GSTDMB 2012: DYNAMICAL MODELLING FOR BIOLOGY AND MEDICINE

Lecture 3
Multi-variable differential equation models

Markus Owen

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 CellDesigner to build and simulate single variable models
- **Next, models with more than one state variable:** more complex dynamics possible, analysis more difficult, often resort to computer simulation

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 (2)

- Rate of reaction proportional to the product of the concentrations of the reactants.
- 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**.
- Another example, the "Brusselator":

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

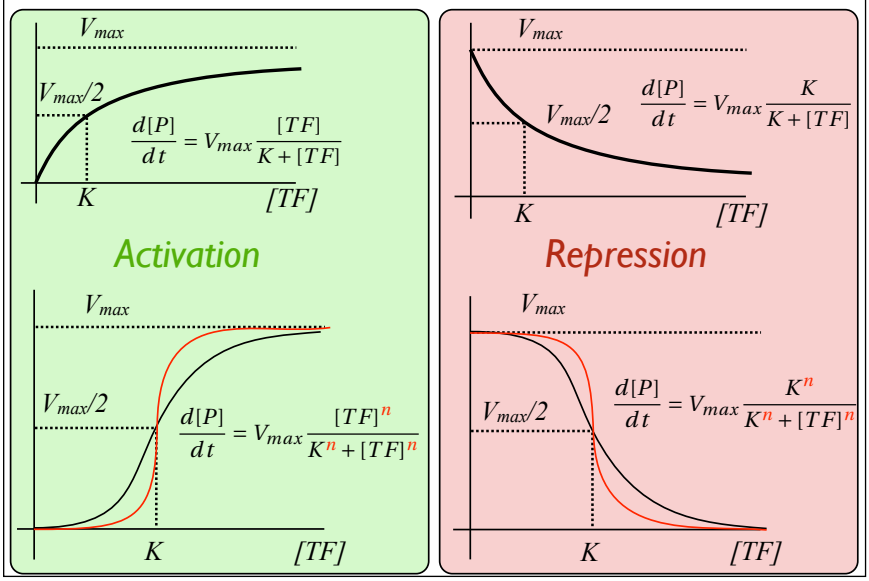
- Assume the concentrations of substrates A and B are constant. E is a product. We are interested in the dynamics of X and Y.
- $x = [X]$, $y = [Y]$, the concentrations of X and Y.
- $\boxed{3}$ has rate $k_3 x^2 y$ which *produces* one molecule of X and consumes one of Y.

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

- This system is a famous example which can have oscillatory solutions (see later).

4

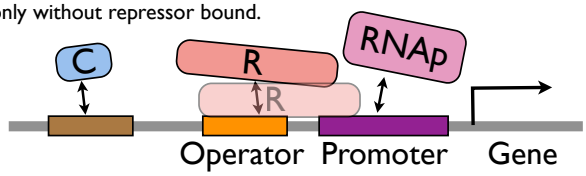
Transcriptional regulation



5

Multiple TFs?

- What about multiple transcription factors? Some activating and some repressing?
- E.g. **Lac-operon**:
  Catabolite activator protein (CAP), $[C]$, activates (promotes binding of RNAp).
  Lac-repressor, $[R]$, blocks the RNAp binding site.
  Transcription only without repressor bound.



- Catalogue all the relevant states and the contribution of each to transcription rate. Transcription only when RNA-polymerase (RNAp) binds to the promoter.
- Write down ODEs and simplify using Michaelis-Menten approach.

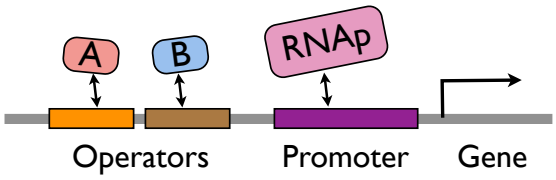
$$\frac{dP}{dt} = \frac{V_{max}(1 + k_1[C])}{1 + k_1[C] + k_2[R] + k_3[R][C]}$$

- or use the Shea-Ackers approach...

6

Shea-Ackers (1)

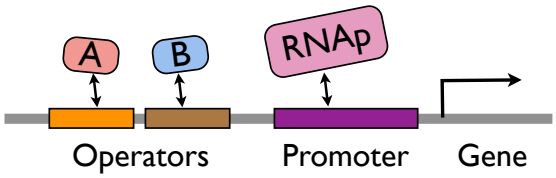
- Method originally developed for the lysis/lysogeny switch in Lambda phage
- Two time scales:
  - Slow: Transcription/Translation/Degradation
  - Fast: Binding/unbinding of TFs to gene – thermal equilibrium
- Possible cases: TF, TF+RNAp, RNAp - probability associated with each
- Enumerate all cases,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



7

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



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

RNAp bound RNAp unbound

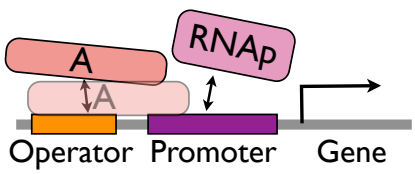
- ***i,j,k*** = ***0*** (unbound) or ***1*** (bound)
- δ_{ijk} related to binding energy, $\delta_{000} = 1$

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

8

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	$\delta_{01}RNAp$
1	1	-

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

- Only the last term corresponds to a transcriptionally active state, so

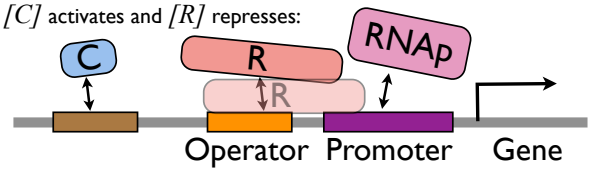
$$T \propto \frac{\delta_{01}[RNAp]}{1 + \delta_{10}[A] + \delta_{01}[RNAp]}$$

- For constant RNAp this is like a decreasing Hill function of order 1.

9

Lac-operon revisited

E.g. Lac-operon: $[C]$ activates and $[R]$ represses:

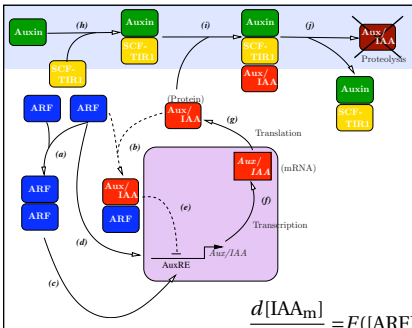


Michaelis-Menten approach: $$\frac{dP}{dt} = \frac{V_{max}(1 + k_1[C])}{1 + k_1[C] + k_2[R] + k_3[R][C]}$$

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

[C]	[R]	[RNAp]	Rate
0	0	0	1
1	0	0	$\delta_{100}[C]$
0	1	0	$\delta_{010}[R]$
0	0	1	$\delta_{001}[RNAp]$
1	1	0	$\delta_{110}[C][R]$
1	0	1	$\delta_{101}[C][RNAp]$
0	1	1	-
1	1	1	-

Auxin model revisited:



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

$$F([\mathrm{ARF}], [\mathrm{IAA_p}], [\mathrm{ARF\text{-}IAA}], [\mathrm{ARF_2}]) = \frac{\lambda_1 \frac{[\mathrm{ARF}]}{\theta_{\mathrm{ARF}}} + \lambda_2 \left(\frac{[\mathrm{ARF_2}]}{\theta_{\mathrm{ARF_2}}} + \frac{[\mathrm{ARF}]^2}{\psi_{\mathrm{ARF}}} \right)}{1 + \frac{[\mathrm{ARF}]}{\theta_{\mathrm{ARF}}} + \frac{[\mathrm{ARF_2}]}{\theta_{\mathrm{ARF_2}}} + \frac{[\mathrm{ARF\text{-}IAA}]}{\theta_{\mathrm{ARF\text{-}IAA}}} + \frac{[\mathrm{ARF}][\mathrm{IAA_p}]}{\psi_{\mathrm{ARF\text{-}IAA}}} + \frac{[\mathrm{ARF}]^2}{\psi_{\mathrm{ARF}}}}$$

Equivalent to Shea-Ackers formulation

Synthetic networks in E. coli

- **Key question:** How do we know that the simple mathematical representations we use are appropriate?
- **One answer:** Test their validity by constructing small networks in cells.
- Measurements on network state can then be compared directly to the behaviour predicted by simple mathematical models.
- Introduce a small number of genes controlled by promoters/repressors.
- Choose regulatory strengths based on a mathematical model of the network.
- A fluorescent reporter gives a read-out of a component of the network.
- The first examples of synthetic networks in *E. coli* were reported in 2000:
  - *Toggle switch* (Gardner, Cantor & Collins)
  - *Oscillator, a.k.a. "Repressilator"* (Elowitz & Leibler)

Toggle switch: two-gene repressor network

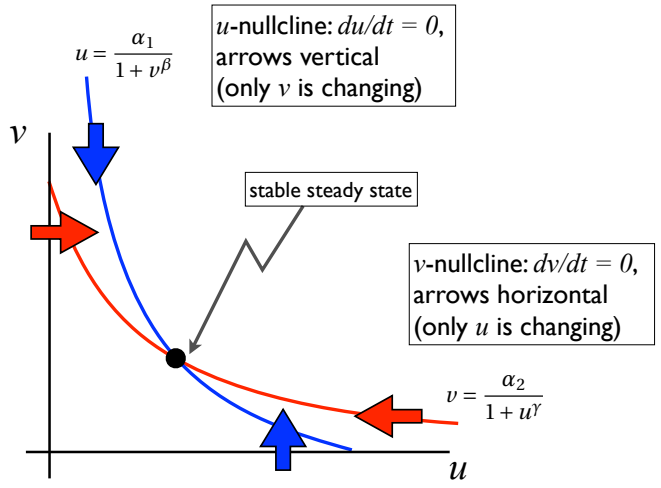
Rate of change of u = production repressed by v - degradation

Rate of change of v = production repressed by u - degradation

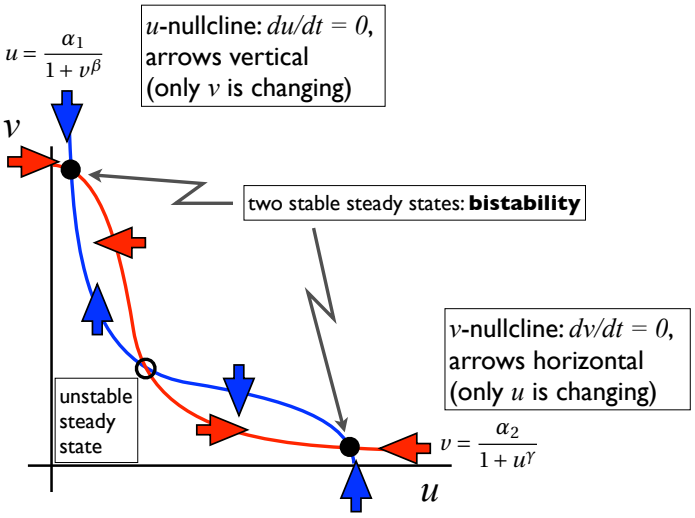
$$\frac{du}{dt} = \frac{\alpha_1}{1+v^\beta} - u, \qquad \frac{dv}{dt} = \frac{\alpha_2}{1+u^\gamma} - v$$

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

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

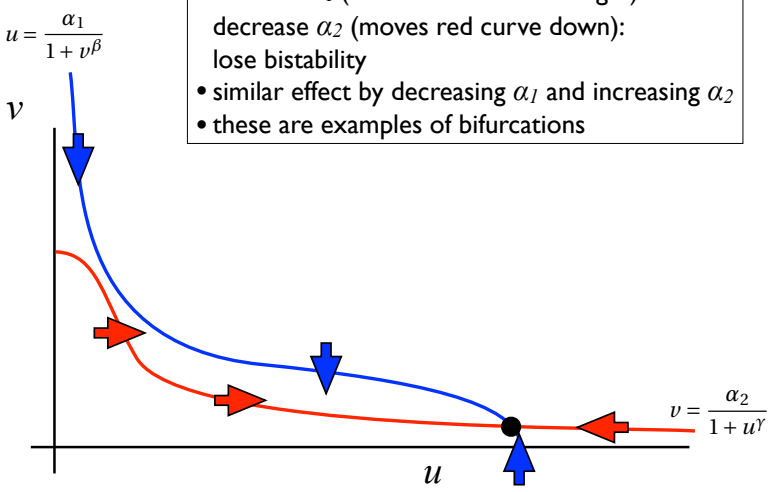


Two-gene repressor network: $\beta, \gamma > 1$

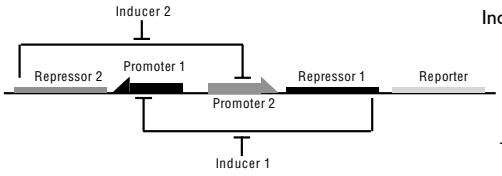


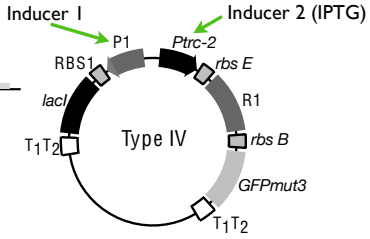
Two-gene repressor network: $\beta, \gamma > 1$

- 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

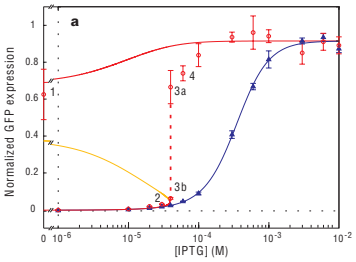


Toggle switch: implementation

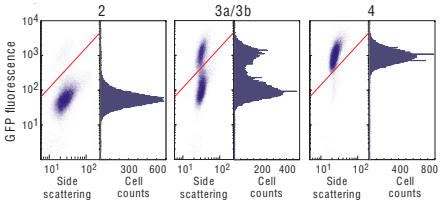




Transitions as predicted by model

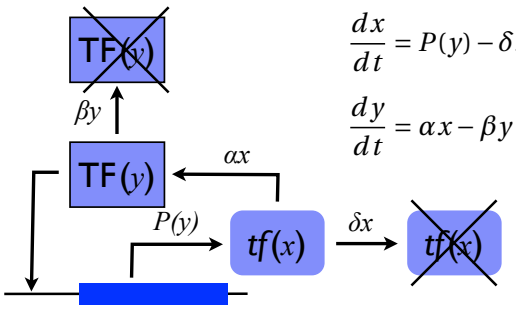


Close to bifurcation, bimodal distribution of cells



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

Transcriptional regulation revisited



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

$$\frac{dy}{dt} = \alpha x - \beta y$$

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

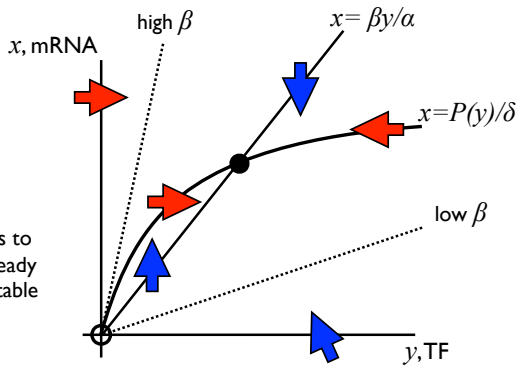
Transcriptional regulation revisited

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

$$\frac{dy}{dt} = \alpha x - \beta y$$

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

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



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

19

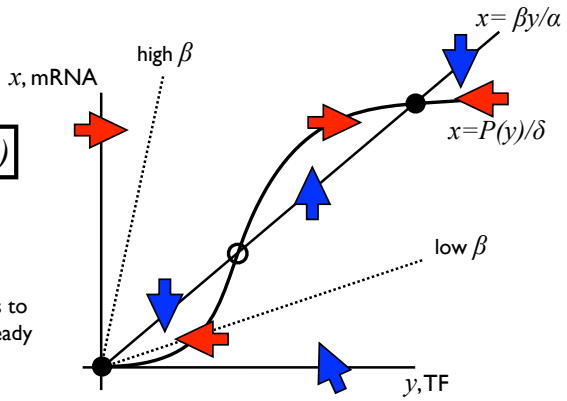
Transcriptional regulation revisited

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

$$\frac{dy}{dt} = \alpha x - \beta y$$

- x-Nullcline is: $x = P(y)/\delta$ and y-nullcline is $x = \beta y/\alpha$
- Easier to think of y as a function of y, 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 β or δ leads to loss of the nonzero steady states.

20

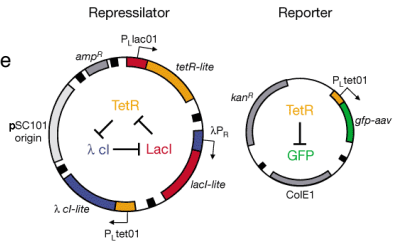
An engineered negative feedback oscillator

The repressilator:

- Transfect *E. coli* with a plasmid containing three repressors:

  $lacI \dashv tetR \dashv cI \dashv lacI$

- Also transfect with a reporter plasmid (visualise TetR expression)



- Represent the system using six variables: three mRNAs and three proteins.
- Linear degradation.
- "Hill function" transcriptional repression.
- Basal transcription.
- Linear translation.

$$\frac{dm_i}{dt} = \alpha_0 + \frac{\alpha}{1+p_j^n} - m_i \qquad i = lacI,\ tetR,\ cI$$

$$\frac{dp_i}{dt} = \beta(m_i - p_i) \qquad j = cI,\ lacI,\ tetR$$

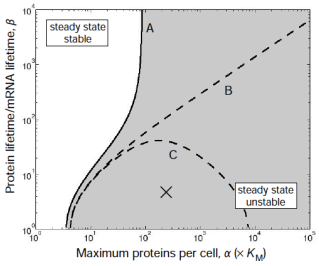
Elowitz, M.B. & Leibler, S. *Nature* **403**, 335 (2000).

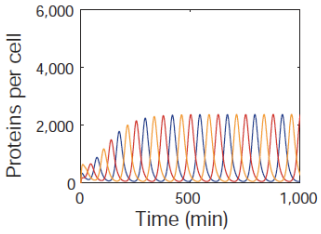
An engineered negative feedback oscillator

$$\frac{dm_i}{dt} = \alpha_0 + \frac{\alpha}{1+p_j^n} - m_i, \quad \frac{dp_i}{dt} = \beta(m_i - p_i)$$

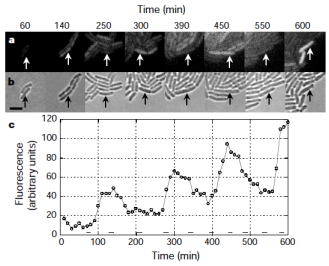
- Use the mathematical model to explore the dynamics as a function of the parameters.
- Engineer the promoters and molecular degradation rates appropriately for oscillations.
- Uses "*LINEAR STABILITY ANALYSIS*"

A: $n = 2.1, \alpha_0 = 0.$ B: $n = 2, \alpha_0 = 0.$ C: $n = 2, \alpha_0/\alpha = 0.001.$





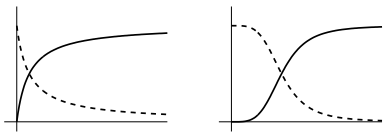
Track bacteria with time lapse over several division cycles (marked with bars in c).



22

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).

23

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

$$S + I + R = N$$

$$S + I \le N$$

24

Epidemiology

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

S-nullcline: $dS/dt = 0$.

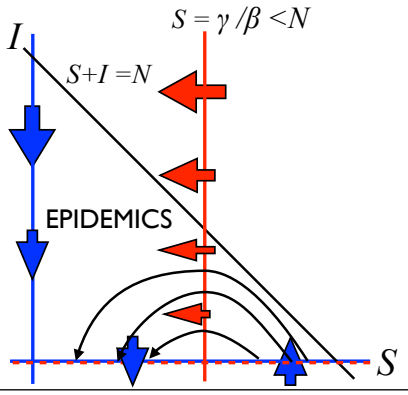
$S = 0$ and $I = 0$.

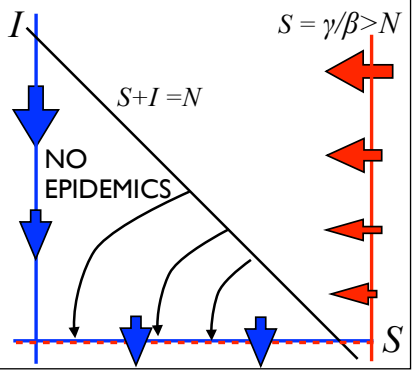
Arrows vertical (only I is changing)

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)





25

Brusselator model

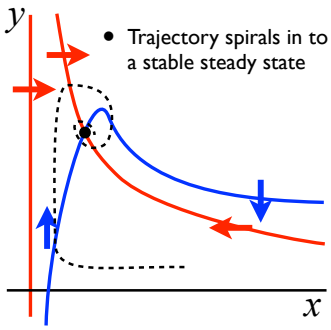
- This system is a famous example which can have oscillatory solutions.

$$\frac{dx}{dt} = k_1 A - (k_2 B + k_4)x + k_3 x^2 y,$$
$$\frac{dy}{dt} = k_2 B x - k_3 x^2 y.$$

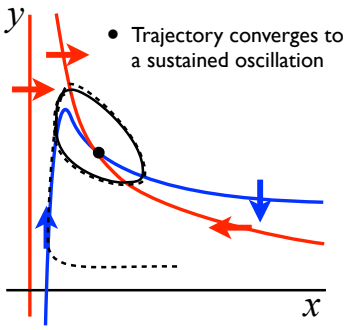
Nullclines

$$\frac{dx}{dt} = 0: \quad y = \frac{(k_2 B + k_4)x - k_1 A}{k_3 x^2}$$

$$\frac{dy}{dt} = 0: \quad y = \frac{k_2 B}{k_3 x} \quad \text{or} \quad x = 0$$



- Trajectory spirals in to a stable steady state



- Trajectory converges to a sustained oscillation

26

Summary

- Gene transcription, mRNA translation, protein interactions, decay, etc, can be described using differential equations.
  There are different approaches to combining multiple transcription factors.
- Mathematical analysis of relatively simple models (with two state variables) can be done using **phase-plane** methods.
  - **phase-plane** represents state of a two-variable system by points on the plane.
  - each point has associated rates of change for each variable, which define a **direction** in the phase-plane (often represented by an **arrow**).
  - sketch the nullclines - curves where one variable is not changing (so there are **two** nullclines if there are **two** variables)
  - steady states are where nullclines cross
- Mutual repression can lead to bistability - but so can positive autoregulation.
- Other simple motifs can be analysed in considerable detail.
- No analogous approach to phase-planes for systems with more than two variables - we rely on more advanced maths (not here!), or computer simulation.
- Network topology *may* be more important than parameter values.
- Similar modelling/analysis applies to other areas of biology and medicine.

27