We consider the dynamics of particles driven by a collection of interacting molecular motors in the context of asymmetric simple exclusion processes (ASEP). The model is formulated to account for i) excluded volume interactions, ii) the observed asymmetry of the stochastic movement of individual motors and iii) interactions between motors and particles. Items (i) and (ii) form the basis of ASEP models and have already been considered in the literature to study the behavior of a collection of interacting motors in the absence of cargo. Item (iii) is new. It is introduced in this context as an attempt to describe explicitly the dependence of particle dynamics on the movement of motors [C.Goldman, E.Sena, Physica A 388(2009)]. The steady-state solutions to this model indicate that the system undergoes a phase transition of condensation type that can explain why, for sufficiently high motor densities, cargo velocity becomes independent of density in concert with the data obtained by Beeg et al [Biophys.J. 94(2008)]. The model predicts also that if more than one cargo-particle is present in the system these may exhibit inversion in movement direction. This means that the model suggests an alternative to explain the origin of the so called bidirectional movement of cargo according to which, inversions may happen in the presence of just one kind of motor.

Financial support: Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP

3752-Pos

Surface Adsorption of Protein Corona Controls the Cell Internalization Mechanism of Multicomponent Lipoplexes in Serum

Giulio Caracciolo[1], Luciano Callipo[1], Sofia Candeloro De Sanctis[1], Chiara Cavaliere[1], Daniela Pozzi[1], Giuseppe Caruso[1], Michelle Digman[2], Susana Sanchez[2], Enrico Gratton[2].
[1]Sapienza University of Rome, Rome, Italy, [2]University of California, Irvine, Irvine, CA, USA.

Designer multicomponent lipoplexes have recently emerged as especially promising transfection candidates, since they are from 10 to 100 times more efficient than binary complexes usually employed for gene delivery purposes. Here, we show, for the first time, that after internalization binary complexes of lower transfection potency remain in compact perinuclear endosomes, while multicomponent systems have intrinsic endosomal rupture properties that allow plasmid DNA to escape from endosomes with extremely high efficiency. Endosomal rupture results in an extraordinarily homogeneous distribution of unbound plasmid DNA throughout the cytoplasm and in the nucleus. Serum has often been reported as a barrier to efficient lipid-mediated transfection. Here we found that the transfection efficiency of multicomponent lipoplexes increases in serum. To provide insight into the mechanism of lipoplex-serum interaction, several state-of-the-art methodologies have been applied. The nanostructure of lipoplexes was found to be serum-resistant as revealed by high resolution synchrotron small angle X-ray scattering, while dynamic light scattering measurements showed a marked size increase of complexes. Proteomics experiments showed that serum proteins competed for the cationic surface of lipid membranes leading to the formation of a rich a 'protein corona'. Combining structural results with proteomics findings, we suggest that such a protein corona can promote large aggregation of intact lipoplexes. According to a recently proposed size-dependent mechanism of lipoplex entry within cells, protein corona-induced formation of large aggregates most likely results in a switch from a clathrin-dependent to caveolae-mediated entry pathway into the cells which is likely to be responsible for the observed transfection efficiency boost. As a consequence, we suggest that surface adsorption of protein corona can have a high biological impact on serum-resistant cationic formulations for in vitro and in vivo lipid-mediated gene delivery applications.

3753-Pos

More Freight, More Engines: Force Scales with Cargo Size in a Living Cell

Bela Dhamankar, Jeneva A. Laib, Robert A. Bloodgood, William H. Guilford.
University of Virginia, Charlottesville, VA, USA.

The protein FMG-1 is transported in a saltatory manner within the flagellar membrane of *Chlamydomonas* by intracellular kinesin-2 and dynein-2 motors. Three models have been proposed to explain how these movements are coordinated: transport complex, biased accumulation, and molecular clutch (Laib et al., PNAS, 2009). To help discriminate between these models, we attached microspheres of varying diameter (0.5-1.4 μm) but identical surface chemistry to *Chlamydomonas* flagella and used a laser trap to determine whether the forces imposed on the microspheres by intracellular motors were dependent upon bead size. Larger microspheres are expected to accumulate larger patches of FMG-1. The transport complex and biased accumulation models suggest that force should be dependent on FMG-1 patch size; in contrast, the molecular clutch model suggests that force should be roughly independent of patch size. Our data shows that larger microspheres result in larger anterograde and retrograde forces. With microspheres ranging from 0.5 to 1.4 um in diameter, anterograde forces respectively ranged from 6.9 to 30.6 pN and retrograde forces from 7.7 to 48.8 pN. This size dependency is consistent with either the transport complex or biased accumulation model, but not with the molecular clutch model. The forces measured at the smallest microsphere size were roughly equivalent to the *in vitro* stall forces of single kinesin and dynein motor molecules. These data show the feasibility of *in vivo* single motor mechanics experiments for understanding motor regulation and coordination.

3754-Pos

In Vivo Organelle Tracking, Calibration, and Force Measurement with an Optical Trap

Benjamin H. Blehm, Yann R. Chemla, Paul R. Selvin.
UIUC, Urbana, IL, USA.

We report our results on calibrating an optical trap in vivo while simultaneously trapping and tracking organelles with it. We have built an optical trap with microsecond time resolution and nm spatial resolution with a trapping laser that is positioned with an Acousto-Optic Deflector (AOD). This AOD is of fundamental importance to the in vivo stiffness calibration technique, as this technique requires high speed oscillation of the trap position (on the order of 10's of kHz), in order to compare an active spectrum (the system's response to laser driving) with a passive spectrum (the system's response to Brownian motion). In addition, we have an AOD in our detection laser's path, allowing us to calibrate our position detection Quadrant Photo Diode's (QPD) volts to nanometers conversion. Our system allows calibration of every necessary trap parameter in the cell on each individual organelle we trap. We currently trap lipid droplets in Cos-7 cells, and are planning on measuring motor stall forces, motor number, and looking at switching between kinesin based transport and dynein based transport.

3755-Pos

Effects of Actin Filaments on NGF Retrograde Transport

Yasuko Osakada, Harsha Mudrakola, Kai Zhang, Bianxiao Cui.
Stanford university, Stanford, CA, USA.

Actin filament is an essential component of the cell cytoskeletal system under the physiological conditions. In addition to their roles in supporting cell shape, actin filaments act as molecular tracks for myosin motors that are involved in the movement of organelles such as mitochondria in the axon. However, how actin filaments regulate axonal transport processes are yet to be fully elucidated.

Nerve growth factor binds and activates its receptor located at axon terminus, which intriguers the complex to be endocytosed sorted into signaling endosomes. NGF-containing endosomes are retrogradely transported from the axon terminus to the cell body. In this study, we investigated the effects of actin filiments on axonal transport by tracking the transport of single NGF modified with the quantum dot using microfluidic device.

Embryonic DRG neurons were cultured in the microfluidic nerve cell chambers made by PDMS. The microfluidic chamber allows us to apply latrunculin B, an actin de-polymerization inhibitor, exclusively to the middle segment of axon. This treatment would not affect signaling processes in the cell body or the endocytosis process that happens at the axon termini. We monitored the retrograde transport of Qdot-NGF in actin-depleted axons using TIRF microscopy. We found that NGF axonal transport continues in axons that are depleted of actin filiments, confirming previous reports that NGF transport is a microtubule-based process. However, we found that the average speed of axonal transport slowed down in Latrunculin B treated axons. Detailed analysis of why actin depolymerization affects axonal transport is still in progress.

3756-Pos

Mean Time and Probability to Reach a Structured Target

Thibault Lagache, David Holcman.
Ecole Normale Supérieure, Paris, France.

In cellular biology, reaching a target before being degraded or trapped is a ubiquitous problem. In particular, the communication between the cytoplasm and the nucleus is ensured by many small nuclear pores located on the nuclear membrane. DNA viruses hijack the cell replication and transport machinery to reach one of these nanopores before being trapped or degraded through the ubiquitin-proteasome system. In this article we provide general formula for the conditioned mean first passage time and the probability a particle (such as a virus) reaches a structured target (such as a nucleus covered by many small absorbing pores). Because the particle can be intermittently actively transported on the microtubules network, the asymptotics formulas account for a drift term in the Langevin description of trajectories. Mean first passage time questions are crucial and will help to understand quantitatively the cell biology at a molecular level.