

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

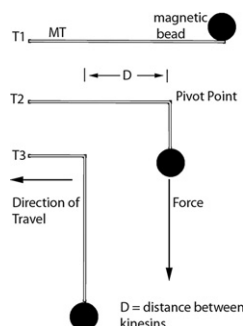
1928-Pos

"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 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 SURFE²R 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 SO₄/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 SO₄/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 H₂O₂ 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 (H₂) 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 H₂ via formate hydrogen lyase (FHL) system probably (1). It was demonstrated that in *E. coli* during fermentation of glucose depending of medium pH H₂ 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 F₀F₁-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 type fermenting glycerol protons expelled via F₀F₁-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* or *ΔhybC* 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 F₀F₁-ATPase activity is low H3 or H4 but not other hydrogenases may participate in the H⁺ extrusion or have proton translocating ability.