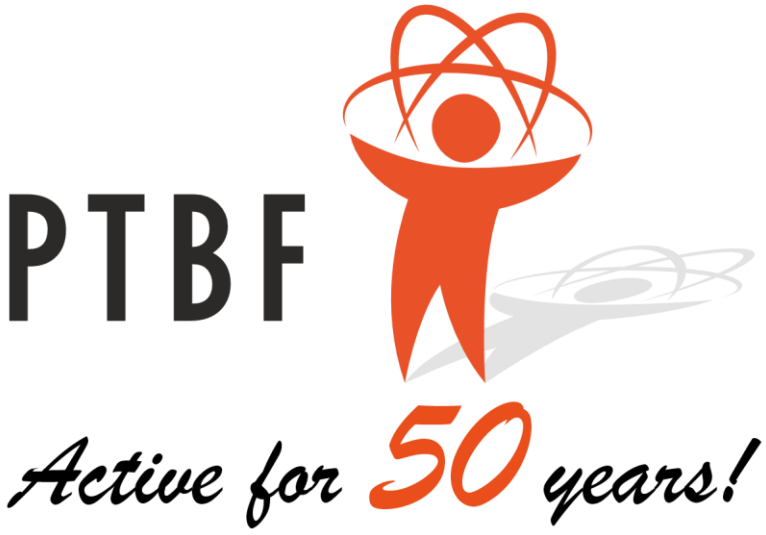
Temporary Abstracts Book



Ultimately, the abstracts will be published in Current Topics in Biophysics

Plenary lectures

WHEN YOU LOOK DEEP INTO THE HUMAN EYE… A BIOPHYSICIST'S PERSPECTIVE.

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When you look deep into a human eye, you will see a yellow spot. When you carefully examine this part of the retina, you will discover that this color comes from lutein and zeaxanthin, yellow xanthophyll pigments, the same ones found in the chloroplasts of plants. In fact, the very same pigment molecules that we owe to our healthy "green" diet may perform important biological functions, similar in both the photosynthetic apparatus and the eye. The results of the recent studies from our laboratory reveal the operation of very interesting and distinctive for the retina molecular mechanisms based upon the trans-cis photo-isomerization of xanthophylls. During my talk, I will provide an overview of these mechanisms, their physiological consequences, and promote a "colorful" diet that is extremely important for your sharp vision throughout the decades of your life.

ROOM TEMPERATURE PHOSPHORESCENCE WITH DIRECT TRIPLET STATE EXCITATION

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Although transitions between states of different multiplicity are strongly forbidden, in many cases they have been observed. For example, triplet-singlet transition is forbidden, but phosphorescence is commonly observed and measured. Usually, phosphorescence requires low temperatures, and most measurements are done in liquid nitrogen or helium. However, immobilization of fluorophores in polymers with a low permeability for oxygen often results in easily observable phosphorescence emission at room temperature, phenomenon called RTP. Interestingly, in many cases RTP can be achieved with the direct triplet state excitation at longer wavelengths than absorption. In this lecture a recent achievements in directly excited RTP will be presented.

SESSION I: Nanomedicine – biophysical aspect

CONJUGATES OF DNA AND BORON CLUSTERS AS BUILDING BLOCKS FOR NANOPARTICLE CARRIERS OF THERAPEUTIC NUCLEIC ACIDS WITH GENE SILENCING ACTIVITIES

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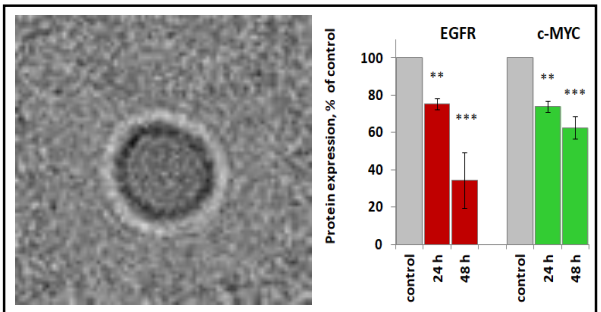
Despite thousands of chemotherapeutic drugs approved for clinical practice, there are still many unmet needs in the prevention and treatment of many diseases. The need to fill this never-shrinking gap is driving search for new drugs, both in the traditional chemotherapeutic category and in newer generation drugs such as biotherapeutics. Still another, emerging recently drug modality are therapeutic nucleic acids (TNAs) that have the potential to address many currently unmet by chemotherapeutic and biotherapeutic drugs needs [1-3]. However, even though already there are over 20 TNAs on the market, TNAs are still a new technology that faces challenges and problems that must to be solved. They include difficulties with delivery, susceptibility to degradation by nucleases, rapid clearance from the body, off-target effects and others. In quest to find solutions to these problems, a number of technologies are tested, one of them is nanotechnology.

For several years, our laboratory has researched modifying nucleic acids with boron clusters, a molecular cages with unique and advantageous properties [4], and studied their applications as probes for molecular diagnostics, boron carriers for boron neutron capture therapy (BNCT) and therapeutic nucleic acids. Continuing involvement in the very hot research area of TNAs we are focusing now on nanoparticle carriers of TNAs based on composites of DNA-oligomers and boron clusters.

Specifically designed conjugates of antisense DNA-oligomers and oligofunctionalized boron clusters are used as building blocks in construction of new class of bionanoparticles capable of silencing the expression of Growth Factor Receptor (EGFR) and c-MYC (myelocytomatosis oncogene) were chosen. Both proteins are overexpressed in several high-mortality human cancers and are proven therapeutic targets. EGFR and c-Myc are associated not only with a low survival rate but also with disease progression among others in metastatic pancreatic cancer therefore, we chose pancreatic cancer as a challenge and cancer type in our studies, and the human pancreatic cancer cell line PANC-1 as an in vitro model.



EGFR and c-MYC silencing nanoparticles.
Chem. *Eur. J. 2024*, 30, e202303531

Herein methods developed in our laboratories for the synthesis of these novel bioinorganic composites, their application for the construction of functional nanoparticles and their therapeutic potential as agents with capacity to silence one or two different oncogenes simultaneously in the same cancerous cell will be presented.

ACKNOWLEDGMENTS

This work was supported by the National Science Center, Poland, grant No 2015/16/W/ST5/00413

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CELL RESPONSE DRIVEN BY SURFACE CHEMISTRY AND CHARGES ON ELECTROSPUN POLYMER NANOFIBERS

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Surface charge is a critical determinant in cell–biomaterial interactions, influencing adhesion, proliferation, and regenerative signaling. Electrospun polymer scaffolds, known for their high surface-area-to-volume ratio, are promising candidates for biomedical applications such as tissue engineering, drug delivery, and skin regeneration [1-2]. However, precise control over surface charge during electrospinning remains underexplored.

A comprehensive investigation of distinct mechanisms to modulate surface potential in electrospun fibers was performed. We examined the influence of polymer chain orientation induced by alternating voltage polarity during electrospinning [3]. We also explored material diffusion between core and shell phases in coaxial fibers [4]. Most recently, we evaluated the impact of incorporating two-dimensional conductive nanomaterials—reduced graphene oxide (rGO) and titanium carbide MXenes ($Ti_3C_2T_x$)—on surface potential of polymer fibers [5].We demonstrate that reversing the polarity of the applied voltage during electrospinning significantly alters the surface potential of poly(L-lactic acid) (PLLA), polycaprolactone (PCL), poly(vinylidene fluoride) (PVDF) fibers, as shown by Kelvin probe force microscopy (KPFM), and this modulation directly enhances osteoblast adhesion [6]. Additionally, we explore how diffusion between core and shell materials affects fibre surface chemistry and charge distribution, shedding light on a previously unexamined factor in coaxial electrospinning. Moreover, we show that embedding rGO and MXene nanosheets within polymer matrices modifies surface potential and bioactivity, even when these nanomaterials are not surface-exposed.

Scaffolds were systematically characterized using KPFM, X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM) and zeta potential measurements to correlate surface properties with cellular responses. Confocal laser scanning microscopy (CLSM) with AiryScan was employed to visualize focal adhesion complexes, offering insights into how surface charge governs outside-in and inside-out signaling pathways.

In summary, our findings highlight the pivotal role of scaffold surface potential in mediating cellular responses and underscore the importance of tailored electrospun fibre design to accelerate tissue regeneration processes.

ACKNOWLEDGMENTS

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REENGINEERING OF A BACTERIAL COMPARTMENT INTO TAILORABLE BIONANOMATERIALS

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Self-assembling protein cages are naturally occurring hollow nanostructures with inherent capabilities for compartmentalizing and organizing biomolecules, making them promising candidates for a wide range of bioengineering applications. A key challenge in this field is precisely controlling their assembly and morphology to tailor function for specific applications. Understanding the molecular mechanisms underlying the polymorphic protein assemblies provides a basis for designing ones with the desired morphology.

Our recent work elucidates fundamental principles governing the self-assembly of a model cage-forming protein, *Aquifex aeolicus* lumazine synthase (AaLS) [1,2]. An engineered, circularly permuted variant of AaLS exhibits remarkable structural plasticity, assembling into diverse, hollow spherical and cylindrical structures in response to subtle changes in ionic strength (Fig. 1). Cryogenic electron microscopy (cryo-EM) reveals that these structures are composed entirely of pentameric subunits, and the dramatic cage-to-tube transformation is mediated by an α-helix domain that is untethered from its native position by circular permutation, a key structural determinant for controlling assembly [3]. The utility of this controlled assembly is demonstrated by the stabilization of labile biomolecules produced in host cells and their efficient retrieval outside biological contexts, showcasing cpAaLS as a versatile platform for nanoscale manipulation and cargo delivery [4].

Our results highlight the potential for broad advancements across biotechnological applications, ranging from bioproduction to nanomedicine. Furthermore, the structural insights gained into the assembly mechanisms and the role of circular permutation in dictating morphology provide a valuable framework for the rational design of novel nanomaterials with tailored properties.

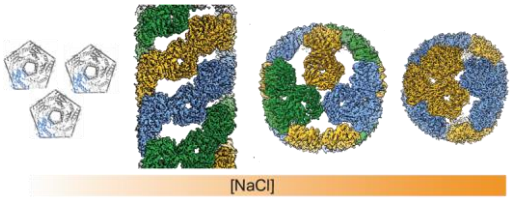

Fig.1. Electron microscopic structures of spherical and tubular assemblies formed by a circularly permuted variant of *Aquifex aeolicus* lumazine synthase. Colors indicate individual threads in the tube or symmetry-related pentamers in the spheres. Transformation between these spheres and tubes occurs solely in response to a change in ionic strength.

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NANOCARRIER-BASED DELIVERY OF ROSE BENGAL FOR ENHANCED PHOTODYNAMIC THERAPY OF BASAL CELL CARCINOMA

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Photodynamic therapy (PDT) is an innovative approach to skin cancer treatment, offering a targeted and less invasive alternative to conventional radiotherapy and chemotherapy while minimizing adverse effects. PDT relies on the activation of a photosensitizing agent by light of a specific wavelength in the presence of molecular oxygen, generating singlet oxygen and reactive oxygen species that induce cell death. The effectiveness of PDT is largely dependent on the properties of the photosensitizer, which can face limitations such as poor solubility, low tumor specificity, and inadequate accumulation at the target site. To overcome these challenges, nanocarriers have been explored as effective delivery systems [1].

This study focuses on the potential of dendrimers [2, 3], dendrimersomes [4], and polymersomes [5] as nanoscale carriers for rose Bengal - a photosensitizer. We evaluated four distinct nanocarriers based on key parameters, including spectral properties, encapsulation efficiency, singlet oxygen generation, intracellular transport, and phototoxicity. By systematically comparing these nanosystems, we assessed their ability to enhance the therapeutic efficacy of rose Bengal in PDT for basal cell carcinoma.

Our findings highlight that the interaction mechanism between rose Bengal and the nanocarrier plays a crucial role in determining its photodynamic efficiency. Among the studied delivery methods, phosphorus dendrimers demonstrated the highest efficacy, significantly improving singlet oxygen production, intracellular transport, and phototoxic effects [2]. These results demonstrate the potential of nanocarrier-based delivery strategies for optimizing PDT outcomes in basal cell carcinoma treatment.

ACKNOWLEDGMENTS

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PEPTIDOLIPOSOMAL FORMULATIONS FOR ANTIVIRAL THERAPIES

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There is an urgent need for the development of new antiviral formulations because humanity is constantly confronted with a range of new, potentially lethal viruses originating from different reservoirs. These formulations should prevent the progression of viremia and disease while being relatively easily adaptable to specific needs and guarantee resistance to mutational changes [1]. Such a goal can be achieved with appropriately functionalized nanoparticles such as liposomes [2].

Our aim was to develop effective therapeutic formulations against coronavirus and influenza infections. Using maleimide-functionalized liposomes as a platform for the immobilization, stabilization and delivery of short peptide sequences with high affinity to viral particles, we focused on fine-tuning the lipid composition and size calibration procedure to achieve selective binding, high homogeneity and excellent long-term stability. We show that the stability of the formulations depends not only on their chemical composition, but more importantly on the particle size calibration technique used in their preparation. The approach based on the widely used extrusion through membranes of defined pores makes it possible to achieve long-term stability. However, a stable and highly homogeneous formulation could also be produced by a high-throughput microfluidic homogenization technique [3]. In a first step towards the creation of nanostructures that recognize and deactivate viral particles, we have demonstrated the robustness and specificity of the prepared nanostructures by measuring the biomolecular interactions using microscale thermophoresis. The inhibitory effect of the obtained preparations against the infection of susceptible cells by pseudoviruses (lentiviruses bearing genes encoding luciferase-conjugated SARS-Cov-2 proteins) was also confirmed. Furthermore, our nanoformulations showed no toxicity either *in vitro* or *in vivo*.

Thus, the developed nanocarrier technology can serve as a platform for virus-inactivating nanoparticles, and its versatility can be ensured by replacing individual components.

ACKNOWLEDGMENTS

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HOTTER TOGETHER: BOOSTING HYPERTHERMIA EFFICIENCY THROUGH INTER-PARTICLE INTERACTIONS

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In recent years, magnetic nanoparticles (MNPs) have attracted growing interest as anticancer agents within the scientific and medical fields. A prominent example is NanoTherm® therapy, primarily applied in treating gliomas, which utilizes magnetically induced hyperthermia [1]. In this therapy, an alternating magnetic field generates heat through Néel and Brownian motions and hysteresis losses (in some instances) of magnetic particles [2].

The chemical composition, size, and shape of the MNPs significantly influence the efficiency of magnetically induced hyperthermia. As such, these parameters are systematically tested to optimize performance [3]. One of the most critical factors is the dispersion concentration of the magnetic particles (C_{magn}). However, the relationship between C_{magn} and the specific absorption rate (SAR) is nonlinear. Moreover, the resulting temperature increase (ΔT) does not directly correlate with SAR values. These phenomena were recently explored in depth by Kim et al. [4], who linked them to inter-particle interactions in magnetic fluids at varying concentrations.

In this study, we focus on these inter-particle interactions by varying the dispersion concentration and modifying the initial nanoparticle interactions during the synthesis process. Tailored synthesis methods allow us to produce a variety of nanoparticle structures, including ultrafine and highly agglomerated particles, large cuboidal particles, multicore MNPs, and ultrafine particles embedded in a polymer matrix.

Each nanoparticle type exhibits distinct behavior when analyzing SAR as a function of concentration. Interestingly, even nanoparticles with identical chemical compositions and similar size and shape can show significantly different heating efficiencies (see Table 1). These variations are attributed to inter-particle interactions, which can be precisely adjusted during synthesis using different surface modifiers.

Table 1. Influence of modifiers on the SAR value measured for dispersion concentration of 1 mg/ml at 386.5 kHz and 26 kA/m

Modifier	NPs size [nm]	SAR [W/g_{Fe3O4}]
D-glucosamine sulfate potassium chloride	7.85±0.5	24.3±3.6
N-Hydroxysuccinimide	12.4±0.9	34.5±2.7
Ethylenediamine	12.2±0.7	60.4±6.2

Notably, a high SAR value does not necessarily result in a corresponding temperature increase. The temperature rise tends to increase with higher dispersion concentrations. Therefore, the optimization of both SAR and ΔT should be carried out simultaneously, considering the potential adverse effects of MNPs on human health [5].

ACKNOWLEDGMENTS

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SESSION II: Life-essential biomolecules

FROM MDA TO KDA – ACROSS THE SCALE OF THE CRYOEM SPA ANALYSIS OF BIOLOGICAL MOLECULES

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Cryo-electron microscopy has gained considerable attention and has proven to be a powerful tool in structural biology. For the past years, scientists worldwide tried to push the boundaries of the technique and used it in their research projects to solve structures of all kinds of biological molecules. Here, in this presentation, I would like to show that size does matter, but there is a way to overcome this issue. Starting from large protein assemblies like synthetic cages of paradoxical geometry derived from bacterial enzyme, virus-like particles of different shapes and sized (both: spherical and rod-like) being in the MDa range (Figure 1),

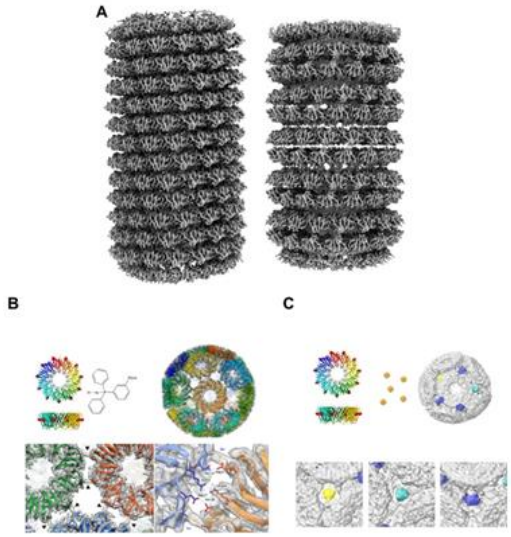

Figure 1. (A) an overview on two polymorphic structures of the modified TMV coat protein assemblies (B) synthetic protein cage made with toroidal-shaped protein and held by Au(I) bridges (C) synthetic protein cage made with toroidal-shaped protein andheld by gold nanoparticles (AuGNPs)

and going down to relatively small multimeric enzymes in complexes with their partners (both: proteins and small molecules), and finally finishing at sub 30-kDa particles like free tRNA molecule, being the smallest molecule so far reconstructed with cryoEM. Despite the broad range of molecular masses, we were able to show some important features of the molecules investigated like the symmetry of the assemblies, paradoxical arrangements of the building blocks, draw some rules about the symmetry breaking, describe the composition of the complexes, decipher the mechanism of their action, get to know the nature of the crucial bonds or even the influence of the introduced modifications on the structure's rigidity (to some extend)(Figure 2).

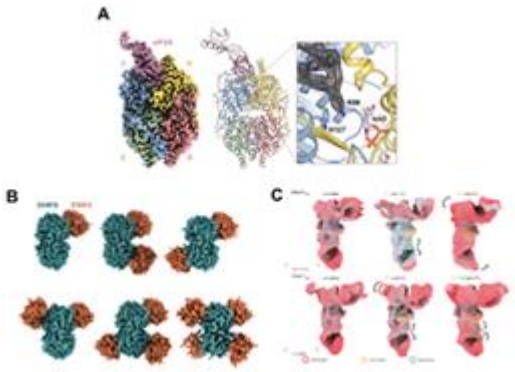

Figure 2. (A) reconstruction of a DHS-eIF5A complex with zoom onto active site (B) different stoichiometry complexes of DHS and its biding partner ERK2 (C) set of structures of free tRNA molecules showing modification sites and thier influence on the flexibility/ridigity

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PH-RESPONSIVE CHLOROPHYLL DERIVATIVES-MODIFIED LIPOSOMES FOR DOXORUBICIN DELIVERY

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Triple-negative breast cancer (TNBC) accounts for approximately 10% of all breast cancer cases and is distinguished by its aggressive clinical behavior and high rates of metastasis. A significant challenge in the treatment of TNBC is the absence of estrogen, progesterone, and HER2 receptors, which excludes the use of receptor-targeted therapies. Thus, systemic chemotherapy with doxorubicin remains a primary treatment approach. The clinical application of doxorubicin is limited due to severe systemic side effects, particularly dose-dependent cardiotoxicity, which often results in therapy discontinuation. Hence, liposomal formulations of doxorubicin have been introduced to reduce off-target toxicity.

Building on our recent findings that sulforaphane (SFN) synergistically enhances doxorubicin efficacy and reduces its toxicity in vivo [1], this study aimed to design a novel pH-sensitive liposomal delivery system that enables targeted release of doxorubicin in the acidic tumor microenvironment while limiting release under physiological pH.

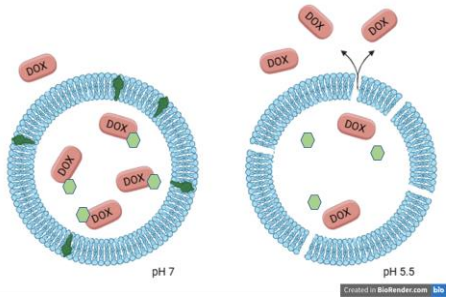

Fig.1. Doxorubicin Release from pH-Sensitive Liposomes Co-Encapsulating Doxorubicin and Chlorophyll Derivatives

The innovative aspect of this approach involves the use of natural, non-toxic chlorophyll derivatives—chlorophyll, chlorophyllin, and pheophytin—as pH-responsive release modulators. The proposed mechanism under acidic conditions involves: the reversible protonation of doxorubicin, which weakens its interaction with chlorophyllin and facilitates its diffusion across the liposomal membrane; degradation of lipophilic chlorophyll, localized within the liposomal lipid bilayer, increasing membrane permeability.

Liposomes were prepared via the passive loading method and characterized by Dynamic Light Scattering (DLS) - size, polydispersity index (PDI), zeta potential, and drug loading efficacy were determined. Next, drug release profiles were evaluated at pH 7.4 (physiological) and pH 6.5 (tumor-mimicking), revealing enhanced doxorubicin release under acidic conditions while restricting release under physiological pH.

Finally, in vitro studies were conducted on MDA-MB-231 TNBC cells using both 2D monolayer cultures and 3D spheroid models. While no significant differences were observed in cell viability between treatment groups in 2D cultures, spheroid assays demonstrated that liposomes containing doxorubicin and chlorophyllin reduced spheroid size and altered morphology compared to doxorubicin-only liposomes. The chlorophyllin-based formulation thus showed superior performance in a physiologically relevant model. The lack of comparable efficacy with chlorophyll and pheophytin under in vitro conditions remains to be elucidated.

ACKNOWLEDGMENTS

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X-RAY SYNCHROTRON AND NEUTRON SCATTERING STUDIES OF BIOMEMBRANE-PROTEIN INTERACTIONS AT AIR-LIQUID AND SOLID-LIQUID INTERFACES

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In nature, lipid membranes perform many living cell functions from selective transport and recognition to simple sequestration. They generally consist of a single phospholipid bilayer or in special cases, such as the lung surfactants, a single monolayer. In the cases discussed here, the lipid membrane will be approximated as a single lipid layer at the air-liquid interface (a surfactant Langmuir layer) or supported single lipid bilayers at the solid-liquid interface. Several surface-sensitive scattering techniques have been developed for probing the structure of such ultra-thin, molecular 2-D arrays of surfactants. These include X-ray reflectometry and in-plane grazing incidence diffraction. Both are particularly challenging to study due to the required horizontality of the sample. I will illustrate the use of X-ray and neutron surface scattering methods to characterize the structures of several types of model membranes. The properties of these soft-condensed, ultra-thin layers are of general interest to a wide scientific audience working in the fields of chemistry and biology since they are relevant to such important areas as bio-mineralization, biosensors, advanced drug delivery systems, and protein-membrane interactions.

LIPID DROPLETS IN VASCULAR DYSFUNCTION

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Introduction: Lipid droplets (LDs) are lipid-rich organelles found in most cells, including endothelial cells—the thin layer that lines the interior surface of blood vessels. Although historically LDs were regarded as passive cytosolic inclusions, their active roles in both physiological and pathological processes are now increasingly recognized.

The formation of vascular LDs, induced by vascular inflammation or lipid overload, is now considered as a key factor in the pathophysiology of diabetes and cardiometabolic diseases. Sodium-glucose co-transporter 2 inhibitors (SGLT2-I) have shown beneficial effects in treating these conditions. Therefore, we hypothesized that SGLT2-I might directly influence the formation of vascular LDs during inflammation or lipid overload.

Methods: To investigate the mechanisms underlying the effects of empagliflozin (representing SGLT2-I) on vascular LDs formation, we used isolated murine aorta from both wild-type and SGLT2 knockout animals. LDs formation was induced by treating the aorta either with tumour necrosis factor (TNF) to mimic vascular inflammation or with oleic acid (OA) to simulate lipid overload. Vascular LDs and associated markers of inflammation were assessed using fluorescence microscopy. Additionally, to explore the underlying mechanisms, we employed pharmacological inhibitors targeting the sodium-hydrogen exchanger 1 (NHE1), endothelial sodium channels (EnNaC), the sodium-calcium exchanger (NCX), protein kinase C (PKC), and NOX1/4.

Results: SGLT2-I effectively inhibited LDs formation in the aorta exposed to TNF or OA. Empagliflozin not only reduced vascular inflammation, as indicated by decreased ICAM-1 expression, but also significantly diminished TNF/OA-induced LDs formation. These effects were consistently observed even in SGLT2-KO mice. Further investigation revealed that inhibiting NHE1, PKC, or NOX1/4 replicated empagliflozin's impact on TNF-induced vascular inflammation, with no additional effect from empagliflozin itself. However, NHE1 inhibition was not involved in empagliflozin's SGLT2-independent reduction of OA-induced LD formation.

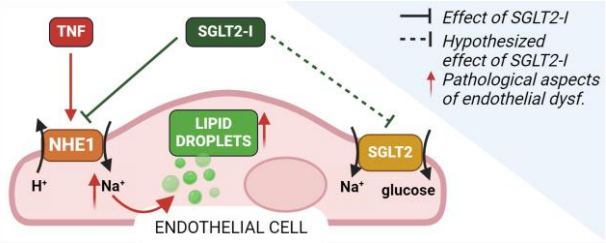

Fig.1. The results showed that empagliflozin's effect on vascular LDs formation was SGLT2-independent in both TNF-induced inflammation and OA-induced lipid overload models. Moreover, NHE1 is key for the SGLT2-I action on vascular inflammation and LDs formation but not for reducing LDs during lipid overload.

Conclusions: This study shows that SGLT2-I prevent the formation of vascular LDs. Specifically, empagliflozin inhibits LDs formation during both vascular inflammation and lipid overload through an SGLT2-independent mechanism. The protective effects of empagliflozin are mediated by the NHE1/PKC/NOX pathway in response to TNF, but this pathway is not involved in the OA-induced LDs formation.

ACKNOWLEDGMENTS

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ANTIGEN BINDING TO SURFACE IMMOBILIZED ANTIBODIES: TOF-SIMS EXAMINATION OF THE IGG ORIENTATION AND IMMOBILIZATION STABILITY

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In most biosensors, selective binding of analytes by detecting biomolecules occurs at the interface, which is then converted into a detection signal. Antibodies are often applied as detecting molecules due to their ability to specifically bind antigens. IgG is the most commonly used antibody, having a characteristic Y-shaped structure, consisting of a constant Fc domain and two Fab domains containing antigen-binding sites. Due to its structure, this antibody can adapt on the surface different orientations, which differ in access to the antigen binding sites and as the result in the efficiency of antigen binding. Therefore, the quality of the biorecognition layer, involving, the surface density of detecting molecules, their biological activity, elimination of nonspecific adsorption and layer stability, is crucial for the effective and reliable performance of a biosensor device [1]. Controlling the orientation of antibodies and molecular composition of biorecognition layer is extremely important but still challenging. The IgG orientation is most commonly inferred from indirect methods, prone to high uncertainty. In contrast, antibody orientation can be resolved with ToF-SIMS mass spectrometry, because of the technique surface sensitivity and discrimination of the Fc and Fab domains with different amino acid composition. This method, however, is limited to comparative analysis between samples that hindering an absolute determination of the antibody dominant orientation. In this work, we present the novel approach of surface density dependent studies of antibody orientation with ToF-SIMS and PCA, which allows for direct tracking of orientation changes induced by the increasing molecules surface amount and for an accurate evaluation of the dominant orientation by estimation of share of molecules with head-on and tail-on alignment (f_{Fc} fraction) [2,3]. We examined the surface density dependent orientation of antibodies immobilized on silane-modified silicon by physical adsorption (APTES layer) and covalent coupling (APTES layer activated with glutaraldehyde, APTES/GA). Differences in dominant vertical orientations are revealed and discussed in terms of relevant molecule–molecule and molecule–surface interactions. Moreover, the impact of the pH of the IgG solution on the dominant vertical orientation of the antibodies immobilized on APTES and APTES/GA is determined and expressed by the f_{Fc} fraction [4]. Additionally, the stability of IgG immobilization on APTES and APTES/GA is examined, depending on the initial IgG surface density, by ToF-SIMS molecular composition analysis and by WLRS real-time monitoring of layer thickness. This analysis surprisingly reveals a partial exchange of IgG molecules with BSA during the surface blocking step [5]. Results of IgG orientation and immobilization stability are juxtaposed with the antigen binding efficiency providing a complete insight into biofunctionalization process.

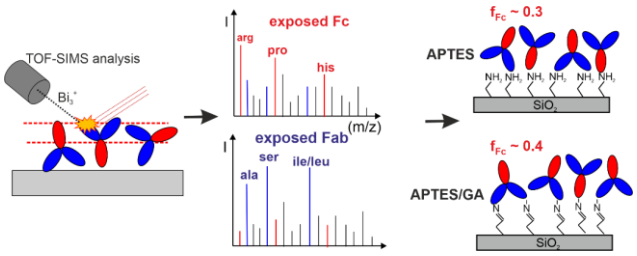

Fig.1. Application of ToF-SIMS with PCA for analysis of the orientation of IgG molecules on the surface.

ACKNOWLEDGMENTS

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HEME AND HEME OXYGENASES – NOVEL ACTIVITIES OF OLD FRIENDS

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Heme is an essential yet potentially cytotoxic molecule that plays a central role in cellular metabolism, signaling, and development. While its biosynthesis is crucial for mitochondrial function and regulation of some transcription factors, excessive or misregulated heme accumulation, especially of intermediates such as protoporphyrin IX (PPIX), can lead to phototoxicity and oxidative stress.

Recent research has shed light on previously underappreciated roles of heme and its degradation enzymes, particularly heme oxygenase-1 (HO-1), in embryonic development, DNA replication, and inflammatory signaling. In mouse preimplantation embryos, increased heme synthesis or inhibition of ferrochelatase disrupts cleavage and sensitizes embryos to light via PPIX accumulation, suggesting that early developmental stages are highly sensitive to perturbations in heme metabolism.

Concurrently, HO-1, traditionally viewed as an anti-oxidative enzyme, is now recognized for its nuclear functions, including its role in resolving DNA G-quadruplex (G4) structures [1]. HO-1 deficiency leads to G4 accumulation, replication stress, and impaired nuclear p53 localization, indicating its protective function in genome stability [2]. Furthermore, studies in HO-1-deficient fibroblasts and knockout mice reveal that although interferon-stimulated gene (ISG) expression is enhanced in vivo, their response to proinflammatory stimuli such as TNFα is paradoxically weakened in vitro, likely due to impaired NF-κB and STAT1 signaling [3]. This disruption correlates with defective nuclear transport mechanisms involving PARP1 [3], suggesting a broader role for HO-1 in regulating nucleocytoplasmic trafficking under stress.

Collectively, these findings uncover a network of heme- and HO-1-mediated processes that extend far beyond their classical roles, positioning them as critical modulators of early development, genomic integrity, and inflammatory homeostasis. These novel insights into "old friends" open promising avenues for understanding diseases linked to metabolic and inflammatory dysregulation.

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SESSION III: Biophysics of biological systems: from cells to tissues

MIND THE GAP! BIOMOLECULES IN PLASMONIC NANOCAVITY

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Despite several decades of intense research, the most significant biomolecules including nucleic acids, proteins, and lipids hide many secrets from us. Due to the methodological limitations such as lack of sufficient sensitivity and spatial resolution of conventional analytical approaches, the properties and functionalities of such molecules, heterogeneous at the nanoscale, remain unclear.

Hence, to achieve significant progress in the characterization of the molecules of life, intense research on the physics and chemistry of processes occurring in plasmonic nano-gap junctions must be performed, providing solid information on the generation of surface plasmons, near-field confinement of the generated electromagnetic field by the nanojunction/cavity, related optical field enhancement in the close vicinity of the characterized biomolecules, as well as the field enhanced molecule vibronic excitations, Raman scattering, and infrared transitions in the nano-gap junction. All those fundamental subjects are at the base of modern molecular nanospectroscopies, such Tip-Enhanced Raman Spectroscopy (TERS), and Fourier Transform InfraRed nano-spectroscopy (nanoFTIR).

Our research involve the nanospectroscopic investigation into the local molecular structure of biologically significant biomolecules. including:

i) aggregating Alzheimer's proteins and peptides to monitor the nanoscale distribution of β-sheet secondary structure for revealing the aggregation pathways [1].

ii) cross-talking amyloid-β and the anti-aggregation drug called bexarotene, which slows down the protein aggregation process via steric effects, largely prohibiting the antiparallel to parallel β-sheet rearrangement [2].

iii) amyloid-β individual aggregates in liquid to improve the TER data quality due to the protective role of solvent, in particular, high heat capacity of liquid reduces the effective temperature of analyte preventing its thermal decomposition [3].

iv) aggregating tau protein for probing the antiparallel to parallel β-sheet rearrangement [4].

v) lipid monolayers for investigating of local molecular distribution, orientation, phase separation, and formation of domains [5].

vi) individual DNA strands to explore nanoscale spectral markers of the Double Strand Breaks formation and DNA conformational transitions

ACKNOWLEDGMENTS

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DEVELOPMENT OF NOVEL OPTICAL MICROSCOPY METHODS TO STUDY CELL MIGRATION

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Cell migration is an important biological phenomenon. It plays a crucial role in many biological processes such as wound healing, tissue engineering, functioning of the immune system or embryo development. However, disruption of molecular mechanisms, controlling cell migration, can lead to pathological conditions such as arthritis, osteoporosis, congenital disorders or cancer metastasis. This highlights that the understanding of molecular mechanisms, that regulate cell migration, can help in the development of novel prevention methods or therapies designed to cure above mentioned diseases. Optical microscopy is one of the major experimental techniques employed in cell migration research. Its main advantage is that it allows studies of live cells behavior in their physiological environment. Recently, a connection of advanced optical microscopy techniques with other experimental methods led to the development of novel experimental approaches to cell migration research. One such development was to combine a confocal microscopy technique, which allows to visualize the cell structure and composition in 3D, providing a look inside the cell, and engineering of elastic, hydrogel cell culture substrates which resulted in the discovery of novel microtubule-based cellular structures [1]. In another similar development, wide-field optical microscopy combined with Optically Detected Magnetic Resonance (ODMR) from microdiamonds and Traction Force Microscopy technique provides the prospect of truly multiparametric investigation of cellular processes in live cells, where the local environmental temperature and cellular tractions can be measured simultaneously [2]. Finally, a combination of wide-field optical microscopy, polymer elastic substrate method and computer aided analysis of large image data sets made it possible to elucidate the complex regulation of cellular morphology [3]. Those developments demonstrate that optical microscopy, in connection with other experimental techniques, can deliver novel, important information about biological systems.

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CORRELATIVE AFM–OPTICAL NANOSCOPY FOR POLYPHARMACY STUDIES IN HEPATIC ENDOTHELIUM

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Polypharmacy, defined as the concurrent administration of five or more pharmaceutical agents, poses a growing health challenge, particularly in aging populations in Europe. The liver, responsible for systemic detoxification, is a key target in understanding the cellular mechanisms underlying adverse drug interactions. In particular, liver sinusoidal endothelial cells (LSECs) mediate hepatic filtration, functioning as the first barrier between circulating blood and the liver parenchyma [1]. To facilitate their sieving function, LSECs are perforated with numerous fenestrations, transcellular pores ranging from 50 to 350 nm in diameter [1]. Fenestrations are dynamic structures that respond rapidly to pharmacological stimuli, making them sensitive indicators of hepatic endothelial function [2,3]. Their size and distribution fall below the resolution limit of conventional light microscopy, necessitating advanced imaging approaches.

To investigate the nanoscale effects of polypharmacy on LSEC phenotype, we have developed a correlative microscopy framework that integrates high-speed atomic force microscopy (AFM) with super-resolution structured illumination microscopy (SR-SIM). Our AFM modality provides sub-50 nm lateral resolution with temporal precision below one second, enabling the real-time observation of fenestration dynamics and cell elasticity. Complementarily, SR-SIM enhances visualization of cytoskeletal architecture, offering insight into drug-induced morphological and mechanical remodeling. We further explore the hypothesis that fenestration deformability is functionally linked to the overall cell elastic modulus, particularly under inflammatory or fibrotic conditions.

Here, we present our latest findings on drug-induced modulation of LSEC nanomechanics and fenestration morphology, as well as the implementation of a custom-designed AFM-SIM correlative platform. This integrative approach provides new opportunities to unravel the biophysical underpinnings of polypharmacy at the cellular and subcellular levels within the hepatic microvasculature (e.g. Fig. 1)[5].

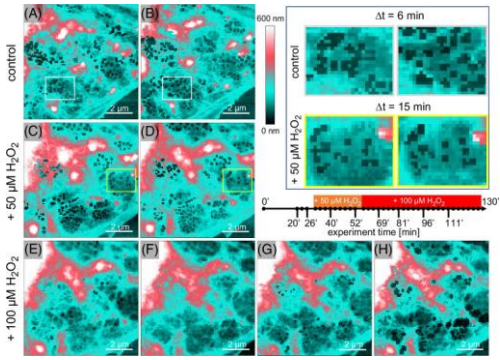

Fig.1. Dynamic defenestration and refenestration in live LSEC treated with hydrogen peroxide. Presented images (A–H) correspond with consecutive time points (20'-111') indicated on a timeline. The whole 130 min long experiment is presented in [5].

ACKNOWLEDGMENTS

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THE IMPACT OF 3D MICROENVIRONMENT RHEOLOGY ON CELL INVASION ACCOMPANIED BY PROTEIN EXPRESSION CHANGES IN CANCER SPHEROIDS

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The mechanical properties of the tumor microenvironment critically influence cancer progression [1-2]. Recent studies have highlighted that, beyond biochemical cues, physical signals such as matrix stiffness can profoundly influence cell behavior, affecting their proliferation and metastatic potential [3].

In this study, we investigate how the rheology of collagen–hyaluronic acid (Col-HA) hydrogels regulates cell migration and which proteins alter their expression in bladder cancer spheroids. By tuning matrix microstructure and viscoelasticity, we established a 3D platform mimicking the conditions of physiologically relevant extracellular matrix (ECM). Spheroids were formed from human non-malignant cancer cells of the ureter (HCV29), transitional cell carcinoma (T24), and bladder carcinoma (HT1376) cells. The hydrogel's microstructure was characterized using scanning electron microscopy (SEM) and fluorescence microscopy, showing the formation of a highly porous fibrillar microstructure with a high level of Col-HA association in the 3D matrix. The hydrogel rheology was measured using a rotational rheometer working in oscillation mode, applying shear strain at the level mimicking physiological mechanical forces (shear strain γ = 1%, and frequency f = 0.1 ÷ 10 Hz). Stiffness-dependent cell migration was recorded using a light microscope. The migration of cells was significantly larger for T24 cells, which are highly invasive compared to HCV29 and HT1376 cells. Cell migration was accompanied by collagen fiber alignment and the formation of microtracks for cell movement. Correspondingly, Western blots revealed stiffness-dependent modulation of key proteins involved in cell migration. Our findings demonstrate that the mechanical properties of the 3D Col-HA hydrogels directly influence cell migration from the spheroids' surface and depend on hydrogel stiffness and cell phenotype. The obtained results might help understand the relationship between physicochemical and biological properties in the tumor–ECM interactions.

ACKNOWLEDGMENTS

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HOW TISSUE STIFFNESS AFFECTS MICROGLIAL MIGRATION AND MORPHOLOGY

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Microglia, the fundamental immune cells of the central nervous system, are highly dynamic and responsive to the brain's microenvironment. One key factor influencing their behavior is the mechanical stiffness of the surrounding tissue, which alters during aging, neuroinflammation, and disease [1,2]. To investigate microglial mechanoresponsiveness, we cultured HMC3 cells on polyacrylamide (PAA) substrates mimicking a range of physiological and pathological brain stiffnesses (0.2 kPa to 23 kPa), as well as glass.

Our study focused on how substrate stiffness affects microglial migration, persistence, and morphology. We classified migrating cells into three distinct fractions based on their mean square displacement (MSD): local (MSD < 20 μm²), moderate (20–350 μm²), and global (>350 μm²) migration. Analysis using a modified persistent random walk (PRW) model [3] revealed that both migration speed and persistence time increased with substrate stiffness, reaching the highest values for substrates with stiffnesses of 5 and 23 kPa. Specifically, cells on stiffer substrates exhibited higher speed (μm/h), greater end-to-end displacement, and longer persistence time, indicating enhanced migratory capacity.

Interestingly, the amoeboid morphology—often associated with cell`s activation—was most prominent at intermediate stiffness (5 kPa), where persistence time was also the highest. Moreover, we observed an increase in population heterogeneity with substrate stiffness: cells appeared more homogeneous on soft substrates, but exhibited greater phenotypic diversity as stiffness increased. These findings suggest that mechanical cues modulate microglial behavior in a stiffness-dependent manner, which may have implications for understanding their role in aging and disease.

An exponential correlation was shown between persistence time and cell speed on different substrates, suggesting some optimization of microglia motility under changing stiffness conditions. These findings highlight the importance of mechanical cues in regulating microglial migratory behavior, with potential implications for neurodegenerative disorders, where altered tissue mechanics may affect nerve cell function and immune response.

ACKNOWLEDGMENTS

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PREDICTING PATIENT HEALTH TRAJECTORIES WITH FOUNDATION MODELS: A NEW FRONTIER IN COMPUTATIONAL MEDICINE

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Recent advances in computational science including applications in biophysics have laid the groundwork for modeling biological systems as dynamic, data-driven processes. Inspired by these principles, we introduce ETHOS (Enhanced Transformer for Health Outcome Simulation) [1], a novel foundation model designed to predict patient-specific health trajectories based on electronic health records (EHRs). ETHOS adapts the transformer architecture [2], originally developed for natural language processing, to analyze Patient Health Timelines (PHTs), which encode heterogeneous clinical events as structured sequences, analogous to physical state transitions in a dynamic system.

Unlike traditional models that require task-specific training or curated labels, ETHOS operates in a zero-shot setting. Once trained on large-scale EHR data, it can forecast future health events such as mortality, readmissions, or length of stay purely based on past information. This forecasting is achieved through generative simulation using Monte Carlo sampling over tokenized timelines, allowing ETHOS to sample a multiverse of plausible patient futures under uncertainty, a concept aligned with probabilistic approaches in statistical mechanics.

Our published results demonstrate that ETHOS achieves state-of-the-art performance on multiple clinical benchmarks, including ICU mortality (AUC = 0.93), hospital readmission (Figure 1). Furthermore, the model retains high fidelity even in noisy, incomplete datasets such as MIMIC-IV, highlighting its robustness to data inconsistencies, a critical requirement for clinical deployment [1,3].

Beyond predictive accuracy, ETHOS is designed with interpretability and scalability in mind. By leveraging token-level attention mechanisms and causal modeling, it allows for event-level explainability and simulates counterfactual trajectories under hypothetical interventions. This framework sets the stage for interactive AI agents in healthcare, capable of offering real-time, personalized guidance to clinicians, similar to digital decision-support systems grounded in physical modeling principles.

ETHOS exemplifies how concepts from biophysics—such as system dynamics, trajectory simulation, and causal inference, can underpin next-generation AI tools in medicine. We propose this model as a foundational computational framework opening new avenues for translational applications in precision health.

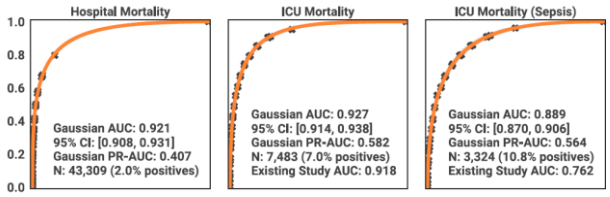

Fig.1. Predictive performance of ETHOS for hospital and ICU mortality outcomes. Receiver Operating Characteristic (ROC) curves for ETHOS predictions on three distinct clinical endpoints: (left) all-cause hospital mortality, (middle) ICU mortality, and (right) ICU mortality in patients with sepsis. For each task, ETHOS achieves high discriminatory performance, with AUC values of 0.921, 0.927, and 0.889, respectively. Confidence intervals (95% CI), Gaussian-smoothed precision-recall AUCs, and cohort characteristics (N and prevalence) are shown in the figure. ETHOS outperforms or matches previously reported models, including a benchmark study for ICU mortality with an AUC of 0.918 and a sepsis-specific ICU mortality study with an AUC of 0.762. These results highlight ETHOS's robust zero-shot predictive capabilities across diverse clinical scenarios.

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I gratefully acknowledge the contributions of my students and colleagues, in particular Pawel Renc.

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PERSPECTIVES OF THE USE OF HEMORHEOLOGICAL STUDIES IN MEDICAL DIAGNOSTICS

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Introduction: Rheology – the science of the flow of matter and the accompanying phenomena of deformation of real bodies – in relation to biological material it is called biorheology, and in relation to blood – hemorheology. Blood flow through blood vessels is a very complex phenomenon due to the physical and physicochemical properties of blood and the structure and properties of the circulatory system. The rheological characteristics of each material depend mainly on two parameters: viscosity and elasticity. Viscosity is a parameter defining the material's resistance to flow, and elasticity expresses the material's resistance to deformation [1,2]. Hemorheological tests are based primarily on measurements of whole blood viscosity. The parameters determining the whole blood viscosity are: plasma viscosity, hematocrit, deformability and aggregability of red blood cells. This paper presents the results of our own research and a review of literature studies indicating that disorders of whole blood viscosity and plasma viscosity may be an indication for expanding diagnostic tests.

Methods: Hemorheological tests are performed "in vitro". Blood is collected in the presence of an anticoagulant (EDTA). Measurements of whole blood viscosity as a function of shear rate are performed using rotational rheometers. Since blood plasma is a Newtonian fluid, its viscosity can be measured using both rotational and capillary viscometers. Additional information about blood rheology can be obtained from non-viscometric oscillatory measurements, also known as dynamic mechanical analysis (DMA). The principle of the oscillatory technique is to determine the amplitude and phase of oscillations of the tested sample subjected to the action of a harmonic force with a controlled amplitude and frequency. The measurement performed using the oscillatory method provides information about the viscoelastic properties of the liquid - two components of the complex blood viscosity. The determination of the aggregability and deformability of blood cells is performed directly using aggregometers and appropriate filters. The deformability of erythrocytes and their ability to orient themselves in the flow are estimated based on measurements of the flow time through capillaries or analysis of the diffraction pattern of transmitted light. Information on the ability of erythrocytes to aggregate and deform can be obtained by mathematical analysis of flow curves based on rheological models containing parameters related to the properties of these erythrocytes. Many models describing fluid flow can be found in the literature. The most commonly used model of the flow curve in hemorheology is the model proposed by Quemada in the late eighties [3]. The advantage of this model is the fact that it was formulated for substances with properties identical to those observed in whole blood, i.e. for a concentrated suspension of particles that can aggregate and does not show the existence of a limiting shear stress. The model takes into account the variability of the shape of blood cells and the formation of aggregates - in both cases due to the change in the maximum packing density. The influence of the specific behavior of erythrocytes is most important in blood flow in the microcirculation.

Results: Hemorheological factors such as whole blood viscosity, plasma viscosity, hematocrit, white blood cell count, fibrinogen, lipids and lipoproteins affect blood flow in both macrovessels and microvessels and are strongly associated with incidental cardiovascular events. Increased whole blood viscosity is observed in some neoplastic diseases despite low hematocrit. In the acute phase of stroke, high plasma viscosity and increased ability of erythrocytes to aggregate are observed with simultaneous increase in erythrocyte stiffness. In the case of diabetes, increased viscosity and aggregability of erythrocytes are observed. Studies of correlations between the thermographic image of blood flow in the upper and lower limbs with blood viscosity tests allowed for detection of the first circulatory disorders. In COVID patients elevated blood viscosity was correlated with increased patients mortality [4,5].

Conclusions: The analysis of changes in physicochemical properties of blood conducted in this study shows how important the hemorheological factor can be in diagnostics. Many aspects of hemorheological functioning of a living organism are not yet known. Measurement techniques are constantly being improved, becoming more accurate, which should enable better use of hemorheological measurements for diagnostics and therapy in the future.

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EXPRESSION AND FUNCTIONAL ROLE OF TRPV1 CHANNELS IN T LYMPHOCYTES: IMPLICATIONS FOR IMMUNE REGULATIONHE

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Transient Receptor Potential (TRP) channels are a diverse family of cation channels involved in sensory perception, including temperature, pain, and pH sensing [1]. Among them, TRPV1, classically known as a thermoreceptor and pain sensor, has recently garnered interest in immunology due to its expression in various immune cell types [2]. Immune cells often operate under changing environmental conditions, such as fluctuations in temperature, local acidosis, or inflammatory signals—factors known to modulate TRPV1 activity [3].

In this study, we focused on the expression and functional relevance of TRPV1 in peripheral blood mononuclear cells (PBMCs), with a particular emphasis on T lymphocytes. Our data demonstrate that TRPV1 is expressed at both mRNA and protein levels in unstimulated PBMCs and T cells. Using Fura-2 calcium imaging, we observed increased intracellular calcium levels upon stimulation with capsaicin (a TRPV1 agonist), indicating the functional presence of TRPV1 channels in these cells.

Interestingly, TRPV1 expression appears to decrease following T cell activation, as evidenced by reduced TRPV1 mRNA levels in qPCR analysis and lower capsaicin-induced calcium influx in activated cells. This suggests that TRPV1 expression and function are dynamically regulated in the course of immune activation.

Our findings support the hypothesis that TRPV1 may contribute to the modulation of immune responses, potentially linking environmental cues such as temperature, pH, or inflammatory mediators to T cell function. Given the complexity of immune regulation, TRPV1 may represent a novel target for immunomodulatory strategies, particularly in conditions associated with inflammation or altered tissue homeostasis.

ACKNOWLEDGMENTS

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TIME-DEPENDENT REVERSAL OF HIGH-FAT DIET-INDUCED INSULIN RESISTANCE, PERIVASCULAR ADIPOSE TISSUE BIOCHEMICAL CHANGES IN RELATION TO ENDOTHELIAL (DYS)FUNCTION

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Background: Perivascular adipose tissue (PVAT) is essential in controlling vascular function. Our previous results[1,2] of short high-fat diet (HFD)-induced obesity indicated significant changes in PVAT lipid composition accompanied by endothelial dysfunction only in the abdominal aorta (AA). In contrast, the thoracic aorta (TA) with brown-like PVAT remained unchanged. Prolonged (8 weeks) HFD feeding causes insulin resistance, endothelial dysfunction in both TA and AA and alters PVAT, but these changes can be restored by HFD replacement with a normal diet.

Methods and results: A multimodal functional, spectroscopic, and molecular characterization of aortas and PVATs was involved to evaluate the effects of diet reversal after eight weeks of HFD (60 kcal% of fat with 1% of cholesterol). After one week of HFD reversal, full reversal of systemic insulin resistance was observed. Using a magnetic resonance imaging (MRI) technique to characterize endothelial function *in vivo*, it was found that the endothelial dysfunction in TA was partially reversed after one week, with full recovery requiring at least six weeks (Fig. 1). Conversely, Raman spectroscopy revealed that the lipid unsaturation degree[3] of AA PVAT fully recovered early, which is reflected in *Scd1* gene expression assessed by qPCR, while recovery in the TA PVAT was only partial. Delayed reversal was associated with transcriptomic alterations in PVAT, not aorta, manifested by altered gene expression of *Insr*, *Irs1/2*, *End1* and *Gucy1b1*, and adipokines (*Lep*, *Nampt and Adipoq*).

Conclusions: We demonstrated that 8-week HFD feeding results in the impairment of the endothelial function in the entire aorta (TA and AA) and alterations in PVAT lipid composition. However, the response to HFD reversal is location-dependent, with the TA showing quicker restoration, associated with brown-like PVAT. In the AA, endothelial function recovery does not appear to depend on systemic insulin resistance, nor is it related to lipid unsaturation in the AA PVAT. Delayed response of AA to HFD withdrawal can be attributed to metabolic alterations in AA PVAT evoked by disruption in adipokine secretion and local insulin resistance attributed to PVAT dysfunction.

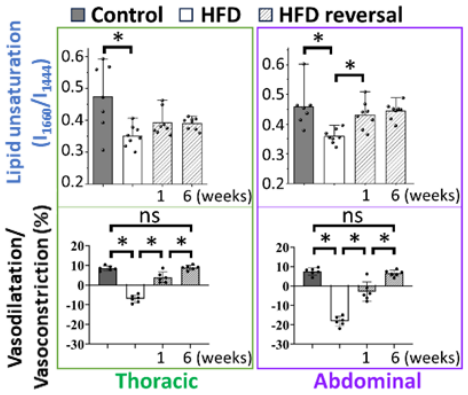

Fig.1. Differential response in aorta in HFD reversal studied by Raman and MRI. Endothelial dysfunction is partially reversed after one week in TA and at least six weeks are needed for full recovery after HFD. Lipid unsaturation fully recovered early and only in AA.

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OXIDATIVE STRESS CAUSED BY ACROLEIN AND GLYOXAL IN MONONUCLEAR HUMAN BLOOD CELLS

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Acrolein (ACR) and glyoxal (GO) are highly reactive aldehydes generated endogenously during lipid peroxidation, glycation reactions, and inflammation. Both toxins are found in environmental sources like cigarette smoke, combustion products, and highly processed food. Scientific research confirms a strong link between elevated levels of ACR and GO in the human body and the pathogenesis of various diseases, including atherosclerosis, diabetic complications, neurodegenerative disorders, and kidney diseases [1,2].

Carbonyl compounds promote oxidative stress by modifying essential biomolecules, forming adducts with proteins and nucleic acids (carbonyl stress), and depleting intracellular glutathione (GSH). Among the amino acids, the most likely to bind to these toxins are the thiol groups of cysteine, the amino groups of lysine or arginine, and the imidazole groups of histidine. Protein modifications can lead to a cascade of effects, including cellular dysfunction, damage to organelles, and ultimately, cell death. Another mechanism of toxicity of both aldehydes involves the induction of the production of ROS in cells, which results in a disturbance of the redox balance in cells and the induction of oxidative stress.

This study investigated how ACR and GO induce oxidative changes in mononuclear blood cells (MNCs). MNCs isolated from the buffy coat were treated with ACR (30, 60, and 90 μM) or GO (2, 5, 10 mM) for 24 h at 37 °C. After incubation, the levels of ROS and RNS, GSH, and free thiol groups in the cells were determined using fluorometric methods. Spectrophotometric techniques were used to determine the level of free amino groups of protein and catalase activity in cells. Statistical analysis of the obtained results was performed using Statistica v. 13.3 (StatSoft Polska, Kraków, Poland).

Exposure to acrolein and glyoxal increased the levels of reactive oxygen nitrogen species in all assays, confirming the induction of oxidative stress. Additionally, we observed a decrease in catalase activity, free amino groups, thiol groups, and GSH levels in cells treated with ACR or GO.

The reduction in thiol groups, GSH content, and catalase activity indicates a compromise in redox homeostasis in MNCs treated with ACR or GO. The loss of free thiol groups in the proteins of MNCs treated with ACR and GO may be attributed to ROS oxidation and the binding of these toxins. Furthermore, the reduction in amino groups suggests protein modification due to carbonyl stress and potential adduct formation with reactive aldehydes.

Our findings support the hypothesis that aldehyde-induced stress disrupts antioxidant homeostasis in the studied cells. Moreover, the obtained research results confirm the results of previous studies conducted with the participation of both toxins in human erythrocytes [3,4]. The increased levels of ROS and RNS, along with modified protein structures, could have implications for chronic inflammatory and metabolic diseases. It is important to note that acrolein is more toxic than glyoxal. While the changes observed in cells were similar for both toxins, ACR induced these effects at micromolar concentrations, whereas GO required millimolar concentrations.

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SESSION V: Ion channel in the cell biophysics

MAPPING SPATIAL ORGANIZATION OF FUNCTIONAL INPUTS IN VALENCE-RELATED AMYGDALO-HIPPOCAMPAL CIRCUITS

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The formation of memories in response to aversive or rewarding stimuli is crucial in guiding avoidance or approach behaviors. Scattered, projection-defined neuronal populations within the basolateral amygdala (BLA) selectively activate during encoding and retrieval of memories associated with either positive or negative valence. Interestingly, BLA neurons projecting to the CA1 area of ventral hippocampus (vCA1) respond to both positive or negative predicting cues with no marked bias, suggesting that, within the whole responding population, two distinct subnetworks relay opposite information to vCA1. However, the mechanism by which vCA1 pyramidal neurons discern between positive and negative-related information remains unclear. The valence information might stay segregated within two distinct neuronal populations in vCA1, or it might also converge onto the same vCA1 neurons, which have the capability to specifically encode negative or positive valence.

We suggest that valence-activated BLA neurons contact vCA1 dendrites in a precise spatial organization that together with inhibitory synapses can generate unique valence-related spiking patterns in the postsynaptic neuron. To validate this hypothesis, we aimed at building a map of the spatial location of functional synaptic inputs from BLA, vCA3 and bistratified interneurons onto vCA1 pyramidal neurons. To this end, we have developed an automated procedure to perform single-spine calcium imaging in the whole vCA1 dendritic arbor exploiting custom made neural network algorithms combined with electrophysiology and optogenetics. This integrated approach allowed to reveal the unique distribution of BLA and vCA3 and inhibitory inputs onto the whole dendritic arbor of vCA1 pyramidal neurons.

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EXCITATORY EFFECTS OF METABOTROPIC RECEPTORS IN NEOCORTICAL VASOACTIVE INTESTINAL POLYPEPTIDE-EXPRESSING INTERNEURONS

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Vasoactive intestinal polypeptide-expressing interneurons (VIP-INs) accounts for approximately 10-15% of inhibitory interneurons in the neocortex. However, VIP-INs play an important role in neuronal networks, creating a disinhibitory circuit by targeting other inhibitory interneurons and thus releasing excitatory neurons from inhibition. On the other hand, VIP-INs also are inhibited by other interneurons. Here, we examined how the activity of VIP-INs is modulated through metabotropic receptors for GABA (GABAbRs). These receptors affect variety of signaling pathways leading to different effects on specific neuronal populations. In layer 2/3 of mouse primary somatosensory cortex, we found that only a subset of vasoactive intestinal polypeptide-expressing interneurons (VIP-INs) is sensitive to GABAbRs but surprisingly pharmacological activation of these receptors had different effects on VIP-IN intrinsic excitability depending on extracellular Ca2+ levels. When electrophysiological recordings were performed in standard conditions with elevated extracellular Ca2+ level, GABAbRs enhanced intrinsic excitability of VIP-INs through indirect inhibition of big conductance voltage- and calcium-activated potassium (BK) channels and by reducing GABAaR-mediated inhibition. However, a classical inhibitory effect of GABAbRs on VIP-INs was observed in recordings with physiological (low) Ca2+ concentration.

In conclusion, we show new mechanisms of GABAbR function in the neocortex. Our findings are crucial for better understanding of mechanisms underlying modulation of neuronal circuits.

ACKNOWLEDGMENTS

This work was supported by the National Science Centre, Poland (2020/39/B/NZ4/01462 to J.U.-C).

A DETERMINISTIC MODEL OF NICOTINIC RECEPTOR FUNCTION: A SHIFT FROM STOCHASTIC PARADIGMS

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We recently presented a novel deterministic model for the operation of the nicotinic acetylcholine receptor (nAChR) [1], offering an alternative to traditional stochastic (Markovian) models [2]. To validate the model predictions, we used ultrahigh-resolution single-channel recordings of the nACH receptor [3]. Unlike these models, the new approach assumes that receptor gating is fully determined by agonist binding, with no random transitions or delays. The model assumes that the agonist molecules bind to the receptor alternately and repeatedly (Fig.1A,C). After dissociating from the receptor, the agonists remain within the binding pocket and participate in subsequent binding events. The receptor remains open as long as at least one agonist molecule is bound (Fig.1C). Thus, prolonged openings occur through repeated binding of both agonist molecules.

The model also accounts for brief openings, which happen when only one agonist molecule is involved in activation, or when the receptor is partially desensitized. We define a partially desensitized receptor as one with a C-loop in the closed (down) position. (Fig.1B). Such a receptor can still open briefly if an agonist binds at the site where the C-loop remains in the open (up) position.

In the Markov models, conformational transitions from the resting state to the open and desensitized states are triggered by an increase in the agonist affinity. In deterministic model the binding site affinity remains constant regardless of whether the second site is occupied or whether the receptor is in the open or closed state.

We provide exact mathematical formulas linking agonist binding times to receptor with measured receptor opening times. This enables quantitative validation of the model and connects directly measurable macroscopic properties with microscopic parameters characterizing receptors.

Furthermore, the model suggests that receptor kinetics can be modulated without direct binding of a modulator molecule, pointing to a novel form of non-classical modulation [4]. The model can help assess whether an ionotropic receptor behaves deterministically.

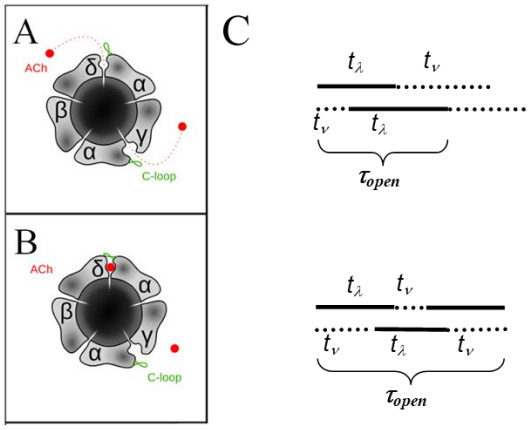

Fig.1. Schematic presentation of nACh receptor in the closed (A) and partially desensitized (B) configurations and gating mechanism of fully functional receptor (C). The open time (τ_{open}) is determined according to the rule: the channel opens at the moment of the first agonist binding and closes at the moment when both binding sites are empty. The length of each line corresponds to the time: how long the agonist is bound (solid, t_λ), detached (dotted, t_ν). Prepared based on [1].

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Professor Manfred Heckmann (Institute of Physiology, Julius-Maximilians-Universität Würzburg) kindly provided ultrahigh-resolution data of the ACh receptor.

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STRUCTURE-FUNCTION RELATIONSHIP OF THE GABA TYPE A RECEPTOR.

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The γ-aminobutyric acid type A receptor ($GABA_AR$) is a pentameric ligand-gated ion channel responsible for mediating inhibitory neurotransmission in the central nervous system. Dysfunction of $GABA_AR$ is implicated in several neurological and psychiatric disorders. Despite substantial progress in understanding $GABA_AR$ architecture and function, the molecular details of its activation mechanism remain incomplete [1]. We aimed to identify and temporally map structural elements of the $\alpha_1\beta_2\gamma_2$ $GABA_AR$ subtype that facilitate the transition from the agonist-bound to the fully open channel state. We applied single-channel recordings with high temporal resolution to wild-type and a wide array of single-point mutants targeting different structural regions of the receptor, followed by kinetic modeling and Φ-value analysis [2] to determine the contribution of each residue to gating transitions.

Mutations were introduced in functionally distinct receptor regions, including the agonist binding site, extracellular-transmembrane domain interface, and ion pore, allowing us to compare their effects on dose-response characteristics and gating kinetics. Notably, mutations in the binding pocket - such as β_2E155, β_2F200, α_1F45 and α_1F64 - produced significant right shifts in EC_{50} values, consistent with disrupted ligand binding but also significantly affected the receptor gating. Conversely, mutations in regions distant from the binding site, especially in the N-terminal region (β_2F31, α_1F14), at the domain interface (β_2V53, β_2P273, α_1H55, α_1P277) and transmembrane helices (β_2H267, β_2E270 β_2L296, α_1G258, and α_1L300), predominantly altered channel opening and closing kinetics without major changes in agonist affinity.

A particularly striking result was that some mutations at the domain interface—such as α_1R220 and β_2R216 in the β10-M1 linker and α_1D54, β_2E52 in loop 2 resulted in a near-complete loss of function despite normal membrane expression. This suggests that these residues are essential for the conformational coupling between extracellular and transmembrane domains. On the contrary a number of the ion pore lining residues mutations (β_2T256, β_2L259, α_1T260, α_1L263) induced the spontaneous activity of the receptor underlining a labile conformation of the channel gate.

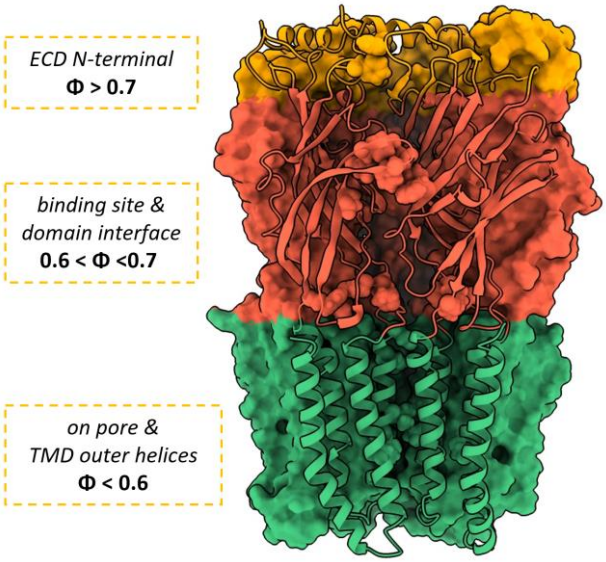

Fig.1. Φ-value map of the $GABA_AR$.

Using Φ-value analysis, we reconstructed a sequential map of conformational changes leading from extracellular domain top to agonist binding through domain interface to pore opening (Fig. 1), supporting a model where early events localize near the binding site and later transitions propagate through the domain interface into the transmembrane helices. Small range of all obtained Φ-values suggest that movement of the respective receptor structures is highly synchronized. This corresponds well with significant effects of almost all of investigated single point mutants on the receptor gating, multiple conjugated interactions between respective residues and also exceptionally broad variety of modulators affecting $GABA_AR$ gating. All those data supports the allosteric character of the receptor.

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THE EFFECTS OF 17β-ESTRADIOL AND PROGESTERONE ON THE BK CHANNEL ACTIVITY IN HUMAN GLIOBLASTOMA CELLS

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Sex differences in glioblastoma incidence and progression suggest a regulatory role of steroid hormones, including 17β-estradiol (E2) and progesterone (P) [1], but the exact mechanism of action is not yet understood. In this study, we examined how E2 and P influence the expression and functionality of large-conductance voltage- and Ca^{2+}-activated potassium channels (BK) channels and evaluated their impact on cell viability and cycle progression in human U87-MG glioblastoma cells.

U87-MG cells were treated with two E2 and P concentrations (i.e., E2: physiological-like 0.0018 μg/ml and pharmaceutical 1.8 μg/ml; P: physiological-like 0.025 μg/ml and pharmaceutical 25 μg/ml) for 24–48h. Cell viability was assessed using the CCK-8 assay. RT-qPCR was used to measure the expression of BK α and β (1–4) subunits. Promoter regions were screened for hormone-responsive elements using the online program AliBaba2.1. Functional activity of gBK channels was evaluated via single-channel patch-clamp method. The recordings were obtained at varied membrane voltages (–50 mV to +75 mV with 25 mV step) and analyzed by kinetic, correlation and nonlinear methods. Flow cytometry was used to assess the effects of E2 and P effects on the cell cycle.

Both E2 and P induced a concentration-dependent inhibition of BK channel activity in U87-MG cells, with a well-pronounced reduction in open-state probability (p_{op}), especially under depolarized conditions, as shown for exemplary potential of 50 mV in Table 1.

Table 1. The values of open state probability (p_{op}) of the BK channels in U87-MG glioblastoma cells at membrane potential of 50 mV at different concentrations of 17β-estradiol (E2) and progesterone (P). The Δp_{op} is given as standard error.

[E2] [μg/ml]	[P] [μg/ml]	$p_{op} \pm \Delta p_{op}$
0	0	0.45±0.06
0.0018	0	0.11±0.03
1.8	0	0.14±0.03
0	0.025	0.07±0.03
0	25	0.08±0.03

The analysis of the dwell-time series of channel states concluded that the hormones change the relative stability of the open and closed states, but not the number of observed states of discernible length. The application of nonlinear methods of patch-clamp series analysis allowed us to formulate running hypotheses about the possible mechanisms of channel-hormone interactions.

Expression analysis revealed hormone-dependent regulation of β subunits (particularly β3 and β4), with mostly evident effects at physiological doses. E2 and P exposure also affected cell cycle and cell viability, indicating their potent modulation of glioblastoma cells' biology.

To conclude, this study reveals novel aspects of glioblastoma biology focusing on the role of K^+ channel modulation by sex hormones. Both 17β-estradiol and progesterone function as effective inhibitors and expression modulators of BK channels in glioblastoma cells, affecting both channel biophysics and cellular processes.

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SESSION VI: Medical significance of proteins and nucleic acids

ATOMIC-RESOLUTION STRUCTURAL INSIGHTS INTO NATURALLY-CRYSTALLINE PROTEINACOUS MOSQUITOCIDES.

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As the vector of malaria, Dengue fever and filariasis, the mosquito is arguably the organism most threatening to human health. Chemical mosquitocides are cost-effective, but they also affect crustaceans, bees and fish, and extensive application results in resistance in the field. Notably, the recent spread of resistance to pyrethroid insecticides threatens efforts to control malaria. To date, the most environmentally-safe alternative to control mosquito populations remains the application of proteinacous mosquitocides produced in the form of naturally-occurring nanocrystals by entomopathogenic bacteria. Notably, *Lysinibacillus shapericus* (*Ls*) produces the binary toxin Tpp1Aa/2Aa (formerly known as BinAB) while *Bacillus thuringiensis israelensis* (*Bti*) produces a cocktail of four naturally-crystalline proteinaceous toxins (Cyt1Aa, Cry11Aa, Cry4Aa, Cry4Ba). The structures of these proteins long remain elusive due to both the minute size of the natural crystals and the difficulty to recrystallize the toxins *in vitro* after their dissolution. We will report on the *in vivo* protoxin structures of *Ls* Tpp1Aa/2Aa as well as *Bti* Cyt1Aa and Cry11Aa, which we solved by applying serial femtosecond crystallography to the naturally-occurring nanocrystals [1-3]. We will present results from structure-guided mutagenesis, which afforded the identification of residues that affect crystal size, pH sensitivity and toxin folding, thus providing insights into each toxin's bioactivation cascade. Altogether, our results open avenues for development of new, rational strategies for improved mosquito control, e.g. by development of recombinant bacterial insecticides combining potent larvicidal proteins of different origins [1-3].

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BK_{CA} CHANNEL AS A NOVEL MODULATOR OF DNA DAMAGE RESPONSE IN HUMAN BRONCHIAL EPITHELIAL CELLS EXPOSED TO PARTICULATE MATTER

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Although particulate matter (PM) is a well-recognized genotoxic environmental agent, the molecular mechanisms underlying its harmful health effects remain poorly understood. The respiratory epithelium, as the primary site of PM deposition, acts as a protective barrier and is enriched in potassium channels that are essential for maintaining airway surface liquid homeostasis. In human bronchial epithelial (HBE) cells, large-conductance calcium-activated potassium (BK_{Ca}) channels—located at the apical plasma membrane and within the inner mitochondrial membrane—play a key role in this regulation.

In this study, we investigated the potential involvement of the BK_{Ca} channel in the cellular DNA damage response (DDR) following PM exposure [1]. While DDR pathways have been extensively characterized, the role of ion channels in these processes remains largely unexplored. To address this, we employed BK_{Ca}-depleted HBE cells (HBE $\Delta\alpha BK_{Ca}$) as a physiological model [2].

Exposure to standardized PM (SRM-2786) in HBE $\Delta\alpha BK_{Ca}$ cells resulted in decreased clonogenic survival, elevated ROS levels, PARP1-dependent apoptosis, cell cycle alterations, and an increase in DNA double-strand breaks compared to wild-type (HBE WT) cells. qPCR analysis revealed upregulation of genes involved in both single-strand break repair (SSBR), such as OGG1 and XRCC1, and double-strand break repair (DSBR), including XRCC3 and PARP3, suggesting a compensatory activation of DDR pathways.

In conclusion, this study provides the first evidence of a critical role for the BK_{Ca} channel in modulating the DNA damage response to particulate matter in bronchial epithelial cells.

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STRUCTURE-GUIDED STABILIZATION OF MEMBRANE-ACTIVE PEPTIDES AS A STRATEGY TO COMBAT ANTIBIOTIC RESISTANCE

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The emergence of multidrug-resistant bacteria poses a serious global health challenge and necessitates the search for alternative antibacterial agents. Membrane-active peptides (MAPs), including antimicrobial peptides (AMPs) and cell-penetrating peptides (CPPs), are promising molecules due to their fast mode of action and low propensity to induce resistance. However, their use is limited by poor structural stability, susceptibility to proteolytic degradation, and possible toxicity toward mammalian cells.

Our work focuses on improving the antibacterial properties of MAPs by stabilizing their biologically active conformations, particularly α-helices (Fig. 1). We employed a hydrocarbon stapling strategy that involves the incorporation of two (*S*)-2-(4′-pentenyl)-alanine residues into the peptide sequence and covalent side-chain cross-linking. This modification locks the peptides into a helical conformation and enhances their structural integrity. Using this method, we successfully modified a range of peptides, including anoplin (a naturally weak AMP) [1], a CPP - $(KFF)_3K$ [2], and *de novo* designed amphipathic peptides rich in lysines and leucines [3].

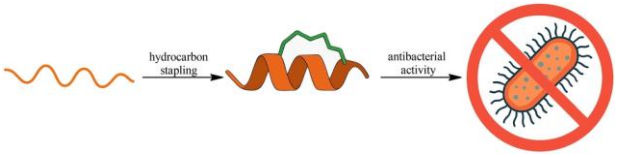

Fig.1. Schematic representation of the structure–activity relationship for membrane-active peptides. Hydrocarbon stapling stabilizes the α-helical conformation of unstructured, linear peptides thereby conferring antibacterial activity.

Stapled versions of these peptides showed significantly improved antibacterial activity, with MIC values ranging from 2 to 4 μM against both gram-positive and gram-negative strains. The stapled anoplin exhibited a 16-fold increase in activity and substantially enhanced proteolytic stability [1]. Similarly, stapled $(KFF)_3K$ gained membrane-permeabilizing properties and potent antimicrobial function [2]. Structural studies confirmed that these peptides adopt membrane-active α-helical conformations. Importantly, none of the stapled peptides exhibited hemolytic activity or cytotoxic effects on mammalian cells [1-3].

In a further step, we designed and synthesized conjugates of the stapled peptides with aminoglycoside antibiotics, such as neomycin and amikacin, using both reducible and non-reducible linkers. These conjugates retained or exceeded the antibacterial activity of the parent compounds and were effective against resistant bacterial strains, highlighting the synergistic potential of peptide–antibiotic hybrids [4].

In conclusion, our strategy of stabilizing secondary structures has proven highly effective in improving the antimicrobial performance and therapeutic potential of MAPs, offering a promising platform for the development of next-generation antibacterial agents.

ACKNOWLEDGMENTS

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BICARBONATE TRANSPORT CORRECTION DRIVES CLINICAL BENEFIT OF ELEXACAFTOR/TEZACAFTOR/IVACAFTOR IN F508DEL-CF

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Cystic fibrosis (CF) is caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), an epithelial ion channel essential for maintaining airway surface homeostasis through the coordinated transport of chloride (Cl^-) and bicarbonate (HCO_3^-) into the airway surface liquid (ASL). Defective CFTR disrupts this ion balance, leading to ASL dehydration, impaired mucociliary clearance, and progressive lung disease. The Elexacaftor/Tezacaftor/Ivacaftor (ETI) triple modulator therapy has demonstrated substantial clinical benefits in people with CF (pwCF) carrying the F508del mutation, primarily attributed to restored Cl^- transport. Emerging evidence indicates that ETI-mediated Cl^- correction is further modulated by airway inflammation. However, the effect of F508del-CFTR rescue on transepithelial electrogenic HCO_3^- secretion — a critical determinant of ASL pH, viscosity, and host defense — remains incompletely characterized, limiting our understanding of the full therapeutic potential of CFTR modulators.

We aimed to characterize CFTR-mediated transepithelial bicarbonate (HCO_3^-) transport following F508del-CFTR rescue, both at baseline and under inflammatory conditions. The extent of CFTR functional correction was correlated with clinical outcomes in people with CF.

Methods

Primary human nasal and bronchial epithelial cells from people with CF (pwCF) carrying at least one F508del-CFTR allele were treated with Elexacaftor/Tezacaftor/Ivacaftor (ETI), both alone and in combination with TNF-α and IL-17 to model an inflammatory microenvironment. CFTR-mediated transepithelial bicarbonate (HCO_3^-) and chloride (Cl^-) transport was assessed by using short-circuit current (Isc) measurements in Cl^--free and HCO_3^--free buffer systems, respectively.

Results

ETI treatment significantly increased F508del-CFTR–dependent bicarbonate (HCO_3^-) and chloride (Cl^-) short-circuit currents (Isc) to a similar extent. The $Isc\ HCO_3^-$ / $Isc\ Cl^-$ ratio in ETI-treated F508del cultures was comparable to that observed in wild-type (WT) epithelia. Exposure to TNF-α and IL-17 further enhanced ETI-corrected CFTR-mediated HCO_3^- and Cl^- transport, without altering their relative permeability ratio. No significant differences in Cl^- or HCO_3^- transport correction were observed between F508del homozygous and heterozygous primary cultures. At the individual patient level, the extent of HCO_3^- transport correction correlated with improvements in FEV_1, while Cl^- transport correction was associated with changes in sweat chloride concentration.

Conclusions

ETI restores chloride (Cl^-) and bicarbonate (HCO_3^-) transport at similar rates across the airway epithelium, with selectivity akin to wild-type CFTR. Both CFTR-dependent HCO_3^- and Cl^- transport independently and additively influence pulmonary disease severity in CF. Incorporating bicarbonate transport assays into clinical trials may enhance the evaluation of modulator efficacy and aid in optimizing personalized treatment strategies for CF.

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METALLIC NANOPARTICLES AS POTENTIAL MODULATORS OF ANTICANCER THERAPY

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Cancer is one of the greatest challenges faced by medicine. World Health Organization estimates that cancer's incidence and death toll approached 20 and 10 million, in 2022, respectively, with the most common being lung, breast, colorectum, and prostate cancers [1].

Although heterogeneity of the disease requires individual approach to each patient, chemotherapy remains one of the most commonly used treatment regimens. Despite its undeniable efficacy, factors such as cancer cells resistance and severe side effects of the therapy significantly limit the potential benefits for the patient. Researchers all around the globe are trying to address these concerns aiming for the therapy combining maximized efficiency with minimal complications of the therapy[2].

Nanomedicine is one of the disciplines heavily involved in this research. The unique properties of nanomaterials, including metallic nanoparticles, make them excellent candidates for the modulators of the classic anticancer drugs used in chemotherapy. The nanoparticles may be used both as the theragnostic agents and drug delivery vessels increasing selectivity of the anticancer drug, reducing cancer cells drug resistance, and contributing to the anticancer activity of the treatment regimen [3].

Therefore, we decided to evaluate the metallic nanoparticles potential to modulate the activity of the anticancer drugs. In our research we employed number of physicochemical methods to analyze interactions between selected metallic nanoparticles and anticancer agents, starting from spectroscopic methods via dynamic light scattering and atomic force microscopy to calorimetric methods. Subsequently, we assessed influence of nanoparticles on the anticancer drugs biological activity in the Ames mutagenicity test, MTT and alamarBlue cytotoxicity tests, and 3D Matrigel test.

Obtained results indicate direct interactions between metallic nanoparticles and anticancer drugs from anthracyclines group. However, there was no conclusive evidence on the cisplatin interactions with nanoparticles. Nevertheless, employed biological assays revealed significant influence of analyzed nanoparticles on the biological activity of all investigated anticancer drugs. Notably, the mutagenic activity of all tested was reduced while cytotoxic activity against chosen cancer cell lines was either not affected or elevated. Moreover, in case of the non-cancerous cell lines we observed protective effects of the tested nanoparticles against evaluated anticancer drugs.

Summing up, the results of our research indicate direct interactions between most of anticancer drugs and metallic nanoparticles. Furthermore, observed interactions affect biological activity of the drugs increasing their anticancer potential and selectivity. Similar effect was observed in case of cisplatin, where no direct interactions were confirmed.

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SESSION VII: Spectroscopy – ideas, methods, and applications in life science

PROTEINS: NEW AVENUES FOR THE DESIGN OF OPTICAL BIOSENSORS

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Since several years, researcher have acknowledged the importance of integrating biological molecules into the design of artificial devices. Biosensors are a combination of signal transducers and biomolecules, and they have a fundamental role in medical diagnosis, food safety and environmental control. The compactness, portability, high specificity, and sensitivity are the motives that the design of biosensors is considered to have a high potential in all analytical practices. Consequently, modern biotechnological strategies are exploiting the use of proteins, enzymes and antibody as components of sensors for analyses of high social interest. In particular, the idea is to take advantage of the extremely wide range of selective affinities sculpted into the various biomolecules by natural biological evolution. The number of potential molecules specifically recognized by different biomolecules is enormous and it sorts from small molecules to macromolecules (including protein themselves). The advantages of using proteins as components of biosensors are presented.

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WHAT VESICLES REMEMBER: NANOSCALE TRACES OF CELLULAR IDENTITY IN PLASMA MEMBRANE MODELS

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Cell-derived plasma membrane vesicles are among the most physiologically relevant model systems for studying native membrane composition and architecture [1]. Structural abnormalities in cell membranes are a hallmark of tumor cells and often accompany neoplastic transformation. Changes in membrane composition can significantly affect its biophysical properties, contributing to increased resistance to anticancer therapies [2]. However, the lack of specific biophysical or biochemical profiles for cancer cell membranes persists, primarily due to limited methods for nanoscale and spatial characterization of such thin and flexible structures.

In this study, we propose the use of atomic force microscopy working in force spectroscopy mode to analyze the nanoscale mechanical properties of plasma membrane vesicles derived from normal (microglia) and cancerous (glioblastoma) cell lines (Fig. 1A). For the first time, we demonstrate that the mechanical properties of plasma membrane vesicles (Fig. 1B) closely resemble those of the cells from which they originate (Fig. 1C). Furthermore, we describe differences in the biomolecular composition of these vesicles using FT-IR spectroscopy combined with principal component analysis (PCA). Finally, we show that integrating atomic force microscopy with infrared spectroscopy for the study of native plasma membranes reveals pronounced local heterogeneity that would otherwise remain undetected (Fig. 1D).

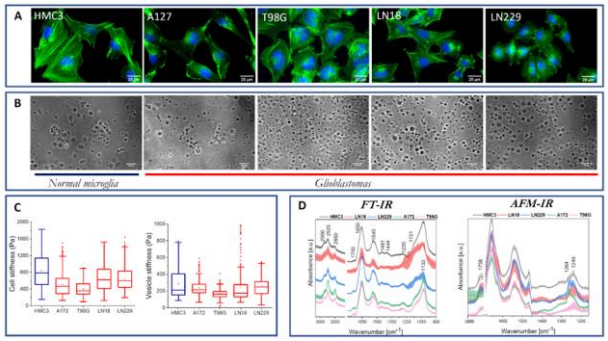

Fig.1. (A) Fluorescence images of normal microglia (HMC3) and glioblastoma cell lines (A172, T98G, LN18, LN229). (B) Optical images of the membrane vesicles isolated from live cells. (C) The mechanical properties of cells and isolated vesicles measured using atomic force microscopy. (D) Averaged FT-IR and AFM-IR absorption spectra of the vesicles isolated from microglia and glioblastoma cells (shading denotes standard deviation).

ACKNOWLEDGMENTS

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PROBING THE IMPACT OF CANNABIDIOL ON CELLULAR LIPID DYNAMICS VIA VIBRATIONAL SPECTROSCOPY

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Lipids, once considered merely as cellular energy reservoirs, are now recognized as dynamic regulators of numerous biological processes, including signal transduction, membrane remodeling, and cellular stress responses.[1] Increasing evidence points to their important role in the development of anti-cancer therapy resistance, including radioresistance. Malignant peripheral nerve sheath tumors (MPNST) are among the most radioresistant types of tumors, with limited treatment options and poor prognosis.[2]

In our study, we investigated the effects of cannabidiol (CBD)—a non-psychoactive compound with anticancer and neuroprotective potential—on the radiosensitivity of normal and cancerous cells of the peripheral nervous system. Our findings reveal that CBD sensitizes MPNST cells to ionizing radiation, while simultaneously protecting normal Schwann cells from radiation-induced damage.[3] Mechanistic insights obtained from vibrational spectroscopy strongly suggest that lipids are key modulators of this differential response.

Using a combination of Raman, FT-IR, and nanoscale AFM-IR imaging, we demonstrated distinct changes in the levels, distribution, and conformation of cellular lipids—especially cholesterol and its esters as well as phospholipids—in both cell types. The high-resolution chemoselective maps obtained by AFM-IR revealed localized lipid accumulation and modifications that correlate with the observed radiobiological effects.

To precisely track lipid alterations at the molecular level, we employed spectroscopically active probes in the form of deuterated lipids, whose unique C–D stretching vibrations appear in the cell-silent region of the spectrum (2000–2300 cm^{-1}), avoiding overlap with endogenous cellular signals.[4] This allowed us to selectively monitor the dynamics and distribution of cholesterol modifications in both cell lines, and its interaction with CBD.

These results highlight the pivotal role of lipid metabolism in modulating cellular responses to therapy and demonstrate the power of combining label-free vibrational spectroscopy with active molecular probes for uncovering treatment-induced biochemical changes. Our approach provides new insights into CBD-mediated modulation of radioresponse and suggests that lipid-targeting strategies could enhance therapeutic outcomes in tumors like MPNST.

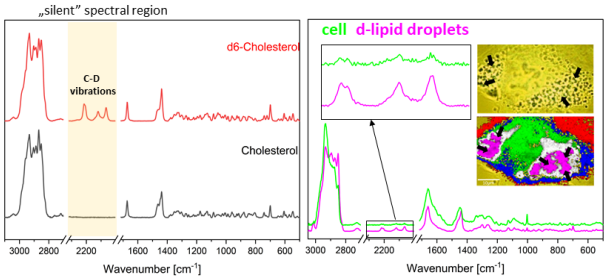

Fig.1.Comparison of Raman spectral profiles of cholesterol and its deuterated analogue (left panel). Cluster analysis of a cell treated with 50 μM d_6-cholesterol reveals regions of deuterated lipid accumulation (pink), confirming the presence of C–D vibrational bands identified in the collected spectra (right panel)

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THE LASING SPECTROSCOPY IN STUDIES ON PROTEIN AGGREGATION LINKED WITH NEURODEGENERATIVE DISEASES

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There is a growing imperative to detect neurodegenerative diseases at their earliest, pre-symptomatic stages if we are to intervene effectively. In these disorders, small, diffusible assemblies of misfolded proteins - amyloid oligomers [1,2], and specific types of fibrils strains [3] are now recognized as the principal culprits driving neuronal damage. While Thioflavin T (ThT) fluorescence has long been a staple for monitoring protein aggregation, it struggles to capture the fleeting, early-stage oligomeric species, and its signal is vague in terms of fibrils structure. Further obscured by subtle microviscosity changes around the assemblies. By contrast, exploiting optical gain through lasing of ThT-labeled oligomers dramatically amplifies these weak emissions, offering a level of sensitivity far beyond conventional fluorescence. We developed a multi-parametric assay that combines enhanced ThT fluorescence, Fabry-Perot cavity lasing, and machine-learning-driven image analysis. By embedding ThT-stained samples in an optical cavity under pulsed excitation, we induce narrowband lasing (FWHM ~2 nm) that amplifies viscosity-modulated emission into sharp spikes. This approach not only reveals the structural rearrangements that accompany disease progression but also discriminates between distinct aggregation states and fibril strains *via* their characteristic lasing thresholds. It shows that lasing should help with early diagnosis and strains recognition of neurodegenerative diseases, potentially before clinical symptoms emerge, which could improve patient outcomes through timely therapeutic intervention.

Fig.1. Illustration of Thioflavin T rotation highlighting the role of microviscosity in protein aggregation.

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THE MOBILITY OF EGFP CHROMOPHORE: ENVIRONMENTAL INFLUENCE ON FLUORESCENCE LIFETIME AND ANISOTROPY DECAY

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Fluorescent proteins (FPs) are nowadays widely used in a variety of spectroscopic methods, especially as biological markers for in vitro and in vivo imaging. [1,2]. One of the interesting applications is monitoring changes in the anisotropy of the green fluorescent protein (GFP) fluorescence in cells and tissues [3,4]
All FPs share common features: the cylindrical form, composition of 11 β-sheets, an α-helical segment buried inside the barrel and the fluorescent chromophore formed autocatalytically from 3 amino acids. [5]

Table 1. Parameters of fluorescence and anisotropy decays of EGFP in solution and PVA film at 20 °C. Fluorescence was excited and observed at 482 nm and 515 nm, respectively.

Sample name	Lifetimes		Anisotropy Decay	
	τ_1 [ns]	τ_2 [ns]	R_0	φ [ns]
EGFP in water	1.07 ±0.35	2.85 ±0.05	0.381	8.65 ±1.27
EGFP in buffer	0.92 ±0.27	2.84 ±0.04	0.387	8.90 ±1.35
EGFP in 2% PVA	0.92 ±0.30	2.81 ±0.05	0.356	9.09 ±1.76
EGFP in 4% PVA	0.84 ±0.32	2.77 ±0.04	0.385	8.71 ±1.79
EGFP in PVA film	-	1.91 ±0.01	0.151	0.869 ±0.066
EGFP dissolved from PVA film	1.17 ±0.48	2.89 ±0.10	0.383	6.09 ±0.76

We performed comparative studies on EGFP (F64L/S65T-GFP) fluorescence properties in different environment – from various solutions to molecule entrapped in the poly (vinyl alcohol) (PVA) film (Table 1).
In contrast to small organic fluorescent molecules stiffened in polymer matrices [6], the immobilisation of EGFP in the PVA film results in a shorter fluorescence lifetime and rotational correlation time (□), as well as lower initial anisotropy (R0). Interestingly, increasing the viscosity of the solution does not affect any of EGFP fluorescence properties.
We suggest that the fast anisotropy decay in PVA film is due to an increase in the mobility of the EGFP chromophore inside the protein after rearrangement of hydrogen bonds during PVA drying.
These findings shed light on the role and importance of structural water in GFP. The revealed unique fluorescent properties of GFP may be used to the development of novel applications in its use as a molecular marker.

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SESSION VIII: Biosensing and microfluidic systems for disease biomarkers detection in biomedical research

FROM CELLS-ON-A-CHIP TO ORGAN-ON-A-CHIP – NEW DEVICES AND TOOLS FOR PRECLINICAL STUDIES

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One of the main goals of cell engineering is to develop advanced, three-dimensional (3D) cell and tissue models that mimic tissue physiology in vivo [1]. Thanks to the use of microsystems, it is possible to mimic the spatial growth of cells, the complex composition of the extracellular matrix or control intercellular interactions in laboratory conditions.

The intensive development of miniaturization, which has been going on for several years, has made it possible to use modern technological solutions in chemical and biological research. Lab-on-a-chip systems are one of new microfluidic technologies that enable the creation of advanced 2D and 3D cell cultures in laboratory conditions [1]. Microfluidic cell culture has significant advantages over conventional macroscopic cell culture techniques and has the potential to improve knowledge in many fields of medicine, biology and chemistry.

Currently, preclinical drug research is based on several commonly used cell models, including two-dimensional (2D) and three-dimensional (3D) models cultured under standard conditions (static cultures, culture plates). Therefore, to reproduce the correct ratio of cell model volume to the external environment and flow conditions, a new approach to the generation and culture of in vitro cell models called the Organ-on-a-Chip (OoC) systems, has been proposed. The real challenge is to choose which cell model would be most suitable for modeling a particular organ using OoC technology.

The lecture will present several applications of cell engineering developed in our research group, i.e. the development and testing of new drugs and testing the effectiveness of various combinations of anticancer therapies (2), the Islet-on-chip system for creating islet cell cultures (3,4).

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ELECTROCHEMICAL BIOSENSORS FOR MULTIPLE BIOMARKERS DETECTION

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The interest in biosensor technology has been constantly growing over the last few years. Designing biosensors capable of detecting two or more analytes in a single measurement remains a significant challenge [1, 2]. Electrochemical methods are frequently used for this purpose, mainly due to the ability to apply two or more different redox labels, each characterized by independent and distinguishable electrochemical signals. Additionally, alongside antibodies, aptamers have been increasingly used as bioreceptors in the construction of such sensors [2]. In our group, we have joined this research line, and within this presentation we report on (I) multianalyte sensing platforms for cardiac biomarkers through the development of aptamer-based electrochemical sensors for brain natriuretic peptide (BNP-32) and cardiac troponin I (cTnI) [3] and (II) simultaneous detection of low density lipoprotein (LDL) and malondialdehyde-modified low density lipoprotein (MDA-LDL) based on the approach involving the formation of two types of specific immunoconjugates consisting of monoclonal antibodies: anti-LDL or anti-MDA-LDL, together with redox-active molecules: ferrocene and anthraquinone, respectively, coated on magnetic beads (MBs) [4]. In the first example, commercial gold-screen printed electrodes were modified electrophoretically with polyethyleneimine/ reduced graphene films. Covalent grafting of propargyl-acetic acid integrates proparyl groups onto the electrode, to which azide-terminated aptamers can be immobilized using Cu(I)-based "click"-chemistry. To ensure low biofouling and high specificity, the cardiac sensor was modified with pyrene anchor carrying poly(ethylene glycol) units. In the case of BNP-32, the sensor developed shows a linear response from 1 pg mL^{-1} to 1 µg mL^{-1} in serum; for cTnI, linearity is observed from 1 pg mL^{-1} to 10 ng mL^{-1}, as required for early-stage diagnosis of heart failure. In the second example, the decrease in redox molecules current in the concentration range of 0.001-1.0 ng mL^{-1} for LDL and 0.01-10.0 ng mL^{-1} for MDA-LDL, registered by square wave voltammetry (SWV), was observed upon the formation of complexes between LDL or MDA-LDL and the appropriate immunoconjugates. The detection limits were estimated to be 0.2 ng mL^{-1} for LDL and 0.1 ng mL^{-1} for MDA-LDL.

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ADVANCED MICROFLUIDIC STRATEGIES FOR DROPLET HANDLING AND BIOMEDICAL APPLICATIONS

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Microfluidics, a multidisciplinary area bridging physics, biology, and chemistry, has grown remarkably due to its ability to manipulate fluids at microscales [1]. Our work emphasizes the design and refinement of microfluidic devices that enable controlled droplet generation and manipulation [2,3], with practical applications in life sciences and chemical analysis.

By utilizing two-phase flows in confined channels, we engineer systems that exploit droplet-based transport, enabling precise sample encapsulation and reagent handling. Innovative passive control elements and capillary-hydrodynamic circuits are incorporated to guide droplet behavior without external actuation, offering compact and programmable platforms [4–7].

Algorithmic strategies complement our physical designs: digital droplet merging and splitting are used to achieve dynamic concentration control, improving reproducibility and flexibility of biochemical protocols [4,8].

Biomedical applications of our research include microfluidic chambers tailored for cell culture under biomimetic mechanical stress [9]. In collaboration with Université Grenoble Alpes, we investigated epithelial tissue mechanics, revealing how curvature modulates calcium signaling and gene expression. Another system, developed with the University of Oxford, allows us to measure oxygen unloading kinetics from erythrocytes using ultra-fast medium exchange and fluorescence microscopy [10].

These tools have proven effective in real-world scenarios, such as evaluating oxygen delivery efficiency during human kidney perfusion in transplant settings [11]. We demonstrate that red blood cell behavior, rather than blood flow alone, governs tissue oxygenation, contributing to a revised understanding of oxygen delivery metrics [12].

Our results underscore the potential of microfluidic systems not only as precise fluid manipulators, but also as transformative platforms for biological experimentation and diagnostics.

ACKNOWLEDGMENTS

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ELECTROCHEMICAL RNA-BASED APTASENSOR FOR NEOMYCIN DETECTION IN MILK SAMPLES

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The overuse of antibiotics in livestock, particularly in dairy cows, has raised significant concerns due to its direct contribution to the emergence of antibiotic resistance in humans. Antibiotic treatments, widely used to prevent and manage bovine mastitis, can promote the selection of resistant bacterial strains that can disseminate through the food chain, the environment, and direct contact with farm workers. This growing public health issue results in antibiotic-resistant infections that are increasingly difficult to treat, leading to higher morbidity, mortality, and economic burdens. Among commonly used veterinary antibiotics, neomycin is frequently administered in veterinary therapy for farm animals. Therefore, the development of simple and cost-effective methods for neomycin detection in food products is of paramount importance.

This study presents the development of a novel electrochemical aptasensor for detecting neomycin in cow's milk. A 2'-O-methylated ssRNA aptamer (APT) was employed as the recognition element and covalently immobilized onto a gold electrode surface, accompanied by co-deposited thiolated molecules (c-DTMs). The influence of different immobilization strategies and c-DTMs on the sensitivity of an impedimetric aptasensor for neomycin detection was systematically investigated. Electrochemical impedance spectroscopy (EIS) was employed, using $Fe(CN)_6^{3-/4-}$ redox probes, to evaluate sensor performance. Three distinct immobilization approaches were compared: (i) co-deposition (one-step) – simultaneous immobilization of APT and c-DTMs; (ii) sequential (two-step) – APT deposition followed by c-DTMs immobilization; and (iii) a hybrid method involving co-deposition followed by sequential modification. The tested c-DTMs included 6-mercaptohexan-1-ol, 4-mercaptophenol, mercapto-polyethylene glycol, and mercaptosulfobetaine methylacrylate.

Our findings demonstrate that the one-step co-deposition of APT and c-DTMs leads to the highest sensor efficiency for neomycin detection. Among the tested c-DTMs, 4-mercaptophenol provided the most effective reduction of nonspecific interactions, thereby improving sensor selectivity. The aptasensor's performance was assessed by monitoring changes in electron transfer resistance upon neomycin binding, recorded using EIS in the presence of $Fe(CN)_6^{3-/4}$ redox couples. The developed aptasensor exhibited high sensitivity, achieving a low detection limit of 36.3 nM in a 10-fold diluted cow's milk sample. Moreover, it demonstrated excellent selectivity for neomycin, effectively distinguishing it from structurally similar aminoglycosides (kanamycin and streptomycin) as well as tetracycline antibiotics (tetracycline and oxytetracycline).

The proposed electrochemical aptasensor provides a user-friendly, scalable, and cost-effective solution for detecting neomycin in milk samples. Its high sensitivity and specificity make it a promising tool for food safety monitoring and quality control in the dairy industry.

ACKNOWLEDGMENTS

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MONITORING OF THE MITOCHONDRIAL NETWORK IN A CELLULAR MODEL OF PARKINSON'S DISEASE UNDER THE INFLUENCE OF MDIVI-1

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Mitochondrial fragmentation is an early hallmark of dopaminergic neurodegeneration in Parkinson's disease (PD)[1-3], yet there is a lack of quantitative, long-term assays for evaluating mitochondria-targeted therapeutics. Here, we present an integrated, reusable microfluidic culture chamber (Fig.1) with fully automated, on-stage fluorescence microscopy, which can track the mitochondrial network of living neurons for ten days — from SH-SY5Y differentiation through toxin injury to pharmacological rescue.

Differentiated SH-SY5Y cells were challenged with the Parkinsonian toxin MPP^+ and subsequently treated with five concentrations of the dynamin-related protein 1 inhibitor Mdivi-1. The microfluidic device delivers programmable pulses of culture medium, BioTracker 488 Green Mitochondria Dye, toxin, and drug with sub-microlitre precision while maintaining a temperature of 37 °C. Custom software triggers time-lapse imaging and streams data directly to ImageJ/MiNA[4] for skeletonisation of the mitochondrial network (Fig.2).

In three repeated biological studies, we observed a 42% decrease in the median mitochondrial branching length (MBL) after exposure to MPP^+ ($p < 0.001$). Mdivi-1 caused a concentration-dependent restoration of MBL, reaching the highest values at a concentration of 40 μM ($p < 0.01$) (Fig.3); total branching length and network area reflected this trend. These results confirm that MBL is a sensitive, information-rich indicator of mitochondrial health and demonstrate that acute mitochondrial fragmentation can be reversed pharmacologically.

Our platform enables the high-throughput, longitudinal interrogation of mitochondrial dynamics under precisely controlled microenvironments. We anticipate that coupling this assay with patient-derived neurons will accelerate the discovery of disease-modifying drugs and inform mitochondrial gene-therapy strategies for PD and related neurodegenerative disorders.

Fig.1.The microfluidic chip. (a) The microfluidic device consists of several layers of polycarbonate that are connected using thin PDMS gaskets. The cells are grown on a microscope cover glass. (b) The chip's appearance after assembly.

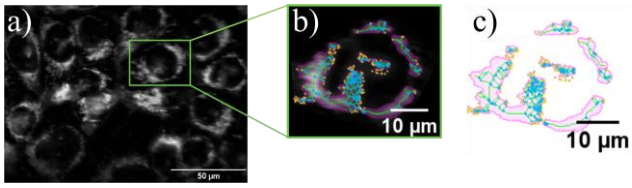

Fig.2. Image processing: (a) Image of undifferentiated SH-SY5Y cells. The mitochondria were stained with BioTracker 488 Green Mitochondria Dye. (b) Skeletal image of the mitochondrial network in a single cell. (c) Binary representation, where pixels are represented as either containing a signal or being background.

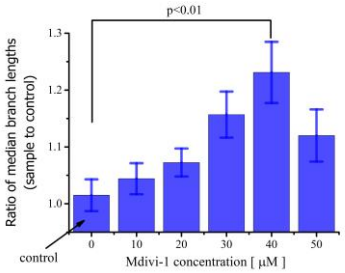

Fig.3. The effect of different concentrations of Mdivi-1 on the change in the ratio of median branch lengths (sample to control) was investigated. Measurements were taken after differentiation and administration of the Parkinsonian toxin MPP+ (control). Statistics were collected from three independent experiments. Confidence levels for the ANOVA test are indicated.

ACKNOWLEDGMENTS

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STUDIES IN YEAST REVEALED A MOLECULAR MECHANISM OF NEURODEGENEERATIVE DISEASES LINKED TO *MT-ATP6* MUTATIONS

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Mutations in the mitochondrial MT-ATP6 gene lead to a deficiency or absence of ATP—the energy-rich molecule synthesized in mitochondria by ATP synthase—and consequently result in mitochondrial diseases. The number of identified variants continues to increase due to the widespread use of next-generation sequencing (NGS) in patient diagnostics, with 962 entries currently listed in the MitoMap database. Assessing the functional consequences and pathogenicity of these variants remains challenging, particularly when they are found in only a small number of patients or coexist with wild-type mitochondrial DNA in cells and tissues (heteroplasmy), a common phenomenon. Taking advantage of the genetic tractability of Saccharomyces cerevisiae and the high instability of heteroplasmy in this organism, we constructed over twenty yeast strains bearing mutations in the ATP6 gene equivalent to those identified in patients. These mutations affect highly conserved residues of subunit a (Atp6) of ATP synthase, a protein essential for proton translocation across the mitochondrial inner membrane, which is coupled to ATP synthesis. Their effects on the function and biogenesis of yeast ATP synthase were analyzed using biochemical and molecular biology techniques. Thanks to the recent availability of high-resolution structures of yeast ATP synthase, we also investigated the structural consequences of these substitutions in silico and proposed molecular mechanisms of pathogenicity for five of the mutations. Furthermore, the identification of genetic suppressors for some of these mutations—located in distal regions of the Atp6 protein—that restored enzymatic activity provides a promising starting point for the development of small molecules that could be used to treat these currently incurable diseases.

I will also report on the engineering of a yeast strain expressing a new type of split-GFP, termed Bi-Genomic Mitochondrial Split-GFP (BiG Mito-Split-GFP). In this strain, the sequence encoding the non-fluorescent GFP1–10 fragment (the first ten β-strands) was integrated into the mitochondrial genome and is thus translated by the mitochondrial machinery, while the complementary fragment (GFPβ11) is fused to a nuclear-encoded protein of interest, translated in the cytosol. The self-assembly of this bi-genomically encoded split-GFP occurs exclusively in mitochondria, and only when the protein of interest is present in the matrix. Therefore, BiG Mito-Split-GFP provides a definitive method for confirming the localization of a given protein within the mitochondrial matrix.

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PUZZLING PATH OF POTASSIUM INFLUX INTO MITOCHONDRIA – THE STORY OF MITOK_{ATP}

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Mitochondrial ATP-sensitive potassium channels (mitoK_{ATP}), first described over three decades ago, seem to play an important role in cardioprotection. Yet, despite extensive efforts, their molecular identity remains unresolved. Initial mitoplast patch-clamp recordings revealed a K^+ conductance inhibited by matrix ATP and glibenclamide, suggesting a mitochondrial counterpart of sarcolemmal K_{ATP} channels [1] . This led to the hypothesis that mitoK_{ATP} comprises a Kir6.x-type pore-forming subunit and a sulfonylurea receptor (SUR2A). However, knockout of Kir6.1/6.2 failed to eliminate mitochondrial K^+ fluxes, prompting a search for alternative candidates.

Three main hypotheses currently dominate. The first posits that the renal potassium channel isoform ROMK2 (Kir1.1), which contains a mitochondrial targeting sequence, forms the channel pore [2]. Overexpression of ROMK2 in cardiomyocytes induces an ATP-inhibited, diazoxide-activated K^+ current and improves resistance to ischemia–reperfusion injury. In our studies, purified ROMK2 reconstituted into planar lipid bilayers generated single-channel activity consistent with mitoK_{ATP} properties [3]. The channel was activated by diazoxide and inhibited by ATP/Mg^{2+} and glibenclamide. In other studies, a 55-kDa mitochondrial splice variant of SUR2A was shown to co-assemble with ROMK2, and SUR2A-55 overexpression enhanced mitoKATP activity and cardioprotection *in vivo*. However, genetic studies produced conflicting results: cardiomyocyte-specific ROMK deletion did not abolish cardioprotection, whereas global ROMK knockout worsened injury.

A second model proposes a dedicated mitochondrial K_{ATP} complex formed by CCDC51 (pore-forming) and ABCB8 (regulatory). A reconstituted CCDC51–ABCB8 channel recapitulates mitoK_{ATP} pharmacology, including diazoxide activation and ATP/glibenclamide inhibition. CCDC51 knockout abrogates diazoxide-induced K^+ uptake and eliminates preconditioning-induced cardioprotection, strongly supporting this complex as a functional mitoK_{ATP} entity [4].

A third hypothesis suggests that the F1Fo-ATP synthase may function as a K^+ channel under stress conditions. Fo subunit can conduct K^+ along with protons, potentially acting as a latent uniporter during ischemia. Curiously, K^+ transport *via* purified ATP synthase exhibits pharmacology of the mitoK_{ATP} channel [5].

Despite these advances, it remains unclear what is the molecular identity of mito$K_{ATP.}$ An important question is whether multiple mitoK_{ATP} entities or regulatory modes exist (potentially unifying Kir/ROMK and CCDC51/mitoSUR paradigms), and how this knowledge can be harnessed therapeutically. Resolving the mitoK_{ATP} mystery will guide new strategies for safeguarding mitochondria under stress.

ACKNOWLEDGMENTS

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ENHANCING ANTIPLATELET EFFICACY THROUGH BIOENERGETIC MODULATION: COMBINED INHIBITION OF GLYCOLYSIS AND OXIDATIVE PHOSPHORYLATION

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Blood platelets play a crucial role in the development of vascular thrombosis, a major complication in patients with cardiovascular and metabolic diseases. The primary strategy to reduce the risk of thrombotic cardiovascular events is antiplatelet therapy. However, this approach is not fully effective in patients with metabolic disorders, where platelets often exhibit hyperreactivity despite pharmacological treatment. This suggests that alterations in platelet energy metabolism may underlie this condition.

Platelet activation is an energy-demanding process that relies on dynamically regulated metabolic pathways, with energy derived from both glycolysis and mitochondrial oxidative phosphorylation (OXPHOS). Such bioenergetic plasticity allows rapid functional responses and can contribute to platelet hyperreactivity in metabolic disorders, often limiting the efficacy of antiplatelet therapies. In this study, we investigated whether mild interference with platelet energy metabolism could enhance the inhibitory effect of cangrelor, a $P2Y_{12}$ receptor antagonist that does not directly affect bioenergetic pathways.

Washed platelets from healthy donors were treated with CORM-A1 (a carbon monoxide-releasing molecule that inhibits both glycolysis and mitochondrial respiration) or a combination of 2-deoxy-D-glucose (2DG, a glycolytic inhibitor) and oligomycin (an ATP synthase inhibitor) at low concentrations, in the presence or absence of cangrelor. Platelet aggregation was assessed using light transmission aggregometry, while the oxygen consumption rate (OCR) and the extracellular acidification rate (ECAR), reflecting real-time metabolic flux, were measured using Seahorse extracellular flux analysis. Intraplatelet ATP levels and reactive oxygen species (ROS) production were quantified using luminescence- and fluorescence-based assays. Targeted liquid chromatography-tandem mass spectroscopy (LC-MS/MS) analysis was employed to assess intracellular metabolites and eicosanoids.

Our results show that dual, partial inhibition of both glycolysis and OXPHOS significantly enhanced the antiplatelet effect of cangrelor. Although CORM-A1 and the combination of oligomycin and 2DG modulated platelet bioenergetics through distinct mechanisms, both strategies led to increased accumulation of adenosine, potentially responsible for the enhanced antiaggregatory effect of cangrelor. In contrast, reductions in ROS and eicosanoid production appeared to be consequences, rather than causes, of diminished platelet activity. Metabolomic profiling revealed that oligomycin alone shifted metabolism toward glycolysis and decreased aspartate. In contrast, 2DG increased erythrose 4-phosphate and aspartate levels, pointing to differential regulation of glycolytic and ancillary pathways. Changes in aspartate levels most clearly distinguished the effects of oligomycin and 2DG. However, under thrombin-stimulated conditions, combined treatment showed that the metabolic effects of 2DG predominated.

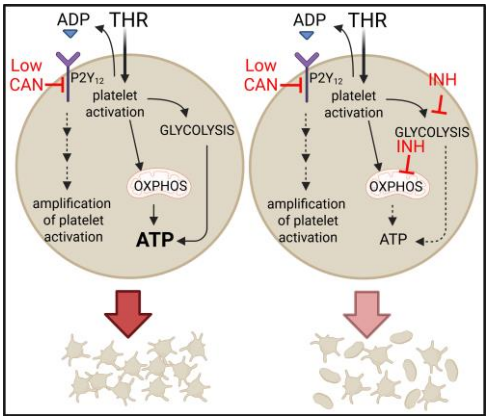

Fig.1. Mild metabolic inhibition enhances cangrelor-induced platelet inhibition (prepared with BioRender).

These findings highlight the central role of bioenergetic pathways in platelet function and suggest that dual, partial inhibition of energy metabolism may represent a novel strategy to enhance antiplatelet therapies – particularly in metabolically dysregulated states.

ACKNOWLEDGMENTS

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UNRAVELLING METABOLIC DISRUPTIONS IN MPAN DISEASE – INSIGHTS FROM PATIENTS FIBROBLASTS

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Neurodegeneration with Brain Iron Accumulation (NBIA) is a rare inherited disease characterized by progressive symptoms associated with excessive and abnormal iron deposition in the brain. Out of the 11 described NBIA subtypes, the most frequently diagnosed are pantothenate kinase-associated neurodegeneration (PKAN), PLA2G6-associated neurodegeneration (PLAN), beta-propeller protein-associated neurodegeneration (BPAN), and mitochondrial membrane-associated neurodegeneration (MPAN). In our study, we focus on the MPAN subtype, which is caused by mutations in the *C19orf12* gene and is one of the most common NBIA subtypes diagnosed in Poland.

The goal of our research is to identify the affected metabolic pathways in fibroblasts derived from MPAN patients with a mutation in the *C19orf12* gene. Our experimental approach is based on growing fibroblasts under both basal and OXPHOS-promoting conditions to better visualize potential mitochondrial metabolic defects.

Fibroblasts derived from MPAN patients are characterized by impaired cellular and mitochondrial processes, such as reduced proliferation, altered metabolic activity, decreased oxygen consumption, and increased ROS levels, when compared with control fibroblasts. These alterations become more apparent under conditions that favor mitochondrial metabolism. Moreover, the exact role of the C19orf12 protein in cellular physiology, as well as the impact of mutations in the *C19orf12* gene, will be evaluated using HEK-T clones with mutated *C19orf12* gene. This will provide deeper insight into the pathomechanism of MPAN disease.

ACKNOWLEDGMENTS

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LIGHT-MEDIATED ACTIVATION OF MITOCHONDRIAL BK_{CA} CHANNEL PROTECTS GUINEA PIG CARDIOMYOCYTES AGAINST HYPOXIC INJURY

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Photobiomodulation is a non-invasive medical intervention based on the regulation of biological systems *via* illumination with infrared light (IRL). The therapeutic potential of IRL includes analgesic, anti-inflammatory, and cytoprotective effects, especially in ischemia-reperfusion processes. One of the proteins that absorbs IRL is cytochrome c oxidase (COX), located in the inner mitochondrial membrane, constituting complex IV of the mitochondrial respiratory chain. In the structure of COX, two copper centres can be distinguished, Cu_A and Cu_B, which absorb light from the red and infrared spectral range. The maximal absorption wavelengths change depending on the redox conditions: in an oxidising environment, they are 820 nm and 680 nm for Cu_A and Cu_B, respectively; in a reducing environment, they are 620 nm and 760 nm for Cu_A and Cu_B, respectively. Previous studies have shown that changes in COX activity affect the opening of the large-conductance Ca^{2+}-activated potassium channel ($mitoBK_{Ca}$) [1]. In turn, it has been shown that stimulation of the $mitoBK_{Ca}$ channel, like other mitochondrial potassium ion channels by chemical compounds, has a cytoprotective effect, especially in the case of hypoxia and ischemia-reperfusion injury [2].

As a research model, we have chosen cardiomyocytes isolated from guinea pigs and mitochondria isolated from them. The quality and purity of isolated mitochondria were confirmed by electron microscopy, functional and biochemical analysis. The Western Blot technique confirmed the presence of the α subunit forming the pore of the $mitoBK_{Ca}$ channel. Patch-clamp studies revealed the presence of a functional channel with the characteristics of the $mitoBK_{Ca}$ channel, including a conductance of about 130 pS, and a voltage dependence and sensitivity to Ca^{2+}. Moreover, inhibition by paxilline (a classical inhibitor of the BK_{Ca} channel) was also observed. The patch-clamp experiments have also shown a regulation of $mitoBK_{Ca}$ channel activity by IRL. Illumination with 820 nm light was able to restore $mitoBK_{Ca}$ channel activity, which had dropped in response to 300 μM $K_3[Fe(CN)_6]$. Moreover, irradiation with 760 nm wavelength reactivated the channel inhibited by reducing agents - ascorbate and TMPD. To investigate the cytoprotective effect of IRL, the cardiomyocytes were subjected to three regimens before hypoxia: glucose deprivation, exposure to infrared light (820 nm), and a combination of both events.

Our results show that in specified redox conditions 820 nm light regulates the activity of the $mitoBK_{Ca}$ channel present in the mitochondria of guinea pig cardiomyocytes and that IRL exposure provides significant cytoprotection against subsequent hypoxic stress.

ACKNOWLEDGMENTS

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SESSION X: Protein dynamics, disorder, and phase separation

THE ROLE OF PHASE SEPARATION IN REGULATING ANIMAL GENE EXPRESSION

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Transcriptional condensates offer a new framework for understanding the organization of genomic activity. These protein-rich, sub-micrometre assemblies of transcription factors and RNA Polymerase II have been observed in mammalian embryonic stem cells and embryos of model organisms. However, their mechanism of formation and physiological function remain topics of debate. Using optical tweezers experiments, we recently demonstrated that a pioneer transcription factor can undergo surface condensation on DNA in a sequence-specific manner [1]. This involved a transition from a thin adsorbed layer to a thick condensed layer, characteristic of a prewetting transition. We are now investigating whether surface condensation could explain the formation of transcriptional condensates in a living animal. We have established *C. elegans* embryo as a model system, where we characterize several condensate-forming transcription factors and study their role in gene regulation during development and stress response. We found that genomic locations with a high local clustering of binding sites promote condensate formation. Deleting one such location reduced condensate numbers and altered the gene expression profile of several genes. Previous work on engineered condensates in mammalian cells showed that phase separation could buffer variation in cellular protein concentration [2]. Our experiments in *C. elegans* suggest that endogenous transcription factor condensates could act as buffers that regulate the level of available transcription factor and fine-tune the organismal response to stress.

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ENHANCING GŌMARTINI 3 APPROACH FOR THE STUDY OF CONFORMATIONAL CHANGES IN LARGE-SCALE BIOMOLECULAR ASSEMBLIES

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Advances in structural biology, particularly through cryo-electron microscopy (cryo-EM), have enabled the high-resolution characterization of increasingly complex biomolecular assemblies. These developments underscore the need for computational methods capable of capturing biologically relevant conformational changes over extended timescales. While atomistic molecular dynamics (MD) offers detailed insights at atomic resolution, it is inherently limited to local structural fluctuations and often fails to capture the large-scale transitions commonly observed in biological systems.

GōMartini 3 is a coarse-grained (CG) approach that enables the simulation of protein-membrane interactions, protein folding or unfolding under mechanical forces, and intrinsically disordered proteins. This method is well-validated for small protein systems, such as those composed of one or two well-structured chains, but its application to large macromolecular assemblies remains limited.

Here, we present an enhanced approach for the study of such complex systems, which integrates information from atomistic MD into the GōMartini 3 model to better capture long-timescale dynamics [1]. Native contacts (NCs) identified from short atomistic MD trajectories are used to inform GōMartini 3, enabling the exploration of conformational transitions with reduced computational overhead. Benchmarking different NC selection strategies revealed that incorporating both high-frequency intra- and inter-chain contacts significantly improves structural flexibility and sampling efficiency. This approach successfully reproduces the conformational landscape of the SARS-CoV-2 spike protein, composed of ∼3000 residues, outperforming the standard implementation. The full framework is available as an open-source resource, offering a scalable tool for simulating complex biomolecular assemblies comprising thousands of residues over timescales reaching hundreds of microseconds.

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BACK TO FIRST PRINCIPLES: MODELS OF INTRINSICALLY DISORDERED PROTEIN CONFORMATIONS

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As much as 50% of proteins in eukaryotes are believed to be intrinsically disordered [1]. While recent breakthroughs in machine learning have enabled computational tools that rapidly predict protein structure from sequence alone—largely thanks to the Protein Data Bank's archive of over 200,000 experimentally determined conformations—these structures represent only a subset of proteins: those with stable, well-defined folds. Predicting the dynamic conformations of intrinsically disordered proteins (IDPs) requires extrapolation beyond AlphaFold's current capabilities and demands more information than the sequence alone can provide.

Data on IDPs remain sparse, and their conformations are sensitive to environmental factors such as ionic strength, temperature and molecular crowding, making model development and evaluation challenging. Because of their extended conformations, direct numerical simulation (both all-atom and coarse-grained) can be prohibitively expensive. Our recent work [2] shows that many phenomenological models of average molecular size overfit, particularly when predicting hydrodynamic size.

While several sequence-based corrections to Gaussian chain models have been proposed, we find that first-principles modeling consistently outperforms them, underscoring the central role of steric interactions in modeling fully disordered and multidomain IDPs. Furthermore, additional data on the dependence of conformation on ionic strength allow us to examine under what conditions screened electrostatic interactions significantly influence protein conformation and when they do not.

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THE DEVIL IS IN DETAILS – PROTEIN HDX REVEALS CRITICAL CHANGES IN DYNAMICS UNDERLYING PROTEIN FUNCTIONAL DIFFERENCES.

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HDX provides unique insight into structural dynamics of proteins. It allows to monitor the relative frequencies of local or global unfolding events, which enable the exchange of main chain amides to solvent deuteria. Over the years HDX studies have shown that the half-lives of such opening events may span from sub-second times to hundreds of hours, so the dynamic axis of protein structurome is long, spanning several orders of magnitude. It has also shown that the dynamic component is quite well represented in typical proteins, and highly stable regions are rather and exception than a rule. Moreover, in some cases of protein variants of the same crystal structures, but different functionalities the detailed analyses of HDX results may provide dynamic rationale of their functional differences.
Two such cases will exemplified in the presentation.

IN OR OUT? GW182 SD JOINS THE BIOMOLECULAR CONDENSATES PARTY IN miRNA-MEDIATED GENE SILENCING

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GW182 is a fuzzy, intrinsically disordered protein that plays a key role in processing bodies (P-bodies)[1]. These biomolecular condensates are responsible for degrading mRNA during post-transcriptional miRNA-mediated gene silencing[2]. The N-terminal region of GW182, called the Ago-binding domain (ABD), binds to Argonaute (Ago), a core component of the miRNA-induced silencing complex (miRISC). The C-terminal region, known as the silencing domain (SD), recruits the CCR4-NOT deadenylase complex to miRISC-targeted mRNAs and enables gene repression.

CCR4-NOT is involved not only in the miRNA-mediated silencing pathway. It also functions in a different post-transcriptional repression mechanism driven by tristetraprolin (TTP)[3], an intrinsically disordered RNA-binding protein that targets AU-rich elements in the 3′ untranslated regions (UTRs) of cytokine mRNAs. Both GW182 and TTP recruit CCR4-NOT to silence gene expression, but they do so through distinct mechanisms. This functional overlap led us to explore whether the two silencing pathways might converge or compete with one another.

Interestingly, GW182 SD can repress gene expression even when tethered to mRNA independently of the ABD. While recent studies have revealed the role of the GW182 ABD in liquid-liquid phase separation (LLPS)[4], the contribution of the SD to this process remains unclear. It is also unknown how other proteins that interact with GW182 might affect the P-body formation.

To address these knowledge gaps, we performed biophysical studies showing that the human GW182 SD can drive LLPS independently from the ABD. Phase diagrams reveal that this phase behaviour is temperature-sensitive and relies on π-π interactions between tryptophan side chains. We also observed that GW182 SD forms multiprotein liquid droplets with a fragment of the CNOT1 subunit of CCR4-NOT that specifically binds to GW182 SD. This interaction points to a host–client relationship[5] between GW182 and CNOT1. Furthermore, the presence of TTP as a third component disrupts the formation of these condensates. This interference suggests that GW182 and TTP are in direct molecular competition for binding to the same region of CNOT1. This could indicate the possibility of the two post-transcriptional gene silencing pathways crossing over[6].

ACKNOWLEDGMENTS

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LOOKING FOR MOLECULAR MECHANISMS OF THE CYTOPROTECTIVE ROLE OF NPAS4

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NPAS4 (Neuronal PAS domain-containing protein 4), which belongs to the bHLH-PAS transcription factors, was discovered in hippocampal neurons [1]. Later, NPAS4 was shown to be induced by activity and stress in pancreatic β-cells for protection from endoplasmic reticulum stress, leading to the suggestion of NPAS4 as a therapeutic target in type 2 diabetes [2] and pancreas transplantation [3]. NPAS4 has neuroprotective effects in the damaged brain after ischemic stroke and has been proposed as a component of novel stroke therapies [4]. Also, NPAS4 has been proposed as a therapeutic target for depression, neurodegenerative diseases associated with synaptic dysfunction [5] and Alzheimer's disease [6]. Despite the presented important functions of NPAS4 and its potential therapeutic application, the mechanism of action of this protein, especially nongenomic way is not understood.

We believe that the multifunctionality of NPAS4 reported in the literature depends on the intrinsically disordered nature of its structure. The conformational plasticity of the long C-terminal region, predicted as IDR (intrinsically disordered region), its sensitivity to environmental changes and ability to interact with multiple partners, could explain documented multiple functions of NPAS4. Recently, the ability to form liquid-liquid phase separation (LLPS) has been proposed to be important for neuronal development and synaptic plasticity. Mutations in areas responsible for LLPS have been shown to lead to pathological aggregation and diseases such as autism or cancer. During this presentation, we will present attempts to clarify the links between NPAS4 and the development of neurodegenerative diseases.

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HOW MACHINE LEARNING ALLOWS TO RECONSTRUCT CARDIOMYOCYTE ACTION POTENTIAL FROM THE SURFACE OF THE BODY

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Motivation and Aim: Interpretation of certain electrocardiographic symptoms is still considered an art. Modern techniques, such as machine learning (ML) can be particularly useful, however following Wittgenstein, their abilities to interpret the world are set by the limits of the language, that has been used in learning process. Our aim is to demonstrate the usefulness of ML techniques based on PhysECG reconstruction model: a new paradigm of ECG interpretation, based on molecular theory of biopotentials. We want to show, that the features of the model have a clear bearing to underlying cardiac anatomy and physiology. Specifically, we show, how the local cardiomyocyte action potential (AP) can be quite reliably reconstructed from the surface ECG. We present the results of a pilot study [1], which are long from being reliably confirmed, on the clinical level, but on the technical level they generate promising results.

Novelty: Application of PhysECG algorithm allows to interpret the ECG within a different dogma. We decompose the passage of an activity wave through the ventricles into two mutually related but functionally disjoint processes: passage of the activation wavefront (P1) and cardiomyocyte response (P2). Starting the analysis from the electronic circuit analysis of the electrode setup, we show how to reconstruct the true unipolar potentials, that reach individual electrodes, and how they account to ECG lead, observed in clinic.

Methods: We have used a ML model trained on 800,000 12-lead ECG recordings of MIMIC database, which contains healthy individuals and patients with various cardiological symptoms. The model performs a two-step reconstruction of the ECG, which is decomposed into activation functions (P1 process) and locally spatially averaged cardiomyocyte response (AP), resolved per electrode. As test data we have used 549 recordings of PTB database, including 80 patients of cardiological norm. For the pilot study we have used 51 recordings of Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) from National Institute of Cardiology.

Main results: We confirm the result, that the Wilson potential is far from being constant and far from zero [2]. The analysis allows us to show correlation between QRS widening and T wave inversion: note, that in clinic the processes of depolarization and repolarization are often treated separately, due to complex spatiotemporal dynamics. We demonstrate a conceptual model of the QRS-T wave relation, showing, that there is a direct link between T wave inversion and wide QRS complex: note, that both these symptoms are observed in the ECG of ARVC patients. Finally, we show, that the possibility to perform a regional resolution of the AP reveals regional changes, which are potentially arrhythmogenic. We show that early results on the assessment of activation duration show correlation with the progression of the disease.

Conclusion: The PhysECG – a physically motivated projection algorithm, on the level of a technical pilot study, is a promising tool, which deserves further validation. The analysis of the results obtained thanks to its utilization suggests, that the phenomena of depolarization and repolarization should not be treated separately. However, there exists a viable possibility to decompose the electric activity into two processes: the spatiotemporal dynamics of the electrical stimulus wavefront and the response of the cardiomyocytes, reflected in their AP parameters.

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DEEP LEARNING FOR MEDICAL DIAGNOSIS

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Deep learning is increasingly redefining diagnostic workflows in medicine, particularly in radiology, where it enables automated, accurate, and scalable analysis of complex imaging data. This talk provides a high-level overview of deep learning approaches in medical diagnostics, with a focused examination of their application to pancreatic tumor detection using computed tomography (CT) imaging. Pancreatic cancer remains one of the deadliest malignancies, largely due to late-stage diagnosis and the subtle, often ambiguous appearance of tumors on imaging. We present recent developments in convolutional neural networks (CNNs) and hybrid architectures trained on CT datasets for the segmentation and classification of pancreatic lesions. These models have demonstrated the potential to detect tumors at earlier stages and differentiate between malignant and benign growths with increasing accuracy. The talk will also cover practical challenges, including limited annotated datasets, variability in scan protocols across institutions, and the need for clinically interpretable outputs. Strategies such as data augmentation, and model tuning are discussed in the context of improving performance and generalizability. We conclude by exploring the clinical implications of AI-assisted radiology, regulatory considerations, and pathways toward integration into real-world diagnostic settings. This session is intended for researchers and clinicians at the intersection of AI and healthcare, highlighting the opportunities and challenges of applying deep learning to high-impact diagnostic problems.

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FROM ROSENBLATT'S PERCEPTRON TO JUMPER'S ALPHAFOLD

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Today, machine learning methods have become part of mainstream everyday use. In the natural sciences, they have emerged as standard research tools across many fields, significantly extending and complementing conventional approaches to modeling complex systems and processes.

When Frank Rosenblatt introduced the first artificial neuron in 1958 [1], his inspiration came directly from biology. That biological grounding continued to shape the development of neural network architectures — for example, convolutional neural networks [2] were modeled after the structure of the cat's visual cortex.

When John Jumper and Demis Hassabis presented AlphaFold2, their neural network model for predicting tertiary protein structure [3], the direction of inspiration had reversed. Their model was built not on biological intuition, but on abstract mathematical constructs such as tensors, attention mechanisms, and the transformer architecture, initially developed for natural language translation. These tools from the world of artificial intelligence were used to address one of the central challenges in biology: protein folding [4].

In this talk, I will outline key aspects of AlphaFold2's inner workings, framing them within the broader historical shift from biologically inspired architectures to mathematically grounded paradigms — a transformation reflected even in the evolving terminology, as tensors replaced neurons along the way. This perspective will be set against the backdrop of recurrent cycles of enthusiasm in neural network research, each periodically constrained by the technological limitations of its time.

This framing is especially relevant today, as society increasingly reflects on the boundaries, potential, and implications of artificial intelligence—in science, across societal structures, and in our individual lives.

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COMPUTATIONAL INSIGHTS INTO TARGETING PTERIDINE REDUCTASE 1, A KEY ENZYME FROM PATHOGENIC TRYPANOSOMATIDS

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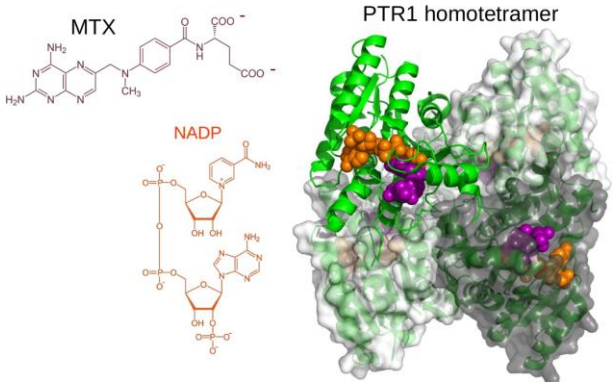

Fig.1. Homotetrameric enzyme pteridine reductase 1 (PTR1) of *Trypanosoma brucei* along with its cofactor NADP and a model antifolate methotrexate (MTX).

Pteridine reductase 1 (PTR1) is a folate pathway enzyme in trypanosomatid parasites that uses an NADP cofactor to reduce folates for DNA synthesis. It contributes to resistance against antifolate drugs like methotrexate (MTX), and thus is considered a promising anti-trypanosomatid drug target.

Using molecular docking simulations, we have developed two compound series combining 2-aminobenzothiazole and 3,4-dichlorophenyl moieties, resulting in several low-micromolar PTR1 and parasite growth inhibitors, less toxic than the parent compound [1]. One compound exhibited inhibition against both *T. brucei* and *Leishmania* species, which is relatively uncommon. Finally, computationally efficient quantum-mechanical calculations enabled us to elucidate the effects of halogen substitutions on inhibitor interactions with PTR1.

These efforts have also shown that drug development targeting PTR1 is hindered by a limited understanding of its structural dynamics, which we have studied using molecular dynamics simulations and related computational techniques [2,3]. We have uncovered an opening movement of the substrate loop that affects interactions of PTR1 with substrates, product, and the model inhibitor MTX. The dynamics of loop-ligand interactions appear critical for understanding binding mechanisms. These findings highlight factors influencing ligand binding to PTR1 and may support further PTR1-targeted drug design.

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SIMDNA: A COARSE-GRAINED METHOD FOR DNA FOLDING SIMULATIONS AND 3D STRUCTURE PREDICTION

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DNA, the blueprint of life, primarily forms a double helix but can also create structures like junctions, triplexes, and quadruplexes. These structures are essential for cellular functions, including gene expression regulation, replication, and genome stability maintenance.

Exploring DNA structure through methods like X-ray crystallography, NMR, and Cryo-EM spectroscopy is crucial but accompanied by challenges. These methods can be costly and time-intensive. X-ray crystallography captures static snapshots of DNA conformations, lacking dynamic insights. Moreover, NMR is restricted in its ability to analyze smaller DNA molecules, while achieving high-resolution Cryo-EM density maps is more common for larger biomolecules, such as those with 150 kDa.

SimDNA is a new computational tool based on SimRNA [1] that addresses these challenges. It predicts DNA 3D structures using a coarse-grained representation and the Metropolis Monte Carlo sampling technique - a statistical mechanics method that efficiently explores conformational spaces of the molecule by sampling from Boltzmann distribution.

This approach allows SimDNA to accurately fold various DNA forms, including duplexes, junctions, and non-canonical structures like triplexes and G-quadruplexes, even without external restraints. Furthermore, SimDNA enables guided simulations using data from experiments or other computational methods, providing a versatile tool for researchers. This flexibility allows user-defined restraints to focus simulations on specific interactions or structural configurations, facilitating the study of transitions between different DNA structures. Overall, SimDNA holds great promise for advancing our understanding of DNA behavior, offering insights into fundamental biological processes, and aiding in biomedical research and therapeutic development.

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Posters

SESSION I: NANOMEDICINE – BIOPHYSICAL ASPECTS

HOW DO PLATINUM NANOPARTICLES AFFECT THE BIOLOGICAL ACTIVITY OF DOXORUBICIN?

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Nowadays, platinum nanoparticles (PtNPs) attract much attention due to their properties, such as various sizes and shapes, surface functionalization, and large surface to volume ratio. Importantly, PtNPs are proven to possess anticancer properties and may be used as drug delivery system to provide more efficient treatment [1]. Doxorubicin (DOX), an anthracycline antibiotic, is widely used in treatment of various cancers such as breast, ovarian or hematological malignances. However its usage is limited due to major side effects, particularly severe cardiotoxicity, and drug resistance [2].

Therefore, in this research we decided to investigate whether PtNPs can interact with DOX and consequently influence the biological activity of the drug. Hence, a broad range of physicochemical methods, such as Atomic Force Microscopy (AFM), Dynamic Light Scattering (DLS), Fluorescence Spectroscopy, and biological methods including Ames mutagenicity test and cytotoxicity assay on both non-cancerous and cancerous cell lines were employed.

Firstly, the DLS and AFM results revealed that DOX triggers PtNPs aggregation. In turn, nanoparticles decreased DOX fluorescence and the effect was dilution-independent. Moreover, the Ames assay, showed that PtNPs decrease DOX mutagenicity. Importantly, the results of AlamarBlue cytotoxicity assay revealed that nanoparticles addition promoted cell viability reduction in cancerous cell line in comparison to DOX alone, while they increased the cell viability in non-cancerous cell line.

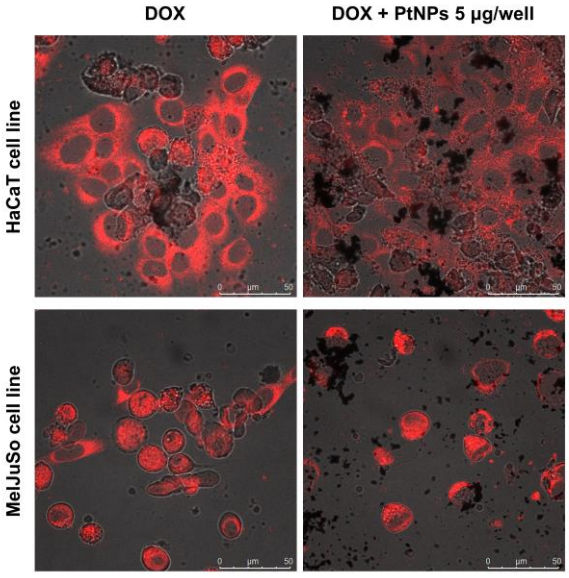

Fig.1. Confocal microscopy images of 70 nm PtNPs influence on DOX cytotoxic effects in HaCaT and MelJuSo cell lines.

The confocal microscopy imaging further confirmed that PtNPs had completely opposite effects in the two cell lines. In case of the cancerous MelJuSo cell line, the nanoparticles addition to DOX resulted in fluorescence quenching and a dramatic change in the morphology of the cells. Most of the cells were circular with approximately 1/3 of them showing membrane blebbing which may suggest apoptosis. However, in case of non-cancerous HaCaT cell line, PtNPs improved cell morphology and density of the cell culture compared to DOX alone [3].

In summary, the results confirmed that interactions between PtNPs and DOX led to promising effect in cytotoxicity against cancer cells, while simultaneously providing a protective effect on healthy tissue.

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INNOVATIVE APPLICATIONS OF KNOWN ANTIOXIDANTS: LIPID NANOCARRIERS WITH α-TOCOPHEROLS - DSC AND AFM ANALYSIS

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Based on literature and our preliminary studies, tocopherols—like cholesterol—can be used to create liposomal nanocarriers with favourable properties, such as uniform size, controlled shape, and high homogeneity, which support their stability and effectiveness in therapeutic applications [1]. The wide biological activity of tocopherols, especially α-tocopherol, makes them appear to be a competitive component of lipid nanocarriers to cholesterol.

The α-tocopherol derivatives can affect the mechanical and structural properties of the lipid bilayer of the nanocarriers, hence the precise determination of these changes is crucial for therapeutic applications of liposomes with tocopherols as a drug delivery system.

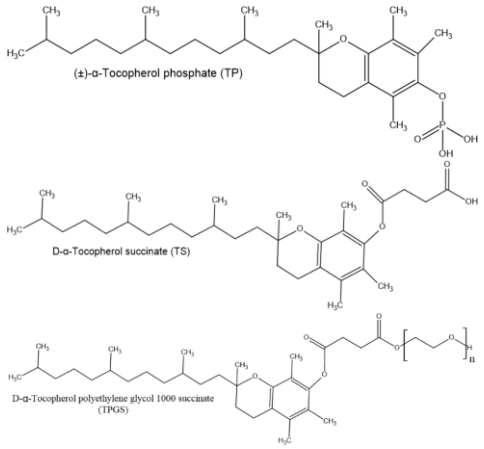

Fig.1. Structure of the α-tocopherol derivatives.

The compounds selected for the study (Fig.1): α-tocopherol phosphate (TP), α-tocopherol succinate (TS) and α-tocopherol succinate-polyethylene glycol conjugate (TPGS) have interesting biological properties beyond their commonly attributed function as antioxidants [2-4], and so far have not been characterized for their role as components of lipid nanocarriers.

In the presented research task, we focused on analyzing and comparing the thermotropic parameters and mechanical properties of three systems: DPPC:TP, DPPC:TS and DPPC:TPGS. We used differential scanning calorimetry (DSC), fluorescence spectroscopy and atomic force microscopy (AFM) to evaluate the physicochemical properties, shape and defects, and topography of the lipid nanocarriers.

Our results show that α-tocopherol derivatives alter the properties and behavior of the lipid bilayer of liposomes in a compound structure-dependent manner. All α-tocopherol derivatives reduce the temperature of the main DPPC phase transition. In contrast to TP, TS increases the stiffness of the lipid bilayer and probably has an effect on reducing its permeability. TPGS stabilizes liposomes, but in the same time induces phase inhomogeneity.

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PLATINUM NANOPARTICLES INTERACT WITH IDARUBICIN AND AFFECT ITS BIOLOGICAL ACTIVITY

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Metallic nanoparticles have attracted the scientific community's interest since the last century, and among them, platinum nanoparticles (PtNPs) have gained significant attention recently. Due to the variety of size, shape, composition, optical properties and possibility of surface functionalization, they exhibit a broad range of features, therefore they were applied clinically as medicinal, antibacterial or anticancer agents, either alone or in conjunction with drugs, serving as drug carriers [1]. Combining them with chemotherapeutics could result in enhancing the efficacy of the drug and possibly reduce the significance of side effects. With that in mind, we assessed the effects of commercially available platinum nanoparticles on idarubicin (IDA), an antibiotic anticancer agent used in treatment of variety of leukaemias [2].

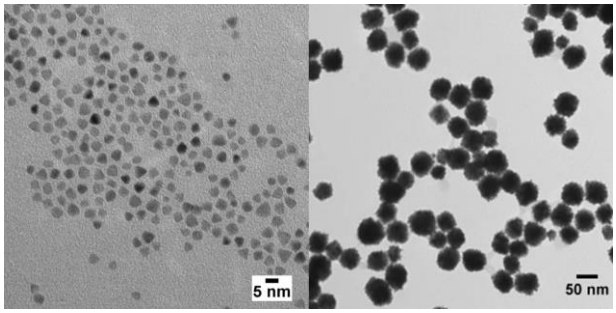

Fig. 1. Representative pictures of 5 nm (left) and 50 nm (right) platinum nanoparticles obtained from the NanoComposix company (www.nanocomposix.com, accessed 07.05.2025).

Firstly, we employed various physicochemical methods, such as dynamic light scattering (DLS) and atomic force microscopy (AFM) to asses the possibility of interactions through aggregation. As IDA is a fluorescent compound we performed spectrofluorimetric analysis to see if there are any close-distance interactions between PtNPs and IDA. Furthermore, we assessed the enthalpy changes using isothermal titration calorimetry (ITC). Finally, the biological effects of PtNPs on IDA were evaluated using Ames Mutagenicity assay with *Salmonella enterica* serovar Typhimurium TA98 strain.

Both DLS and AFM showed formation of aggregates upon addition of IDA to PtNPs. Through spectroscopic analysis we observed a significant quenching of IDA's fluorescence upon titration with PtNPs, where this effect was more intense than the one observed upon titrating with identical volumes of the buffer. The ITC showed that the interactions between PtNPs and IDA are of endothermic manner, with the enthalpy in the range of 1.2 kcal/mol. The mutagenicity assay revealed that platinum nanoparticles significantly lower the mutagenicity of idarubicin in all tested concentrations, proving that the discovered interactions influence the biological activity of the drug.

The overall results of the aforementioned analyses provide valuable insight into possible modulation of drug activity using platinum nanoparticles and point out the need to further study this combination, especially in eukaryotic *in vitro* analysis.

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NEXT-GENERATION CELL SHEET ENGINEERING VIA SMART POLYMER BRUSH COATINGS

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The future of regenerative medicine hinges on smart materials that enable precise, non-invasive control of cell behavior. In our work, we develop and characterize advanced temperature- and pH-responsive polymer brush coatings tailored for cell sheet engineering - platforms that not only support robust cell culture but also allow for gentle, enzyme-free detachment of intact cell layers.

We have synthesized thermoresponsive copolymer brushes, such as poly(N-isopropylacrylamide-*co*-2-hydroxyethyl methacrylate) [P(NIPAM-co-HEMA)] and poly(oligo(ethylene glycol) methacrylate-*co*-2-hydroxyethyl methacrylate) [P(OEGMA-co-HEMA)], which exhibit tunable lower and upper critical solution temperatures [1]. These coatings enable precise modulation of cell adhesion and spontaneous detachment without enzymatic intervention, preserving cell viability and extracellular matrix integrity. Additionally, we have explored the temperature-responsive properties of pH-sensitive poly(methacrylic acid) (PMAA) grafted brush coatings [2]. These surfaces exhibit controlled wettability, supporting fibroblast culture and highlighting their potential in tissue engineering applications. In our latest work, we have developed Cu-nanoparticle-loaded poly(4-vinylpyridine) (P4VP) brush coatings that integrate antibacterial and thermoresponsive functionalities [3]. These coatings facilitate the harvesting of retinal cell sheets while providing antibacterial properties, demonstrating their potential in ophthalmic regenerative therapies.

Altogether, these smart brush coatings offer a modular and responsive toolkit for next-generation biointerfaces - platforms that meet the growing demand for safer, smarter, and more efficient cell sheet technologies.

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NUCLEOBINDIN-2 AS A POTENTIAL MODULATOR OF BIOMINERALIZATION

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Nucleobindin-2 (Nucb2) is a multifunctional calcium- and DNA-binding protein implicated in various physiological processes, including energy homeostasiss [1], stress response [2], and cancer progression [3, 4]. Structurally, Nucb2 contains EF-hand motifs that confer high affinity for divalent cations, particularly calcium (Ca^{2+}), which is crucial for its conformational stability and biological activity [5]. In addition to Ca^{2+}, Nucb2 can bind other metal ions such as zinc (Zn^{2+}) and magnesium (Mg^{2+}) [6], influencing its intracellular localization and interactions with molecular partners. Ion binding alters the secondary structure of the protein and may modulate its function in calcium-dependent signaling pathways. Recent studies suggest that Nucb2 possesses unique structural features—especially its EF-hand motifs and a putative acidic domain—that may implicate it in biomineralization processes. Biomineralization refers to the biologically controlled deposition of minerals, such as hydroxyapatite in bone or calcium carbonate in marine organisms, which requires tightly regulated ion transport and protein-mineral interactions. The ability of Nucb2 to bind Ca^{2+}, Mg^{2+}, and Zn^{2+} positions it as a potential modulator of mineral nucleation and growth. Its ion-induced conformational changes may facilitate the spatial organization of ions into stable nucleation sites or influence vesicular transport of mineral precursors. Furthermore, Nucb2 has been detected in tissues undergoing active mineralization, supporting its potential physiological relevance. Although direct evidence for the involvement of Nucb2 in mineral scaffolding remains limited, its structural parallels with other mineralization-associated proteins, such as osteopontin and calmodulin, suggest a possible regulatory function. Here, we present preliminary observations indicating that Nucb2 is involved in mineral-associated cellular processes, possibly through its interaction with divalent metal ions relevant to biomineral formation, and may directly regulate the morphology of the resulting calcium carbonate biocrystals.

ACKNOWLEDGMENTS

The work was supported by a subsidy from the Polish Ministry of Science and Higher Education for the Faculty of Chemistry of Wroclaw University of Science and Technology.

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INSIGHT INTO THE OLIGOMERIC STATE OF THE NUDT12 NUDIX PROTEIN

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Nudt12 is a member of the NUDIX protein superfamily that is characterized by a highly conserved NUDIX motif (GX5EX5[UA]XREX2EEXGU, where U is hydrophobic and X any amino acid). The glutamic acid residues within the NUDIX sequence REUXEE play role in the binding of divalent metal ions required for the catalytic activity of NUDIX enzymes [**1**]. Nudt12 was initially identified as NADH diphosphatase [**2**]. It hydrolyses also structures present on 5' end of RNA (standard m^7GpppN cap, and a "metabolite" cap structures as NAD or dpCoA [**3, 4**]), and is active towards a set of dinucleotide analogs of the standard mRNA cap structure, differing in methylation status of the initial guanosine and the type/methylation of the adjacent nucleotide [**5**, **6**].

Human Nudt12 and its murine homologue are both dimeric proteins with 88% amino acid identity. Resolved crystal structures of hNudt12 and mNudt12 showed the presence of two distinct N- and C-terminal domains, and bound divalent metal ions (Mg^{2+} or Cd^{2+}) in NUDIX motif [**3**, **7**]. Dimerization of Nudt12 is essential for its catalytic activity and stability *in vivo*, as was demonstrated for the human protein: a designed monomeric mutant of hNudt12 was inactive in decapping assays [**3**].

The dimeric form of wild-type hNudt12 was confirmed *in vitro* by size exclusion chromatography (SEC) and analytical ultracentrifugation (AUC) [**3**]. However our initial AUC experiment for the murine protein mNudt12 showed a dimer-monomer equilibrium. Here, we report SEC analysis of mNudt12 oligomeric states under different experimental conditions (e.g. the presence of divalent ions or increasing protein concentration). Preliminary results confirmed the existence in solution dimeric forms of hNudt12, and a dimer-monomer equilibrium for mNudt12 that could be shifted in the presence of magnesium ions. As mentioned earlier, the monomeric form of hNudt12 is catalytically compromised; therefore, the influence of the oligomeric state of murine Nudt12 on its enzymatic activity and stability needs further investigation.

ACKNOWLEDGMENTS

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COOPERATIVITY BETWEEN THE mRNA 5'CAP AND 4E-BP BINDING SITES IN eIF4E EXPLORED VIA TRYPTOPHAN MUTAGENESIS AND FLUORESCENCE LIFETIME ANALYSIS

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Specific recognition of the mRNAs 5' terminal cap structure by the eukaryotic initiation factor eIF4E is the first, rate-limiting, step in the cap-dependent translation [1]. Small 4E-binding proteins (4E-BP1, 4E-BP2, and 4E-BP3) inhibit the translation initiation process by competing with eIF4G initiation factor for the same binding site and by blocking the assembly of the translation machinery [1]. Although the cap and 4E-BP binding sites in eIF4E are spatially distant (Fig. 1), they do not act independently. According to previous studies, the cap binding to eIF4E makes the affinity of eIF4E to 4E-BP1 significantly stronger, while binding of 4E-BP1 to the cap-eIF4E complex makes the cap dissociation slightly easier [2]. This finding indicates that the binding of either cap or 4E-BP1 induces conformational changes in eIF4E, not only in the region of a given binding site, but also in a distant region encompassing the binding site of the other ligand.

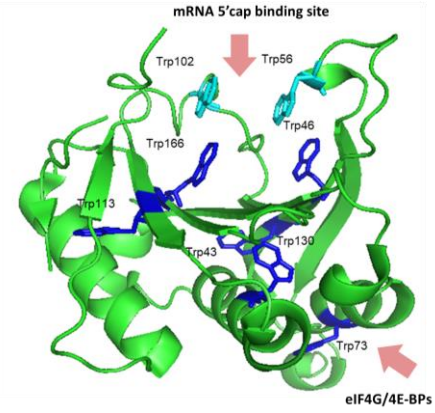

Fig.1. X-ray structure of eIF4E (PDB: 1IPC). The tryptophan residues are marked in blue and cyan

To elucidate the structural basis of the cooperativity between the cap- and 4EBP binding sites in eIF4E, we used emission spectroscopy, based on the intrinsic fluorescence of eIF4E originating from tyrosine and tryptophan residues. The eIF4E sequence contains eight tryptophans. Two of these (Trp 56 and 102) are located in the cap-binding site, and a third (Trp 73) is found on the 4E-BP-binding surface (Fig. 1). We investigated the effects of 4E-BP1 and a cap analogue (m7GTP) on the fluorescence lifetimes of eIF4E. Experiments were conducted on wild-type (WT) eIF4E, as well as on tryptophan-deficient mutants at the cap (W56A, W102A, W56A-W102A) and 4E-BP (W73F) binding sites, and for the triple mutant (W56A-W102A-W73F). For all eIF4E variants, complex formation with cap and/or with 4E-BP1 results in shorter fluorescence lifetimes compared to lifetimes of apo eIF4E. Furthermore, a substantial enhancement in eIF4E lifetimes is observed as the emission wavelength increases, for both the apo eIF4E and the binary and ternary complexes (Fig. 2). However, for mutants lacking Trp 56 (W56A, W56A-W102A and W56A-W102A-W73F), this increase in the presence of 4E-BP1 is less than for Trp 56-containing variants (WT, W73F and W102A).

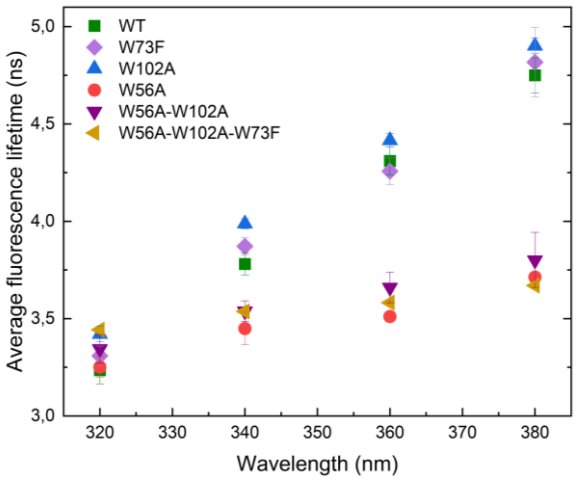

Fig. 2. The average fluorescence lifetimes of eIF4E variants in the presence of 4E-BP1, measured at different emission wavelengths for excitation at 284 nm

The results obtained suggest that Trp 56 may be one of the amino acids involved in the communication between the distant cap- and 4E-BP binding sites in eIF4E.

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TREATMENT OF FLEXIBILITY OF PROTEIN BACKBONE IN SIMULATIONS OF PROTEIN-LIGAND INTERACTIONS USING STEERED MOLECULAR DYNAMICS

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To ensure that an external force can break the interaction between a protein and a ligand, the steered molecular dynamics simulation requires a harmonic restrained potential applied to the protein backbone. A usual practice is that all or a certain number of protein's heavy atoms or Cα atoms are fixed, being restrained by a small force. This present study reveals that while fixing both either all heavy atoms and or all Cα atoms is not a good approach, while fixing a too small number of few atoms sometimes cannot prevent the protein from rotating under the influence of the bulk water layer, and the pulled molecule may smack into the wall of the active site. We found that restraining the Cα atoms under certain conditions is more relevant. Thus, we would propose an alternative solution in which only the Cα atoms of the protein at a distance larger than 1.2 nm from the ligand are restrained. A more flexible, but not too flexible, protein will be expected to lead to a more natural release of the ligand.

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DEPENDENCE OF THE FLUORESCENCE QUANTUM YIELD OF INDIVIDUAL TRYPTOPHAN RESIDUES IN A PROTEIN ON THE EXCITATION WAVELENGTH

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Sodium dodecyl sulfate (SDS) is an anionic surfactant that induces changes in both the secondary and tertiary structure of proteins. When examining such changes by fluorescence detection in the protein α-chymotrypsin, it was observed that the fluorescence of the protein, both in the presence and absence of SDS, depends not only on the presence of the surfactant itself but also on the excitation wavelength[1]. In the fluorescence spectrum measurements, a 320 nm cutoff filter was used, meaning that the detected protein fluorescence originated only from tryptophan residues.

Accordingly, a series of fluorescence spectrum measurements were carried out for both α-chymotrypsin and another protein, α-chymotrypsinogen, in the presence and absence of SDS, at four selected excitation wavelengths: 222, 260, 280, and 295 nm

We hypothesized that the fluorescence emission of individual tryptophan residues in the protein depends on the excitation wavelength. The fluorescence spectra of the proteins were analyzed according to a method found in the literature[2], where the authors presented fluorescence spectra as relative, normalized spectra: the curve for protein + SDS was subtracted from the curve for protein + buffer, and the resulting relative spectrum was then normalized at the short-wavelength minimum to –100 units. The obtained fluorescence spectra are presented in Figure 1. This way of presenting the spectra allows for the analysis of the total effect of the signal change compared to the initial value.

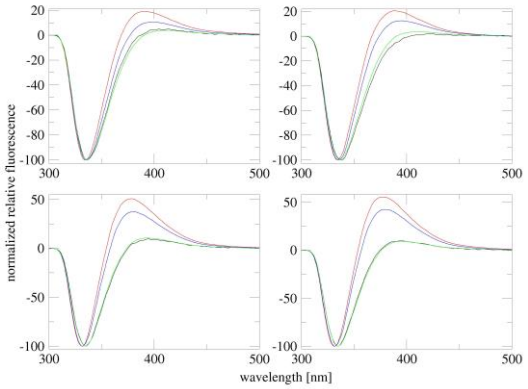

Fig.1. Normalized relative fluorescence spectra. Top: 5 μM α-chymotrypsin with 20 mM SDS (left) and 40 mM SDS (right); Bottom: 5 μM α-chymotrypsinogen with 20 mM SDS (left) and 40 mM SDS (right). Excitation wavelengths: 222 nm – black, 260 nm – red, 280 nm – blue, 295 nm – green.

When analyzing the spectra, clear differences in their appearance depending on the excitation wavelength can be seen. For both proteins, it is evident that the size of the positive limb is largest at 260 nm excitation, slightly smaller at 280 nm, and smallest at 222 nm and 295 nm. Moreover, in the case of α-chymotrypsin, the negative limb predominates at all excitations, meaning that in an experiment using the stopped-flow fluorescence detection method, a decrease in fluorescence should be observed — and this is indeed the case, as shown in the upper part of Figure 2.

In the case of α-chymotrypsinogen, although the negative limb also predominates for all excitations (as with α-chymotrypsin), but at 260 nm and 280 nm excitations the positive limb is considerably larger than at 222 nm and 295 nm. This difference is sufficient that for 260 nm and 280 nm excitations, an increase in the fluorescence signal over time is observed in kinetic measurements, whereas at 222 nm and 295 nm, a decrease is seen — as shown in the lower part of Figure 2.

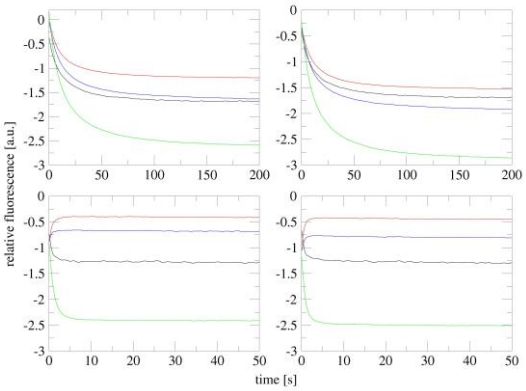

Fig.2. Relative reaction progress curves measured via fluorescence. Top: 5 μM α-chymotrypsin with 20 mM SDS (left) and 40 mM SDS (right); Bottom: 5 μM α-chymotrypsinogen with 20 mM SDS (left) and 40 mM SDS (right). Excitation wavelengths: 222 nm – black, 260 nm – red, 280 nm – blue, 295 nm – green..

In summary, the obtained results clearly suggest that the contribution of fluorescence emitted by individual tryptophans in a protein to the total, registered fluorescence depends on the excitation wavelength. We believe this is due to the fact that the non-radiative energy dissipation pathways of tryptophans, during the transition from higher excited states to the lowest excited singlet state (the state from which fluorescence occurs), occur with varying probabilities[3].

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BIOPHYSICAL ASPECTS OF ADIPOSE TISSUES REMODELING DURING OBESITY DEVELOPMENT

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During the development of chronic obesity, adipose tissue undergoes significant remodeling, which can result in chronic inflammation leading to fibrosis. This may cause local tissue damage and ultimately initiate dysfunction of multiple organs [1-4].

The aim of this study was to determine the effect of extracellular hyaluronan removal using hyaluronidase on the rheological properties of 3T3-L1 cells during their differentiation process into adipocytes, as well as to perform rheological studies on a lipid-rich adipose tissue hydrogel model. A NanoWizard 4 BioScience AFM (Bruker Nano GmbH, Berlin, Germany), operating in Force Spectroscopy mode, was used to measure the stiffness of confluent cell culture. The Young's modulus (E) was determined by analyzing force–indentation curves and fitting the data to the Hertz contact model. Rheological characteristics of hydrogels with added lipid elements were evaluated using a strain-controlled Anton Paar MCR702e rheometer (Anton Paar GmbH, Graz, Austria) with a parallel plate setup. The tests quantified the storage modulus (G′) and loss modulus (G″) by measuring the stress required to induce deformation. Two types of shear tests were performed: (1) oscillatory shear strain tests at 1 Hz frequency and 1% amplitude under compressive strain levels of ε = 0%, 10%, 20%, 30%, and 40%; and (2) strain amplitude sweep tests ranging from 0.1% to 100% at a constant frequency of 1 Hz.

The obtained results indicate that the removal of extracellular hyaluronan affects the mechanical properties of adipocytes. The results may contribute to a better understanding of the complex mechanics of the extracellular matrix of adipose tissue, which may affect the process of cell differentiation.

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EFFECTS OF LPS AND FPR2 AGONIST (IG4) ON THE MECHANICAL PROPERTIES OF MICROGLIAL CELLS.

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Alzheimer's disease is a common neurodegenerative disease characterized by chronic inflammation and the accumulation of beta-amyloid (Aβ) in the brain. Microglial cells, resident macrophages in the central nervous system, are thought to play a significant role in the development of this disease. These cells have both neurotoxic and neuroprotective effects. [1]

The presented studies aimed to investigate microglia's biomechanical properties (cell rigidity) in inflammatory conditions. The primary microglial cells obtained from the knock-in murine model of late-onset Alzheimer's disease ($APP^{NLF/NLF}$) and wild-type mice (WT) isolated from 1- or 2-day-old mice were used in experiments. Isolated cells were cultured in the presence or absence of bacterial endotoxin (lipopolysaccharide, LPS). Moreover, the impact of an agonist of formyl peptide receptor 2 (FPR2) (IG4) in basal and LPS-stimulated conditions was investigated. The biomechanical measurements were performed using atomic force microscopy (AFM), which worked in force spectroscopy mode. Data were collected over the nucleus region, and the Hertz-Sneddon model was used to evaluate the mechanical properties of cells.

Significant changes in the morphology of LPS-treated microglial cells from WT or $APP^{NLF/NLF}$ mice, contrary to non-stimulated cells, were observed. In basal conditions, WT microglia's mechanical properties differed from $APP^{NLF/NLF}$ microglia. LPS significantly increased the elastic modulus for microglial cells in both models. In basal condition IG4 agonist did not affect the biomechanical properties of microglial cells from WT and $APP^{NLF/NLF}$ mice. However, after immunoactivation evoked by LPS stimulation, this agonist has varied effects. In microglia cultures obtained from WT mice, IG4 significantly increases the LPS-evoked increase in Young's modulus. In the case of $APP^{NLF/NLF}$ microglia, IG4 lowered Young's modulus enhancement evoked by LPS.

In summary, our results indicate that inflammation, mimicked by LPS treatment, affects the biomechanical properties of mouse microglial cells. Moreover, the FPR2 agonist compound IG4 enhances the effect of LPS in cells isolated from WT mice, while reducing the LPS effect in cells isolated from APP mice.

ACKNOWLEDGMENTS

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THE EFFECT OF POLLUTION ON THE ELECTROPHYSIOLOGY OF EPITHELIUM – INSIGHTS FROM CACO-2 CELL MODEL

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The problem of plastic pollution and its impact on living organisms has now become a critical environmental concern. The breakdown of plastic debris produces micro- and nanoparticles which are ingested by living organisms and interact with the intestinal barrier. However, our understanding of their effects on human epithelial tissues and transepithelial water and ion transport remains limited. The aim of this study was to investigate the influence of polystyrene nanoplastics: PS-NPs (100 nm diameter) on the human intestinal epithelial cell line Caco-2.

This research focused on the observed increased mucus secretion displayed by Caco-2 cells in response to PS-NPs treatment. Utilizing Ussing chamber studies, we deduced that PS-NPs alter ion transport across cell monolayers. The presence of nanoplastics decreased CFTR channel activity, however, increased the activity of CaCC channels, e.g. TMEM16a. The TMEM16a channel involvement was verified using both ion transporting proteins modulators and the Fura-2 calcium indicator. The study also verified the cytotoxic properties of PS-NPs and its influence on TEER (Transepithelial Electrical Resistance).

This research validates that the elevated TMEM16a activity was responsible for the observed increased mucus secretion, acting as a recently discovered defence mechanism of Caco-2 cells against PS-NPs

ACKNOWLEDGMENTS

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SUBSTRATE VISCOSELASTICITY AND ADHESIVE LIGANDS AS REGULATORS OF GLIOMA CELL MIGRATION

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Gliomas are highly invasive brain tumors with a poor prognosis due to their ability to adapt to the unique mechanical and biochemical properties of the brain, which are largely defined by the extracellular matrix (ECM) [1]. While the role of ECM stiffness in tumor progression has been relatively studied [2], the contribution of ECM viscoelasticity to glioma cell behavior is poorly characterized. The presence of specific ECM adhesion ligands plays a key role in modulating cell-substrate interactions [3] and may further influence glioma cell migration which is a fundamental event in cancer progression [4, 5, 6].

This study aims to investigate how the viscoelastic properties of ECM, in combination with specific adhesive ligands (collagen I, fibronectin, laminin), modulate the migration dynamics of glioma cells.

Human glioma cells were cultured on polyacrylamide-based hydrogels with constant storage (G') and varying loss module (G"), mimicking brain tissue viscoelasticity [7]. Hydrogels and glass surface which serve as a control were functionalized with collagen I, fibronectin or laminin [3]. Cell migration was assessed using time-lapse microscopy and image analysis using ImageJ (Fiji).

We observed that ECM viscoelasticity along with adhesive ligand type modulates glioma cell motility. Understanding the complex interactions between mechanical and biochemical cues in the tumor microenvironment may be used to develop new therapeutic strategies aimed at reducing glioma invasiveness.

ACKNOWLEDGMENTS

This research was partially supported by National Science Center of Poland: UMO-2023/51/B/NZ7/00910 and by the Medical University of Białystok (B.SUB.25.403).

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IMIX AS A PARAMETER USED IN THE EVALUATION OF COLLAGEN TREATMENT FOR LOWER LEG ULCERS

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Leg ulcers affect over 1% of the adult population. In individuals over the age of 80 it's about 3%. Various treatment methods exist for leg ulcers. One of them is collagen therapy. In this method, patients applied collagen to the skin surrounding the ulcer by massaging it in daily for a period of 12 weeks. 89 patients diagnosed with unilateral lower leg ulcers were included in the study. Patients were assigned to the treatment or control group

The condition of the skin during therapy was assessed using electrical parameters, including the IMIX parameter. This parameter is one of the Ollmar parameters and reflects changes in skin reactance. It is defined as the ratio of the imaginary component of impedance at 20 kHz to the real component of impedance at 500 kHz. Measurements were taken at weeks 0, 4, 8, 12, and again at week 24 (after the collagen treatment) in both the treatment and control groups.

The results demonstrated that electrical parameters, including IMIX, may be useful in evaluating skin condition. This conclusion is supported by the presence of statistically significant differences between the treatment and control groups at three stages of the collagen therapy. Therefore, the IMIX parameter may be considered a valuable tool for monitoring skin condition during collagen treatment in the management of lower leg ulcers.

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NEW 1,2-BENZOTHIAZINE DERIVATIVES AS INHIBITORS OF CYCLOOXYGENASE WITH MEMBRANE PERTURBING POTENCY

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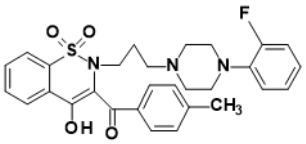
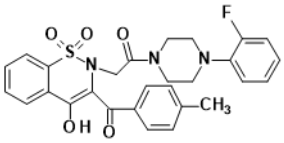
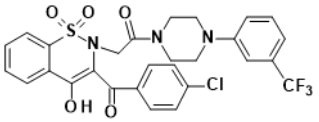
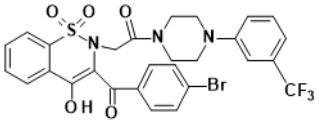
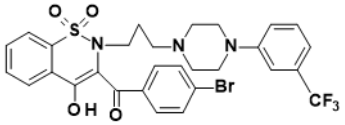
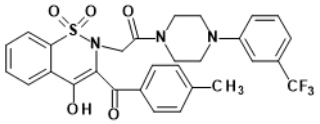
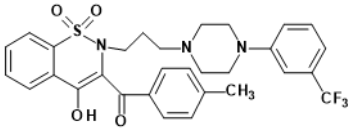
Cyclooxygenase (COX) is an enzyme located in the lumen of the endoplasmic reticulum, the Golgi apparatus and the nuclear membrane of the cell, embedded with hydrophobic fragments in the lipid bilayer of the membrane. COX catalyzes the process of prostanoid synthesis, i.e. prostaglandins, prostacyclin and thromboxane from arachidonic acid [1]. However, since inflammation has been linked to cancer development, the search for new, safer anti-inflammatory drugs has become even more important [2,3].

In our previous studies, we obtained a series of 1,2-benzothiazine derivatives with anti-inflammatory and analgesic activity confirmed in *in vivo* tests on mice [4].

In this study, a series of 1,2-benzothiazine derivatives (Tab. 1) were evaluated. In particular we investigated the interactions of the potential drugs with lipid bilayers, an important consideration for membrane permeability and overall pharmacokinetics. We have used differential scanning calorimetry method (DSC) to determine the interactions of studied compounds with phospholipid bilayers as models of biological membranes [5]. All examined compounds decreased the main transition temperature of phospholipid used to obtain the model membranes (DMPC) in a concentration-dependent manner. The addition of 1,2-benzothiazine derivatives to phospholipid also resulted in broadening of the transition peaks. Moreover, all examined compounds decreased the enthalpy of the DMPC main phase transition. It was therefore concluded that all the compounds interacted with phosphatidylcholine model membrane affecting its thermotropic properties. Although the greatest impact on the main transition temperature change was observed for the compounds PR25, PR49 and PR50. In addition, we evaluated the ability of studied compounds to inhibit COX-1 and COX-2 activity and selectivity using cyclooxygenase inhibition assay.

Table 1. Chemical structure of 1,2-benzothiazine derivatives

Compound symbol	Chemical structure
PR24	
PR25	
PR45	
PR47	
PR48	
PR49	
PR50	

Our findings suggest that the 1,2-benzothiazine derivatives could serve as potential lead candidates for the development of safer anti-inflammatory agents.

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TOXICITY ASSESSMENT OF CHITOSAN-BASED FILMS MODIFIED WITH QUERCETIN AND METALS - PRELIMINARY WOUND HEALING STUDIES

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Chitosan has found applications in various industries such as medicine, agriculture, textiles, food and environmental protection due to its many beneficial properties. In medicine, chitosan-based materials are used as dressings to speed up the healing process of injuries and burns. In skin and bone tissue engineering, chitosan is used to produce a variety of materials including membranes, hydrogels, sponges and fibres. Scientific literature also indicates the potential of chitosan and chitosan-engineered materials as drug carriers, including anti-cancer drugs, intranasal drugs, gene delivery drugs, prenatal drugs and ocular drug delivery systems [1-3].

Quercetin is a flavonoid with potent antioxidant and anti-inflammatory properties. As an antioxidant, it protects cells from oxidative stress, which promotes tissue regeneration. In addition, quercetin has anti-inflammatory effects by inhibiting the activity of enzymes and reducing the secretion of pro-inflammatory cytokines, which further promotes the healing process, especially during the inflammatory phase [4-5].

Based on their individual properties, we concluded that the combination of quercetin and chitosan films may induce synergistic effects, combining the properties of both substances for more effective wound healing. As a prelude to wound healing, we are evaluating the effects of these films on erythrocytes, peripheral blood mononuclear cells (PBMC) and human dermal fibroblasts (BJ line) in vitro.

The aim of this study is to evaluate the biocompatibility of chitosan films modified with quercetin and metals (Ag, Au, Cu, Bi) with blood cells and skin fibroblasts (BJ line) by assessing haemotoxicity, cytotoxicity and genotoxicity.

Chitosan films showed low toxicity to human erythrocytes, around 5%. Silver-containing nanomaterials showed higher toxicity (above 10%), depending on the silver content of the particle. In cytotoxicity analyses, silver-containing nanocomposites were more toxic than other chitosan films. Other variants showed a favourable toxicity profile, suggesting further research into their potential use in wound healing. Analysis of damage to genetic material showed low levels of damage. The addition of quercetin, a natural antioxidant, may partially mitigate the adverse effects of silver. Therefore, chitosan-quercetin metal films may have reduced cytotoxicity to skin fibroblasts and other human cells, increasing their potential for use in tissue engineering and wound healing.

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PHYSICOCHEMICAL DESCRIPTORS OF HALOGENATED FLAVONOIDS: INSIGHTS INTO THEIR ANTIBACTERIAL POTENTIAL

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Flavonoids are natural compounds found in plants, known for their anti-inflammatory, cytotoxic, and bactericidal properties. The analysis of flavonoid descriptors is essential for developing new derivatives, predicting biological properties, and understanding their mechanisms of action. With the rise in antibiotic-resistant bacteria, exploring flavonoid derivatives containing halogen atoms (bromine, chlorine), has become increasingly important. These substituents can significantly enhance the antibacterial properties of flavonoids, making them promising candidates for future therapeutic applications [1,2]. *In silico* studies, leveraging computational methods, offer a powerful approach to elucidate the structure-activity relationships of flavonoids, predict their interactions with biological targets, and explore their pharmacokinetic properties [3].

This study focused on three halogenated flavonoid compounds: 3'-bromo-5'-chloro-2'-hydroxychalcone, 8-bromo-6-chloroflavanone, and 8-bromo-6-chloroflavone. Laboratory experiments and SwissADME *in silico* analyses were conducted to examine their physicochemical properties, including melting point, molecular weight, polarity, log P, and others. Although the compounds have similar molar masses, differences in structure, bond flexibility, and insaturation were found to influence their biological activity [4].

The results showed that 8-bromo-6-chloroflavanone effectively inhibited the growth of pathogenic bacteria without significantly impacting probiotic bacteria, while 3'-bromo-5'-chloro-2'-hydroxychalcone, and 8-bromo-6-chloroflavone suppressed both probiotic and pathogenic bacteria. The presence of bromine and chlorine atoms enhanced the bactericidal effects compared to quercetin, a commonly studied natural compound [4].

These findings highlight the potential of halogenated flavonoids as alternatives to current natural products, particularly in regulating intestinal microbiota. Further research will explore their mechanisms of action and include additional *in vitro* and *in vivo* studies, focusing on their effects on HCT 116, FHC, and Caco-2 cell lines.

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ACTIVITY OF IMMUCILLINS ON THE PURINE NUCLEOSIDE PHOSPHORYLASE (PNP) FROM *H. PYLORI* AND ON THE BACTERIAL GROWTH

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Helicobacter pylori is a gram-negative, microaerophilic bacterium which colonizes the gastric and duodenal mucosa of half of the world's human population, and its presence may cause serious diseases, such as stomach and duodenal ulcers and stomach cancer. Unfortunately, the available therapies fail in 20% of patients due to increasing resistance to the antibiotics used. Therefore, it is very important to search for new molecular targets to design new drugs enabling eradication of *H. pylori* [1].

Immucillins are a group of synthetic compounds that are analogues of purine nucleosides. They inhibit PNP-catalyzed reactions by imitating their transition state [2]. Kicska et al. [3] have shown that immucillin H (Imm-H) is an inhibitor of PNP from *P. falciparum* (causing malaria in humans), which, like *H. pylori*, does not synthesize purines and purine nucleosides *de novo*, suggesting that it may also inhibit the *H. pylori* PNP enzyme and the replication of this bacterium. Therefore we decided to characterize interactions of Imm-H and other immucillins with PNP from *H. pylori* 26695 strain, and their influence on the replication of *H. pylori*.

Immucillins, which form a strong but slowly forming complex with PNP [2], were incubated with the enzyme in a reaction mixture lacking substrate, and then the reaction was initiated by adding the missing substrate (m^7Guo). We showed that immucillin A (Imm-A) (K_i = 1.3 ± 0.2 nM) is a potent inhibitor of *H. pylori* 26695 PNP, similar to Imm-H (K_i = 9.8 ± 0.8 nM). In contrast, MT-DADMe-ImmA does not inhibit *H. pylori* PNP.

We determined minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) values for immucillins against the reference strain *H. pylori* 26695 using the double serial dilution method in liquid medium [4]. Imm-A inhibits the growth of *H. pylori* (MIC = 80 μM (21 μg/ml)) in contrast to Imm-H, which does not affect the growth of this pathogen. However, Imm-A has no bactericidal effect on *H. pylori*, while the MBC value for MT-DADMe-ImmA is 5 μM (1.47 μg/ml), but its target is a different *H. pylori* enzyme.

Our studies show that among the compounds from the immucillin group, the most promising for use in the eradication of *H. pylori* in humans is Imm-A, which, as an adenosine analogue, does not interact significantly with the host PNP.

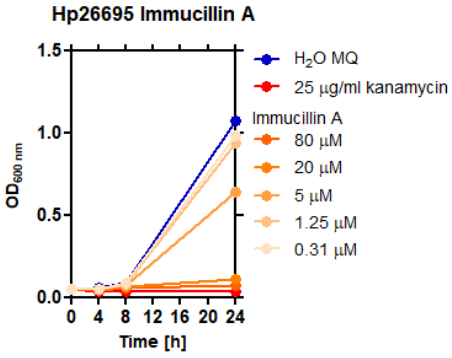

Fig.1. The growth curve of *H. pylori* 26695 strain in bacterial culture in the presence of various concentrations of immucillin A.

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SESSION V: ION CHANNEL IN THE CELL BIOPHYSICS

THE ROLE OF THE MITOCHONDRIAL BKCA CHANNEL IN THE PHYSIOLOGY AND DAMAGE OF RESPIRATORY EPITHELIAL CELLS INDUCED BY URBAN PARTICULATE MATTER

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Recently, it has been shown that the inner mitochondrial membrane's potassium channels (mitoK) are involved in cytoprotection. Therefore, protecting epithelial cells from particulate matter (PM)-induced damage may be related to activating potassium channels in the mitochondria.

To verify the role of mitochondrial large-conductance Ca^{2+}-regulated potassium (mitoBK$_{Ca}$) channel in cytoprotection in response to stress induced by PM, we performed a series of experiments using patch-clamp, transepithelial electrical resistance assessment, mitochondrial respiration measurements, fluorescence methods for the ROS level and mitochondrial membrane potential assessment, and cell viability measurements using trypan blue staining. In the human bronchiolar lung epithelial cell damage model (16HBE14o- wt), particulate matter 4 μm in diameter was used (PM4.0).

We observed that PM decreased the transepithelial electrical resistance in HBE cells dose-dependently. The effect was partially abolished by quercetin, mitoBK$_{Ca}$ channel activator. Penitrem A (BK$_{Ca}$ channel inhibitor) reversed the effect of quercetin. The patch-clamp findings confirmed that the effect is associated with channels. Quercetin activated the mitoBK$_{Ca}$ channel, abolishing the effect of penitrem A. The results were compatible with mitochondrial membrane and respiration measurements. Quercetin decreased the mitochondrial membrane potential and increased mitochondrial respiration. The effect was abolished by penitrem A only in whole-cell respiration measurements. PM-induced ROS levels are reduced at the cellular and mitochondrial levels. It correlates with cell viability results for quercetin, which increases HBE cell viability after PM administration. The toxic effect was also shown at the mitochondrial level. The PM incubation with the cells substantially reduced the mitochondrial function, which was measured as respiration control with fully uncoupled mitochondria compared to the inhibited electron transport chain.

To support our data, we used an analog of quercetin-isorhamnetin, a substance that has one hydroxyl group changed to a methoxy group. After its application, Isorhamnetin has no effect on the mitoBK$_{Ca}$ channel activity, respiratory rate, and mitochondrial membrane potential. Additionally, we used CRISPR/Cas9 technology in 16HBE14o- cells to generate cell lines lacking the alpha subunit of the BK$_{Ca}$ channel encoded by the KCNMA1 gene. Mitochondrial patch-clamp experiments showed the absence of an active mitoBK$_{Ca}$ channel in knockout cells (HBE ΔBK).

A better understanding of the relationship between mitochondrial metabolism and cell pathophysiology could aid in the search for effective cytoprotection strategies. Perhaps, by using naturally derived mitochondrial BK$_{Ca}$ channel activators, we will learn to support and induce these mechanisms to counteract the consequences of PM-induced damage.

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RADIOGENIC EFFECTS ON ION CHANNEL FUNCTION: INVESTIGATING THE ROLE OF BK_{CA} POTASSIUM CHANNEL IN DNA DAMAGE RESPONSE

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Glioblastoma multiforme (GBM) is the most common and aggressive primary brain tumor arising from astrocytes and is classified as WHO grade 4 astrocytoma. Standard treatment includes surgical resection, chemotherapy with temozolomide, and radiation therapy, but the low survival rate of patients highlights the urgency for innovative and more effective therapeutic tools [1]. The response of this type of tumor to chemoradiotherapy is poor, possibly due to a higher repair activity of the genetic material, among other causes. DNA double-strand breaks (DSBs) are an essential type of lesion to the genetic material, which have the potential to trigger processes of cell death or cause gene aberrations that promote tumorigenesis. Radiotherapy eliminates tumor cells by causing DSBs. Therefore, targeting the cellular DNA damage response is a promising strategy to enhance tumor radiosensitivity.

Ionizing radiation (IR) has been shown to stimulate ion transport, which is crucial for the DNA damage response (DDR) [2]. The DNA damage response is a highly coordinated cellular defense mechanism that sustains genomic integrity by detecting and repairing DNA lesions. The pivotal role of DDR in cellular function and survival is emphasized by the association of DDR defects with many human disorders, including cancer, aging, and neurodegenerative diseases. Although the DDR mechanisms have been extensively studied, most research has focused on cytosolic or nuclear proteins rather than biological membrane-present ion channels. Recently, potassium channels have been described as 'oncochannels' involved in tumor progression and treatment resistance in many cancers, highly expressed in bone, breast, ovary, and prostate cancer and glioma [3]. Due to the high drug sensitivity of these channels, targeting them may represent a new approach to treating glioma. According to the latest reports, oncochannels contribute to glioblastoma stem cell properties, program and execute cell migration and invasion, regulate the cell cycle, and confer therapy resistance. Cell migration and invasion in glioblastoma are critically dependent on changes in the level of Ca^{2+}. Moreover, overexpression of large-conductance Ca^{2+}-regulated potassium channel (BK_{Ca}) in glioblastoma can promote tumor progression and therapy resistance, which also presents an opportunity for developing novel therapeutic strategies. BK_{Ca} channels play a significant role in regulating mitochondrial function and redox homeostasis. These channels are modulated by ROS and other redox-active molecules, which influence their function and, consequently, the redox state of the cell [4]. Modulating BK_{Ca} potassium channels could alter cancer cells' response to radiotherapy and potentially overcome treatment resistance. Therefore, the primary goal is to investigate the mechanism of the BK_{Ca} channel in DNA damage response in modulation, enhancing the radiosensitivity of cancer cells and overcoming treatment resistance using ionizing radiation and pharmacological approaches.

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THE ROLE OF THE POTASSIUM AND CHLORIDE TRANSPORT IN THE DEVELOPMENT OF INFLAMMATION INDUCED BY PARTICULATE MATTER

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Particulate matter (PM) poses an increasing threat to human health. Their effects on the human body include the development of inflammation [1]. PM has been shown to exhibit immunomodulatory properties in bronchial epithelial cells by inducing the production of cytokines such as tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6) [2].

To investigate the role of ion channels in the development of inflammation, bronchial epithelial cell lines were used: wild-type (HBE WT), with a deletion in the gene encoding the α subunit of the large-conductance potassium channel (HBE $\Delta\alpha BK_{Ca}$), and with a mutation of the gene encoding the CFTR channel (CFBE). Each cell line was exposed to various concentrations of PM and assessed for changes in reactive oxygen species production, proinflammatory cytokine secretion (IL-6, TNF-α), mitochondrial respiration (via oxygen consumption rate), intracellular calcium levels, and transepithelial electrical resistance (TEER).

PM exposure significantly increased ROS synthesis and amplified IL-6 and TNF-α release, particularly in HBE $\Delta\alpha BK_{Ca}$ and CFBE cells. TNF-α induced the highest inflammatory response in HBE $\Delta\alpha BK_{Ca}$ and CFBE cells compared to HBE WT cells, as measured by IL-6 quantification, suggesting a role for ion channels in the inflammatory response. Mitochondrial function was also adversely affected, as evidenced by reduced maximal respiratory capacity in both HBE $\Delta\alpha BK_{Ca}$ and CFBE cells relative to HBE WT. Additionally, depending on its concentration, PM increased intracellular calcium ion levels in all cell lines. Finally, PM exposure led to a pronounced reduction in TEER, with CFBE monolayers displaying the most significant susceptibility to barrier disruption.

These studies highlight the vulnerability of potassium and chloride transport disorders in airway epithelial cells to PM-induced injury, which encompasses oxidative and inflammatory stress, mitochondrial dysfunction, and compromised epithelial barrier integrity. Targeting BK_{Ca} channel modulation and mitigating oxidative/inflammatory pathways could represent promising therapeutic strategies to protect airway health against environmental pollutants.

ACKNOWLEDGMENTS

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POLYSTYRENE NANOPARTICLES INTERFERE WITH DNA REPAIR MECHANISMS IN HUMAN INTESTINAL CACO-2 CELL LINE MODEL

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Nanoplastic (NP) occurs ubiquitously in aquatic and terrestrial environments, and its harmful biological effects have been observed in a variety of organisms, i.e. bacteria, plants, and animals [1]. Studies on human cells provide fundamental information on key mechanisms of toxicity that will provide answers to the question of whether and how NP poses a health risk. Mechanisms of toxicity mainly include membrane disruption, and production of reactive oxygen species, and may induce DNA damage including oxidative DNA damage (single-strand breaks, SSBs) and DNA double-strand breaks (DSBs) [2]. The emerging concern over environmental nanoparticles, particularly polystyrene (PS) nanoparticles, involves that there is some evidence suggesting that PS particles may be genotoxic in mammalian cells, however, the molecular basis is unclear [3].

This study investigates the interactions between NPs and an immortalized cell line of human colorectal adenocarcinoma cells (Caco-2) by exposing them to various NPs concentrations 50, 100, 400, 800, and 1200 μg/mL). The Caco-2 human epithelial cell line is a prevalent model for studying the intestinal epithelial barrier [4]. Derived from colon cancer, a noteworthy feature of this cell line is its spontaneous differentiation into a monolayer that closely resembles absorptive enterocytes functionally and morphologically, reflecting those found in the intestine.

We assessed potential cytotoxicity using the clonogenic assay and examined NP genotoxicity using the alkaline comet assay and flow cytometry assays (PARP1-dependent apoptosis and cell cycle changes). Our findings indicate a moderate level of NP cytotoxicity observed in the clonogenic assay. Moreover, our preliminary results showed no changes in cell cycle distribution and a minimal increase in the level of apoptotic cells. In addition, no single or double DNA strand breaks were observed. Since we did not observe direct effects, we expect indirect effects mediated by other molecules (e.g., induction of reactive oxygen species (ROS), inhibition of DNA repair mechanisms). To test the likely production of reactive oxygen species by Caco-2 cells in the presence of PNP, DCFDA fluorescent probe staining was performed to determine the level of ROS. After exposure to polystyrene nanoparticles, induction of oxidative stress was observed. We also performed RNA isolation and cDNA synthesis for qPCR assay, which allowed us to check the expression of characteristic genes involved in DNA repair pathways. In particular, critical genes involved in the base excision repair (BER) and DSB repair pathways were downregulated, suggesting a potential impairment of the cell's ability to repair oxidative DNA damage.

This study highlights the sublethal effects of nanoplastics on intestinal barrier cells. It underscores the possible risks of chronic exposure to these environmental contaminants, which can lead to genome instability and other long-term health consequences.

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EFFECT OF MUTATIONS IN THE *KCNMA1* GENE ON BK CHANNEL ACTIVITY

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Large-conductance calcium-activated K^+ channel (BK) gating involves three major molecular processes: voltage sensor movement, sensor-pore coupling, and pore opening. Recently, a mechanism for BK channel gating was proposed, suggesting that the structure of the pore does not undergo significant physical movement but, like many other ion channels, undergoes relatively small structural changes accompanied by hydrophobic gating. The deep-pore region of the human BK channel (residues G310-P320) undergoes hydrophobic dewetting transitions in the calcium-free state, which prevents the permeation of water and small ions. The barrier to K^+ permeation arises from the vapor gap that separates the selectivity filter from the bulk solution [1].

The cytoplasmic entry to the BK pore expands to a large central cavity that turns into the selectivity filter. The walls of the central cavity are primarily hydrophobic. Mutations in this region change the open probability of the BK channel, increasing or decreasing it depending on the polarity and hydrophobicity of substituted amino acids [2]. The same region is involved in channel blocking by paxilline [3].

The study aimed to investigate the effect of the point mutation in the *KCNMA1* gene, encoding the pore-forming alpha subunit, on BK channel function. We examined three substitute mutations, which decrease the hydrophobicity of amino acids lining the pore's central cavity: L312A, F315D, and A316G. The HEK293 cells were transfected with plasmids encoding either the wild-type (WT) or one of the mutated alpha subunits. All mutants formed functional ion channels. The properties of the channels were analyzed in cell-attached and inside-out configurations using the patch clamp technique. Compared to the WT, all three mutants exhibited increased channel activity (Fig.1). Studied mutations affected channel unitary amplitude, kinetics, and sensitivity to Ca^{2+}, indicating that alterations in hydrophobicity within the central cavity modulate the energy landscape of the gating process.

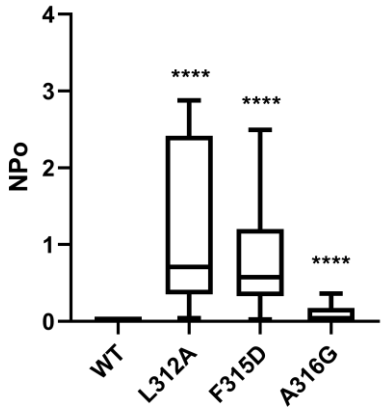

Fig.1. N-channel open probability (NPo) of WT and mutant BK channels at -50 mV in cell-attached configuration. WT n=21, L312A n=10, F315D n=16, A316G n=21; ****p<0.0001 Mann-Whitney test.

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ELECTROPHYSIOLOGICAL ASSESSMENT OF BK CHANNEL ACTIVITY IN LRRC26-POSITIVE CELLS

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The auxiliary γ1 subunit of the BK channel, LRRC26, is known to dramatically shift the activation voltage of BK channels by approximately 140 mV toward hyperpolarization, enabling channel opening at resting membrane potentials and in the absence of Ca^{2+} [1,2]. While LRRC26 expression has been previously reported in epithelial cells, vascular smooth muscle cells, and certain cancer lines, it has not been observed in neuronal cells.

We investigated, using immunofluorescence and the patch-clamp technique, the expression and functional impact of LRRC26 in various cell types, including breast cancer cells (T47D), prostate cancer cells (LNCaP), rat cerebellar Purkinje neurons, and HEK293 cells co-transfected with BK and γ1 cDNAs. Immunofluorescence confirmed LRRC26 presence across all cell types, including Purkinje neurons, where its expression had not been previously reported.

Single-channel patch-clamp recordings under symmetric K^+ and 1 EGTA conditions revealed functional BK-γ1 complexes only in LNCaP cells and HEK293 cells expressing both BK and γ1. T47D cells exhibited only rare BK activity, with no evidence of γ1-mediated modulation, even after BK channel transfection. Similarly, Purkinje neurons displayed BK activity only under depolarizing or Ca^{2+} presence conditions, with no signatures of γ1-associated hyperpolarized activation. To examine whether the formation of BK-γ1 complexes could be influenced by metabolic conditions, we investigated the effects of glucose availability in Purkinje cells. However, under glucose-limited conditions, no enhancement in BK-γ1-type activity was observed.

Our findings suggest that while LRRC26 protein is detectable in multiple cell types, its functional coupling with BK channels may be cell-type specific and condition-dependent. This implies that LRRC26 may serve additional, possibly non-membrane-associated, cellular roles beyond its established function as a BK channel auxiliary subunit.

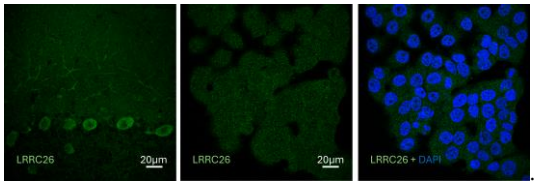

Fig.1. Immunostaining with anti-LRRC26 antibody (green) in Purkinje cells (left) and T47D cells (middle and right). Signal intensity is higher in Purkinje cell bodies and dendrites compared to surrounding cells. DAPI staining (right) shows nuclei in T47D cells.

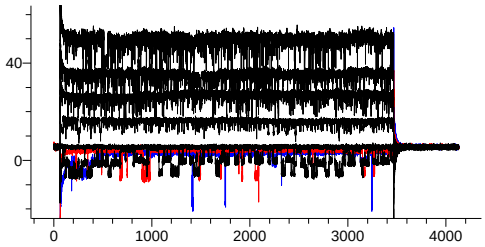

Fig.2. BK channel activity in LNCaP cells in inside-out configuration with symmetrical K^+ and 1 mM EGTA. Voltage steps from -80 mV to 100 mV in 20 mV increments. Blue: currents at -80 mV; red: currents at -40 mV; X-axis: mV; Y-axis: ms. Cell culture methods are described in [3].

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THE INHIBITORY EFFECT OF RESVERATROL ON KV1.3 CHANNELS IN JURKAT T CELLS – A PUTATIVE ROLE IN ANTI-CANCER ACTIVITY

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Resveratrol (3,4',5 – trihydroxystilbene) is a biologically-active plant-derived polyphenol, which exerts anti-viral, anti-bacterial, anti-fungal, anti-inflammatory, anti-atherosclerotic, anti-cancer and neuroprotective effects. It is also a modulator of activity of various types of potassium channels. Our previous studies have shown that resveratrol is also an inhibitor of voltage-gated potassium channels Kv1.3 in human T lymphocytes [1].

Voltage-gated potassium channels Kv1.3 encoded by the KCNA3 gene are widely present among different tissues [2]. The channels may be expressed not only in the plasma membrane, but also in the inner mitochondrial membrane (mito Kv1.3 channels) [3]. The channels' activity plays a significant role in a regulation of proliferation and apoptosis of Kv1.3 channel-expressing cells [3]. The channels' expression may be significantly changed in some cancer disorders [3].

Inhibitors of the channels may putatively find clinical application in therapy of various diseases, including some cancer disorders characterized by an over expression of Kv1.3 channels, such as melanoma, pancreatic ductal adenocarcinoma (PDAC), multiple myeloma and B-type chronic lymphocytic leukaemia (B-CLL) [3].

It is known that some lipophilic small-molecule organic inhibitors of Kv1.3 channels may exert anti-proliferative and pro-apoptotic activity on Kv1.3 channel-expressing cancer cells, selectively eliminating them while sparing the normal ones [3].

To the group of lipophilic small-molecule organic inhibitors of Kv1.3 channels in cancer cells belong also some compounds from the groups of flavonoids, chalcones and statins [3]. The inhibitory effect on the channels may significantly be augmented upon a co-application of flavonoids and chalcones with the statins: simvastatin and mevastatin [3]. The augmented inhibitory effect on the channels may be co-related to an improved pro-apoptotic activity of these compounds, applied in a combination, on Kv1.3 channel-expressing cancer cells [3].

This study reports an inhibitory effect of resveratrol on Kv1.3 channels endogenously expressed in a Kv1.3 channel-expressing cancer cell model system – human leukemic T cell line Jurkat T

The study was performed applying the whole-cell "patch-clamp" technique [4].

Obtained data provide evidence that an application of resveratrol at the concentrations of 4.5 μM, 7.5 μM, 15 μM, 30 μM nad 60 μM caused a dose-dependent inhibition of the whole-cell potassium currents to about 0.52 of the control value. The inhibitory effect on the channels was accompanied by a significant slowing of the currents' activation rate, without any significant change of the inactivation rate. The inhibitory effect of resveratrol on the channels was reversible.

The inhibitory effect of resveratrol on Kv1.3 channels in cancer cells was weaker than the effect observed earlier for Kv1.3 channels expressed in normal human T lymphocytes [1]. Moreover, in contrast to what was observed in case of human T lymphocytes [1], no additivity was observed upon a co-application of resveratrol with genistein, both compounds at 30 μM concentration.

On the other hand, the inhibitory effect of resveratrol on Kv1.3 channels in Jurkat T cells was significantly augmented upon a co-application of resveratrol at 30 μM concentration with mevastatin or simvastatin, applied at 6 μM concentration. The currents were reduced to about 0.31 of the control value upon a co-application of resveratrol with mevastatin and to about 0.14 upon a co-application of resveratrol with simvastatin. This may indicate that the inhibitory effects on the channels may be additive or synergistic upon a co-application of resveratrol with the statins.

The inhibition of Kv1.3 channels may be involved in anti-cancer activity of resveratrol on Kv1.3 channel expressing cancer cells [3], especially upon a co-application of resveratrol with the statins.

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ASSESSMENT OF TRPM2 EXPRESSION IN LYMPHOCYTES T UNDER HYPOXIC CONDITIONS

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The immune system plays a crucial role in maintaining homeostasis, with T lymphocytes orchestrating adaptive immune responses through cytokine production and cytotoxic activity [1]. Effective T cell activation is essential for combating infections and malignancies. However, in pathophysiological environments such as the tumor microenvironment (TME), factors like hypoxia and oxidative stress can impair T cell function, reducing their immunological efficacy [2, 3].

The TRPM2 (Transient Receptor Potential Melastatin 2) ion channel is a redox-sensitive, non-selective cation channel activated by ADP-ribose, reactive oxygen species (ROS), TNF-α, and Concanavalin A. It has been implicated in immune regulation by modulating T cell activation, proliferation, and calcium signaling, particularly under oxidative stress. Nevertheless, its role under hypoxic conditions remains incompletely understood.

This study aimed to assess TRPM2 gene expression in peripheral blood lymphocytes (PBLs) cultured under hypoxia (1% O_2) and chemically induced hypoxia ($CoCl_2$), following CD3/CD28-mediated activation. Gene expression of TRPM2, along with activation markers CD25 and CD69, was analyzed using quantitative RT-PCR.

Our results demonstrate increased TRPM2 expression in activated lymphocytes under both hypoxic conditions, suggesting its involvement in adaptive responses to low-oxygen stress. Activation markers confirmed T cell stimulation; however, their expression was attenuated in hypoxia, particularly under chemical hypoxia, indicating impaired full activation. These findings suggest that TRPM2 may play a role in T cell adaptation to hypoxic stress and could represent a potential target in modulating immune responses within hostile microenvironments.

ACKNOWLEDGMENTS

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THE ROLE OF ION TRANSPORT INDUCED BY MODIFIED IONOPHORES AND COMPOUNDS OF NATURAL ORIGINS

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Cystic fibrosis is the most common genetic disease among rare diseases. It is caused by mutations in the *cftr* (cystic fibrosis transmembrane conductance regulator) gene, which encodes the CFTR anion channel. The CFTR channel defect results in abnormal transport of ions and water across the epithelium, leading to thick and sticky lingering mucus in the affected organs [1]. The activity of the CFTR channel is dependent on ATP molecules. Therefore, increasing cellular ATP levels can promote chloride secretion and contribute to better epithelial hydration.

Increasing cellular ATP levels can be induced using mitochondria-directed ionophores capable of transporting ions across biological membranes. Their activity can lead to depolarization of the inner mitochondrial membrane and increase of cellular respiration. In addition, ionophores can affect the electrophysiology of epithelial tissues by participation in various signaling pathways. The same effect can be achieved by compounds of natural origin available in a varied diet. Examples of such compounds are flavonoids, which are capable to activate ion channels and modulating metabolic pathways [2].

The ability of the ionophores used in the study to transport ions across biological membranes and the effects on the electrophysiology of cellular monolayers were studied in Ussing chamber and by Black Lipid Membrane technique. The effects of the tested compounds on changes in the cellular respiration level were studied in Oroboros system. Transepithelial chloride transport was determined in an Ussing chamber. ATP levels were determined by the commercially available bioluminescent assay. In addition, migration assays, viability assays, ROS level and transepithelial electrical resistance of the monolayers were performed. The assays were conducted on respiratory cell lines such as the cancer cell line A549 and the bronchial epithelial cell line 16HBE14o-.

The results of the experiments showed that the targeted compounds exhibited the ability to transport ions across biological membranes, and in a concentration-dependent manner to increase cellular respiration. Ussing chamber experiments showed that the tested mitochondria-directed ionophores did not affect the chloride current flowing through cell monolayers. On the other hand, the experimentally selected flavonoid- luteolin appeared to increase intracellular ATP concentration, transepithelial electrical resistance, affect metabolism, proliferation and modulate chloride secretion.

These findings suggest that luteolin may strengthen the barrier function of human bronchial epithelial (HBE) cells and holds potential to support epithelial hydration mechanisms—offering a promising perspective for therapeutic strategies in cystic fibrosis.

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SESSION VI: MEDICAL SIGNIFICANCE OF PROTEINS AND NUCLEIC ACIDS

OLIGOMER FORMATION BY THE IMMUNE RESPONSE PROTEIN IFIT1: A BIOCHEMICAL AND BIOPHYSICAL STUDY

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There are many ways for a eucaryotic organism to defend against pathogenes. One of these is the ability to recognise foreign genetic information, hinder its expression, and target it for degradation. Proteins from the IFIT family play a significant role in this process. Eucaryotic mRNA is equipped with a special cap structure at the 5' end, consisting of m^7Gppp and additional methylations at the first and second nucleotides of the mRNA chain. RNA without these modifications is recognised and bound by the IFIT1 protein, preventing the association with the eucaryotic translation initiation factor eIF4E and, consequently, arresting the translation of foreign proteins [1].

Recently, many efforts have been devoted to introducing specific modifications to the cap structure to increase its affinity towards eIF4E and decrease its affinity towards IFIT1 [2, 3]. This was predominantly done for medical purposes, to obtain an even stronger affinity for modified mRNA than for mRNA with native caps and to promote the translation of particular proteins that could serve as vaccines, including anty-tumour ones.

In order to study the affinity of IFIT1 for differentially modified caps, as well as the dynamics of these interactions, it was necessary to use IFIT1 in monomeric form so that the protein-cap interactions could be examined at a 1:1 ratio. The aim of the presented study was to obtain monomeric IFIT1 protein by introducing mutations to the C-terminal domain responsible for the homodimerisation process, and further to investigate and compare the biochemical and biophysical properties of the wild-type and mutated proteins [4].

For the preliminary characterization, the wild-type and mutated IFIT1 proteins were subjected to observation by differential light scattering (DLS) and differential scanning fluorimetry (DSF). All of the proteins studied produced advanced oligomeric forms, which nevertheless differed in both structure and stability. The most stable forms were obtained for the native IFIT1 protein, and the least stable for the double mutant protein. Moreover, the native IFIT1 protein was found to be the most prone to forming high-order oligomers. The stability of the aggregates formed during incubation of proteins at 37°C was tested using proteinase K digestion and further observation of the degradation products. Again, the most stable were the aggregates formed by the native IFIT1 protein, while the aggregates formed by both mutants were much more susceptible to proteolytic degradation.

During the studies, it was not possible to obtain monomeric particles, which suggests that the introduced mutations did not sufficiently inhibit the homodimerisation process. Nevertheless, the mutagenesis compromised the structural integrity of IFIT1, affecting its ability to maintain stable oligomeric assemblies under physiological conditions. Studying the oligomerisation of the IFIT1 protein and the role of the structures formed during this process opens up a new, previously unknown area in the broader immune response.

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SESSION VII: SPECTROSCOPY – IDEAS, METHODS, AND APPLICATIONS IN LIFE SCIENCE

MOLECULAR SPECTROSCOPY AS A POWER TOOL FOR STUDYING THE PORPHYRIN–DNA INTERACTION

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The porphyrins are macrocyclic compounds with unique spectroscopic and photophysical properties. They are widely used as photosensitizers in anticancer photodynamic therapy, probes for the structure and dynamics of nucleic acids, anti-viral and antimicrobial agents, and carriers of antisense oligonucleotides for their delivery, stabilizers of G-quadruplexes of telomeric DNA etc. The interest in studying porphyrin-DNA interaction is caused by the great potential of the data obtained for biomedical application, nanotechnology, and molecular electronics.

In this work we discuss the application of various spectroscopic techniques (absorption spectroscopy, polarized fluorescence, absorption and fluorescence melting, fluorimetric titration, and resonance light scattering) to study the binding of two cationic *meso*-porphyrins (Fig. 1) and its conjugates with phenazine dye to nucleic acids of different primary and secondary structure, including single-stranded [1–3], double-stranded [4–6], and quadruplex [4, 6–8] ones.

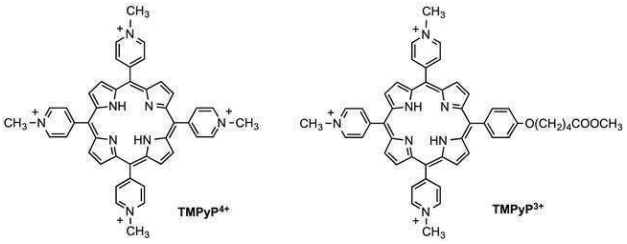

Fig.1. Molecular structures of two cationic *meso*-porphyrins.

Using the spectroscopic methods, the binding modes of the porphyrins to nucleic acids were identified, the thermodynamic parameters of binding were obtained, the formation of porphyrin aggregates on the surface of the biopolymer was revealed, and their size was determined.

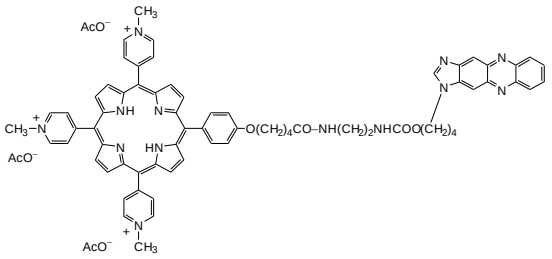

Fig.2. Molecular structure of porphyrin-imidazophenazine conjugate.

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BOUND PROTEIN INFLUENCES TRIPLET STATE RELAXATION TIME OF AF488 FLUORESCENT PROBE

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Fluorescent dyes are commonly used to study the properties and interactions of biomolecules. While the spectral parameters of the Alexa Fluor series are well known, the triplet state lifetimes are usually assumed to be in the range of microseconds without further specification.

Here we investigate the triplet state dynamics of a bright and photostable dye, AF488 (an isomerically pure 5-analogue of Alexa Fluor 488), when conjugated to acid-rich intrinsically disordered and standard globular proteins using fluorescence correlation spectroscopy (FCS). By varying the laser excitation power and the viscosity of the environment, we accurately characterize the changes in the triplet state relaxation time. While AF488 typically exhibits a stable triplet lifetime over a range of environments, we observe a significant shortening of the triplet state lifetime when the dye is bound to highly acidic IDPs.

SESSION VIII: BIOSENSING AND MICROFLUIDIC SYSTEMS FOR DISEASE BIOMARKERS DETECTION IN BIOMEDICAL RESEARCH

QUARTZ CRYSTAL MICROBALANCE WITH IMMOBILISED MITOCHONDRIA AS A LABEL-FREE BIOSENSOR FOR RAPID SCREENING OF NEUROPROTECTIVE DRUGS

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Disturbed mitochondrial morphology and dynamics are now recognised as key factors in neurodegenerative disorders such as Parkinson's and Alzheimer's diseases[1,2]. Therefore, quantifying these alterations quickly and without fluorescent labels is essential for the discovery of truly disease-modifying therapies.

Herein, we present a label-free biosensor utilizing a quartz crystal microbalance (QCM) for real-time monitoring of morphological changes in isolated mitochondria from SH-SY5Y neuroblastoma cells. The mitochondria are immobilized onto a cysteamine-functionalized, 10 MHz quartz crystal housed within a polycarbonate microfluidic chip. This chip is securely sealed with O-rings and four thumb screws, allowing the piezoelectric crystal to be easily replaced within two minutes, facilitating rapid serial measurements without risk of cross-contamination.

Our results demonstrate that exposure of differentiated SH-SY5Y cells to the Parkinson's toxin MPP^+ (100 μM) resulted in a 138 ± 25 Hz reduction in frequency, reflecting mitochondrial matrix contraction and fragmentation, which is consistent with observations in live-cell studies[3]. Subsequent perfusion with the dynamin-related protein 1 inhibitor Mdivi-1 (10 μM) increased the frequency by 312 ± 24 Hz, indicating the effective restoration of mitochondrial integrity, which supports the previously documented anti-fragmentation effects[4]. Control experiments using empty crystals exhibited a drift of less than 1 Hz, confirming that the observed frequency variations specifically reflect changes in mitochondrial morphology.

The presented QCM-microfluidic platform offers a powerful, high-throughput route to identifying mitochondria-targeted neuroprotective agents and tracking therapeutic efficacy in neurodegenerative disease research by coupling nano-sensitive gravimetric sensing with a rapidly disassembled microfluidic chip.

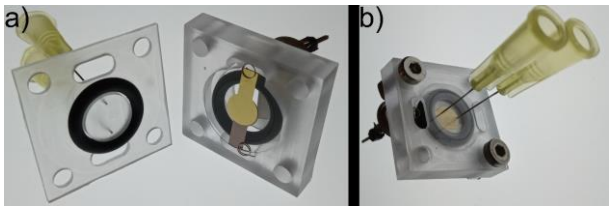

Fig.1. The figure illustrates the microfluidic system in two configurations: disassembled (a) and assembled (b). The system contains a piezoelectric quartz element that is used to monitor the morphological changes that mitochondria undergo as a result of drug exposure.

ACKNOWLEDGMENTS

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ADVANCING BIOMEDICAL RESEARCH THROUGH MICROFLUIDICS: MICROFLUIDIC SYSTEMS FOR SINGLE-CELL ANALYSIS, HIGH-PRECISION OXYGEN RELEASE IMAGING, MECHANOBIOLOGY STUDIES, AND DYNAMIC CELL CULTURE

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Microfluidic systems offer precise control over fluid flow and microscale environments, making them powerful tools for high-throughput analysis, manipulation, and imaging of cells or droplets. A central aim of our research is to develop microfluidic platforms tailored to the specific needs of biological and medical research.

One of our most advanced technologies, currently in commercial prototype development, is a microfluidic system for single-cell oxygen saturation and release imaging. Developed in close collaboration with the University of Oxford, it enables a detailed assessment of red blood cell function [1, 2]. The system was successfully applied to study human kidneys perfused with stored blood during transplantation, where organ respiration was monitored under cold perfusion [3]. The findings revealed a strong correlation between kidney oxygen consumption and erythrocyte oxygen-release capacity – challenging the conventional notion that oxygen delivery is determined solely by blood flow and oxygen content.

In parallel, we developed microfluidic devices capable of generating mechanical gradients in epithelial tissues through controlled deformation. In partnership with the University Grenoble Alpes, we investigated how curvature influences calcium signaling and gene expression in epithelial monolayers, providing new insights into tissue morphogenesis and mechanotransduction [4].

We also developed systems for single-cell immobilization and manipulation, enabling long-term observation of isolated cells or spheroids in individual droplet incubators or microscale cell traps, with several hundred replicates achievable in a single experiment. These cell traps were used to study the formation of immunosuppressive niches in Hodgkin lymphoma by analyzing interactions between CAR-T lymphocytes and cancerous B-cells at single-cell resolution.

Finally, we present a static droplet microfluidic incubator designed for dynamic, long-term culture of bacterial and mammalian cells with real-time, single-cell monitoring. Utilizing a controlled coalescence mechanism, the device supports versatile, automated nutrient delivery and waste removal protocols, which can be tailored to the specific requirements of different cell types. *In vitro* studies confirmed its effectiveness in sustaining long-term cultures of *E. coli* and A549 epithelial cells under optimized shear stress conditions. The results showed improved cell growth, as well as controllable cell organization—supporting the formation of either confluent monolayers or 3D spheroid-like structures. The device's modular design allows seamless integration with upstream and downstream microfluidic components, as well as closed-loop feedback control systems.

Together, these advances highlight the potential of microfluidics to investigate complex biological phenomena with high precision and throughput, supporting diverse applications in cell biology, transfusion medicine, organ preservation, immunology, and drug discovery.

ACKNOWLEDGMENTS

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BIOSENSORS AS DIAGNOSTIC TOOLS FOR EARLY DETECTION OF CANCER BIOMARKERS

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Cancer diseases are one of the leading causes of death worldwide. Tumors arise as a result of uncontrolled proliferation and abnormal cell growth, which can lead to the invasion of surrounding tissues and metastasize. Early diagnosis is the most important strategy against cancer, in which molecular biomarkers playing an increasingly important role. Cancer biomarkers are molecules present in the patient's body whose levels correlate with the presence, stage, or progression of the cancer disease [1-2].

Among the biomarkers, survivin and EphA2 can be distinguished, which have significant diagnostic and prognostic value. Survivin, encoded by the *BIRC5* gene, has a dual function: it inhibits programmed cell apoptosis and regulates cell division. Moreover, EphA2 is a tyrosine kinase receptor from the ephrin family involved in cell adhesion, migration, and invasion processes. It should be noted that overexpression of these biomarkers has been demonstrated in several tumors including breast, lung, colorectal and prostate cancers. High levels are associated with aggressive disease progression, resistance to chemotherapy and radiotherapy, and poor prognosis. In healthy tissues, their expression is minimal. Both biomarkers are currently investigated as targets for novel anticancer therapies [3-4].

In recent years, a great interest in the use of biosensors has been made for the detection of cancer biomarkers. Biosensors are analytical devices that integrate biological elements (such as enzymes, antibodies, nucleic acids) with physical, chemical or optical transducers, enabling the detection of the presence of a specific analyte. Among the numerous classes of biosensors, optical and electrochemical biosensors have gained particular importance due to their sensitivity, selectivity and potential for miniaturization and point-of-care applications [5].

ACKNOWLEDGMENTS

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STUDY OF THE EXPRESSION OF CANCER BIOMARKERS - EPHA2 AND SURVIVIN USING AUTOMATED CAPILLARY ELECTROPHORESIS

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Poland has one of the highest rates of lung cancer incidence and mortality in Europe. Currently, the mortality rate is around 40 per 100,000 people. One of the main problems of oncology is late diagnosis and the difficult monitoring of changes in the disease during treatment. This is due to the type of therapy undertaken and the way in which cancer cells respond to the treatment. Monitoring of the biomarkers can then be significantly difficult due to differences in gene expression [1]. Therefore, in order to design a sensitive exosome-based biosensor to monitor the patient's condition from liquid biopsy, it is necessary to examine the expression of proteins in response to treatment, as protein levels in exosomes are derivative of expression levels in their origin cells.

In this study, we utilized a drug used in lung cancer therapy - paclitaxel (PTX)[2]. First, we determined the concentration of PTX necessary to induce changes in healthy alveolar cell line CI-huArlo and the non-small cell lung cancer A549 cell line. For this purpose, we conducted an AlamarBlue viability test. The selected concentration correlated with that obtained in blood flow during chemotherapy. Early and late apoptosis as well as necrosis assays were performed with flow cytometry technique using Annexin V and Draq7 labeling. To check the apoptotic activity of the cells, PARP1 protein expression was investigated, and then the expression of survivin and EphA2 proteins was measured in both cell types, before and after paclitaxel treatment, respectively. It was shown that there is a significant difference in survivin expression between non-treated CI-huArlo and A549 cells. The significant difference in EphA2 expression between treated and non-treated healthy as well as cancer cells was also presented.

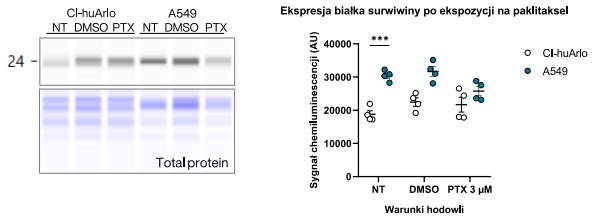

Fig.1. Expression of survivin protein in CI-huArlo and A549 cells before and after treatment with 3 µM PTX.

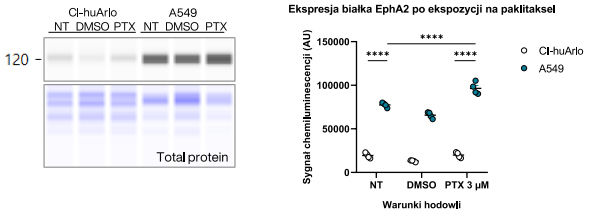

Fig.2. Expression of EphA2 protein in CI-huArlo and A549 cells before and after treatment with 3 µM PTX.

Obtained results indicate a great potential of EphA2 based biosensor for early and late detecting (under treatment) lung cancer and survivin based biosensor for early lung cancer diagnosis.

ACKNOWLEDGMENTS

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ELECTROCHEMICAL DNA BIOSENSORS FOR THE DETECTION OF SURVIVIN CANCER BIOMARKER

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Due to the rising numbers of new cancer cases around the world and specific features of this disease, such as the high mortality rates, fast tumor growth, invasion into neighboring cells and cancer cells' migration, the early diagnosis and prompt effective cancer treatment strategies become crucial in the fight against cancer [1-2].

Survivin (Sur) is the smallest member of the inhibitor of apoptosis proteins (IAP) family. It became a diagnostic and prognostic cancer biomarker and potential therapeutic target owing to its strong expression in malignant tumors and very weak expression in normal differentiated cells [3].

The goal of this work was to design and test electrochemical DNA biosensors for the detection of mRNA survivin in cancer cells and exosomes as well as for the investigation of human colorectal cancer cells metastasis with different metastatic potential.

The biosensor was based on redox-labelled molecular beacons immobilized on the gold electrodes via thiol groups. In the presence of complementary oligonucleotides, the analytical signal from electrochemical marker decreased due to the higher rigidity of DNA duplex and longer distance to the electrode surface.

The consecutive steps of the gold electrodes modification were characterized using a cyclic voltammetry technique. The efficiency of developed biosensors in real samples, containing lysate from cancer cells was also investigated.

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FUNCTIONAL RECONSTITUTION OF DIACYLGLYCEROL KINASE EPSILON AND ITS COMPLEX WITH ROMK2 POTASSIUM CHANNEL IN NATIVE COPOLYMER NANODISCS

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Diacylglycerol kinase epsilon (DGKε) catalyzes phosphorylation of 1-stearoyl-2-arachidonoyl (18:0/20:4) diacylglycerol (SAG) thus converting it into 1-stearoyl-2-arachidonoyl phosphatidic acid (SAPA). Our previous studies using the Turbo-ID method have demonstrated that DGKε is a member of the ROMK2 potassium channel proxisome. Moreover, direct interaction of these two proteins has been confirmed by co-immunoprecipitation. Additionally, phosphatidic acid, being DGKε product, has been shown to stimulate the activity of ROMK2 in artificial lipid bilayers [1].

In the present work, we demonstrate that both ROMK2 and DGKε can be efficiently solubilized from mammalian cells using nanodisc-forming copolymers of various structures. Additionally, as revealed by fluorescent assay followed by TLC separation, the activity of DGKε is retained in many nanodiscs. Of the copolymers examined, the low-charged zwitterionic Sulfo-Cubipol proved to be the most effective in solubilization of both proteins, as well as in maintaining the activity of DGKε. Moreover, this copolymer enabled to assess the impact of ROMK2-DGKε interaction on the kinase activity.

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WHAT IS THE ROLE OF MITOBK$_{CA}$ CHANNEL IN BRONCHIAL EPITHELIUM EXPOSED TO PARTICULATE MATTERS (PMS)?

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Bronchial epithelial cells, which line the lower respiratory tract, form a monolayer that serves as a natural barrier between the external environment and the internal milieu of the body. These cells are continually exposed to harmful agents such as pathogens, allergens, and air pollutants, all of which can contribute to respiratory diseases and increased mortality. Potassium channels play a key role in lung physiology, including ion homeostasis, mucus secretion, and maintenance of epithelial integrity. Among them, mitochondrial potassium (mitoK) channels facilitate potassium ion influx into mitochondria, leading to reduced mitochondrial membrane potential, enhanced respiratory chain activity, and increased mitochondrial respiration, ultimately influencing reactive oxygen species (ROS) production. Activation of these channels also modulates mitochondrial matrix volume and prevents calcium ion overload within mitochondria. Our previous research identified the presence of mitochondrial large-conductance Ca^{2+}-activated potassium channels (mitoBK$_{Ca}$) in human bronchial epithelial (HBE) cells. In this project, we investigated the role of mitoBK$_{Ca}$ in mitochondrial physiology under exposure to particulate matters (PMs). Using CRISPR/Cas9 genome editing, we established a novel 16HBE14o$^-$ cell line with a disruption of the KCNMA1 gene (HBE Δα), which encodes the pore-forming α subunit of the BK$_{Ca}$ channel. In HBE Δα cells, neither the α subunits nor channel activity was detectable. Notably, these cells exhibited impaired mitochondrial function, characterized by reduced cellular respiration and altered OXPHOS-dependent ATP production. Additionally, we observed a reorganization of the respiratory chain in the absence of the BK$_{Ca}$ channel. To further elucidate the molecular mechanisms underlying these effects, we performed RNA sequencing to analyze transcriptomic profiles of both wild-type and HBE Δα cells following short- and long-term exposure to low and high concentrations of PMs. The absence of the BK$_{Ca}$ channel resulted in significant transcriptional alterations in genes associated with mitochondrial function. Moreover, PMs exposure induced pronounced changes in the transcriptome, with a distinct response observed between the two cell lines. These findings underscore the critical role of BK$_{Ca}$ channels in maintaining mitochondrial function and cellular homeostasis in bronchial epithelial cells, particularly under conditions of environmental stress such as PM exposure.

ACKNOWLEDGMENTS

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PROXIMITY LABELING REVEALS NEW INTERACTORS OF MITOCHONDRIAL BK_{CA} CHANNELS

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Mitochondria play a vital role in cell function, particularly in ATP production through oxidative phosphorylation, enabled by the membrane potential across the inner mitochondrial membrane. Potassium channels identified in this membrane regulate mitochondrial activity by influencing membrane potential, respiration rate, and ROS production. Their activity has also been linked to cytoprotection. One of the best-characterized channels is the mitochondrial large-conductance calcium-activated potassium (mitoBK_{Ca}) channel, similar in structure and function to plasma membrane BK_{Ca} channels.

In this study, we aimed to identify protein interactions of BK_{Ca}/mitoBK_{Ca} channel subunits, including mitochondrial partners. We used the TurboID technique, which labels nearby proteins via biotinylation, generating a construct with the β4 subunit of the BK_{Ca} channel fused to TurboID ligase. Additional constructs targeted TurboID to specific cellular compartments to control for nonspecific labeling. Experiments were performed in HEK293T and U-87 MG astrocytoma cells.

Mass spectrometry of biotinylated proteins from mitochondrial fractions and whole-cell lysates showed that the β4 subunit labeled proteins from the ER, cytosol, plasma membrane, nucleus, and mitochondria. Among mitochondrial proteins, we identified MICOS complex components and proteins involved in complex IV assembly. However, co-immunoprecipitation did not confirm direct interactions with these mitochondrial proteins.

The confirmed interactor was TMX1, a protein mainly localized to the ER, especially in mitochondrial-associated membranes (MAMs). TMX1 interacted with both β4 and α subunits of the BK_{Ca} channel. As an oxidoreductase, TMX1 may modulate BK_{Ca} function through redox regulation. Previous research also showed that TMX1 regulates calcium transfer between the ER and mitochondria, affecting cellular metabolism. This suggests that TMX1–BK_{Ca} interaction may also influence this process.

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SESSION X: PROTEIN DYNAMICS, DISORDER, AND PHASE SEPARATION

LIQUID–LIQUID PHASE SEPARATION OF A HIGHLY CHARGED CORAL PROTEIN REGULATES CALCIUM CARBONATE FORMATION

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Non-classical crystallization theory challenges traditional models by proposing that crystal formation proceeds through intermediate states such as amorphous calcium carbonate (ACC) or polymer-induced liquid precursors (PILPs) [1]. Although ACC has been identified in the coral *Stylophora pistillata* [2], direct evidence for PILPs remains limited, with most insights inferred from the analysis of solid phases. In the context of biomineralization in living organisms such as corals, the polymers that are thought to be involved in skeleton formation are coral acid-rich proteins (CARPs) [3], which are secreted at the coral tissue–skeleton interface. These proteins have been shown to bind calcium and influence crystal morphology [3] and polymorph selection [4].

In this study, we demonstrate that the aspartic- and glutamic acid-rich protein (AGARP), an intrinsically disordered protein with an exceptionally high charge of -148 e per molecule and the first cloned CARP from the model coral species, *Acropora millepora*, modulates calcium carbonate formation *via* liquid-liquid phase separation (LLPS) [5].

Using fluorescence correlation spectroscopy, we observed that AGARP and Ca^{2+} ions form early aggregates in non-crowded, water-like solutions prior to the emergence of ACC, as confirmed by scanning electron microscopy with energy-dispersive X-ray spectroscopy.

On the other hand, under molecular crowding conditions that mimic the endoplasmic reticulum and extracellular matrix environments, where AGARP is processed after biosynthesis and exported, respectively, AGARP forms liquid protein–calcium condensates (LPCCs) through LLPS, as revealed by confocal laser scanning fluorescence microscopy and fluorescence recovery after photobleaching experiments. When exposed to carbonate ions, these LPCCs serve as crystallization precursors, and the resulting $CaCO_3$ phases exhibit smooth edges that differ markedly from the sharp edges formed in the absence of AGARP.

Our findings suggest that the LPCCs could be biologically relevant precursors in calcium carbonate biomineralization and highlight the importance of LLPS and macromolecular crowding in this process. This study provides a new perspective on the processes involved in the skeleton formation and offers valuable insights for designing bioinspired materials.

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CONDENSATION OF GALECTIN-3 N-TERMINAL DOMAIN IN MARTINI 3 COARSE-GRAINED SIMULATIONS

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Galectin-3 is a protein comprising two distinct domains: a N-terminal domain (NTD) of approximately 120 amino acid residues and a carbohydrate-recognition domain (CRD) that binds specific carbohydrates. The NTD is characterised by a high content of proline, glycine, and tyrosine residues, contributing to its flexibility and lack of a well-defined structure. It plays a crucial role in the ability of galectin-3 to self-associate and form transient multimers, which is essential for many of its biological functions, including cell adhesion, signaling, and immune regulation. The NTD has also been linked to various pathological conditions, including cancer, inflammation, and fibrosis. Recent experimental studies have shown that both the full-length galectin-3 and its NTD can form biomolecular condensates in a process of liquid-liquid phase separation (LLPS).

To investigate LLPS of the NTD with near-atomic resolution, we employed molecular dynamics simulations using the Martini 3 coarse-grained force field [1]. We systematically fine-tuned the solute-solvent interactions within the Martini 3 force field to achieve an optimal agreement with the experimental phase diagram of the NTD, ensuring the accuracy and relevance of our simulations. Our simulations revealed a concentration-dependent condensation of the NTD, and we observed significant differences in the geometric properties of the NTD chains between the condensed and dilute phases. Specifically, parameters such as the radius of gyration, the maximum interatomic distance, and the end-to-end distance were found, on average, to be higher for the NTDs in the condensates than for the dilute NTDs in an ionic solution, which indicates that the NTD chains adopt more extended conformations within the condensates. Furthermore, the auto-correlation time of the end-to-end distance was higher in the condensates than in the dilute phase, suggesting slower conformational dynamics of the NTD within these structures. As a matter of fact, the diffusion coefficient of the NTD in the condensates was found to be approximately two times lower than in the dilute phase. In addition, analysis of intra-molecular contacts revealed that tyrosine and tryptophan residues interact with the rest of the NTD chain about two times more frequently than other residues, suggesting their key role in driving the NTD condensation. Furthermore, analysis of inter-molecular contacts demonstrated that these interactions, on average, are an order of magnitude stronger in the condensates than in the dilute phase. The condensed NTDs were found to exhibit reduced amounts of contacts with both ions and water molecules, with an average of 1.45 ± 0.02 ions and 39.8 ± 0.6 water molecules within its contact distance. This is in marked contrast to dilute NTDs, which displayed significantly higher contact numbers (6.2 ± 0.6 ions and 163 ± 10 water molecules, respectively). This observation suggests that the NTD condensation is accompanied by a decrease in the NTD solvent accessibility and by a preference for protein-protein interactions over protein-solvent interactions. Taken together, our results provide detailed insights into the molecular mechanisms underlying the NTD condensation, highlighting the crucial role of interactions involving tyrosine and tryptophan residues, reduced solvent accessibility, and altered conformational dynamics within the condensates. These findings could provide a foundation for future research aimed at modulating the NTD condensation for therapeutic purposes, impacting our understanding and treatment of galectin-3 related diseases.

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THE INTRINSICALLY DISORDERED AB REGION: A KEY MODULATOR OF THE MOLECULAR PROPERTIES OF HUMAN RXRγ

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The human retinoid X receptor γ (*h*RXRγ) is a ligand-dependent transcription regulator that belongs to the nuclear receptor superfamily. It is characterized by conserved structural domains and a unique, intrinsically disordered N-terminal AB region. While the AB region is known to modulate the transcriptional activation of target genes, its structural role within the full-length receptor remains poorly understood. Here, we show that the AB region shapes the structural organization of *h*RXRγ. Comparative analyses of the full-length receptor (*h*RXRγ) and a deletion mutant lacking the AB region (ΔAB*h*RXRγ) reveal that the AB region modulates oligomerization, stability, and conformational heterogeneity. Rather than acting independently, the AB region integrates with the receptor core, fine-tuning its structural variability and enhancing its responsiveness to environmental conditions. These findings position the AB region as a key modulator of *h*RXRγ's structural plasticity and, potentially, its transcriptional activity.

INVESTIGATION OF AGGREGATION PROPERTIES OF YEAST DCS1 PROTEIN

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Decapping Scavenger (DcpS) enzyme was initially identified as one of the factors playing role in mRNA turnover. DcpS proteins have been found in eukaryotes of varying complexity, including yeast, nematodes, and humans. DcpS belongs to the superfamily of HIT (Histidine Triad) proteins that utilize the evolutionary conserved motif (His-X-His-X-His-X, where X denotes hydrophobic amino acids) to cleave the 5',5'-triphosphate bridge within a mRNA 5'cap structure, releasing m7GMP and ppN or diphosphate terminated oligoribonucleotide [1].

In addition to its role in mRNA cap metabolism, human DcpS has been reported to function in the pre-mRNA splicing and the regulation of miRNA turnover [2, 3], as a potential therapeutic target in specific cancer types [4], and in the development of nervous system [5]. Interestingly, several mutations in DcpS have been linked to neurological disorders, e.g. ARS syndrome [6].

Recently we have shown that human DcpS protein undergoes aggregation into a beta-sheet-like amyloid fibrils *in vitro* under physiological temperature, what may be connected to its function in neurological disfunctions development. Moreover, the nematode DcpS (*Cenorhabditis elegans* DcpS) has also been shown to form amyloid-like fibrils under the same experimental conditions [7].

Here, we investigated the aggregation properties of DCS1 protein – a DcpS homologue from the unicellular eukaryote (*Saccaromyces cerevisiae*). DCS1 share 32,55% or 31,42% identity of amino acid sequence with human DcpS and *C.elegans* DcpS, respectively, and overlap in the structural alignment with hDcpS and CeDcpS. Bioinformatic analysis of potential aggregation-prone regions with WALTZ and Cordax prediction models revealed the presence of two such motifs in the DCS1 amino acid sequence, similarly to human and nematode homologs. However, initial screening of the aggregation process using a Thioflavin T (ThT) assay, in which an increase in ThT dye fluorescence is observed upon binding to the stacked beta-sheets present in amyloid fibrils, showed no such effect for DCS1. Also, the DCS1 solution remained transparent over the assay period, in contrast to the CeDcpS solution under the same experimental conditions. In summary, the preliminary results obtained here for DCS1 suggest a lack of, or much weaker, aggregation propensity for this DcpS homologue from a lower unicellular eukaryote, which requires further investigation.

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DISTINGUISHING SPECIFIC AND NONSPECIFIC CATION INTERACTIONS WITH ACID-RICH PROTEINS USING FCS

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Biomineralisation in corals is modulated by the secretion of proteins into the extracellular skeletal organic matrix. A subclass of these proteins, the coral acid-rich proteins (CARPs), are thought to regulate calcium carbonate formation by binding to calcium ions. However, the exact nature of this interaction remains unclear. In particular, the selection of the calcium carbonate polymorph depends on the ionic composition of the environment, since the presence of Mg^{2+} cations is necessary to obtain the naturally occurring aragonite. We investigate the conformational behaviour of two highly acidic intrinsically disordered proteins (IDPs) upon interaction with counterions, Ca^{2+} and Na^{+}, by measuring the changes in their hydrodynamic size with increasing salt concentrations using fluorescence correlation spectroscopy (FCS). By varying both the ionic strength and the identity of the cation, we measure the influence of ionic conditions on protein dimensions. Our results show a strong dependence of the hydrodynamic size on the cation type and concentration. While the changes observed for the monovalent cation are consistent with the predictions of Debye screening, the results obtained for the divalent cation differ significantly, suggesting counterion-specific interactions beyond simple electrostatics. We are able to separate the overall screening from putative site-specific cation binding and show that only a subset of the observed behaviour can be fully explained by classical Debye-Hückel theory.

SESSION XI: THEORETICAL, COMPUTATIONAL, AND DATA-DRIVEN ADVANCES IN BIOPHYSICS

IS THE DEVELOPMENT OF MOLECULAR BIOPHYSICS METHODS SUBJECT TO THE LAWS OF EVOLUTION?

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The development and selection of methods, models, and theories in time from the point of view of their usefulness in scientific research resembles the process of biological evolution with its Darwinian selection mechanisms. The question is, how does the evolutionary tree of useful and functional experimental and theoretical methods of molecular biophysics look? Below, I present a hierarchical tree applied to AI/ML molecular biophysics problems. The analysis and the trees were created using the Anthropic Claude application [1]. For a general overview, see e.g. [2]. During the presentation, I will present the full tree, which accounts for the evolution of experimental and theoretical biophysics methods. Also, among other things, a flowchart tree will be presented. In addition to their cognitive value, such trees can be very useful in teaching biophysics methods, because they provide a global view of the current state of knowledge and research methodologies.

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AI/ML MOLECULAR BIOPHYSICS APPLICATIONS
├── Traditional ML (2000s-2015)
│   ├── Support Vector Machines (2000s)
│   ├── Random Forests (2005s)
│   ├── Principal Component Analysis (2000s)
│   └── Hidden Markov Models (2000s)
├── Deep Learning (2010s-present)
│   ├── Convolutional Neural Networks (2010s)
│   │   ├── Image Classification (2012)
│   │   ├── Cryo-EM Analysis (2015)
│   │   └── Medical Imaging (2017)
│   ├── Recurrent Neural Networks (2010s)
│   │   ├── Sequence Analysis (2015)
│   │   └── Time Series Prediction (2018)
│   ├── Transformer Models (2017-present)
│   │   ├── Protein Language Models (2019)
│   │   ├── ESM Models (2021)
│   │   └── ProtTrans (2021)
│   └── Graph Neural Networks (2018-present)
│       ├── Molecular Property Prediction(2018)
│       ├── Protein Function Prediction (2020)
│       └── Drug-Target Interaction (2021)
├── Structure Prediction (2018-present)
│   ├── AlphaFold (2018)
│   │   ├── AlphaFold 1 (2018)
│   │   └── AlphaFold 2 (2020)
│   ├── ColabFold (2021)
│   ├── ChimeraX AlphaFold (2021)
│   └── ESMFold (2022)
├── Dynamics Prediction (2019-present)
│   ├── Boltzmann Generators (2019)
│   ├── Neural ODEs (2020)
│   ├── Graph-based Dynamics (2021)
│   └── Diffusion Models (2023)
└── Drug Discovery (2015-present)
    ├── Virtual Screening (2015)
    ├── Generative Models (2018)
    ├── Molecular Optimization (2020)
    └── Target Identification (2022)
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PREDICTING STRUCTURE AND PROPERTIES OF POLYTRYPTOPHAN CRYSTALS

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Polymers containing aromatic sidechains can exhibit interesting charge transport properties. Theoretical investigations suggest they may even become high-temperature superconductors under specific conditions [1]. Aligning polymer chains in a periodic lattice (or even chaotic, quasi-periodic structure [2]) may help in forming a stable transport network. For this reason I studied polytryptophan chains (containing 12 or less monomers). As very hydrophobic molecules, they are hard to crystallize under typical conditions (it was never done, so the possibility of achieving a periodic structure is only hypothetical). Their crystallization is itself a challenge, but possible crystal structures are worth considering (due to interesting properties they may exhibit).

Polymer Structure Predictor (PSP) [3] was used to construct infinitely long polymer chains, and then align them in parallel in a crystal lattice. The method included inserting new chain in different configurations with the respect to the existing one, then repeating the process until a stable crystal structure was found (see Figure 1). This is a great simplification, but aligning 12-monomer molecules consisting of hundreds of atoms proved to be too complex for crystal structure prediction algorithms.

This simplified model of a polytryptophan crystal allowed for DFT calculations [4]: the Fermi energy and band structure were calculated. Surprisingly, no band gap was found, which suggests such a crystal would be a conductor (but no sign of superconductivity was found).

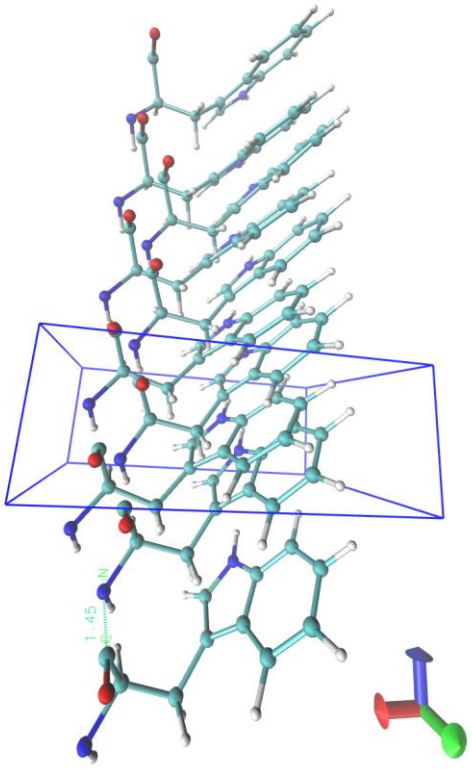

Fig.1. Two infinite polytryptophan chains generated using Polymer Structure Predictor. Atoms are colored using the CPK scheme. The unit cell is shown in blue, crystallographic axes are in the corner.

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IN SILICO DESIGN OF MOLECULARLY IMPRINTED POLYMERS FOR REMDESIVIR AND ITS ACTIVE METABOLITE

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Remdesivir is a broad-spectrum antiviral drug that gained significant attention during the COVID-19 pandemic due to its activity against RNA viruses, including SARS-CoV-2. After administration, remdesivir is metabolized in the body into its pharmacologically active analogs, such as GS-441524 (Fig.1), which inhibit viral RNA polymerase, thereby disrupting viral replication. Monitoring the concentrations of remdesivir and its metabolites in patients' body fluids is crucial for assessing therapeutic efficacy and ensuring safe, individualized dosing. [1] Despite the existence of methods for determining remdesivir, few have been adequately validated, highlighting the need to develop new, reliable methods for quantifying these compounds.

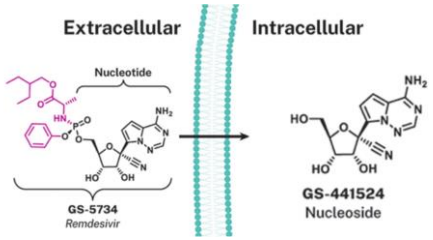

Fig.1. Structures of remdesivir and its metabolite GS-441524.

Molecularly imprinted polymers (MIPs) are a promising tool for the selective extraction of remdesivir and its metabolites from biological fluids. MIPs are synthetic materials with binding sites specifically designed for target molecules. Their selectivity, stability, and reusability make them ideal for detecting and extracting small molecules from biological samples. In personalized medicine, MIPs can be used to monitor therapeutic drug levels, enabling accurate and cost-effective detection of pharmaceuticals and their metabolites. [2]

This study focused on applying computational chemistry tools to the synthesis of molecularly imprinted polymers (MIPs) for antiviral drug recognition. Molecular modeling techniques can significantly accelerate MIP development by enabling rational design prior to experimental synthesis. Quantum chemical calculations (DFT) and molecular mechanics were employed to investigate the interactions within pre-polymerization complexes of remdesivir, its active metabolite GS-441524, and a set of selected functional monomers. The primary objective was to identify the most suitable monomers exhibiting the strongest interactions with these compounds in the pre-polymerization complex and to determine the optimal monomer-to-template molar ratio for designing and synthesizing MIPs capable of selectively recognizing remdesivir and its metabolite in patients' blood samples. The theoretical analysis of the MIP binding site considered carbazole-based monomers and a solvent mixture of acetonitrile:DMSO (8:2, v/v).

The results revealed significant differences in the binding affinities of the selected monomers, identifying promising candidates for MIP design.

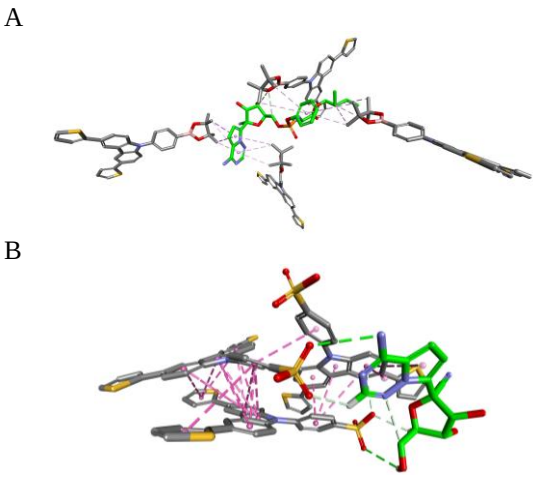

Fig.2. The most stable pre-polymerization complex, comprising remdesivir (A), its metabolite (B), and four molecules of the selected functional monomer.

Based on the calculations of Gibbs free energy values, a molar ratio of 1:4 (template:functional monomer) was identified as optimal for forming stable pre-polymerization complexes involving remdesivir, its metabolite, and selected functional monomers (Fig. 2). These findings provide a theoretical foundation for developing molecularly imprinted polymers for remdesivir and its metabolite, GS-441524, for antiviral drug detection, with potential applications in personalized medicine.

ACKNOWLEDGMENTS

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INSIDE THE PROJECT AGRITECH: DIFFERENT APPROACHES FOR THE EVALUATION OF SAFETY PARAMETERS ALONG THE AGRI-FOOD SUPPLY CHAIN

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The National Center for the Development of New Technologies in Agriculture (Agritech)[1] is an initiative in Italy funded by the Italian Ministry of University and Research under the National Recovery and Resilience Plan (PNRR). One of the primary objectives of this project is to promote the advancement of agri-food production, with a strong focus on safety, traceability, and security throughout the supply chain. This approach aligns with the core principles of the European Green Deal, which aims to create fair, healthy, and environmentally friendly food systems[2].

One of the significant challenges in managing the food supply chain is the risk of microbiological and chemical contamination. Food inspections are conducted at production sites and processing plants to meet regulatory requirements. These inspections typically involve random sampling and laboratory analysis, which can take two or more days to provide results. The time and cost involved in each analysis often lead to reduced oversight, increasing health risks. Furthermore, due to these time and cost constraints, analyses cannot be performed at every stage of the supply chain, allowing products to reach grocery stores before thorough checks are made. This scenario underscores the urgent need to enhance food safety, and the limitations of current analysis methods motivate us to propose new approaches for detecting contaminants along the food supply chain.

In this project, we are working on three different methodologies to detect specific contaminants that may be present in the cereal, dairy, and wine value chains. Our targets are aflatoxin B1 (AFB1), aflatoxin M1 (AFM1), and ovalbumin (OVA). AFB1 is one of the most widespread and hazardous mycotoxins that can contaminate various foods, including cereals, before and after harvest. AFM1 is a by-product of the hepatic metabolism of AFB1 and is frequently found in the milk of animal species fed with AFB1-contaminated fodder[3]. Lastly, OVA, one of the oldest fining agents used in winemaking, is also an allergen. Traces of OVA[4] that potentially remain in wine after filtration can trigger allergic reactions in sensitive consumers.

To detect these contaminants, we are working on three distinct methodologies: Surface Plasmon Resonance, Fluorescence Immunoassay, and Electrochemical Impedance Spectroscopy. The common principle behind these methods is to monitor the binding interaction of specific antibodies with the selected targets.

A more detailed overview of these three approaches will be presented, subdividing them according to the respective food chain and the analyte target.

ACKNOWLEDGMENTS

This work has been funded within the project AGRITECH Spoke 9 – Codice progetto MUR: AGRITECH ”National Research Centre for Agricultural Technologies” - CUP CN00000022, of the National Recovery and Resilience Plan (PNRR) financed by the European Union ”Next Generation EU”.

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IDENTIFICATION OF VOLATILE ORGANIC COMPOUNDS (VOCs) AS MARKERS OF GOAT CHEESES RIPENING: PERSPECTIVES AND APPLICABILITY IN BIOSENSOR DESIGN

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Ripening is a complex and dynamic process that determines the final organoleptic characteristics of cheese. The production of volatile organic compounds (VOCs) is among the most informative parameters related to maturation. These metabolites are generated by the enzymatic degradation of lipids, proteins, and carbohydrates, through proteolysis, lipolysis, and the metabolism of citrate and lactate [1,2]. VOCs, besides playing a fundamental role in defining the flavour characteristics of cheese, act as molecular indicators of specific stages of maturation. Accurate analytical information about cheese ripening is essential for several applications: optimization of the ripening conditions, early intervention in case of defects, and tailoring of the development of new cheese varieties with customize aromatic profiles [3]. In this perspective, the identification of one or more volatile components as specific biomarkers of ripening will allow for a rapid, and non-destructive control of the cheese production processes [4]. For this purpose, goat cheeses were analysed by headspace solid-phase micro extraction (HS-SPME) coupled to gas chromatography-mass spectrometry (GC-MS) to characterize the VOCs profile and evaluate its evolution during the maturation process. The first objective was to identify VOCs that could be utilized as molecular markers capable of discriminating between early, intermediate and advanced ripening cheese stages [5]. This selection represents the first step towards the design of a dedicated biosensor, capable of selectively detecting these VOCs. The biosensor will be designed to operate in real conditions, ensuring sensitivity, specificity and robustness. This can contribute to the automation and improvement of quality in the dairy value chain.

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MULTISTEP LOSS OF CATALYTIC ACTIVITY AND LIGAND BINDING ABILITY OF HEXAMERIC PURINE NUCLEOSIDE PHOSPHORYLASE FROM *E. COLI*

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It is commonly accepted paradigm that enzymes exist in two states, active or inactive and that enzyme catalysis is such a complicated process, that a change in any biophysical property of an enzyme leads to a total loss of catalytic ability. What's more, the catalytically disabled protein is often considered as unable to bind ligands. Here however, we present purine nucleoside phosphorylase (PNP), which breaks this paradigm.

PNPs are key proteins in the purine salvage pathway, that enables cells to recover purine bases and pentose-1-phosphate from used nucleosides. PNPs are widely studied for their potential medical applications, e.g. as targets in antitumour therapies, while inhibitors of human PNP are considered immunosuppressive agents [1]. Lack of *de novo* nucleosides synthesis pathway in some bacteria makes PNP a target for potential drugs in designing therapies against such organisms [2].

But PNPs are also remarkable molecular machines, especially hexameric PNP from *E. coli*, which in the course of our studies, turned out to be, itself, an interesting research object. *E. coli* PNP in the apo form is a homohexamer, both in terms of sequence and three dimensional structure of monomers. However when it comes to symmetry it is trimer of dimers (Fig. 1, top). Upon binding of phosphate, one of the substrates, in some subunits conformational change occurs: segmentation of H8 helix and movement of its N-terminal part towards the active site, partially closing the entrance. According to the flip-flop model, catalysis occurs in the closed active sites, while substrates bind also to the adjacent open active site [3].

We have discovered that this enzyme does not inactivate in one step, but rather goes through several intermediate states, with various distribution of the closed active sites, which is reflected in various catalytic and phosphate binding abilities of such intermediates. Following the activity decay over time and measuring a dependence of dissociation constants for phosphate on the activity of the enzyme sample, we have shown that a gradual loss of the catalytic activity towards natural substrates correlates with the reduced ability to bind phosphate. Taking into account these data and known crystallographic structures of *E. coli* PNP, we were able to identify specific intermediates on the pathway from the fully active to the inactive enzyme (Fig. 1, bottom).

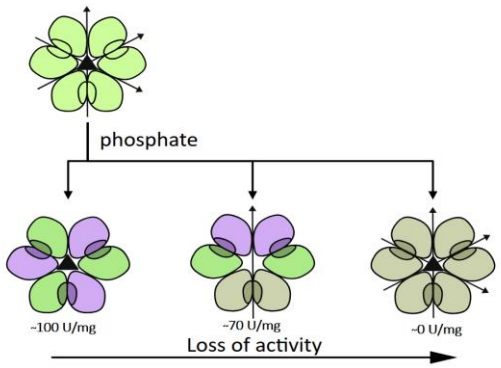

Fig.1. Intermediates in the inactivation path of hexameric molecule of E. coli PNP. (Top) apo form (PDB 1ecp) with all subunits in the same, open, conformation. Active sites position at the inner interfaces of dimers, and the symmetry of the molecule are shown. Upon binding of ligands some of the subunits close entry to the active site. Distribution of subunits with open and closed active sites depends on the protein activity (bottom from the right) 1. fully active form, with every second subunit in the closed conformation (violet), trimer of three identical open-closed dimers; 2. protein with ~2/3 of its maximal activity, with two open-closed dimers and one open-open dimer, the latter is inactive towards natural substrates; 3. form with all subunits unable to close active sites, incapable of catalysing reaction with natural substrates.

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ILLUMINATING CANCER: BIOIMAGING POTENCY OF 3-(1,1-DICYANOETHENYL)-1-PHENYL-4,5-DIHYDRO-1H-PYRAZOLE (DCNP)

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Optical imaging serves as a valuable method for observing and comprehending biological processes in both *in vitro* and *in vivo* systems. It provides a powerful means of visualizing key chemical and biological activities in living cells in real-time. Thus, it offers several advantages for disease diagnosis, including high spatial resolution, quick data acquisition, no ionizing radiation risks, affordability and/or minimal invasiveness. Optical imaging relies on the use of luminogens, which have the ability to absorb light and convert it into detectable fluorescence. To achieve high-quality fluorescence bioimaging, it is essential to use organic exogenous contrast agents that offer biocompatibility, brightness, and photostability. Unfortunately, commonly used organic dyes exhibit non-emissive properties during aggregation, making them less suitable for medical applications. Aggregation-induced emission (AIE) molecules have been identified as ideal candidates for fluorescence bioimaging, mostly because the hydrophilic feature of the bio-environment can cause almost instant aggregation for typically hydrophobic organic dyes. Thus, AIE luminogens offer benefits such as brighter emission from the aggregates than in the dilute solution, and the potential to exhibit large Stokes shifts and high resistance to photobleaching.

3-(1,1-dicyanoethenyl)-1-phenyl-4,5-dihydro-1H-pyrazole, known as DCNP, comprises organic "push–pull" molecule with nonlinear optical characteristic [1]. In our studies, DCNP was obtained through a three-step synthesis. The first step involved double N-alkylation of phenylhydrazine derivatives, carried out in an aqueous medium under microwave irradiation to accelerate the reaction [2]. The second step consisted of Vilsmeier–Haack formylation, while the final step was a Knoevenagel condensation, leading to the formation of the target molecule. In the next step, AIE behaviors of the compound were evaluated. Increased fluorescence intensity of DCNP was observed in solutions where the ratio between solvent and nonsolvent were changing. *In vitro* experiments were undertaken to investigate the bioimaging potency of DCNP, focusing on the cancer illumination. We used sulforhodamine assay (SRB) to assess the influence of the compound on the viability of selected cancer cell lines derived from different origin. Our results showed low cytotoxicity of DCNP suggesting its high potential for use with living cells. The staining potency of selected AIE compound was examined by fluorescence microscopy and spectrofluorimetric method. We found the strong enhancement of fluorescence intensity of DCNP in the presence of model lipid bilayers what suggested high visualization capacity of the compound. Time- and concentration-dependent accumulation of the probe was investigated and its ability to localize into the cells was examined. Due to the important role of fluorescent dyes in monitoring cell death, the biodistribution of DCNP in cells undergoing apoptosis was also examined. Further studies will be conducted to increase the specificity of the tested AIE compound towards selected cell lines.

ACKNOWLEDGMENTS

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NEW APPROACHES FOR MILK QUALITY MONITORING

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Milk is a fundamental food source, valued globally for its rich nutritional content, including proteins, fats, vitamins, and essential minerals that benefit all age groups. Monitoring milk quality is critical in order to maintain food safety and human health[1].

Therefore, there is an urgent need for the development of fast, sensitive, reliable and cost-effective methods and sensor systems for milk quality monitoring.

Volatile Organic Compounds (VOCs) are important indicators of milk quality and origin, reflecting factors such as animal metabolism, diet, geographic region, and grazing conditions, which can differentiate milk samples from various farming systems or regions. There is high evidence that the VOCs profile of milk from grazing cattle is different from that of cows fed indoor[2].

The aim of this work was to design and to develop an innovative "impinger" biosensor that utilizes Molecular Recognition Elements (MRE) such as Odorant-Binding Proteins (OBPs) to detect VOCs in milk, to differentiate milk samples from intensive versus extensive farming systems. The impinger captures the VOCs released from milk, which then are collected in a liquid phase. This liquid sample was transferred to the biosensor chamber, where OBPs bind selectively to VOCs. The binding event triggers a Förster Resonance Energy Transfer (FRET) signal that is proportional to the VOCs concentration, allowing the quantification of specific compounds and it is indicative of milk quality. The binding between pOBP and VOCs was investigated using Head Space Solid-Phase Microextraction coupled with Gas Chromatography-Mass Spectrometry (HS-SPME/GC-MS), confirming the specificity and efficiency of OBP-VOC interactions. This research provides a foundation for future advancements in biosensor technology for food quality monitoring.

This real-time approach provides a sensitive and cost-effective solution for VOCs monitoring.

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POLYSTYRENE NANOPARTICLES AND THEIR EPIGENETIC EFFECTS IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS

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The potential effect of PS-NPs on the molecular markers and determinants of the carcinogenesis process was investigated. We studied the effects of non-functionalized polystyrene nanoparticles (PS-NPs) of varying diameters (29 nm, 44 nm, and 72 nm) on specific epigenetic modifications and gene expression profiles in human peripheral blood mononuclear cells (PBMCs) *in vitro*. We used concentrations ranging from 0.001 to 100 μg/mL and cells were incubated for 24 hours. We analysed the level of 5-metyl-2'-deoxycytidine (5-mdC) by mass spectrometry method, methylation in the promoter regions of suppressor genes TP53 (P53), CDKN2A (P16), and CDKN1A (P21) and proto-oncogenes (CCND1, BCL2, BCL6), along with their expression profile by Real-Time PCR assays.
The results revealed no significant changes in global DNA methylation/demethylation levels in PBMCs after short-term exposure to non-functionalized PS-NPs. None of the PS-NPs caused a change in the methylation pattern of the promoter regions of the TP53, CDKN2A, CDKN1A, CCND1, BCL2 and BCL6 genes. However, gene profiling indicated that PS-NPs with a diameter of 29 nm and 44 nm altered the expression of TP53 gene. The smallest PS-NPs with a diameter of 29 nm increased the expression of the TP53 gene at a concentration of 10 μg/mL, while PS-NPs with a diameter of 44 nm did so at a concentration of 100 μg/mL. An increase in the expression of the CDKN2A gene was also observed when PBMCs were exposed to PS-NPs with 29 nm in diameter at the highest concentration. Our study elucidates the limited epigenetic effect of PS-NPs on human PBMCs under the examined conditions.
Funding: The research was conducted within the framework of the National Science Centre, Poland, Project No. 2021/41/N/NZ7/02049.

REVEALING CANCER DYNAMICS: THE APPLICATION OF PYRIMIDINEBASED FLUORESCENT COMPOUND FOR ANALYZING ORGANELLE ACCUMULATION WITH NO TOXICITY IN GLIOMA AND COLON CANCER CELLS.

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This study presents a newly developed pyrimidine-based compound that exhibits imaging capabilities without cytotoxic effect. Fluorescence-based compounds represent a powerful tool for non-invasive bioimaging of living systems in real time, which is crucial for analyzing cancer dynamics. Contemporary cancer treatment, including glioma and colon cancer, relies on advanced diagnostic methods and therapies tailored to individual patient needs [1].

Fluorescent compounds, particularly those containing nitrogen heteroatoms, have the ability to accumulate in different parts of biological tissue, and their exceptional optoelectronic properties and biocompatibility make them useful for applications in cancer studies.

In our research, we synthesized 4,4'-(2,2'(pyrimidine-4,6-diyl)bis(hydrazine-2-yl-1ylidene)bis (methanylylidene)) bis (*N*,*N*-diphenylaniline) employing a two-step synthesis, where the key step was the hydrazone condensation reaction. Optical characterization of the compound was performed using UV-Vis spectrometers, revealing interesting properties dependent on the molecule's environment, including polarity and pH. The absence of toxicity was confirmed using the sulforhodamine B (SRB) assay, a widely accepted method for evaluating cell viability. This non-toxic profile is a significant advantage, especially for applications involving mitochondrial fluorescence visualization, where maintaining cellular integrity is crucial. Additionally, mitochondrial membrane potential assays were conducted as part of the study. This aspect of the investigation was essential to check the absence of cytotoxic effects suggested by preliminary viability tests. Since mitochondria play a central role in cellular energy metabolism and apoptotic signaling, assessing mitochondrial function provides a more sensitive and mechanistically relevant indicator of toxicity. Given that many hydrazone and pyrimidine derivatives can pose cytotoxic risks, the development of a safe compound suitable for live-cell imaging represents a meaningful step forward. Importantly, the studied pyrimidine based fluorescent compound does not induce changes in mitochondrial membrane potential, preserving the functional state of mitochondria during imaging.

Hydrazone-based probes are especially useful due to their ability to detect cations, anions, aldehydes, and ketones, which can be used to label biomolecules such as oxidized proteins or saccharides.

ACKNOWLEDGMENTS

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FLUORESCENCE CORRELATION SPECTROSCOPY ASSAY TO DETECT THE PRESENCE OF TOXIC MICROCYSTIN-LR MOLECULES IN WATER

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Fluorescence correlation spectroscopy (FCS) is a quantitative technique that allows the determination of concentration and diffusion properties of fluorescently labeled species with single-molecule sensitivity. In a standard confocal optical set-up, a laser is focused on a diffraction-limited spot using a high numerical aperture objective lens. The emitted light is collected through a confocal pinhole, defining a small observation volume (femtoliters). When the fluorescent species diffuse through this observation volume, the photons emitted are recorded over time, generating fluorescence fluctuations[1].

These fluctuations can be analyzed using two different methods: autocorrelation analysis (AC) and photon counting histogram analysis (PCH). In the case of the autocorrelation analysis, it is possible to study the diffusion rate and concentration of the fluorescent species over time, while the photon counting histogram analysis (PCH) allows us to quantify the concentration and molecular brightness of the species based on the analysis of their amplitude [1].

Several studies have shown the effectiveness of FCS to address fundamental questions in biology[2-5]. Furthermore, some studies have used this technique in sensing to identify specific molecules, such as allergens, toxins and antibiotics, in various matrices[6,7]. This method relies on the variation of the fluorescence fluctuation, associated with the formation of a molecular complex between a specific biomolecule (antibody, binding protein, peptide, etc.) and its fluorescent target analyte, in the absence and presence of the analyte. In this study, we present the application of the FCS method to detect the presence of toxic microcystins-LR (MC-LR) in water. For this purpose, we used a fluorescence-labeled conjugate (BSA-MC-LR) and monoclonal antibody to develop a competitive assay for the sensitive and accurate detection of MC-LR. The obtained results will be presented and discussed.

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SYNERGY IN ANTIPROLIFERATIVE ACTIVITY BETWEEN VARIOUS MODULATORS OF CELLULAR CHOLESTEROL HOMEOSTASIS IN COLON CANCER CELLS

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Cholesterol (Chol) is an important component of cellular membranes affecting their fluidity and permeability. Its altered levels are associated with various pathologies, e.g., atherosclerosis and cardiovascular disease [1]. Additionally, disturbed Chol and lipid homeostasis are observed in cancer [2].

The interaction of simvastatin, the anti-hyperlipidemic drug with three antidepressant and antipsychotic drugs was studied. All of them are known to affect Chol homeostasis. Statins are inhibitors of main enzyme in Chol biosynthesis, HMG-CoA reductase [3]. Common side effect of antipsychotic drugs is weight gain accompanied by metabolic syndrome [8]. Some antipsychotics also up-regulate expression of genes engaged in Chol biosynthesis, being controlled by SREBP transcription factor [4]. SREBP is main regulator of lipid homeostasis. Additionally, statins and many antipsychotic drugs possess anticancer activity [5]. Disruption of Chol homeostasis has been suggested to be responsible for their cytotoxicity [5].

Antiproliferative activity of imipramine, flupentixol, and trifluperazine was corroborated in two human colon cancer cell lines. All studied compounds exhibited significant degree of selectivity towards cancerous *versus* non-cancerous cells.

The activity of the studied drugs combined with low concentration of simvastatin was also investigated. Synergistic interaction between them was discovered and analyzed in detail with the use of mathematical models (Chou-Talalay & HSA method).

To better understand mechanism of drugs' cytotoxicity their influence on cellular level of Chol was studied and the most active drugs turned out to decrease its amount. The studied drugs induced also the enhancement of expression of SREBP-controlled genes encoding HMG-CoA reductase and LDL receptor.

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TRANSPORT MECHANISM OF PARACETAMOL IN POLYMER NANOCOMPOSITE MATERIALS

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In this work, the new class of hybrid materials - polyurethane/Cloisite® 30B (PU/PEO Cloisite® 30B) nanocomposite hydrogel systems for paracetamol drug delivery were studied. We present the swelling and release properties of these drug delivery systems depending on clay - Cloisite® 30B (CLO) concentration, as well as crosslinking agent type.

The transport mechanism, swelling and release processes of the active substance in nanocomposite matrix, were studied using gravimetric and UV-Vis spectroscopic methods.

Swelling and release processes depend on the amount of clay nanoparticles in these systems and the degree of crosslinking of PU/PEG/Cloisite® 30B hydrogel nanocomposites. The presence of clay causes, on the one hand, a reduction in free volumes in the polymer matrices, making the swelling process less effective, on the other hand, the high swelling and self-aggregation behavior of Cloisite® 30B and the interactions of paracetamol both with it and with the matrix, cause a change in the transport mechanism from anomalous diffusion to Fickian-like diffusion.

It has been also proven that in the case of modification of polymer matrices with nanoparticles, the appropriate selection of their concentration is crucial, due to the potential possibility of controlling the swelling and release processes in drug delivery patches [1]. Analyzing the data obtained from the steady-state spectroscopic and gravimetric measurements one can state that exceeding a certain critical value of the nanofiller concentration (0.5%), leads to its aggregation, which causes decreasing free volumes in the system and decreasing of swelling, in the case of more cross-linked systems but also relaxation rates decrease due to the barrier effect and increase due to the swelling of the aggregated clay. The relaxation rate increases slightly with increasing paracetamol concentration in the presence of nanoparticles and decreases when they are not present in the system, because clays promote swelling of the polymer matrix. Release relaxation rates increase with increasing CLO concentration, the nanoparticles presence in the system increase the efficiency of hydrogel expansion during the swelling processes and by clay-matrix interaction. In the case of too high concentration of Cloisite® 30B, the diffusion rate decreases due to the barrier effect corelated with clay aggregation, because the diffusion pathlength increases and causes decrease of drug concentration gradient. Diffusion coefficients of paracetamol molecules in the release process increase with increasing concentration of Cloisite® 30B in all matrices, diffusion coefficients D_{short_app} and D_{long_app} [2] are of the same order and are higher for long-time approximation (Fig.1).

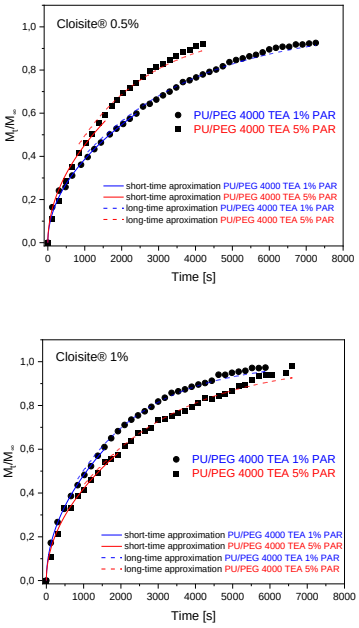

Fig. 1. Determination of diffusion coefficients from release curves for PU/PEG 4 000 TEA hydrogels with 0.5% and 1.0% CLO.

In matrices characterized by a high degree of crosslinking anomalous diffusion occurs; the presence of Cloisite® 30B nanoparticles in the system additionally inhibits the sorption process due to the barrier effect; the swelling rates, as well as the maximum swelling value, are lower than that observed for lower degrees of crosslinking systems.

The theoretical analysis of the swelling and release processes showed that for pure hydrogels anomalous diffusion transport mechanism occurs and changes to "Near-Fickian" or "Less Fickian" diffusion for matrices with Cloisite® 30B filler. In this work, it would be proposed to name the "Near Fickian" diffusion process for which the swelling and release exponent n is in the range of 0.50-0.55 due to a "double swelling" phenomenon related to the diffusion as a consequence of concentration gradient and the swelling of nanoparticles (Fig.2).

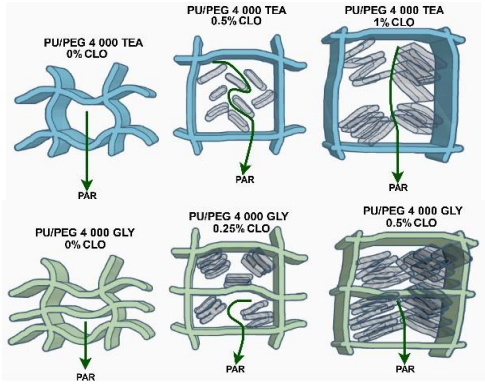

Fig. 2. Schematical model of "double swelling", barrier effect and aggregation of clay – influence on paracetamol release process.

The analysis of transport mechanisms in polyurethane nanocomposites containing Cloisite® 30B nanoparticles showed that the drug-release process can be controlled by the concentration of nanoparticles in the system and the degree of crosslinking of the polymer matrix. The presence of paracetamol doesn't change significantly the swelling process, however, the matrix degree of crosslinking increase, resulting in a decrease of its swelling. This can be used to produce new patches with a controlled drug release process.

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DIFFERENTIAL ANALYSIS OF MALE AND FEMALE CUCUMBER LINES AT mRNA AND miRNA LEVELS

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Cucumber (Cucumis sativus) serves as a model species for investigating the processes related to sex determination in plants [1]. While the development of flower organs is known to be regulated at both mRNA and microRNA (miRNA) levels, the precise interactions governing these processes remain insufficiently understood [2].

This study aimed to elucidate regulatory pathways involved in flower development by analyzing differentially expressed genes (DEGs) identified via RNA sequencing (RNA-seq) of flower buds from three cucumber lines: 2gg (gynoecious), Gy3 (gynoecious and weak) and B10 (monoecious) [3]. In parallel, small RNA (sRNA) sequencing was employed to identify differentially expressed miRNAs and their corresponding target genes. Gene ontology (GO) enrichment analysis was performed to study the molecular functions and biological processes associated with these genes. Additionally, protein-protein interaction network analysis was conducted to identify key pathways affected by DEGs. As a result, several candidate genes were highlighted as potentially crucial regulators of sex determination in cucumber flowers.

These findings contribute to a better understanding of the complex genetic and epigenetic mechanisms involved in floral development.

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