Kinesin walks on microtubules

- step size: 8 nm
- velocity: 0.8 µm/s
- force: 6 pN

... and consumes ATP

http://valelab.ucsf.edu
Other Motor Proteins

Dynein
Moves cargo towards the minus ends of microtubules

Kinesin
Typically moves cargo towards the plus ends of microtubules
(some kinesin motors move toward the minus end)

Joseph Roland 2004
Question: Alone or in Groups?
Our live-cell imaging: DIC
(differential interference contrast)
Normal DIC Microscopy Image

50x50 μm
Orca ER camera
125 ms exposure
60X water immersion
NA 1.0
Nikon Eclipse
Chick motoneurons
glass-bottom dish
Measuring Vesicle Diameter

A.

B.

50x50 µm, Orca ER camera, 125 ms exposure, 60X water immersion
NA 1.0, Nikon Eclipse, NT2 cell, glass-bottom dish
Vesicle “Volume” Measurements

Works below diffraction limit!

Figure 1

Peak to Trough (µm)

Bead Diameter (from label, µm)

Integral Value (a.u.)

Shtrideman et al. 2009
How does MEDIC work?
(motion-enhanced DIC)

Hill, Macosko, Holzwarth 2008
Normal DIC Microscopy Movie

50x50 μm
Orca ER camera
8 frames/s
60X water immersion
NA 1.0
Nikon Eclipse
Chick motoneurons
glass-bottom dish
MEDIC movie of a Motoneuron

50x50 μm
Orca ER camera
8 frames/s
60X water immersion
NA 1.0
Nikon Eclipse
Chick motoneurons
glass-bottom dish
Simultaneous MEDIC+fluorescence

50x50 μm
Orca
125 ms
60X oil
NA 1.4
Nikon Ti-E
Mouse fibroblast on glass
Stained with LysoTracker

Hirokawa, Science 1998
Computerized Vesicle Tracking

50x50 μm
Orca ER camera
8 frames/s
60X water immersion
NA 1.0
Nikon Eclipse
Chick motoneurons
glass-bottom dish
Obtaining trajectories ($x$-$y$ → $y'$-$t$)

**A.**
- $t = 0$ s
- $t = 2$ s
- $t = 4$ s
- $t = 6$ s

**B.**
- $X^2_{\text{red}} = 1.09$
- $\sigma = 0.05$

Orca ER camera, 125 ms exposure, 60X water immersion
NA 1.0, Nikon Eclipse, NT2 cell, glass-bottom dish, scale bar 5 $\mu$m

Shtridelman et al. 2008
Slope of Distance vs Time is Velocity

\( \chi^2_{\text{red}} = 1.1296 \)
\( \sigma = 0.05 \)

1.8 \( \mu \text{m/s} \)
1.3 \( \mu \text{m/s} \)
0.4 \( \mu \text{m/s} \)
1.7 \( \mu \text{m/s} \)
0.67 \( \mu \text{m/s} \)
2.4 \( \mu \text{m/s} \)  \(\rightarrow\) Very fast!

Hill et al. 2004 (PC12 cells)
How does kinesin move so fast in a cell (although it’s highly viscous)?
Larger Cargo Moves Slower

Shtridelman et al. 2009
Larger Cargo Often Moves Retro

Macosko et al. 2008

![Graph showing the distribution of average velocity for small and large cargo](image_url)
Cargo Slows 1→3 Days in Culture

Macosko et al. 2008
Velocity histograms give insight

Chick neuron

Macosko et al. 2008
Same Pattern as in PC12 cells

Number of Occurrences

\( \frac{v}{v_0} \)

1 motor

2 motors

3 motors

4 motors

Hill et al. 2004
And in NT2 Cells...
Other Organisms As Well...

Worm
Zahn, 2004
Kural, 2006

Frog
Levi, 2006

Chick
Breuer, 1975!

Rat
Hill, 2003

Fruit fly
Hypothesis: more motors = more speed

Recap:

- Get slopes

- Plot histogram

Explain peaks by number of motors (from many files)
Stokes’ Law: $F$ and $v$ linearly related
(get viscosity from Stokes-Einstein)
Measuring Intracellular Viscosity
Model that may explain hypothesis

A

Drag force ($F_d$) or motor force ($F_m$), in pN

B

Velocity ($\mu$m/s) vs Diameter ($\mu$m)
Model that may explain hypothesis

(adding curves for 2 and 3 motors)

data would not yield multimodal velocity

data do yield multimodal velocity histogram

Shtridelman et al. 2008
Latest data from NT2 cells

Shtridelman et al. 2009
Vesicle Transport: Physicists’ View

- $F_{\text{Drag}}$
- $F_k$
- $\sim nV$
- $nF_k$
- $F'_{\text{Drag}}$
Summary of single molecule studies

- Live-cell imaging: MEDIC, sub-diffraction sizing
- “Single” molecule extended to groups: force-velocity
- Insights from polymer physics: Stokes, viscosity

How could this help **whole** cell studies?

Example: Vesicle transport in rat cortical neurons was found to increase with increasing plating density. I.e. MEDIC and vesicle tracking reveals unnoticed differences in cell cultures.

Bauer et al. 2008 (Neuroscience Lett)
In Vitro single molecule experiments

Viscous drag
Magnetic drag

Magnetic tweezers

Changing gears: drug discovery
2nd project: discovering cancer drugs

Holy grail in cancer therapy: **Targeted cancer therapeutics**

Need to find molecules that only target cancer cells

Two key insights:

1. Using single molecule techniques we can, for the first time, observe *individual* binding events.

2. Then: using an AFM* we pick the *single* high affinity binding molecules from a large pool of uninteresting molecules.

*It may turn out to be cheaper or easier to use other methods

However, we have a problem:

1. Single molecule binders are hard to identify, even with PCR.

2. An additional insight: Put *multiple* copies of the *same* molecule on each bead to amplify the identification process.