To go with the flow: Molecular motors are a drag
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Summary

Why this thesis?

All organisms (plants, animals, fungi and bacteria) are built up from cells. These are the smallest building blocks of an organism that contains its genetic material (DNA). In eukaryotic cells the DNA is stored in the cell nucleus. Eukaryotic cells are membrane bound structures that are filled with cytoplasm and a cytoskeleton. The latter consists of microtubules and filaments that determine the internal organisation of the cell and gives the cell its shape. The cytoplasm consists of cytosol (the liquid component) and membrane bound organelles e.g. vesicles, mitochondria, chloroplasts, peroxisomes, lysosomes, golgi apparatus, cell nucleus, etc.

In this thesis I study the influence of the cytosol on organelle transport in the eukaryotic cell. In particular, I am interested in the phenomena cytoplasmic and axoplasmic streaming. In both these phenomena, many organelles are transported in the same direction over relatively long distances. This molecular cargo transport is powered by molecular motors. These molecular motors are transport proteins that literally walk along the cytoskeleton while carrying a cargo such as an organelle. When a cargo is dragged through the cytosol it will experience resistance in the form of an opposing fluid friction force. The magnitude of this force depends upon the size and shape of the cargo as well as on the viscosity of the fluid. The latter is a physical material property that indicates how strongly a fluid resists deformation. In the cytosol the viscosity is a factor 1000 larger than in water. This means that, in the cell, molecular motors need to deliver a much larger force to obtain the same velocity as in water. However, the motor-cargo velocities measured in vivo (in a living cell) are similar to, or even larger than, the single motor velocities from in vitro (laboratory environment) experiments in water. The goal of this thesis is to explain the underlying mechanism that makes this possible and to provide a possible explanation for cytoplasmic and axoplasmic streaming.

Molecular Motors

In chapter 1, the molecular motors are introduced. These are proteins that, by hydrolysis of ATP, are capable of converting chemical energy into mechanical work. I am
interested in linear motors that walk along cytoskeletal tracks while carrying cargoes such as vesicles or other organelle types. This is called active transport. There are three different families of motor proteins: kinesin and dynein that are associated with microtubules and myosin that walks on actin filaments. In the chapter results from both in vitro and in vivo experiments are discussed.

The physics

In chapter 2 the fluid dynamics equations are introduced that can be used to describe the motion of molecular motors with cargoes (organelles) in a fluid medium. These equations describe both the transport in solution and along the cytoskeleton. In addition, the forces that are acting upon the motor-organelle complexes and the transport properties are discussed. The latter is treated using dimensionless numbers such as the Reynolds (inertia versus friction forces), Péclet (diffusion versus directed transport) and Stokes (sensitivity to a flow field) numbers. For small objects, such as molecular motors and their cargoes, the friction and thermal forces dominate over inertial forces and over the influence of gravity. This is called a low Reynolds number environment, where the fluid motion can be described using the Stokes equation. In the course of (active) directed organelle transport along the cytoskeleton, there will be collisions between the organelles and the surrounding fluid molecules. During these collisions momentum is transferred from the organelle to the fluid molecules. The fluid molecules in turn will collide with their neighbours, transfer momentum, etc., etc. This momentum transfer does not continue indefinitely as at each collision a small part of the energy is dissipated. I assume that cytosol is a Newtonian fluid, in this type of fluid the magnitude of the momentum transfer decreases linearly with the inverse of the distance. The motion of other organelles, either suspended or bound to the cytoskeleton, will be influenced by the momentum transport via the fluid and vice versa. When many organelles are simultaneously transported in the same direction by molecular motors along the cytoskeleton, then the momentum transfer to the fluid molecules will give rise to a directed fluid flow. This fluid flow will give the bound motors a ‘push in the back’ making them walk faster along the cytoskeleton. In addition, the suspended organelles will go with the flow resulting in directed motion. In this thesis I investigate if this flow field, driven by momentum transfer, can explain biological phenomena such as cytoplasmic streaming in plants and axoplasmic streaming in neurons. Mathematically, the flow field can be modelled using a hydrodynamic interaction tensor (e.g. using the Oseen or the Rotne-Prager tensor). Finally, at the end of the chapter, a Langevin type equation is introduced. This equation describes the motion of suspended and bound motor-organelle complexes and the hydrodynamic interactions between them. This equation will be solved using computer simulations in chapters 4 and 6.
Models For Molecular Motor Transport

The theoretical models that inspired the simulation models that are introduced in this thesis are reviewed in chapter 3. From these models is learned that a model for molecular motors should include the following features:

- Directed motion of molecular motors along the cytoskeleton.
- Thermal fluctuations (diffusion) of motors attached to the cytoskeleton or in solution.
- Adsorption of motors on, and desorption from, the cytoskeleton.

However, all the models that are discussed neglect the effects of momentum transfer via the fluid. In chapters 4 and 6 two new simulation models are introduced that include all the above features and additionally include the hydrodynamic interactions via the fluid. The two models are a lattice model and a Brownian dynamics model.

Results

In chapters 4 and 6 computer simulations are presented of a Newtonian fluid embedded between two parallel sections of cytoskeleton with identical polarisation. In the system, the molecular motors with cargoes alternate between periods of directed active transport when bound to the filament and periods of passive diffusion in solution. The switching between these states is driven by the fact the motors can detach from and (re)attach to the cytoskeleton, thus taking the motor processivity into account. In the simulations the motor-cargo complexes are modelled either as hard-spheres (chapter 4) or as soft repulsive hard-spheres (chapter 6). Hence, it is prevented that two motors can occupy the same volume at a given moment in time i.e. excluded volume is taken into account.

The simulations show that the collective effect of the hydrodynamic interactions leads to a substantial increase in the average velocity of motors attached to a filament. This effect is enhanced when the number of active motors in the system increases. Moreover, the momentum transfer leads to a non-negligible, directed flow of suspended organelles. The hydrodynamic coupling is robust enough for suspended objects to flow across gaps in the cytoskeleton. Naturally, the more ordered the environment in the cell, the stronger the effect of the hydrodynamic coupling. Such an ordered environment can be found in cytoplasmic strands that cross the central vacuole in mature plant cells and in axons.

That hydrodynamic interactions can give rise to a flow of suspended material is confirmed in chapter 5 where experiments on the stamen hair cells of the flower Tradescantia
virginiana and of cells of Nicotiana tabacum are presented. In these cells cytoplasmic streaming takes place in cytoplasmic strands which are essential transport routes for the distribution of organelles and metabolites. In the strands active directed organelle transport takes place powered by molecular motors. When the molecular motor activity is inhibited then the directed transport changes to a diffusive motion. Using a technique called Fluorescence Recovery After Photobleaching (FRAP) it can be determined if there is any fluid flow (and in which direction) present in the cytoplasmic strand. The technique consists of injecting Green Fluorescent Proteins (GFP) into the cell’s cytosol. Next, the fluorescent material in a small area is destroyed using a laser. Subsequently, the fluorescent recovery in the area is followed i.e. the entry of active GFP. The direction from which the GFP enter the area is an indicator of the direction of fluid flow. If there is no directed fluid flow, the recovery will occur with equal velocity from all directions. However, in the cytoplasmic strands the recovery is observed to take place in the same direction as the active transport. More importantly, suspended organelles are observed to move in the same direction. Thus, both simulations and experiments suggest that momentum transfer through the solution, coming from actively transported cargoes, is the underlying mechanism of cytoplasmic streaming. This is a novel transport mechanism additional to diffusion and active motor transport.

**Outlook**

The reader is presented with a potential follow-up project in chapter 7. In addition to the linear hydrodynamic interactions between the organelles via momentum transfer, there exist rotational hydrodynamic interactions. Following the same physical mechanism, these interactions give rise to a rotational fluid flow field surrounding the organelles. This mechanism could e.g. be used to explain biological phenomena such as the rotating chloroplasts that have been reported in cytoplasmic drops. Additionally, the mechanism could make accessible all kinds of novel applications for microscopic devices that are interesting for both 'proof-of-principle’ experiments and technological applications.

Finally, the results presented in this thesis could be useful for medical researchers to better understand the influence of the fluid medium on organelle transport. This may be important as axonal transport deficiencies are linked to neurodegenerative conditions such as Alzheimer’s decease.