Inferring gene networks with single-cell data: from mechanistic modelling to statistics

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Biohasard

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Biological context

How do cells make decisions?

Example of decision making:

Differentiation: “stem” cell $\longrightarrow$ “mature” cell

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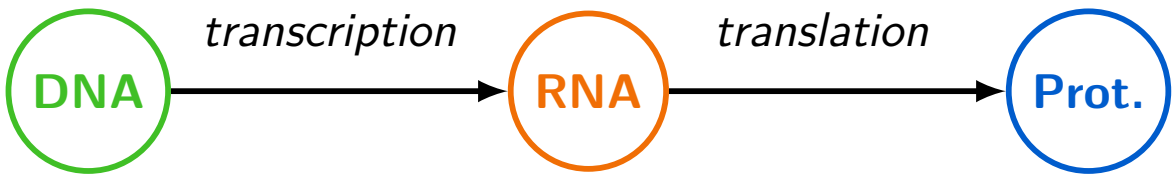

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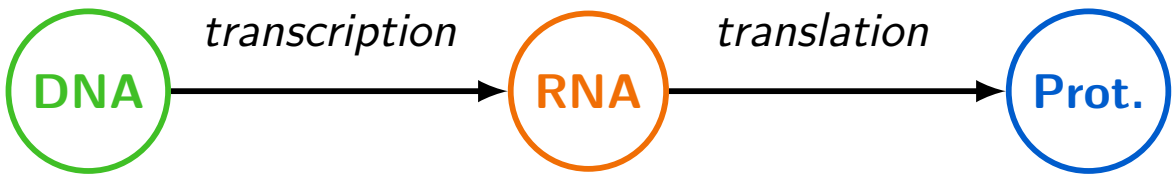


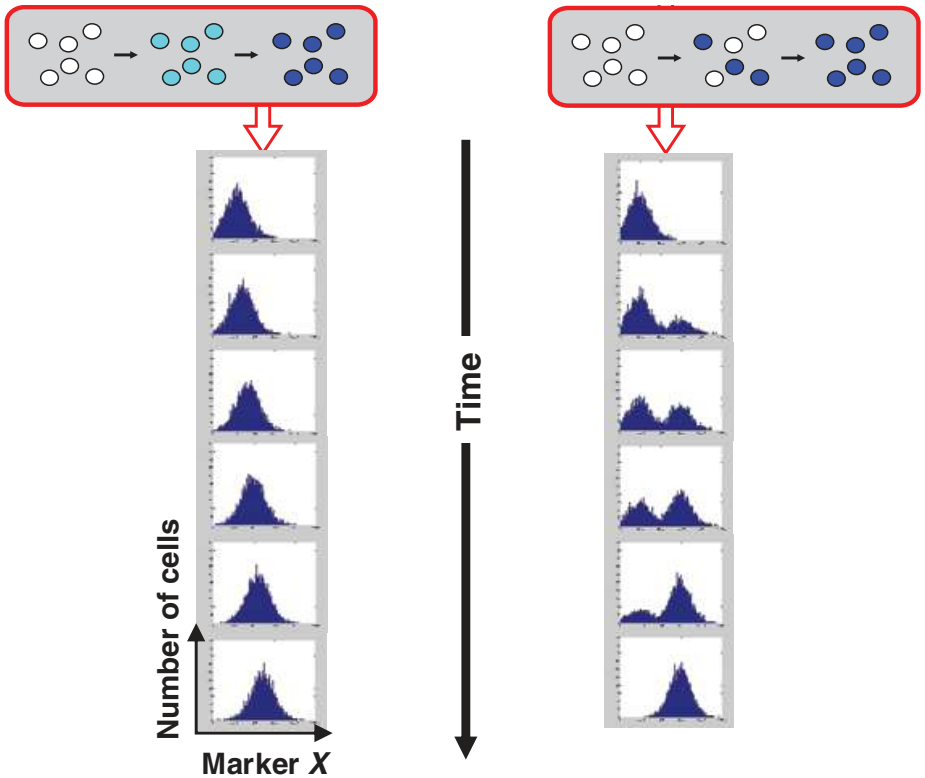
Basic idea of systems biology:

The behaviour of a cell emerges from interactions between genes

1. Why a stochastic model?

Differentiation: change of paradigm

« old school »



« new school »

S. Huang, Non-genetic heterogeneity of cells in development: more than just noise.

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Differentiation: change of paradigm

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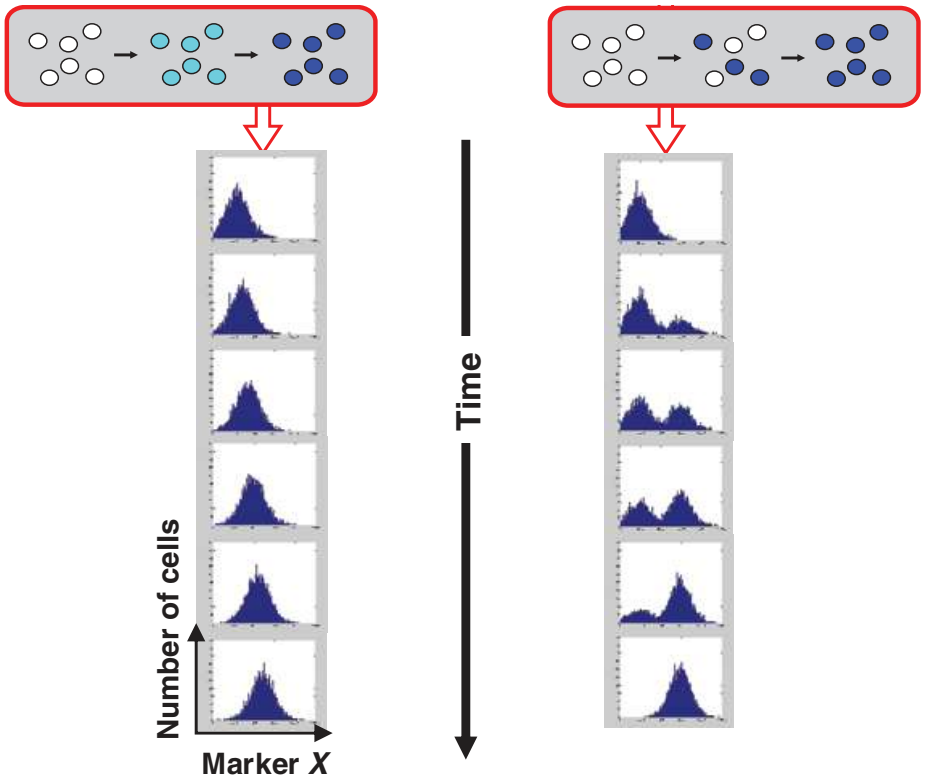

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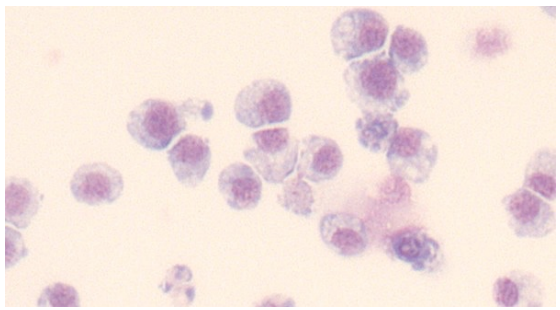
Remark

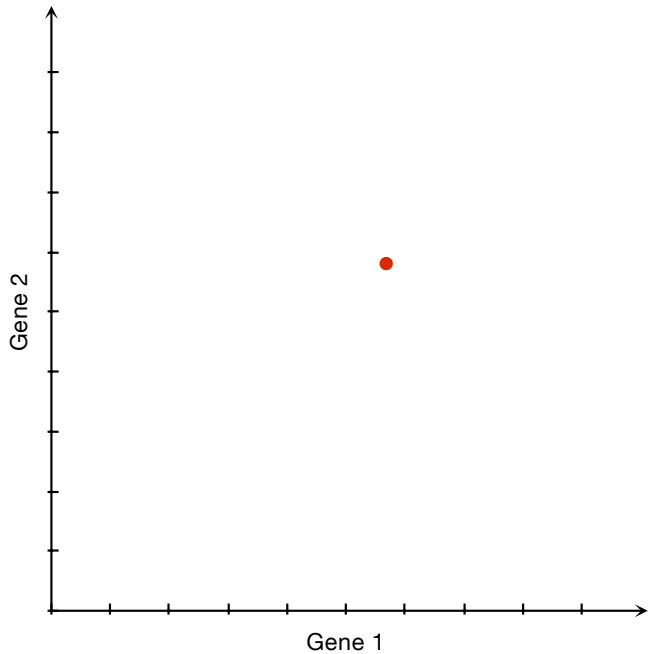
Seen on average (aka population or "bulk" data) as historically, these two paradigms are not distinguishable.

Population data

Cell population



Population average



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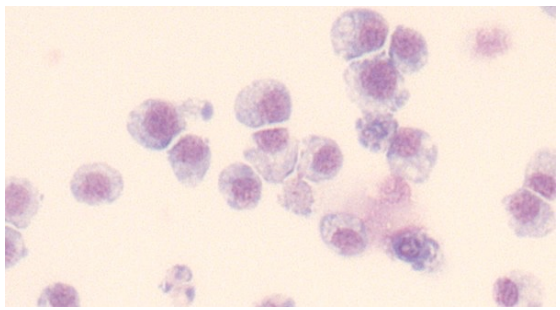
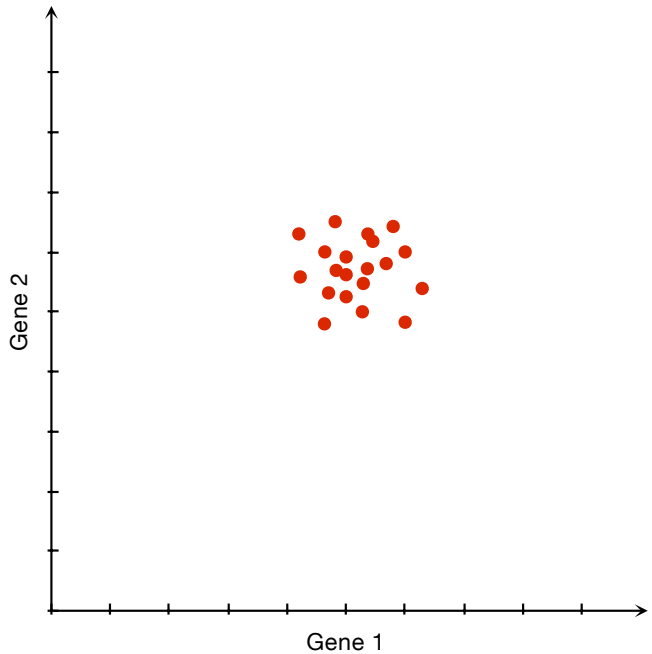


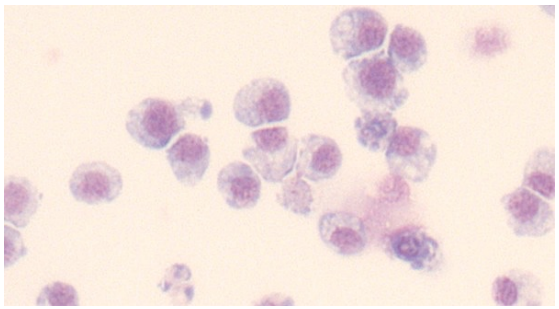
Repeats



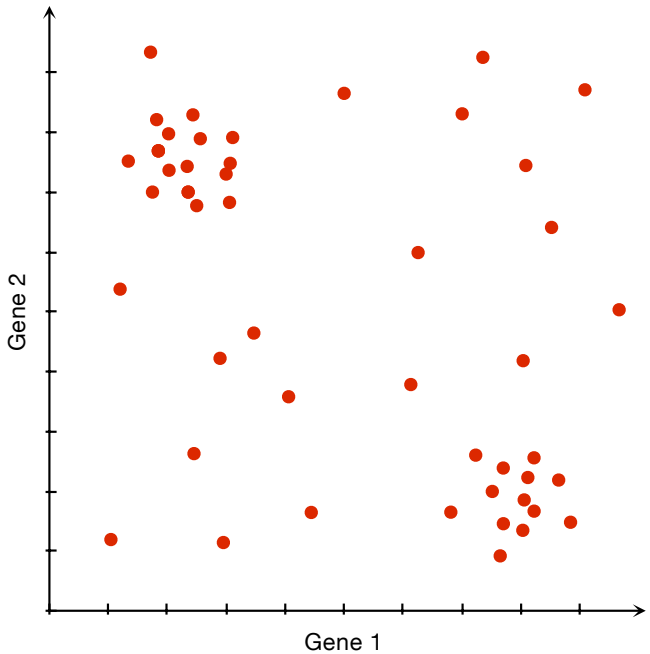
- Repeats only reveal technical noise
- To get biological variability, one has to *change conditions*

Single-cell data

Individual cells



Single cells, 1 exp.



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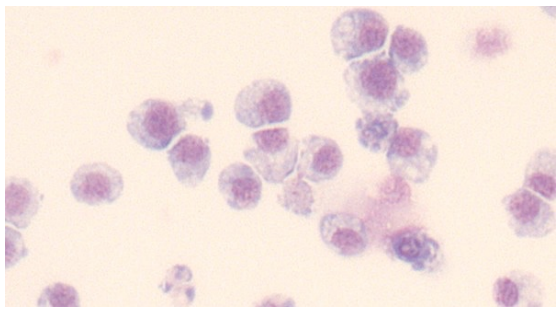

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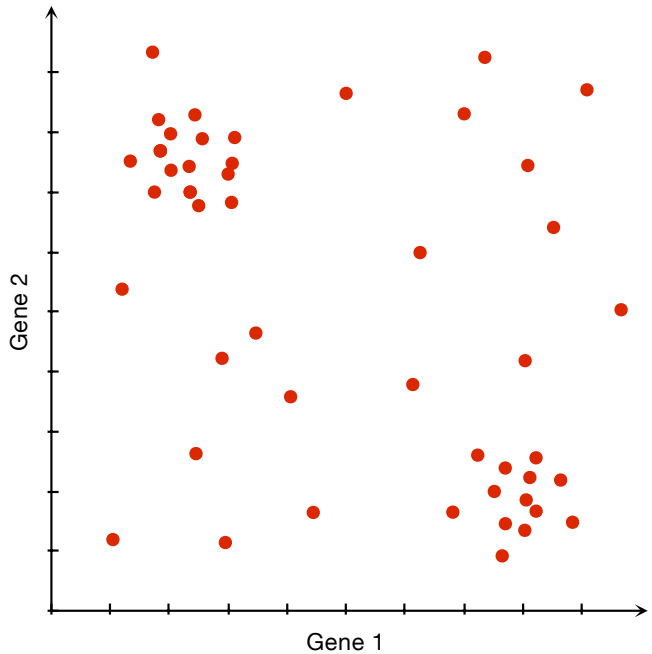
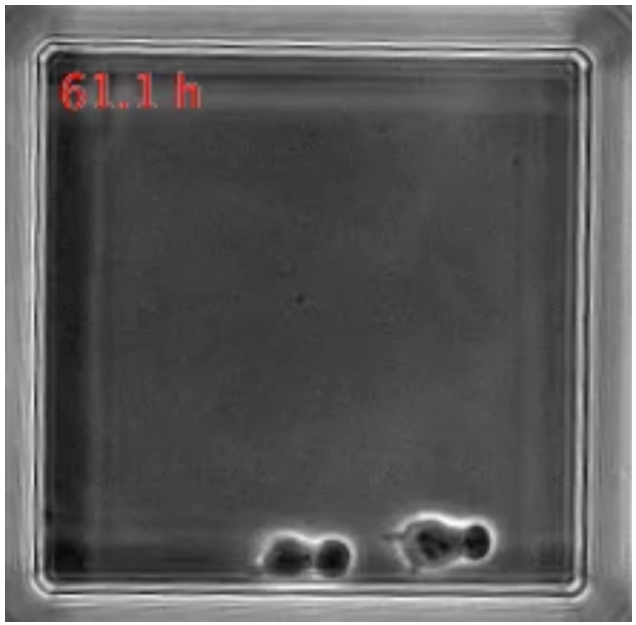


- Variability looks important...
- Sub-populations may appear: “molecular phenotypes”

A visual example



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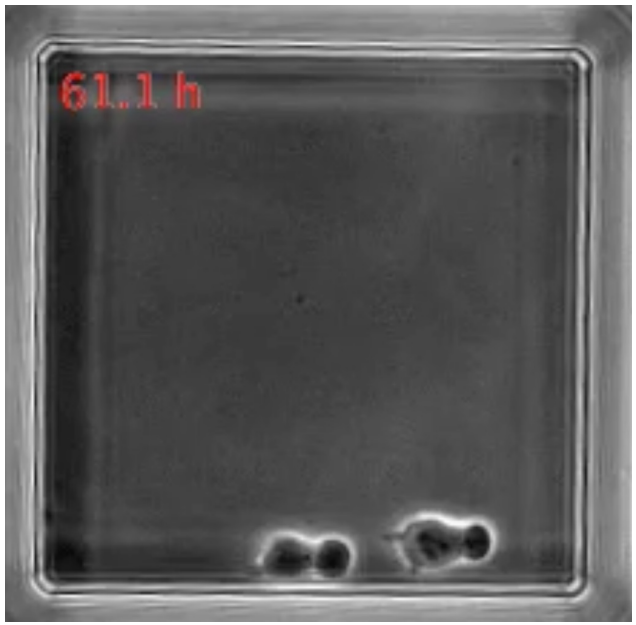

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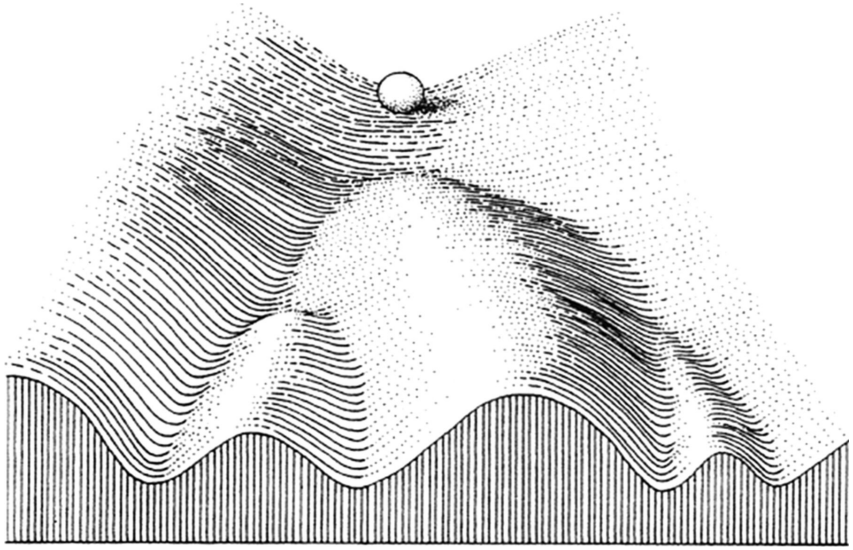
New paradigm

Gene expression is a stochastic phenomenon!

A modern view of Waddington landscapes



$\leftarrow$ 1942 !!!

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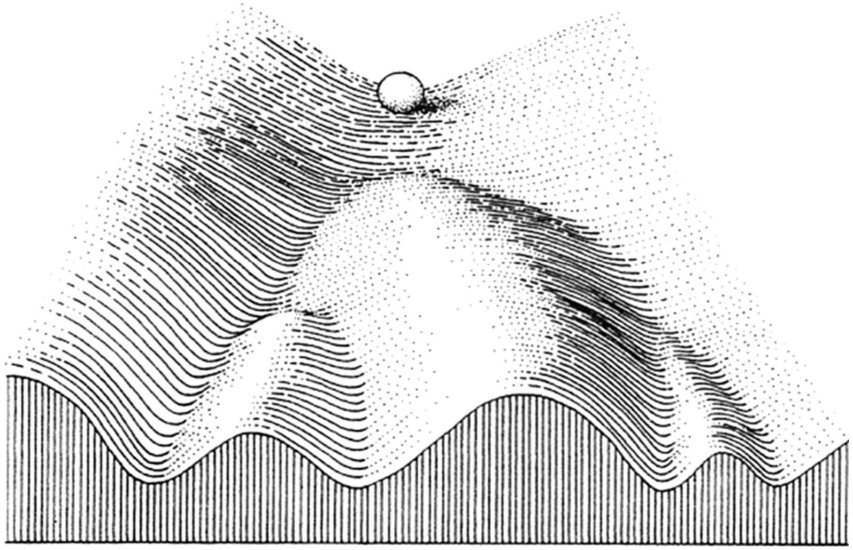

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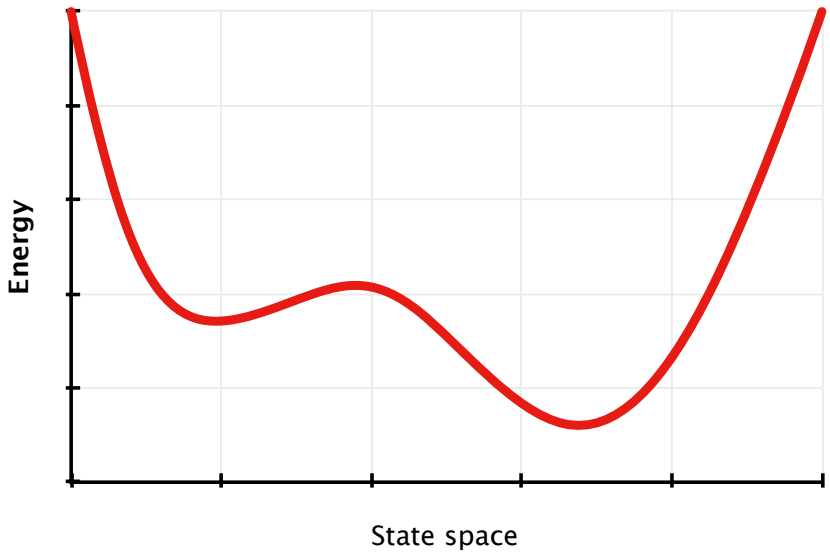

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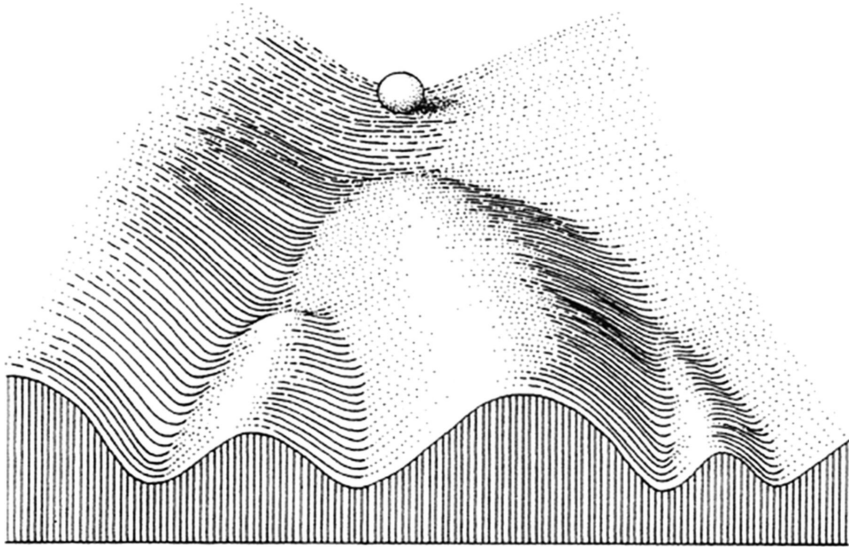

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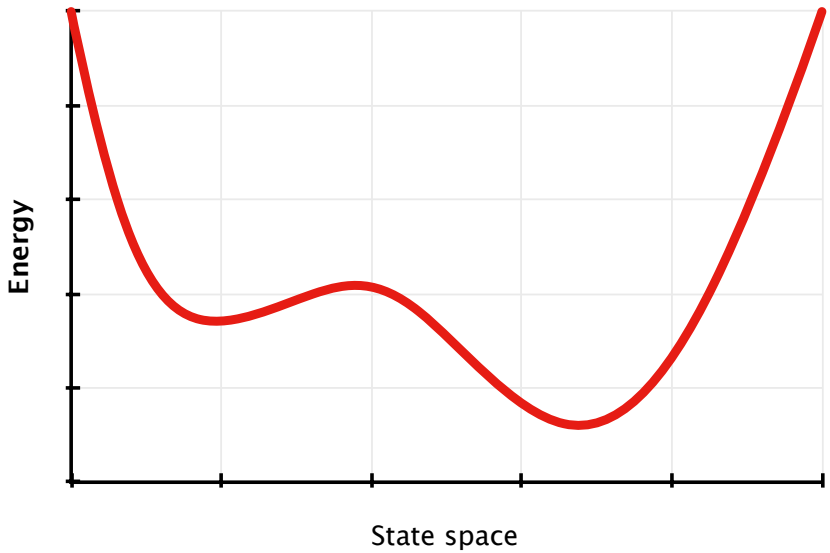
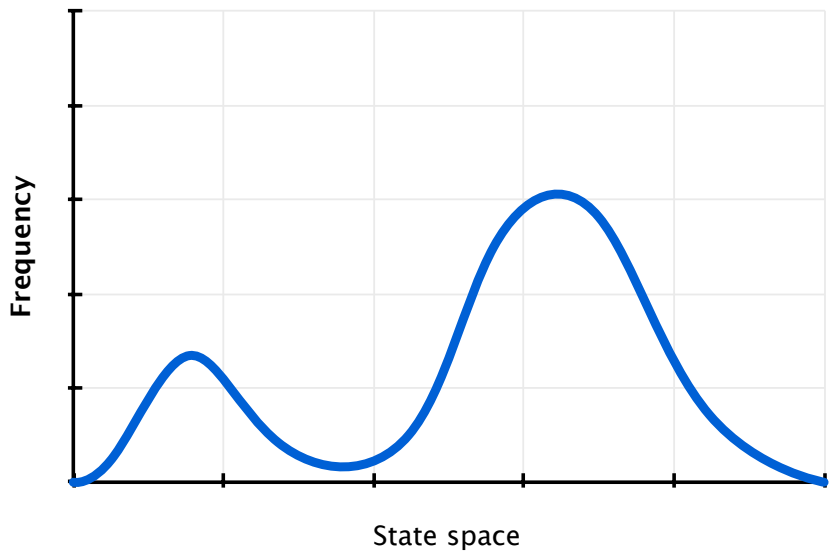
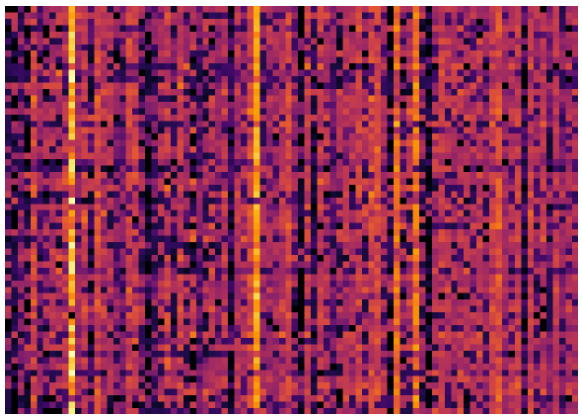




Statistical question

Gene expression levels



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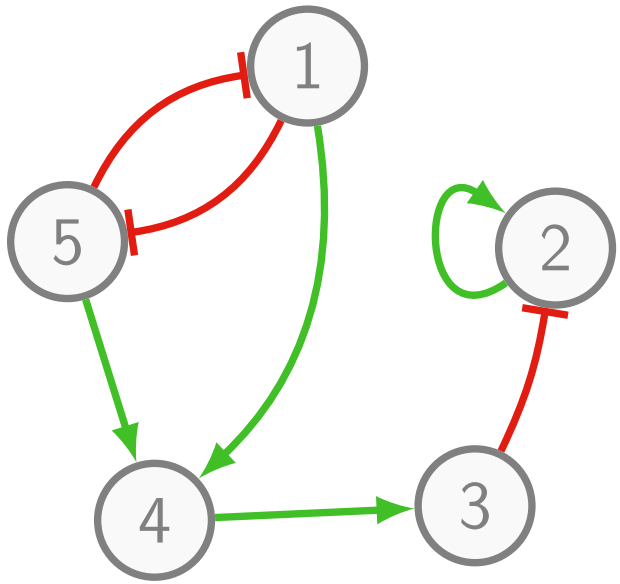
Gene regulatory network



"Gold standard" dilemma: use real data (*uncertain network*) or simulated data (*known network but unrealistic data*)?

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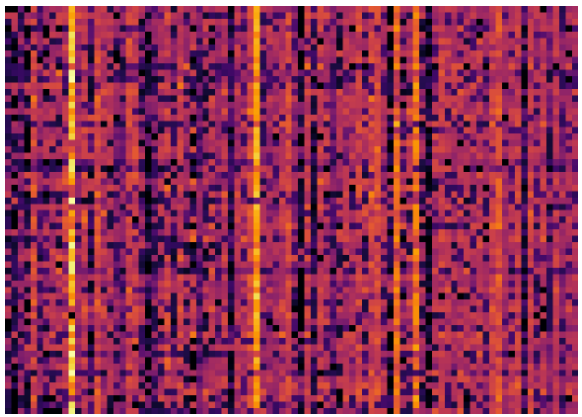

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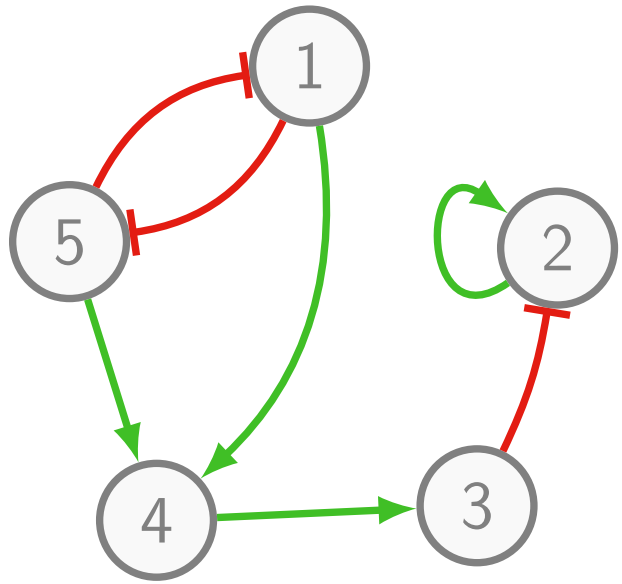
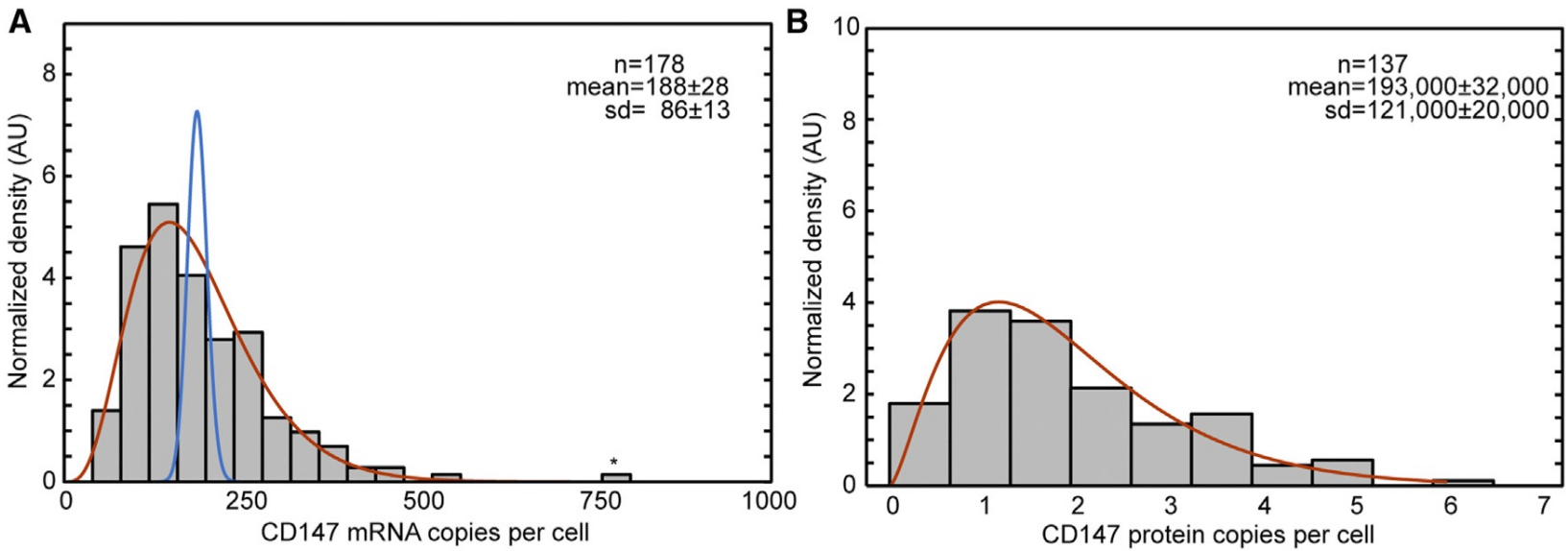


"Gold standard" dilemma: use real data (*uncertain network*) or simulated data (*known network but unrealistic data*)?

Our approach

1. Build a mechanistic (*hence stochastic*) gene network model
2. **Calibrate** the model, which will correspond to **infer** a network

What kind of stochasticity?



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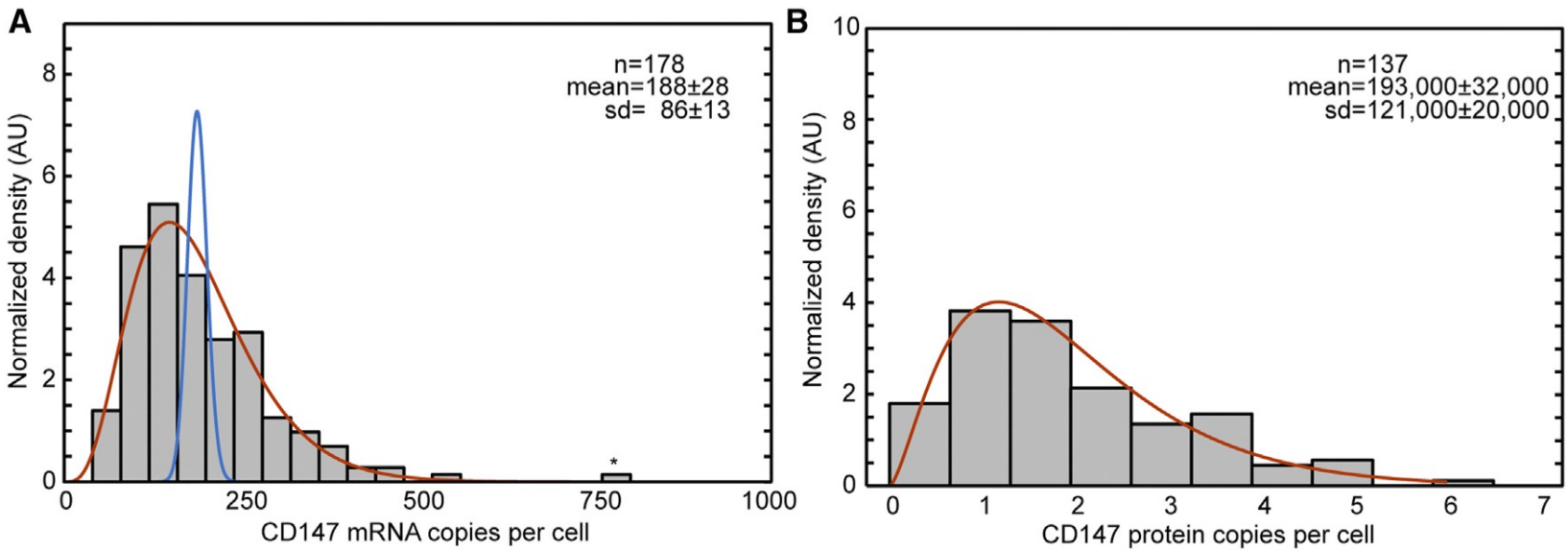

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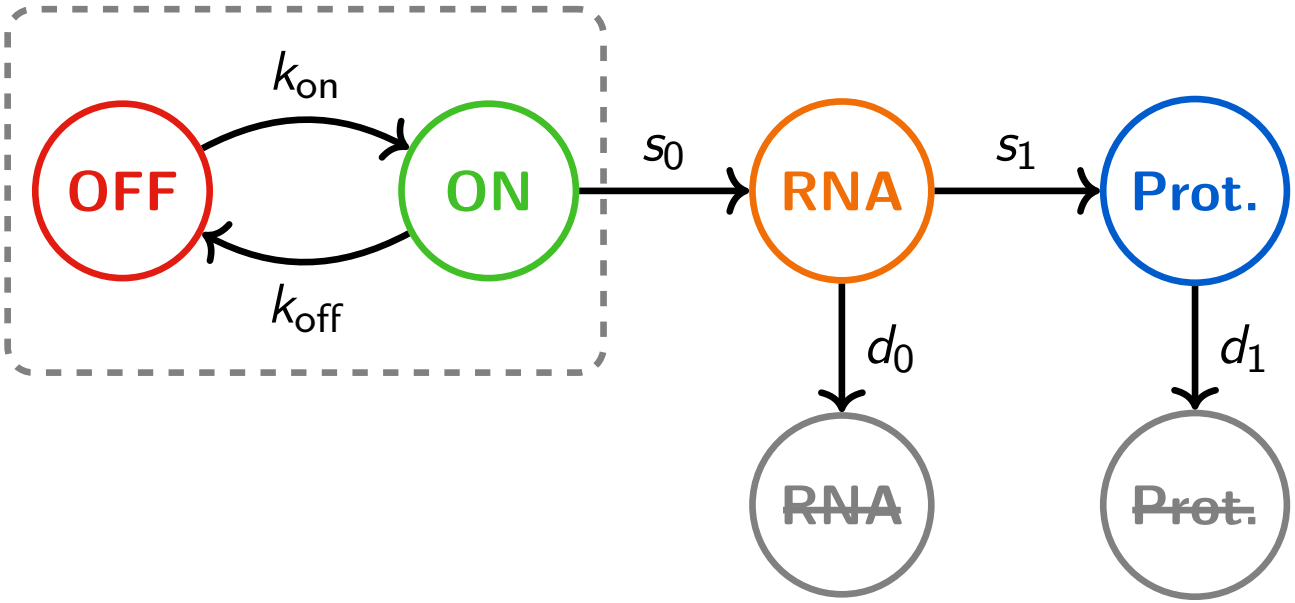
Remarks

- Typical distributions are **not Poisson** but rather **Gamma**
- Sometimes they appear as **mixtures** of Gamma distributions
- For this gene: $\langle \mathrm{mRNA} \rangle \approx 10^2$ and $\langle \mathrm{Protein} \rangle \approx 10^5$ copies/cell

2. Mechanistic network model

Building block for gene networks



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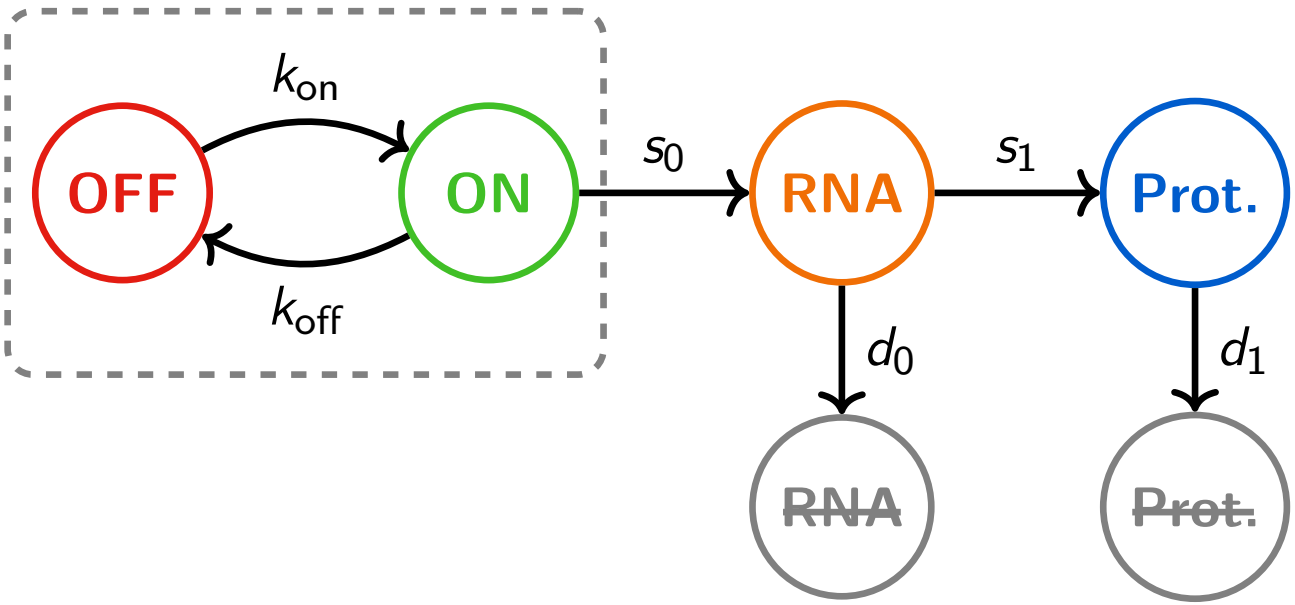

Remarks

- This model is **simple enough** to tackle it mathematically
- Rates k_{on}, k_{off} represent a set of many **underlying reactions**
- Can **reproduce data** when set in "bursty" regime ($k_{off} \gg k_{on}$)

Keeping only the most important noise

The only rare species is the **promoter state** $E(t) = 0$ or 1.

$$E(t) : 0 \xrightarrow{k_{\text{on}}} 1, \quad 1 \xrightarrow{k_{\text{off}}} 0$$

$$M'(t) = s_0 E(t) - d_0 M(t)$$

$$P'(t) = s_1 M(t) - d_1 P(t)$$

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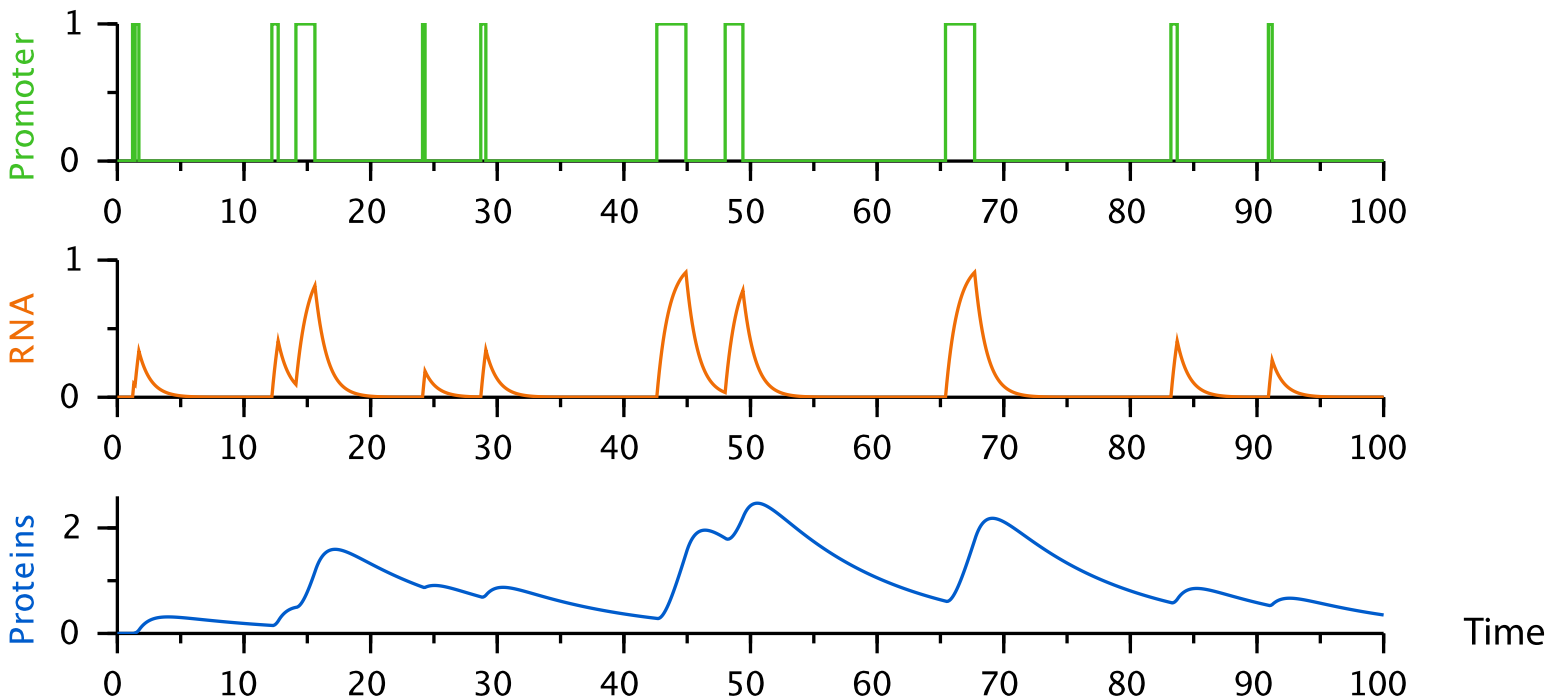
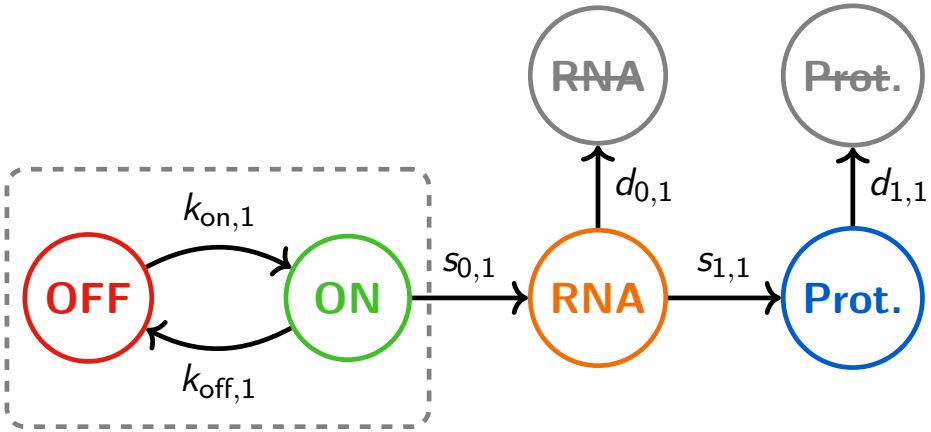
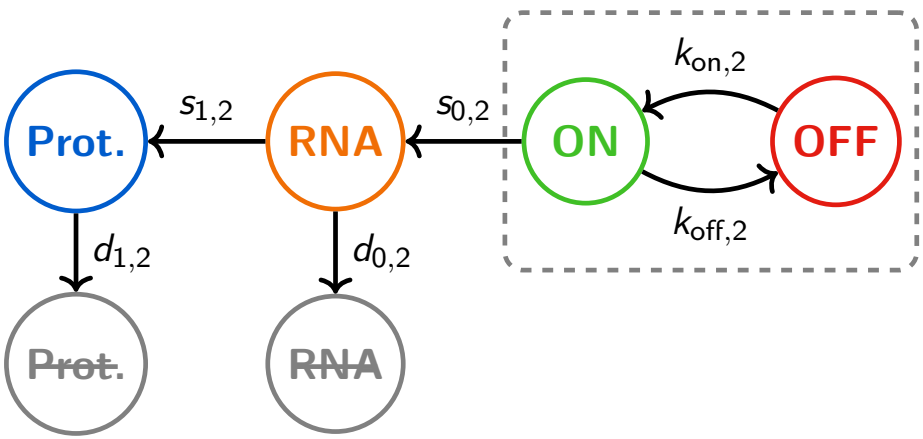


Interacting genes





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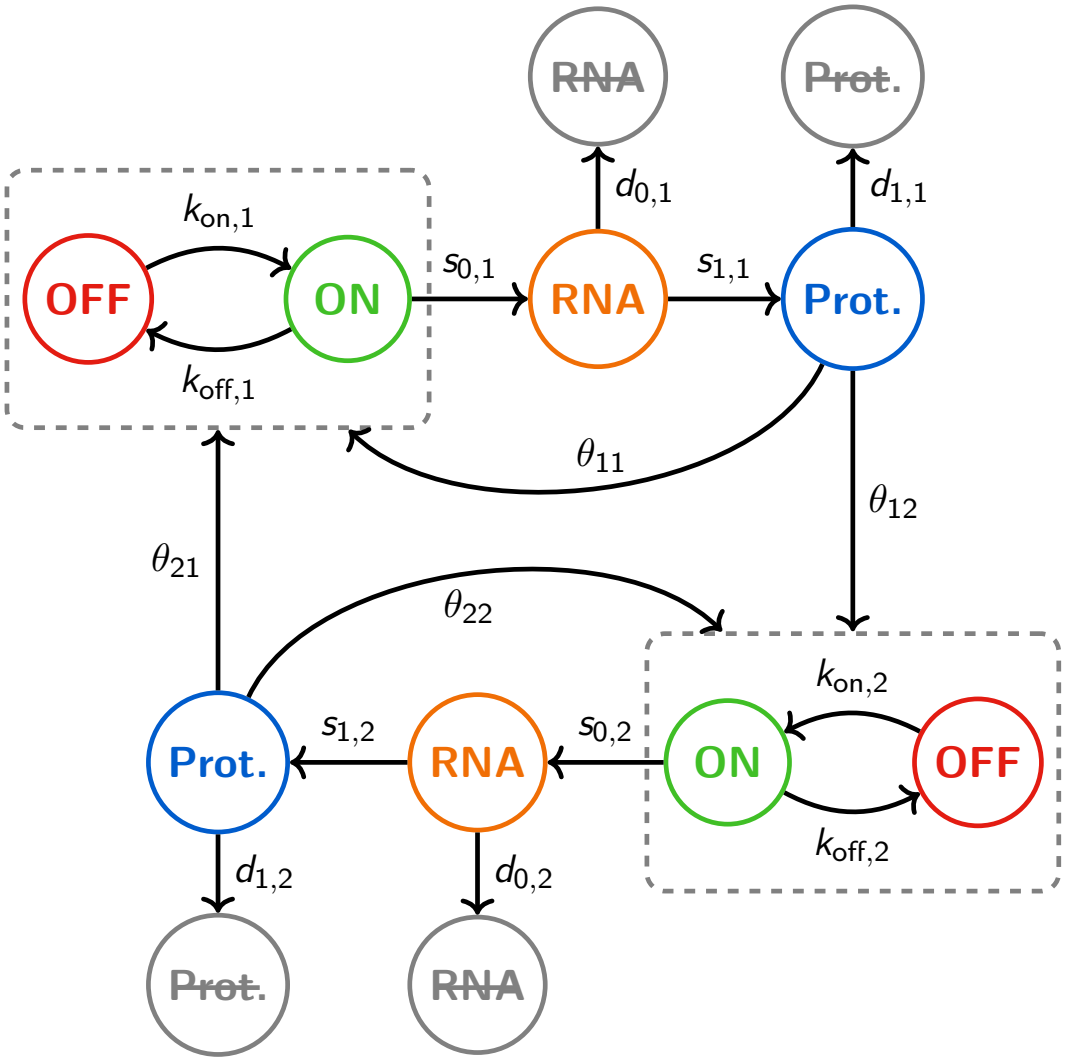

Principle: $k_{\text{on},i}$, $k_{\text{off},i}$ functions of proteins parameterized by $\theta = (\theta_{ij})_{1 \leqslant i,j \leqslant n}$

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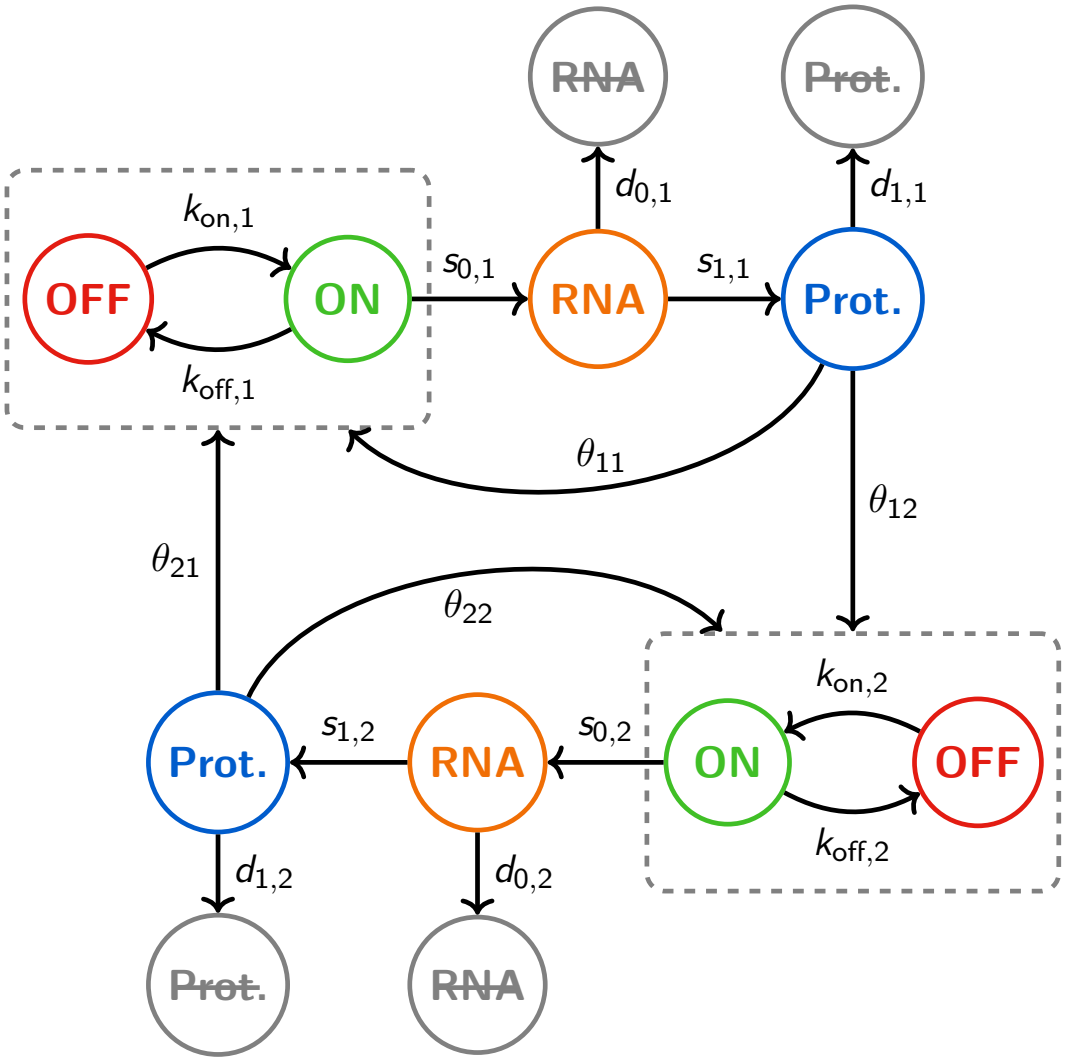

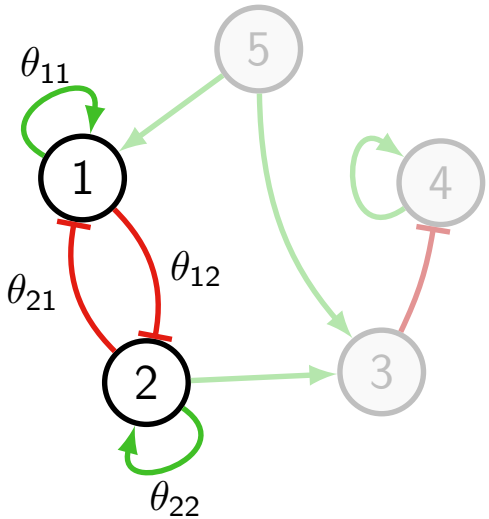

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Network model (dimensionless version)

We note n the number of genes in the network and write:

- $\mathbf{E} = (E_1, \ldots, E_n) \in \{0,1\}^n$ (promoters)
- $\mathbf{M} = (M_1, \ldots, M_n) \in [0,1]^n$ (mRNA)
- $\mathbf{P} = (P_1, \ldots, P_n) \in [0,1]^n$ (proteins)

We then consider the process $(\mathbf{E}(t), \mathbf{M}(t), \mathbf{P}(t))_{t \geqslant 0}$ defined by:

$$\forall i \in [\![1, n]\!], \quad \begin{cases} E_i(t) : 0 \xrightarrow{k_{\text{on},i}(\mathbf{P}(t))} 1, \quad 1 \xrightarrow{k_{\text{off},i}(\mathbf{P}(t))} 0 \\ M_i{}'(t) = d_{0,i}(E_i(t) - M_i(t)) \\ P_i{}'(t) = d_{1,i}(M_i(t) - P_i(t)) \end{cases}$$

Some known results

Theorem (Benaïm, Le Borgne, Malrieu and Zitt, 2015)

Suppose that the functions $k_{\mathrm{on},i}$ and $k_{\mathrm{off},i}$ are continuous and > 0 on $[0,1]^n$. Then $(\mathbf{E}(t), \mathbf{M}(t), \mathbf{P}(t))_{t \geqslant 0}$ is an **ergodic PDMP**.

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When promoters and mRNA are faster than proteins:

Deterministic limit (Faggionato, Gabrielli and Crivellari, 2010)

At the limit $[d_{1,i} / \min(d_{0,i}, k_{\mathrm{on},i}, k_{\mathrm{off},i})] \to 0$, proteins follow

$$\frac{\mathrm{d}\mathbf{P}}{\mathrm{d}t} = \Phi(\mathbf{P}) \quad \text{where} \quad \Phi_i(\mathbf{P}) = d_{1,i} \left(\frac{k_{\mathrm{on},i}(\mathbf{P})}{k_{\mathrm{on},i}(\mathbf{P}) + k_{\mathrm{off},i}(\mathbf{P})} - P_i \right).$$

There is also a diffusion limit (Pakdaman, Thieullen and Wainrib, 2012)

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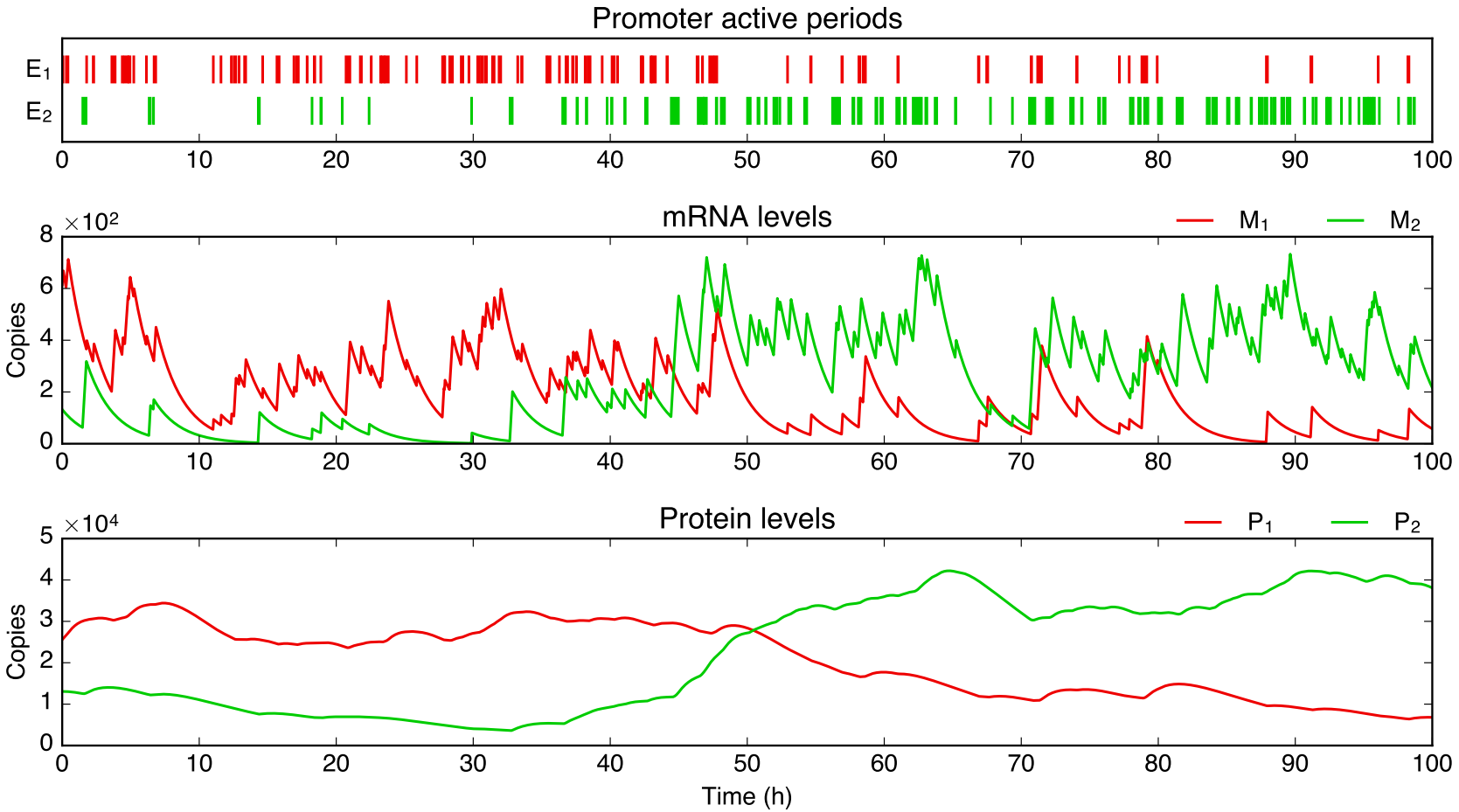
There is also a diffusion limit (Pakdaman, Thieullen and Wainrib, 2012)

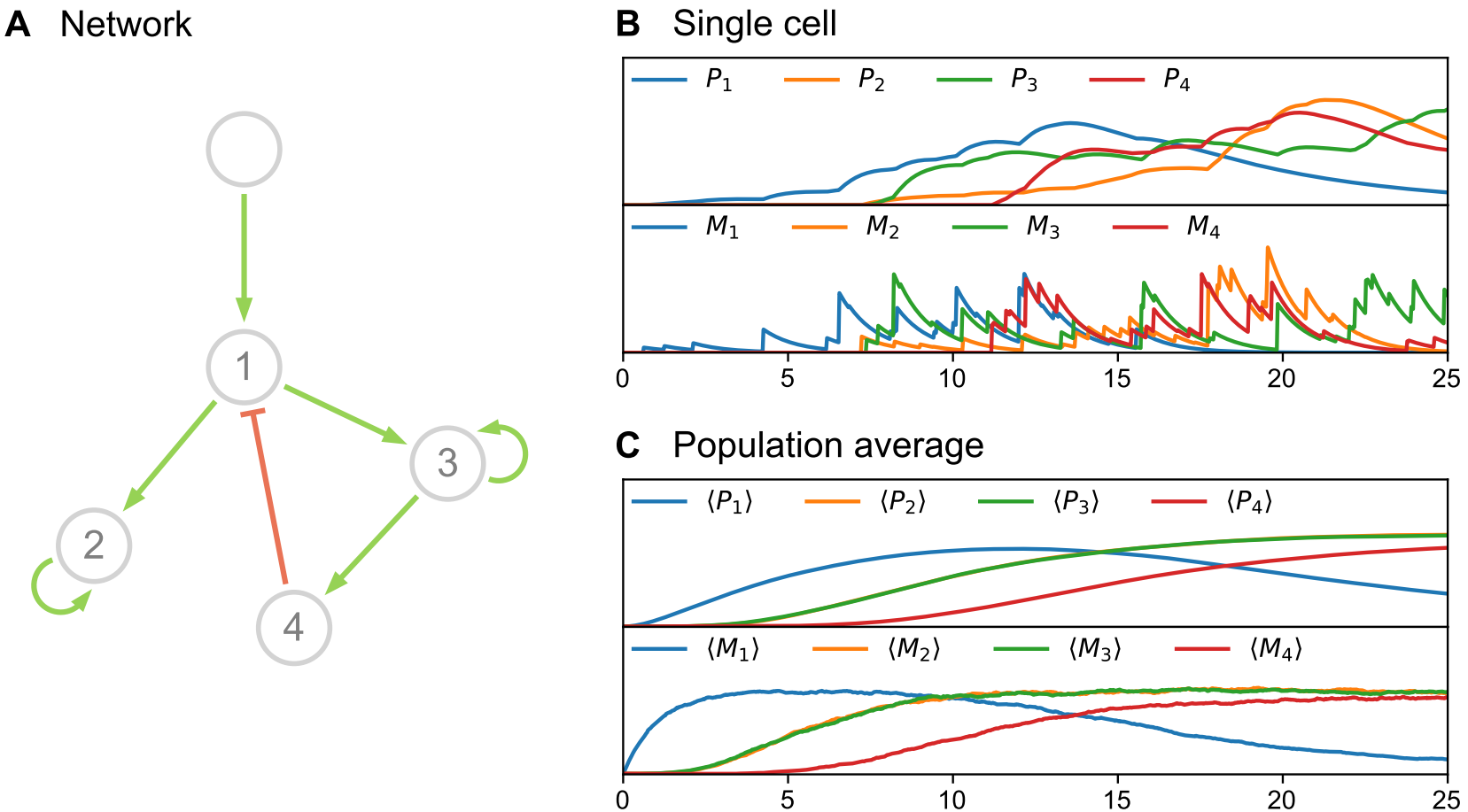
Idea: *we place ourselves in the case* $d_{1,i} \ll \min(d_{0,i}, k_{\mathrm{on},i}, k_{\mathrm{off},i})$, *but without passing directly to the limit* (in practice $d_{1,i}/d_{0,i} \approx 0.2$).

Example 1: two-gene "toggle switch"

$$\theta = \begin{pmatrix} + & - \\ - & + \end{pmatrix}$$



Example 2: four genes with stimulus



Two crucial simplifications

1. When $d_{1,i} \ll d_{0,i}$, we have an "intermediate" simplification:

$$\forall i \in [\![1, n]\!], \quad \begin{cases} E_i(t) : 0 \xrightarrow{k_{\text{on},i}(\mathbf{P}(t))} 1, \quad 1 \xrightarrow{k_{\text{off},i}(\mathbf{P}(t))} 0 \\ {P_i}'(t) = d_{1,i}(E_i(t) - P_i(t)) \end{cases}$$

2. We can then define (when $k_{\text{on},i} \ll k_{\text{off},i}$) :

$$\mathcal{L}(\mathbf{M}|\mathbf{P}) = \bigotimes_{i=1}^{n} \text{Gamma}\left(\frac{k_{\text{on},i}(\mathbf{P})}{d_{0,i}}, \frac{k_{\text{off},i}(\mathbf{P})}{d_{0,i}}\right)$$

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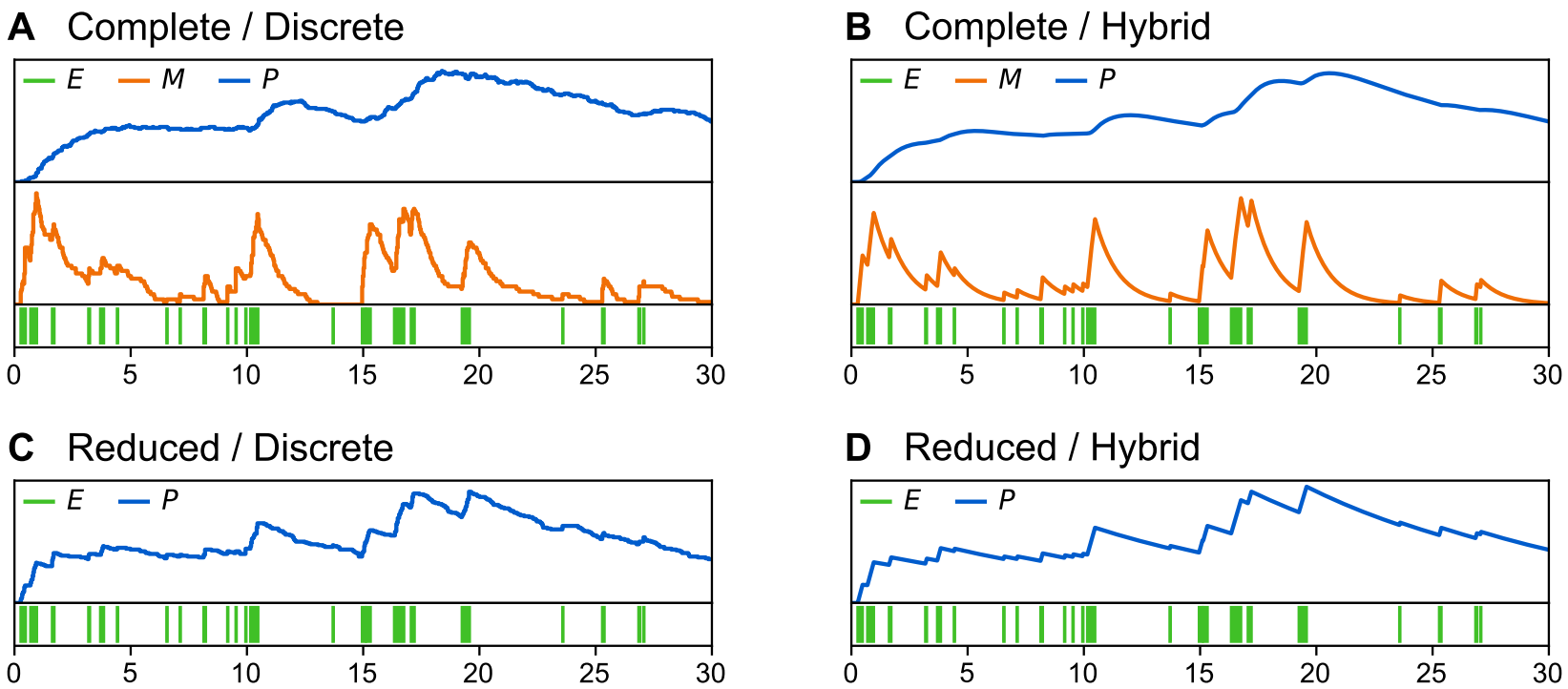
Remark

The reduced model $(\mathbf{E}(t), \mathbf{P}(t))_{t \geqslant 0}$ is still a PDMP with the same properties (ergodicity, same deterministic limit).

Comparing models



Remark

We shall use:

- Model A or B for **data simulation** ("gold standard")
- Model C or D for **building inference algorithms**

3. Deriving a statistical model

Inference strategy

1. Obtain a simple **analytical approximation** of the stationary distribution $p(\mathbf{x}, \mathbf{y}|\theta)$ of mRNA $\mathbf{x} = (x_i)$ and proteins $\mathbf{y} = (y_i)$
2. Replace $\theta = (\theta_{ij})$ by a **variational parameter** $\alpha(t) = (\alpha_{ij}(t))$
3. Use $p(\mathbf{x}, \mathbf{y}|\alpha(t))$ as a **statistical likelihood** to be maximized

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Statistical model for cell k observed at time t_k

$$p(\mathbf{y}_k) = \prod_{i=1}^{n} {y_{ki}}^{c_i\sigma_{ki}-1} e^{-c_i y_{ki}} \frac{{c_i}^{c_i\sigma_{ki}}}{\Gamma(c_i\sigma_{ki})}$$

$$p(\mathbf{x}_k|\mathbf{y}_k) = \prod_{i=1}^{n} {x_{ki}}^{a_i\sigma_{ki}-1} e^{-b_i x_{ki}} \frac{{b_i}^{a_i\sigma_{ki}}}{\Gamma(a_i\sigma_{ki})}$$

Inference strategy

1. Obtain a simple **analytical approximation** of the stationary distribution $p(\mathbf{x}, \mathbf{y}|\theta)$ of mRNA $\mathbf{x} = (x_i)$ and proteins $\mathbf{y} = (y_i)$
2. Replace $\theta = (\theta_{ij})$ by a **variational parameter** $\alpha(t) = (\alpha_{ij}(t))$
3. Use $p(\mathbf{x}, \mathbf{y}|\alpha(t))$ as a **statistical likelihood** to be maximized

Statistical model for cell k observed at time t_k

$$p(\mathbf{y}_k) = \prod_{i=1}^{n} {y_{ki}}^{c_i\sigma_{ki}-1} e^{-c_i y_{ki}} \frac{{c_i}^{c_i\sigma_{ki}}}{\Gamma(c_i\sigma_{ki})}$$

$$p(\mathbf{x}_k|\mathbf{y}_k) = \prod_{i=1}^{n} {x_{ki}}^{a_i\sigma_{ki}-1} e^{-b_i x_{ki}} \frac{{b_i}^{a_i\sigma_{ki}}}{\Gamma(a_i\sigma_{ki})}$$

Interaction function (choice of $k_{\mathrm{on},i}$)

$$\sigma_{ki}(\mathbf{y}_k) = \frac{\exp(\beta_i + \sum_j \alpha_{ji}(t_k) y_{kj})}{1 + \exp(\beta_i + \sum_j \alpha_{ji}(t_k) y_{kj})}$$

Self-consistent field approximation

Aim: approximate the stationary distribution $p(\mathbf{y})$ of $(\mathbf{P}(t))_{t \geqslant 0}$

Hartree approximation (Walczak, Sasai and Wolynes, 2005)

Locally independent promoters but which are subject to a common "proteomic field": in other words $p(\mathbf{y}) \approx h(\mathbf{y})$ with

$$h(\mathbf{y}) = \prod_{i=1}^{n} \frac{{y_i}^{a_i(\mathbf{y})-1}(1-y_i)^{b_i(\mathbf{y})-1}}{\mathrm{B}(a_i(\mathbf{y}), b_i(\mathbf{y}))}$$

where $a_i(\mathbf{y}) = k_{\mathrm{on},i}(\mathbf{y})/d_{1,i}$ and $b_i(\mathbf{y}) = k_{\mathrm{off},i}(\mathbf{y})/d_{1,i}$.

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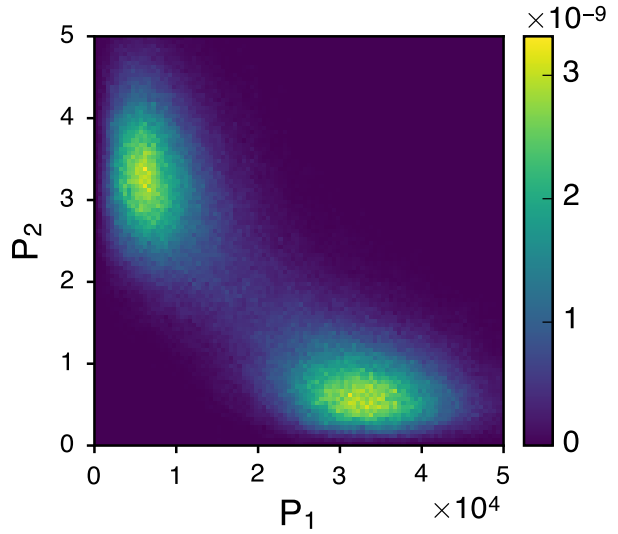
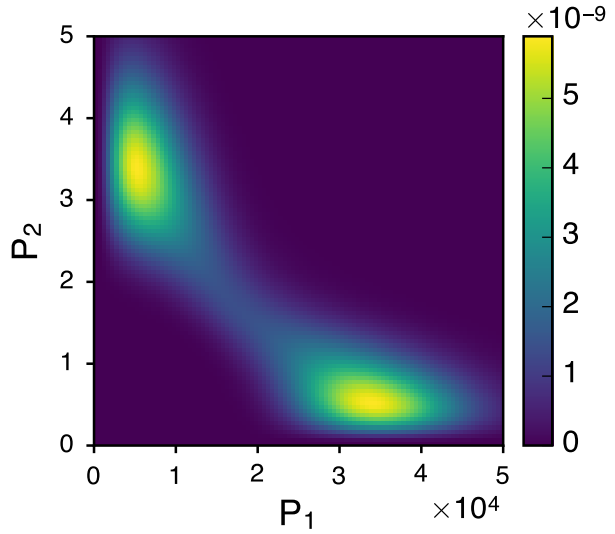
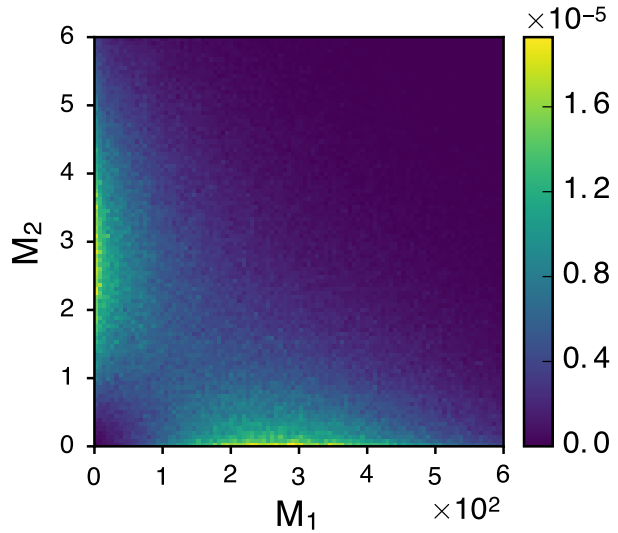
Why it should work: a concentration result

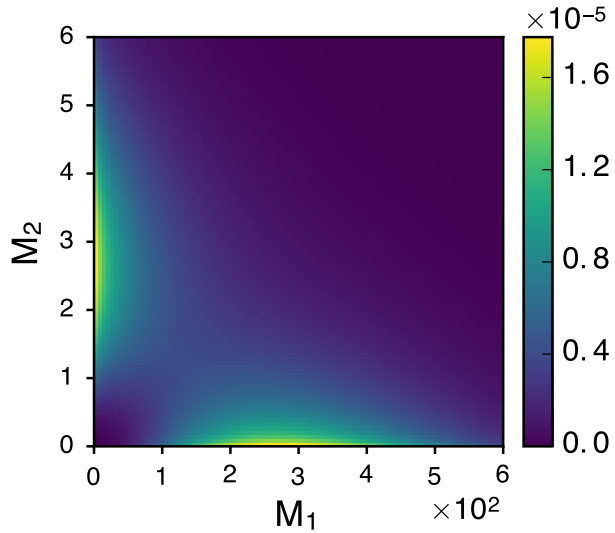
At the limit $d_{1,i} \ll \min(d_{0,i}, k_{\mathrm{on},i}, k_{\mathrm{off},i})$, the function h converges to a sum of Dirac measures $\delta_{\bar{\mathbf{y}}_k}$ where the $\bar{\mathbf{y}}_k$ are exactly the **fixed points of the previous deterministic system** (i.e. $\Phi(\bar{\mathbf{y}}_k) = 0$).

Distributions: exact vs. approximate

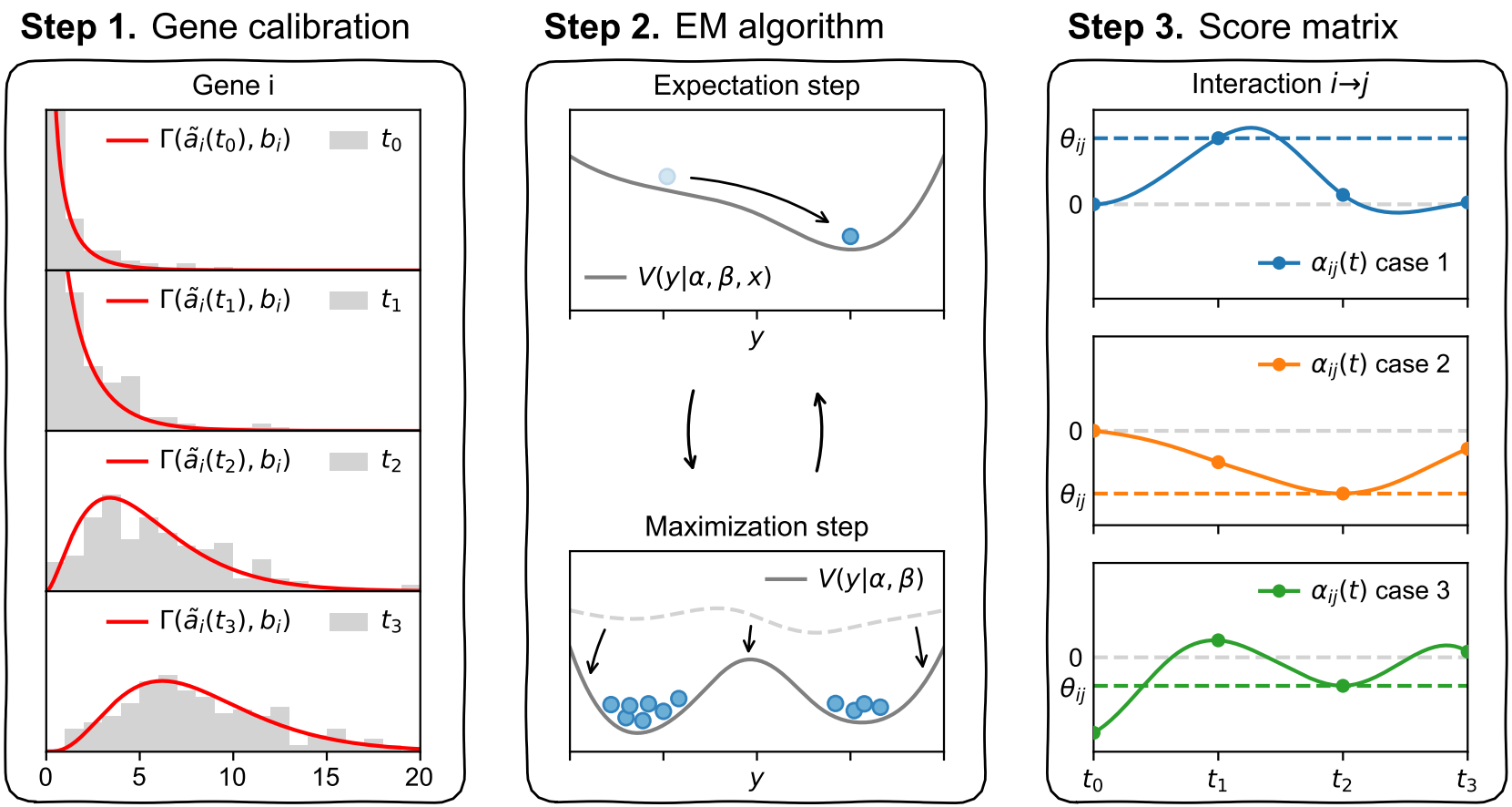




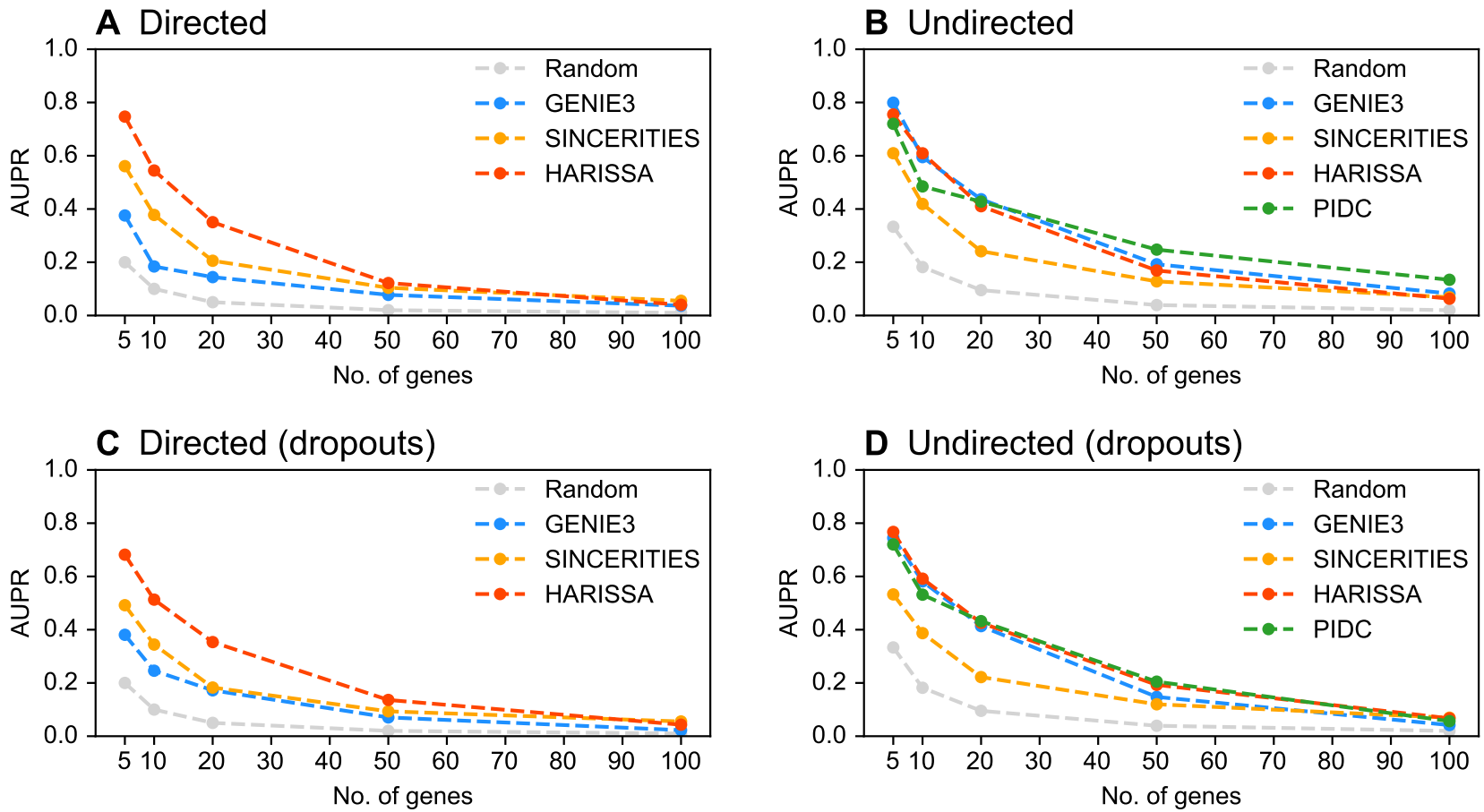




Inference in practice

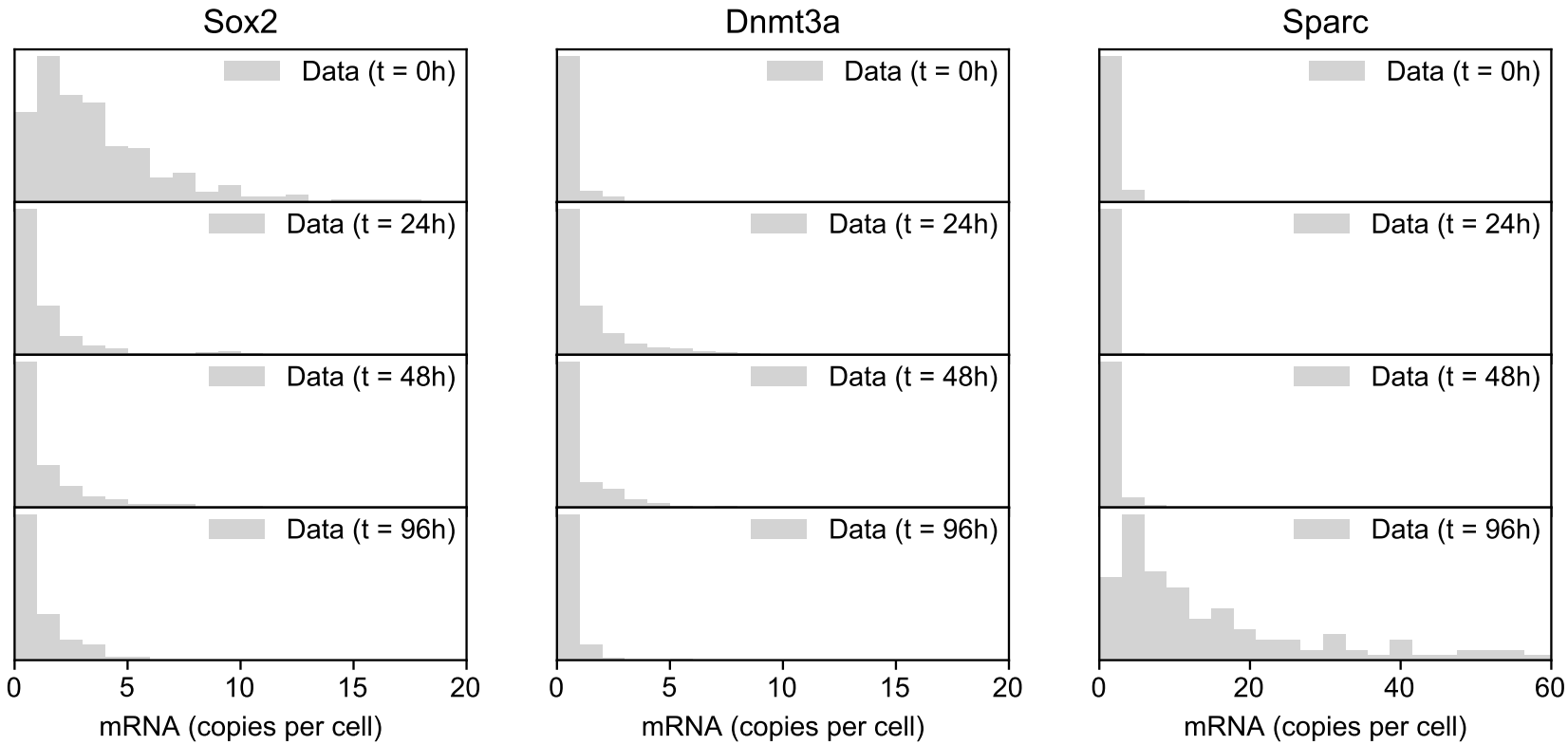


Small benchmark (PDMP network model)



Data: 10 time points with 100 cells per time point (1000 cells sampled per data set)
Networks: random directed trees (uniform distribution) with stimulus and activations

Real data

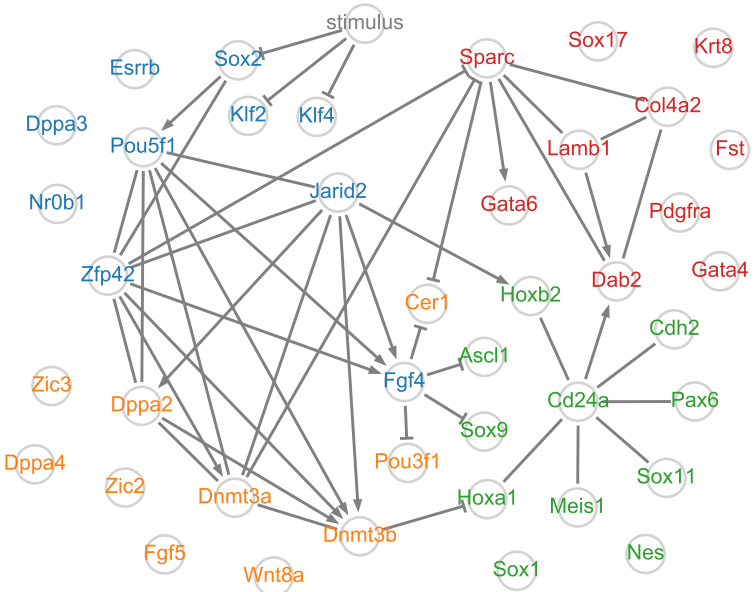


Semrau *et al.*, Dynamics of lineage commitment revealed by single-cell transcriptomics of differentiating embryonic stem cells. *Nature Communications*, 8(1)2017

Data: 9 time points with 272 cells on average per time point (between 137 and 335)
Inference: particular subset of 41 genes considered in [Semrau *et al.*, 2017]

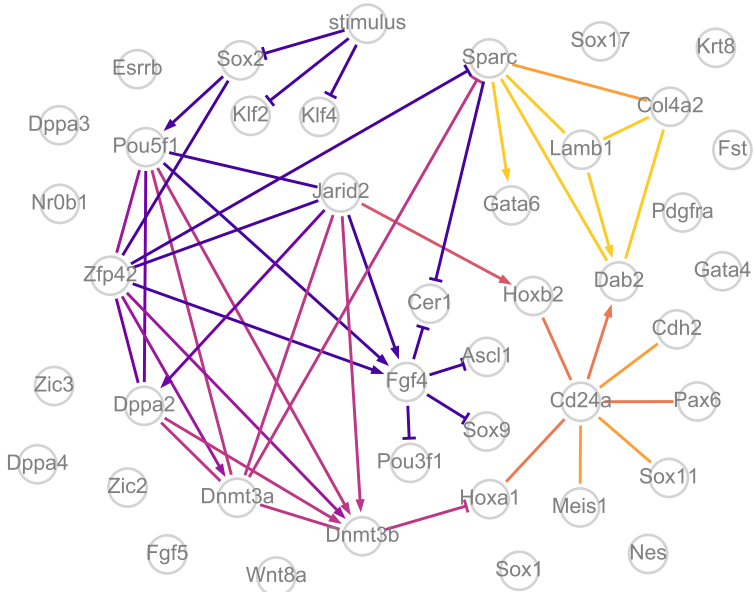
First result: two viewpoints

A Inferred network



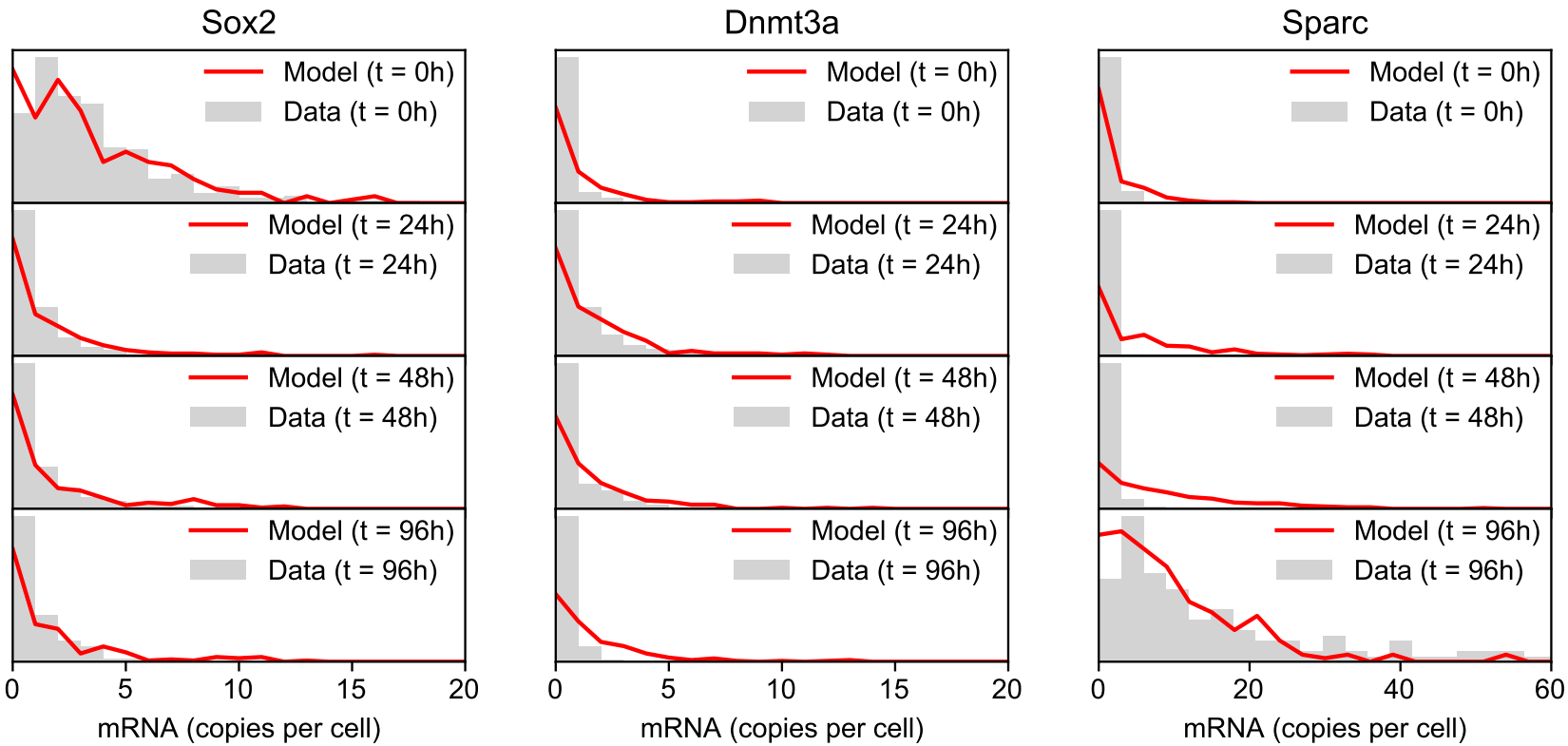
Pluripotency
Extraembryonic endoderm
Post-implantation epiblast
Neuroectoderm

B Time decomposition



6h 12h 24h 36h 48h 60h 72h 96h

Back to the mechanistic model

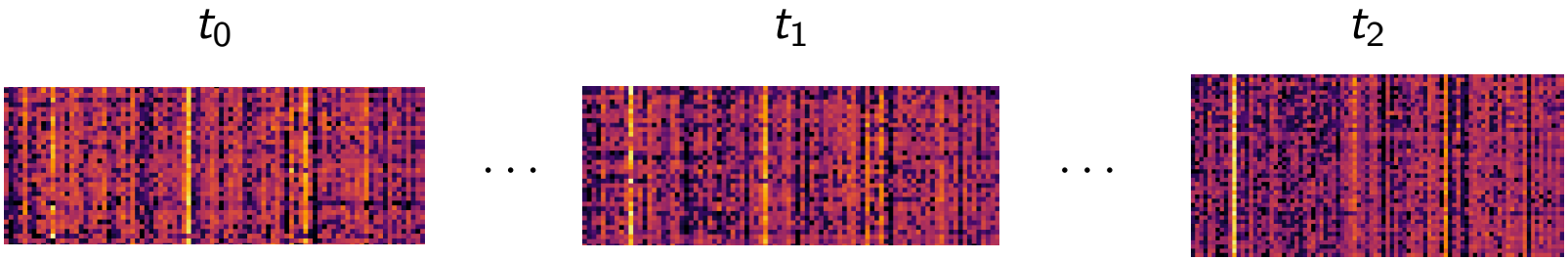


Prospects

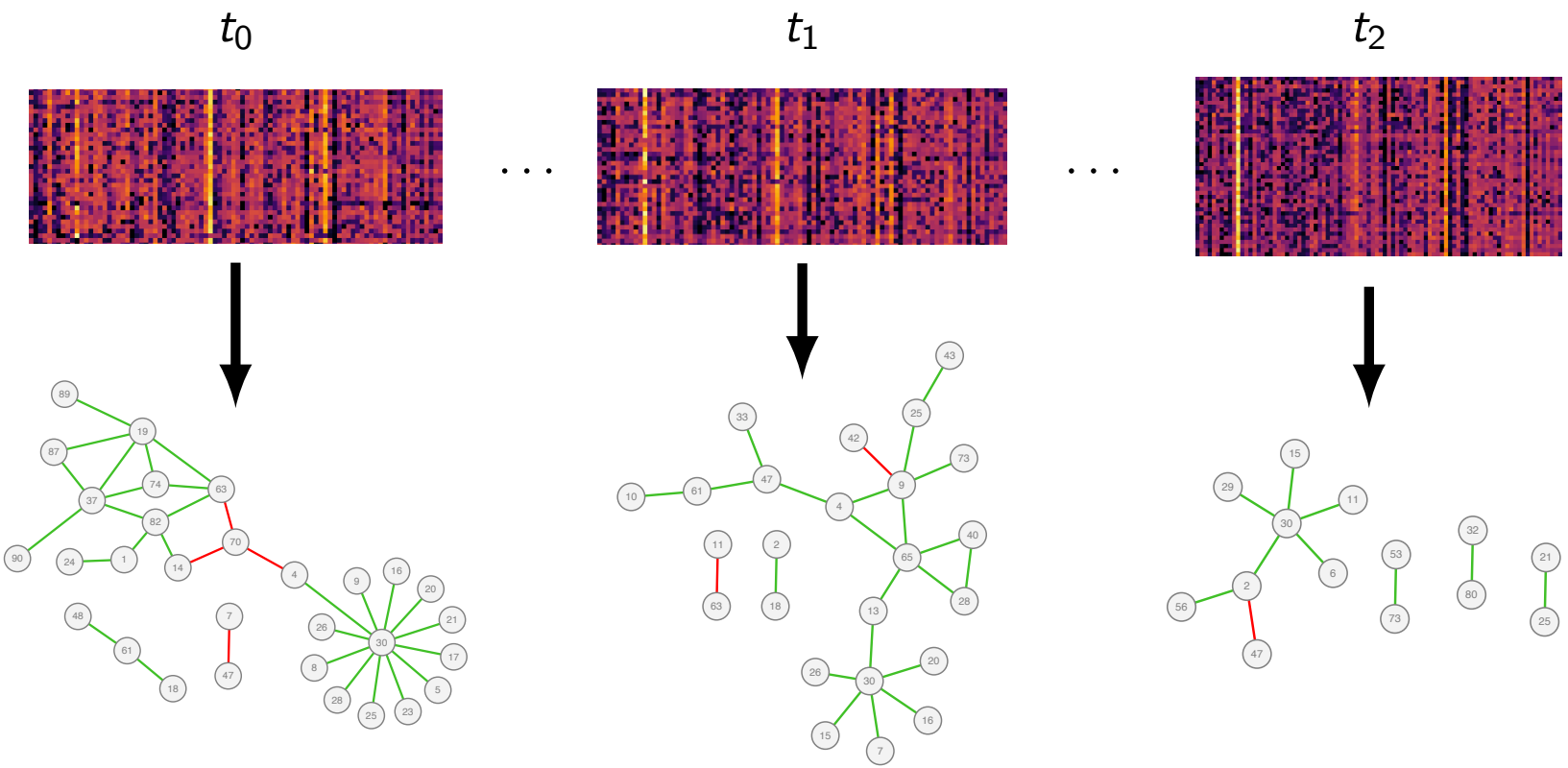
Calibration seems not too bad. Can now make **predictions**...

An open question...

How to optimally exploit the time information?



How to optimally exploit the time information?



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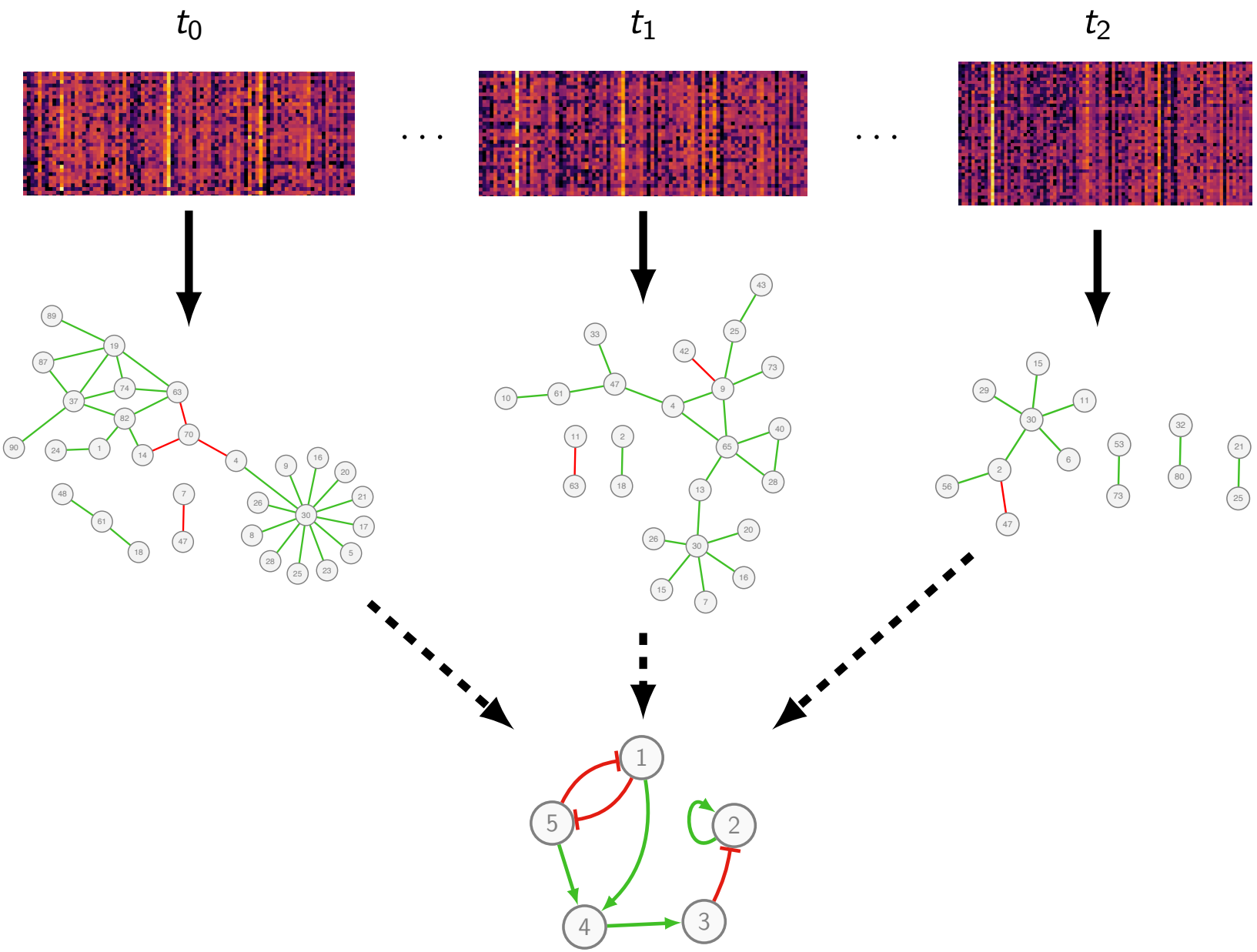

Thank you!

Herbach, U., Bonnaffoux, A., Espinasse, T., and Gandrillon, O. (2017).

Inferring gene regulatory networks from single-cell data: a mechanistic approach.

BMC Systems Biology, 11(1):105.

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