UNIVERSITAT POLITÈCNICA DE VALÈNCIA

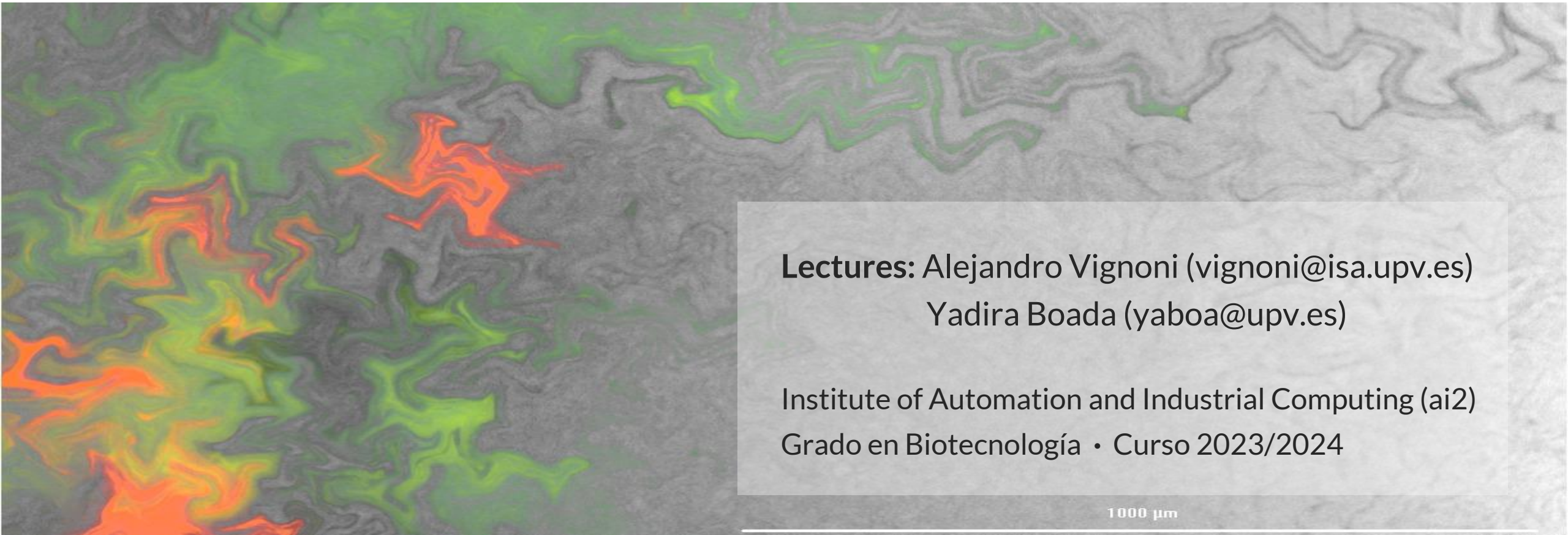
ai2 INSTITUTO DE AUTOMÁTICA E INFORMÁTICA INDUSTRIAL

sb2cl Lab

Synthetic Biology and Biosystems Control Lab

Synthetic Biology

Modeling Genetic Circuits



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Institute of Automation and Industrial Computing (ai2)

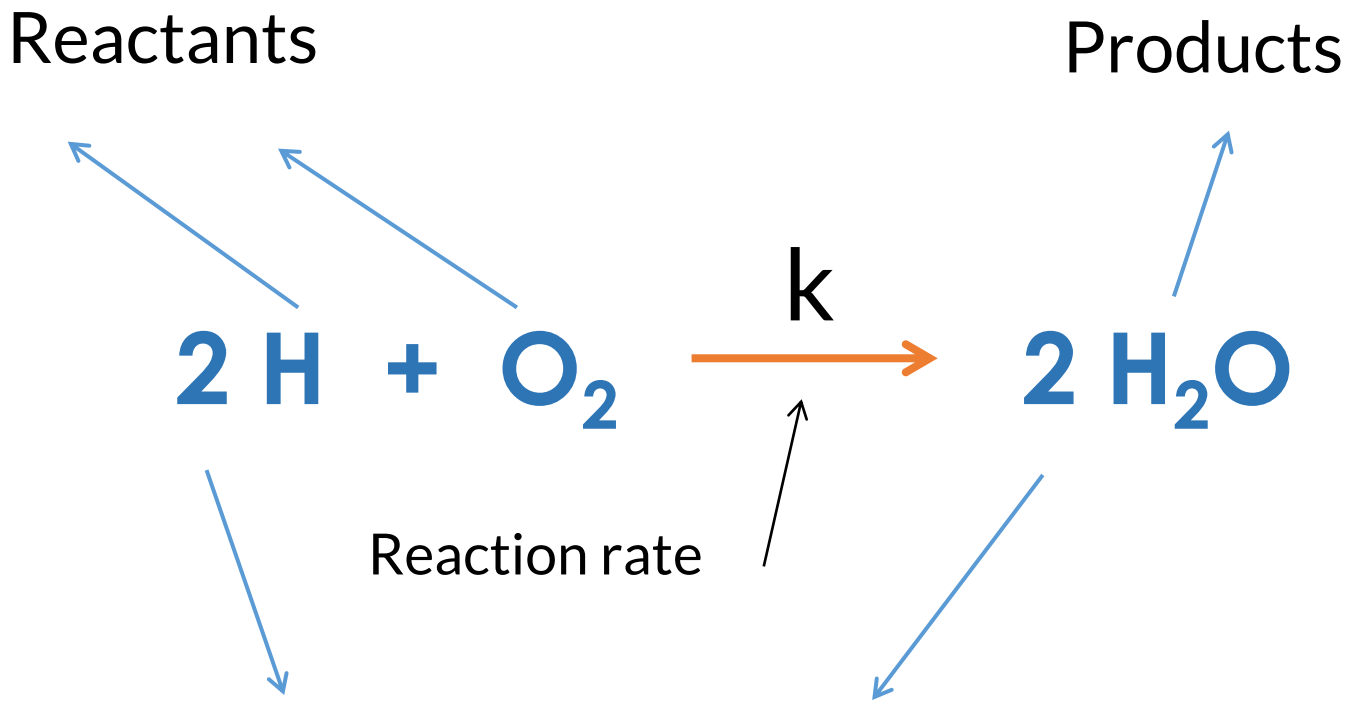
Grado en Biotecnología • Curso 2023/2024

Content

1. Law of Mass Action
2. The central dogma of molecular biology
3. Examples of dynamic models in biology

1. Reminder: Law of Mass Action and kinetic equations

Example: Reaction of Water



I. Reaction of Water – Kinetics of H_2

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

Rate of change for $[H]$ over time:

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

decrease

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

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

II. Reaction of Water – Kinetics of O_2

Rate of change for $[O]$ over time:

decrease

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

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

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

III. Reaction of Water – Kinetics of H_2O

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

Rate of change of $[H_2O]$ over time:

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

increase

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

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

Summing up

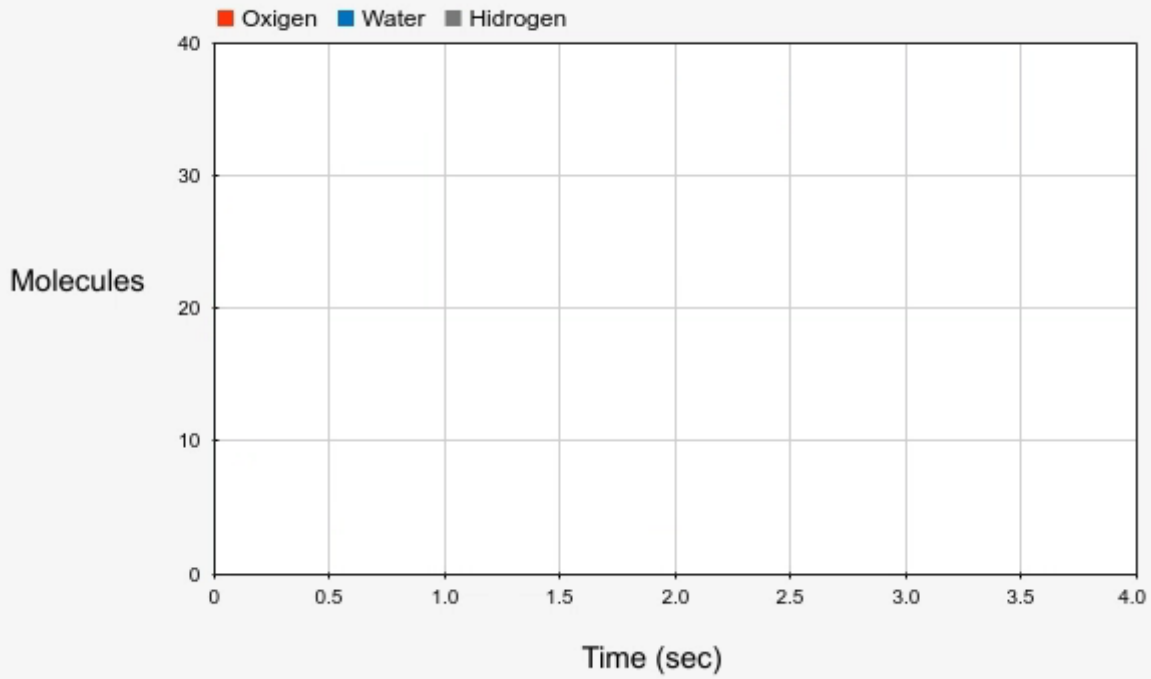
1. Biochemical reactions

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

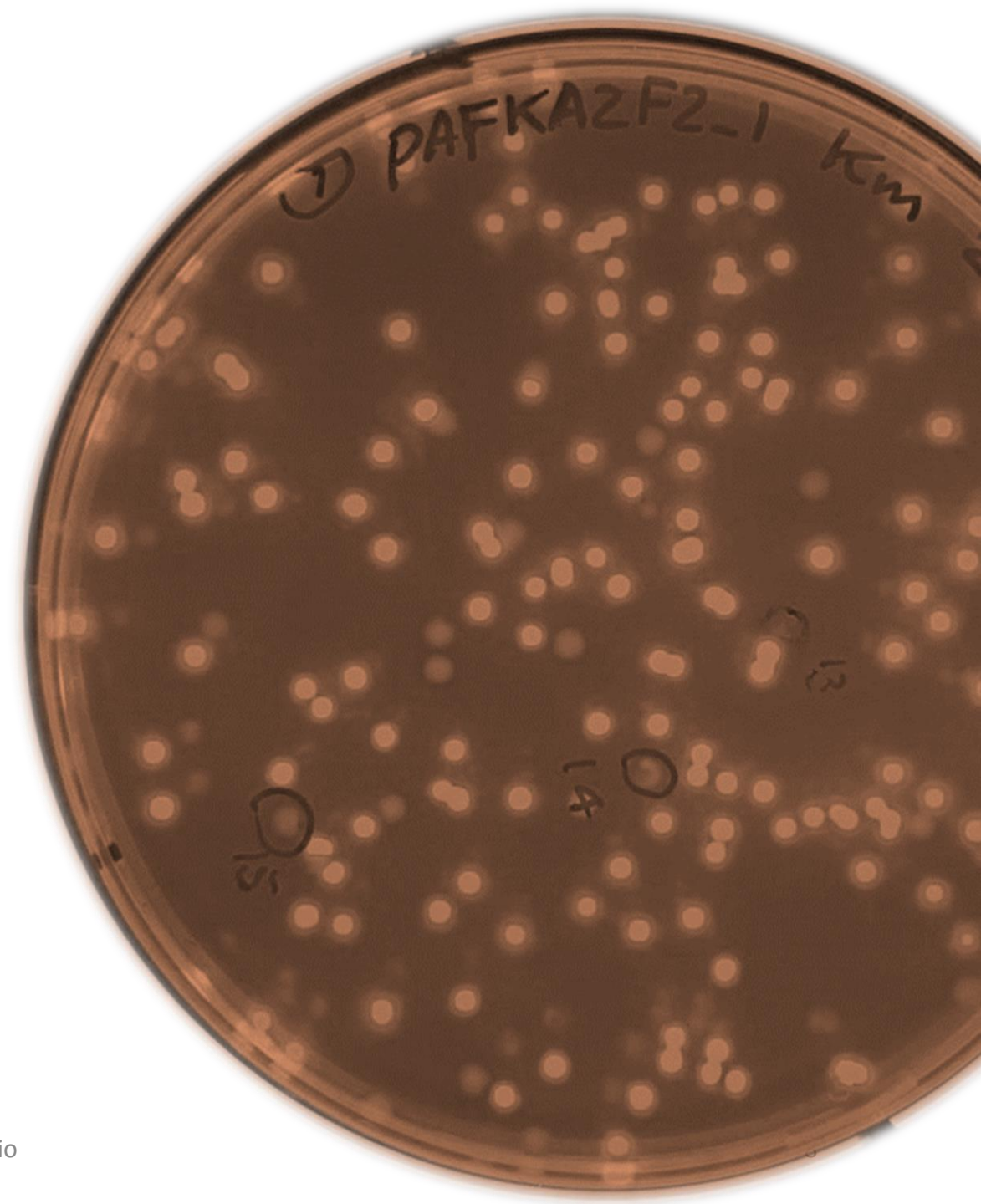
2. Kinetic model (ODEs)

$$\begin{cases} [\dot{H}_2] = -2k[H_2]^2[O_2] \\ [\dot{O}_2] = -\,k[H_2]^2[O_2] \\ [\dot{H_2O}] = 2k[H_2]^2[O_2] \end{cases}$$

3. Temporal dynamic simulation

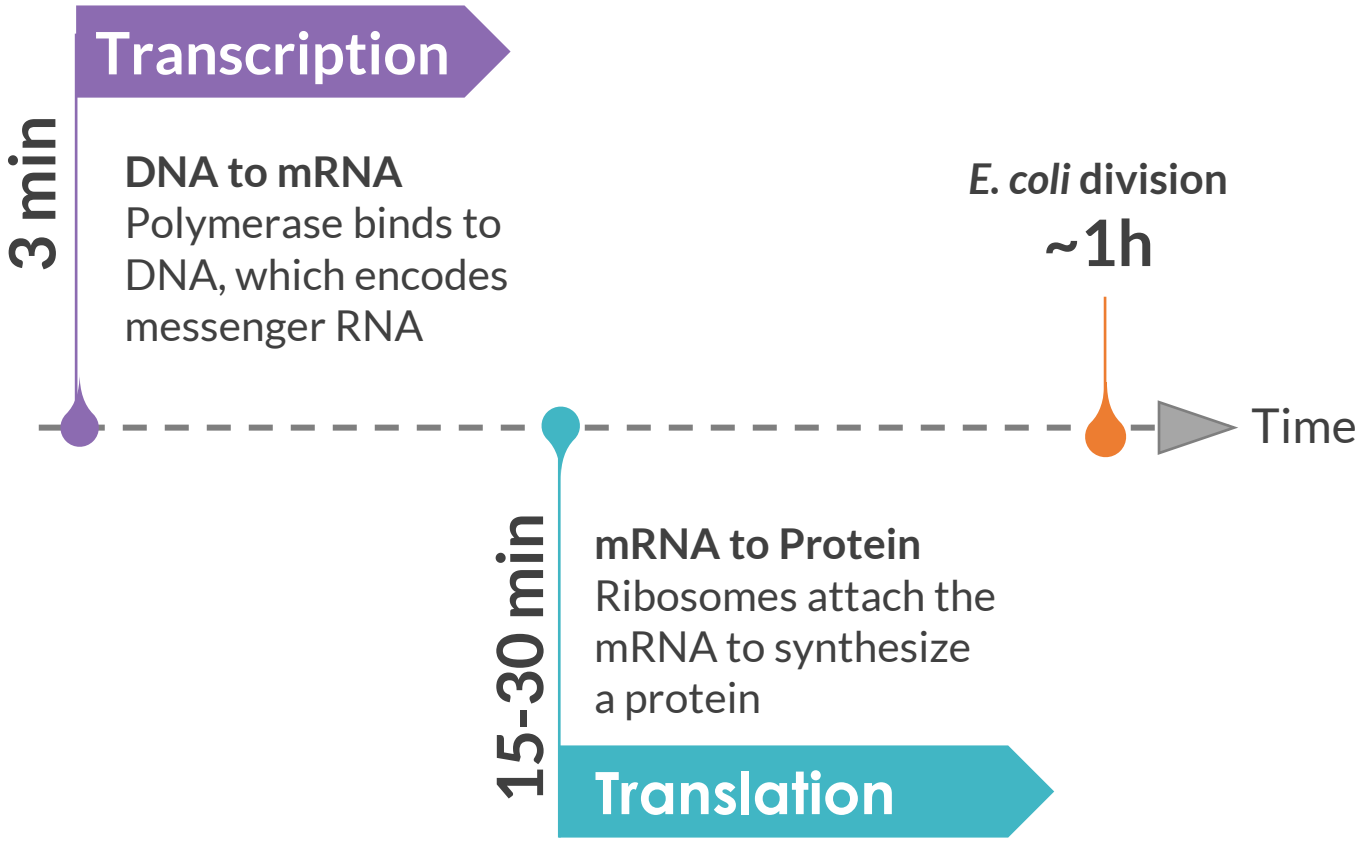


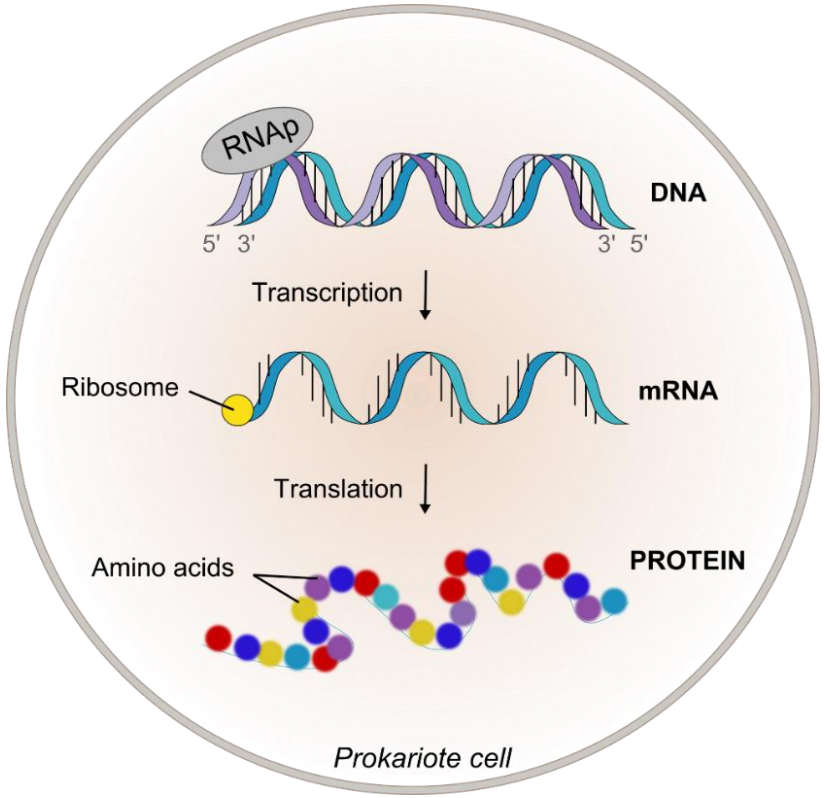
The central dogma of molecular biology



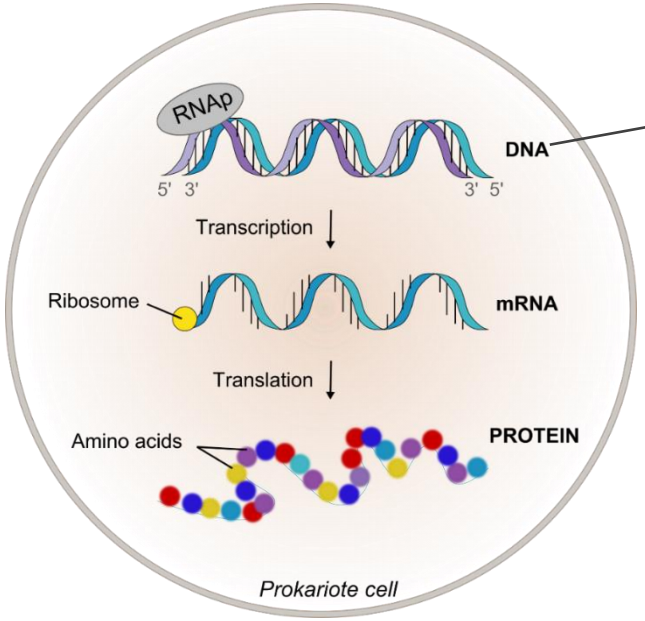
The central dogma of molecular biology

To produce a protein:

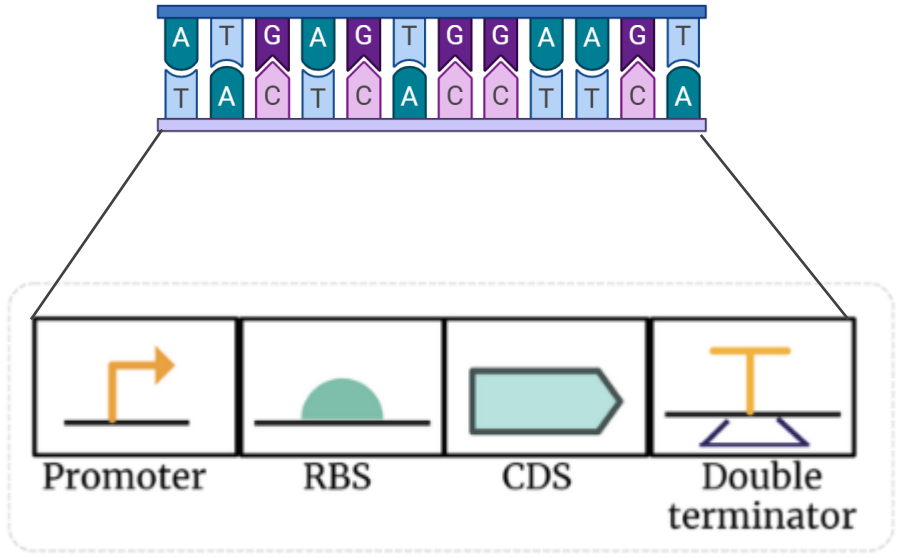




The transcriptional unit



DNA sequence



A **transcriptional unit** encodes one protein*

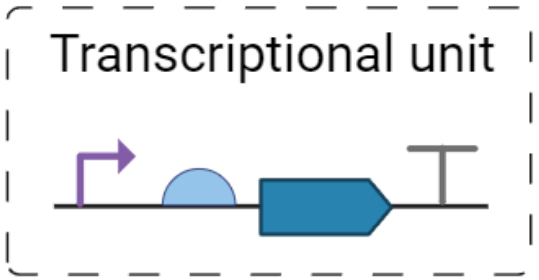
*SBOL diagram (Synthetic Biology Open Language) https://sbolstandard.org

2 ways of produce a protein (gene expression)

A transcriptional unit produces a protein when several biochemical reactions occur:

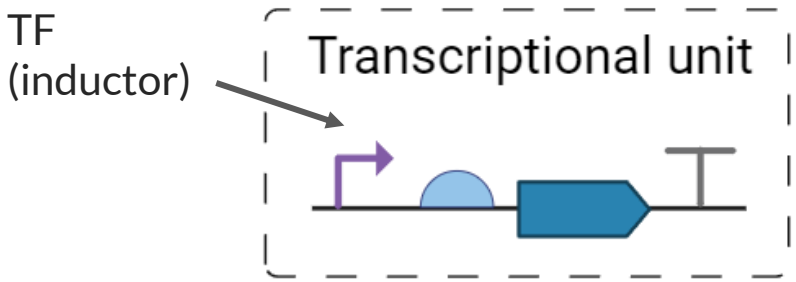
1. Constitutive gene expression

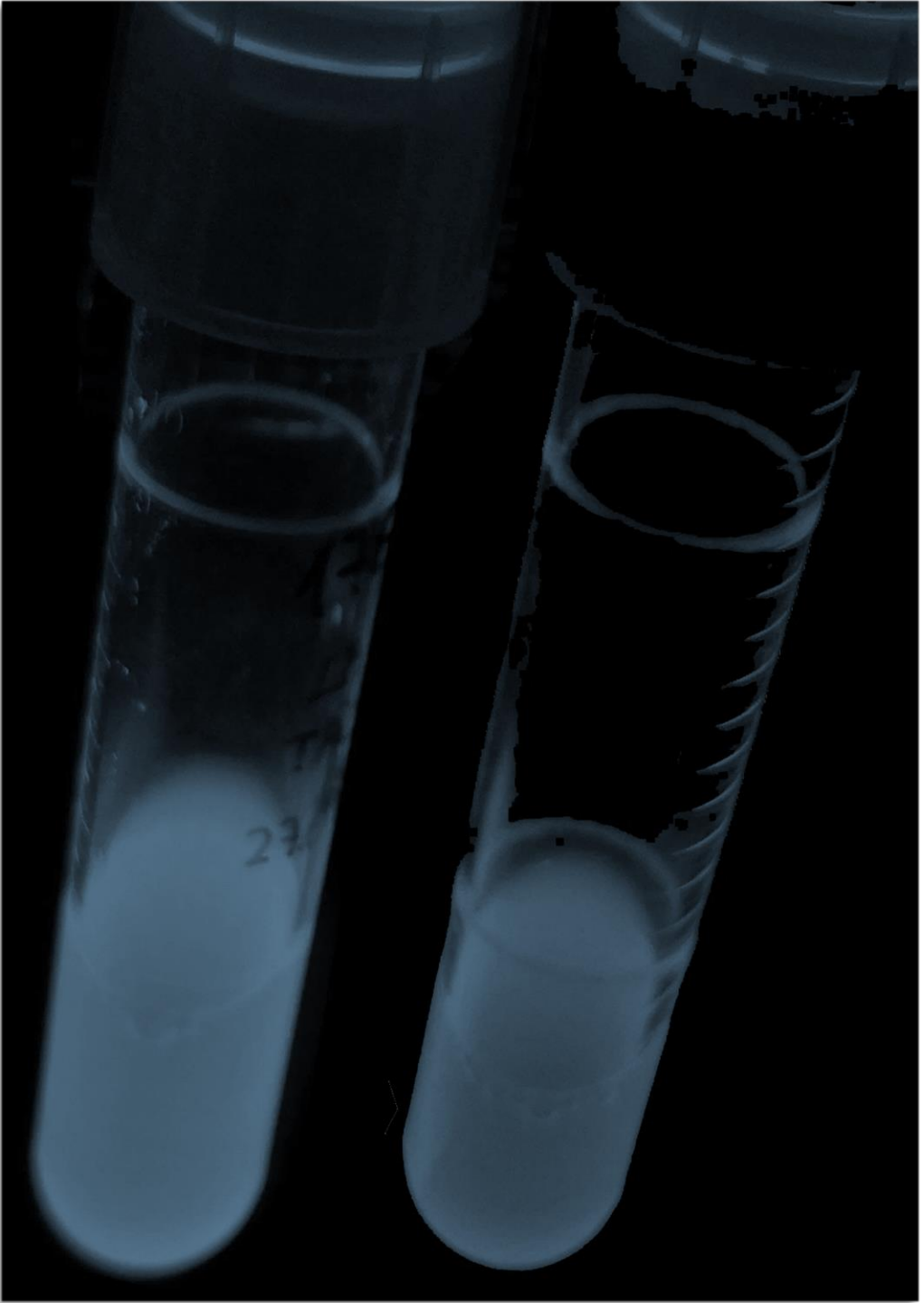
- No regulation of protein production
- No external inductors



2. Regulated gene expression

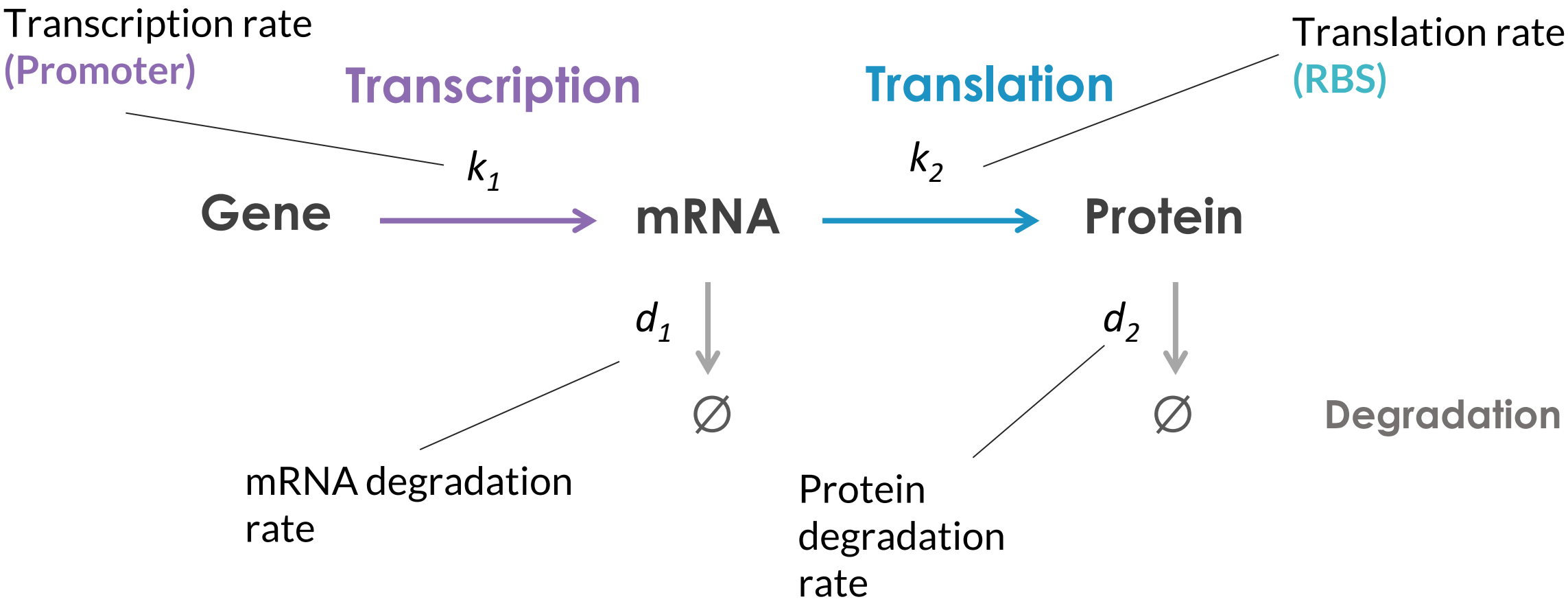
- Activation or repression of production
- Other inductors needed
- The inductor is called transcription factor (TF)



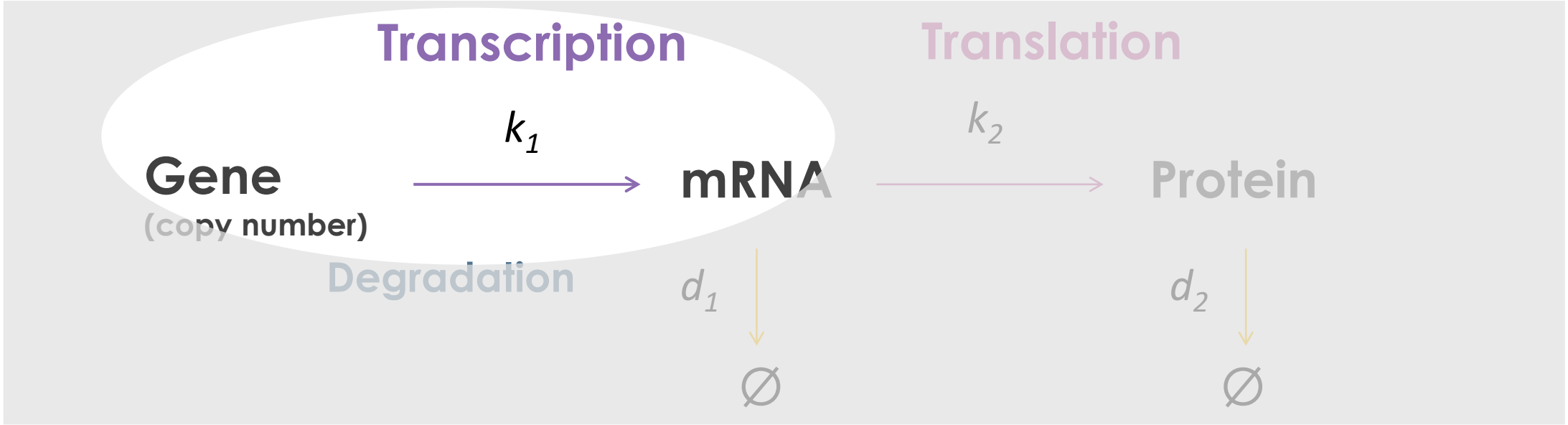


Modelling constitutive gene expression (simplified version)

Constitutive gene expression (Simplified version)

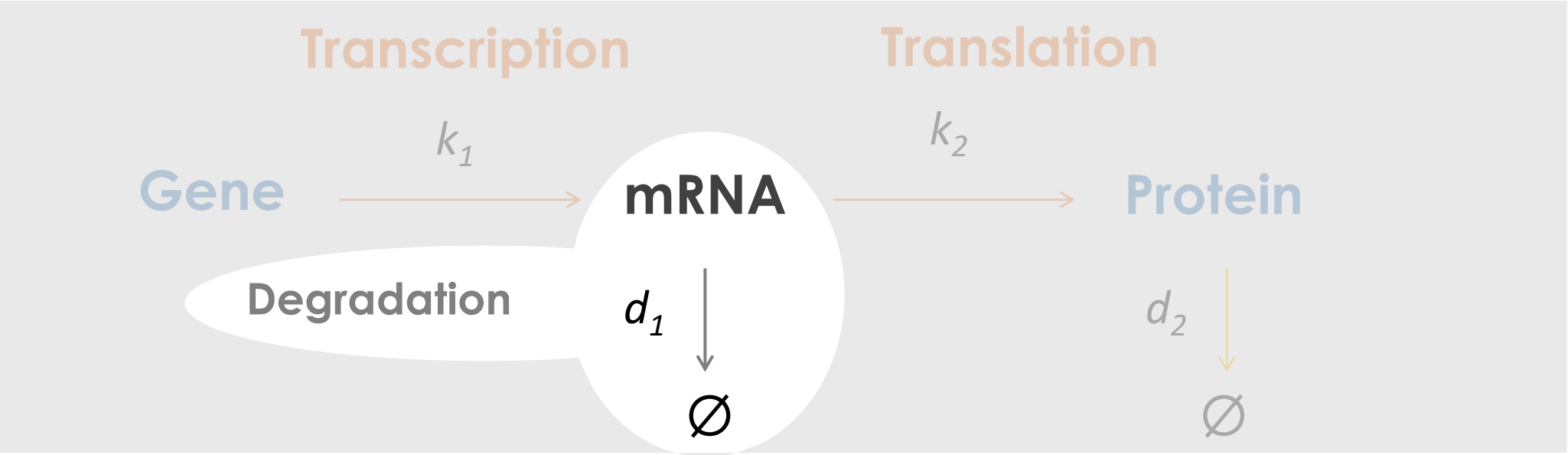


Constitutive gene expression (Simplified version)



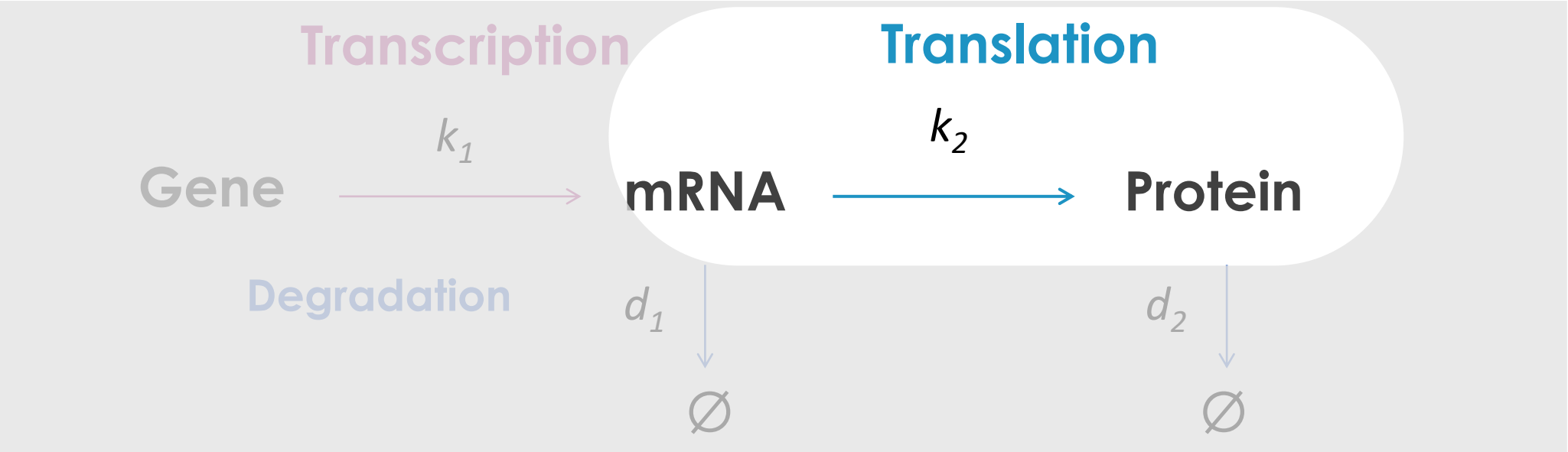
$$[\dot{\mathrm{mRNA}}] = k_1[\mathrm{Gene}]$$

Constitutive gene expression (Simplified version)



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

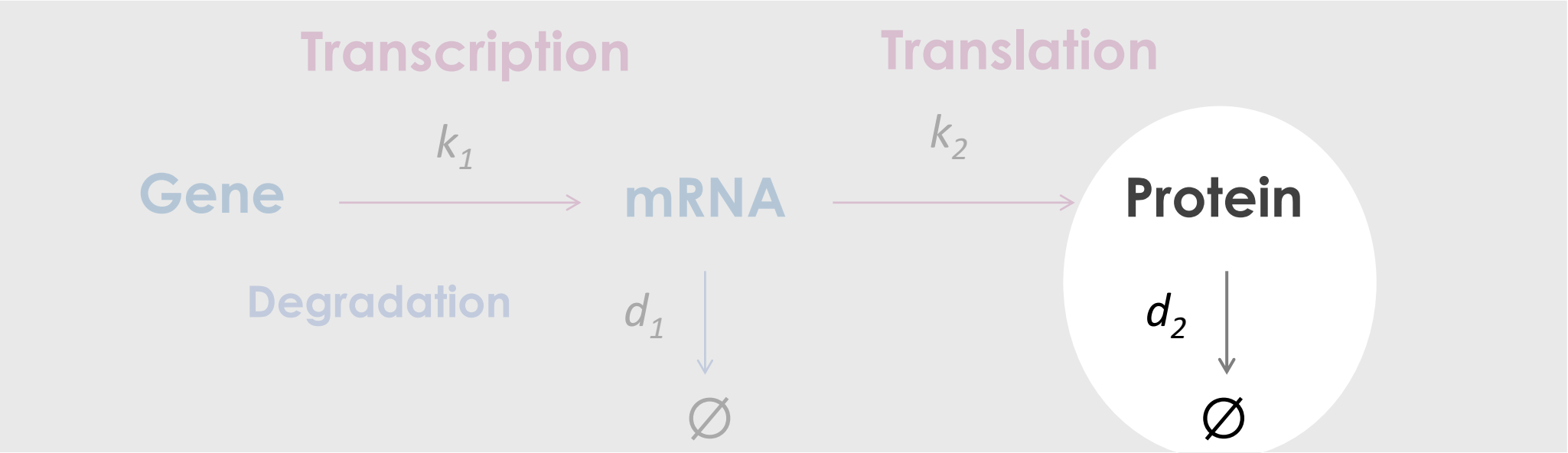
Constitutive gene expression (Simplified version)



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

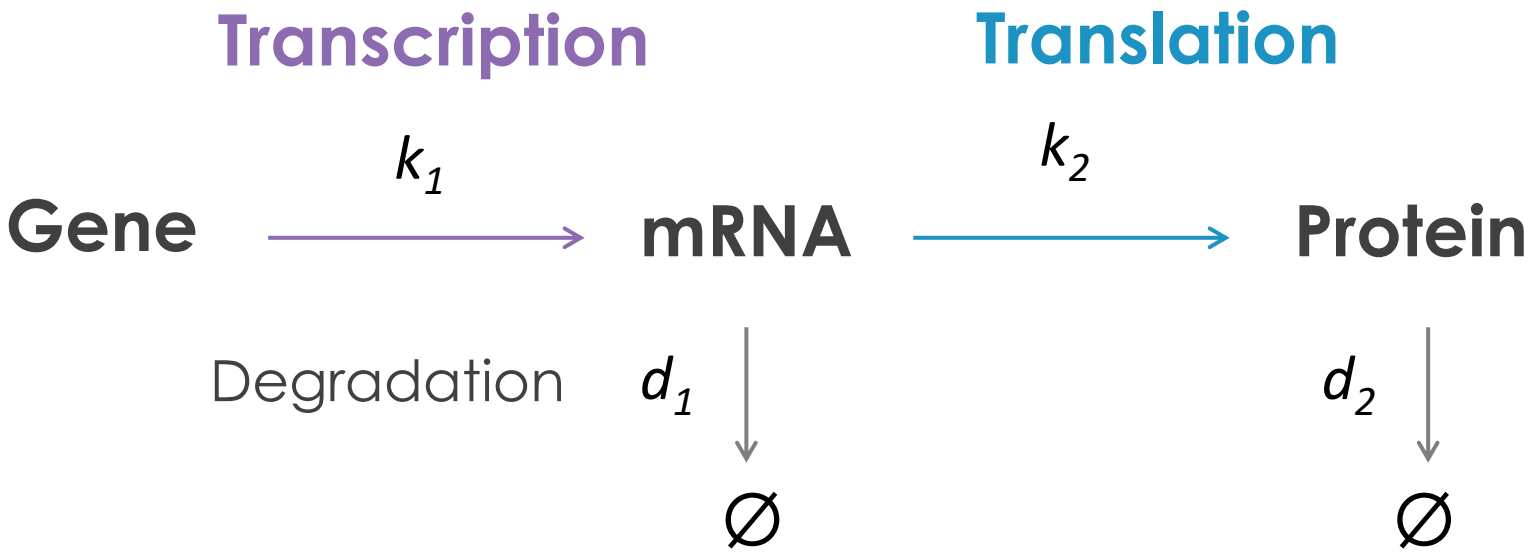
$$[\dot{\text{Protein}}] = k_2[\text{mRNA}]$$

Constitutive gene expression (Simplified version)



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

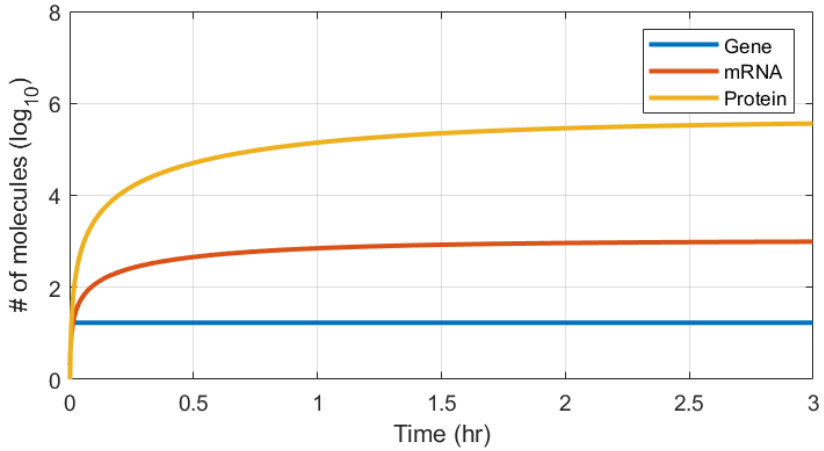
$$[\dot{\text{Protein}}] = k_2[\text{mRNA}] - d_2[\text{Protein}]$$



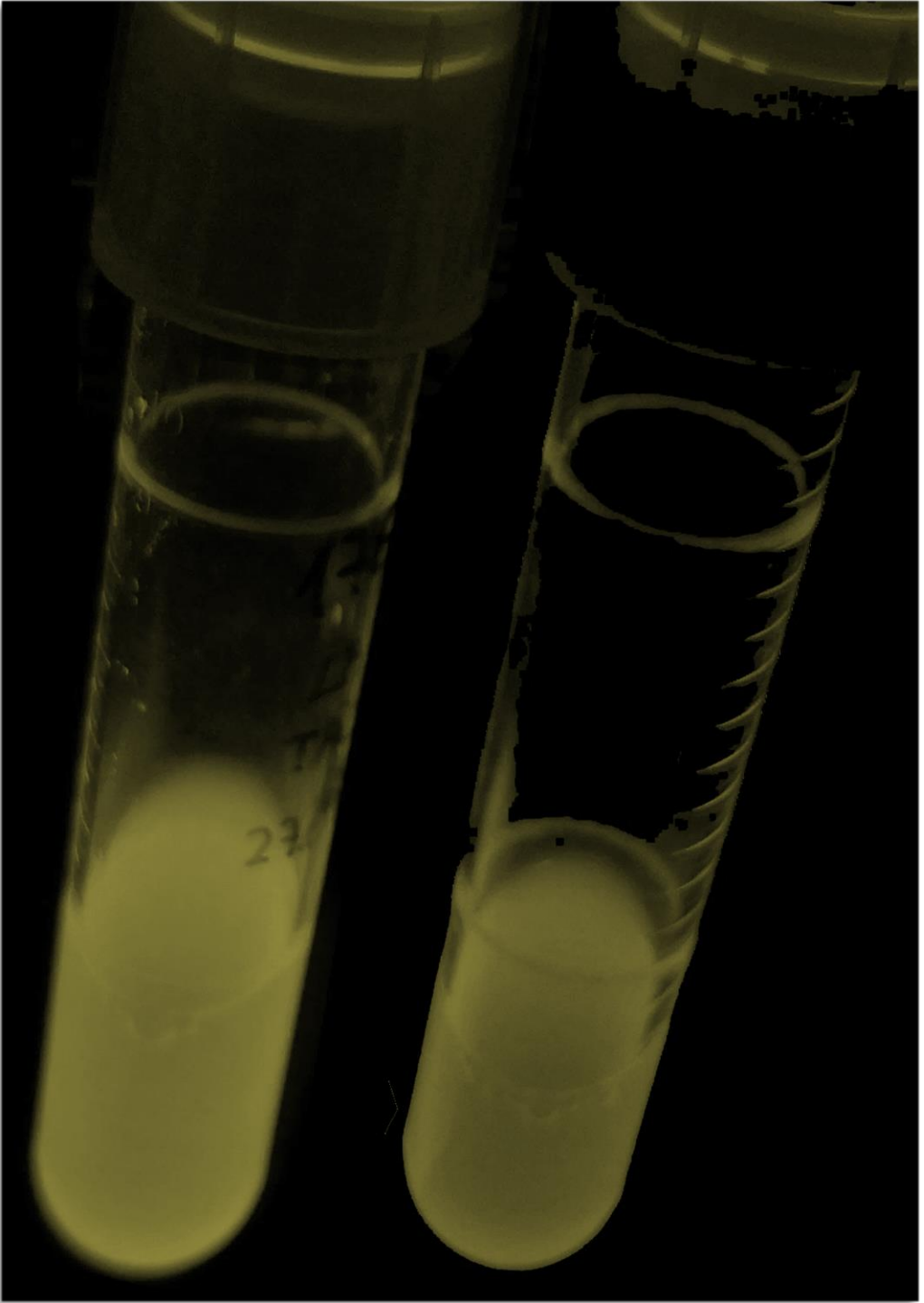
$$\begin{cases} [\dot{\text{mRNA}}] = k_1[\text{Gene}] - d_1[\text{mRNA}] \\ [\dot{\text{Protein}}] = k_2[\text{mRNA}] - d_2[\text{Protein}] \end{cases}$$

Remarks

$$\begin{cases} \dot{[\text{mRNA}]} = k_1[\text{Gene}] - d_1[\text{mRNA}] \\ \dot{[\text{Protein}]} = k_2[\text{mRNA}] - d_2[\text{Protein}] \end{cases}$$

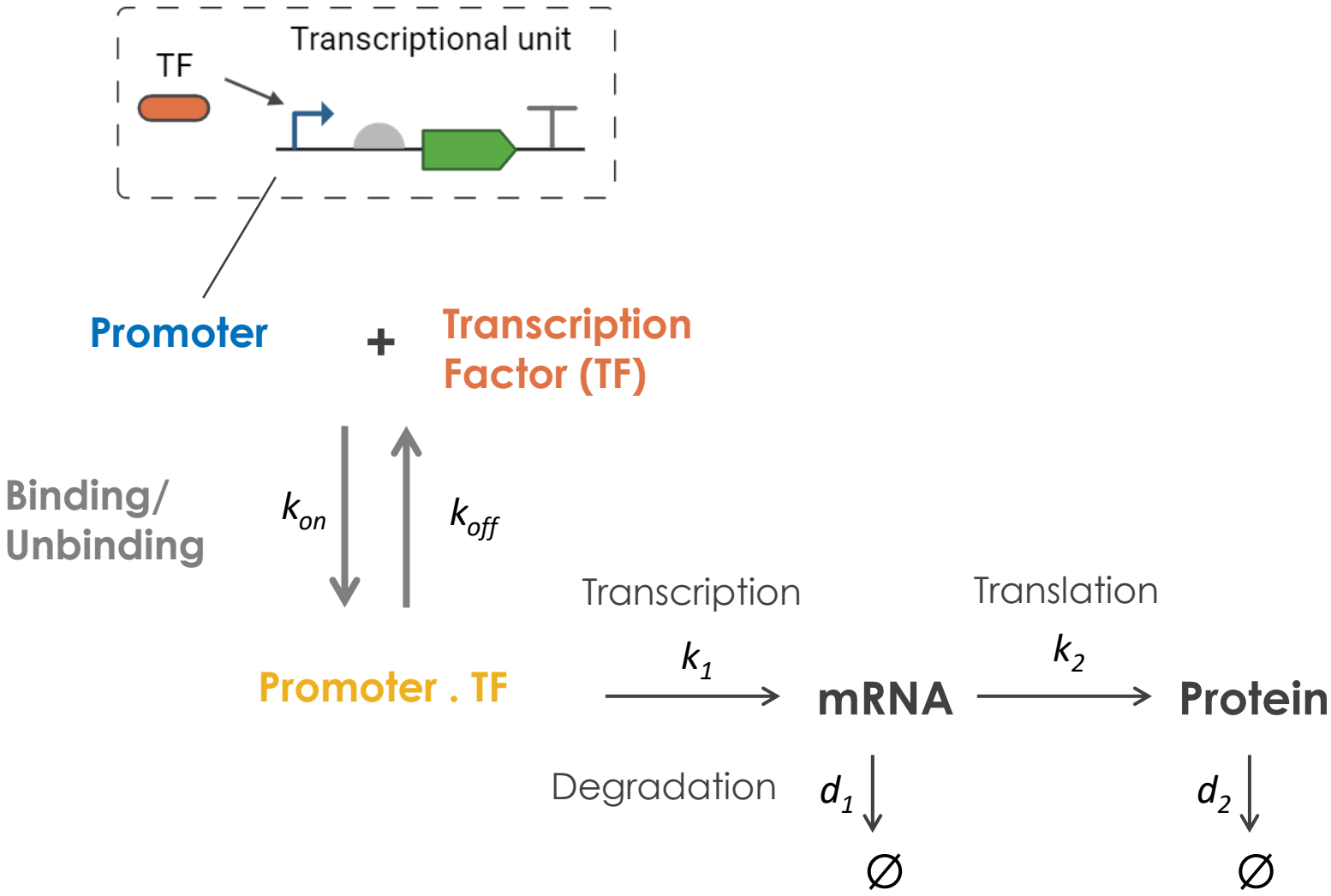


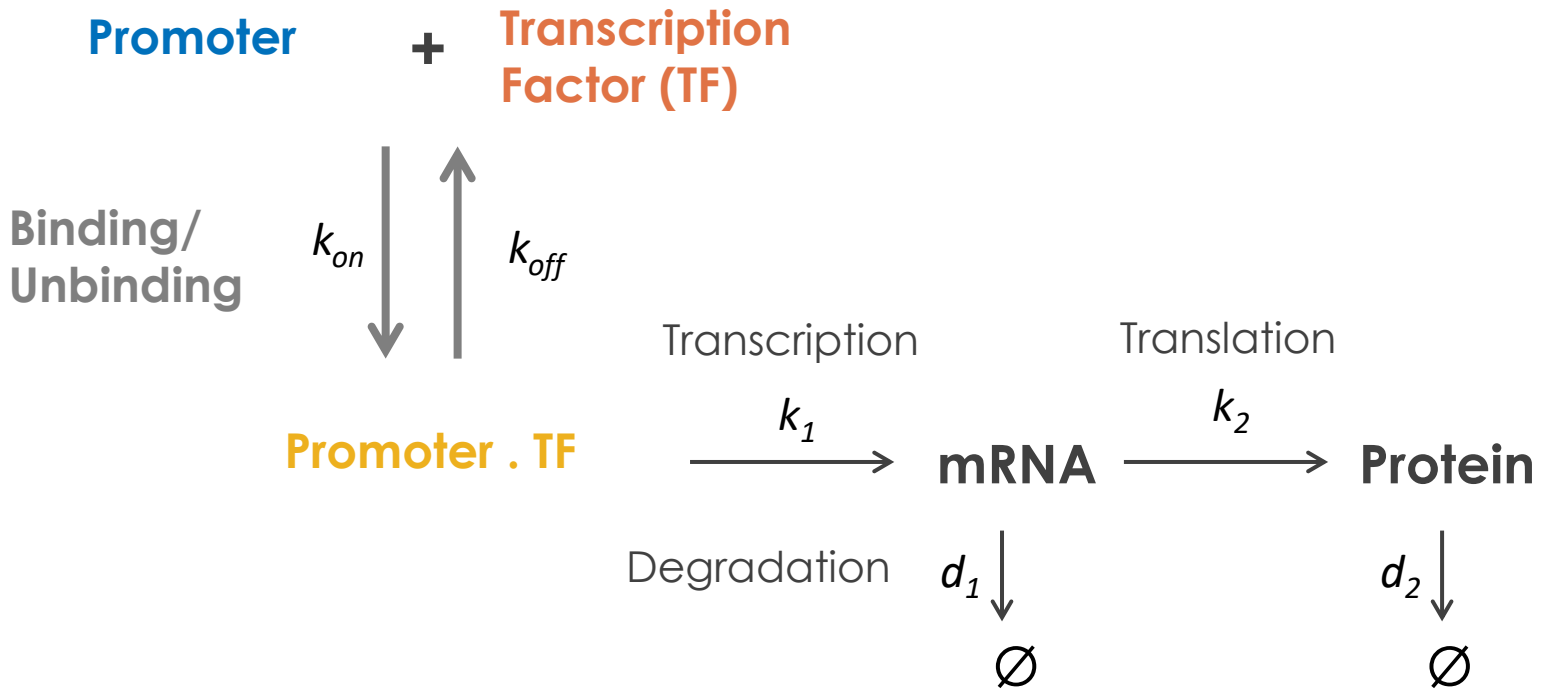
- [Gene] is considered a constant value and depends on:
  - the Origin of Replication and
  - the **Plasmid Copy Number (C_N) where the Gene is cloned.**
- We are considering:
  a. RNA polymerase and Ribosomes are in **sufficiently enough** amount so that they are not limiting the kinetics.
  b. Binding/Unbinding processes are much faster than transcription and translation.



Modelling gene expression regulated by Transcription Factors (TF)

Gene expression regulated by Transcription Factors (TF)





We will obtain a model: 5 Equations with 7 parameters

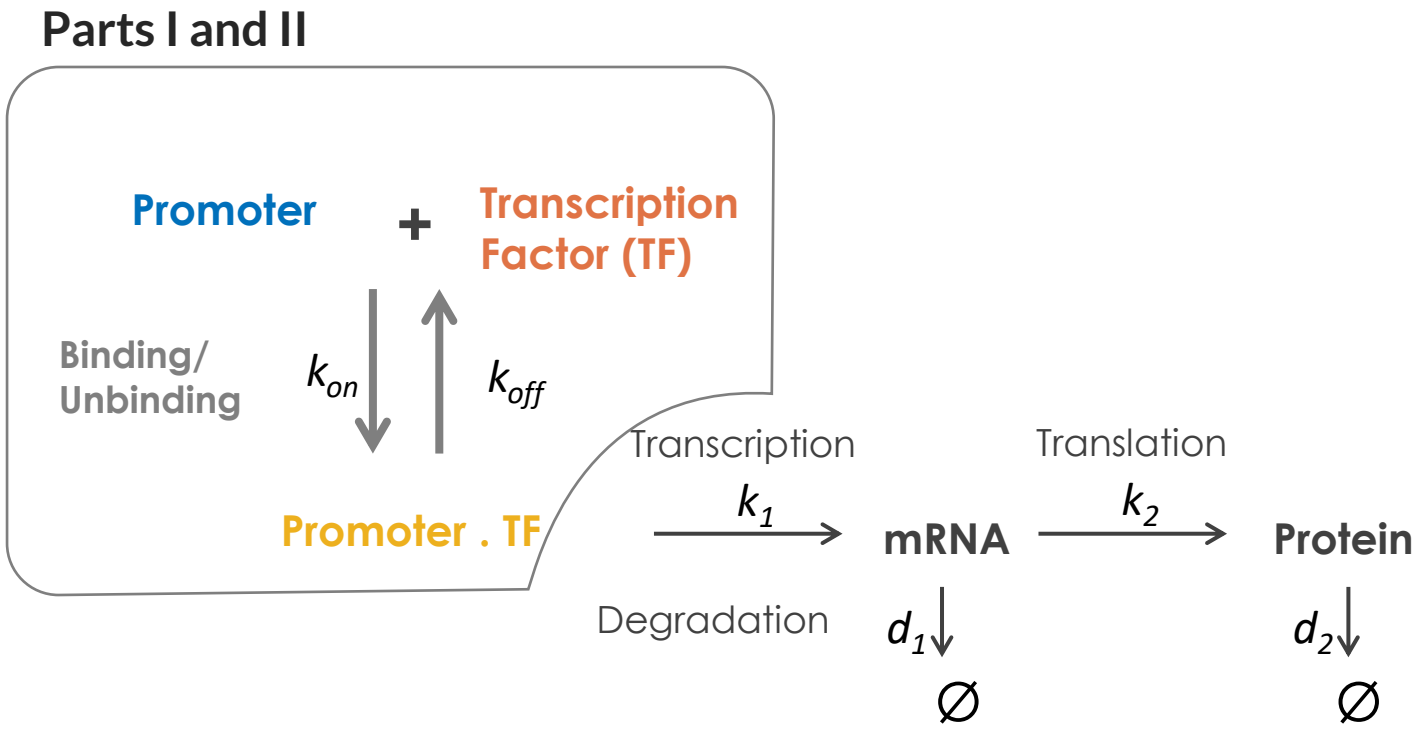
Problems

1. k_1, k_2, d_1 and d_2 become indistinguishable when we measure only the protein amount.
2. k_{on}, k_{off}, are very difficult to measure.

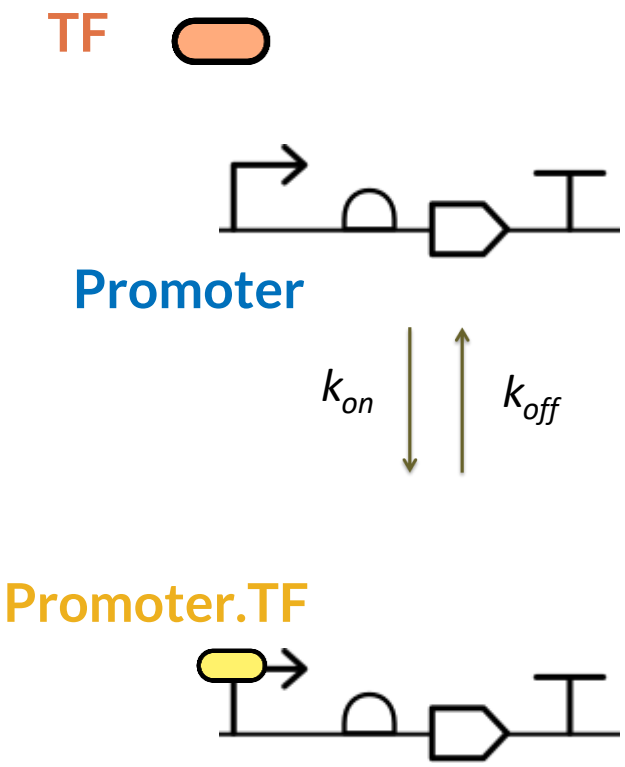
Simplifying the regulation of gene expression

To obtain a model to relate with experimental data:

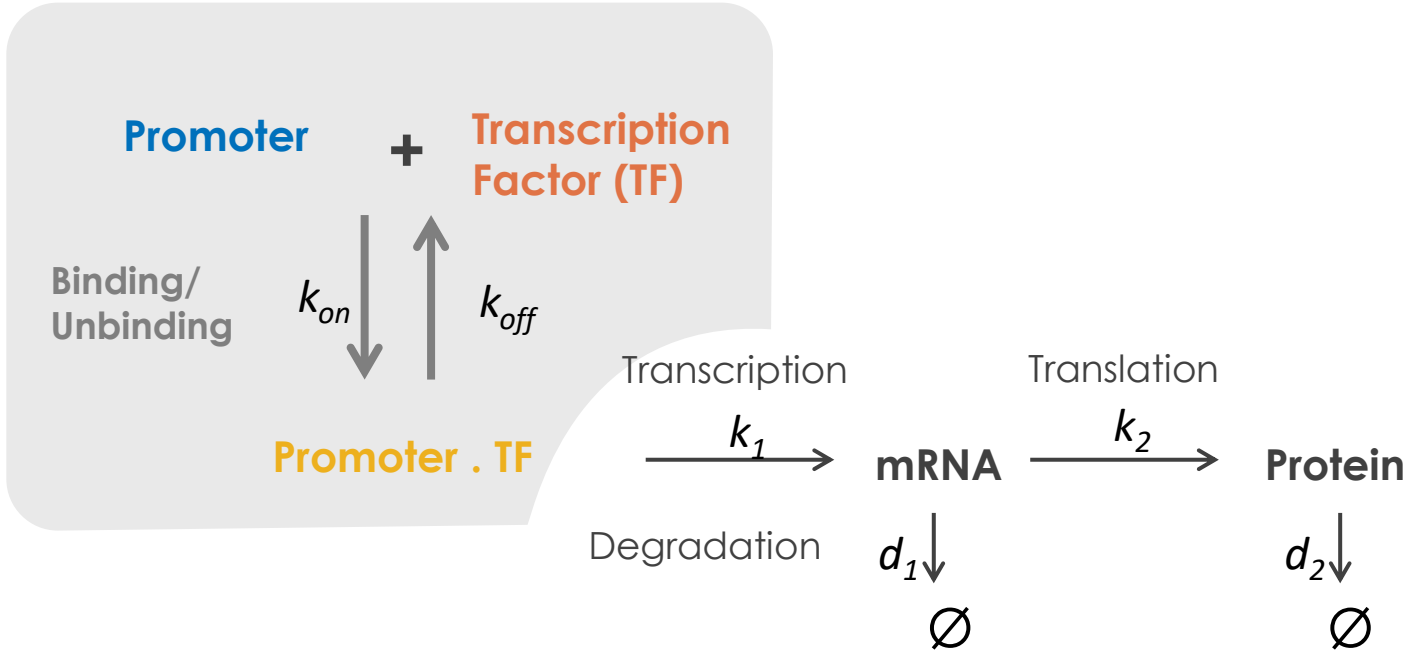
Part I. Getting the model with TF
Part II. Model reduction
Part III. Modelling gene expression + Hill function



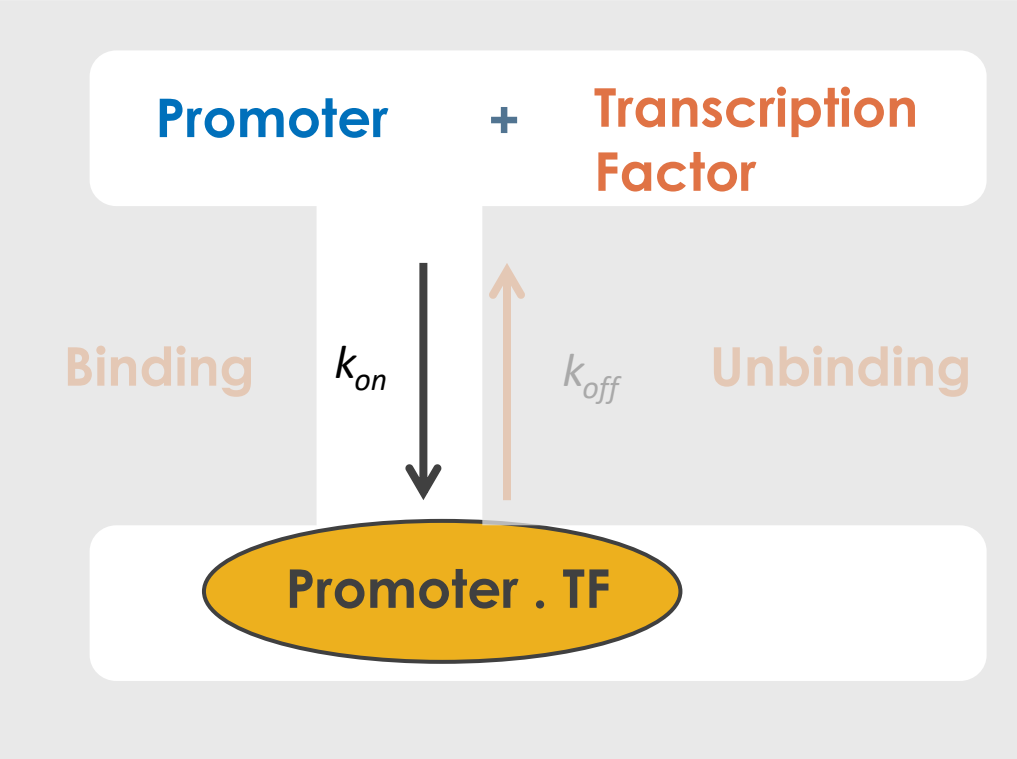
Part I: Getting the model with TF



Schematic diagram SBOL

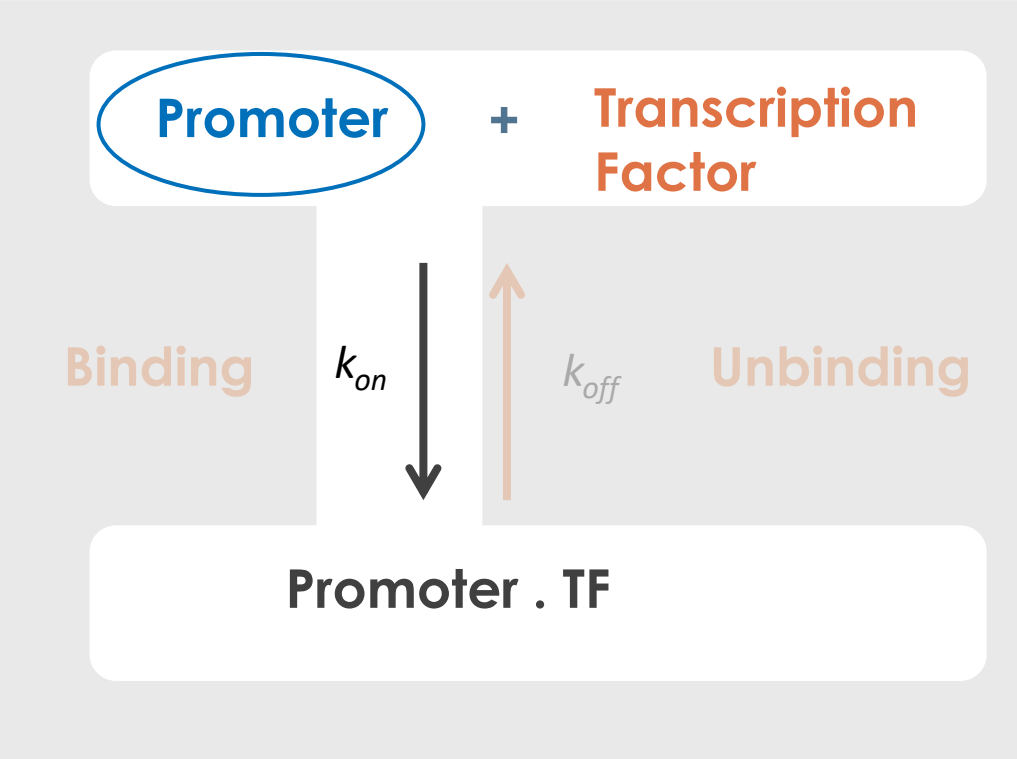


Part I: Getting the model with TF



$$[\dot{\text{Prom.TF}}] = k_{on}\,[\text{Prom}][\text{TF}]$$

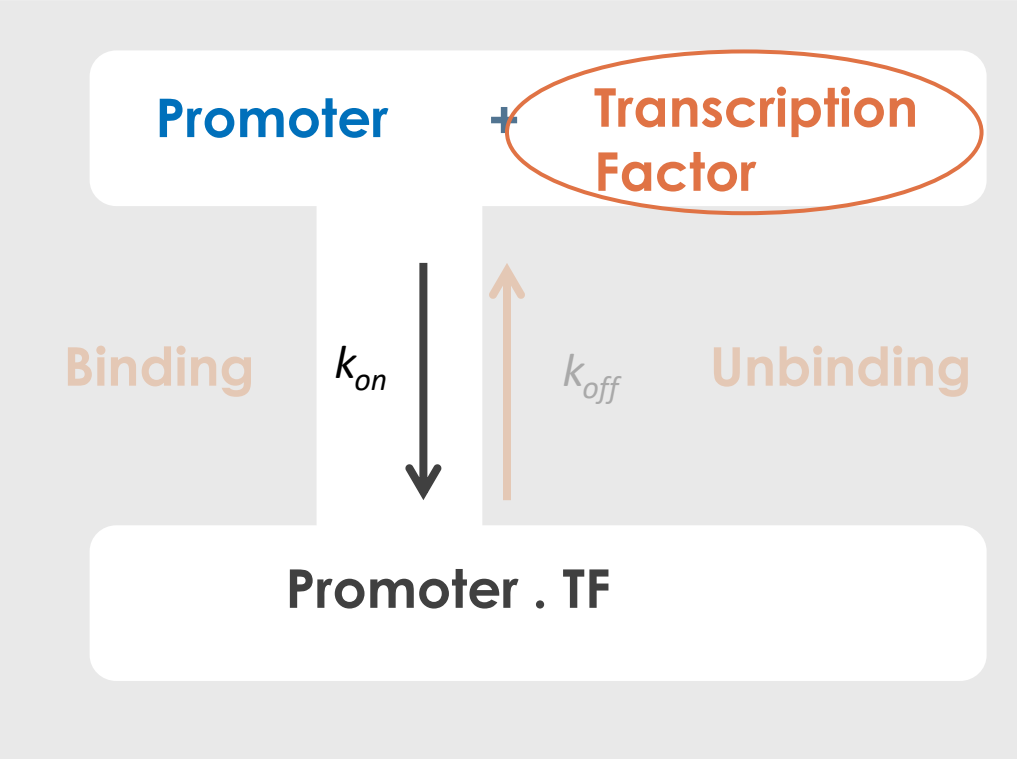
Part I: Getting the model with TF



$$[\dot{\text{Prom.TF}}] = k_{on}\,[\text{Prom}][\text{TF}]$$

$$[\dot{\text{Prom}}] = -k_{on}\,[\text{Prom}][\text{TF}]$$

Part I: Getting the model with TF

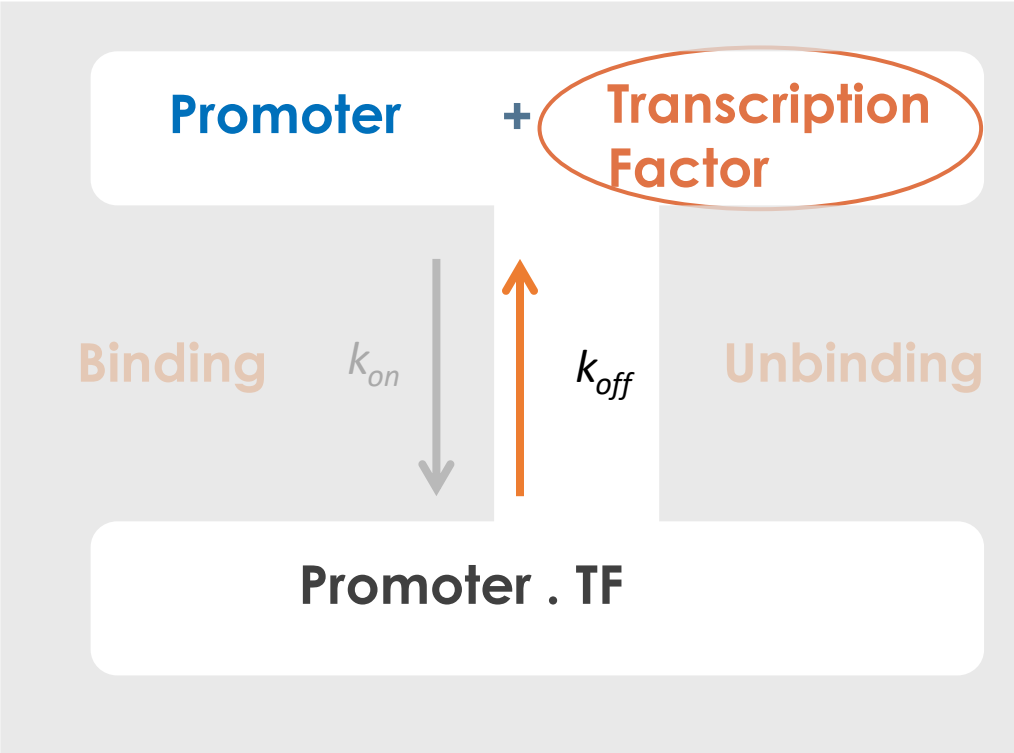


$$[\dot{\text{Prom.TF}}] = k_{on}\,[\text{Prom}][\text{TF}]$$

$$[\dot{\text{Prom}}] = -k_{on}\,[\text{Prom}][\text{TF}]$$

$$[\dot{\text{TF}}] = -k_{on}\,[\text{Prom}][\text{TF}]$$

Part I: Getting the model with TF

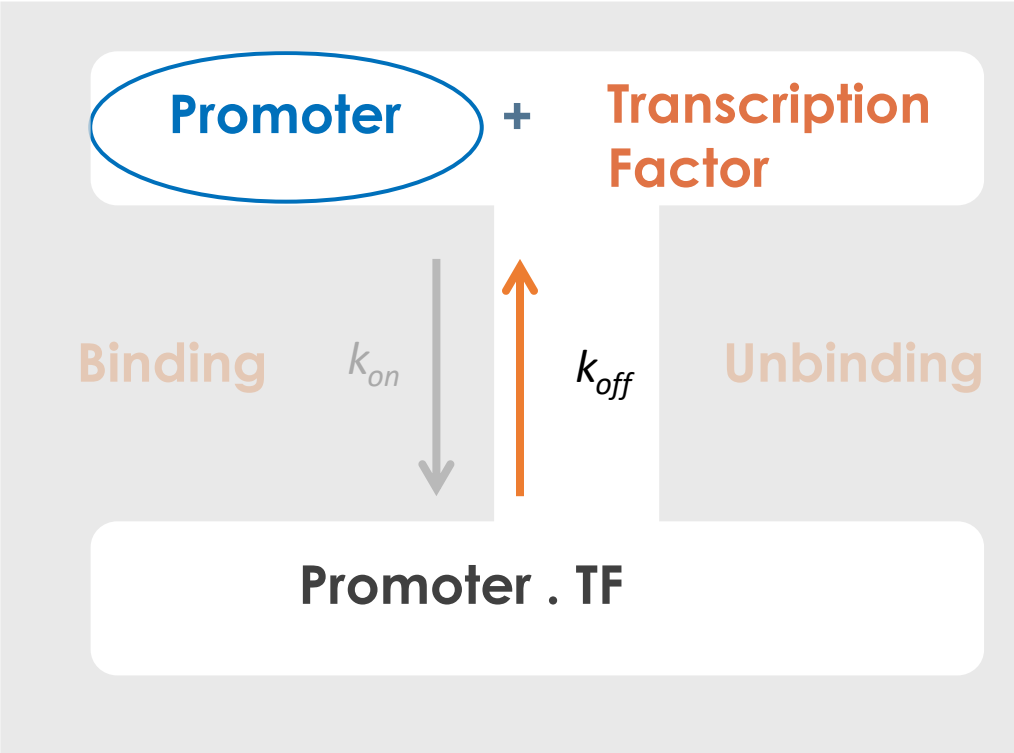


$$[\dot{\text{Prom. TF}}] = k_{on}\,[\text{Prom}][\text{TF}]$$

$$[\dot{\text{Prom}}] = -k_{on}\,[\text{Prom}][\text{TF}]$$

$$[\dot{\text{TF}}] = -k_{on}\,[\text{Prom}][\text{TF}] + k_{off}[\text{Prom. TF}]$$

Part I: Getting the model with TF

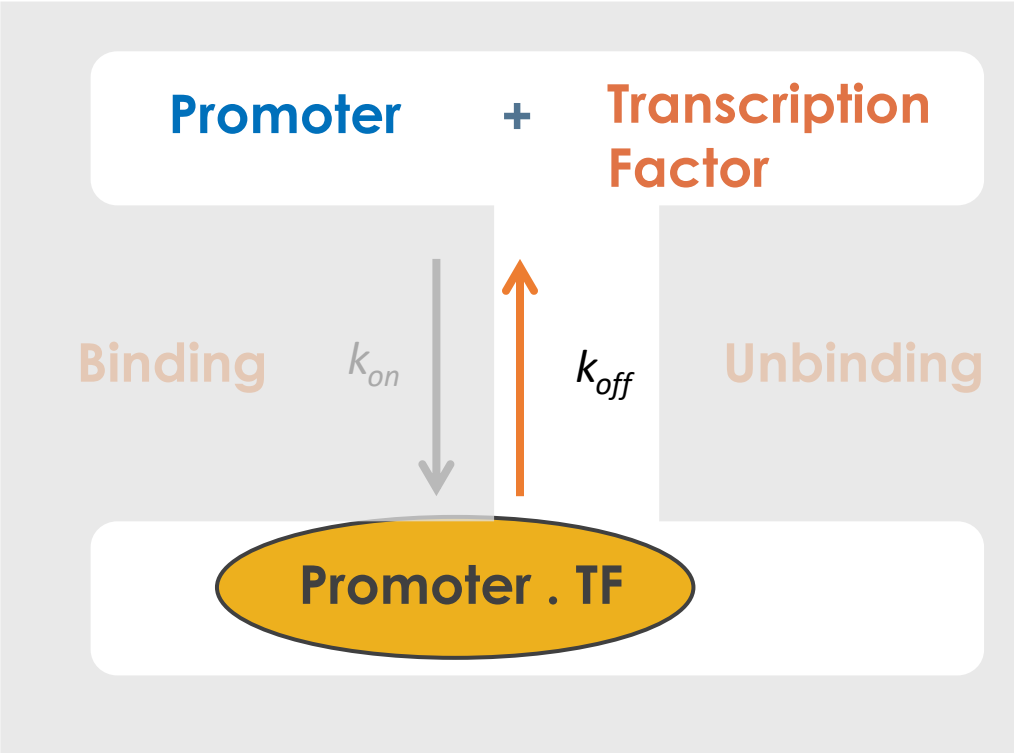


$$[\dot{\text{Prom. TF}}] = k_{on}\,[\text{Prom}][\text{TF}]$$

$$[\dot{\text{Prom}}] = -k_{on}\,[\text{Prom}][\text{TF}] + k_{off}[\text{Prom. TF}]$$

$$[\dot{\text{TF}}] = -k_{on}\,[\text{Prom}][\text{TF}] + k_{off}[\text{Prom. TF}]$$

Part I: Getting the model with TF



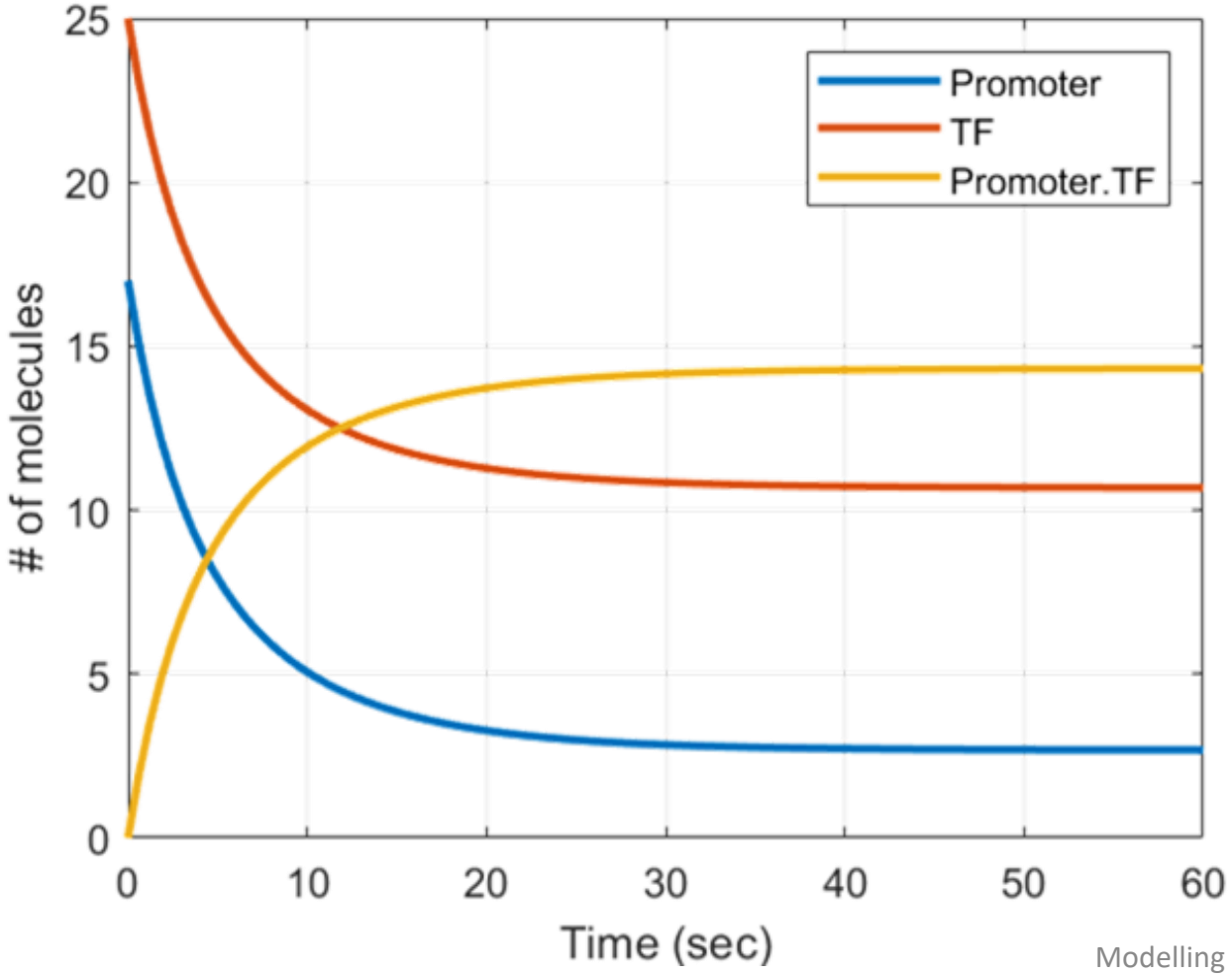
$$[\dot{\text{Prom.}}\,\text{TF}] = k_{on}\,[\text{Prom}][\text{TF}] - k_{off}[\text{Prom.}\,\text{TF}]$$

$$[\dot{\text{Prom}}] = -k_{on}\,[\text{Prom}][\text{TF}] + k_{off}[\text{Prom.}\,\text{TF}]$$

$$[\dot{\text{TF}}] = -k_{on}\,[\text{Prom}][\text{TF}] + k_{off}[\text{Prom.}\,\text{TF}]$$

Part I: Getting the model with TF

Temporal simulations MATLAB



$$\dot{[\text{Prom}]} = -k_{on}\,[\text{Prom}][\text{TF}] + k_{off}[\text{Prom.TF}]$$

$$\dot{[\text{TF}]} = -k_{on}\,[\text{Prom}][\text{TF}] + k_{off}[\text{Prom.TF}]$$

$$\dot{[\text{Prom.TF}]} = \quad k_{on}\,[\text{Prom}][\text{TF}] - k_{off}[\text{Prom.TF}]$$

Starting with:

17 Promoters (Plasmid copy number)

25 molecules of Transcription Factor (TF)

k_{on} = 0.5 molecules^{-1} min^{-1}

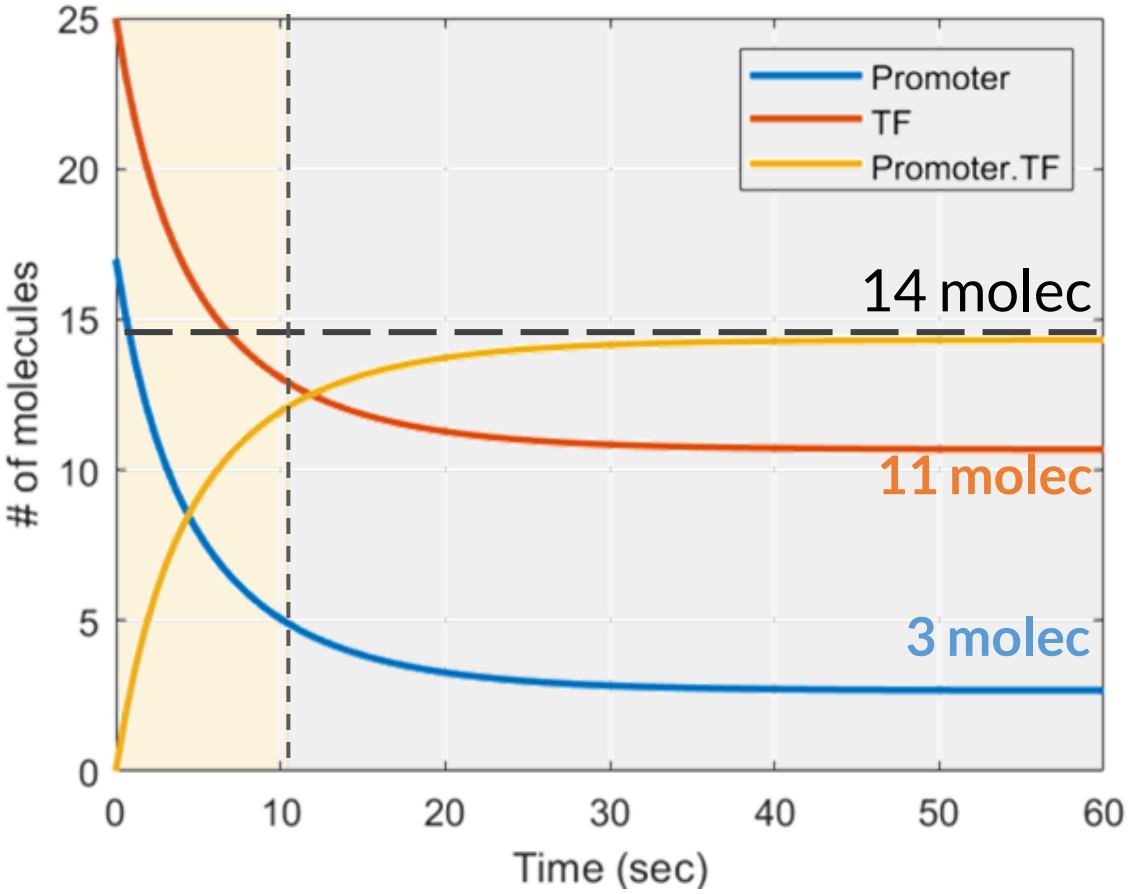
k_{off} = 1 min^{-1}

Genetic_circuit_model_simple.mlx

Simulation_Gene_Expression_pyLab.ipynb

Part I: Getting the model with TF

Temporal simulations MATLAB



$$\begin{aligned}
[\dot{\text{Prom}}] &= -k_{on}\,[\text{Prom}][\text{TF}] + k_{off}[\text{Prom.TF}] \\
[\dot{\text{TF}}] &= -k_{on}\,[\text{Prom}][\text{TF}] + k_{off}[\text{Prom.TF}] \\
[\dot{\text{Prom.TF}}] &= \;\; k_{on}\,[\text{Prom}][\text{TF}] - k_{off}[\text{Prom.TF}]
\end{aligned}$$

Remarks

- First two equations are equal (**Blue** and **Orange**)!
- The sum of the first ODE and the third one is identically zero (**Blue** and **Yellow**)!
- **Use this fact (promoter invariance) to simplify the equations and reduce the model.**

Part II: Model reduction

Promoter invariance (Plasmid Copy Number) because DNA is a constant

$$\dot{[\text{Prom}]} = -k_{on}\,[\text{Prom}][\text{TF}] + k_{off}[\text{Prom.TF}]$$

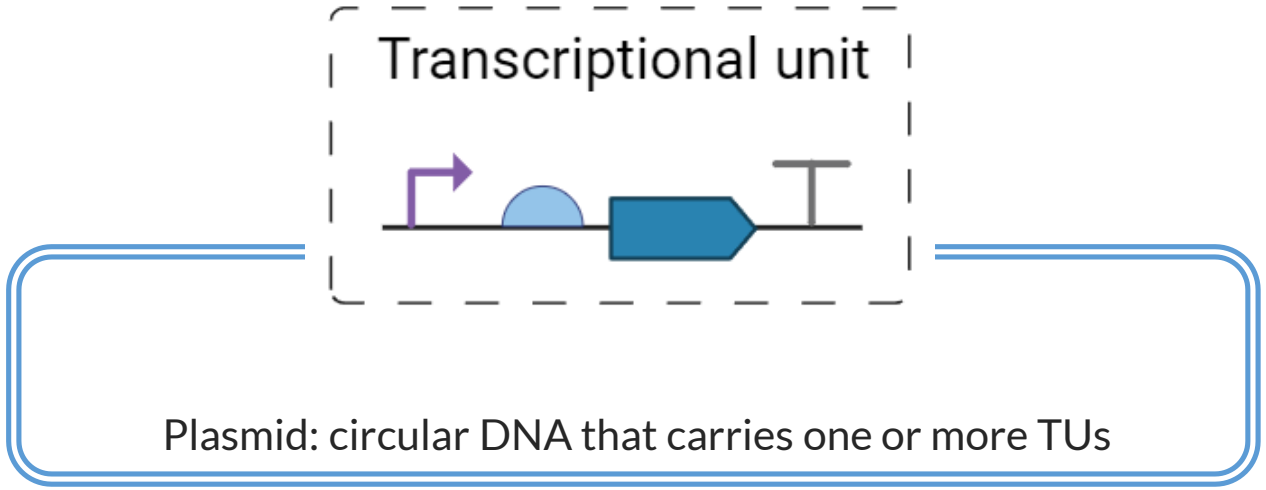
$$+ \qquad \dot{[\text{Prom.TF}]} = \quad k_{on}\,[\text{Prom}][\text{TF}] - k_{off}[\text{Prom.TF}]$$

$$\dot{[\text{Prom.TF}]} + \dot{[\text{Prom}]} = 0$$

Integrating this: $[\text{Prom.TF}] + [\text{Prom}] = C_N$ ← Plasmid Copy Number

$[\text{Prom}] = C_N - [\text{Prom.TF}]$ **Save this one, we will use it later.**

Part II: Model reduction

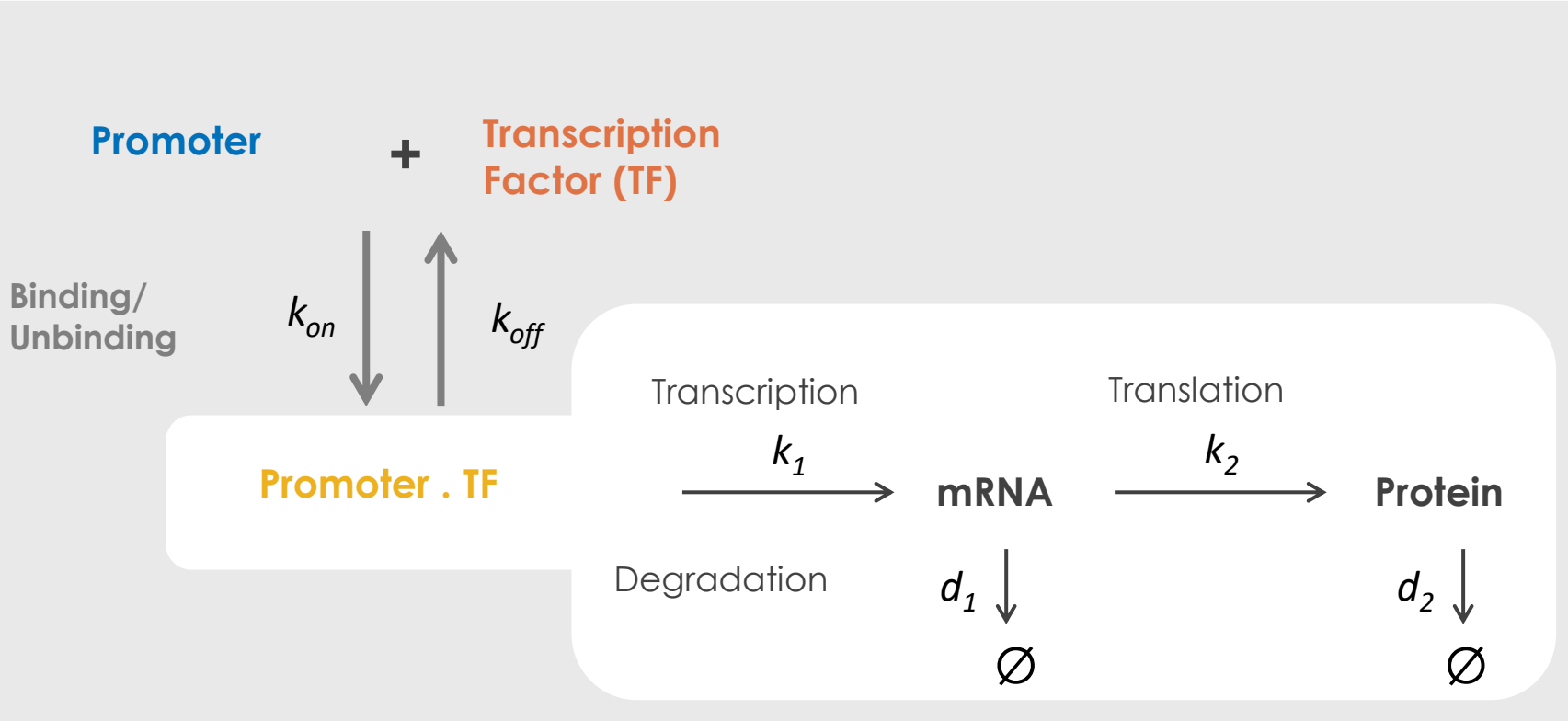


$$[\text{Prom. TF}] + [\text{Prom}] = C_N$$ ← Plasmid Copy Number

$$[\text{Prom}] = C_N - [\text{Prom. TF}]$$ **Save this one, we will use it later.**

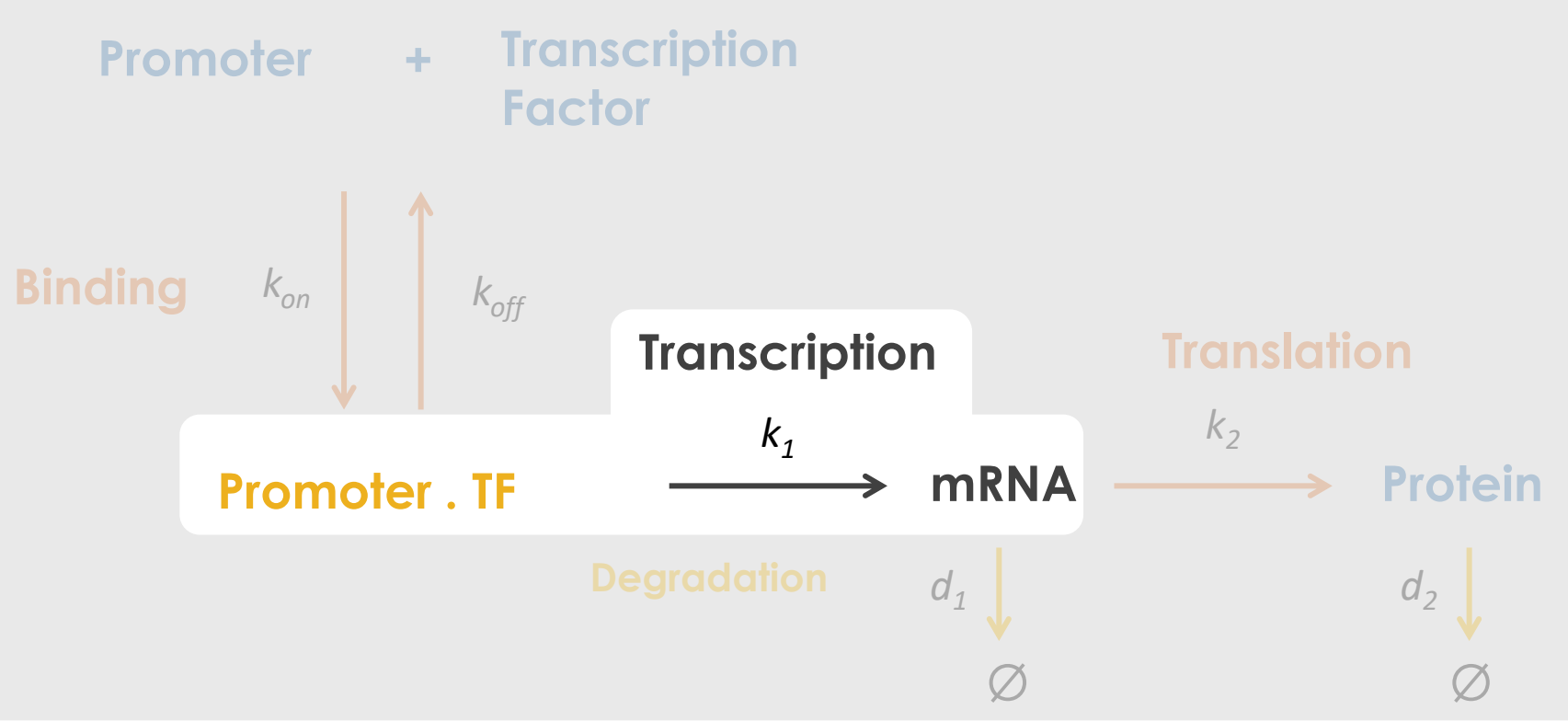
Section 3: Derivation of the Hill function

Part III: Derivation of the Hill function



$$[\dot{\mathrm{mRNA}}] = k_1[\mathrm{Prom.TF}]$$

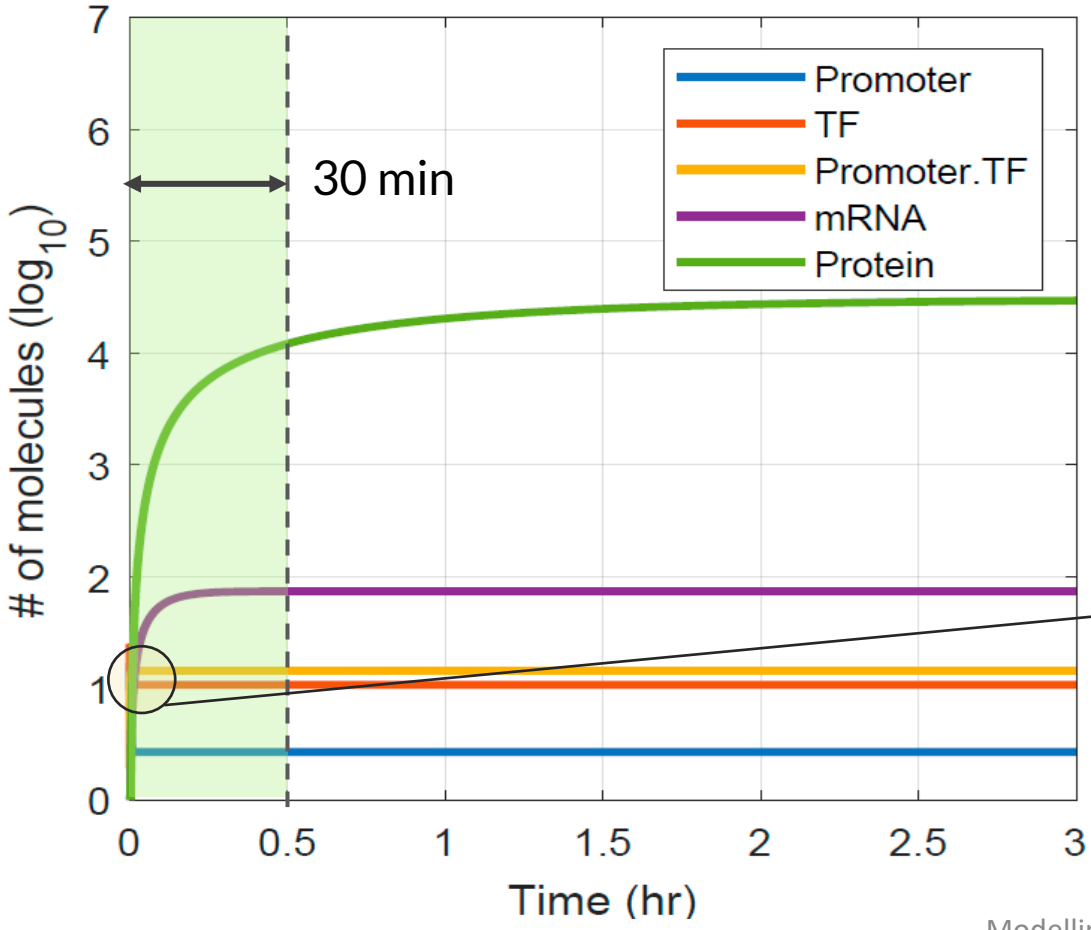
Part III: Derivation of the Hill function

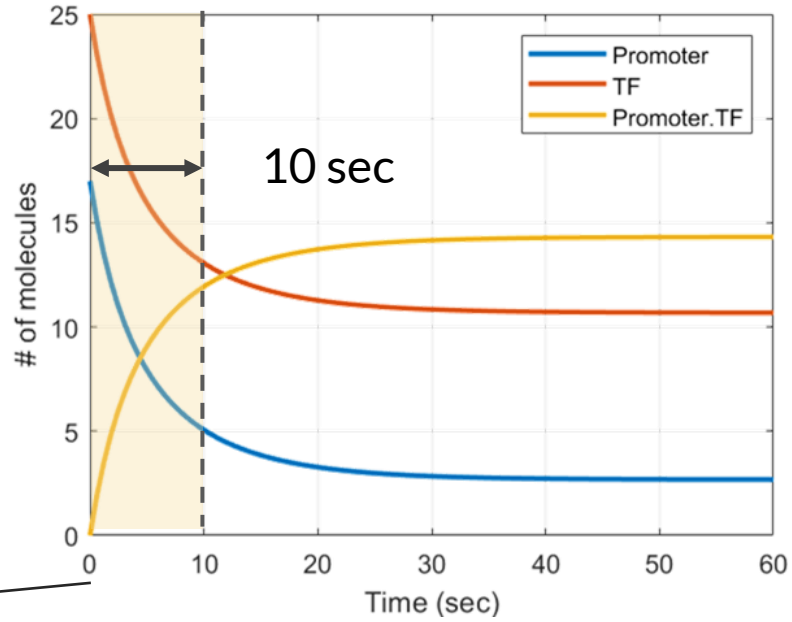


$$[\dot{\mathrm{mRNA}}] = k_1[\mathrm{Prom.\,TF}]$$

Part III: Derivation of the Hill function

Difference in time scales: Binding in seconds, transcription/translation from minutes to hours.





Genetic_circuit_model_TF.mlx

Simulation_Gene_Expression_pyLab.ipynb

Part III: Derivation of the Hill function

Quasi Steady State Approximation (QSSA)
TF rapidly binds to the promoter and this reaction reaches equilibrium very fast.

$$\dot{[\text{Prom.TF}]} \approx 0$$

In the 3rd equation $\dot{[\text{Prom.TF}]} = k_{on}\ [\text{Prom}][\text{TF}] - k_{off}[\text{Prom.TF}]$

$$0 = k_{on}\ [\text{Prom}][\text{TF}] - k_{off}[\text{Prom.TF}]$$

From invariance (previous slide):

$[\text{Prom}] = C_N - [\text{Prom.TF}]$ ⟶ Using these two, we will derive the **Hill function**

Part III: Derivation of the Hill function

Replacing the free promoter equation into the TF bound Promoter one:

$$[\text{Prom}] = C_N - [\text{Prom. TF}]$$

$$0 = k_{on}\ [\textbf{Prom}][\text{TF}] - k_{off}[\text{Prom. TF}]$$

$$0 = k_{on}\ (C_N - [\textbf{Prom.}\ \boldsymbol{TF}])[\text{TF}] - k_{off}[\text{Prom. TF}]$$

Solving for the TF bound Promoter $[\textbf{Prom.}\ \boldsymbol{TF}]$:

Part III: Derivation of the Hill function

Solving for the TF bound Promoter $[\mathbf{Prom.}\,\boldsymbol{TF}]$:

$$k_{on}\,(C_N - [\text{Prom. TF}])[\text{TF}] = k_{off}[\text{Prom. TF}]$$

$$k_{on}\,[\text{TF}]C_N - k_{on}\,[\text{TF}][\text{Prom. TF}] = k_{off}[\text{Prom. TF}]$$

$$k_{on}\,[\text{TF}]C_N = k_{on}\,[\text{TF}][\text{Prom. TF}] + k_{off}[\text{Prom. TF}]$$

$$k_{on}\,[\text{TF}]C_N = \left(k_{on}\,[\text{TF}] + k_{off}\right)[\text{Prom. TF}]$$

A bit of algebra…

$$[\text{Prom. TF}] = C_N \frac{k_{on}[\text{TF}]}{k_{on}\,[\text{TF}]+k_{off}} = C_N \frac{[\text{TF}]}{\frac{k_{off}}{k_{on}}+[\text{TF}]} = C_N \frac{[\text{TF}]}{K_d+[\text{TF}]}$$

Part III: Derivation of the Hill function

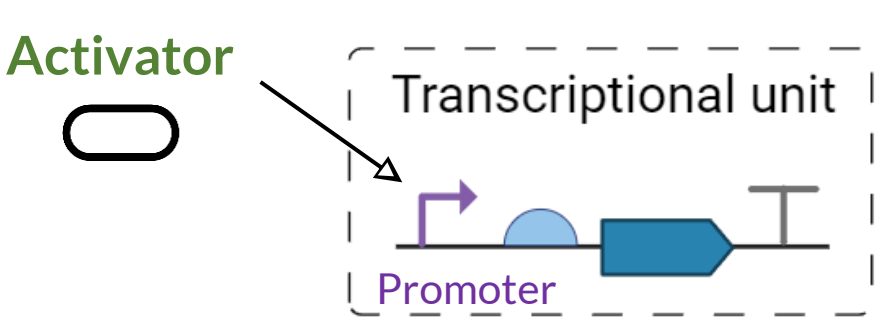
The Hill function represents how protein production depends on the transcription factor (TF)

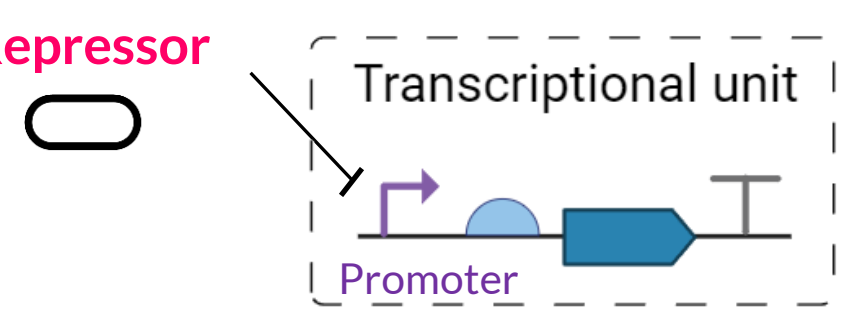
$$[\text{Prom. TF}] = C_N \frac{[\text{TF}]^n}{K_d^n + [\text{TF}]^n}$$

$K_d = \frac{K_{off}}{K_{on}}$ dissociation constant

n Hill coefficient

Two types of TFs for production:

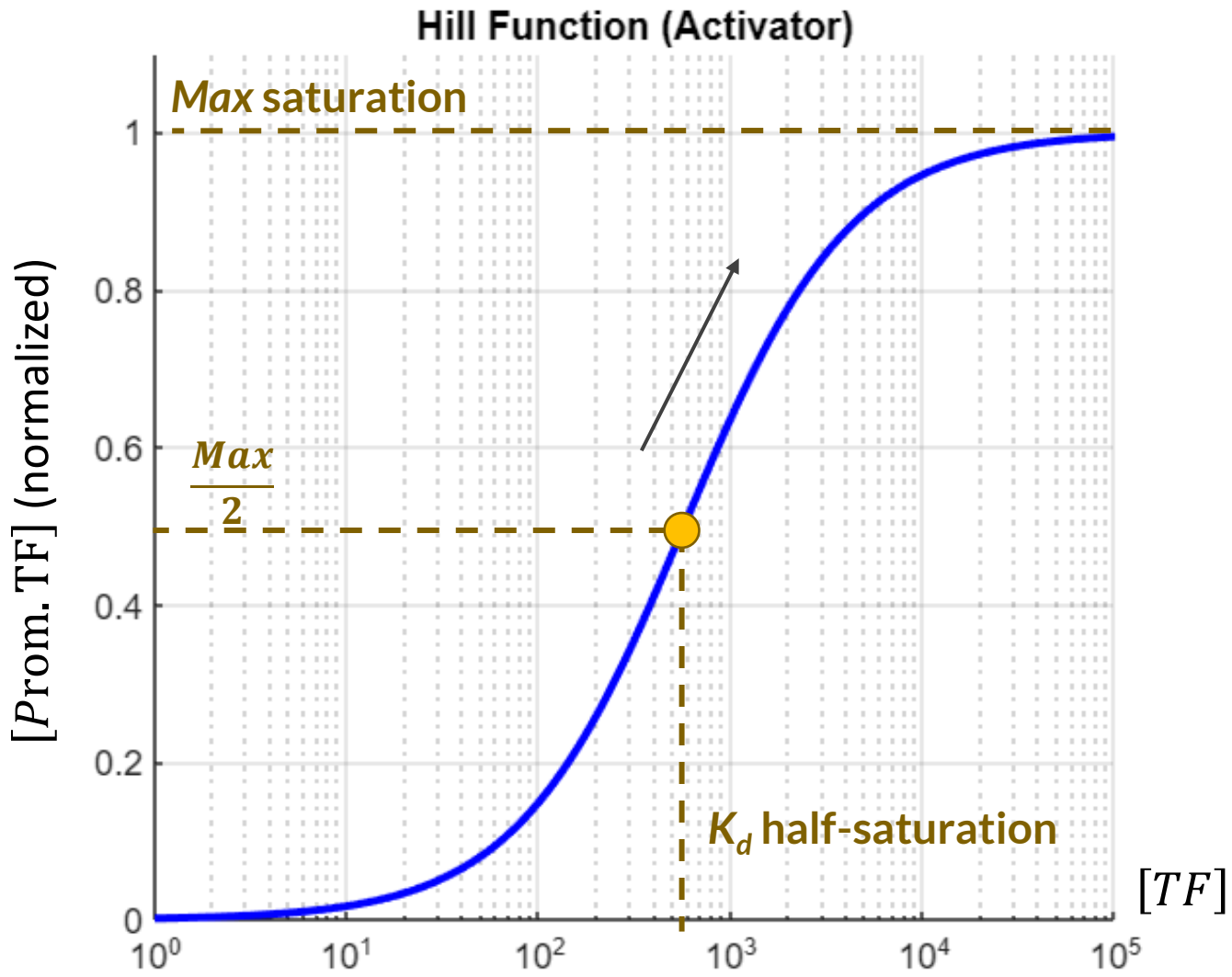




Hill function of an activator

$$[\text{Prom. TF}] = C_N \frac{[\text{TF}]^n}{K_d^n + [\text{TF}]^n}$$

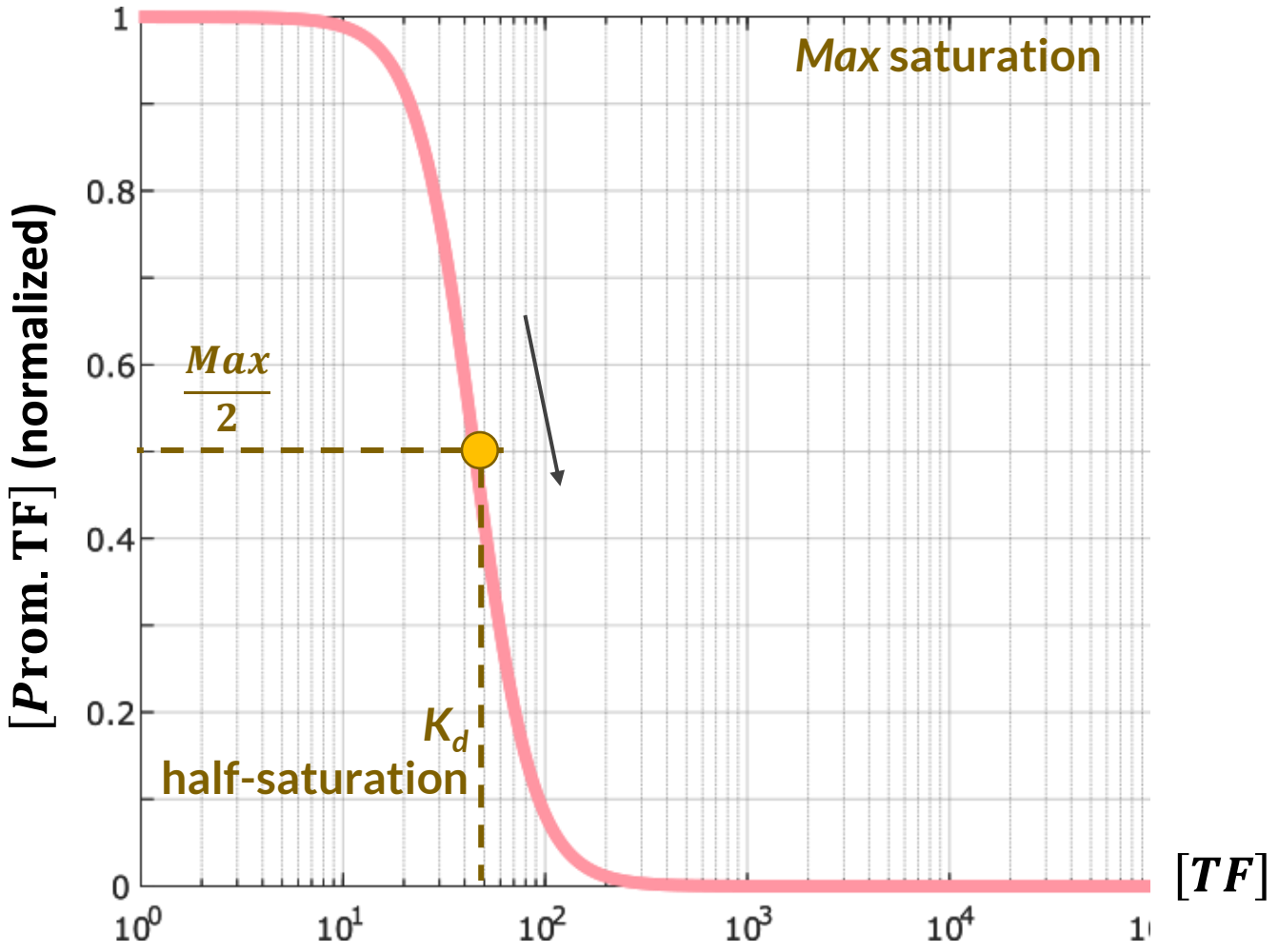
C_N = 1 molecule of DNA
K_d = 500 molecules
n = 1 molecules of TF bound themselves (sensitivity)



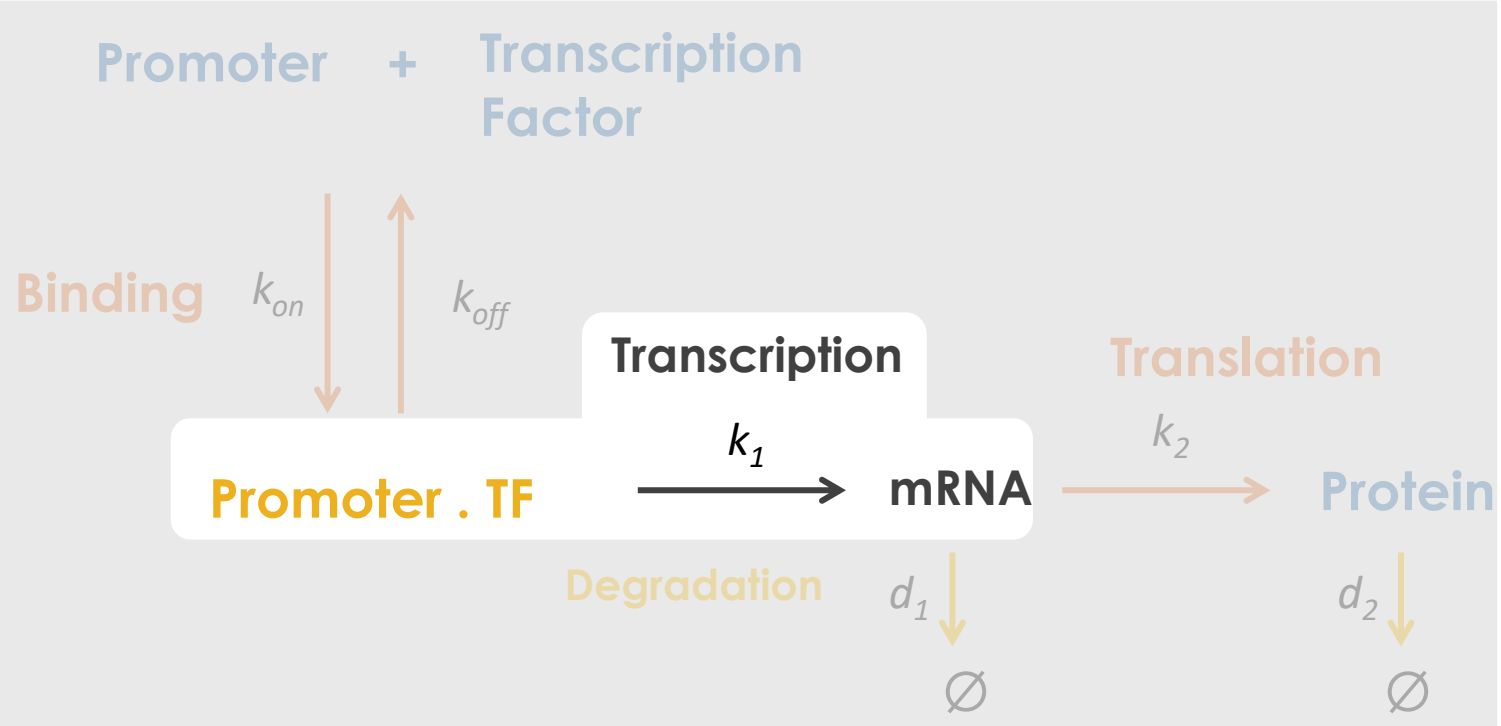
Hill function of a repressor

$$[\text{Prom. TF}] = C_N \frac{K_d^n}{K_d^n + [\text{TF}]^n}$$

C_N = 1 molecule of DNA
K_d = 50 molecules
n = 3 molecules of TF bound themselves (sensitivity)



Part III: Derivation of the Hill function



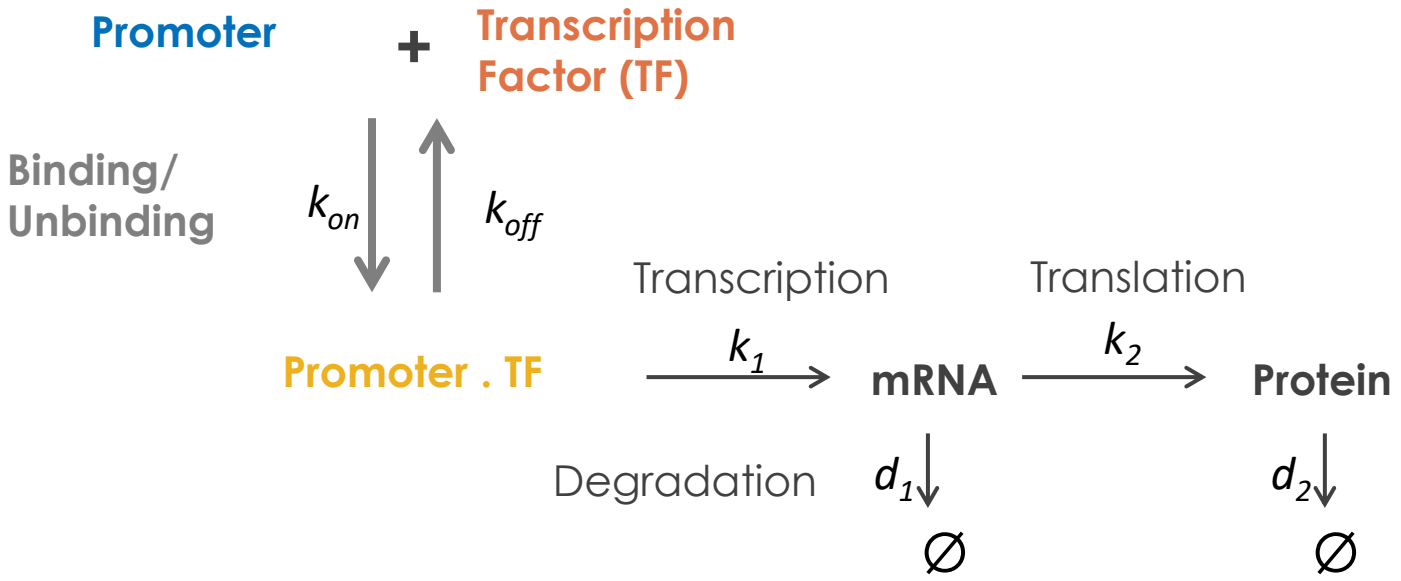
$$[\dot{\mathrm{mRNA}}] = k_1[\mathrm{Prom.\,TF}]$$

$$[\mathrm{Prom.\,TF}] = C_N \frac{[\mathrm{TF}]}{K_d + [\mathrm{TF}]}$$

The complete equation of the mRNA (activator with n=1):

$$[\dot{\mathrm{mRNA}}] = k_1\ C_N \frac{[\mathrm{TF}]}{K_d + [\mathrm{TF}]} - d_1[\mathrm{mRNA}]$$

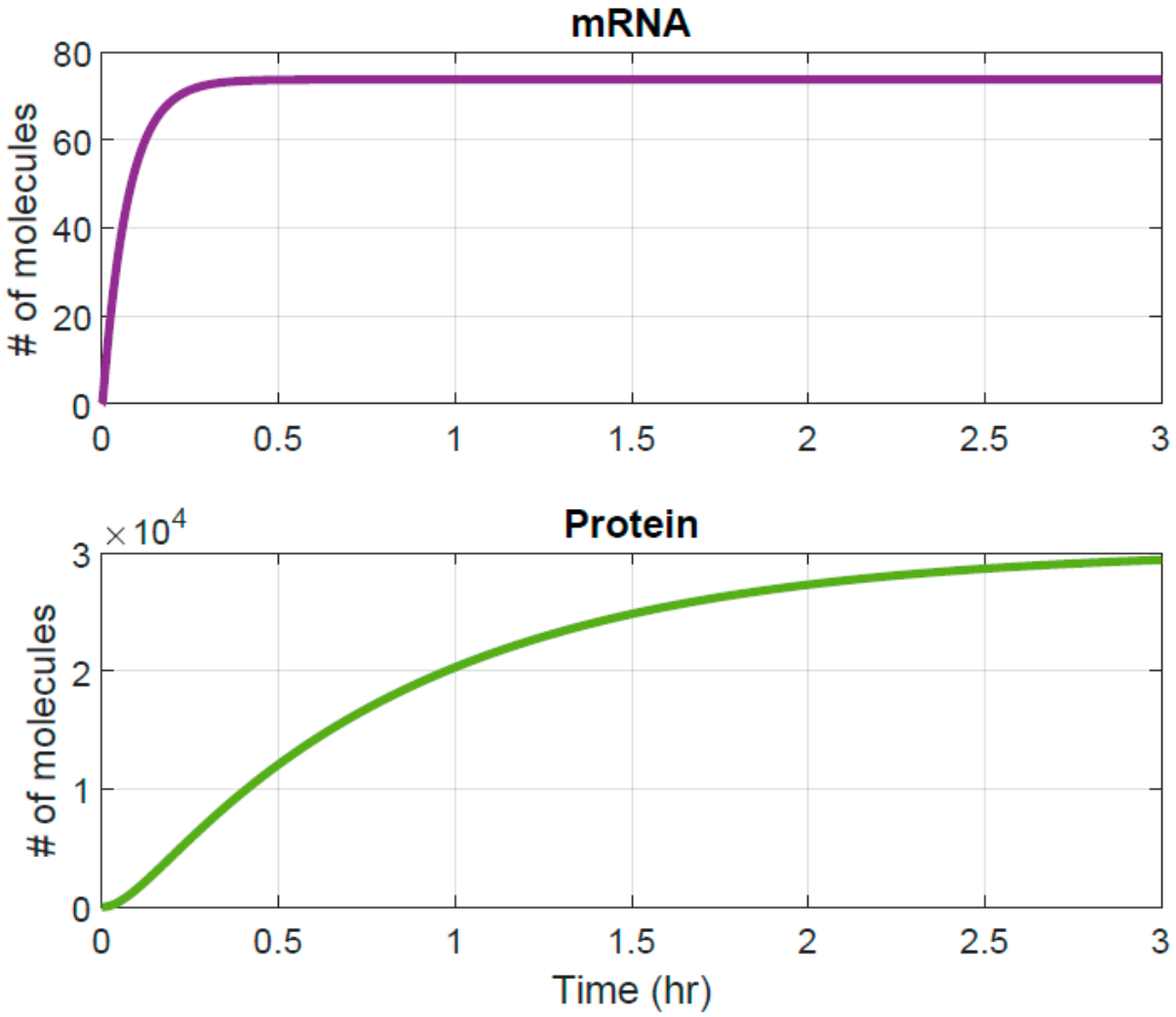
Part III: Derivation of the Hill function



Dynamic Model

$$\begin{cases} \dfrac{d[\mathrm{mRNA}]}{dt} = [\dot{\mathrm{mRNA}}] = k_1 \; C_N \dfrac{[\mathrm{TF}]}{K_d + [\mathrm{TF}]} - d_1[\mathrm{mRNA}] \\ \dfrac{d[\mathrm{Protein}]}{dt} = [\dot{\mathrm{Protein}}] = k_2[\mathrm{mRNA}] - d_2[\mathrm{Protein}] \end{cases}$$

Temporal simulations



$$\begin{cases} [\dot{\mathrm{mRNA}}] = k_1 \; C_N \dfrac{[\mathrm{TF}]}{K_d + [\mathrm{TF}]} - d_1[\mathrm{mRNA}] \\ [\dot{\mathrm{Protein}}] = k_2[\mathrm{mRNA}] - d_2[\mathrm{Protein}] \end{cases}$$

Parameters:

C_N = 17 molecules (Plasmid copy number)

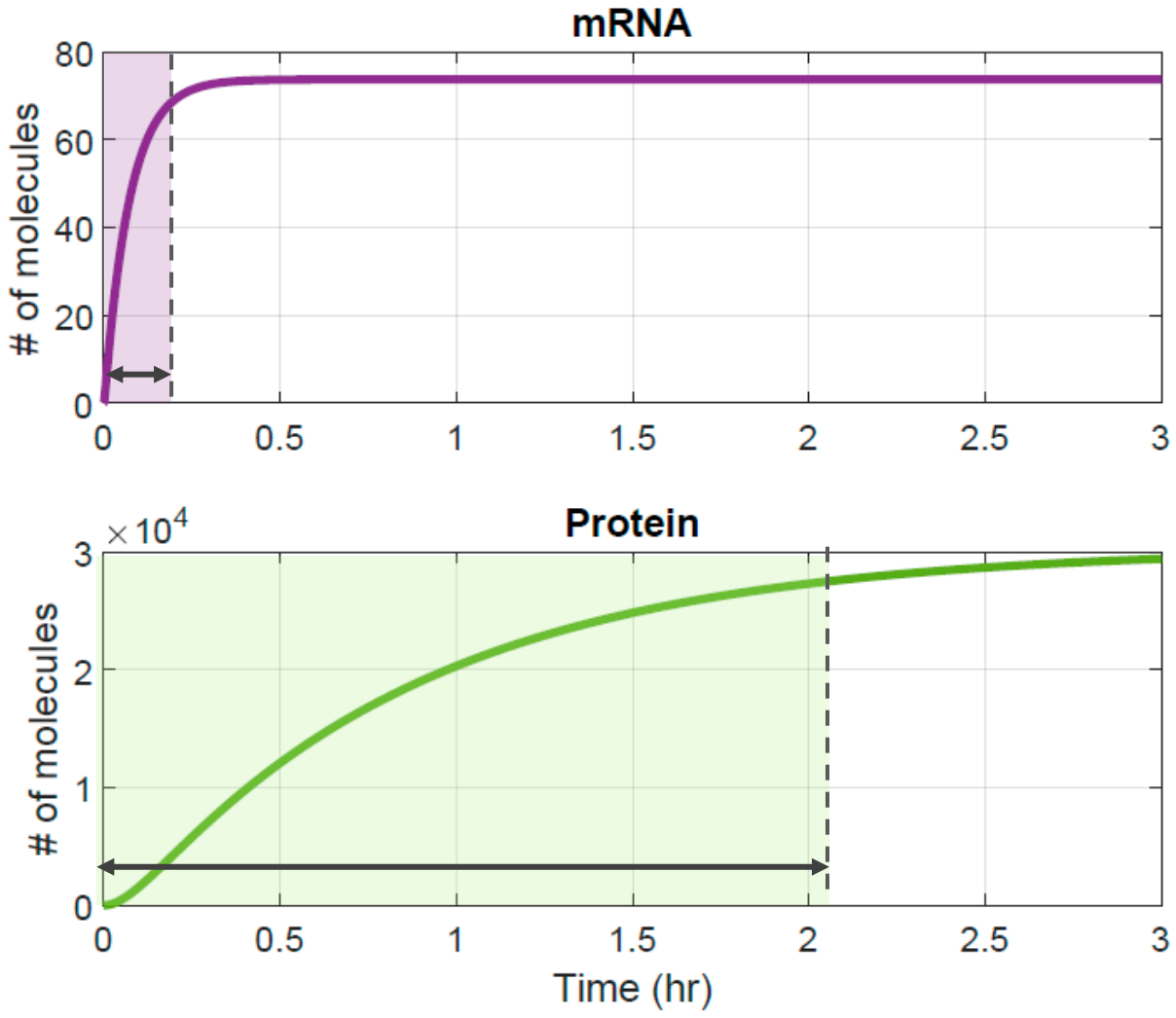
Kd = 2 molecules

TF = 25 molecules (Transcription Factor)

The other parameters same than constitutive

Genetic_circuit_model_TF.mlx

Simulation_Gene_Expression_pyLab.ipynb



Difference in time scales:
transcription (mRNA) in minutes

translation (Protein) takes ~2 hours

Remarks: Transcription is faster than Translation

mRNA is much faster than Protein production, we use the **same trick than before (QSSA):**

$$\frac{d[\mathrm{mRNA}]}{dt} = [\dot{\mathrm{mRNA}}] \approx 0$$

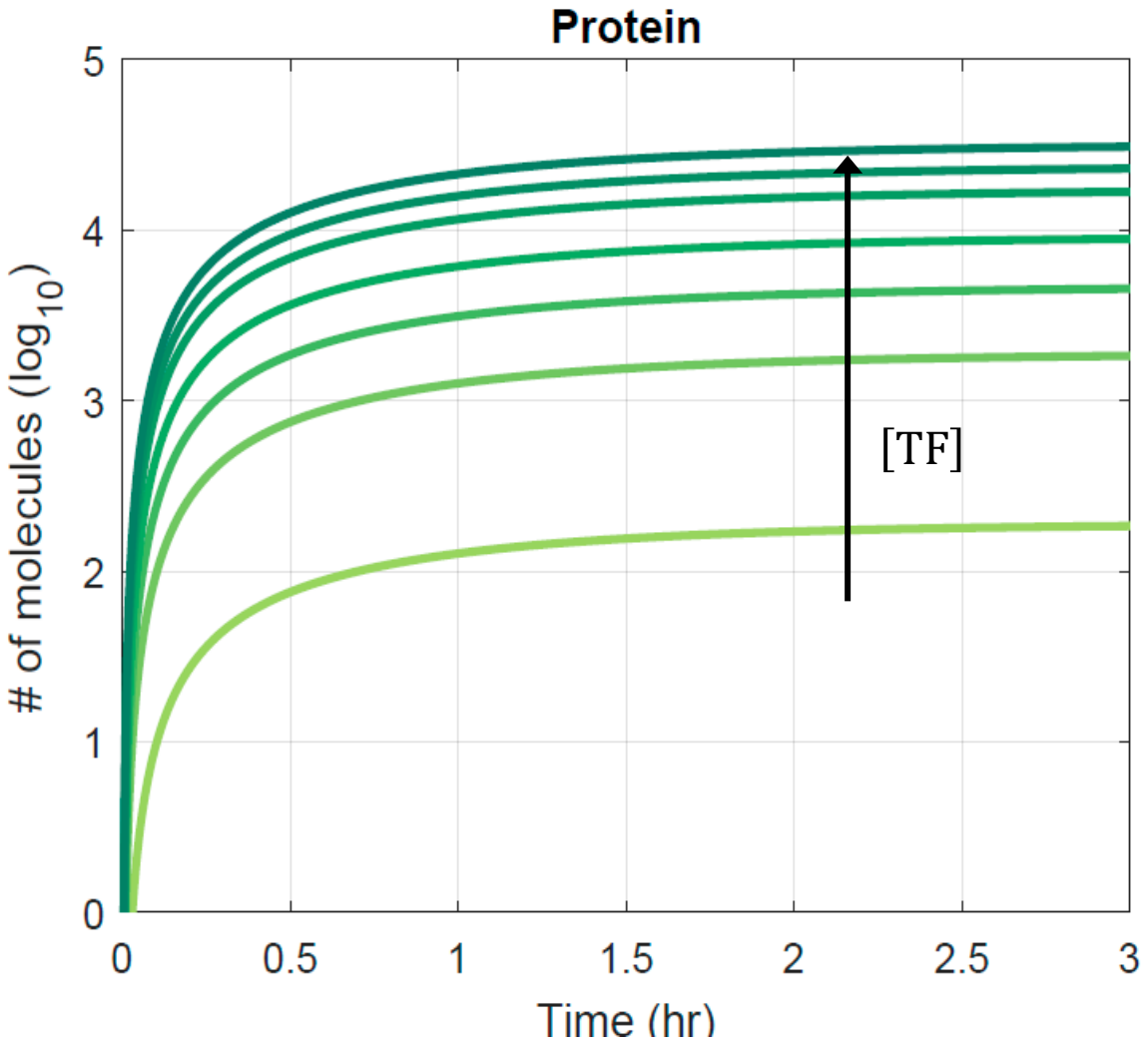
$$0 = k_1\ C_N \frac{[\mathrm{TF}]^n}{K_d^n + [\mathrm{TF}]^n} - d_1[\mathrm{mRNA}] \quad \rightarrow \quad [\boldsymbol{mRNA}] = \frac{k_1}{d_1}\ C_N \frac{[\mathrm{TF}]^n}{K_d^n + [\mathrm{TF}]^n}$$

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

$$[\dot{\mathrm{Protein}}] = \alpha \frac{[\mathrm{TF}]^n}{K_d^n + [\mathrm{TF}]^n} - d_2[\mathrm{Protein}]$$

Where $\alpha = k_2 \frac{k_1}{d_1} C_N$

Simulating only translation



$$\dot{[\text{Protein}]} = \alpha \frac{[\text{TF}]^n}{K_d^n + [\text{TF}]^n} - d_2[\text{Protein}]$$

$$\alpha = k_2 \frac{k_1}{d_1} C_N$$

With:
α = 720 molecules min^{-1}
K_d = 2 molecules
d_2 = 0.02 min^{-1}
(this means 34 min of doubling time)

[TF]: from 0.1 molecule to 25 molecules of Transcription Factor

Experimental data of translation

To analise the final protein production, we use the **same trick (QSSA)**:

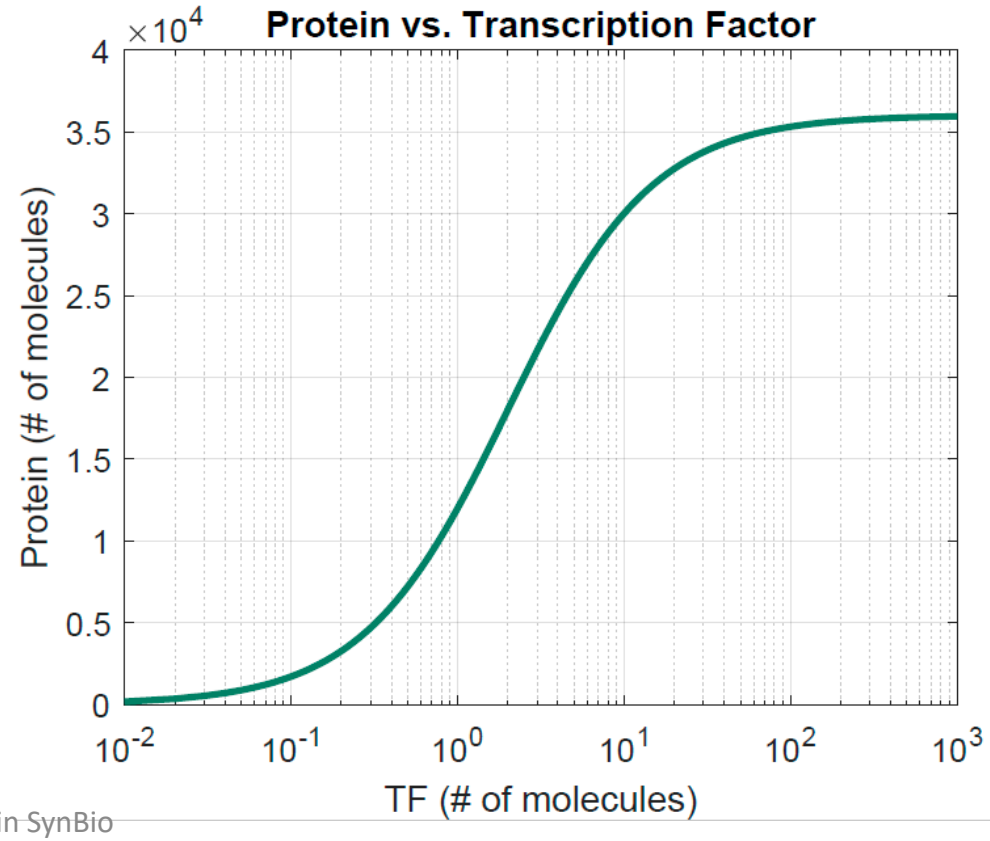
(equilibrium expression of protein ⟶ data at the end of the experiment)

$$\frac{d[\text{Protein}]}{dt} = [\dot{\text{Protein}}] \approx 0$$

$$[\text{Protein}] = \frac{\alpha}{d_2} \frac{[\text{TF}]^n}{K_d^n + [\text{TF}]^n}$$

$$with\ \alpha = k_2 \frac{k_1}{d_1} C_N$$

Protein concentration is an algebraic equation



MATLAB

MATLAB

How do I relate my model to the **lab parts?**

Use of the Matlab Apps varying the slides in the simulations

Measurements Calibration

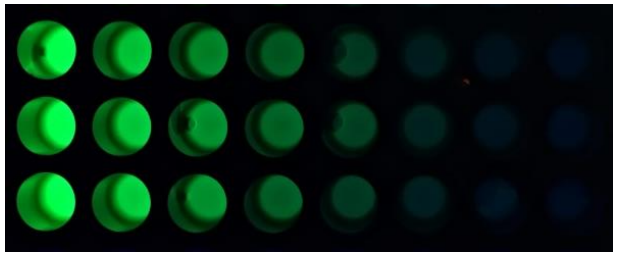
We need to get experimental data

Protein measurement

Plate Reader

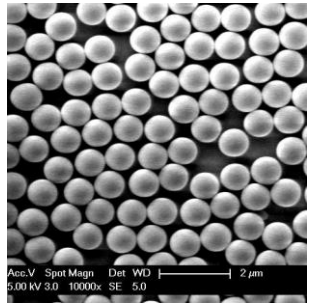
Calibrated measurement to validate our model with experiments

Fluorescein Sodium Salt



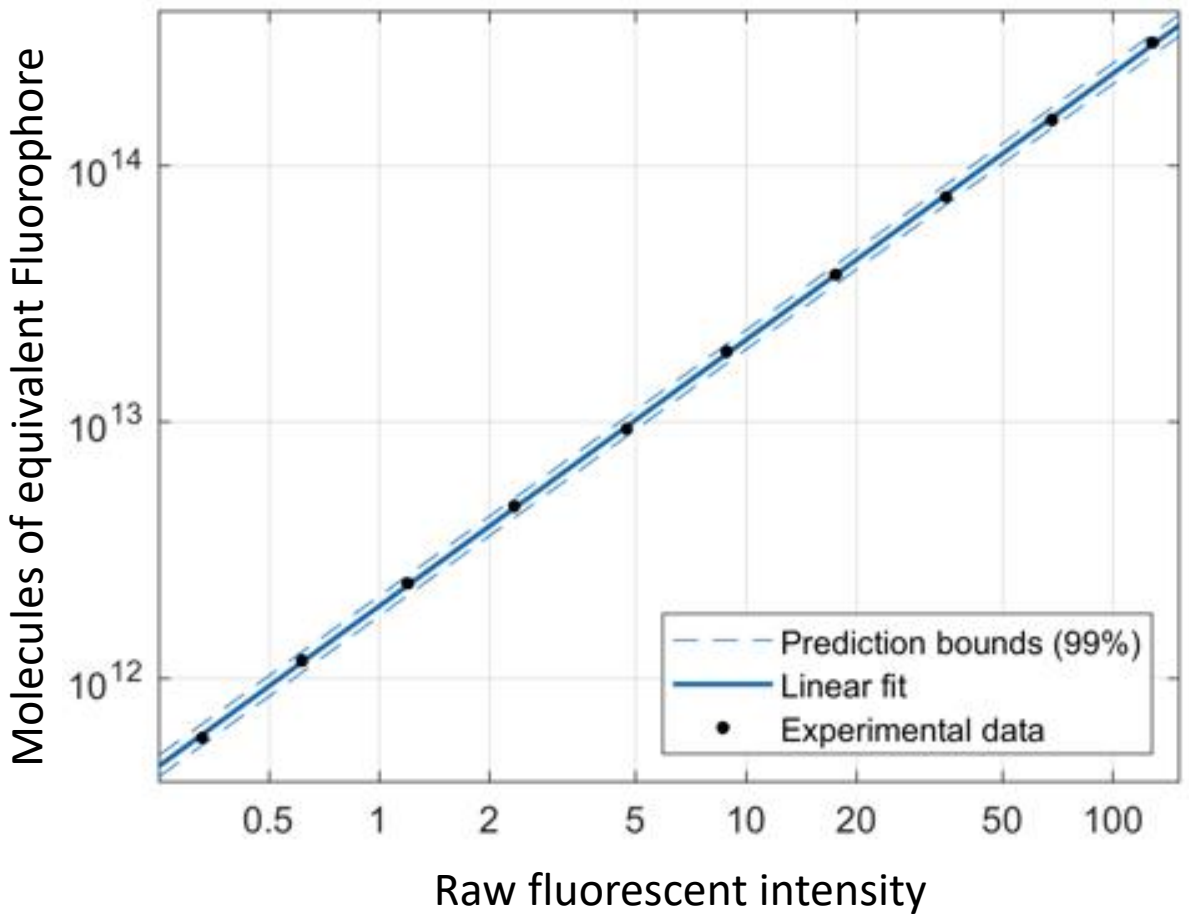
Sulforhodamine 101

Silica Beads



Multicolor plate reader fluorescence calibration, Synthetic Biology, Oxford 2022.

Calibration of protein expression: Fluorescein Sodium Salt to Molecules of equivalent Fluorophore (MEFL)



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PLATERO: A calibration protocol for plate reader green fluorescence measurements

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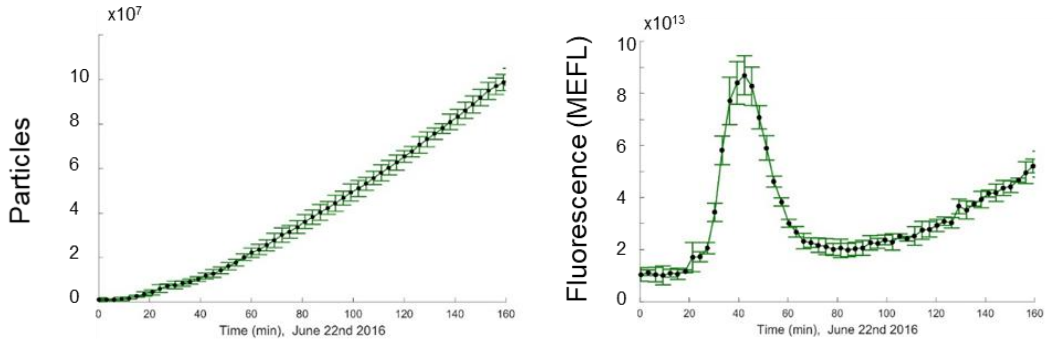
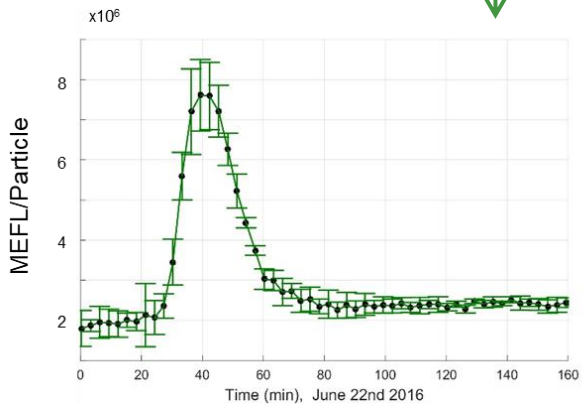
MEFL/Particle unit is equivalent to number of molecules/cell from the dynamic model (ODE)





$$
\begin{cases}
\frac{d[R]}{dt} = \frac{p_R C_N k_R}{dm_R + \mu} - (d_R + \mu)[R] \\
\frac{d[cI]}{dt} = \frac{p_{cI} C_N k_{cI}}{dm_{cI} + \mu}\left(\alpha + (1-\alpha)\frac{\frac{1}{k_{dlux}}\left(\frac{[R][A]}{k_{d2} C_N}\right)^2}{1 + \frac{1}{k_{dlux}}\left(\frac{[R][A]}{k_{d2} C_N}\right)^2}\right) - (d_{cI} + \mu)[cI] \\
\frac{d[GFP]}{dt} = \frac{p_G C_N k_G}{dm_G + \mu}\left(\alpha + (1-\alpha)\frac{\frac{1}{k_{dlux}}\left(\frac{[R][A]}{k_{d2} C_N}\right)^2}{1 + \frac{1}{k_{dlux}}\left(\frac{[R][A]}{k_{d2} C_N}\right)^2}\frac{1}{1 + \frac{[cI]^2}{k_{dcI} C_N}}\right) - (d_G + \mu)[G] \\
\frac{dN}{dt} = \mu N\left(1 - \frac{N}{N_{max}}\right)
\end{cases}
$$