Lab 6: Complicated Kinetics

Setup

Follow the link from the course schedule to a "jupyter" notebook describing chemical kinetics.

- Look at the second cell (starts with "# define a reaction"). Draw the reaction scheme, with rate constants, on a piece of paper.
- You can run the code in each cell by selecting the cell and clicking the "Run" button above. Run all cells up to the one labeled "*Compare stochastic and deterministic simulations.*"
  - Can you rationalize the shapes of the curves given the reaction scheme?
  - Can you rationalize any difference(s) between the Markov and Gillespie simulations?
- Run the cell labeled "*Compare stochastic and deterministic simulations*" and the one immediately thereafter.
  - Can you figure out what the graph shows given the code?
  - What do you conclude about the differences and similarities between the Markov and Gillespie simulations?

Michaelis-Menten Enzymes

We are now going to use the Gillespie simluator to test the validity of the assumptions we made when we derived the Michaelis-Menten equation. We made two assumptions:

- There is a steady state population of $E \cdot S$.
- Product formation is irreversible.

To test the effects of these simulations:

- Find the cell labeled "*Create your own gillespie reaction below.*"
- Enter the following reaction describing a Michaelis-Menten enzyme in for the *rxn*:
  - $S + E \rightleftarrows^{k_f}_{k_r} ES \rightarrow^{k_3} E + P$
  - $k_f = 1\ s^{-1} \cdot molecules^{-1}$
  - $k_r = 1\ s^{-1}$
  - $k_{cat} = 0.1\ s^{-1}$

  - 10 *enzyme molecules*
  - $1,000$ *substrate molecules*
  - 0 *product molecules*
  - 0 $enzyme \cdot substrate$ *complexes*
  - $10,000$ *steps*
- Run the cell and note the output.
- From the output, is the steady-state assumption justified?
- Now modify the reaction, adding reversibility ($ES \leftarrow E + P$).
  - What happens to the entire reaction?
  - Are there any points on the curve where the reversible and irreversible curves overlap?
  - Can these differences be justified in molecular terms?
- Do you still believe Michaelis-Menten analyses after this work?

Actin polymerization

Another useful feature of a Gillespie simulator is the ability so simulate arbitrarily complicated kinetics. We are going to use the simulator to explore the actin polymerization reaction. This is a high-order reaction that is difficult to model analytically.

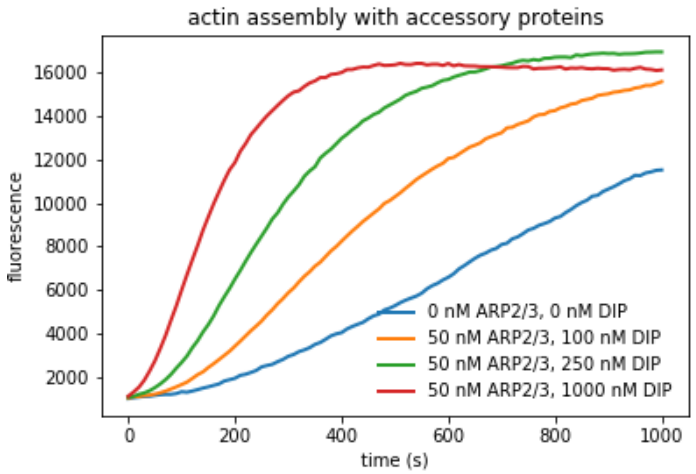
- Find the cell labeled "*The actin polymerization reaction.*"
- Run the cell beginning with `rxn_lines = ["A1 + A1 -> A2; 0.001"]`. Draw the mechanism described by the scheme printed out below the cell. (`A1` is an actin monomer, A2 is an actin dimer, etc.)
- Now run the set of cells up to the cell labeled "*Effector mechanisms.*" This should spit out a graph that shows the number of actin monomers in filaments with more than four subunits vs. time.
  - What is the shape of the curve?
  - Can you justify it in terms of the underlying reaction?

Effectors:

Biologically, actin polymerization can be tuned by effector molecules. Two such molecules are ARP2/3 and DIP. We are going to explore two possible mechanisms by which these molecules may regulate actin polymerization.

- In the literature, there are two possible interaction mechanisms proposed for ARP2/3 and DIP's interaction with actin. Draw the two mechanisms described by "mech1" and "mech2" as reaction schemes.

- Connor Balzer, a student in Brad Nolen's lab, collected actin polymerization data using concentrations of actin, ARP2/3, and DIP that are similar to those we are simulating. In his assay, fluorescence intensity is proportional to the number of polymerized actin subunits. Does his data make you favor one of the proposed mechanisms over the other? What features of the graphs distinguish the models?



- Connor did not collect data without ARP2/3 but with DIP. Fortunately, we have a computational model where we can vary the concentration of ARP2/3. What happens to the curves for your favored model when you set $[ARP2/3]$ to 0? What does this tell you about the "logic" of actin polymerization as encoded by DIP and ARP2/3?

- In fission yeast, actin monomers are found all over the cell. Arp2/3 and DIP are found at discrete locations in the cell. One of the few places ARP2/3 and DIP are found together is near sites of vesicle formation. Given this and your favored kinetic model, propose a biological function for these patches of overlapping APR2/3 and DIP localization.