Universal Biology - Report on cell differentiation (古澤 topic 3)

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A Jupyter notebook containing animations and interactive figures can be found at github.com/japhba/UB

This report is mainly focused around the reconsideration and discussion of *(Suzuki, Furusawa, and Kaneko 2011)*. First, the fundamental characteristics of stem cells are reviewed. To find a mathematical description of the mechanisms involved, the relevant interactions are formulated and subsummarized in a model. Those dynamics of the GRN are then implemented and simulation of different setups is carried out. In the context of the state space involved, the roles of fundamental modules of stemness dynamics, switches and oscillators are considered. From this, a connection to the model of Isologous Differentiation *(Kaneko and Yomo 1997)* is drawn. A minimal two-protein model is examined for the bifurcation mechanism arising from asymmetric oscillatory dynamics. To judge the relevance of this approach, it is helpful to compare it to purely noise driven dynamics. In an appendix, we provide a further simulation on the stability of the switching mechanism of the Turing model around a fixpoint.

Cell differentiation is the mechanism in biology giving rise to specialization amongst the cells in the developing body. Whereas in early stages, organisms only consist of a single cell type (the stem cell), in later development stages cells diversify towards such that are commited to muscular, digestial or neuronal tasks.

At first, the embryonic stem cell bears the potential to ultimately produce any cell in the adult organism and is thus termed *totipotent*. Upon further evolution of the organism, cells start to specialize into subsequent categories, loosing some of their potential, becoming *multipotent*. At the final stage of cell development, cells enter a stage at which they no longer differentiate, but have reached a largely irreversible point of commitment, fulfilling their specific task within the body. The determinism at which this occurs is surprising, as identical initial conditions lead to a variety of cells.

Stem cells found in nature typically are observed to have the following properties:

Proliferation (or self-renewal)
The ability of a (stem) cell to reproduce itself while keeping its properties in a fraction of its offspring, especially its potential to develop into multiple types.

Differentiation (or potency)
The ability of a cell specialize into a variety of subtypes, given only one genetic configuration (regulatory network). Typically, during diversification, plasticity is lost in differentiated cells.

In addition to these requirements, stem cell division dynamics should fulfil two more properties, namely robustness against environmental changes and irreversibility of the differentiation process:

Robustness
When an organism develops, its stem cells differentiate into various subtypes. It is observed that each cell type appears with frequency of small variance: For example, the respective number of muscular and neuronal cells need to obey a rather fixed proportion, otherwise the organism would not be able to prosper. Hence, the question arises how such a regulation on number ratios is biologically realized and found in a possible model.

Irreversibility
The stem cell transformation occurs only in one direction in nature: An already differentiated cell has lost its potential to take on all the variants that its parent cell could.

It is interesting to note that the last point is no strict classification. Indeed, wherever an organism is severely distorted and lacks a certain type of cell, some already specialized cells may indeed find a way of *de-differentiation* and are endowed with stemness again. This is observed from transplantation of cells into an environment lacking their respective type. From this, one can expect a controlling effect of the environment onto cell development.

Modeling a stem cell

Upon searching a model for the description of cell dynamics with the aim of finding a possible reduced mechanism, it is essential to isolate the basic principles that govern the cell's evolution. It is helpful to distinguish different contributions to the overall cell dynamics.

Intra-cellular dynamics
The mechanics of a cell are primarily governed by the network of gene expressions, which actively controls the proteins expression levels.

Inter-cellular dynamics
A cell is, for example after division, constantly surrounded by other cells. These may influence the expression dynamics by diffusion processes by biosignalling, effectively coupling the systems of single cells.

Environmental dynamics
Lastly, all the cells are surrounded in by a medium, which is, in contrast to the second point, a quasi infinite reservoir of certain resources, thermodynamically speaking.

As will be examined below, especially the second point of interaction between cells is essential to give rise to stemness behavior, and necessary for robustness to changes in the number ratio of stem cells.

Mathematical description

Biological background

The cellular mechanism is known as gene expression. In the process of protein biosynthesis, the mRNA that has been transcribed from a piece of static DNA, is translated by ribosomes which generate proteins. In the synthesis of mRNA however, several chemicals may bind onto the DNA as transcription factors, regulating the mRNA and therefore the protein expressions. Thus, a mutual feedback between chemicals and mRNA presence in the cell is created.

A cell's activity is then primarily characterised by the abundance of proteins i fulfilling different functions. In this description, we will denote this $p_i^l(t)$, where the upper index l denotes a specific cell of an ensemble. We are interested in the time evolution of these abundances. A gene regulatory network (GRN) is then the instance governing the time evolution of $p_i^l(t)$.

GRN equations

The system we consider can be stated in terms of network flow dynamics. These are given in form of the following equations:

$$\dot{m}_i^l = \gamma_m(F_i^l - m_i^l)$$
$$\dot{p}_i^l = \gamma_n(p_i^l - m_i^l) + D_i(\bar{p}_i - p_i),$$

where

$$F_i^l = f\left(\sum_j J_{ij} p_j^l - \theta_i\right)$$

and

$$f(x) = \frac{1}{1+\exp(-\beta x)}.$$

These equations contain two main variables: The mRNA expression m and the protein abundance concentration p for each cell l and each protein i. These two quantities stand in biological relation: The GRN, encoded by the coupling matrix J, determines whether a gene is being expressed, depending on the current protein abundance. This is additionally mediated by a threshold θ, at which a certain protein abundance triggers the process. Thus, expression is, choosing the Hill coefficient β in the activator function f large, a rather binary process, that sets in abruptly once a certain limit is crossed. The activator function introduces a nonlinearity to the dynamics, thereby making the network sensitive towards small variations around the threshold and giving rise to a lot of complex and potentially chaotic behavior.

The protein abundances p, on the other hand, depend on the expression rate of mRNA, as it governs the synthetization process, that is taken to be continuous and needs no activation. Additionally, here, a diffusion term is added to allow certain proteins (where D_i is different from 0) to penetrate through the cell membranes. Thereby, the internal concentrations tend towards the cell average $\bar{p}$. Note however that this is a simple picture for interaction, besides passive diffusion there might be a plentitude of other biosignalling mechanisms. Further, we consider no time shift of the protein dependencies in $\bar{p}$, which means a rapid diffusion and discarding of spatial dependencies. The medium is also taken to be comparatively small in volume compared to the cell size, so that its protein concentrations are roughly given as the cell average.

Both protein expression $\dot{p}$ and mRNA abundance $\dot{m}$ rates are reduced by the dilution terms $-m$ or $-p$, because the cell is expected to enlarge its volume proportionally to those substances' presence, with respective proportionality constants γ_p and γ_m.

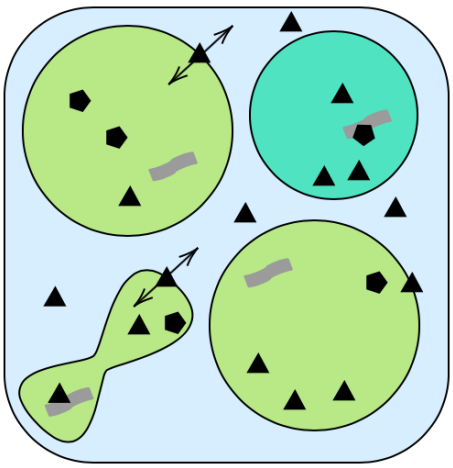


Figure 1: Illustration of the setup with the gene sequences partly controlled by the proteins (pentagon and triangles), the latter one being diffusive through the cells into the small bath (blue). A cell division with splitting of the proteins is shown in the lower left. The turquoise indicates a novel cell type having differentiated upon the process.

At some time, the cell will, driven by its growth, undergo division. In this process, we will assume that the protein concentrations almost split symmetrically between the daughter cells, and are only slightly perturbed by a noise drawn from an uniform, symmetric distribution around 0 of width σ.

We can regard a cell as having differentiated if its phenotype (protein expressions) is different and stable from the stem cell's phenotype. Further, we can restrict this to discard back transitions to the original state, accounting for the observed lower plasticity of specialized cells.

Mechanisms for Stemness

In this section, we attempt to achieve stemness by analysis some basic network configurations and their behavior under cell division. Then, by combining them, a simuation of a more complicated network is conducted, exhibiting stem cell dynamics.

Switch mechanism: Turing type network

In the appendix of this notebook, an investigation and simulation of the properties of Turing type networks and their role in forming the so named patterns in presented.

Layout

We consider a model type Turing system with two states, A and I. A is an activator that activates the expression of itself *and* I, while I is an inhibitor, repressing its and the activators expression.

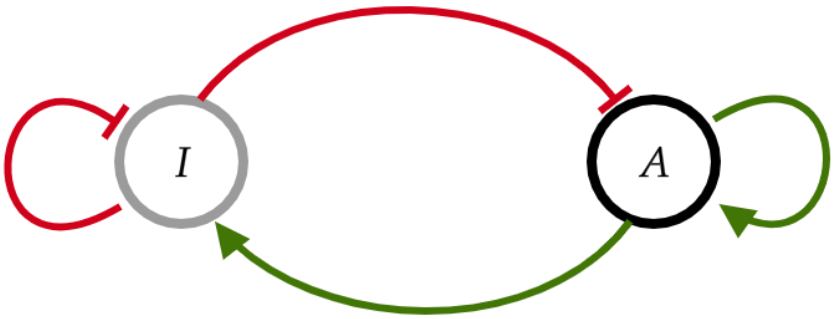


Figure 3: Turing network consisting of a (pn) loop with self-feedbacks. Inhibitions are coloured in red, activations in green. The diffusive protein I is indicated by a grey boundary.

This is the most simple case of a Turing mechanism, working as described above. By the feedback mechanisms involved, a fixpoint state is well imaginable. To see this, consider first the situation without the self-feedback. Then, we have in absence of diffusion

$$\begin{aligned} \dot{p}_i &= m_i - p_i \\ \dot{m}_i &= F_i(m) - m_i. \end{aligned}$$

Looking for a fixpoint, we set the time derivatives on the left hand side to zero. Then

$$\begin{aligned} p_i &= m_i \\ m_i &= \left(1 + \exp\left(\sum_j J_{ij} m_j\right)\right)^{-1}, \end{aligned}$$

where the threshold was taken to be zero, as this will shift only slightly shift the below curve. By drawing the graph of the second, nonlinear equation it is quickly seen that there exists a fixpoint solution with $m_i < 1$, in the presence of self-coupling:

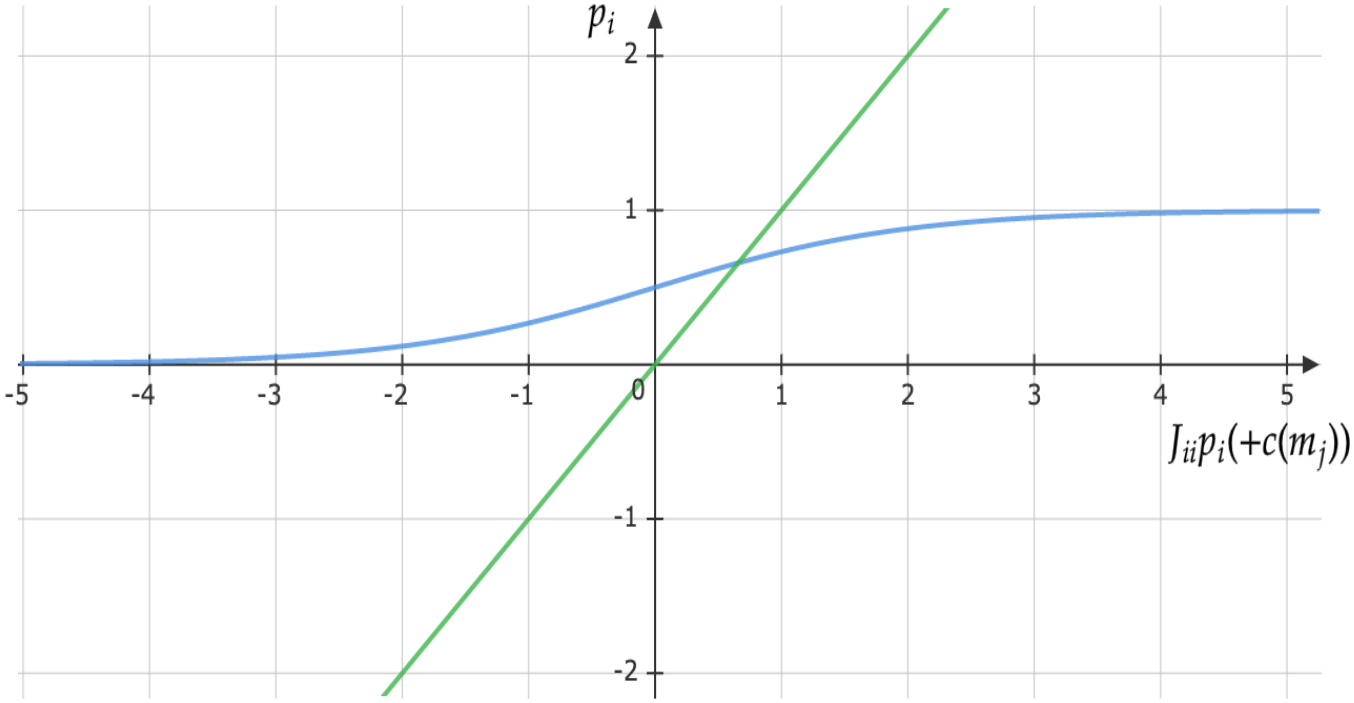


Figure 2: Illustration of fixpoint for self-coupling Turing type network as introduced above. Here, for illustration purposes, β was set to 1.

Relation of Turing model to cell differentiation

Consider a mutual network of two states A and I. As we have seen above, it is possible for a suitable network to evolve a Steady State. This state represents the configuration of the stem cell.

The single cell dynamics react upon an increase of A with increased expression of A, but also I. This subsequently couples back on A, which is then limited in its growth, thus being stabilized. Likewise, if I were to increase, A would decrease, thereby countering the initial perturbation. The same applies for the opposite process.

What happens when the cell divides? Upon division, proteins in the daughter cells that are diffusive may penetrate towards the other cell. Therefore, due to a slight asymmetry in concentrations after division, one daughter cell might hold a higher concentration of A than the other one. We may examine the effect of this as a small perturbation around the former fixpoint:
As the inhibitor I is essentially held constant by the diffusion constraint, the activator abundances in both cells are stabilized no longer and vary into the direction of their perturbation, until they possibly fall into distinct, *differentiated* stable states. The instability thus is only introduced upon division of the cell, which makes cell-cell interaction a mandatory component for diversification.

Simulation parameters

A simulation is carried out below to show this process for the network at hand. The parameters used are

- $\beta = 40$

- $\theta = [-0.01, -0.03, 0.02, 0.01, -0.02]$
- $D = 0.4$
- $\gamma_m = 6, \gamma_p = 1$
- $\sigma = 10^{-5}$

as the result of the extensive simulations carried out in *(Suzuki, Furusawa, and Kaneko 2011)*, where especially the threshold values must be chosen with care. The role of the diffusion parameter strength will be discussed below. If not stated differntly or manually altered below, these values will be chosen for all simulations below.

An implementation of the protein dynamics simulation is found in the file `cell.py` and is being loaded into the present notebook.

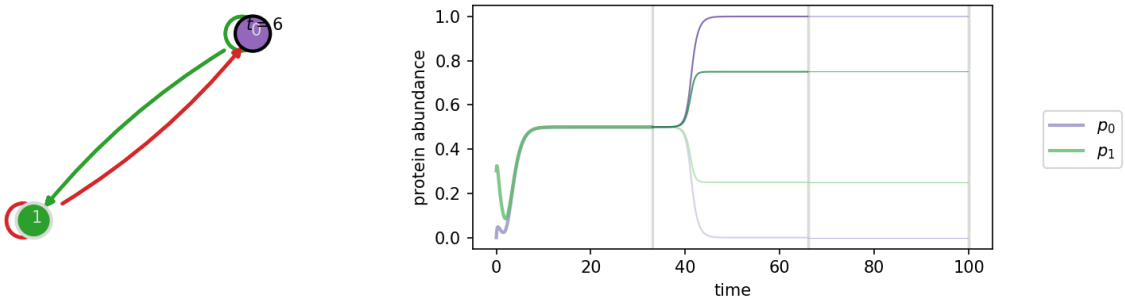
In [80]:

```
import numpy as np
import matplotlib.pyplot as plt
%load_ext autoreload
%autoreload 2
%matplotlib notebook
%matplotlib notebook
from cell import Cell
from networkDefs import *
from plotGraph import plotDevelopment, plotProj, proteinsInCells
```

```
The autoreload extension is already loaded. To re
load it, use:
  %reload_ext autoreload
```

In [81]:

```
# set initial conditions on proteins and mRNA
np.random.seed(1)
dim = 2
m_ini = np.random.uniform(0, 1, (dim,L))
p_ini = np.random.uniform(0, 1, (dim,L))

# construct cell and modify GRN JT self couplings
JT[0,0] = +1
JT[1,1] = -1

# the cell class takes coupling J, diffusion D and threshold T as inputs
# further, the network is initalized with random states
Cell_T = Cell(J = JT, D = DT, T = [0,0], m = m_ini, p = p_ini, name = "Turing type r
ani = plotDevelopment(Cell_T, divs = 2, tmax = 100, proteins = [0,1])
plt.show(block = False)
```



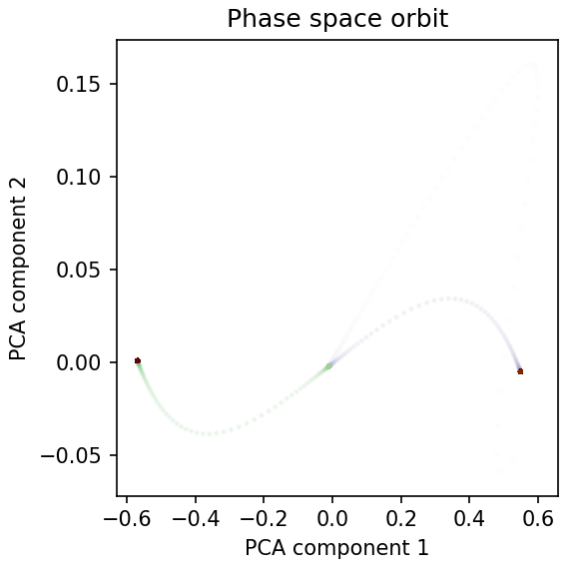
Simulation 1.1: A (pn) loop with symmetric self-feedback, also known as Turing mechanism. The particular GRN layout used here and after are

containted in `networkDefs.py` and have been imported into the notebook. The left frame shows an animation of the network inhibition and activation mechanisms for one of the cells. The size of the filled circles indicate the momentary protein abundance of the respective type, while the arrow thickness indicate the strength at which the other proteins regulate the mRNA sequence by which this state is synthesized. In the upper right corner, the time passed is indicated.
The right panel shows the time series of the respective protein abundances. A vertical line indicates the time at which a cell was split, adding and substracting noise to the daughter cells. After each splitting, the line opacity is put to half its former value. The proteins in the two daughter cells are indicated by a slightly lighter and a slightly darker color, respectively, thereby expressing similarity to the former state.
The code for the left animation is adapted from 古澤 先生's Python tutorial.

In the above simulation, the initalization was done with a random state on $(0, 1)$. It is a remarkable property of dynamical systems with an attractor that the subsequent dynamics are to a certain degree (the size of basin of the attractor) robust against perturbation.

In [9]:

```
plotProj(Cell_T)
```



Simulation 1.2: Phase space plot of the above dynamics. The colors denote different cells I instead of different proteins, contrary to the above plot. Darker colors indicate that the state lies further in time.

In phase space, we can see the binary diversification well projected (even for dimensions larger 2) by a principle component analysis, as the parent and the two daughter cells span a two dimensional plane even in high dimensional spaces that is well projectable onto the 2D plane.

Upon the examination of the time series data, it can be observed that the differentiated cells have both left the state of the initial stem cell, so that it can't be recovered during evolution and further divisions. This makes the Turing mechanism on its own an unsuitable model for a complete description of cell division.

Oscillation: Negative feedback

When defining the properties of stem cells, it was stated that it is important for cells to feature both proliferation *and* differentiation abilities.

We have seen that a Turing mechanism, consisting in its simplest form of a (pn) module with positive and negative self-feedback may give rise to the splitting observed in FIGURE 1 and therefore provide a mechanism of differentiation upon cell division. For the question of proliferation of the original cell type, we need to take into account an additional mechanism. The Turing module basically consisted of a positive feedback loop, that became unstable upon a slight asymmetry introduced by cell division, as discussed above. In this process, the initial state got completely lost.

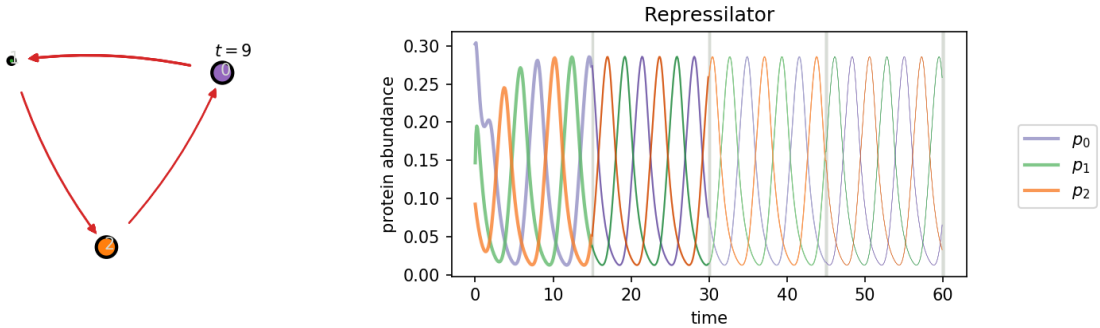
It would be helpful to find a mechanism that is able to retain the initial state. This could be realized by allowing only a fraction of cells to transcend to the differentiated type. For this, it is somehow necessary to create an asymmetry in the cells, so that their fate is divided. A static fixpoint as seen in the Turing model can't provide such dynamics. Hence, it may be helpful to include a non-static mechanism.

Such an oscillatory mechanism can be created by a negative feedback loop mechanism. Suitable models are encoutered in the form of, for example, a repressilator *(Elowitz and Leibler 2000)*, which consists of a negative feedback loop between three genes. (Indeed, the introduction of oscillatory dynamics is the key point for the dephasing which will give rise to the differences in fate, as is discussed below in the model of Isologous Differentiation.)

We construct and observe the oscillatory dynamics of the repressilator network:

In [82]:

```
# set initial conditions on proteins and mRNA
np.random.seed(1)
dim = 3
m_ini = np.random.uniform(0, 1, (dim,L))
p_ini = np.random.uniform(0, 1, (dim,L))

# construct cell
Cell_R = Cell(J = JR, D = DR, T = [0,0,0], m = m_ini, p = p_ini, name = "Repressilat
ani = plotDevelopment(Cell_R, divs = 3, tmax = 60, proteins = [0,1,2])
plt.show(block = False)
```

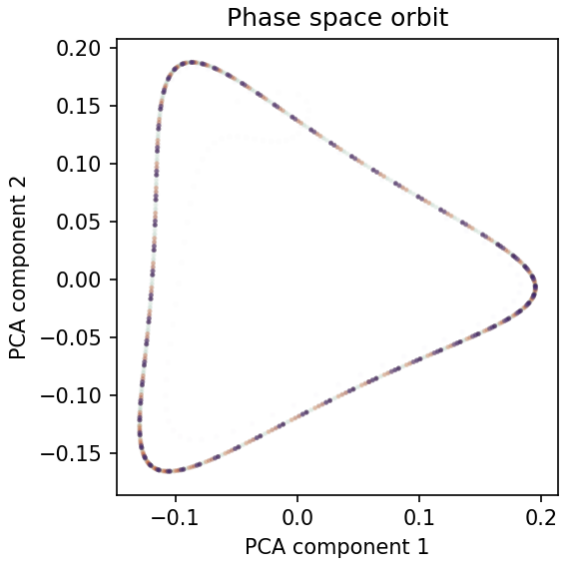


Simulation 2.1: An animation of the network dynamics of the repressilator and its time series until $t = 60$.

Note that due to the absence of diffusion and thus interaction, the division of does not affect the overall expression pattern, and adding noise does not effect the stability of the system.

In [30]:

```
plotProj(Cell_R)
```



Simulation 2.2: Orbit in phase space of the Repressilator oscillator. This is an example of a limit cycle in phase space, a kind of non-fixed point attractor.

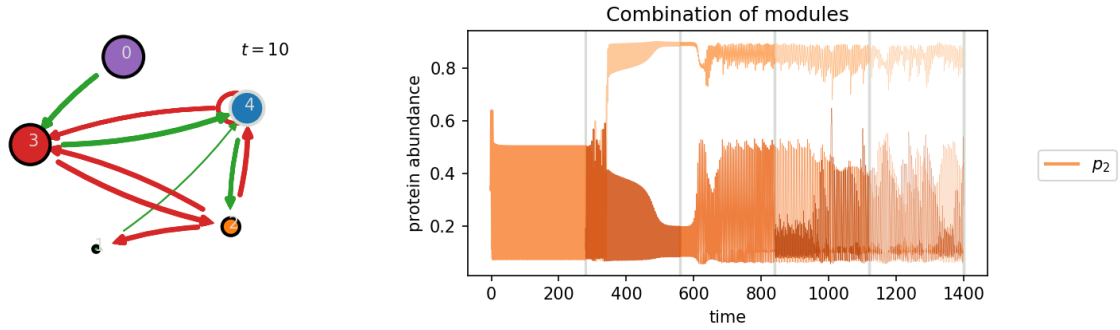
Both the positive feedback of the Turing module for switching to differentiated states and the negative feedback of repressilator like networks may be neccessary for achieving stem cell dynamics. Note that other negative feedback loops which contain not necessarily a repressilator may in combination with other network components still give rise to oscillation.

Combination of modules

We can consider a more complex network, featuring both modules of Turing type switch and negative feedback loop oscillator type, as can be observed in the network below.

In [83]:

```
# set initial conditions on proteins and mRNA
np.random.seed(1)
dim = 5
m_ini = np.random.uniform(0, 1, (dim,L))
p_ini = np.random.uniform(0, 1, (dim,L))

# construct cell
Cell_3 = Cell(J = J3, D = D3, m = m_ini, p = p_ini, name = "Combination of modules")
ani, Cell_3 = plotDevelopment(Cell_3, divs = 4, tmax = 1400, proteins = [2])
plt.show(block = False)
```

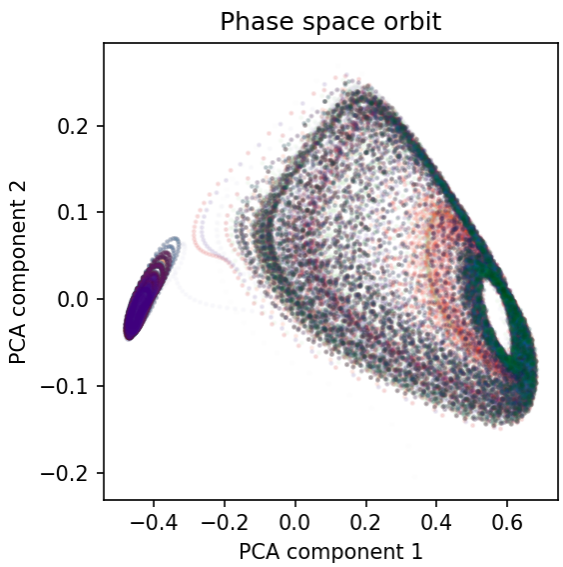


Simulation 3.1: Network animation and time series data for the protein 2 for the network to the left. In some areas of the plot, the oscillation is hardly resolvable due to the tight scaling.

We see that after some divisions, a new cell type emerges due to the presence of multiple cell interaction and persists even after further divisions. Note that its oscillation amplitude is significantly reduced compared to the original state.

In [79]:

```
plotProj(Cell_3)
```



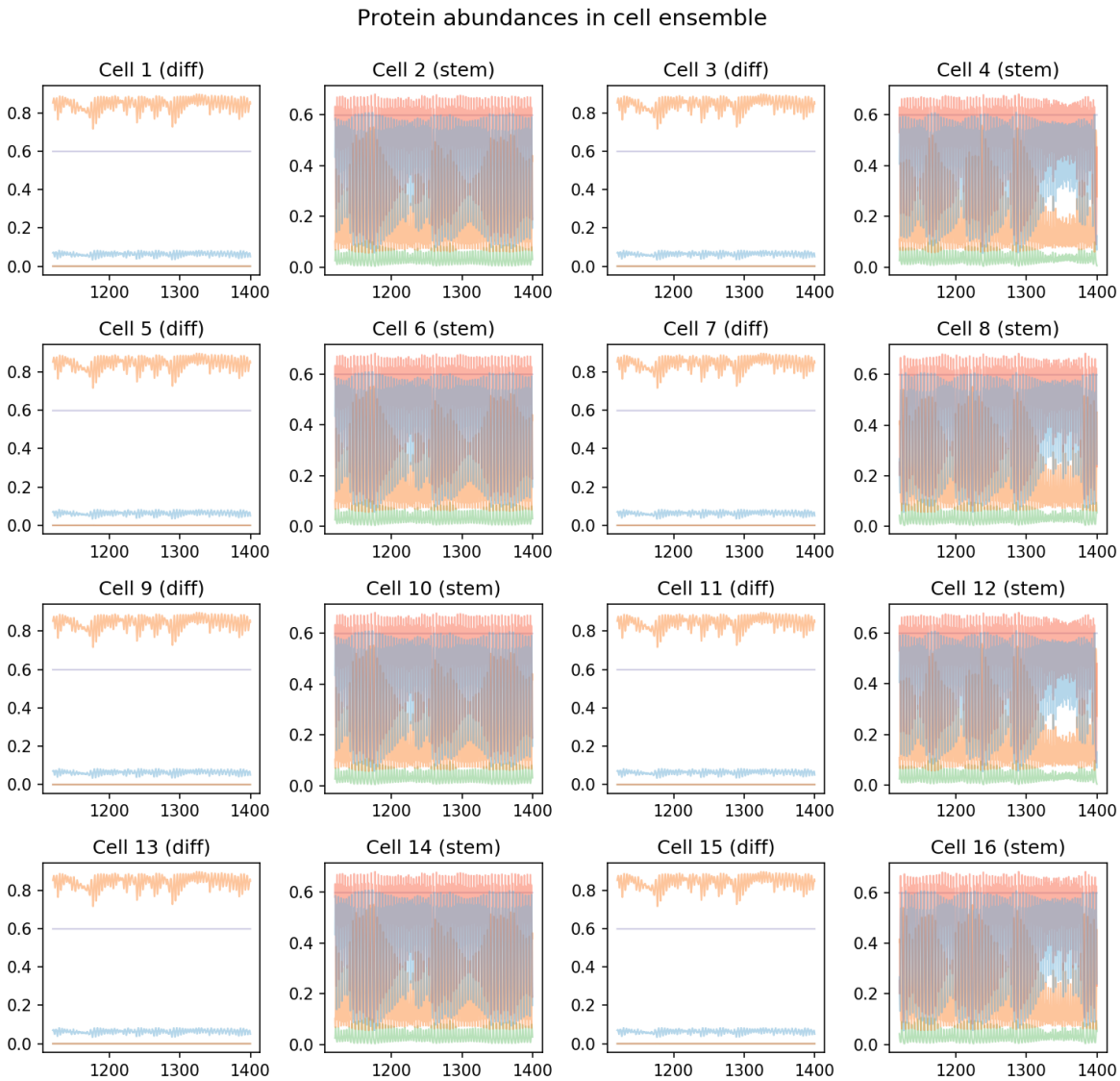
Simulation 3.2: Orbit in phase space of the complicated, combined network.

In the phase space trajectory, we can make out a big oscillatory cycle, corresponding to the stem cell totipotent state. We see the appearence of one smaller attractor, which emerge as soon as the network evolves

in time. This correponds to the lower oscillation amplitude observed in the above time series data.

In [78]:

```
proteinsInCells(Cell_3)
```



Simulation 3.3: Protein abundances across the daughter cells after the last division. From the above breakdown of the protein abundances across the cells, we see that in the last generation of the divisions, there are indeed a set of cells that have differentiated *(diff)* and a set that still exhibits large amplitude oscillation *(stem)*. This illustrates the reduced complexity upon differentiation. The classification of cells is conducted by giving a measure for the oscillation amplitude, and thereby to some degree its complexity. Taking the L_2 norm of each protein time series and summing them serves as such an indicator.

Note on construction of differentiation dynamics

The procedure that we followed seems straightforward: Search for a mechanism that fulfils the differentiation requirement (Turing switch), then try to amend its shortcomings by combining it with a complementary type, the negative feedback oscillatory module. However, while this seemed to work straightforwardly, it has to be noted that working stemness dynamics is relatively rare in networks, so that only a selection of networks indeed shows this behavior.

Interpretation in phase space

The discussed simulations identify a Turing switch mechanism in combination with an oscillatory component as the mandatory components to observe stemness. We can attempt to get a better understanding of these mechanisms by considering the cell's protein abundances in a phase space, similar to what the plots show above. Time evolution of the system forms a trajectory in this space.

A dynamical system as introduced above may have regions of phase space that act as attractor to the trajectory, corresponding to a distinct cell type. As in the first example of the Turing network, the system might quickly enter the attractors center and stay at this fixpoint. However, there are also non-stationary, stable configurations: An oscillatory attractor, as in the negatively coupled Repressilator systems may keep the trajectory periodically moving, but stable in its configuration in space, similar to what is shown in the figure below.

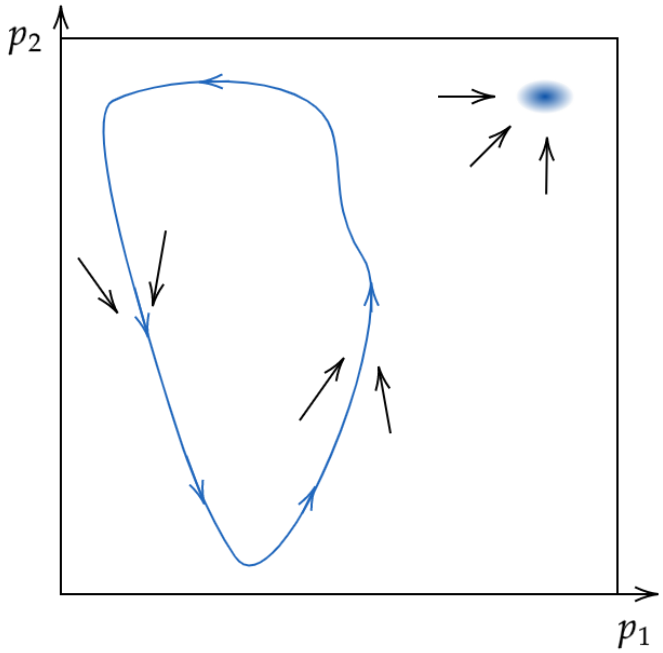


Figure 3: Sketch of the phase space for a two dimensional system with protein expression levels p_1, p_2. On the left hand side, a limit cycle is shown that may give rise to dynamics as in the Repressilator, similar to what is shown in **Simulation 3.2**. In the upper right corner lies a fixed point attractor, that could correspond to a Turing type mechanism.

Neither of these configurations or attractors by itself is able to give rise to a diversification and proliferation simultaneously. For this, the combination of both mechanisms was required.

For finding stemness, we would expect either both daughter cells or at least one of them to persist in the multipotent state. This succeeds with the introduction of the oscillator module. Now we observe that only one daughter cell leaves the parent configuration and becomes a commited state. The Turing module then realizes the requirement of irreversibility: The created, commited cell state usually finds itself in a smaller basin of attraction, and typically shows oscillation behavior with smaller amplitude. This limits this new cells ability to differentiate again, it continues to proliferate without changing its type any more. The Turing module thereby acts as a stabilizer of the differentiated state.

Comparison to Isologous Diversification

The phase space evolution of cell differentiation has been proposed to follow a model of several steps of diversification, which is briefly reviewed here and put into relation to the need for asynchroneous oscillation dynamics that were found in simulations. A detailed description is found in *(Kaneko and Yomo 1997)*. This study focusses on a general mechanism to produce differentiated cells, even for the same internal and external initial conditions. The process evolves without the need to take into account active controlling mechanism.

The stem cell offspring is pictured as being in a periodic orbit in protein state space. To transit to the differentiated states, the following steps are taken:

1. Synchronous oscillation
   All daughter cells are in periodic orbit in phase space, and especially their phases are in synchronization. This state persists until the small fluctuations introduced at each division amplify.

2. Clustering of oscillation phases
   The diffusive coupling of state of cells gives rise to an instability in the phases between their oscillations, which has been studied as Dynamic Clustering. This effect of grouping as opposed to random decoherency is due to the strong intercellular interaction, a general feature of coupled nonlinear oscillators. Biologically, this exchange is amounted to biochemicals, acting as signal transmitters in between cells. Note that at this stage, the cells are to be considered of the same type, as the time averaged concentration $\langle p_i(t) \rangle$ is the same for all clusters.

3. Differentiation in metabolite composition
   As each cluster undergoes further divisions, the average concentration of proteins start to differ in between clusters. This can be understood by examining the underlying GRN: Due to the dephasing in 2.), the chemical concentrations between cells differ at each point in time. The interaction by diffusion leads to chemical fluxes in between cells, thereby forming instability, similar to what was discussed with the simple Turing model. In state space then, the dephased orbits have transited to distinct areas and have formed a new type of cell with distinct average concentrations.

4. Determination of differentiated cells
   In this stage, cells manifest their newly gained dynamics, that is they become robust towards the interactions with their surroundings. This introduces irreversibility into the model by making the differentiated cell stable as well as allowing it to produce progeny of its own type, without transiting back.

At the core of this process lies the mechanism of intercellular communication, which gives rise to every step of this chain depending on the number of cells present.

Entropic view on differentiation

This paragraph only mentions the concept, as I lack the dynamical systems theory knownledge for an adequate description.
The potential diversity of stem cells is often expressed in high dimensional protein dynamics. This characteristic is partially lost upon cell division, as is seen in **Simulation 3.2** and **3.3**. In the time series data, the oscillation amplitude was reduced or small around a constant value. In the phase space picture, the diversified orbit occupied a smaller region of phase space.
These to notions can be brought together by assigning a cells state an entropy, corresponding to its orbital complexity. A measure for this phase space volume is the Kolmogorov-Sinai entropy *(Sinai 2009)*. In **Figure 6** below, I attempted to incorparate a measure for orbital complexity into the stem and differentiated subsystems. I am however not sure if it can be sensibly related to attractor steepness, that is whether a lower entropy system is the irreversible state (contrary to what thermodynamics suggests). I try to come back to this in the discussion section.

Minimal model for differentiation mechanism

The stages of the Isologous Diversification mechanism in combination with the state space concept of multiple attractor gives (at least in the detail given here) a qualitative understanding on why differentiation occurs. However, it is hard to identify the role that oscillations and dephasing take in this process. To get a closer understanding on this, it is helpful to go back to an elementary model and study its properties under division. This is done in detail in *(Goto and Kaneko 2013)*, from which we want to link the central thoughts to the simulations carried out above.

The most simple case is the two state system encountered in **Simulation 1**. We have already seen why such a system has a fixpoint of single-cell dynamics. For a description, a reduced formulation of the biologically motivated model introduced in the beginning is aimed for.

Layout

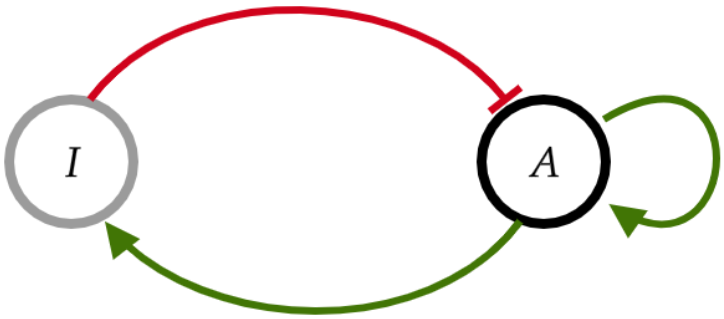


Figure 5: Layout of a minimal, two dimensional asymmetric oscillator. The inhibitory protein is marked gray.

In the adiabatic limit of fast mRNA expression compared to protein abundance changes, we can discard the mRNA variable and consider only the p.

Let $x = p_1$ and $y = p_2$, where y be diffusive. The dynamics for diffusion only between those cells are then

$$\begin{aligned}\dot{x}_1 &= f(J_{xy}y_1 + J_{xx}x_1 - \theta_x) - x_1 \\ \dot{y}_1 &= f(J_{yx}x_1 + J_{yy}y_1 - \theta_y) - y_1 \underbrace{+D(y_2 - y_1)}_{+\alpha}\end{aligned}$$

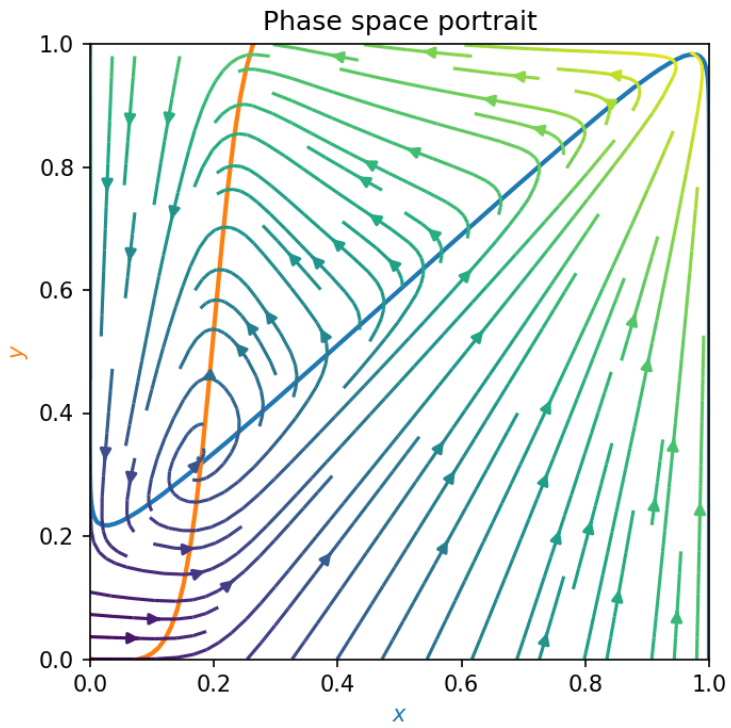
and

$$\begin{aligned}\dot{x}_2 &= f(J_{xy}y_2 + J_{xx}x_1 - \theta_x) - x_2 \\ \dot{y}_2 &= f(J_{yx}x_2 + J_{yy}y_2 - \theta_y) - y_2 \underbrace{-D(y_2 - y_1)}_{-\alpha}.\end{aligned}$$

Note especially the sign difference in the diffusion term α. As this system is two dimensional, the phase space can be examined graphically.

In [38]:

```
from plotGraph import phasePlot2D, bifurcation, Ddependence
phasePlot2D()
```



α

Simulation 4.1: Phase space portrait of the two-dimensional minimal system that we introduced above. The axes show the concentrations of the proteins, respectively. It shows the *single cell* phase space flow $\frac{dx}{dt}(x,y)$, $\frac{dy}{dt}(x,y)$. Hue indicates the magnitude of the flow vectors. The solid blue and orange line indicate the nullclines $\frac{dx}{dt} = 0$ (blue), $\frac{dy}{dt} = 0$ (orange). The slider below the plot allows for adjusting the constant diffusion strength α between the cells.

There are in general two steady states: One is the limit cycle surrounding the oscillatory attractor in the lower left, characteristic for the stem cell oscillation (unfortunately, I wasn't able to find a way to determine its location reproducibly, as the calculations are quite intense). The other, fixpoint-like steady state lies in the upper right corner of the state space and depends on the sign of the coupling parameter α of the system, which can be adjusted with the slider.

Upon cell division, α will have a finite value with opposite sign for both cells. For the cell with positive α, at the intersection of the x and y nullcline a new fixpoint arises, which is distant from the limit cycle that had been the original stem cell attractor. This corresponds to the differentiated cell type. The other cell stays however bound to the oscillatory attractor. A bifurcation of this type is known as *Saddle Node in Limit Cycle* (SNIC) in Dynamical Systems theory.

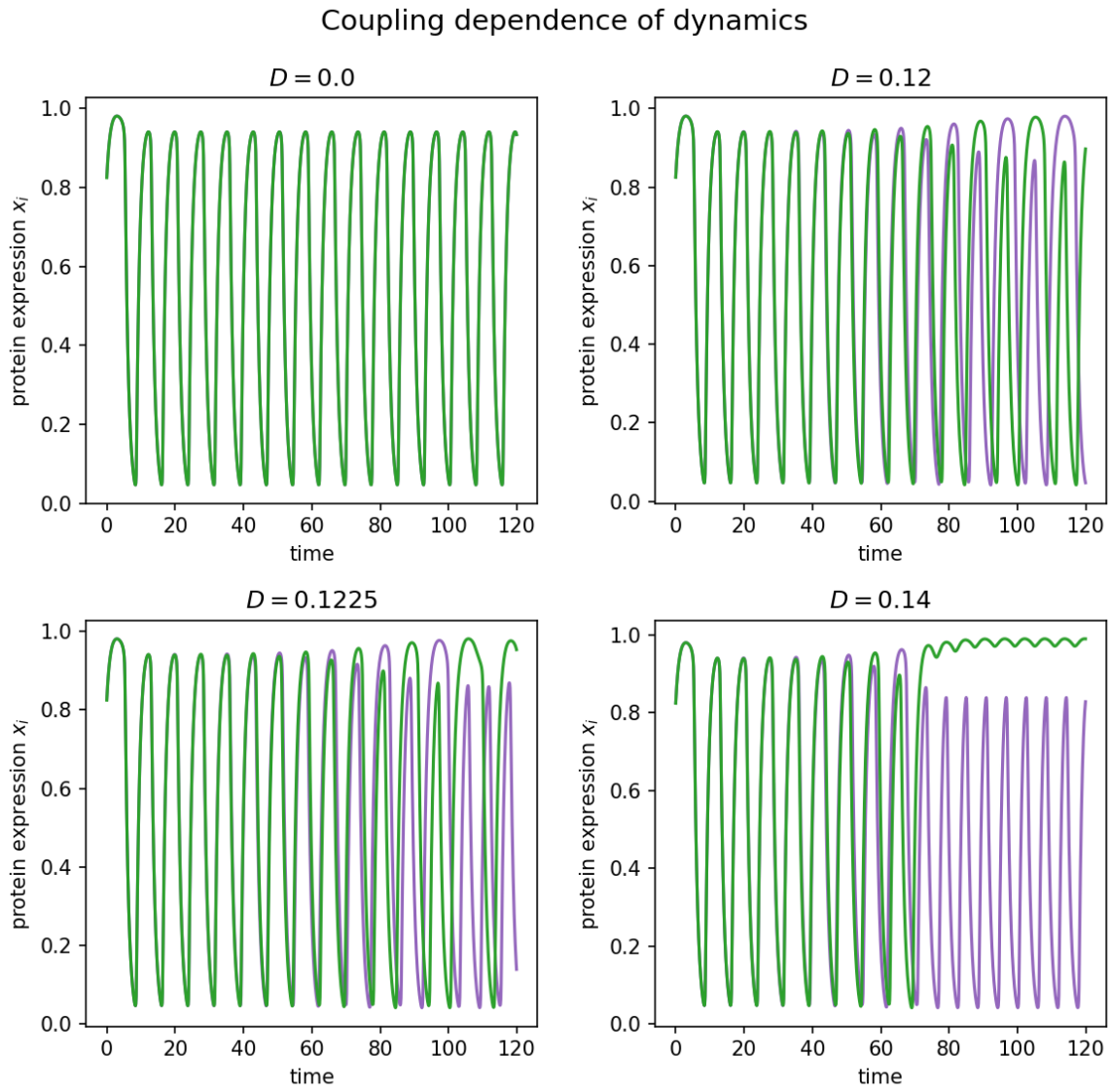
In this reasoning, it was assumed that α is constant, whereas in fact it is a dynamical quantity dependent on the difference in states in both cells, staying driven by the oscillation of the limit cycle attractor cell. The phenomenon of emergence of the fixpoint can be regarded to remain valid if the nullcline intersection is present

for a larger period than it is absent. Due to the time dependence of α, one can expect a small persisting oscillation even in the differentiated cell. The opposite sign in α between $\dot{y}_1$ and $\dot{y}_2$ splits their behaviour, such that one oscillation proceeds faster and they become dephased. Note that the original cell has slower oscillation, in accordance with the general tendency of higher dimensional systems (the stem cell) to have slower dynamics.

In this discussion the importance of the coupling parameter for the differentiation was discussed. Below, the time series data for different coupling strenghts (D is the coupling *constant*) is plotted.

In [59]:

```
Ddependence(ds = [0.0, 0.12, 0.1225, 0.14], t = np.arange(0, 120, .1))
```



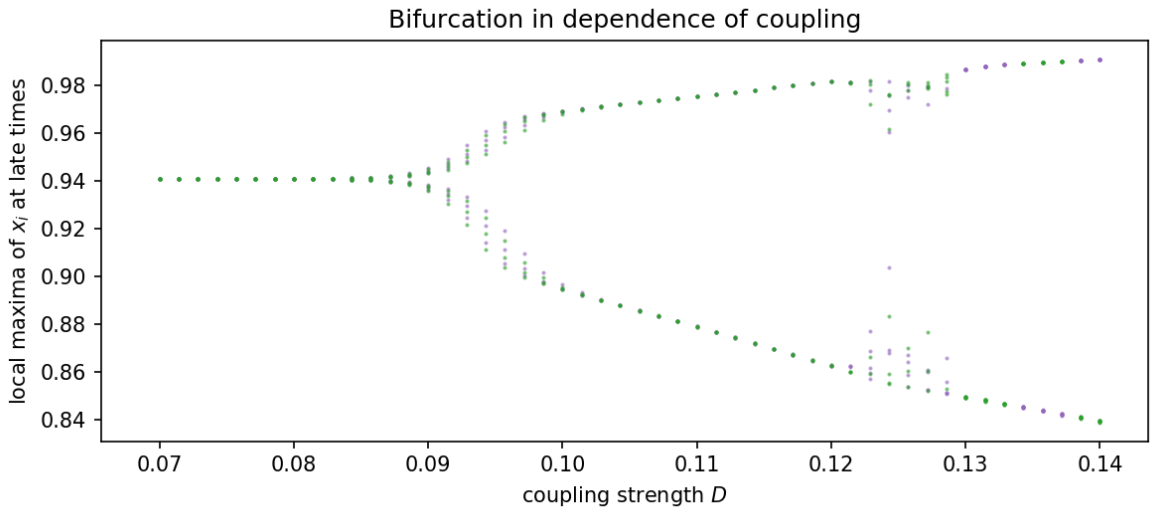
Simulation 4.2: Coupling dependence of time evolution of the asymmetric two dimensional oscillator. As the coupling strength increases, one can observe dephasing and varing amplitude, until beyond a critical coupling strength, differentiation takes place.

The above parametric dependence is for dynamical systems often represented in a bifurcation plot, which condenses the behavior of a phase transition parameter in dependence of the bifurcation variable. The latter is the interaction strength D, while the former can be taken to be the local amplitude maxima of the two

dimensional system under consideration. The amplitude was a characteristic measure of the orbital complexity of the system. From the above figure one can observe its dependence with the interaction strength. By examining its asymmetry in late development stages of the system, we may conclude on its splitting behavior.

In [84]:

```
bifurcation(supportPoints = 50)
```



Simulation 4.3: Bifurcation plot of the amplitudes of the protein variables x_1 and x_2. The data was obtained by initializing a divided cell with a small uniform noise on the interval $[-10^{-3}, 10^{-3}]$. The system was integrated until time $t = 2500$ and for the last 500 time steps (corresponding to a steady state, as verified manually), the maximum values for each time series of the x_i were determined using the `scipy.peakfinder` method on default settings. *As this is a heavy calculation that takes around a minute, one might try reducing the number of support points*.

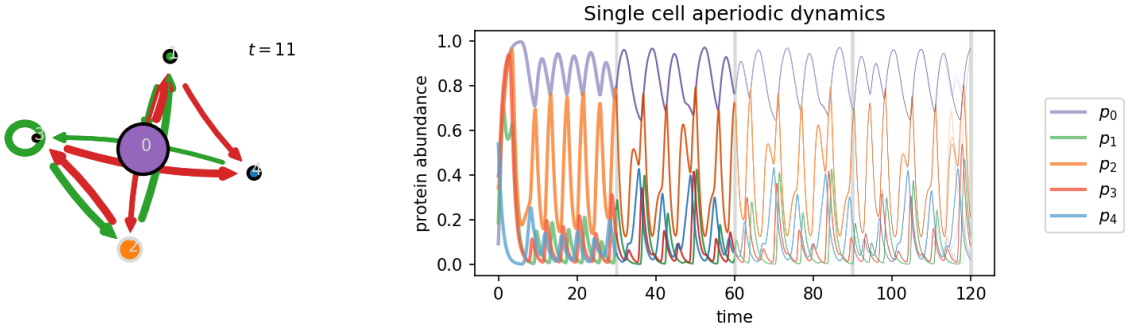
The above bifurcation plot shows different stages in the system development which have not been clearly observable from the above plots. At $D = 0.8$, the amplitude becomes instable and the system is in superposition of oscillations. Their amplitude difference enlarges as the system size is increased. At $D = 0.12$, the system enters a critical region, where the amplitude isn't clearly split into two, but has multiple values. Only after D has trespassed $D = 0.13$, stable differentiation behavior emerges: The higher amplitude is exclusively taken by one cell, whereas the lower maxima corresponds to the the original stem cell. This is different form the behavior in the former regime: Now, not two oscillations are superimposed, but one state oscillates with small amplitude at a high value, while the other maintains its stem cell large amplitude oscillation, as in the last pane of **Simulation 4.2**. The switching of colors in this region appears because due to the symmetric noise, sometimes one and sometimes the other cell takes on the differentiated state.
The results here differ slightly from the ones in *(Goto and Kaneko 2013)*, perhaps because here for this simulation, the difference to the average concentration $\bar{y} = (y_1 + y_2)/2$ was taken.

Side note on chaotic dynamics

We saw that in a certain parameter regime, a coupled system might be subject to chaotic (also known as aperiodic) behavior. However, there are systems in which such dynamics, due to the nonlinearity, can already arise in single cell dynamics, which is only briefly mentioned here.

In [76]:

```
# set initial conditions on proteins and mRNA
np.random.seed(1)
dim = 5
m_ini = np.random.uniform(0, 1, (dim,L))
p_ini = np.random.uniform(0, 1, (dim,L))

# construct cell
Cell_4 = Cell(J = J4, D = D4, m = m_ini, p = p_ini, name = "Single cell aperiodic dy
ani = plotDevelopment(Cell_4, divs = 3, proteins = [0,1,2,3,4])
plt.show(block = False)
```



Simulation 5: Aperiodic behavior for a cell even before its division.

Hierarchical stem cells and hematopoietic systems

In addition to the previously considered case of emergence of a new class of cells, the question may arise whether further hierarchical stages, that is cells with different degress of specialisation, can be modeled.

The two modules of switches and oscillators provide the basic building blocks for such systems. The combination of an aperiodic oscillation (AO) with Turing type switch mechanisms (TS) can enable hierarchical differentiation as the cells multiply.

In the minimal picture, two modes of combination for more complex networks are possible; a parallel mode of stacking and a sequential mode. Both can be described for diversification of a stem cell A into B and C.

Parallel stacking
A asymmetric module as considered above is combined with a Turing switch, so that the model constitutes four proteins in total. When the asymmetric differntiation occurs, the differentiated state exhibts a almost constant, high protein expression, as seen in the phase space diagram. This is then negatively coupled to the inhibitory module of the TS, thereby triggering its diversification mechanism into B and C.

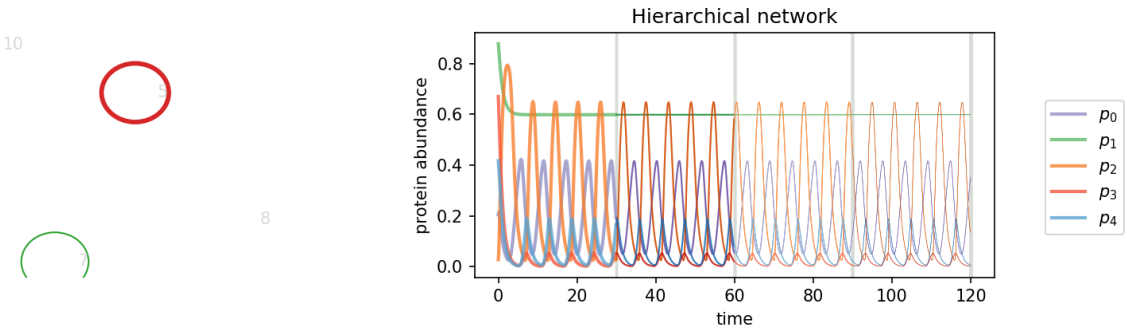
Sequential stacking
A TS is placed in between two AO modules, with six proteins in total. Again, the characteristic bifurcation leads to a constant expression of one protein in the first AO, which inhibits one part of the negatively coupled TS, leading to a differentiation in its two proteins. So far, the situation is identical to the first case. As the protein of the intermediate TS that was inhibited is itself negatively coupled to the second AO, the differentiation mechanism in its proteins is induced.

The key difference between these mechanism is that in parallel stacking, the differentiation of A to B and C occurs simulatiously. In the sequential mode on the other hand, the intermediate Turing motif needs first to have been adressed until the diversification to cell type C can take place. I find it however still difficult to grasp the relation between the modules.

I try to simulate such a system, however, I lack a sufficient understanding of the mechanisms involved, so that I struggle to give a good interpretation of the result. I think that choosing an appropriate threshold depending on the function of several components in the network may improve the results.

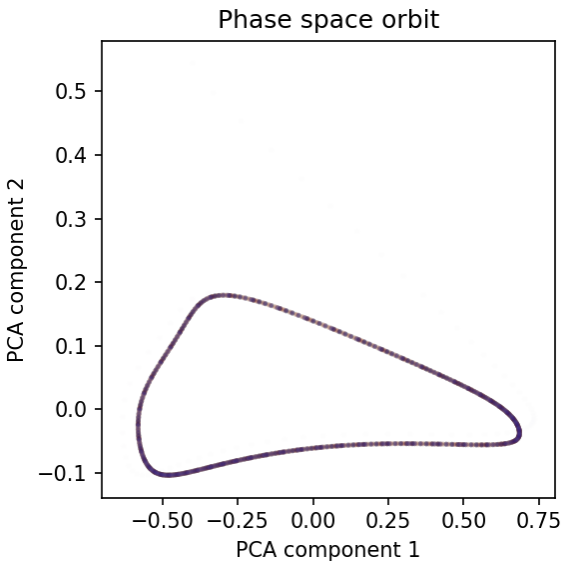
In [73]:

```
np.random.seed(1)
dim = 12
m_ini = np.random.uniform(0, 1, (dim,L))
p_ini = np.random.uniform(0, 1, (dim,L))

# construct cell
Cell_H = Cell(J = JH, D = DH, T = np.full(12, -0.01), m = m_ini, p = p_ini, name =
ani = plotDevelopment(Cell_H, divs = 3, proteins = [0,1,2,3,4])
plt.show(block = False)
```



In [74]:

```
plotProj(Cell_H)
```



Simulation 6: Attempted simulation of a hierarchical system, which was not successful due most likely due to poorly chosen thresholds. Further testing could provide more insight on this, which is unfortnately not possible in this project.

Comparison to noise as a source for differentiation

The systems we considered required only a small introduction of noise upon cell division (it is however necessary to make the systems any different at all!). This seems natural, as a division is rather (depending on the number of proteins) unlikely to produce perfect symmetry. However, there exist models that focus on noise as the main mechanism driving differentiation, originating for example from molecular (internal) or environmental (external) fluctuations. Those do not necessarily depend on intercellular interaction or the division process. The basic mechanism may be readily understood in the phase space picture, as was initially proposed with Waddington's landscape.

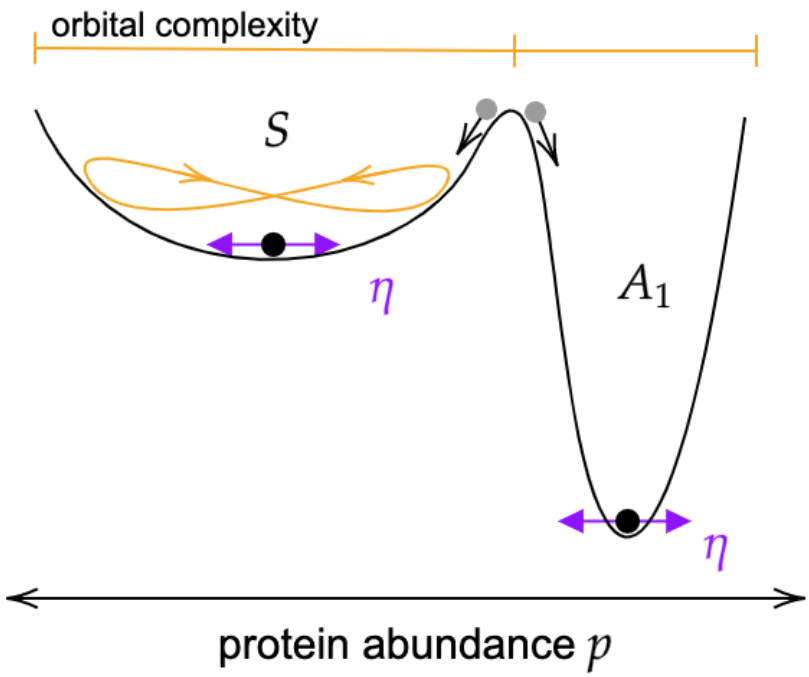


Figure 6: Sketch of Waddington's potential 'landscape', here for the heavily reduced one dimensional case. On the left, the stem cell attractor is shown, while the right basin corresponds to a specialised state. A periodic orbit and a measure for the orbital complexity are indicated in orange. In terms of the above discussed entropy picture, the stem state is expected to feature the larger basin due to its higher protein dimensionality and thus orbital complexity. The relative of the wells, in an energy picture indicates that the backwards transition from the differentiated state is much harder, thus encoding irreversibility. The purple arrows indicate noise in the system. The grey state indicate the splitting of two daughter cells due to a small instability upon cell division.

For simplicity, let's consider a single cell in state space. If its state is stable, it will have its dynamics bound to an attractor. Upon the introduction of noise to time evolution, the cell might however acquire a sufficiently large perturbation to leave this basin of attraction and transit to a secondary state.

When looking back to the requirements for stemness stated in the beginning, the noise driven model however has some shortcomings:

Robustness
As the origin for cell division is stochastic in nature, we can only formulate the expectation value of cells that will have made the transition after some time, or more precisely the ratio of stem cells to differentiated cells. However, depending on the phase space's structure and the noise amplitude, that ratio may carry rather large variance if being stochastically driven.
Experiments show that the differentiation process is robust around a certain number ratio of differentiated cells.

If one type of cells is decimated in an organism, the cells strive to restore this difference. This sensitivity to surrounding cells of the cellular dynamics stress the importance of including interaction into the dynamics, otherwise purely environmental (medium) changes could not realize such regulation.
Another aspect of robustness is the time dependency of differentiation. The oscillatory dynamics model is closely determined in the time point that a cell leaves the initial attractor (due to the periodicity), while this time is random for the noise driven process.

Irreversibility
In order to realize the directedness of the differentiation process, the attractors in state space have to be different in characteristics. While the stem cell attractor is in general more plastic and allows for plural differentiations, the commited cell state lacks this flexibility and is closely confined. Thus, intuitively we may regard the stem attractor as a rather large, shallow basin while the commited cell's attractor is small in size. To establish a distinction in transtition rates between those states, the system needs to be tuned to the right noise level: If the perturbation strength is too large, the cell will switch between the states with near identical likeness. On the other hand, the fluctuation needs to be sufficiently large to leave the initial basin in the first place. This requirement for tuning might be more severe if the system is to differentiate to multiple hierarchical attractors. This issue is not initially present for the oscillatory dynamics model.

Discussion of the results

Biological relevance

The considered model shows, in a given parameter range, differentiation behavior that fulfils the initial postulates of diversification and proliferation, where the latter relied heavily on oscillatory dynamics inside the cell. Such a phenomenon, on first glance seeming odd for a system that is thought to be in a sort of balance, is in fact found to occur in multiple biological systems, as has been pointed out by Goodwin.

Here, mostly low dimensional models have been focussed on. In reality, cells constitute a number of proteins in the order of thousands. By combination of elementary network constituents, an expectation would be that the basic stem dynamics still apply, however due to the higher dimensionality a plentitude of further attractors emerges in state space, corresponding to multiple stable states.

To experimentally verify such a mechanism, it would somehow be required to obtain a picture of the regulatory pathways of the cell, and probe if couplings consisting of the elementary modules found above indeed constitute a substantial fraction.
Previously, there is indeed evidence of the presence of positive and negative feedback loops, for example in known core embryonic stem cell (ES) GRNs or in neuronal regulatory mechanisms where central proteins such as Hes1 can be determined to be relevant to control.

All our considerations where taken with dimensionless parameters. On the one hand, it would be interesting how the frequency determining parameters γ with the values chosen would relate to a real world oscillations timescale, which for cells is in the range of minutes or hours. On the other hand, it would provide novel insights to see find the types of proteins that have the diffusion strength in the range of the model considered here, and therefore conclude on their relevance to diversification.

Conclusion

The simulations showing stemness behavior were a small selection of possible GRN configurations that were selected on their properties relating to the initially stated mandatory characteristics for stemness. This stresses indeed the point that there are several configurations of the combination of switch and oscillation modules able to produce stemness dynamics, however, this is not a sufficient criterion. The cell-cell interaction is of essential importance for the splitting of states, and the stabilization of the new state, giving rise to robustness.

One is able to identify quasi necessesary modules to generate stemness in cells, however, a completely reduced model like a simple Turing loop fails to show proliferation in its dynamics. A minimal model of an asymmetric oscillator did show a lot of the characteristics necessary, and exhibits robustness, which wasn't taken up in the report.
We attempted to describe some of the mechanisms observed in the high dimensional simulations in the low phase space picture. There, we had to further simplify by introduction of adiabacy assumption.
The simple picture of the Waddington landscape, as suggested in **Figure 6** however, has difficulties to explain all the phenomena observed. Especially the relevance of oscillatory dynamics and the limit cycle interaction that proofed to be so essential for a non-noise centered model are not easily framed in these terms.

In the nonlinear, chaotic and often very high dimensional behavior of complex systems, these models however often provide a good guide to their analysis and understanding, as long as they are treated in awareness of their shortcomings. The robustness and complexity of a stem cell on multiple hierarchical stages is, in its full complexity, an emergent and marvellous property of complex biological systems.

Although complex systems seemed to me to some degree an unapproachable field, I enjoyed learning about the dynamical systems and found it quite enlightening to find a connection to some biological concepts encountered in my school time.

When trying to print the report, it turned out far longer than intended... My apologies for that!

Appendix

Side note on Turing patterns

As this is such an essential type of network, we aim for a more detailed description here, by examining the behaviour of a Turing type system around a fixpoint.

In a nutshell, the Turing model consists of multiple chemical compound concentrations $c_i = c_i(\vec{x}, t)$, obeying the differential equations

$$\dot{c}_i = \underbrace{f_i(\{c_j\})}_{\text{reaction}} - \underbrace{D_i \Delta c_i}_{\text{diffusion}}$$

for each i. Δ denotes the Laplace operator, which is the second derivative in a one dimensional case.

We assume the simplest form of a Turing model, featuring two species only, call A and B:

$$\dot{c}_a = f_a(c_a, c_b) - D_a \Delta c_a$$
$$\dot{c}_b = f_b(c_a, c_b) - D_b \Delta c_b.$$

In the lecture, we have analysed this kind of system, and we will here briefly recapitulate the results.

As we will be particularly interested in solutions that oscillate in space, we perform a fixpoint analysis of the system. Assume the system has a fixpoint c^*. Consider then the transformed equations for the new coordinates δx around the fixpoint via $c = c^* + \delta c$. To first order in the new coordinates δc, the equations become

$$\dot{\delta c}_a = \sum_j J_{aj} \delta c_j - D_a \Delta c_a$$
$$\dot{\delta c}_b = \sum_j J_{bj} \delta c_j - D_b \Delta c_b,$$

which can be written in matrix form

$$\dot{\delta \mathbf{c}} = (J - D\Delta)\delta \mathbf{c},$$

where J is the Jacobian at $\mathbf{c}^*$ of the function vector $\mathbf{f} = \{ f_i \}$ and D summarizes the diffusion constants.

where J is the Jacobian of $\mathbf{f}$ at the fixed point $\mathbf{c}^*$ and D summarizes the diffusion constants.

We expand the solution into a Fourier series in space, and are thus able to evaluate the equation in Fourier space:

$$\delta\dot{\mathbf{c}} = \underbrace{(J + D\kappa^2)}_{M}\delta\mathbf{c},$$

where κ is the spatial frequency of a Fourier mode.

We call the system stable if it is convex, i.e. its coupling matrix M has the real parts of its Eigenvalues λ smaller than 0. This is equivalent to the solving Eigenmodes $\delta\mathbf{x}$ being harmonic oscillators, which are stable around their resting position. As the full solution will be composed from a superposition of the Eigenmodes, we demand the amplitude of each mode to be bounded.

Depending on the eigenvalues of M, we may make conclusions about the behaviour of the solution by investigating an elementary solution in real space;

$$\begin{aligned}\delta\mathbf{c} &= \mathbf{E}_\lambda e^{i\kappa x} e^{\lambda t} \\ &= \mathbf{E}_\lambda e^{i(\kappa x + \Im\lambda t)} e^{\Re\lambda t},\end{aligned}$$

with the solutions eigenmode specifing vector $\mathbf{E}_\lambda$ and $\Re$, $\Im$ denoting real- and imaginary part of the eigenvalue.

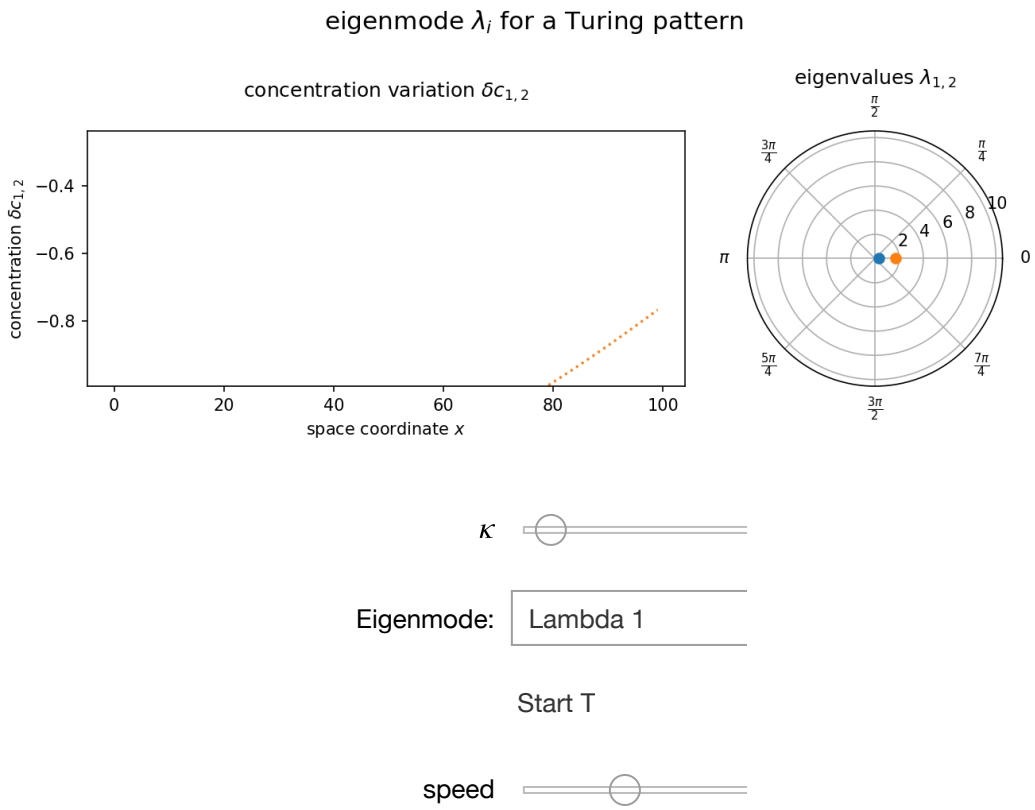
The general solution then follows as a linear combination of these modes. When $\Re\lambda < 0$, such modes decay and the system is indeed stable, as claimed above. If $\Im\lambda \neq 0$ on the other hand, the system oscillates over time and the pattern is expected to slowly wander as a wave, as is observed for some animals.

In this case, a fixpoint $\mathbf{c}^*$ is indeed stable over time and a persistent pattern can form.

I include here a somewhat unfinished simulation of the spatial and temporal decay of such modes, depending on the matrix. The source code is found in `turing.py`.

In [68]:

```
### solve the EV problem for the coupling matrix

from turing import *

J11 = -1
J12 = +1
J21 = -1/2
J22 = +1

Da  = 1
Db  = 1

J = np.array([[J11, J12], [J21, J22]])
D = [Da, Db]

ani = turingAnimation(J,D)

#plt.ylim(-100,100)
plt.suptitle("eigenmode $\lambda_i$ for a Turing pattern", size = 15)
plt.show(block = False)
```



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