Synthetic Biology and Biosystems Control Lab
Valencia UPV

2020
Measurement

Modeling: ODEs and Hill Functions

Section 2: Derivation of the Hill Function

by Alejandro Vignoni (alvig2@upv.es)

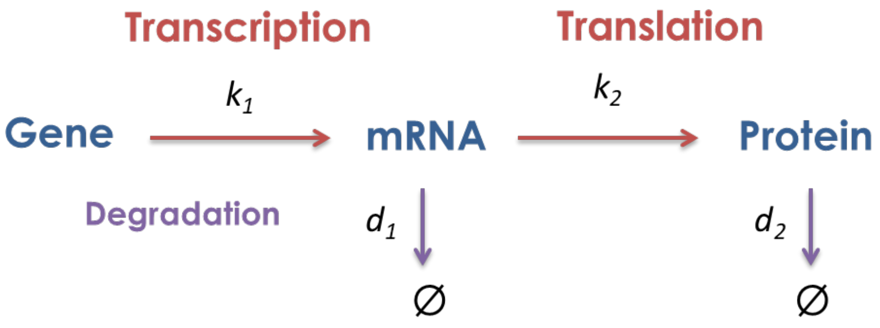
An iGEM Measurement Committee Webinar
Week 2, June 23rd, 2020

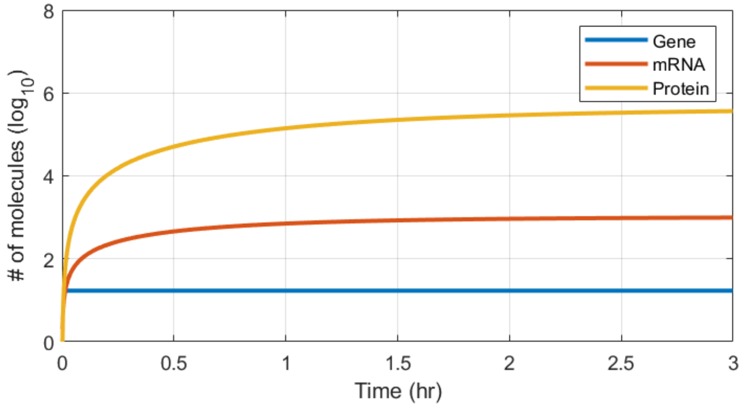
$$\begin{cases} \dfrac{d[LuxI]_i}{dt} = b_i + u([AHL]_i) - \gamma_I\,[LuxI]_i \\ \dfrac{d[AHL]_i}{dt} = K_A\,[LuxI]_i + d([AHL]_i - [AHL]_e) - \gamma_A\,[AHL]_i \end{cases}$$

Today Webinar’s Topics

- 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)

Remember: Constitutive gene expression

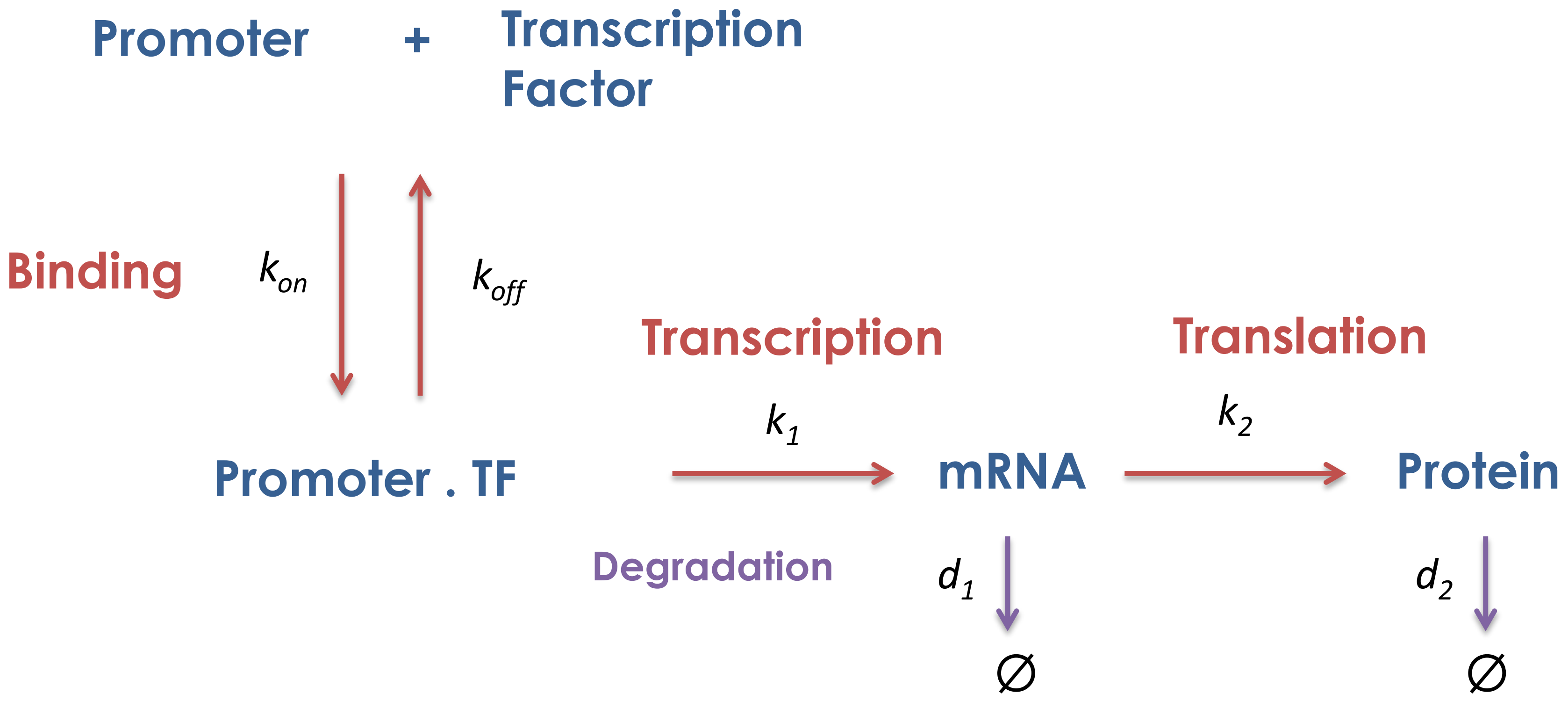




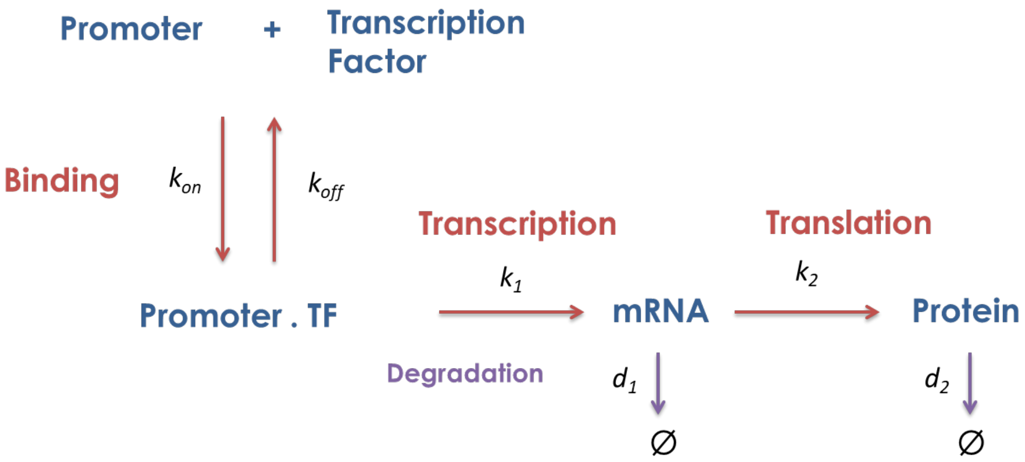
$$\dot{[\mathrm{mRNA}]} = k_1[\mathrm{Gene}] - d_1[\mathrm{mRNA}]$$

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

Gene expression regulation by Transcription Factors (TF)



Gene expression regulation by Transcription Factors (TF)



We will get: -5 Equations
-with 7 parameters

Problems:

k_1, k_2, d_1 and d_2 become indistinguishable when we measure only the protein amount.

k_{on}, k_{off}, are very difficult to measure.

Gene expression regulation by Transcription Factors (TF)

We want to approximate and simplify the problem and obtain a model easier to relate with experimental data:

1. We will obtain all the equations.
2. Approximate and reduce them.

We will

guishable

ein

Problems: → k_{on}, k_{off}, are very difficult to measure.

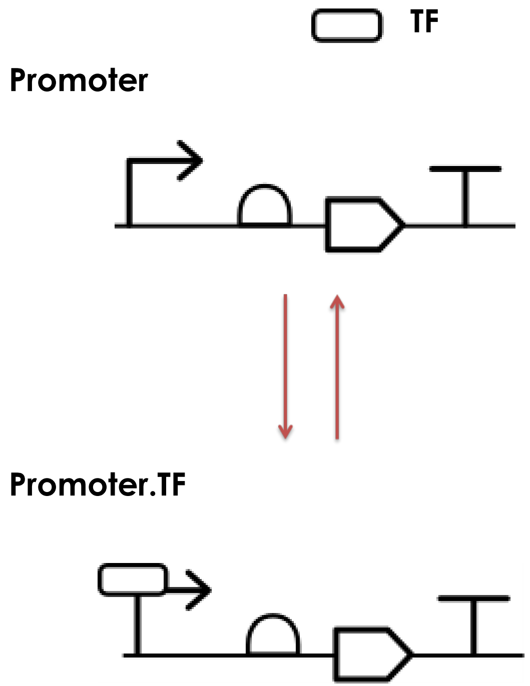
Gene expression regulation by Transcription Factors (TF)

Part I: Getting the model

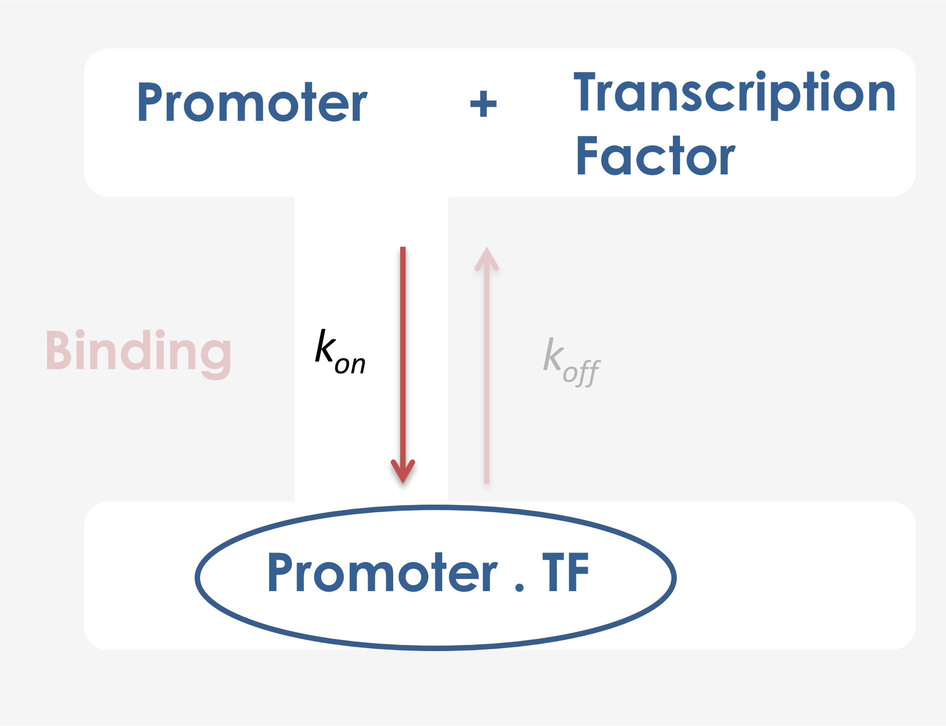
Promoter + **Transcription Factor**

Binding k_{on} $\downarrow\uparrow$ k_{off} **Unbinding**

Promoter . TF

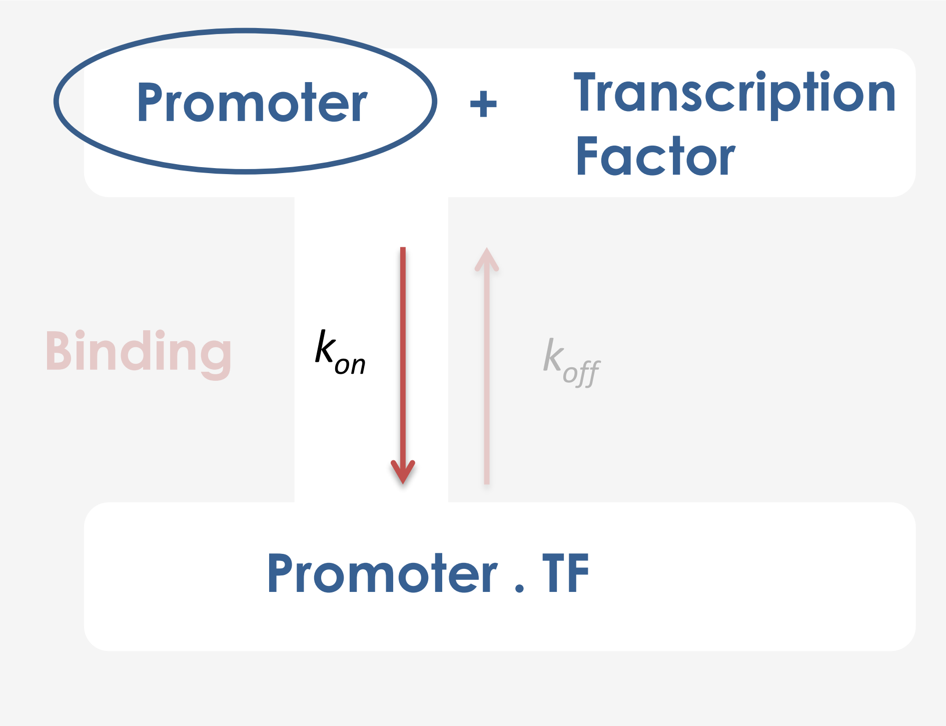


Gene expression regulation by Transcription Factors (TF)



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

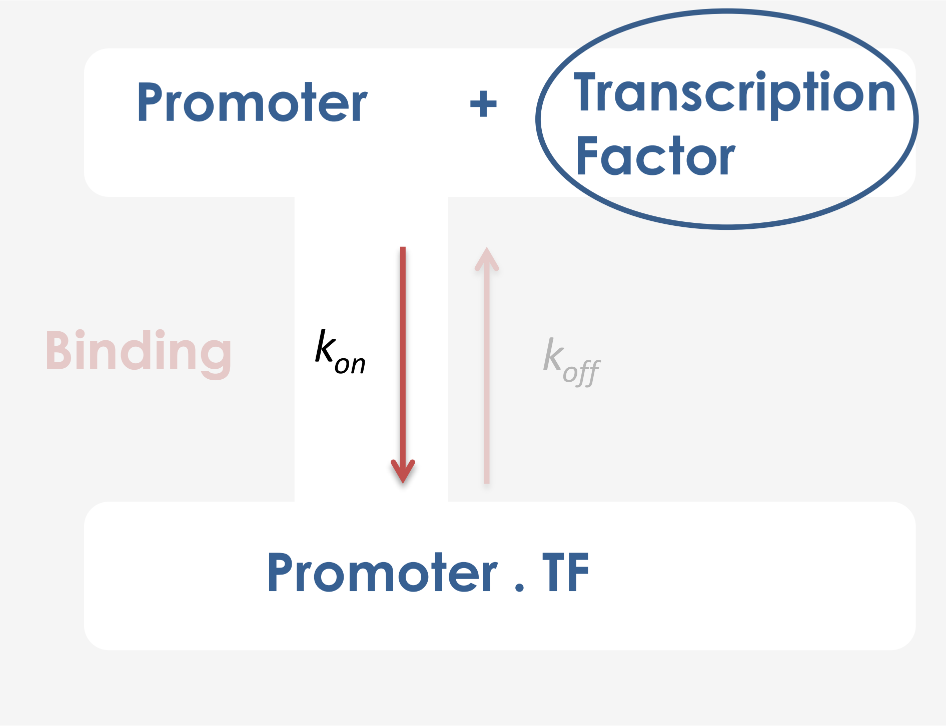
Gene expression regulation by Transcription Factors (TF)



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

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

Gene expression regulation by Transcription Factors (TF)

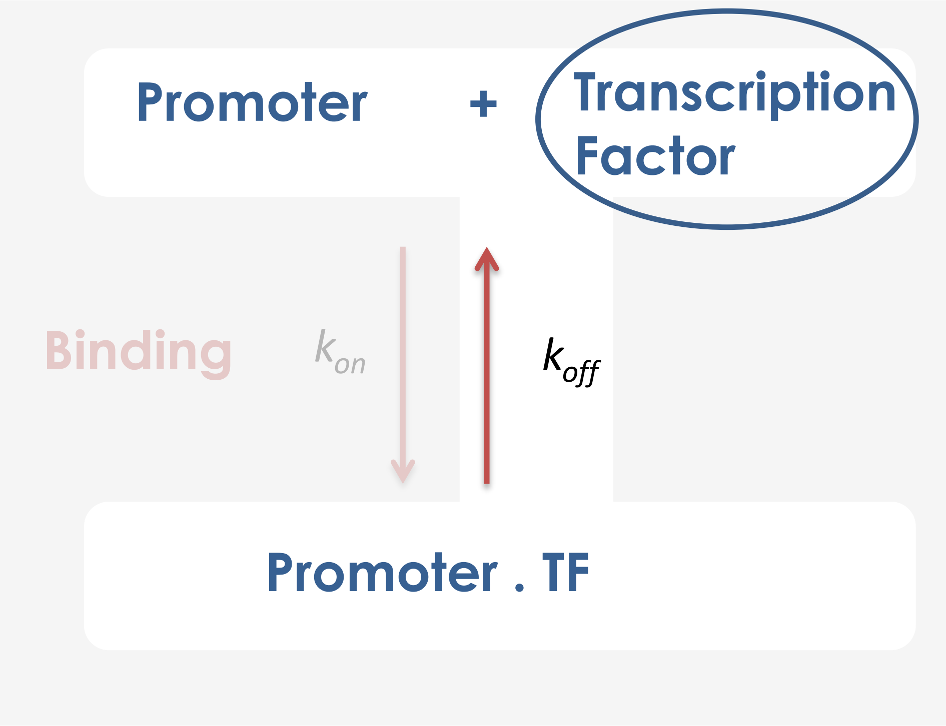


$$\dot{[\mathrm{Prom.TF}]} = k_{on}\,[\mathrm{Prom}][\mathrm{TF}]$$

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

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

Gene expression regulation by Transcription Factors (TF)

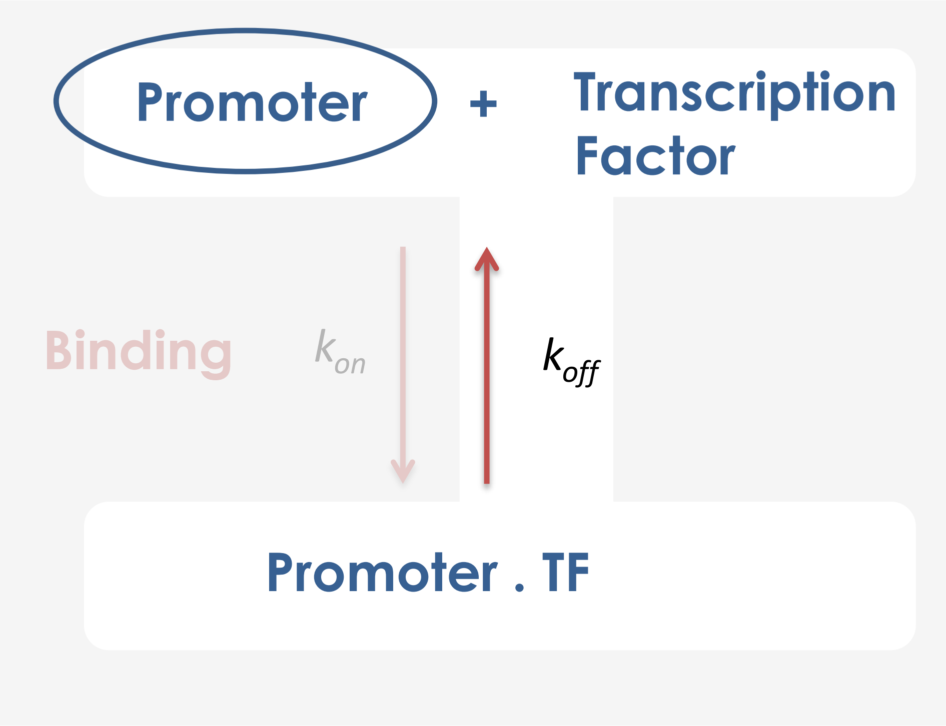


$$\dot{[\text{Prom.TF}]} = k_{on}\,[\text{Prom}][\text{TF}]$$

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

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

Gene expression regulation by Transcription Factors (TF)

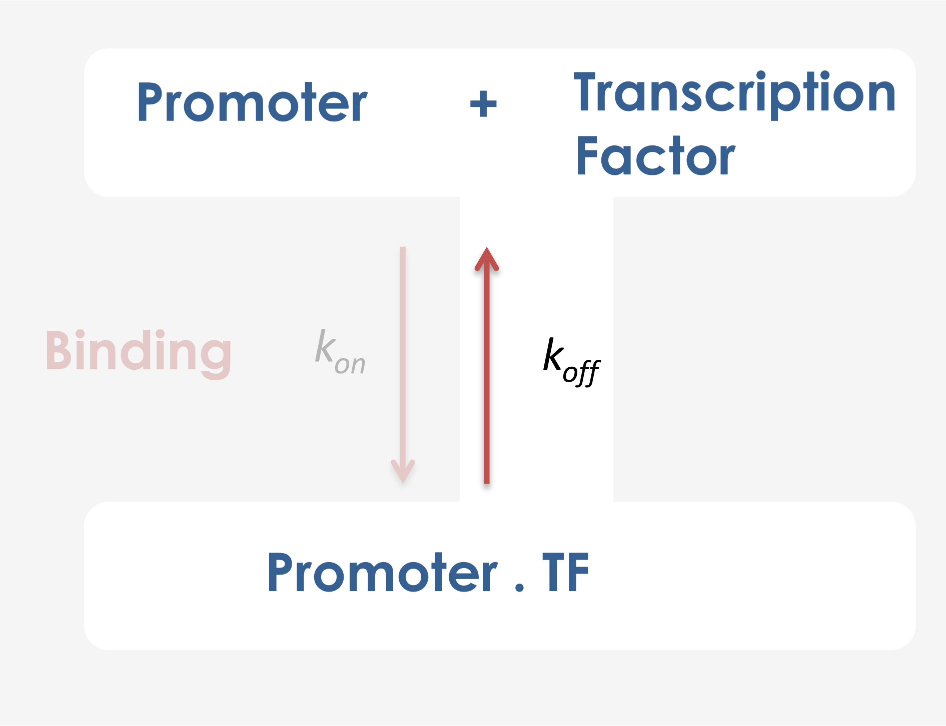


$$\dot{[\mathrm{Prom.TF}]} = k_{on}\,[\mathrm{Prom}][\mathrm{TF}]$$

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

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

Gene expression regulation by Transcription Factors (TF)



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

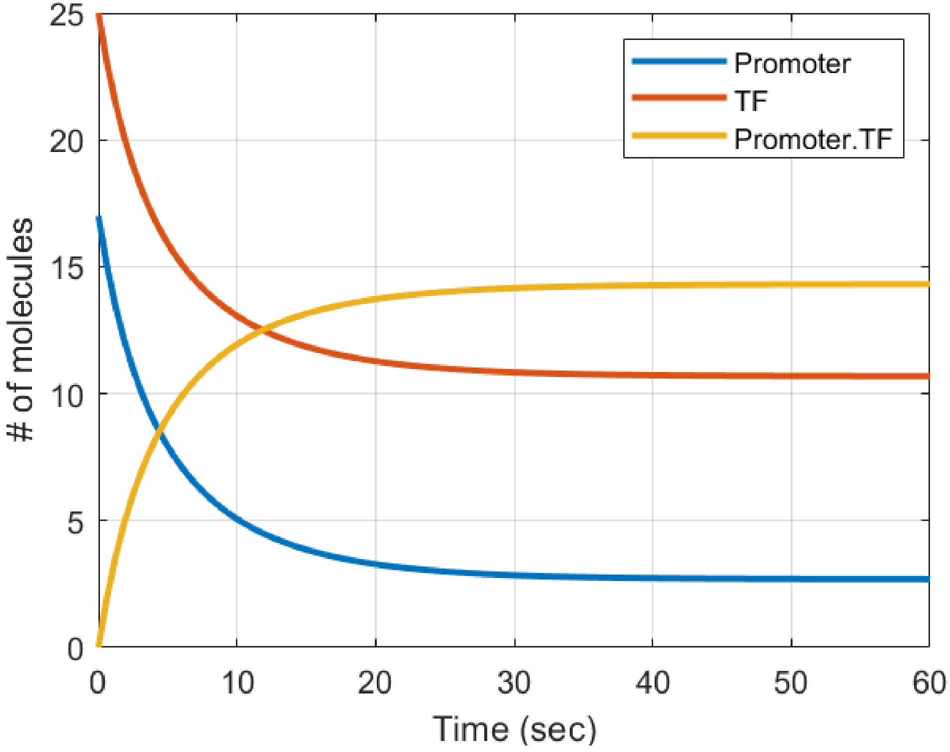
$$\dot{[\mathrm{Prom}]} = -k_{on}\,[\mathrm{Prom}][\mathrm{TF}] + k_{off}[\mathrm{Prom.TF}]$$

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

Gene expression regulation by Transcription Factors (TF)

Simulation

MATLAB



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

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

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

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}

`Main_TF.m`

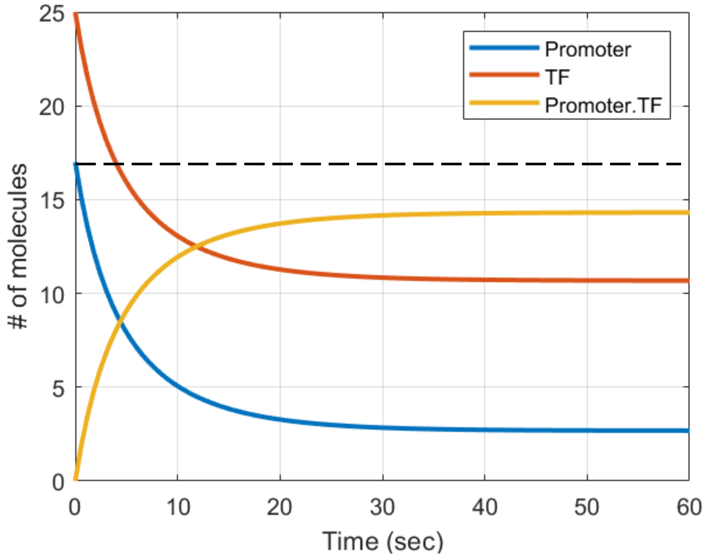
Gene expression regulation by Transcription Factors (TF)

Part II: Model Reduction

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

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

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



Remarks

- First two equations are equal (Blue and red)!
- The sum of the first one and the third one is identically zero (Blue and yellow)!
- We can use this fact (promoter invariance) to simplify the equations and reduce the model.

Gene expression regulation by Transcription Factors (TF)

Promoter invariance (constant Plasmid Copy Number)

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

+

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

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

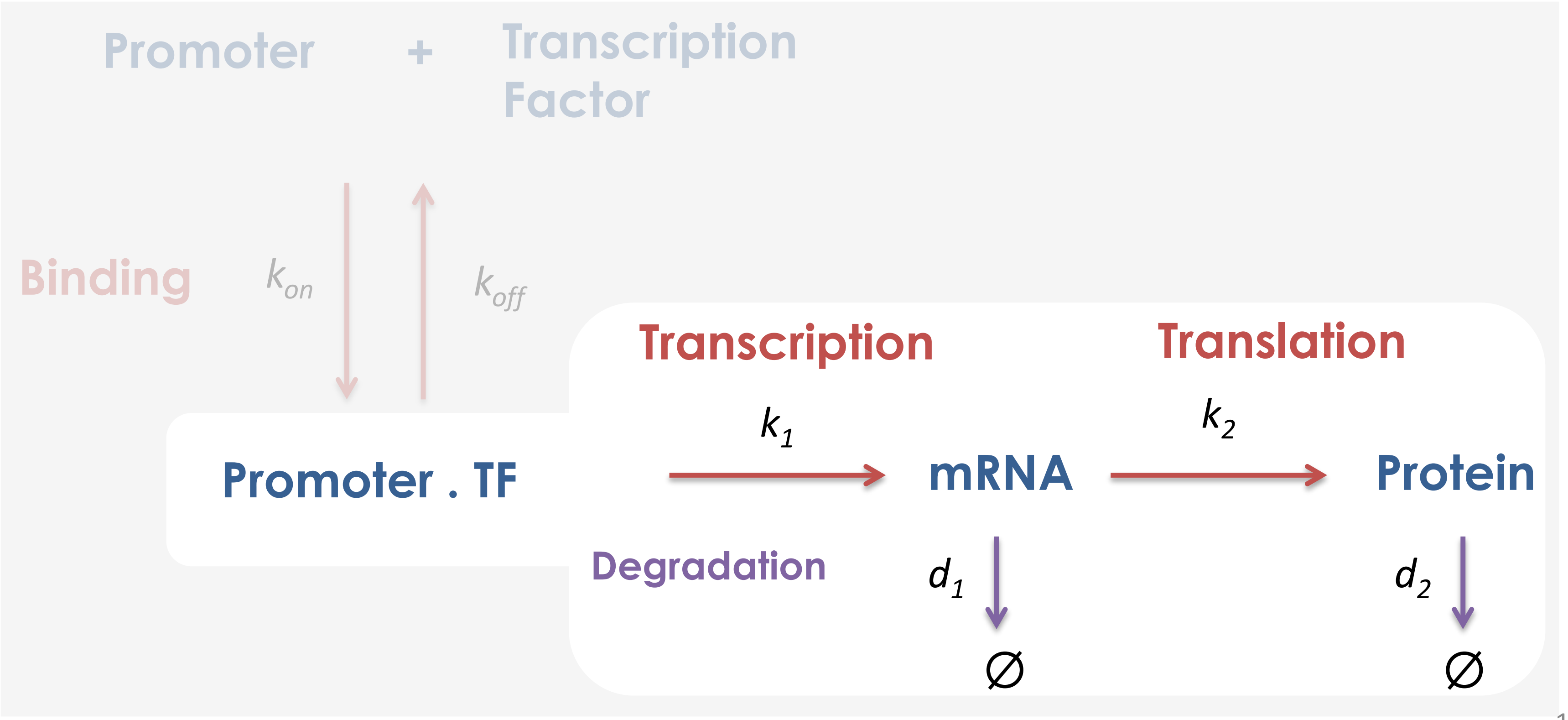
$$[\text{Prom.TF}] + [\text{Prom}] = C_N$$ ← Plasmid Copy Number

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

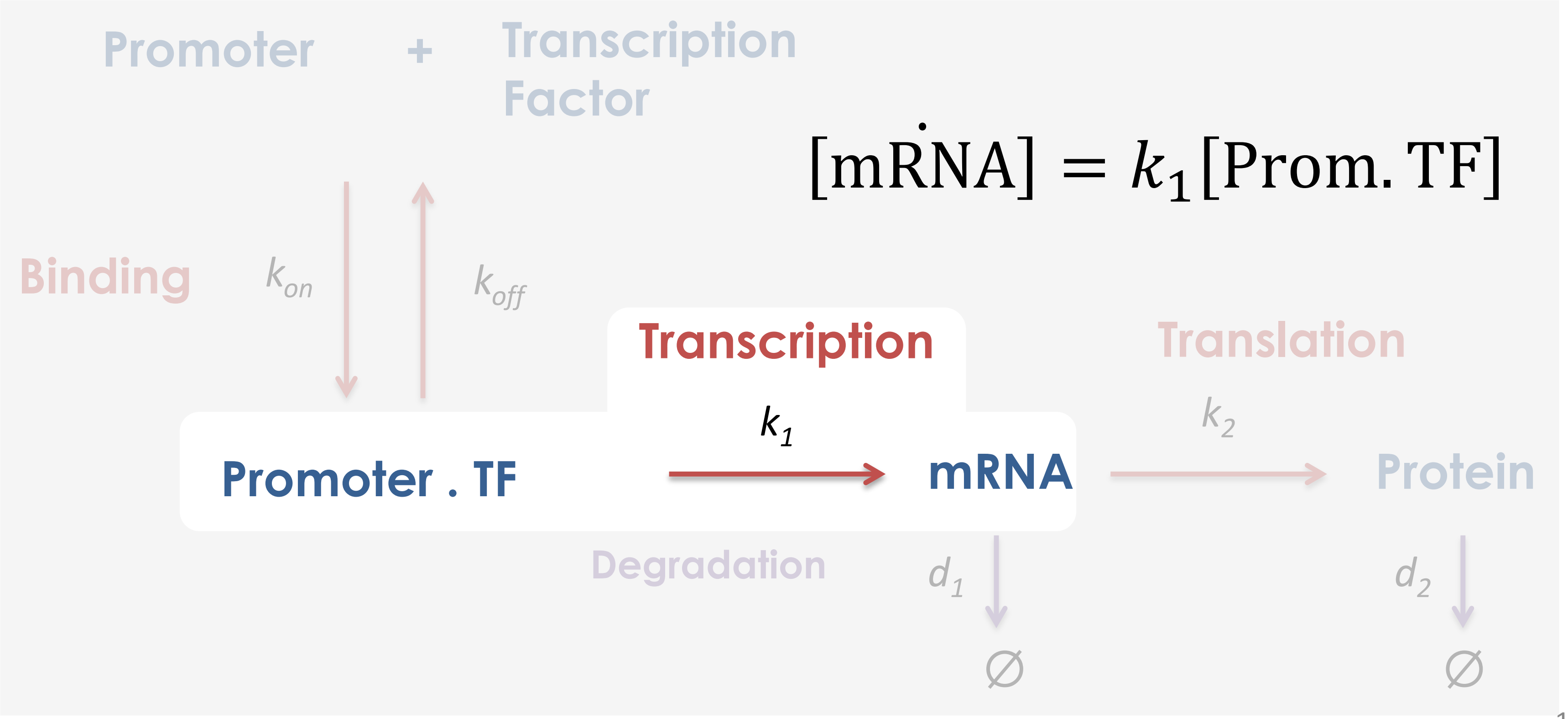
Save this one, we will use it later.

Gene expression regulation by Transcription Factors (TF)

Part I: Getting the model



Gene expression regulation by Transcription Factors (TF)



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

Gene expression regulation by Transcription Factors (TF)

Fast Transcription Factor – Promoter binding

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

Because of the difference in time scales: Binding in the seconds, transcription/translation from minutes to hours; we can say that TF rapidly binds to the promoter and this reaction reaches equilibrium very fast.
This is called Quasy Steady State Approximation (QSSA).

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

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

From invariance (previous slide)

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

Gene expression regulation by Transcription Factors (TF)

Fast Transcription Factor – Promoter binding

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

Because of the difference in time scales: Binding in the seconds, transcription/translation from minutes to hours; we can say that TF rapidly binds to the promoter and this reaction reaches equilibrium very fast.
This is called Quasy Steady State Approximation (QSSA).

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

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

From invariance (previous slide)

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

Using these two, we will derive the Hill function

Gene expression regulation by Transcription Factors (TF)

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

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

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

$$0 = k_{on}\,(C_N - [\mathrm{Prom.TF}])[\mathrm{TF}] - k_{off}[\mathrm{Prom.TF}]$$

Gene expression regulation by Transcription Factors (TF)

Solving for the TF bound Promoter:

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

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

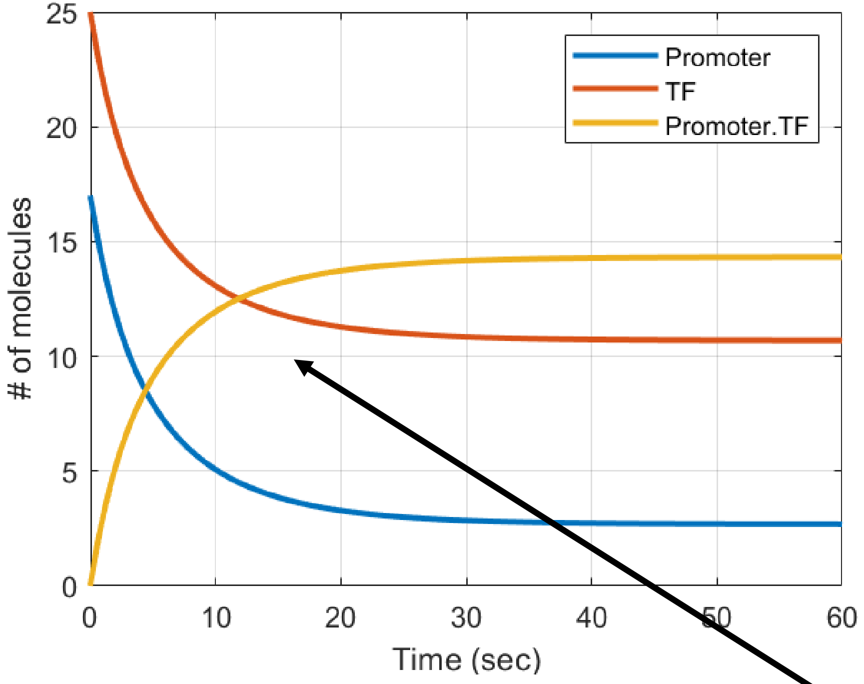
$$k_{on}\,[\mathrm{TF}]C_N = k_{on}\,[\mathrm{TF}][\mathrm{Prom.TF}] + k_{off}[\mathrm{Prom.TF}]$$

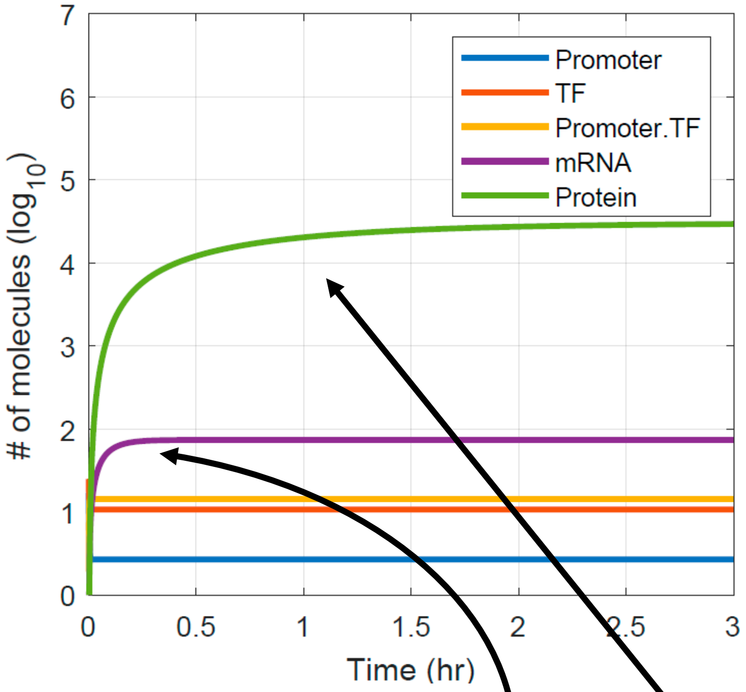
A bit of algebra...

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

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

Gene expression regulation by Transcription Factors (TF)





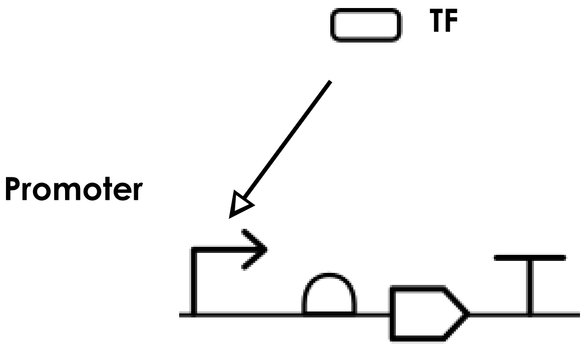
Note the difference in time scales: Binding in the seconds, transcription/translation from minutes to hours.

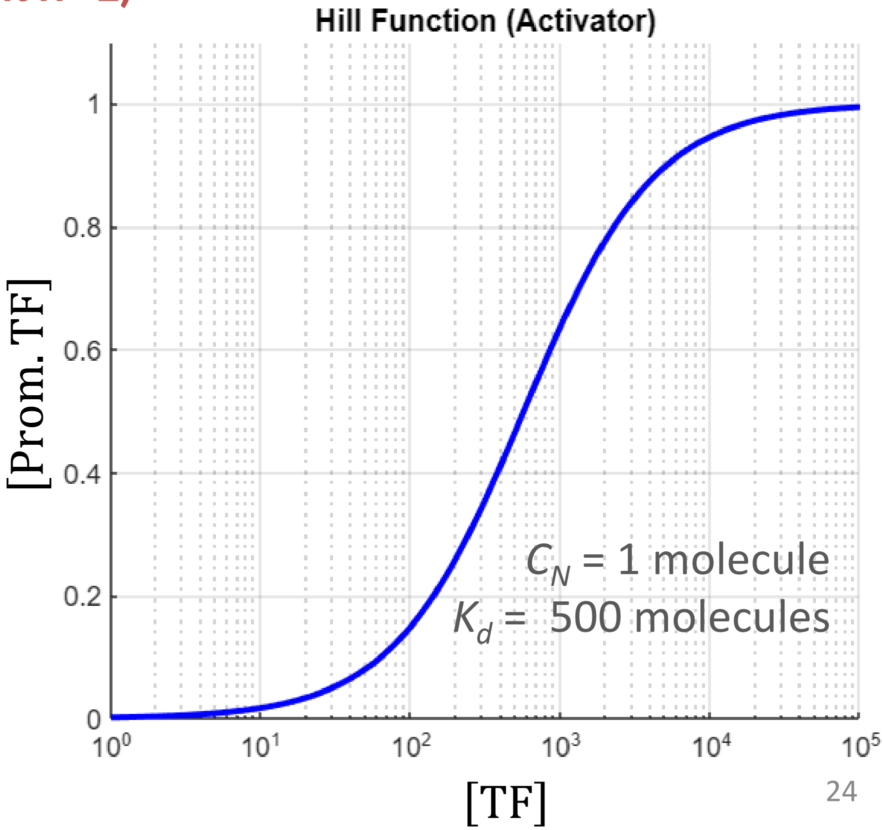
Gene expression regulation by Transcription Factors (TF)

We get the Hill function (with Hill coefficient n=1)

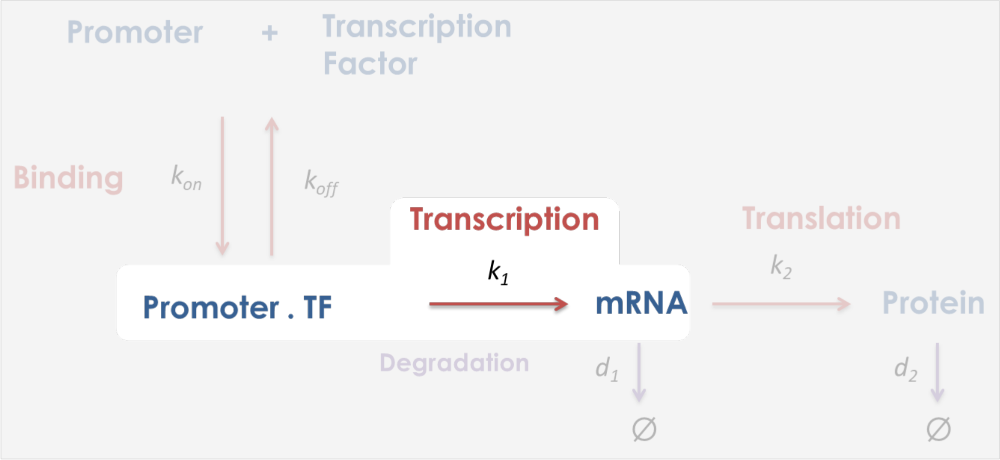
$$[\text{Prom.TF}] = C_N \frac{[\text{TF}]}{K_d + [\text{TF}]}$$

Activator





Gene expression regulation by Transcription Factors (TF)



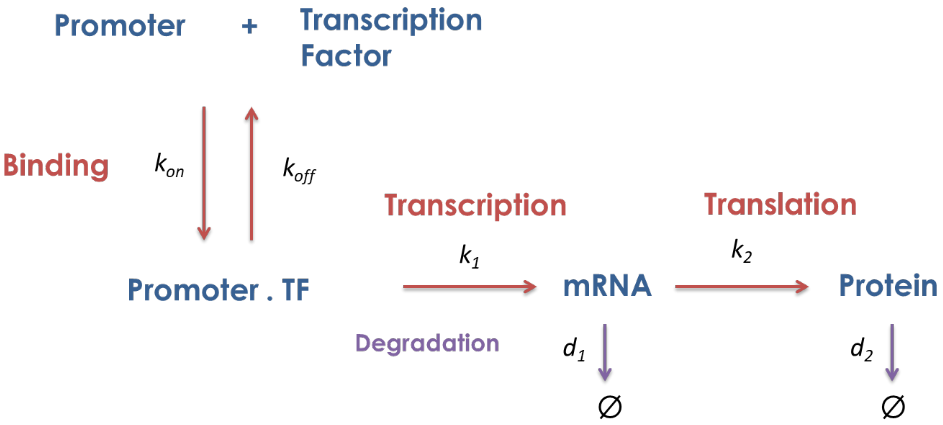
$$[\mathrm{Prom.TF}] = C_N \frac{[\mathrm{TF}]}{K_d + [\mathrm{TF}]}$$

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

The complete equation for the mRNA

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

Gene expression regulation by Transcription Factors (TF)

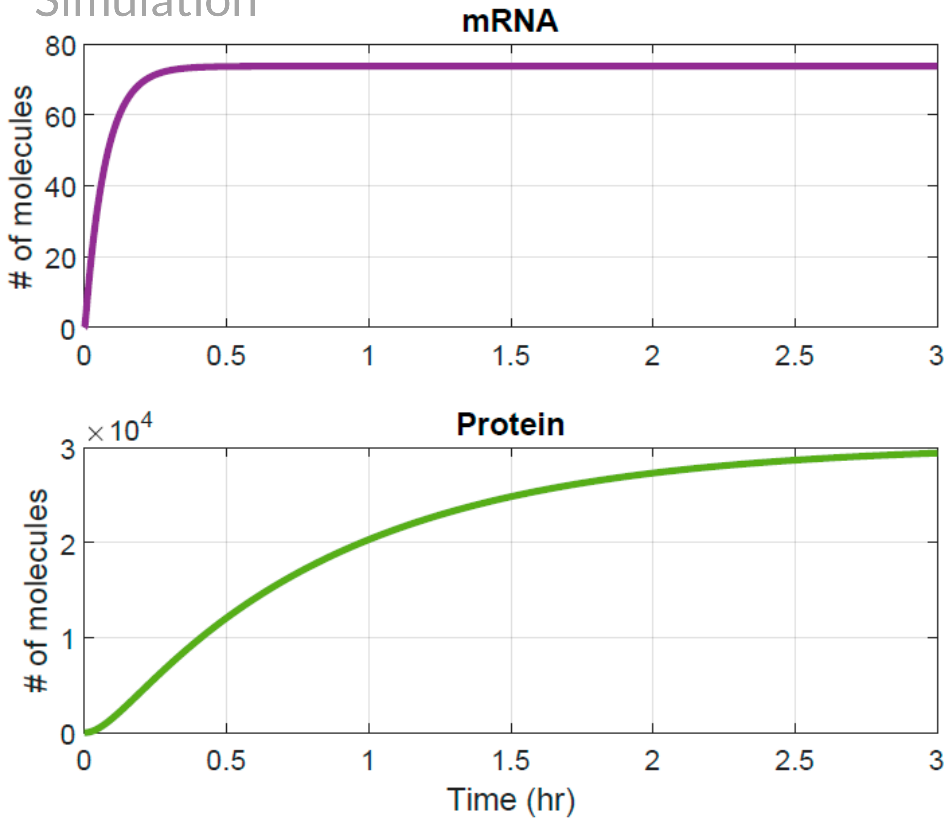


$$\frac{d[\mathrm{mRNA}]}{dt} = [\dot{\mathrm{mRNA}}] = k_1 \; C_N \frac{[\mathrm{TF}]}{K_d + [\mathrm{TF}]} - d_1[\mathrm{mRNA}]$$

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

Gene expression regulation by Transcription Factors (TF)

Simulation



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

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

Parameters:

CN = 17 molecules (Plasmid copy number)

Kd = 2 molecules

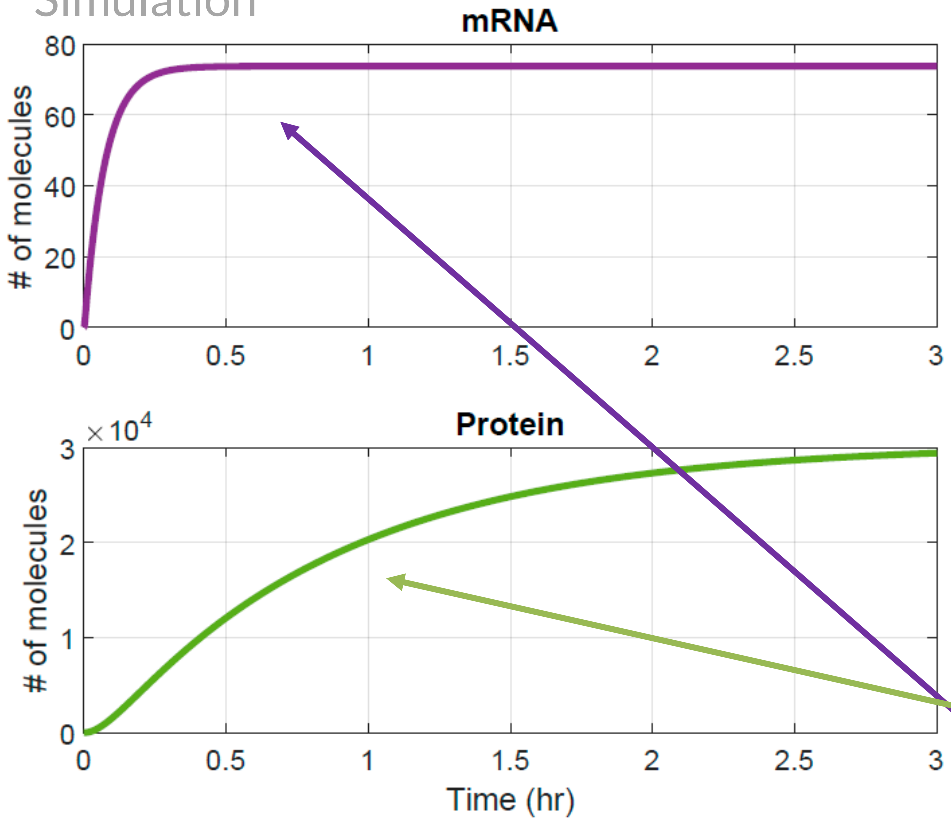
TF = 25 molecules (Transcription Factor)

The other parameters same than constitutive

`Main_TF.m`

Gene expression regulation by Transcription Factors (TF)

Simulation



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

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

Parameters:

CN = 17 molecules (Plasmid copy number)

Kd = 2 molecules

TF = 25 molecules (Transcription Factor)

The other parameters same than constitutive

Note the difference in time scales: transcription (mRNA) in minutes, translation (Protein) hours.

Gene expression regulation by Transcription Factors (TF)

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

$$\dot{[\mathrm{mRNA}]} \approx 0$$

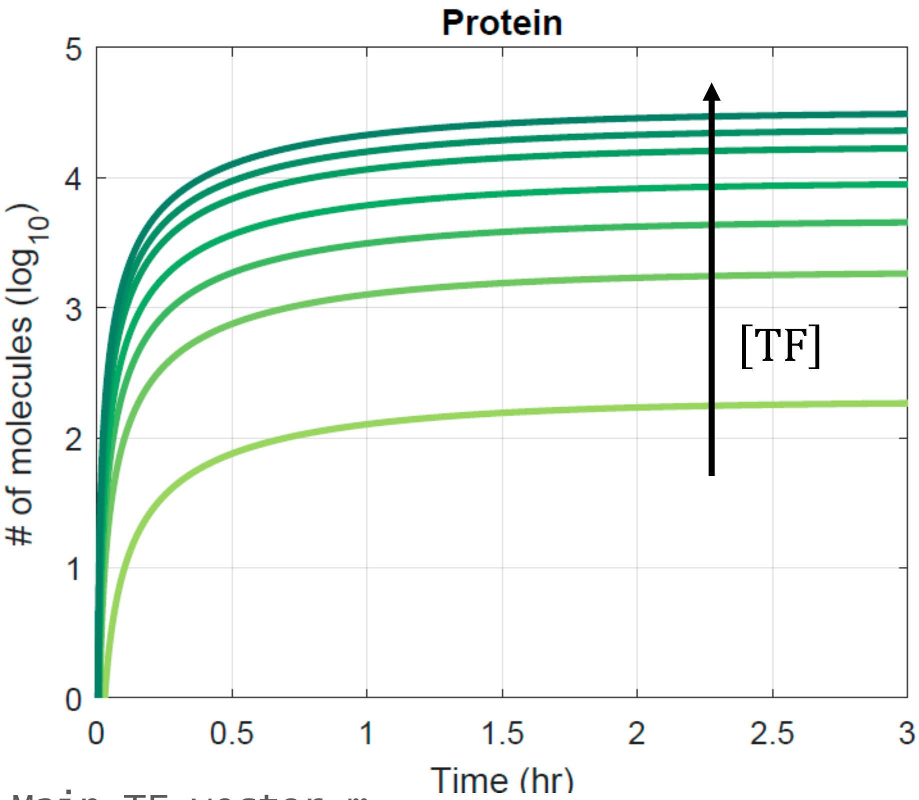
$$0 = k_1\, C_N \frac{[\mathrm{TF}]}{K_d + [\mathrm{TF}]} - d_1[\mathrm{mRNA}] \Longrightarrow [\mathrm{mRNA}] = \frac{k_1}{d_1} C_N \frac{[\mathrm{TF}]}{K_d + [\mathrm{TF}]}$$

$$\boxed{\frac{d[\mathrm{Protein}]}{dt} = \dot{[\mathrm{Protein}]} = \alpha\, \frac{[\mathrm{TF}]}{K_d + [\mathrm{TF}]} - d_2[\mathrm{Protein}]}$$

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

Gene expression regulation by Transcription Factors (TF)

Simulation



Main_TF_vector.m

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

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

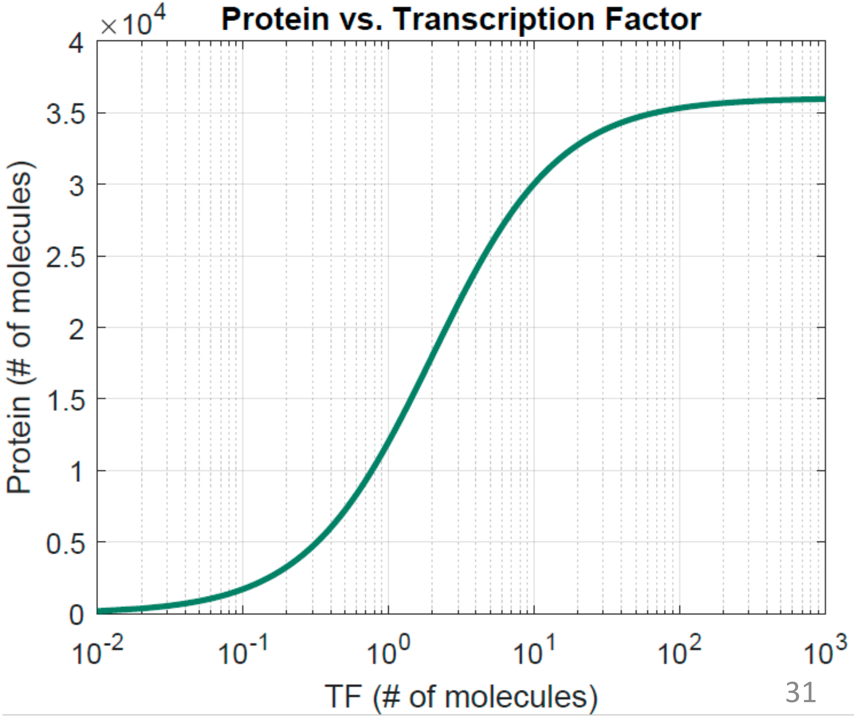
[TF]: from 0.1 molecule to 25 molecules of Transcription Factor

Gene expression regulation by Transcription Factors (TF)

Now, if we want the steady state we can use the same trick (QSSA) that we used before (equilibrium expression of protein, data at the end of the experiment)

$$[\dot{\text{Protein}}] \approx 0$$

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



protein_vs_TF.m

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

Stay tuned, next Section 3:

Hill function examples and intuitions: effects of parameters on activators, repressors, hybrid promoters, using a Matlab exploration package.