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Plenary lectures

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

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

ROOM TEMPERATURE PHOSPHORESCENCE WITH DIRECT TRIPLET STATE EXCITATION

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

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

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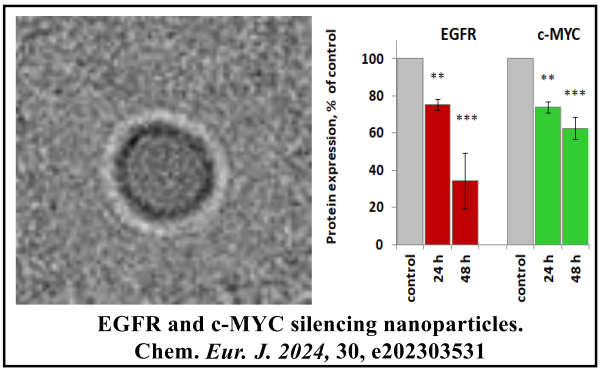
Despite thousands of chemotherapeutic drugs approved for clinical practice, there are still many unmet needs in the prevention and treatment of many diseases. The need to fill this never-shrinking gap is driving search for new drugs, both in the traditional chemotherapeutic category and in newer generation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

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

Specifically designed conjugates of antisense DNA-oligomers and oligofunctionalized boron clusters are used as building blocks in construction of new class of bionanoparticles capable of silencing the expression of selected target genes [5,6]. As model targets, Epidermal Growth Factor Receptor (EGFR) and c-MYC (myelocytomatosis oncogene) were chosen. Both proteins are overexpressed in several high-mortality human cancers and are proven therapeutic targets. EGFR and c-Myc are associated not only with a low survival rate but also with disease progression among others in metastatic pancreatic cancer therefore, we chose pancreatic cancer as a challenge and cancer type in our studies, and the human pancreatic cancer cell line PANC-1 as an in vitro model.



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

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

ACKNOWLEDGMENTS

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

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

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

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

In summary, our findings highlight the pivotal role of scaffold surface potential in mediating cellular responses and underscore the importance of tailored electrospun

fibre design to accelerate tissue regeneration processes.

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REENGINEERING OF A BACTERIAL COMPARTMENT INTO TAILORABLE BIONANOMATERIALS

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

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

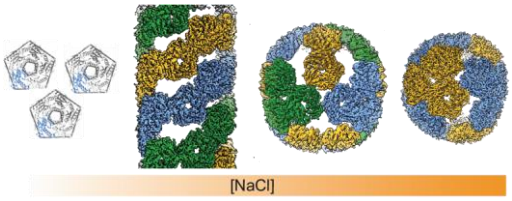

Fig.1. Electron microscopic structures of spherical and tubular assemblies formed by a circularly permuted variant of *Aquifex aeolicus* lumazine synthase. Colors indicate individual threads in the tube or symmetry-related pentamers in the spheres. Transformation between these spheres and tubes occurs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

Our results highlight the potential for broad advancements across biotechnological applications, ranging from bioproduction to nanomedicine. Furthermore, the structural insights gained into the

assembly mechanisms and the role of circular permutation in dictating morphology provide a valuable framework for the rational design of novel nanomaterials with tailored properties.

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

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

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

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

ACKNOWLEDGMENTS

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PEPTIDOOLIPOSOMAL FORMULATIONS FOR ANTIVIRAL THERAPIES

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

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

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

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HOTTER TOGETHER: BOOSTING HYPERTHERMIA EFFICIENCY THROUGH INTER-PARTICLE INTERACTIONS

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

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

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

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

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

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

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

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

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

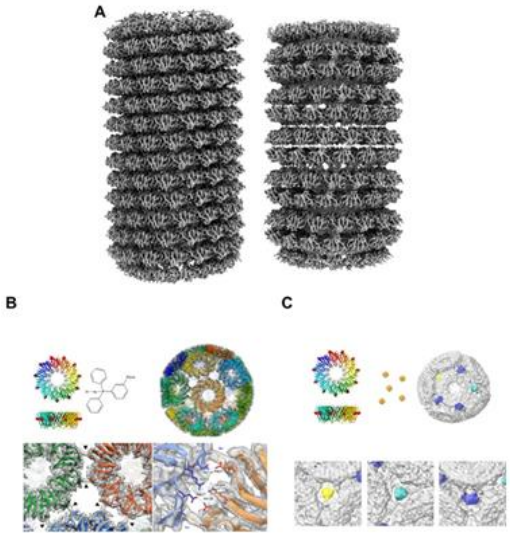

Figure 1. (A) an overview on two polymorphic structures of the modified TMV coat protein assemblies (B) synthetic protein cage made with toroidal-shaped protein and held by Au(I) bridges (C) synthetic protein cage made with toroidal-shaped protein andheld by gold nanoparticles (AuGNPs)

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

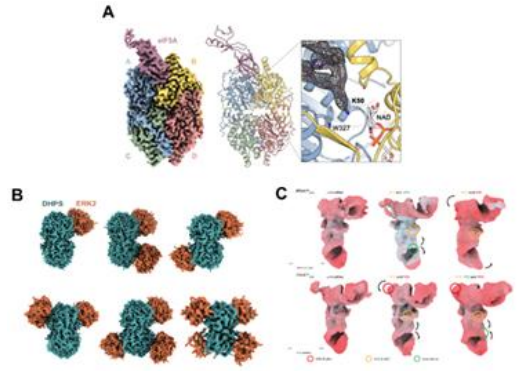

Figure 2. (A) reconstruction of a DHS-eIF5A complex with zoom onto active site (B) different stoichiometry complexes of DHS and its biding partner ERK2 (C) set of structures of free tRNA molecules showing modification sites and thier influence on the flexibility/ridigity

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PH-RESPONSIVE CHLOROPHYLL DERIVATIVES-MODIFIED LIPOSOMES FOR DOXORUBICIN DELIVERY

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

introduced to reduce off-target toxicity.

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

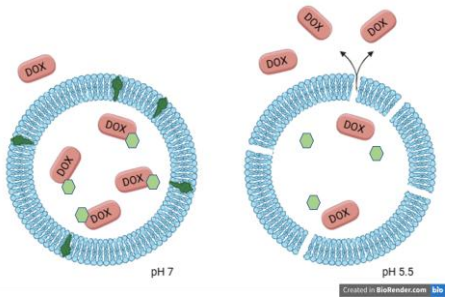

Fig.1. Doxorubicin Release from pH-Sensitive Liposomes Co-Encapsulating Doxorubicin and Chlorophyll Derivatives

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

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

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

ACKNOWLEDGMENTS

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

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

LIPID DROPLETS IN VASCULAR DYSFUNCTION

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

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

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

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

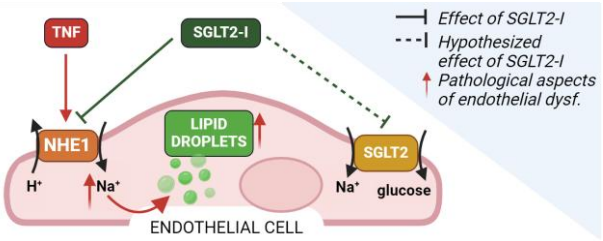

Fig.1. The results showed that empagliflozin's effect on vascular LDs formation was SGLT2-independent in both TNF-induced inflammation and OA-induced lipid overload models. Moreover, NHE1 is key for the SGLT2-I action on vascular inflammation and LDs formation but not for reducing LDs during lipid overload.

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

ACKNOWLEDGMENTS

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

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In most biosensors, selective binding of analytes by detecting biomolecules occurs at the interface, which is then converted into a detection signal. Antibodies are often applied as detecting molecules due to their ability to specifically bind antigens. IgG is the most commonly used antibody, having a characteristic Y-shaped structure, consisting of a constant Fc domain and two Fab domains containing antigen-binding sites. Due to its

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

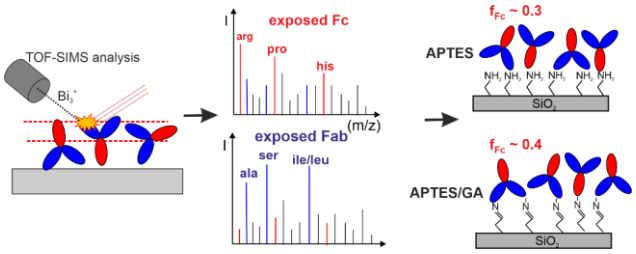

Fig.1. *Application of ToF-SIMS with PCA for analysis of the orientation of IgG molecules on the surface.*

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HEME AND HEME OXYGENASES – NOVEL ACTIVITIES OF OLD FRIENDS

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Heme is an essential yet potentially cytotoxic molecule that plays a central role in cellular metabolism, signaling, and development. While its biosynthesis is crucial for mitochondrial function and regulation of

some transcription factors, excessive or misregulated heme accumulation, especially of intermediates such as protoporphyrin IX (PPIX), can lead to phototoxicity and oxidative stress.

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

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

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

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MIND THE GAP! BIOMOLECULES IN PLASMONIC NANOCAVITY

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

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

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

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

ii) cross-talking amyloid-β and the anti-aggregation drug called bexarotene, which slows down the protein aggregation process via steric effects, largely prohibiting the antiparallel to parallel β-sheet rearrangement [2].
iii) amyloid-β individual aggregates in liquid to improve the TER data quality due to the protective role of solvent, in particular, high heat capacity of liquid reduces the effective temperature of analyte preventing its thermal decomposition [3].
iv) aggregating tau protein for probing the antiparallel to parallel β-sheet rearrangement [4].
v) lipid monolayers for investigating of local molecular distribution, orientation, phase separation, and formation of domains [5].
vi) individual DNA strands to explore nanoscale spectral markers of the Double Strand Breaks formation and DNA conformational transitions

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DEVELOPMENT OF NOVEL OPTICAL MICROSCOPY METHODS TO STUDY CELL MIGRATION

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

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CORRELATIVE AFM–OPTICAL NANOSCOPY FOR POLYPHARMACY STUDIES IN HEPATIC ENDOTHELIUM

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

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

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

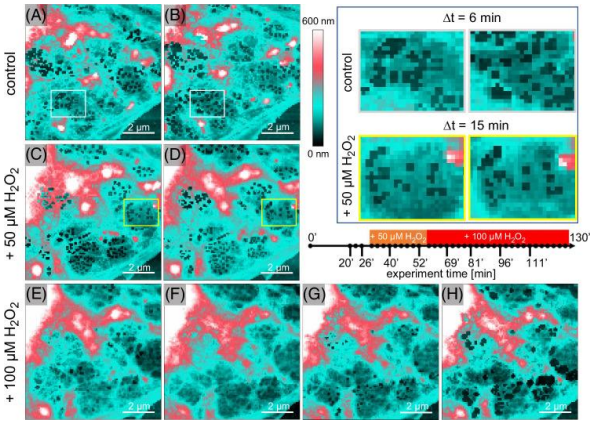

Fig.1. Dynamic defenestration and refenestration in live LSEC treated with hydrogen peroxide. Presented images (A–H) correspond with consecutive time points (20'-111') indicated on a timeline. The whole 130 min long experiment is presented in [5].

ACKNOWLEDGMENTS

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

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

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

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HOW TISSUE STIFFNESS AFFECTS MICROGLIAL MIGRATION AND MORPHOLOGY

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

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

cells on stiffer substrates exhibited higher speed (μm/h), greater end-to-end displacement, and longer persistence time, indicating enhanced migratory capacity.

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

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

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

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

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

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

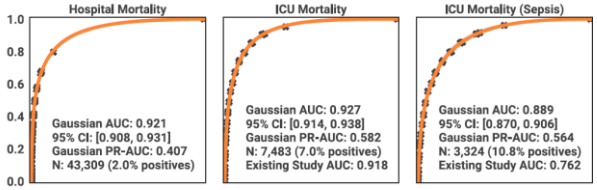

Fig.1. Predictive performance of ETHOS for hospital and ICU mortality outcomes. Receiver Operating Characteristic (ROC) curves for ETHOS predictions on three distinct clinical endpoints: (left) all-cause hospital mortality, (middle) ICU mortality, and (right) ICU mortality in patients with sepsis. For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

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

modeling principles.

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

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PERSPECTIVES OF THE USE OF HEMORHEOLOGICAL TESTS IN MEDICAL DIAGNOSTICS.

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Introduction: Rheology – the science of the flow of matter and the accompanying phenomena of deformation of real bodies – in relation to biological material it is called biorheology, and in relation to blood – hemorheology. Blood flow through blood vessels is a very complex phenomenon due to the physical and physicochemical properties of blood and the structure and properties of the circulatory system. The rheological characteristics of each material depend mainly on two parameters: viscosity and elasticity. Viscosity is a parameter defining the material's resistance to flow, and elasticity expresses the material's resistance to deformation. Hemorheological tests are based primarily on measurements of whole blood viscosity. The parameters determining the whole blood viscosity are: plasma viscosity, hematocrit, deformability and aggregability of red blood cells. Purpose: This paper presents the results of our own research and a review of literature studies indicating that disorders of whole blood viscosity and plasma viscosity may be an indication for expanding diagnostic tests. Methods: Hemorheological tests are performed "in vitro". Blood is collected in the presence of an anticoagulant (EDTA). Measurements of whole blood viscosity as a function of shear rate are performed using rotational rheometers. Since blood plasma is a Newtonian fluid, its viscosity can be measured using both rotational and capillary viscometers. Additional information about blood rheology can be obtained from non-viscometric oscillatory measurements, also known as dynamic mechanical analysis (DMA). The principle of the oscillatory technique is to determine the amplitude and phase of oscillations of the tested sample subjected to the action of a harmonic force with a controlled amplitude and frequency. The measurement performed using the oscillatory method provides information about the viscoelastic properties of the liquid - two components of the complex blood viscosity. The determination of the aggregability and deformability of blood cells is performed directly using aggregometers and appropriate filters. The deformability of erythrocytes and their ability to orient themselves in the flow are estimated based on measurements of the flow time through capillaries or analysis of the diffraction pattern of transmitted light. Information on the ability of erythrocytes to aggregate and deform can be obtained by mathematical analysis of flow curves based on rheological models containing parameters related to the properties of these erythrocytes. Many models describing fluid flow can be found in the literature. The most commonly used model of the flow curve in hemorheology is the model proposed by Quemada in the late eighties. The advantage of this model is the fact that it was formulated for substances with properties identical to those observed in whole blood, i.e. for a concentrated suspension of particles that can aggregate and does not show the existence of a limiting shear stress. The model takes into account the variability of the shape of blood cells and the formation of aggregates - in both cases due to the change in the maximum packing density. The influence of the specific behavior of erythrocytes is most important in blood flow in the microcirculation. Results: Hemorheological factors such as whole blood viscosity, plasma viscosity, hematocrit, white blood cell count, fibrinogen, lipids and lipoproteins affect blood flow in both macrovessels and microvessels and are strongly associated with incidental cardiovascular events. Increased whole blood viscosity is observed in some neoplastic diseases despite low hematocrit. In the acute phase of stroke, high plasma viscosity and increased ability of erythrocytes to aggregate are observed with simultaneous increase in erythrocyte stiffness. In the case of diabetes, increased viscosity and aggregability of erythrocytes are observed.

Studies of correlations between the thermographic image of blood flow in the upper and lower limbs with blood viscosity tests allowed for detection of the first circulatory disorders. In COVID patients elevated blood viscosity was correlated with increased patients mortality. Conclusions: The analysis of changes in physicochemical properties of blood conducted in this study shows how important the hemorheological factor can be in diagnostics. Many aspects of hemorheological functioning of a living organism are not yet known. Measurement techniques are constantly being improved, becoming more accurate, which should enable better use of hemorheological measurements for diagnostics and therapy in the future.

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

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

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

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

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

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

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Background: Perivascular adipose tissue (PVAT) is essential in controlling vascular function. Our previous results[1,2] of short high-fat diet (HFD)-induced obesity indicated significant changes in PVAT lipid composition accompanied by endothelial dysfunction only in the

abdominal aorta (AA). In contrast, the thoracic aorta (TA) with brown-like PVAT remained unchanged. Prolonged (8 weeks) HFD feeding causes insulin resistance, endothelial dysfunction in both TA and AA and alters PVAT, but these changes can be restored by HFD replacement with a normal diet.

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

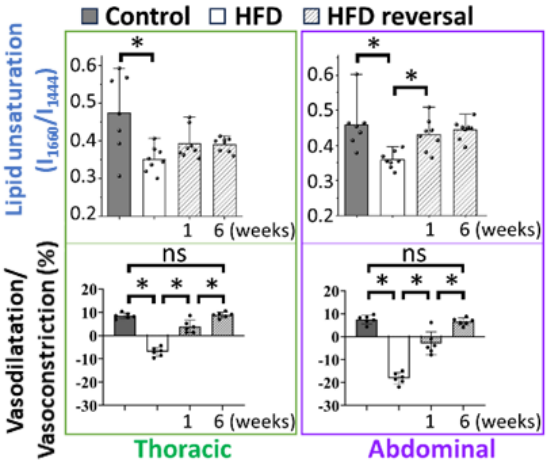

Fig.1. Differential response in aorta in HFD reversal studied by Raman and MRI. Endothelial dysfunction is partially reversed after one week in TA and at least six weeks are needed for full recovery after HFD. Lipid unsaturation fully recovered early and only in AA.

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

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

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

Carbonyl compounds promote oxidative stress by modifying essential biomolecules, forming adducts with proteins and nucleic acids (carbonyl stress), and depleting intracellular glutathione (GSH). Among the amino acids, the most likely to bind to these toxins are the thiol groups of cysteine, the amino groups of lysine or arginine, and the imidazole groups of histidine. Protein modifications can lead to a cascade of effects,

including cellular dysfunction, damage to organelles, and ultimately, cell death. Another mechanism of toxicity of both aldehydes involves the induction of the production of ROS in cells, which results in a disturbance of the redox balance in cells and the induction of oxidative stress.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

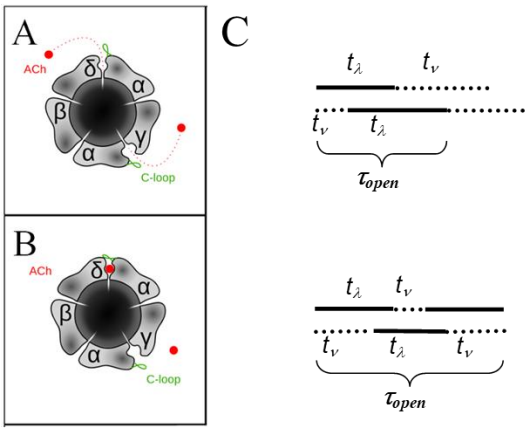

Fig.1. Schematic presentation of nACh receptor in the closed (A) and partially desensitized (B) configurations and gating mechanism of fully functional receptor (C). The open time (τ_{open}) is determined according to the rule: the channel opens at the moment of the first agonist binding and closes at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

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

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STRUCTURE-FUNCTION RELATIONSHIP OF THE GABA TYPE A RECEPTOR.

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

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

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

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

supports the allosteric character of the receptor.

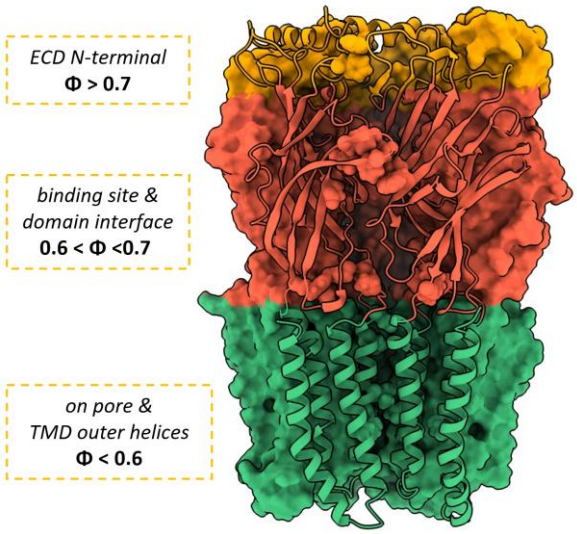

Fig.1. Φ-value map of the $GABA_AR$.

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

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

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

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

Table 2. The values of open state probability (p_{op}) of the BK channels in U87-MG glioblastoma cells at membrane potential of 50 mV at different concentrations of 17β-estradiol (E2) and progesterone (P). The Δp_{op} is given as standard error.

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

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

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

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

processes.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Methods Primary human nasal and bronchial epithelial cells from people with CF (pwCF) carrying at least one F508del-CFTR allele were treated with Elexacaftor/Tezacaftor/Ivacaftor (ETI), both alone and in combination with TNF-α and IL-17 to model an

inflammatory microenvironment. CFTR-mediated transepithelial bicarbonate (HCO_3^-) and chloride (Cl^-) transport was assessed by using short-circuit current (Isc) measurements in Cl^--free and HCO_3^--free buffer systems, respectively.

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

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

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METALLIC NANOPARTICLES AS POTENTIAL MODULATORS OF ANTICANCER THERAPY

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

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

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

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

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

Summing up, the results of our research indicate direct interactions between most of anticancer drugs and metallic nanoparticles. Furthermore, observed

interactions affect biological activity of the drugs increasing their anticancer potential and selectivity. Similar effect was observed in case of cisplatin, where no direct interactions were confirmed.

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PROTEINS: NEW AVENUES FOR THE DESIGN OF OPTICAL BIOSENSORS

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

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

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

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

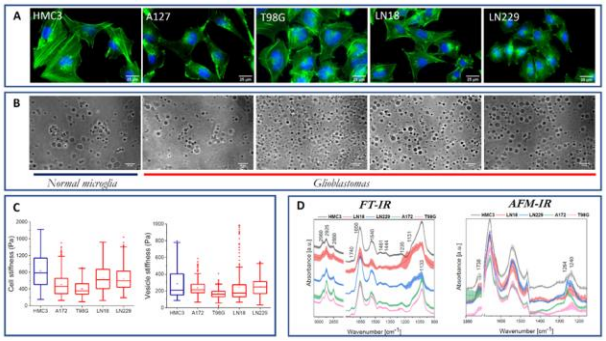

Fig.1. (A) Fluorescence images of normal microglia (HMC3) and glioblastoma cell lines (A172, T98G, LN18, LN229). (B) Optical images of the membrane vesicles isolated from live cells. (C) The mechanical properties of cells and isolated vesicles measured using atomic force microscopy. (D) Averaged FT-IR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

ACKNOWLEDGMENTS

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

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

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

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

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

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

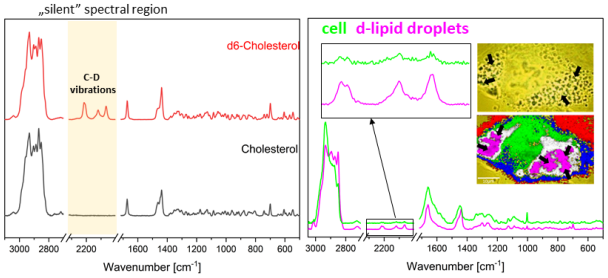

Fig.1.Comparison of Raman spectral profiles of cholesterol and its deuterated analogue (left panel). Cluster analysis of a cell treated with 50 μM d_6-cholesterol reveals regions of deuterated lipid accumulation (pink), confirming the presence of C–D vibrational bands identified in the collected spectra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

ACKNOWLEDGMENTS

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

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

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

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

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Fluorescent proteins (FPs) are nowadays widely used in a variety of spectroscopic methods, especially as biological markers for *in vitro* and *in vivo* imaging. [1,2]. One of the interesting applications is monitoring changes in the anisotropy of the green fluorescent protein (GFP) fluorescence in cells and tissues [3,4]

All FPs share common features: the cylindrical form, composition of 11 β-sheets, an α-helical segment buried inside the barrel and the fluorescent chromophore formed autocatalytically from 3 amino acids. [5]

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

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

We performed comparative studies on EGFP (F64L/S65T-GFP) fluorescence properties in different environment – from various solutions to molecule entrapped in the poly (vinyl alcohol) (PVA) film (Table 1).

In contrast to small organic fluorescent molecules stiffened in polymer matrices [6], the immobilisation of EGFP in the PVA film results in a shorter fluorescence lifetime and rotational correlation time (j), as well as lower initial anisotropy (R_0). Interestingly, increasing the viscosity of the solution does not affect any of EGFP fluorescence properties.

We suggest that the fast anisotropy decay in PVA film is due to an increase in the mobility of the EGFP chromophore inside the protein after rearrangement of hydrogen bonds during PVA drying.

These findings shed light on the role and importance of structural water in GFP. The revealed unique fluorescent properties of GFP may be used to the development of novel applications in its use as a molecular marker.

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

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One of the main goals of cell engineering is to develop advanced, three-dimensional (3D) cell and tissue models that mimic tissue physiology in vivo [1]. Thanks to the

use of microsystems, it is possible to mimic the spatial growth of cells, the complex composition of the extracellular matrix or control intercellular interactions in laboratory conditions.

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

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

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

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ELECTROCHEMICAL BIOSENSORS FOR MULTIPLE BIOMARKERS DETECTION

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

were estimated to be 0.2 ng mL^{-1} for LDL and 0.1 ng mL^{-1} for MDA-LDL.

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ADVANCED MICROFLUIDIC STRATEGIES FOR DROPLET HANDLING AND BIOMEDICAL APPLICATIONS

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

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

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

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

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

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

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ELECTROCHEMICAL RNA-BASED APTASENSOR FOR NEOMYCIN DETECTION IN MILK SAMPLES

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

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

Our findings demonstrate that the one-step co-deposition of APT and c-DTMs leads to the highest sensor efficiency for neomycin detection. Among the tested c-DTMs, 4-mercaptophenol provided the most effective reduction of nonspecific interactions, thereby improving sensor selectivity. The aptasensor's performance was assessed by monitoring changes in electron transfer resistance upon neomycin binding, recorded using EIS in the presence of $Fe(CN)_6^{3-/4}$ redox

couples. The developed aptasensor exhibited high sensitivity, achieving a low detection limit of 36.3 nM in a 10-fold diluted cow's milk sample. Moreover, it demonstrated excellent selectivity for neomycin, effectively distinguishing it from structurally similar aminoglycosides (kanamycin and streptomycin) as well as tetracycline antibiotics (tetracycline and oxytetracycline).

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

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

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

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

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

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

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

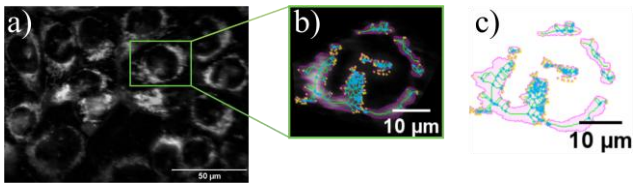

Fig.2. Image processing: (a) Image of undifferentiated SH-SY5Y cells. The mitochondria were stained with BioTracker 488 Green Mitochondria Dye. (b) Skeletal image of the mitochondrial network in a single cell. (c) Binary representation, where pixels are represented as either containing a signal or being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

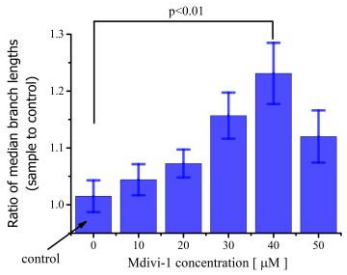

Fig.3. The effect of different concentrations of Mdivi-1 on the change in the ratio of median branch lengths (sample to control) was investigated. Measurements were taken after differentiation and administration of the Parkinsonian toxin MPP+ (control). Statistics were collected from three independent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

ACKNOWLEDGMENTS

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

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

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

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

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Mitochondrial ATP-sensitive potassium channels (mitoK_{ATP}), first described over three decades ago, seem to play an important role in cardioprotection. Yet, despite extensive efforts, their molecular identity remains unresolved. Initial mitoplast patch-clamp recordings revealed a K^+ conductance inhibited by matrix ATP and glibenclamide, suggesting a

mitochondrial counterpart of sarcolemmal K_{ATP} channels [1] . This led to the hypothesis that mitoK_{ATP} comprises a Kir6.x-type pore-forming subunit and a sulfonylurea receptor (SUR2A). However, knockout of Kir6.1/6.2 failed to eliminate mitochondrial K^+ fluxes, prompting a search for alternative candidates.

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

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

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

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

ACKNOWLEDGMENTS

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

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

Platelet activation is an energy-demanding process that relies on dynamically regulated metabolic pathways, with energy derived from both glycolysis and mitochondrial oxidative phosphorylation (OXPHOS). Such bioenergetic plasticity allows rapid functional responses and can contribute to platelet hyperreactivity in metabolic disorders, often limiting the efficacy of antiplatelet therapies. In this study, we investigated whether mild interference with platelet energy

metabolism could enhance the inhibitory effect of cangrelor, a $P2Y_{12}$ receptor antagonist that does not directly affect bioenergetic pathways.

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

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

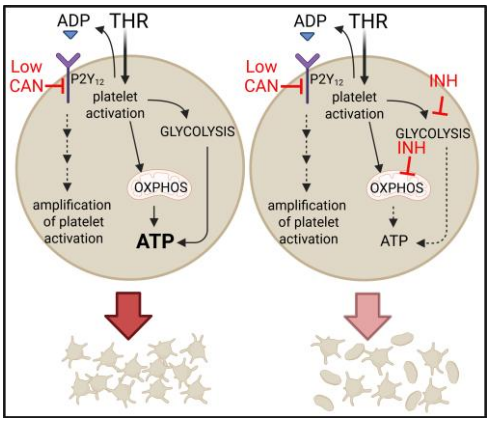

Fig.1. Mild metabolic inhibition enhances cangrelor-induced platelet inhibition (prepared with BioRender).

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

ACKNOWLEDGMENTS

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UNRAVELLING METABOLIC DISRUPTIONS IN MPAN DISEASE – INSIGHTS FROM PATIENTS FIBROBLASTS

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

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

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

ACKNOWLEDGMENTS

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LIGHT-MEDIATED ACTIVATION OF MITOCHONDRIAL BKCA CHANNEL PROTECTS GUINEA PIG CARDIOMYOCYTES AGAINST HYPOXIC INJURY

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Photobiomodulation is a non-invasive medical intervention based on the regulation of biological systems via illumination with infrared light (IRL). The therapeutic potential of IRL includes analgesic, anti-inflammatory, and cytoprotective effects, especially in ischemia-reperfusion processes. One of the proteins that absorbs IRL is cytochrome c oxidase (COX), located in the inner mitochondrial membrane, constituting complex IV of the mitochondrial respiratory chain. In the structure of COX, two copper centres can be distinguished, CuA and CuB, which absorb light from the red and infrared spectral range. The maximal absorption wavelengths change depending on the redox conditions: in an oxidising environment, they are 820 nm and 680 nm for CuA and CuB, respectively; in a reducing environment, they are 620 nm and 760 nm for CuA and CuB, respectively. Previous studies have shown that changes in COX activity affect the opening of the large-conductance Ca2+-activated potassium channel (mitoBKCa) [1]. In turn, it has been shown that stimulation of the mitoBKCa channel, like other mitochondrial potassium ion channels by chemical compounds, has a cytoprotective effect, especially in the case of hypoxia and ischemia-reperfusion injury [2].

As a research model, we have chosen cardiomyocytes isolated from guinea pigs and mitochondria isolated from them. The quality and purity of isolated mitochondria were confirmed by electron microscopy, functional and biochemical analysis. The Western Blot technique confirmed the presence of the α subunit forming the pore of the mitoBKCa channel. Patch-clamp studies revealed the presence of a functional channel with the characteristics of the mitoBKCa channel, including a conductance of about 130 pS, and a voltage dependence and sensitivity to Ca2+. Moreover, inhibition by paxilline (a classical inhibitor of the BKCa channel) was also observed. The patch-clamp experiments have also shown a regulation of mitoBKCa channel activity by IRL. Illumination with 820 nm light was able to restore mitoBKCa channel activity, which had dropped in response to 300 μM K3[Fe(CN)6]. Moreover, irradiation with 760 nm wavelength reactivated the channel inhibited by reducing agents - ascorbate and TMPD. To investigate the cytoprotective effect of IRL, the cardiomyocytes were subjected to three regimens before hypoxia: glucose deprivation, exposure to infrared light (820 nm), and a combination of both events.

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

ACKNOWLEDGMENTS

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THE ROLE OF PHASE SEPARATION IN REGULATING ANIMAL GENE EXPRESSION

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

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

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

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

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

residues over timescales reaching hundreds of microseconds.

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BACK TO FIRST PRINCIPLES: MODELS OF INTRINSICALLY DISORDERED PROTEIN CONFORMATIONS

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

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

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

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

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

Two such cases will exemplified in the presentation.

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

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

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

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

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

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LOOKING FOR MOLECULAR MECHANISMS OF THE CYTOPROTECTIVE ROLE OF NPAS4

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

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

presentation, we will present attempts to clarify the links between NPAS4 and the development of neurodegenerative diseases.

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

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

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

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

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

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

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DEEP LEARNING FOR MEDICAL DIAGNOSIS

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

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FROM ROSENBLATT'S PERCEPTRON TO JUMPER'S ALPHAFOLD

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

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

When John Jumper and Demis Hassabis presented AlphaFold2, their neural network model for predicting tertiary protein structure [3], the direction of inspiration

had reversed. Their model was built not on biological intuition, but on abstract mathematical constructs such as tensors, attention mechanisms, and the transformer architecture, initially developed for natural language translation. These tools from the world of artificial intelligence were used to address one of the central challenges in biology: protein folding [4].

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

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

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

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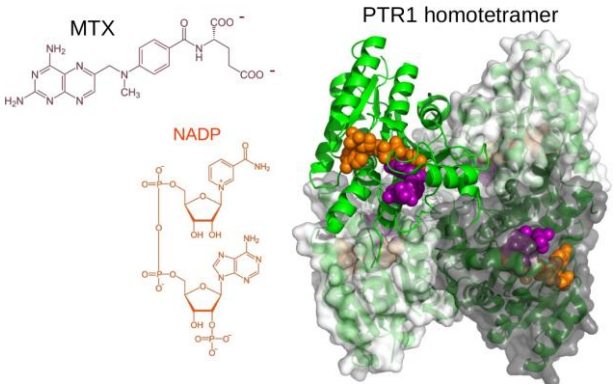

Fig.1. Homotetrameric enzyme pteridine reductase 1 (PTR1) of *Trypanosoma brucei* along with its cofactor NADP and a model antifolate methotrexate (MTX).

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

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

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

further PTR1-targeted drug design.

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

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

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

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

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

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