is essential for the synthesis of phospholipids, the main component of cell membranes, and abnormal choline accumulation in cancer cells is strongly correlated with malignant tumor growth. The choline transporter is responsible for the transport of choline into cells and is thought to be a target molecule for cancer therapy. In this study, we analyzed the functional expression of the choline transporter in human hepatocellular carcinoma cells and investigated the antitumor effects of its inhibitors.

Choline transporter-like protein 1 (CTL1) was highly expressed in human hepatocellular carcinoma cell line, HuH-7 and localized to the cell membrane. [3H]Choline uptake in HuH-7 cells was Na+-independent and pH-dependent, via a single transport system. These choline transport properties in HuH-7 cells were similar to those of CTL1. In choline-deficient cultures, decreased cell viability, increased caspase-3/7 activity, and the appearance of annexin V-positive cells were observed. The novel choline uptake inhibitors Amb4269951 and Amb4269675 decreased cell viability and increased caspase-3/7 activity. These choline uptake inhibitors activated the sphingomyelinase/ceramide pathway, and ceramide inhibited cell viability and increased caspase-3/7 activity.

CTL1 is responsible for the transport of extracellular choline, and inhibition of CTL1 function induces apoptosis, suggesting that CTL1 may be a new target molecule for cancer therapy. Furthermore, CTL1 inhibitors induce apoptotic cell death by a novel mechanism of action, suggesting that they may be novel therapeutic agents for liver cancer.

1-21 Neutralizing ability of a single domain VNAR antibody: In vitro neutralization of SARS-CoV-2 variants Delta and Omicron

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The high transmission rate of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to the emergence of new variants harboring different mutational patterns that have been circulating around the world since the beginning of the COVID-19 pandemic. The new variants of SARS-CoV-2 have challenged the development of effective therapies based on neutralizing antibodies targeting the spike protein. Variable new antigen receptors (VNARs) are an antibody technology that has been introduced into the list of possible therapeutic options to counteract SARS-CoV-2. Their unique qualities, such as high affinity for the target molecule, paratope reformatting, and greater stability make them attractive molecules to counteract emerging SARS-CoV-2 variants. In this work, we characterized a VNAR antibody (SP240) that was isolated from a synthetic phage library of VNAR domains and screened against the receptor-binding domain (RBD) of the spike protein. In the phage display we used plasma with high neutralizing antibody titers against SARS-CoV-2 to selectively displace the VNAR antibodies bound to the antigen SARS-CoV-2 RBD. The selected VNAR SP240 was modeled in silico, and it has been suggested that the SP240 possesses a high affinity to the spike protein of the Omicron (-41.06 REU) and Delta (-41.97) variants. Additionally, it was unveiled that SP240-binding epitopes are located within the ACE2-binding interface of the spike protein (437-508 aa), indicating that the neutralizing mechanism of SP240 is through the direct blocking of the spike-ACE2 interaction. The SP240-neutralizing ability was tested against the live SARS-CoV-2 virus, Delta and Omicron. The VNAR antibody SP240 cleared the infection of both variants in the lung cell line A549-ACE2-TMPRSS2 (>90%), giving an NT50 of 0.1833 ug/ml for Omicron, and 0.8818 ug/ml for Delta. This study highlights the potential of VNAR as a neutralizing antibody and the probable resilience of VNAR SP240 to emerging SARS-CoV-2 variants.

2-1 Molecular Dynamics Modelling and Structural Analysis of FH2 Domain of Formin DAAM (Drosophila Melanogaster)

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In previous studies, some FH2- and WH2-domain-containing formins were shown to behave as intrinsically disordered proteins. The aim of this research was to study the structural dynamics of the FH2 domain of formin DAAM.

To model the structural dynamics of the FH2 domain, all-atom explicit simulations of chain A of FH2 domains of DAAM, DAAM1 and mDia1 solvated in a water box were conducted at temperatures ranging from 293.15 to 353.15 K using VMD 1.9.2, NAMD 2.14 and Amber Tools 21. To investigate the structural features of the FH2 domain, measurement of the intrinsic tryptophan fluorescence of the FH2 domain of DAAAM was conducted using a Jobin Yvon spectral fluorimeter (Horiba) at temperatures ranging from 293.15 to 353.15 K. G-actin and SALS WH2 were used as controls of ordered and disordered protein with well-described structural behavior. Fluorescence was excited at 295 nm and recorded in the range of 300–450 nm (slit width 7 nm). Correction for the inner filter effect was also performed using a UV2700 spectrophotometer (Shimadzu).

Our RMSD and peptide bond energy analyses of molecular dynamics of FH2 domains, chain A, of all studied formins revealed insignificant changes in RMSD values at temperatures ranging from 293.15 to 353.15 K, similar to SALS WH2. In contrast, RMSD values of G-actin showed considerable increases at 328 K (55 oC), which corresponds to the denaturation of G-actin molecule at this temperature and its transition from a native and ordered to denatured and disordered state, which is well-described in the literature. Our fluorescence spectrometry study of max wavelengths of fluorescence intensities for FH2 DAAM showed a broader temperature range of transition on its melting curve from a native to unfolded state during its thermal denaturation if compared to G-actin, but completely different from SALS WH2, which can be attributed to a large variance in temperature points of denaturation of different secondary structures in FH2 domain, which supports the conclusion on the mostly disordered nature of the FH2 domain of DAAM lacking well-defined tertiary structures.

Poster 2-2

2-2 Unravelling the potential to form amyloid-like aggregates in proteins of Zika virus

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Human-encoded soluble proteins have the ability to form amyloid-like fibrils. This generic phenomenon causes severe human illnesses such as Alzheimer's disease, Parkinson's disease, and several taupathies. Such disease-causing proteins/peptides are composed of certain fragments known as aggregation-prone regions (APR) that drive their misfolding, ultimately leading the assembly into highly ordered β-sheet-rich fibrils that have distinct soluble states compared to the native proteins. Similarly, viral proteins have been found to form such insoluble aggregates in vitro as well as in vivo. Previously, the capsid anchor region of Zika virus (ZIKV) was found to highly aggregate in in vitro conditions. Here, we show that ZIKV consists of several other such aggregation-prone hotspots spread across its entire proteome. By employing high-accuracy prediction tools, we identified APRs in both structural and non-structural proteins of ZIKV. Furthermore, using a combination of dye-based assays (ThT and ANS), Raman spectroscopy, and X-ray diffraction, we establish the in vitro formation of amyloid-like fibrils by ZIKV proteins. The morphological features of these fibrils are investigated using microscopy techniques such as HR-TEM and AFM. We found the full-length envelope protein, its fragmented envelope domain III (EDIII), NS3 helicase protein, and cytosolic region of NS4B protein to be highly aggregating in the experimental setup. Furthermore, these aggregates are cytotoxic in nature. Our findings pave the way for an extensive and detailed functional analysis of these predicted APRs in the future to enhance our understanding of the role played by amyloids in the pathogenesis of flavivirus.

2-3 A proposed computational target fishing and target identification framework revealed potential drug targets of Rooibos in Diabetes Mellitus

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Introduction: Modern drug discovery's focus is moving away from the traditional "one target one drug" approach. Considering that natural products (NPs) act via the modulation of multiple proteins, opposed to a single target, drug discovery based on NPs is an attractive re-emerging research field. Here, we present a computational workflow, integrating selected in silico target fishing and target identification platforms for improved drug target and drug-target-pathway identification of query compounds towards a specific disease of interest.

Methodology: The literature reports Rooibos tea to exert beneficial health benefits for Type 2 Diabetes Mellitus (T2DM) patients. Twelve natural compounds previously isolated from aqueous Rooibos extracts (representing Rooibos tea's NPs), were screened via the proposed in silico workflow to identify potential drug leads and mechanisms of action towards T2DM.

Results: Initial target fishing predicted 740 protein targets of interest. Each of the 12 Rooibos compounds was associated with one or more of these protein targets. Target identification reduced the protein targets to 20. The most promising drug target proteins were IGF1R, CYP7A1, and IFNG.

Discussion: Structure-activity relationships of the query Rooibos compounds revealed the flavonoid compounds, over the dihydrochalcones, to potentially modulate certain signaling pathways associated with T2DM. Of note, the PPAR (via CYP7A1) and TGF-beta (IFNG) signaling pathways modulate pathways related to T2DM's main disease pathway. The drug-target-pathways AMPK (via IGF1R) and PPAR (via CYP7A1) signaling pathways are associated with the anti-diabetic drug class, Thiazolidinedione. Although some of the findings are supported via previous literature reports, further experimentation for confirmation is warranted.

Conclusion: In conclusion, target fishing combined with target identification computational biology tools may provide insight into previously unknown drug targets and drug-target-pathways of query compounds. Thus, the proposed computational workflow is worthy to assist with the drug discovery process of naturally derived compounds.

2-4 NRF2 gene knockout contributes to mTOR signaling modulation, cell cycle arrest, and cisplatin sensibilization in DU145 prostate cancer cells

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Prostate cancer represents one of the main causes of mortality in men, and it still does not have established effective treatments, making necessary the identification of target molecules that lead to improved sensitivity to treatments. NRF2 (Nuclear factor erythroid 2-related factor 2) is a key protein involved with drug efflux, detoxification, and oxidative stress balance. The relation between NRF2 and the mTOR pathway in cancer development is ambiguous since it can be either activated or inhibited by rapamycin. On the other hand, NRF2 can activate the mTOR pathway, showing a complex relationship between these two proteins. Here, we performed the NRF2 knockout (NKO) in DU145 cells, using the CRISPR/Cas9 system, which was confirmed by Sanger sequencing and the downregulation of targets NQO1 and HO1. Besides presenting lower contents of Cyclin D1 and CDK2 proteins, NKO cells showed cell cycle arrest through accumulation in S and G2 phases. The content of total mTOR and S6 proteins had a tendency to decrease in NKO cells, which was followed by a lower ability of these cells to form colonies in a clonogenic assay. NKO cells also showed a reduced migration rate compared to wild-type cells in a scratch assay. Finally, the cisplatin treatment induced the cleavage of PARP1 in NKO cells, indicating that cisplatin led to apoptosis in these cells. Taken together, these results show that NRF2 represents a mediator of oncogenesis and can be a potential target to sensitize prostate cancer cells to chemotherapy.

2-5 Elucidating the amyloid aggregation propensity of zika virus transmembrane domains Capsid Anchor and 2K

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Zika virus (ZIKV), a member of Flavivirus genus, is an arthropod-borne human pathogen. ZIKV infection has been linked with severe diseases such as Guillain-Barré syndrome, microcephaly and myelitis. Its genetic material is a positive-sense, single-stranded, enveloped RNA (+ssRNA) that encodes a single polyprotein. Translation of the genome occurs close to the host cell endoplasmic reticulum (ER) in a manner that the polypeptide chain is weaved back and forth through multiple transmembrane domains (TMD). Capsid-anchor (CA) and 2K peptide of ZIKV are small, single-pass transmembrane sequences that separates Capsid from prM and NS4A from NS4B, respectively. These are also the only TMD's that carry cleavage sites specific for both host viral peptidase and viral protease enzyme. Upon cleavage,other viral proteins mature, leaving behind CA and 2K embedded within the ER membrane. It is a well-established fact that viruses hijack the machinery of infected cells for their own benefit, thus disrupting normal functions, including the ER-associated protein degradation (ERAD) pathway. So, question arises about the fate of these TMDs. Both CA and 2K perform multiple roles in the virus assembly and maturation but in isolation, whether they further contribute to cellular pathology is unknown. Disease caused by aggregation of viral proteins is a relatively new and exciting concept that is being explored worldwide now. Therefore, in this study, we investigated the amyloid-forming propensity of CA and 2K peptide at physiological conditions. We observed their aggregation behavior using dye binding assays and kinetics. The morphological analysis explored by high-resolution microscopy (TEM and AFM) revealed characteristic amyloid-like fibrils. Further, the effect on mammalian cells exhibited the cytotoxic nature of the CA amyloid-fibrils. Our findings collectively shed light on the amyloidogenic phenomenon of flaviviral protein, which may contribute to their infection and unlock a novel paradigm in virus-host relations.

2-6 Selection and analysis of multitarget designed ligands able to inhibit Protein Tyrosine Phosphatase 1B and Aldose Reductase as new drugs for treatment of Type 2 diabetes and its complications.

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Type 2 diabetes (T2D) is a chronic disease that is expanding around the world and is characterized by alterations in glucose, lipid and protein metabolism caused by the body's inability to use or produce adequate amounts of insulin. Although T2D patients may rely on different types of oral antihyperglycemic drugs, many of them see their health condition deteriorate over time due to the inability of drugs to mimic the physiological role of insulin. The economic and social cost of such pathology is raising every year, with the risk of becoming unsustainable in the next few decades. Therefore, the development of new drug therapies for the treatment of this disease will be a priority for the next few years.

The tyrosine phosphatase 1B (PTP1B) and aldose reductase (AR) are considered promising pharmacological targets for treatment of T2D and some related pathologies such as cataract/blindness and nephropathy.

To address the need to improve glycemic control and delay the onset of diabetes-related complications, we studied the activity of a series of novel multi-target drugs specifically designed to target both PTP1B and AR. Preliminary assays revealed that CA4, SP4, CA8 and SP3 compounds were the most active against both targets. Afterwards, kinetic analyses showed that CA4 and SP4 molecules acted as pure non-competitive inhibitors, whereas CA8 and SP3 acted as mixed-type non-competitive inhibitor of PTP1B. Moreover, both CA4 and CA8 behaved as mixed non-competitive inhibitors, while CA4 behaved as a tight binding inhibitor of AR. Docking analyses were carried out to highlight the interaction mode of such molecules with targets. Finally, tests carried out with C2C12 cells confirmed that treatment with CA4 and CA8 compounds enhanced phosphorylation of Akt (negatively regulated by PTP1B) and improved glucose uptake, suggesting them as leading compounds for development of new antidiabetic drugs.

2-7 Functionalization of PAMAM dendrimers by the Protein Corona

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Nanoparticles are increasingly used in nanomedicine due to their unique physicochemical properties. An essential aspect of the practical application of nanoparticles is understanding the mechanism of their interaction with blood plasma proteins, of which there are over 2,932. The interaction mechanism of small "soft" nanoparticles with body fluids differs significantly from the behavior of hard particles. Therefore, poly(amidoamine) (PAMAM) dendrimers containing various functional groups in their structure were selected for the research. PAMAM dendrimers are synthetic, non-immunogenic, hyperbranched polymer systems with nanometric dimensions, the synthesis of which can be precisely controlled in terms of size, shape, molecular weight, composition, and reactivity1.

The properties of the corona protein on the surface of the dendrimers were monitored for the PAMAM /BSA and PAMAM/fibrinogen systems. UV-Vis spectrophotometry and laser Doppler velocity (LDV) allowed for determining the efficiency of forming the dendrimer-protein complex. The conformational stability of the considered systems and the aggregation process were controlled using the Dynamic Light Scattering (DLS) method. Potential protein structural changes induced by complex formation were assessed on the basis of circular dichroism (CD) spectra. Quartz microbalance (QCM-D) was used to monitor the kinetics of bilayer formation on the gold surface and control the effectiveness of surface passivation in selecting plasma proteins.

Changes in the zeta potential of the dendrimers clearly indicate the formation of a stable PAMAM-protein complex. The formed albumin corona on the surface of the dendrimer effectively prevents adsorption of fibrinogen and, consequently, the potential process of opsonization of the system. In the range of low concentrations of dendrimers relative to the protein, no significant changes in the secondary structure of the protein are observed.

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2-8 Effect of NADPH oxidase (NOX2) inhibition in a resistant amebic experimental model

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Background. Entamoeba histolytica is the causal agent of intestinal amoebiasis; it is the third-highest cause of death from parasitic disease in the world, which can cause a serious condition called amebic liver abscess (ALA). The BALB/c mouse was determined to be a model of resistance, in which amoebae are eliminated by the fourth day after inoculation. Several works have analyzed the role of neutrophils in amebiasis, how neutrophils interact with amoebas, and the enzymes that are present in neutrophils, which are serine proteases, myeloperoxidase (MPO), NADPH oxidase (NOX2), and superoxide dismutase (SOD). Currently, there is no information about the enzyme NOX2 in ALA. Objective. The aim of this study was to analyze the role of the enzyme NOX2 in the resolution of ALA in Balb/c mice. Methodology. Male Balb/c mice were divided into two groups: I) amoeba-inoculated animals (CT) and 2) amoeba-inoculated animals treated with apocynin, a NOX2 enzyme inhibitor (0.41 mg/25 g). The animals were euthanized at 3, 6 and 12 h post-infection. In the ALA samples, the ALA percentage, histological changes during the evolution of ALA, and number of amoebas were determined. Results. A significant increase in the percentage of lesions at 3 and 6 h was observed in the group treated with NOX2 inhibitor, and morphologic changes such as smaller inflammatory foci with larger areas of ischemia were also observed, in contrast to the control group. In the group treated with the NOX2 inhibitor, it was observed that the number of amoebas without apparent damage increased compared to the CT group. Conclusions. The results obtained show that the inhibition of NOX2 in the ALA resistance model increases the liver parenchymal damage at early stages.

2-9 ATRAID, a highly glycosylated protein localized to membranous cellular compartments

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All-trans retinoic acid-induced differentiation factor (ATRAID) is a well-conserved, poorly characterized gene. Its expression is induced by all-trans retinoic acid. Two transcripts are listed in NCBI: transcript 3 (isoform C) and transcript 1 (isoform A). The difference between them is lacking N-terminal signal peptide in isoform A. Both isoforms are predicted to contain several N-glycosylation sites. ATRAID binds to NELL-1 at the nuclear envelope of human osteoblasts and dental pulp cells, decreasing proliferation by reducing the expression of cyclin D1 and increasing differentiation. In ARPE-19 cells, ATRAID accelerates cellular senescence. Its expression increases in RPE-cells derived from aged mice. ATRAID locates to the mitochondria of ARPE-19 cells and binds to NRF2. Its overexpression induces morphological changes and functional disruption in mitochondria. Moreover, it is shown that ATRAID binds with lysosomal proteins, LAMP1 and SLC37A3. The complex of ATRAID/SLC37A3 is required for releasing nitrogen-containing bisphosphates (N-BPs) from the lysosome to the cytoplasm.

In this study, we aim to find out more about ATRAID. We produce transgenic cell lines expressing ATRAID with a c-terminal Flag-tag. Western blotting reveals ATRAID is highly N- glycosylated. The combination of subcellular fractionation and ICC shows that ATRAID is absent from the cytosol and the nucleus and is located in the plasma membrane and intracellular vesicles or compartments.

2-10 The Effects of Human Menstrual Blood Mesenchymal Stem Cell Growth Factor Proteins on Cartilage Tissue Regeneration in Vitro

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Growth factors play an important role in regulating cellular functions, stimulating metabolic activity and promoting stem cell differentiation. These proteins are frequently analyzed in mesenchymal stem cells (MSCs), as MSCs have a strong capacity of differentiating into adipose and skeletal tissues. Bone marrow MSCs (BMMSCs) are a classic type of stem cell that are mostly studied and used for cartilage tissue repair after trauma or osteoarthritis (OA). Menstrual blood MSCs (MenSCs) are much less characterized; however, they are known to possess more pronounced stem cell properties, as compared to classical BMMSCs. Moreover, MenSCs possess a large amount of growth factors responsible for effective endometrium regeneration, which might be a potential co-stimulant for other tissue regeneration purposes. Although MenSCs are attractive alternatives to BMMSCs, their use for cartilage tissue regeneration is still under consideration and research.

The aim of the study is to evaluate the potential of MenSC growth factors in stimulating BMMSCs' chondrogenic differentiation and their role in protecting cartilage from degradation under inflammatory conditions in vitro.

MenSCs and BMMSCs were characterized according to stem cell markers (flowcytometry) and differentiation capacity. Chondrogenic differentiation of co-cultured cells was evaluated according to cartilage extracellular matrix protein formation (histology) and the secretion of TGF-β1, activin A, IGF-1 and BMP2 (ELISA). The results demonstrated that the chondrogenic differentiation of BMMSCs was more pronounced in co-culture with MenSCs, as compared to single BMMSC cultures. The secretion of all growth factors was increased in cell co-cultures, as compared to single populations. In addition, human cartilage explants co-cultured with MenSCs revealed increased growth factors and reduced cartilage extracellular matrix protein release under inflammatory conditions.

In conclusion, MenSCs may turn out to be a promising population of stem cells for their rich secretion of growth factors and capacity to stimulate BMMSC differentiation and prevent cartilage from degradation.

Poster 2-11

2-11 Riluzole antiamebic activity against *Entamoeba histolytica* trophozoites

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Background: Amebiasis is caused by the parasite Entamoeba histolytica (E. histolytica); this affects millions of people throughout the world. Metronidazole (MTZ) is currently used for the treatment against this protozoan. MTZ can have different adverse effects in humans, such as genotoxic, teratogenic, mutagenic and carcinogenic effects in animals. In addition, the resistance of E. histolytica trophozoites with this compound has been described. Therefore, it is necessary to test new antiamebic drugs to avoid these effects. There are some studies that show that Riluzole has activity against some protozoan parasites. Objective: The aim of the present study was evaluated for the first time, the in vitro antiamebic activity of Riluzole on E. histolytica. Methodology: Trophozoites were treated with different concentrations of Riluzole after 5 h, compared with MTZ at the same concentrations. The viability was determined using the WST-1 cell proliferation reagent, and the 50% inhibitory concentration (IC50) was obtained by linear regression between the concentration of the compound and the percentage of inhibition. Also, the genic expression of amebic enzymes such as Thioredoxin (Trx), Peroxiredoxin (Prx), Rubrerythrin (Rr), Thioredoxin reductase (TrxR) was evaluated. Results: Our results showed a decrease in amoebic viability from a concentration of 30 µM to 480 µM, the latter being the one that presented a greater antiamoebic activity, observing around 51% of dead amoebae. The IC50 was 319.5 µM at 5 h. The gene expression analysis showed a significant overexpression of Prx and TrxR enzymes, and it does not show a difference in Trx and Rr enzymes gene expression. Conclusion: Our results demonstrated that Riluzole has an antiamebic effect against E. histolytica and Prx and TrxR enzymes are the antiamebic targets. This work was supported by SAPPI-IPN, Proyecto Multidisciplinario Módulos 20221473, 20221426, 20221514 and 20220507.

2-12 A new approach to dissect the molecular basis of abnormal brain intercellular communication in schizophrenia

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Alterations in brain intercellular communication mechanisms during schizophrenia have been extensively unveiled in neuroimaging studies. However, the molecular basis of these alterations remains poorly understood. In this work, we use state-of-the-art clinical neuroproteomics and systems biology to define the proteome compositions of brain extracellular vesicles (EVs), as mediators of intercellular communication, between specific brain regions (prefrontal cortex, caudate and hippocampus). Post-mortem brain tissues of patients with a schizophrenia diagnostic and age-matched controls were profiled. The obtained results indicate changes in the expression of multiple EV proteins, including a significant modulation of synaptic-specific proteins in the hippocampus. Moreover, the proteomic profiling of brain EVs also indicated the down-regulation of the proteins myelin-associated protein MBP and DPYSL2 in the prefrontal cortex. Of note, alterations in the levels of DPYSL2 have been directly linked to the onset of schizophrenia. Additionally, some of these proteins were revealed here as connected to an altered EVs-mediated interregional brain communication system in psychotic patients. The data described in this work represent the first global analysis of the proteome compositions of brain EVs in patients with schizophrenia and lay the groundwork for new therapeutic targets in this disease.

2-13 Inhibition of NADPH oxidase (NOX2) in a susceptible amebic experimental model.

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Background. Amebiasis is an intestinal infection caused by Entamoeba histolytica (E.h) that affects millions of people in Latin America, Africa and Asia. Amebic liver abscess (ALA) is the most common complication of amebiasis. Studies conducted using a susceptibility model of ALA demonstrate that neutrophils (NPs) are the first cells to contact E.h at the liver parenchyma. In a previous report, our group demonstrated that in an in vivo hamster model, myeloperoxidase (MPO), an enzyme secreted by NPs, showed inadequate activation due to a significant decrease in enzymatic activity and expression. Moreover, NPs produce and release reactive oxygen species (ROS) such as NADPH oxidase (NOX2), an ROS-producing oxidase enzyme. There is no information regarding the presence and role of NOX2 in ALA evolution. Objective: The aim of the current work was to analyze the in vivo role of NOX2 in an ALA susceptibility model. Methodology: Male hamsters were randomly distributed into two groups: 1) a group inoculated with amebas and 2) a group inoculated with amebas and treated with apocynin (AP) NOX2 inhibitor (1.64 mg of AP). Animals were sacrificed at 3, 6 and 12 h post-infection. In samples of ALA, we determined: 1) the percentage of lesions, the morphologic changes during ALA evolution and quantification of the number of E.h in ALA. Results: A significant difference between the two groups in the percentage of lesions was determined. Additionally, morphologic changes were observed between both groups in the ALA. Finally, hamsters treated with AP showed significant differences in the percentage of viable E.h compared with the untreated hamsters. Conclusions: Our results show that during the pathogenesis of ALA, the absence of NOX2 favors the resolution of ALA, probably due to the lack of ROS.

2-14 Antiamebic effect of kaempferol against *Entamoeba histolytica* through dysregulation of amebic enzymes

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Background: Entamoeba histolytica (E. histolytica) is a protozoan parasite in humans that provokes amebiasis. The most widely employed drug against E. histolytica is metronidazole; however, studies have reported that it induces genotoxic effects, DNA damage, and furthermore, some ameba strains are resistant to metronidazole. Due to this, it is necessary to explore new compounds that can eliminate E. histolytica. Flavonoids are natural compounds that have demonstrated an inhibition of amebic growth and dysregulation of different amebic enzymes. Despite the knowledge acquired, its target and action mechanisms are not completely dilucidated. Objective: The present work evaluates the effect of kaempferol against E. histolytica trophozoites. Methodology: Trophozoites were incubated with kaempferol or metronidazole (reference drug) for 90 min at different concentrations for: viability analysis using WST-1 and genic expression of amebic enzymes as Thioredoxin (Trx), Peroxiredoxin (Prx), Rubrerythrin (Rr), Thioredoxin reductase (TrxR). Results: Our study demonstrated a significant reduction of amebic viability of trophozoites incubated with kaempferol at 130, 140, and 150 mM concentrations, compared with metronidazole at the same concentrations (p0.0001). The gene expression analysis showed a significant downregulation of Prx and Rr enzymes. No differences were observed in Trx and TrxR enzymes gene expression. Conclusion: Our results revealed that kaempferol has an anti-amebic activity associated to the downregulation of amebic gene expression like Prx and Rr enzymes, which participate in detoxification of ROS and defense of parasite. This work was supported by SAPPI-IPN, Proyecto Multidisciplinario Módulos 20221473, 20221426, 20220507 and 20221514.

2-15 The Search of New Small Molecules for the Treating of Cardiac Amyloidosis by Molecular Docking into Target Protein Receptors

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Cardiomyopathies are severe diseases that affect 2.5 million people per year.
Cardiac amyloidosis, associated with the deposition of the abnormal protein amyloid in the cardiac muscle and surrounding tissues, is currently a difficult and challenging disease, which leads to the impairing of heart structure and function, resulting in heart failure syndrome and death.
The number of medications for the treating of cardiac amyloidosis is currently extremely limited, and all of them have non-selective action modes with significant side effects.
Molecular docking of small molecules into the target proteins allows us to find a set of perspective organic molecules which may result in the effective therapy with significantly less side effects.
Regularised models of the spatial structures of 136 core receptor proteins in the PDBbind database were built from the existing crystal structures of these proteins in the PDB database using standard protocols and tools from ICM-Pro (Molsoft LLC, USA). The spatial structures of the 10 small molecules were either taken from the ZINC database (http://zinc.docking.org) or the corresponding spatial structures of potential ligands were constructed using the built-in ICM-Pro molecular editor.
The paper presents the results of the virtual screening allowing the identity of best predicted ligands and the results of the molecular docking of 10 small molecules into the active centers of 136 proteins. Moreover, the side effects of pharmaceutical agents currently used in clinical practice, as well as potential drugs with known cardioprotective activity confirmed in animal models, have been evaluated.
This research is expected to broad the fundamental knowledge about the interaction between potential small molecules with the target receptors in the field of cardiac amyloidosis treatment.
This research was funded by Russian Science Foundation, project number 21-74-20093.

2-16 Identification of the Structure and Genomic Variants in Amyloid Protein Fibrils by Next-Generation Sequencing

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The most common protein components of fibrils in cardiac amyloidosis are the light chains of immunoglobulin (AL) and amyloid transthyretin (ATTR).

Protein aggregate myopathies may also result in a hypertrophic phenotype and represent a group of hereditary or acquired muscle disorders that are morphologically characterized by an abnormal accumulation of proteins in muscle fibers. Hereditary aggregate myopathies are caused by mutations of cytoskeletal and sarcomeric proteins. Myofibrillary myopathies, actinopathy, and myosin aggregate accumulation disease are well-known examples of this group of diseases.

During this work, we realized:

1) A search and compilation of a database of patients with hypertrophic phenotype with suspected transthyretin amyloidosis of the heart, in whom the results of the initial diagnosis of this disease were questionable or weakly positive (a total of 53 patients were examined). Echocardiography, 99Tc-DPD-myocardial scintigraphy, control of clinical blood analysis, and control of biochemical blood analysis (ALT, AST, lipidogram, creatinine, potassium, bilirubin, INR, troponin) were conducted.

2) Performing parallel exomic high-performance next-generation sequencing with an Illumina Nexseq instrument using a set of target exomic probes.

As a result, patients with suspected amyloid myocardial damage were identified, and next-generation sequencing was performed to identify the genomic variants and structure of amyloid fibrils.
This research was funded by the Russian Science Foundation, project number 21-74-20093.

2-17 The Sars-CoV and Sars-CoV-2 spike protein interactions with the human protein receptor ACE2 and the impact of zinc ions on that interaction

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Understanding the role of SARS peak glycoprotein and the nature of its interaction with a host cell receptor protein remains fundamental to understanding viral infectivity and pathogenesis. One hypothesis explaining the higher SARS-CoV-2 transmission rate compared to SARS-CoV is the genetic recombination of the S.1 MS protein, X-ray crystallography and cryoelectron microscopy have provided important insight into SARS-CoV-2 interaction with the host cell receptor by elucidating three-dimensional structures Spike viral and ACE2.2 host proteins. Our in-depth thermodynamic and spectroscopic studies have revealed an interesting feature of these interactions, namely that the binding strength to the human receptor is very similar for both viruses, but they differ in their enthalpy and entropy changes. Since the ACE2 protein is a zinc metalloenzyme, we also investigated the effect of zinc (II) ions on the binding of the S protein to the receptor protein in two different buffers.

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2-18 Characterisation of the Plasmodium falciparum 90 kDa heat-shock protein

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Plasmodium falciparum (Pf) parasites are the causative agents of the most lethal form of Malaria. Despite progress made towards eradicating the disease, antimalarial drug resistance is an emerging threat to these efforts. To survive the drug pressure, the parasites up-regulate the expression of heat-shock proteins (Hsps). Hsps are molecular chaperones that are essential for the survival of the parasites and are thus desirable drug targets. Hsps are crucial for folding numerous secreted proteins and for maintaining cellular homeostasis. The 90 kDa Hsps (Hsp90) play a central role in protein quality control. There are four members of Hsp90s expressed by the parasites that are localised in different cell compartments. Among these, both the cytosolic (PfHsp90) and the endoplasmic reticulum resident (PfGrp94) are essential for parasite survival. The PfHsp90 is potentially important for chaperoning parasite proteins that are in the cytosol and PfGrp94 for proteins secreted to the host's red blood cells. Therefore, this study elucidated the structure and functional features of PfHsp90 and PfGrp94 that make these proteins amenable for antimalarial drug targeting.

2-19 Exploring the chaperoning activity and ligandability of the most versatile human protein carrier, the HSA

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Human serum albumin (HSA) is the main zinc (II) and Fatty acids (FAs) carrier in blood plasma. The HSA site with the strongest affinity for zinc (II), multi-metal binding site A, is disrupted by the presence of fatty acids (FAs). Therefore, the FA concentration in the blood influences zinc distribution, which may affect both normal physiological processes and a range of diseases. Fluctuation in zinc (II) ions homeostasis is a causative agent of Parkinson's disease and other neurodegenerative diseases due to the direct interaction of zinc (II) ions with α-synuclein (AS) and promoting AS aggregation. Based on the current knowledge of HSA's structure and its coordination chemistry with zinc (II), we investigated zinc (II) interactions and the effect of various FAs on HSA structure, stability, and zinc (II) binding. We also examined how HSA, the most abundant protein in human blood, binds to AS and protect it from aggregation by zinc (II). Our results show the existence of one high-affinity zinc (II) binding site and multiple low-affinity sites present on HSA. Upon the binding of FAs to HSA, we observed a range of behaviors in terms of zinc (II) affinity, depending on the type of FA. Then We conclude that zinc (II) enhances the anti-aggregation chaperoning role of HSA that relies on protecting the hydrophobic N-terminal and NAC regions of AS, rather than the polar negatively charged C-terminus. This suggested a previously undocumented role of zinc (II) in HSA function and AS aggregation.

2-20 EGF receptor signaling inhibits type I interferon response in models of oncolytic therapy by vesicular stomatitis virus

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Oncolytic viruses (OV) represent a new era of cancer therapy as the antiviral response is usually impaired in cancer cells [1][2]. OV therapy is considered a promising option for the treatment of neoplasms, alone or in combination with other approaches [3]. Among viruses considered attractive platforms for developing OV strains is Vesicular stomatitis virus (VSV), a relatively small single-strand RNA genome member of the rhabdovirus family [4] with proved antitumor efficacy [5]. The aim of the study is to determine the differences in the sensitivity of tumor cells to VSV in order to predict the efficiency of viral therapy. We tested a panel of cancer cell lines (DBTRG-05MG, HOS, U251, A172, HEK293T, RD, primary glioma cells) from our collection in order to assess its sensitivity to VSV after the pretreatment with Type I interferon and anti-EGFR drug Gefitinib. Our results showed a significant difference between cell lines in intensity of its response to interferon treatment. Some of cells were protected from VSV infection. HER2 protein was significantly overexpressed in some cell lines with potentially defected interferon system, supporting recent reports that EGF receptor signaling attenuates interferon responses via HER2 co-receptor activity [6]. We showed that gefitinib influences the type I interferon response in VSV sensitive cell lines, enhancing their protection from VSV infection. Accordingly, the combined treatment of cells with EGF receptor inhibitors such as gefitinib and type I interferon increases the resistance of sensitive cell lines to VSV. These gefitinib effects led us to the clinically significant conclusion that the combination of anti-EGFR therapy with interferon-sensitive oncolytic viruses is not effective for tumors with higher HER2/neu expression. More studies are needed to confirm our findings and suggestions in the follow-up efforts on larger sets of tumor samples.

2-21 Effects of DGAT1 deficiency on keratinocyte growth and function

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The epidermis is comprised of several layers of keratinocytes at different stages of differentiation. The topmost layer is formed by keratinized and anucleated cells that are cross-linked and embedded in a lipid-rich matrix, forming an impermeable protective barrier. This epidermal extracellular matrix is delivered by lamellar bodies produced by keratinocytes, and contains various lipids, antimicrobial peptides and proteolytic enzymes. Among the enzymes involved in the production of epidermal lipids, DGAT1 (acyl-CoA:diacylglycerol acetyltransferase 1) is believed to play a significant role. Its ability to catalyze the synthesis of a broad spectrum of lipids, retinoids and waxes contributes to the maintenance of the proper barrier function of the skin. Murine DGAT1 deficiency is mainly characterized by lower fat-pad weight, resistance to diet-induced obesity, and the absence of milk production, but Dgat1KO mice also exhibit hair loss, sebaceous gland atrophy and increased trans-epidermal water loss. Skin abnormalities in DGAT1 null mice might be an effect of the disturbed lipid synthesis in keratinocytes; however, it remains unclear how DGAT1 absence directly affects these cells. In addition to the synthesis of triacylglycerols, DGAT1 is known to take part in retinoid metabolism due to its acyl-CoA:retinol acyltransferase (ARAT) activity. Moreover, DGAT1's physiological function in esterifying retinol has been observed in mouse skin. It has also been shown that retinoids can regulate keratinocyte proliferation and differentiation which, taken together, led us to investigate the role of DGAT1 in maintaining proper keratinocyte function. We performed the RNA-Seq analysis of Dgat1KO and Dgat1WT epidermal transcriptome, followed by functional assays on primary mouse keratinocytes. As a result, we determined the impact of DGAT1 deficiency on the keratinocyte proliferation, differentiation, molecular signatures and biological processes. Overall, this study shows that DGAT1 modulates keratinocyte function in the epidermis and plays a vital and multifunctional role in skin protection.