Stellenbosch University – Biochemistry Honours 2025 **27 March 2025**

Mini-course 3: Systems Biology (Biochemistry 714)

Assessment 1: Tutorial test

Examiners: Dr DD van Niekerk and Prof JL Snoep Time: 2.5 hours (14h00–16h30)
Marks: 50

Instructions

This is an **open-book** assessment. You may make use of your notes as well as online tutorial material including Mathematica notebook memos. However, **communication between students and the use of AI coding tools are not allowed** - you have to submit your own work.

Hand in a Mathematica notebook in which you answer the questions below. The notebook should be submitted via the dedicated link on STEMLearn, and the file should be named as follows:
`studentnumber_Lastname_Initials.nb` (e.g. `12345678_VanNiekerk_DD.nb`)

Background

Glucose 6-phosphate dehydrogenase (G6PDH) is an enzyme that catalyses the following reaction:

$$\text{G6P} + \text{NADP} \longrightarrow \text{6PGL} + \text{NADPH}$$

The enzyme was characterised kinetically by determining the initial rate of the formation of the product NADPH through spectrophotometric measurement at 340 nm. The experiment was repeated for various concentrations of both substrates.

The rate of the enzyme-catalysed reaction can be described by the following rate equation:

$$v = \frac{V_f a \cdot b}{(K_a + a)(K_b + b)} \tag{1}$$

where a and b refer to the concentrations of the substrates G6P and NADP, respectively, V_f is the maximal (limiting) rate, and K_a and K_b are the respective Michaelis constants for the substrates.

Tasks

All analyses should be performed in a Mathematica notebook, and at the end you should only submit this one file. You can insert comments as required for explanations using:
`(* comment *)`.

In the Download section of the course website (folder `Tutorial_assessment`) you will find data files from the kinetic experiments. These are named `AxxByy.csv`, where `xx` refers to the concentration of A (in mM) for that particular experiment, and `yy` to the concentration of B. The files are comma-delimited and contain two columns: time in minutes, and concentration of NADPH in mM.

1. Visualise the data by plotting the time courses as **scatter plots**, as follows: [15]

   - On the first graph, plot all the experiments where a was varied and b kept constant at 24 mM, i.e. $a = 0.5, 1, 2, 4$ and 8 mM. Plot these on the same set of axes, using different colours to distinguish the datasets. Label the axes and provide a legend.
   - On a second graph, do the same for the experiments where b was varied and a kept constant at 8 mM, i.e. $b = 1.5, 3, 6, 12$ and 24 mM. Format the graph in the same way as the first.

2. Fit a line to each of the datasets to determine the initial rates. [10]

3. Create a new 2-dimensional list that combines the data. The entries of the list should be in the form $\{a, b, \text{rate}\}$. [5]

   If you did not manage to perform step 2, you can use the following data to carry on with the rest of the assignment. Note that these are *not the correct values*, so only use these if you could not obtain the real values!

   Backup initial rate data

a (mM)	b (mM)	rate (mM min^{-1})
0	0	0
0.5	24	0.512
1	24	0.816
2	24	1.202
4	24	1.561
8	24	1.747
8	1.5	0.603
8	3	0.912
8	6	1.267
8	12	1.558

4. Fit equation (1) to the data to obtain estimates for the parameters V_f, K_a and K_b. Perform a *global fit* on *all the data* simultaneously. Provide error estimates for each of the parameters and give their units! [10]

5. Plot the fit. The graphs should contain the fitted function (as a line) and the experimental data (as points). Provide two graphs: [10]

   - *rate* vs. a (with b fixed at its highest value)
   - *rate* vs. b (with a fixed at its highest value)

   You will therefore use only a part of the data for each plot. Label the axes (including units) and provide a legend for the lines/points.