From *single-cell* to *multi-cell* systems space-time differentiation: a case study

Irene Zorzan

Department of Information Engineering - University of Padova

Project PROACTIVE "From Single-Cell to Multi-Cells Information Systems Analysis"

May 10, 2019

Outline

- Biological motivations
- Case study: a mathematical model for Central Nervous System differentiation — **Single-cell level**
- A multi-cell model capturing spatio-temporal pattern formation in a cell population — **Multi-cell level**
- Rigorous theoretical analysis of the minimal regulatory network — **Single-cell level**
- Ongoing and future work

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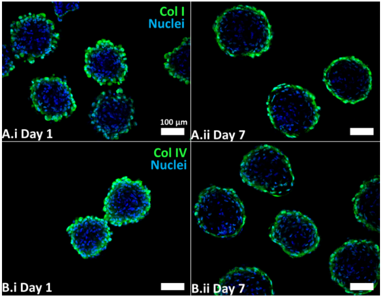

D.G. Belair, C.J. Wolf, C. Wood, H. Ren, R. Grindstaff, W. Padgett, et al., *Engineering human cell spheroids to model embryonic tissue fusion in vitro*, PLOS ONE, 12(9):1-31, September 2017. Immunofluorescence staining for extracellular matrix proteins collagen I and collagen IV in human cell spheroids on day 1 and day 7.

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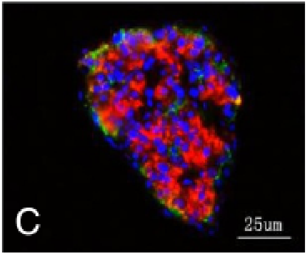

Zhang et al., *Evaluation of islets derived from human fetal pancreatic progenitor cells in diabetes treatment*, Stem Cell Research & Therapy, 4(6):141, 2013. Differentiation of pancreatic progenitor cells and formation of islet-like structures. Islet immunofluorescence stained for insulin (red) and glucagon (green), DAPI used for nuclei staining (blue).

Biological inspiring questions

How can cells orchestrate responses as a whole?

Which molecular mechanisms are responsible for cellular patterning? Lateral stabilization, lateral inhibition?

Which is the role of (positive and negative) feedback?

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Can we control or redirect the differentiation process?

We need to understand regulatory mechanisms both at single-cell level and at population level.

A control theoretic approach

How can we tackle these questions
from a control engineering perspective?

1) Dynamic model capturing pattern formation in multi-cell systems
2) Theoretical analysis of the model (stability, structural properties)
3) Hypothesis testing through model simulations

Case study: Central Nervous System differentiation

PLOS | ONE

OPEN ACCESS Freely available online

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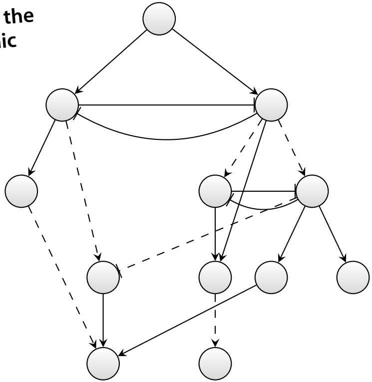
Regulatory network with 12 genes



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i) From undifferentiated *stem cell* into neuron or glia;

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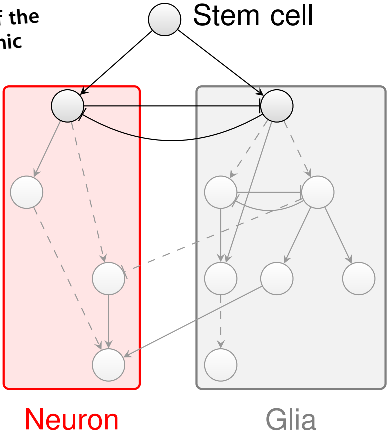

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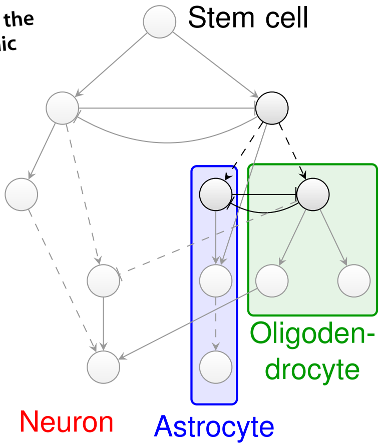
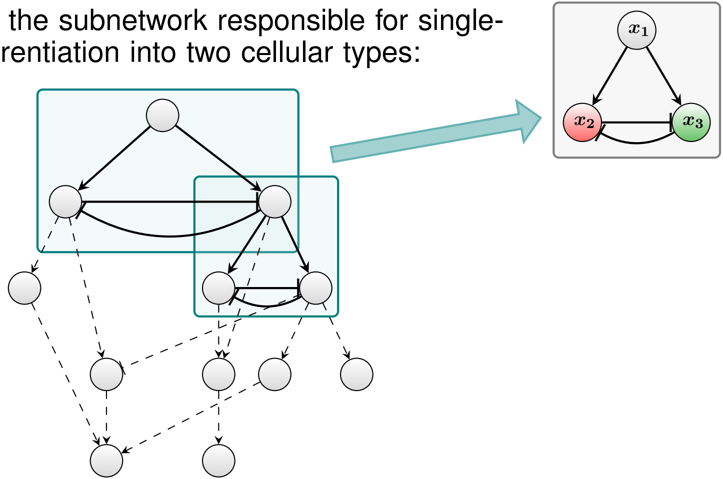


Towards a population model . . .

- Focus on the subnetwork responsible for single-level differentiation into two cellular types:



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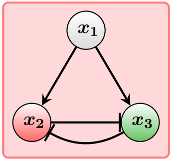

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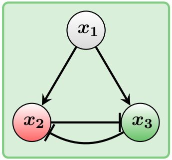

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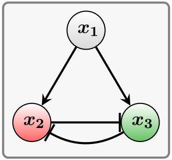
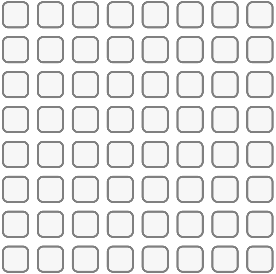
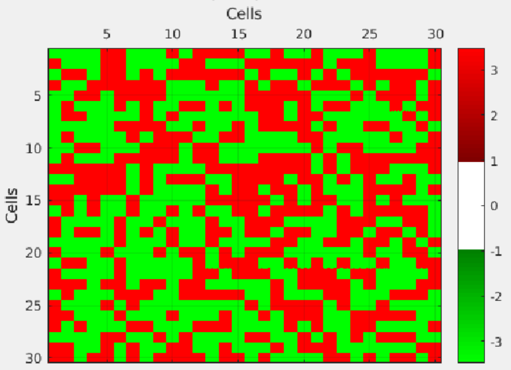


- Grid of cells modelling a monolayer cell culture



Simulations

1) If we don't model cell-cell interactions and each cell behaves independetly of its neighbours, the result is unrealistic!



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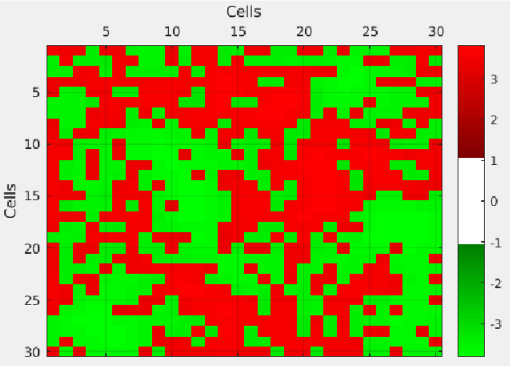

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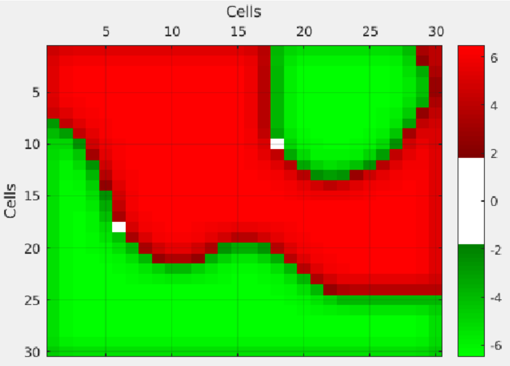

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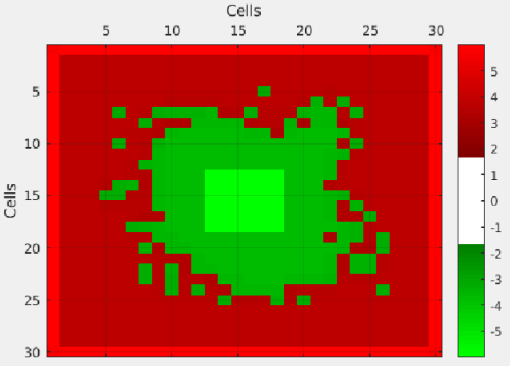
3) Cell-environment interactions: local mechanical stimuli enforce cells differentiation to a specific type.
Border effect: outer border forced to Type 2, inner square to Type 3



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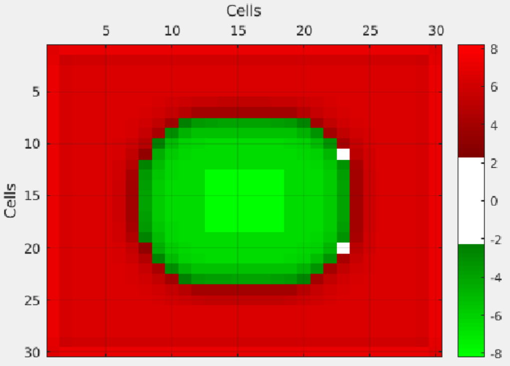

Strong cell-cell interactions

A multi-cell model

From a qualitative point of view the model is able to capture different macroscopic behaviours (e.g., islet formation, sharp differentiation) of multi-cell systems.

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- As the feedback intensity parameter varies, different spatio-temporal patterns arise:
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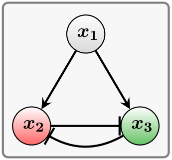
- As the feedback intensity parameter varies, different spatio-temporal patterns arise:
  - Weak cell-cell interactions lead to jagged borders between cell populations;
  - Strong cell-cell interactions result in sharper differentiation bounds.
- Enforced patterns mimicking the effect of external stimuli acting locally (border effect) can be identified.

Theoretical analysis

Single-cell level

3 gene regulatory network

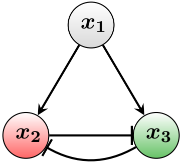


When is differentiation possible?

When does the activator gene triggers the differentiation process?

Do cooperativity of the activator and cooperativity of the repressors play equal roles?

Minimal gene regulatory network



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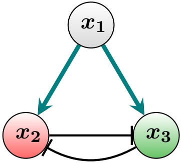

- Activation from Gene 1 to both Gene 2 and Gene 3

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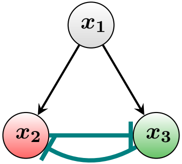

- Activation from Gene 1 to both Gene 2 and Gene 3
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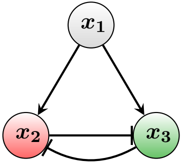

$$\dot{x}_1 = -\gamma(x_1 - \alpha)$$
$$\dot{x}_2 = a\frac{x_1^m}{1 + x_1^m + x_3^n} - kx_2$$
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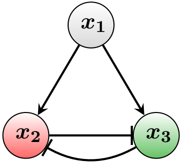

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generalized Hill function

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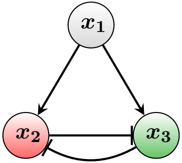

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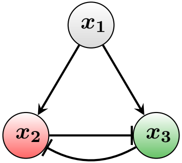

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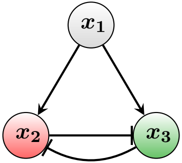

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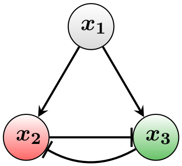

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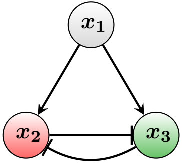

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- α – steady-state expression level of Gene 1 ($\alpha \in \mathbb{R}_+$)
- a – production rate ($a \in \mathbb{R}_+$)
- k – first order degradation rate ($k \in \mathbb{R}_+$)

Asymptotic behaviour

Structural properties of the Jacobian:

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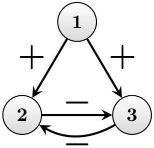

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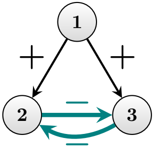
All cycles are positive

If unstable dynamics appear, it is solely due to real unstable eigenvalues.

No limit cycles are possible!



Equilibrium points

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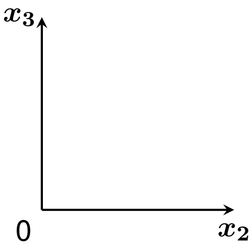

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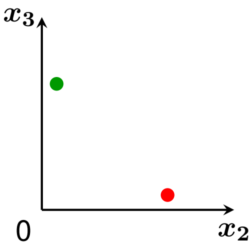

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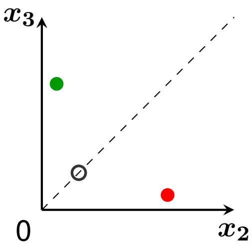

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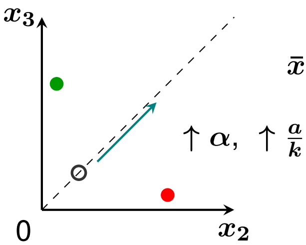

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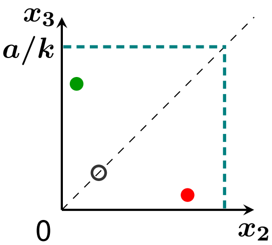

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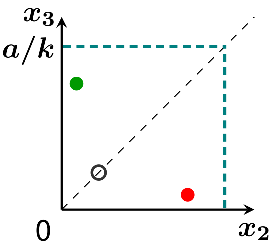

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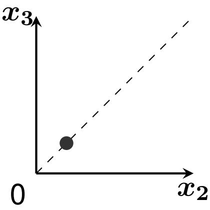
There exist either 3 distinct equilibria or a unique equilibrium.

Stability of equilibria

We assume that $J(\mathbf{x})$ evaluated at $\mathbf{x}^{\mathbf{eq}}$ is invertible.

1) If $\bar{\mathbf{x}}$ is the unique equilibrium, it is asymptotically stable.



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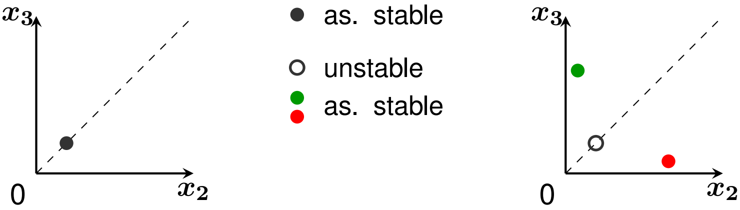

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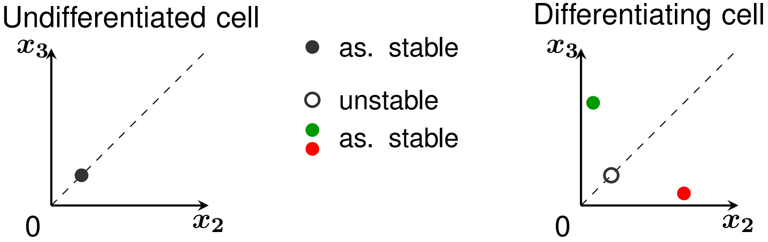

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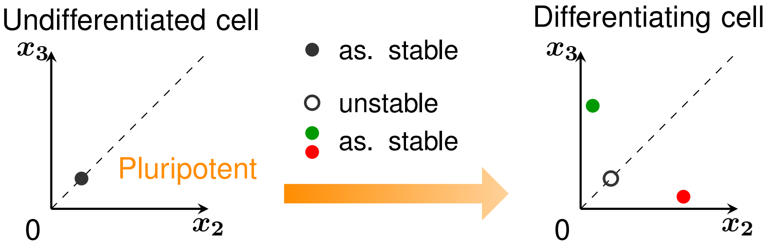
We assume that $J(\mathrm{x})$ evaluated at x^{eq} is invertible.

1) If $\bar{\mathrm{x}}$ is the unique equilibrium, it is asymptotically stable.
2) If 3 equilibria exist, $\bar{\mathrm{x}}$ is unstable while the other two equilibria are asymptotically stable.



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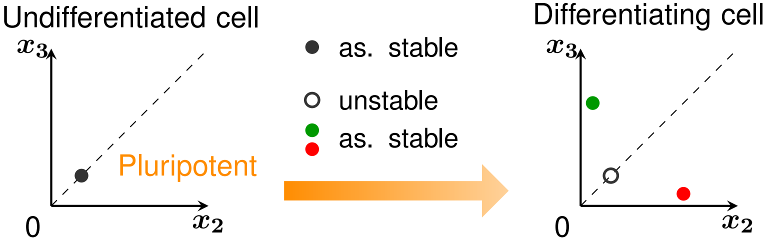

Which region of the parameter space corresponds to pluripotency?
Given a pluripotent cell, when does Gene 1 *induce* differentiation?

Non-differentiating vs pluripotent cell

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n – repressors Hill coeff.
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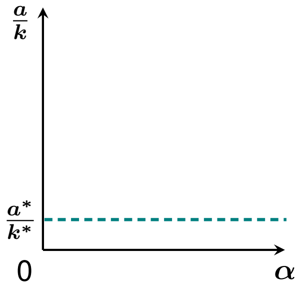

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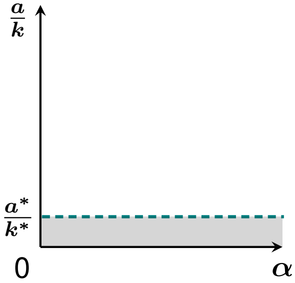

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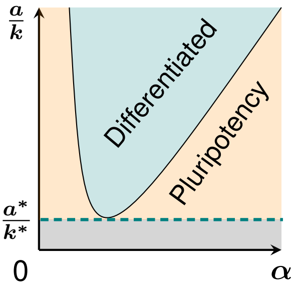
   If $\frac{a}{k} > \frac{a^*}{k^*}$, the cell undergoes differentiation when α belongs to a specific range of values.



Pluripotet vs differentiated state

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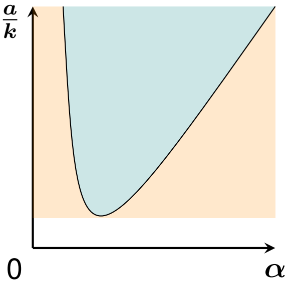
$$\alpha^* := \sqrt[m]{\frac{n-1}{2}\left[1 + \left(\frac{a}{k}\frac{n-1}{n+1}\right)^n\right]}$$



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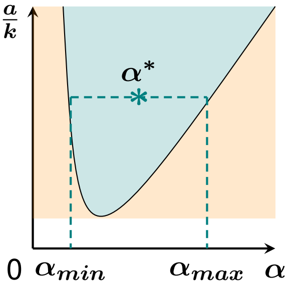
There exist $\alpha_{min} \in (0, \alpha^*)$ and $\alpha_{max} \in (\alpha^*, +\infty)$ such that for $\alpha \in (\alpha_{min}, \alpha_{max})$ the cell is in differentiated state, and is in pluripotent state otherwise.



Biological implications

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- Gene 1 represents the triggering gene: appropriate expression levels are required to induce differentiation.
- Repressor cooperativity and activator cooperativity play different role: the first one is crucial to control differentiation.

Ongoing and future work

Aim: analysing the behaviour of *interacting* pluripotent cells

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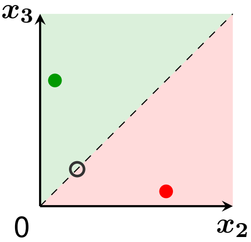
In a deterministic setting . . .

$$\dot{x}_2 = \mathcal{H}(x_1, x_3) - k x_2 + u_2$$
$$\dot{x}_3 = \mathcal{H}(x_1, x_2) - k x_3 + u_3$$



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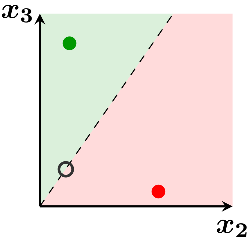
It is (not so) easy to prove that for $u_2 \ll u_3$:

- equilibrium points are shifted
- basins of attraction vary in amplitude



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What's the probability of jumping from one basin of attraction to the other?

It is

for u

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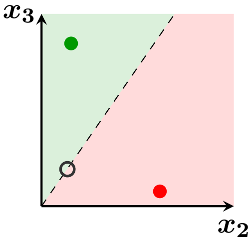

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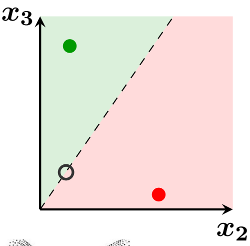
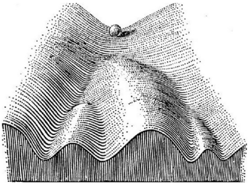


Waddington's epigenetic landscape: hilltop represents pluripotent state, valleys represent differentiated states

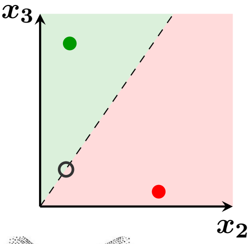


Ongoing and future work

Aim: analysing the behaviour of *interacting* pluripotent cells

In a ~~deterministic~~ stochastic setting ...

- diffusive noise
- stationary distribution



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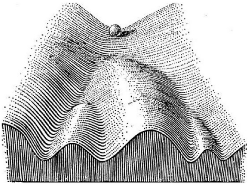
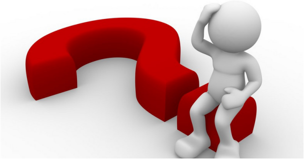


Thanks for your attention!

Questions?



Possible collaborations?