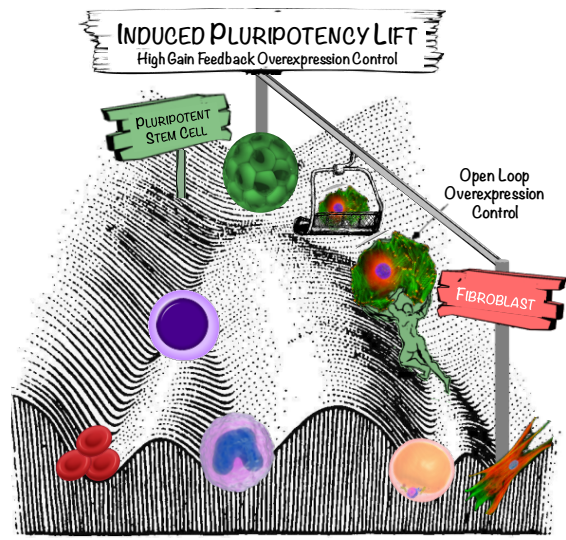


A mathematical model of cell fate reprogramming: From open loop to closed loop feedback control

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Outline

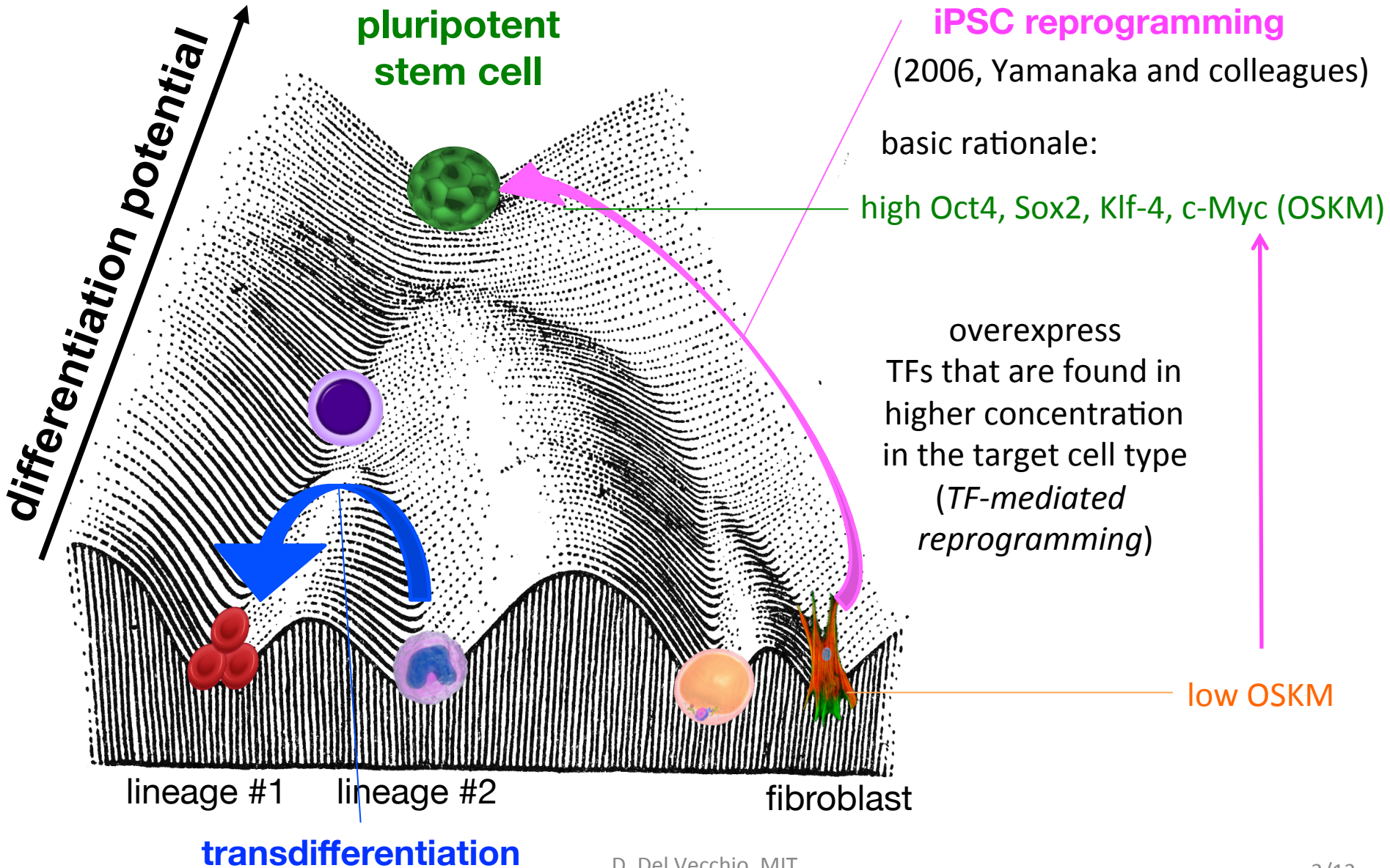
Background on cell fate reprogramming

Reprogramming GRNs through prefixed overexpression

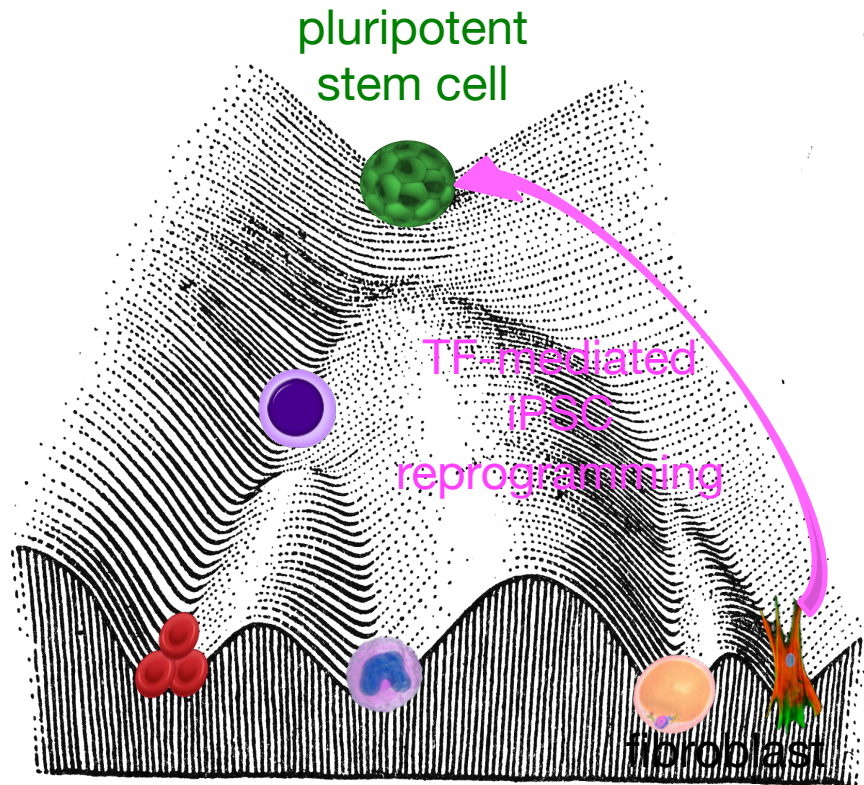
Reprogramming GRNs through feedback overexpression

A blueprint of a synthetic genetic feedback controller implementing feedback overexpression

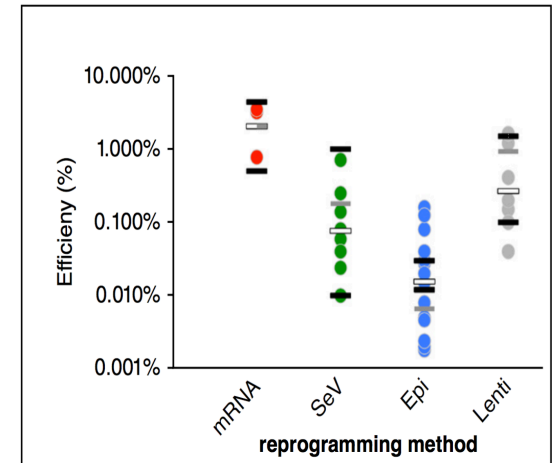
Background on cell fate reprogramming



Background on cell fate reprogramming



- Today: efficiency remains below 1% across a range of transfection methods



Schlaeger and Daheron (2015)

- Majority of resulting cells become partially reprogrammed – overexpressed TFs fail to have the pluripotent concentrations (Morris and Daley, 2013; Chan et al. 2009)
- TF stoichiometry highly affects efficiency (Papapedrou et al. 2009)

- Can reprogramming failure be attributed to the structure of the core GRN that is responsible for pluripotent (PL) cell fate determination and maintenance?
- If GRN structure can be implicated in these failures, what reprogramming approach can be considered that is highly independent of the endogenous GRN?

Outline

Background on cell fate reprogramming

Reprogramming GRNs through prefixed overexpression

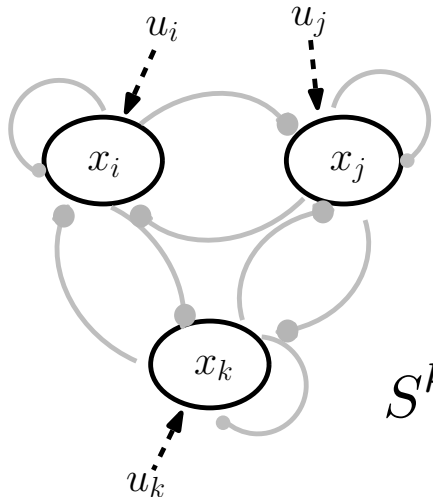
Reprogramming GRNs through feedback overexpression

A blueprint of a synthetic genetic feedback controller implementing feedback overexpression

A mathematical model of cell fate reprogramming

Gene regulatory network (GRN) x_i concentration of TF x_i $x = (x_1, \dots, x_n)$ state of the GRN

$u_i \geq 0$ external stimuli (overexpression)

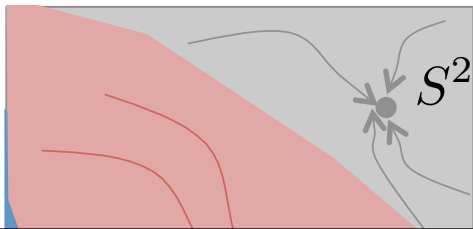


$$\Sigma_u : \frac{dx_i}{dt} = f_i(x, u_i) = \underbrace{H_i(x)}_{\text{Hill function}} - \underbrace{\gamma_i x_i}_{\text{decay}} + u_i \quad \Sigma_0 : u = 0$$

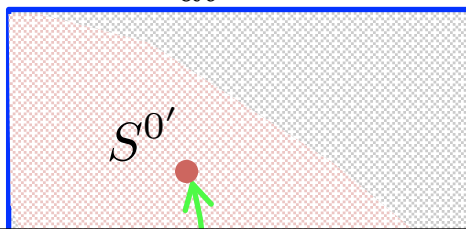
system without overexpression

S^k stable steady state of Σ_0 $R_0(S^k)$ region of attraction of S^k
 $f(S^k, 0) = 0$

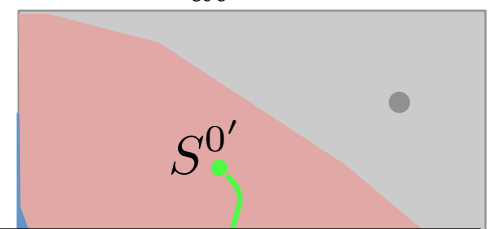
$$\Sigma_0 : \frac{dx}{dt} = f(x, 0)$$



$$\Sigma_u : \frac{dx}{dt} = f(x, u)$$



$$\Sigma_0 : \frac{dx}{dt} = f(x, 0)$$



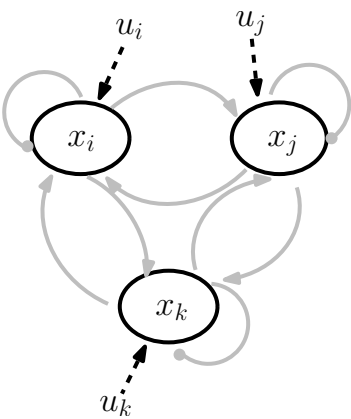
The GRN is **strongly reprogrammable** to S^0 provided there is a u such that Σ_u has the attracting set contained in $R_0(S^0)$

S^1 $x(0)$

$x(0)$

Reprogramming cooperative GRNs

Cooperative GRN

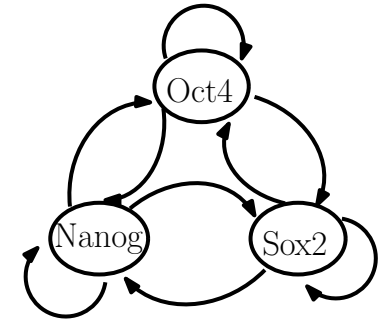


$$\frac{dx_i}{dt} = f_i(x, u_i)$$

$$\frac{\partial f_i}{\partial x_j} \geq 0, \quad i \neq j \quad \text{positive mutual regulation}$$

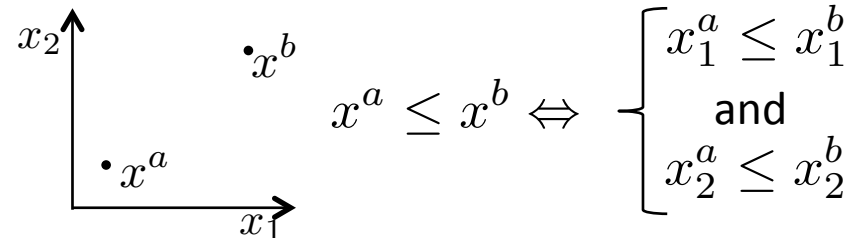
$$\frac{\partial f_i}{\partial u_i} \geq 0 \quad \text{positive stimulation}$$

core pluripotency GRN



Boyer et al. (2005)

Fact 1: The set of stable steady states in a cooperative GRN has always a maximal element



Theorem: A cooperative GRN is strongly reprogrammable only to its maximal stable steady state

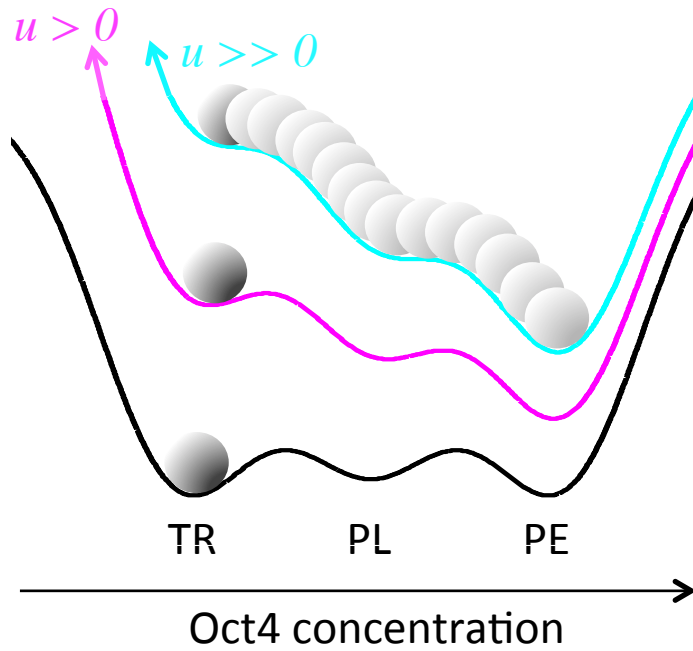
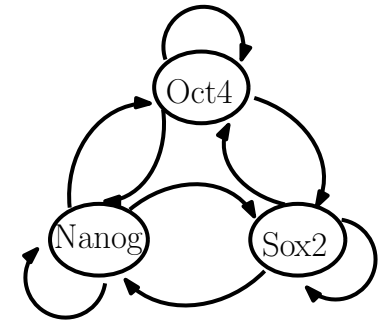
→ A cooperative GRN is *not strongly reprogrammable* to stable steady states that have intermediate values of some of the TF concentrations x_i

Reprogramming cooperative GRNs

Oct4 concentration { *low* in trophectoderm (TR)
intermediate in pluripotent cells (PL)
high in primitive endoderm (PE)

Niwa et al. (2000)

→ Core pluripotency GRN is not strongly reprogrammable to PL



Overexpression u destabilizes TR

But as u is increased, PL valley may disappear before the TR valley

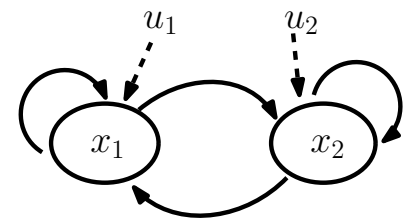
PE is always an attractor for all u

Reprogramming cooperative GRNs

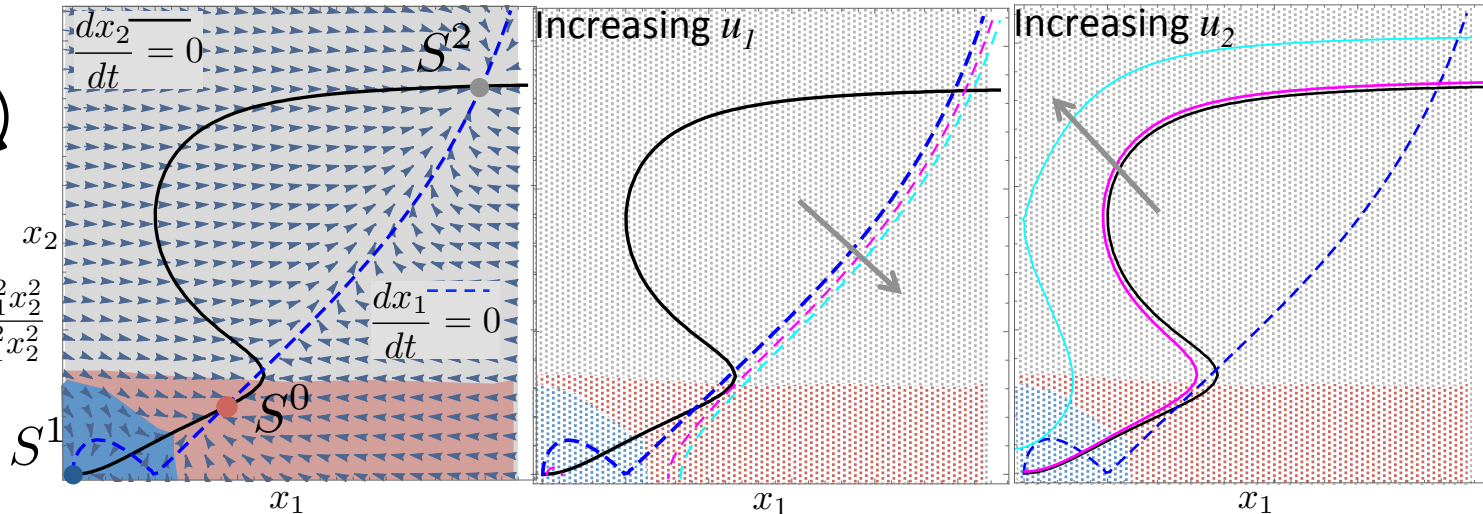
A GRN is **weakly reprogrammable to S^0 from x^0** if there is an input u such that the attracting set of x^0 is contained in the region of attraction of S^0

Fact: A cooperative GRN may be weakly reprogrammable to an intermediate state from a state lower than it. *But, whether an input u exists is highly dependent on the GRN*

Example:

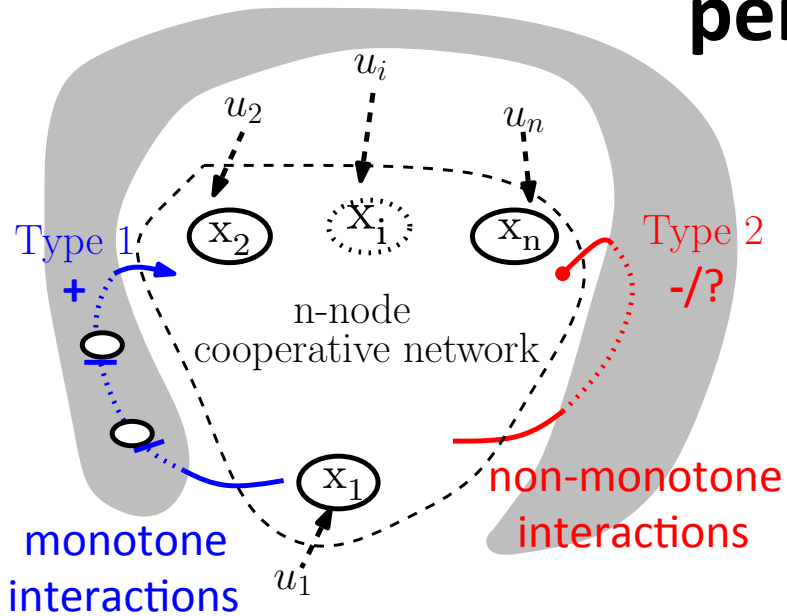


$$H_i(x) = \frac{a_i x_1^2 + b_i x_2^2 + c_i x_1^2 x_2^2}{1 + x_1^2 + x_2^2 + d x_1^2 x_2^2}$$



Preset overexpression is a robust approach to reprogram the GRN to the maximal state but a **fragile approach to reprogram the GRN to intermediate states**

Reprogramming cooperative GRNs subject to perturbations



When a core GRN is embedded in a larger GRN:

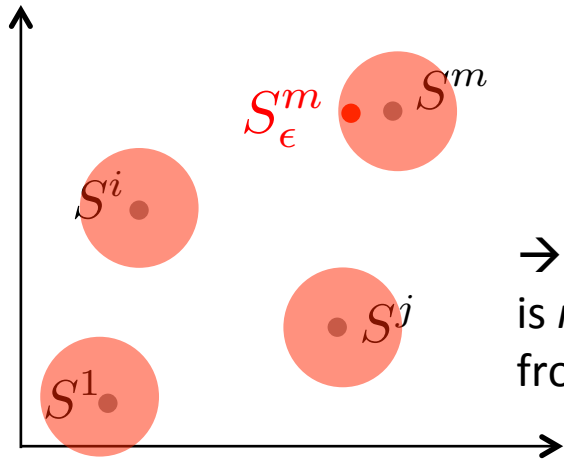
monotone interactions do not change the reprogramming properties

non monotone interactions:

$$\frac{dx}{dt} = f(x, u) + \epsilon d(x), \quad \|d(x)\| \leq d_M$$

cooperative vector field non-monotone perturbation

for ϵ small, the perturbed stable steady states are in ϵ -balls around the nominal ones

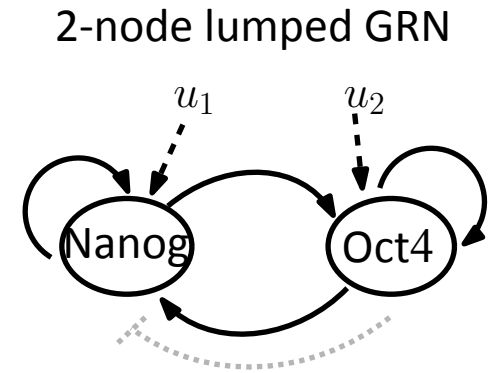
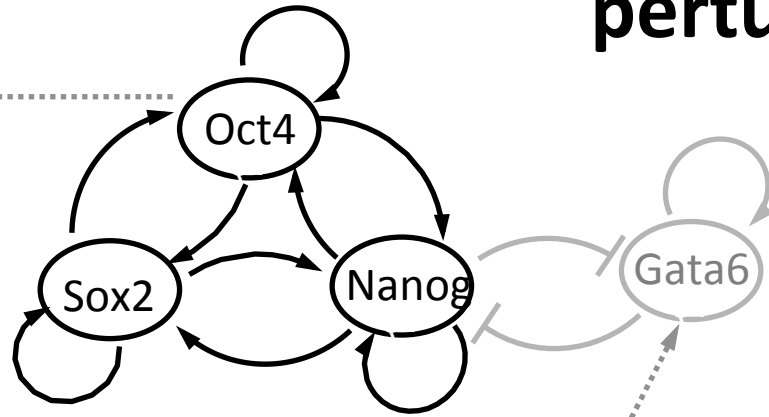


Theorem: For ϵ sufficiently small, the perturbed cooperative GRN is strongly reprogrammable only to S^m_ϵ (the perturbation of the maximal stable steady state S^m)

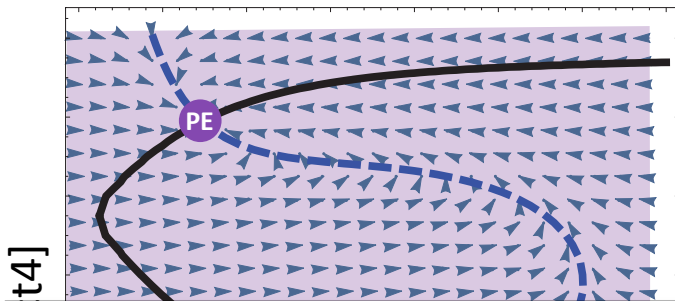
→ A cooperative GRN subject to small non-monotone perturbations is *not strongly reprogrammable* to stable steady states that arise from the perturbation of intermediate ones

Reprogramming cooperative GRNs subject to perturbations

Chickamane et al, 2008



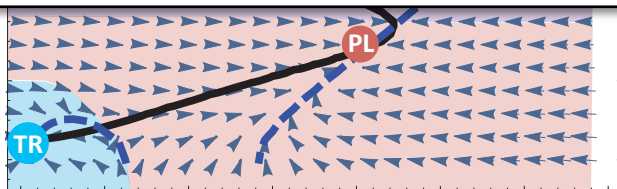
Non-monotone perturbation



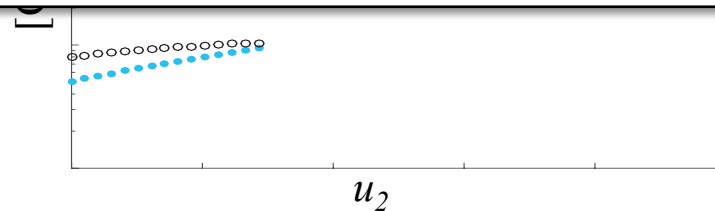
Oct4 overexpression: PL disappears before TR cannot reprogram the GRN from TR to PL. GRN strongly reprogrammable to PE only

● TR ● PL ● PE ○ unstable

Preset overexpression of TFs does not have sufficient control over TF concentrations to enforce their target (PL) concentrations within the GRN



[Nanog]



Outline

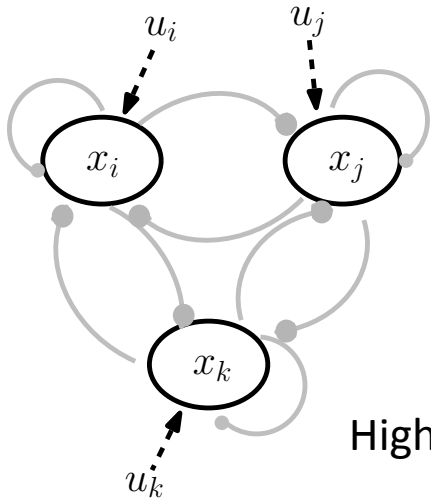
Background on cell fate reprogramming

Reprogramming GRNs through prefixed overexpression

Reprogramming GRNs through feedback overexpression

A blueprint of a synthetic genetic feedback controller implementing feedback overexpression

High-gain feedback globally stabilizes any target state



$$\Sigma_u \quad \frac{dx_i}{dt} = H_i(x) - \gamma_i x_i + u_i, \quad u_i = G(x_i^* - x_i) \quad \begin{matrix} x_i^* \\ \text{target} \\ \text{state} \end{matrix}$$

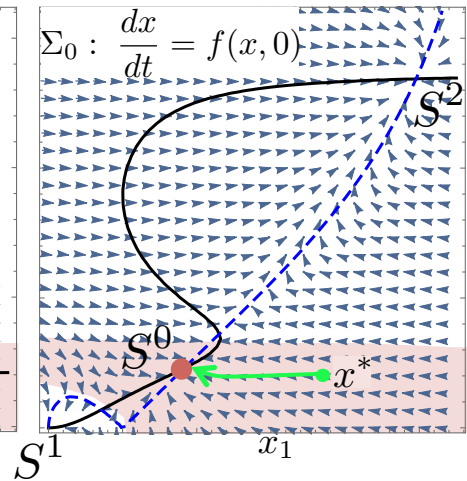
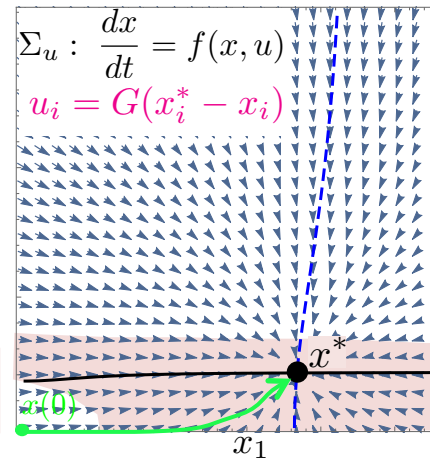
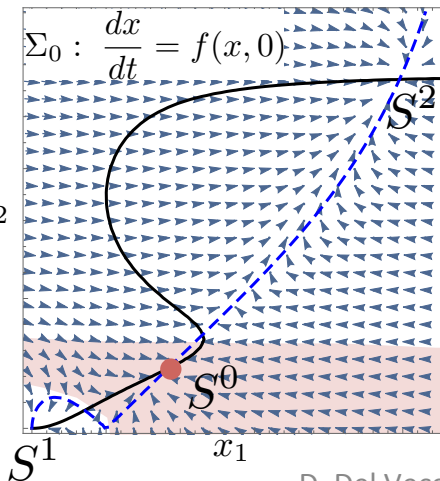
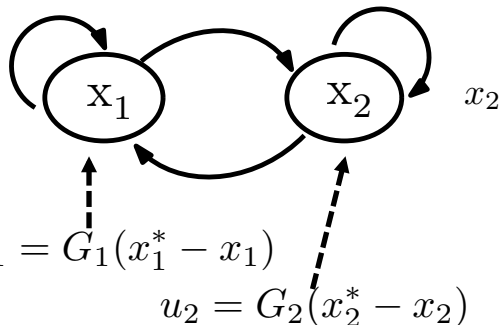
the overexpression level is not preset but it is adjusted based on the proximity to the target state

High-gain: $Gx_i^* \gg H_i(x)$, $G \gg \gamma_i \rightarrow \frac{dx_i}{dt} \approx G(x_i^* - x_i)$ x_i^* is unique steady state

Using tools from contraction theory prove that $\limsup_{t \rightarrow \infty} \|x_i(t) - x_i^*(t)\| \rightarrow \frac{(H_M + \gamma_i x_i^*)}{G + \gamma_i}$

Example:

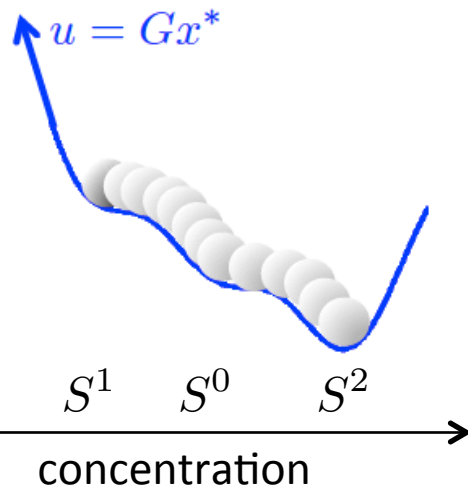
Pick x^* in region of attraction of S^0



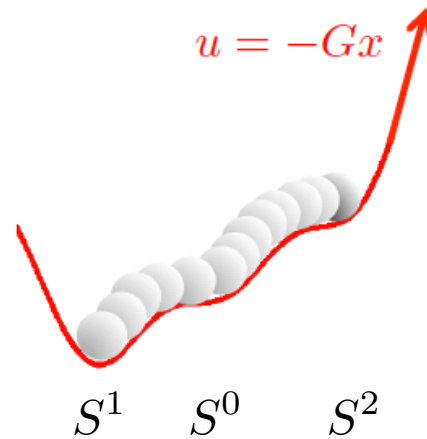
High-gain feedback globally stabilizes any target state

Qualitatively:

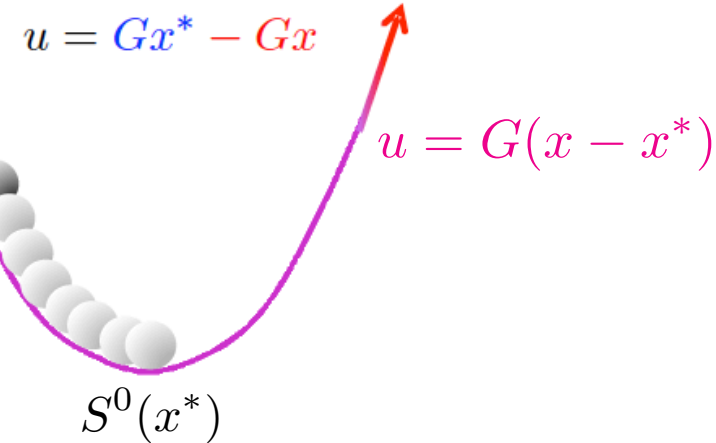
preset overexpression



enhanced degradation



combined



We can physically interpret this controller as applying large overexpression combined with similarly large enhance degradation of the transcription factor

We look for a synthetic genetic circuit design capable of

- Large overexpression of endogenous TF, which is tunable through inducers
- Enhanced degradation of both endogenous and synthetically produced TF

Outline

Background on cell fate reprogramming

Reprogramming GRNs through prefixed overexpression

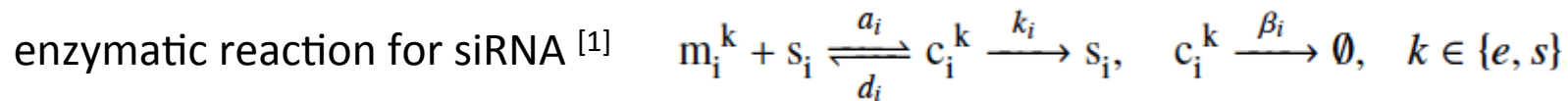
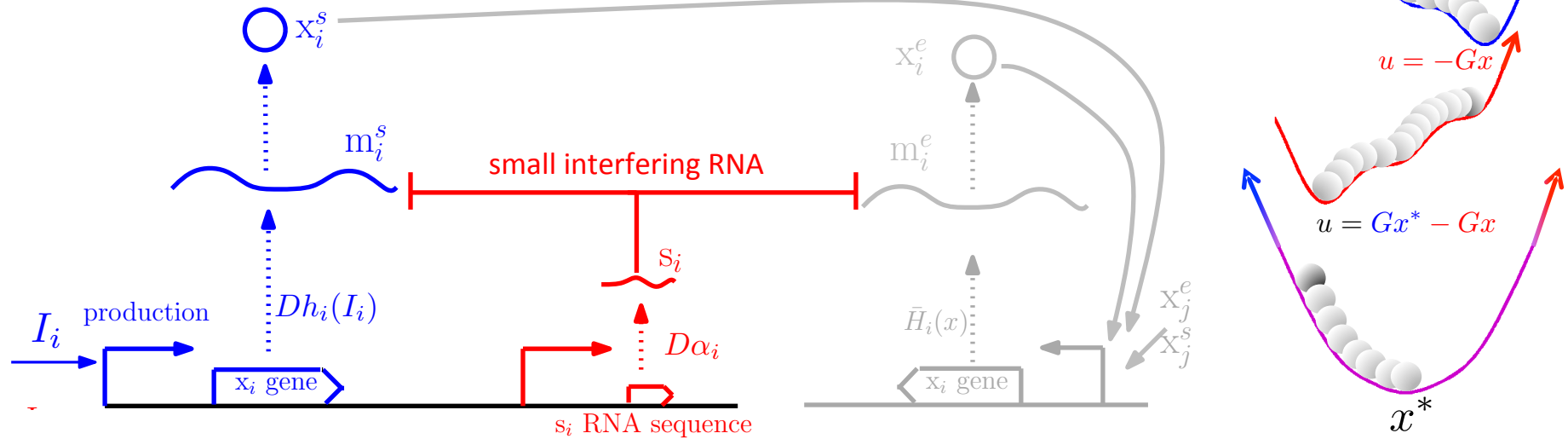
Reprogramming GRNs through feedback overexpression

A blueprint of a synthetic genetic feedback controller implementing feedback overexpression

Synthetic genetic circuit implementing feedback control of TF overexpression

Synthetic circuit

Endogenous system



total species concentration (endogenous plus synthetic)
$$m_i = m_i^e + m_i^s \quad x_i = x_i^e + x_i^s$$

assumption^[1] $m_i \ll K_i$ (M-M constant)

$$\frac{dm_i}{dt} = (\bar{H}_i(x) - \delta_i m_i + G_i(m_i^* - m_i)) \left(\frac{1}{1 + \bar{s}_i/K_i} \right)$$

$$\frac{dx_i}{dt} = \kappa_i m_i - \gamma_i x_i \quad \bar{s}_i = D\alpha_i/\beta_i$$

DNA copy number

$$G_i = D \frac{k_i \alpha_i}{K_i \beta_i} \quad \text{feedback gain}$$

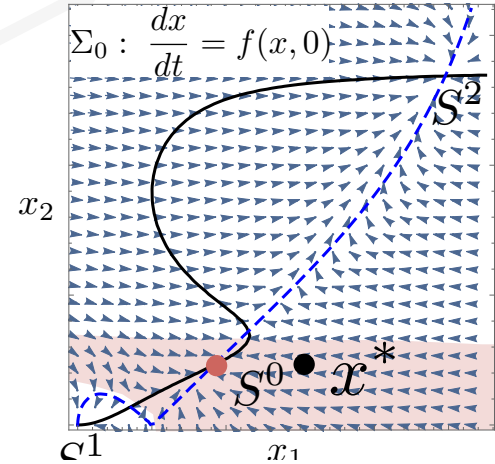
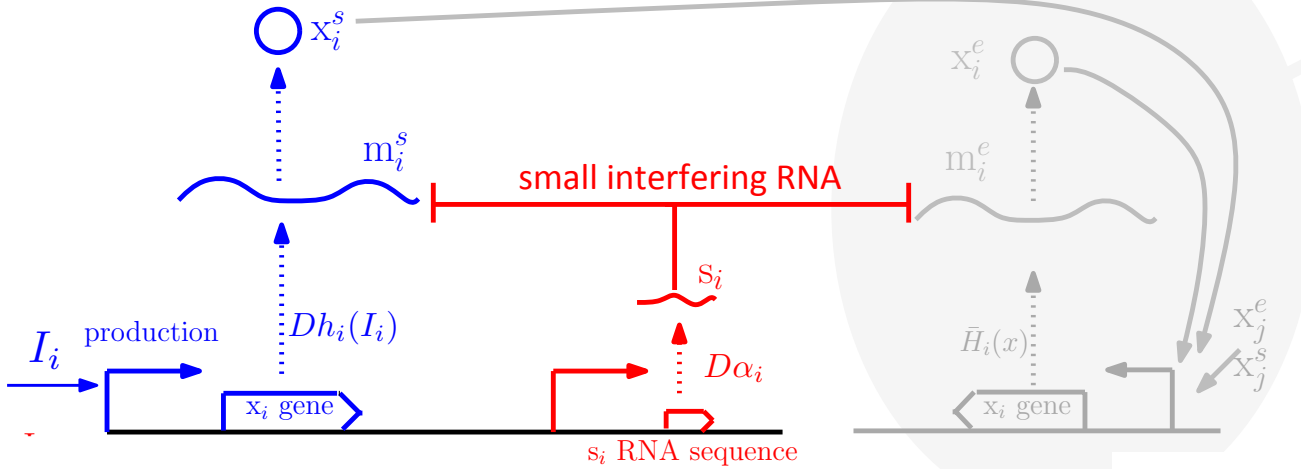
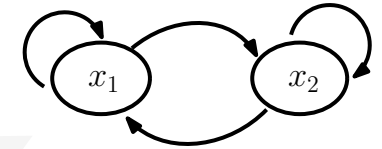
$$m_i^* = \frac{\gamma_i}{\kappa_i} x_i^* \quad x_i^* = F(I_i^*)$$

target encoded by inducer

Synthetic genetic circuit for feedback control of TF overexpression

Synthetic circuit

Endogenous system



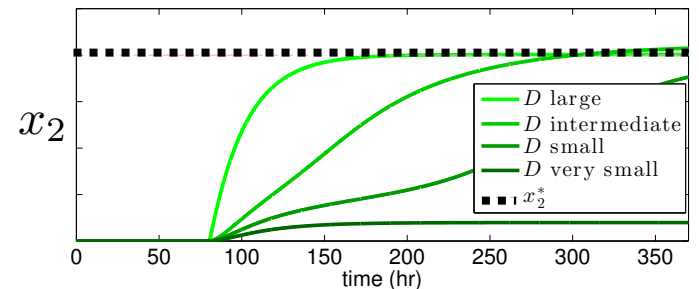
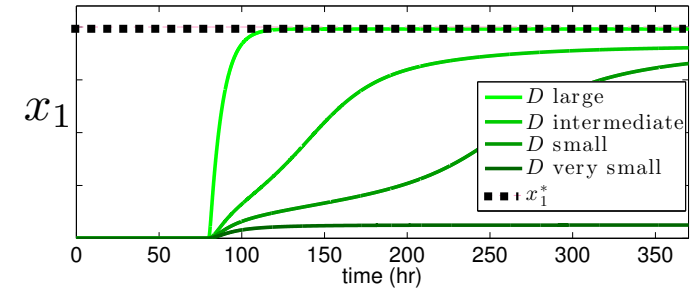
$$G_i \gg \delta_i, \quad G_i m_i^* \gg \bar{H}_i(x) \quad (\text{DNA copy number } \sim 1-5)$$

$$\frac{dm_i}{dt} \approx G_i(m_i^* - m_i) \left(\frac{1}{1 + \bar{s}_i/K_i} \right)$$

$$\frac{dx_i}{dt} = \kappa_i m_i - \gamma_i x_i \quad G_i = D \frac{k_i \alpha_i}{K_i \beta_i}$$

$$m_i(t) \rightarrow m_i^* \quad x_i(t) \rightarrow x_i^*$$

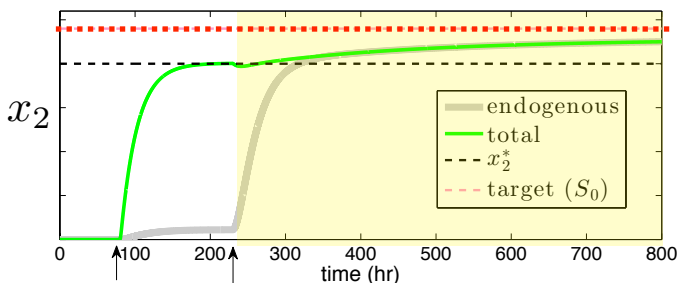
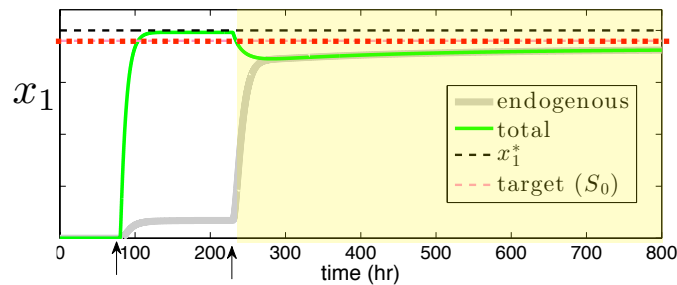
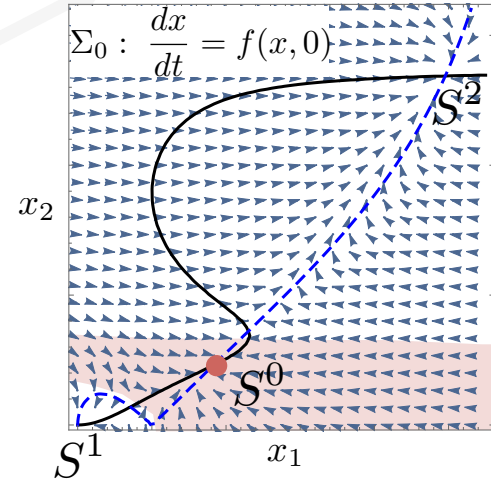
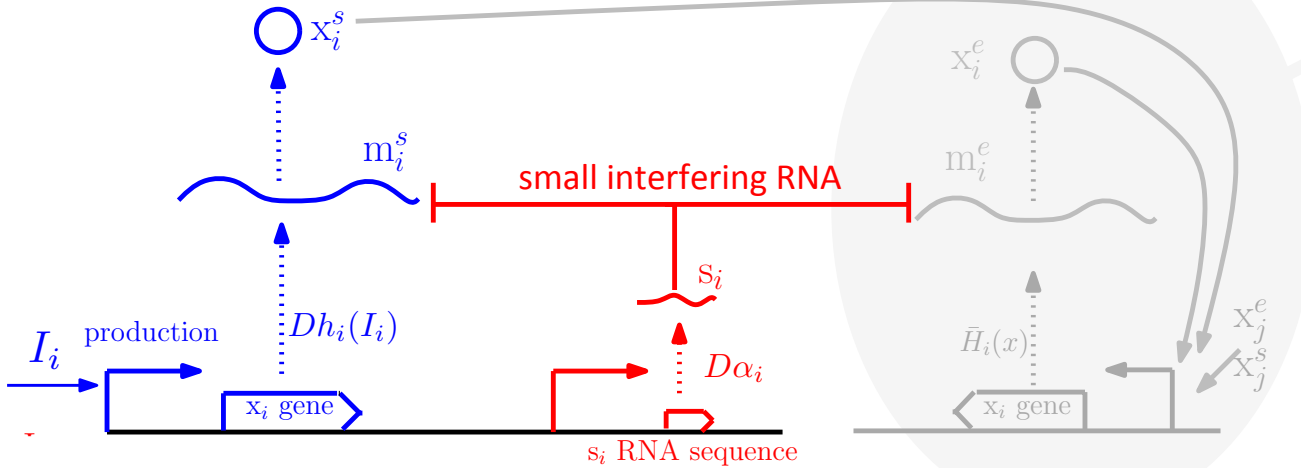
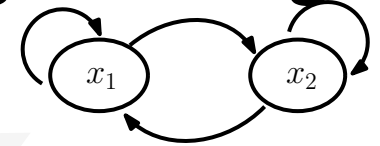
convergence speed for mRNA $\sim 1/k_i$



Using the genetic controller for reprogramming

Synthetic circuit

Endogenous system



when the controller is shut down, the endogenous species take over

$$\frac{dm_i}{dt} = (\bar{H}_i(x) - \delta_i m_i)$$

$$\frac{dx_i}{dt} = \kappa_i m_i - \gamma_i x_i$$

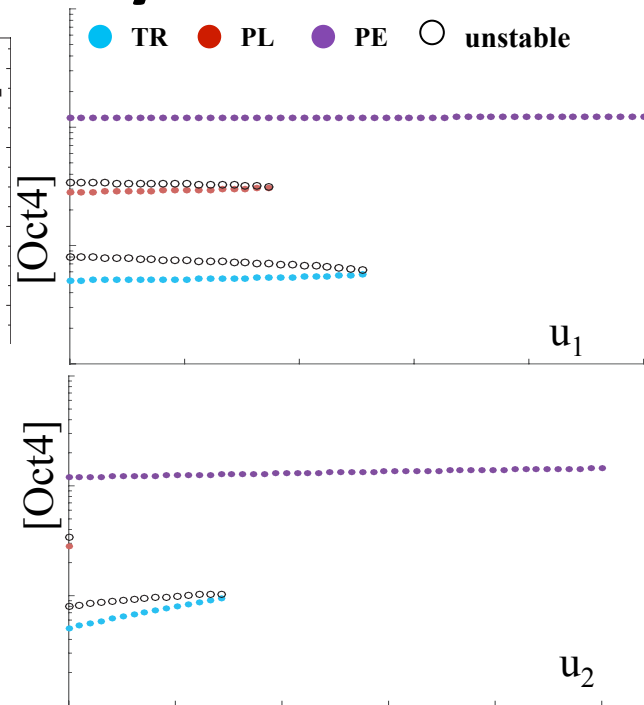
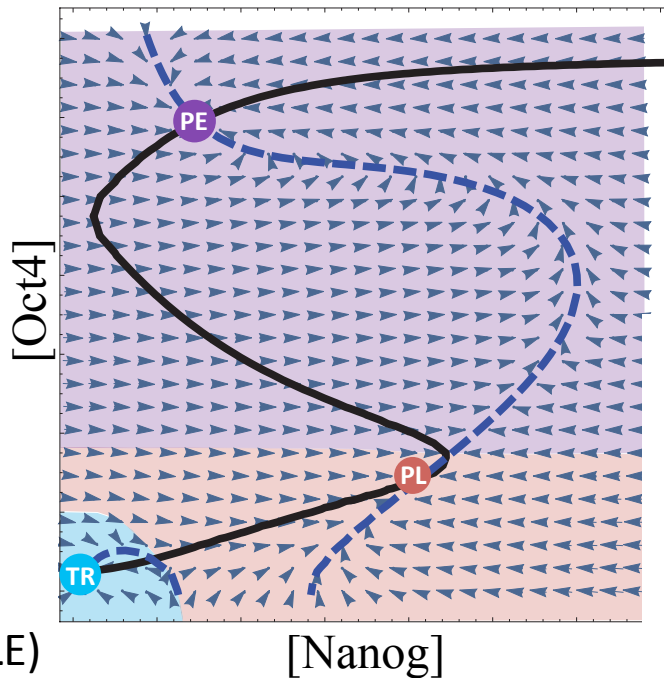
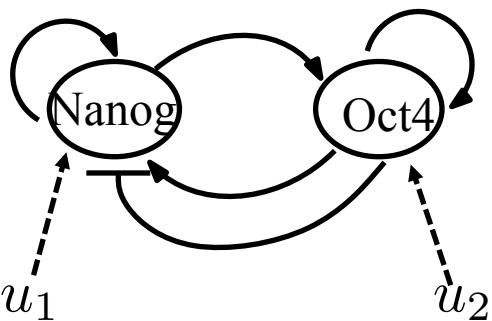
$$x(0) \approx x^*, m(0) \approx m^*$$

in the region of attraction of $S^0 \Rightarrow (x(t), m(t)) \rightarrow S^0$

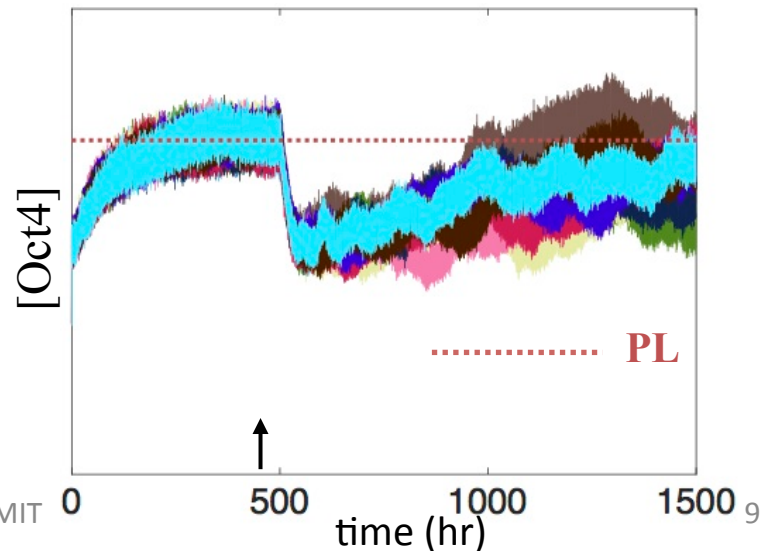
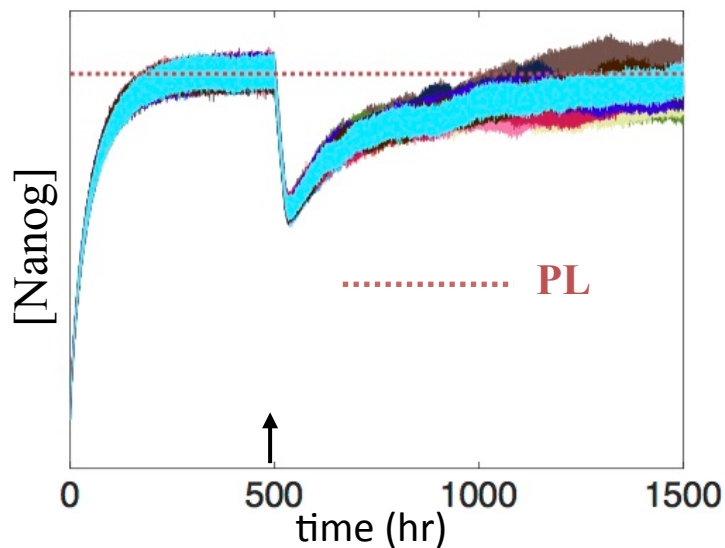
since $\frac{dm_i^s}{dt} = -\delta_i m_i^s \Rightarrow (x^s(t), m^s(t)) \rightarrow 0$

$$\Rightarrow (x^e(t), m^e(t)) \rightarrow S^0$$

Reprogramming the pluripotency network



stochastic simulations (CLE)

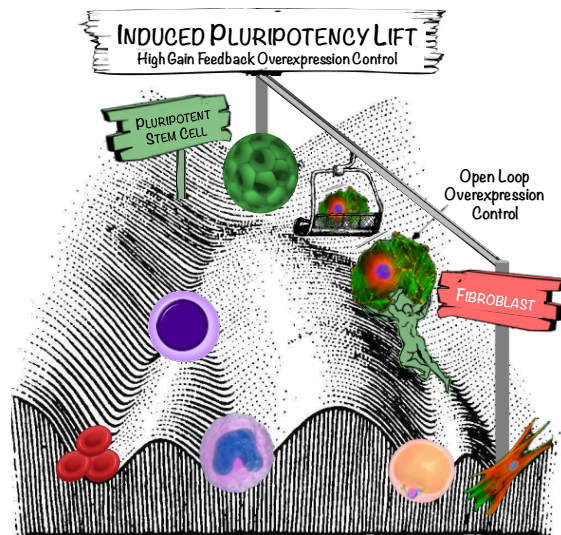


Summary

We proposed a mathematical model that implies network structure in reprogramming failure

We proposed a closed loop feedback control approach of overexpression to over-rule network's dynamics

[Del Vecchio et. al. *Cell Systems*, 2017](#)



On-going and future work

Mathematical framework to determine how intrinsic noise in the low molecule count regime affects reprogramming

Mathematical model of how chromatin dynamics affects reprogramming

Implementation of the genetic feedback controller in reprogrammable cell lines

Thanks to...



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