# The Role of Casein in Supporting Operation of Surface Bound Kinesin


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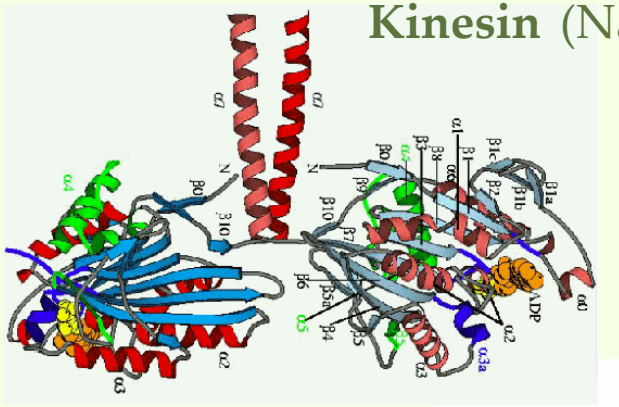
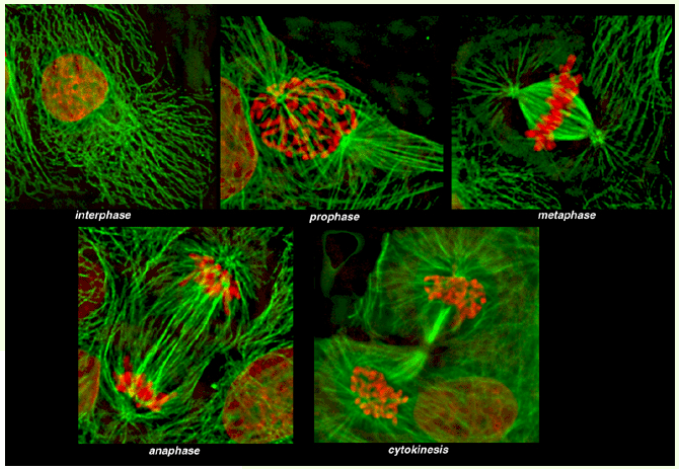
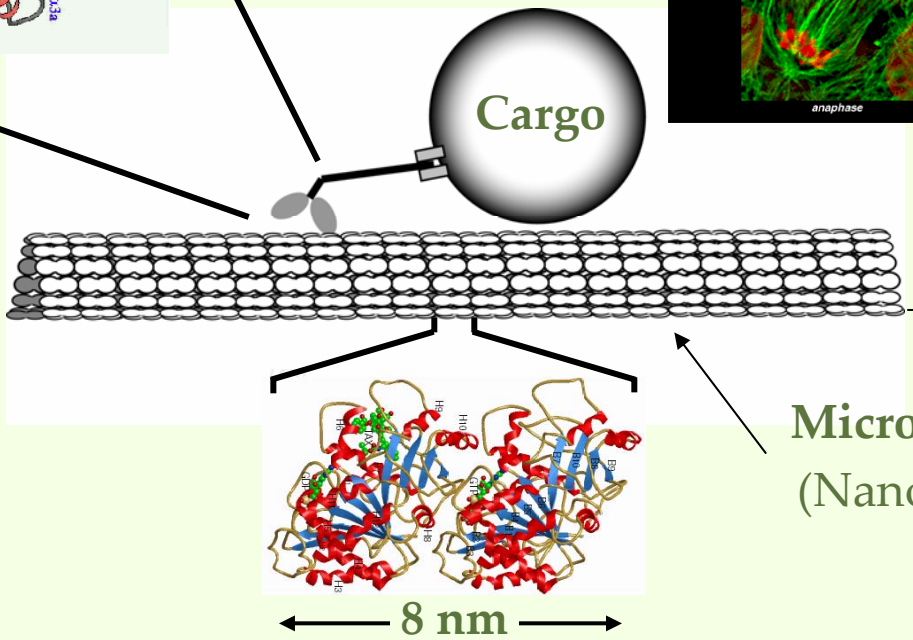
# Kinesin and Microtubule

**Kinesin** (Nano trucks)






Kozielski et al.

Nogales et al.

http://web.bio.ed.ac.uk/research/groups/earnshaw/mitosisGrL.gif

# Casein

- Comprises 80% of proteins in milk and cheese
- Family of phosphorylated proteins ($\alpha_{s1}$, $\alpha_{s2}$, $\beta$, and $\kappa$)
- Part of milk transport system
- Used in food industry for nutritional, dispersing, and binding properties

# Motility Assay

- Incubate casein

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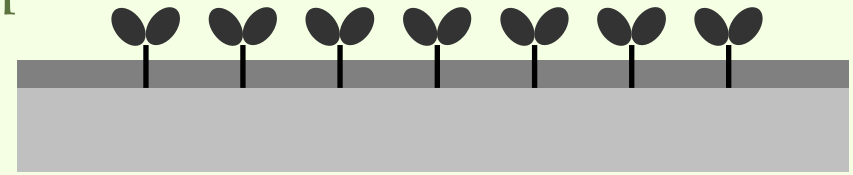
*Glass Substrate* *Casein*

# Motility Assay

- Incubate casein
- Incubate kinesin in 1% casein



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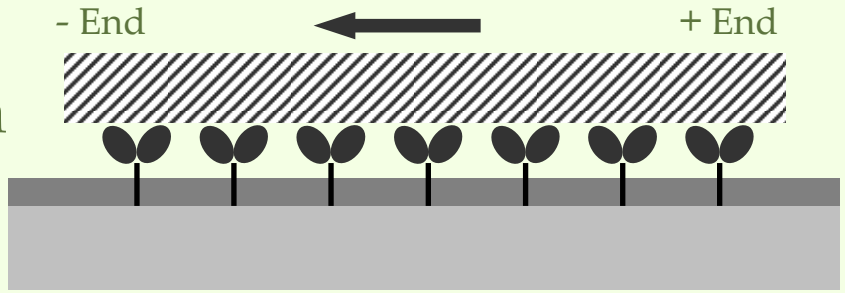
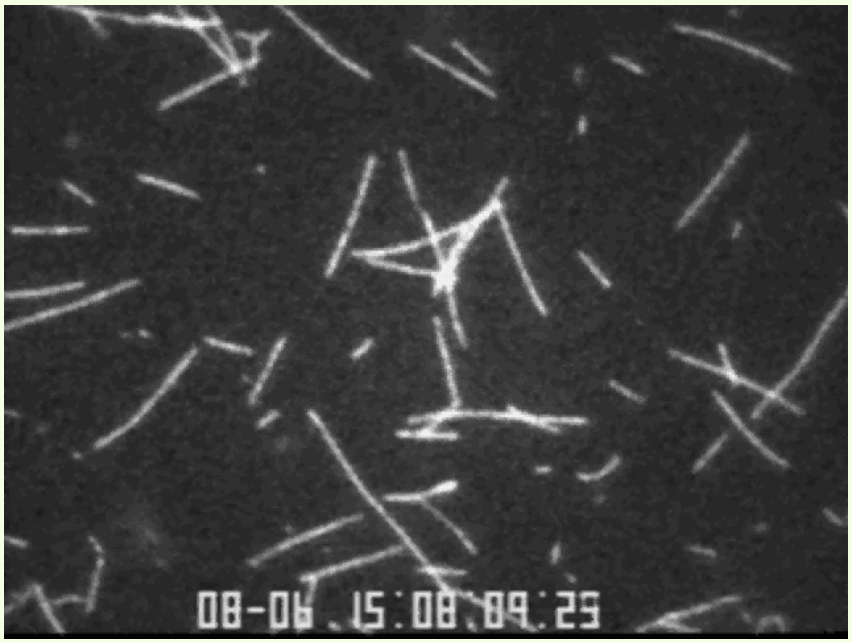
*Glass Substrate* *Casein* *Kinesin*

# Motility Assay

- Incubate casein
- Incubate kinesin in 1% casein
- Flow microtubules in 1% casein
- Check for motility of microtubules

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*Glass Substrate* *Casein* *Kinesin* *Microtubule* *Direction of motion*

# Motility Assay

- Incubate casein
- Incubate kinesin in 1% casein
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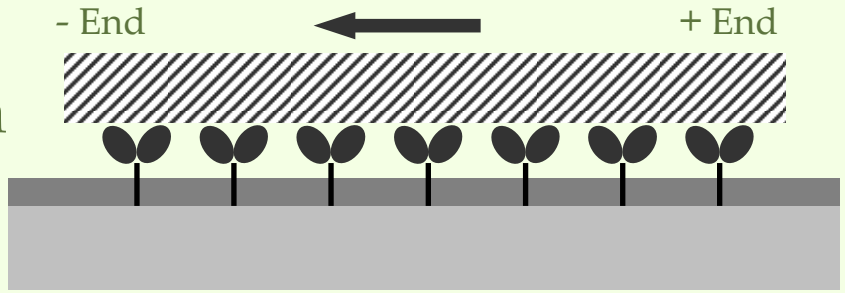
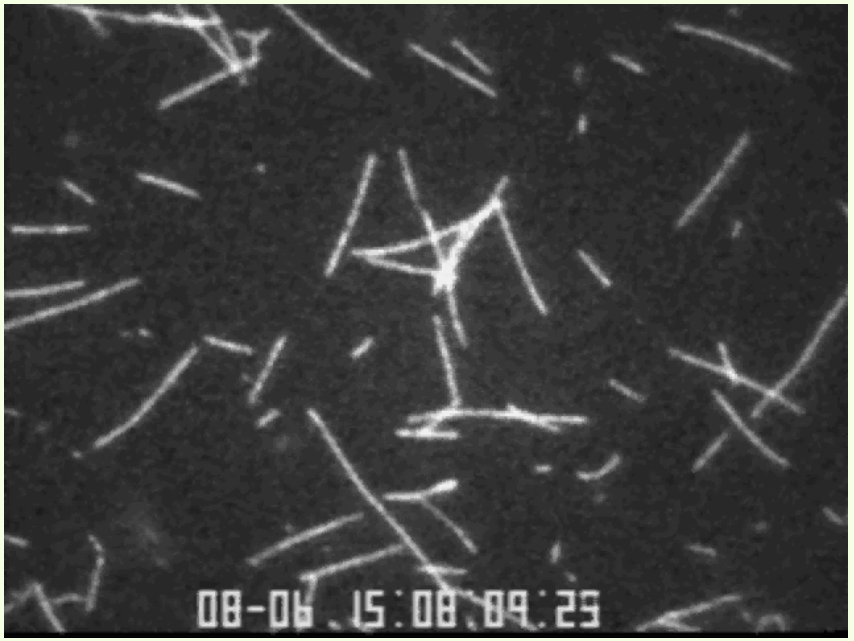
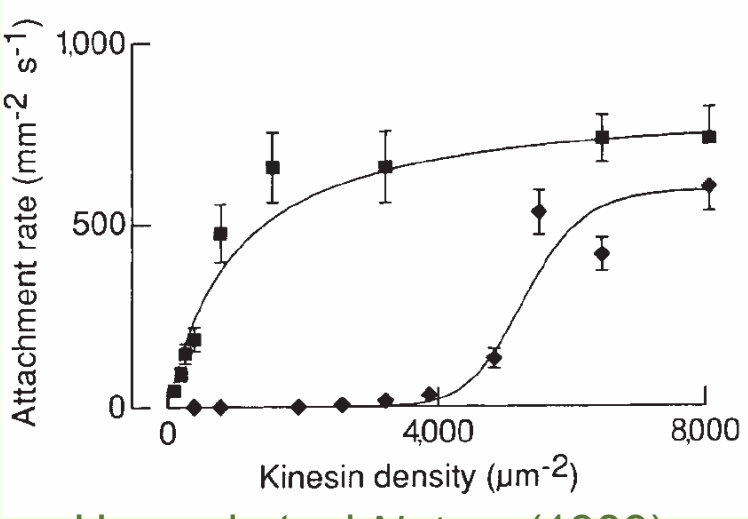


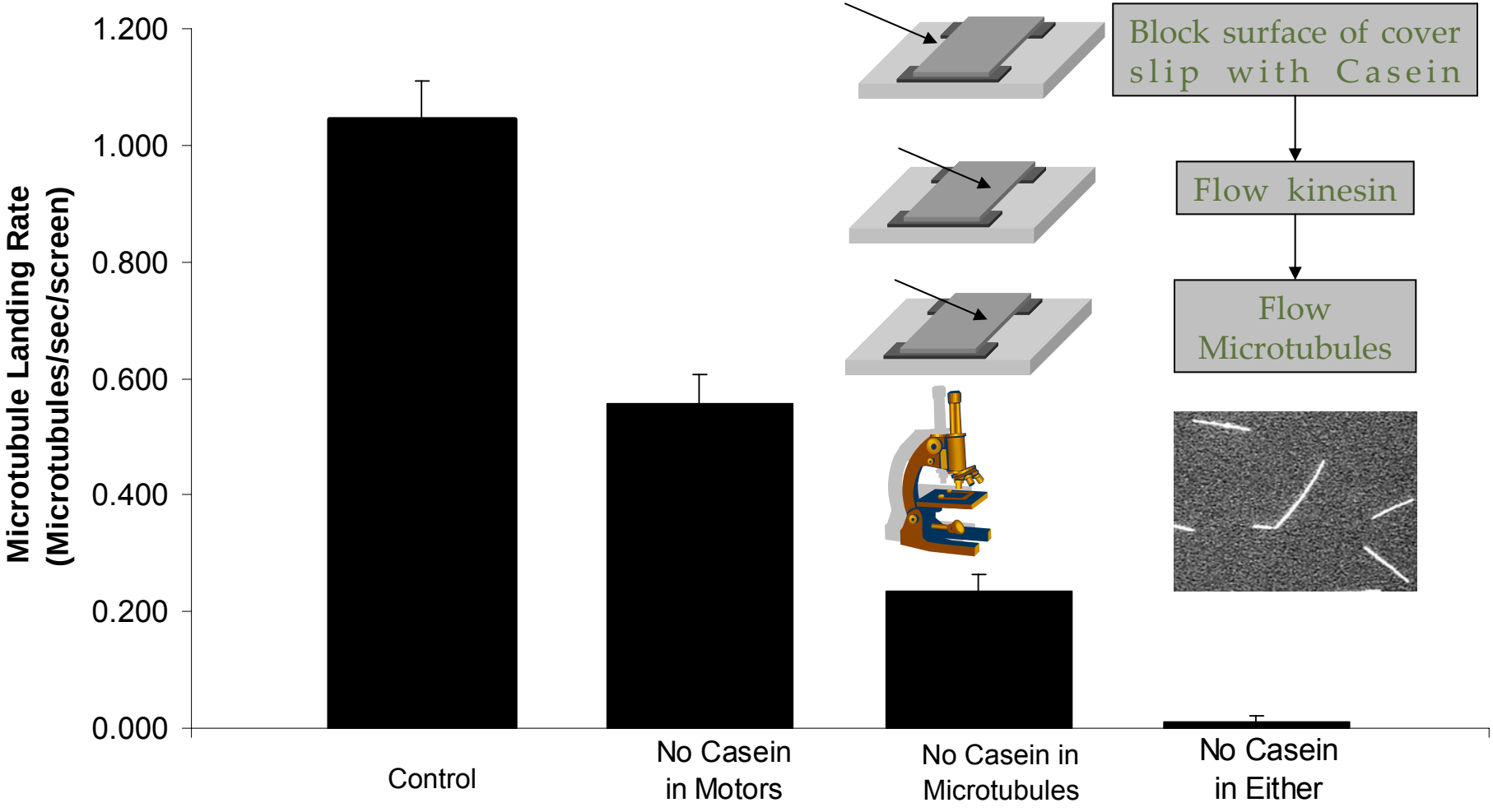




Howard et. al *Nature* (1989)



*Glass Substrate* *Casein* *Kinesin* *Microtubule* *Direction of motion*

# Casein Maximizes Kinesin Activity



# Casein Maximizes Kinesin Activity

| Casein blocking | Kinesin density | Casein in solution | Casein in MT solution | Average Number of Microtubules (±~30%)* |
|---|---|---|---|---|
| No casein | Low (spec) | No | No | 0 |
| | | | Yes | 0 |
| | | Yes | No | 1.4 |
| | | | Yes | 4.4 |
| | High (spec) | No | No | 0 |
| | | | Yes | 0 |
| | | Yes | No | 9.4 |
| | | | Yes | 60 |
| Casein | Low (spec) | No | No | 0 |
| | | | Yes | 0 |
| | | Yes | No | 3.6 |
| | | | Yes | 9.2 |
| | High (spec) | No | No | 13.6 |
| | | | Yes | 48 |
| | | Yes | No | 20 |
| | | | Yes | 80 |

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*average number of moving microtubules (~5-20μm in length) observed over 6 observation fields (~50μm square)

# Initial Casein Block has no Significant Impact on Motility

| Casein blocking | Kinesin density | Casein in solution | Casein in MT solution | Average Number of Microtubules (±~30%) |
|---|---|---|---|---|
| No casein | Low (spec) | No | No | 0 |
| | | | Yes | 0 |
| | | Yes | No | 1.4 |
| | | | Yes | 4.4 |
| | High (spec) | No | No | 0 |
| | | | Yes | 0 |
| | | Yes | No | 9.4 |
| | | | Yes | 60 |
| Casein | Low (spec) | No | No | 0 |
| | | | Yes | 0 |
| | | Yes | No | 3.6 |
| | | | Yes | 9.2 |
| | High (spec) | No | No | 13.6 |
| | | | Yes | 48 |
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# Casein in Final MT Solution significantly improves motility

| Casein blocking | Kinesin density | Casein in solution | Casein in MT solution | Average Number of Microtubules (±~30%) |
|---|---|---|---|---|
| No casein | Low (spec) | No | No | 0 |
| | | | Yes | 0 |
| | | Yes | No | 1.4 |
| | | | Yes | 4.4 |
| | High (spec) | No | No | 0 |
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| | High (spec) | No | No | 13.6 |
| | | | Yes | 48 |
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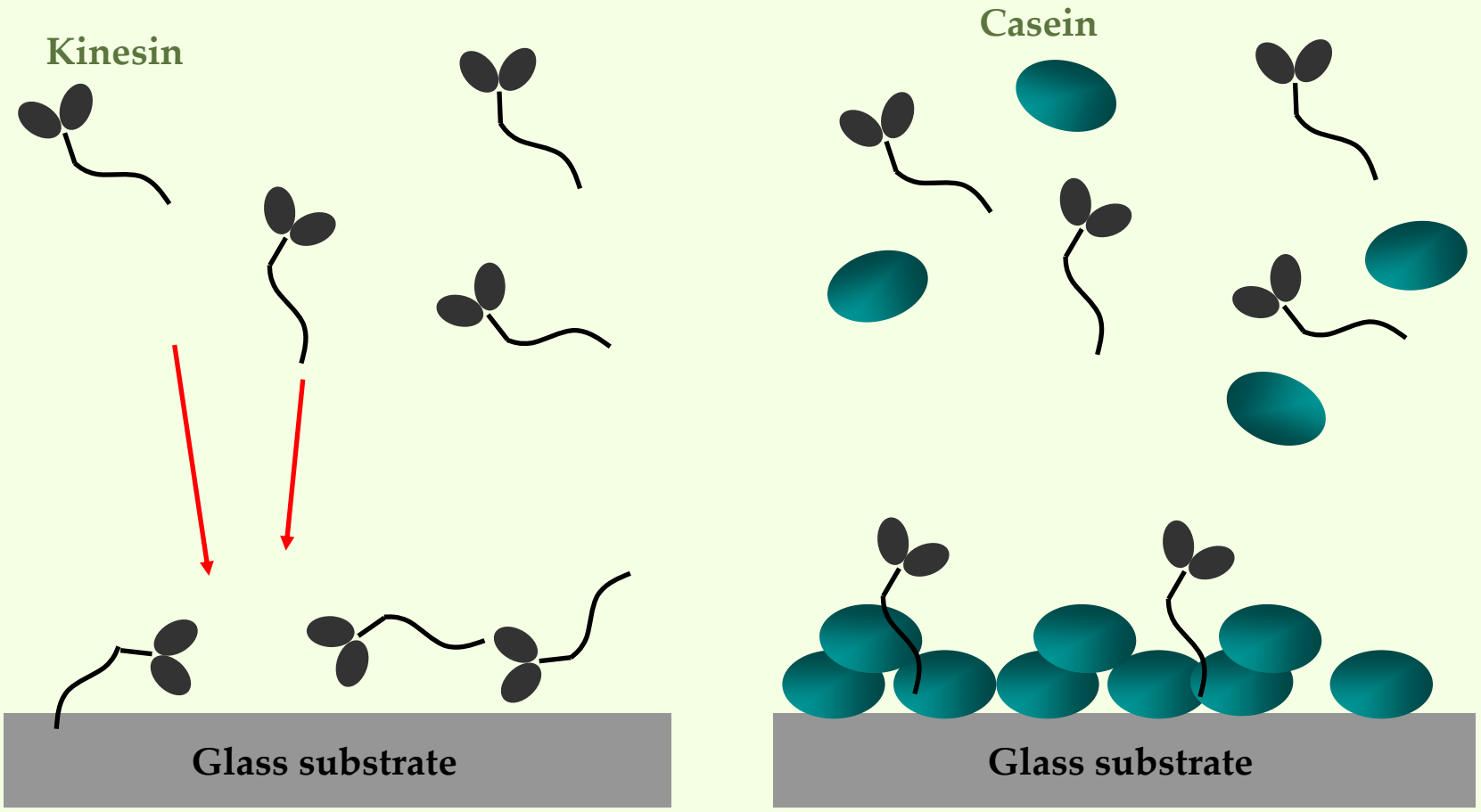
# Experiments

- If there is no casein blocking step and in the motor solution then no microtubules are observed
- The initial casein blocking step does not significantly impact the microtubule motility when casein is included in the subsequent kinesin adsorption solution
- The inclusion of casein in the microtubule solution always increases the observed number of microtubules

# New Model for Kinesin-Casein Asssembly



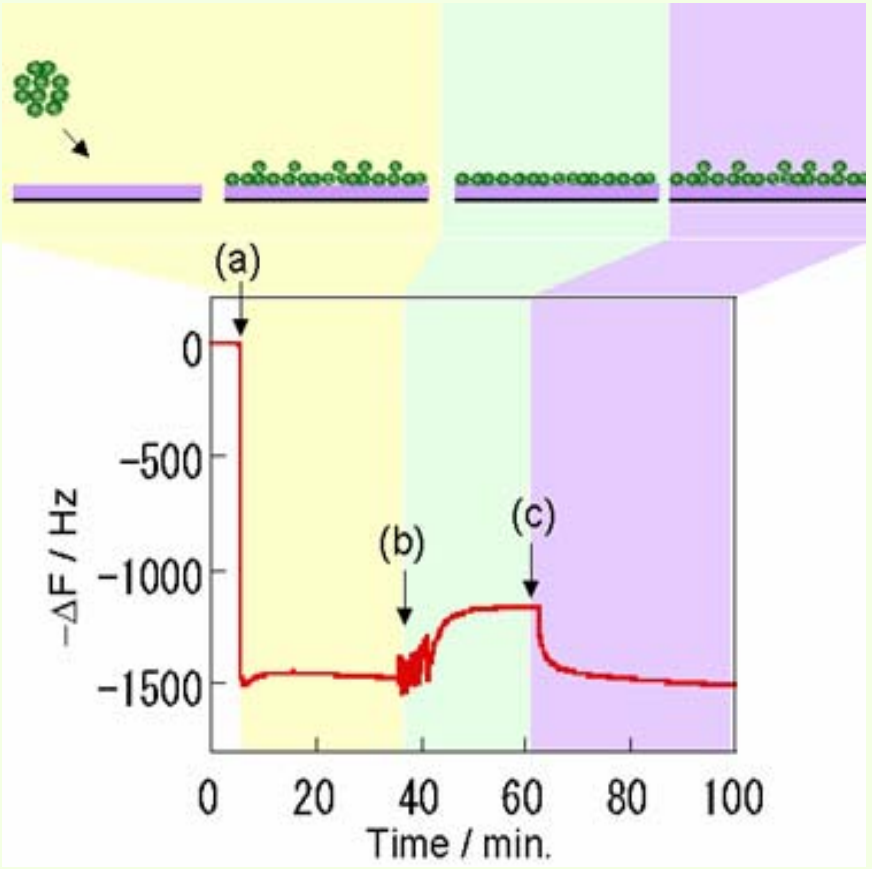

V. Verma, W. O. Hancock and J. M. Catchmark. *Submitted to Journal of Biological Engineering*

# Quartz Crystal Microbalance (QCM)

- QCM can detect binding of proteins to surfaces down to nanogram (submonolayer) levels
- Resonance frequency of crystal changes linearly with protein adhesion
- Decrease in resonance frequency correlated to mass of protein binding

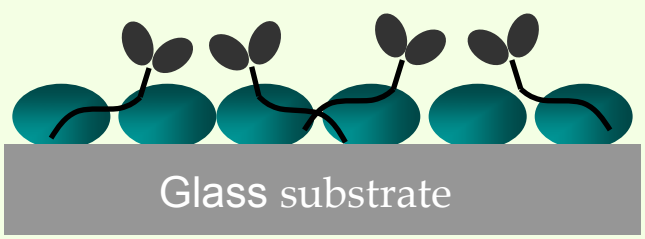
# Casein Binding to Surface as Measured by QCM

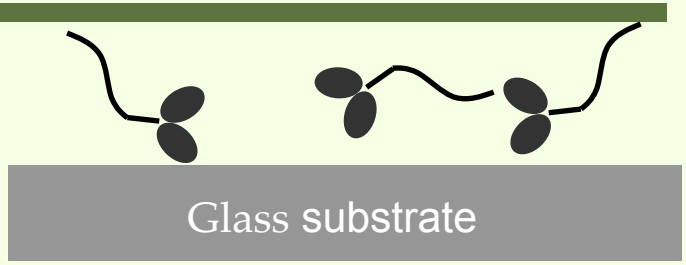
- Casein incubation results in sharp drop in resonant frequency
- Frequency increases when casein washed out
- Corresponds to weakly bound casein leaving
- Resonant frequency falls again on adding casein
- Casein has bilayer assembly on surface

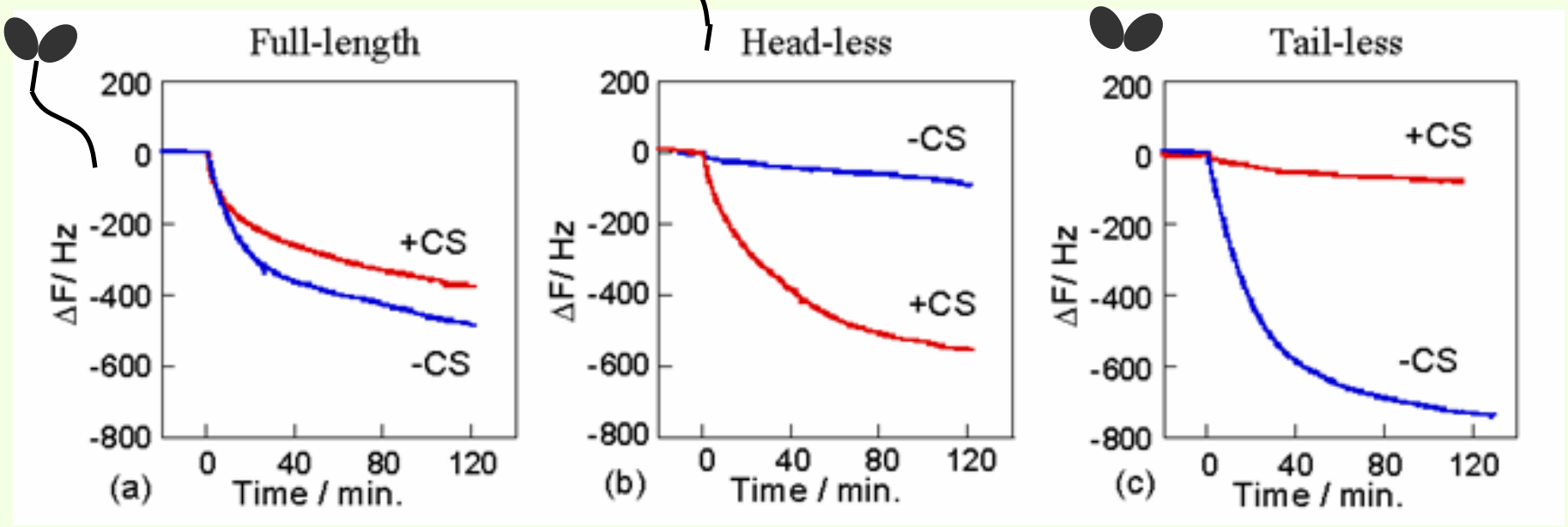



T. Ozeki, V. Verma, et. al (Manuscript in progress)

# Measuring Kinesin Surface Binding by QCM



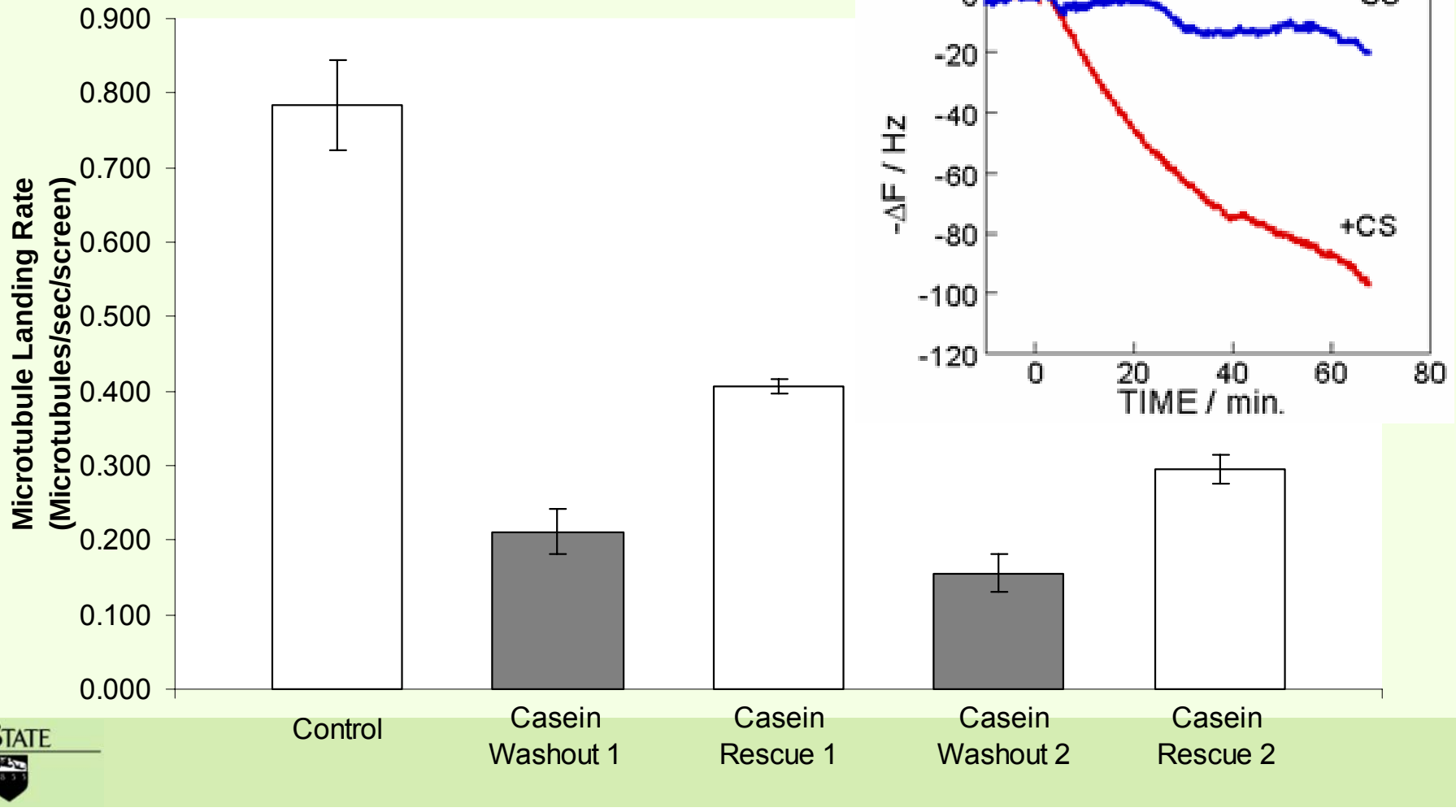




Full length kinesin binds more in presence of casein

Kinesin tail binds better to surface in presence of casein

Kinesin head binds better to surface in absence of casein

# Weakly Bound Casein Enhances Kinesin Function

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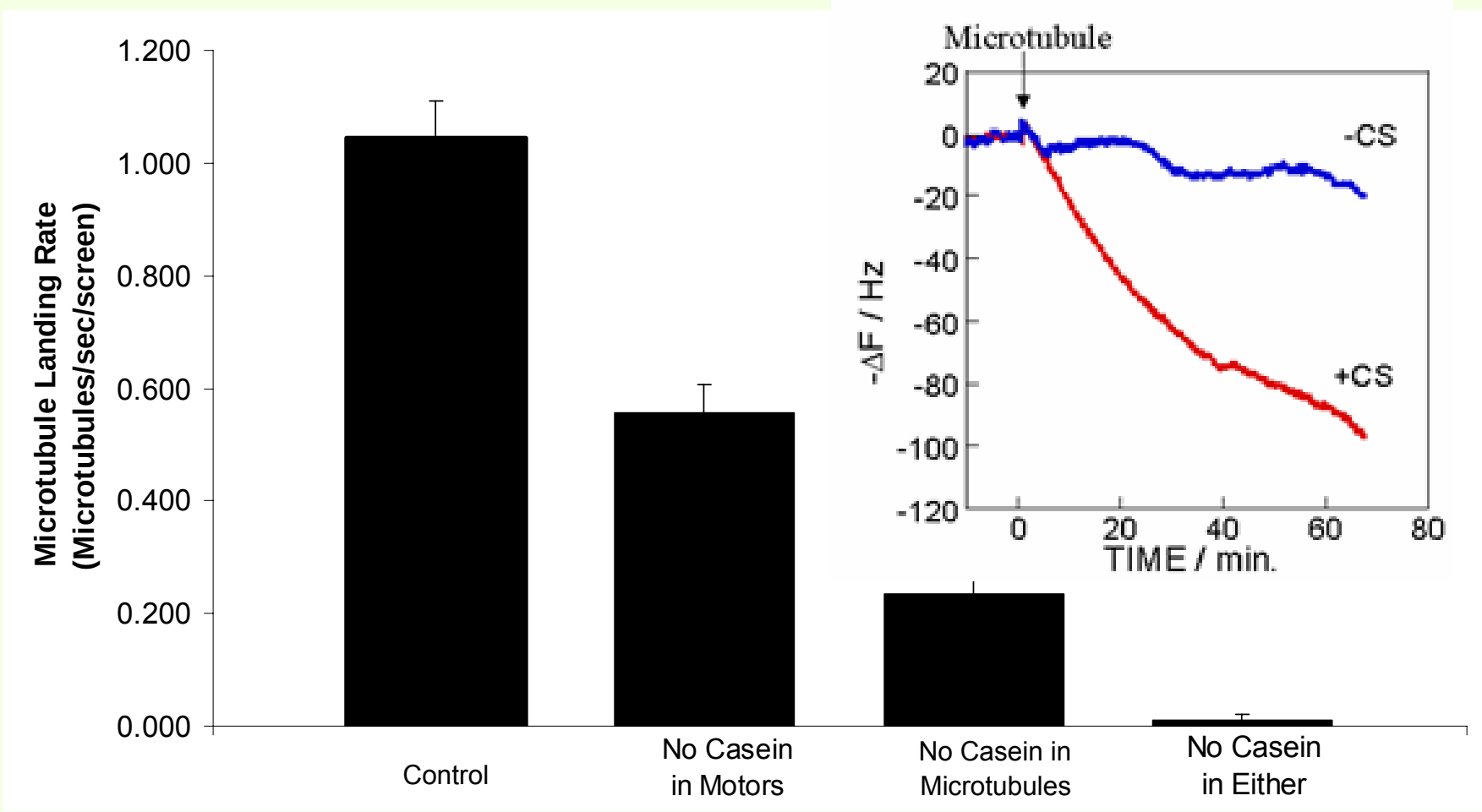
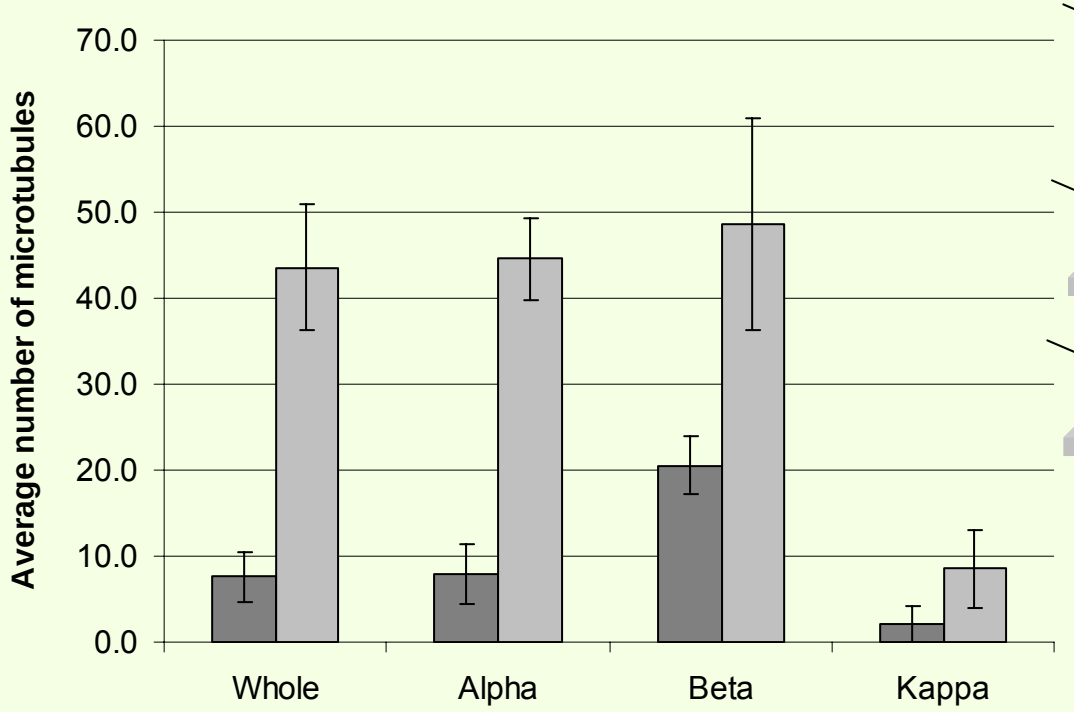
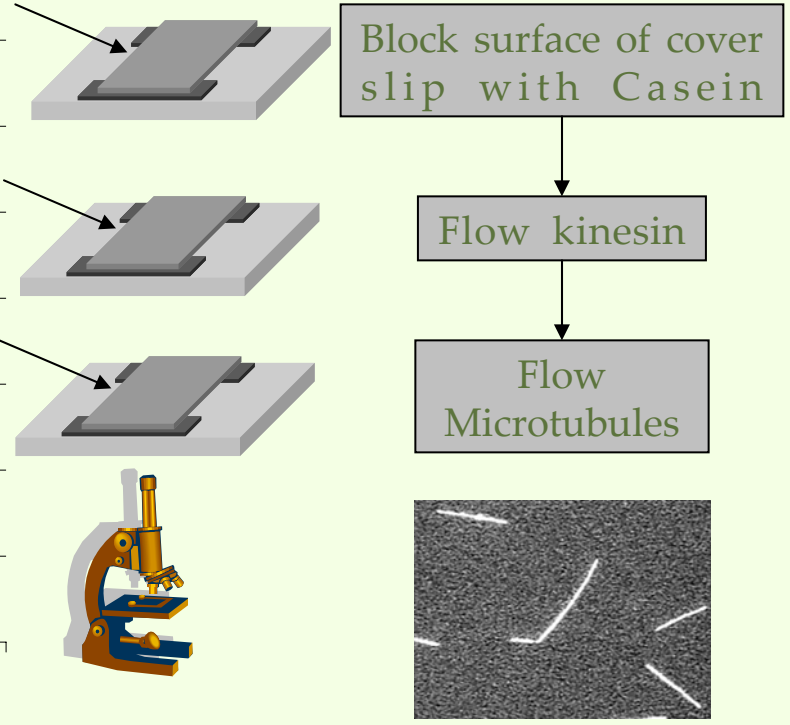
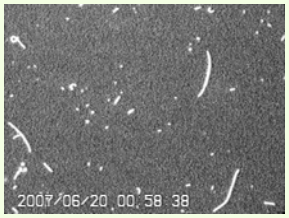
# Casein Effecting Kinesin MT Interaction



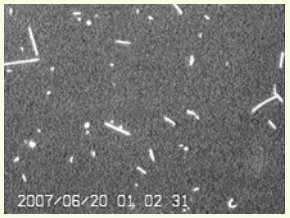
# Effectiveness of Different Casein Subunits on Kinesin Function




Whole casein

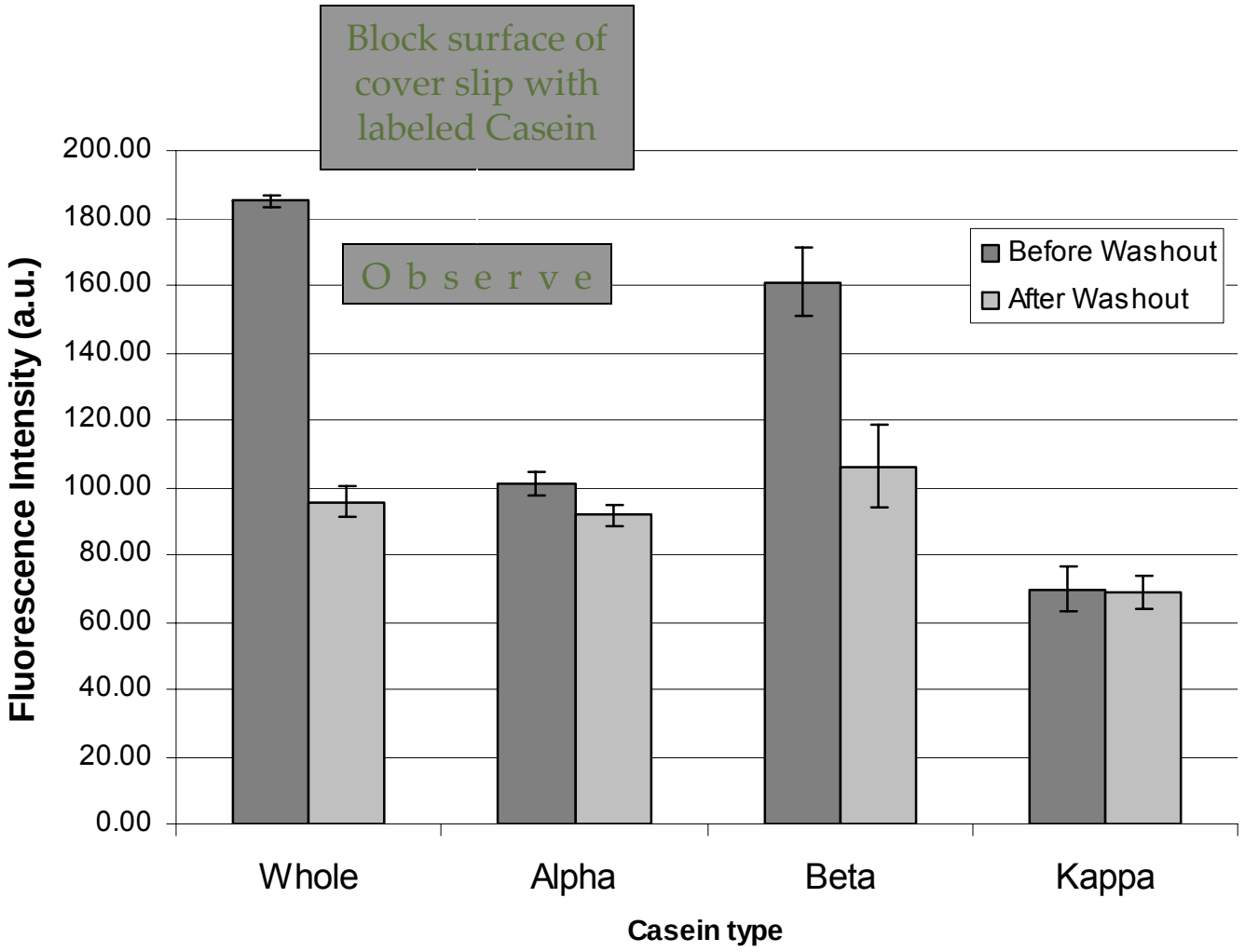



α casein


β casein


κ casein

# Casein Bi-layer Formation




Block surface of cover slip with Casein

Block surface of cover slip with labeled Casein

Observe

# Conclusions

- Casein binds to $SiO_2$ surfaces as a tightly bound and a reversibly bound layers
- Reversible bound casein enhances motor function
- Casein prevents kinesin heads from binding to $SiO_2$ surface
- Casein has an affinity for both $SiO_2$ and kinesin that anchor kinesin on surface
- β casein is found to be the best at promoting kinesin-microtubule interaction while κ casein is found to be the worst
- Identifying methods for anchoring functional kinesin onto surfaces may lead to practical hybrid biological devices employing kinesin

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