sb2cl Lab

INSTITUTO DE AUTOMÁTICA E INFORMÁTICA INDUSTRIAL

UNIVERSIDAD POLITECNICA DE VALENCIA

Synthetic Biology and Biosystems Control Lab
Valencia UPV

iGEM 2020 Measurement

Modeling: ODEs and Hill Functions

Section 1: ODEs, Law of mass action and the central dogma

by Alejandro Vignoni (alvig2@upv.es)

An iGEM Measurement Committee Webinar

Week 2, June 23rd, 2020

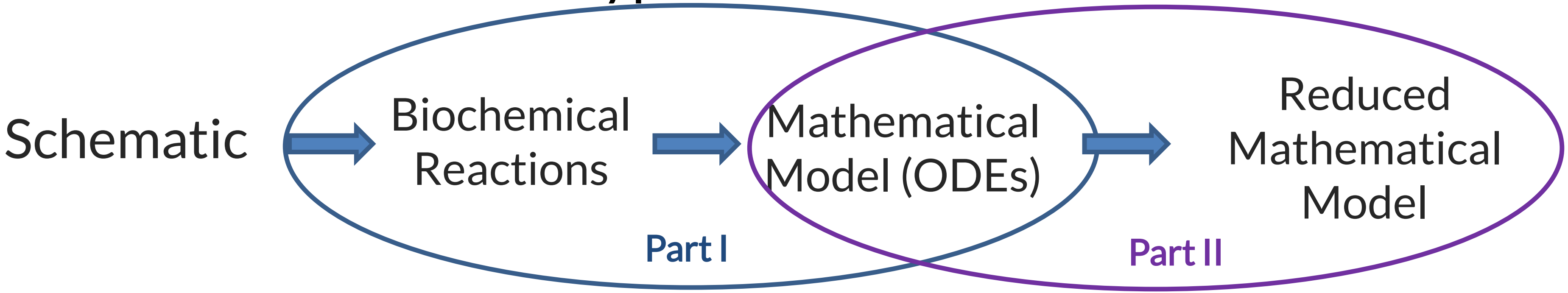
$$\begin{cases} \dfrac{d[LuxI]_i}{dt} = b_i + u([AHL]_i) - \gamma_I\,[LuxI]_i \\ \dfrac{d[AHL]_i}{dt} = K_A[LuxI]_i + d([AHL]_i - [AHL]_e) - \gamma_A\,[AHL]_i \end{cases}$$

Today Webinar’s Topics

2020
Measurement

- Section 1: ODEs, the law of mass action, and the central dogma (15 min)
- Section 2: Derivation of a Hill function from the law of mass action (15 min)
- Section 3: Hill function examples and intuitions: effects of parameters on activators, repressors, hybrid promoters, using a Matlab exploration package. (15min)
- Q&A – (at the end of each 15 minutes block, total 15 min)

Types of models



Schematic

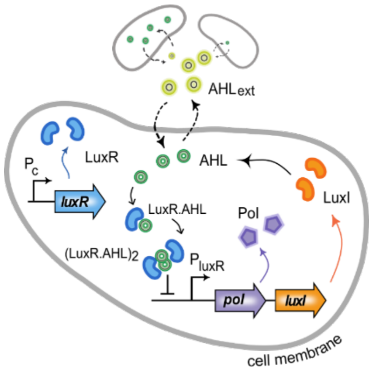
Biochemical Reactions

Mathematical Model (ODEs)

Reduced Mathematical Model

Part I

Part II



$$\xrightarrow{\mathrm{C_R}} \mathrm{mR}$$
$$\mathrm{gPI} \xrightarrow{\mathrm{k_{e_I}}} \mathrm{gPI} + \mathrm{mPI}$$
$$\mathrm{mR} \xrightarrow{\mathrm{p_R}} \mathrm{mR} + \mathrm{R}$$
$$\mathrm{mPI} \xrightarrow{\mathrm{p_I}} \mathrm{mPI} + \mathrm{PI}$$
$$\mathrm{I} \xrightarrow{\mathrm{k_A}} \mathrm{A} + \mathrm{I}$$
$$\mathrm{R} + \mathrm{A} \underset{\mathrm{k_{-1}}}{\overset{\mathrm{k_{-1}/k_{d1}}}{\rightleftharpoons}} (\mathrm{R}\cdot\mathrm{A})$$
$$_2(\mathrm{R}\cdot\mathrm{A}) \underset{\mathrm{k_{-2}}}{\overset{\mathrm{k_{-2}/k_{d2}}}{\rightleftharpoons}} (\mathrm{R}\cdot\mathrm{A})_2$$
$$\mathrm{gPI} + (\mathrm{R}\cdot\mathrm{A})_2 \underset{\mathrm{k_{lux}}}{\overset{\mathrm{k_{lux}/k_{dlux}}}{\rightleftharpoons}} \mathrm{gPI}\cdot(\mathrm{R}\cdot\mathrm{A})_2$$
$$\mathrm{gPI}\cdot(\mathrm{R}\cdot\mathrm{A})_2 \xrightarrow{\alpha \mathrm{k_{e_I}}} \mathrm{gPI}\cdot(\mathrm{R}\cdot\mathrm{A})_2 + \mathrm{mPI}$$
$$\mathrm{A} \underset{\mathrm{DV_c}}{\overset{\mathrm{D}}{\rightleftharpoons}} \mathrm{A_{ext}}$$

$$\dot{n}_1^i = \mathrm{k_{e_I}} n_7^i + \alpha \mathrm{k_{e_I}} n_8^i - \mathrm{d_{m_I}} n_1^i$$
$$\dot{n}_2^i = \mathrm{C_R} - \mathrm{d_{m_R}} n_2^i$$
$$\dot{n}_3^i = \mathrm{p_I} n_1^i - \mathrm{d_I} n_3^i$$
$$\dot{n}_4^i = \mathrm{p_R} n_2^i + \mathrm{k_{-1}} n_5^i - \mathrm{d_R} n_4^i - \frac{\mathrm{k_{-1}}}{\mathrm{k_{d1}}} n_9^i n_4^i$$
$$\dot{n}_5^i = 2\mathrm{k_{-2}} n_6^i + \frac{\mathrm{k_{-1}}}{\mathrm{k_{d1}}} n_9^i n_4^i + \left(-\mathrm{k_{-1}} - \mathrm{d_{RA}} - 2\frac{\mathrm{k_{-2}}}{\mathrm{k_{d2}}} n_5^i\right) n_5^i$$
$$\dot{n}_6^i = \mathrm{k_{lux}} n_8^i + \frac{\mathrm{k_{-2}}}{\mathrm{k_{d2}}} {n_5^i}^2 + \left(-\mathrm{k_{-2}} - \mathrm{d_{RA_2}} - \frac{\mathrm{k_{lux}}}{\mathrm{k_{dlux}}} n_7^i\right) n_6^i$$
$$\dot{n}_7^i = \mathrm{k_{lux}} n_8^i - \frac{\mathrm{k_{lux}}}{\mathrm{k_{dlux}}} n_6^i n_7^i$$
$$\dot{n}_8^i = -\mathrm{k_{lux}} n_8^i + \frac{\mathrm{k_{lux}}}{\mathrm{k_{dlux}}} n_6^i n_7^i$$
$$\dot{n}_9^i = \mathrm{D}\left(\mathrm{V_c} n_{10} - n_9^i\right) - \left(\frac{\mathrm{k_{-1}}}{\mathrm{k_{d1}}} n_4^i + \mathrm{d_A}\right) n_9^i + \mathrm{k_{-1}} n_5^i + \mathrm{k_A} n_3^i$$
$$\dot{n}_{10} = \mathrm{D}\left(-N\mathrm{V_c} n_{10} + \sum_{i=1}^{N} n_9^i\right) - \mathrm{d_{A_e}} n_{10}$$

$$\dot{n}_1^i = \frac{\mathrm{C_I p_I}}{\mathrm{d_{m_I}}}\left(\frac{\mathrm{k_{dlux}} + \alpha n_3^i}{\mathrm{k_{dlux}} + n_3^i}\right) - \mathrm{d_I} n_1^i$$
$$\dot{n}_2^i = \frac{\mathrm{C_R p_R}}{\mathrm{d_{m_R}}} + \mathrm{k_{-1}} n_6^i - \left(\frac{\mathrm{k_{-1}}}{\mathrm{k_{d1}}} n_4^i + \mathrm{d_R}\right) n_2^i$$
$$\dot{n}_3^i = \frac{\mathrm{k_{-2}}}{\mathrm{k_{d2}}} (n_6^i)^2 - (\mathrm{k_{-2}} + \mathrm{d_{RA_2}}) n_3^i$$
$$\dot{n}_4^i = \mathrm{k_{-1}} n_6^i + \mathrm{k_A} n_1^i + \mathrm{D}\left(\frac{n_5}{\mathrm{V_c}} - n_4^i\right) - \left(\frac{\mathrm{k_{-1}}}{\mathrm{k_{d1}}} n_2^i + \mathrm{d_A}\right)$$

Easier to use and to relate with experimental data

From
Y. Boada (2018)

But what is an Ordinary Differential Equations (ODE)?

These are equations with variables and their derivatives

If we have any function (the typical one):

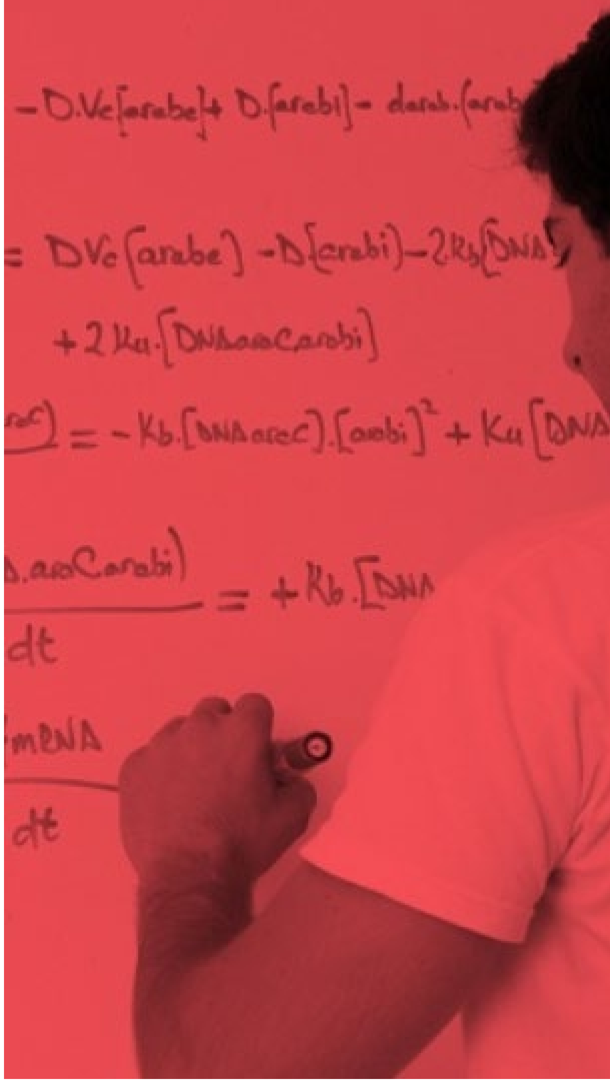
$y = f(t)$ (y only depends on the variable t, but we could have $y = f(t, x_1, x_2, \ldots x_n)$)

Do you remember the definition of the derivative of a function?

$$\dot{y} = \frac{df(t)}{dt} = \lim_{h \to 0} \frac{f(t+h) - f(t)}{h}$$

(we can have higher order derivatives y'', y''', $y^{(n)}$)

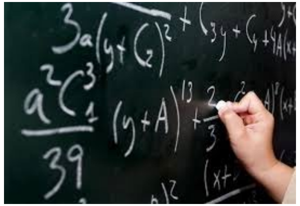
But they can be very challenging and difficult to solve!!


−D.Vc[arabe] + D.[arabi] − darab.(arab
= DVc(arabe) − D[arabi] − 2Kb[DNA
+ 2 Ku.[DNAaraCarabi]
= − Kb.[DNAaraC].[arabi]^2 + Ku[DNA
.araCarabi)
= + Kb.[DNA
dt
mRNA
dt

But if they're so complicated... how do we solve them?

We can solve differential equations in two ways:

- **Analytically:** solving for the unknown…



- **Numerically:** in an approximate way.

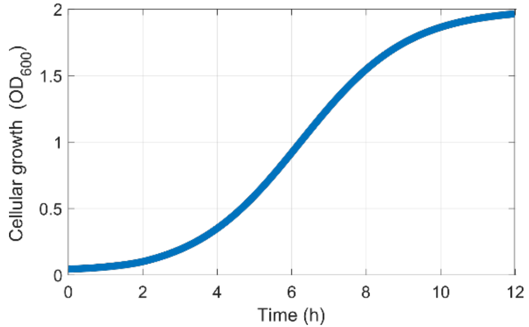
$$\dot{y} \simeq \frac{f(h+h)-f(x)}{h}$$

(with an h very small)

Why do we use them?

Differential equations describe biological behaviour, physical laws, human activities, and much more….

$$\frac{dx}{dt} = \mu x \left(1 - \frac{x}{x_{max}}\right)$$

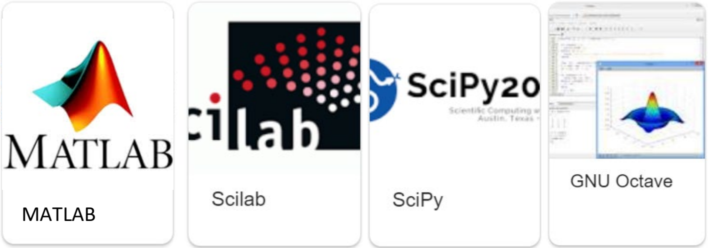


And the set of equations that describe a system, a phenomenon... is known as ODE model

Software for Ordinary Differential Equations (ODEs) solving

•MATLAB, a technical computing application (MATrix LABoratory)

FREE LICENSE WITH iGEM and the Measurement Committee has some software programed in MATLAB for flow cytometry and plate reader data analysis and calibration.



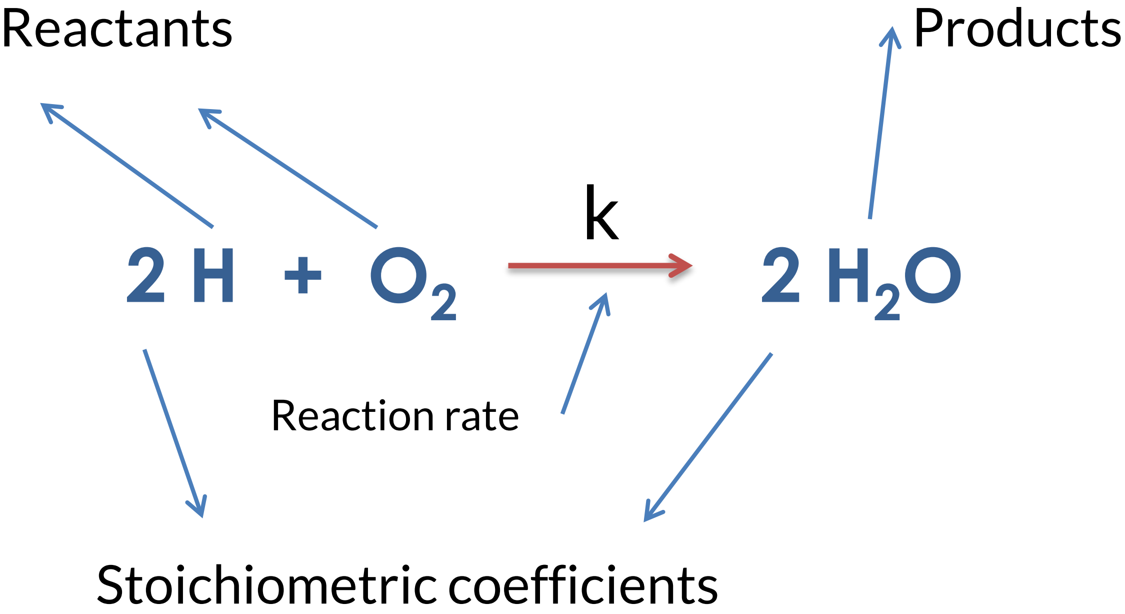
•Maxima, an open-source computer algebra system.
•COPASI, a free software package for the integration and analysis of ODEs.
•GNU Octave, a high-level language, basically a open-source versión of MATLAB.
•Scilab, an open source application for numerical computation.
•Maple, a proprietary application for symbolic calculations.
•Mathematica, a proprietary application primarily intended for symbolic calculations.
•Julia (programming language), a high-level language primarily intended for numerical computations.
•SageMath, an open-source application that uses a Python-like syntax with a wide range of capabilities spanning several branches of mathematics.
•SciPy, a Python package that includes an ODE integration module.
•GNU R, an open source computational environment primarily intended for statistics, which includes packages for ODE solving.

Let us begin this journey **Part I** from:

Biochemical Reactions → Mathematical Model (ODEs)

Reminder: Law of mass action and kinetic equations

Example: Reaction of Water



Reactants

Products

$$2\,H + O_2 \xrightarrow{k} 2\,H_2O$$

Reaction rate

Stoichiometric coefficients

Law of mass action and kinetic equations

Reaction of Water – Kinetics of H_2

$$2\,H_2 + O_2 \xrightarrow{k} 2\,H_2O$$

Rate of change of $[H]$

Decreases or it is consumed

$$[\dot{H}_2] = -\underbrace{2k}\underbrace{[H_2]^2[O_2]}$$

Stoichiometric coefficient of $[H_2]$ times the reaction rate *k*

product of the concentrations of the reactants ($[H_2] \times [H_2] \times [O_2] = [H_2]^2[O_2]$)

Law of mass action and kinetic equations

Reaction of Water – Kinetics of O_2

$$2\,H_2 + O_2 \xrightarrow{k} 2\,H_2O$$

Rate of change of $[H]$

Decreases or it is consumed

$$[\dot{O}_2] = -\ \underbrace{k}_{\substack{\text{Stoichiometric}\\ \text{coefficient of } [O_2] \text{ times}\\ \text{the reaction rate } k}}\underbrace{[H_2]^2[O_2]}_{\substack{\text{product of the concentrations of the}\\ \text{reactants (} [H_2] \times [H_2] \times [O_2] = [H_2]^2[O_2] \text{)}}}$$

Law of mass action and kinetic equations

Reaction of Water – Kinetics of H_2O

$$2\,H_2 + O_2 \xrightarrow{k} 2\,H_2O$$

Rate of change of $[H_2O]$

Increases or it is produced

$$\dot{[H_2O]} = +\underbrace{2k}\underbrace{[H_2]^2[O_2]}$$

Stoichiometric coefficient of $[H_2O]$ times the reaction rate k

product of the concentrations of the reactants ($[H_2] \times [H_2] \times [O_2] = [H_2]^2[O_2]$)

Law of mass action and kinetic equations

Reaction of Water – Kinetics

$$2\,H_2 + O_2 \xrightarrow{k} 2\,H_2O$$

$$[\dot{H}_2] = -2k[H_2]^2[O_2]$$

$$[\dot{O}_2] = -\ k[H_2]^2[O_2]$$

$$[\dot{H_2O}] = 2k[H_2]^2[O_2]$$

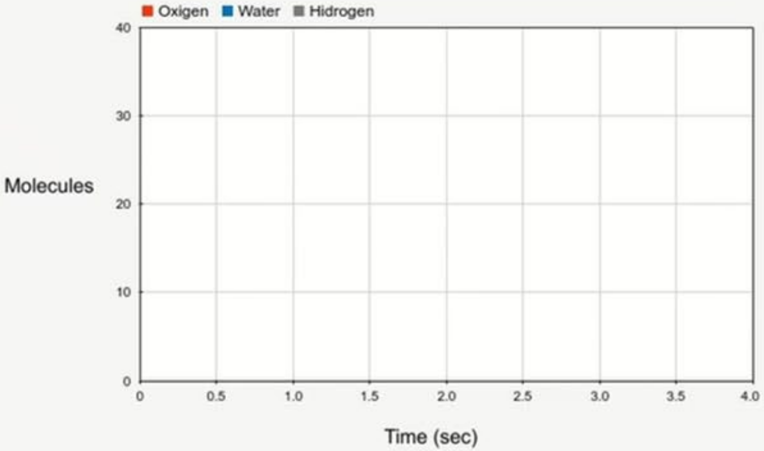
Reaction of Water – Kinetics

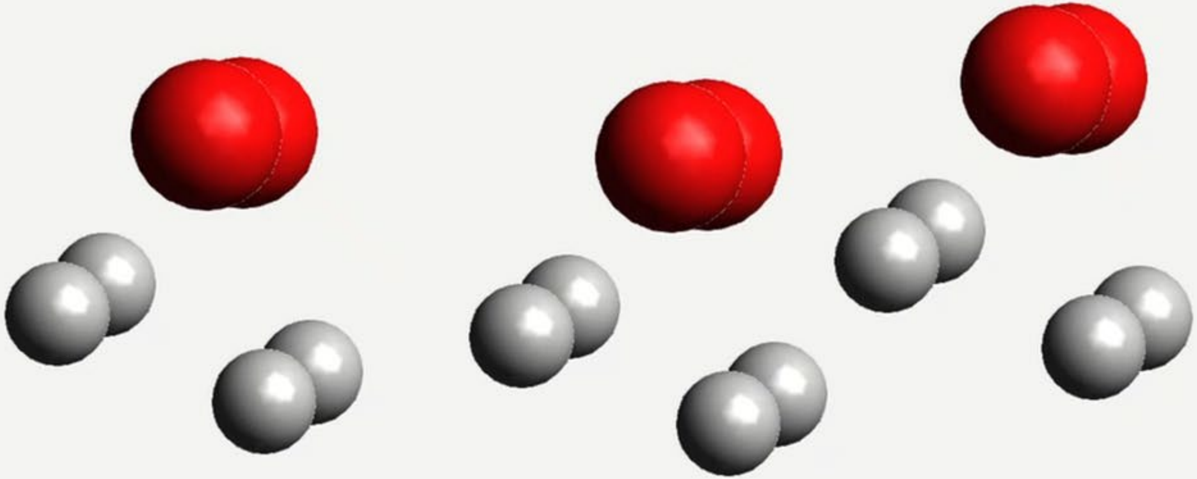
$$2\,H_2 + O_2 \xrightarrow{k} 2\,H_2O$$

$$[\dot{H_2}] = -2k[H_2]^2[O_2]$$

$$[\dot{O_2}] = -\ k[H_2]^2[O_2]$$

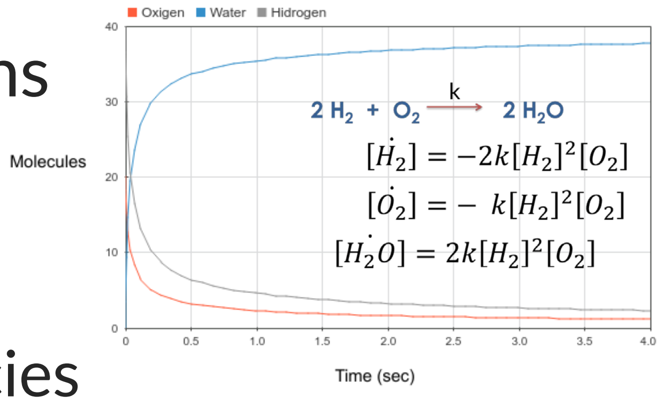
$$[\dot{H_2O}] = 2k[H_2]^2[O_2]$$





Check video on the repository!!

Law of mass action and kinetic equations



$$2\,H_2 + O_2 \xrightarrow{k} 2\,H_2O$$

$$[\dot{H}_2] = -2k[H_2]^2[O_2]$$

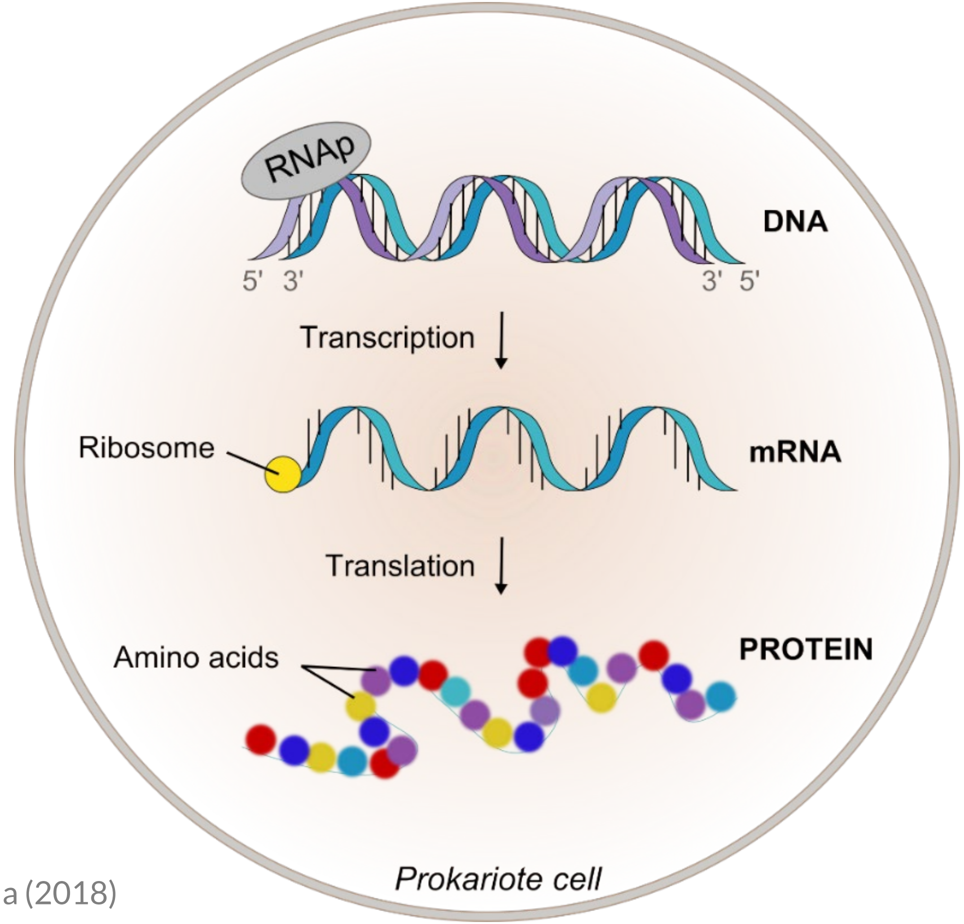
$$[\dot{O}_2] = -\ k[H_2]^2[O_2]$$

$$[\dot{H_2O}] = 2k[H_2]^2[O_2]$$

Summary

- One equation for each one of the species
- Rate of change of $[A]$ (concentration of species A) is proportional to:
  - Stoichiometric coefficient of A time reaction rate k
  - The product of the concentrations of the reactants

The central dogma of molecular biology

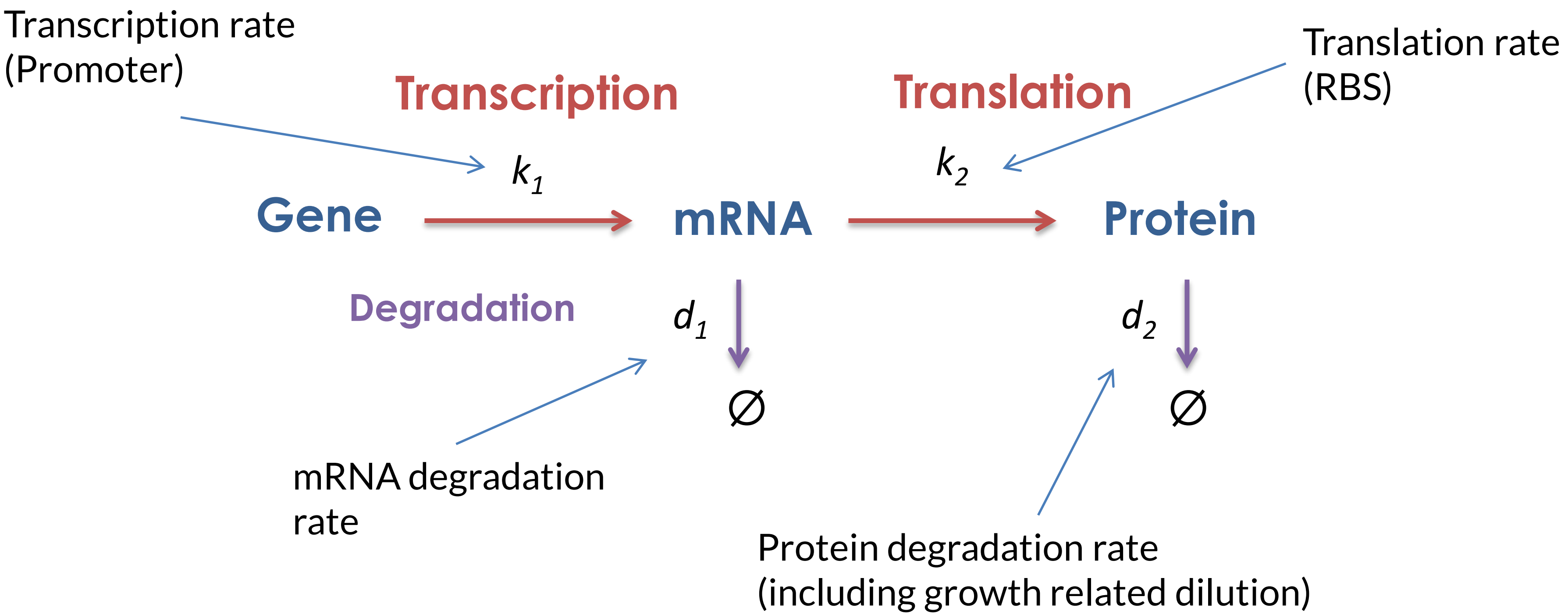


Transcription of DNA by RNA polymerase

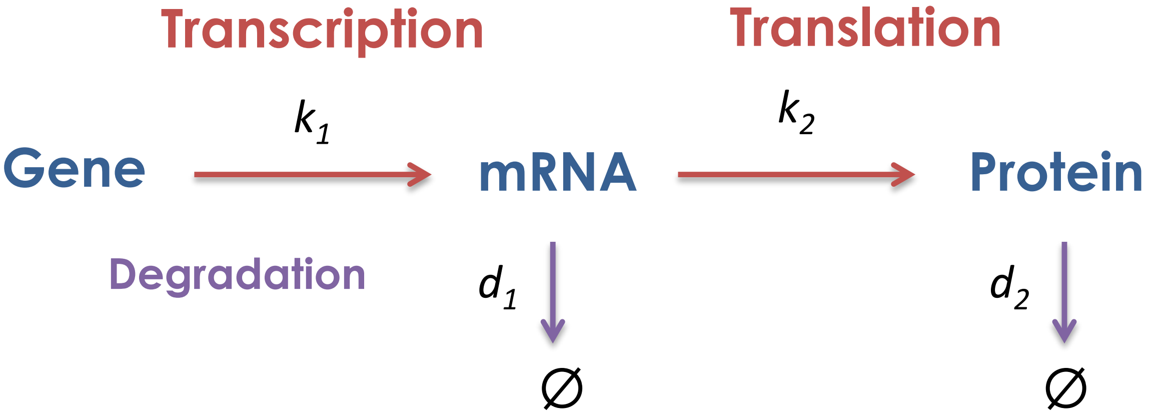
Translation of mRNA by Ribosomes

From
Y. Boada (2018)

Constitutive gene expression (Simplified version)



Constitutive gene expression (Simplified version)



Transcription

Translation

k_1

k_2

Gene

mRNA

Protein

Degradation

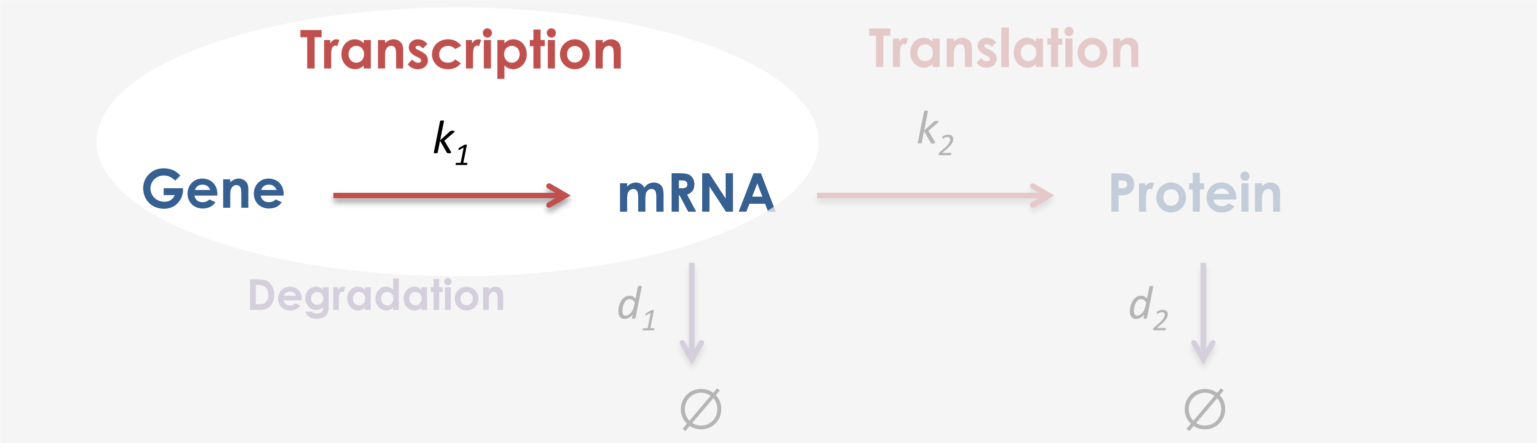
d_1

d_2

$\varnothing$

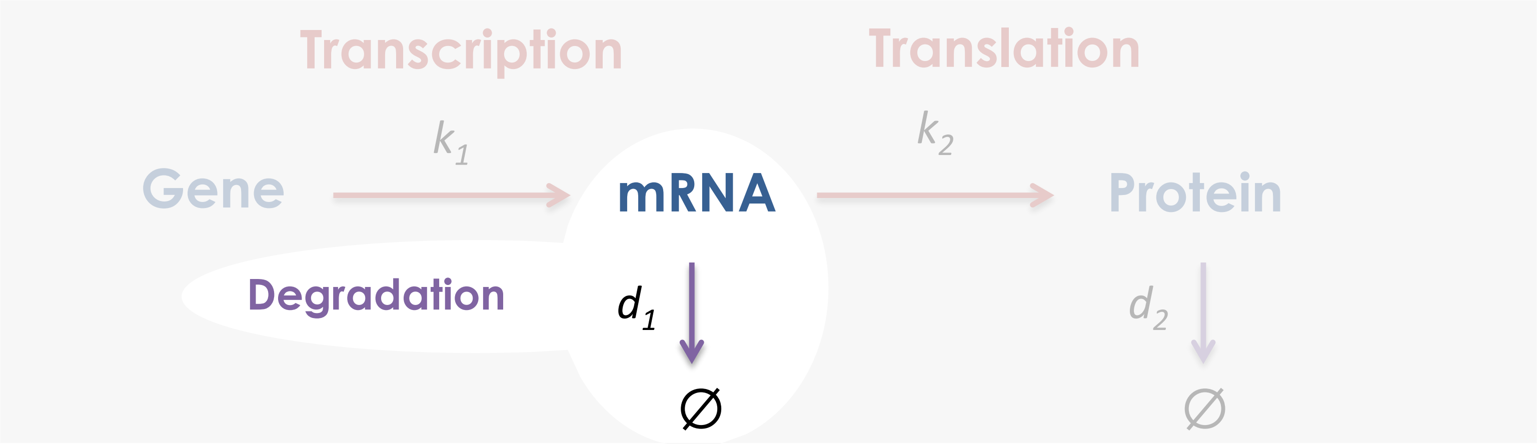
$\varnothing$

Constitutive gene expression (Simplified version)



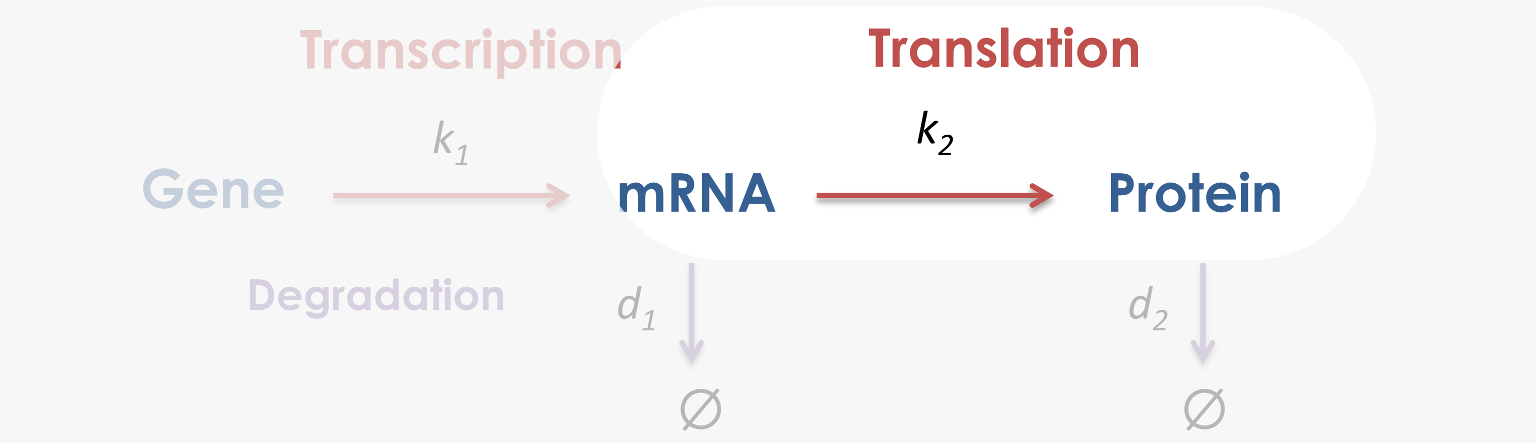
$$[\dot{\mathrm{mRNA}}] = k_1[\mathrm{Gene}]$$

Constitutive gene expression (Simplified version)



$$[\dot{\text{mRNA}}] = k_1[\text{Gene}] - d_1[\text{mRNA}]$$

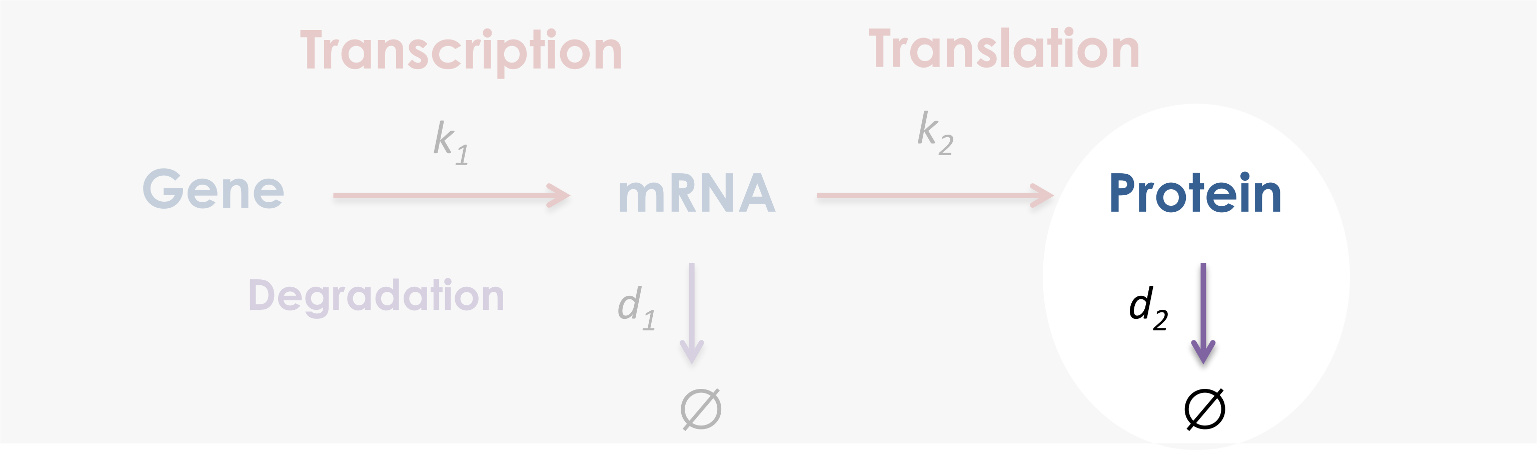
Constitutive gene expression (Simplified version)



$$\dot{[\mathrm{mRNA}]} = k_1[\mathrm{Gene}] - d_1[\mathrm{mRNA}]$$

$$\dot{[\mathrm{Protein}]} = k_2[\mathrm{mRNA}]$$

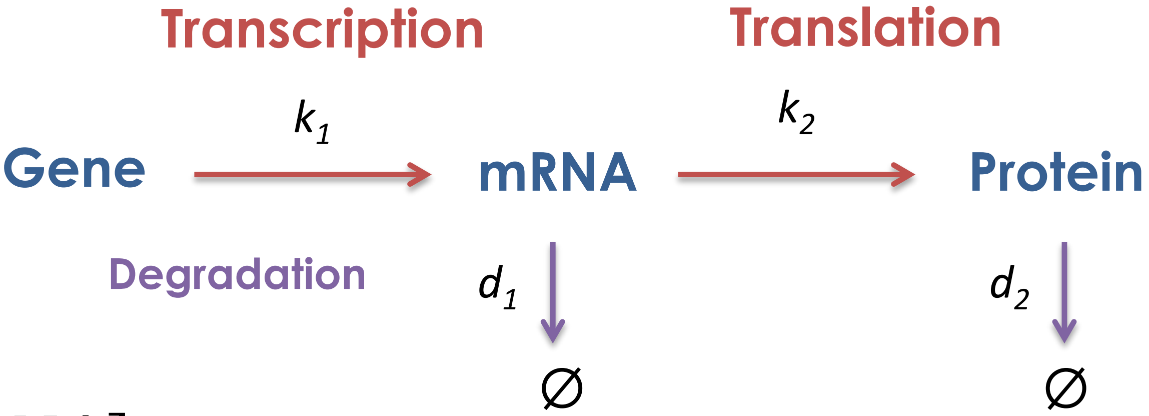
Constitutive gene expression (Simplified version)



$$\dot{[\mathrm{mRNA}]} = k_1[\mathrm{Gene}] - d_1[\mathrm{mRNA}]$$

$$\dot{[\mathrm{Protein}]} = k_2[\mathrm{mRNA}] - d_2[\mathrm{Protein}]$$

Constitutive gene expression (Simplified version)



$$\frac{d[\mathrm{mRNA}]}{dt} = \dot{[\mathrm{mRNA}]} = k_1[\mathrm{Gene}] - d_1[\mathrm{mRNA}]$$

$$\frac{d[\mathrm{Protein}]}{dt} = \dot{[\mathrm{Protein}]} = k_2[\mathrm{mRNA}] - d_2[\mathrm{Protein}]$$

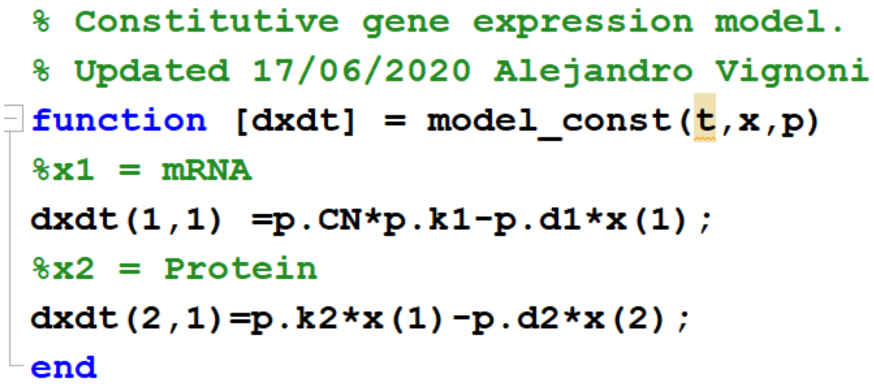
Constitutive gene expression - Simulation

ODE model

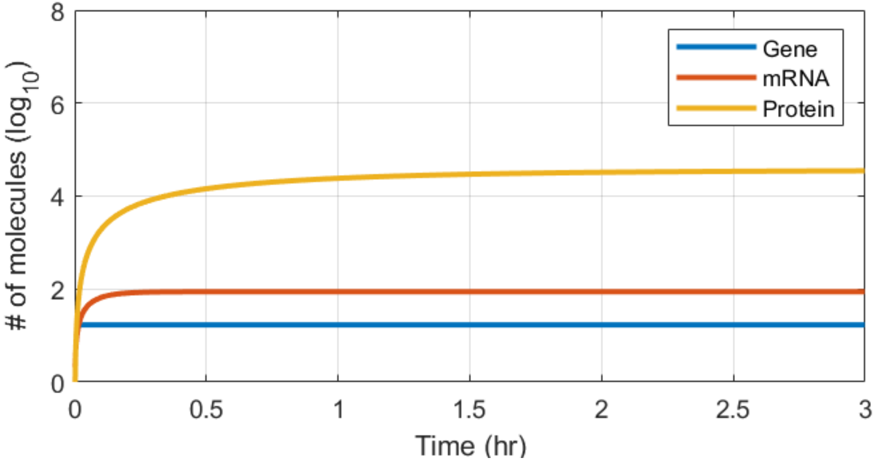
$$[\dot{\text{mRNA}}] = k_1[\text{Gene}] - d_1[\text{mRNA}]$$

$$[\dot{\text{Protein}}] = k_2[\text{mRNA}] - d_2[\text{Protein}]$$

Function defines the ODE model



```
% Constitutive gene expression model.
% Updated 17/06/2020 Alejandro Vignoni
function [dxdt] = model_const(t,x,p)
%x1 = mRNA
dxdt(1,1) =p.CN*p.k1-p.d1*x(1);
%x2 = Protein
dxdt(2,1)=p.k2*x(1)-p.d2*x(2);
end
```



Main_const.m

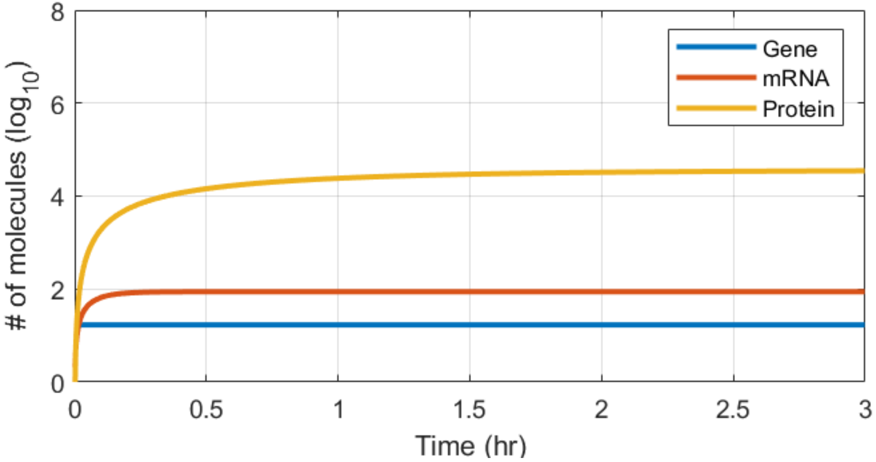
Constitutive gene expression - Simulation

Parameters definition

```
%Parameters
p.CN = 17;         % plasmid number  pACYC184 (17 copies/cell)
p.d1 = log(2)/3;   % mRNA degradation rate  [1/min]
p.d2 = 0.02;       % degradation rate [1/min]
p.k2 = 8.23;       % translation rate  [1/min]
p.k1 = 1.19;       % transcription rate [1/min]
```

Simulation configuration and execution

```
tfin = 60*3;    %simulation final time
step = 0.1;     %simulation step
tspan = 0:step:tfin-step;
% options for ode function
opti = odeset('AbsTol',1e-8,'RelTol',1e-6);
Init = [0 0];  %initial conditions
```



```
[t0,x0] = ode23t(@(t,x) model_const(t,x,p),tspan, Init, opti);
```

https://blogs.mathworks.com/cleve/2014/06/09/ordinary-differential-equations-stiffness/

Constitutive gene expression - Simulation

Parameters definition

```
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Simulation configuration and execution

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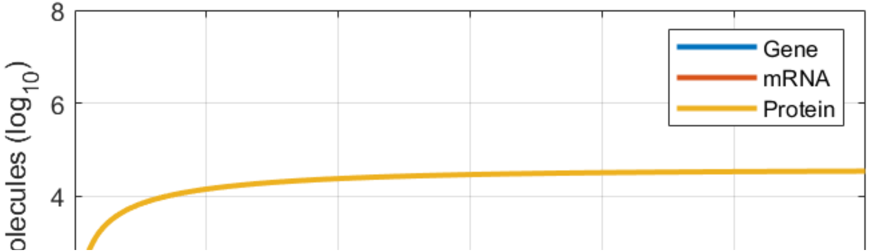
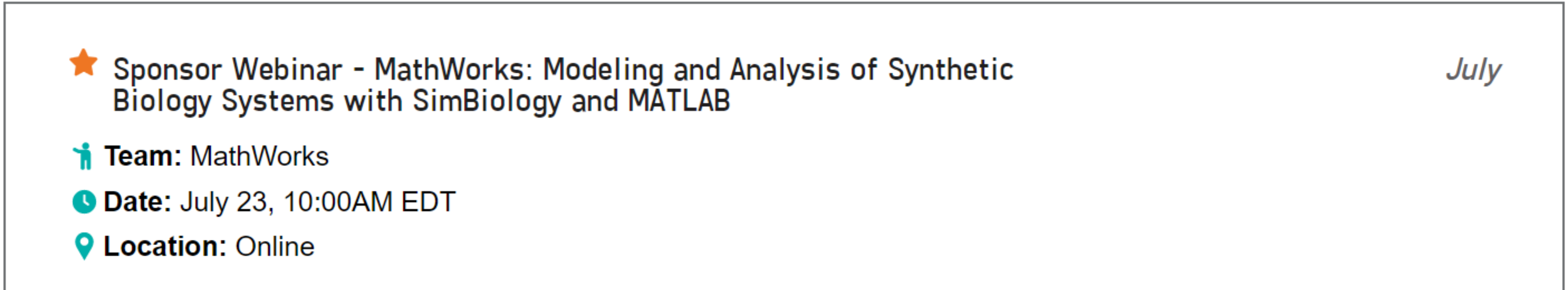



★ Sponsor Webinar - MathWorks: Modeling and Analysis of Synthetic Biology Systems with SimBiology and MATLAB

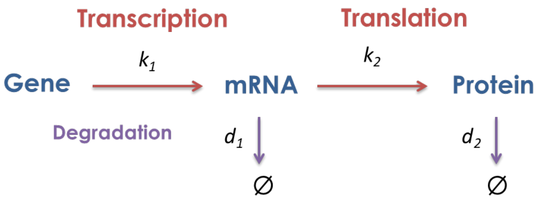
July

Team: MathWorks

Date: July 23, 10:00AM EDT

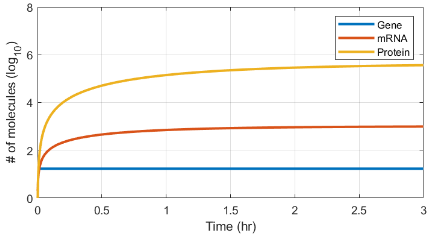
Location: Online

Constitutive gene expression - Remarks



$$\dot{[\mathrm{mRNA}]} = k_1[\mathrm{Gene}] - d_1[\mathrm{mRNA}]$$

$$\dot{[\mathrm{Protein}]} = k_2[\mathrm{mRNA}] - d_2[\mathrm{Protein}]$$



$[\mathrm{Gene}]$ is considered a constant value and depends on: the Origin of Replication and the Plasmid Copy Number where the Gene is cloned.

We are considering:

- RNA polymerase and Ribosomes are sufficient enough so that they are not limiting the kinetics.
- Binding/Unbinding processes are much faster than transcription and translation.
- Protein degradation includes growth asociated dilution.

Questions?
Ask writing in the chat or contact me by email (alvig2 [at] upv [dot] es)

Stay tuned, next Section 2:

Derivation of a Hill function from

the law of mass action (15 min)