to casein proteins for surface passivation. We report preliminary results on the surface passivation performance of lipid molecules and other materials in gliding motility assays. [1] Vivek Verma, William O Hancock, Jeffrey M Catchmark, ''The role of casein in supporting the operation of surface bound kinesin,'' J. Biol. Eng. 2008; 2: 14. PMID: 18937863

1928-Pos

"Popoffs" Under a Transverse Force Reveal the Number and Location of Active Kinesin Motors During Motility Assays

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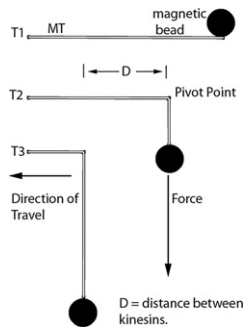
Determination of the number of active motors pulling a single MT or bead during motility assays has proven difficult. Traditional protein concentration assays, such as Bradford, cannot distinguish between active and inactive motors. We attach a superparamagnetic bead to the (+) end of a microtubule. When placed in a magnet with uniform magnetic field gradient, the bead pulls on the MT with a controllable 0-10 pN force. If the force is perpendicular to the gliding direction of the MT, a short section of the MT ''pops off'' the surface every 2 to 5 s, as shown in the diagram. This detachment is characterized by rapid motion of the superparamagnetic bead in the direction of higher magnetic field gradient followed by normal microtubule gliding velocity when the MT is pulled taut. The length of the short section between ''popoffs'' is the distance between active kinesins along a microtubule.



1929-Pos

Calcium Dependent Regulation of Kinesin Function using Binding System of CaM and M13 Peptide

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Kinesin is known as a dimeric motor protein, which carries cellular cargoes along microtubules by hydrolyzing ATP. Calmodulin (CaM) is a calcium binding protein that participates in cellular regulatory processes. CaM undergoes a conformational change upon binding to calcium, which enables it to bind to specific proteins for a specific response. We have previously demonstrated that kinesin fused with CaM at the C-terminal binds reversibly to M13-Qdots in a calcium dependent manner. In this study, we have tried to make the calcium dependent reversible dimerization of kinesin utilizing CaM- target peptide M13 binding system in order to control motility of kinesin. First we have designed and prepared the cDNA of the truncated kinesin (355 amino acids) that does not form dimer. Subsequently we prepared the cDNA encoding two kinesin chimeric proteins in which C-terminal of kinesin355 was fused with calmodulin (kinesin355-CaM) and fused with M13-GFP (Kinesin355-M13-GFP). The cDNAs of the kinesin chimeras were cloned into expression vector pET21a and transformed into E.coli BL21. The kinesin chimeras were successfully expressed and purified by Co-Chelate column. These kinesin chimeras showed normal ATPase activities. Furthermore, K355-CaM bound to M13-YFP in a calcium dependent manner. And the calcium dependent interaction between kinesin355-CaM and kinesin355-M13-GFP was examined.

Membrane Transport

1930-Pos

The Sodium-Glucose Co-Transporter SGLT1 - Could Light Help Prevent Type II Diabetes?

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The sodium-glucose co-transporter SGLT1 is responsible for the active transport of glucose in small intestine and kidney. Consuming food with high degrees of carbohydrates and glucose leads to a temporary, rapid increase of blood glucose levels via the absorption of glucose and galactose through SGLT1. This influences the glucose homeostasis and increases the insulin resistance of peripheral tissue. The subsequent ''glucose-toxicity'' leads to degeneration of beta-cells and, in last consequence, to the generation of type II diabetes. The reduction of oral glucose availability by inhibition of SGLT1 with flavonoids or other ''nutraceuticals'' might be one possibility to prevent type II diabetes.

The transport of glucose via SGLT1 is electrogenic and coupled to the co-transport of sodium ions. Its features are examined using cell-free, solid-supported-membrane-based electrophysiology, namely the SURFE^{2}R technology platform (IonGate Biosciences), where transporter-containing membrane fragments or vesicles are mechanically and electrically coupled to a gold-coated biochip. For SGLT1, it is important to establish a membrane potential prior to substrate application, to enhance the sensitivity of the assay. This potential can be built up via a SO_4/Cl^- gradient across the membrane. The following detectable transport activity is in the range of 300-1000 pA.

To avoid unspecific side effects and to speed up screening, the electrochemical SO_4/Cl^- gradient can be replaced by a light-driven gradient. Therefore, we generated cell lines with light polarizable membranes, where the application of light generates a membrane potential. With this technique it is possible to achieve higher throughput and a better signal-to-noise ratio in drug screening.

1931-Pos

Adaptation of Animals to Different Types of Oxidative Stress: The Role of Mitochondrial Potassium Transport Systems

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We studied parameters of the ATP-dependent influx of potassium into mitochondria, which were isolated from rats varying in their resistance to ischemia and from hypoxia-adapted animals. It has been found that in the heart and liver mitochondria, the rates of the ATP-dependent potassium influx and H_2O_2 production (in case of ATP-inhibited transport) are higher in the hypoxia-resistant rats, as compared to those in the hypoxia-sensitive animals. When adapted to low oxygen, the hypoxia-sensitive rats demonstrated rates of the both processes increasing to the levels observed in the hypoxia-resistant animals. However, the concentration of potassium in the mitochondria of hypoxia-resistant and adapted animals decreased. This indicates that adaptation to hypoxia stimulates not only the influx of potassium into mitochondria, but also K^+/H^+ exchange. The activation of such a potassium cycle can lower the production of ROS, which plays a crucial role in the lethal cell injury associated with cardiac ischemia and reperfusion. It has been further found that uridine and UMP (precursors of UDP, a metabolic activator of mitoK_{ATP}) greatly decreased the index of ischemic alteration upon 60-min acute ischemia, as well as the size of infarction zone under ischemia-reperfusion conditions. The inhibitors of K_{ATP} channels (glibenclamide and 5-HD) reversed the anti-ischemic effect of uridine and UMP. These agents also exerted an anti-arrhythmic effect, which was completely abolished by glibenclamide but not 5-HD. It should be noted that uridine and UMP recovered the levels of ATP, phosphocreatin and glycogen, which were decreased during ischemia, while glibenclamide and 5-HD eliminated these effects. Also demonstrated was the effectiveness of uridine in the reduction of lipopolysaccharide-induced inflammation (another model of oxidative stress).

1932-Pos

Investigation of Proton-Potassium Exchange During Fermentation of Glycerol by Bacteria *escherichia Coli* at Alkaline and Acidic pH

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Production of molecular hydrogen (H_2) by bacteria from a variety of renewable, cheap and abundant carbon sources is a developing new area of technology. Recently it has been shown that bacteria *Escherichia coli* is able to ferment glycerol and produce H_2 via formate hydrogen lyase (FHL) system probably (1). It was demonstrated that in *E. coli* during fermentation of glucose depending of medium pH H_2 produces via two forms of FHL-1 and FHL-2, constituted by formate dehydrogenase H and hydrogenase 3 (H3) or hydrogenase 4 (H4): at alkaline pH FHL-2 was shown to relate with the proton translocating F_0F_1-ATPase and potassium uptake TrkA system (2).

In this study it's shown that at acidic (pH 5.5) and alkaline (pH 7.5) medium in *E. coli* wild typefermenting glycerol protons expelled via F_0F_1-ATPase with low rate compared with the glucose fermentation. The potassium uptake was very low. During fermentation of glycerol at alkaline pH H^+ extrusion was stimulated in *ΔhyfG or ΔfhlA* (with defective H4 or activator of FHL, respectively)and not markedly changed in *ΔhyaB orΔhybC* mutants (with defective H1 or H2). The H^+ extrusion was almost the same in all these mutants at acidic pH. The results indicate that at alkaline pH when F_0F_1-ATPase activity is low H3 or H4 but not other hydrogenases may participate in the H^+ extrusion or have proton translocating ability.

1. *Dharmadi,Y., Murarka, A. and Gonzalez, R.* (2006) Biotechnol. Bioeng. 94, 821-828.
2. *Poladyan, A. and Trchounian, A.* (2009) In Bacterial Membranes. Ed. A. Trchounian, Research Signpost, Kerala (India), pp.197-231.

1933-Pos

Molecular Dynamics Simulations Reveal TolC Flexibility in the AcrB Interface Region

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Over-production of multi-drug efflux pumps is a prominent example of how bacteria gain resistance against antibiotics. In Escherichia coli the AcrA/B-TolC efflux pump is capable to expel a broad range of drugs, using the energy of proton-motive force. The detailed functional mechanism of this efflux system is not fully understood yet. While AcrB is the engine in this system, the outer membrane protein TolC acts as an efflux duct that also interacts with a numerous other inner membrane translocases. TolC occurs in at least two states, one that is impermeable for drugs and one where drug passage is possible. To gain insight into TolC ground state dynamics, we performed a series of 5 independent, unbiased 150ns MD simulations of closed state wild type TolC (PDB ID 1EK9) in a phospholipid/water environment at 0.15M NaCl concentration. Simulations were performed using GROMACS 4.0.3 and G53a6-GROMOS96 force field. While TolC remains closed between a ''bottleneck region'' outlined by Asp-374 &371 and above, we observe opening and closing motions in the AcrB interface region near Gly-365. This local flexibility could be of functional relevance in the AcrB-TolC complex formation. In all simulations the Asp-371&374 aspartate ring region was stable, displaying no fluctuations in the cross-sectional area of the TolC channel. Whereas previous studies found potassium ions to bind frequently, stabilizing a closed TolC conformation in the AcrB interface region, we observe frequent and unhindered passage of sodium ions. However, in one simulation a consecutive binding event of two sodium ions occurs between Gly-365 and Asp-374, stabilizing a similarly closed conformation for more than 15 ns. We introduce a new tool to analyze protein-internal cavities and record pore profiles based on time-averaged water & protein residence probabilities.

1934-Pos

Plant Aquaporins Co-Expression Senses Differentially the Intracelluar pH

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The plant plasma membrane (PM) expresses two types of aquaporins: PIP1 and PIP2. These PIP are characterized by: i- the faculty to reduce water permeation through the pore after cytosolic acidification as a consequence of gating process, ii- the ability to modulate membrane water permeability by co-expression of both types.

We investigated if these functional characteristics of PIP can act together to give a new and relevant modulation response to acidification. To test our hypothesis we used PIP1 and PIP2 from different plant sources (Beta vulgaris roots and Fragaria x ananassa fruits). The experimental approach used was to perform a functional study of PIP by means of the heterologous expression system Xenopus oocytes and analyzed the oocyte PM water permeability coefficient (Pf) when PIP are injected.

Briefly, the Pf was increased ten-fold by PIP2, but it remained low for both control oocytes and PIP1 injected ones. Moreover, when oocytes expressed PIP2, a partial (70%) pH inhibitory response under cytosolic acidification (pH 6) was detected.

When PIP1-PIP2 co-expression was assayed, Pf was enhanced seven-fold in comparison with Pf obtained by PIP2 expression alone. Furthermore, the pH dependent behavior showed that PIP1-PIP2 co-expression accounts for different pH sensitivity by shifting the EC50 of the inhibitory response from pH 6.1 to pH 6.9, compared to PIP2.

Our results show that: i- PIP co-expression impacts on the membrane water permeability not only by modulating the water transport capacity but also the pH regulatory response, improving in this way membrane plasticity, ii- this PIP behavior is not a tissue specific and/or species-dependent response but a more general one.

In conclusion, aquaporin co-expression widens and enhances regulatory properties that control adjustment of water movements which might be of great importance to react to variable osmotic and pH stress.

1935-Pos

Membrane Transport of Hydrogen Sulfide: No Facilitator Required

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Hydrogen sulfide (H_2S) has emerged as a new and important member in the group of gaseous signalling molecules. However, the molecular transport mechanism has not yet been identified. Prediction of its actual membrane permeability, P_M, according to Overton's rule (1) is hampered by the fact that the partition coefficient into the organic phase is not known. Because of structural similarities with H_2O, it was hypothesized that aquaporins may facilitate H_2S transport across cell membranes. We tested this hypothesis by reconstituting the archeal aquaporin AfAQP from sulfide reducing bacteria *Archaeoglobus fulgidus* into planar membranes and by monitoring the resulting facilitation of osmotic water flow and H_2S flux. To measure H_2O and H_2S fluxes, respectively, sodium ion dilution and buffer acidification by proton release were recorded in the immediate membrane vicinity. Both [Na^+] and pH were measured by scanning ion selective microelectrodes. A lower limit of $P_{M,H2S} > 0.5 \pm 0.4$ cm/s was calculated by numerically solving the complete system of differential reaction diffusion equations and fitting the theoretical pH distribution to experimental pH profiles. Even though reconstitution of AfAQP significantly increased water permeability through planar lipid bilayers, $P_{M,H2S}$ remained unchanged. The fact that cholesterol and sphingomyelin reconstitution did not turn these membranes into a H_2S barrier indicates that H_2S transport through epithelial barriers, endothelial barriers and membrane rafts also occurs by simple diffusion and does not require facilitation by membrane channels (2).

1. Missner, A & Pohl, P (2009) 110 years of the Meyer-Overton rule: Predicting membrane permeability of gases and other small compounds ChemPhysChem 10:1405-1414.
2.Mathai, JC, Missner, A, Kügler, P, Saparov, SM, Zeidel, ML, Lee, JK, Pohl, P (2009) No facilitator required for membrane transport of hydrogen sulfide Proc.Natl. Acad. Sci. USA 106:16633-16638.

1936-Pos

Protein Transport Through the Anthrax Toxin Channel: Molecular Mechanisms

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Bacillus anthracis, the causative agent of anthrax, produced a toxin composed of a translocase heptameric channel, $(PA_{63})_7$, which allows its two substrate proteins, lethal and edema factor (LF and EF), to translocate across a host cell's endosomal membrane, disrupting the cell's normal function. Protein translocation through the channel, reconstituted in lipid bilayers, is driven (N-terminal end first) by a proton electrochemical potential gradient. The $(PA_{63})_7$ channel strongly disfavors the entry of negatively charged residues on proteins, and hence the acidic side chains on LF_N (the N-terminal 263 residues of LF) enter protonated; these protons are released into the *trans* solution upon exiting the channel, thereby making this a proton-protein symporter. Consistent with this idea, a single SO_3^-, which is essentially not titrateable, introduced at most positions in LF_N, drastically inhibited voltage-driven LF_N translocation. The lumen of the $(PA_{63})_7$ 14-strand β barrel is ~15 Å wide and can barely accommodate an alpha-helix with its side chains. Translocation through the lumen thus requires the substrates to unfold. Here we present an approach using biotin-streptavidin chemistry to determine the length of the translocating polypeptide chain within the channel as it is traversing the $(PA_{63})_7$ channel lumen, with the goal of shedding light on the structure of the polypeptide chain as it crosses the channel. We created a stopper at the LF_N C terminus and attached a biotin at the N terminus. Translocation proceeds until the C terminus reaches the channel's *cis* entrance, and binding of the N-terminal biotin with streptavidin added to the *trans* side of the membrane, locks the polypeptide chain within the channel. By reducing the distance between the N-terminal biotin and the C-terminal stopper by deletion constructs, we can determine the minimum length that allows streptavidin to grab the N-terminal biotin.