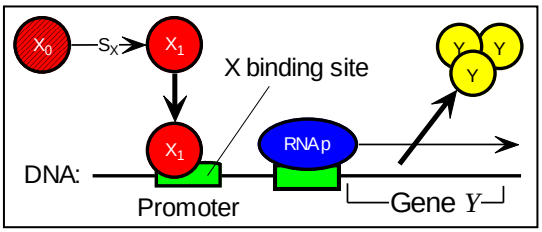
205. Biochemical cognition: Building a brainy bacterium

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):

$$(1) \quad G_Y + X \underset{k_{bwd}}{\overset{k_{fwd}}{\rightleftarrows}} XG_Y \xrightarrow{k_{cat}} G_Y + Y$$

(i) Draw an SPD of this model.

(ii) Study and run the lactose breakdown model in **CellularCognition**. Use the rate values $k_{fwd} = 2\ \mathrm{M^{-1}s^{-1}}$, $k_{bwd} = 1\ \mathrm{s^{-1}}$ and $k_{cat} = 1.5\ \mathrm{s^{-1}}$, with initial concentrations $[X] = 8\ \mathrm{M}$ and $[G_Y] = 4\ \mathrm{M}$, and generate a BOTG over 5 seconds.

(iii) 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:

(iv) 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:

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

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

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

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

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

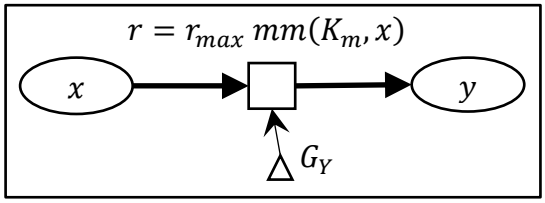
This gives us an approximate value for the rate of the reaction:

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

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 ***saturating*** 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***:

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

(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 $t \to \infty$, 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. (Hint: To start, keep the degradation rate fairly low.)

(x) When you have discovered the nature of the GFP behaviour from the previous exercise, experiment with the Hill constant K: what condition must K necessarily fulfil in order for the three genes A, B, C to generate this specific behaviour?