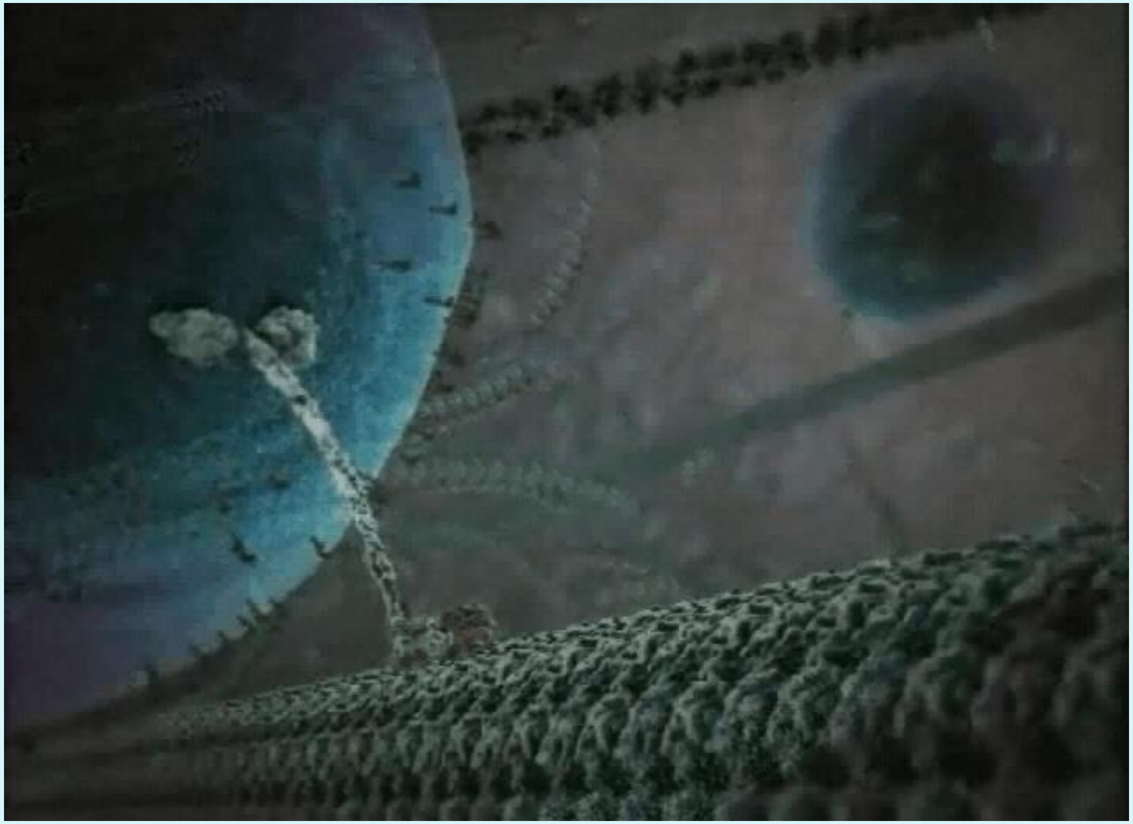
How is motor transport regulated? Discovery of a new kinesin-family regulatory pathway, with an associated detour into heterogeneity

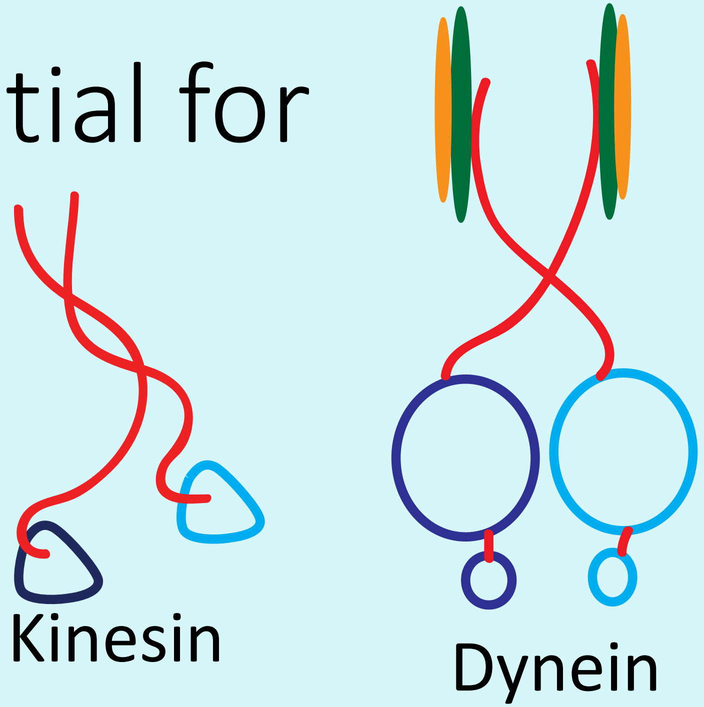


Steven Gross & **Jing Xu** & **Michelle Mattson Hoss** & **Yonggun Jun** & **Babu Reddy**

University of California, Irvine & Korean Institute for Advanced Study (KIAS)

Work Supported by National Institute of Health

Kinesin & Dynein essential for neuronal function



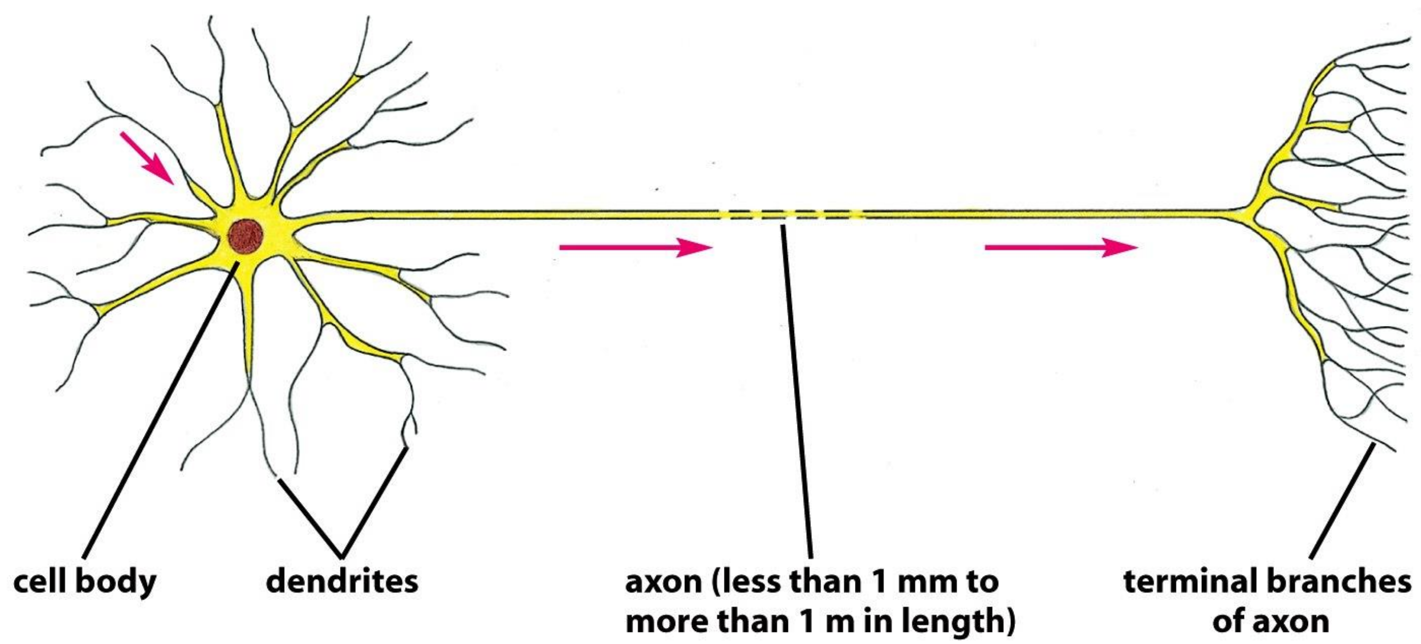


Figure 11-28 Molecular Biology of the Cell 5/e (© Garland Science 2008)

…And for neurotropic viruses such as herpes

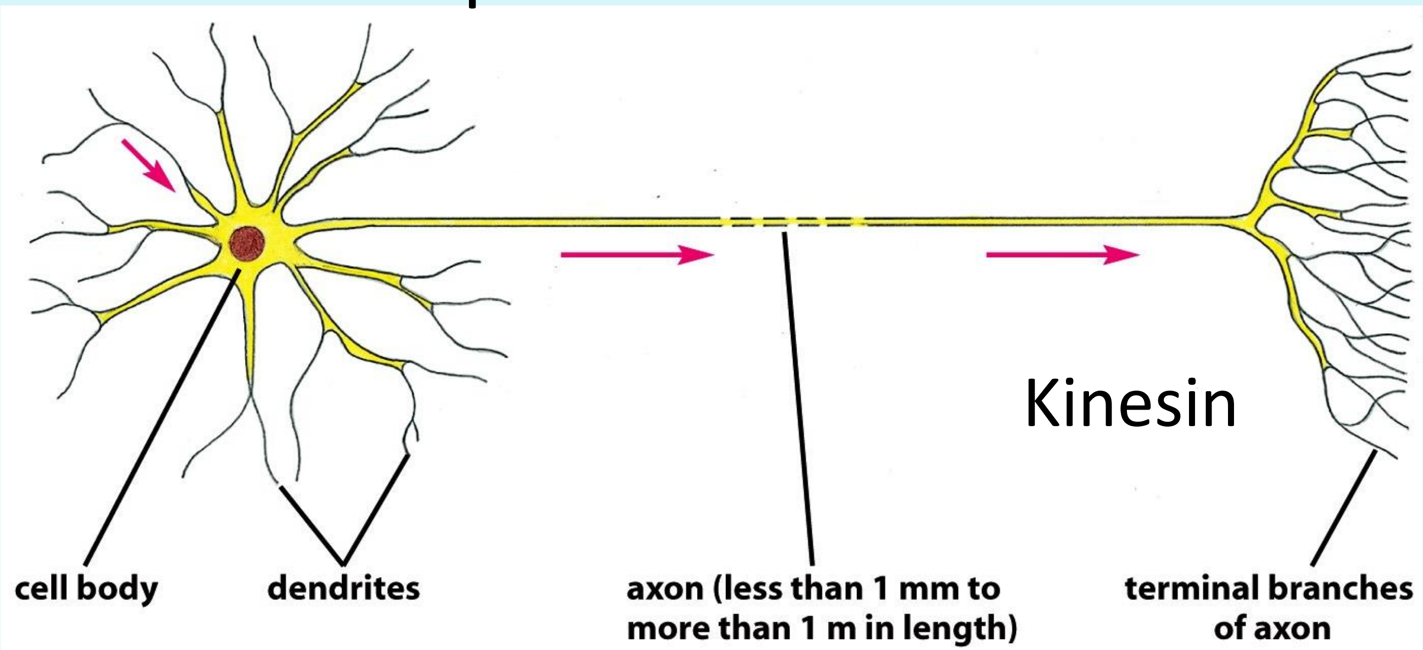


Figure 11-28 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Motors also critical for Cellular Organization and Active Transport

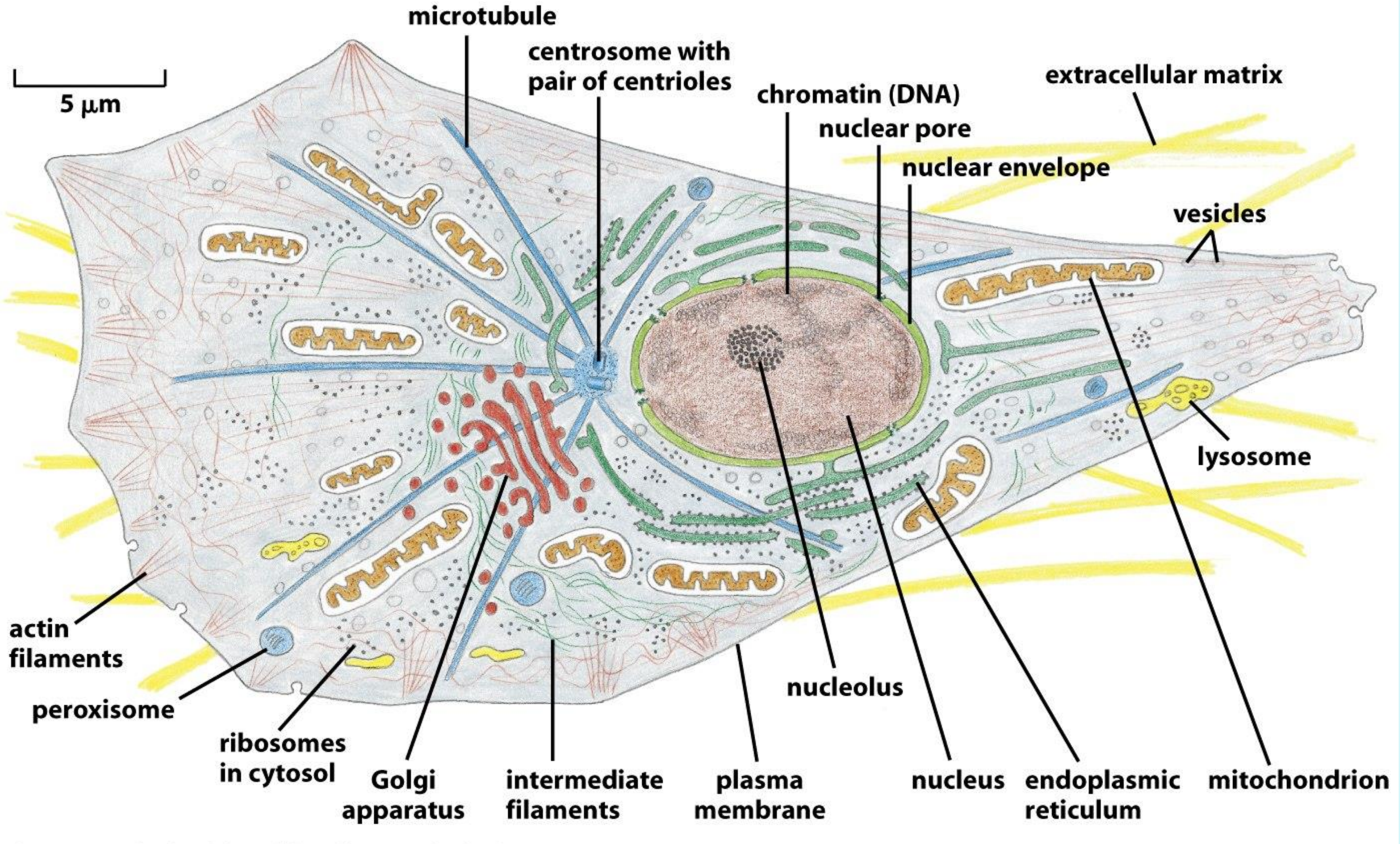
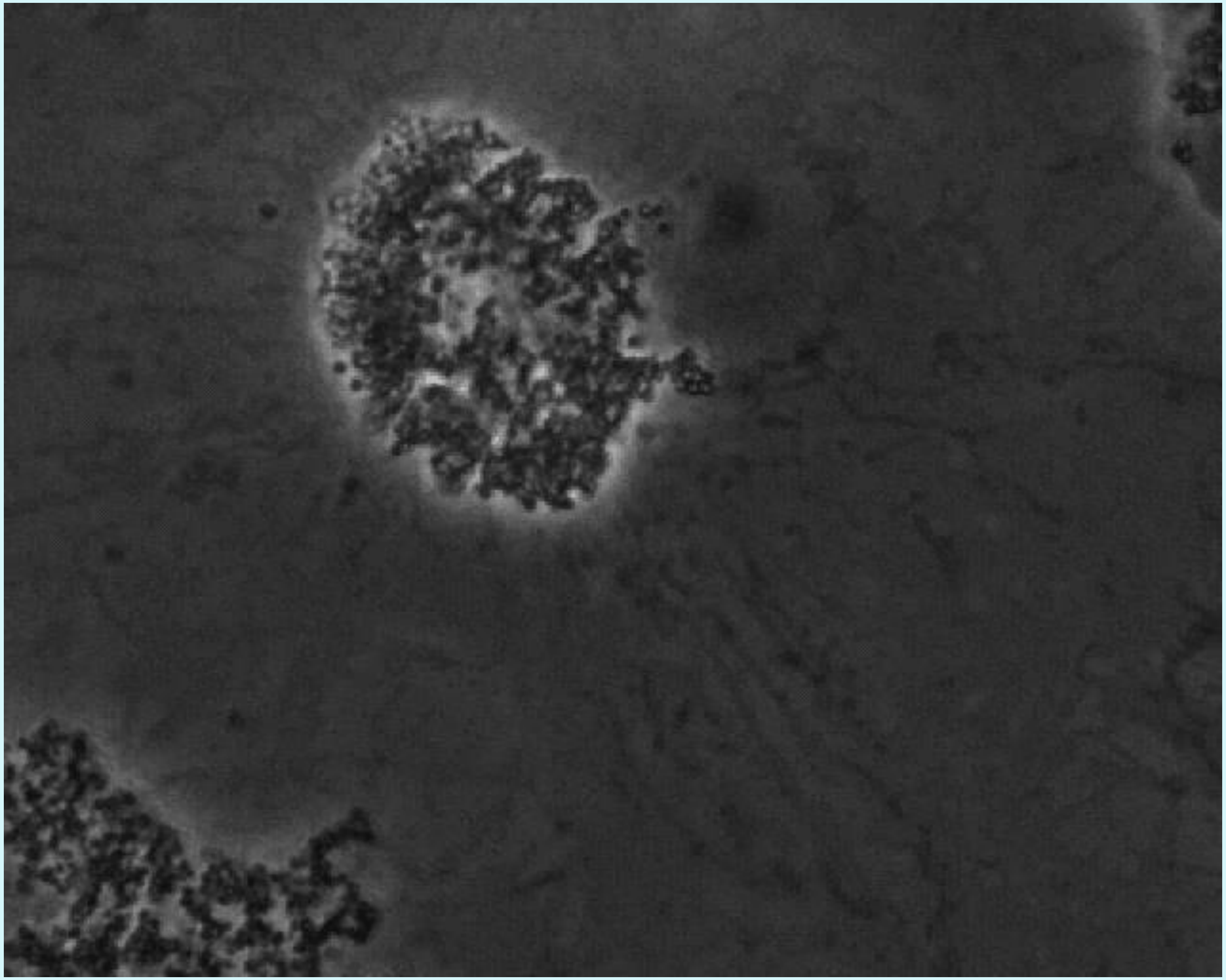
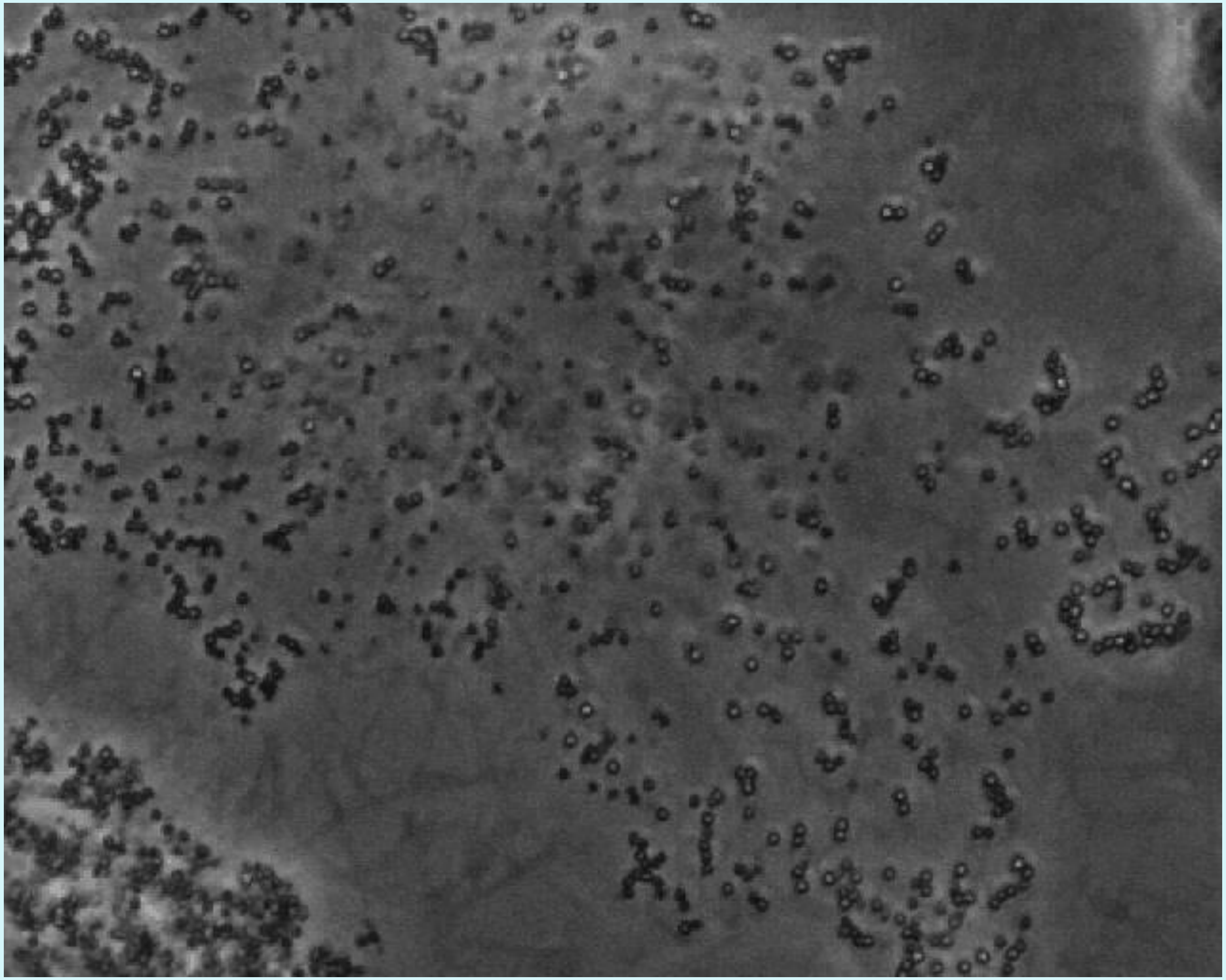


Figure 1-30 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Cellular transport demands change→ regulation of transport is critical



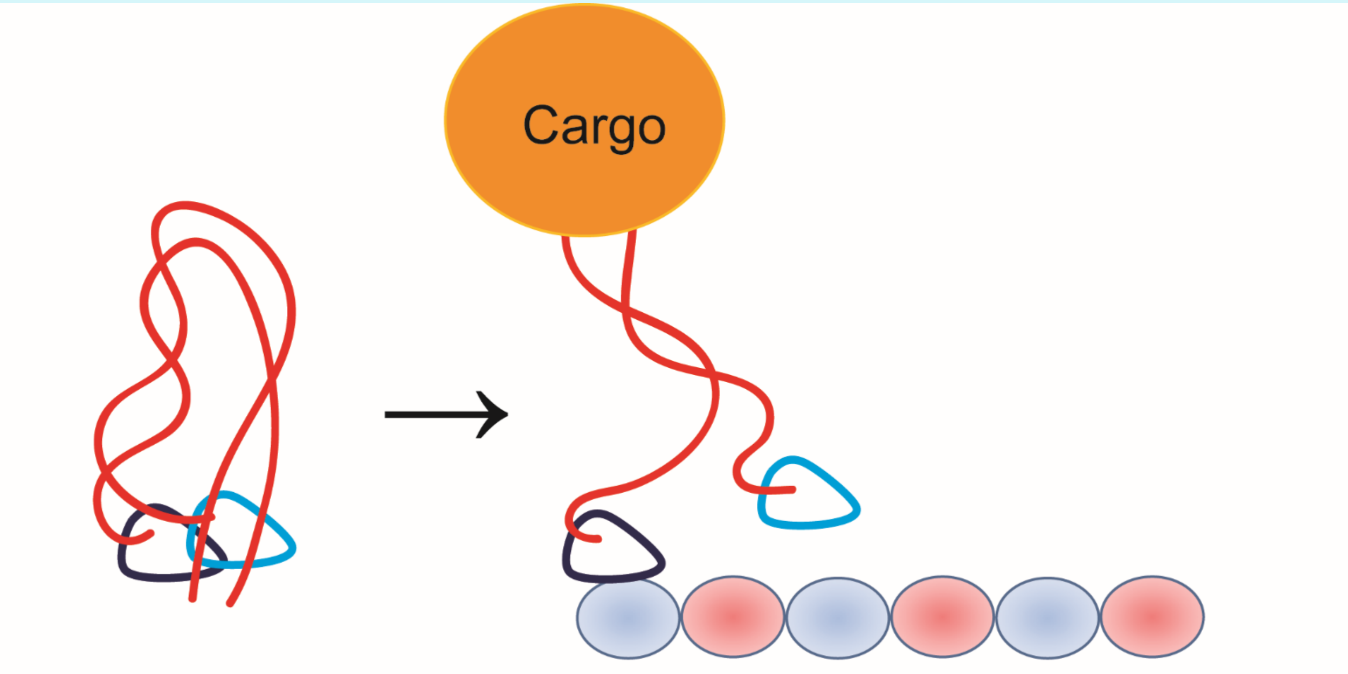
Cellular transport demands change→ regulation of transport is critical



Cellular transport demands change→ regulation of transport is critical

However, we know very little about how motors are regulated…

Dominant paradigm: head-tail inhibition



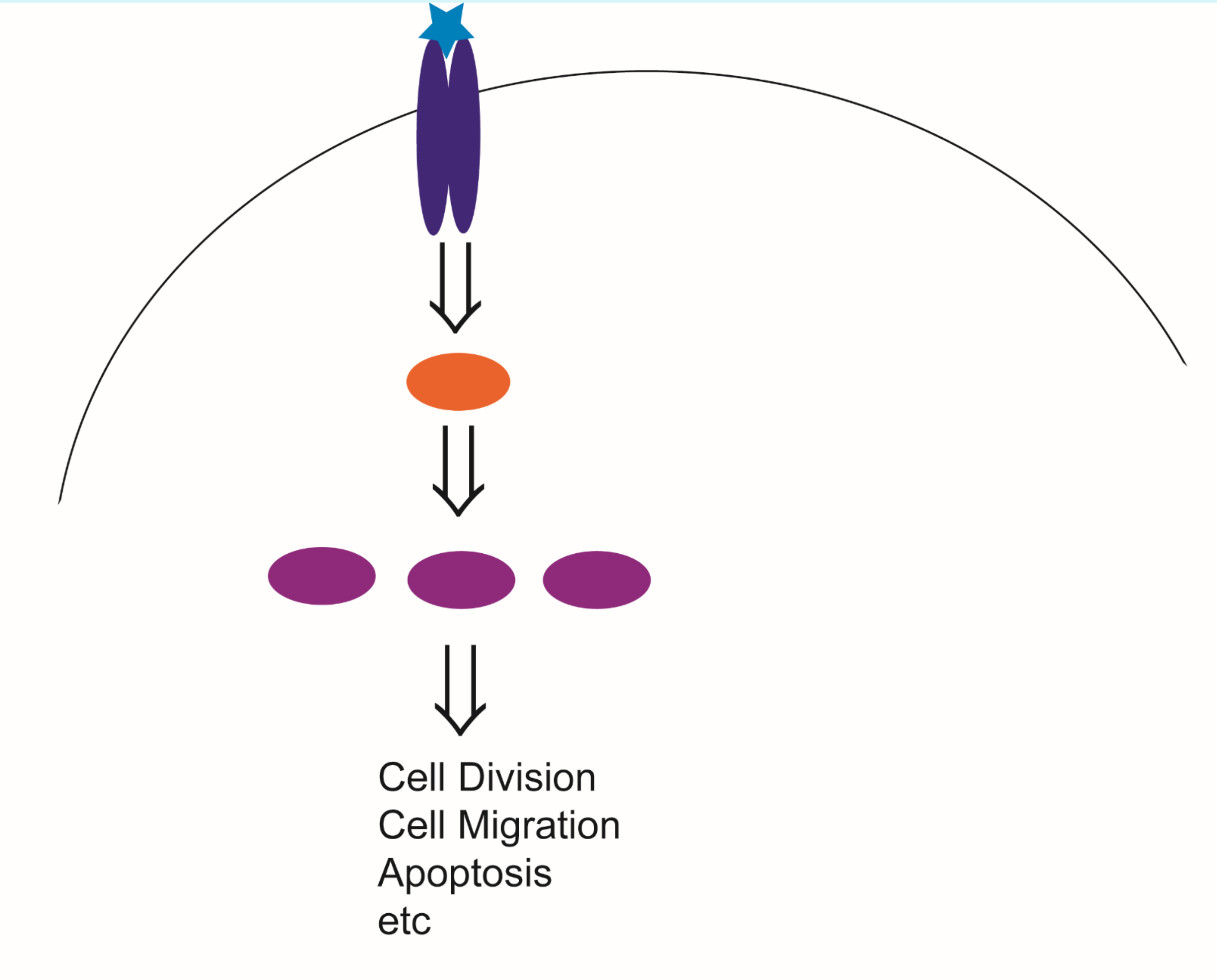
Motor inhibition likely released when motor recruited to cargo…

There is also on-cargo regulation…
(just saw 2 examples)

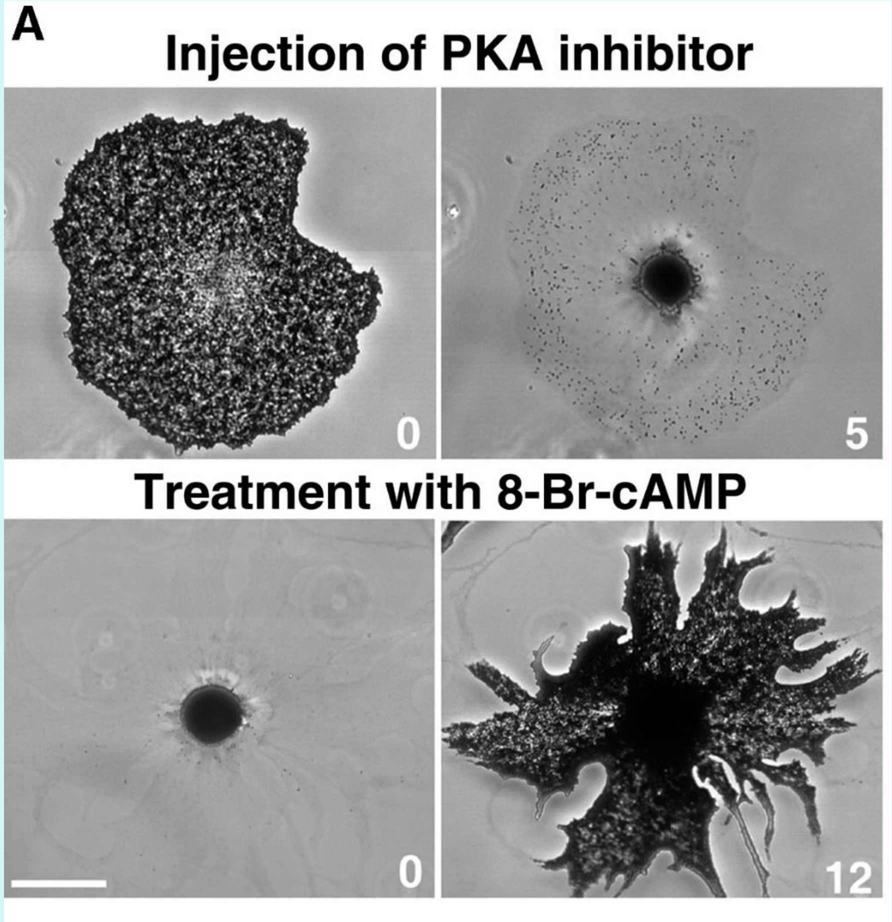
uncertain mechanism:

no recruitment or release of motors!

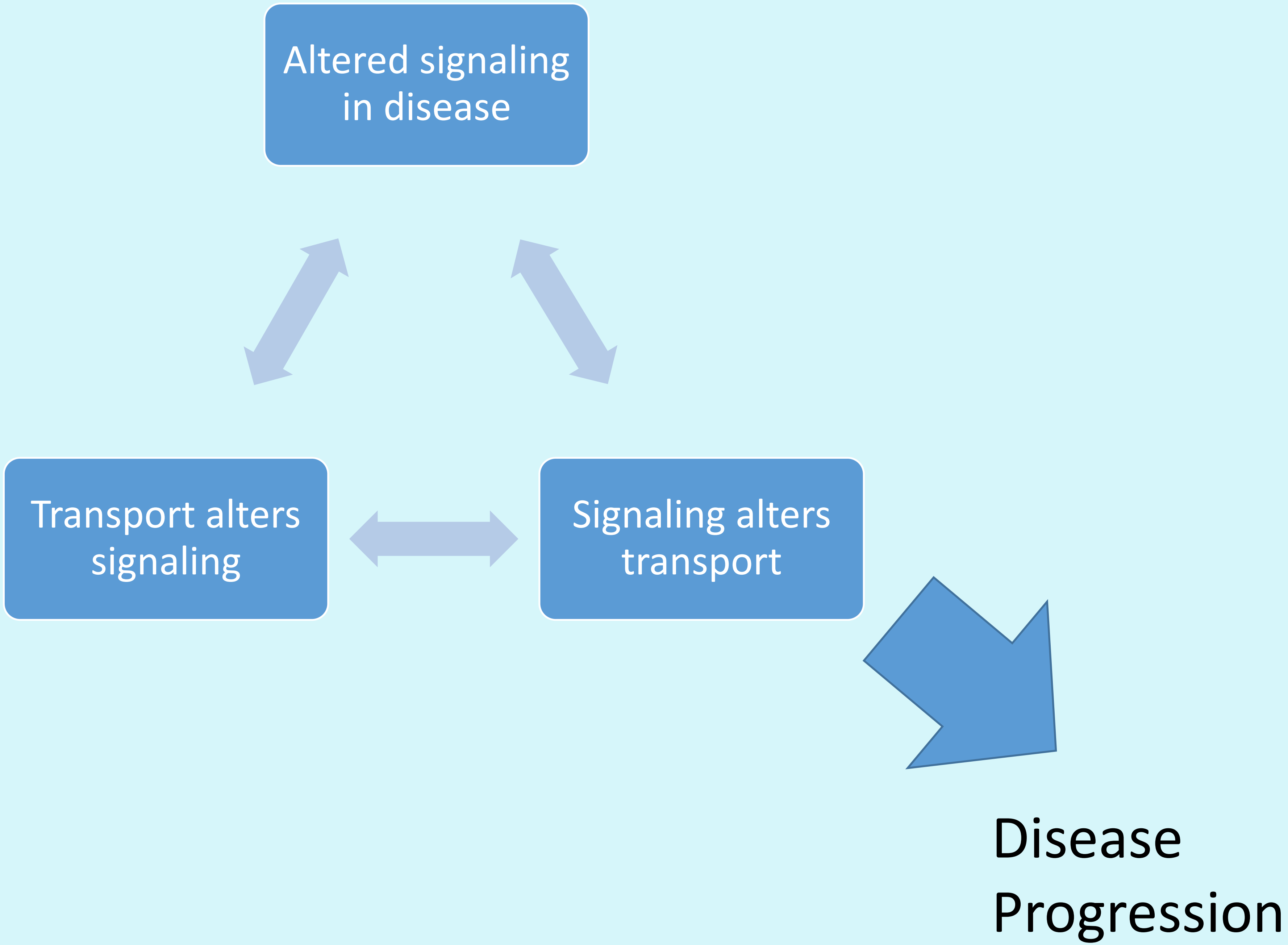
Hypothesis: Transport controlled by Cell Signaling



Signaling → Transport



Rodionov, V. *et al. Current Biology.* (2003).

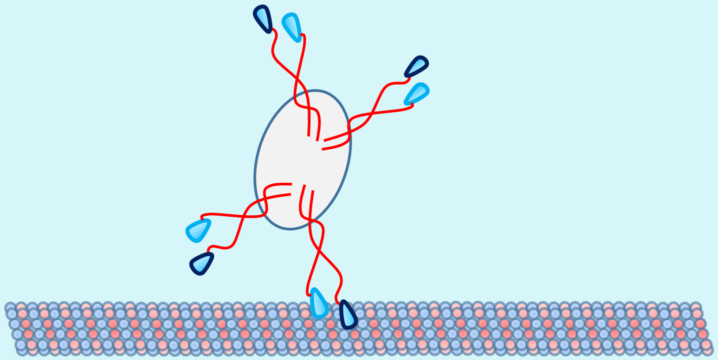

Altered signaling in disease
Transport alters signaling
Signaling alters transport
Disease Progression

Search for novel motor regulators…

Test effects of Signaling Kinases Implicated in Transport

- JNK Family
  - Higher activity in Alzheimer's and Huntington's
- GSK3β
  - Higher activity in Alzheimer's and Huntington's
- **CK2**
  - Lower activity in Alzheimer's, higher in cancers

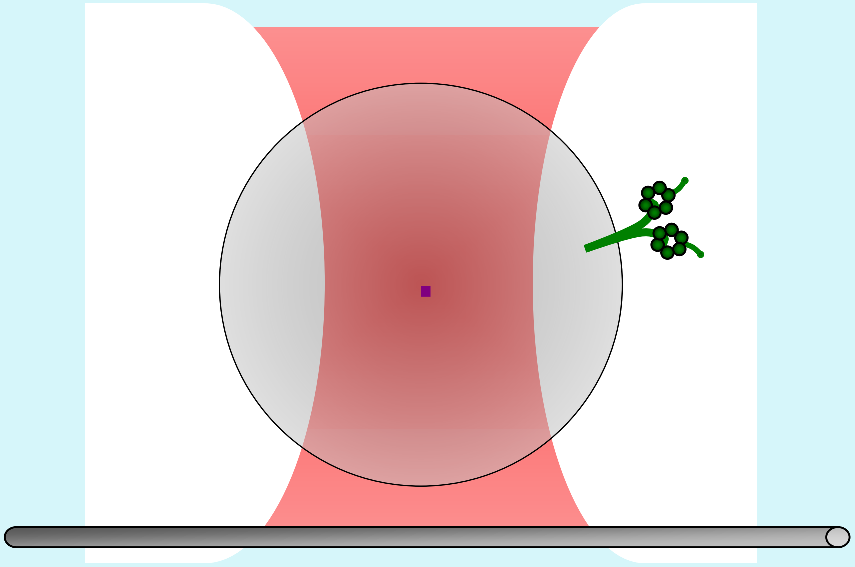
Do Kinases Alter Kinesin Function?

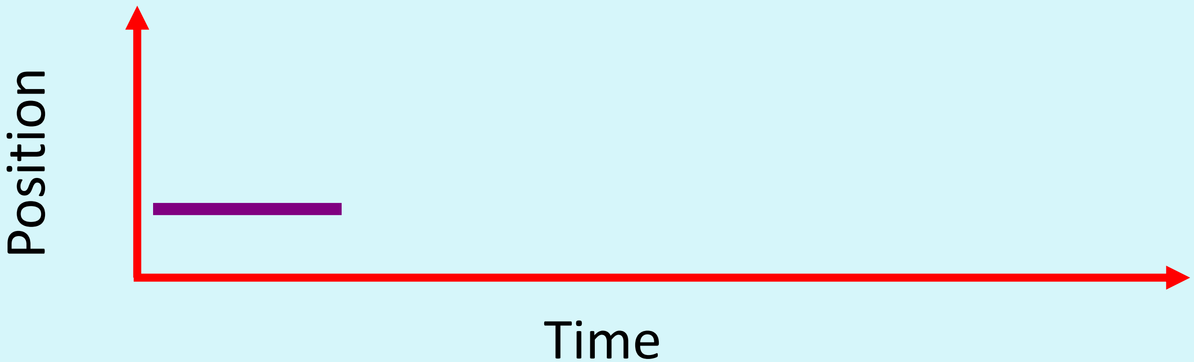


What we measure –

- Distance
- Speed (Velocity)
- Force Production

The basics of the *in vitro* assay: Optical Trap →Measure Motor Motility (Velocity, Travel)





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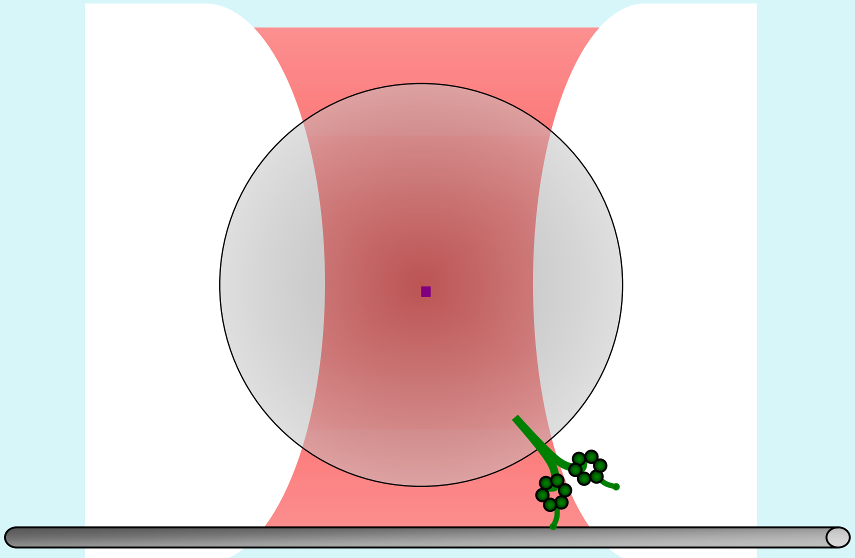
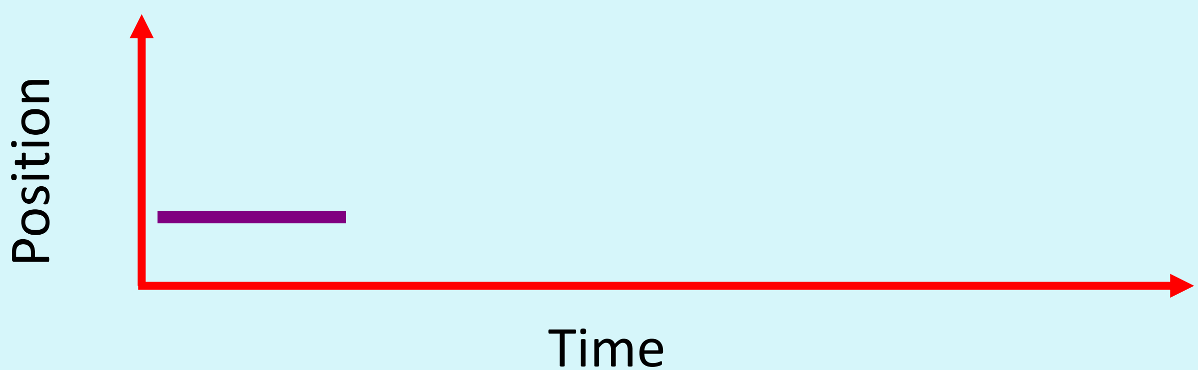
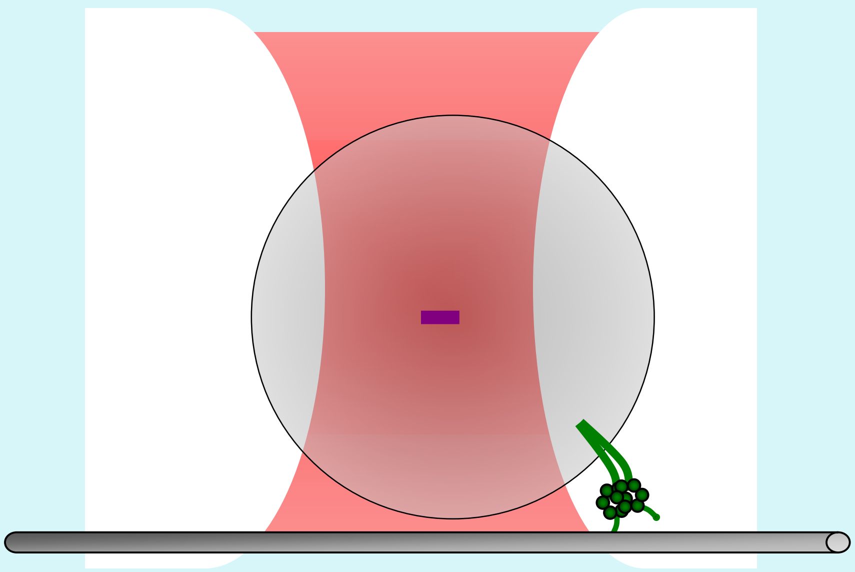
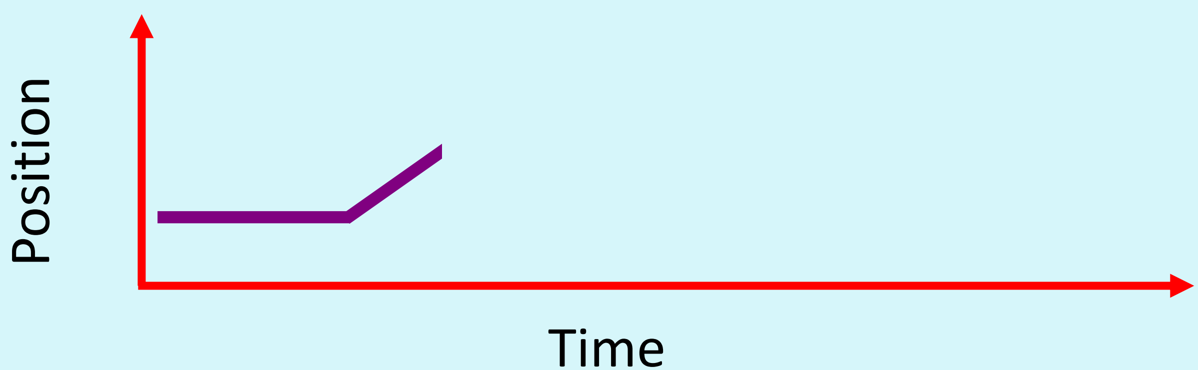


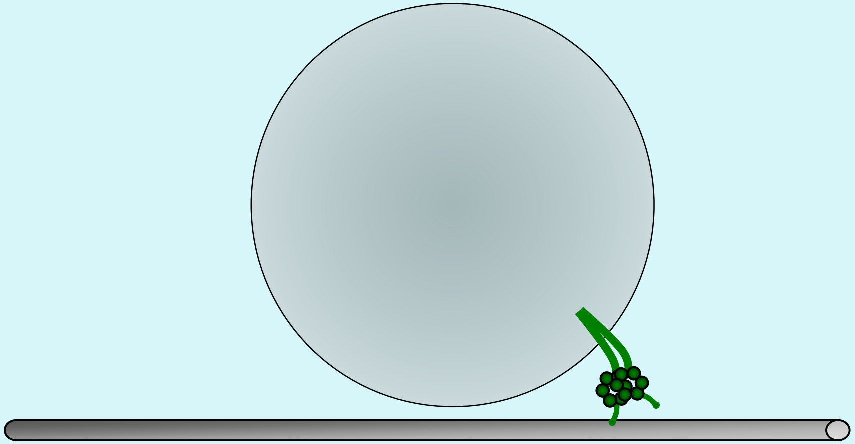


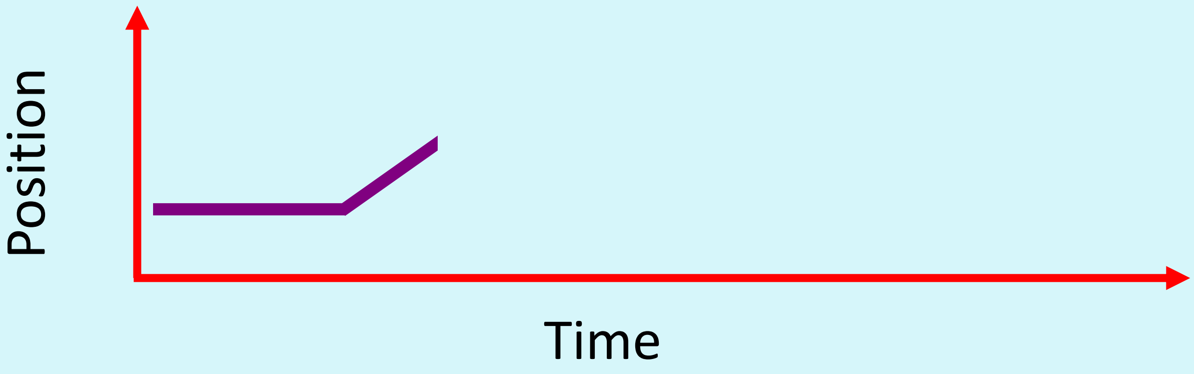
The basics of the *in vitro* assay: Optical Trap →Measure Motor Motility (Velocity, Travel)





The basics of the *in vitro* assay: Optical Trap →Measure Motor Motility (Velocity, Travel)





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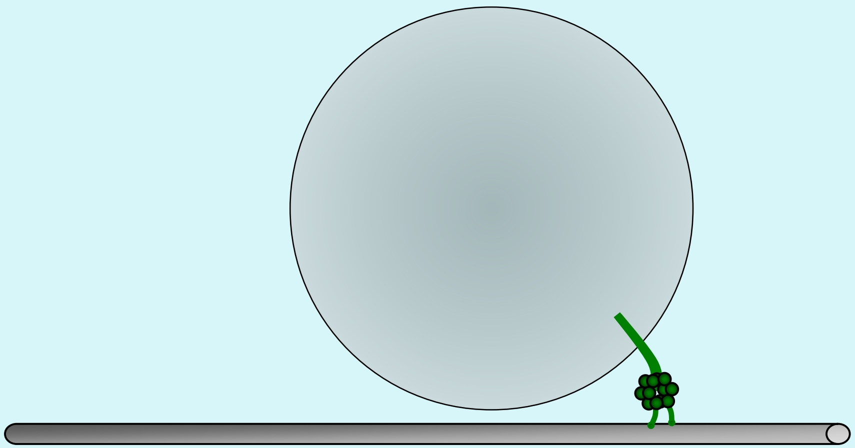
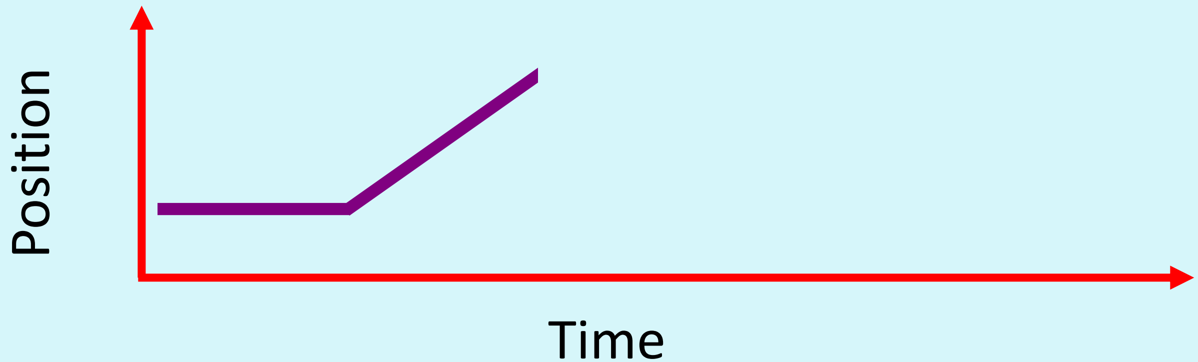
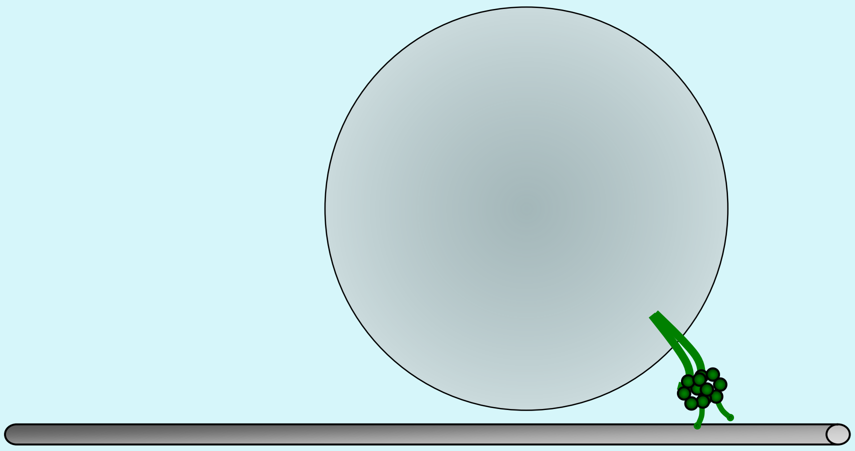
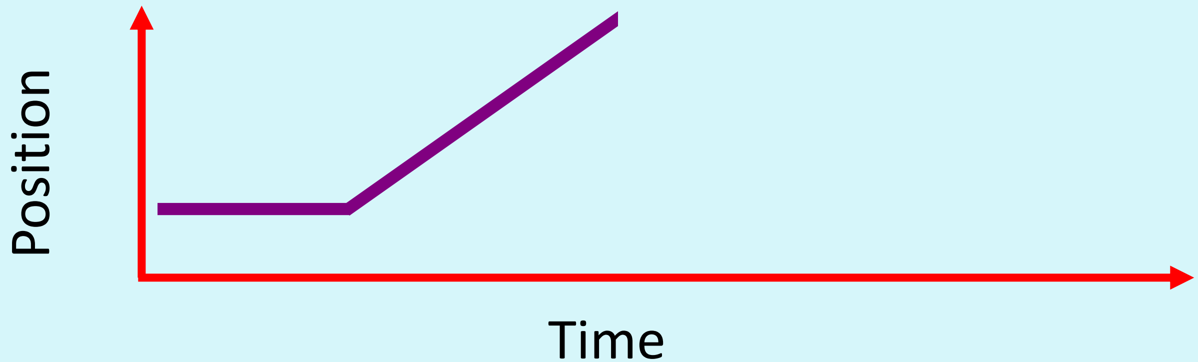


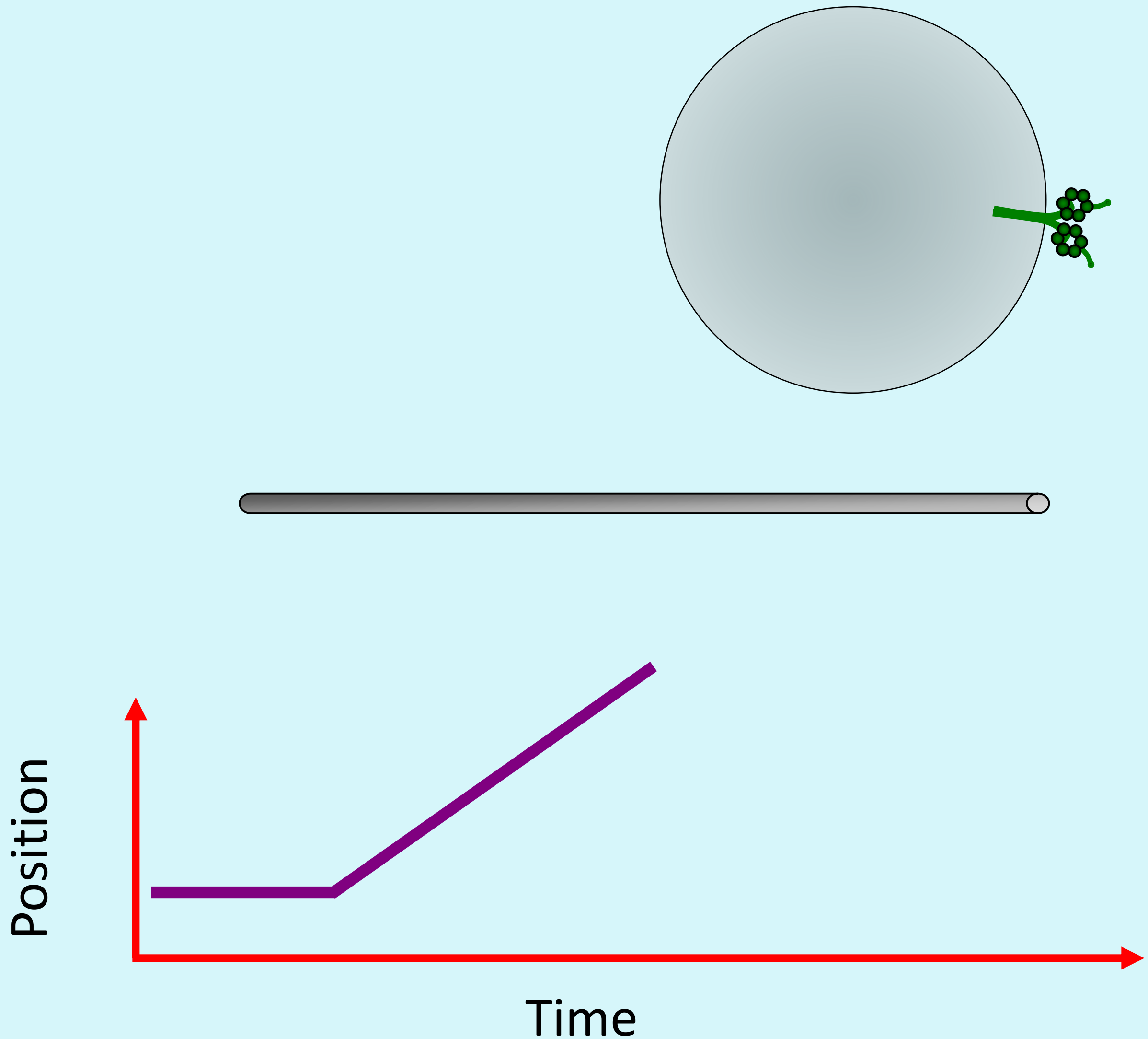


The basics of the *in vitro* assay: Optical Trap →Measure Motor Motility (Velocity, Travel)



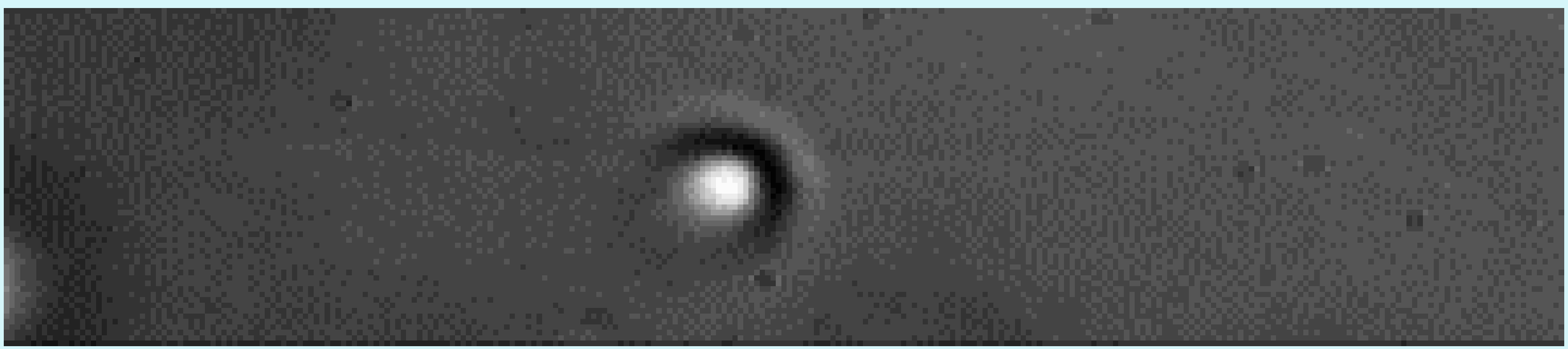


The basics of the *in vitro* assay: Optical Trap →Measure Motor Motility (Velocity, Travel)



Kinesin walking on MT *in vitro*

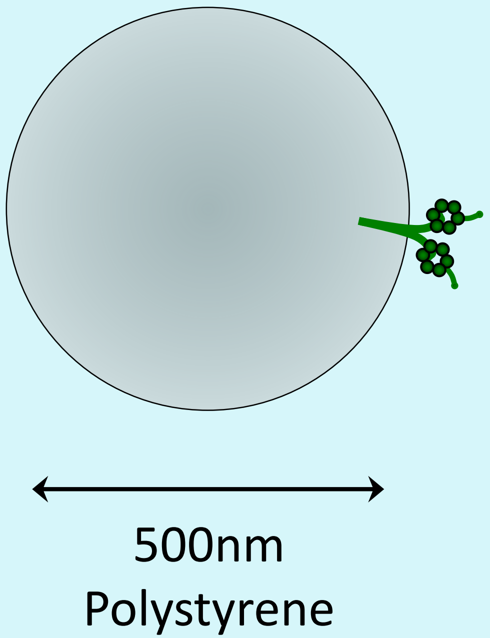
+



d

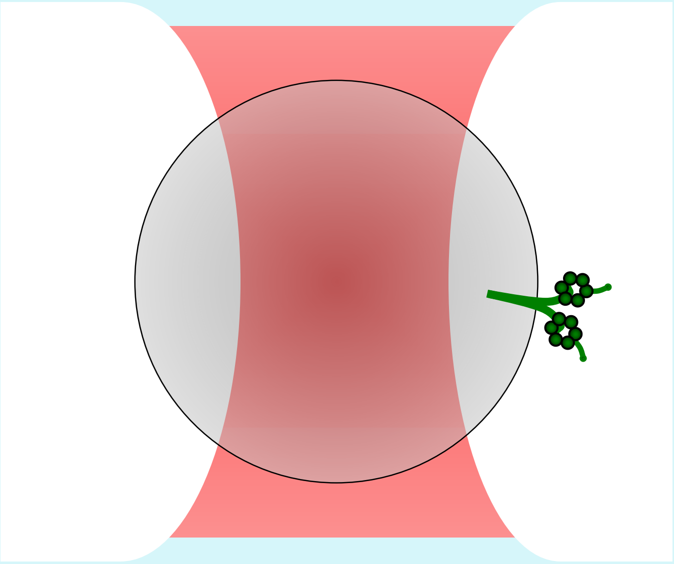
Suvranta Tripathy

Can also measure forces with the Optical Trap



Ashkin et *al*., *Opt. Lett.* (1986), Block et *al*, *Nature* (1990)

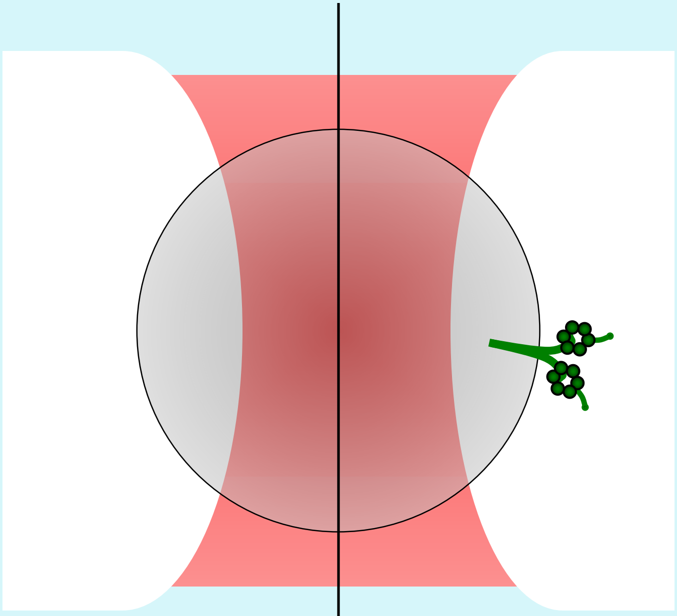
Optical Trap: Single-Beam Gradient Force Trap



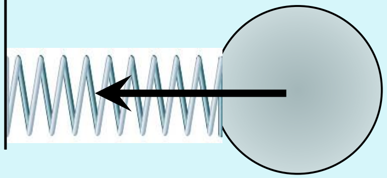
980nm
Single Mode Diode

Ashkin et *al*., *Opt. Lett.* (1986), Block et *al*, *Nature* (1990)

Optical Trap: Single-Beam Gradient Force Trap



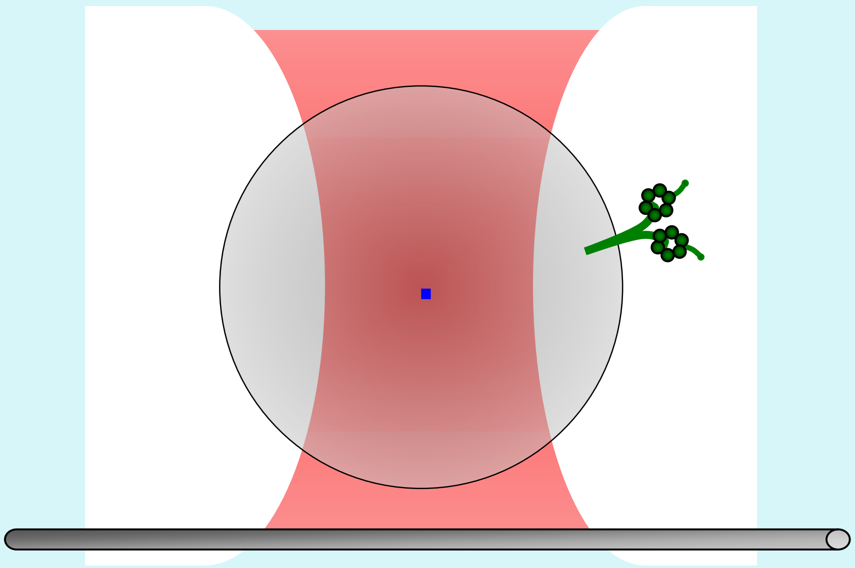
980nm
Single Mode Diode

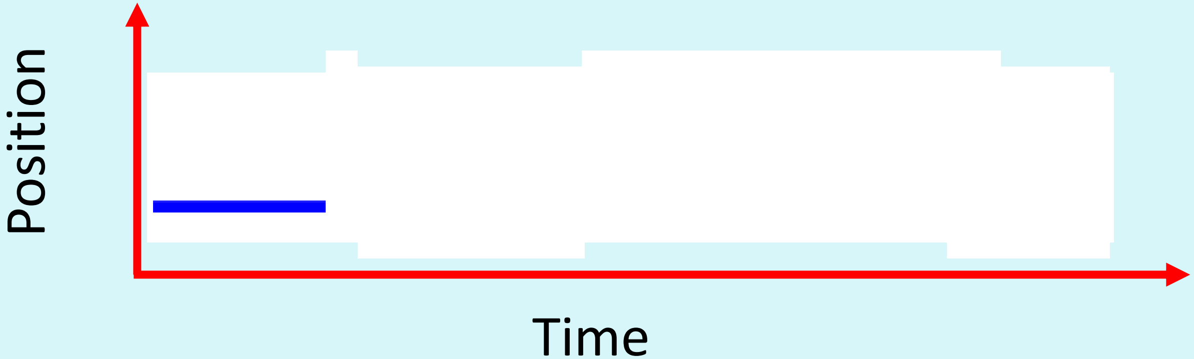


Force = k x Position

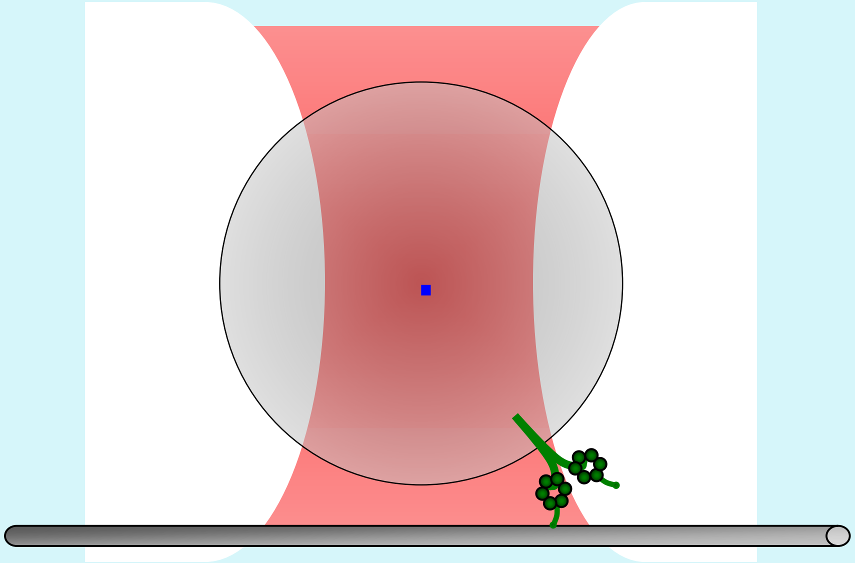
Ashkin et *al.*, *Opt. Lett.* (1986), Block et *al*, *Nature* (1990)

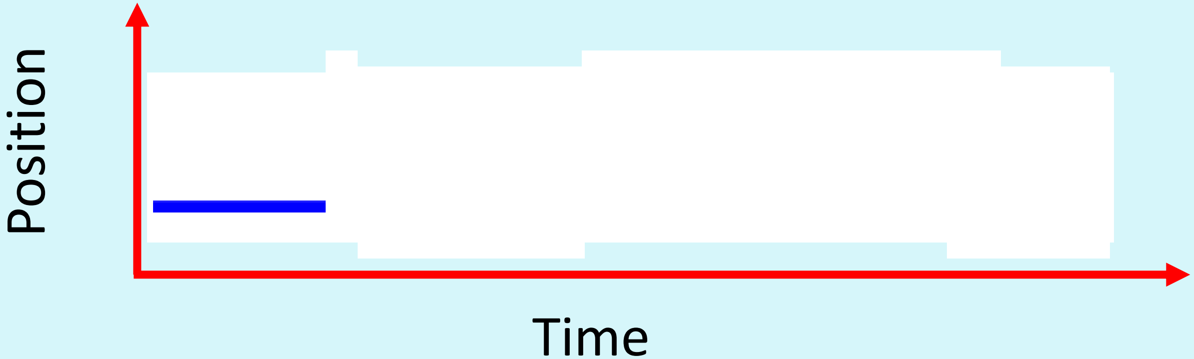
Optical Trap Measures Motor Force Production





Optical Trap Measures Motor Force Production





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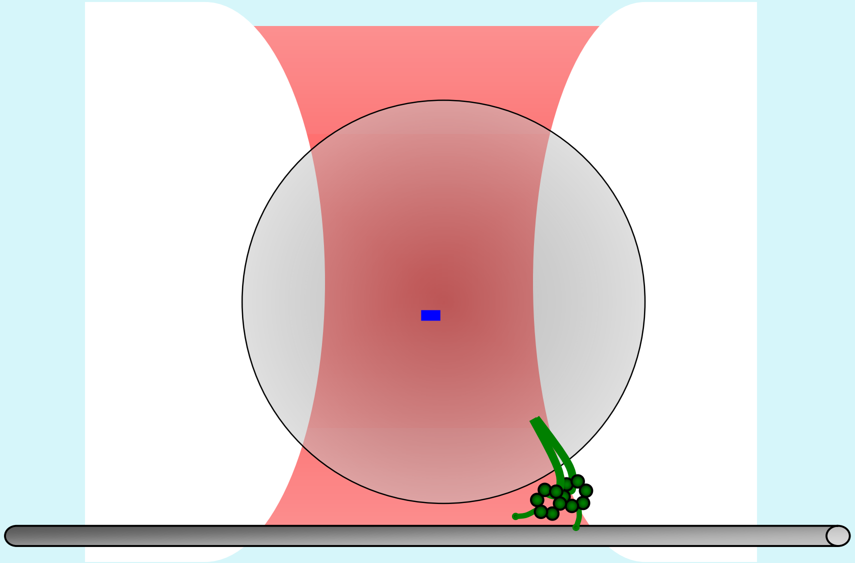

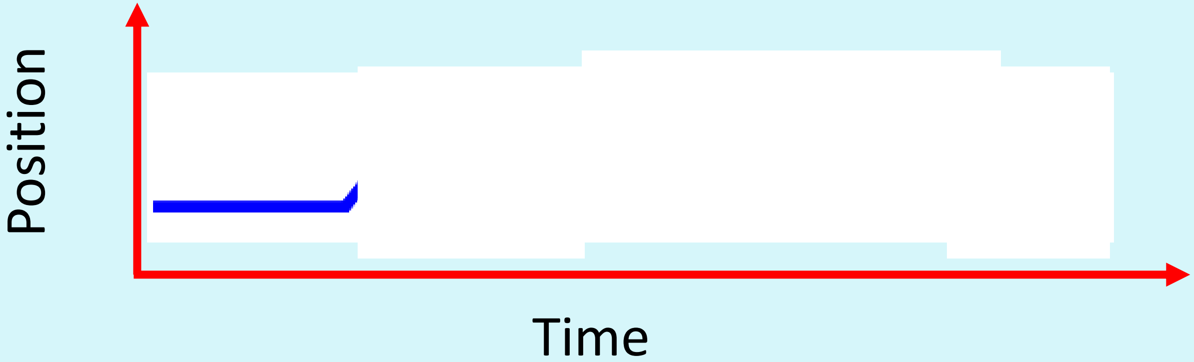

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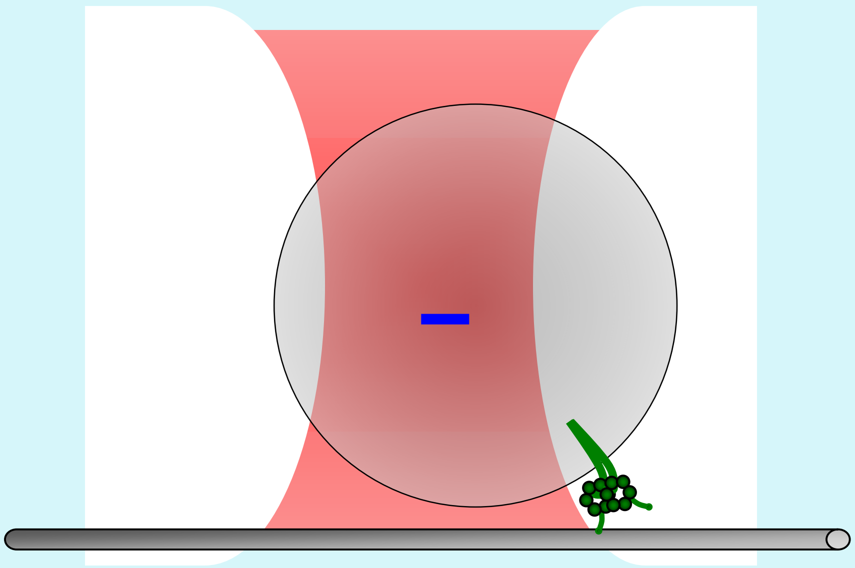

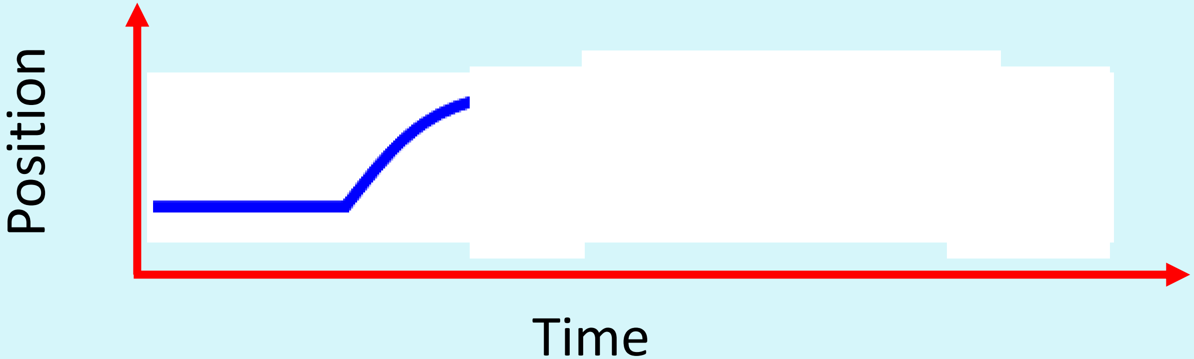

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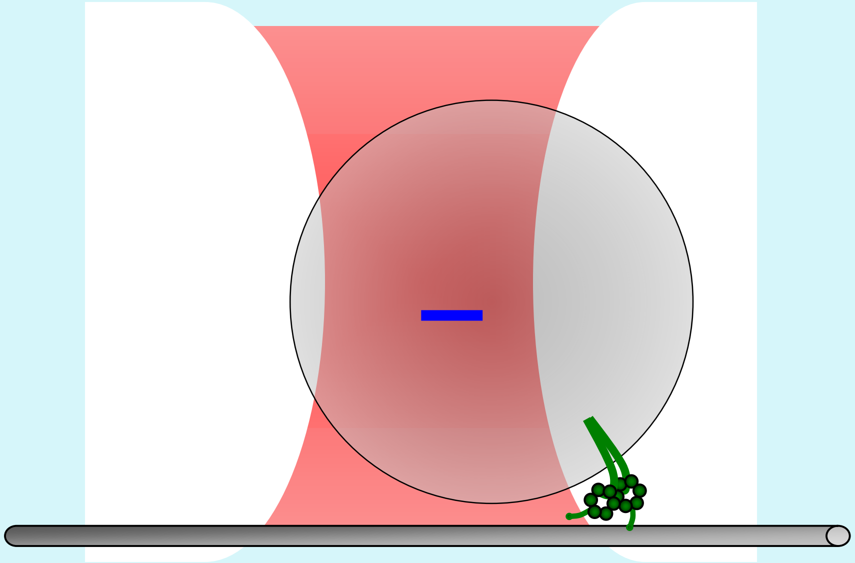

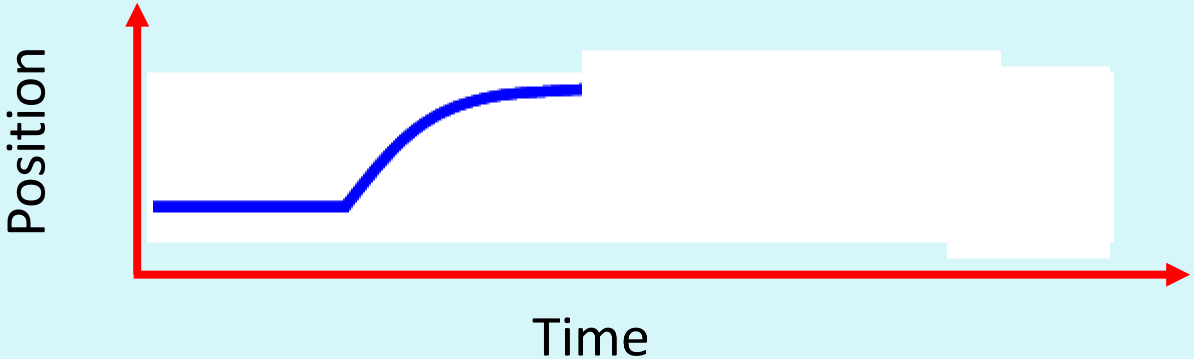

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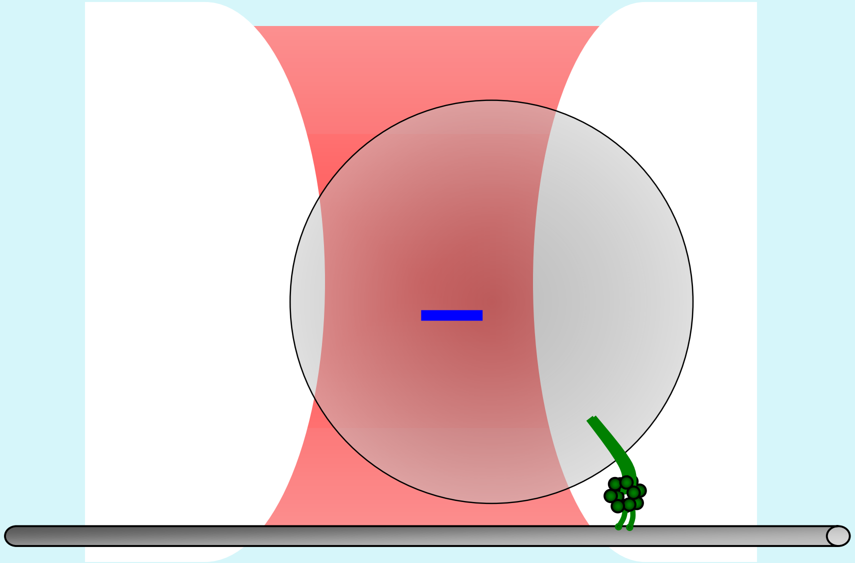

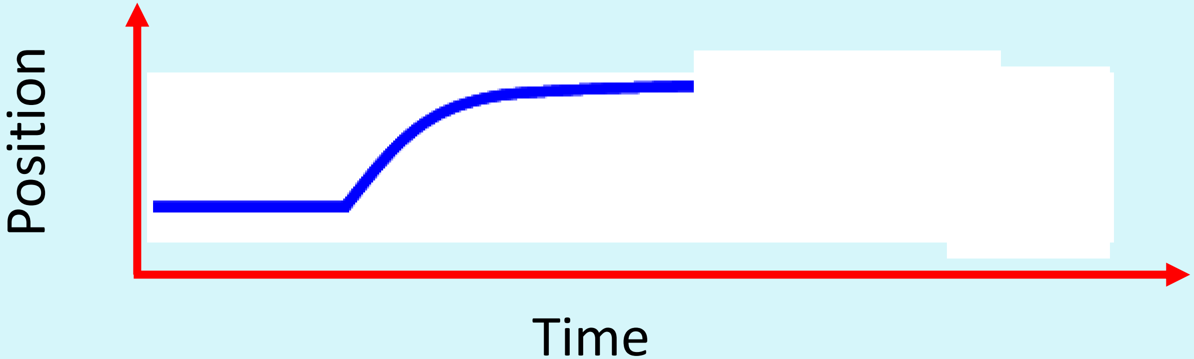

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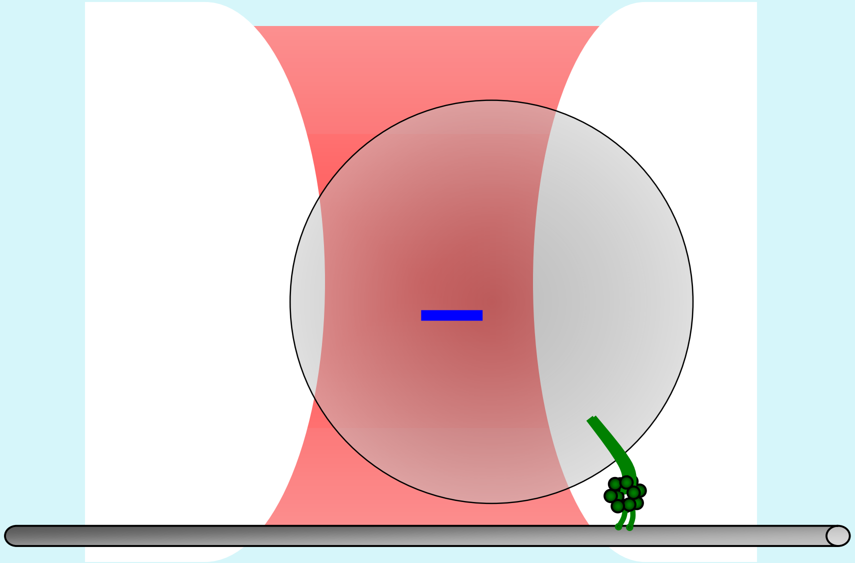

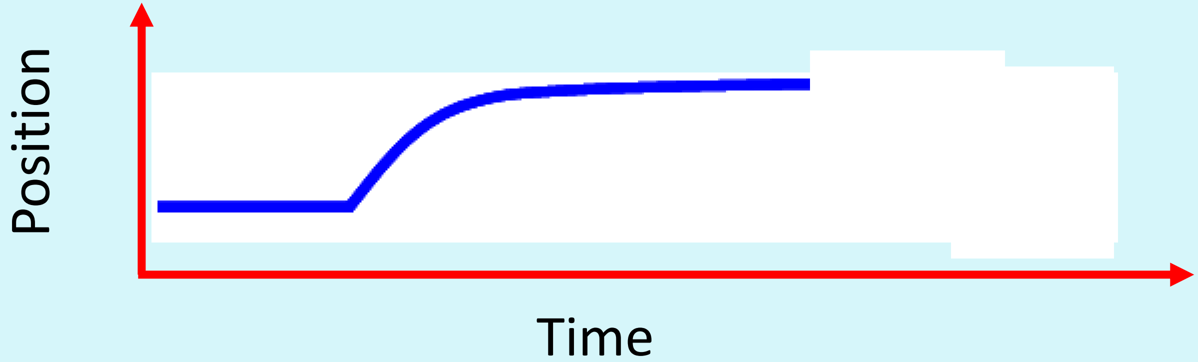

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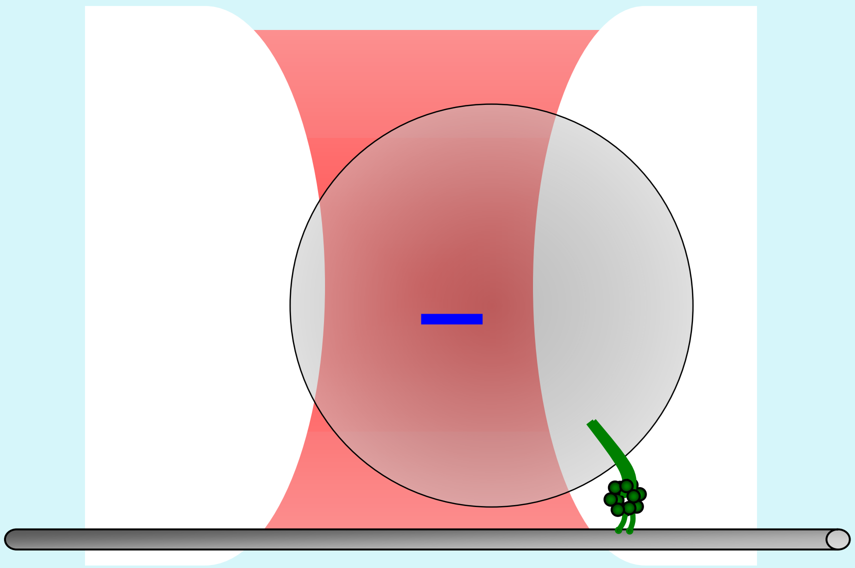

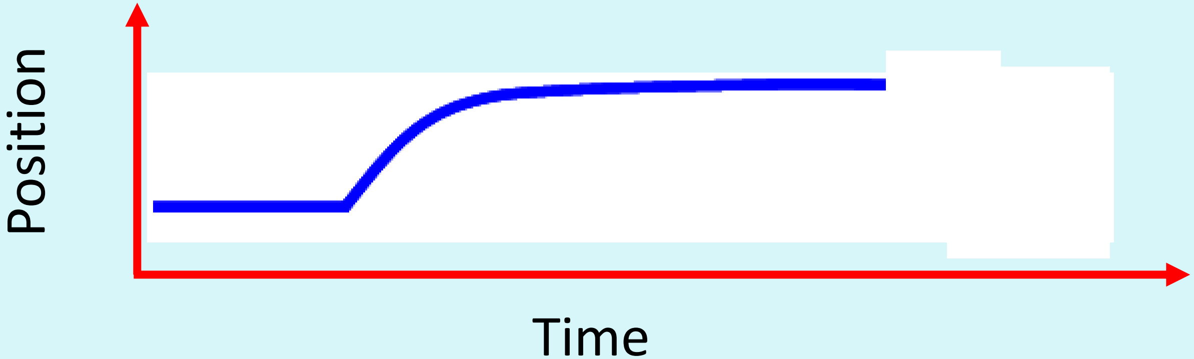

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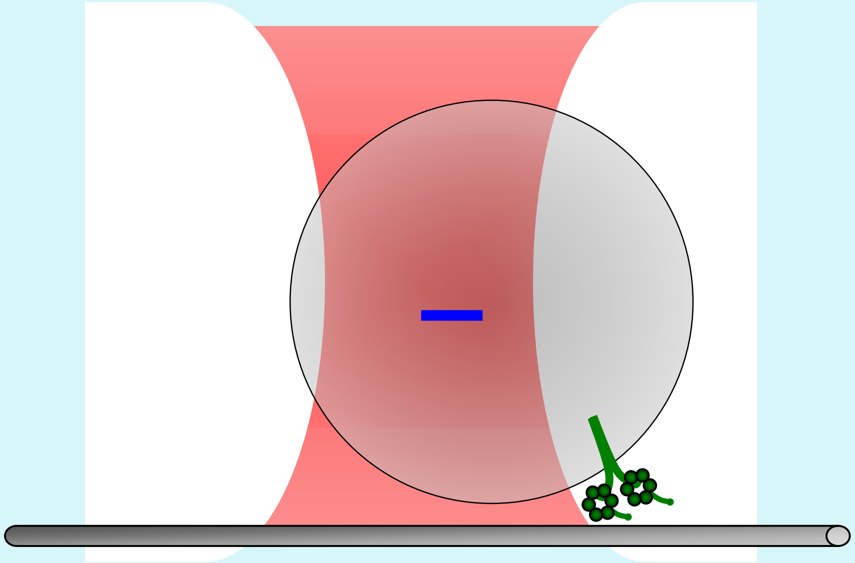

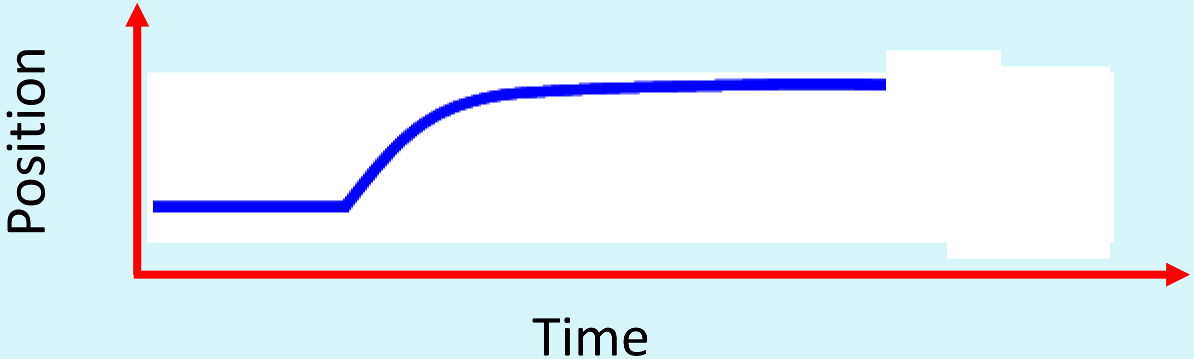

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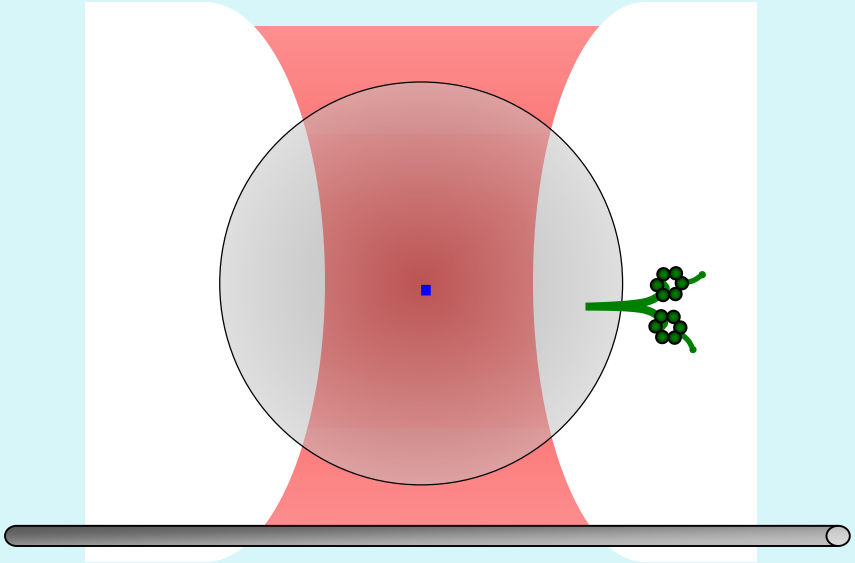

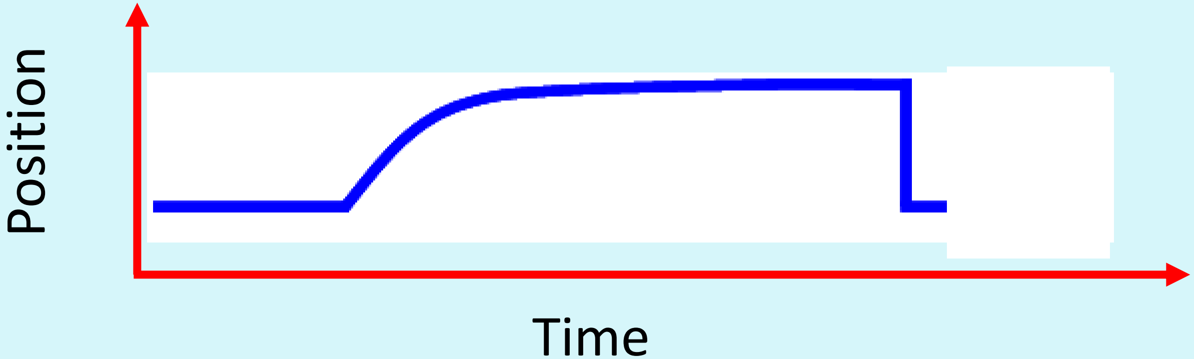

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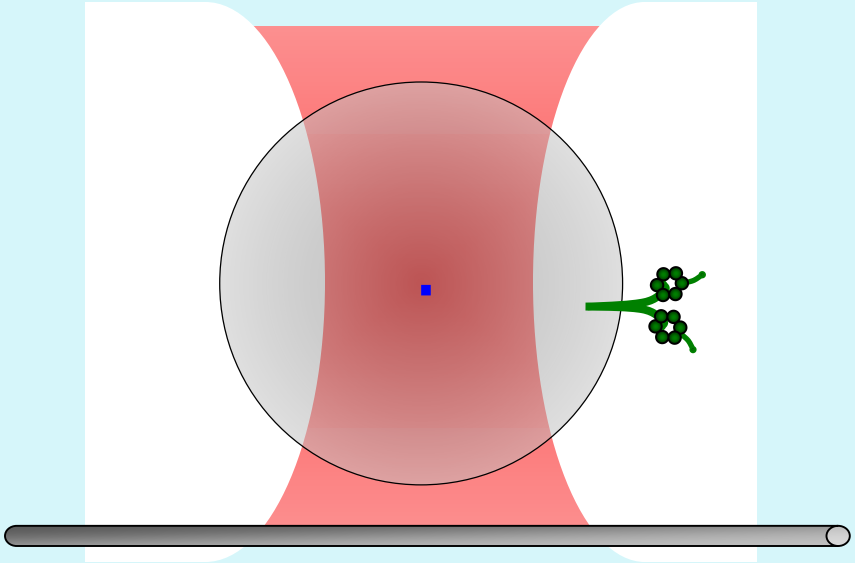

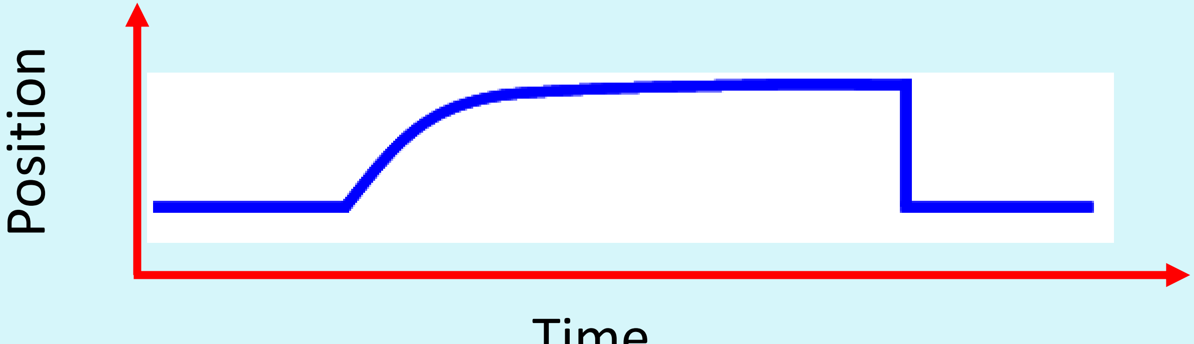

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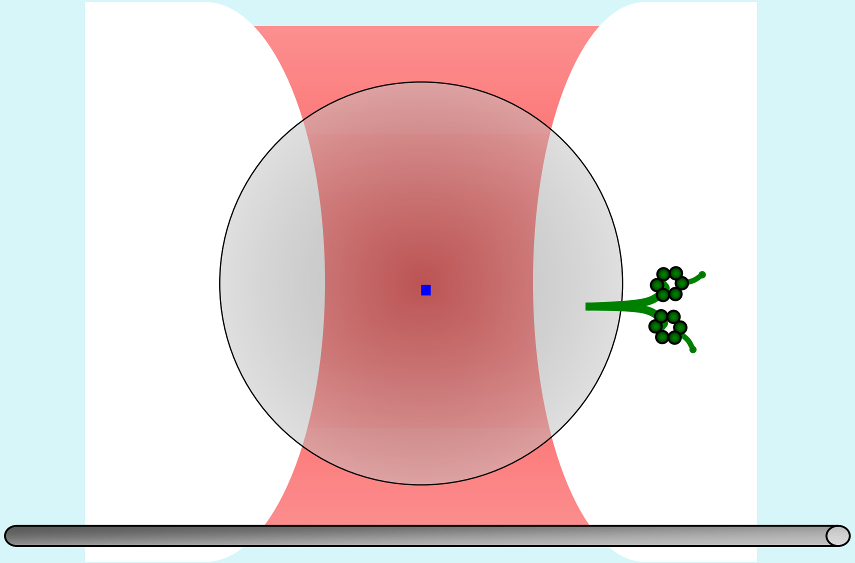
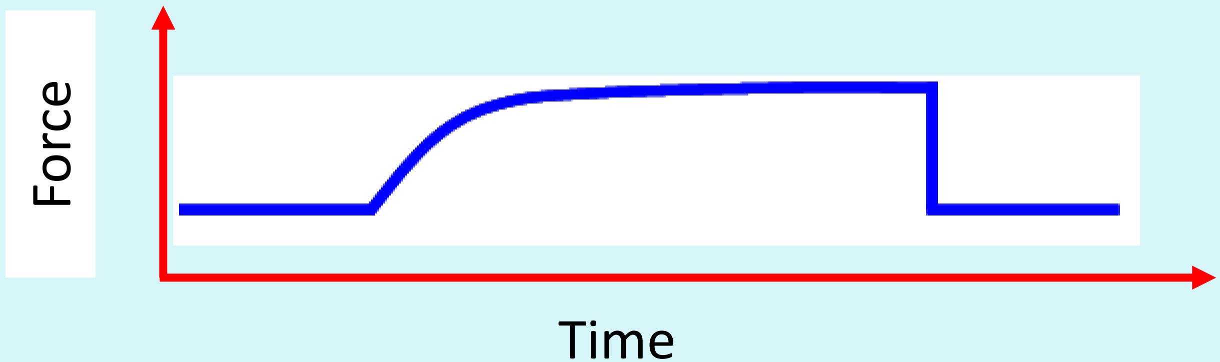


Force = k x Position



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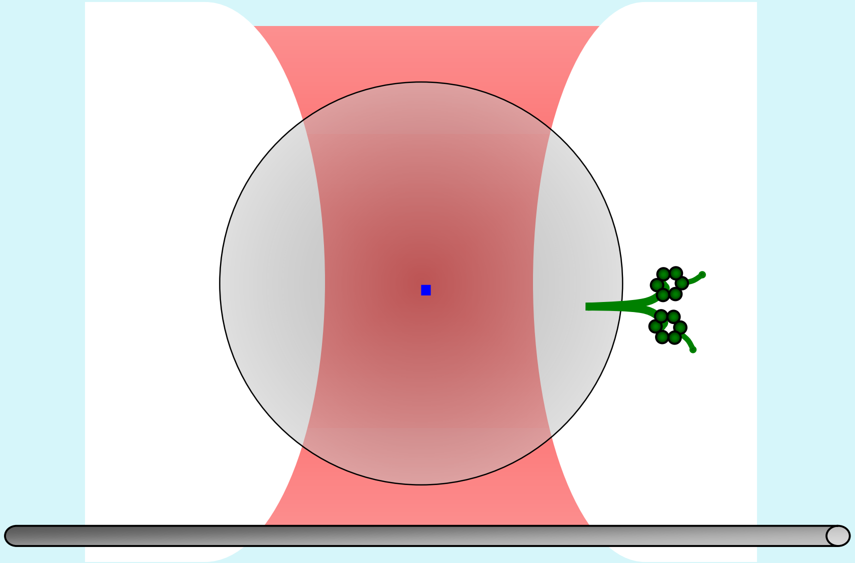

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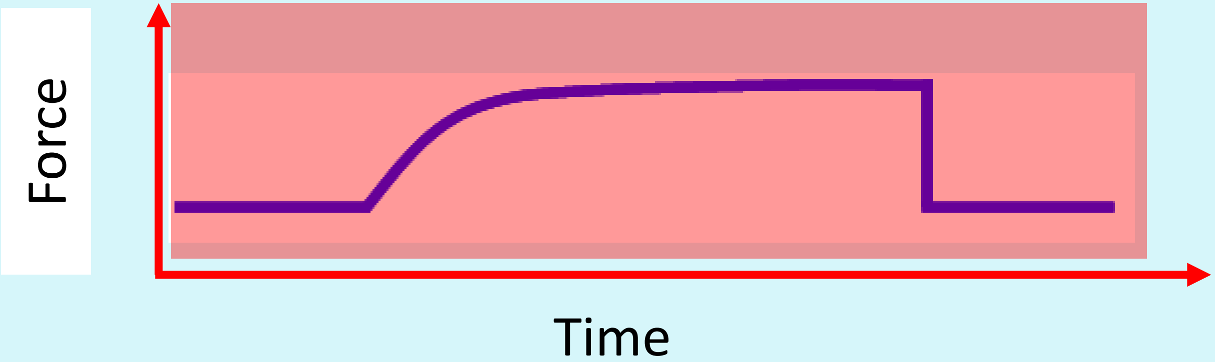
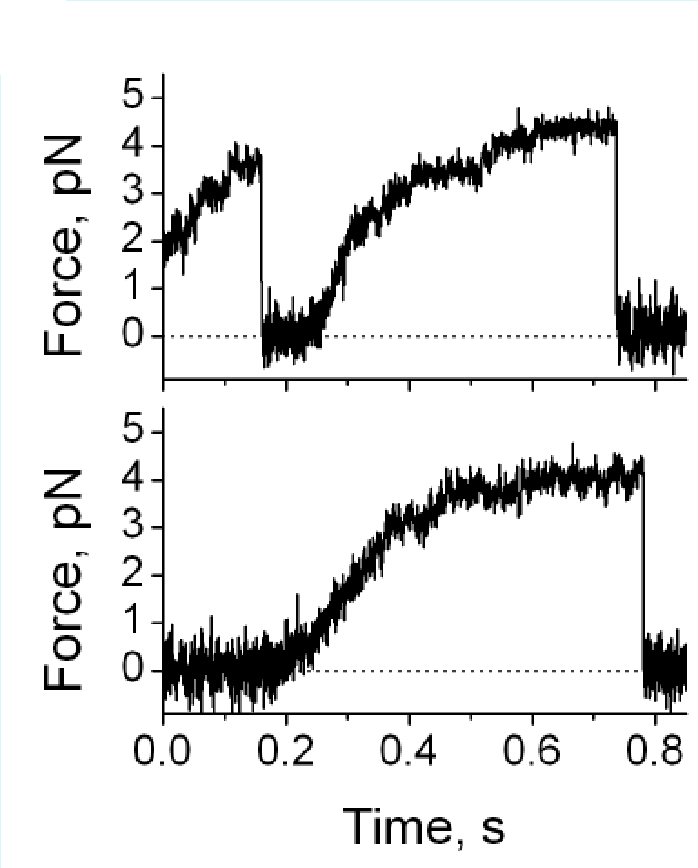


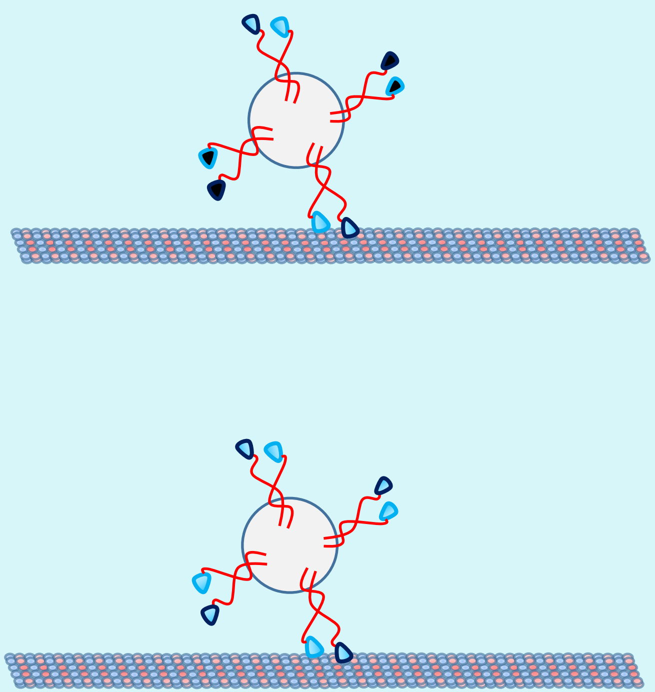
Quadrant Photodiode (2 kHz)

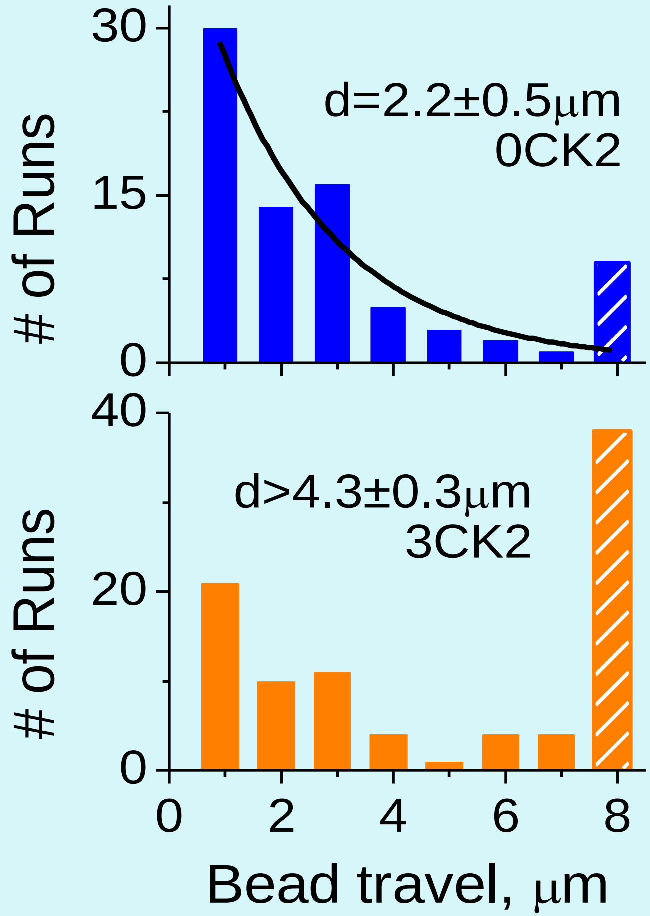
Example kinesin force production traces



Jing Xu

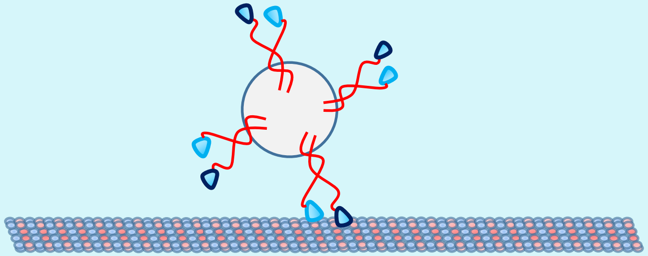
Experiment: Treat kinesins with CK2 → quantify function in single-molecule type assays.

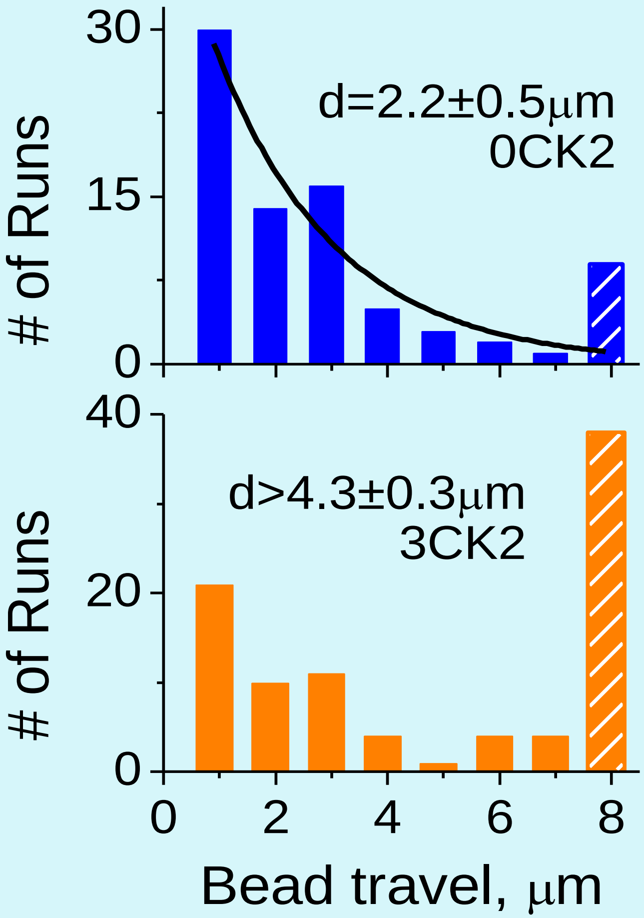




Xu, J., *et al*. *Nat. Commun.* (2012)

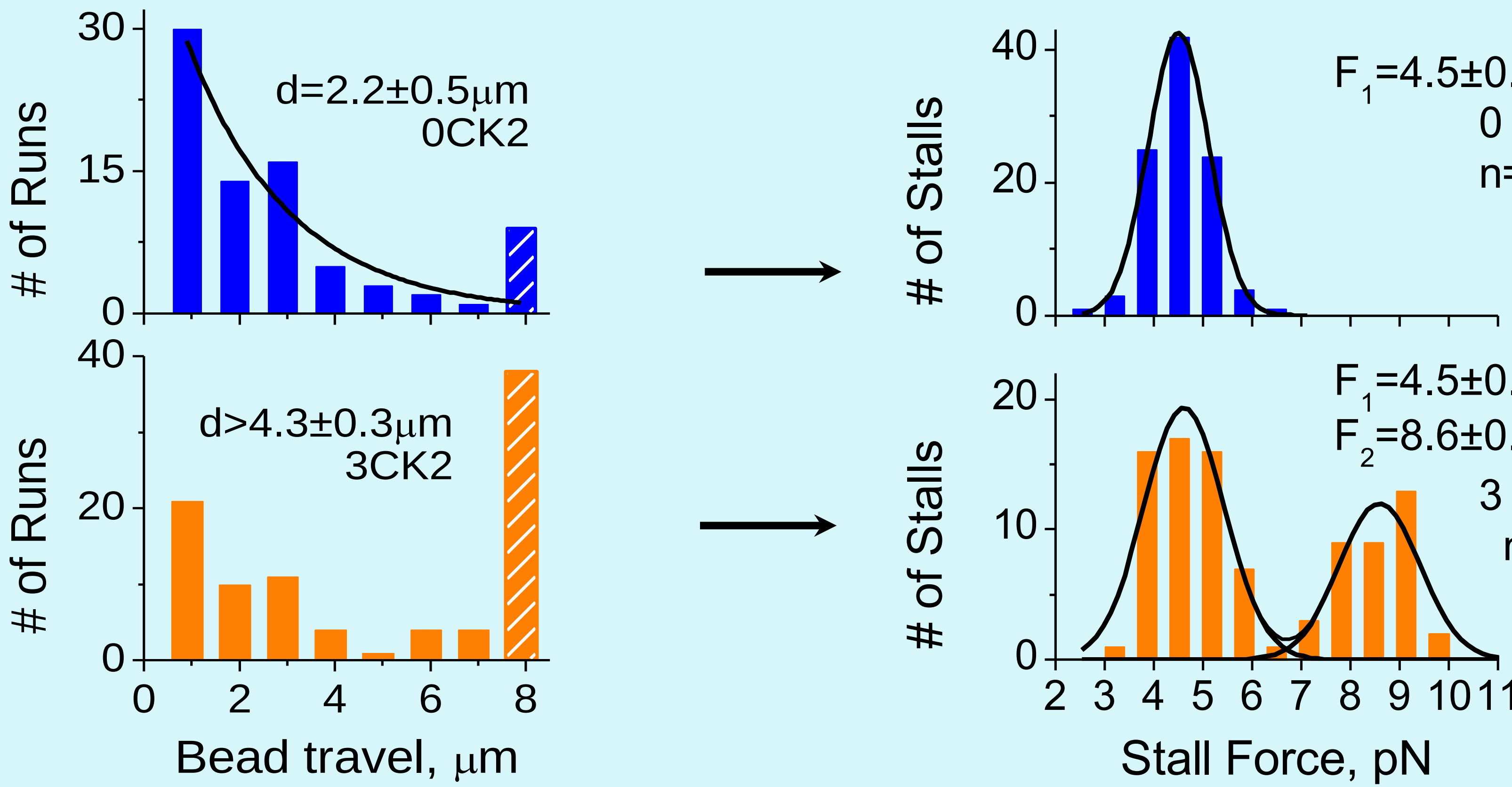
Possible interpretations:
a) Increased processivity
b) more motors active





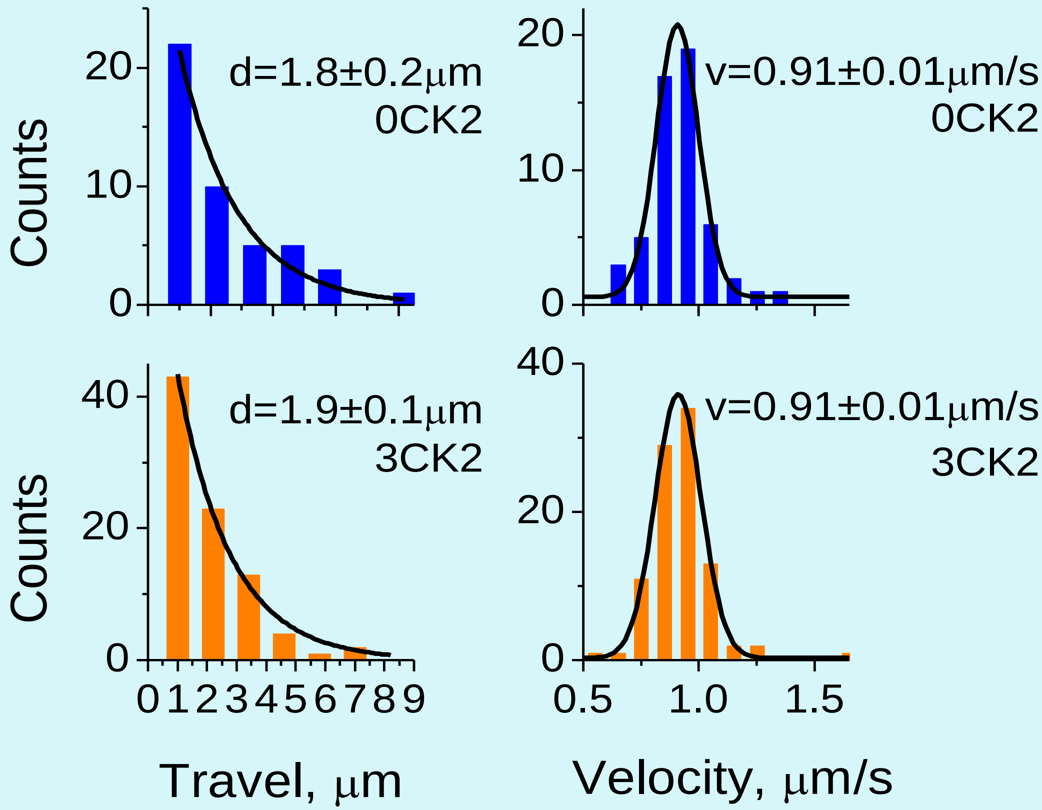
Xu, J., *et al*. *Nat. Commun.* (2012)

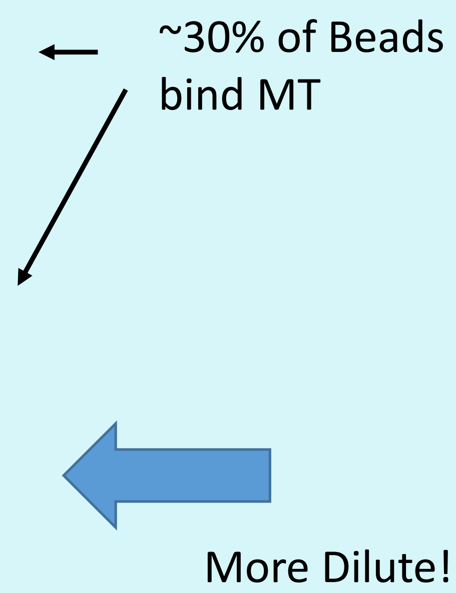
More motors active !



Xu, J., *et al*. *Nat. Commun.* (2012)

CK2 Does Not Change single-motor Properties





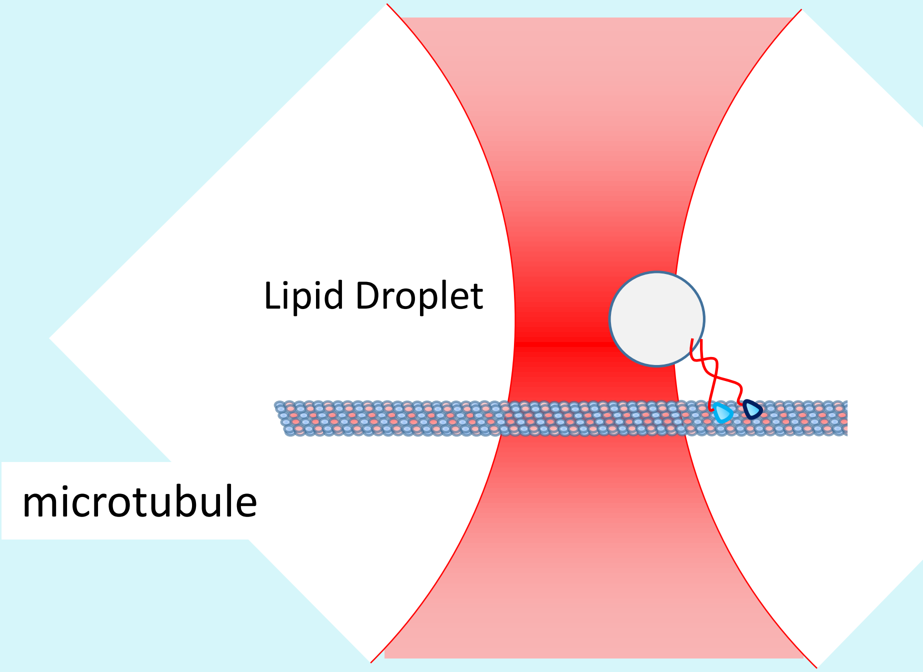
Xu, J., *et al*. *Nat. Commun.* (2012)

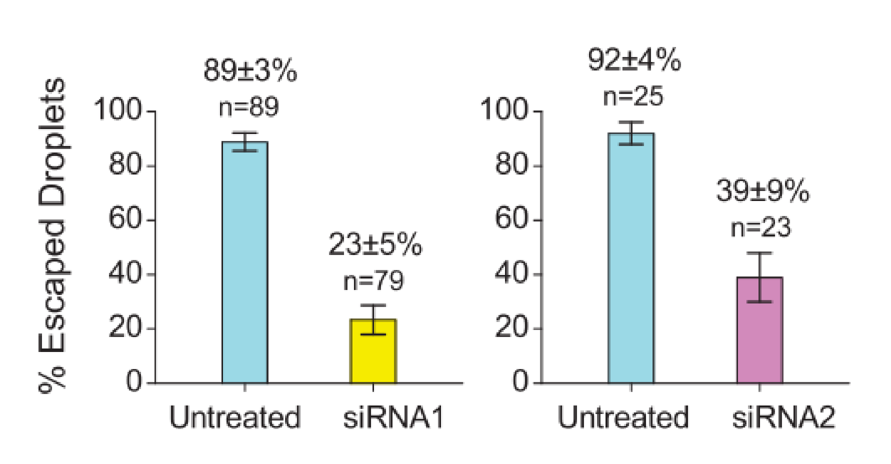
Emerging Questions:

1. In vitro artifact, or relevant *in vivo*?

2. What is the nature of the effect ?

CK2/Kinesin Interaction Involved in Lipid Droplet Force Production





Yonggun Jun, Babu JN Reddy

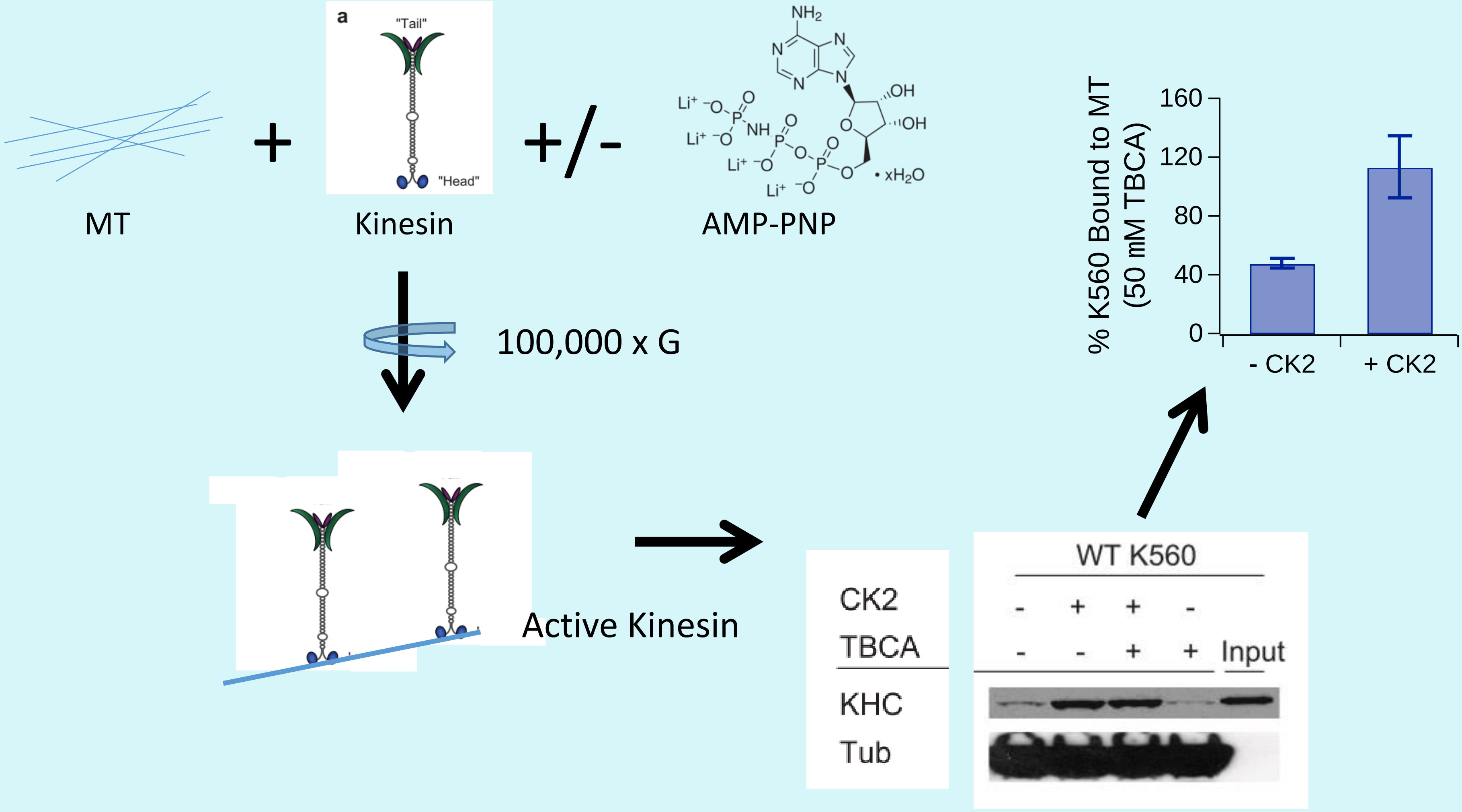
Xu, J., *et al*. *Nat. Commun.* (2012)

Conclusion: CK2 relevant for both in vivo and in vitro function

How is it changing the amount of active motors?

Is it changing motor activity itself (on-rate or overall number of active motors), or recruitment of motors to cargos?

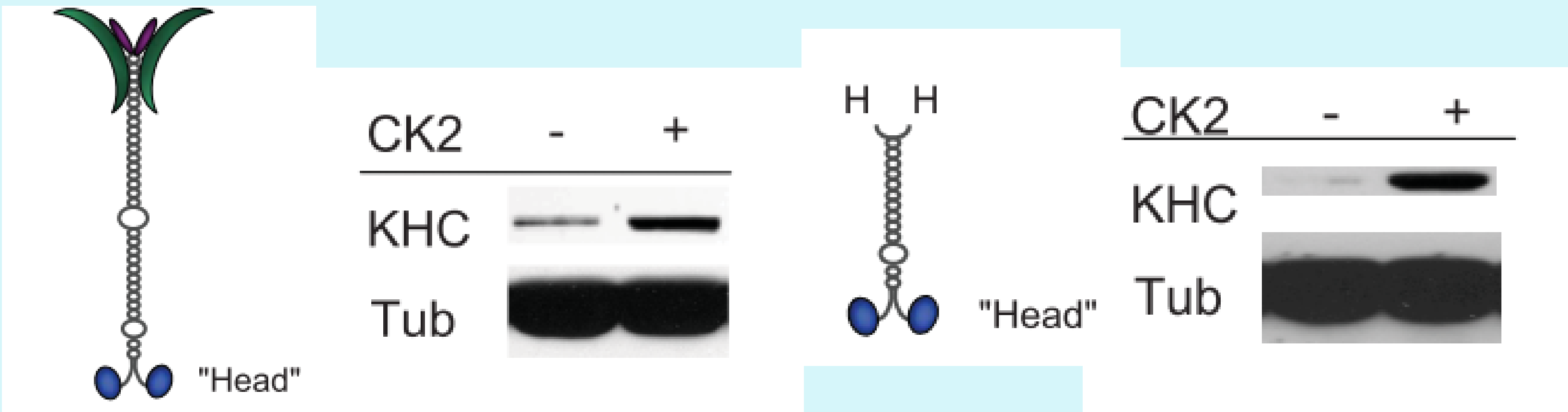
In-Vitro: Active Motor Assay—NO CARGO



What’s going on? Models….

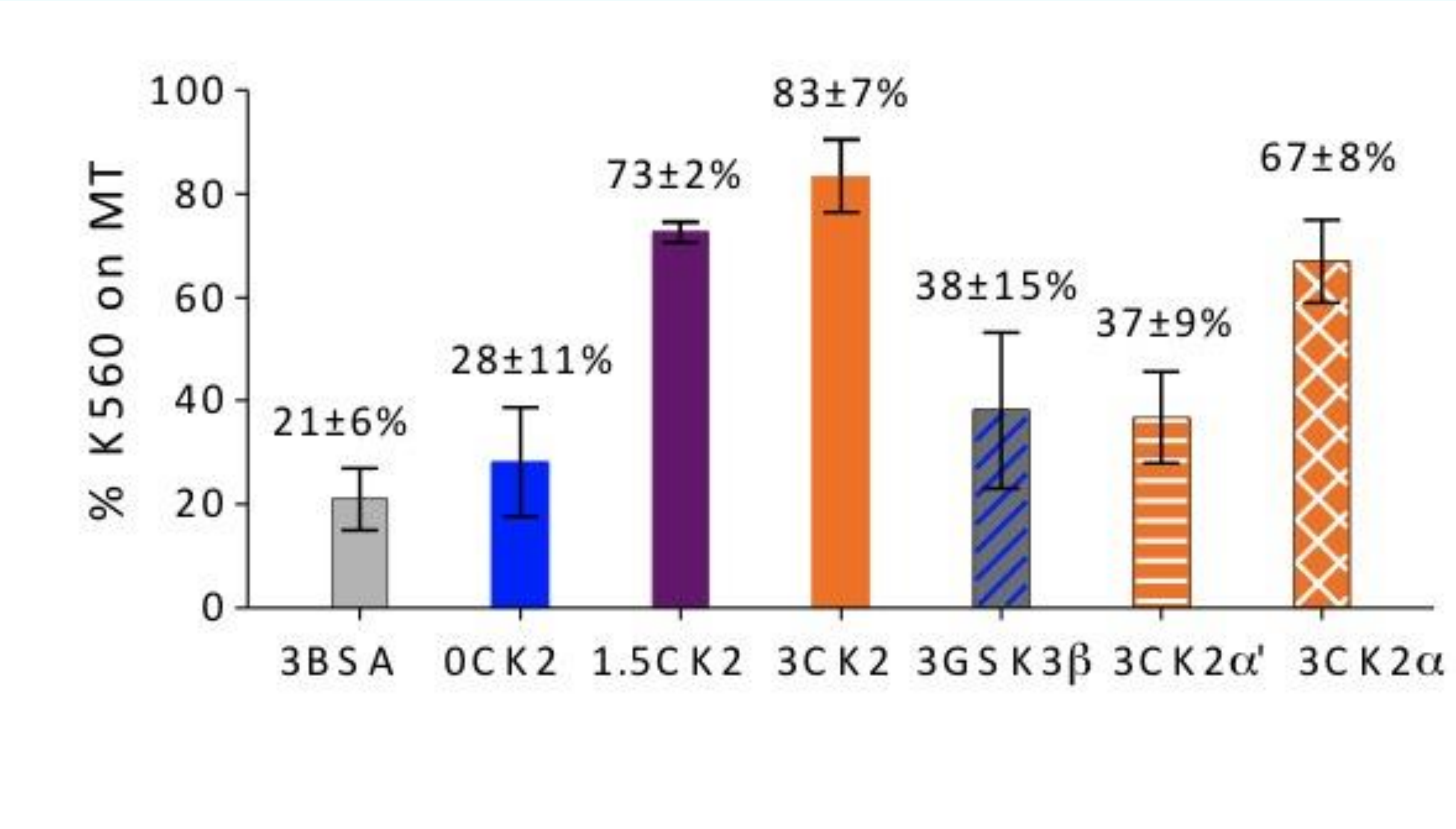
1. CK2 releases head-tail inhibition.
2. CK2 functions as a crowding agent (prevents denaturation)
3. CK2 Phosphorylating Kinesin (changing motor on-rate)
4. More interesting/unexpected possibilities

Kinesin Inactivation (& CK2 Reactivation) Independent of Head-Tail Inhibition



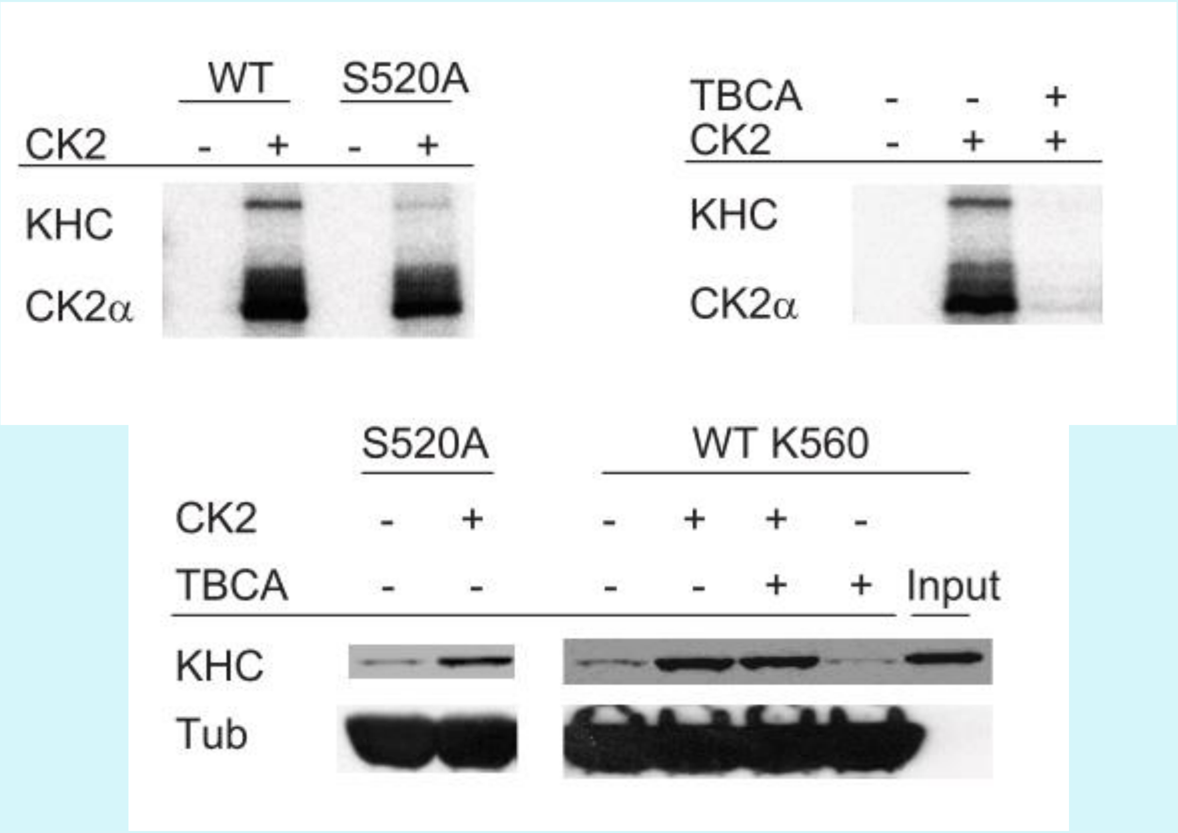
Xu, J., *et al*. *Nat. Commun.* (2012)

CK2α Specific (not simple crowding)



Xu, J., *et al*. *Nat. Commun.* (2012)

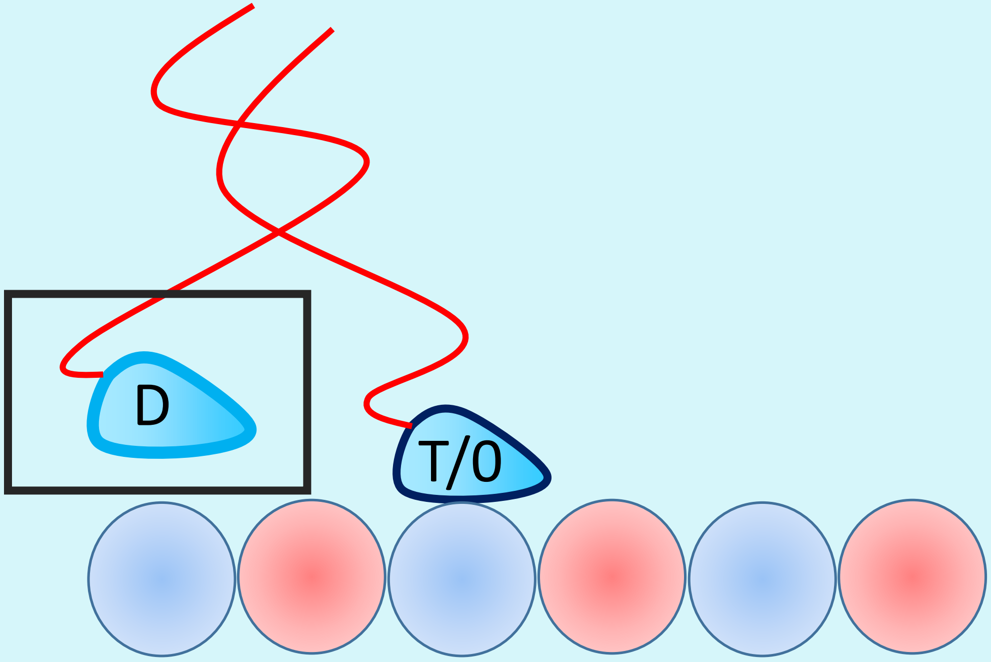
Activation Independent of kinase activity



Xu, J., *et al*. *Nat. Commun.* (2012)

Part 2:Mechanism of regulation

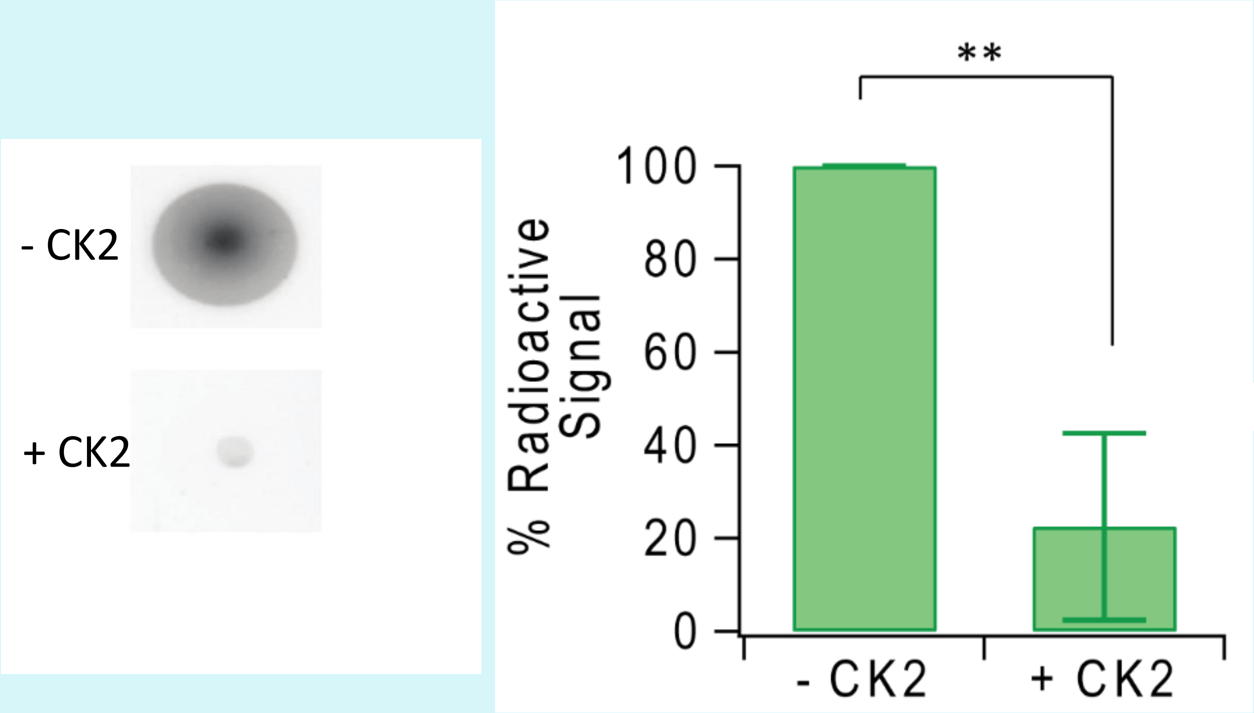
Kinesin MT Binding States



2 Models for Mechanism

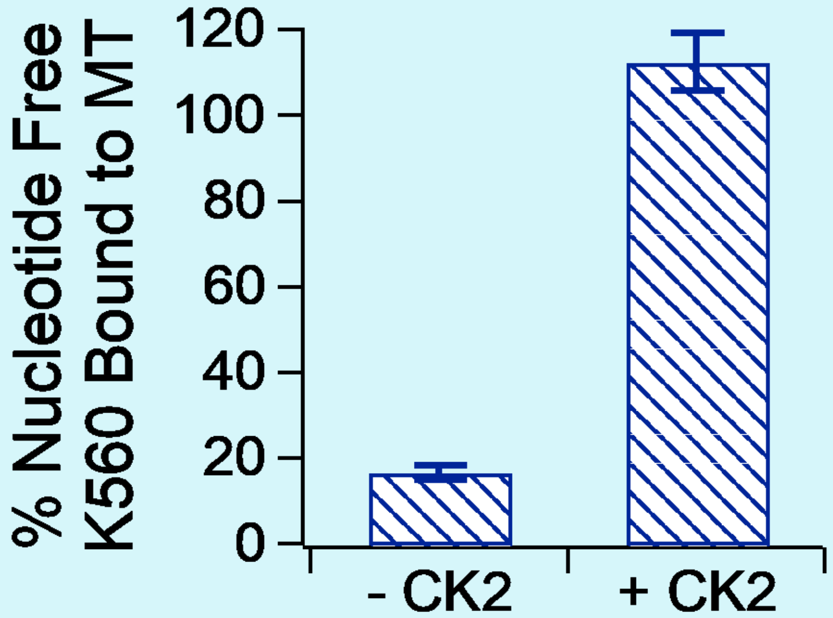
1. Heads trapped in nucleotide state, causing low microtubule affinity
2. Other conformational change resulting in low microtubule affinity

CK2 Accelerates Nucleotide Exchange



Mattson-Hoss *et al.*, PNAS, 2014

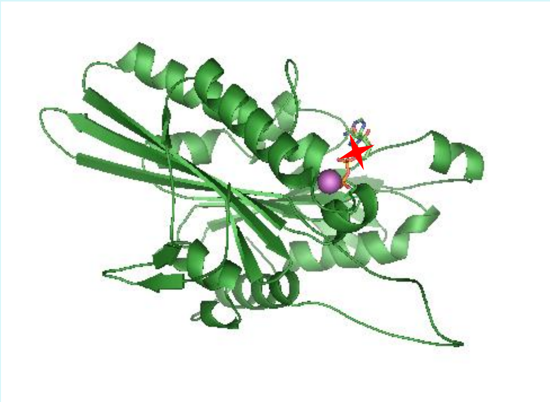
Nucleotide State Does Not Affect Reactivation

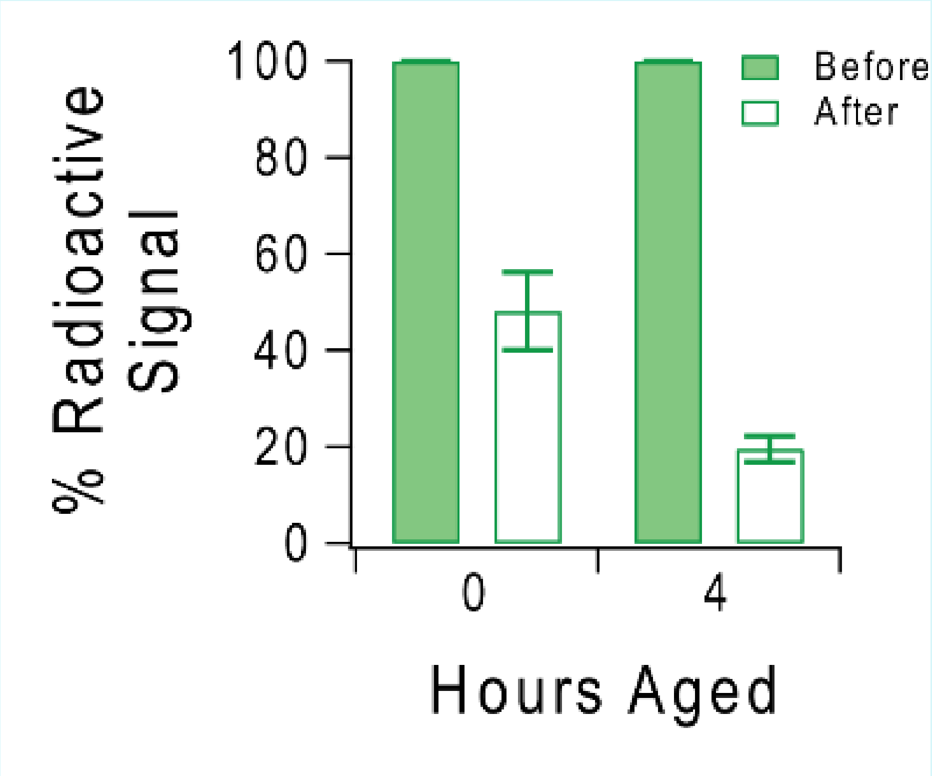


Mattson-Hoss *et al.* PNAS 2014

Inactive Kinesin is Not Trapped in the Weak MT Affinity State

= P32

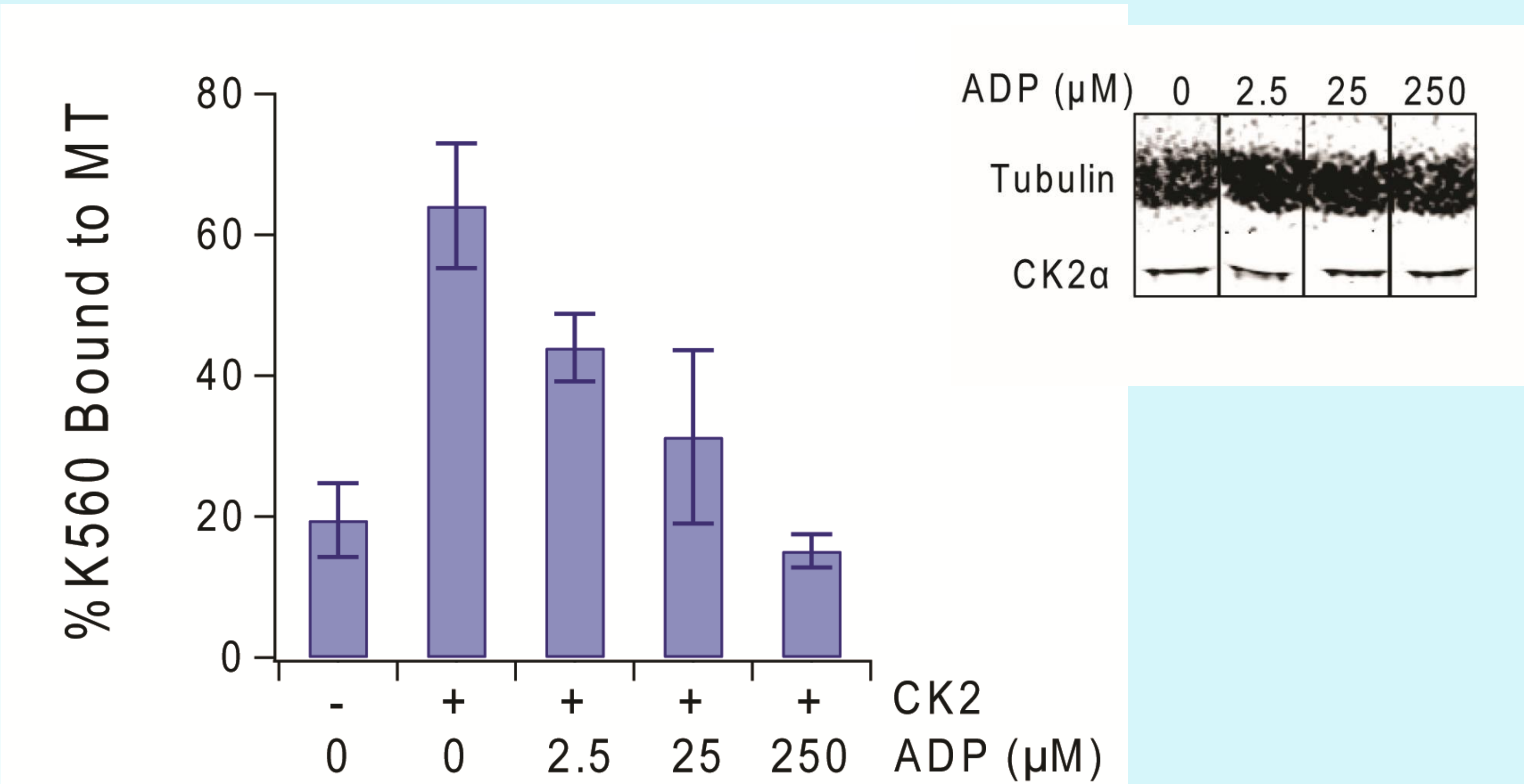




Can exchange ADP for ATP in pulse-chase experiment, even when inactive!

Mattson-Hoss *et al.* PNAS

Kinesin's Binding to MT is Independent of CK2-MT Complex Formation

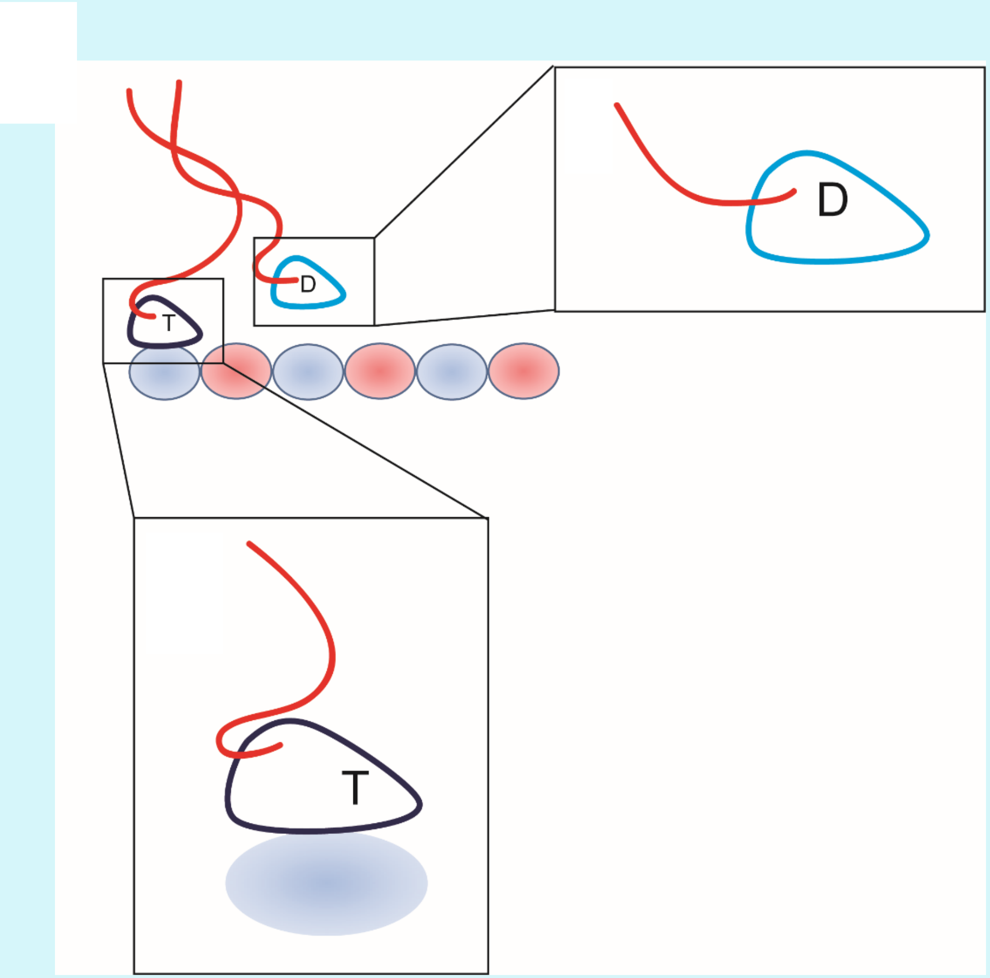


Mattson-Hoss *et al.*,. PNAS, 2014

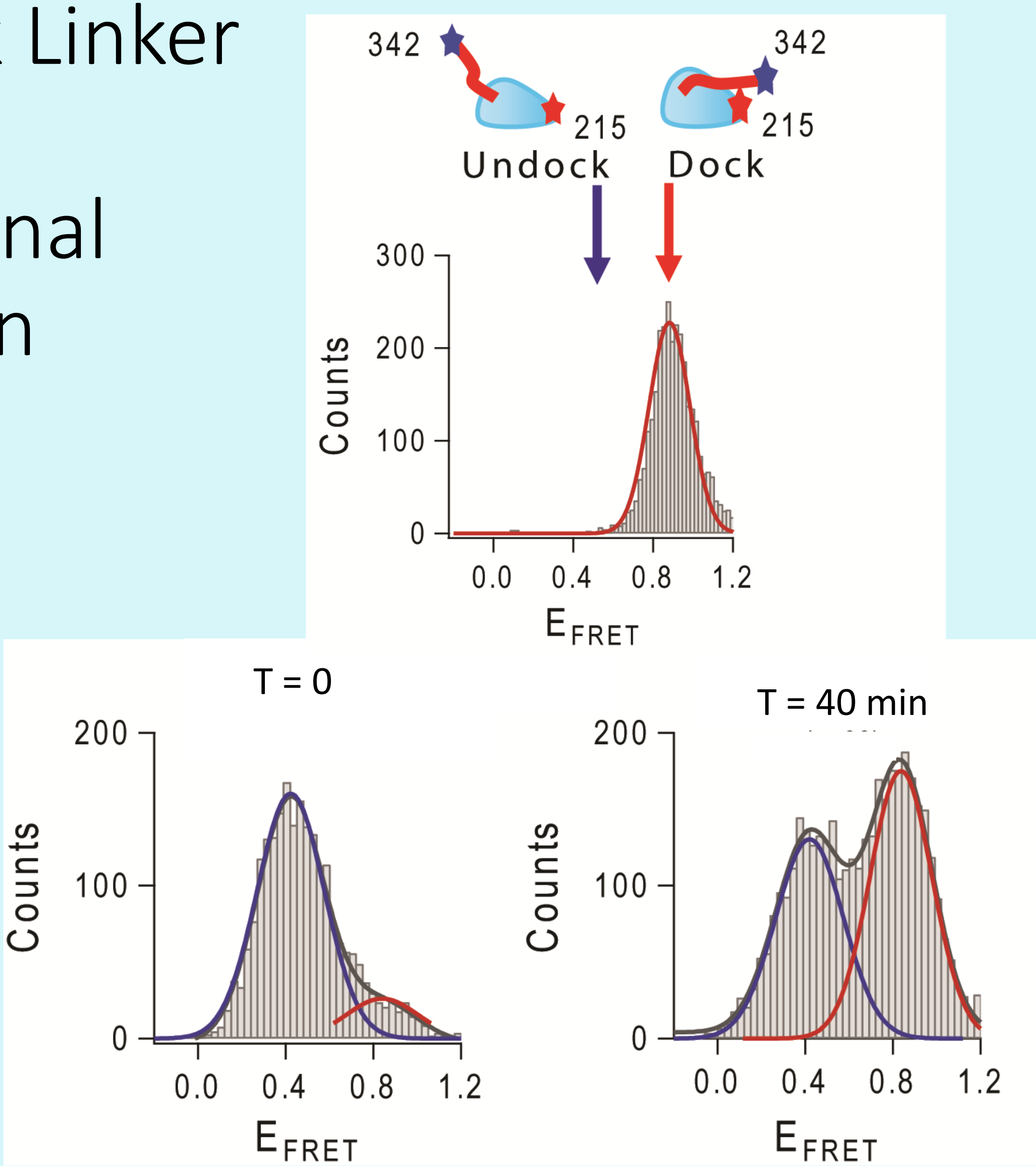
Conclusion: reject Model 1

- Causes conformational change
- Nucleotide release is secondary
- What is primary conformational change?

Kinesin Neck Linker Motion



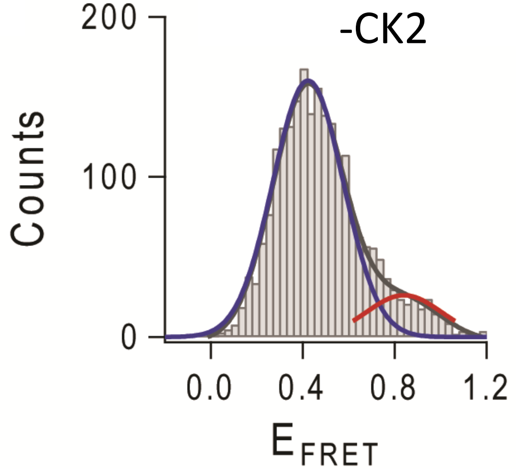
Kinesin Neck Linker Undergoes a Conformational Change when Inactive



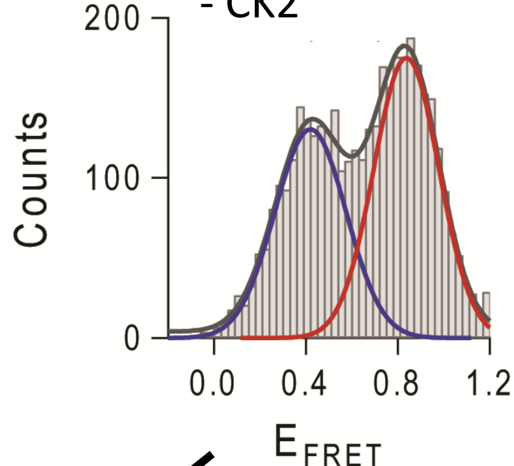
Mattson-Hoss *et al.* , PNAS 2014

CK2 Reverses Docking of the Neck Linker

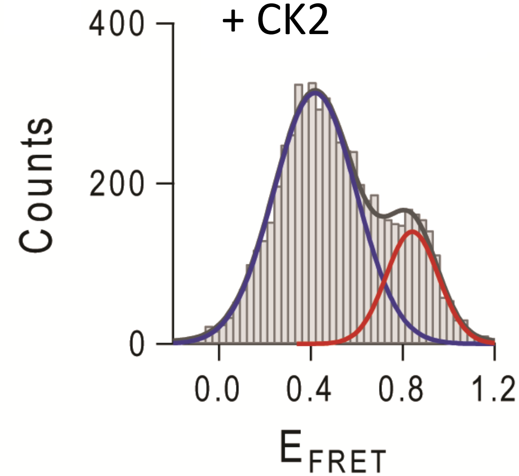
T=0
-CK2



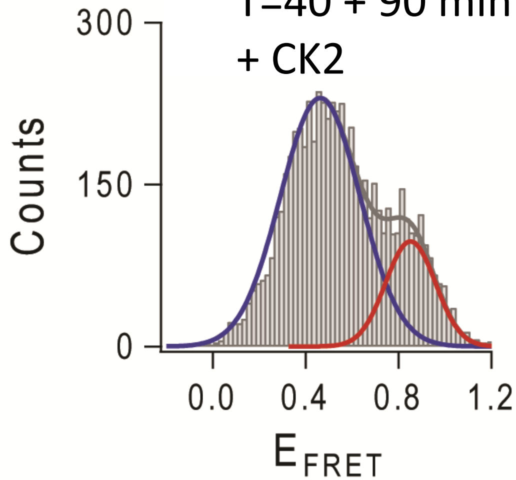
T=40 min
- CK2



T=40 min
+ CK2



T=40 + 90 min
+ CK2



Aged first, then CK2 added

Mattson-Hoss *et al.* PNAS

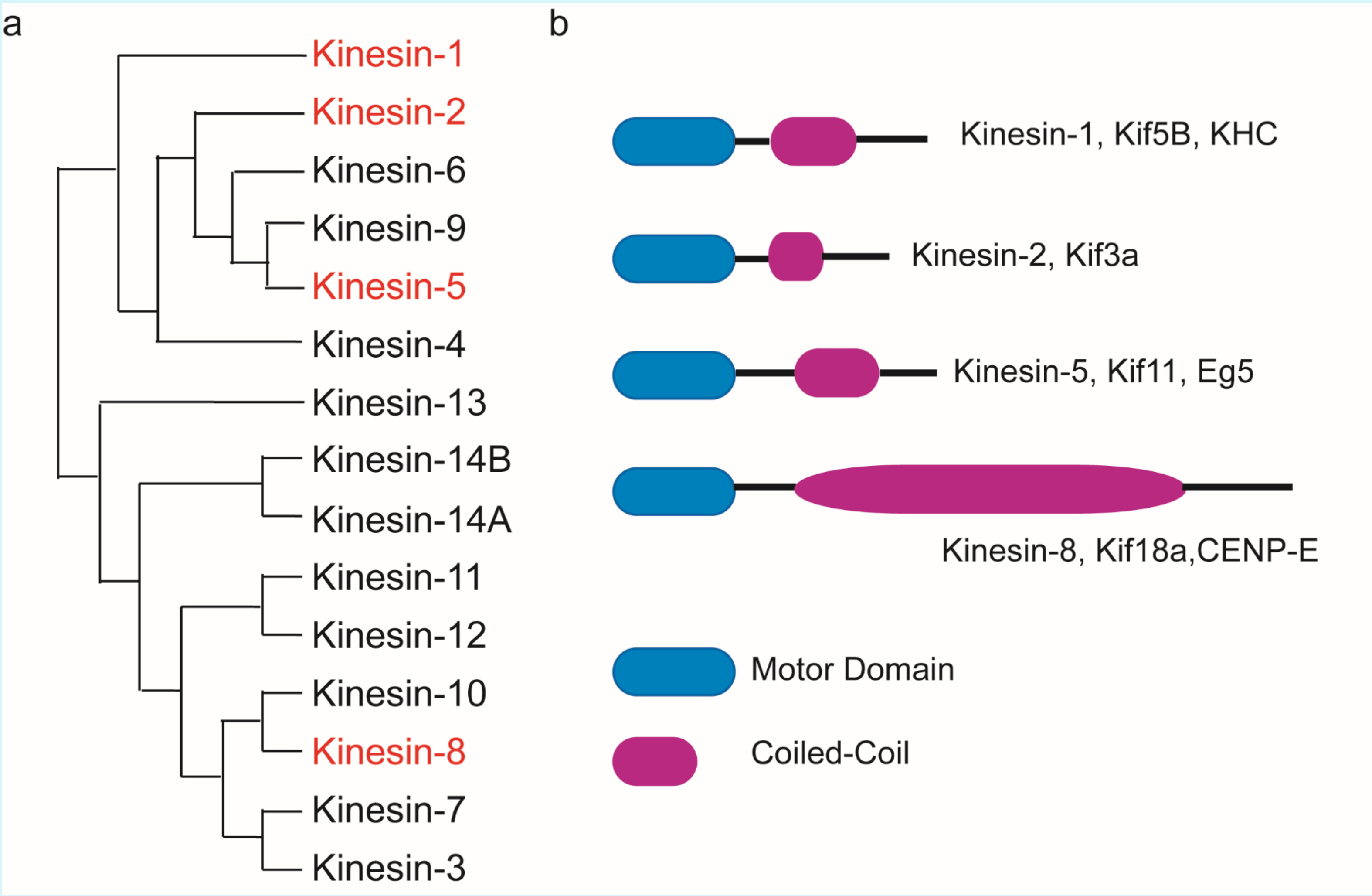
Conclusions

- Treatment with CK2
  - Facilitates nucleotide exchange
  - Loss of activity corresponds to movement of the neck linker
  - CK2 reverses motion of the neck linker
  - Reactivation does not require presence of MT

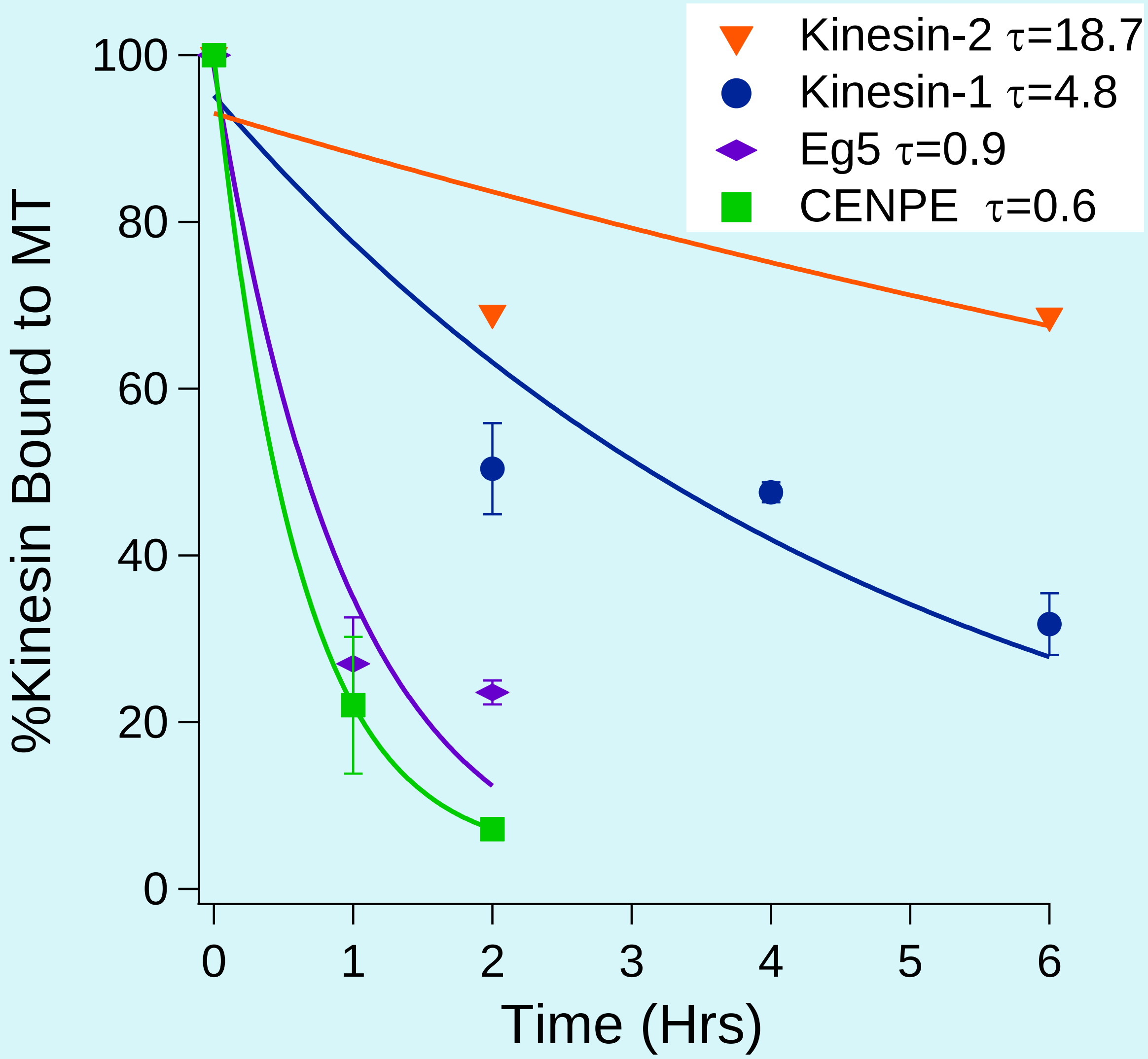
- Future Directions –
  - Understand how CK2 causes motion of the neck linker, and how this controls motor activity. Theorists…where are you…..?

Part 3: Regulation of OTHER Kinesin-Family Members

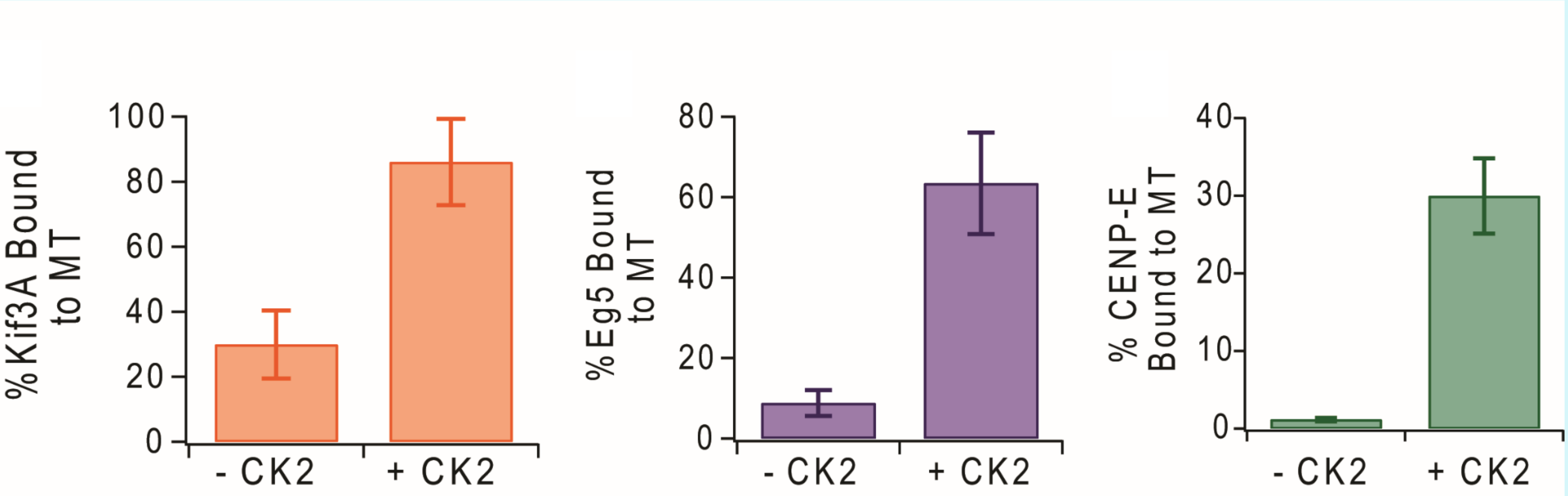
Kinesin Family



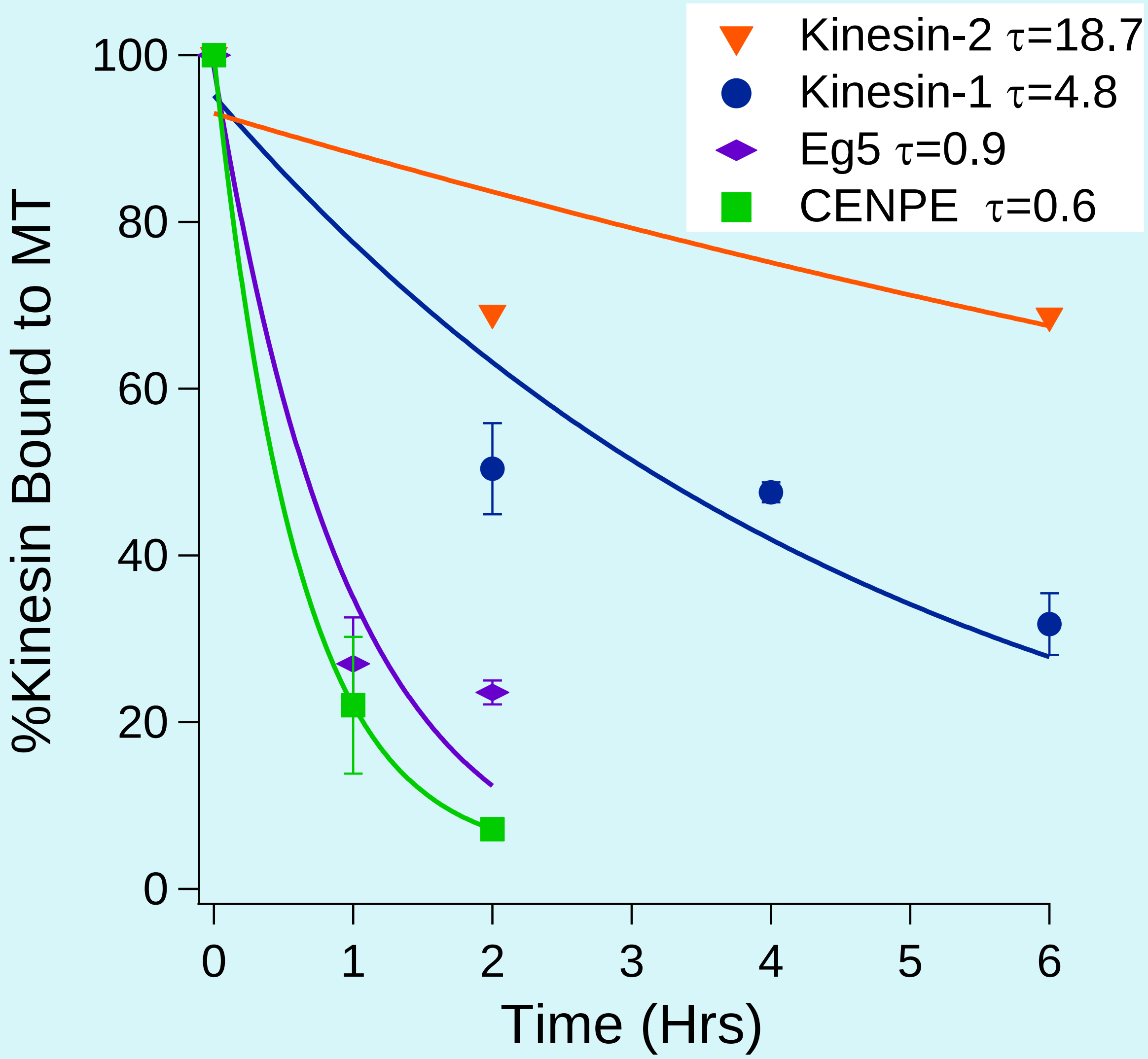
Kinesin Family Members Also Go Inactive



Kinesin Family Members Are Rescued by CK2 *in Vitro*



Different Inactivation Rates Dependent on Function

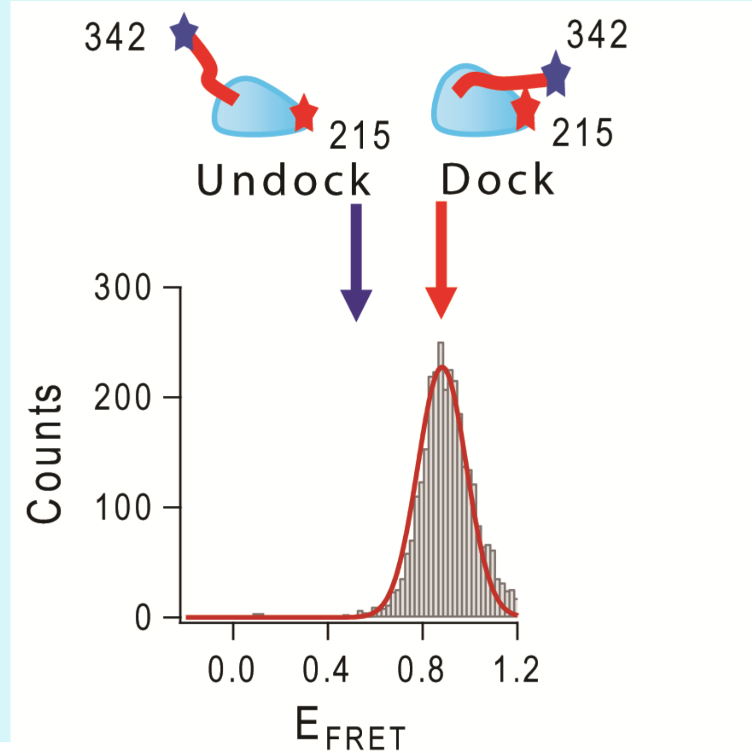


Conclusions

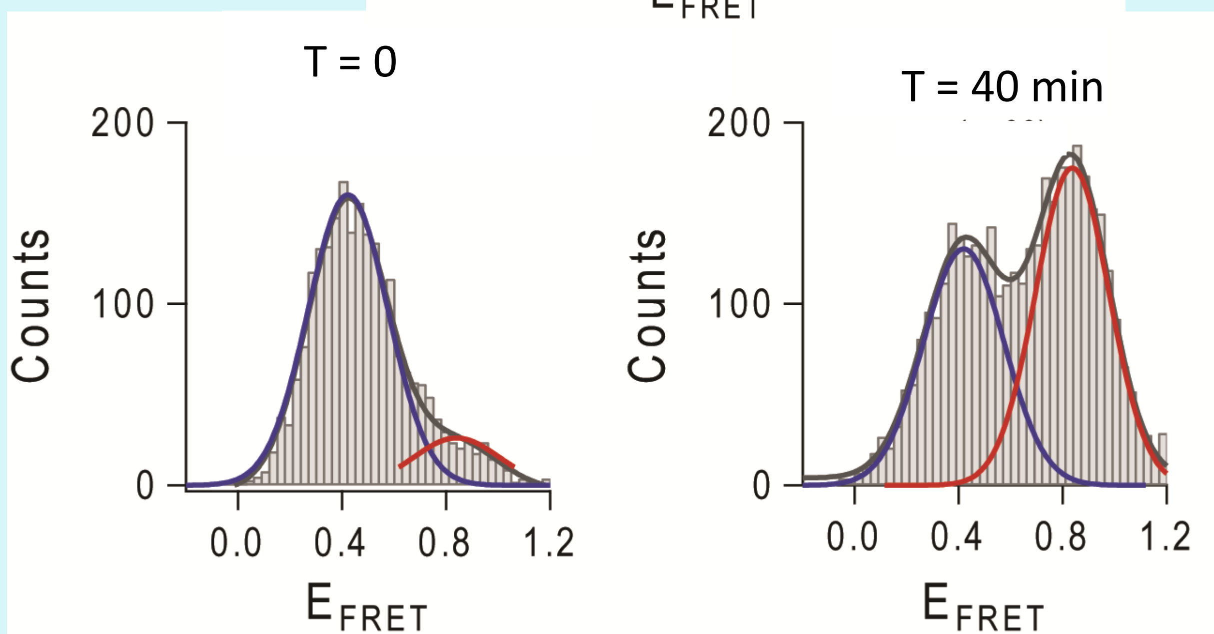
- Kinesin-Family goes inactive
  - **Function may dictate inactivation of the kinesin motor**
  - Variability in reactivation → structure related

- Future Directions –
  - Test other kinesin family members
  - Test in cells interaction between other motors and CK2

Kinesin Neck Linker Undergoes a Conformational Change when Inactive



N.B.: high Fret—held close together→ very ordered. **Not unfolded!**



Mattson-Hoss *et al.* , PNAS 2014

Overall Observations about Kinesin inactivation/ CK2-mediated reactivation

1. Kinesin inactivation occurs for both endogenous full-length kinesin, and also truncated (tail-less) dimers, and for single headed monomers

2. CK2 can reactivate each of the above

3. CK2 doesn't change the single-molecule properties of the 'active' motors—just the overall number of active motors

4. CK2-mediated reactivation of kinesin is independent of CK2's kinase activity

See Xu et al, "Casein kinase 2 reverses tail-independent inactivation of kinesin-1", Nature Communications (2012)).

This suggests that, from a protein-folding point of view, that the low energy 'ground state' is NOT the active state.

The Active state is a long-lived intermediate !!

Conclusion 1

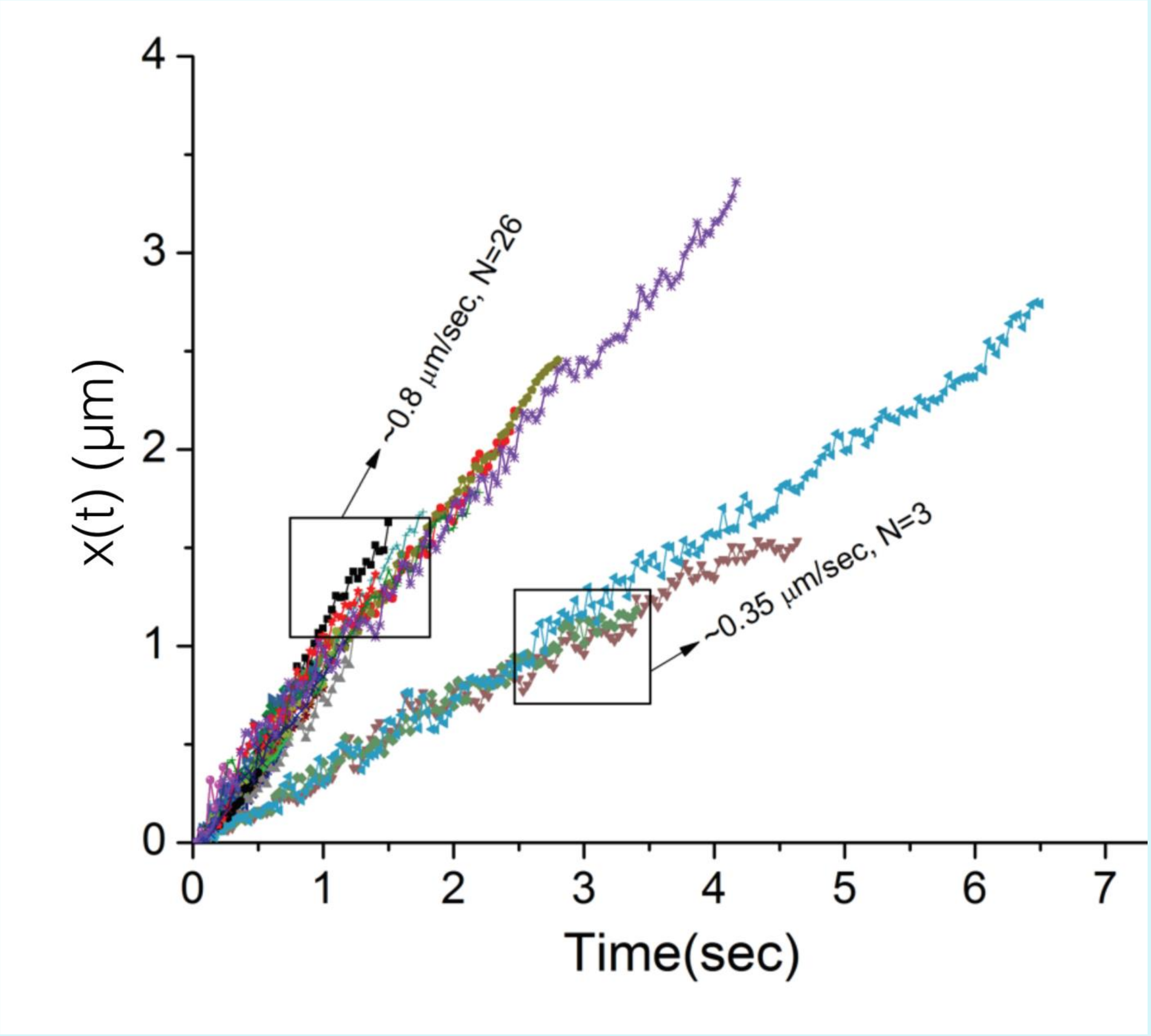
The ‘ground state’ of kinesin is a folded state, but is NOT the active state—doesn’t interact with microtubules

The ‘functional, active state’ is some sort of long-lived intermediate state.

Part 4: Heterogeneity

Question: Is there only a single intermediate?

Are all motors the same?

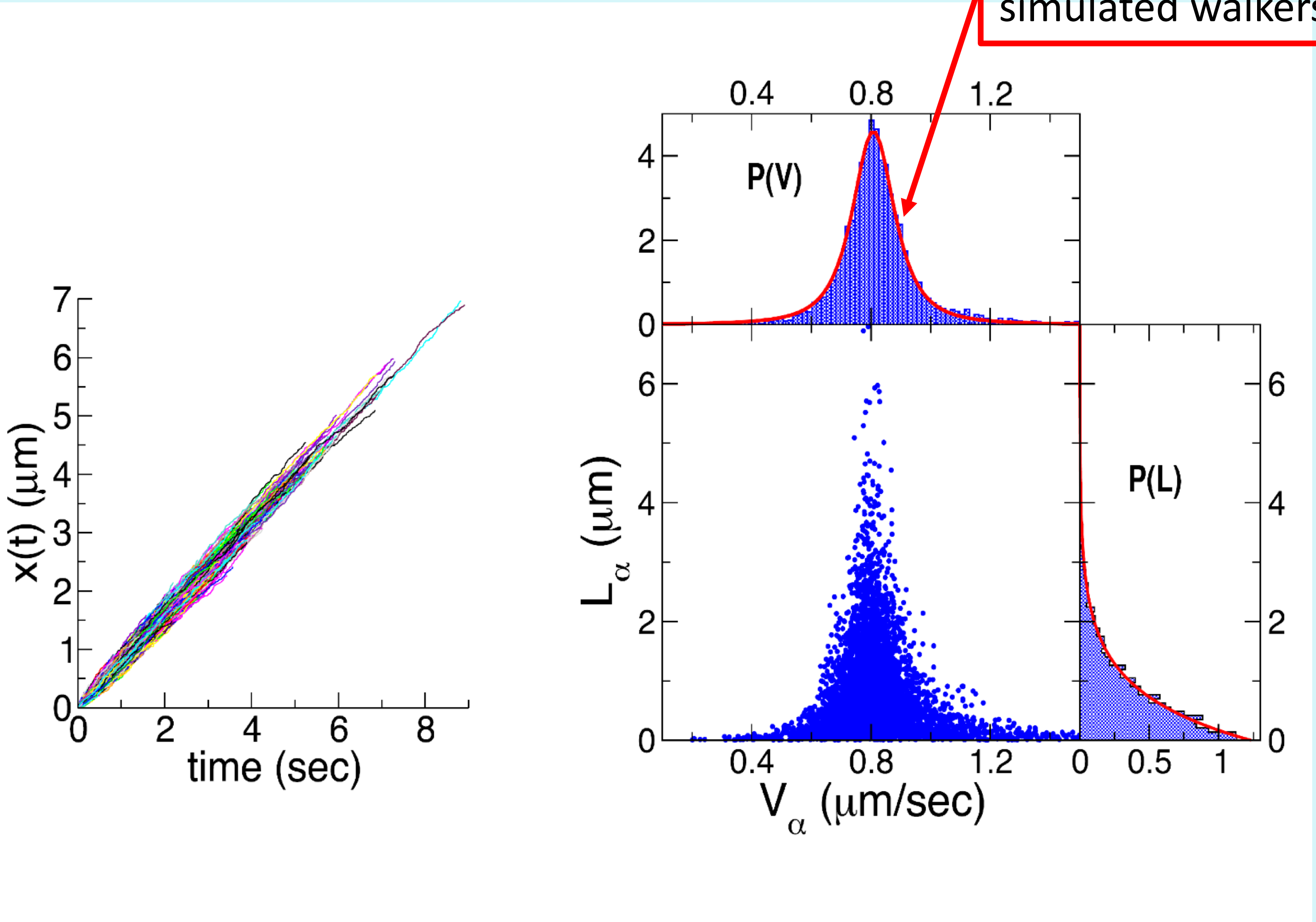


Surprising: expect “identical” proteins to function the same way

Possible Explanations:

1. They are functioning identically; the differences are simply due to expected stochastic variation in individual steps of the enzymatic cycle
2. May reflect an unknown chemical change due to purification—may not happen in vivo
3. They are not identical motors—some are post-translationally modified
4. They have different amounts of head-tail interactions (the tail is auto-inhibitory)
5. They are moving along different microtubules (with different amounts of post-translational modifications)
6. The differences reflect surface effects—some are moving along a microtubule protofilament close to surface, others further away
7. Differences result from differences in how motors are attached to the artificial cargos
8. Could really reflect something interesting—perhaps two different functional folded states?

Simulated motors (stochastic steppers) that are all the same….



Model captures observed variation in simulated walkers

Possible Explanations:

1. They are functioning identically; the differences are simply due to expected stochastic variation in individual steps of the enzymatic cycle
2. **May reflect an unknown chemical change due to purification—may not happen in vivo**
3. They are not identical motors—some are post-translationally modified
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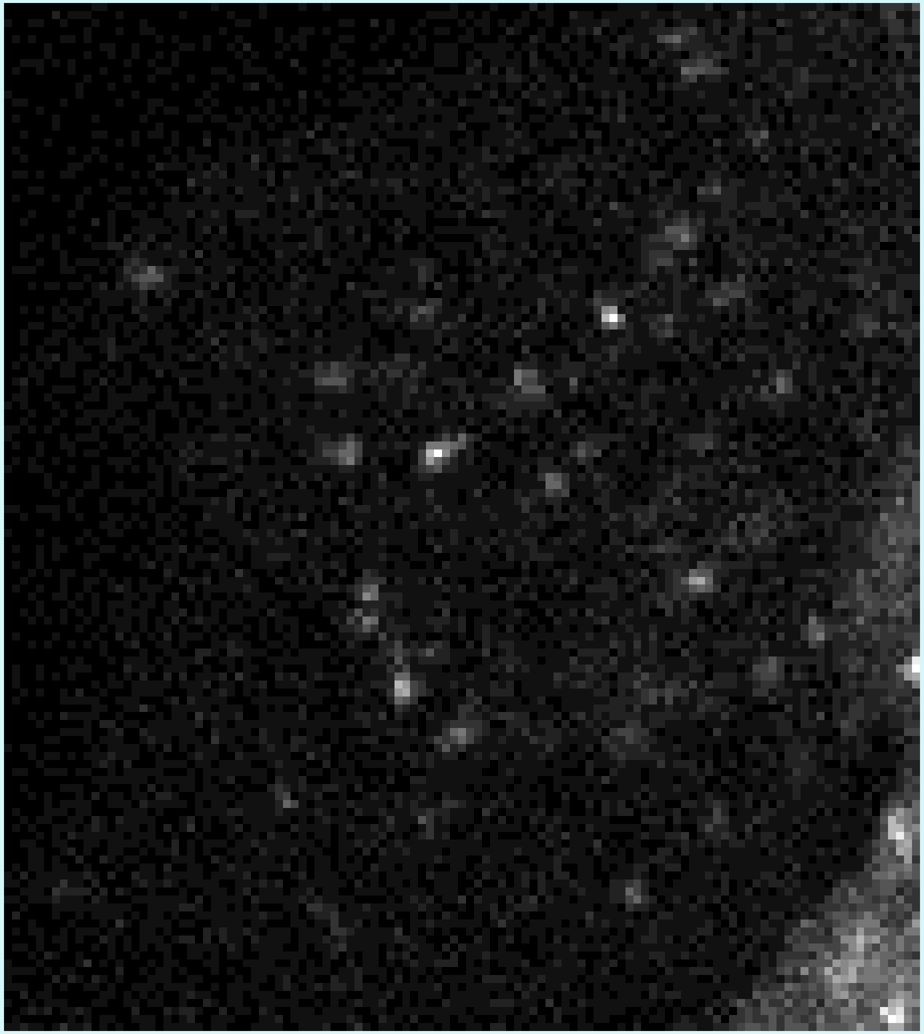
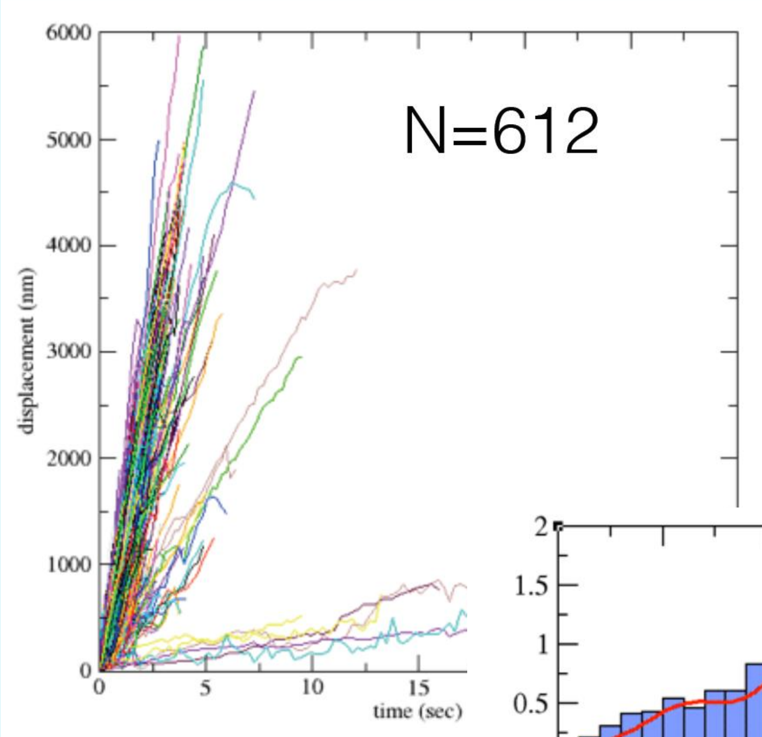
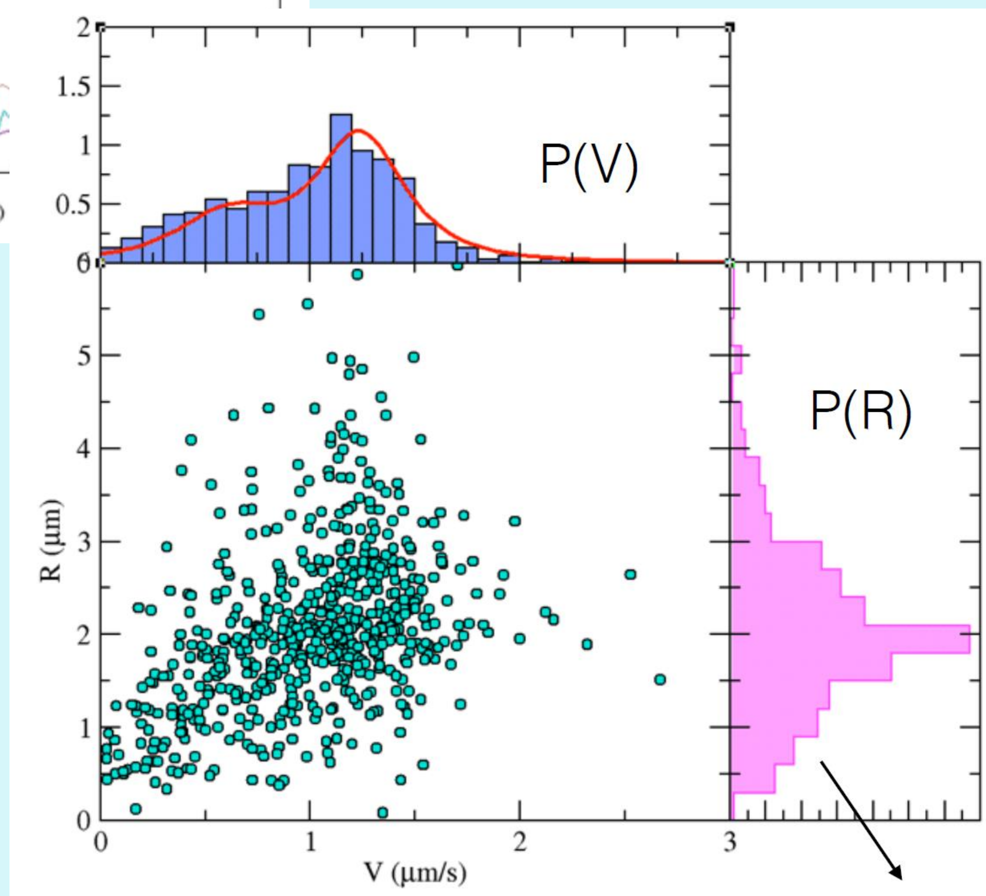
In vivo, we observe differences in single-molecule function



How significant are the differences in velocity?





in vivo traces
by Marvin group
K560-GFP

P(V) fit parameters :
V1 = 1.23696
L1 = 0.80157
D1 = 0.0173648
V2 = 0.601843
L2 = 2.00431
D2 = 0.118586
phi= 0.699506

Possible Explanations:

1. They are functioning identically; the differences are simply due to expected stochastic variation in individual steps of the enzymatic cycle
2. May reflect an unknown chemical change due to purification—may not happen in vivo
3. **They are not identical motors—some are post-translationally modified**
4. **They have different amounts of head-tail interactions (the tail is auto-inhibitory)**
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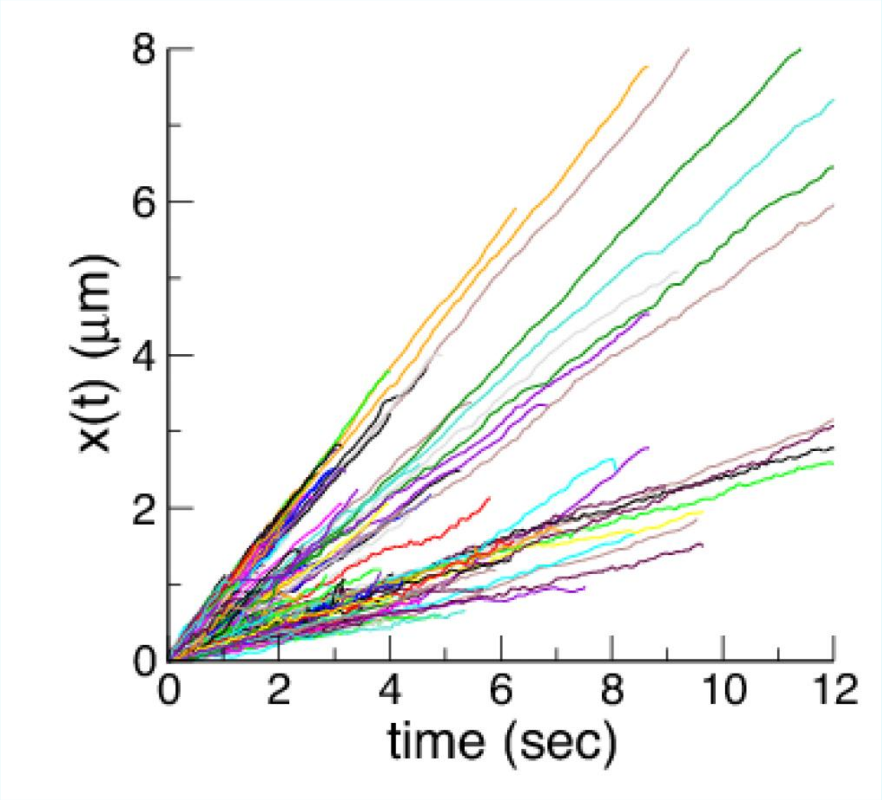
6. The differences reflect surface effects—some are moving along a microtubule protofilament close to surface, others further away
7. Differences result from differences in how motors are attached to the artificial cargos
8. Could really reflect something interesting—perhaps two different functional folded states?

In vivo, there could be post-translational modifications.

Make recombinant motors in e.coli



Single K560 molecules with QDs attached via anti-HA antibodies, moving in vitro on surface-immobilized MTs

Possible Explanations:

1. They are functioning identically; the differences are simply due to expected stochastic variation in individual steps of the enzymatic cycle
2. May reflect an unknown chemical change due to purification—may not happen in vivo
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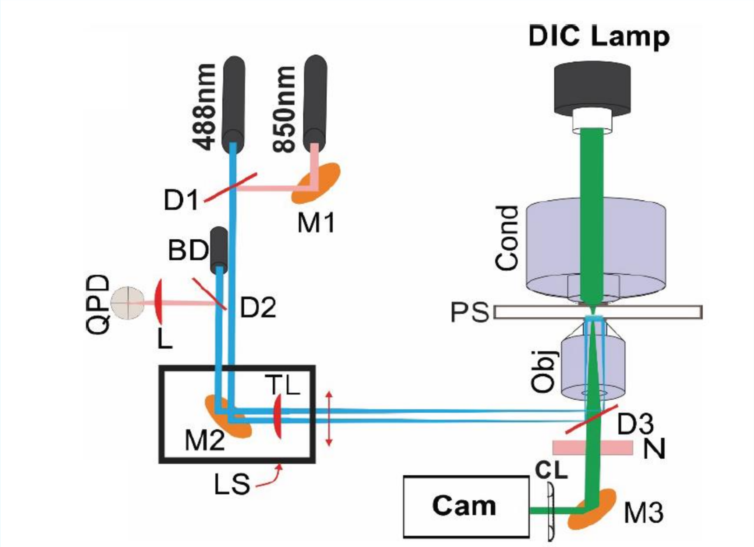
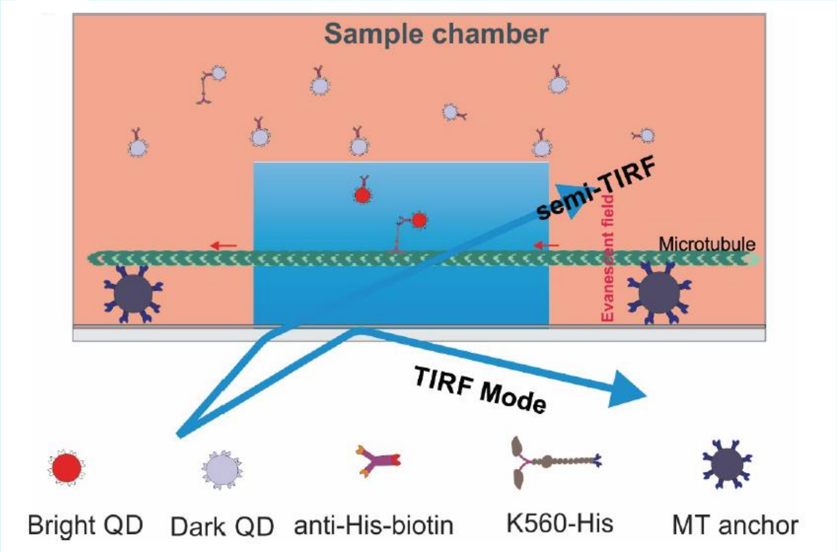
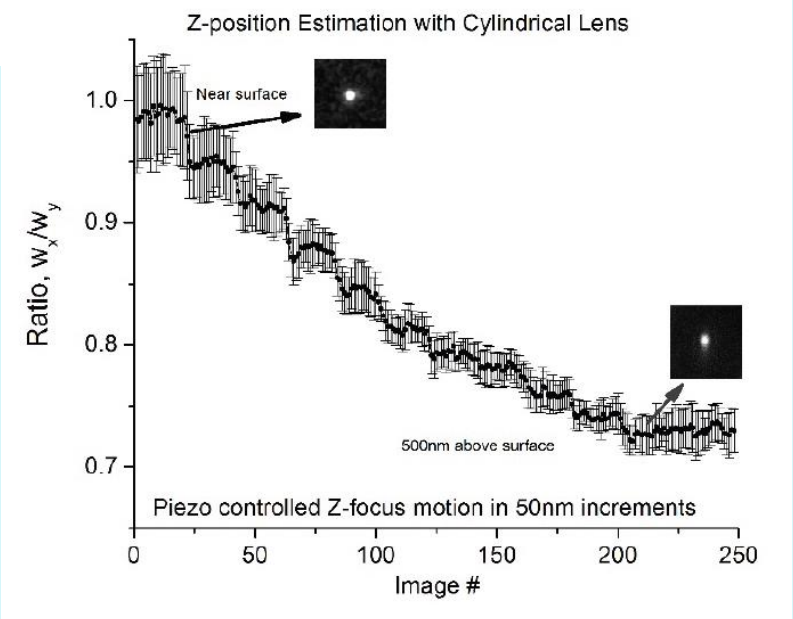
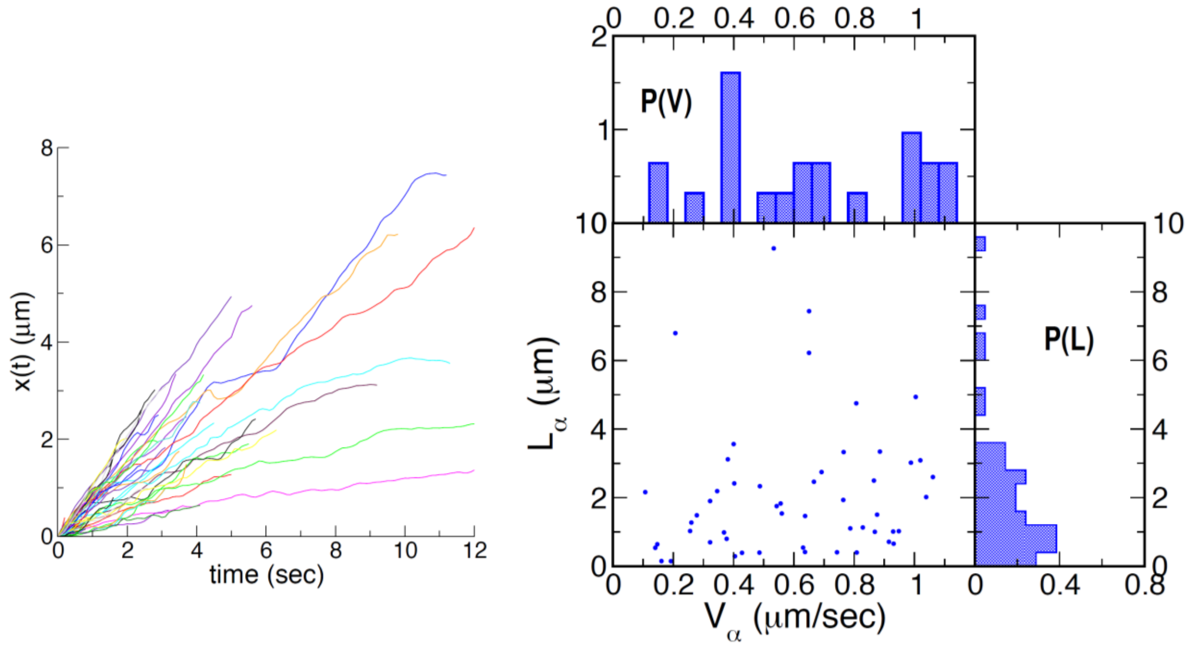
Off-Surface Measurements







Off-Surface tracks: still heterogeneous!



Possible Explanations:

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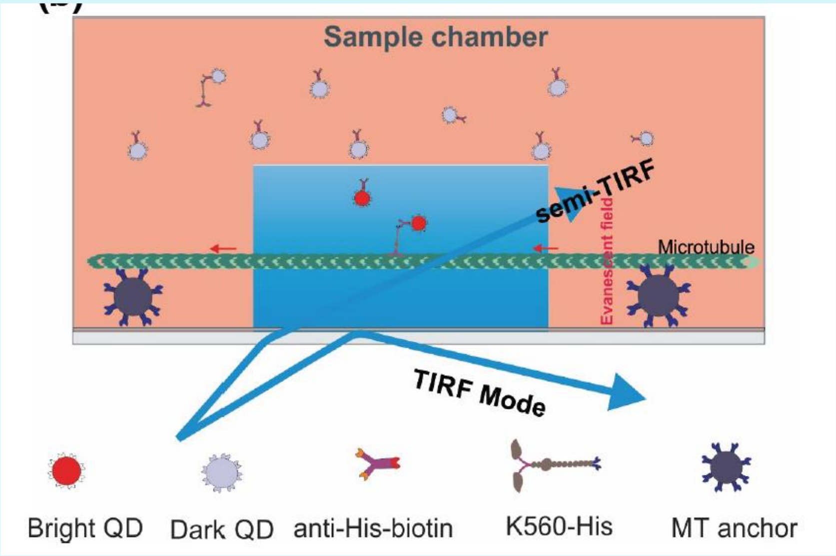
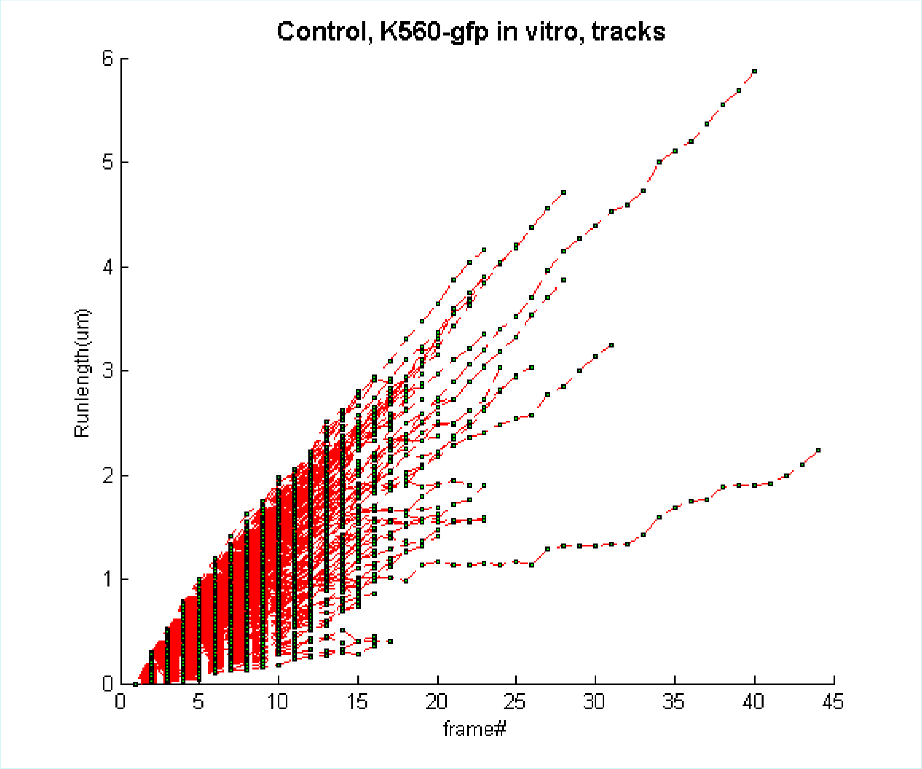
7. **Differences result from differences in how motors are attached to the artificial cargos**
8. Could really reflect something interesting—perhaps two different functional folded states?

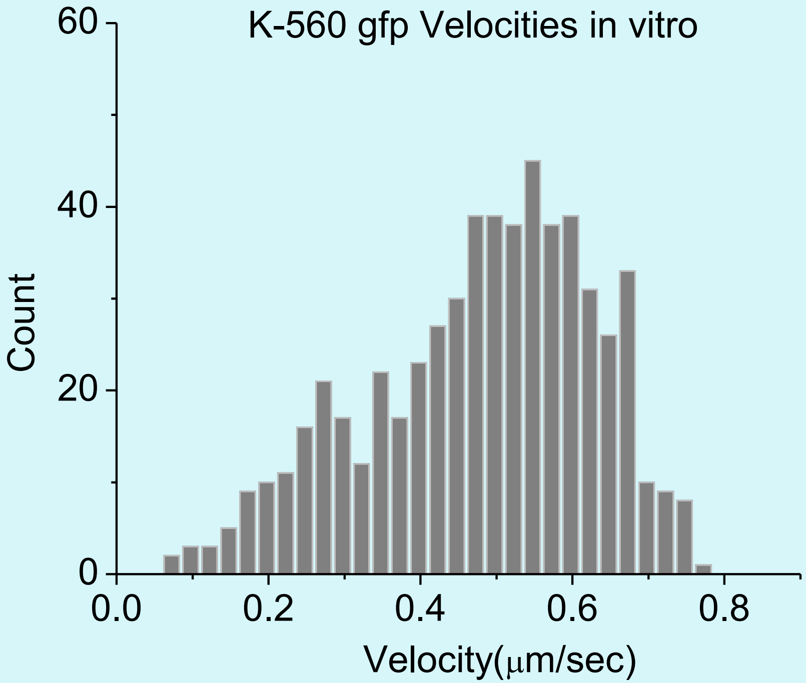
7. Differences result from differences in how motors are attached to the artificial cargos

Use TIRF imaging, but now with GFP-K560 (no cargos)

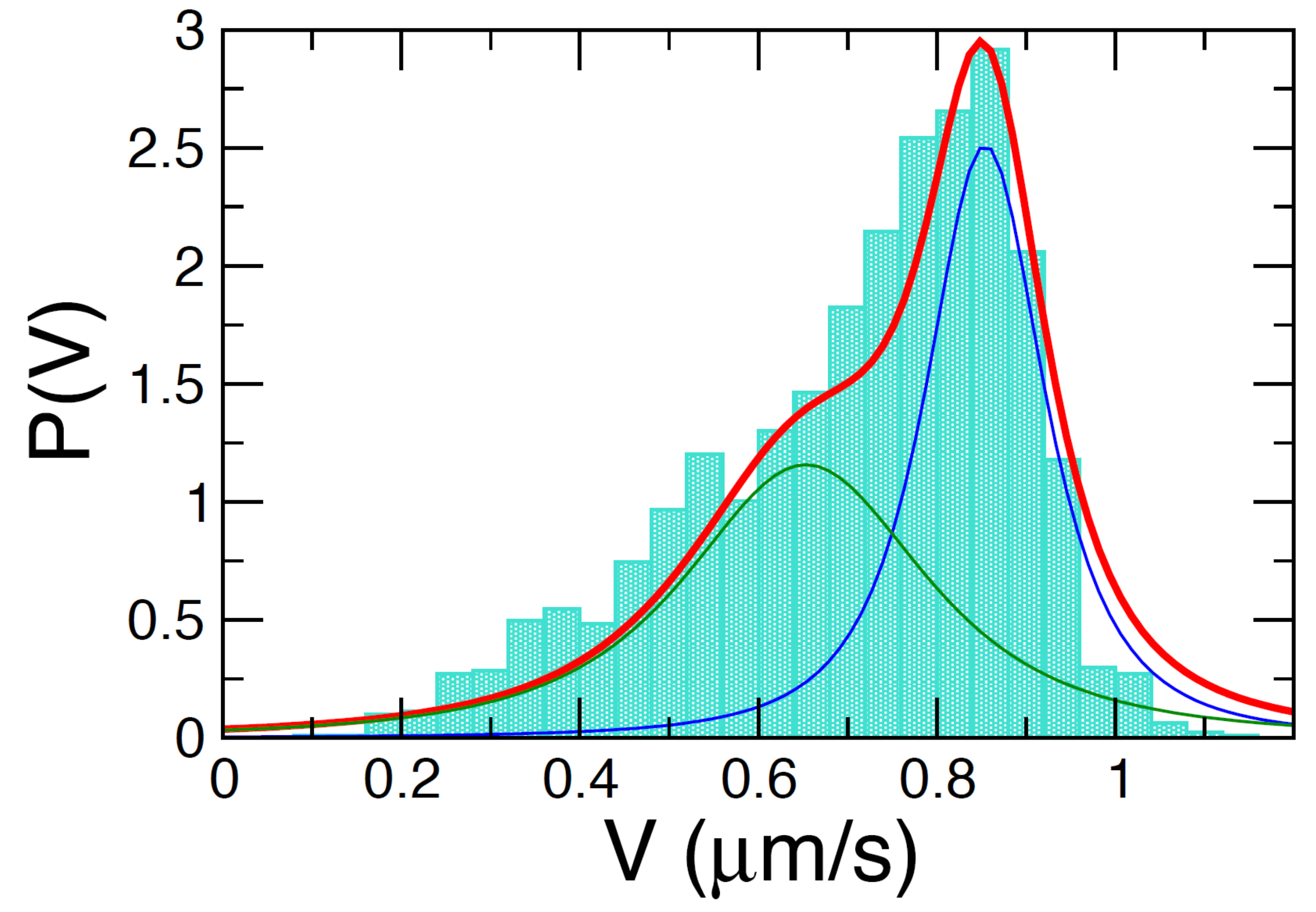


GFP-K560 Fusion proteins: many measurements, no cargos, still heterogeneous!





Velocity distribution of runs from GFP-K560 Fusion proteins



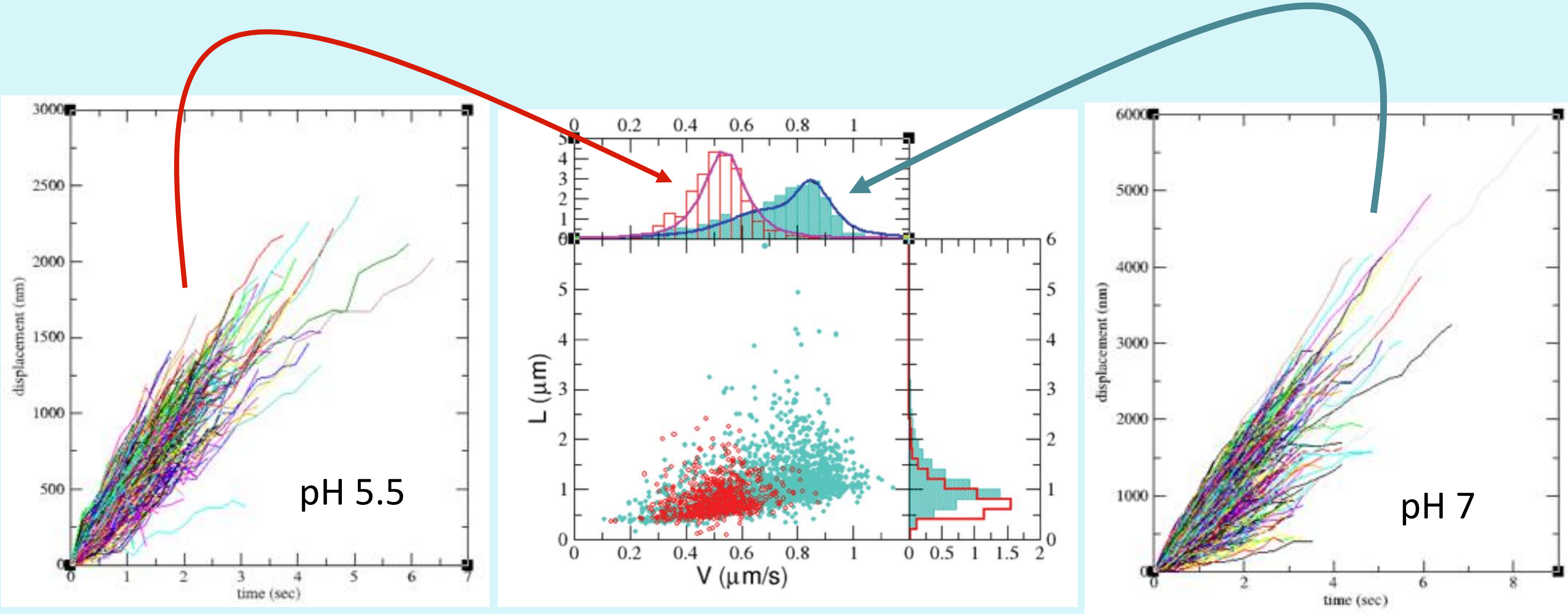
8. Could really reflect something interesting—perhaps two different functional folded states?

If multiple folded states, would like to selectively alter one.

Approach 1 (suggested by Bernie Brooks): change pH

8. Could really reflect something interesting—perhaps two different functional folded states?

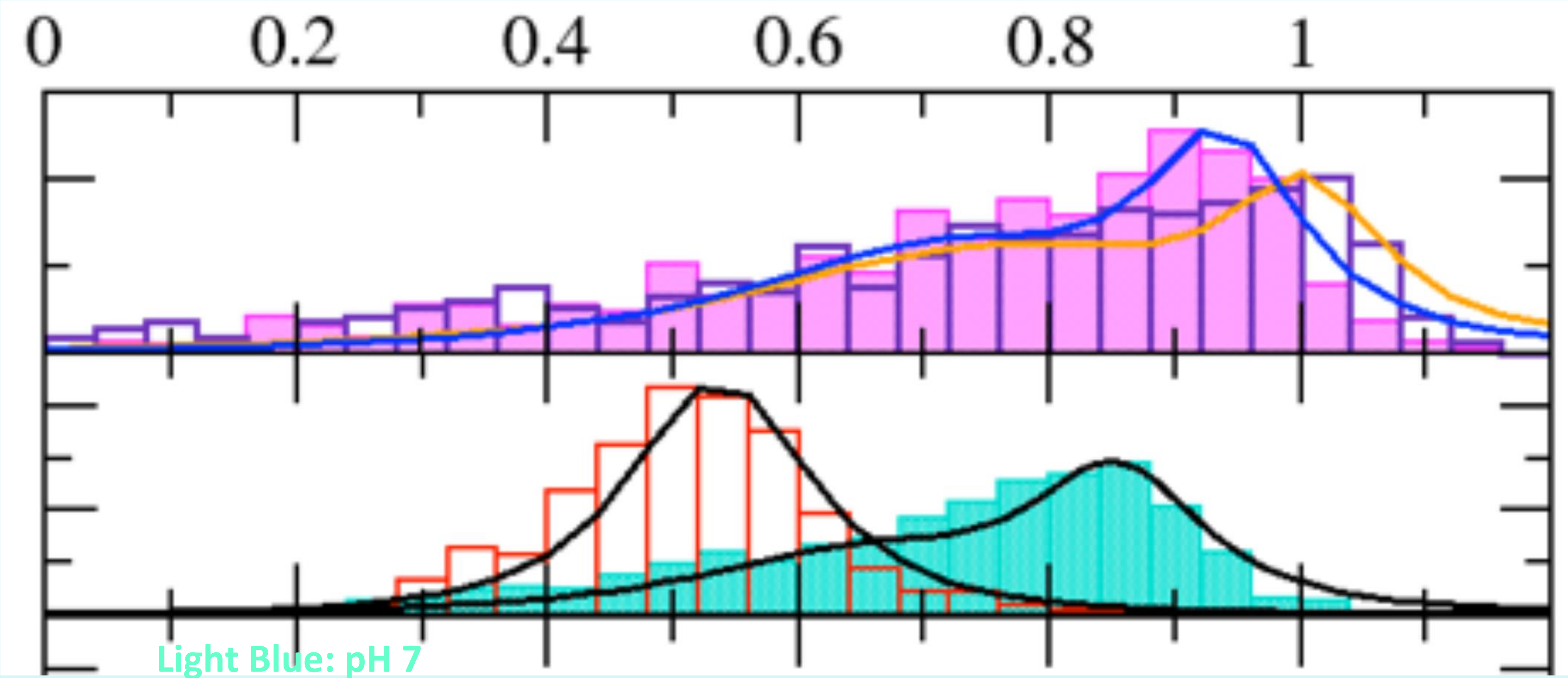
If multiple folded states, would like to selectively alter one.
Approach 1 (suggested by Bernie): change pH



pH 7 is 'wildtype'; low pH (5.5) eliminates the slow population

8. Could really reflect something interesting—perhaps two different functional folded states?

Compare effects of low and high pH



Light Blue: pH 7
(Black fit curve, bottom right)

Pink: pH 8.1
(blue fit curve, top)

Red: pH 5.5
(black fit, lower left)

Purple: pH 10.85
(orange fit curve,top)

Percentage of total in the 'Fast' population:

pH 5.5	100%
pH 7	52%
pH 8.1	37%
pH 10.85	27%

Conclusion: we think there are two or more different functional folded states for kinesin

Overall Conclusions

- Novel form of kinesin regulation → dependent on signaling kinase
  - Independent of auto-inhibition
  - Independent of CK2's kinase activity
- Model of regulation
  - CK2 regulates based on localization
- Functional state not lowest energy state→ possibility of multiple different intermediate folded states
  - This may explain observed functional heterogeneity

Awaiting theoretical input:

a) **Ramifications of the heterogeneity**
b) **Why the neck-linker docking leads to inactivation**

Acknowledgements

Steven Gross

Current Gross Lab Members:

- Yonggun Jun
- **Babu JN Reddy**
- Abdo Durra

Previous Lab Members:

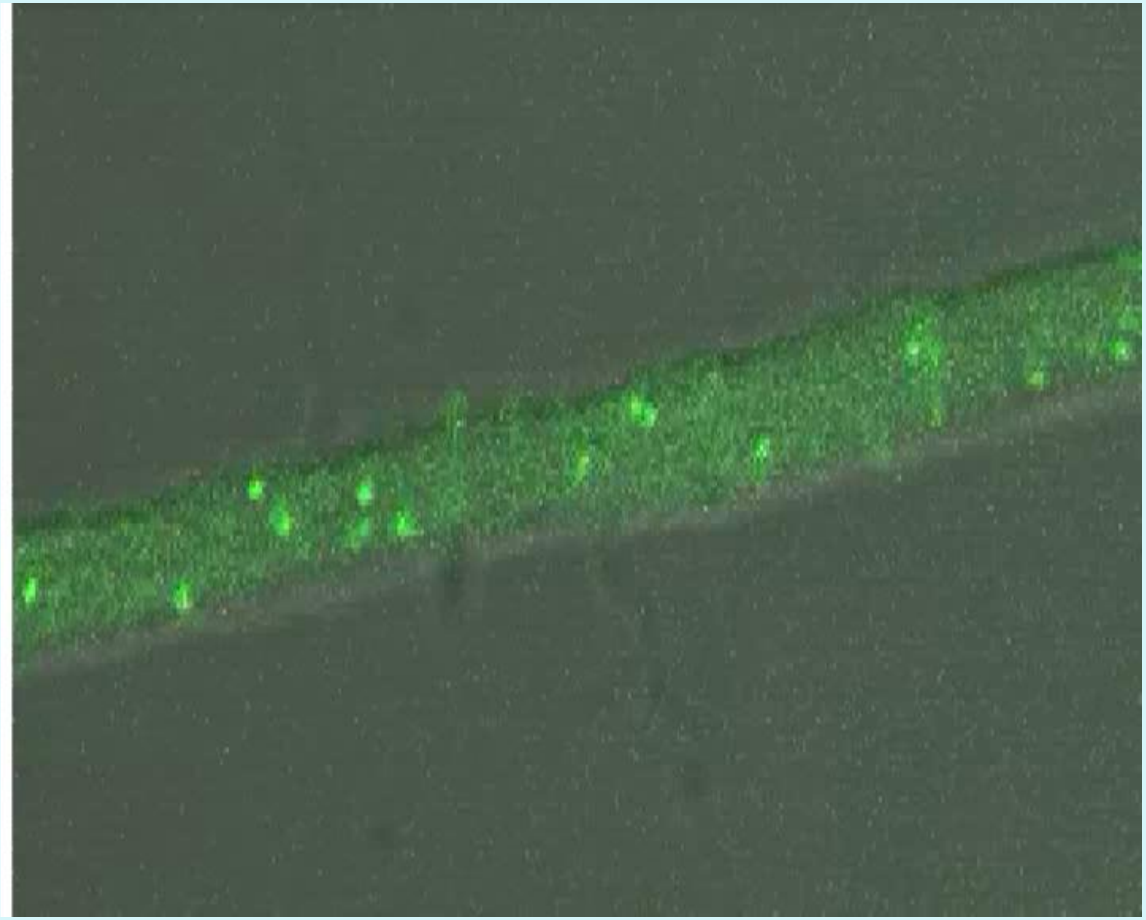
- Jing Xu
- Suvranta Tripathy

Collaborators:

- Michio Tomishige
- Yamato Niitani
- Elizabeth Gordon
- Lee Bardwell
- Steven Rosenfeld
- Les Wilson
- Herb Miller
- William Hancock
- Tom Poulos

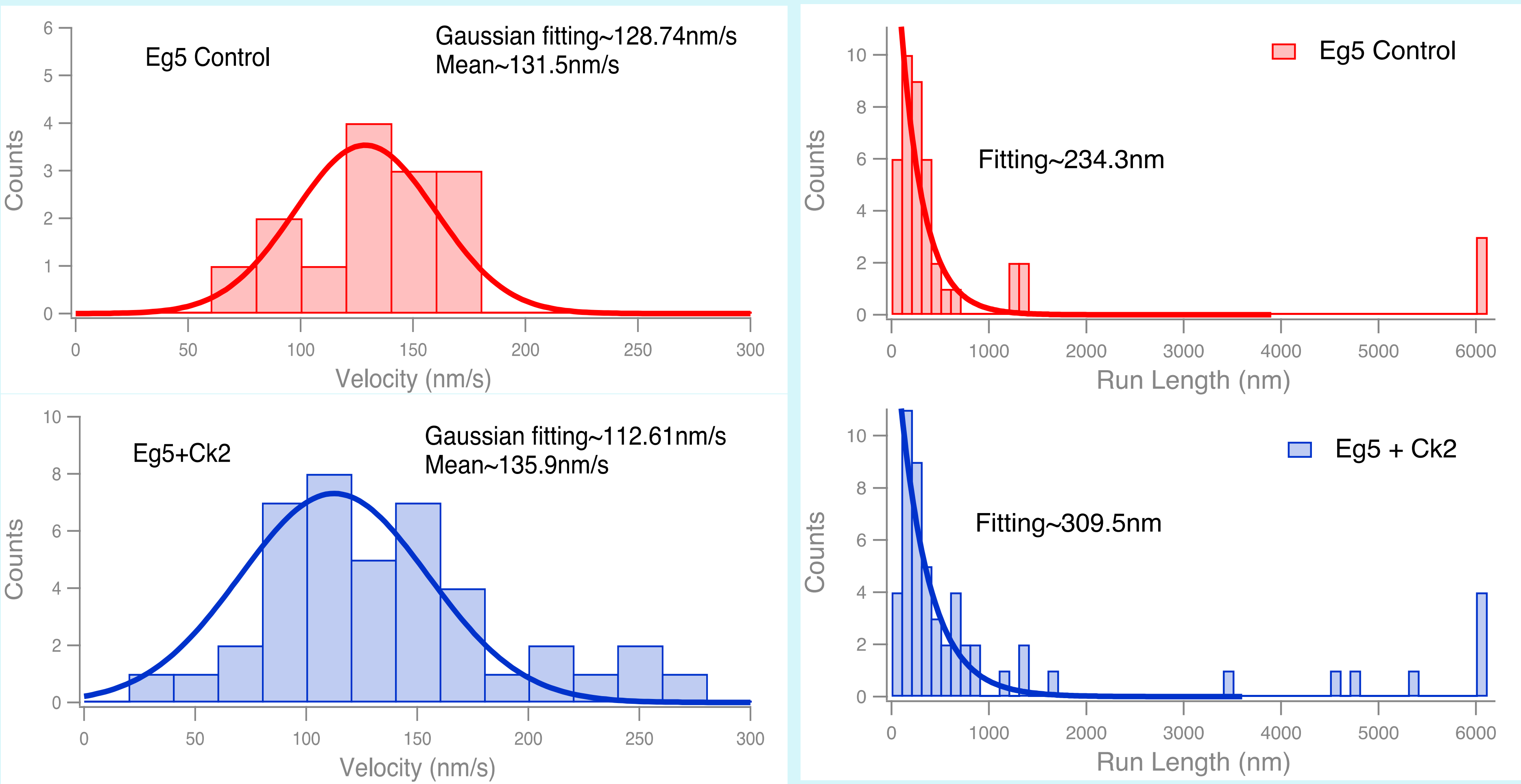
NIH

Active Transport of Viral Particles in Neurons



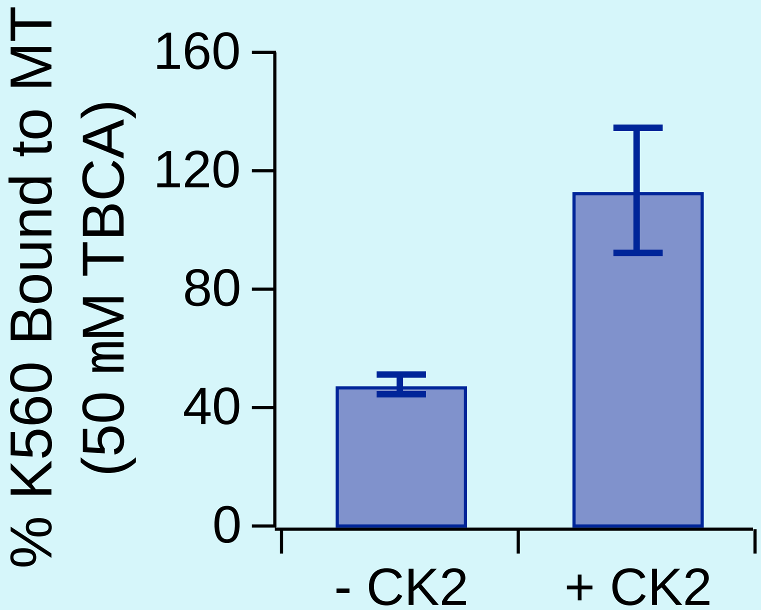
G.A. Smith*, S.P Gross*, and L.W. Enquist, "Herpesviruses use bidirectional fast-axonal transport to spread in sensory neurons", PNAS 98 3466-70 (2001)

CK2 Does Not Change Eg5 Motor Properties



Yonggun Jun

CK2 Does not Act Like a Nucleotide Sink

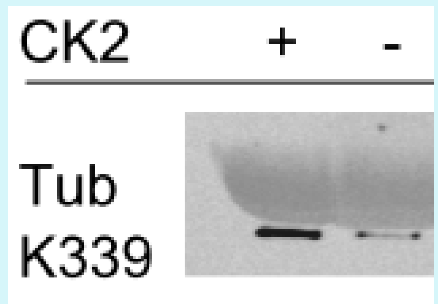


Conclusion – Part II

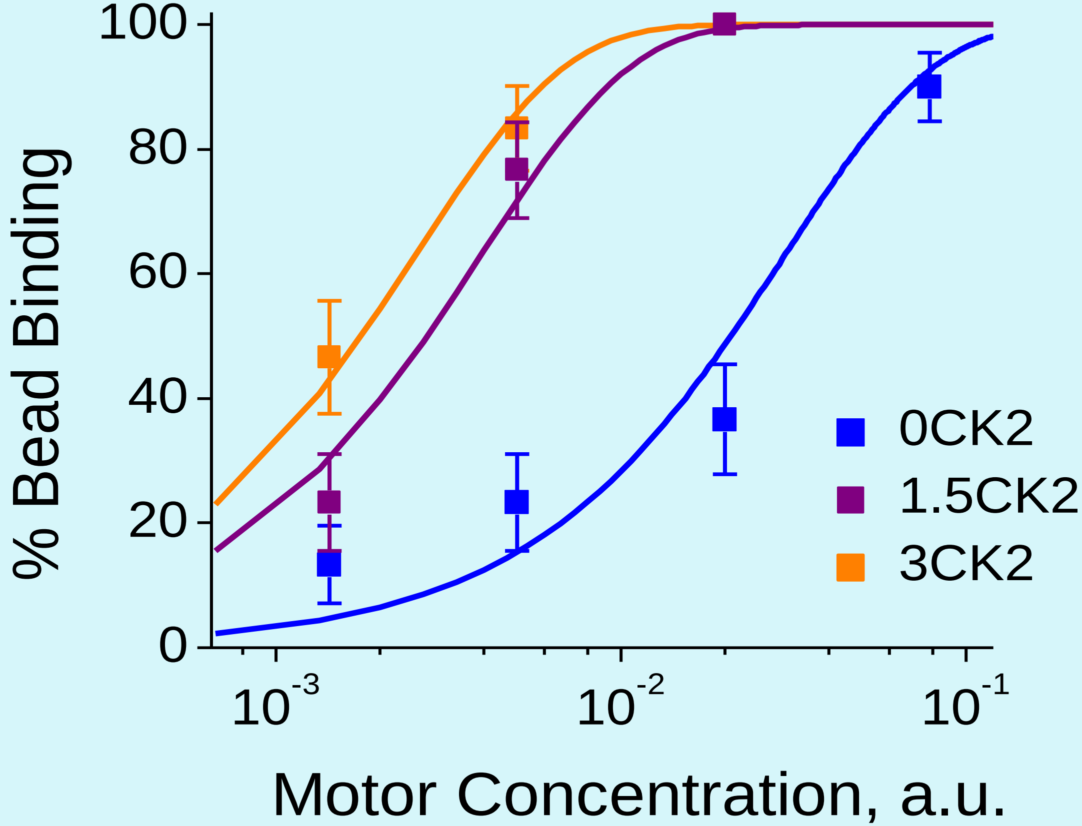
- Presence of CK2 in cells alters Eg5 function

- Future Directions
  - Overexpress CK2 and use kinase inhibitor
  - Overexpress kinase dead CK2
  - Eg5 KD
  - Is Eg5 inactivation the same as kinesin 1?

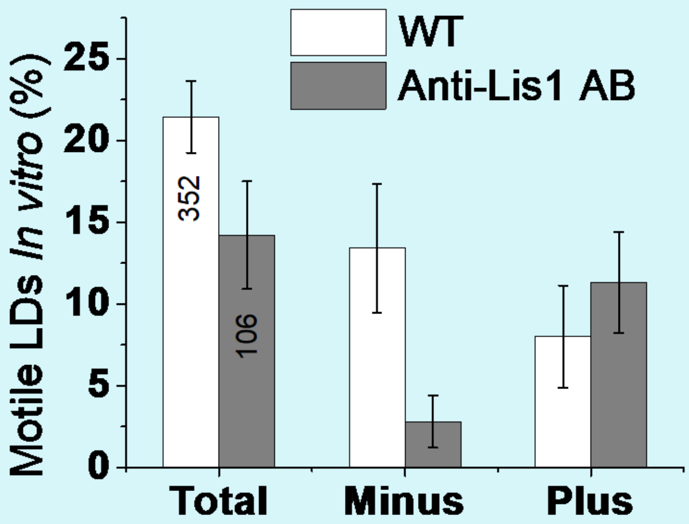

"Head"
H

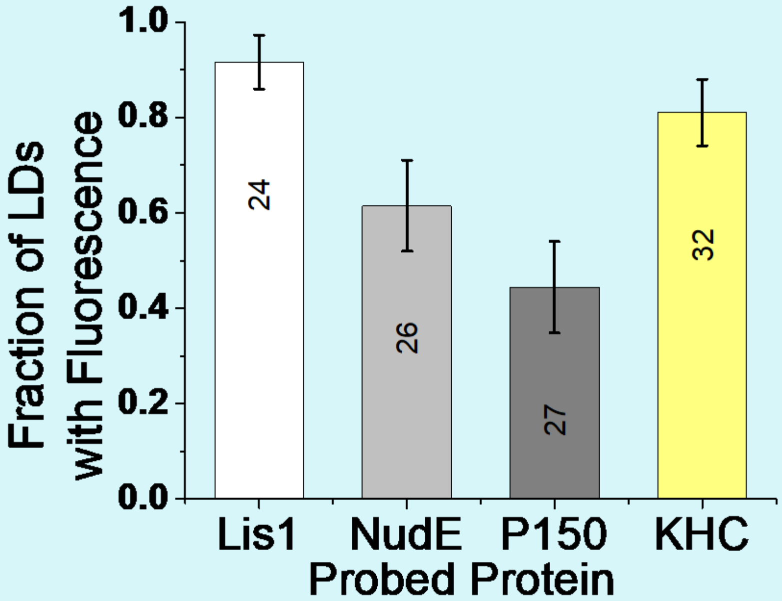

CK2
+
-
Tub
K339

1:1 Ratio of CK2:Kinesin Required



Kinesin Present on Cargos but not Active





Babu J.N. Reddy