∘ CCBS. 2025 Fall. Lecture 3. Dynamical systems and diversity of bioregulation.

① Dynamics of 1D, 2D systems.

② Local stability analysis

③ Polynomial dynamical systems by CRNs

④ Time scale separation and archetypal behaviors of bioregulation.

(⑤. "LEGO" of bioregulation.
for homework.)

Last time. we showed that CRNs in general have

dynamics $\frac{dx}{dt} = \Gamma v$. $x \longleftarrow v$

And if we know the mechanisms. so we write down

only elementary reactions. we have

$$\frac{dx}{dt} = \Gamma v(x) = \Gamma \underbrace{\Lambda_k}_{\text{diag}(k)} x^{\alpha} \quad \times \quad v$$

$\alpha \in \mathbb{Z}_{\geq 0}^{n \times m}$ reactant stoichiometry matrix.

This is a subset of $\dot{x} = f(x)$. autonomous dynamical systems.

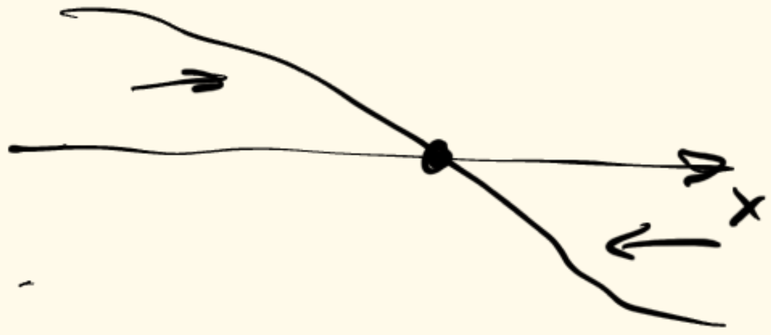
How to analyze its dynamics?

Let's start simple — 1D and 2D

① Dynamics of 1D and 2D systems.

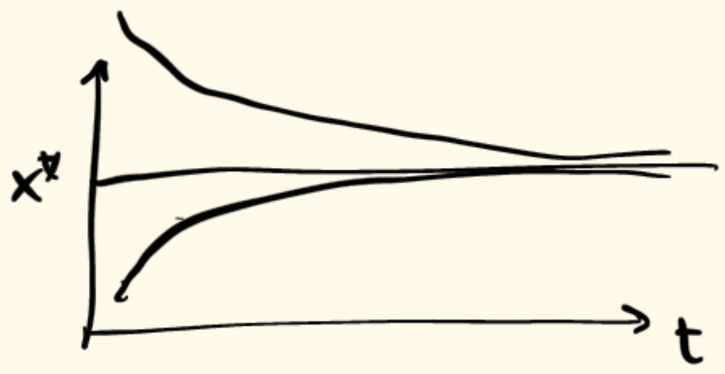
1D system on a line : $x \in \mathbb{R}$.

$\dot{x} = f(x)$. $\begin{cases} f(x) > 0. & \text{right} \\ f(x) < 0 & \text{left.} \\ f(x) = 0 & \text{don't move.} \end{cases}$



Def A fixed point is x^* s.t. $f(x^*) = 0$.

So you can never cross a fixed pt.



Def. A fixed point is (locally) stable

if starting from $x(0)$ close to x^*, $x(t) \longrightarrow x^*$ as $t \to \infty$

Exp 1. $\frac{dx}{dt} = f(x) = -x$

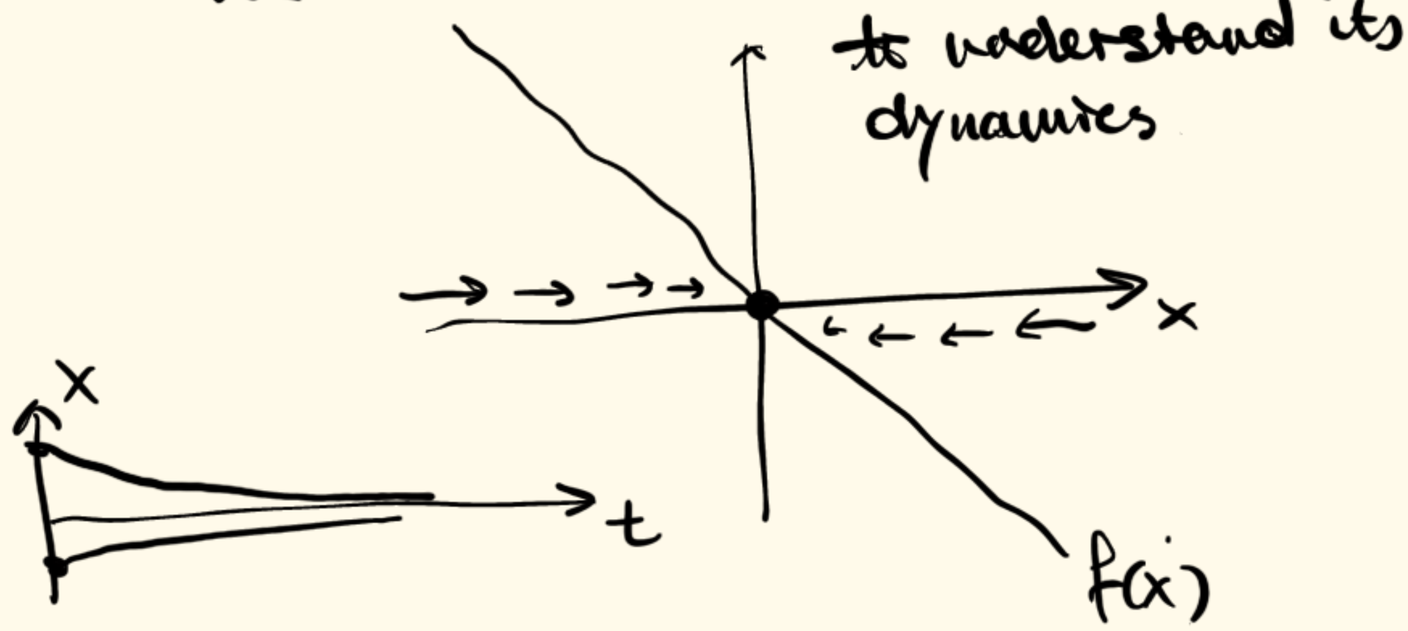
Solve: $\frac{1}{x}\frac{dx}{dt} = -1$

$$\frac{d \log x}{dt} = -1$$

$$\Rightarrow \log x(t) = -t + \log x(0)$$

$$\Rightarrow x(t) = x(0)\, e^{-t}$$

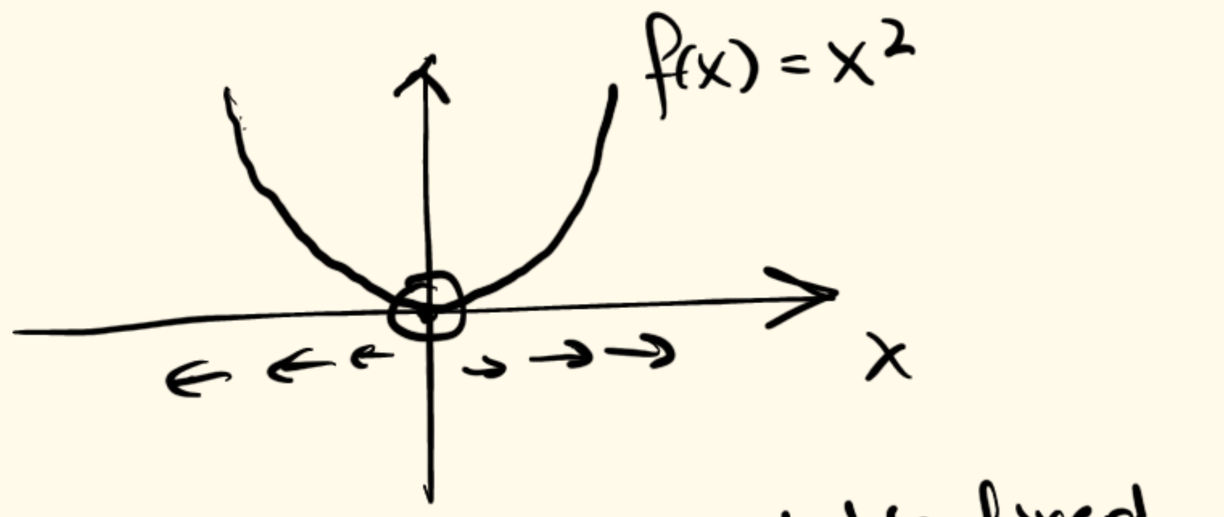
We can also draw a phase portrait to understand its dynamics



Def Phase portrait : for every x, draw $f(x)$. (vector field)

This gives us a geometric way of reasoning about dynamics. independent of details.

$$\frac{dx}{dt} = x^2.$$



So we see $x^* = 0$ is unstable

We use ● to denote stable fixed point
○ to denote unstable.

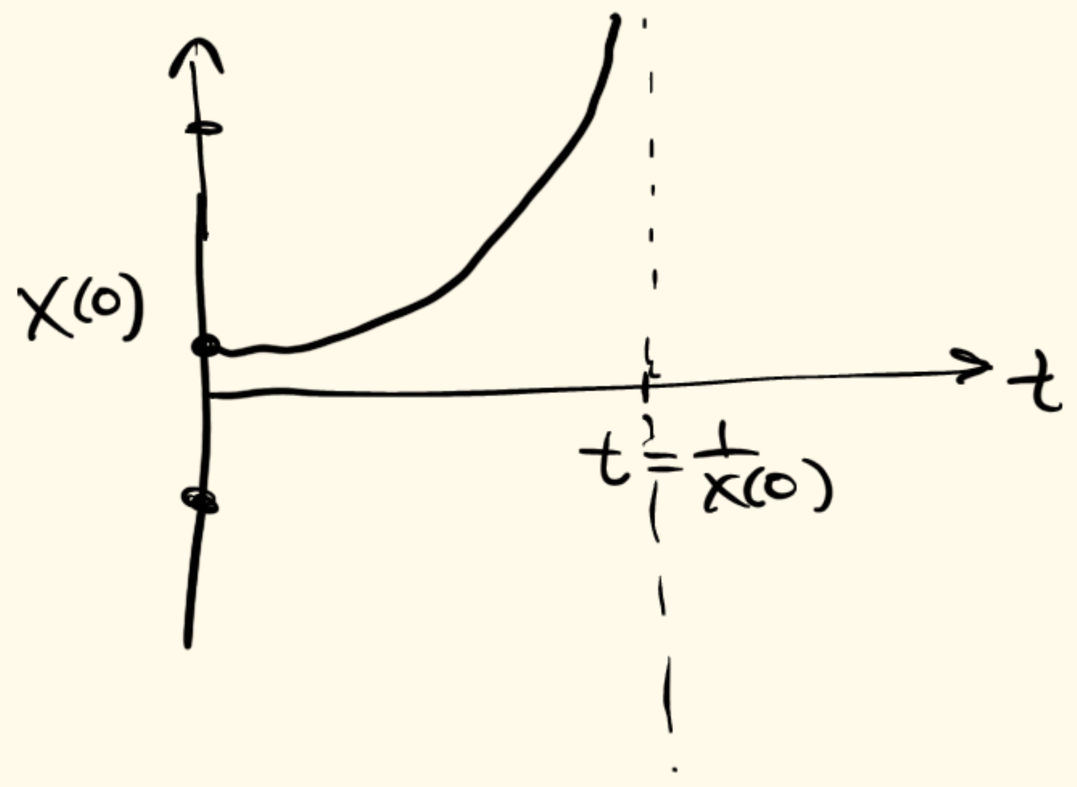
Solve : $\frac{1}{x^2}\frac{dx}{dt} = 1.$

$$\frac{d\,-\frac{1}{x}}{dt} = 1$$

$$\Rightarrow -\frac{1}{x} = t - \frac{1}{x(0)}$$

$$\Rightarrow x(t) = \frac{1}{\frac{1}{x(0)} - t}$$

$$x(t) = \frac{x(0)}{1 - x(0)t}$$

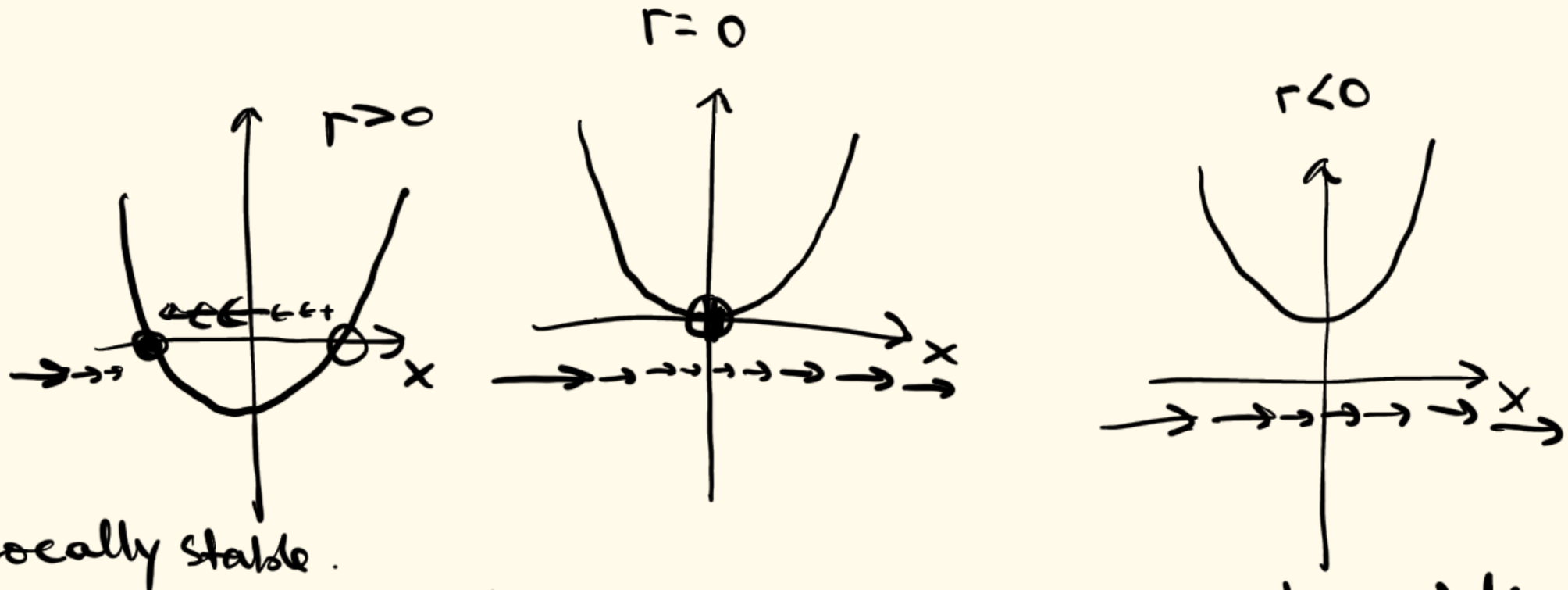


Finite time blow up!

— This is rather pathological, unrealistic.
esp. when close to $t = \frac{1}{x(0)}$!

— "Geometric thinking" can't distinguish such edge cases.
But works well within "normal / regular" behaviors.

Exp 3. $\frac{dx}{dt} = x^2 + r$



Locally stable.

Stable in a region.

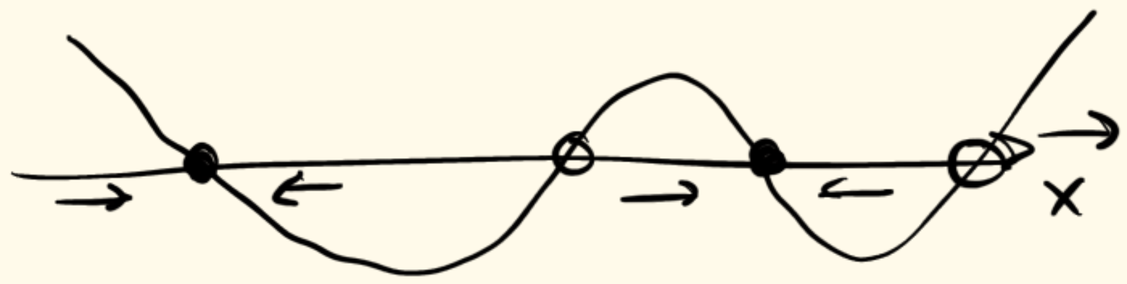
$\dot{x}^* = 0$ is a fixed pt.

Unstable.

= Half stable".

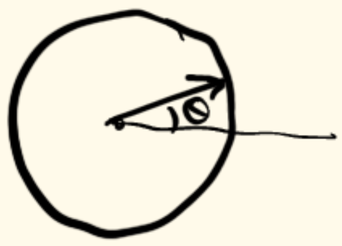
nowhere stable.

Exp 4. Multistability.



"Geometric thinking" works well!

Exp 5. 1D can also be a circle.

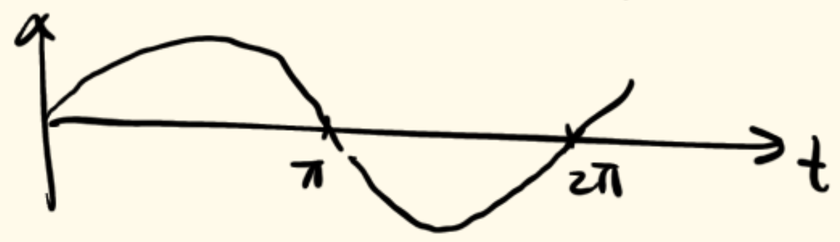


$\theta \in [0, 2\pi)$

$\dot{\theta} = 1$ rotates with a period of 2π.

$y = \sin\theta$

$\dot{y} = \cos\theta$.



— You can also have fixed points on the circle...

e.g. $\dot{\theta} = -\theta$.

- 2D system. $\dot{x}_1 = f_1(x)$, $\dot{x}_2 = f_2(x)$

  — on top of fixed points. x^* s.t. $f_1(x^*) = f_2(x^*) = 0$.

  we have <u>nullclines</u>:

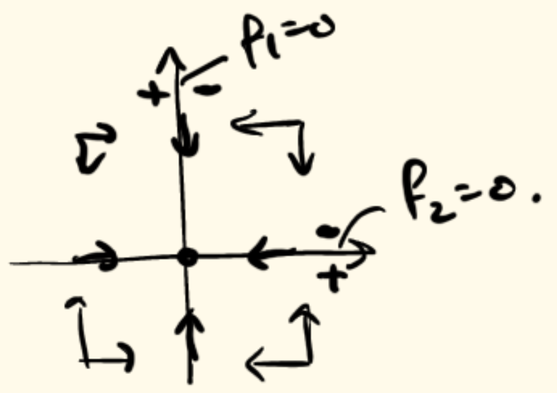
  Def: nullcline of x_i is $\{x \in \mathbb{R}^2 : f_i(x) = 0\}$. $i = 1, 2$.

  — x_i doesn't change on nullclines of x_i.

  Like fixed points in 1D. nullclines partition phase space.

  In 2D. <u>on nullcline of x_1, only x_2 can change!</u>

<u>Exp 1</u>. $\dot{x}_1 = -x_1$, $\dot{x}_2 = -x_2$



Phase portrait.

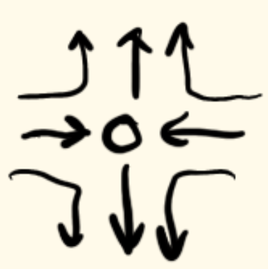
$\frac{d}{dt}\begin{bmatrix} x_1 \\ x_2 \end{bmatrix} = \begin{bmatrix} -1 & -1 \end{bmatrix}\begin{bmatrix} x_1 \\ x_2 \end{bmatrix}$

(linear system notation $\dot{x} = Ax$)

By combining two 1D dynamics. we can get

Stable

unstable



("Saddle").

— Dynamics around fixed points

Anything <u>new in 2D</u>? — Rotations. around fixed points

spirals in

Stable.

spirals out

unstable.

rotates periodically.

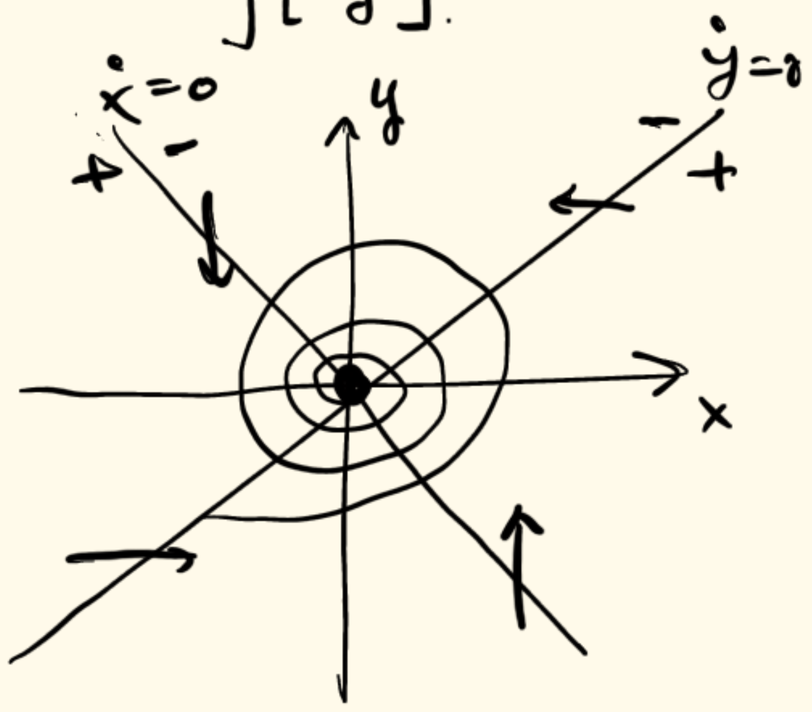
<u>center.</u>

Exp spiral in. $\dot\theta = 1$, $\dot r = -r$. (make it up by composing ID!)

$$\begin{bmatrix}\dot x\\ \dot y\end{bmatrix} = \begin{bmatrix}-1 & -1\\ 1 & -1\end{bmatrix}\begin{bmatrix}x\\ y\end{bmatrix}.$$

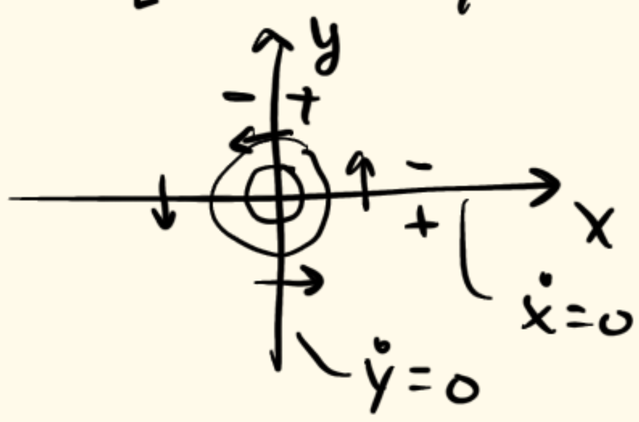
$\Rightarrow$ $x = r\cos\theta$, $y = r\sin\theta$.

$\Rightarrow$
$$\dot x = \dot r\cos\theta - r\sin\theta = -r\cos\theta - r\sin\theta = -x - y.$$
$$\dot y = \dot r\sin\theta + r\cos\theta = -r\sin\theta + r\cos\theta = x - y$$



Exp center. $\dot\theta = 1$, $\dot r = 0$ $\Rightarrow$ $\dot x = -r\sin\theta = -y$, $\dot y = r\cos\theta = x$.

$$\begin{bmatrix}\dot x\\ \dot y\end{bmatrix} = \begin{bmatrix}0 & -1\\ 1 & 0\end{bmatrix}\begin{bmatrix}x\\ y\end{bmatrix}$$

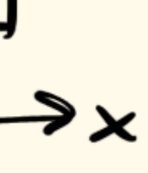


To build intuition on such behaviors.

here is its mechanical implementation.

Exp Mass on a spring.

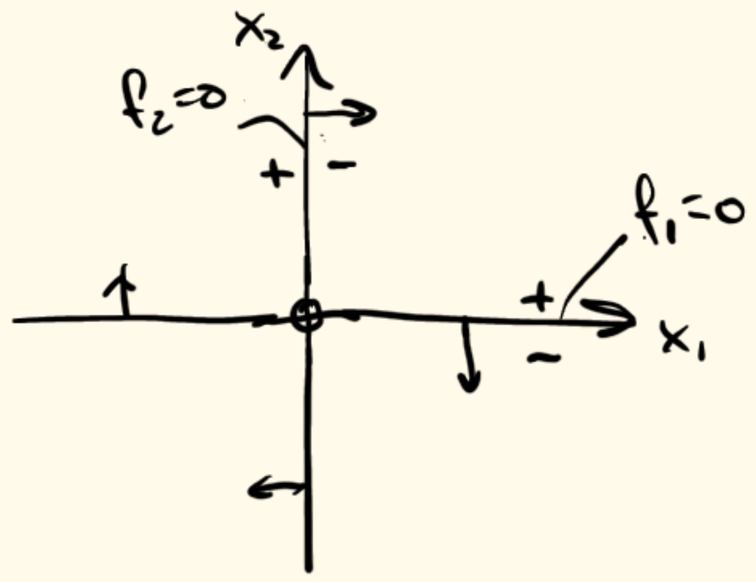
$F = m\ddot x$, $F = -kx$.



$\Rightarrow$ $m\ddot x + kx = 0$

$\Rightarrow$ $x_1 = x$, $x_2 = \dot x$. so $m\dot x_2 + kx_1 = 0$.

$$\frac{d}{dt}\begin{bmatrix}x_1\\ x_2\end{bmatrix} = \begin{bmatrix}0 & 1\\ -\frac{k}{m} & 0\end{bmatrix}\begin{bmatrix}x_1\\ x_2\end{bmatrix}$$

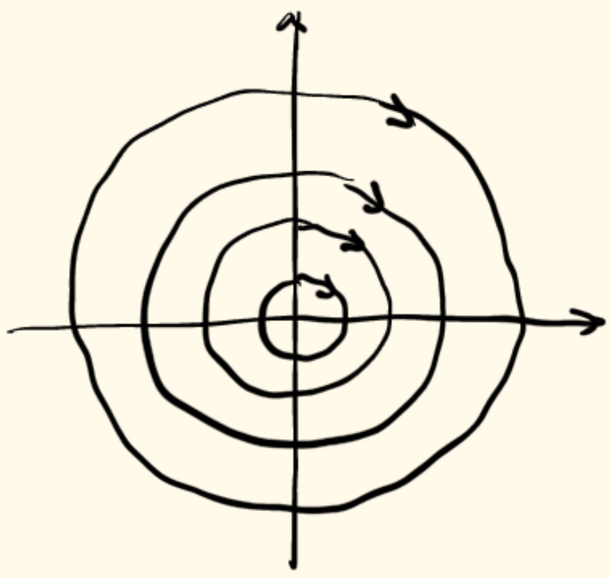


Does traj. spiral in or out?

Neither.

$x = 0$ is a <u>center</u>. not ~~stable~~. (traj. doesn't converge to it.)

How to see this? In this case. we can look at <u>energy.</u>

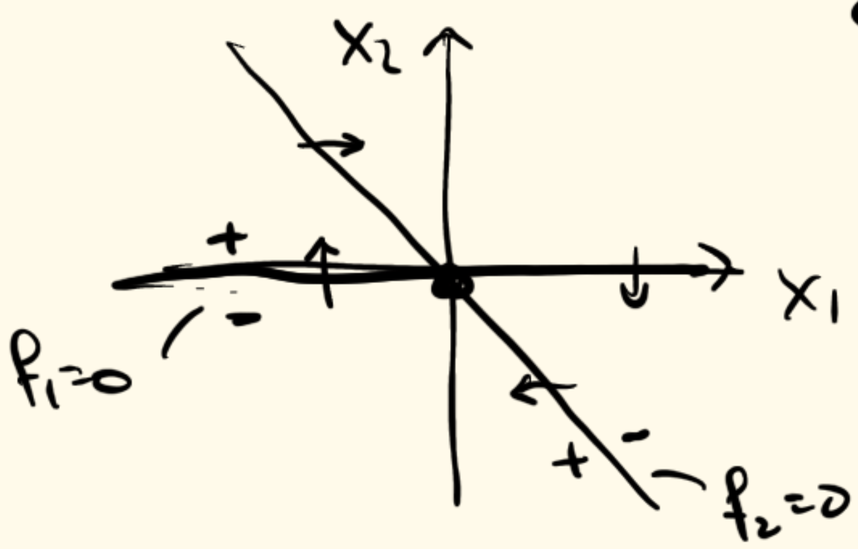


Energy $\frac{1}{2}mx_2^2 + \frac{1}{2}kx_1^2 = E$ is <u>conserved.</u>

$$\left[\begin{aligned}\frac{dE}{dt} &= mx_2\dot x_2 + kx_1\dot x_1\\ &= -mx_2\tfrac{k}{m}x_1 + kx_1x_2\\ &= -kx_1x_2 + kx_1x_2\\ &= 0\end{aligned}\right]$$

— Add friction. $m\ddot{x} + k_f\dot{x} + k_s x = 0$

$$\Rightarrow \frac{d}{dt}\begin{bmatrix} x_1 \\ x_2 \end{bmatrix} = \begin{bmatrix} 0 & 1 \\ -\frac{k_s}{m} & -\frac{k_f}{m} \end{bmatrix}\begin{bmatrix} x_1 \\ x_2 \end{bmatrix}$$



Spiral in or out? $\Rightarrow$.

$$E = \frac{1}{2}mx_2^2 + \frac{1}{2}k_s x_1^2$$

$$\frac{dE}{dt} = m x_2\left(-\frac{k_s}{m}x_1 - \frac{k_f}{m}x_2\right) + k_s x_1 x_2$$

$$= -k_f x_2^2 \leq 0 \text{ unless } x_2 = 0.$$

= Circle shrinks over time..."

$\Rightarrow$.

— New in 2D : limit cycles. (a "fixed orbit").

Stable

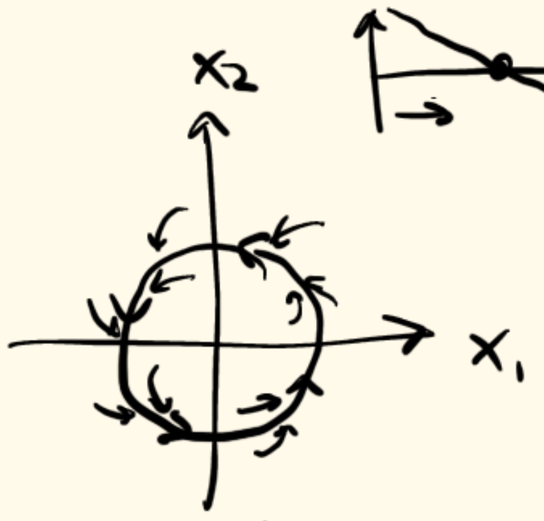
Unstable

e.g. compose two 1D systems in polar coordinates.

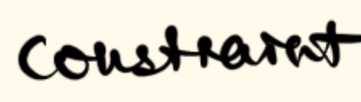
$$\dot{\theta} = k.$$

$$\dot{r} = 1 - r.$$

$\Rightarrow$.



To analyze such. usually it's easier to just simulate:

- 3D and beyond.
  - Traj can roam freely. no constraint due to dimensions anymore. e.g. 
  - Chaos, etc... Nothing very useful can be said.

— Best to just simulate and look.

— "Lack of structure" is not a structure.
Not useful.

— To say something for ≥3 dim. still by dimension reduction.

→. Reduce to 0-d : fixed point. stable manifold.

Reduce to 1-d : fixed point, limit cycles.

Reduce to 2-d : then analyze on 2d —

(2). Fixed point local dynamics in general. ⇒ Local is always Linear

$\frac{dx}{dt} = f(x)$. ⇒. consider small perturbation. Δx. around x.

$\frac{d}{dt}(x+\Delta x) = \frac{d}{dt}\Delta x = f(x+\Delta x) \approx f(x) + \frac{\partial f}{\partial x}(x)\Delta x$.

At $x = x^*$, a fixed point. so $f(x^*)=0$.

⇒. $\frac{d\Delta x}{dt} = A\Delta x$. $A = \frac{\partial f}{\partial x}(x^*)$.

— Linear dynamics $\frac{dx}{dt} = Ax$. fixed pt. $x=0$.

• Scalar $\frac{dx}{dt} = ax$. ⇒ $x(t) = x(0)e^{at}$.

• Eigenvector. (λ, v) s.t. $Av = \lambda v$.

⇒. let $x(0) = y(0)v$. then.

$\frac{1}{\Delta t}(x(\Delta t) - x(0)) = Ax(0)$

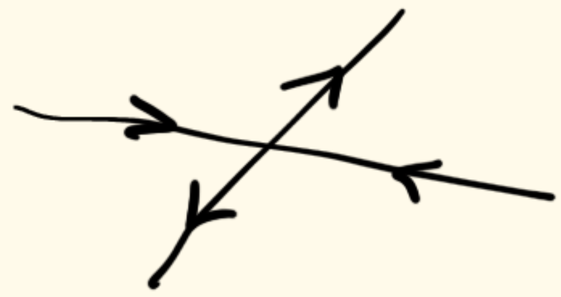
$\frac{y(\Delta t) - y(0)}{\Delta t} v = Av\, y(0) = \lambda v\, y(0)$

⇒. $\frac{dy}{dt} = \lambda y$ ⇒. $y(t) = y(0)e^{\lambda t}$.

⇒. $x(t) = y(0)e^{\lambda t}\, v$.

- So behaviors can be decomposed in terms of eigenvectors and eigenvalues.
  (Start on an eigenvector. Stay on the eigenvector.)

real part. — $Re(\lambda) > 0$. — unstable
$Re(\lambda) < 0$. — stable.



- Imaginary eigenvalue?
  — Rotations. (pairs of eigenvectors.)
  ↳ Rotation on the plane.

A is Hurwitz: $Re(\lambda) < 0$. for all $\lambda \in$ Eigenvalue(A).
This guarantees $x=0$ is stable.

— 2D. $A = \begin{bmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{bmatrix}$.

$\lambda_1 + \lambda_2 = \operatorname{tr} A = a_{11} + a_{22} < 0$.

$\lambda_1 \lambda_2 = \det A = a_{11}a_{22} - a_{12}a_{21} > 0$.

This is necessary and suff. for $Re(\lambda_i) < 0$.

Rules to determine fixed point stability in terms of A in general are called. Routh - Hurwitz criterion.

③. CRNs as polynomial dynamical systems.

- Now we've learned how to analyze dynamics, let's look at dynamical systems from CRNs.
  — They have further structure.

$$\dot{x} = \Gamma v(x) = \Gamma \Lambda_k x^{\alpha} = f^+(x) - f^-(x).$$

Rule [P]

- Positivity : — variables x_j are positive.
  — fluxes $v_i(x)$ are positive.

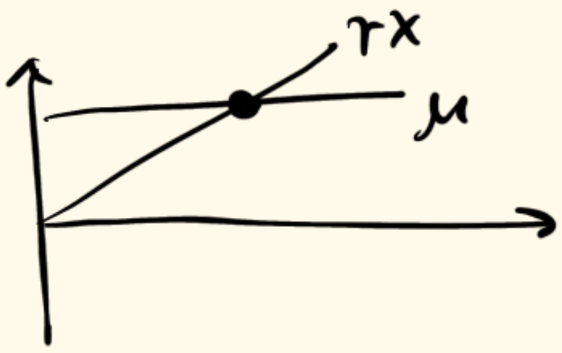
Rule [PU]

- Parameter Uncertainty : reaction rate constants $k_i \neq k_{i'}$ for different reactions $i \neq i'$. = can't perfect match!''

e.g. both increase/decrease of x_j are by comparison between production f_j^+ and degradation f_j^-.

$$\dot{x}_j = f_j = f_j^+ - f_j^-$$

e.g. $\dot{x} = \mu - \gamma x$



then because μ and γx are two different processes.. so the fixed point $x^* = \frac{\mu}{\gamma}$ is the result of two processes pushing on each other.

So. it "shakes". it's uncertain.

the value of x^* is not structural.

- Positivity implies constraint on polynomial dynamics.

Rule [CP] (constrained polynomial)

$$\dot{x}_j = \sum_{i:\, \nu_{ij} > 0} \nu_{ij} v_i - \sum_{i:\, \nu_{ij} < 0} |\nu_{ij}| v_i$$

$$\longrightarrow k_i x_1^{\alpha_{i1}} \cdots x_n^{\alpha_{in}}$$

For a reaction degrading x_j.

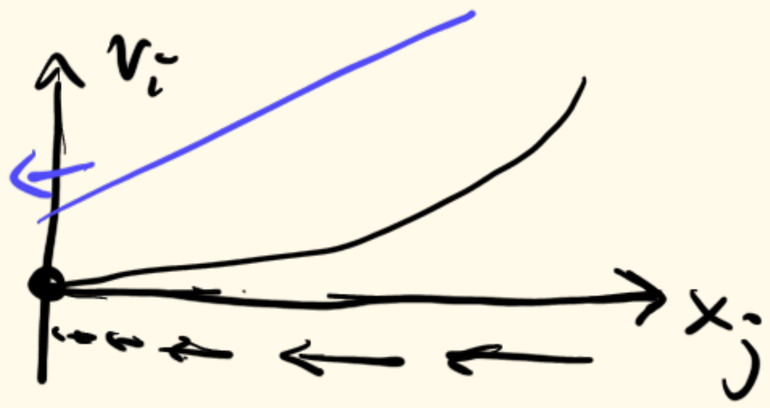
we need $\nu_{ij} < 0$.

since $\alpha_{ij} \geq 0$, $\beta_{ij} \geq 0$. this requires $\underline{\alpha_{ij} \geq 1}$.

so. flux $v_i \propto x_j^{\alpha_{ij}}$. $\alpha_{ij} \geq 1$.

Reasonable from positivity:

$v_i = 0$ et $x_j = 0$.



otherwise x_j becomes negative!

— This constraint keeps the positive orthant
= forward invariant". (Starts in here. always in here.)

- Examples of constrained polynomial dynamics of CRNs

① $\dot{x} = x - \mu$. is this possible?

② $\dot{x}_1 = \mu_1 - x_2$

$\dot{x}_2 = x_1 - \mu_2$.

$\left(\begin{bmatrix} \dot{x}_1 \\ \dot{x}_2 \end{bmatrix} = \begin{bmatrix} 0 & -1 \\ 1 & 0 \end{bmatrix} \begin{bmatrix} x_1 \\ x_2 \end{bmatrix} \right)$.

Is this possible?

Optional

③ Rössler attractor. $\dot{x} = -z - y$.

$\dot{y} = x + ay$

$\dot{z} = b + z(x - c)$.

Q: Are there ways to circumvent such constraints?

- Virtual dynamics by coordinate transformation.

  — For example. Dual rail representation.

$$\dot{x}_+ = f^+ - \eta x_+ x_- \qquad x = x_+ - x_-$$
$$\dot{x}_- = f^- - \eta x_+ x_- \quad \Rightarrow \quad \dot{x} = f^+ - f^-.$$

  — But. because f^+, f^- are fluxes,

  they are polynomials of x_+, x_-,

  so, not natural polynomials of $x = x_+ - x_-$

  e.g. $\dot{x} = \mu - kx$ requires.

$$\dot{x} = \mu - k(x_+ - x_-) = \underbrace{\mu + kx_-}_{f^+} - \underbrace{kx_+}_{f^-}$$

  This means

  we require two reactions' $\quad X_- \xrightarrow{k_1} X_- + X_+$

  reaction rate constants $\quad X_+ \xrightarrow{k_2} X_+ + X_-$

  k_1 and k_2 perfectly matches!

This is not structural. (violate Rule 1PC1).

Q: is there a structural way to go beyond constrained polynomials?

A: Yes. Time-scale separation.

④. Diverse bioregulation by time-scale separation.

- As we estimated before.

Enzymatic reaction $E + S \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} C \overset{k_{cat}}{\rightsquigarrow} E + P$

Typically. $k_{on} \sim 1\ s^{-1} nM^{-1}$.

For metabolite $S \sim 1\,mM$.

$\Rightarrow E + S \xrightarrow{k_{on}} C$ about $k_{on} S \sim 10^6\ s^{-1}$.

while typical enzymes react around $\underbrace{10 - 10^2\ s^{-1}}_{k_{cat}}$. k_{cat} (approx.) as one step.

So. maybe. <u>binding much faster than catalysis!</u>

Optional ↓

- Quasi-steady state assumption. (QSSA).

— Dynamics of C reaches steady state!

$$0 = \frac{dC}{dt} = k_{on} E S - (k_{off} + k_{cat}) C.$$

— When is this true?

Consider one example scenario.

If we assume substrate is overabundant, $S_{tot} \gg E_{tot}$.
($S_{tot} = S + C$, $E_{tot} = E + C$)

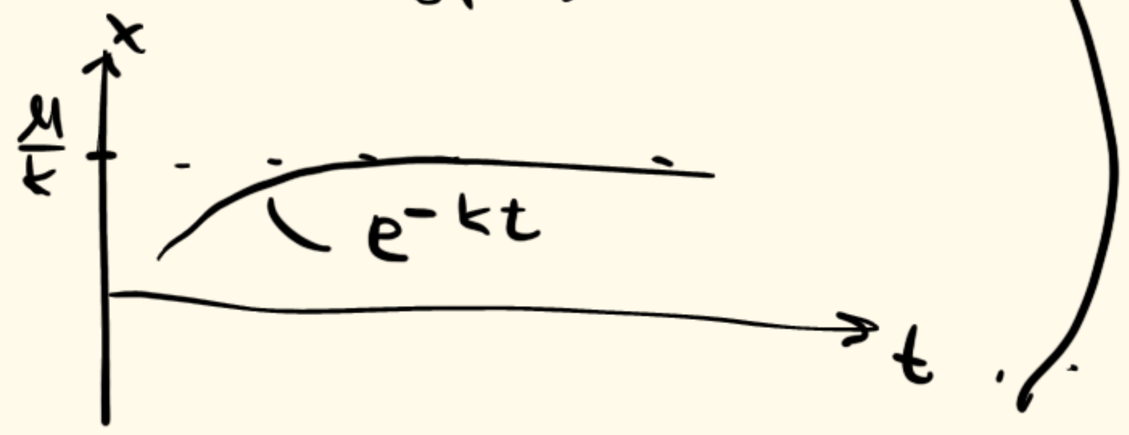
then. $S_{tot} \hat{\approx} S$. so.

(e.g. metabolite 1mM. enzyme 1μM.)

$$\frac{dC}{dt} \hat{\approx} k_{on} (E_{tot} - C) S_{tot} - (k_{off} + k_{cat}) C$$

$$\hat{=} k_{on} E_{tot} S_{tot} - (\underbrace{k_{on} S_{tot}}_{10^6 s^{-1}} + k_{off} + \underbrace{k_{cat}}_{10^1 - 10^2 s^{-1}}) C.$$

(This is of the form $\frac{dx}{dt} = \mu - kx$.

Solution : 

.)

So. the timescale we care about satisfies any of below.

- slower than k_{cat}
- slower than $k_{on} S_{tot}$.

(- slower than k_{off} ← k_{off} usually small..)

then QSSA holds.

- Example exception : k_{cat} is 10 min^{-1}

Cas9. and. $k_{on} S_{tot}$ is 10 min^{-1}.

which is slower than gene expression. (small.

(Unless E_{tot} is large. e.g. 100 μM.

then $k_{on} E_{tot}$ is 1 s^{-1}.).

- What New behavior does binding faster than catalysis give us?

Start simple: $0 = \frac{dC}{dt} = k_{on} ES - (k_{off} + k_{cat}) C$.

$\Rightarrow$. $C = \frac{ES}{K}$. $K = \frac{k_{off} + k_{cat}}{k_{on}}$.

So. we have $v = k_{cat} C(E_{tot}, S_{tot})$.

and C relates to (total) concentrations of catalysis

by $\begin{cases} C = \frac{ES}{K}. \\ E_{tot} = E + C \\ S_{tot} = S + C. \end{cases}$

We can solve this directly.

$$KC = ES = (E_{tot} - C)(S_{tot} - C)$$

$$\Rightarrow \quad E_{tot} S_{tot} - (E_{tot} + S_{tot} + K) C + C^2 = 0.$$

$$\Rightarrow \quad C = \frac{1}{2}\left[(E_{tot} + S_{tot} + K) \mp \sqrt{(E_{tot} + S_{tot} + K)^2 - 4 E_{tot} S_{tot}}\right]$$

Ok. this is a new functional form. not monomial.

But what = behavior" could we have?

- Consider dominance regimes.

i.e. what dominates the totals.

Where Do They Hold?

Dom Reg 1. $E_{tot} \sim E.$ $S_{tot} \sim S.$ $\longleftrightarrow$ $E \gg C.$ $S \gg C.$

$\Rightarrow C = \frac{ES}{K} \approx \frac{E_{tot} S_{tot}}{K}.$ $(\approx)$ $E_{tot}, S_{tot} \gg \frac{E_{tot} S_{tot}}{K}.$

$\Leftrightarrow K \gg S_{tot}, E_{tot}.$

Just mass-action like before

Dom Reg 2. $E_{tot} \sim E.$ $S_{tot} \sim C.$ $\longleftrightarrow$ $E \gg C.$ $C \gg S.$

$\Rightarrow C \sim S_{tot}$ $(\approx)$ $E_{tot} \gg S_{tot}$ $S_{tot} \gg \frac{CK}{E} = \frac{S_{tot} K}{E_{tot}}$

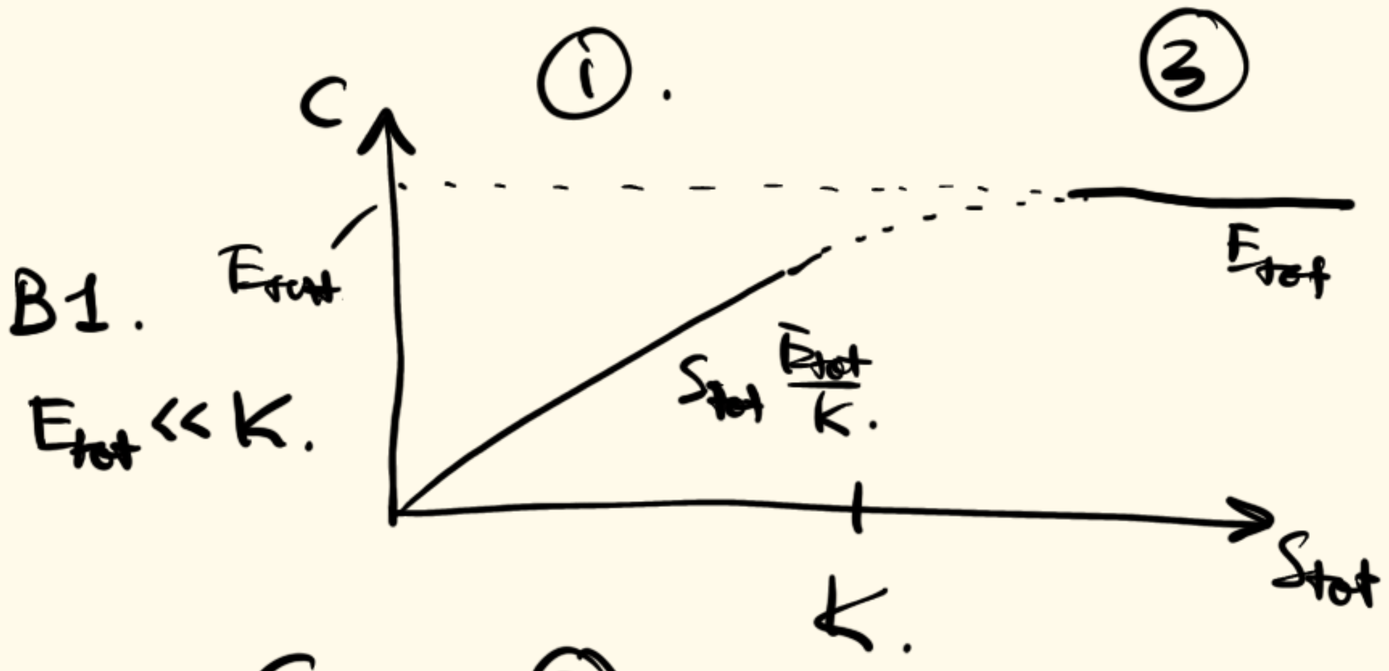
"Saturates". that's new! $\Leftrightarrow E_{tot} \gg S_{tot}, K.$

Dom Reg 3. $E_{tot} \sim C.$ $S_{tot} \sim S.$ $\longleftrightarrow$ $S_{tot} \gg E_{tot}, K.$

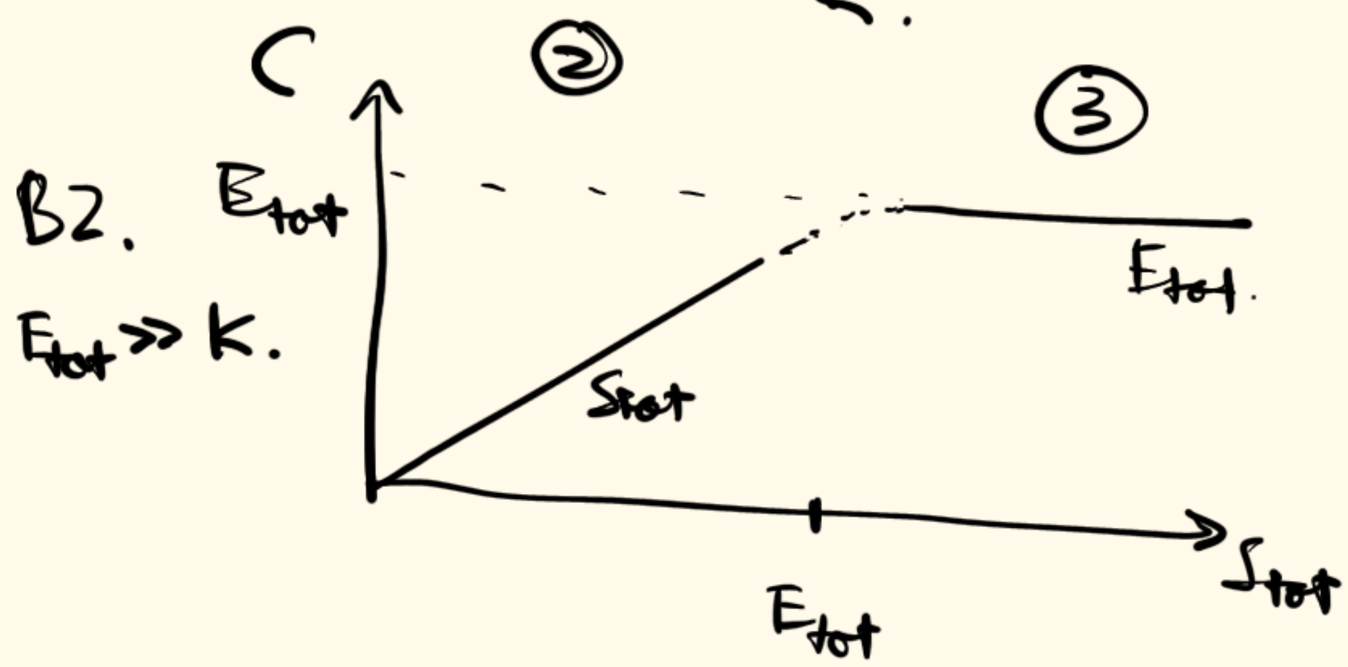
$\Rightarrow C \sim E_{tot}.$

- Behavior of reaction flux

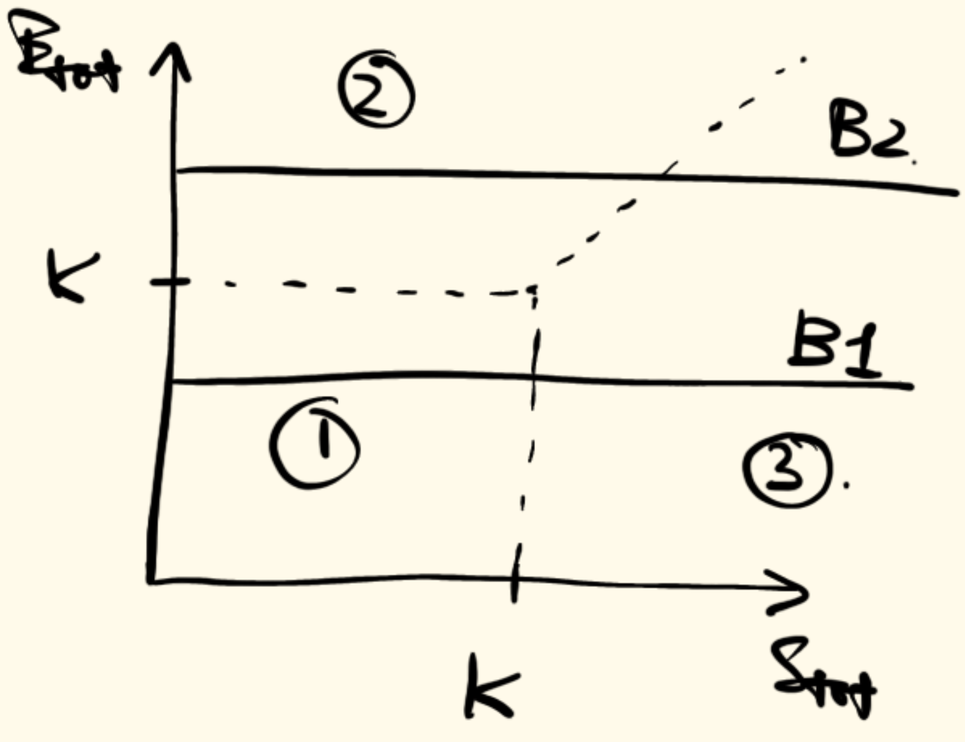
$v = k_{cat} C$. as we change S_{tot}?



B1. $E_{tot} \ll K$.



B2. $E_{tot} \gg K$.



Link them?

$$C = E_{tot} \frac{S_{tot}/K}{1 + S_{tot}/K}.$$

= Saturation".

$C = \min\{S_{tot}, E_{tot}\}$

= Bottleneck".

- What if S is inhibitory?

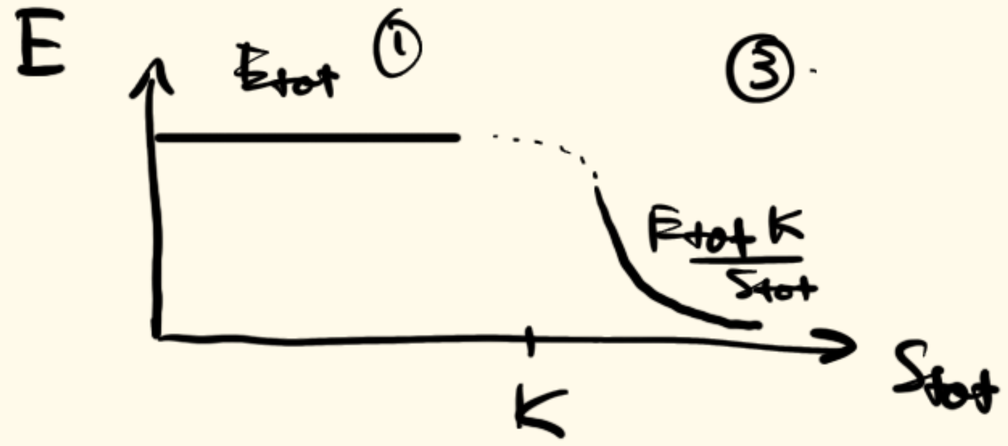
$E + S \rightleftharpoons C$.

$v = k_{cat} E$.

⇒. Dom Reg 1. $E \sim E_{tot}$

Dom Reg 2. $E \sim E_{tot}$.

Dom Reg 3. $E = \frac{CK}{S} \sim \frac{E_{tot} K}{S_{tot}}$

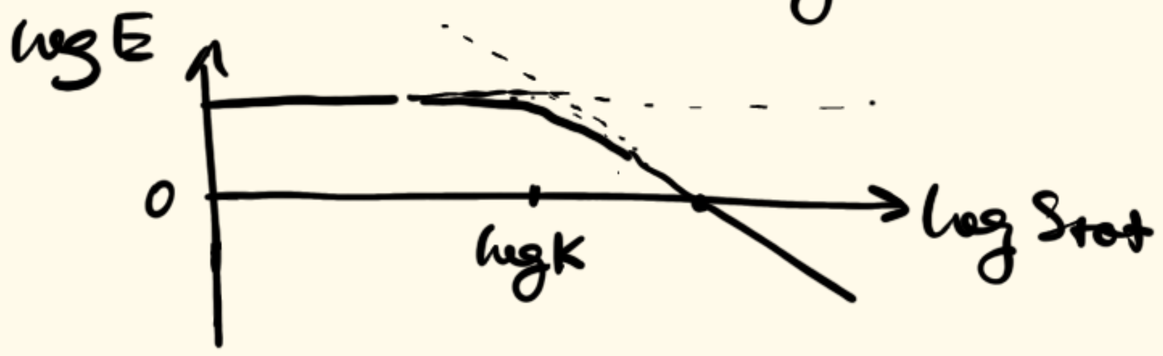


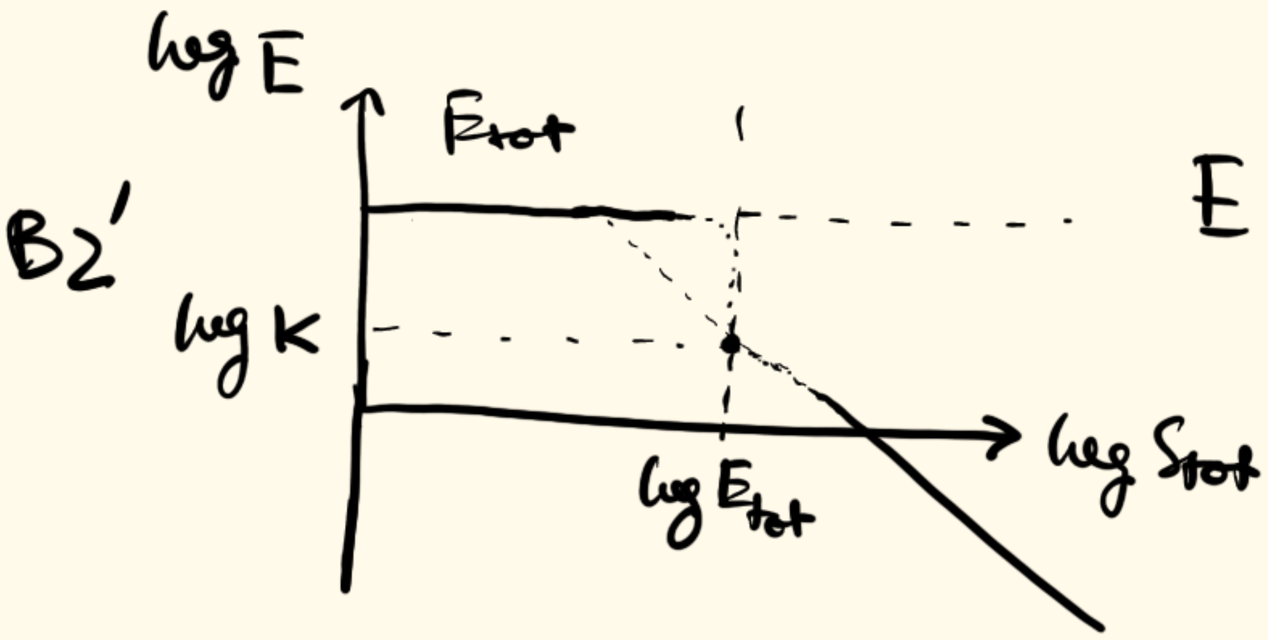
B1'

$$E = E_{tot} \frac{1}{1 + \frac{S_{tot}}{K}}.$$

= Saturation."

This is clearer in log scale





$$E = \begin{cases} E_{tot} & S_{tot} < E_{tot} \\ \frac{E_{tot} K}{S_{tot}} & S_{tot} > E_{tot} \end{cases}$$

= "Ultrasensitivity".

- So. Just from <u>one binding reaction</u>

by time-scale sep. we can create

<u>3 behaviors</u> beyond constrained polynomials.

namely. { Saturation.
Bottleneck.
Ultrasensitivity.

that holds in certain <u>regimes</u> of concentration space.

(In retrospect. Mass Action for enzymatic reaction rates also only holds in DomReg 1. $C \sim \frac{E_{tot} S_{tot}}{K}$.)

- These form building blocks for

diverse bioregulation that are <u>Structural</u>

e.g. Now. is $\dot{x} = x^{-1} - \mu$ possible ?.

Yes. under certain regimes ...

HW problem. ①. Hill function. $\frac{x^n}{1+x^n}$.

Regimes: $G_{tot} = G + C$

$R_{tot} = R + nC$. from $G + nR \rightleftharpoons C$. $v = k_{cat} C$

$R_{tot} \sim R$;

② Competitive binding.

$G + R_1 \rightleftharpoons C_1$ $G + R_2 \rightleftharpoons C_2$. $v = k_{cat} C_1$.

Assume both binding are tight?

$C_1 = \min\{G + C_1, R_{1,tot}\}$.

$R_{2,tot} > G + C_2$. so.

$C_2 \approx G + C_2$.

Assume G is limiting.

then. $R_{1,tot} \approx R_1$. $R_{2,tot} \approx R_2$

Also. $v = k_{cat} G$?

$C_1 + C_2 = \min\{G_{tot}, R_{1,tot} + R_{2,tot}\}$.

③ Titration sponge.

$G + R \rightleftharpoons C_{GR}$ $R + T \rightleftharpoons C_{RT}$.

$v = k_{cat} G$. ↳ titrator.

titration sponge.

G+R. Overabundance.

R+T. tight binding. T less.

Reference: repressilator.

rep_initiation.

$$G = G_{tot} \frac{1}{1 + \frac{R'_{tot}}{K_{GR}}}.$$ and. $C_{RT} \approx T_{tot}$.

$$\Rightarrow \boxed{G = G_{tot} \frac{1}{1 + \frac{R_{tot} - T_{tot}}{K_{GR}}}}$$

$\Rightarrow$. $R_{tot} = R'_{tot} + C_{RT}$

$= R + C_{GR} + C_{RT}$

$\Rightarrow R'_{tot} = R_{tot} - C_{RT}$.

$= R_{tot} - T_{tot}$

Conditional range?

④. Gf