Identifying Mechanisms of Cargo Selection by Kinesin Motor Proteins

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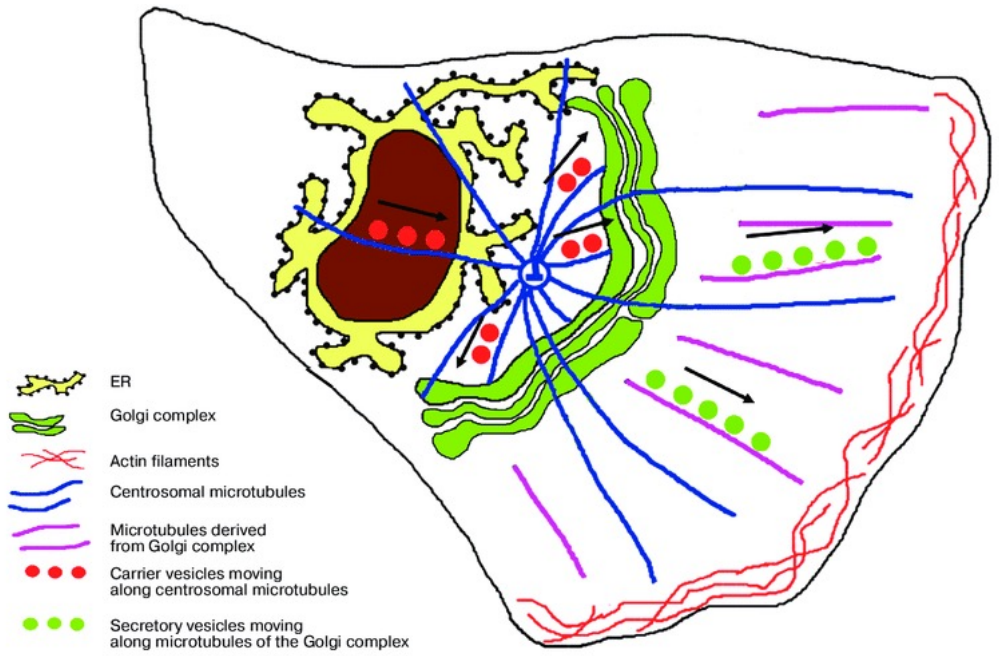
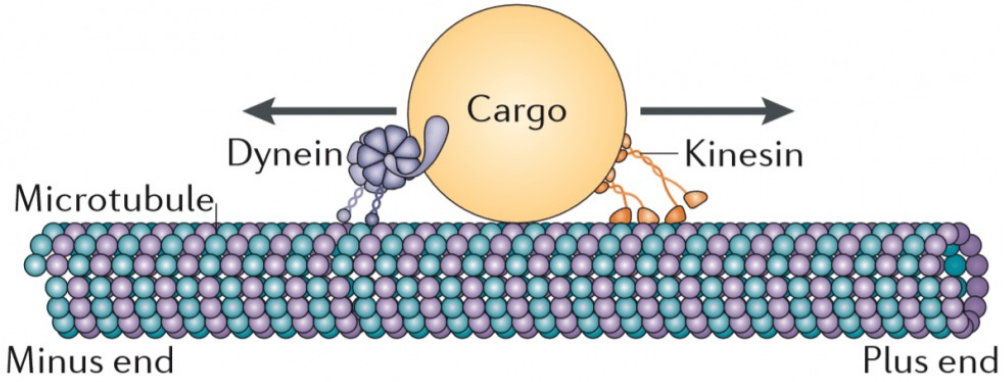
There are ~5,500 plasma membrane and secreted proteins in cells.

How do they get delivered from sites of synthesis to sites of action correctly?



Microtubules (blue) organize components of the cytoplasm, and individual microtubules act as highways for the trafficking of molecules destined for the plasma membrane or secretion.

Two classes of Microtubule Motor Proteins Move Cargo through Cells.



Kinesins and Dynein walk along MTs to transport organelles, cytoplasmic proteins, vesicle-bound proteins, lipids, mRNA, and RNA-protein complexes.

Kinesins are responsible for the trafficking of newly synthesized proteins through the secretory pathway to their final destinations.

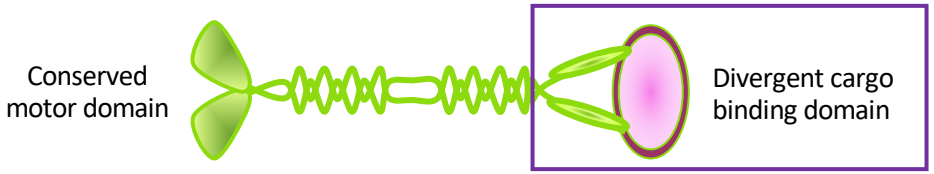
Animation of kinesin carrying a vesicle and “walking along” MTs

There are 45 Human Genes encoding distinct kinesin family motor proteins. Each kinesin can recognize and transport multiple types of cargo, but they do not do this randomly – instead, they select certain cargo over others for transport.

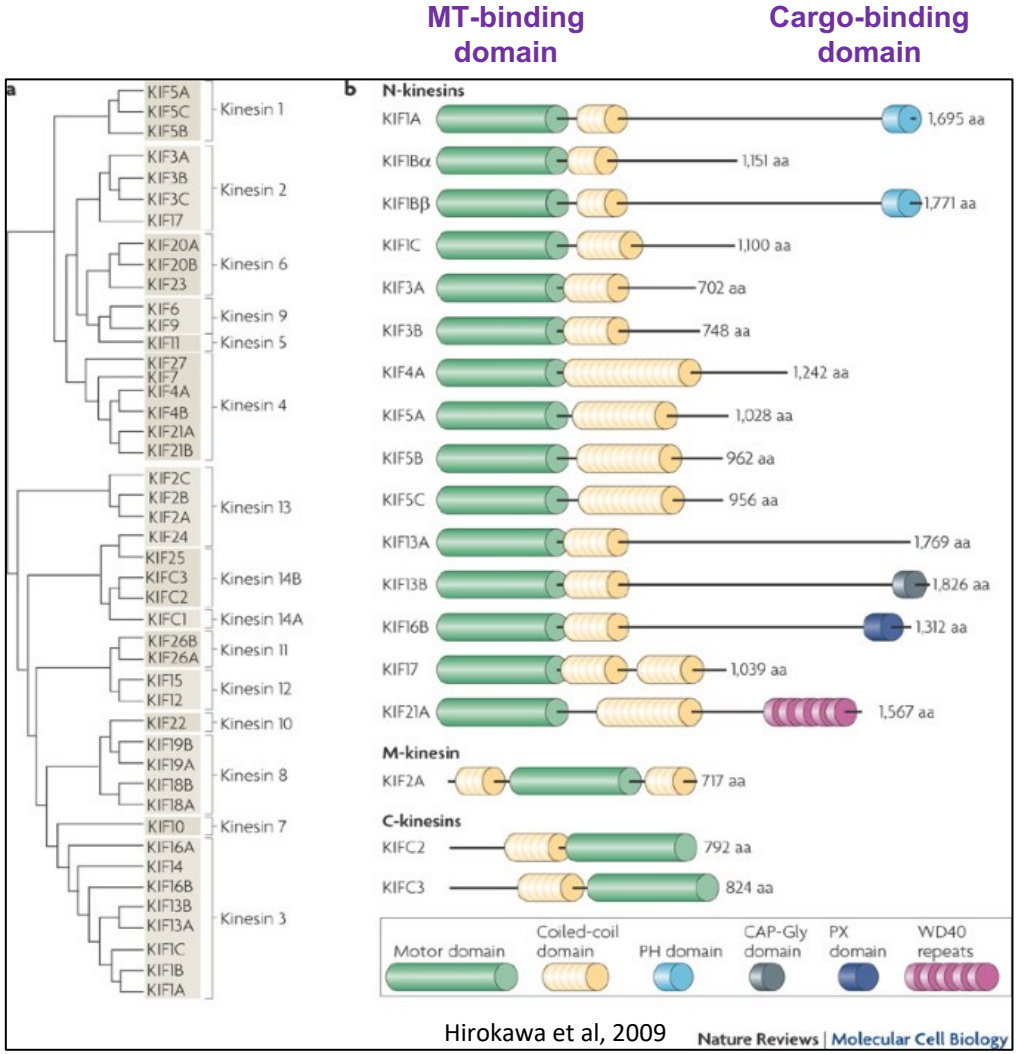
How do kinesins recognize cargo selectively and deliver them to where they function in cells?

- To address this question, I am using time-lapse imaging to monitor trafficking of Aquaporins, a family of related proteins, from the Golgi to the plasma membrane in single cells.

- To identify which kinesin is responsible for the trafficking of these plasma membrane proteins, I am expressing dominant-negative kinesin tail domains that bind cargo but cannot bind or move on microtubules (MT).



- I will use the information gained from my experiments to try and identify the molecular signatures of cargo that allow them to interact selectively with different kinesin motor proteins.

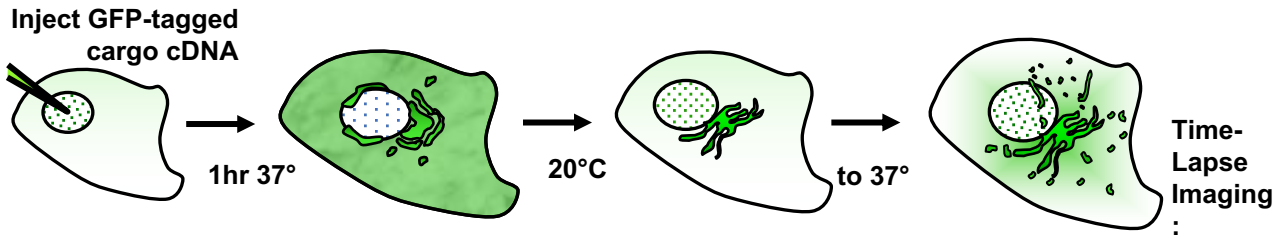


Kinesin family motors share conserved MT-binding domains but have unique cargo-binding tail domains

Overview of Methods

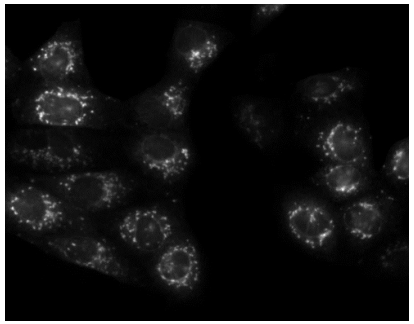
1

- Microinject cDNA encoding AQP1-GFP (Aquaporin 1 with green fluorescent protein tag) into MDCK II cells



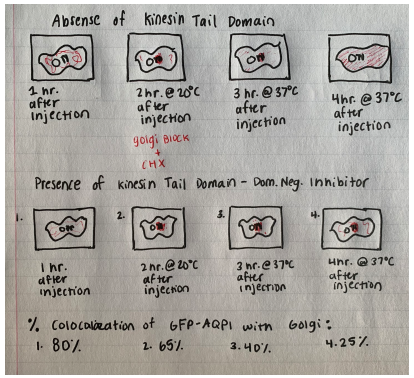
2

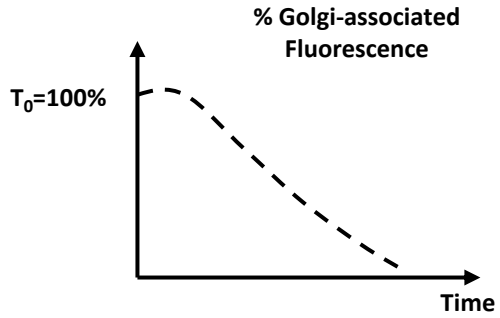
- Time-lapse imaging to monitor trafficking of AQP1-GFP in the absence or presence of co-expressed kinesin dominant-negative tail domains



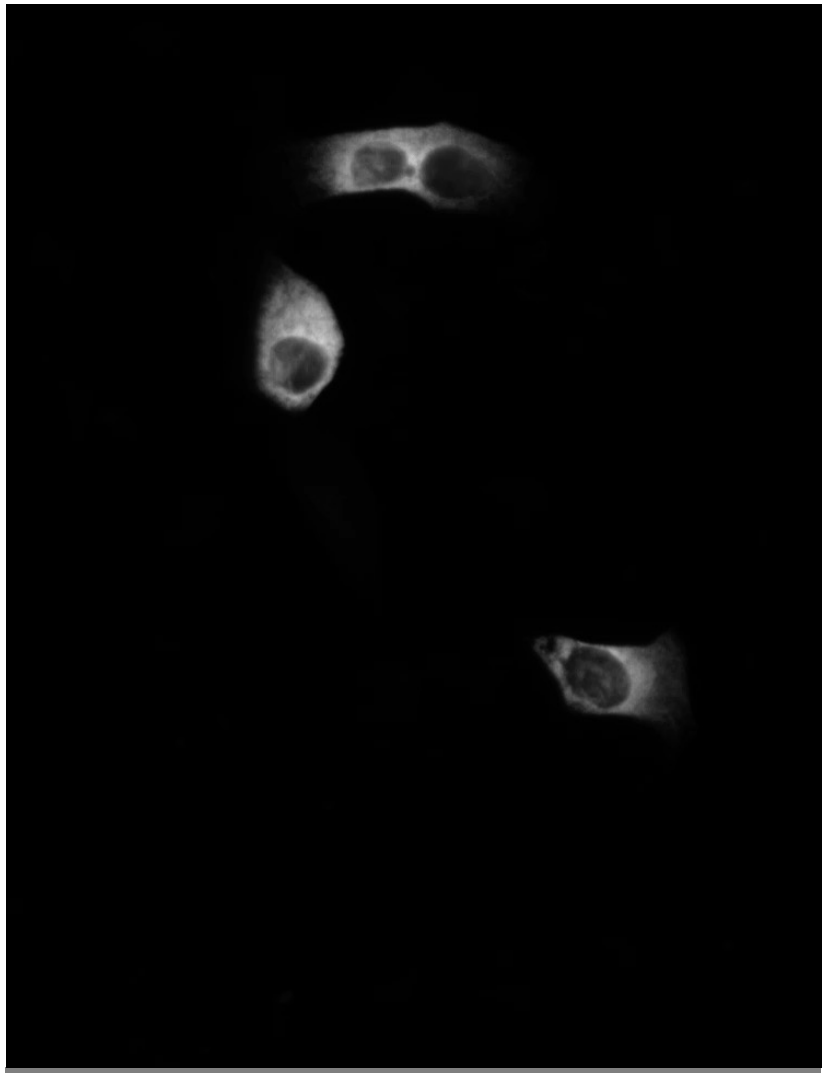
3

- Analysis: Quantify amount of Golgi-associated AQP-GFP fluorescence over time after exit from the Golgi.



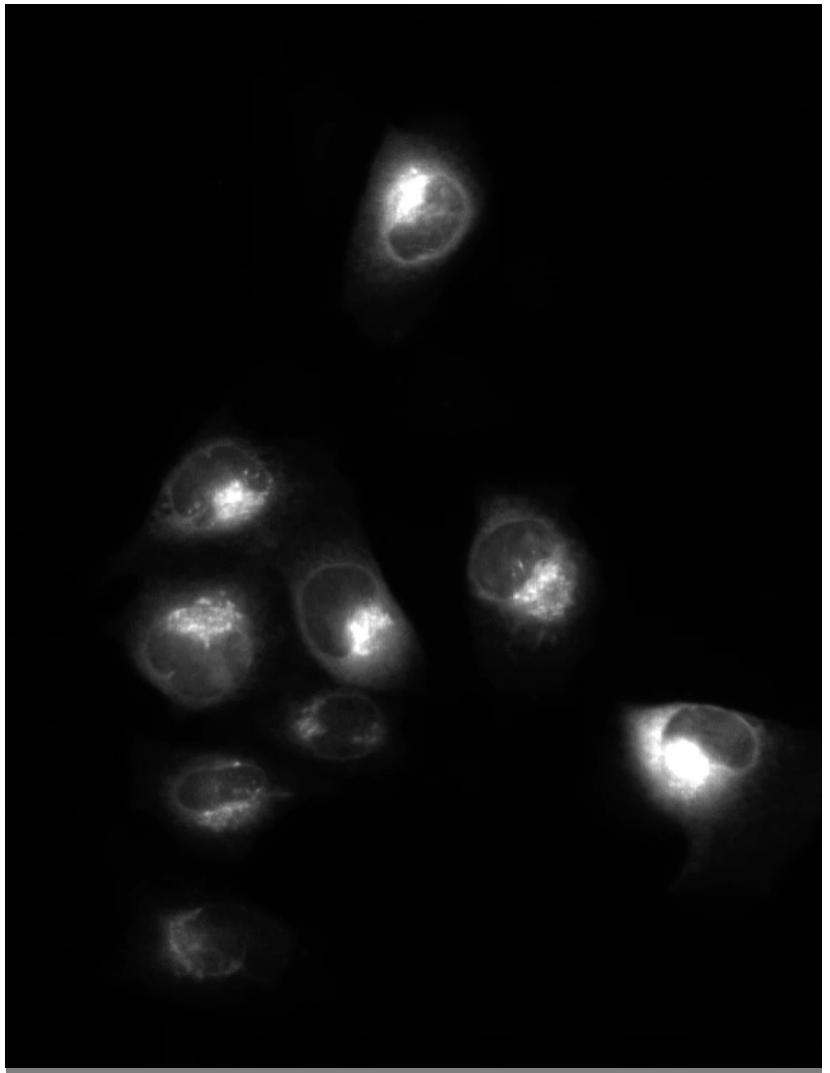


Trafficking of newly synthesized AQP1-GFP in MDCK cells



AQP1

Cells injected with AQP1-GFP cDNA in absence of a kinesin inhibitor

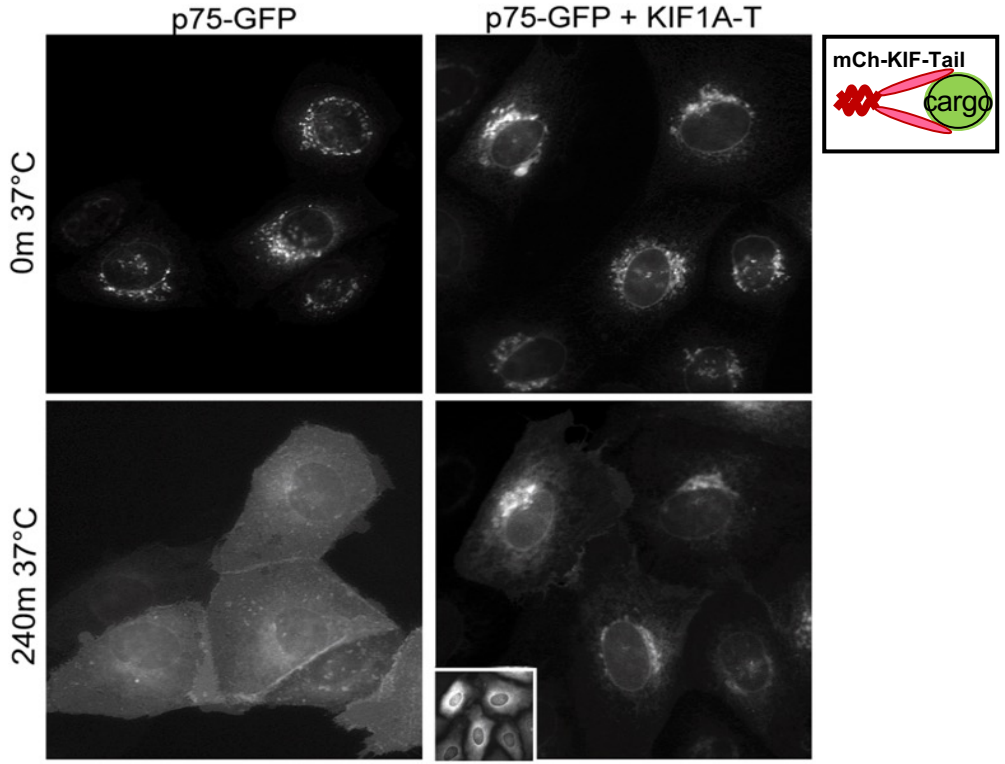


AQP3

Cells injected with AQP3-GFP cDNA in absence of a kinesin inhibitor

- This video is an example of a time-lapse recording showing MDCK II cells microinjected with cDNA encoding a GFP-tagged membrane protein, and how the protein moves from the Golgi to plasma membrane.

Example of expected outcome when kinesin dominant-negative tail domain inhibits cargo transport



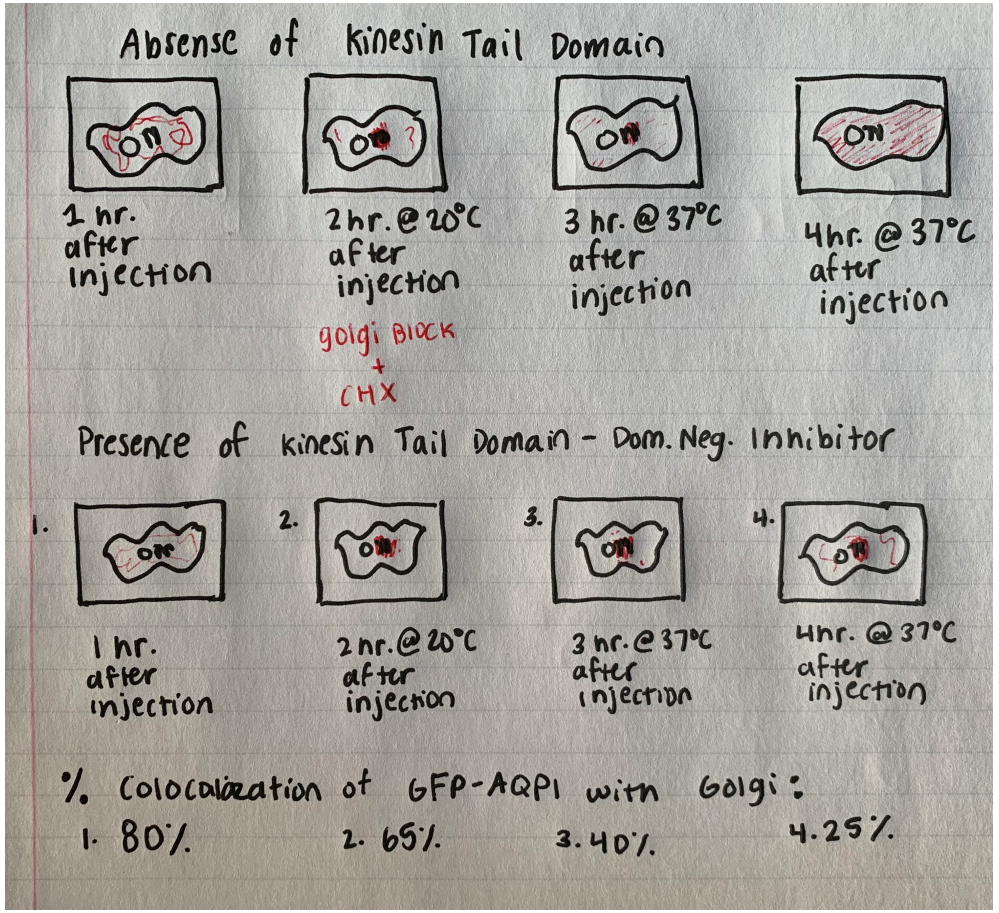
Example showing protein trafficking in cells in the absence (left panels) or presence (right panels) of a kinesin dominant-negative inhibitor

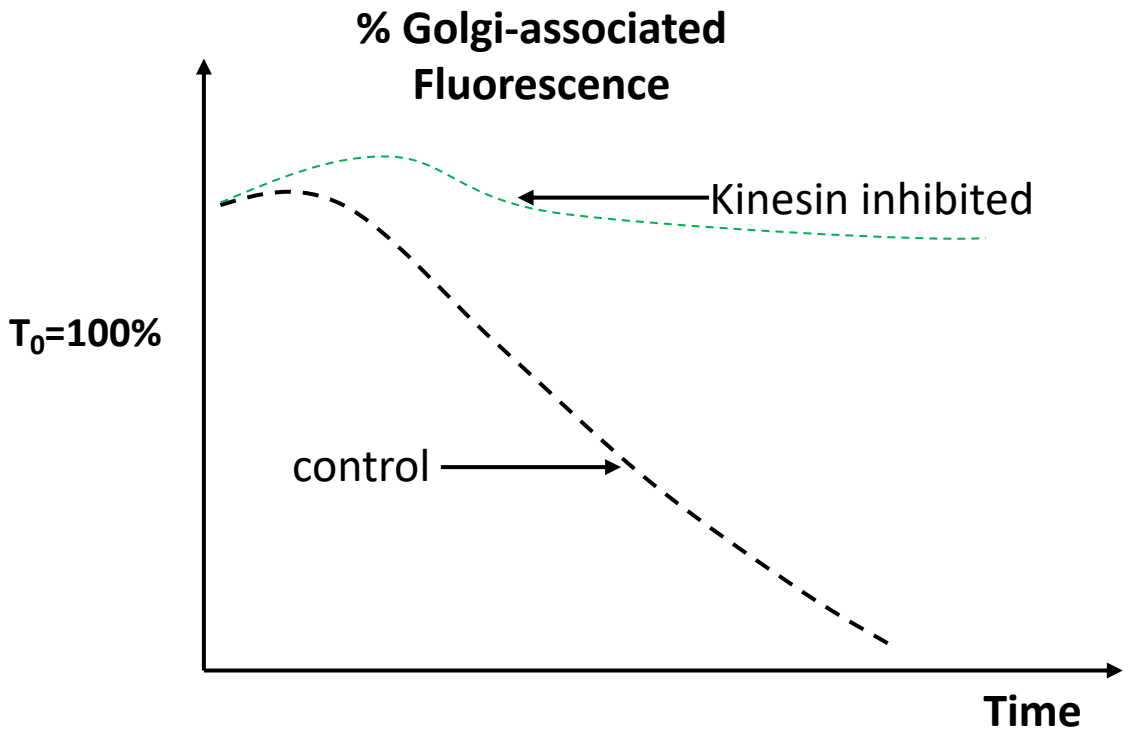
- Determine which kinesin causes a change in kinetics of the AQP trafficking
- Co-inject the AQP with kinesin inhibitors
- The inhibitors are are dominant-negative kinesin C-terminal tail domains – these domains are unique for each kinesin, while the MT binding motor domains are highly conserved). When overexpressed in cells, they dominate over specific endogenous kinesins and block their function.
- Use quantitative analysis to see where the fluorescence from GFP goes in the cell and note changes in the mechanics of the AQP trafficking

Methods

3. Analysis: Quantify amount of Golgi-associated AQP-GFP fluorescence over time after exit from the Golgi.

- Measure Total and Golgi-associated AQP1-GFP fluorescence at each time point in the live-cell recording. Graph the ratio of Golgi/Total AQP1-GFP fluorescence over time to determine extent of transport inhibition by kinesin dominant-negative.

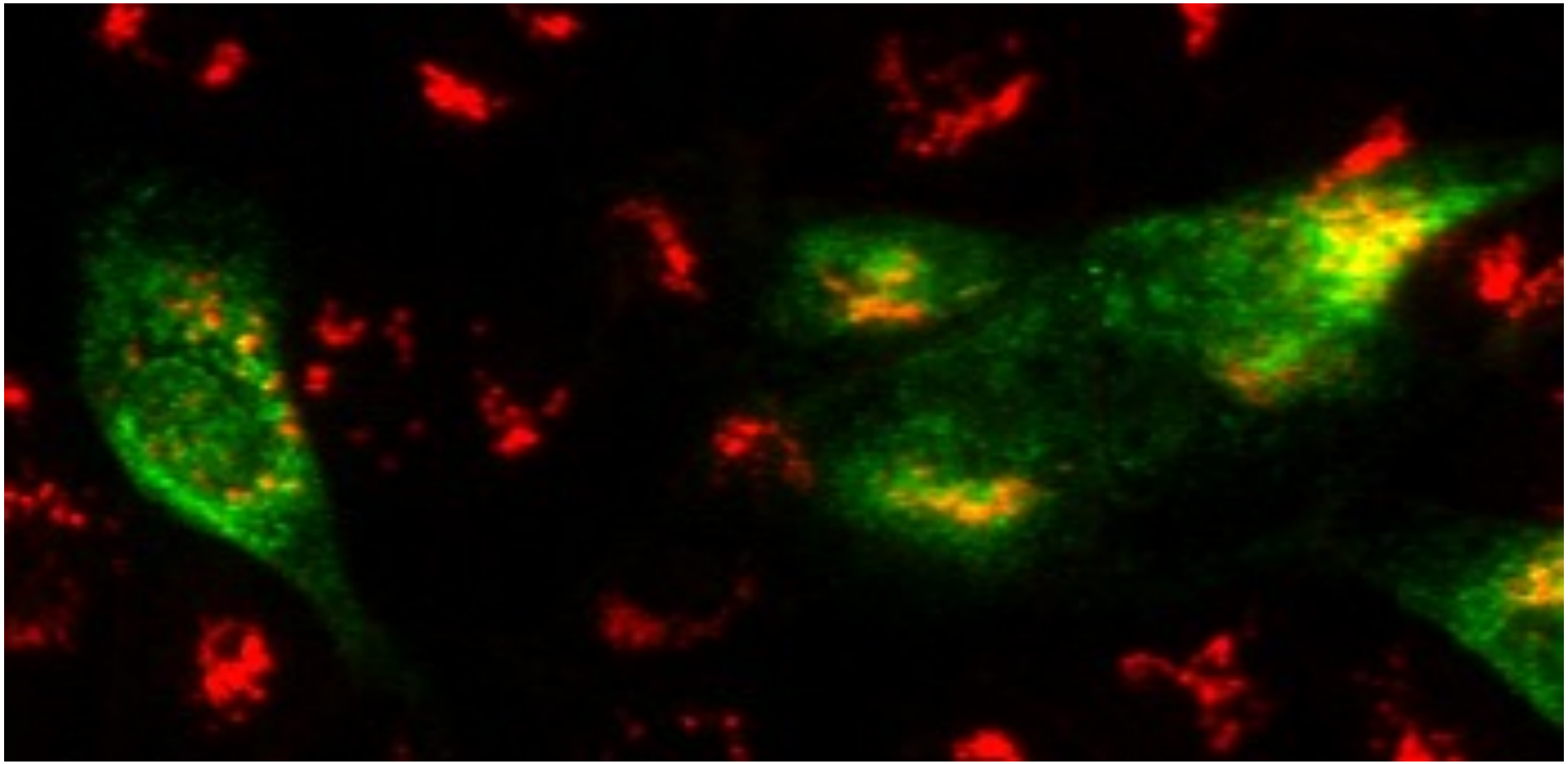




Summary and Future Directions

- To date, I have characterized the trafficking of AQP1 and compared it with the trafficking of AQP3.
- AQP1 and AQP3 appear to behave differently in the secretory pathway. This suggests that they utilize different motor machinery to move from one place to another in the cell.
- Over the next semester, I plan on testing different kinesins, figure out which ones play a role in the trafficking of which membrane proteins, and further investigate the specifics of that membrane protein's mislocalization in hope of finding more information on correcting it.
- I will use the information gained from my experiments to try and identify the molecular signatures of cargo that allow them to interact selectively with different kinesin motor proteins.

Alternative method using fixed cells – analyze the colocalization of the AQP cargo with Golgi to determine how much is in the Golgi over time.



Red: GM-130 Golgi Marker

Green: AQP-GFP Marker

Image of cells at time point 60 minutes after injection