

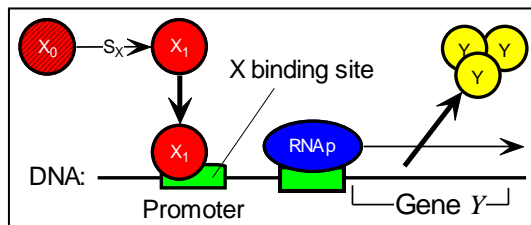
205. Biochemical cognition: Building a brainy bacterium

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.

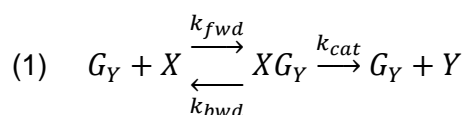
Why do I need these skills?

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 10^{-6} m long bag of around 4000 different proteins of about 1000 molecules each. It senses its environment via signalling proteins S_X that enter the cell, and responds by expressing **regulator** proteins that either act on the environment or change the cell's internal state. First, S_X binds to the regulator X_0 , thereby activating it into a state X_1 that binds to the promoter of a target gene G_Y to regulate the expression rate of another protein Y . 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 G_Y as a catalyst that computes the rate of production of Y as a function of the concentration of X . To model this, let's first look at the second-order catalytic reaction implemented in **CellularCognition**, where the enzyme galactosidase (G_Y) first binds reversibly to lactose (X), then breaks into galactosidase (G_Y) plus glucose and galactose (Y):



- Draw an SPD of this model.
- Study and run the lactose breakdown model in **CellularCognition**. Use the rate values $k_{fwd} = 2 \text{ M}^{-1}\text{s}^{-1}$, $k_{bwd} = 1 \text{ s}^{-1}$ and $k_{cat} = 1.5 \text{ s}^{-1}$, with initial concentrations $[X] = 8 \text{ M}$ and $[G_Y] = 4 \text{ M}$, and generate a BOTG over 5 seconds.
- Compare the curves for G_Y and for XG_Y : 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 $[G_Y]$ is usually much smaller than the substrate concentration $[X]$, which changes the shape of the curves:

- Change the initial concentration $[G_Y]$ of the unbound enzyme to 0.5, and rerun your simulation. What is the approximate rate of change of the concentration $[XG_Y]$ 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 $[XG_Y]$ is constant. The balance (inputs–outputs) equation for XS is then:

$$\frac{d[XG_Y]}{dt} \equiv k_{fwd}[X][G_Y] - k_{bwd}[XG_Y] - k_{cat}[XG_Y] = 0$$

$$\Rightarrow [XG_Y] = \frac{k_{fwd}[X][G_Y]}{k_{bwd} + k_{cat}} \equiv \frac{[X][G_Y]}{K_m}$$

where $K_m \equiv (k_{bwd} + k_{cat})/k_{fwd}$ is the **Michaelis constant** for the catalytic reaction (1). But the total enzyme concentration (bound + unbound) is a constant $[G_Y]_0 = [G_Y] + [XG_Y]$, in which case

$$[XG_Y] = \frac{([G_Y]_0 - [XG_Y])[G_Y]}{K_m}$$

$$\Rightarrow [XG_Y] = \frac{[G_Y]_0 [X]}{K_m + [X]}$$

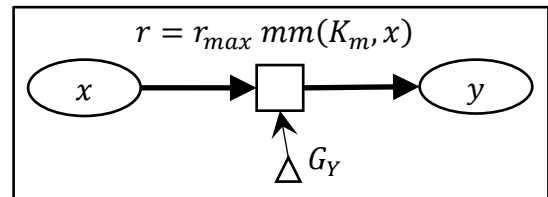
This gives us an approximate value for the rate of the reaction:

$$r = \frac{d[Y]}{dt} = k_{cat}[XG_Y] = k_{cat}[G_Y]_0 \left(\frac{[X]}{K_m + [X]} \right) \equiv r_{max} \left(\frac{[X]}{K_m + [X]} \right)$$

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

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

- (vi) Calculate the Michaelis constant K_m and the maximum rate r_{max} 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 G_Y now as the gene for protein Y , together with its promoter. When a regulator molecule X binds to this promoter, it activates or inhibits the gene by forming the new complex XG_Y . This is exactly analogous to our lactose-breakdown situation above, and again we can describe this regulation of G_Y using the same kind of saturating function that you explored in exercise (v):

- The factor $\frac{x}{K+x}$ describes **activation** of G_Y from 0 to 1 with increasing concentrations of X .
- The factor $\frac{K}{K+x}$ describes **inhibition** of G_Y from 1 to 0 with increasing concentrations of X .

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

$$hill(x, K = 1, n = 1) = \begin{cases} \frac{x^n}{K^n + x^n} & (K \geq 0) \\ \frac{|K|^n}{|K|^n + x^n} & (K < 0) \end{cases}$$

- (vii) Implement the Hill function in Julia: positive half-response values K 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) $hill(x, K, 1)$ implements the above

activation and inhibition factors; (b) K is the half-response level of x ; (c) n (the **cooperativity**) controls the abruptness of the Hill function's step-like shape.

How can I deepen my practice of the skills?

- (viii) The concentration of all gene products decreases over time due to dilution and breakdown. Suppose β is the *constant* expression rate of some gene product X , and that α is the breakdown rate of X . Derive the differential equation $\dot{x} = \beta - \alpha x$ for the concentration $x \equiv [X]$, and prove that as $t \rightarrow \infty$, x approaches the stable equilibrium value $\bar{x} = \beta/\alpha$.

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

- (ix) In a certain experiment, researchers inserted three regulators into the DNA of *E. coli* and connected them as an inhibition cycle: A inhibits B , which inhibits C , which inhibits A . C also activates the Green Fluorescent Protein gene *GFP*. In an experiment, protein A is initially present in the cell, while the concentrations of B and C are zero. A , B and C all have identical values for the degradation/dilution rate α , maximum expression rate β , half-response constant K and cooperativity n . Build these parameter values into a new **KineticModel** in **CellularCognition** to discover what behaviour the researchers observed.