# Biochemical cognition: Building a brainy bacterium

## What new skills will I possess after completing this laboratory?

* **Generalising** Michaelis-Menten kinetics to Hill functions;
* **Applying** ODEs to chemical balance equations;



* **Developing** designs for a genetic switch.

## Why do I need these skills?

In this lab, we use the module **CellularCognition** to study how biochemistry implements ***cognition***: organisms’ ability to *choose*. This diagram illustrates how an *E. coli* cell makes ***choices*** (survival-relevant decisions) in relation to problems posed by its environment.

A bacterial cell is a long bag of around 4000 different proteins of about 1000 molecules each. It senses its environment via signalling proteins that enter the cell, and responds by expressing ***regulator*** proteins that either act on the environment or change the cell's internal state. First, binds to the regulator , thereby activating it into a state that binds to the promoter of a target gene to regulate the expression rate of another protein . The cell’s internal state is the set of instantaneous concentrations of about 300 different regulators that regulate protein expression.

So the 'brain' of a cell consists of a ***regulatory network*** that works exactly like other structural processing systems such as neural or immune networks. It contains thousands of chemical species that react with each other. Think of the gene as a catalyst that computes the rate of production of as a function of the concentration of . To model this, let’s first look at the second-order catalytic reaction implemented in **CellularCognition**, where the enzyme galactosidase () first binds reversibly to lactose (), then breaks into galactosidase () plus glucose and galactose ():

1. Draw an SPD of this model.
2. Study and run the lactose breakdown model in **CellularCognition**. Use the rate values , and , with initial concentrations and , and generate a BOTG over 5 seconds.
3. Compare the curves for and for : why do they have this particular relationship?

## What is the structure of the skills?

The dynamics of exercise (ii) are typical of catalytic reactions, but the enzyme concentration is usually much smaller than the substrate concentration , which changes the shape of the curves:

1. Change the initial concentration of the unbound enzyme to 0.5, and rerun your simulation. What is the approximate rate of change of the concentration of the bound substrate-enzyme complex for most of the simulation run?

This discovery helps us to model catalysis more efficiently by assuming that reaction **Error! Reference source not found.** has settled into a steady state in which is constant. The balance (inputs–outputs) equation for XS is then:

where is the ***Michaelis constant*** for the catalytic reaction (1). But the total enzyme concentration (bound + unbound) is a constant , in which case

This gives us an approximate value for the rate of the reaction:

where is the maximum reaction rate. This is the ***Michaelis-Menten equation*** for reaction (1).

1. Create in the julia console a function that implements the bracketed expression in the Michaelis-Menten (MM) equation. Then use **GLMakie.lines()** to plot the function against , and so verify each of the following three statements: (a) is a ***saturating*** function of ; (b) the ***least upper bound*** of is 1; (c) is the ***half-response*** value of in the function .

1. Calculate the Michaelis constant and the maximum rate for our lactose breakdown model. On the right is an SPD of the MM model: implement it in **CellularCognition** as a second **KineticModel**. Discuss the behaviour differences between this model and that of exercise (ii).

## How can I extend my skills?

There is another way of viewing the MM equation. Imagine now as the gene for protein , together with its promoter. When a regulator molecule binds to this promoter, it activates or inhibits the gene by forming the new complex . This is exactly analogous to our lactose-breakdown situation above, and again we can describe this regulation of using the same kind of saturating function that you explored in exercise (v):

* The factor describes ***activation*** of from 0 to 1 with increasing concentrations of .
* The factor describes ***inhibition*** of from 1 to 0 with increasing concentrations of .

In general, however, several () regulator molecules may need to cooperate in order to regulate a gene, and we describe this more general situation using the saturating ***Hill function***:

1. Implement the Hill function in Julia: positive half-response values denote activation, while negative values denote inhibition. Test your function at the console by plotting graphs to verify the following statements about the Hill function: (a) implements the above activation and inhibition factors; (b) is the half-response level of ; (c) (the ***cooperativity***) controls the abruptness of the Hill function's step-like shape.

## How can I deepen my practice of the skills?

1. The concentration of all gene products decreases over time due to dilution and breakdown. Suppose  is the *constant* expression rate of some gene product , and that is the breakdown rate of . Derive the differential equation for the concentration , and prove that as , approaches the stable equilibrium value .

Exercise (viii) shows us the importance of degradation for cellular information processing: the combination of constant expression with exponential degradation constant *always* leads to a constant concentration for gene products; this value is then stored in the 'brain' of the cell.

1. In a certain experiment, researchers inserted three regulators into the DNA of *E. coli* and connected them as an inhibition cycle: inhibits , which inhibits , which inhibits . also activates the Green Fluorescent Protein gene *GFP*. In an experiment, protein is initially present in the cell, while the concentrations of and are zero. , and all have identical values for the degradation/dilution rate , maximum expression rate , half-response constant and cooperativity . Build these parameter values into a new **KineticModel** in **CellularCognition** to discover what behaviour the researchers observed. (Hint: To start, keep the degradation rate fairly low.)
2. When you have discovered the nature of the GFP behaviour from the previous exercise, experiment with the Hill constant : what condition must necessarily fulfil in order for the three genes , , to generate this specific behaviour?