BE/CS 196a Lab 03: Protocol design and rate measurement of a DNA-strand-displacement reaction

Objective

(i) simulate the kinetics of a DNA-strand displacement reaction, (ii) understand the basics for designing an experimental protocol, and (iii) estimate the reaction rate constant by comparing simulation with experimental data.

Definitions

Avogadro constant: $N_A = 6.02214076 \times 10^{23}$
mol: mole, a unit of amount; 1 mol = N_A molecules
L: liter, a unit of volume; 1 L = 1000 cm^3 (i.e. a cube with 10 centimeter sides)
M: molar, a unit of concentration, 1 M = 1 mol/L
mM: millimolar, 1 mM = 10^{-3} M
µM: micromolar, 1 µM = 10^{-6} M
nM: nanomolar, 1 nM = 10^{-9} M

1 Modeling and simulation (40 min)

A chemical reaction (or chemical reaction network) can be modeled using ordinary differential equations based on mass-action kinetics.

For example, $R1 + R2 \xrightarrow{k} P1 + P2$ can be modeled as

$$\begin{aligned}
\frac{d[R1]}{dt} &= -k[R1][R2] \\
\frac{d[R2]}{dt} &= -k[R1][R2] \\
\frac{d[P1]}{dt} &= k[R1][R2] \\
\frac{d[P2]}{dt} &= k[R1][R2]
\end{aligned}$$

where $[R1]$ and $[R2]$ are the concentrations of the first and second reactant, respectively, $[P1]$ and $[P2]$ are the concentrations of the first and second product, and k is a bimolecular reaction rate constant with unit /M/s.

Model and simulate a DNA-strand-displacement reaction in Mathematica:

- Look up the built-in function NDSolve.
- Define a function that finds a numerical solution to the set of ordinary differential equations that model a bimolecular reaction $R1+R2 \xrightarrow{k} P1+P2$ with variable rate constant and initial concentrations of the reactants, assuming that the initial concentrations of the products are 0.
- Look up the built-in function Plot. Check out Options PlotLabel, FrameLabel, PlotLegends, and LabelStyle – they are useful for adding necessary information that clarifies the content of a plot.
- Simulate and plot the kinetics of the reaction shown in Fig. 1, with initial concentrations of Rep6 = 100 nM and w5,6 = 0/20/40/60 nM, and $k_{rep} = 5 \times 10^4$ /M/s, for 8 hours.

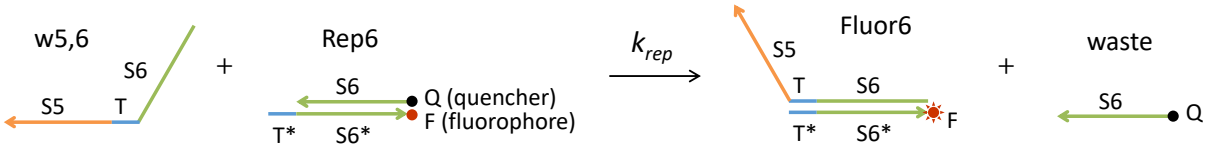

Figure 1: **Domain-level diagram of a DNA strand displacement reaction.** A domain is a string of nucleotides that are functionally independent. Colored lines represent DNA strands at the domain level, with arrowheads marking their 3' ends and colors indicating distinct DNA sequences. S5 and S6 are 15-nucleotide branch migration domains. T is a 5-nucleotide toehold domain. Star indicates Watson-Crick complementarity. A fluorophore is a fluorescent chemical compound that can re-emit light upon light excitation. A quencher is a chemical compound that absorbs excitation energy from a fluorophore. When a fluorophore and a quencher are close together, such as in Rep6, the fluorophore's emission is suppressed, resulting in low fluorescence. When a fluorophore and a quencher are separated apart, such as in two different molecules Fluor6 and waste, the fluorophore's emission results in high fluorescence.

2 Protocol design (20 min)

Design an experimental protocol to measure the rate of the strand-displacement reaction shown in Fig. 1, with Rep6 = 20 nM and w5,6 = 0/5/10/15 nM.

2.1 Prepare buffer

1×TE buffer solution contains 10 mM Tris and 1 mM EDTA. Tris and EDTA protects DNA from degradation. 100×TE is 100 times concentrate compared to 1×TE. We will use off-the-shelf 1× and 100×TE.

Question

What is the concentration of Tris and EDTA in 100×TE buffer solution?

1×TE/10×Mg^{2+} buffer contains 125 mM Mg^{2+}. Positively charged magnesium (Mg^{2+}) helps bring together negatively charged DNA strands, and thus is needed for all hybridization and strand displacement processes. We will make 1×TE/10×Mg^{2+} buffer using 100×TE, 1 M $MgCl_2$, and water (H_2O).

Facts about mixing

concentration × volume = constant

Question

What are the volumes of 100×TE, 1 M $MgCl_2$, and H_2O, respectively, that should be mixed together to create 5 mL of 1×TE/10×Mg^{2+} buffer?

2.2 Anneal reporter complex

Annealing is a process where single-stranded DNA molecules are mixed together, and heated up and cooled down in a thermal cycler, allowing any complementary sequences to hybridize to each other and form a double-stranded complex.

When two molecules are mixed together in a test tube, it is practically impossible to have exact stoichiometry (e.g. exactly the same amount of each molecule), because of pipetting error and concentration inaccuracy – each of which can typically introduce a 10% difference between the target and actual amounts of a molecule.

Question

Of the two strands consisting Rep6 (refereed to as top and bottom strands), which one(s) can be in excess of the other without introducing any problems in measuring the rate of the strand displacement reaction shown in Fig. 1?

The top and bottom strands in Rep6 are named Rep6-t and Rep6-b, respectively, and ordered from IDT DNA at 100 µM in 1×TE buffer. The desired buffer condition for annealing is 1×TE and 1×Mg^{2+}, which can be achieved by mixing Rep6-t, Rep6-b, 1×TE/10×Mg^{2+}, and 1×TE together with an appropriate volume each.

Question

What are the volumes of Rep6-t, Rep6-b, 1×TE/10×Mg^{2+}, and 1×TE, respectively, that should be mixed together to anneal 100 µL of 10 µM Rep6?

2.3 Dilute signal strand

Signal strand w5,6 is also ordered from IDT DNA at 100 µM in 1×TE buffer.

Question

What is the volume of 100 µM w5,6 that is required to achieve 5 nM w5,6 in 500 µL?

Pipetting error typically increases with decreased volume. To avoid significant errors, we set 1 µL to be the minimum pipetting volume.

Question

Is the volume of w5,6 calculated above smaller or larger than the minimum pipetting volume?

Dilution can be used to bring the concentration of a molecule to the desired range.

Question

What are the volumes of 100 µM w5,6 and 1×TE, respectively, that should be mixed together to create 200 µL of 2.5 µM w5,6?

2.4 Mix reporter with varying amounts of signal strand

The accuracy with which we can prepare sample concentrations is limited because DNA molecules stick to the walls of pipette tips and test tubes. To minimize this issue, we use Lo-Retention tips and Lo-Bind tubes. On top of that we use 20T, a single strand with 20 nucleotides of T's, at a much higher concentration compared to other DNA molecules such that it sticks to the tips and tubes more preferably and thus reduces the loss of other DNA molecules.

Question

What is the pipetting volume of each following ingredient for creating 20 nM Rep6 and 0/5/10/15 nM w5,6 in a 500 µL solution?
1×TE, 1×TE/10×Mg^{2+}, 1000 µM 20T, 10 µM Rep6, and 2.5 µM w5,6.

2.5 Automated protocol design (homework)

Write Mathematica functions for automatically generating the above protocols with variable species names, concentrations, and volumes.

3 Data analysis (30 min)

Download two data files and an example data analysis Mathematica notebook provided with this lab assignment. The data files are from an experiment of the strand-displacement reaction shown in Fig. 1, with Rep6 = 20 nM and w5,6 = 0/5/10/15 nM.

3.1 Analyze a near-perfect dataset

Fix the following issues in the Mathematica notebook to appropriately analyze data1.

- In Simulation, examine the plot and compare it with your plot from section 1 above. Does the molecular kinetics in all four trajectories look the same? If not, use it as a reference to debug your simulation code as part of your homework.

  Now, revise the concentrations of the initial species in the example notebook to simulate the experiment of which the data file was collected from. Remember to change the information for the plot to agree with the revised simulation.

- In Data analysis → Plot raw data, revise the delay time to 5 minutes, which was recorded from the experiment.

- In Data analysis → Plot normalized data, revise the reference list to include two more reference points: the average of the last 5 data points of the 2nd trajectory should be 5 nM, and the average of the last 5 data points of the 3rd trajectory should be 10 nM.

- In Data analysis → Plot normalized data overlaid with simulation, revise the definitions to include the changes that you have made in the simulation above, and revise the time range to display just the part of the trajectories that are relevant for finding a reaction rate constant.

  Does the simulation agree with the experimental data? If not, find a rate constant k_{rep} that fits the data reasonably well.

- Export a plot of normalized data overlaid with simulation, specifying the k_{rep} that you found.

3.2 Analyze a dataset that contains experimental errors

Apply the same data analysis that you did with data1 to data2.

- Does tuning the rate constant k_{rep} alone allow good agreement between normalized data and simulation? If not, identify which aspect(s) of the data appear to be problematic.

- Try removing any problematic trajectories from the reference list used for data normalization and re-comparing the data with simulation. Does tuning the rate constant k_{rep} now allow better agreement between the two?

- Come up with a hypothesis for what might have happened during the experiment that could explain the difference between the expected molecular behavior suggested by simulation and the observed molecular behavior from experimental data.
- Try revising the reference list again to reflect your hypothesis about possible experimental errors, re-normalizing the data, and re-comparing the data with simulation. Does tuning the rate constant k_{rep} now allow good agreement between the two?