Lab Report

Biomolecular Motors

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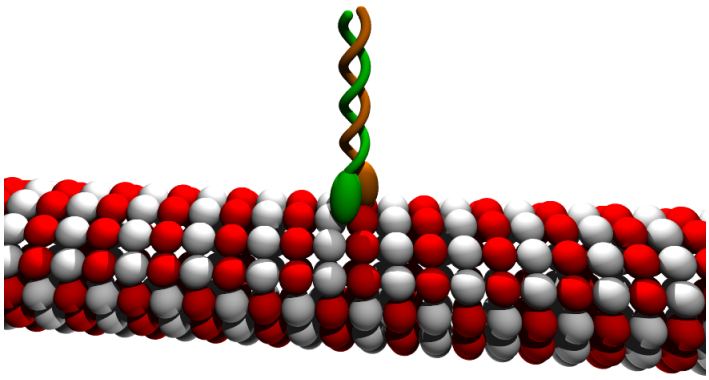

Figure 1: Kinesin-1 walking on a microtubule. [4]

1 Introduction and Theory

Molecular motors are important parts of the cells e. g. in our body. They transport everything inside the cell, either from the centre of the cell towards the periphery or vice versa (e. g. the examined Kinesin-1) or vice versa (e. g. dyneins). The motors play an important role in the cell division, they separate the copied chromosomes to form the new cells. In addition to chromosomes they transport also vesicles, organelles, and protein complexes (see [3]).

The proteins use microtubules (MTs) as tracks and move along these. MTs consist of α- and β-tubulin. α-tubulin has a negative end, and β-tubulin a positive. Positive and negative do not mean charged but a structural polarity. Most kinesins move from the minus end to the plus end. Together the tubulins form a hollow polymer-cylinder as it is shown in figure 1.

These fundamentals are well understood, but the precise biochemical transitions are still object of research (see [3]).

The energy for the movement comes from the hydrolization of ATP (adenosine triphosphate) molecules:

$$\mathrm{ATP} \rightarrow \mathrm{ADP} + \mathrm{P_i} + 10.1 \times 10^{-20}\,\mathrm{J} \tag{1}$$

with ADP: adenosine diphosphate and $\mathrm{P_i}$: Phosphate.

One ATP molecule results in one step of $8\,\mathrm{nm}$ length and a a force of $5\,\mathrm{pN}$. [1] The work is

$$W = F \cdot s = 4 \times 10^{-20}\,\mathrm{J}, \tag{2}$$

which means about $40\,\%$ of the energy input is used.

An interesting aspect is the energy efficiency compared to a combustion engine in the car. A

standard engine in a car has the same energy efficiency as a Kinesin-1 protein. Both are not very energy efficient. The velocity is, compared to a car, rather slow $0.8\,\mu m\,s^{-1}$. Each step has a length of 8 nm, the repeating periodicity of the tubulin dimers.

To observe the proteins a microscope with a high resolution is needed. For this practical course a TIRFM (total internal reflection fluorescence microscope) was used. It is based on fluorescence and uses a laser to excite the molecules. Since the molecules emit light by themselves the observation is easier and one can use different fluorescent molecules to mark the examined structures. In this experiment the MTs are labeled with Rhodamine and the Kinesin-1 with green fluorescent proteins (GFP).

The total internal reflection reduces a lot of background signal, because of the evanescent field, which reaches only 100 nm into the specimen. The rest of the radiation is totaly reflected.

An anti-fade cocktail helps to reduce photo bleaching and protein damage by reducing the amount of free oxygen

$$\text{Glucose} + \mathrm{O_2} + \mathrm{H_2O} \overset{\text{Glucose oxidase}}{\rightarrow} \text{Gluconate} + \mathrm{H_2O_2} \tag{3}$$

$$2\,\mathrm{H_2O_2} \overset{\text{Catalase}}{\rightarrow} 2\,\mathrm{H_2O} + \mathrm{O_2}. \tag{4}$$

2 Experimental Procedure

The first step was to prepare the specimen. Therefore two different coverslips were used: hydrophobic at the bottom and on top hydrophil glass. The glass needs to be hydrophic to allow the anti-bodies to bind to it, the hydrophil glass is used to simplify the process of adding water based solutions between the coverslips. The two layers were hold on distance with three stripes of Parafilm to form and seal up two channels.

After this the solutions had to be prepared: anti-tubulin, microtubules, BRB80T, kinesin dilution solution, first kinesin dilution, second kinesin dilution and finally the kinesin solution. Every solution with proteins had to be chilled to avoid a depolymerization.

Anti-tubulin was added between the two coverslips. After 5 min. the coverslips got washed with BRB80. BRB80 is a buffer solution and it helps later to polymerize the MTs. Then F127 was used to block the rest of the surface, to avoid any attraction between glass and microtubules.

With BRB80T the depolymerization of the MTs can be stopped, because it contains taxol. With this solution the coverslips were washed ca. 90 min. after the F127 was added. The MTs were added, and they bound to the antibodies. A second wash with BRB80T followed to remove all remaining free MTs and finally the kinesin solution was added. The kinesin solution contained the energy source, ATP, and anti-fade cocktail.

The exact preparation can be found in [2].

For the following examination the TIRFM was used. The magnification was $100\times$, the numerical aperture 1.46. To catch more light and improve the resolution, some oil was put between the objective and the bottom coverslip—we used a inverted fluorescence microscope.

Five streams were recorded, each one at a different position, to reduce photo-bleaching of the fluorescent molecules. Every stream contained 1500 frames, taken every $100\,\mathrm{ms}$. The photos of the MTs were taken under "normal light" (lamp) (see Fig. 2), the streams under the laser $\lambda = 488\,\mathrm{nm}$, with a power of $38\,\mathrm{mW}$. The used camera had a pixel size of $16\,\mu\mathrm{m} \times 16\,\mu\mathrm{m}$.

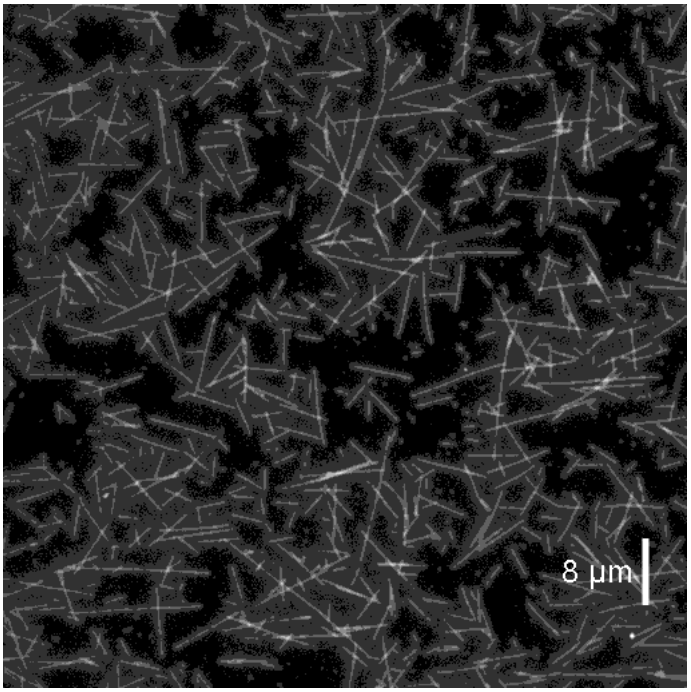

Figure 2: Microtubules in the specimen under a fluorescence microscope.

The MTs were visible as short, straight white lines, which were not moving, whereas the Kinesin-1s were small moving appearing and disappearing dots. On a second specimen the MTs were moving too, a reason why it was not examined further—the binding with the anti-bodies did not work.

3 Results

To get velocity and run length from the streams the software *FIESTA* [5] was used. With the numbers of pixel and the size of every image pixel the software can calculate the real values.

Each pixel of a frame corresponds to an area of $16\,\mu m/100 \times 16\,\mu m/100 = 160\,nm \times 160\,nm$.

On a combined image of all frames of one stream the Kinesin-1 traces were marked. In a second step FIESTA showed the traces in a coordinate system with time as horizontal axis and the distance as vertical axis. The traces were marked again and the software calculated the distance and velocity.

To examine the distribution of the velocity and the run length the data was plotted in two histograms. For the results the data of $N = 232$ molecular motors were used.

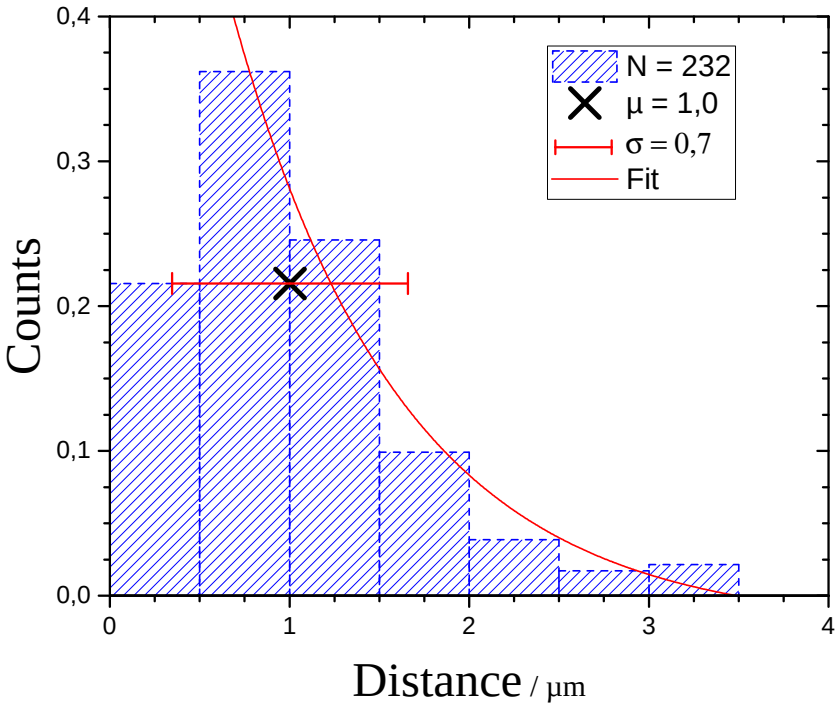

Figure 3: Distribution of the "walked" distance of the Kinesin-1 proteins with an exponential fit.

Figure 3 shows the distribution of the "walked" distances of the Kinesin-1. The mean distance is $(1.0 \pm 0.7)\,\mu m$. The histogram shows the expected exponential distribution. To move this distance the Kinesin-1 needs $\frac{1.0\,\mu m}{8\,nm} = 125$ steps. In [1] the average number of steps is 100.

The mean velocity of the Kinesin-1 is $(0.9 \pm 0.4)\,\mu m\,s^{-1}$. A histogram of the velocities is shown in figure 4. It seems to have the form of a gaußian distribution, but one recognize that the histogram is not symmetric, and it has a sharp peak. Therefore the underlying distribution has to be something different.

Some statistical test were made to support the visual impression. The tests are listed in table 1 and show with a significance level of 0.05, that the underlying distribution of the velocity is *not* a gaußian.

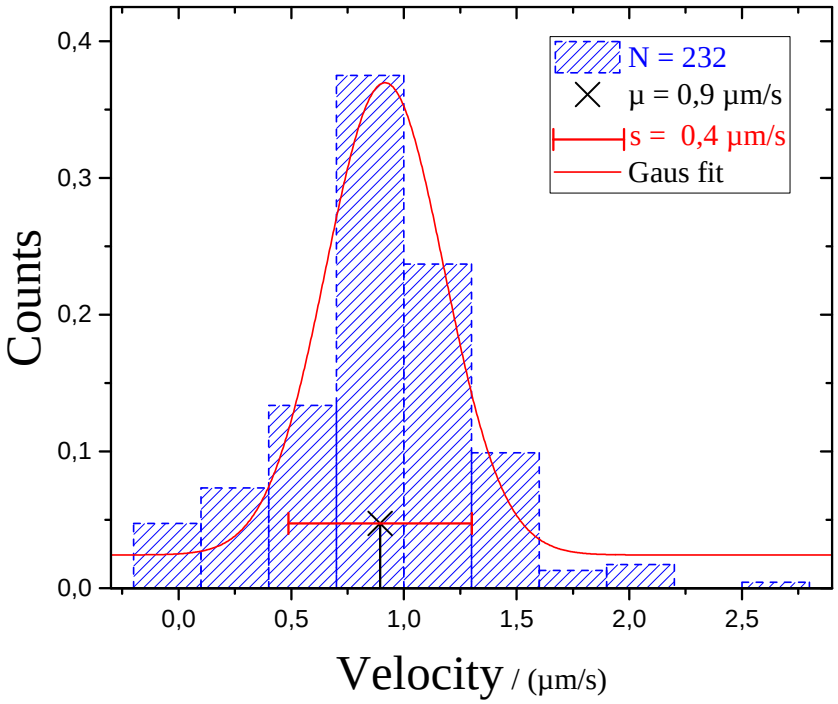

Figure 4: Distribution of the velocity of the Kinesin-1 proteins with a gaußian fit.

Table 1: Tests for the velocity distribution (each with significance level 0.05).

Test	p-value	gaußian distribution
Shapiro-Wilk	1.76×10^{-11}	no
Lilliefors	4.91×10^{-10}	no
Kolgomorov-Smirnov	8.34×10^{-4}	no
Anderson-Darling-Test	2.59×10^{-13}	no

4 Discussion

The measured mean velocity of the Kinesin is $(0.9 \pm 0.4)\,\mu m\,s^{-1}$ and higher than the literature value of $0.8\,\mu m\,s^{-1}$. Since they are not compatible, there is maybe a not examined dependence. Further experiments could show if there is a dependence between velocity and temperature or ATP concentration or another parameter.

The number of steps before detaching is also higher 125 (experiment) compared to 100 (literature), if one takes a step size of $8\,nm$. Therefore it would be interesting to measure the step length or the probability of detaching.

It is very important to be very careful with the preparation, otherwise one will not see the movement of the Kinesin-1.

A higher number of examined Kinesin-1 tracks would have been better, but the recorded frames did not contain more data to analyse, since the density of the MTs was very high (see Fig. 2), causing a lot of crossings. At these crossing it was not possible to decide which way the Kinesin-1 took. A lower density would have improved the measurement.

References

[1] Instruction, *Biomolecular Motors: From Cellular Function to Nanotechnology*, B CUBE, MPI of Molecular Cell Biology and Genetics, 2015.

[2] Stefan Diez, Felix Ruhnow, Till Korten, *GFP-Kinesin Stepping Assay*, B CUBE, `https://www.bcube-dresden.de/w/index.php?title=GFP-Kinesin_Stepping_Assay&oldid=1497`, 2011.

[3] Manfred Schliwa (Editor), *Molecular Motors*, ISBN: 3-572-30594-7, Weinheim: Wiley VCH Verlag, 2003.

[4] Kebes, CC-BY-SA 3.0, `https://de.wikipedia.org/wiki/Datei:Kinesin_cartoon.png`, 3rd Dec. 2015.

[5] Software, *FIESTA*, B CUBE, `https://www.bcube-dresden.de/fiesta/wiki/FIESTA`.