Experiment protocol

Biomolecular Motors: From Cellular Function to Nanotechnology

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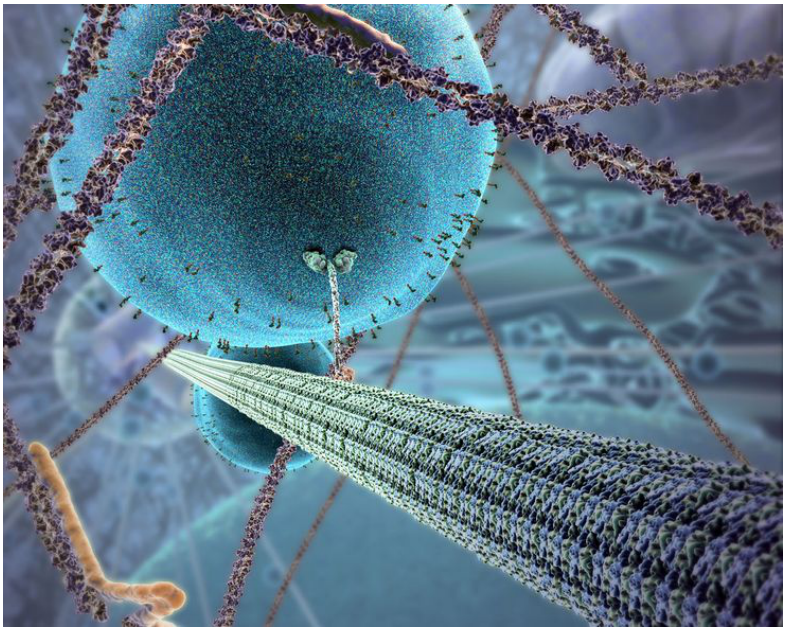

Figure 1: Kinesin-1 with cargo on microtubules [1]

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1 Basics

Proteins are the basis of every living organism. They perform all types of work inside the cells. In this experiment, the interaction of the cytoskeletal motor protein kinesin-1 with microtubule filaments is investigated at two different temperatures.

1.1 Microtubules

Microtubules are hollow polymer-cylinders. They are highly dynamic filaments, always shrinking or growing, which serve as tracks for active intercellular transport. Microtubules consists of tubulin subunits, which give the tubules a plus and minus end, which allow a directed transport.
In this experiment, the dynamic of the tubules is stopped by Taxol. The microtubules are labeled with rhodamine to allow red fluorescence.

1.2 Kinesin-1

Kinesin-1 is a motor protein which transports cargo around the cell. It consists of two identical, connected subunits which both have a head, a stalk and a tail, as can be seen in figure 1. The head serves as motor, the tail as cargoholder.
The movement of kinesin-1 is accomplished through bipedal walk along microtubules: one head moves in front, the other stays connected to the microtubule. For each step, the kinesin-1 "consumes" one ATP-molecule to reach the needed energy. In this way the protein manages to accomplish a velocity of $0.8\,\mu m/s$. However, with a probability of 1% both heads disconnect, resulting in a detachment of the microtubule after an average way length of $0.8\,\mu m$.
In this experiment, the kinesin-1 proteins are labeled with GFP to provide green fluorescence.

1.3 Fluorescence microscopy

To investigate the proteins fluorescence microscopy is used. The rhodamine and the GFP are excited by light: One of their electrons enters an excited orbital. This electron has the opposite spin of the remaining electron in the ground-state orbital. The molecules begin to vibrate and lose thermic energy in the process. After around 10^{-8} seconds the excited electron jumps back to the ground state and emits a photon. Because of the lost energy, the emitted photon has a larger wavelength then the absorbed one and can be distinguished from the laser light. Through this the tubules and proteins with the excited molecules can be observed.
To limit the fluorescing volume, Total Internal Reflection Fluorescence Microscopy is

used. The exciting laser lights are totally reflected before they enter the object. However, an evanescent wave enters the object. Like this, only the first few microtubules and proteins are illuminated.

2 Experimental procedure

The movement of kinesin-1 has to be researched at two different temperatures: 25°C and 37°C. At first one prepares a flow-cell: two pieces of glass – one hydrophob, one hydrophil to provide a better flow of the liquids – are fused with 3 pieces of parafilm to form two channels, one as a backup. Then the channels are flushed with BRB80 – this is done after every flushing, to wash the channels, keep them wet and provide a good environment for the experiment. Then Anti-Tubulin antibodies, to bind the microtubules in the channels, and F127, to prevent the binding of the kinesin-1 with the glass, are put inside. After this the microtubules, are flushed in. After a waiting time of around 1h the kinesin-1 final solution, which includes the kinesin-1, nutrients for both the microtubules and the kinesin-1 and ATP for the movement of the kinesin-1, is flushed in.
To see the behaviour the samples are researched with a TIRF-microscope with a magnification of 100x and a camera pixel-size of $16\,\mu m^2$. At first a photo of the microtubules is taken at normal light, to determine the position of the microtubules. After this a 1000-frames video is taken under green laser light, to see the movement of the kinesin-1. The data-analysis is done using the FIESTA-program which is contributed. Here the lines of the microtubules are marked as can be seen in figure 2 and, after the computer marked tracks of moving particles on this lines, for each temperature 200-500 recognizable tracks of the kinesin-1 are marked. The program calculates the velocity and run-length of this marked tracks.

3 Data and analysis

After the laserlight shone on the probes, the microtubules dissolved. This happened both for the main and the backup-channel of the flow-cell. Because time was short, the experiment couldn't be redone.
The reason for this problem was probably the kinesin-1 final solution, which contained nutrients and stabilizers, even though this solution was reproduced after the first failure. Because the second group, which did their experiment on the same day, had the same problems, the basic liquids for the solution were probably tarnished.
As we had no experimental data of ourselves, the following data-analysis is performed with data of another group. The extraction of the data by using FIESTA was performed by us.

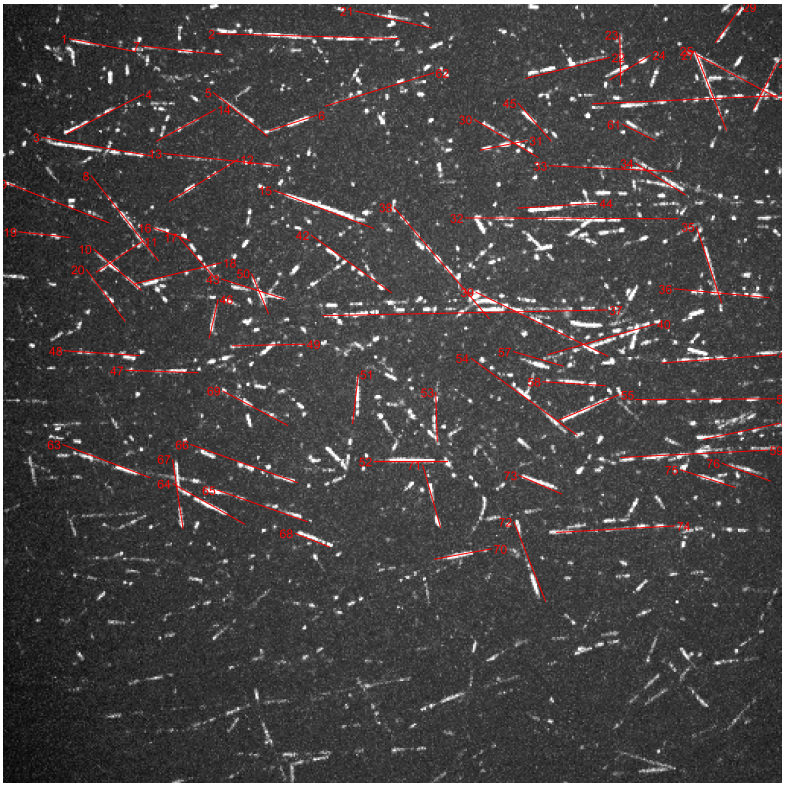

Figure 2: Marking of microtubules in FIESTA for analysis

We acquired the following amount of movement incidents:

$$\begin{aligned} 25^\circ C &: 546 \\ 37^\circ C &: 513 \end{aligned}$$

3.1 Velocity

Histograms of the velocities are shown in figure 3. They resemble normal distributions, however, the performance of a Shapir-Wilk-test gives back a value of $W = 0.8$, which isn't enough to verify this claim. To make sure that this distributions are normal distributions, a greater amount of incidents would be necessary. The calculated mean velocities with their standard deviations as errors are:

$$\begin{aligned} v_{25^\circ C} &= (1.0 \pm 0.5)\,\mu m/s \\ v_{37^\circ C} &= (1.4 \pm 0.6)\,\mu m/s \end{aligned}$$

A comparison of the two mean velocities leads to the claim, that the true mean velocities differ for $0.4\,\mu m/s$. The performance of a Welch-test leads to a one-sided p-value of 0.47

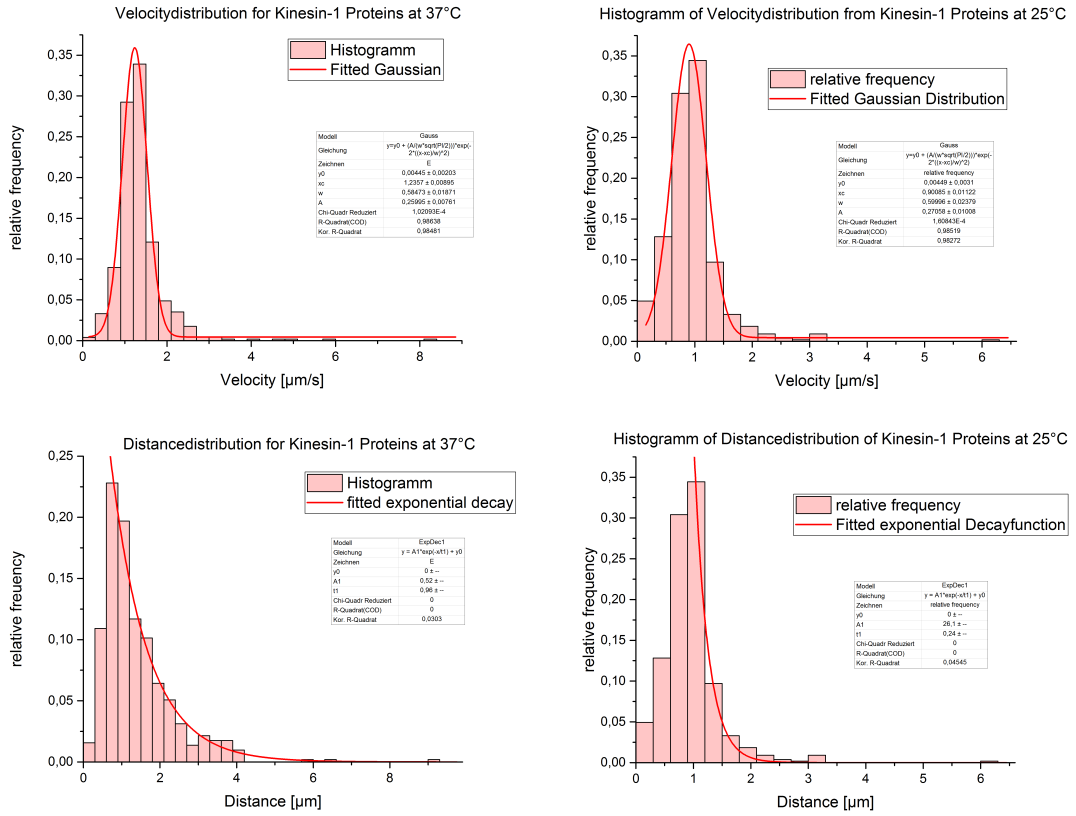

Figure 3: top-left: Normalized velocity of kinesin-1 at 25° C; top-right: Normalized velocity of kinesin-1 at 37° C; mid-left: Normalized run-length of kinesin-1 at 25° C; mid-right: Normalized run-length of kinesin-1 at 37° C; bottom-left: Cumulative run-length of kinesin-1 at 25° C; bottom-right: Cumulative run-length of kinesin-1 at 37° C

and a two-sided p-value of 0.93, which are both bigger than 0.01. Therefore, the claim cannot be falsified.
TIRF-microscope is a very exact measurement, the gained pictures are very accurate, as can be seen in figure 2. Errors come mainly from the lack of sufficient incidents and the evaluation with FIESTA. Because the marking of microtubules and of kinesin-1-tracks was done manually, errors are inevitable. However, most of them should cancel each other statistically. The biggest error of the analysis should therefore be the lack of marked slow proteins: As very slow moving particles weren't marked, because they could be dead proteins or errors of the picture, very slow moving kinesin-1 are ignored.

3.2 Run-length

The histograms of the run-length can also be found in figure 3. These distributions can be fitted with an exponential fit. We fitted without using the first bin three bins for $25°C$ and without using the first two bins for $37°C$. The first bins aren't reliable, as the slowest moving kinesin-1 weren't marked, as explained above. The formula for the fit is:

$$y = a(1 - e^{-bx})^c$$

The calculated mean run-lengths with their standard deviation as errors are:

$$\begin{aligned} l_{25°C} &= (1.2 \pm 0.9)\,\mu m \\ l_{37°C} &= (1.4 \pm 0.9)\,\mu m \end{aligned}$$

Again the claim is, that the true mean velocities differ for the same value than the measured mean velocities, in this case $0.2\,\mu m$. The Welch-test gives a one-sided p-value of 0.44 and a two-sided p-value of 0.88. Because of this, the claim also cannot be neglected.

To achieve an exacter value and lessen the error we now calculate the cumulative distribution function for the run-lengths and fit them. The formula for the fit is:

$$y = 1 - e^{-k(x-x0)}$$

For this formula the mean value is calculated like this:

$$\bar{x} = \ln(2)\frac{1}{k}$$

And it's error with the Gauss' uncertainty propagation:

$$\Delta x = |\ln(2)\frac{-1}{k^2}\Delta k|$$

Thus, the following values are achieved:

$$\begin{aligned} \bar{l}_{25°C} &= (1.2 \pm 0.9)\,\mu m \\ \bar{l}_{37°C} &= (1.4 \pm 0.9)\,\mu m \end{aligned}$$

To solidify our claim, we also plotted the accumulated run-lengths of the kinesin-1. To grant a better visibility of the result, the chosen bin size is much smaller. The plots can be seen in figure 3. It can also be seen, that the chosen cumulative distribution fit is very good, as it is almost an envelope to the accumulated run-lengths.

4 Conclusion

References

[1] https://de.pinterest.com/pin/565905509397043812/