Modeling Pre-lab Homework Assignment
Binding Affinity of Actin Binding Protein - Profilin

Homework: This assignment is to be done individually and uploaded ***BEFORE*** *the start of lab. A video is posted on Canvas that covers the coding syntax you need in order to complete this assignment. It is required to watch the video before you start the assignment.* ***The Quantitative Biosciences Center will be open Sunday – Thursday from 7-9pm (Chicago Time) on Zoom*** *for assistance.* ***Please see Canvas for details on hours and for the Zoom link and sign-in****.*

Organize your code and answers clearly in one .Rmd file. Enter all answers to boldface questions as comments in the code.

This is an individual assignment, but you are allowed to work together in groups and discuss coding and answers. That said, you are responsible for all of the material in this homework assignment. ***DO NOT COPY*** *from anyone that you work with. You are NOT allowed to share code. You need to write the code and answer the questions yourself. Try the coding yourself first before seeking help.*

Be sure to include your name in the file name as follows: lastname_firstname_labday .Rmd. Also type your full name as the first comment in your .Rmd file. Upload 1) the .Rmd file and 2) the knitted html file to Canvas before the start of your lab.

Due: BEFORE the start of your lab period in week 8
TOTAL: 10 points

If you have any questions, please do not hesitate to ask. Best of luck!

We will investigate the actin-binding protein, profilin, and its binding affinity for G-actin. The binding affinity can be expressed as the dissociation constant, K_d, which is the concentration of reactants divided by the concentration of products. We will explore what the binding affinity and the best fit line can tell us about the mechanism of binding of profilin to actin.

PART I: Read in and Plot Experimental Data for a Range of Profilin Concentrations

One way to biochemically determine the K_d is to 1) gather fluorescence vs. time data with varying profilin concentrations, 2) determine the slopes of the linear (elongation) portion, 3) fit the resultant assembly rates vs. concentration range of profilin, and 4) perform a nonlinear fit of the data to extract the binding affinity of profilin for G-actin.

First, read in the .csv file downloaded from canvas and assign each column from the file to a variable in order to further manipulate and plot the data. We will explore assumptions made from fitting our binding affinity data for profilin.

Handling Experimental Data
Import the data file you downloaded from Canvas – **SAVE** the file on your computer and set the working directory through R.

- In RStudio → session → set working directory → choose directory (choose directory where you saved the experimental file – the DESKTOP (or wherever you saved the experimental file)).
- In the console window type: **`getwd()`** – this will give you the exact pathway to your file. Copy and paste this pathway when you read in your .csv file.

    - Variablename=read.csv("/Users/elizabethkovar/Desktop/ Profilin_AssemblyCurves.csv") # data frame
- This tells the software exactly where the file lives to be read in.
- Read in the file by assigning the whole .csv file to a variable name – which will be a matrix with dimensions of 201x12 (201 rows and 12 columns) – this is called a data frame.
- Assign each column to a specific variable
    - Variable_name_column=Variablename$columnName → where columnName is the name of the column explicitly written in the .csv file. You need to look at the csv file to see the tag for each column. Once the file is read into R, you can double click on the name found in environment.

Choose between the following 2 ways to work with your data

1. **Assign each column to a specific variable**
    - Variable_name_column=Variablename$columnName → where columnName is the name of the column explicitly written in the .csv file. You need to look at the csv file to see the tag for each column.
2. **Work with the matrix itself** – remembering the order of the columns and what each column corresponds to. You will can use for loops to iterate over all positions in the column. Look at the video for more specifics.

You can now use these variables (Variable_name_column) in your .Rmd file for plotting or whatever.

1. **Plot the data from Profilin_AssemblyCurves.csv as lines (type="l", include axes labels with units, title, and add a legend). The concentration for actin is 2.5 μM. Remember to assign a color to each of the 11 curves – then you can add a legend.**

2. **Describe what you see. Use comments in the .Rmd file. How do the assembly curves of actin assembly change based on changing the profilin concentration?**

PART II: Linear Portion of the Experimental Curves

We want to look at the elongation rates for each of the 11 curves. To do this, we need to look at the linear portion for all 11 curves in time – this section corresponds to the elongation phase of the curve. We will take the linear portion of all 11 curves and extrapolate the **slope – assembly rate** – during lab.

Choose a linear portion in all the data from the experimental data you just plotted in #1. Choose a portion in time that is linear. You will take all the variables and restrict the data to encompass only that time range that is linear. For example, if you choose 735 s – 1335 s you would grab the information from time[50:90]. This will grab 735 – 1335 s from your time array you read in from the experimental file. When accessing information from an array, you choose a particular index value which will return the actual value in that index number (integer values starting at 1). So, you will take the 50^{th} – 90^{th} positions in the rest of the variables to get the actual fluorescence readings from the varying profilin concentrations to correspond to 735-1335 s.

3. **Plot the linear portion of all 11 curves (type="l", include axes labels with units, title, and add a legend).**

4. **Describe what you see. Use comments in the .Rmd file.**

During lab, we will calculate the individual slopes for each assembly rate vs. profilin concentration and extract the binding affinity of profilin for G-actin.

Upload both your 1) .Rmd and 2) knitted html files to Canvas.

Please make sure to visit the Quantitative Biosciences Center for additional help (details on our Canvas page) or visit https://college.uchicago.edu/academics/quantitative-biosciences-center

If you have further questions, please email me: Elizabeth Kovar ewkovar@uchicago.edu