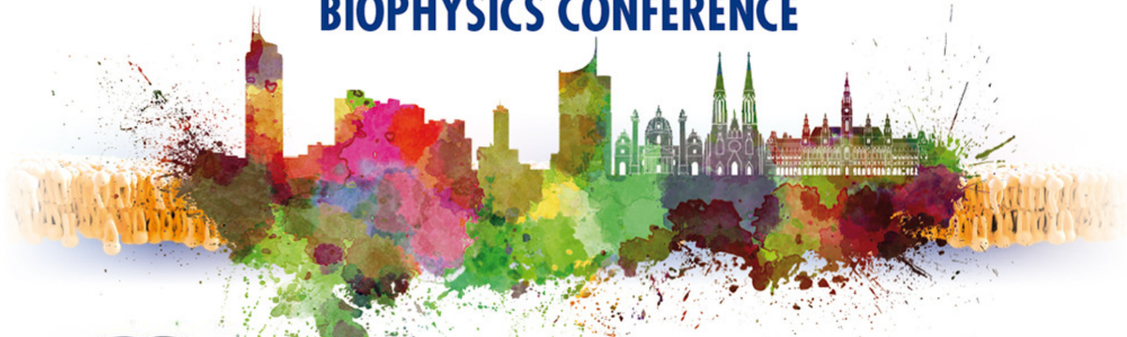

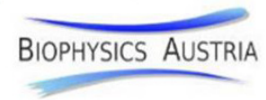

13th EBSA congress

July 24–28, 2021, Vienna, Austria

— *Late Abstracts* —

Oral Presentations

– Late abstracts –

O-168

Cell surface mechanics beyond the bounds of adhesion and tension

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Cell-cell contacts are major determinants of tissue mechanics during morphogenesis and homeostasis. I will show our attempts to measure the mechanics of cell-cell contacts in vivo using different approaches including optical manipulation and high-resolution imaging. I will discuss key aspects of junctional mechanics: the importance of geometry, the time dependence of material properties, and feedback between adhesion and cytoskeletal mechanics.

O-169

Single particle cryo-electron microscopy: A method of choice for studying mRNA translation regulation

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Functions of biomolecules are directly tied to their structures. Solving structures of increasingly large molecules is very challenging, especially when the structures exhibit intrinsic structural heterogeneity. Today, single-particle cryogenic electron microscopy (cryo-EM) became incontestably the way to go in solving the structures of various molecular complexes and nanomachines, especially when the size surpasses several hundred kilo Daltons. Historically, the development of cryo-EM was linked to several molecules such as the ribosomes, which were used to develop, benchmark and test the technique. Indeed, the ribosome provided a sturdy large nano object that is abundant and easy to obtain from most cells. However, it was rather surprising to slowly realize, step by step, the paramount number of different complexes and the incalculable multiplicity of conformations, orientations and shapes that this central molecule can adopt on its long path of mRNA translation into proteins.
Here, I will present few molecular stories from the book of mRNA translation where the technique has proven to be the best storyteller.

O-170

From Ions to Cells

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From the plethora of correlative imaging modalities, SR techniques were most frequently combined with electron microscopy to provide protein-ultrastructure relationships at nanometer-scale resolution. At the other forefront of methods development, scanning probe microscopy techniques aim to capture nanoscale topographical dynamic changes of cells under physiological conditions. To avoid membrane deformation and to provide a method that could unlock long-term monitoring of the biological processes, we recently implemented SICM. The method currently experiences vast leaps in performance due to instrument developments and the ability to fabricate capillaries below tens of nanometers reliably.
In contrast to AFM, SICM is truly non-contact, and represents the soft cell surface much more faithfully. In addition to providing accurate topographic imaging with nanometer resolution, SICM can be used to measure membrane stiffness surface charges and allows local delivery of material (e.g. fluorescent probes). The use of self-blinking dyes in SR microscopy permitted imaging conditions such as low laser excitation intensities and negligible bleaching that are ideal for live-cell imaging. In addition, the high SNR and photophysical properties of self-blinking dyes allow us to extend multiplane cross-correlation analysis to the 4th order using 8-plane volumetric imaging, achieving up to 29 planes. Finally, with a combined SICM-SR setup we demonstrate long-term correlated live-cell imaging.

Abstracts
– Late session –

L-397

Substituent and molecular aggregation-related dual fluorescence effects in the selected 2-amino-1,3,4-thiadiazol derivatives bearing the hydroxypheny

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This study deals with the spectroscopic investigations of *2-amino-1,3,4-thiadiazole* derivatives, namely *2-amino-5-phenyl-1,3,4-thiadiazole* (**TB**), *2-amino-5-(2-hydroxyphenyl)-1,3,4-thiadiazole* (**TS**), and *2-amino-5-(2-hydroxy-5-sulfobenzoyl)-1,3,4-thiadiazole* (**TSF**). The steady fluorescence spectra of **TS** recorded in solvents revealed the presence of dual fluorescence effect. Identical effect was observed upon incorporation of the derivatives mentioned in a model liposomal systems. Depending on the substituent type present at the 5-phenyl ring either two partially overlapped, yet distinct fluorescence bands or only a single emission band was observed. This study revealed that the dual fluorescence effects in TS is most likely associated with the specific molecular aggregation model, which in turn depends on the substituents type and generates the excited state proton transfer processes (ESIPT).
The study reports on the fluorescence effects originating from the presence of the phenolic –OH group, which resides in close proximity to one of the thiadiazole N atoms. The fluorescence effects observed show similarities to those reported for other 1,3,4-thiadiazole analogues and those occurring in liposomal, micellar, and crystal systems. Interestingly, the fluorescence effects mentioned may be relatively easily observed in the molecules tested, which makes the fluorophore systems of 1,3,4-thiadiazole derivatives particularly attractive as potential new fluorescent probes.

L-399

Transient Optoplasmonic Detection of Single Proteins with Sub-Microsecond Resolution

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Optoplasmonic methods capable of single protein detection so far rely on analyte immobilization in order to facilitate detection. These detection schemes, even if they facilitate transient single-molecule detection via consequent formation and cleavage of chemical bonds, typically exhibit time resolutions on the order of milliseconds. The need for analyte immobilisation is a direct consequence of the minuscule dimensions of plasmonic near fields typically providing sub-attolitre-sized detection volumes which in turn demand sub-microsecond temporal resolution for the direct detection of proteins in motion. Here we show that such temporal resolution can indeed be achieved. We demonstrate the observation of single proteins as small as Hemoglobin (molecular weight: 64 kDa) as they traverse plasmonic near fields of gold nanorods and interact with their surface, all while maintaining signal-to-noise ratios larger than 5 and an unprecedented temporal resolution well below microseconds. This method enables the label-free observation of single-molecule dynamics on previously unaccessible timescales.

L-398

SSM-based Electrophysiology meets SGLT1 and GAT1

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We developed functional assays for the human Na^+/GABA and Na^+/glucose co-transporters GAT1 and SGLT1, using solid supported membrane -based electrophysiology (SSME). In conventional electrophysiology voltage steps are used to trigger pre steady-state (PSS) and transport currents, which are commonly recorded in whole cells at a defined holding potential. Transport and PSS electrogenicity in SGLT1 and GAT1 triggered by voltage steps is postulated to be a result from transitions within the substrate-free carriers, e.g. the alternating access of the charged sodium binding sites within the empty carrier. In contrast, SSME utilizes membrane vesicles at 0 mV and the transport cycle is triggered by applying a substrate concentration gradient as the main driving force. Using SSME, we observed substrate-induced PSS currents, likely representing conformational transitions within the substrate-loaded carrier, which are not observed with conventional electrophysiology. We examined the impact of different driving forces on influx, efflux, and PSS currents, focusing on sodium gradients and membrane voltage. We found that internal accumulation of sodium reduces V_{max}, rendering sodium release rate limiting at 0 mV. Application of membrane voltage affected the apparent K_M in SGLT1, but V_{max} in GAT1. We also found that transport properties in influx and efflux modes are highly asymmetric for GAT1, while being more similar for SGLT1.

L-400

Heterotrimeric concatamers of ionotropic P2X4 and P2X7 receptors

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P2X4 and P2X7 receptors are cation channels activated by binding ATP released from damaged and inflamed tissue and are involved in inflammation and pain sensation. Whether P2X4 and P2X7 receptors assemble exclusively as homotrimers or also as P2X4-P2X7 heterotrimers is controversial. To clarify this, we cloned concatamers encoding heterotrimers of P2X4 and/or P2X7 subunits in permutated arrangement. We expressed these concatamers in *X. laevis* oocytes and investigated their function by recording ATP-induced whole cell currents using TEVC. We also visualized their biochemical expression. Heterotrimeric P2X4-P2X7 concatamers have ATP binding site affinities intermediate between those of non-concatenated P2X4 and P2X7 receptors, which have high and low ATP affinity, respectively. The pKd values for ATP depended on the order of the P2X4 and P2X7 subunits in the heterotrimer. Whole-cell currents of non-concatenated P2X4 receptors inactivate, whereas non-concatenated P2X7 receptors show slowly increasing currents upon longer ATP application. Heterotrimeric concatamers showed current kinetics in between the P2X4 and P2X7 phenotype, also depending on the P2X4-P2X7 subunit order. We conclude that P2X4 and P2X7 heterotrimers are functional, with their ATP dependence and current kinetics depending on the specific order of the P2X4-P2X7 subunits.

Abstracts
– Late session –

L-401

Mechanism-based Chronic Inflammation Prediction for Inhaled Particles

Aleksandar Sebastijanovic [1,3], Carola Voss [2,3], Iztok Urbancic [1], Janja Bozic [3], Daniel Svensek [3], Tilen Koklic [1,3], Janez Strancar [1,3]
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We are daily exposed to a multitude of health hazardous airborne particulate matter with notable deposition in the fragile alveolar region of our lungs. Hence, there is a great need for identification and prediction of material-associated diseases, currently hindered due to the lack of in-depth understanding of causal relationships, in particular between acute exposures and chronic symptoms. By applying advanced microscopies and omics to *in vitro* and *in vivo* systems, together with *in silico* molecular modelling, we have here discovered new molecular events such as nanomaterial quarantining and nanomaterial cycling between different lung cell types that determine the long-lasting response to a single nanomaterial exposure. This new insight allows us to predict the spectrum of lung inflammation associated with materials of interest using only *in vitro* measurements and *in silico* modelling potentially relating outcomes to material properties for large number of materials. Because of its profound implications for animal-free predictive toxicology, our work, published in Advanced Materials recently (`https://doi.org/10.1002/adma.202003913`), paves the way to a more efficient and hazard-free introduction of numerous new advanced materials into our lives.

L-402

Azobenzene as light-inducible switch in vesicles, nanodiscs and solvents

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Controlling of physiological membrane properties is an auspicious approach towards the treatment of various illnesses, e.g. Alzheimer disease, which is assumed to be strongly affected by the membrane thickness. Azobenzene (AB) decorated lipids are a strategy to manipulate membranes through the AB trans-cis-isomerization.
Here we investigate the light induced switching mechanism via femtosecond UV/Vis transient absorption (TA) spectroscopy on 4-butyl-azo-4:0-acid-1 (trans-FAAzo-4) and 18:0-azo-phosphatidylcholin (trans-azo PC), in unilamellar phosphatidylcholine (POPC) vesicles, diisobutylene/maleic acid lipid particles (POPC-DIBMALPs) and in solvents.
Our TA measurements yield indications for the assumed isomerization reaction, as well as significant differences between solvent and membrane reaction dynamics. All systems reveal ultrafast excited state dynamics (≤ 1 ps) for both solvent and lipid environment, but a signifcant difference in long time behavior (3 - 30 ps) in the later.

L-403

O to bR transition in bacteriorhodopsin occurs through a proton hole mechanism

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Extensive classical and QM/MM molecular dynamics simulations are used to establish the structural features of the O state in bacteriorhodopsin and its conversion back to the bR ground state. The computed free energy surface is consistent with available experimental data for the kinetics and thermodynamics of the O to bR transition. The simulation results highlight the importance of the proton release group (PRG, consisting of Glu194/204) and the conserved arginine 82 in modulating the hydration level of the protein cavity. In particular, in the O state, deprotonation of the PRG and downward rotation of Arg82 lead to elevated hydration level and a continuous water network that connects the PRG to the protonated Asp85. Proton exchange through this water network is shown by ~ 0.1 μs semiempirical QM/MM free energy simulations to occur through the generation and propagation of a proton hole, which is relayed by Asp212 and stabilized by Arg82. This mechanism provides an explanation for the observation that the D85S mutant of bacteriorhodopsin pumps chloride ions. The electrostatics–hydration coupling mechanism and the involvement of all titration states of water are likely applicable to many biomolecules involved in bioenergetic transduction.

L-404

On the permeation pathway of glycine-gated receptors

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Glycine receptors (GlyR), alongside with $GABA_A$ receptors, are key players of the fast-inhibitory communication in the central nervous system. Their dysfunction is linked to numerous diseases such as hyperekplexia and epileptic disorders. Cryo-EM or X-ray atomic structures have allowed solving several conformations of these pentameric ligand-gated ion channels (pLGICs). Nonetheless, the mechanism of chloride permeation as well as the molecular determinants for asymmetric conductance and rectification evidenced by single-channel electrophysiology have remained elusive. By combining computational electrophysiology based on all-atom molecular dynamics and single-channel measurements in HEK293 cells, we show that the main ion translocation pathway proceeds through unanticipated lateral fenestrations between subunits in the extracellular domain. Mutations of the lateral portals yield strongly rectifying channels endowed with a marked decrease in inward conductance of chloride ions. The latter demonstrates a central role of such lateral fenestration in the function of these important receptors.

Abstracts
– Late session –

L-405

Conformational changes of the alpha-1 glycine receptor studied by Voltage-Clamp Fluorometry

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The alpha-1 homomeric glycine receptors (alpha-1GlyR) is a pentameric ligand-gated ion channel playing an essential role in the synaptic transmission in the nervous system. It is composed of an extracellular domain binding the neurotransmitter and a transmembrane domain forming the ion channel. To study the allosteric pathway of activation, we used voltage-clamp fluorometry that allows measuring concomitantly receptor activation by electrophysiology and conformational changes by fluorescence. We labelled the receptor with the fluorophore TAMRA (through reaction with an engineered cysteine), and introduced nearby the quenching residue Trp to generate fluorophore-quenching pairs reporting on the change in distance between the two positions. Mutational screening allows identification of a pair at the interface between the extracellular and transmembrane domain that shows a robust change in fluorescence upon agonist application. Systematic investigation of antagonists (strychnine), partial agonists (beta-alanine) and allosteric mutations shows that the pair reports on an early motion preceding channel opening. Data are discussed in line of the various cryo-EM and X-ray structures of the receptor revealing several distinct allosteric conformations.

L-406

Peptide Sequence Recognition by Nanopores

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Protein sequencing has been an important goal in proteomics and diagnostics for decades. Current techniques rely on cost intensive large-scale equipment, such as HPLC coupled to mass-spectrometry and subsequent *in-silico* big-data-analysis. These approaches are also time consuming and error prone. Here, we present a novel approach for peptide sequence recognition as a major step towards sequencing in a derivatization-free single molecule experiment using the wt-aerolysin (wt-AeL) nanopore. We follow a bottom-up peptide ladder strategy, which we validate using six different peptide ladder-like sample pools. Each pool is based on a hetero-deca-peptide consisting of a scrambled heptameric sequence of 5 amino acids (S,R,K,Y,A) followed by a C-terminal tri-arginine carrier and –in addition to the full length peptide– contains six fragments shortened by one to six amino acids starting at the N-terminus. We show that using this strategy, sequences can be identified on the basis of resistive pulse analysis using the wt-AeL pore. We also provide evidence that the influence of a single N-terminal amino-acid (aa) removal/addition on resistive pulse depth is sensitive to both its species and that of its nearest neighbor, thus opening an avenue towards peptide sequencing by nanopore.

L-407

In vitro investigation of the voltage-gated prokaryotic sodium channel NavMs

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Structural investigations and molecular dynamics simulations suggest that the bacterial sodium selective channel NavMs differs from potassium channels in its selectivity and transport mode. Instead of channeling water molecules and ions in a single file fashion through the selectivity filter, NavMs allows water molecules to overtake ions and other water molecules *in silico*. Here we wanted to establish an experimental system to test this prediction. First, we expressed the channel in E. coli and transformed the bacterial cells into spheroplasts. Yet, the abundantly overexpressed sodium channel did not insert into the plasma membrane but preferentially adopted a near membrane localization - as revealed by confocal fluorescence microscopy. Fluorescence correlation spectroscopy confirmed this conclusion by showing an exceedingly high NavMs mobility. Next, we purified the overexpressed channel from E. coli cells and reconstituted it into lipid vesicles. Electrophysiological single-channel recordings obtained after fusing proteoliposomes to planar lipid bilayers confirmed NavMs' functionality. Measurements of scattered light intensity in conjunction with fluorescence correlation spectroscopy experiments determine single-channel water flow in the absence and presence of luminal ions.

L-408

It's in the details: Structural diversity in aquaporins defines their stability, oligomerization and selectivity

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Aquaporins (AQPs) are passive membrane-bound facilitators expressed in all kingdoms of life. Each protomer in the homotetrameric complex can facilitate water and partially other neutral solutes selectively and at extraordinary high rates. As AQPs are expressed in virtually all living cells, they are involved in a wide range of physiological functions. This renders them interesting drug targets and potential candidates addressing drought resistance in plants via genetic engineering. Furthermore, aquaporins are subjects of intense research concerning water filtration and as templates for the design of artificial water channels.
While an ideal water channel must withstand adverse physical and chemical conditions, it needs to maintain high permeability and selectivity. Before the AQP scaffold can be altered significantly to uncover novel aspects of AQP stability and selectivity, its structural peculiarities need to be examined and understood.
We subjected all 20, non-redundant AQP structures to a vigorous evaluation to provide a copious overview of the differences in scaffold and amino acid distribution, focusing on monomer/tetramer stability, pore geometry and channel functionality. The analysis will guide to engineer an improved AQP water channel, suitable for filter membranes.

Abstracts
– Late session –

L-409

The effect of membrane composition on proton migration along the membrane-water interface

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Protons are essential for many transport events, e.g., for the uptake of cations, sugars, amino acids, vitamins, as well as for bioenergetics, e.g., ATP synthesis. Their ability to migrate along the membrane surface, i.e., between a proton source and a proton sink, is often crucial for the efficiency of these processes. Interfacial diffusion seldom involves hopping between titratable residues at the membrane surface since H^+ release may be too slow[1]. We now monitored diffusion along lipid bilayers of different compositions to test the prevalence of entropy for H^+ membrane affinity[2]. We used a caged compound for H^+ release from a several micrometer wide spot of a free-standing planar bilayer and recorded H^+ arrival in a distant membrane patch. Changing enthalpic H^+-lipid interactions by introducing negative or positive charges to the membrane surface merely delayed or accelerated H^+ surface to bulk release, respectively, but did not abolish interfacial H^+ diffusion. In addition, we monitored the effect changes in membrane stiffness exert on H^+ membrane interactions. Our results agree with an interfacial entropic H^+ trap that opposes irreversible H^+ surface to bulk release.

1.Springer, et al. Proc. Natl. Acad. Sci. U.S.A, 2011, 108, 14461-14466.
2.Weichselbaum, et al. Scientific Reports, 2017, 7, 4553.

L-410

Scattering versus fluorescence self-quenching: more than a question of faith for the quantification of water flux in large unilamellar vesicles?

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Water homeostasis is fundamental to all forms of life and plays a major role in human health and disease, plant growth and bacterial survival. The permeation of water is driven by osmotic imbalances of solutes and occurs in either unfacilitated fashion through the cell membrane or in a facilitated one through membrane spanning protein channels. To quantify the effect of the lipid or polymer membrane on channel function, to expedite the development of specific water flow inhibitors or facilitate the design of artificial water channels and aquaporins, accurate ways of determining water permeabilities (P_f) are necessary. A commonly used method is to reconstitute membrane channels into large unilamellar vesicles (LUVs) and to subject these vesicles to an osmotic gradient in a stopped-flow device. Fast recordings of either scattered light intensity or fluorescence self-quenching signals are taken as a readout for vesicle volume change, which in turn can be recalculated to P_f values. By means of computational and experimental data, we extensively discuss advantages and disadvantages of using scattering and self-quenching experiments. We for the first time thoroughly assess the effect of LUVs size distribution and remaining detergent after protein reconstitution on P_f values. Furthermore, we demonstrate the impact of channel distribution between proteoliposomes (PLs) on P_f and the activation energy (E_A) of water permeation, which can be calculated from temperature dependent P_f measurements.

L-411

Molecular motors from a 3D perspective: motion and torque generation of kinesins

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Kinesin motor proteins organize the mitotic spindle by crosslinking and sliding microtubules, which orchestrates the complex process of cell division. Some kinesins, such as kinesin-5 and kinesin-14, not only move straight along a microtubule, but exhibit a lateral stepping component. This results in a sideward motion, which has not been fully investigated so far due to technical limitations. Here we explore how kinesin-5 and kinesin-14 slide microtubules and estimate the rotational forces (torques), that the motors produce. Using a 3D motility assay, we show that both kinesin-5 and kinesin-14 drive the rotation of short microtubules around long, suspended microtubules along helical trajectories. Further, we develop a microtubule coiling assay in which sliding by kineisn-5 and kinesin-14 twists microtubules, indicative of torque generation. A theoretical simulation allows the quantification of the torque from the observed microtubule bending. These results reveal that kinesin-5 and kinesin-14 both rotate microtubules around each other and generate torque. We hypothesize that this behavior serves to organize spindle fibers, to circumnavigate roadblocks and to break symmetry through chirality of the mitotic spindle.

L-412

T-Rec – topology-oriented reconstitution

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The reconstitution of integral membrane proteins (IMP) or membrane protein complexes into a minimalistic system has long been deemed mandatory for unambiguously demonstrating protein function. The preceding overexpression and purification steps crucially depend on the right choice of the host organism and solubilizing detergent. However, the substitution of the detergent for carefully chosen lipids, i.e., reconstitution of the native lipid environment, is often accompanied by a loss of lipid asymmetry and an undesired random IMP orientation. Here we describe a cheap reconstitution method that establishes a unique IMP topology - even though we are missing the natural IMP insertion machinery, i.e., translocons. Therefore, we exploit the same affinity tags we use for IMP purification. Fluorescence correlation spectroscopy serves to control reconstitution efficiency.

Abstracts
– Late session –

L-413

Structural mechanism of GTPase-powered ribosome-tRNA movement

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GTPases are ubiquitous regulators of cell signaling which cycle between their active GTP-bound and inactive GDP-bound forms. Among translational GTPases, elongation factor G (EF-G) stands out, because it generates force to promote the movement of the ribosome along the mRNA at the cost of GTP hydrolysis. The key unresolved question is how GTP hydrolysis drives forward tRNA movement. Here we visualize the GTPase-powered step of ongoing translocation using time-resolved cryo-electron microscopy (cryo-EM). In the EF-G–GDP–Pi state captured on the ribosome prior to translocation, the GTPase switch 1 (sw1) region forms a compact bridge between the small and large ribosomal subunits (SSU and LSU), inducing subunit rotation and twisting of the sarcin-ricin loop (SRL) of the LSU. Remodeling of sw1 upon Pi release loosens the SSU contacts, which triggers a large-scale rigid body rotation of EF-G pivoting at the SRL. The global motion of EF-G relative to the ribosome exerts force to pry apart the SSU head and body domains, resulting in forward movement of the tRNAs on the SSU. The findings demonstrate how an unconventional GTPase cycle synchronizes spontaneous thermal fluctuations of the ribosome into force-generating molecular movement.

L-415

Antibodies against tumor-associated carbohydrate antigens

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Protein glycosylation has received much attention due to its many roles in normal physiological and pathological conditions. Aberrant glycosylation is increasingly being recognized as a hallmark of the epithelial mesenchymal transition. Recently paucimannosides have been definitively added to the list of aberrantly expressed glycans during cancerogenesis.
Paucimannose is the tri-, di-, or monomannosyl structure of *N*-glycans in its unsubstituted form.
IgM antibodies display a higher diversity in their recognition of antigens than the commonly studied IgGs. Through their oligomeric form, they apply cooperative multivalency to bind with a high affinity their antigens. For this reason, carbohydrate antigens are generally recognized by IgM antibodies.
Mannitou is a monoclonal IgM antibody that can specifically bind paucimannose-carrying proteins on human cancer tissues, adult pancreatic stem cells, inflamed mouse pancreata and colorectal cancer cells. Interaction studies of Mannitou antibody with different paucimannosidic glycoproteins gave the necessary insights to better understand its specificity: Mannitou IgM recognizes Man3GlcNAc2 and its a1,6 corefucosylated variant and requires a non-substituted a1,3-linked mannose branch of paucimannose.
We expressed and purified Mannitou in order to prepare complexes with the paucimannosylated receptors for structural biological studies. The upregulation of paucimannosylation under tumorigenic conditions makes Mannitou antibody a promising diagnostic tool to distinguish between cancer and healthy cells.

L-414

CYP2C8 pharmacogenetic risk profiles affecting orthodontic treatment and periodontal remodeling in adverse drug reactions

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Background
Pharmacogenetic polymorphisms in drug metabolizing enzymes as cytochrome P450 monooxygenases affect individual drug exposure. In genomewide association studies, the *CYP2C8* locus has been reported to be associated with bisphosphonate related osteonecrosis of the jaw (ONJ). CYP2C8 activity is determined by a genetic polymorphism leading to decreased metabolism of drugs and endogenous CYP2C8 substrates.
Aim of this study was to elucidate the putative pathomechanism explaining the association between the variant alleles *CYP2C8*2* and **3* causing low CYP2C8 activity, and disturbed remodeling in periodontal fibroblasts (PDL) from patients undergoing orthodontic treatment.
Methods
Assessing the effect of bisphosphonate treatment in PDL as well as in isolated CYP2C8 enzymes expressing *CYP2C8*2* and **3* we studied the effects on H_2O_2 production and lack of substrate oxidation, hence reaction uncoupling.
Results
CYP2C8 activity, enzyme expression and substrate metabolism were detected in human PDL cultures. Zoledronic acid caused ROS production in PDLs, which could partly be due to extrahepatic CYP2C8 metabolism. Enhanced bisphosphonate induced uncoupling of the CYP2C8 enzyme was detected in *CYP2C8*3* resulting in increased H_2O_2 production and lowered amodiaquine oxidation. Substrate (amodiaquine) addition led to decreased H_2O_2 production in isolated CYP2C8 enzymes, but in CYP2C8*3 enzyme, increased H_2O_2 was still detected, especially in presence of inflammatory markers such as arachidonic acid.
Conclusion
ROS production in extrahepatic tissues such as PDLs may further explain periodontal dysfunction of inflammatory processes and alveolar remodeling. *CYP2C8* variants leading to decreased enzyme activity in substrate oxidation may enhance ROS production by reaction uncoupling, contributing to the risk of ONJ.

Abstracts
– Late session –

L-416

Single mechanism of chronic inflammation shared among brain and lungs for inhaled metal-oxides

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Introduction
Chronic diseases such as lung cancer and neurodegenerative diseases develop from chronic inflammation and are associated with the air pollution. We have previously shown that the causal relationship between the chronic inflammation and acute exposure of lung cells to inhaled metal-oxide nanoparticles stems from the interplay of the discovered nanomaterial quarantining and nanomaterial cycling between different lung cell types, driven by the pro-inflammatory secretion profile of involved cells.
Results
After inhalation, particulate matter covers the olfactory epithelia – a bridge to the hippocampus via olfactory nerves. We demonstrate that neurons readily transport TiO2, model metal-oxide nanomaterial, through axonal network over large distances. This establishes nose-brain route where TiO2 either physically disrupts the neural network integrity or gets quarantined through interactions with neurons. Such quarantined material localizes to the spatially narrow section of the cell surface, where it is accessible to the microglia. They endeavour to ingest and destroy invading particles but only manage to degrade biological quarantine, resulting in repeated exposure of bare nanoparticle surface, and their death. Particles get released and the cycle starts over.
Conclusions
Therefore, we hypothesize that onset of chronic inflammation after exposure to metal-oxides in the brain is caused by the same experimentally measurable events as in the lungs. We suggest that nanoparticles that meet criteria of 1) axonal transportability, 2) structural network impairment and,
3) pro-inflammatory signalling should be perceived as potential instigators or aggravators of neurodegenerative diseases.

L-417

Structural mechanisms of muscarinic acetylcholine receptors´ inhibition by low weight molecular compounds

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Hyperactivity of muscarinic acetylcholine receptors (mAChRs) in the smooth muscles of airways leads to a decrease in the airway size and chronic pulmonary hypoventilation that is one of the key causes of asthma, and under adverse conditions leads to chronic obstructive pulmonary disease (COPD) which is the third most deadly disease in the world. The rational discovery/design of new selective drugs against COPD with anti-mAChRs mechanism of action requires a detailed understanding of the structural background and regularities of their interaction with mAChRs. To investigate them the spatial structure of full-size mAChRs M3 and M2 that are the most abundant in the airways smooth muscles was reconstructed. The 7 and 5 regions for specific binding of low-weight molecular compounds were identified on the M3 and M2 surface, respectively. Two of them are known sites of acetylcholine and antagonists binding of the corresponding receptors, the other areas have been identified for the first time and given the peculiarities of their structural organization can be considered as potential sites of allosteric regulation of mAChRs activity. The activity of several compounds predicted *in silico* on the basis of our structural data is suggested in tensometric assays.

L-418

DNA opening during transcription initiation by RNA polymerase II in atomic detail

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RNA polymerase II (RNAPII) is a macro-molecular complex that synthesizes RNA by reading the DNA code, a process called transcription. During the first step of the transcription called “initiation”, the RNAPII opens the double stranded DNA in order to read the DNA code. Since the formation of the DNA transcription bubble remains poorly understood, we used molecular dynamics simulations to provide atomic-level insight into this crucial step of transcription. However, simulating such large-scale, highly non-linear transitions in atomic detail is computationally challenging. Here, by steering the simulation with a combination of (i) guided DNA rotation and (ii) a path collective variable, we obtained for the first time continuous atomic trajectories of the complete DNA opening process. The simulations provide insight into the role of loop dynamics and protein-DNA interactions during DNA opening.

L-419

Rigidity percolation uncovers a structural basis for embryonic tissue phase transitions

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Embryo morphogenesis is impacted by dynamic changes in tissue material properties, which have been proposed to occur via processes akin to phase transitions (PTs). Here, we show that rigidity percolation provides a simple and robust theoretical framework to predict material/structural PTs of embryonic tissues from local cell connectivity. By using percolation theory, combined with directly monitoring dynamic changes in tissue rheology and cell contact mechanics, we demonstrate that the zebrafish blastoderm undergoes a genuine rigidity PT, brought about by a small reduction in adhesion-dependent cell connectivity below a critical value. We quantitatively predict and experimentally verify hallmarks of PTs, including power-law exponents and associated discontinuities of macroscopic observables. Finally, we show that this uniform PT depends on blastoderm cells undergoing meta-synchronous divisions causing random and, consequently, uniform changes in cell connectivity. Collectively, our theoretical and experimental findings reveal the structural basis of material PTs in an organismal context.

Abstracts
– Late session –

L-420

SERS study of glyphosate: Looking for an optimal detection method

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Glyphosate ((N-phosphonomethyl)glycine) is a non-selective, broad-spectrum herbicide. It is an active ingredient in commercial Roundup that is applied very often against weeds. In the 20th century, its use increased dramatically due to the introduction of genetically modified crops resistant to glyphosate which has caused its accumulation in the environment and inedible products. Glyphosate was originally considered to be of low toxicity to humans and animals. However, in recent years a large number of scientific studies have been published on the possible harmful effects of glyphosate (as well as its metabolites and associated compounds) which reveal that this herbicide has a possible association with human carcinogenesis and other chronic diseases, including mental and reproductive behaviours, which is mainly associated with its accumulation in water in the environment. Its use is not yet prohibited, but the question of its monitoring is increasingly important also from the point of view of the EU and its environmental policy. Current European legislation allows the use of glyphosate until December 2022. In this context, it is significantly important and challenging to have a detection method on hand to perform a simple, cost-effective and rapid analysis, also usable *in situ* to monitor and even quantify the application of glyphosate directly in the field. Methods such as GC, HPLC, UV absorption and fluorescence spectroscopies have been used quite frequently and successfully to determine glyphosate even at low concentrations. However, these techniques require a complex cleaning process and multiple derived operating steps, resulting in slow and costly detection and quantification of glyphosate. On the other hand, surface-enhanced Raman spectroscopy (SERS) has already been used as a powerful tool for ultra-sensitive chemical analysis, it does not require sample pre-treatment and there are also portable measurement equipments. With this technique, Raman scattering signal can be enhanced more than a million times through the use of noble metal nanostructures, making it a sufficiently sensitive technique to detect glyphosate molecules. At present, there are several theoretical and experimental studies focused on the Raman and SERS spectra of glyphosate. However, the main drawback of this technique is caused by glyphosate polarity that is too high, so thus, the sensitivity achieved from the SERS experiments is still quite low. Therefore, our main objective is to develop an optimal and simple methodology based on increasing the affinity of the glyphosate molecule towards the surface of the nanostructured metal, and thus, to selectively trap glyphosate and allow its sensitive and selective detection.

Acknowledgement
This work is supported by the Scientific Grant Agencies of the Ministry of the Education of Slovak Republic (APVV-19-0580 and VEGA 1/0557/20) and by the grant of Faculty of Science, P. J. Šafárik University in Košice (VVGS-PF-2021-1770). This work has also been financially supported by MINECO (Project FIS2017-84318-R).

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