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Quantum Dot Motion on Microtubules

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Kinesin is one of the numerous motor proteins present in the cell. Its function is to transport cargo along microtubules, which serve as tracks, by efficiently making use of chemical energy. Here, we have studied the translocation of quantum dots by kinesin. Kinesin was bacterially expressed and conjugated to quantum dots, thus allowing observation of the motion of the quantum dots along the microtubules by fluorescence microscopy. The velocity was determined by a kymograph, and was in agreement with expectations.

Where there is life, there is motion. Our own movements, for example, are the result of motor proteins. One of the best studied examples, kinesin, "walks" along biopolymer tracks termed microtubules, and facilitates intracellular cargo transport (Figure 1).

Kinesin is a heterotetramer constructed from two light chains and two heavy chains, which dimerize to form the stalk. They are terminated by a motor head, which binds specifically to the tubulin, and forms the constitutive element of the microtubule, providing the step-like walking motion. The biological track is a continuously evolving structure and displays polarity. It polymerizes rapidly at the "plus" end and slowly at the "minus" end.[1] The majority of kinesin motors such as kinesin-1, move toward the plus end.

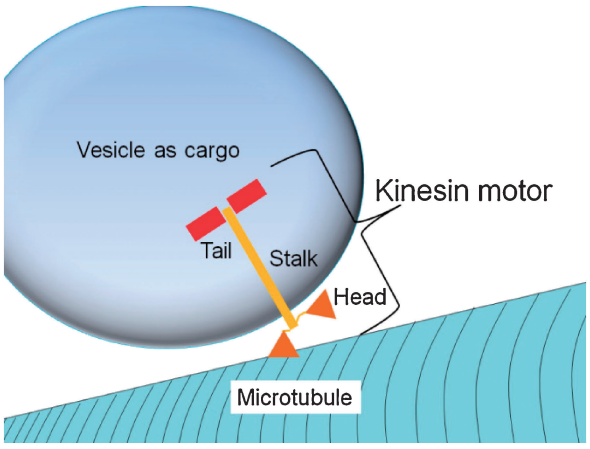

Figure 1. Illustration of the kinesin tetramer transporting cargo along a microtubule, which is a 25 nm diameter hollow cylinder. The cargo binds to the kinesin thanks to the two light chains in red. The motion is generated by a conformation change in the two heavy chains (orange) which fold to form the motor heads.

The mechanical energy required for the walking motion is fuelled by adenosine triphosphate (ATP) hydrolysis, where a single ATP molecule is consumed for each 8 nm step.[1,2] The chemical energy is converted into mechanical energy with a high efficiency (ca. 50%).[3] Furthermore, kinesin can translocate several piconewtons of load over relatively long distances, and its activity is barely limited by cargo size.[4] The velocity can be controlled by the ATP concentration.[5] Overall, these properties make kinesins interesting macromolecules for lab-on-a-chip devices. In the present report, the transport of streptavidin-coated quantum dots (QDs) by kinesin is studied. The advantage of QDs is their small size (ca. 20 nm) and enhanced photostability as compared to conventional fluorophores,[6] thus allowing the direct confirmation of both the activity of the expressed kinesin and its ability to translocate cargo.

Truncated and biotinylated kinesin-1 (length: 400 amino acids) was expressed in *E. coli* using a standard protocol,[2] with histidine tags to facilitate purification. To enable cargo attachment, the strong binding interaction between streptavidin and biotin was exploited.[7] The microtubules were labeled with rhodamine to allow their identification with fluorescence microscopy. An Olympus IX-71 microscope, equipped with a Hamamatsu ImagEM CCD camera, was used for the observations. Streptavidin-coated QDs were purchased from Invitrogen. Unlabeled tubuline and rhodamine-labeled tubulin were purchased from Cytoskeleton Inc.

A schematic representation of the motility assay, showing the QDs that are attached to the kinesin, is presented in Figure 2. In the presence of ATP, the motor will spontaneously attach to the microtubule and "walk" along its length.

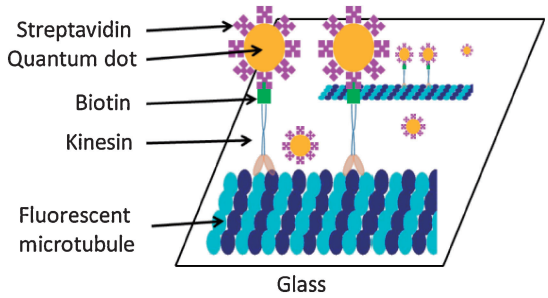

Figure 2. Schematic of the streptavidin-coated QD transport along a microtubule. Light and dark blue represent α and β tubulin, the two components of the tubulin dimer.

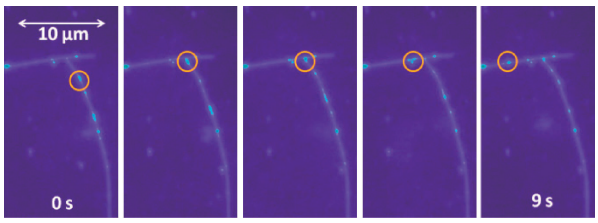

Figure 3. Microscope images of quantum dots moving on rhodamine-labeled microtubules (1 mM ATP). The circle follows one of the transiting QD.

Antifade solutions and microtubules solutions were prepared following the Koch protocol.[8] The microtubules solution is first inserted into a standard flow cell,[9] and incubated for 5 min. The cell is then flushed with BRB80 buffer,[10] containing 10 µM taxol, antifade solution, and 0.2 mg mL^{-1} casein, to remove unbound microtubules and prevent QD adherence. At the same time, a kinesin solution (40 µg mL^{-1}) is incubated for 5 min in BRB80 containing QDs (2 nM), casein (0.2 mg mL^{-1}), taxol (10 µM), antifade (2.5% v/v), and ATP (1 mM), after which it is introduced into the flow cell for observation.

Figure 3 shows snapshots of the QDs positioned on the microtubule, as a function of time. Each QD moves in the same direction, confirming the nondiffusive motion under these conditions.[11] Fast cargo transfer at the intersection of the two microtubules was observed. This type of transition was previously reported by Böhm et al. for 100 nm polystyrene beads.[4] This phenomenon is exemplary for the relatively high surface density of kinesin at the cargo beads, and consistent with the current experimental conditions, where the QD surface is entirely covered with kinesin.

When studying the motion of several QDs over time, it appeared that their velocities were not identical. To measure the velocities, several kymographs (i.e., time–space plots) were created (Figure 4b). A superposition of 600 video-frames is shown in Figure 4a.

Each line in Figure 4b corresponds to a time vs. distance plot of a quantum dot trajectory. The steeper the slope, the lower is the velocity. The resulting data are summarized in a histogram in Figure 5.

The average velocity and the velocity distribution are comparable to that found in previous experiments, performed in BRB80[12] or TC12 buffers,[13] where TC12 has a lower ionic strength than BRB80. A decrease in ionic strength is expected to maximize the interaction between microtubules and kinesin, but may also lead to a decrease in velocity.[5,12] Nevertheless, we measured the same velocity as Seitz et al. for kinesin in TC12. We hypothesize that the difference results from the change in ATP concentration, which was 2 mM in the Seitz experiment. Moreover, the velocity may be affected by defects on the microtubule, which could result from the binding of other molecules. Such defects may affect the friction encountered by the kinesin.[14] In addition to the specific experimental conditions, the velocity distribution may be influenced by several factors such as microtubule configuration, kinesin/QD ratio, kinesin conformation, and its location on the microtubule. In the following discussion, we have assumed that only the last two factors are relevant.

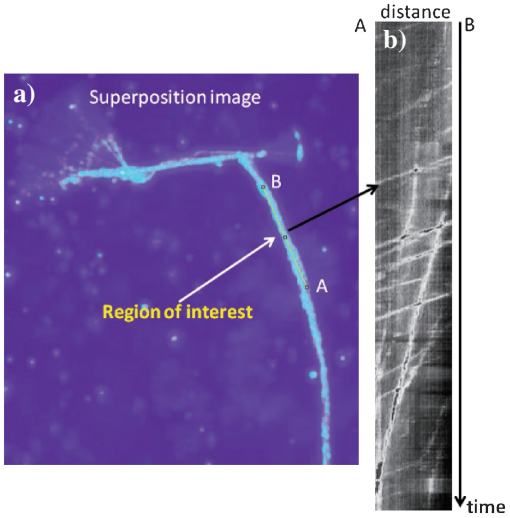

Figure 4. a) Superposition of the 600 frames of the video and b) extracted kymograph from the segment AB. For each frame, the segment AB is recorded and stacked in order to create the space–time image.

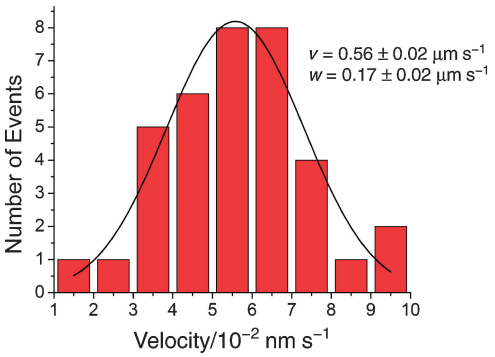

Figure 5. Velocity distribution of quantum dot–kinesin conjugate. Average velocity v is calculated using a Gaussian fit. w is the width.

Our measurements were performed on several short and straight segments, i.e., the intersection between point A and point B in Figure 4. In our particular case, the radius of curvature of the microtubule is large compared to the kinesin step length. Thus, there should not be any local geometrical effect on the velocity of the cargoes. Moreover, we did not observe any change in average velocity before and after the microtubule intersection. Because each cargo moves at a relatively constant velocity (cf. kymograph in Figure 4), each individual kinesin molecule behaves significantly different. Therefore, the speed measured must be influenced by factors intrinsic to the microtubule–kinesin–QD system. Among the possible explanations, the effect of the number of kinesins bound to the QD is negligible, since this should follow a Poisson distribution,[15] and Beeg et al. have shown that the velocity does not depend on the number of motors.[16] The dispersion of velocities may, however, be explained by two other intrinsic factors. First, it is very unlikely that proteins fold in exactly the same way.[17] As a result, the kinesin molecules may display some minor conformational variation, which could modify trans-

location velocity. Second, the velocities may be influenced by the position of the kinesins, which stay on the same protofilament during motion.[18] As each QD conjugate is exposed to different flow and friction forces, owing to microtubule shielding and substrate proximity, their velocities may vary with the corresponding protofilament. The current experimental approach does not allow a distinction between the aforementioned explanations.

In summary, we have demonstrated the transport of QDs by kinesins along microtubules. QDs allow the convenient visualization of the transport process. We foresee that, by tagging kinesins to other molecules of interest, a nanoscale transport system for lab-on-a-chip applications could be generated.

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