(RyR2) function via regulation of both $[Zn^{2+}]_i$ and $[Ca^{2+}]_i$ homeostasis in hyperglycemic cardiomyocytes. We used freshly isolated cardiomyocytes from a rat model of chronic diabetes induced by streptozotocin either treated or untreated with an antioxidant N-acetyl cysteine (NAC; 1.5 mg/kg, daily; for 4 weeks). We used cytosolic Ca^{2+} and Zn^{2+} dyes (Fluo-3AM and FluoZin-3AM, respectively) to measure both Zn^{2+} and Ca^{2+} sparks, their transient changes under electrical or caffeine stimulation, and the resting levels of both $[Zn^{2+}]_i$ and $[Ca^{2+}]_i$ in the loaded cells. We obtained significantly increased resting levels of $[Zn^{2+}]_i$ and $[Ca^{2+}]_i$, impairments in the parameters of both global and transient fluorescence changes in diabetic cardiomyocytes while these were found to be preserved in the *in vivo* and *in vitro* NAC-treated diabetic cardiomyocytes. Free protein-sulfhydryls in isolated diabetic cardiomyocytes were measured to be significantly decreased compared to those of the both controls and NAC-treated diabetics. Furthermore our results have demonstrated that intracellular Zn^{2+} induces marked phosphorylation and dysfunction in the RyR2, while these were preserved with NAC-treatment. Overall, the present data suggest that the changes under hyperglycemia not only in $[Ca^{2+}]_i$ but also $[Zn^{2+}]_i$ play important role in RyR2 function, in part via hyperglycemia-induced increased oxidative stress and depressed antioxidant-defence system in cardiomyocytes. (Supported by TUBITAK-SBAG-109S267&111S042)

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Evaluation of On-Chip Quasi-In Vivo Cardiac Toxicity Assay for Direct Prediction of TdP Occurrence using Closed-Loop-Shaped Cardiomyocyte Network

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Re-entry of excitation in the heart is one of the abnormal phenomena to cause the lethal arrhythmia such as torsade de pointes (TdP) and is thought to be induced by the looped structure of excitation conduction pathway. For adaptable *in vitro* preclinical strategies to evaluate global cardiac safety, an *on-chip* quasi-*in vivo* cardiac toxicity assay for lethal arrhythmia measurement using circuit-shaped cardiomyocyte network model has been developed. The electrocardiogram (ECG)-like field potential data, which includes the information both of the repolarization and of the conductance abnormality, was acquired from the self-convoluted extracellular field potential (FP) profiles of a lined-up cardiomyocyte network on a ring-shaped electrode in an agarose microchamber. When E-4031 (hERG blocker) or Astemizole (TdP positive but false-negative drug traditional *in vitro* assay) were applied to the closed-loop cardiomyocyte network, self-convoluted FP profile of normal beating changed into an abnormal waveform, including early afterdepolarization (EAD) like or TdP-like abnormal waveform at 1 μM in either case (2 out of 8 samples in E-4031 and 8 out of 10 samples in Astemizole), and especially followed by fibrillation-like arrhythmia and arrest in Astemizole. Self-convoluted FP duration prolongation and its fluctuation increase were also observed according to the increase of E-4031 concentration ($188 \pm 21\%$ prolongation of FPD corrected with beating rate and $168 \pm 42\%$ increase of STV at 1 μM, mean ± SE, n=6). The results indicate that the self-convoluted FPs of the ring-shaped cardiomyocyte network assay could be used for quasi-ECG measurement to estimate QT prolongation and arrhythmic potential including the effects on both repolarization process and the conductance abnormality of cardiomyocyte.

Membrane Transporters & Exchangers II

1463-Pos Board B355

Regulation of Ammonium Transport Proteins

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Ammonium, the most reduced form of nitrogen is a primary nutrient for several organisms (archea, bacteria, fungi and plants) and its uptake from the environment is accomplished by dedicated Ammonium Transport (Amt) proteins. In mammals, Amts are crucial for acid-base and pH homeostasis processes in kidney and liver tissues and are generally called Rhesus proteins. Organized as stable trimers, Amt proteins span the membrane with 11 or 12 helices that surround a substrate translocation pore in each of the three monomers [1].
Regulation of assimilatory Amt transport by direct protein-protein interaction involves members of the P(II)-protein family, known as GlnK proteins [2]. GlnK sense the intracellular energy, nitrogen and carbon levels by direct binding effector molecules such as ATP, ADP and 2-oxoglutarate (2-OG) [3-6].
In spite of their highly conserved structure, P(II)-proteins vary strongly in their effector binding kinetics and in the resulting response modulation and interacting partners. The range of downstream target proteins, and the underlying, molecular regulatory processes are still not fully understood [2,7]. We are investigating the role of the three GlnK proteins of the hyperthermophilic euryarchaeon Archaeoglobus fulgidus, each of which is encoded in a transcriptional unit with a distinct amt gene for an ammonium transporter [1,5,6].
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1464-Pos Board B356

Two Distinct Transport Mechanisms in AmtB and RhCG Proteins

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The Amt/Rh family of membrane proteins facilitates the diffusion of ammonium across cellular membranes. Functional data show that Amt proteins, notably found in plants, transport ammonium ion (NH4+) while human rhesus (Rh) proteins transport ammonia (NH3). Comparison between the X-ray structures of the prokaryotic AmtB and the human RhCG reveals important differences at the level of their pore. Despite these important functional and structural differences between Amt and Rh proteins, studies of the AmtB transporter have led to the conclusion that proteins of both sub-families work according to the same mechanism and all transport ammonia. We performed molecular dynamics simulations of the AmtB and RhCG proteins under different conditions. Our free energy calculations suggest that the probability of finding NH3 molecules in the pore of AmtB is negligible in comparison to water and refute a single file diffusion of ammonia in AmtB. The pore lumen of RhCG is found to be more hydrophobic due to the presence of a phenylalanine conserved among Rh proteins. Simulations of RhCG also reveal that one of the signature histidine residues is occasionally exposed to the extra-cellular bulk, which is never observed in AmtB. These quite different hydration patterns are consistent with the idea that permeation in Amt and Rh proteins are not functionally equivalent and take place according to two distinct mechanisms. In both cases, after binding to the histidine dyad, ammonium deprotonates and diffuse down the pore as ammonia. Hydration of the pore in Amt proteins allows proton diffusion, which results in electrogenic transport. Such mechanism cannot take place in Rh proteins due to lack of pore hydration. Our QM/MM simulations rather show that the excess proton is released back to the extracellular bulk through a network of H-bonds, resulting in net NH3 transport.

1465-Pos Board B357

Computational Investigation of Charge Transfer Mechanisms in Ammonium Transporters

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The mechanism by which proteins of the Amt/MEP/Rh family transport ammonium remains largely unknown. No convincing mechanistic picture has emerged yet as to whether it is an electroneutral NH3 transport or an electrogenic $NH3/H^+$ co-transport. using free energy calculations with polarizable force fields and with hybrid QM/MM descriptions, we have investigated the binding of ammonium and its transport across the pore of Escherichia coli's AmtB protein. The simulations reveal a novel cotransport mechanism in which the NH4+ substrate binds deeply into the pore and translocates as separate NH3 and H^+ fragments. Critical to the cotransport mechanism is a pair of highly-conserved histidine residues, that enhance NH4+ binding, form a ''proton wire'' for charge transfer, and provide a scaffold for a water chain in the pore lumen.

1466-Pos Board B358

Multidrug Binding Properties of the AcrB Efflux Pump Characterized by Molecular Dynamics Simulations

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Multidrug resistance in Gram-negative bacteria, to which multidrug efflux pumps such as AcrB makes a major contribution, is becoming a major public health problem. Unfortunately only a few compounds have been cocrystallized with AcrB, and thus computational approaches are essential in elucidating the interaction between diverse ligands and the pump protein. We used molecular dynamics simulation to examine the binding of 9 substrates, 2 inhibitors, and 2

non-substrates to the distal binding pocket of AcrB, identified earlier by X-ray crystallography. This approach gave us more realistic views of the binding than the previously-used docking approach, as the explicit water molecules contributed to the process and the flexible binding site was often seen to undergo large structural changes. We analyzed the interaction in detail in terms of the binding energy, hydrophobic surface matching, and the residues mostly stabilizing the complex. We found that all substrates tested bound to the pocket, whereas the binding to this site was not preferred for the non-substrates. Interestingly, both inhibitors (Phe-Arg-β-naphthylamide and 1-(1-naphtylmethyl)-piperazine) tended to move out of the pocket at least partially, getting into contact with a glycine-rich loop that separates the distal pocket from the more proximal region of the protein and is thought to control the access of substrates to the distal pocket.

1467-Pos Board B359
Coarse-Grained Simulations of the MexAB-OprM Multidrug Resistance Efflux Pump
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Multidrug resistance (MDR) efflux pumps are tripartite membrane protein complexes that confer antibiotic resistance in gram-negative bacteria by active transportation of antibiotic substrates from the periplasmic space to the extracellular milieu. While complete structural characterization of MDR efflux pumps via experimental techniques has remained elusive so far, modeling studies guided by available structural data have hypothesized several possible models of the full tripartite pump assembly. Based on these data, we have assembled several models of the MexAB-OprM efflux pump from Pseudomonas aeruginosa and performed coarse-grained molecular dynamics simulations on these various models in order to further elucidate the mechanisms of pump function and to discern any functional differences that arise in the various models.

1468-Pos Board B360
Functional Investigation of the MexA-MexB-OprM Efflux Pump of Pseudomonas Aeruginosa
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Among the various mechanisms developed by the bacteria to counter to the effect of antibiotics, active efflux is on the front line. In Pseudomonas aeruginosa, a Gram-negative bacteria, efflux transporters are organized as multicomponent systems where MexB, the pump located in the inner membrane, works in conjunction with MexA, a periplasmic protein, and OprM, an outer membrane protein. MexB is a proton motive force-dependent pump with broad substrate specificity.
We describe an original activity assay for MexB and MexA. The pump is co-reconstituted into proteoliposomes together with bacteriorhodopsin (BR), a light-activated proton pump [1]. In this system, upon illumination with visible light, the photo-induced proton gradient created by the BR is shown to be coupled to the active transport of substrates through the pump. This test makes the investigation of the pump possible. In the absence of MexA, MexB has a basal activity which is not substrate-dependent. Once MexB is reconstituted together with MexA, its activity is specific and substrate dependent.
We are working on the reconstitution of the whole efflux pump. To that purpose we reconstitute MexB and OprM in respective proteoliposomes. MexA will be reconstituted either in proteoliposomes (together with MexB) or will be present in the buffer as we have purified a soluble version of this lipid-anchored periplamsic protein. Upon mixing, the tripartite protein complex should form, making it possible to study transport through the whole efflux pump by use of carefully chosen fluorescent reporters.
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1469-Pos Board B361
All-Atom Molecular Dynamics Simulations of Multidrug Efflux Transporter AcrB
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The multidrug transporter AcrB, which forms a part of the tripartite multidrug efflux system (AcrB/AcrA/TolC) in E. coli., actively exports a wide variety of noxious compounds using the proton-motive force as an energy source. Recently, x-ray structures provided that AcrB adopts an asymmetric structure comprising three protomers with different conformations that are sequentially converted during drug export. These cyclic conformational changes for drug export are called the functional rotation. To investigate functional rotation driven by the proton-motive force, a series of all-atom molecular dynamics simulations were performed. Our simulations demonstrate that alternating the protonation states in the transmembrane domain induces functional rotation in the porter domain that is primarily responsible for drug transport.

1470-Pos Board B362
Serotonin and Dopamine Transporter Homology Models Provide a Look Inside the Active Site during Three Stages of Transport
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The central nervous system is controlled by electrochemical impulses propagated through neurotransmitters, whose concentration and length of presence in the synapse determines the duration and the strength of the stimuli. Clearing of the neurotransmitters is the responsibility of specific transporters. The main ones being Serotonin, Dopamine, and Norepinephrine Transporters (SERT, DAT, and NET). As a class of secondary transporters the sodium: neurotransmitter symporters utilize a sodium ion gradient to co-transport a neurotransmitter molecule against its gradient. The coupling of the ions favourable free energy to the unfavourable recycling of the neurotransmitters is the crucial step in deciphering the mechanism of action. Our interests also expand into the substrate interaction with the transporter at the active site. Studying not just the interaction of the protein with the native substrate, but some known inhibitors will provide even more insight into the interactions at the active site. The inhibitors we are interested in are both of pharmaceutical and psychedelic origins. The interesting idea behind looking at both of these is the difference in addictiveness of one over the other. Different inhibitors will also have different modes of action and by exploiting these we can come up with more effective and less addictive drugs for treating many mood and neurodegenerative disorders. The first step in our approach will be to create hSERT and hDAT homology models using LeuT as a template. Each homology models will be modelled in three different states. The open-to-out, open-to-in, and fully occluded states will provide us with the ''snap shots'' during the transport. By analyzing the differences and similarities in ion coupling and substrate/inhibitor interactions clues to the overall mechanism will be obtained.

1471-Pos Board B363
Molecular Dynamics Studies on the GLYT1b Transporter
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We have constructed a homology model of the outward- and inward facing states of the glycine transporter GLYT1b and have modelled the transitions between these with targeted MD. In the inward-facing state we observe Na2, chloride and substrate dissociating from the transporter.

1472-Pos Board B364
Proton-Coupled Transition between Outward-Facing and Inward-Facing States in the Uracil Transporter
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The bacterial nucleobase-ascorbate transporters are proton-driven membrane symporters. The solved structure of the bacterial uracil transporter, which demonstrates an inward-facing open state, is spatially organized into a core domain and a gate domain. Two glutamate residues (Glu241 and Glu290) anchor the substrate uracil in its binding site at the interface between the two domains. However, the outward-facing conformation and the conformational transition between the inward-facing and the outward-facing states are completely unknown. Furthermore, and mechanistically more important, how protons drive this transition remains also elusive. In the present study, we apply molecular dynamics simulations and free energy perturbation (FEP) to address some of these questions. The results of equilibrium simulations show that the empty transporter adopts an outward-facing open conformation through a rigid-body movement of TM5 and TM12. Uracil binding and protonation of Glu241 lead to an occluded state of the transporter. Deprotonation of Glu241 and protonation of His245 (as a result of proton transfer between these side chains) induce the displacement of TM12 from the core domain, exposing the bound uracil to the cytoplasm. Our FEP calculations show that the energy in the uracil bound state with protonated His245 is ~25.9 kcal/mol lower than that with protonated Glu241, suggesting a favorable proton transfer from Glu241 to His245. Lastly, the release of the proton from protonated His245 in the uracil-bound state causes a large separation of TM12 from the core domain, thus allowing the substrate to move out of its binding site and into the cytoplasm. Based on our simulations, we propose that TM5 and TM12 play the role of extracellular and intracellular gates, respectively, and that proton transfer between Glu241 and His245 regulates the conformational transition between the inward-facing and outward-facing states.