Report for the Course Modelling in Computational Science, HT23

Project 2: Cell reprogramming

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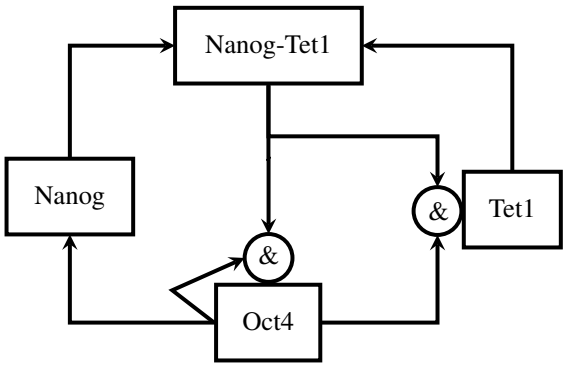

Figure 1: A flowchart showing the dependence of the transcription factors.

Introduction

The following report is part of the second project of the course Modelling in Computational Science, BERN01, taken at Lund university. In this project we model the interplay of three transcription factors which are relevant for mouse embryonic stem cells to reach induced pluripotency. For this we will discuss the setup of the model in the first part of the report and then reproduce the results from [1] in the second part. The code to the project was implemented in `python`. The project report and code can be found online under [2].

The setup

According to [1] the transcription factors Nanog and Oct4 are crucial in establishing pluripotency of a cell. In our simplified model the dependence of the transcription factors Nanog, Oct4 and Tet1 is given by figure 1. Note that Nanog and Tet1 form a complex Nanog-Tet1 together and the & symbol denotes that two transcription factors are required for activation. The concentration of the Nanog-Tet1 complex depends on the concentrations of Nanog and Tet1. By [1] it can be given explicitly by the formula

$$\text{N-T} = \frac{K_d + \text{N} + \text{T}}{2} - \sqrt{\left(\frac{K_d + \text{N} + \text{T}}{2}\right)^2 - \text{N} \cdot \text{T}} \tag{1}$$

where the dimerisation constant K_d is given in table 1. Here N, T and N-T denote the concentration in the cell of Nanog, Tet1 and Nanog-Tet1 respectively[1]. With the

[1]Here we deviate from the notation in [1] by omitting the concentration brackets and subscript.

Parameter	value
Over expression N_{over}	0
Over expression O_{over}	0
Over expression T_{over}	0.05
Probability p_N	1
Probability p_O	1
Probability p_T	1
Hill coefficient n	2
Leukaemia inhibiting factor LIF	0
Dimerisation constant K_d	0.1
Dissociation constant K_{NT}	0.2
Dissociation constant K_O	0.3
Dissociation constant K_{NT}	0.2

Table 1: Default parameter choices. Taken from [1]

Michaelis-Menten model one obtains the ordinary differential equation

$$\begin{bmatrix} \mathrm{N} \\ \mathrm{O} \\ \mathrm{T} \end{bmatrix}' = \begin{bmatrix} N_{\text{over}} + \mathrm{LIF} + p_N \cdot \frac{\mathrm{O}/K_O}{1+\mathrm{O}/K_O} - \mathrm{N} \\ O_{\text{over}} + \mathrm{LIF} + p_O \cdot \frac{\mathrm{O}/K_O}{1+\mathrm{O}/K_O} \cdot \frac{(\mathrm{N\text{-}T}/K_{NT})^n}{1+(\mathrm{N\text{-}T}/K_{NT})^n} - \mathrm{O} \\ T_{\text{over}} + p_T \cdot \frac{\mathrm{O}/K_O}{1+\mathrm{O}/K_O} \cdot \frac{(\mathrm{N\text{-}T}/K_{NT})^n}{1+(\mathrm{N\text{-}T}/K_{NT})^n} - \mathrm{T} \end{bmatrix}. \tag{2}$$

In this equation O stands for the Oct4 concentration. The other parameters are given for mouse embryonic fibroblast steady state (MEF) by table 1. We note that parameter LIF models the effect of the leukaemia inhibiting factor in promoting the differentiation of the cells. The parameters N_{over}, O_{over} and T_{over} describe how much Nanog, Oct4 and Tet1 are over-expressed in the cell. We also note that the system (2) has two equilibrium configurations. One where N, O and T are all greater than 0.5 one where all are small. In the former state the cell reaches pluripotency while in the latter it fails. We will inspect this behaviour more closely in our simulation experiments.

The experiments

In the following part of the report we will discuss our reproduction of some figures given in [1]. To solve the system (2) we used the built-in solver from `Scipy` with default parameters since this yielded satisfactory results. We note that our figures differ a little from [1] in that we let the simulation run for longer to reach the steady state. This explains why our charts are not exact clones.

Varying Nanog, Oct4 and Tet1 separately

Our first experiment series was taken from [1]. The results can be seen in figure 2. This figure consists of 3 subplots for each of which the x-axis represents time and the

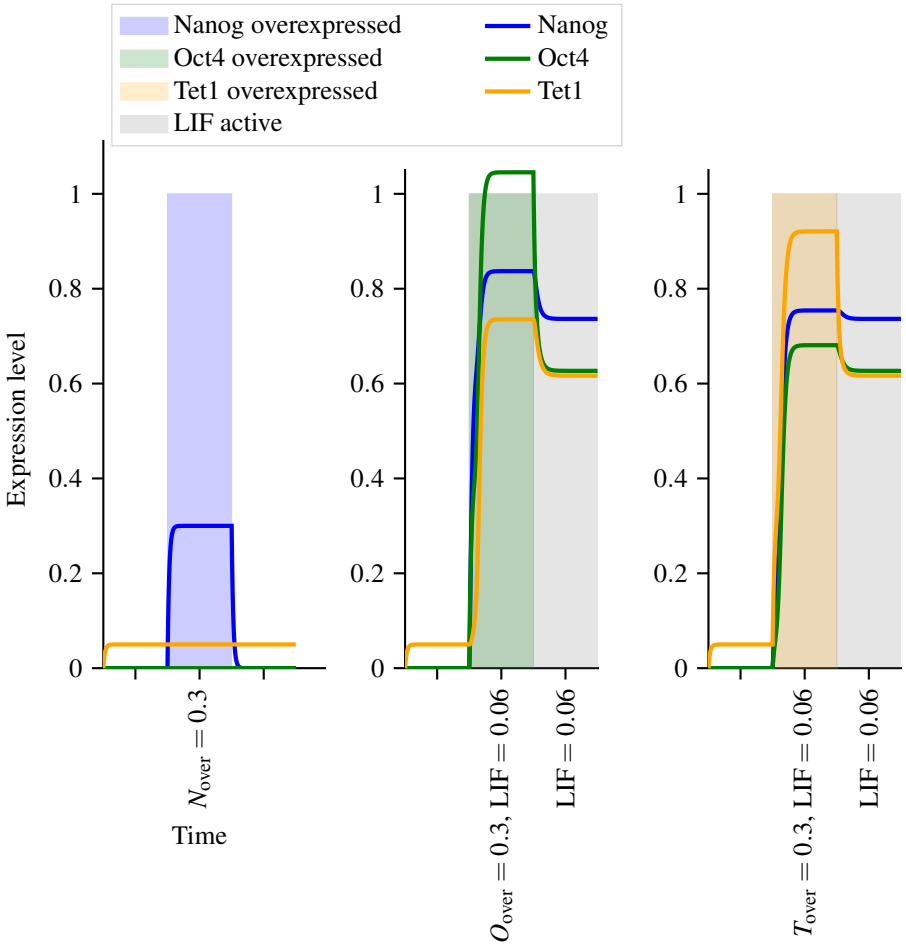

Figure 2: Over-expression of 0.3.

y-axis represents the concentration of the corresponding parameter O_{over}, N_{over} and T_{over}. Each simulation in the 3 subplots consists of 3 parts in which the parameters given in table 1 are varied. The parameters which deviate from this standard choice are given on the x-axis. In the first part of each simulation the cell is allowed to reach the MEF steady state. In the second part one of the transcription factors is over-expressed. In the third part the simulation returns to the MEF steady state or the pluripotent state which corresponds to the MEF steady state with LIF set to 0.06. The choice of the final state depends on whether the cell succeeded in reaching a pluripotent state. In the first subplot one sees that setting N_{over} alone to 0.3 is insufficient to reach pluripotency. In the second and third subplots one sees that setting O_{over} and T_{over} respectively to 0.3 is sufficient to reach pluripotency. Thus in these cases LIF is activated. All of this agrees with what we expect from the diagram and — according to [1] — laboratory experiments.

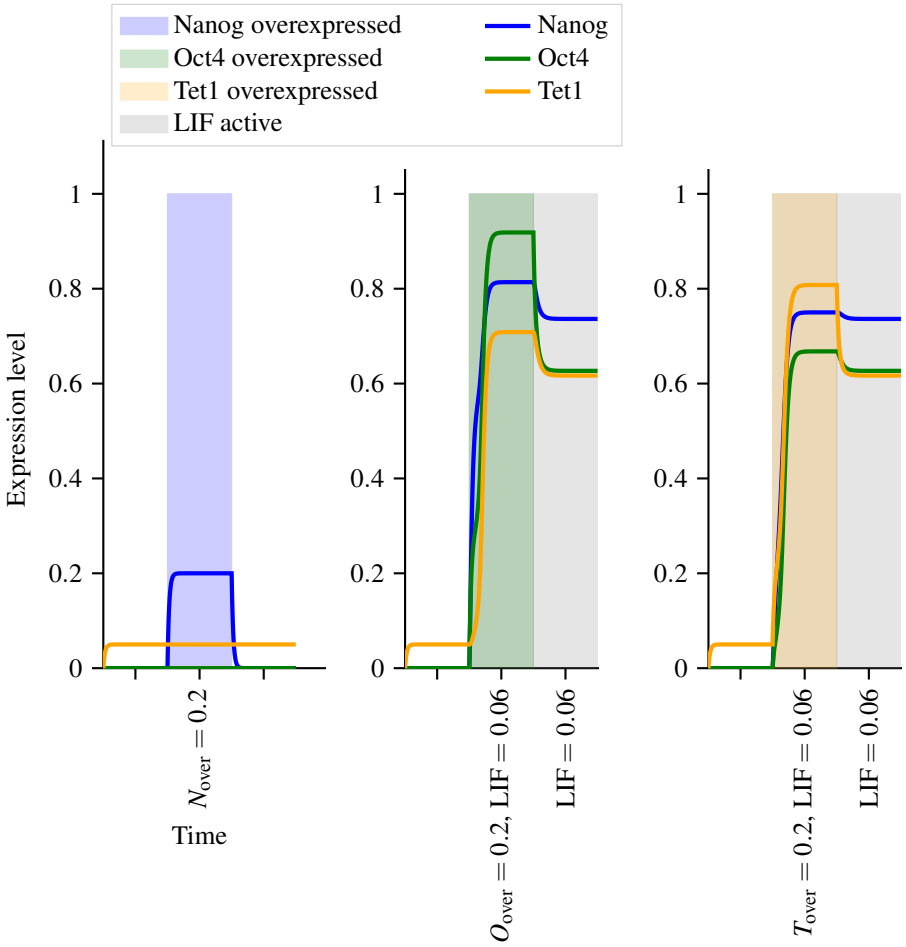

Figure 3: Over-expression of 0.2.

The second experiment is analogous to the first with the difference that we now set N_{over}, O_{over} and T_{over} to 0.2 in the respective experiments. Figure 3 shows the results. Once again we see that for Oct4 and Tet1 the cells reach pluripotency whilst it fails to do so for Nanog. If we compare figure 3 with figure 2 we see that the concentrations during the over-expression phase of the experiment are lower than for the previous experiment. This is to be expected since there are no repressors in this particular model. Also as expected the steady states in the first and third part of each simulation are identical to the previous experiment.

Figure 4 gives the results with an over-expression of 0.13 and yields the same result as the previous experiment. Once again the concentration levels are reduced for the over-expression phase.

In a final experiment of this series the over-expression was set to 0.1. Figure 5

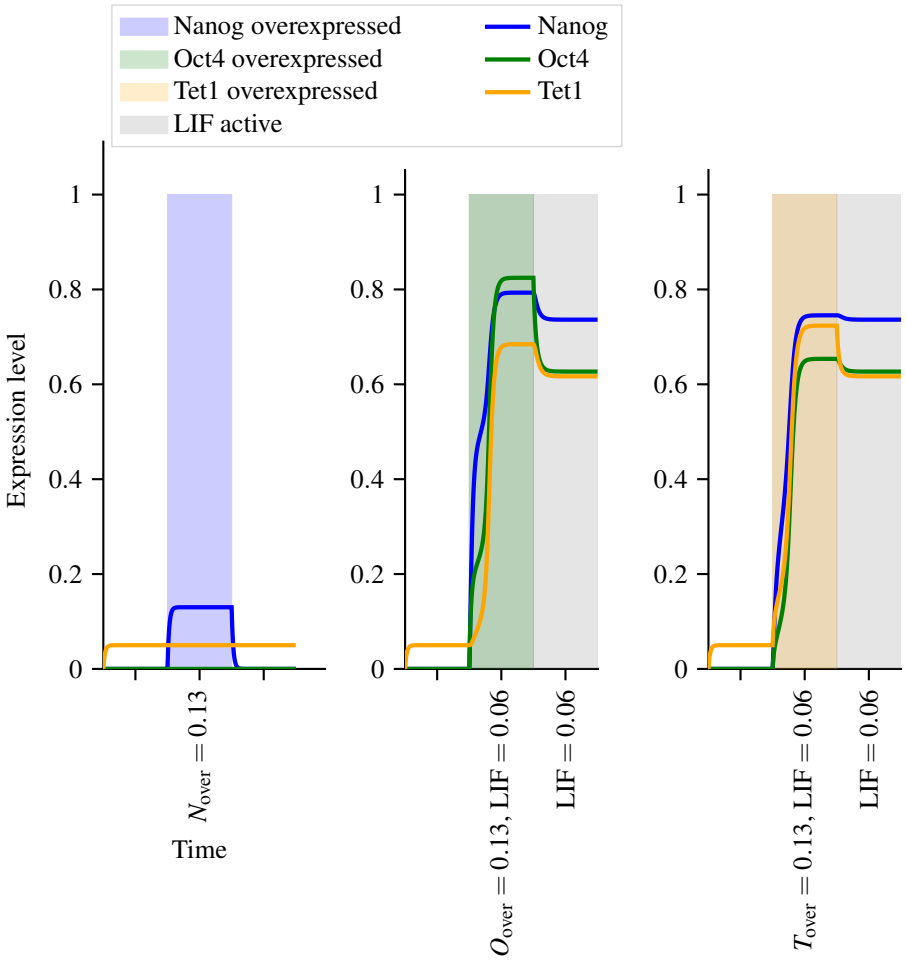

Figure 4: Over-expression of 0.13.

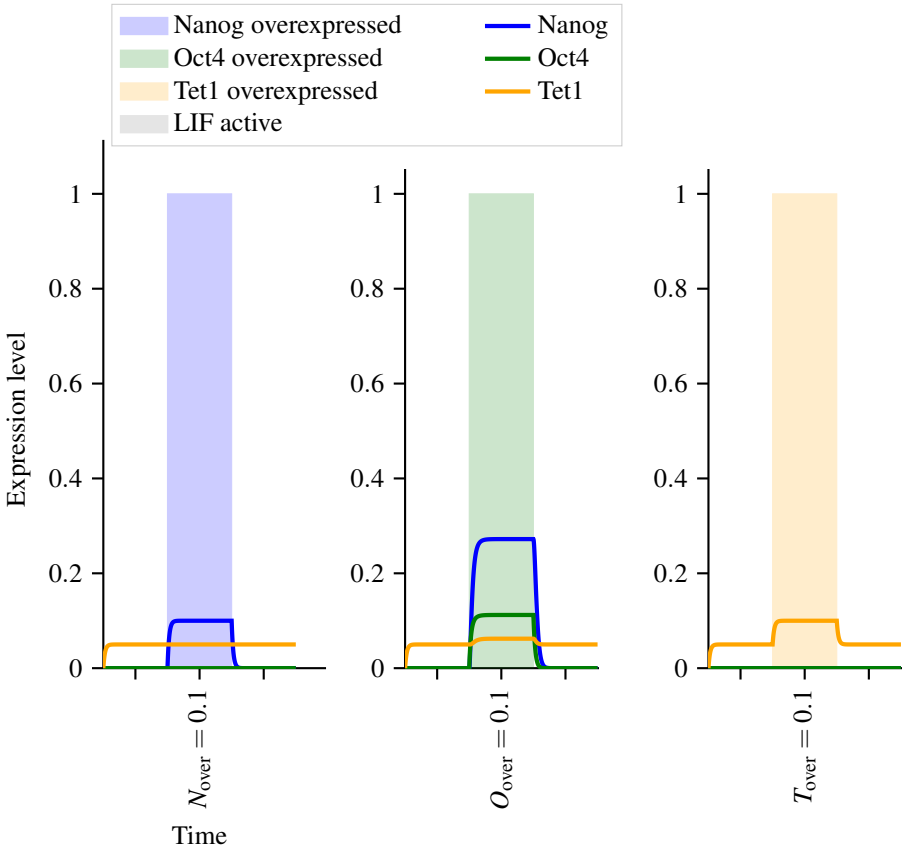

Figure 5: Over-expression of 0.1.

shows the results. We see here that in all cases the cells fail to reach pluripotency as the concentrations of Nanog, Oct4 and Tet1 fail to meet the threshold.

Inducing Pluripotency with Nanog and Oct4

Our next experiment deals with the fact that according to [1] Nanog and low levels of Oct4 are sufficient to reach pluripotency even though the same levels of Nanog and of Oct4 separately are insufficient. For this we set O_{over} to 0.1 and N_{over} to 0.2. The results are shown in figure 6. The first two subplots show that the cells fail to reach pluripotency if either Oct4 or Nanog is overexpressed. In the third plot we see that over-expressing Oct4 and Nanog simultaniously yields a pluripotent cell as would be expected from the diagram 1.

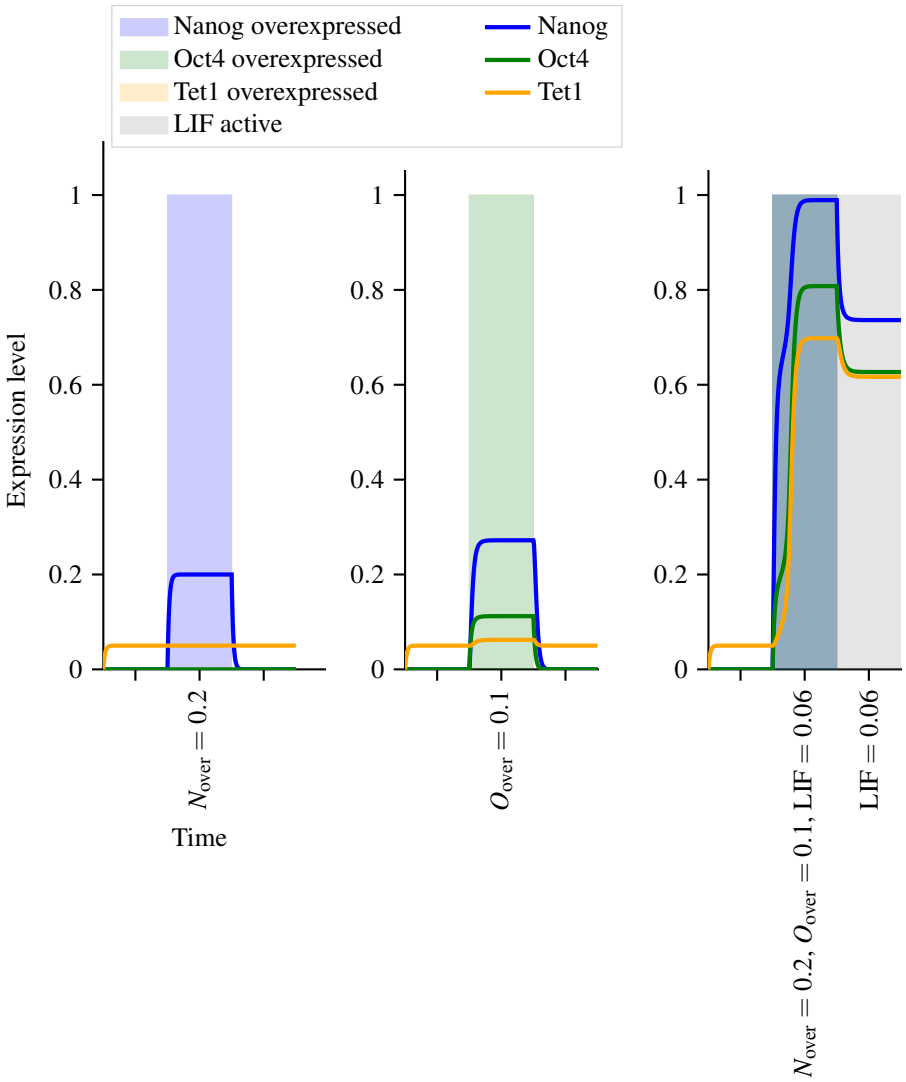

Figure 6: Nanog and Oct4 active.

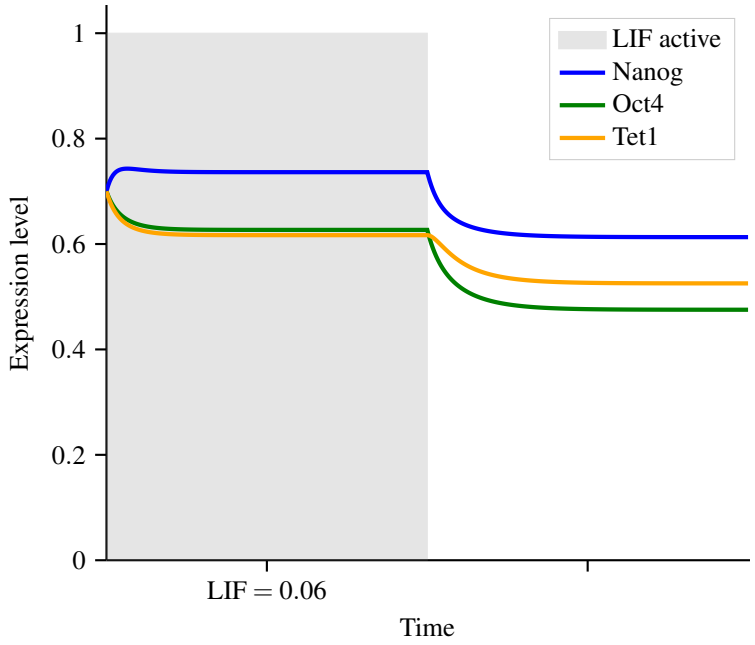

Figure 7: Withdrawing LIF.

Withdrawing LIF

In a final experiment we simulated how a cell in pluripotent state reacts under withdrawal of LIF. For this N, O and T are initialised at 0.7 and the cells are allowed to reach an equilibrium. In the second phase of the experiment LIF is turned off. The result is shown in figure 7. The cell remains pluripotent contrary to labatory evidence. To obtain the figure given in [1] we had to additionally set T_{over} to 0 in the second part. Then indeed the cell becomes somatic.

Modifying the model

In the final part of the project we modified the diagram to allow take into the account that if one adds too much Oct4 to the cells they fail to reach pluripotency. In other words the parameter O_{over} has a Goldilocks zone. Since the cell only reaches pluripotency if Nanog and Oct4 are expressed above a certain threshold and Oct4 is increased we must have that the concentration of Nanog is small if Oct4 is large. Thus we have to have some kind of repressor present in the network. Our final solution is depicted in figure 9. Here the 'n' stands for negation or repression. The reason for introducing two additional new factors rather than only one lies in the fact that the desired effect in the simulation is larger if we have two rather than only one factor. Corresponding to this flowchart we

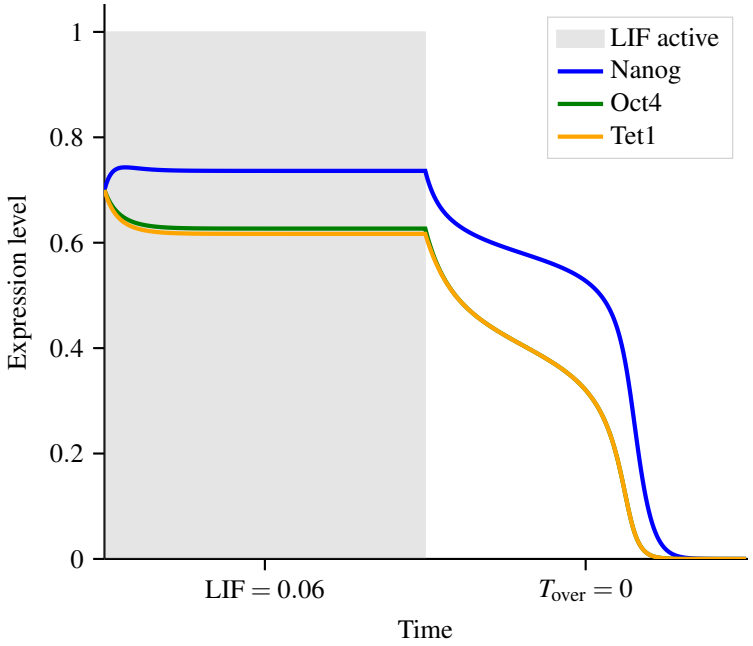

Figure 8: Withdrawing LIF.

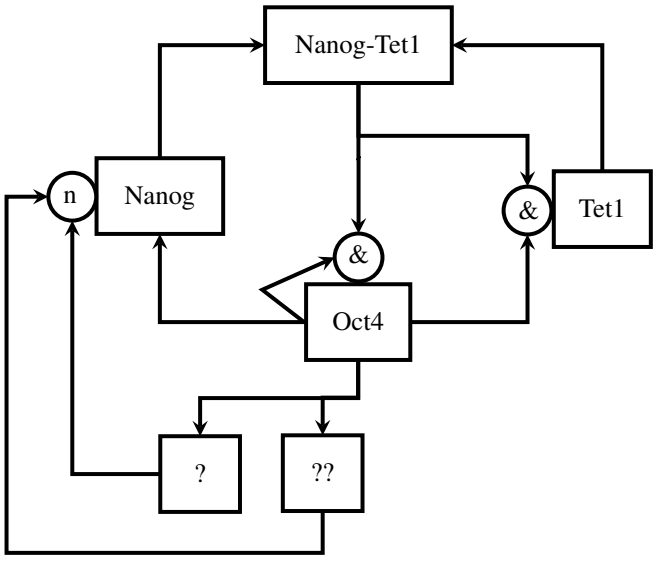

Figure 9: A flowchart showing the dependence of the transcription factors.

Parameter	value
Probability $p_?$	1
Probability $p_{??}$	1
Probability p_N	5
Dissociation constant $K_?$	0.2
Dissociation constant $K_{??}$	0.2
Dissociation constant $K_{O,?}$	0.3
Dissociation constant $K_{O,??}$	0.3

Table 2: Default parameter choices for the extension of the model.

obtain the equation system

$$\begin{bmatrix} \mathrm{N} \\ \mathrm{O} \\ \mathrm{T} \\ ? \\ ?? \end{bmatrix}' = \begin{bmatrix} N_{\text{over}} + \mathrm{LIF} + p_N \cdot \frac{\mathrm{O}/K_O}{1+\mathrm{O}/K_O} \cdot \frac{1}{1+?/K_?} \cdot \frac{1}{1+??/K_{??}} - \mathrm{N} \\ O_{\text{over}} + \mathrm{LIF} + p_O \cdot \frac{\mathrm{O}/K_O}{1+\mathrm{O}/K_O} \cdot \frac{(\text{N-T}/K_{NT})^n}{1+(\text{N-T}/K_{NT})^n} - \mathrm{O} \\ T_{\text{over}} + p_T \cdot \frac{\mathrm{O}/K_O}{1+\mathrm{O}/K_O} \cdot \frac{(\text{N-T}/K_{NT})^n}{1+(\text{N-T}/K_{NT})^n} - \mathrm{T} \\ p_? \cdot \frac{\mathrm{O}/K_{O,?}}{1+\mathrm{O}/K_{O,?}} - ? \\ p_{??} \cdot \frac{\mathrm{O}/K_{O,??}}{1+\mathrm{O}/K_{O,??}} - ?? \end{bmatrix}. \tag{3}$$

where we used once again the Michaelis-Menten approach according to [3] and set the decay rate of the system to be 1 for both factors '?' and '??'. Analogously to the previous model we chose the new parameters according to table 2. We set the parameter p_N to 5 so that we would not have to rescale the plot in the following experiment but the result is qualitatively similar for p_N equal to 1. The other parameters are in the range of the previous experiment.

In a final experiment we wanted to highlight that this modified model indeed gives the desired results. For this we plotted the evolution of the system for O_{over} equal to 0.01, 0.1 and 0.8 in the over-expression phase. Indeed, one can see in figure 10 that the concentration of Nanog is small for O_{over} equal to 0.01 or 0.8. We also see that it is larger for O_{over} equal to 0.1. As desired the Oct4 concentration has a Goldilocks zone with regard to the Nanog concentration. Figure 10 also shows that the Nanog concentration initially overshoots its equilibrium state as it takes a while for the concentrations of the repressors '?' and '??' to rise in the simulation of the equations (3).

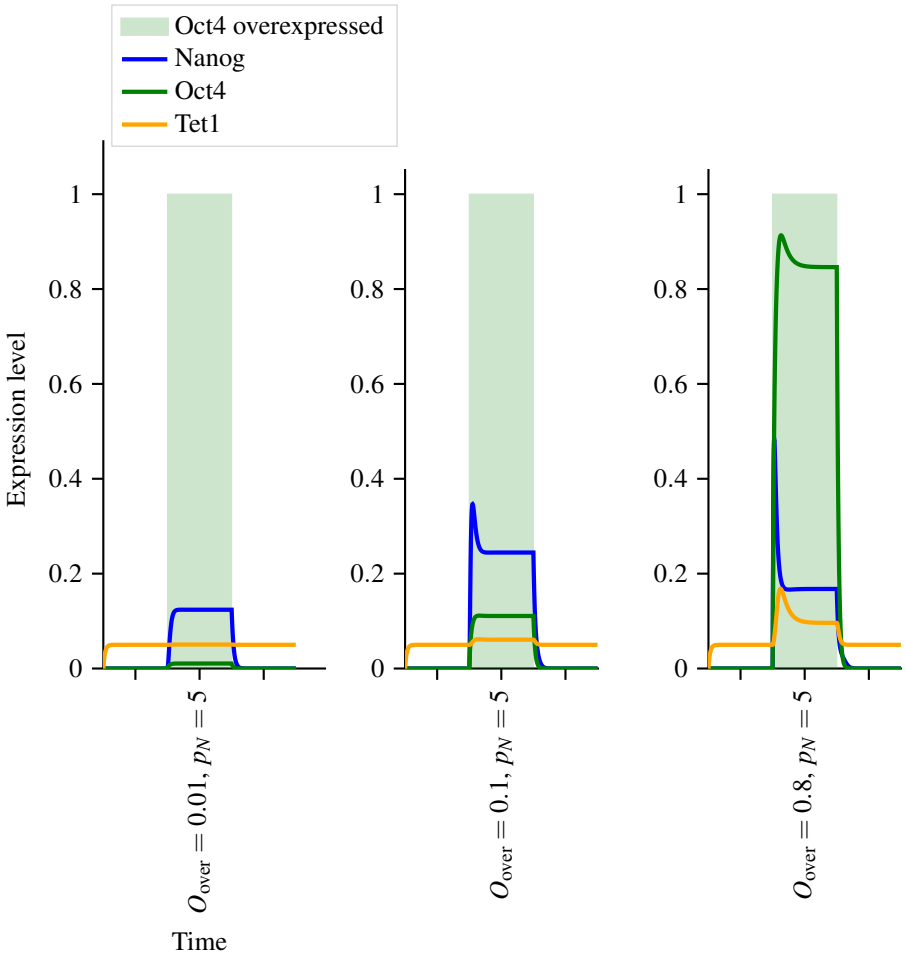

Figure 10: Nanog and Oct4 active.

Conclusion

We have seen that the simple model involving the transcription factors Tet1, Nanog, Oct4 and Nanog-Tet1 can be used to simulate some elementary processes involved for the cell to reach pluripotency. We have seen that in the model the cells reach pluripotency if Oct4 levels or Tet1 levels are higher than a certain threshold. We have also seen that the cell obtains pluripotency if Nanog and Oct4 are combined although they might fail if Nanog and Oct4 are added seperately. We have seen that under the removal of LIF and Oct4 the cell becomes somatic. The additional part of the project made us aware of how fiddly choosing the different parameters in a model can be. This type of model without any finetuning of the parameters gives the desired qualitative behaviour. The quantitative predictions however are very limited in scope. It also made us aware of how important the assumptions are that one makes when setting up the model and how relatively unimportent the actual implementation and the numerical optimisation are for the results.

Bibliography

[1] V. Olariu, C. Lövkvist, and K. Sneppen, "Nanog, oct4 and tet1 interplay in establishing pluripotency," *Scientific Reports*, vol. 6, no. 1, p. 25 438, May 2016, ISSN: 2045-2322. DOI: 10.1038/srep25438. [Online]. Available: https://doi.org/10.1038/srep25438.

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