Posters

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.

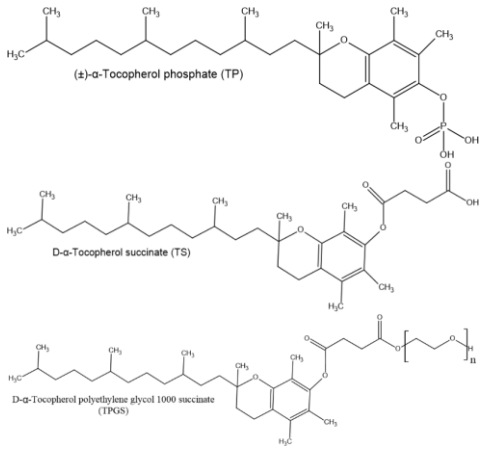

Fig.1. Structure of the α-tocopherol derivatives.

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.

ACKNOWLEDGMENTS

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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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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].

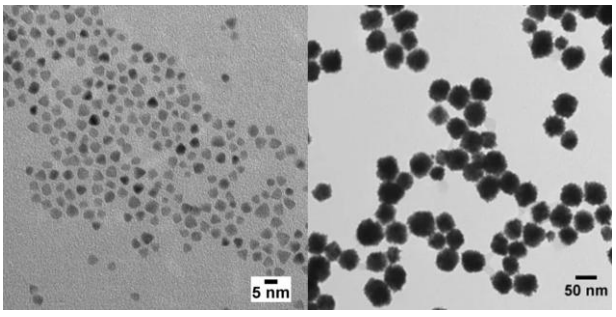

Fig. 1. Representative pictures of 5 nm (left) and 50 nm (right) platinum nanoparticles obtained from the NanoComposix company (www.nanocomposix.com, accessed 07.05.2025).

Firstly, we employed various physicochemical methods, such as dynamic light scattering (DLS) and atomic force microscopy (AFM) to assess 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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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.

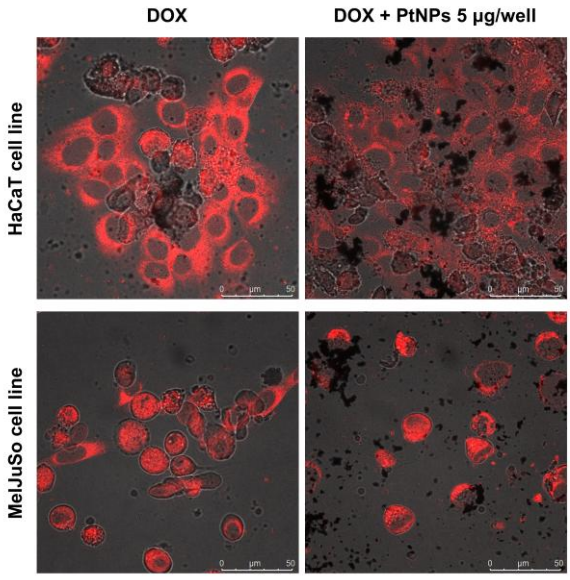

Fig.1. Confocal microscopy images of 70 nm PtNPs influence on DOX cytotoxic effects in HaCaT and MelJuSo cell lines.

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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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.

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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.

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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.

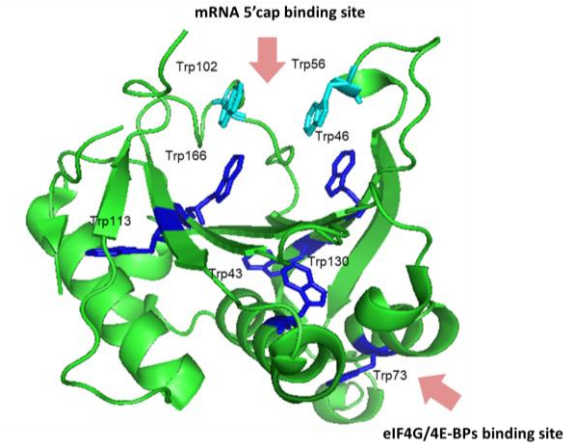

Fig.1. X-ray structure of eIF4E (PDB: 1IPC). The tryptophan residues are marked in blue and cyan

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).

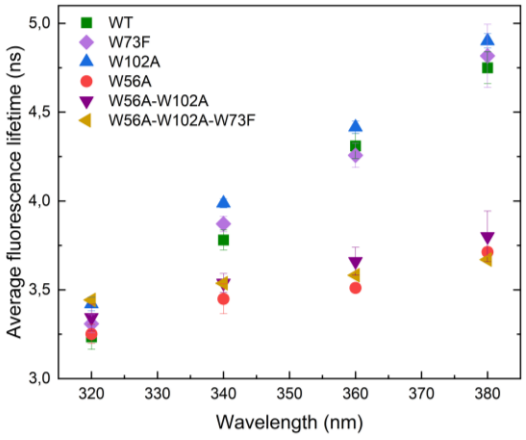

Fig. 2. The average fluorescence lifetimes of eIF4E variants in the presence of 4E-BP1, measured at different emission wavelengths for excitation at 284 nm

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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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.

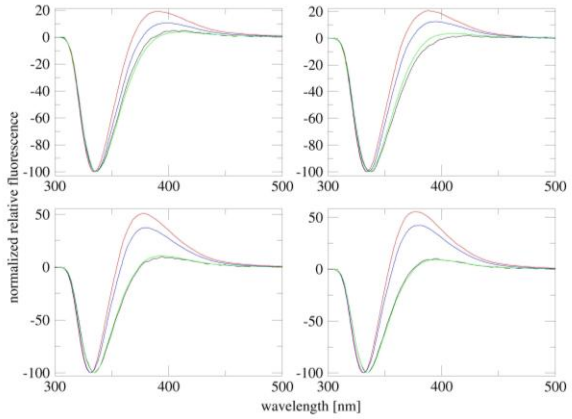

Fig.1. Normalized relative fluorescence spectra. Top: 5 μM α-chymotrypsin with 20 mM SDS (left) and 40 mM SDS (right); Bottom: 5 μM α-chymotrypsinogen with 20 mM SDS (left) and 40 mM SDS (right). Excitation wavelengths: 222 nm – black, 260 nm – red, 280 nm – blue, 295 nm – green.

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.

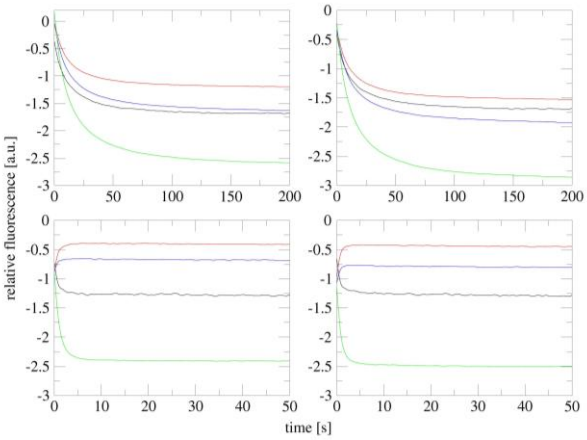

Fig.2. Relative reaction progress curves measured via fluorescence. Top: 5 μM α-chymotrypsin with 20 mM SDS (left) and 40 mM SDS (right); Bottom: 5 μM α-chymotrypsinogen with 20 mM SDS (left) and 40 mM SDS (right). Excitation wavelengths: 222 nm – black, 260 nm – red, 280 nm – blue, 295 nm – green..

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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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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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.

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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.

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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

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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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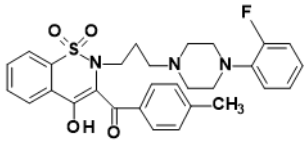
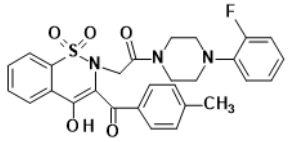
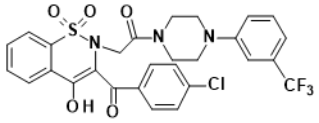
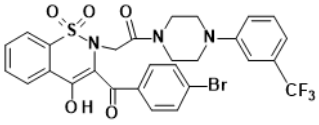
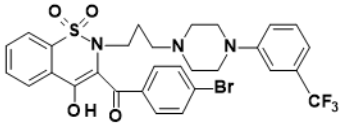
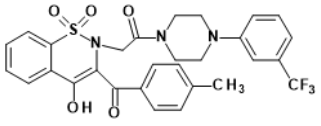
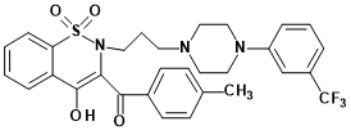
Cyclooxygenase (COX) is an enzyme located in the lumen of the endoplasmic reticulum, the Golgi apparatus and the nuclear membrane of the cell, embedded with hydrophobic fragments in the lipid bilayer of the membrane. COX catalyzes the process of prostanoid synthesis, i.e. prostaglandins, prostacyclin 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.

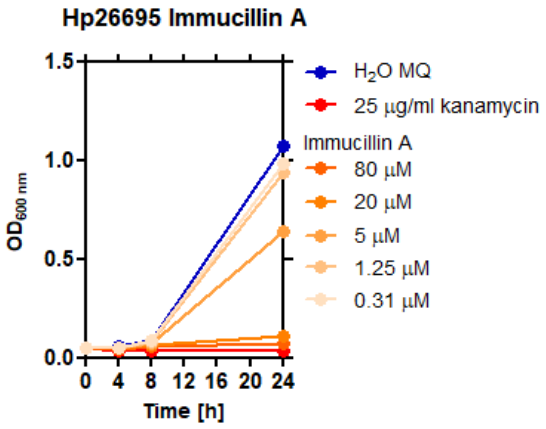

Fig.1. The growth curve of *H. pylori* 26695 strain in bacterial culture in the presence of various concentrations of immucillin A.

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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 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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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.

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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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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.

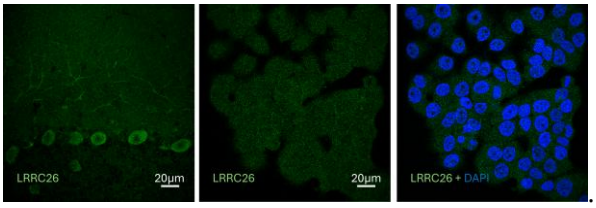

Fig.1. Immunostaining with anti-LRRC26 antibody (green) in Purkinje cells (left) and T47D cells (middle and right). Signal intensity is higher in Purkinje cell bodies and dendrites compared to surrounding cells. DAPI staining (right) shows nuclei in T47D cells.

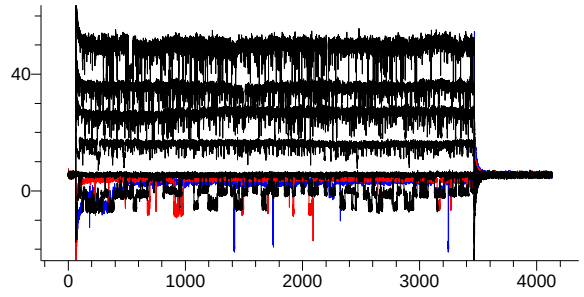

Fig.2. BK channel activity in LNCaP cells in inside-out configuration with symmetrical K^+ and 1 mM EGTA. Voltage steps from -80 mV to 100 mV in 20 mV increments. Blue: currents at -80 mV; red: currents at -40 mV; X-axis: mV; Y-axis: ms. Cell culture methods are described in [3].

ACKNOWLEDGMENTS

This study was supported by a student research grant no. Nr. FW3/3/F/MG/N/22 awarded to A.A. by the Medical University of Warsaw. Sz.T. is a member of the Cerebrum Student Science Club. pCDNA6-LRRC26-V5-His was a gift from Jiusheng Yan [2].

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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.

ACKNOWLEDGMENTS

This study was supported Polish Ministry of Research and University Education funds for Wrocław Medical University – project No SUBZ.A400.25.044.

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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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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 Ca2+, indicating that alterations in hydrophobicity within the central cavity modulate the energy landscape of the gating process.

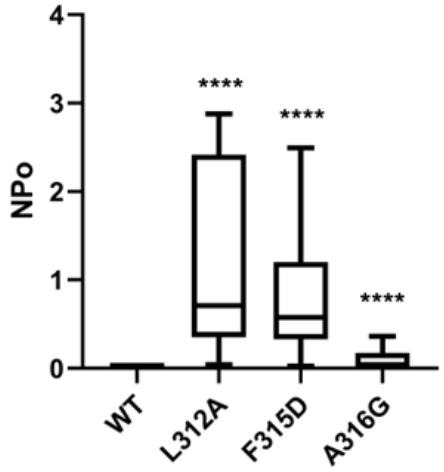

Fig.1. N-channel open probability (NPo) of WT and mutant BK channels at -50 mV in cell-attached configuration. WT n=21, L312A n=10, F315D n=16, A316G n=21; ****p<0.0001 Mann-Whitney test.

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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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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.

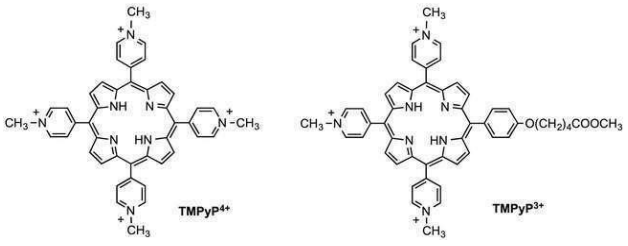

Fig.1. Molecular structures of two cationic *meso*-porphyrins.

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.

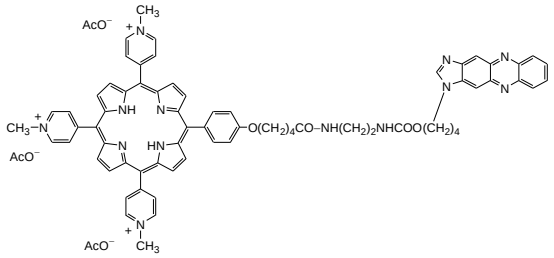

Fig.2. Molecular structure of porphyrin-imidazophenazine conjugate.

ACKNOWLEDGMENTS

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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.

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.

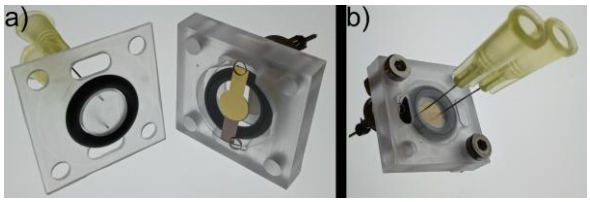

Fig.1. The figure illustrates the microfluidic system in two configurations: disassembled (a) and assembled (b). The system contains a piezoelectric quartz element that is used to monitor the morphological changes that mitochondria undergo as a result of drug exposure.

ACKNOWLEDGMENTS

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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.

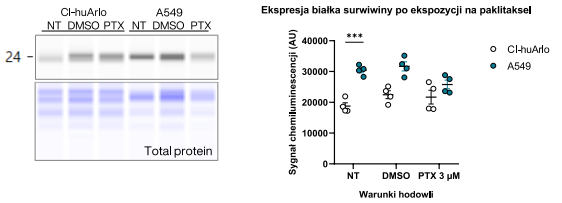

Fig.1. Expression of survivin protein in CI-huArlo and A549 cells before and after treatment with 3 µM PTX.

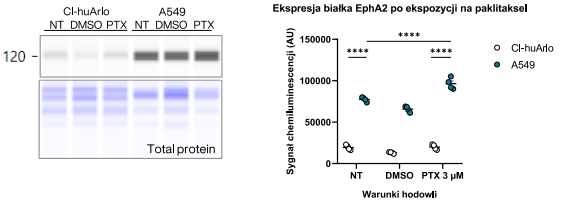

Fig.2. Expression of EphA2 protein in CI-huArlo and A549 cells before and after treatment with 3 µM PTX.

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.

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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.

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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].

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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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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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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.

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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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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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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 Mg2+ 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, Ca2+ 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.

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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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.

```
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)
```

ACKNOWLEDGMENTS

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PREDICTING STRUCTURE AND PROPERTIES OF POLYTRYPTOPHAN CRYSTALS

Łukasz Mioduszewski

.

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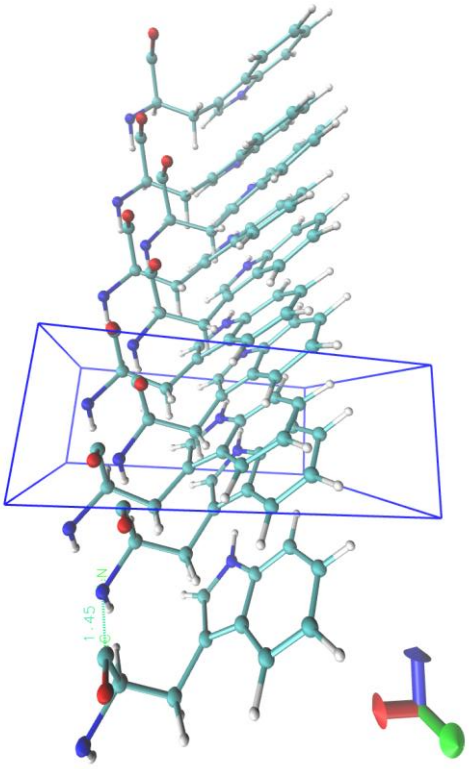
Polymers containing aromatic sidechains can exhibit interesting charge transport properties. Theoretical investigations suggest they may even become high-temperature superconductors under specific conditions [1]. Aligning polymer chains in a periodic lattice (or even chaotic, quasi-periodic structure [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).



[3] 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.

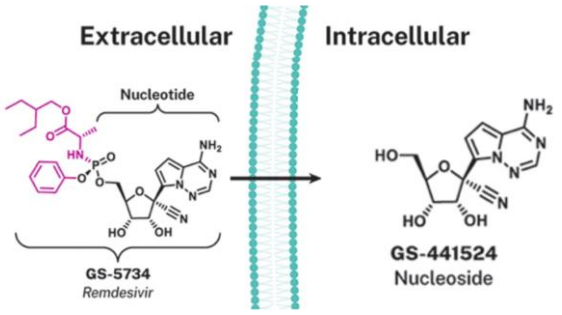


Fig.1. Structures of remdesivir and its metabolite GS-441524.

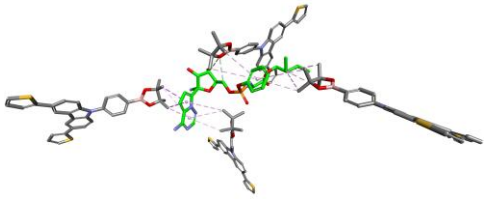
Molecularly imprinted polymers (MIPs) are a promising tool for the selective extraction of remdesivir and its metabolites from biological fluids. MIPs are synthetic materials with binding sites specifically designed for target molecules. Their selectivity, stability, and reusability make them ideal for 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.

A



B

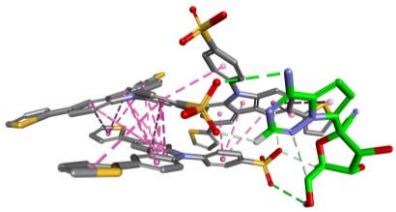


Fig.2. The most stable pre-polymerization complex, comprising remdesivir (A), its metabolite (B), and four molecules of the selected functional monomer.

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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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).

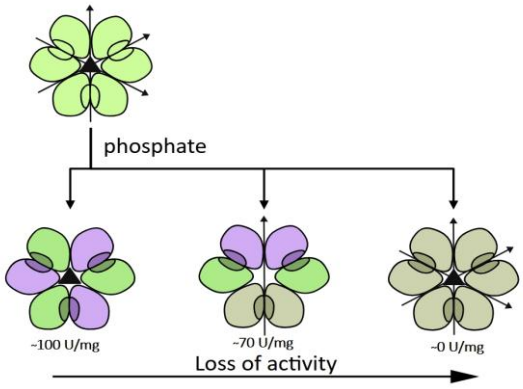

Fig.1. Intermediates in the inactivation path of hexameric molecule of E. coli PNP. (Top) apo form (PDB 1ecp) with all subunits in the same, open, conformation. Active sites position at the inner interfaces of dimers, and the symmetry of the molecule are shown. Upon binding of ligands some of the subunits 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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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.

ACKNOWLEDGMENTS

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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.

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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).

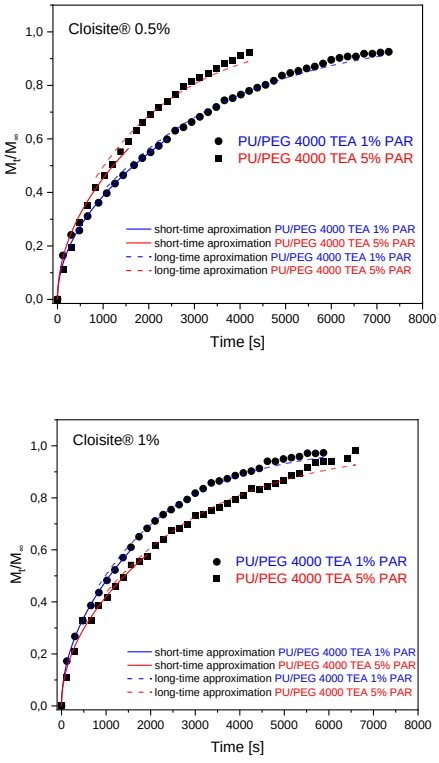

Fig. 1. Determination of diffusion coefficients from release curves for PU/PEG 4 000 TEA hydrogels with 0.5% and 1.0% CLO.

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).

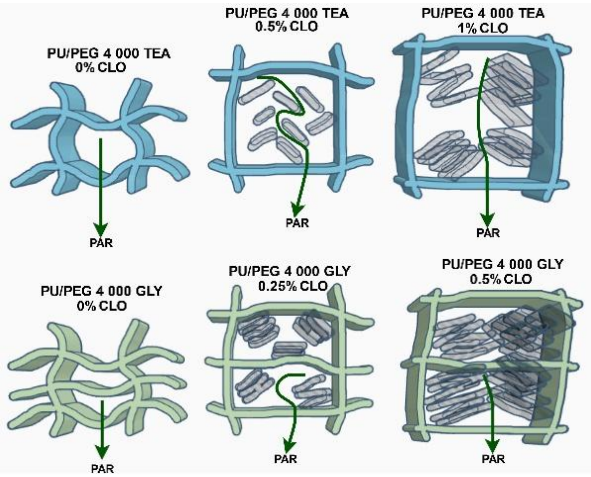

Fig. 2. Schematical model of "double swelling", barrier effect and aggregation of clay – influence on paracetamol release process.

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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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.

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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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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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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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