**Group member first names:**

**Introduction to Part 1**

As you have learned in lecture and in the pre-work, the microtubules of the cytoskeleton play a crucial role in transport of molecules and organelles within the cell as well as cell mobility. Microtubules are especially instrumental in the movement of chromosomes during mitosis of the cell cycle.

In this first module activity, we will be quantifying the dynamics of microtubule polymerization and depolymerization over a single event of both during one round of the cell cycle.

**Exercises - Part 1**

**Students: To receive credit for this exercise, provide short answers and calculations for all questions below.**

The graph below identifies a single polymerization and depolymerization event of a microtubule during one round of the cell cycle.

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**Figure 1. Changes in microtubule filament length (μm) over time.**

1. On the graph above, circle the group of data points that represent polymerization and then do the same for the data points that represent depolymerization. Make sure you delineate which grouping represents each process. If you are unable to draw on this document, explain the answer using time point ranges.
2. How much does the filament length change over the time interval of polymerization and over the time interval of depolymerization?
3. Before you do any calculations, describe which event (polymerization or depolymerization) occurs at a faster rate and how you were able to identify that based on the data.

1. Using the data on the scatter plot, calculate the rate of polymerization in micrometers per minute.

1. Using the data on the scatter plot, calculate the rate of depolymerization in micrometers per minute.
2. Each tubulin dimer subunit is 8.0 nm in length. Using the depolymerization rate from step d above, calculate how many subunits (tubulin dimers) were lost per second on one protofilament of the microtubule with this depolymerization event. How many were lost per second for the entire microtubule, which contains 13 protofilaments?

 Conversion reminders:

* 1000 nm in 1 μm

1. Using the information above with tubulin dimer length and your calculated rate of polymerization, how much GTP is hydrolyzed per second for all 13 protofilaments in the polymerization process shown on the graph? Remember that one GTP is hydrolyzed for each tubulin dimer added to the growing microtubule.

1. As you can see from the graph, the rapid depolymerization results in the complete reduction of the microtubule length. This is referred to as a microtubule catastrophe. Based on what you know about depolymerization, what is occuring with the microtubule filaments that results in this dynamic instability?

Just a note for clarification: Microtubule depolymerization events do not always result in a catastrophe. Microtubules can shrink a small amount and then recover to start polymerizing again, which is called a “rescue”. We are not examining those dynamics within this module.

1. Based on what you know about the stages of the cell cycle and the role of microtubules during those stages, provide a hypothesis for how these different rates of depolymerization and polymerization relate to the role of microtubules during mitosis.

**Exercise Part 2 - Applications with Cancer Treatments**

As you will learn in our course, cancerous development occurs as a result of multiple disruptions within the cell cycle. Mutations in genes associated with cell cycle progression and cell cycle checkpoints can lead to initiation and progression of cancer. As a result, cancerous cells become capable of dividing uncontrollably and bypassing the normal checkpoints that might lead to apoptosis.

Therefore, many chemotherapy treatments target components of the cell cycle to inhibit cancerous cells from continuously undergoing cell division. We will be talking about two common chemotherapy drug treatments that are both plant alkaloids: taxol and vinblastine. Both of these drugs specifically target microtubules and disrupt their function, but do so in two different ways.

Taxol, or paclitaxel, was originally identified from the bark of the Pacific yew tree,*Taxus brevifolia*. Taxol binds directly to a hydrophobic pocket of the beta-tubulin portion of the microtubule subunit. Cells exposed to taxol will have abnormal mitosis, which leads to the triggering of apoptosis.

Vinblastine was originally extracted from the periwinkle plant, *Catharanthus rosea*. Vinblastine binds directly to tubulin as well and has a high affinity for the ends of the microtubules. The impact of vinblastine on microtubules appears to vary by concentration.

We will be examining the impacts of both taxol and vinblastine on microtubule dynamics in the questions below.

1. Compare the changes to microtubule length below for a cell treated with 100 nM taxol to the above graph of an untreated cell. Which stage is most impacted: polymerization or depolymerization? What is the new rate (μm/min) for the stage that changes?

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**Figure 2. Changes in microtubule filament length (μm) over time when treated with 100 nM taxol.**

1. Based on the observed changes, hypothesize how taxol binding to beta tubulin is physically impacting or changing the components of microtubules.
2. Compare the changes to microtubule length below for a cell treated with 400 nM vinblastine to the above graph of an untreated cell. Which stage is most impacted: polymerization or depolymerization? What is the new rate (μm/min) for the stage that changes?



**Figure 3. Changes in microtubule filament length (μm) over time when treated with 400 nM vinblastine.**

1. Based on the observed changes, hypothesize how vinblastine binding to beta tubulin is physically impacting or changing the components of microtubules.
2. Considering what you know about microtubules and their use in the cell cycle, explain in your own words why it would be a good thing for microtubules to be disrupted by the methods used by taxol and vinblastine in a person who has developed cancerous growth.

**Challenge/Sponge Question if time:**

1. Using the graph below showing just the depolymerization, first estimate the half-life of this microtubule decay. Then, calculate the half-life of this microtubule decay using the equation below. Follow the procedures discussed in your pre-work and show your work. How does your estimate compare with the calculated value?

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**Figure 2. Changes in microtubule filament length (μm) from 4 - 4.25 minutes only.**

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