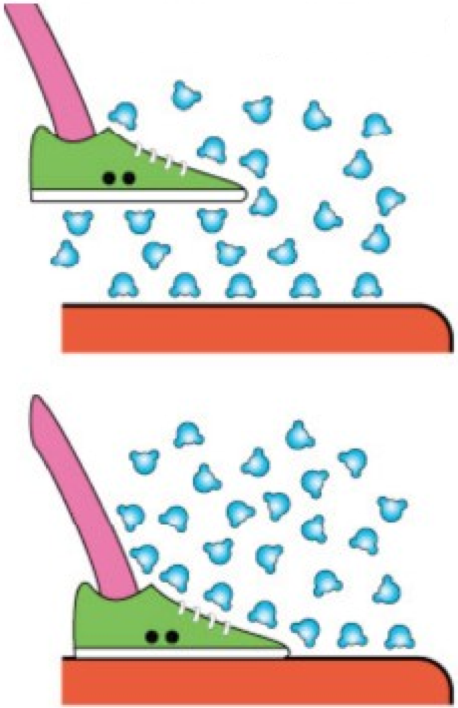
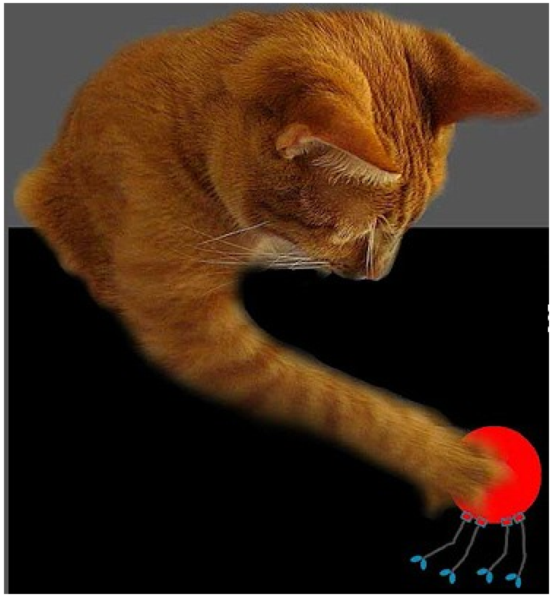
Osmotic stress and water isotope effects in kinesin-1 gliding motility assays

Steve Koch, U. New Mexico Dept. Physics and
Center for High Technology Materials (CHTM)



Thermodynamics and kinetics of molecular motors workshop
Santa Fe, May 20, 2010



Open Notebook Science

Acknowledgments

Larry Herskowitz
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Brian Josey
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Pranav Rathi
Optics Ph.D. Student

Collaborations

Susan Atlas—Lead of the DTRA project
UNM Physics / Cancer Center / Director of CARC

Haiqing Liu (G. Mantano lab)—Microdevice applications of kinesin
LANL & Center for Integrated Nanotechnology (CINT)

Postdoc training

George Bachand—Microdevice applications of kinesin
Sandia & Center for Integrated Nanotechnology (CINT)

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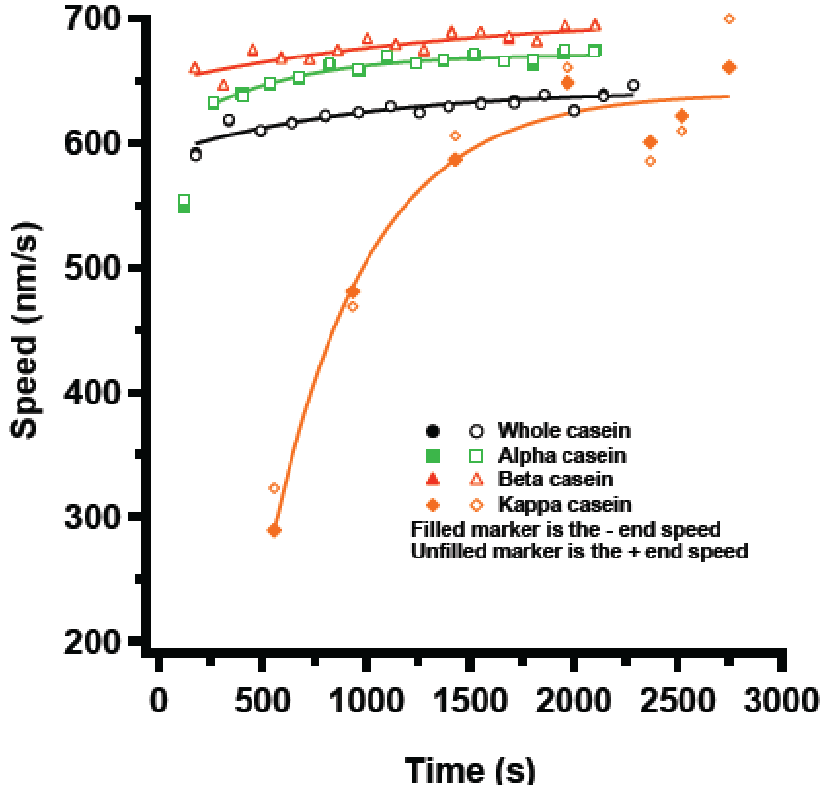
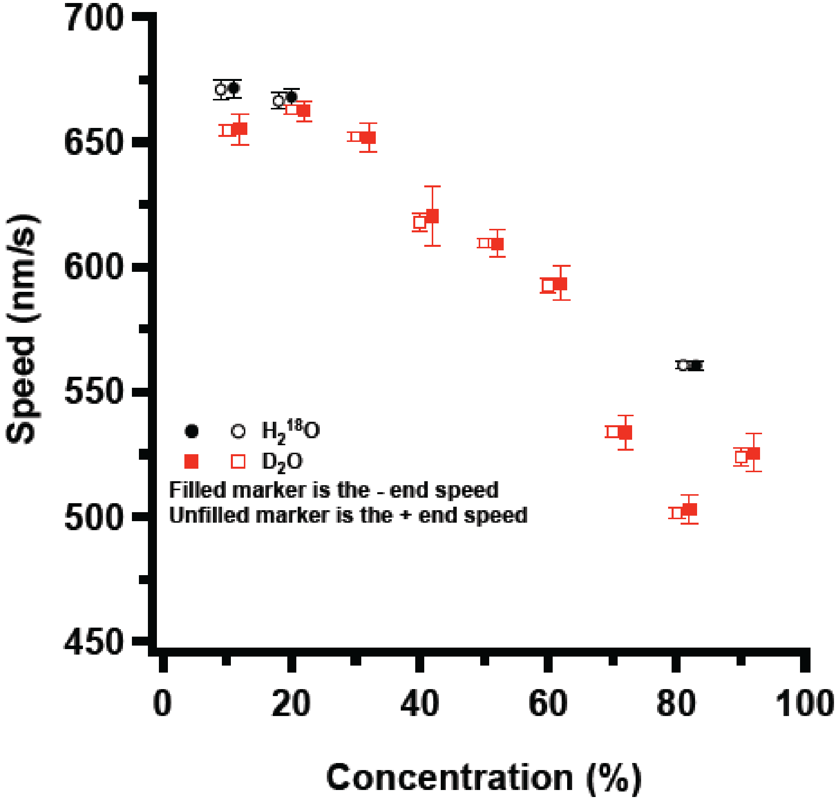
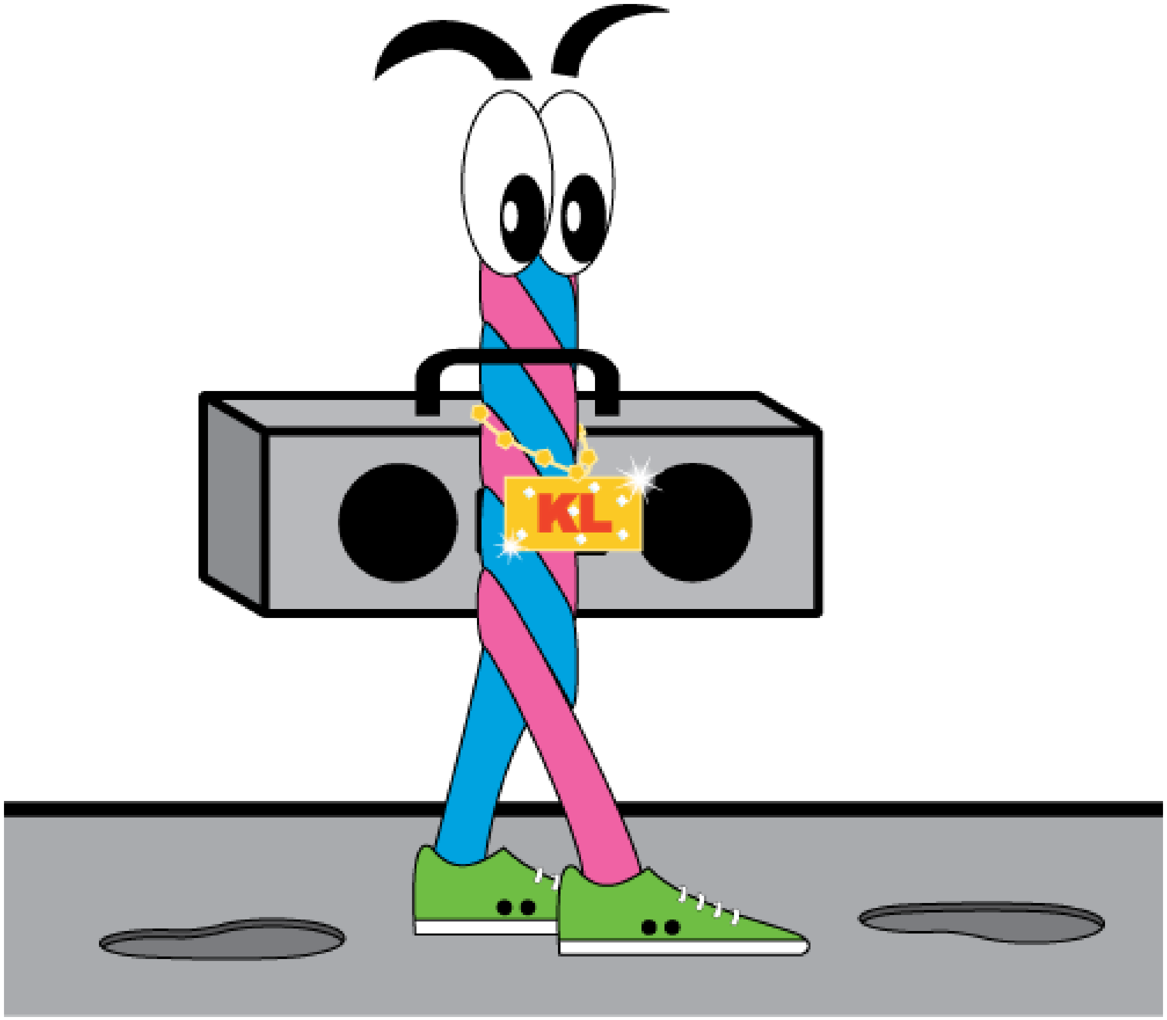
1. Overview of our gliding assay approach



2. Preliminary results isotope and osmotic stress effects in kinesin-1 gliding assays.

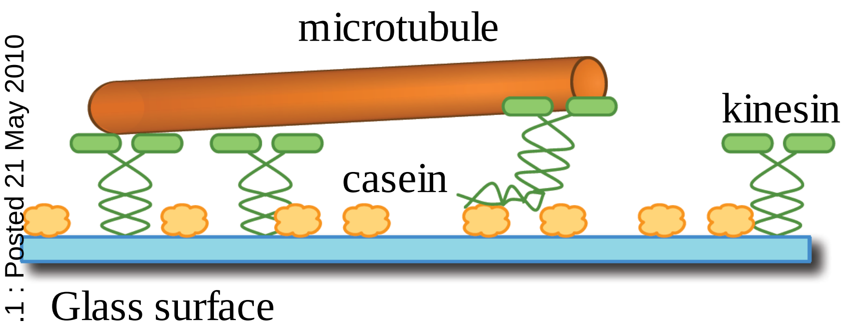


Kinesin / MT introduction



"Kiney"

Gliding motility assay



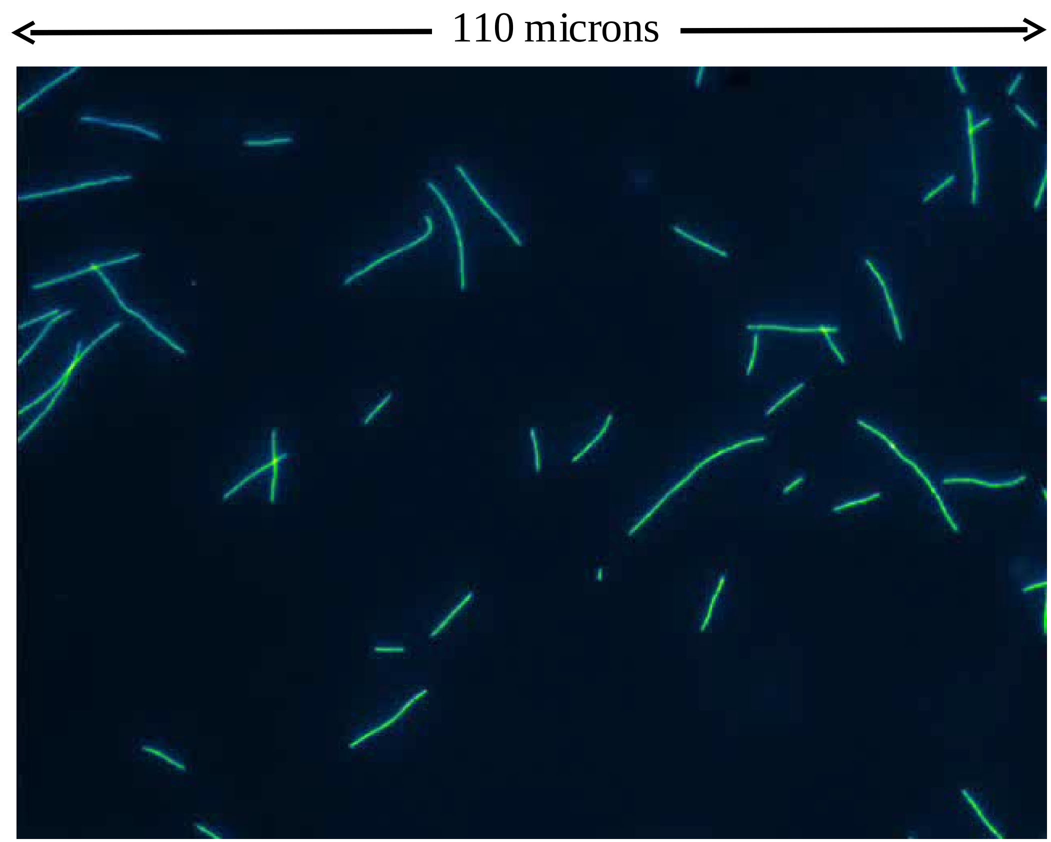
Andy Maloney
Gliding motility assays
- Caseins
- Heavy Water
- Osmotic Stress

Assay details

Dmk401 30 ug / ml

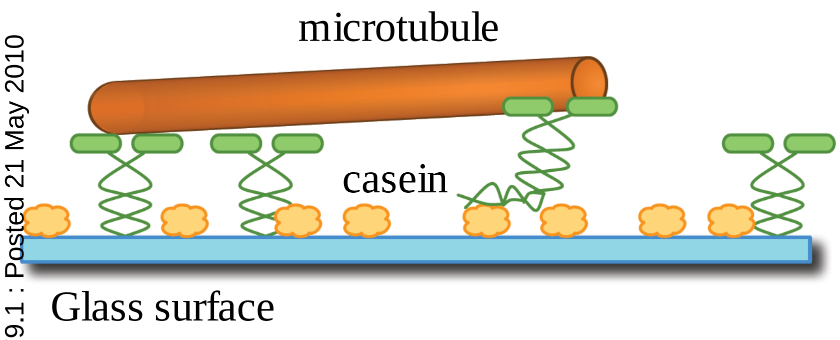
MTs 29% TRITC cytoskeleton bovine

Room temp. (See Andy's poster for more details)



Video: Beta casein
6x speed; false-colored

Gliding motility assay



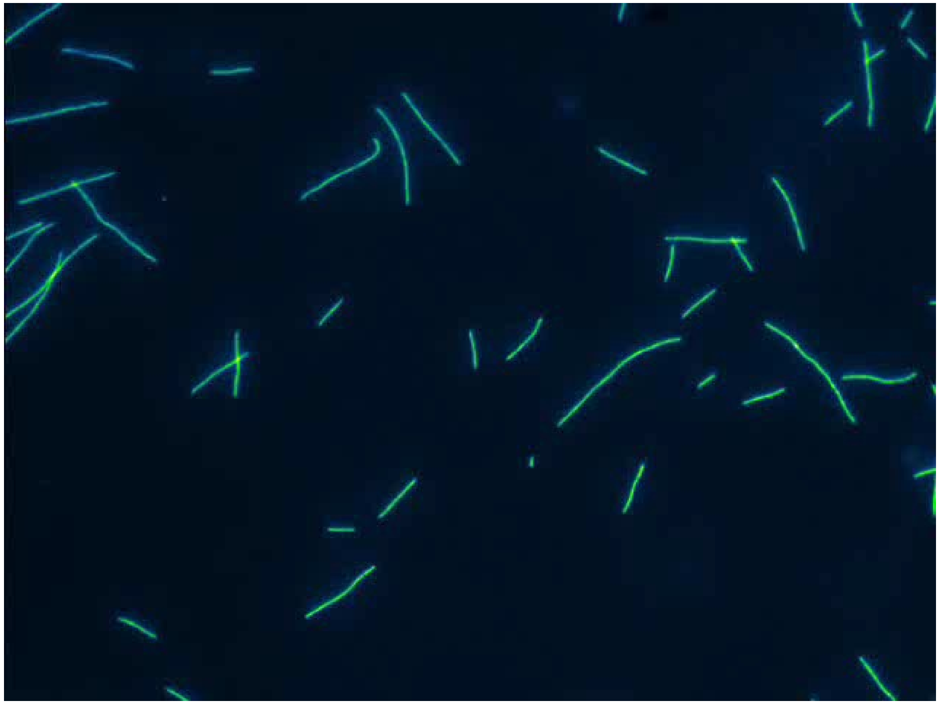
Andy can finish one sample in
1 hour from start to finish

30 minutes of data, ~9,000 images

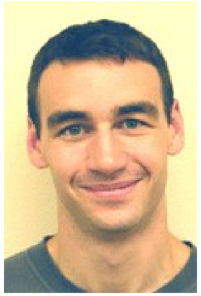
Primary measurement: speed

Also other qualitative measurements

Advantage: lots of data quickly;
robust assay



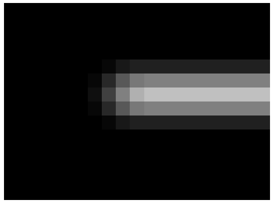
We've developed an open-source, automated microtubule tracking and speed analysis platform



Larry Herskowitz
Image tracking
Stochastic Simulation
Image Simulation

1. Automated Tracking of MTs

Template for pattern matching

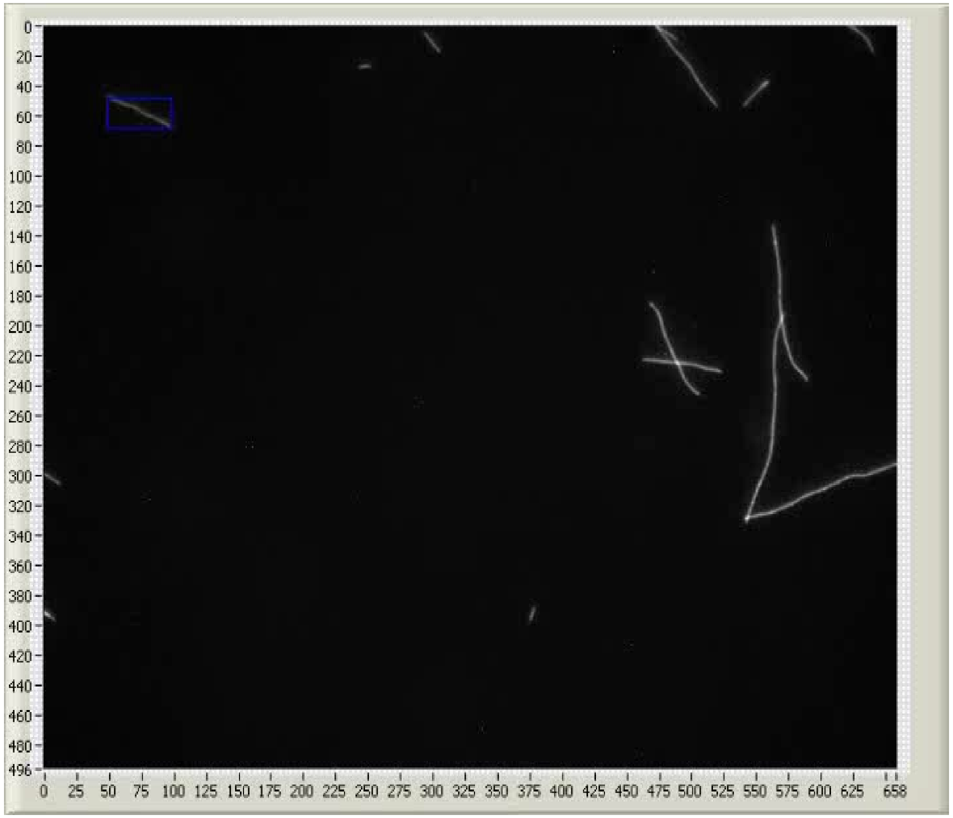


MTs identified with NI Vision 7.1
Segmenting Routines

Position and Angle of MT ends
found via image
pattern matching

X,Y position versus time recorded
for all MTs in folder heirarchy

30 minutes of raw data processed
in approx.
1 hour using 4 cores



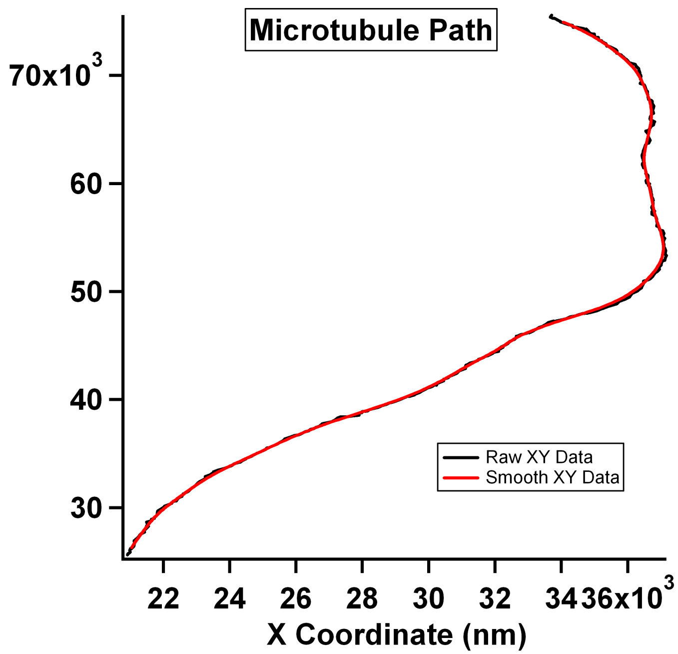
See Larry's poster today for details

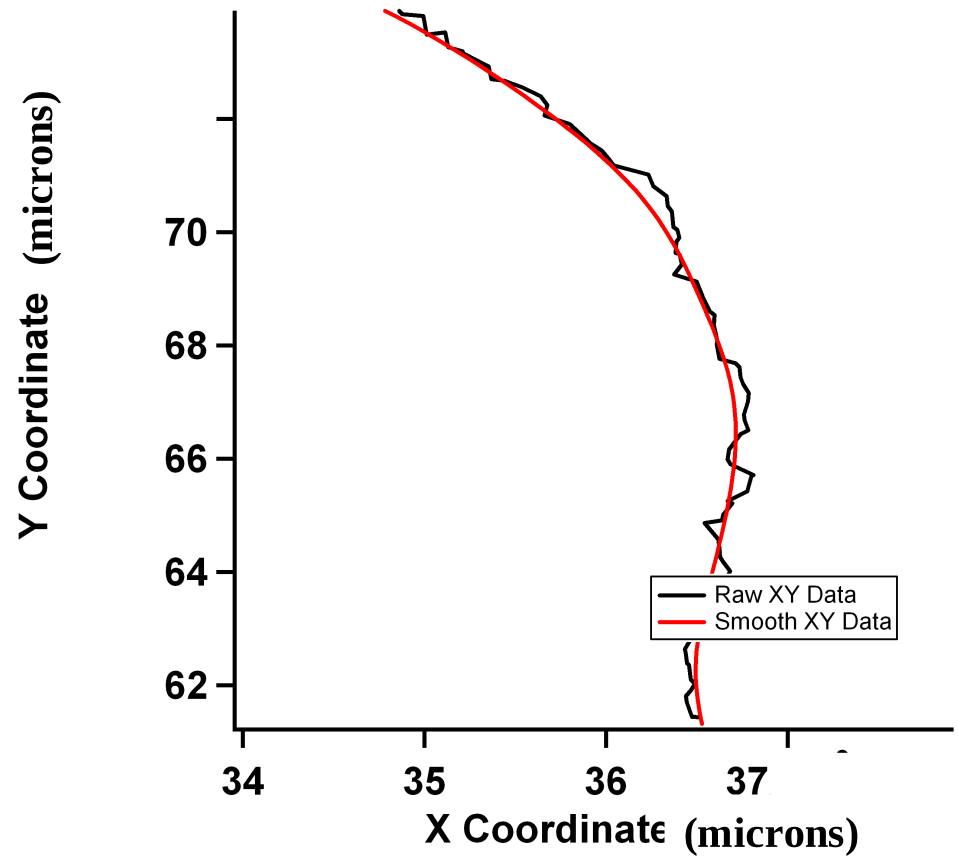
2. Automated Speed Analysis

A. Track smoothing

X, Y data are noisy; dominated by Brownian motion of MT ends

Smoothed with Gaussian-weighted 2 second window





2. Automated speed analysis

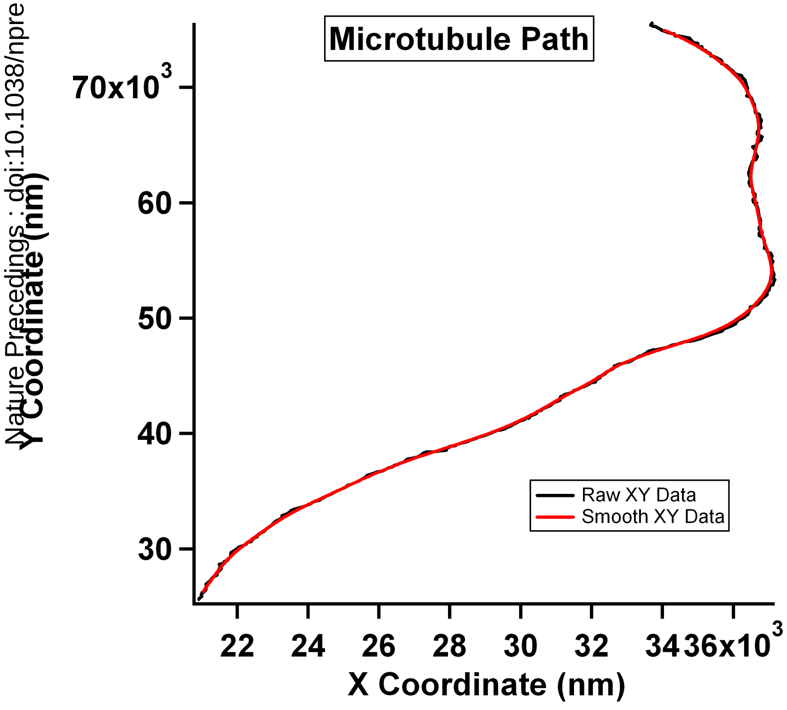
B. Instantaneous speed

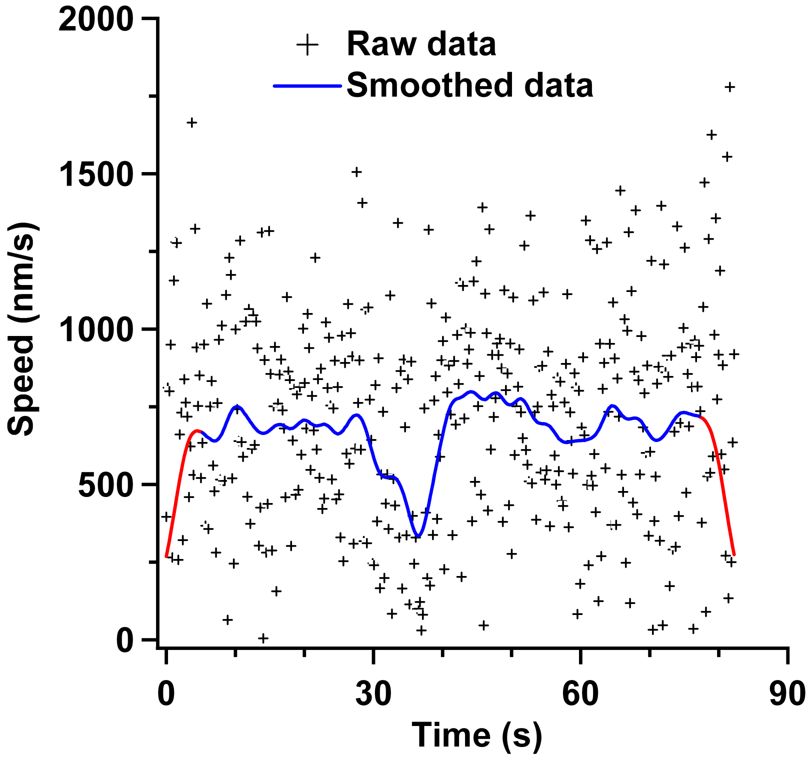
$$Speed_i = |\Delta r_i| / \Delta t_i$$

Alternative to fitting paths to simple shapes

Careful for smoothing errors

-> *What is gliding speed anyway?*



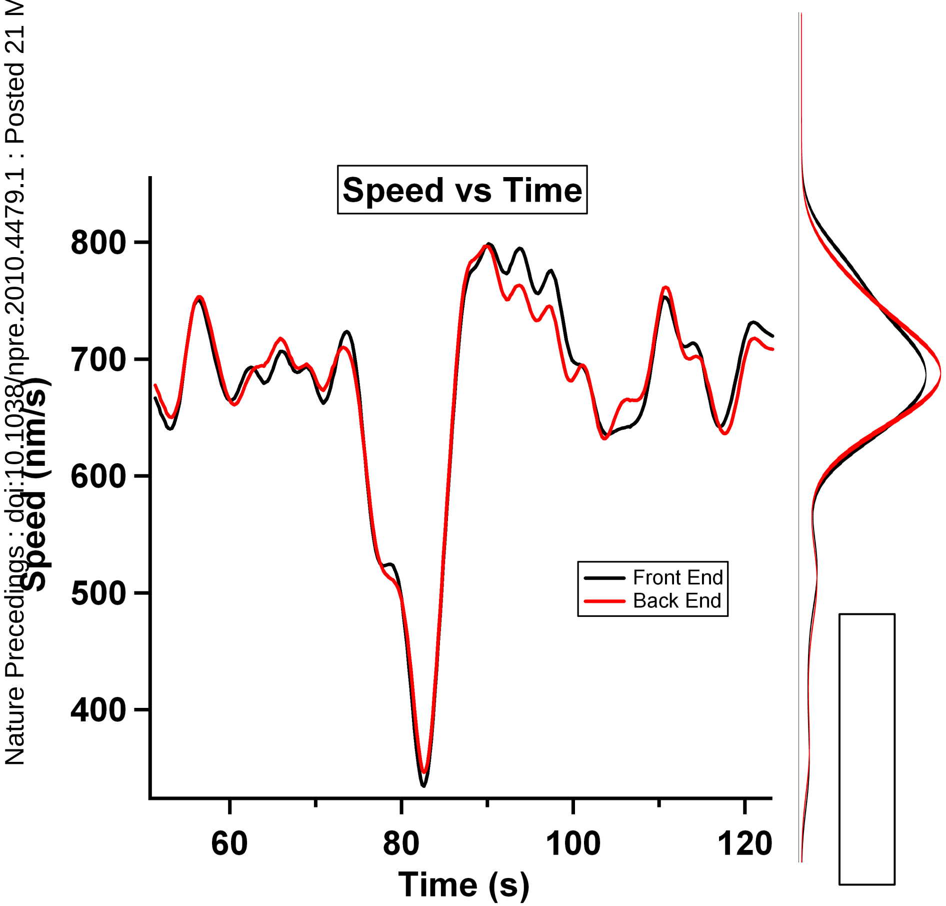


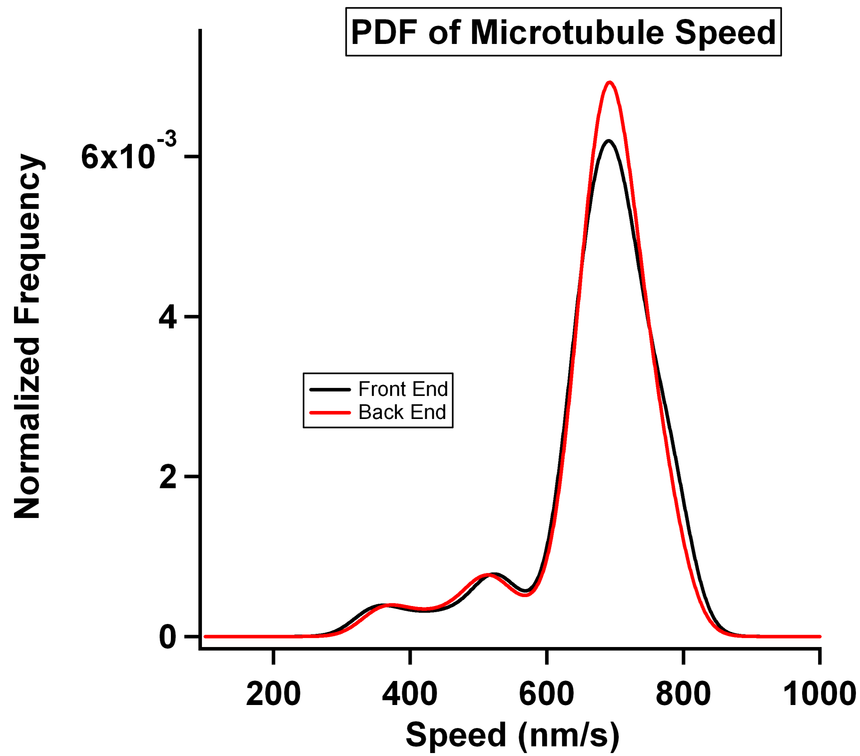
2. Automated speed analysis

C. Kernel density estimation of speed probability distribution function (PDF)

Gaussian kernel, bandwidth 50 nm / s

Silverman 1986 Density Estimations



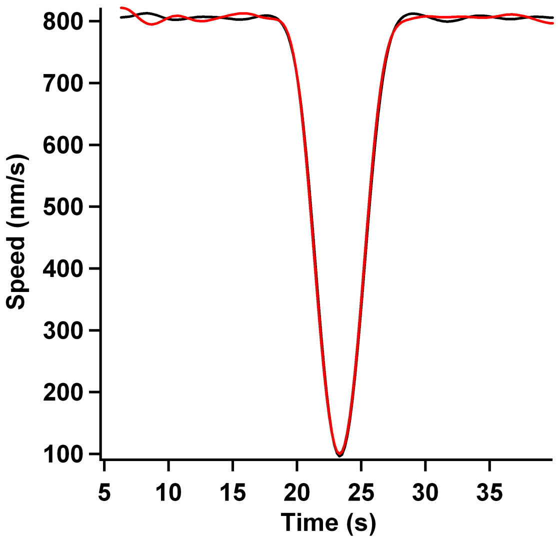


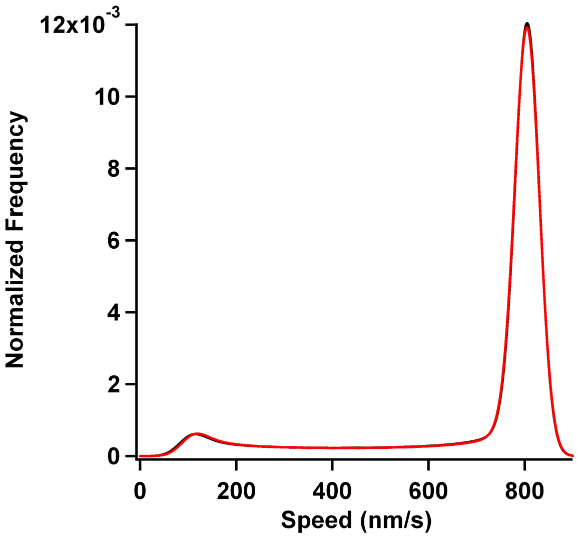
2. Automated speed analysis

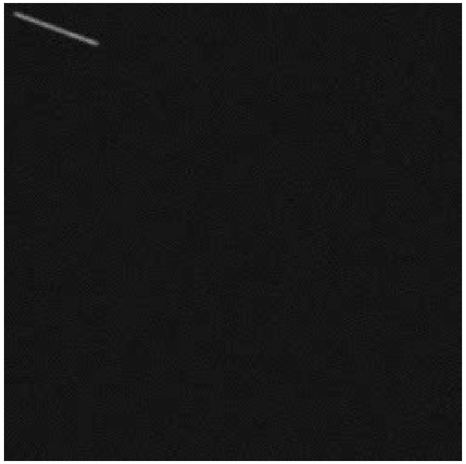
C. Kernel density estimation of speed probability distribution function (PDF)

We checked our algorithm using simulated microtubule image series*

constant gliding speed w/ pause of random duration







* Herskowitz and Koch (2010) Nature Precedings http://dx.doi.org/10.1038/npre.2010.4374.1

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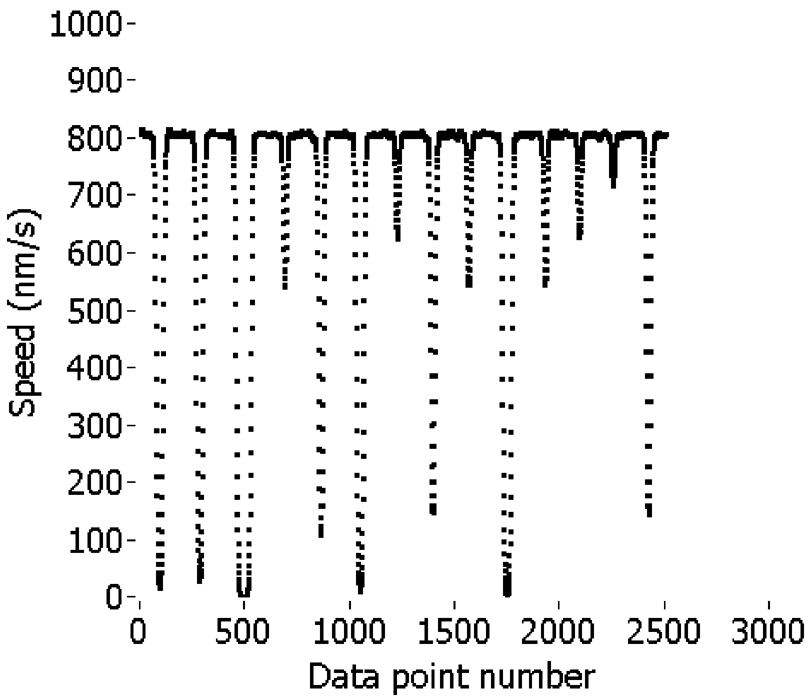
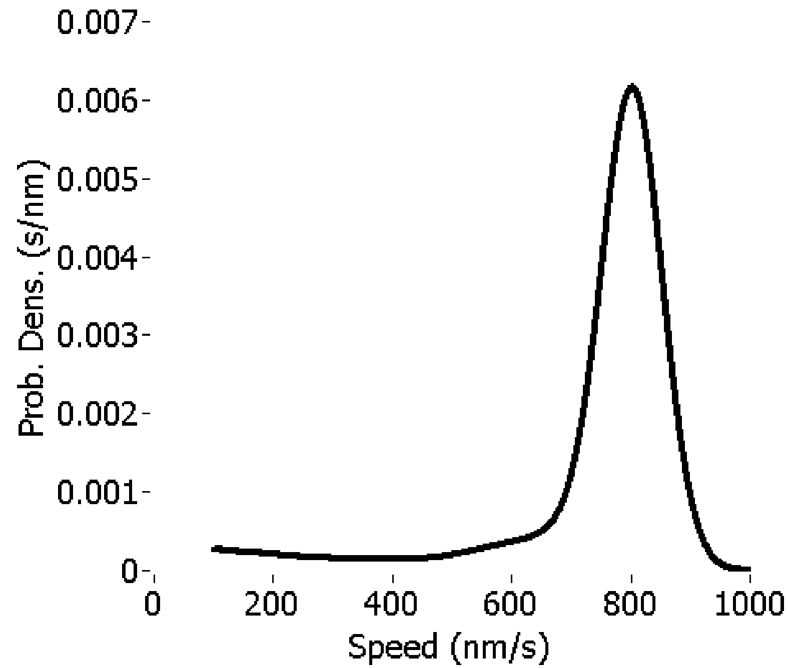




Calculated speed 805.4 +/- 0.2 nm/s (N=15)

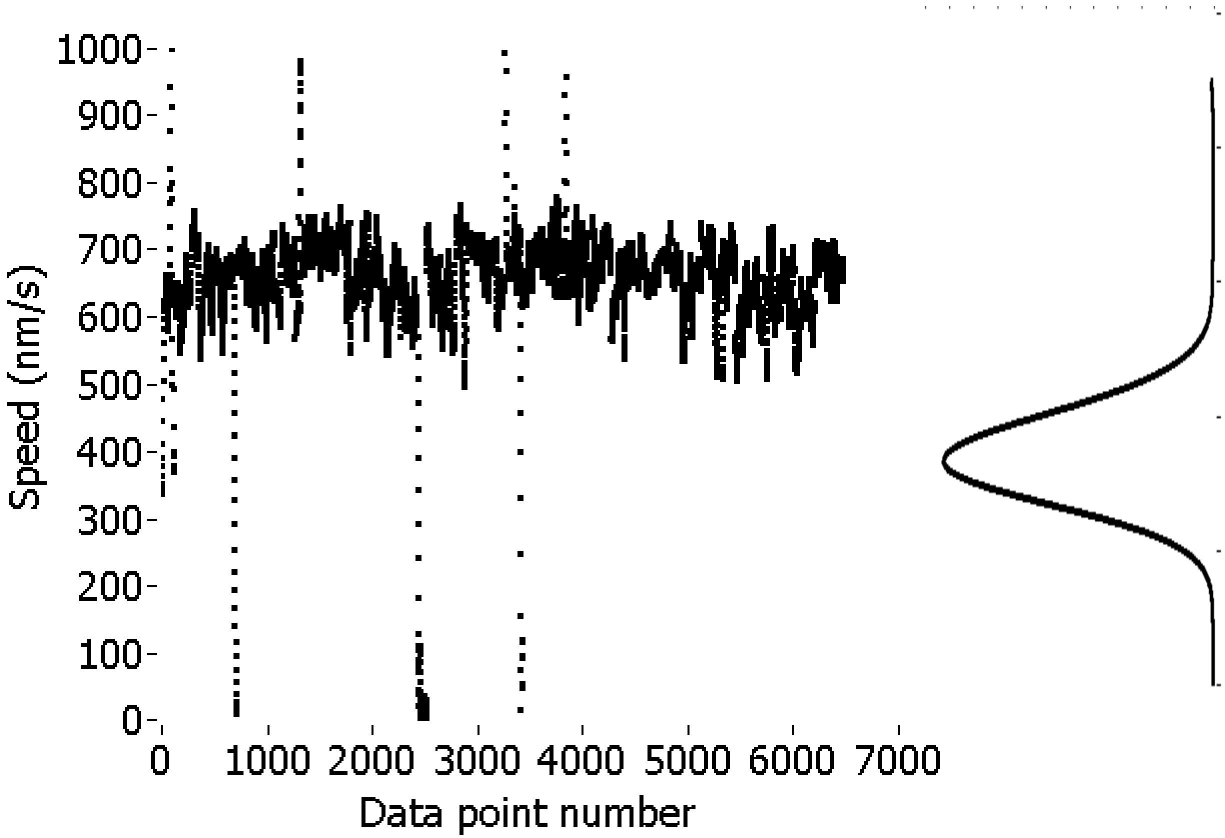
Actual gliding speed 807 nm/s

Pauses probably bias data a bit … more significant with wider bandwidth

2. Automated speed analysis

C. Speed PDF analysis not greatly affected by pauses or tracking errors

Permits automated speed determination for pooled MT tracks for given condition



Most likely speed
670 +/- 5 nm / s

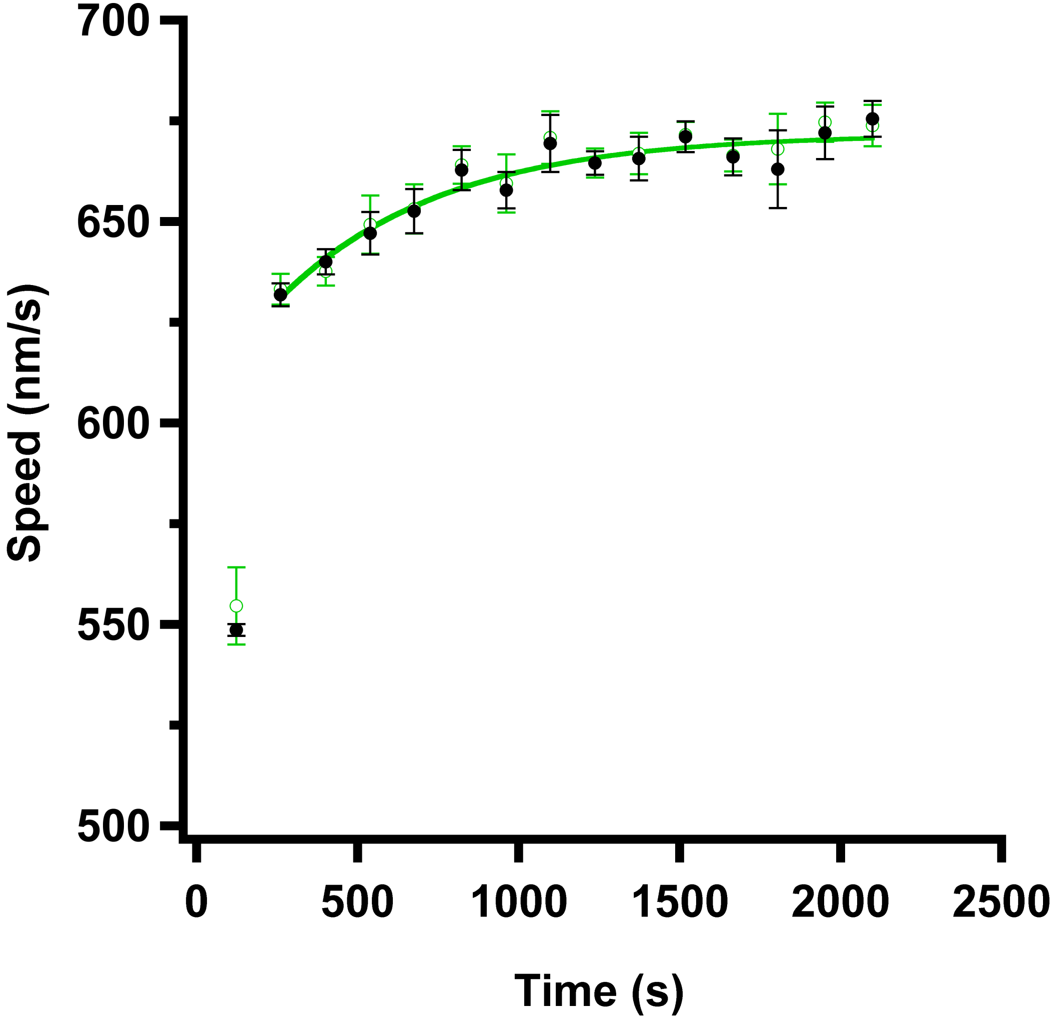
N = 46 microtubule tracks

Two minutes, 600 images, alpha casein data

2. Automated speed analysis

C. Speed PDF analysis not greatly affected by pauses or tracking errors

Permits automated speed determination for pooled MT tracks for given condition



Alpha casein passivation

N=710 MT tracks

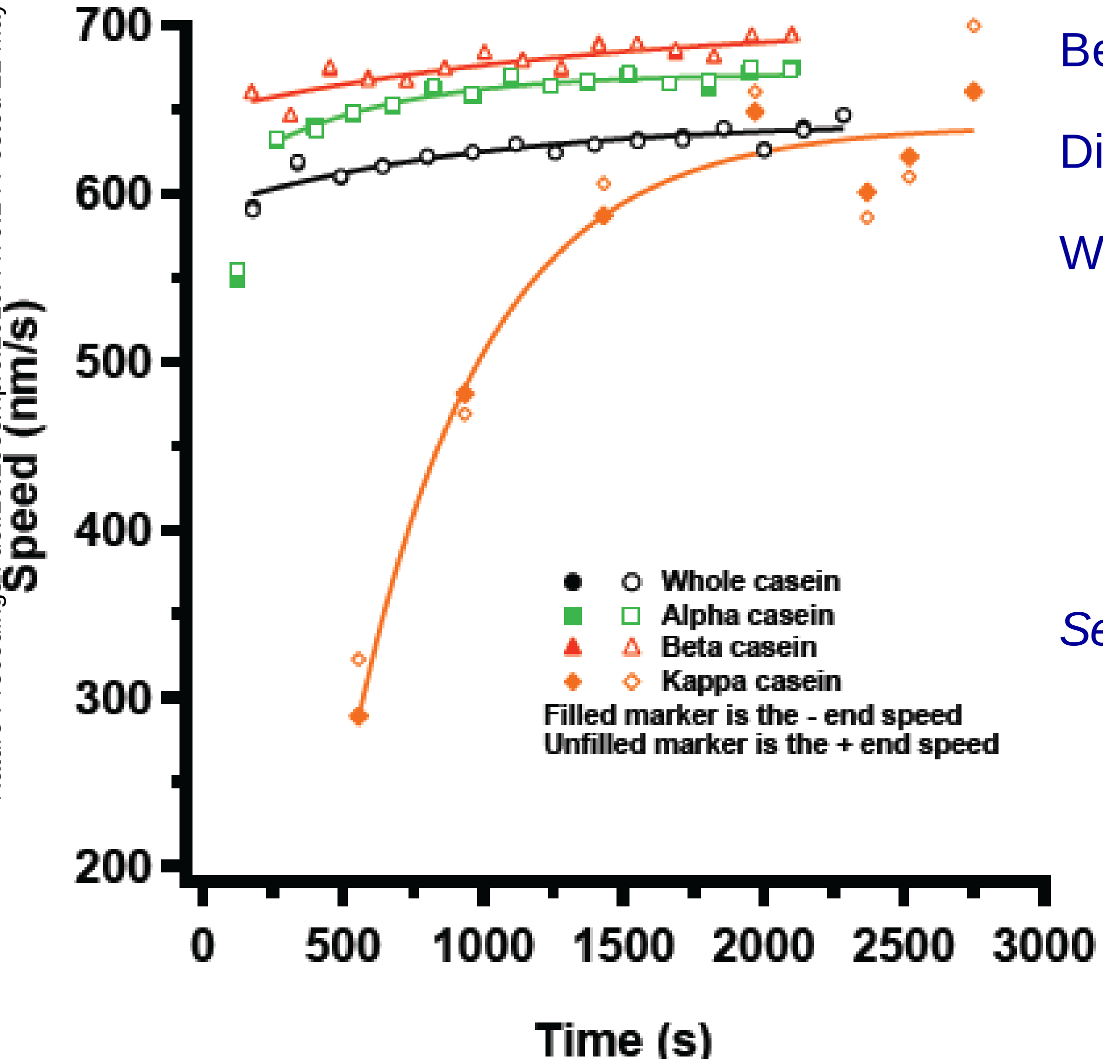
~30 minutes assay time

Analysis time ~10 minutes on one core

2. Automated speed analysis

Speed gradually increases with time for all casein varieties

Intrinsic speed differences between varieties



Beta casein highest speed

Differences in active motor density?

We chose alpha casein and these analysis methods for subsequent isotope and osmotic stress studies

See Andy Maloney's poster for more details

See also: Verma, V., Hancock, W. O., & Catchmark, J. M. (2008). *Journal of biological engineering*, *2*, 14. doi: 10.1186/1754-1611-2-14.

Varying the properties of water may be a useful knob for studying kinesin

Water isotope effects

Heavy-hydrogen water (D_2O)

Heavy-oxygen water ($H_2{}^{18}O$)

Osmotic stress

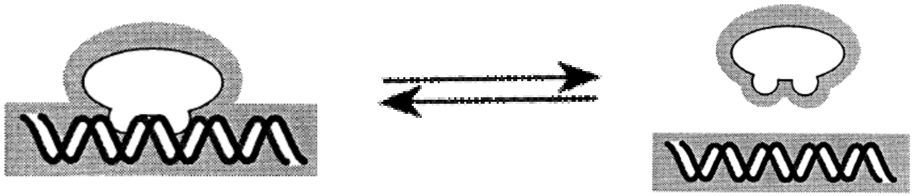
Betaine, Future work: other osmolytes: proline, sucrose, etc.

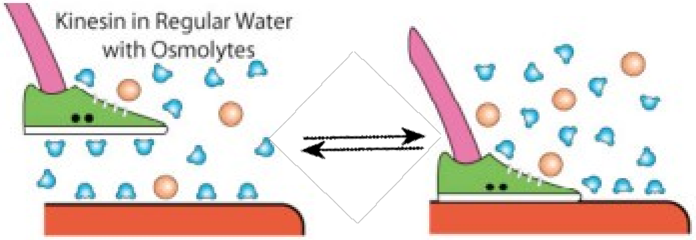
Future: denaturants

May be a method for probing large-surface area interactions between kinesin and tubulin

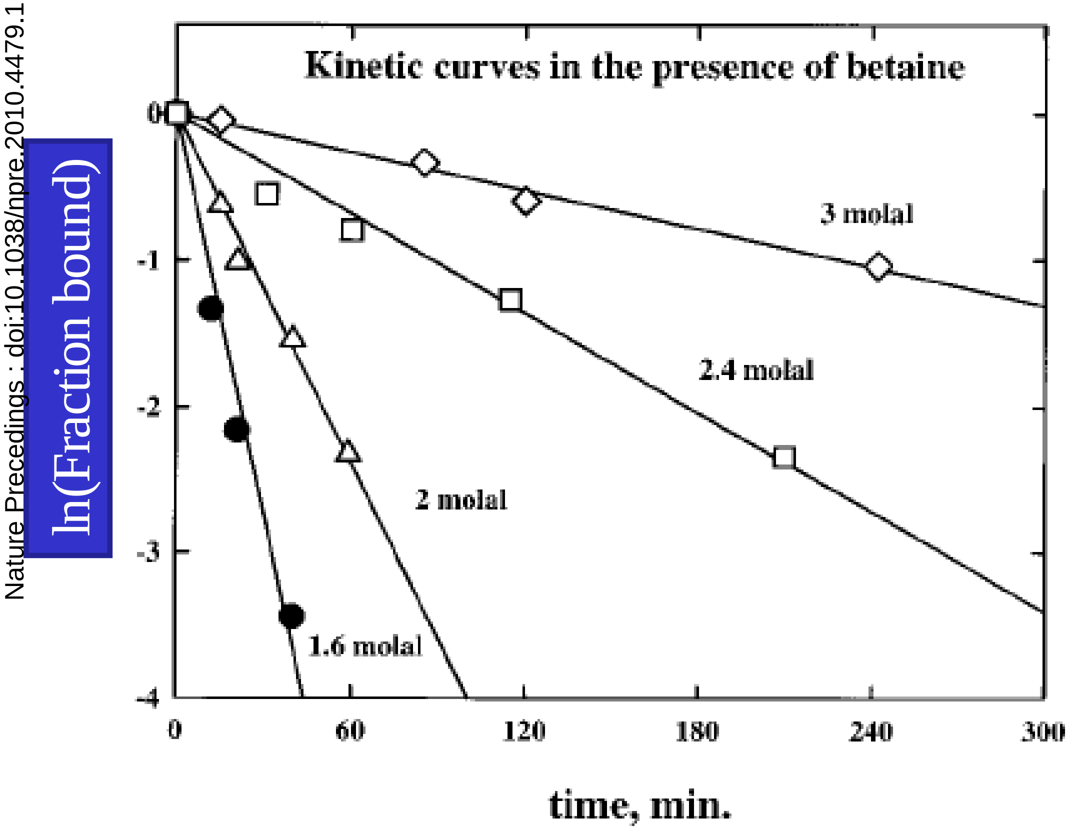
Osmotic stress dramatically increases lifetime of bound molecular complexes

An example from protein-DNA interactions





No kinesin studies yet;
A few myosin studies



Sidorova and Rau
Biopolymers, Vol. 53, 363–368 (2000)

$$\frac{d[\ln(k_{\text{off}})]}{d[\text{osmolal}]} = -\frac{\Delta N_w}{55.6}$$

Off rate reduced by >100 fold

Heavy-hydrogen water (D2O) has been used to probe the actomyosin system

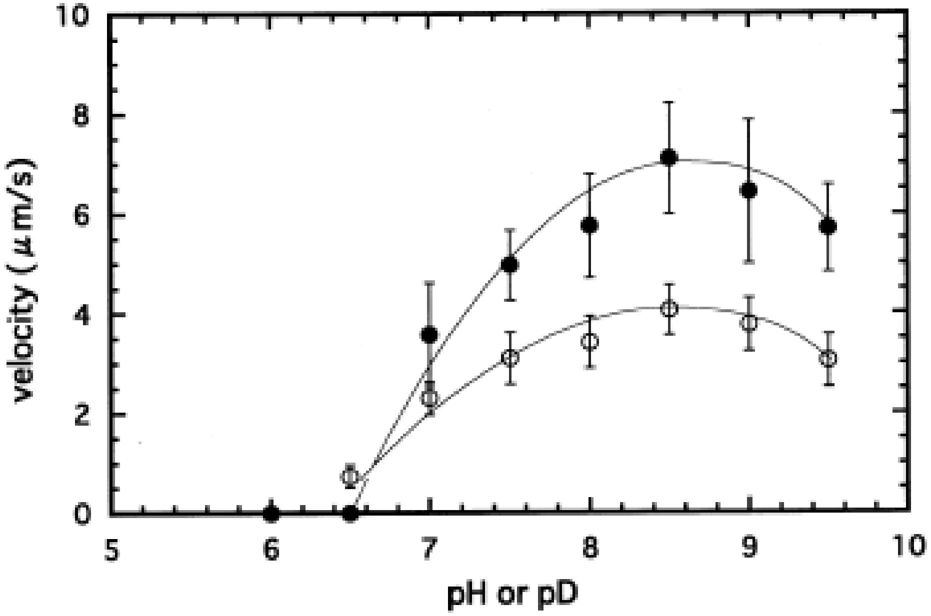


Fig. 1. Dependence of the actin sliding velocity on pH (●) and pD (○) of the H_2O and D_2O assay solution, respectively. Each data point represents the average velocity of 11 different filaments, and the vertical bar shows the standard deviation. The curves were drawn by eye.

Chaen, S., et al (2001). Effect of deuterium oxide on actomyosin motility in vitro. *Biochimica et biophysica acta*, *1506*(3), 218-23. Pub med: 11779555.

The authors list among many possible explanations, an effect on myosin-actin affinity.

How should water isotopes affect kinesin motility?

Answers from molecular dynamics?

Another reason we're looking at water effects is our partnership with Atlas / Valone labs

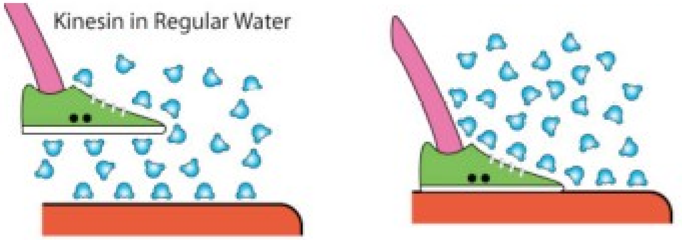
- Susan Atlas (UNM; PI of our DTRA project) and Steve Valone (LANL) are developing the "Charge-Transfer Embedded-Atom Model (CT-EAM)" force field for MD simulations of kinesin and other biomolecules.

  Incorporates "correct" quantum mechanics so that charge transfer can be handled.

  One of the first benefits of CT-EAM will be better simulation of water dynamics…including for differing isotopes.

- Big disconnect between length and time scales for gliding assay and MD

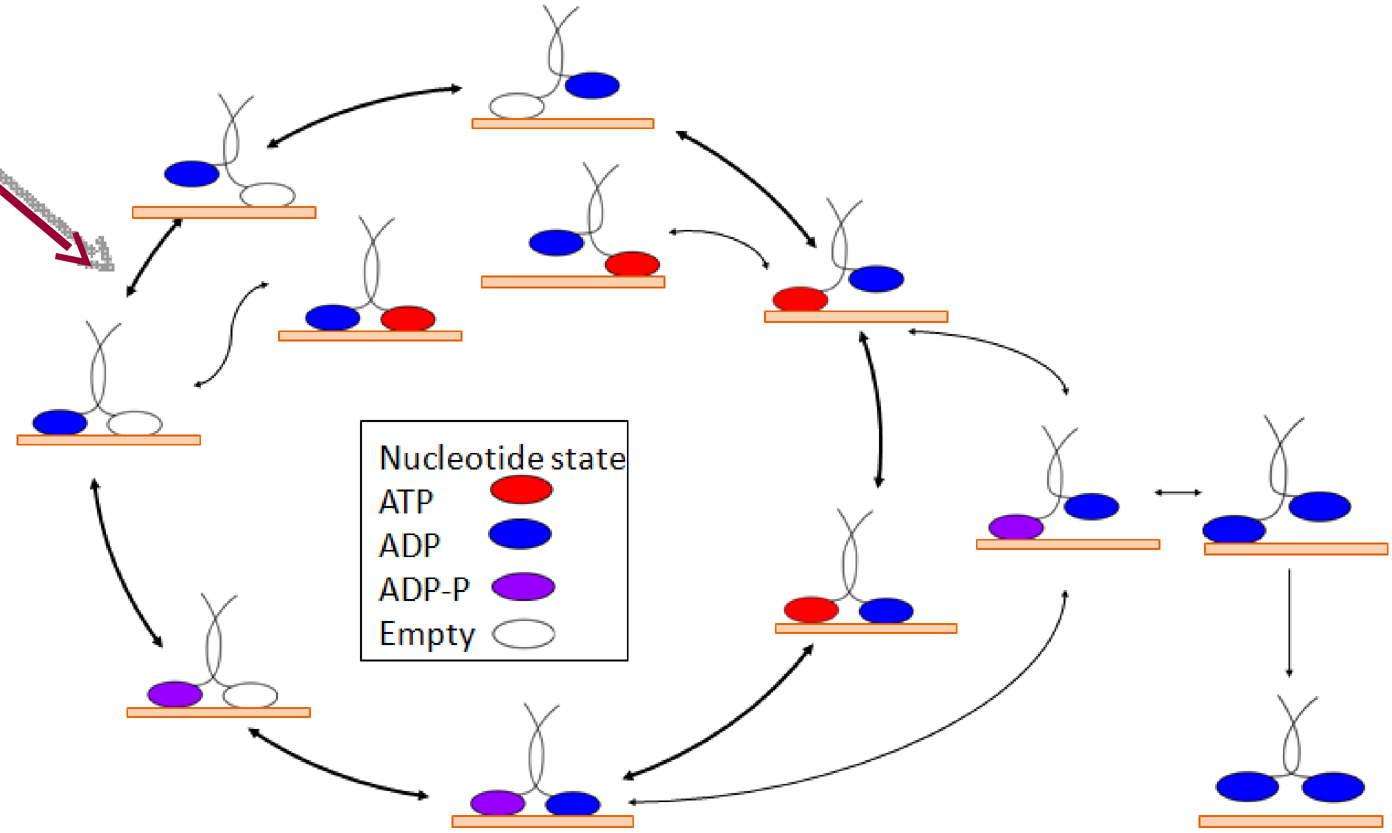
  But both can look at water dynamics.
  MD: How many hydrating water molecules?
  How do water dynamics change with isotope?



Stochastic simulation can help us understand how water might affect kinesin motility

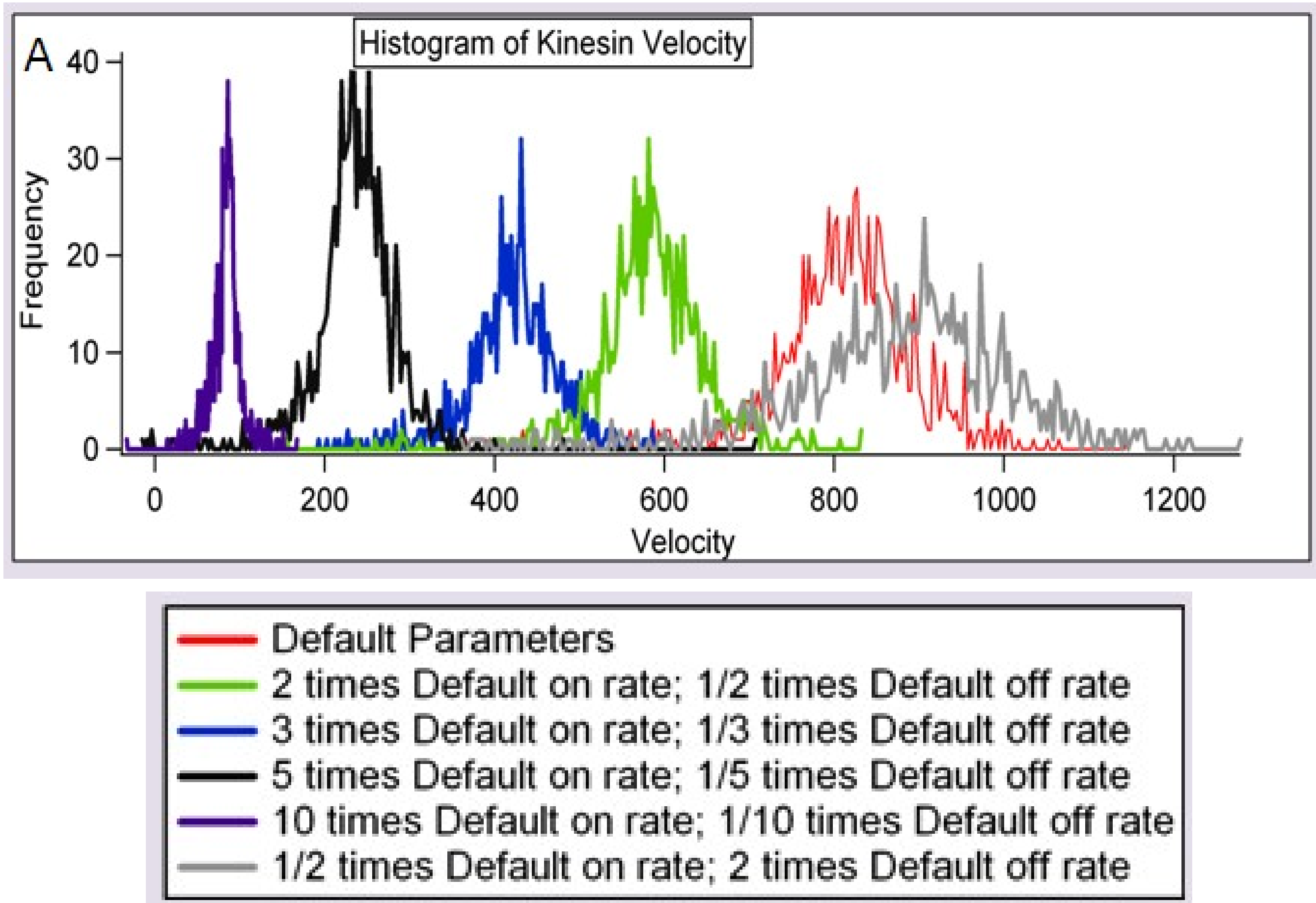
We can ask question: "What will happen if Kinesin-MT off rate decreases?"

The answers aren't always obvious when pathway can branch.



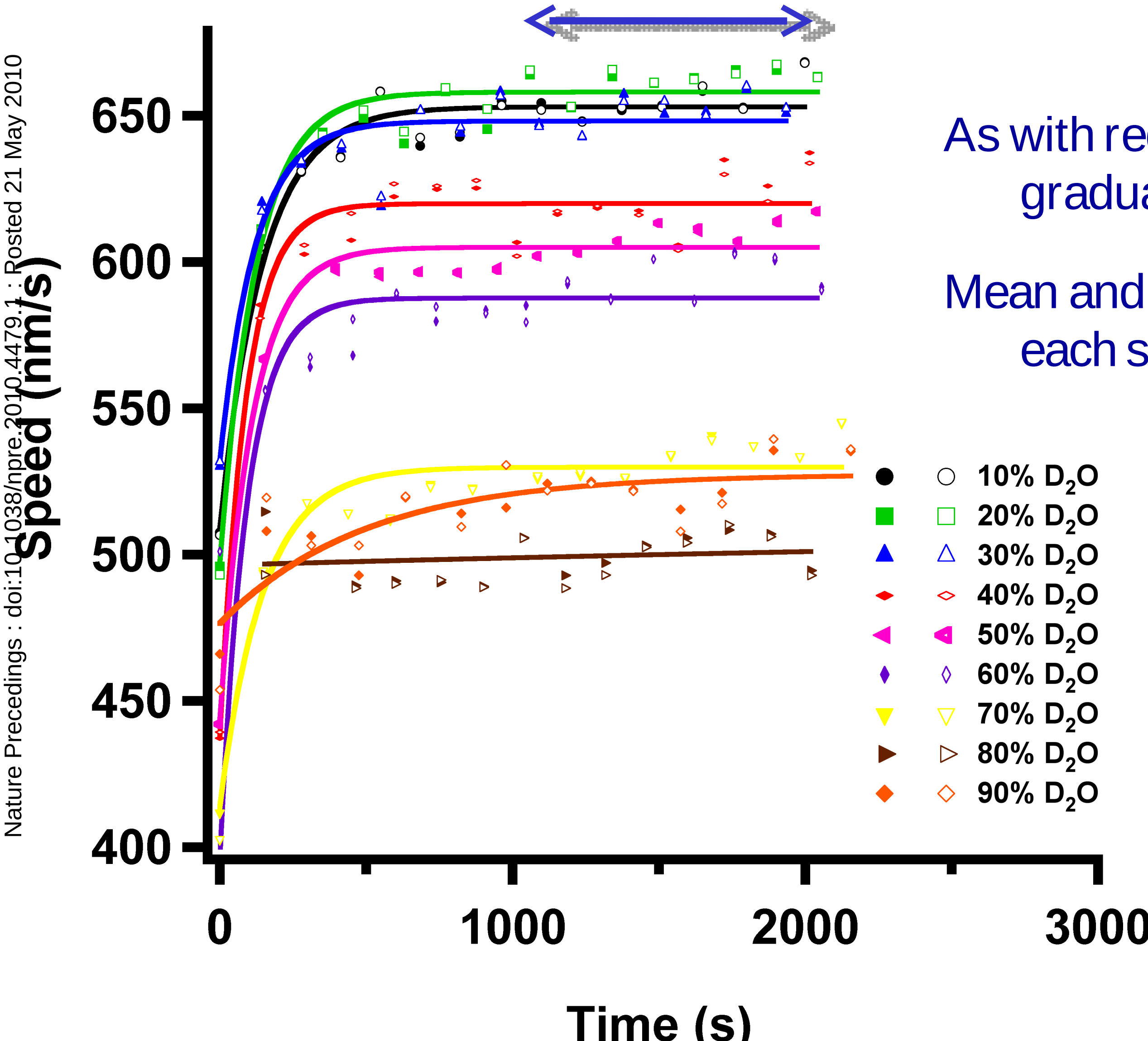
Larry Herskowitz has developed an agent-based stochastic simulation to help us interpret and guide experiments
more info at his poster

Stochastic simulation can help us understand how water might affect kinesin motility



Stochastic simulations tentatively indicate that increased osmotic pressure or increased water mass would decrease kinesin speed.

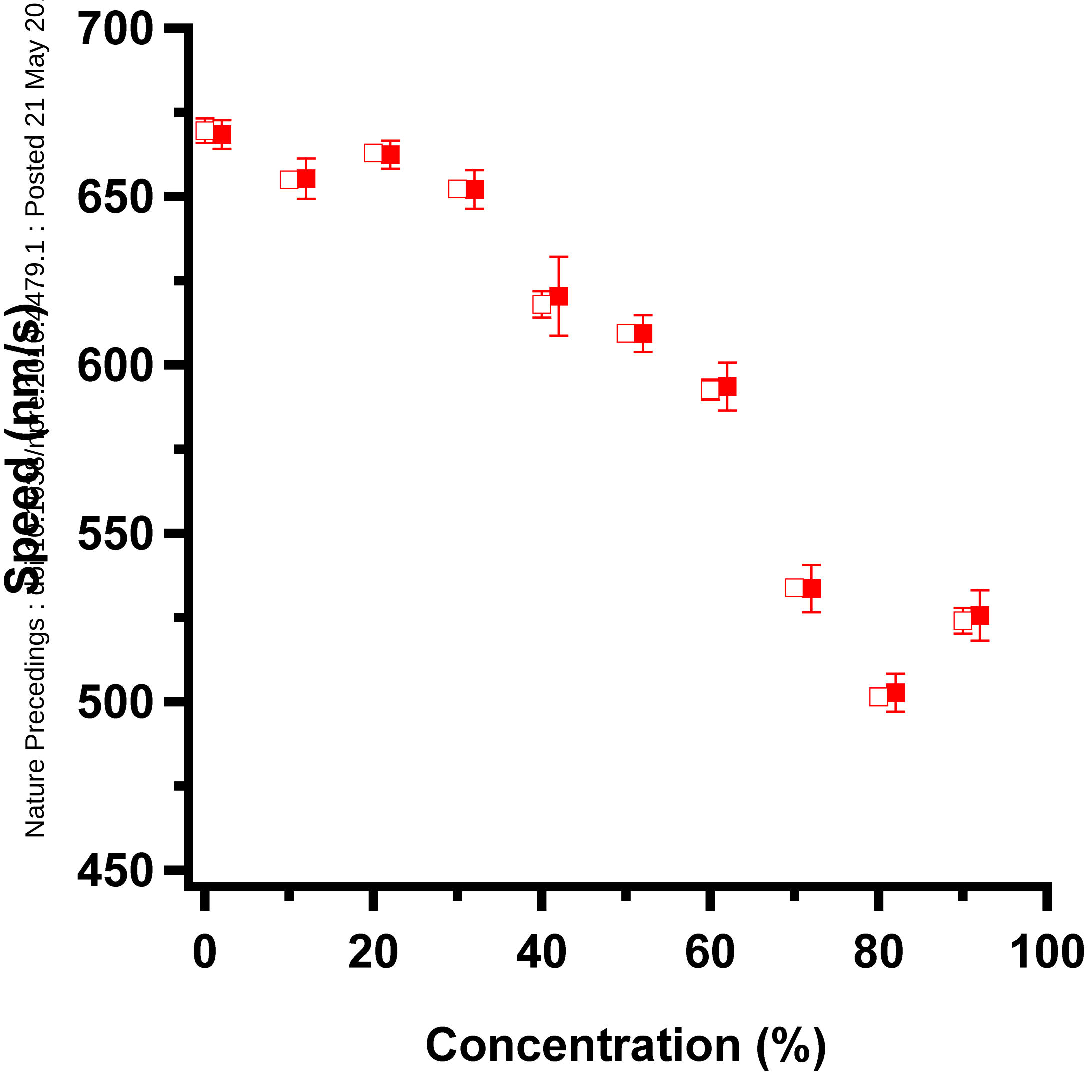
Heavy-hydrogen water reduces MT gliding speed.



As with regular water, speed gradually increases with time

Mean and SEM for last 8 points of each set.

Heavy-hydrogen water reduces MT gliding speed.



Speed steadily decreases as deuterium conc. Increases.

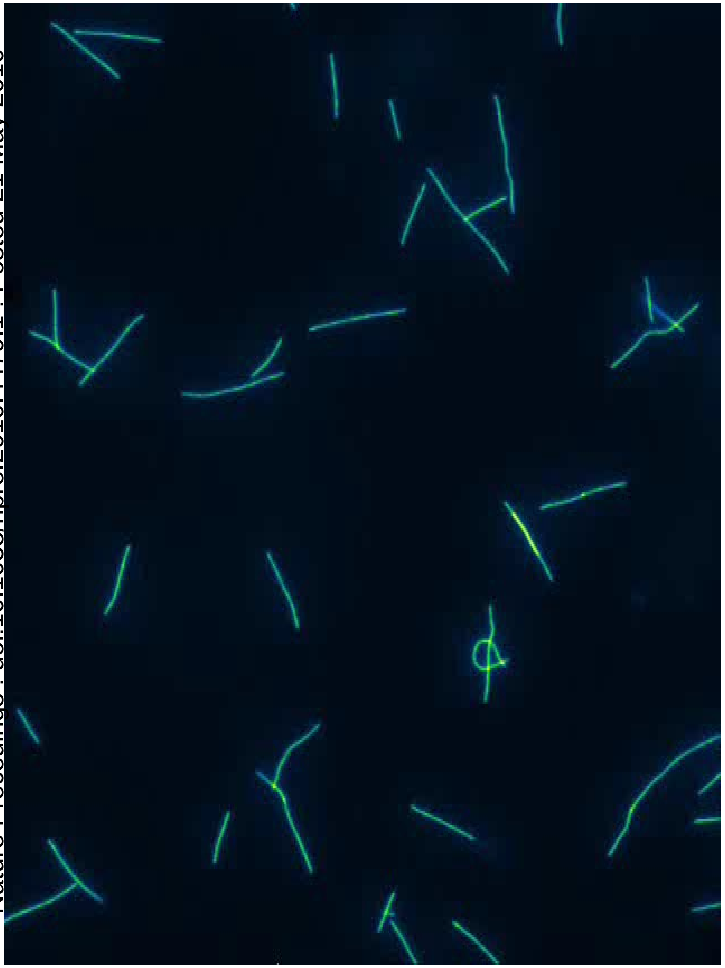
Also, noticed other features (preliminarily):

Reduced photobleaching

Longer-lasting MTs

MT-MT sticking

Other features seen in D2O assays



Also, noticed other features (preliminarily):

Reduced photobleaching

Longer-lasting MTs

MT-MT sticking

It turns out there is existing literature to support all of these observations

Heavy-hydrogen water has significantly different properties from “regular” water

Naturally abundant 1 / 6600 hydrogen molecules is deuterium

17 mM deuterium in “standard mean ocean water”

11% denser than H_2O. Freezes at 3.8C. D-bonds stronger.

Toxic to eukaryotes. The toxic effects are similar to chemotherapeutic drugs.

D_2O has been used to stabilize viral vaccines.

D_2O stabilizes tubulin and microtubules.

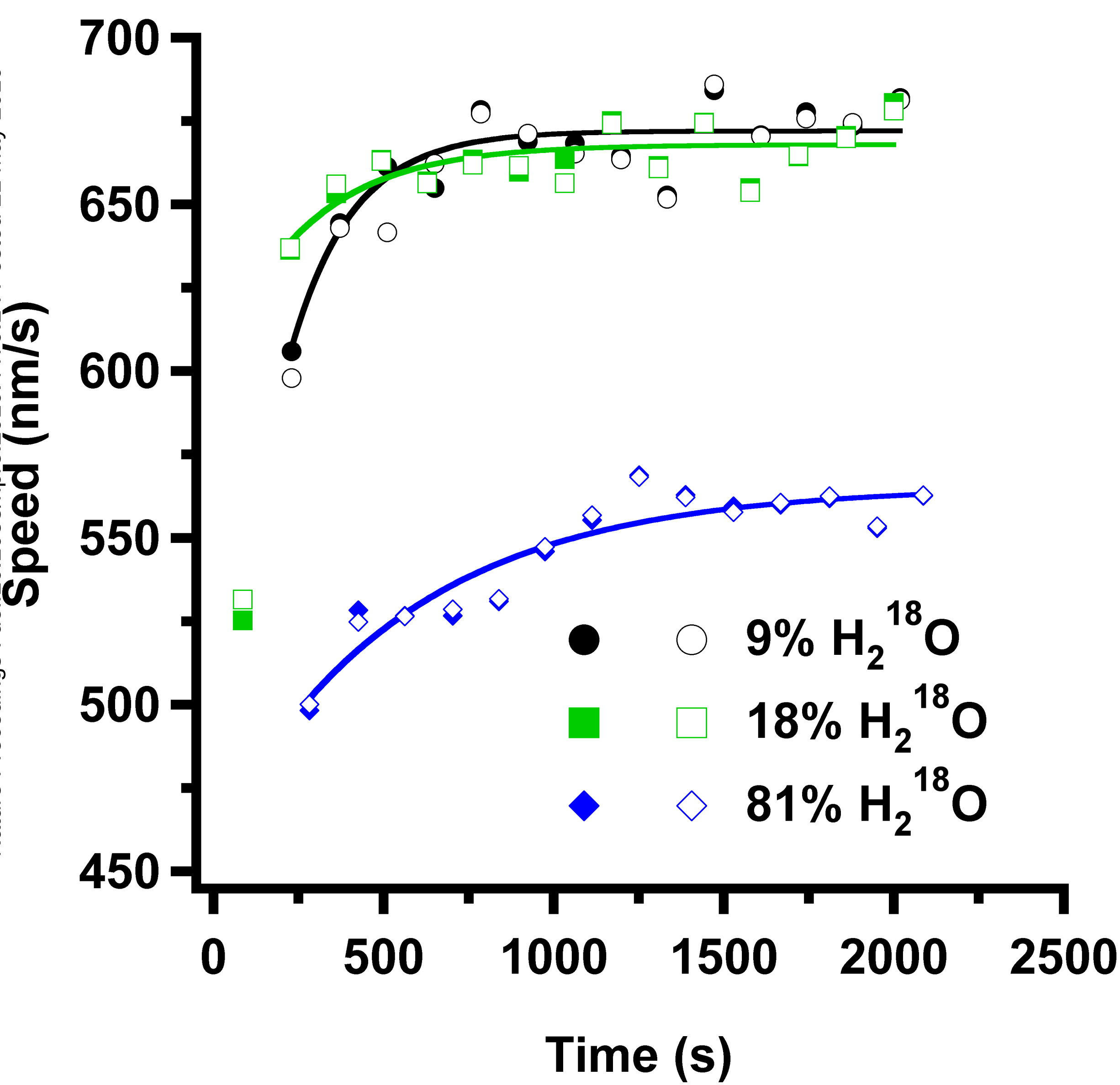
D_2O stimulates tubulin assembly formation.

(Other fascinating factoids…)

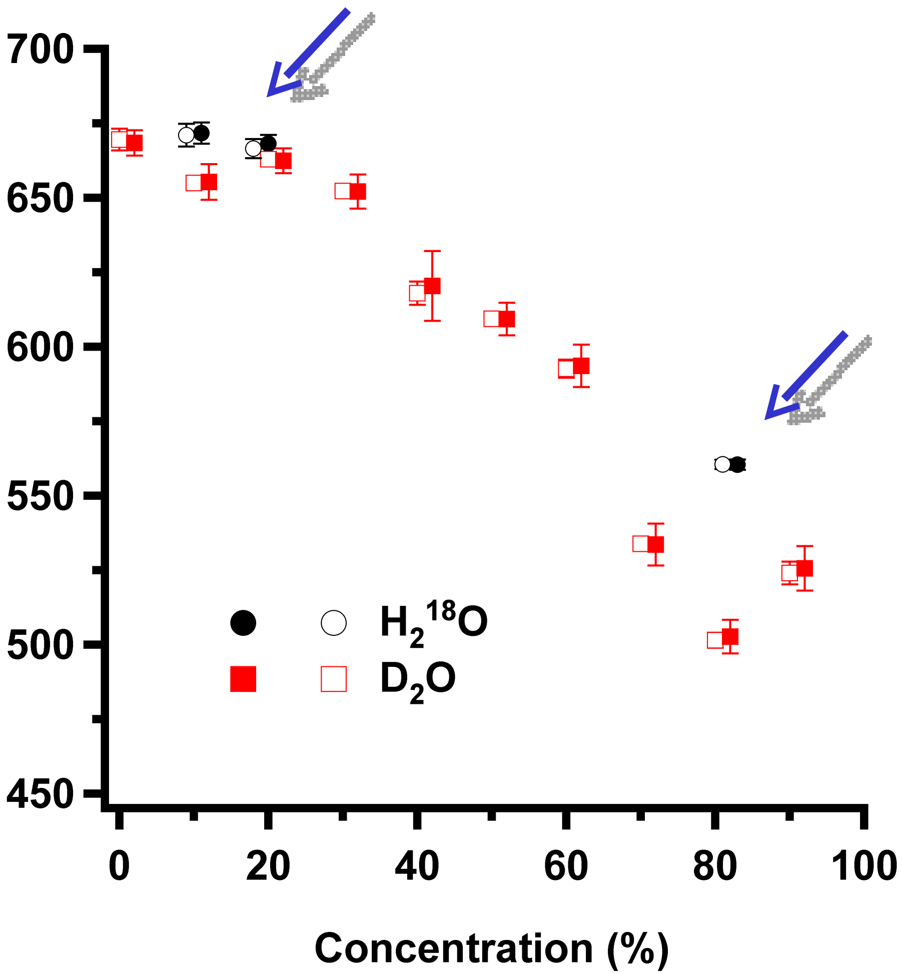
Effects on kinesin motility has not yet been studied

Interesting … but very complicated

Heavy-oxygen water



Surprisingly, heavy-oxygen water has similar effect on gliding speed



Chemistry of oxygen-18 water similar to regular water

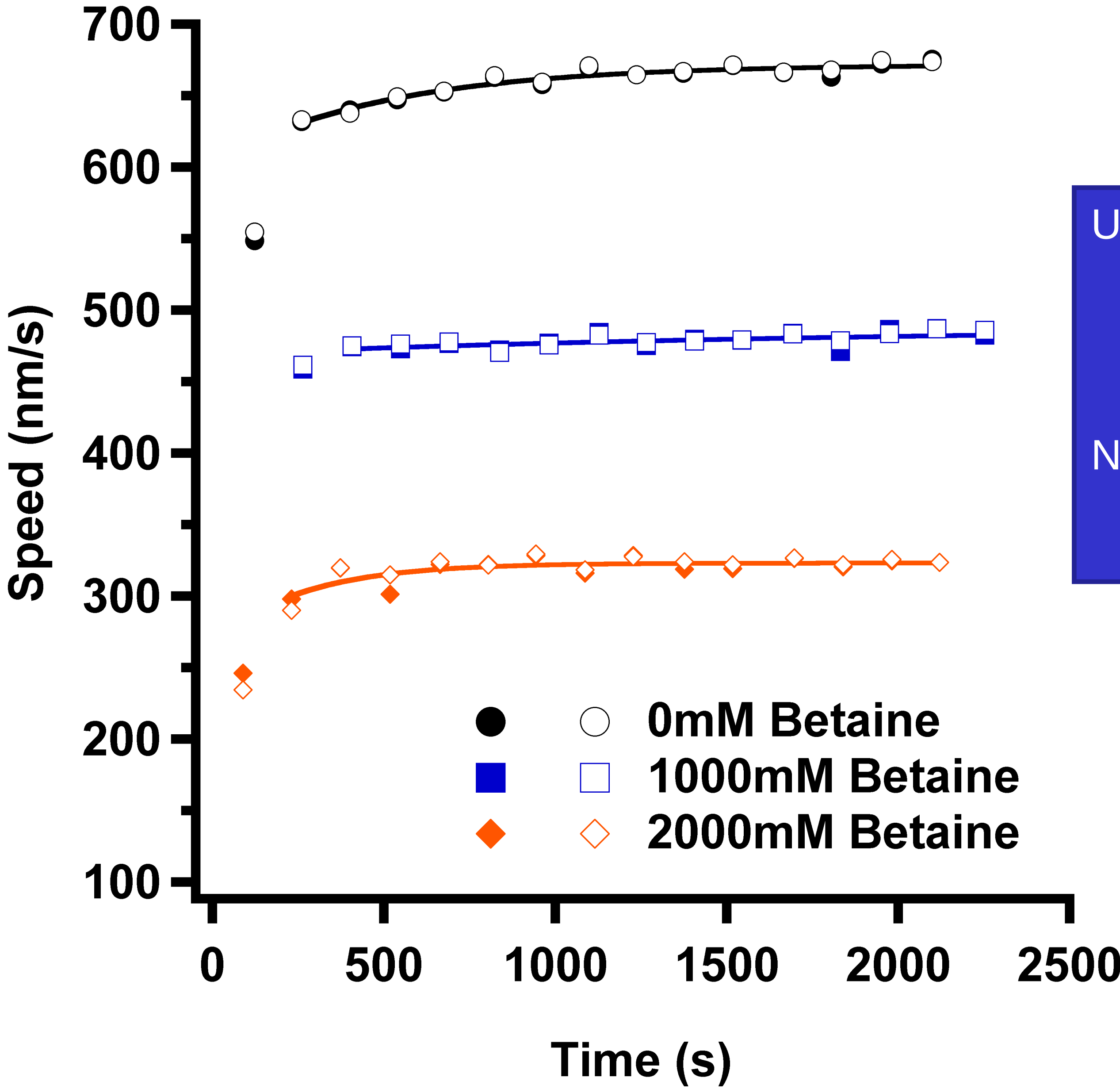
Viscosity of oxygen-18 water only 5% higher than regular water (23% for D2O)*.

Heavy-O water much less confusing, possibly more tractable theoretically. Expensive, though ($1,000 / gram)

Possible that Heavy-O water is a good probe of kinesin-MT surface interaction

*Viscosity: Kudish et al. ***J. Chem. Soc., Faraday Trans. 1***, 1972, **68**, 2041 - 2046, **DOI:** 10.1039/F19726802041

Betaine (osmolyte) substantially reduces gliding speed



Unsure how important viscosity increase is.

Need to repeat with other osmolytes

Current conclusions from water and other experiments

Heavy-oxygen water looks like promising probe of hydration effects

Heavy-hydrogen water may also be interesting probe, but more complicated. May have benefits for stabilization of protein in the lab or in microdevices.

Betaine has strong effect on gliding speed. Viscosity a confounding variable. Need to compare with other osmolytes.

Need a better grasp of solution thermodynamics and kinetics and effect on motor proteins.

Type of surface passivation affects gliding speed. Gliding speed also increases with time.

Open Data, Open-source Software

All data presented here is CC0-licensed and available online:

http://openwetware.org/wiki/Koch_Lab:Data/MT_Gliding_Assay_Readme_File

We're still working on the organization and how to best share. In the meantime:

If useful to you, get in touch and we can help you with the software and / or figuring out the data sets!

Acknowledgments

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Share your science.

Funding

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