GSTDMB 2010: DYNAMICAL MODELLING FOR BIOLOGY AND MEDICINE

Lecture 2.1
Multi-variable differential equation models

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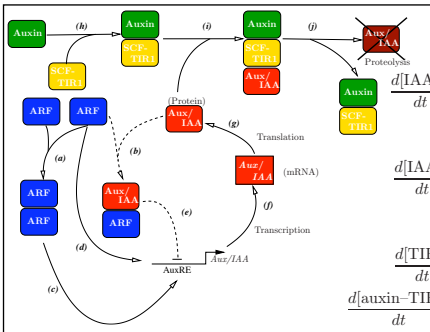
Recap...

- We have introduced simple ordinary differential equation (ODE) models for single state variables.
- **Steady states** and their **stability** are crucial determinant of system dynamics.
- Changes in number or stability of steady states are called **bifurcations**.
- For 1st order **autonomous** ODEs, the **phase-line diagram** can tell us most of the qualitative information we'd like to know about the system dynamics:
  - if you can sketch the graph, you can sketch the dynamics...
  - steady states, stability AND qualitative solution behaviour (fast, slow, increasing, decreasing, etc), bifurcations.
  - solutions cannot oscillate
- For 1st order **non-autonomous** ODEs (e.g. circadian models with time dependent parameters) solutions can oscillate (driven by e.g. day-night cycle)
- We used MATLAB to help sketch phase-line diagrams and simulate ODEs
- **Next, models with more than one state variable:** more complex dynamics possible, analysis more difficult, often resort to computer simulation



$$\frac{d[\mathrm{IAA_m}]}{dt} = \lambda_1 F_1([\mathrm{ARF}],[\mathrm{IAA_p}],[\mathrm{ARF\text{-}IAA}],[\mathrm{ARF_2}])$$

$$+ \lambda_2 F_2([\mathrm{ARF}],[\mathrm{IAA_p}],[\mathrm{ARF\text{-}IAA}],[\mathrm{ARF_2}]) - \mu_{\mathrm{IAA_m}}[\mathrm{IAA_m}],$$

$$\frac{d[\mathrm{IAA_p}]}{dt} = \delta[\mathrm{IAA_m}] - l_\mathrm{a}[\mathrm{IAA_p}][\mathrm{auxin\text{–}TIR1}] + l_\mathrm{d}[\mathrm{auxin\text{–}TIR1\text{–}IAA}] -$$

$$p_\mathrm{a}[\mathrm{IAA_p}][\mathrm{ARF}] + p_d[\mathrm{ARF\text{-}IAA}],$$

$$\frac{d[\mathrm{TIR1}]}{dt} = -k_\mathrm{a}[\mathrm{auxin}][\mathrm{TIR1}] + k_\mathrm{d}[\mathrm{auxin\text{–}TIR1}],$$

$$\frac{d[\mathrm{auxin\text{–}TIR1}]}{dt} = k_\mathrm{a}[\mathrm{auxin}][\mathrm{TIR1}] - k_\mathrm{d}[\mathrm{auxin\text{–}TIR1}]$$

$$+ (l_\mathrm{d} + l_\mathrm{m})[\mathrm{auxin\text{–}TIR1\text{–}IAA}] - l_\mathrm{a}[\mathrm{auxin\text{–}TIR1}][\mathrm{IAA_p}]$$

$$\frac{d[\mathrm{auxin\text{–}TIR1\text{–}IAA}]}{dt} = l_\mathrm{a}[\mathrm{IAA_p}][\mathrm{auxin\text{–}TIR1}] - (l_d + l_\mathrm{m})[\mathrm{auxin\text{–}TIR1\text{–}IAA}],$$

$$\frac{d[\mathrm{IAA^*}]}{dt} = l_\mathrm{m}[\mathrm{auxin\text{–}TIR1\text{–}IAA}] - \mu_{\mathrm{IAA^*}}[\mathrm{IAA^*}],$$

$$\frac{d[\mathrm{ARF}]}{dt} = -2q_\mathrm{a}[\mathrm{ARF}]^2 + 2q_\mathrm{d}[\mathrm{ARF_2}] - p_\mathrm{a}[\mathrm{ARF}][\mathrm{IAA_p}] + p_\mathrm{d}[\mathrm{ARF\text{-}IAA}],$$

$$\frac{d[\mathrm{ARF\text{-}IAA}]}{dt} = p_\mathrm{a}[\mathrm{ARF}][\mathrm{IAA_p}] - p_\mathrm{d}[\mathrm{ARF\text{-}IAA}],$$

$$\frac{d[\mathrm{ARF_2}]}{dt} = q_\mathrm{a}[\mathrm{ARF}]^2 - q_\mathrm{d}[\mathrm{ARF_2}],$$

$$\frac{d[\mathrm{auxin}]}{dt} = \omega + k_\mathrm{d}[\mathrm{auxin\text{–}TIR1}] - k_\mathrm{a}[\mathrm{auxin}][\mathrm{TIR1}] - \mu_\mathrm{Auxin}[\mathrm{auxin}].$$

Signalling networks

- Central dogma of molecular biology:
  - DNA transcribed to RNA (regulated by transcription factors),
  - RNA is translated into Protein.
- Proteins interact, can regulate translation, RNA stability, and transcription.
- RNA can also modulate transcription.
- Signalling networks: interactions between these elements, typically complex and extensive.
- Fundamental approach: decompose into modules that are sufficiently separate from other pathways to be considered on their own.
- Mathematical models: prediction of network behaviour with given topology and interactions.
- Ideas don't just apply to "gene networks", but to many kinds of network: Physiological models, metabolic networks, ecological networks, epidemiology...

The law of Mass Action (1)

- The law of mass action states that the rate of a chemical reaction is proportional to the product of the concentrations of the reactants.
- It is based on the assumptions of i) a well stirred solution and ii) low molecular concentrations, where the probability of diffusing molecules to get close enough, for a reaction to occur, is proportional to the concentrations.
- A rate parameter is used to define the 'probability' of a reaction to occur if two molecules approach each other.
- The mass action formalism has been validated in many experimental settings.
- Given: $n_1 S_1 + n_2 S_2 + \cdots \xrightarrow{k_f} m_1 P_1 + m_2 P_2 + \cdots$
- The reaction rate is $k_f \left(S_1^{n_1} \cdot S_2^{n_2} \cdots \right)$
- The rate of change of a species depends on the rate of reaction and **the net change in the number of molecules** of that species.
- In reality, all reactions should be broken down into bimolecular steps.

The law of Mass Action (2)

- Consider a simple example: $A + B \underset{k_b}{\overset{k_f}{\rightleftharpoons}} C$
- This is two reactions.
- The forward reaction has reactants A and B, and rate $k_f[A][B]$.
  It consumes one molecule of A and B, and produces one molecule of C.
- The reverse reaction has a single reactant C, and rate $k_b[C]$.
  It consumes one molecule of C, and produces one molecule of A and B.

$$\frac{d[A]}{dt} = -k_f[A][B] + k_b[C]$$

$$\frac{d[B]}{dt} = -k_f[A][B] + k_b[C]$$

$$\frac{d[C]}{dt} = +k_f[A][B] - k_b[C]$$

Forward Reverse

The law of Mass Action (3)

- Another example, the "Brusselator":

$$A \xrightarrow[1]{k_1} X, \quad B + X \xrightarrow[2]{k_2} Y + D, \quad 2X + Y \xrightarrow[3]{k_3} 3X, \quad X \xrightarrow[4]{k_4} E$$

- We assume the concentrations of substrates A and B are constant.
- E is a product. We are interested in the dynamics of X and Y.
- $x = [X]$, $y = [Y]$, the concentrations of X and Y.
- 3 has rate $k_3 x^2 y$ which *produces* one molecule of X and consumes one of Y.

$$\begin{aligned} \frac{dx}{dt} &= \overset{1}{k_1 A} - (\overset{2}{k_2 B} + \overset{4}{k_4})x + \overset{3}{k_3 x^2 y}, \\ \frac{dy}{dt} &= \overset{2}{k_2 B x} - \overset{3}{k_3 x^2 y}. \end{aligned}$$

- This system is a famous example which can have oscillatory solutions.

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Michaelis-Menten enzyme kinetics

- S, substrate; E, enzyme; P, product: $S + E \underset{2}{\overset{1}{\rightleftharpoons}} SE \xrightarrow[3]{k_3} P + E$

- Three reactions, + law of mass action:

$$\begin{aligned} \frac{d[S]}{dt} &= -\overset{1}{k_1}[S][E] + \overset{2}{k_2}[SE] \\ \frac{d[E]}{dt} &= -\overset{1}{k_1}[S][E] + \overset{2}{k_2}[SE] + \overset{3}{k_3}[SE] \\ \frac{d[SE]}{dt} &= \overset{1}{k_1}[S][E] - \overset{2}{k_2}[SE] - \overset{3}{k_3}[SE] \\ \frac{d[P]}{dt} &= \overset{3}{k_3}[SE] \end{aligned}$$

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Michaelis-Menten kinetics $S + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} SE \xrightarrow{k_3} P + E$

- Constant total enzyme: $[E] + [SE] = E_0$
- Substrate assumed in excess, $d[S]/dt = 0$
- $[SE]$ assumed to be at **quasi-steady state**

$$0 \approx \cancel{\frac{d[SE]}{dt}} = k_1[S][E] - k_2[SE] - k_3[SE]$$

- A bit more algebra, using the definition: $K = k_1/(k_2 + k_3)$

$$\begin{aligned} [SE] = K[S][E] &= K[S](E_0 - [SE]) \\ [SE]\,(1 + K[S]) &= KE_0[S] \\ [SE] = \frac{KE_0[S]}{1 + K[S]} &= \frac{E_0[S]}{(1/K + [S])} \end{aligned}$$

Finally: $\dfrac{d[P]}{dt} = \dfrac{V_{max}[S]}{K_m + [S]}$ $\qquad V_{max} = k_3 E_0$, $K_m = 1/K$

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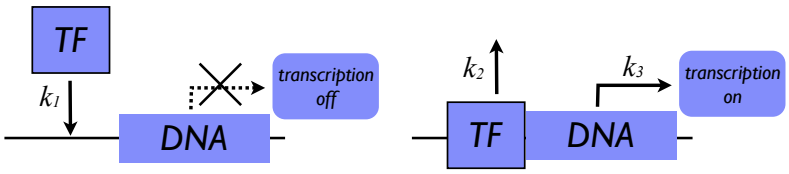
Transcriptional/translational activation

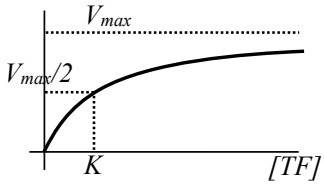
- TF binds to DNA, this complex activates production of protein P.



$$TF + DNA \underset{k_2}{\overset{k_1}{\rightleftharpoons}} TFDNA \overset{k_3}{\rightarrow} P + TFDNA$$

- Assuming TF binding is fast enables use of Michaelis-Menten approach.
- DNA acts as *enzyme*, *[DNA] + [TF-DNA] = 1*

$$\frac{d[P]}{dt} = V_{max}\frac{[TF]}{K + [TF]}$$



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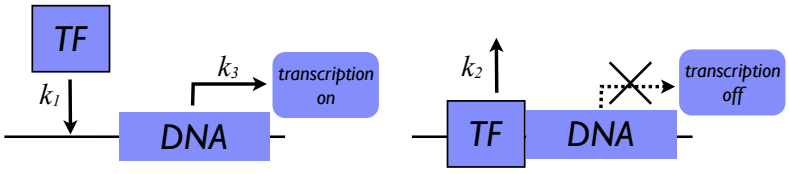
Transcriptional/translational repression

- TF binds to DNA, blocking production of protein P.

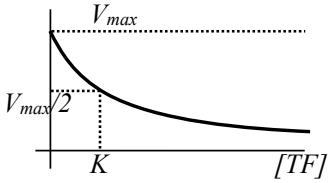


$$TF + DNA \underset{k_2}{\overset{k_1}{\rightleftharpoons}} TFDNA$$

$$DNA \overset{k_3}{\rightarrow} P + DNA$$

- This time, synthesis is a decreasing function of TF concentration:

$$\frac{d[P]}{dt} = \frac{V_{max}K}{K + [TF]}$$



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Hill functions

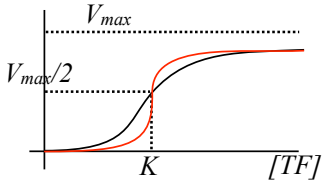
- With the previous expression, the response to changes in substrate (TF) concentration is weak.
- Cooperativity can lead to sharper responses.
- Suppose n molecules of substrate bind to the DNA:

$$n\,TF + DNA \underset{k_2}{\overset{k_1}{\rightleftharpoons}} TF_nDNA \overset{k_3}{\rightarrow} P + TF_nDNA$$

- After some mass action and some algebra...

$$\frac{d[P]}{dt} = V_{max}\frac{[TF]^n}{K^n + [TF]^n}$$

- Larger n → steeper switch
- Same idea for repression



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Multiple Transcription Factors?

- What about multiple transcription factors?
- What if some are activating and some repressing?
- Basically need to catalogue all the relevant states and the contribution of each to transcription rate.
- Write down ODEs and simplify using Michaelis-Menten approach.
- E.g. Lac-operon: *[I]* activates and *[R]* represses:

$$\frac{dP}{dt} = \frac{V_{max}(1 + k_2[I] + k_3[R][I])}{1 + k_1[R] + k_2[I] + k_3[R][I]}$$

- or use the Shea-Ackers approach...

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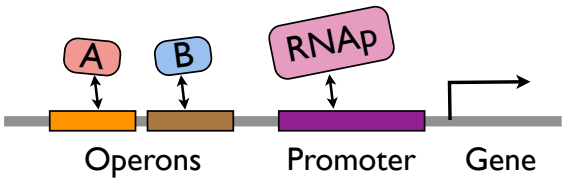
Shea-Ackers (1)

- Method originally developed for the lysis/lysogeny switch in Lambda phage
- Two time scales:
  - Slow: Transcription/Translation/Degradation
  - Fast: Binding/unbinding of TFs to gene – thermal equilibrium
- Possible cases: TF, TF+RNAp, RNAp - probability associated with each
- Enumerate all cases, compute probability of bound RNAp
- Transcription rate is proportional to promoter occupancy



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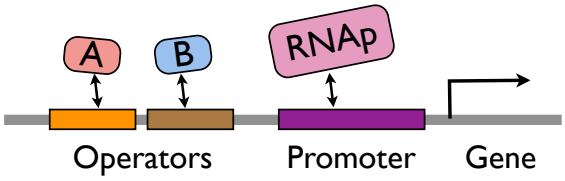
Shea-Ackers (2)

- Example: two transcription factors, A and B
- Enumerate all possibilities - binding/unbinding of A,B and RNAP
- The "partition function" Z contain $2^3 = 8$ terms



$$Z = \sum_{ijk} [A]^i [B]^j [R]^k \delta_{ijk} = Z_{on} + Z_{off}$$

RNAp bound RNAp unbound

- ***i,j,k*** = ***0*** (unbound) or ***1*** (bound)
- δ_{ijk} related to binding energy, $\delta_{000} = 1$

Transcription rate proportional to: $\frac{Z_{on}}{Z_{on} + Z_{off}}$

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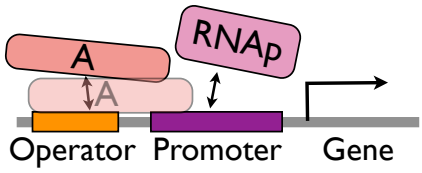
Shea-Ackers: simple example

- The *trp* operon of E. coli is regulated by the TrpR repressor protein A.
- Tryptophan binds the TrpR repressor enabling TrpR to bind the *trp* operator.
- This prevents transcription: the *trp* operator overlaps the RNAp binding site. A and R cannot be simultaneously bound:



[A]	[RNAp]	Rate
0	0	1
1	0	$\delta_{10}A$
0	1	$\delta_{01}R$
1	1	-

$$Z = [A]^0[R]^0\delta_{00} + [A]^1[R]^0\delta_{10} + [A]^0[R]^1\delta_{01}$$

- Only the last term corresponds to a transcriptionally active state, so $T \propto \dfrac{\delta_{01}[R]}{1+\delta_{10}[A]+\delta_{01}[R]}$
- For constant RNAp (R) this is like a decreasing Hill function of order *1*.

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Lac-operon revisited

- Write down ODEs and simplify using Michaelis-Menten approach.
- E.g. Lac-operon: *[I]* activates and *[R]* represses:

$$\frac{dP}{dt} = \frac{V_{max}(1 + k_2[I] + k_3[R][I])}{1 + k_1[R] + k_2[I] + k_3[R][I]}$$

- or use the Shea-Ackers approach... assuming [RNAp] is constant yields the same form as above

[I]	[R]	[RNAp]	Rate
0	0	0	1
1	0	0	$\delta_{100}[I]$
0	1	0	$\delta_{010}[R]$
0	0	1	$\delta_{001}[RNAp]$
1	1	0	$\delta_{110}[I][R]$
1	0	1	$\delta_{101}[I][RNAp]$
0	1	1	$\delta_{011}[R][RNAp]$
1	1	1	$\delta_{111}[I][R][RNAp]$

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Two-gene repressor network

$$\frac{du}{dt} = \frac{\alpha_1}{1+v^\beta} - u$$
$$\frac{dv}{dt} = \frac{\alpha_2}{1+u^\gamma} - v$$

- Can behave as a bistable switch, depending on Hill coefficients
- **Phase-plane analysis** very useful
- Nullclines are curves on which one variable is not changing
  - u-nullcline: $du/dt = 0$, here $u = \dfrac{\alpha_1}{1+v^\beta}$
  - v-nullcline: $dv/dt = 0$, here $v = \dfrac{\alpha_2}{1+u^\gamma}$
  - Steady states where nullclines cross
  - Stability requires more maths - linear algebra, eigenvalues, etc ...

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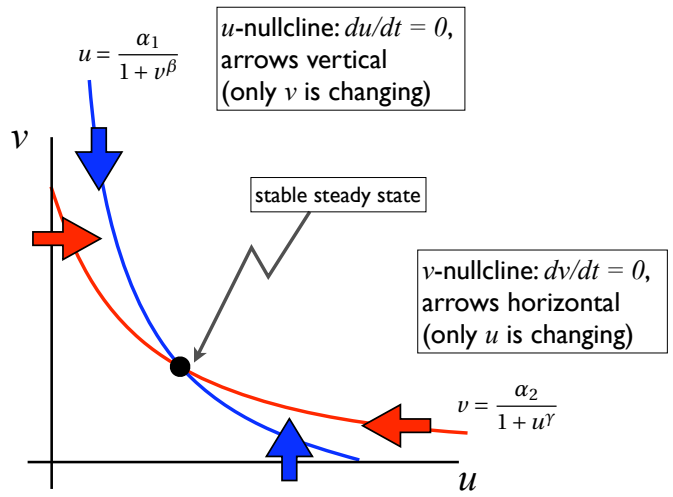
Two-gene repressor network: $\beta = \gamma = 1$



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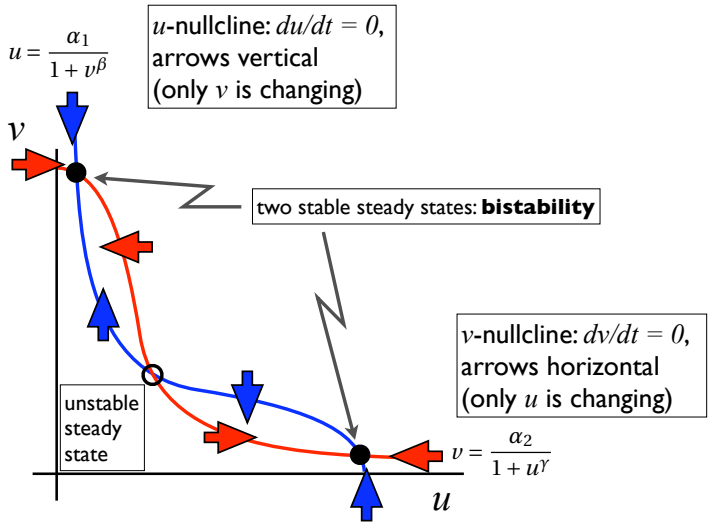
Two-gene repressor network: $\beta, \gamma > 1$



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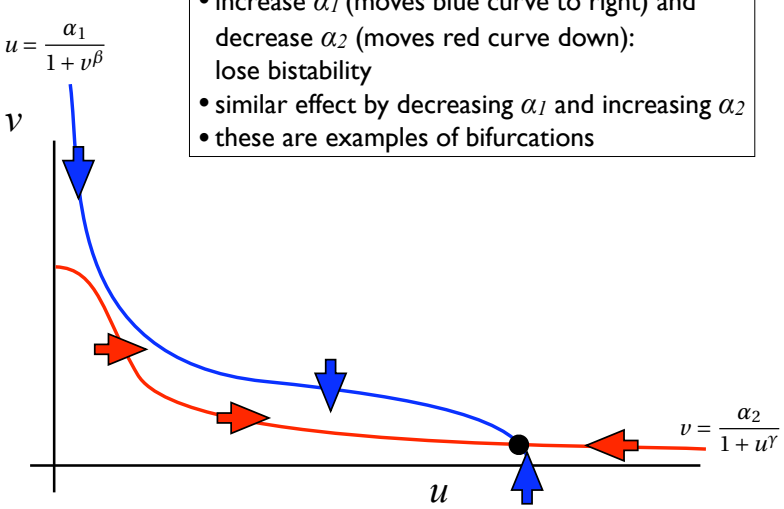
Two-gene repressor network: $\beta, \gamma > 1$

- increase α_1 (moves blue curve to right) and decrease α_2 (moves red curve down): lose bistability
- similar effect by decreasing α_1 and increasing α_2
- these are examples of bifurcations



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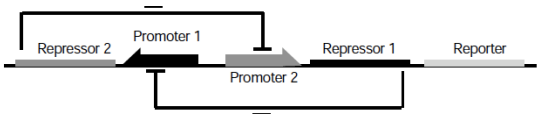
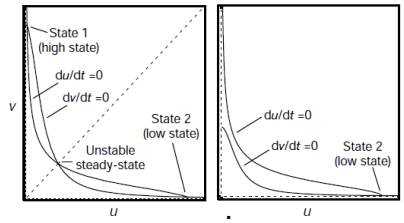
A synthetic toggle switch

Regulatory logic:



$$\frac{du}{dt} = -\delta_1 u + \frac{\alpha_1}{1+v^m}$$
$$\frac{dv}{dt} = -\delta_2 v + \frac{\alpha_2}{1+u^n}$$



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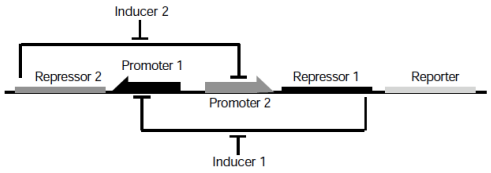
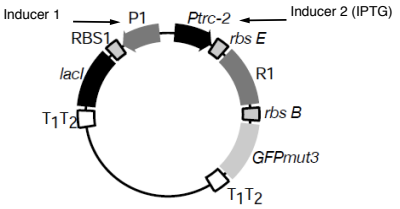
A synthetic toggle switch

External inducers:



Implementation:



Gardner, T.S., Cantor, C.R. & Collins, J.J. (2000). *Nature* **403**, 339–342.

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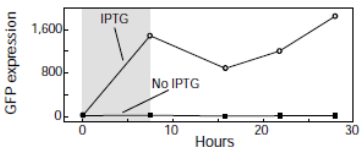
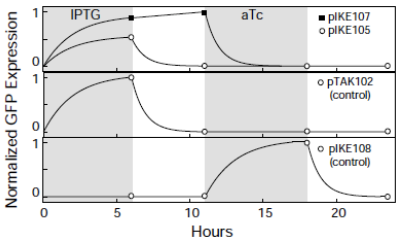
A synthetic toggle switch

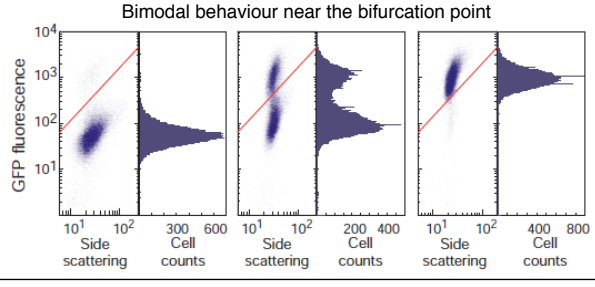
Bistability of steady states



Induced switching



Bimodal behaviour near the bifurcation point



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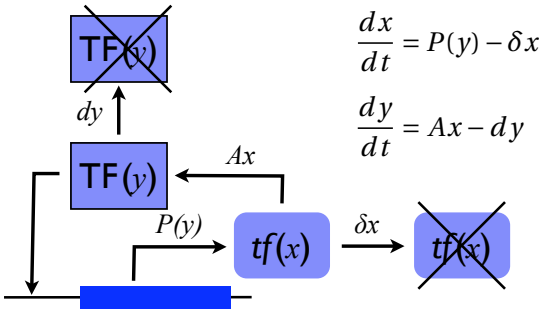
Transcriptional regulation revisited



$$\frac{dx}{dt} = P(y) - \delta x$$

$$\frac{dy}{dt} = Ax - dy$$

- Protein synthesis requires transcription and translation.
- Phase plane analysis quite straightforward.

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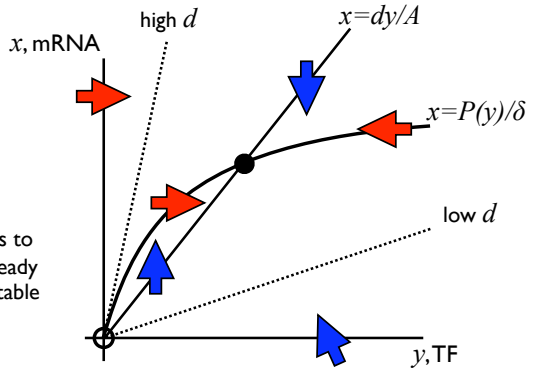
Transcriptional regulation revisited

$$\frac{dx}{dt} = P(y) - \delta x$$

$$\frac{dy}{dt} = Ax - dy$$

- x-Nullcline is: $x = P(y)/\delta$ and y-nullcline is $x = dy/A$
- Easier to think of y as a function of x, otherwise we have $y = P^{-1}(\delta x)$ where P^{-1} is the inverse function...

$P(y)=Ay/(h+y)$



- Increasing d or δ leads to loss of the nonzero steady state, $(0,0)$ becomes stable

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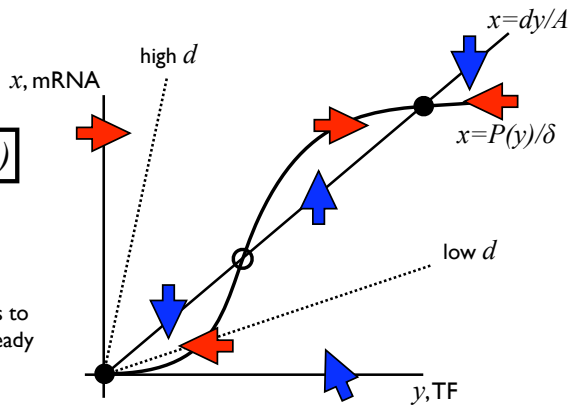
Transcriptional regulation revisited

$$\frac{dx}{dt} = P(y) - \delta x$$

$$\frac{dy}{dt} = Ax - dy$$

- x-Nullcline is: $x = P(y)/\delta$ and y-nullcline is $x = dy/A$
- Easier to think of y as a function of x, otherwise we have $y = P^{-1}(\delta x)$ where P^{-1} is the inverse function...

$P(y)=Ay^2/(h^2+y^2)$



- Bistability again ...
- Increasing d or δ leads to loss of the nonzero steady states.

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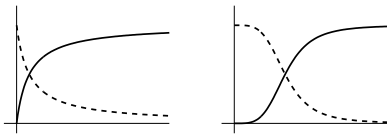
Gene network modelling

- Variables: mRNAs and proteins.
- ODE models: mass action, sigmoidal transcriptional activation and repression, linear decay and translation.



$$\frac{dx}{dt} = \text{synthesis} - \text{decay} \pm \text{transformation} \pm \text{transport}$$

- Parameters:
  - Thresholds for the sigmoidal functions;
  - effective co-operativities, can be high for indirect pathways;
  - half-lives;
  - relative contributions of multiple transcriptional regulators;
  - transfer rates, e.g. cytosol to cell surface;
  - transformation rates, e.g. cleavage, phosphorylation, binding.
- intracellular species: single equation per cell
- cell-surface: multiple equations per cell (e.g. six if we assume hexagonal cells).

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Epidemiology

Simplest model: SIR model.

- Closed population. Individuals do not enter, and leave only by death due to disease.
- Population in 3 compartments: Susceptible, Infective, or Removed (cured and now immune, or dead).
- No spatial effects (uniform mixing), and no heterogeneity in activity (important in, e.g., STDs such as AIDS).
- Negligible incubation time.
- Susceptibles move into Infective class at rate proportional to number of contacts between Susceptibles and Infectives (like law of mass action).
- Infectives removed at some rate into Removed class (*which decouples*).
- An EPIDEMIC if $I(t)>I(0)$ for some $t>0$ (i.e. if the number of infectives goes up)

$$\frac{dS}{dt} = -\beta SI$$

$$\frac{dI}{dt} = \beta SI - \gamma I$$

$$\frac{dR}{dt} = \gamma I$$

- Constant total population

$$S + I + R = N$$

$$S + I \le N$$

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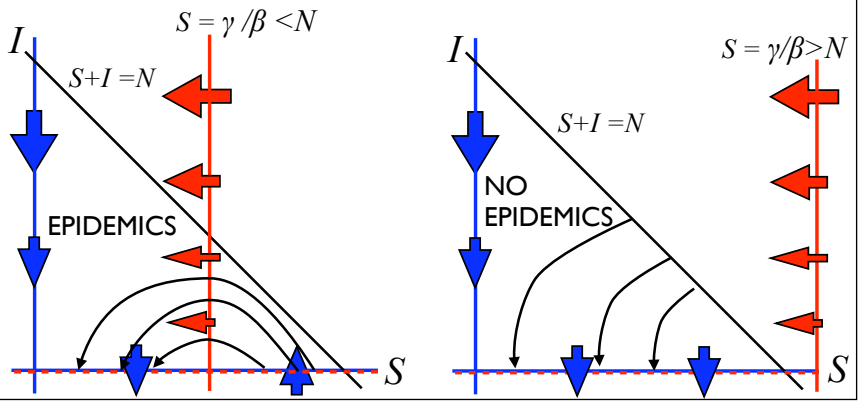
Epidemiology $\frac{dS}{dt} = -\beta SI, \quad \frac{dI}{dt} = \beta SI - \gamma I$

S-nullcline: $dS/dt = 0$.
$S = 0$ and $I = 0$.
Arrows vertical (only I is changing)

I-nullcline: $dI/dt = 0$.
$I = 0$ or $S = \gamma/\beta$, but $S <= N$, so only relevant if $\gamma/\beta < N$.
Arrows horizontal (only S is changing)



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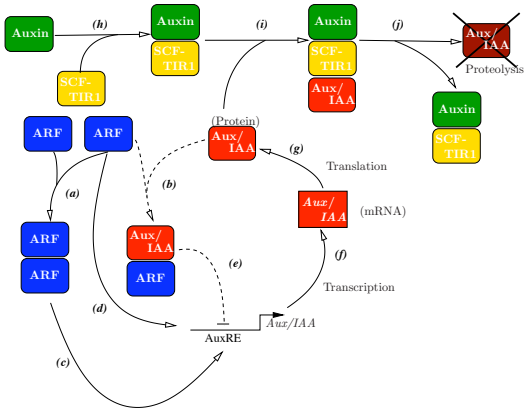
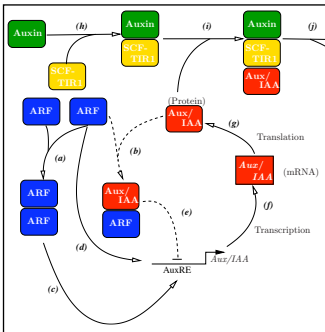
Discussion

- Mathematical models can encode our knowledge about signalling networks.
- Gene transcription, mRNA translation, protein interactions, decay, etc, can be described using differential equations.
- There are different approaches to combining multiple transcription factors.
- Simple and complex models can be used to test hypotheses.
- Mathematical analysis of relatively simple models can be done using **phase-plane** methods.
- Mutual repression can lead to bistability - but we have also seen that cooperative positive autoregulation can lead to bistability.
- Other simple motifs can be analysed in considerable detail.
- Complex signalling networks often require a more computational approach.
- Network topology *may* be more important than parameter values.
- Similar modelling and analysis techniques apply to other areas of biology and medicine.

ODE Example - Auxin signalling



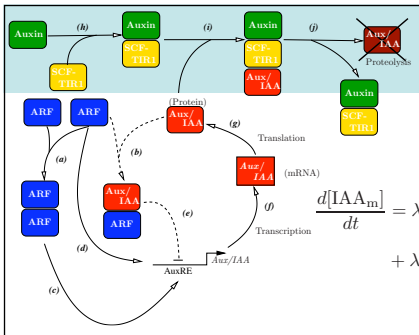
- Auxin is a plant hormone, which stimulates degradation of Aux/IAAs.
- Aux/IAAs repress their own transcription.
- Hence Auxin stimulates Aux/IAA transcription.



$$\frac{d[\mathrm{IAA_m}]}{dt} = \lambda_1 F_1([\mathrm{ARF}],[\mathrm{IAA_p}],[\mathrm{ARF\text{-}IAA}],[\mathrm{ARF_2}])$$
$$+ \lambda_2 F_2([\mathrm{ARF}],[\mathrm{IAA_p}],[\mathrm{ARF\text{-}IAA}],[\mathrm{ARF_2}]) - \mu_{\mathrm{IAA_m}}[\mathrm{IAA_m}],$$
$$\frac{d[\mathrm{IAA_p}]}{dt} = \delta[\mathrm{IAA_m}] - l_\mathrm{a}[\mathrm{IAA_p}][\mathrm{auxin\text{–}TIR1}] + l_\mathrm{d}[\mathrm{auxin\text{–}TIR1\text{–}IAA}] -$$
$$p_\mathrm{a}[\mathrm{IAA_p}][\mathrm{ARF}] + p_d[\mathrm{ARF\text{-}IAA}],$$
$$\frac{d[\mathrm{TIR1}]}{dt} = -k_\mathrm{a}[\mathrm{auxin}][\mathrm{TIR1}] + k_\mathrm{d}[\mathrm{auxin\text{–}TIR1}],$$
$$\frac{d[\mathrm{auxin\text{–}TIR1}]}{dt} = k_\mathrm{a}[\mathrm{auxin}][\mathrm{TIR1}] - k_\mathrm{d}[\mathrm{auxin\text{–}TIR1}]$$
$$+ (l_\mathrm{d} + l_\mathrm{m})[\mathrm{auxin\text{–}TIR1\text{–}IAA}] - l_\mathrm{a}[\mathrm{auxin\text{–}TIR1}][\mathrm{IAA_p}]$$
$$\frac{d[\mathrm{auxin\text{–}TIR1\text{–}IAA}]}{dt} = l_\mathrm{a}[\mathrm{IAA_p}][\mathrm{auxin\text{–}TIR1}] - (l_d + l_\mathrm{m})[\mathrm{auxin\text{–}TIR1\text{–}IAA}],$$

$$\frac{d[\mathrm{IAA^*}]}{dt} = l_\mathrm{m}[\mathrm{auxin\text{–}TIR1\text{–}IAA}] - \mu_{\mathrm{IAA^*}}[\mathrm{IAA^*}],$$
$$\frac{d[\mathrm{ARF}]}{dt} = -2q_\mathrm{a}[\mathrm{ARF}]^2 + 2q_\mathrm{d}[\mathrm{ARF_2}] - p_\mathrm{a}[\mathrm{ARF}][\mathrm{IAA_p}] + p_\mathrm{d}[\mathrm{ARF\text{-}IAA}],$$
$$\frac{d[\mathrm{ARF\text{-}IAA}]}{dt} = p_\mathrm{a}[\mathrm{ARF}][\mathrm{IAA_p}] - p_\mathrm{d}[\mathrm{ARF\text{-}IAA}],$$
$$\frac{d[\mathrm{ARF_2}]}{dt} = q_\mathrm{a}[\mathrm{ARF}]^2 - q_\mathrm{d}[\mathrm{ARF_2}],$$
$$\frac{d[\mathrm{auxin}]}{dt} = \omega + k_\mathrm{d}[\mathrm{auxin\text{–}TIR1}] - k_\mathrm{a}[\mathrm{auxin}][\mathrm{TIR1}] - \mu_\mathrm{Auxin}[\mathrm{auxin}].$$

Transcriptional regulation:



$$\frac{d[\mathrm{IAA_m}]}{dt} = \lambda_1 F_1([\mathrm{ARF}],[\mathrm{IAA_p}],[\mathrm{ARF\text{-}IAA}],[\mathrm{ARF_2}]) + \lambda_2 F_2([\mathrm{ARF}],[\mathrm{IAA_p}],[\mathrm{ARF\text{-}IAA}],[\mathrm{ARF_2}]) - \mu_{\mathrm{IAA_m}}[\mathrm{IAA_m}]$$

$$F_1([\mathrm{ARF}],[\mathrm{IAA_p}],[\mathrm{ARF\text{-}IAA}],[\mathrm{ARF_2}]) = \frac{\frac{[\mathrm{ARF}]}{\theta_{\mathrm{ARF}}}}{1+\frac{[\mathrm{ARF}]}{\theta_{\mathrm{ARF}}}+\frac{[\mathrm{ARF_2}]}{\theta_{\mathrm{ARF_2}}}+\frac{[\mathrm{ARF\text{-}IAA}]}{\theta_{\mathrm{ARF\text{-}IAA}}}+\frac{[\mathrm{ARF}][\mathrm{IAA_p}]}{\psi_{\mathrm{ARF\text{-}IAA}}}+\frac{[\mathrm{ARF}]^2}{\psi_{\mathrm{ARF}}}}$$

$$F_2([\mathrm{ARF}],[\mathrm{IAA_p}],[\mathrm{ARF\text{-}IAA}],[\mathrm{ARF_2}]) = \frac{\frac{[\mathrm{ARF_2}]}{\theta_{\mathrm{ARF_2}}}+\frac{[\mathrm{ARF}]^2}{\psi_{\mathrm{ARF}}}}{1+\frac{[\mathrm{ARF}]}{\theta_{\mathrm{ARF}}}+\frac{[\mathrm{ARF_2}]}{\theta_{\mathrm{ARF_2}}}+\frac{[\mathrm{ARF\text{-}IAA}]}{\theta_{\mathrm{ARF\text{-}IAA}}}+\frac{[\mathrm{ARF}][\mathrm{IAA_p}]}{\psi_{\mathrm{ARF\text{-}IAA}}}+\frac{[\mathrm{ARF}]^2}{\psi_{\mathrm{ARF}}}}$$