• CCBS. 2025 Fall. Lecture 4. Diversity of bioreg. and Adaptation biomachines.

① Timescale separation and archetypal behaviors of bioregulation.

(1*). "LEGO" of bioregelation.
for homework.

Biomachine I: Adaptation biomachines.

② Adaptation in biology and engineered systems.
(phenomenon, examples in bio and engineered systems)

③ Adaptation in control theory
(plant–controller split.
Examples of trying to adapt.).
⇒. PID control

④ Integral feedback in biology as engineered systems.

⑤ Incoherent feedforward reveals nonlinear structure of biology.

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, $S_{tot} \gg E_{tot}$. ($S_{tot} = S + C$, $E_{tot} = E + C$)

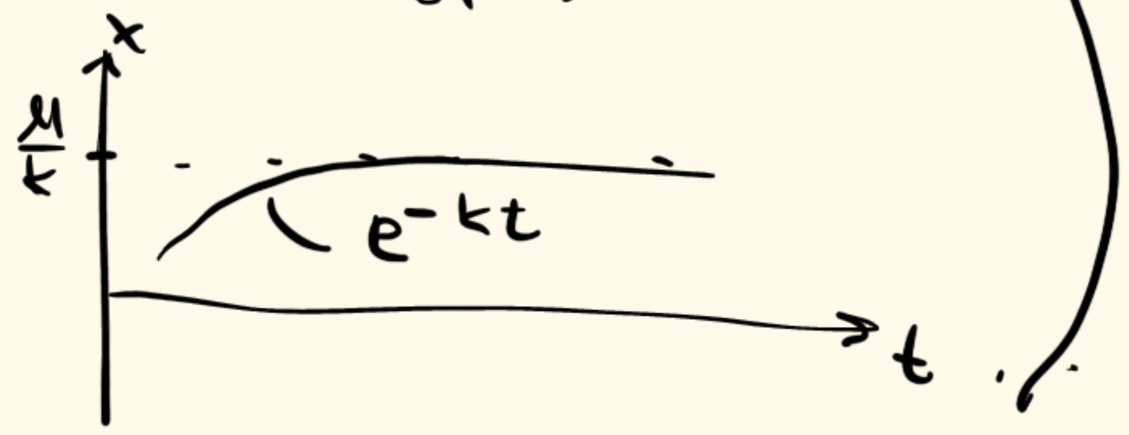
then. $S_{tot} \hat{=} S$. so.

(e.g. metabolite 1mM. enzyme 1µM.)

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

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

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

Control diagram:

(input, system, output).

We can solve this directly. for $C(E_{tot}, S_{tot}, K)$.

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

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

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

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

But what "behavior" could we have?

- Consider dominance regimes.
  - Choose one species dominating each total
  - dominant species can't be repeated

Dominance Conditions i.e. Where Do They Hold?

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

$$\Rightarrow \quad C = \frac{ES}{K} \approx \frac{E_{tot} S_{tot}}{K}.$$

$(\approx)$ $E_{tot}, S_{tot} \gg \frac{E_{tot} S_{tot}}{K}$.

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

Validity conditions.

Just mass-action like before

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

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

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

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

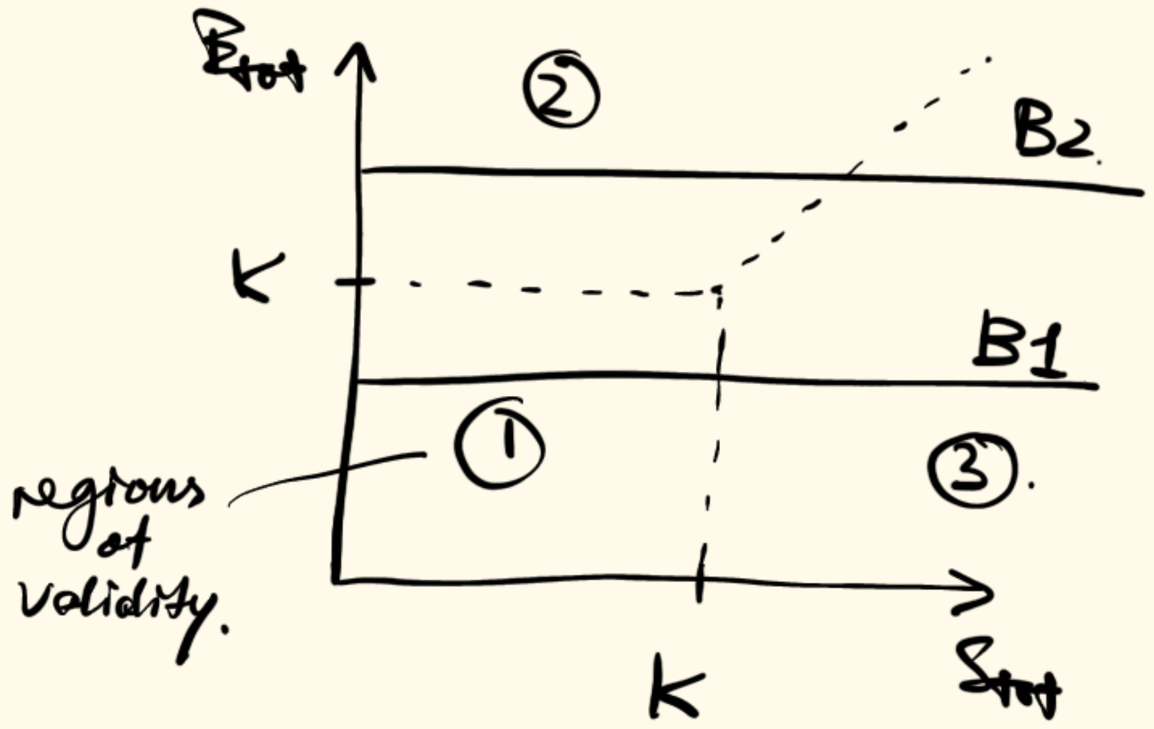
"Saturates". that's new!

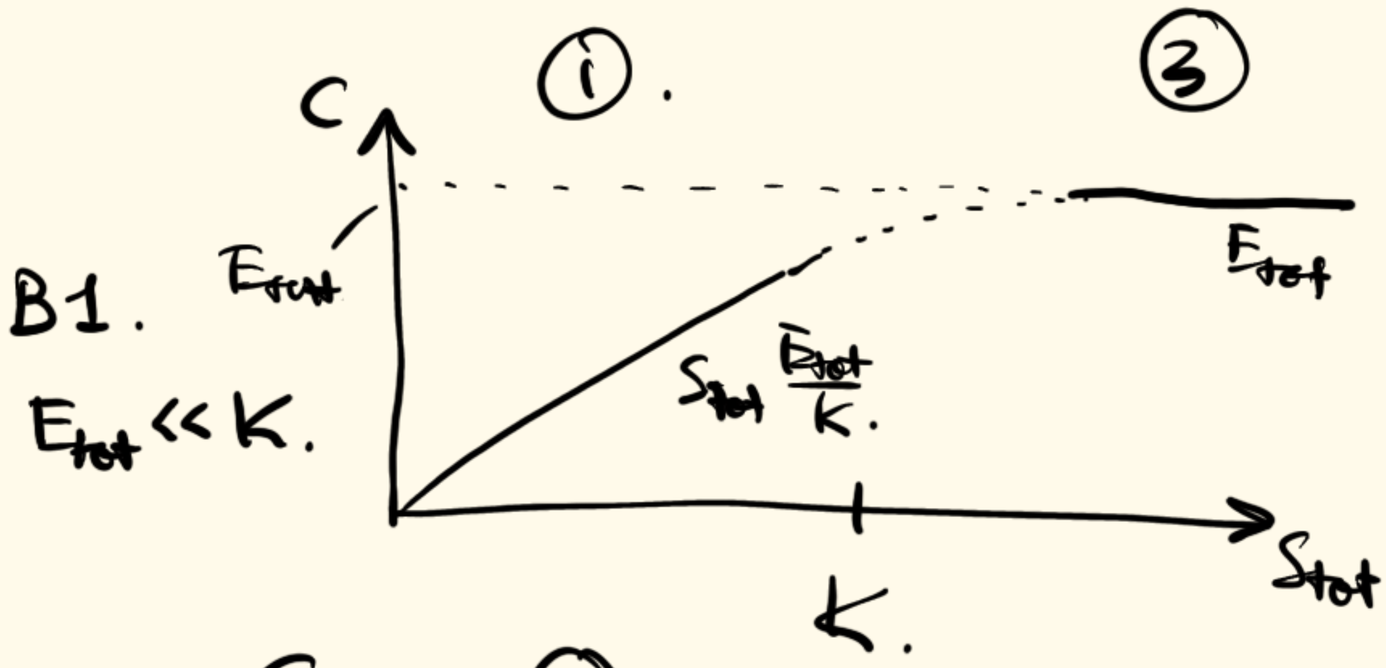
Dom Reg 3. $E_{tot} \sim C$. $S_{tot} \sim S$. $\longleftrightarrow$ $S_{tot} \gg E_{tot}, K$.

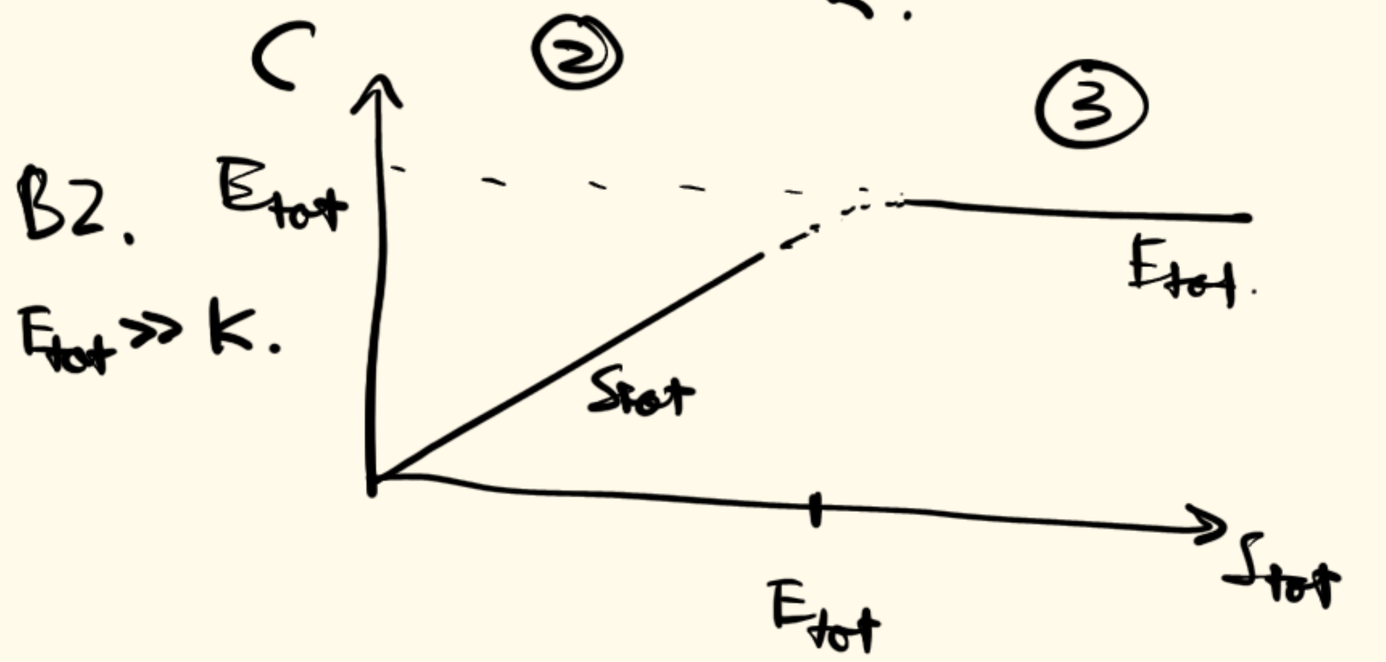
$\Rightarrow$. $C \sim E_{tot}$.

- Behavior of reaction flux

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







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?

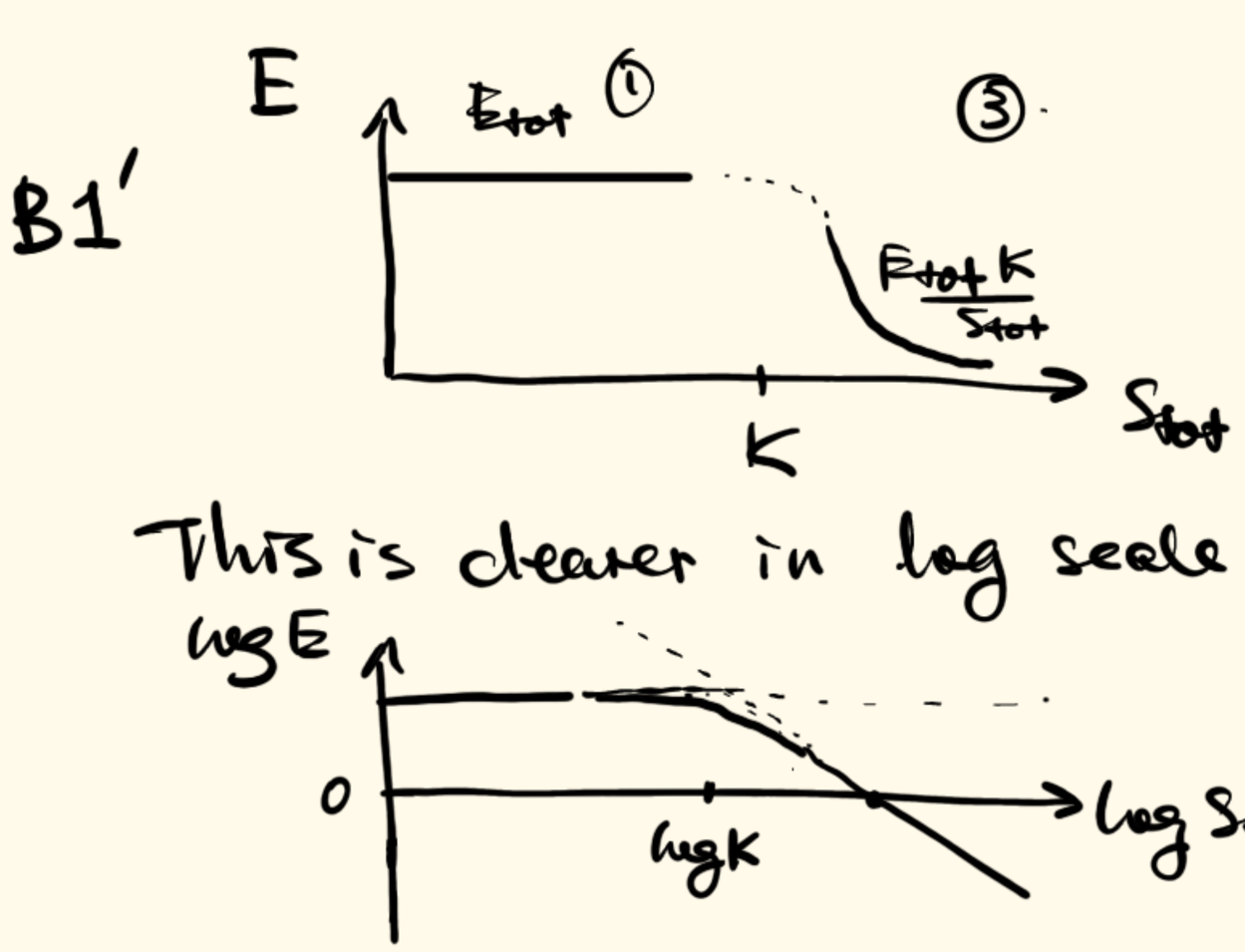
⇒. Dom Reg 1. $E \sim E_{tot}$

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

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

$E + S \rightleftarrows C$.

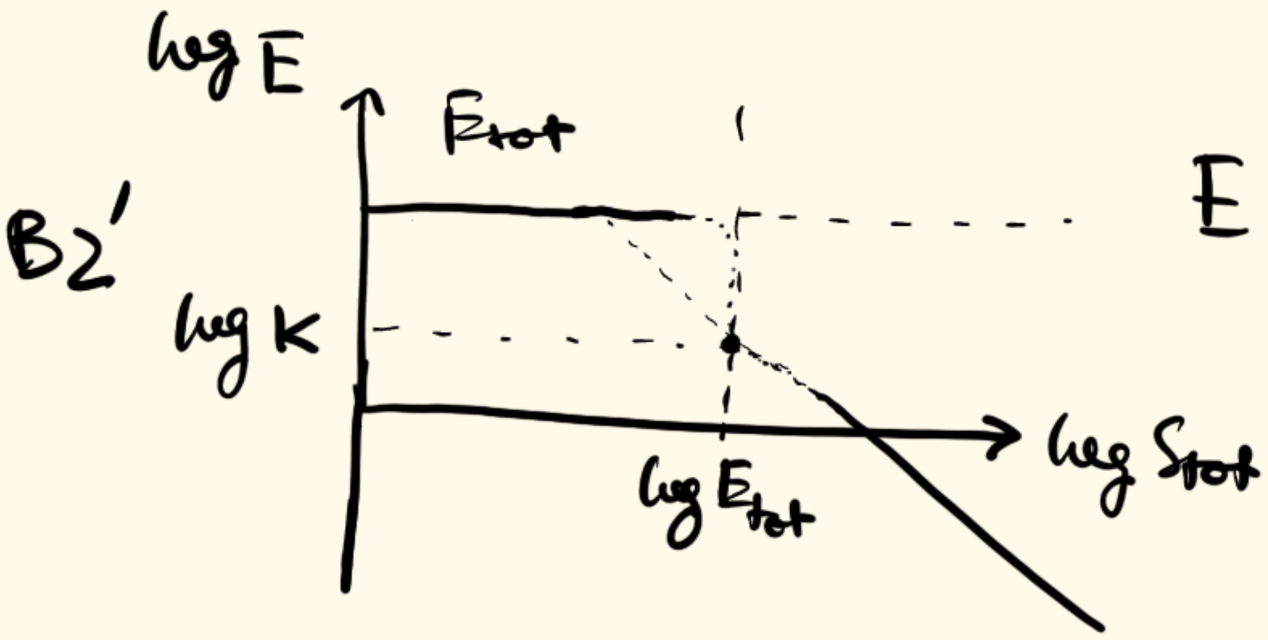
$v = k_{cat} E$.



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

= 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 (-like behavior) 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 ...

- So. what is the class of dynamical systems that biological reaction networks (BRN) can have?

— binding and catalysis reactions

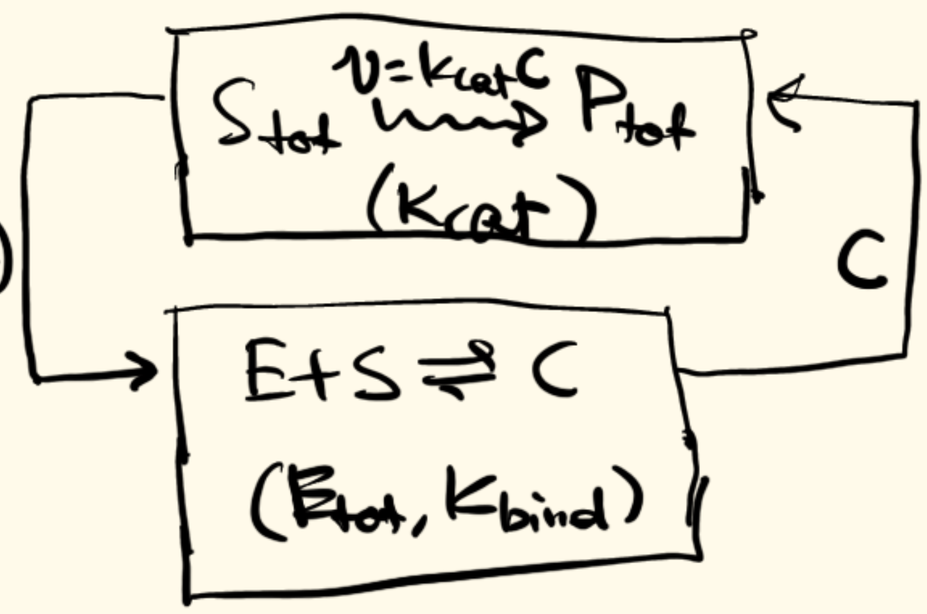
$E+S \rightleftharpoons C \rightarrow E+P$ { binding: $E+S \rightleftharpoons C$; catalysis: $S_{tot} \overset{\nu}{\longrightarrow} P_{tot}$.

binding is elementary (mass-action), fast

catalysis is composite (could be non-mass action), slow.

— This gives us a "two layer system".

binding regulates catalysis.



— Bioregulations are determined by binding reactions.

e.g. $S_{tot}, P_{tot} \rightarrow$ [$E+S \rightleftharpoons C$ (K_{bind})] $\xrightarrow{C}$ $C(S_{tot}, P_{tot}, K_{bind})$.

— Catalysis network. $\frac{dx^{cat}}{dt} = \underbrace{S}_{\text{catalysis stoichiometry.}} v$. e.g. $\frac{d}{dt}\begin{bmatrix} S_{tot} \\ P_{tot} \end{bmatrix} = \begin{bmatrix} -1 \\ +1 \end{bmatrix} v$.

$v_i = k_i^{cat} \underbrace{c_i}_{\text{catalytic active species.}} \Rightarrow \frac{dx^{cat}}{dt} = S \Lambda_{k^{cat}} c$.

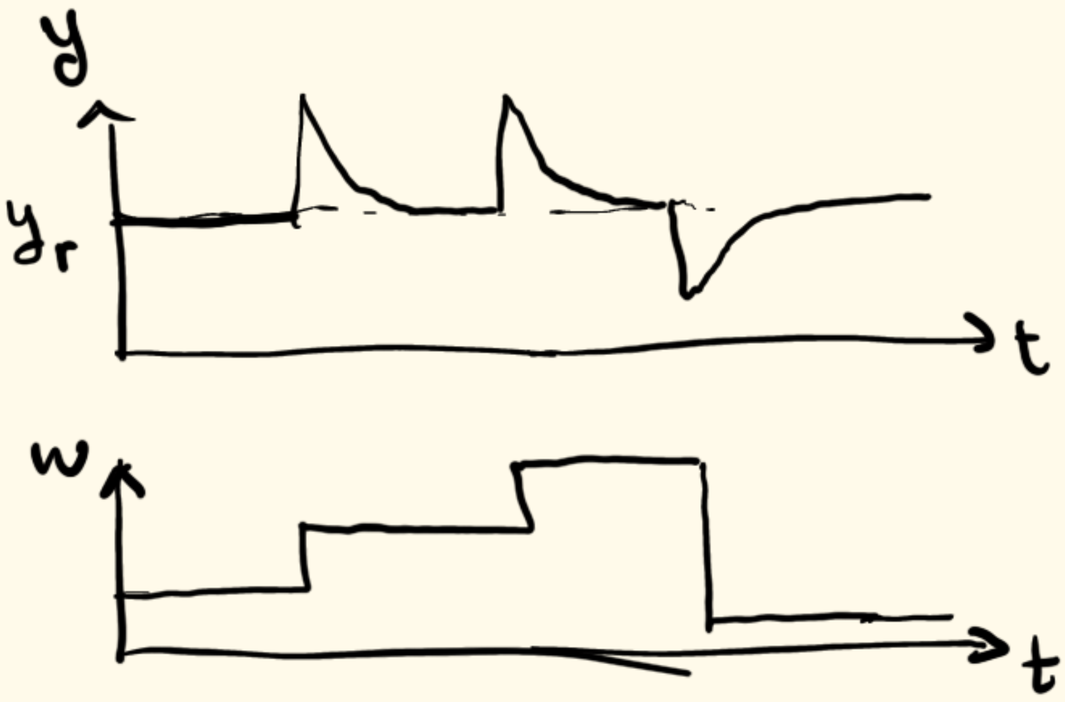
Binding network determines $c(x^{cat})$.

$\Rightarrow \frac{dx^{cat}}{dt} = S \Lambda_{k^{cat}} c(x^{cat})$.

and $c(x^{cat})$ can be diverse, non-mass action, with <u>three</u> simplest building block behaviors.

Next. what are some interesting dynamics that biology cares about?

② Adaptation



Examples in bio.

— body temperature.

— blood sugar/calcium/--- level.

— ATP conc. in cells.

— Standing up.

Homeostasis.

Maintaining an output at a reference value at steady state despite persisting perturbations.

— A hallmark of biology and autonomous machines.

Examples in engineering.

— Watt's improved steam engine.

— 瀹氭簮闸.

— Cars — rejecting bumps on the road

— tracking human input

— audio..

(Through examples. motivate = tracking" is the same as homeostasis. instead of $y=x$. take $y=\frac{x}{w}$. for example.)

So. we could view biological systems as adaptation machines. achieving the same function as engineered systems. so they satisfy the same functional principles but achieve it via different mechanisms.

— The discipline formalizing such functional principles for systems with input and output is control theory

(3). How is adaptation understood in control theory?

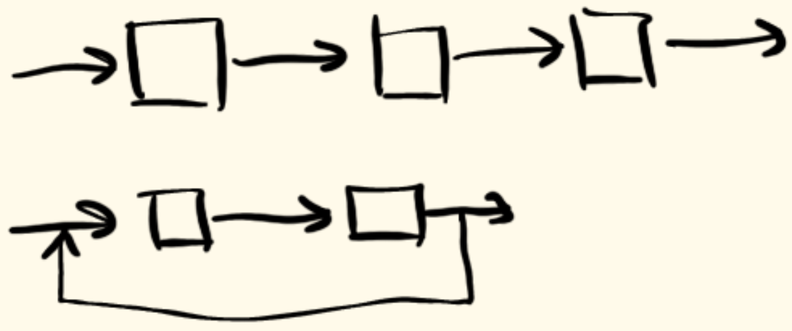
- A control system is described as

e.g. $\dot{x} = f(x, w)$

$y = h(x, w)$.

(<u>state</u> captures all info of the system at present. so you don't need the past to predict its future)

- Control systems can be interconnected.

e.g. 

feed <u>forward</u>

feed <u>back</u>.

- Perfect adaptation is called (step) disturbance rejection

$y \longrightarrow 0$ as $t \longrightarrow +\infty$ for any constant W.

— reference value.

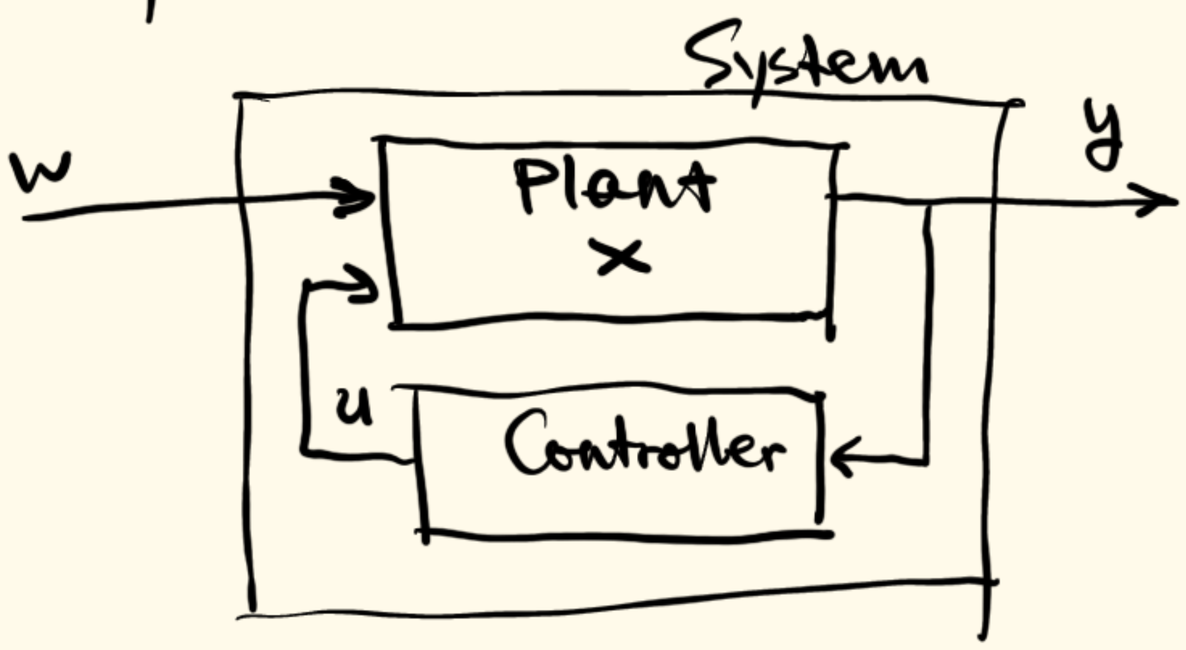
e.g x_1 invariant to W. take $y = x_1 - x_{1,r}$

x_1 tracks W, take $y = x_1 - W$ or $y = \frac{x_1}{W}$.

- We are given a system that previously don't have a property (e.g. adaptation) and make it have this property by adding a controller.

is called a <u>controller design problem</u>.

- A system is split into two subsystems. plant and controller



$\dot{x} = f(x, u, w)$.

$y = h(x, w)$

- We can design the controller,
  but we can't change the plant.

– Controller design for adaptation:

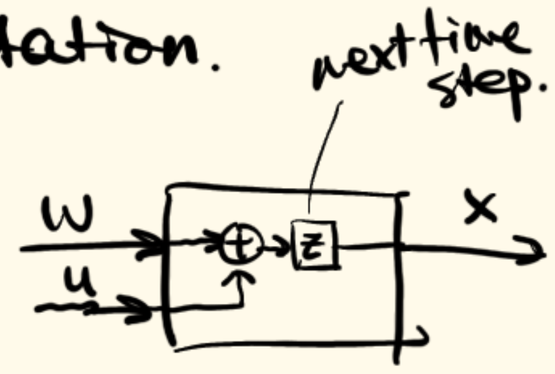
design $y \longrightarrow u$ map such that

$y \longrightarrow 0$ as $t \to +\infty$ for all constant w.

* An example for intuition.

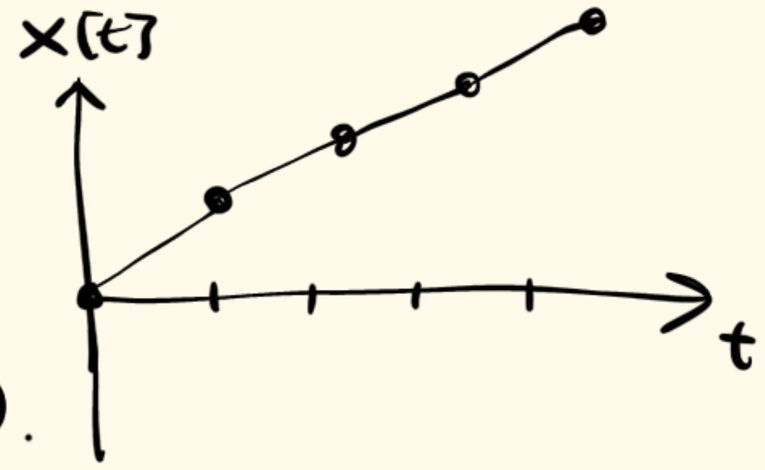
– Let's consider a very simple problem to build some intuition on controller design for adaptation.

Scalar plant, discrete time.



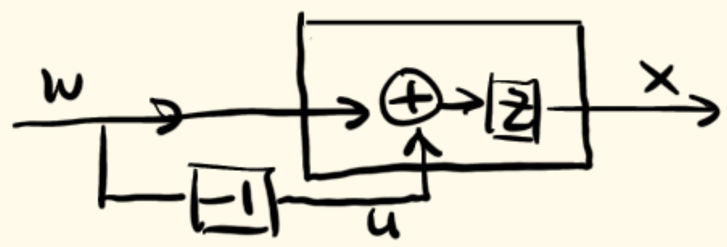
$$X[t+1] = X[t] + \underbrace{w}_{\text{constant. say } w=1.} + u[t].$$

- If no control. $u[t]=0$. then
  x increases linearly
  with increments of w. (const.).



- What should u be to get rid of deviations caused by w?

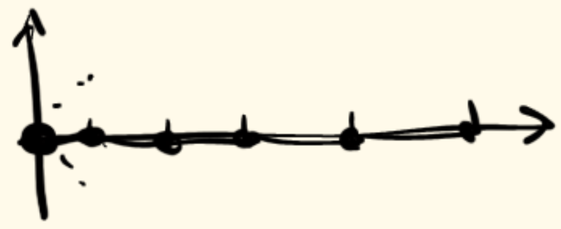
One idea: let u cancel w. e.g. $u = -w$.



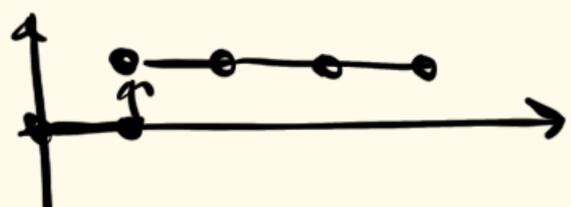
This is feed forward.

$\Rightarrow$ $X[t+1] = X[t] + w - w. = X[t]$.

So. if start at 0. then stays at 0.



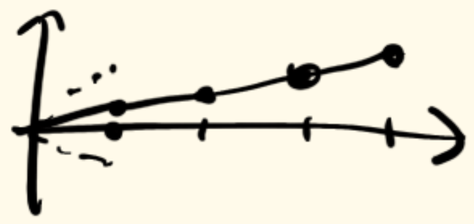
But (1). if any other perturbation. error persists.



(2). Remember rule [PU] ?

In general. hard to make two processes perfectly match in magnitude. ($w+u=0$).

Slight errors will accumulate.

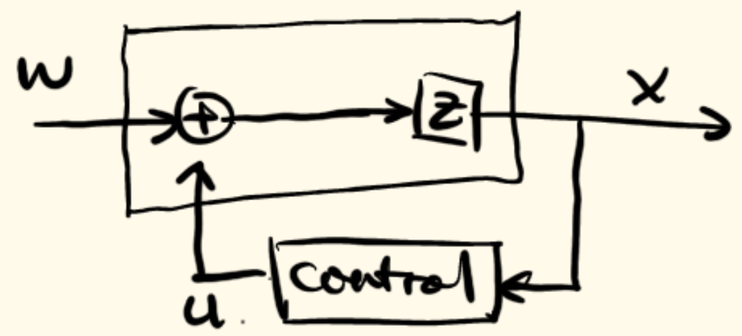


Therefore. feedforward can't achieve perfect adaptation without perfect matching! often unrealistic..

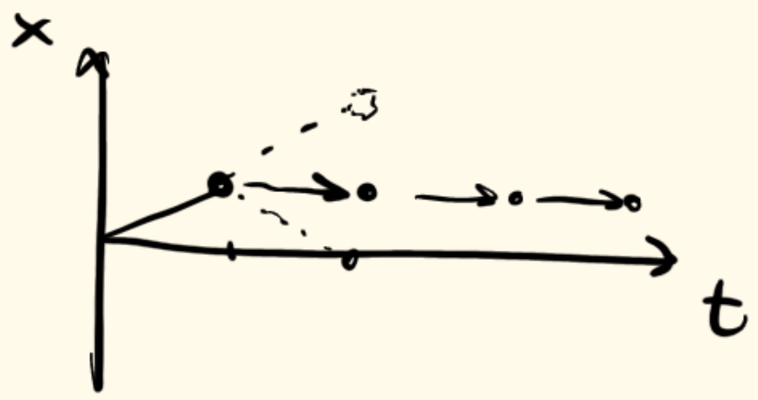
- A better idea: if errors are made, we should be able to observe it in x, then react accordingly.

  i.e. use the power of time. Feedback.

Make u dependent on x.



- First try: push x down by letting $u=-x$.

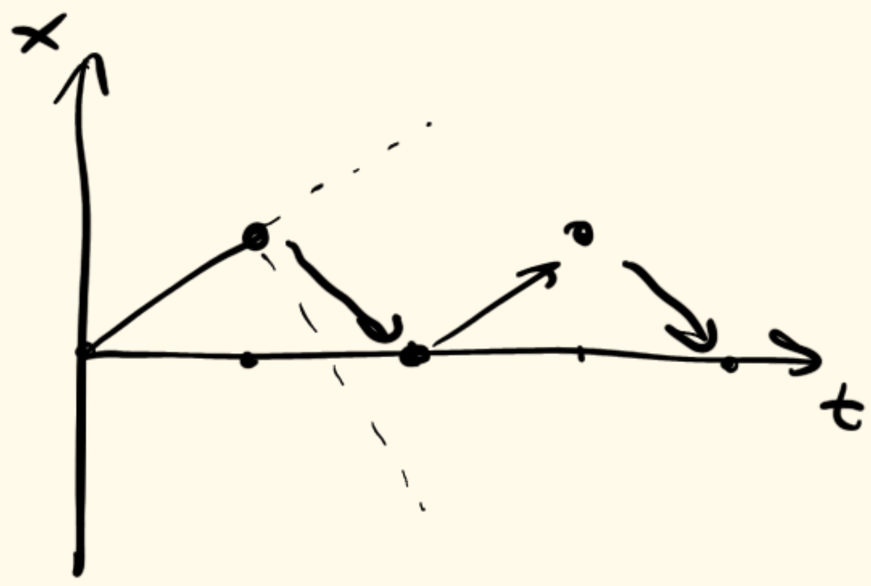


- But this is not perfect adaptation.

  Second try: push down harder. $u=-2x$.

But it oscillates!

We want x to stay at 0.

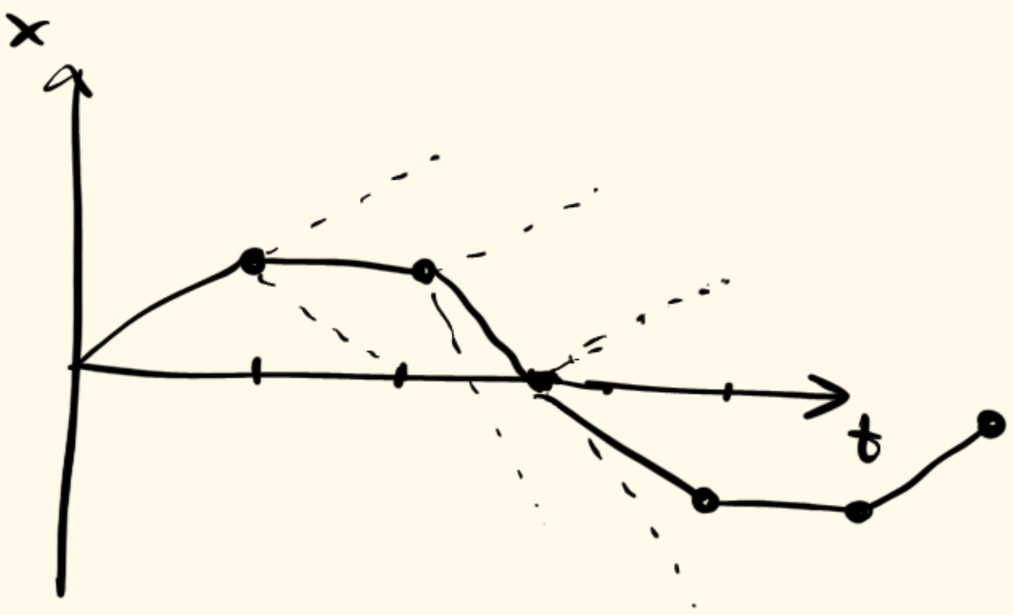


Intuition: once x gets back to 0,
controller forgets to keep pushing down. so x needs to learn from the past.

- Idea: accumulate error from the past:

$$u[t] = -\sum_{t'=0}^{t} x[t']$$

⇒ This is called integral controller.
since it integrates the past error.

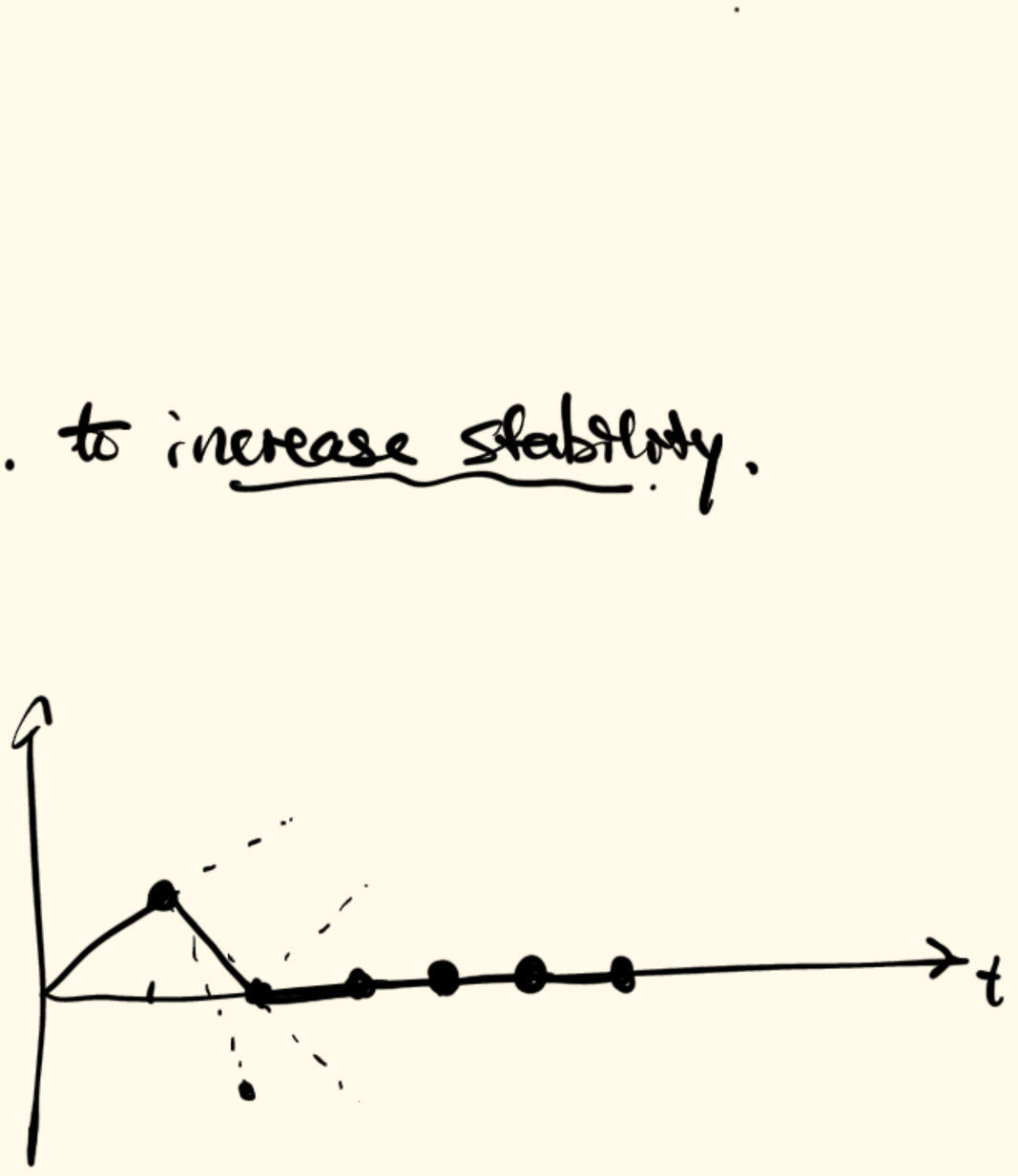


⇒ Still oscillates!

Intuition: there is some delay in control action, causing this oscillation. If we could push down immediately. to increase stability. that could help.

- Idea: combine the two.

$$u[t] = -x[t] - \sum_{t'=0}^{t} x[t'].$$



Perfectly adapts!

- This is an example of a PID controller.

$$u(t) = \underbrace{k_p y}_{\text{proportional}} + k_I \int_0^t y(t')dt' + k_D \frac{dy}{dt}(t).$$

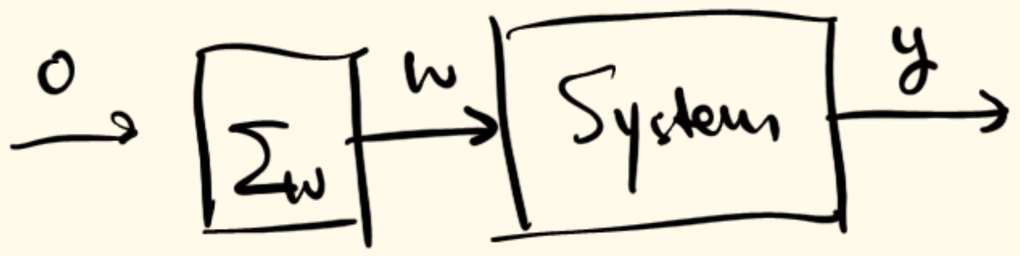
proportional = "present"
integral = "past"
derivative = "future".

- Great majority of controllers in our engineered systems are PID controllers. Great for stabilization and adaptation.
- Stability is very hard in general. since a system can break due to failure of any of its components.

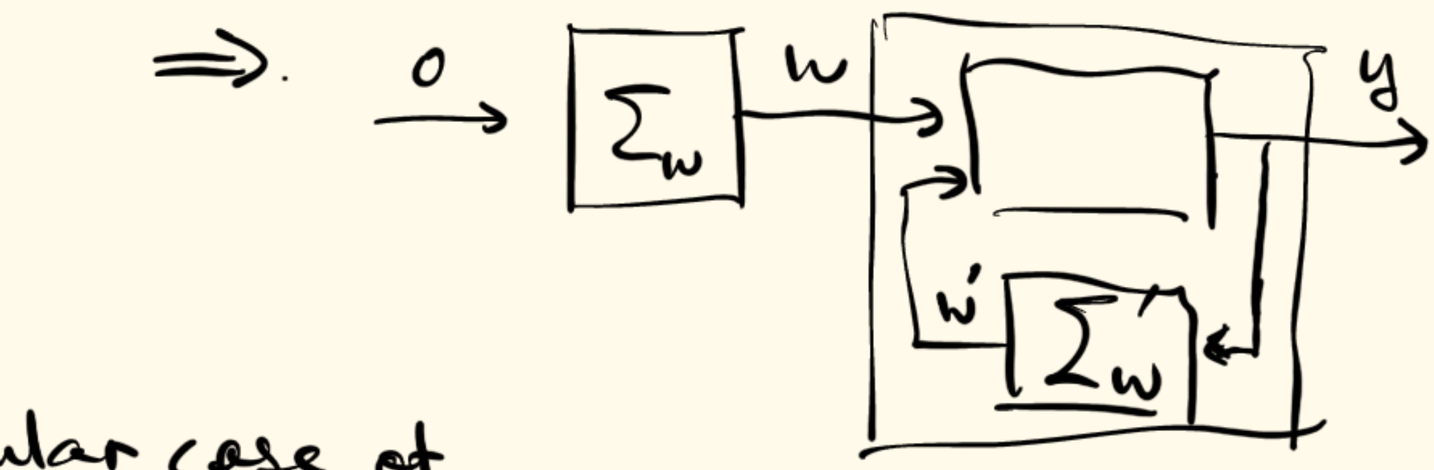
But adaptation, turns out. is "easy".

- In fact. a control ~~theory~~ result, internal model principle

says = "if you want to reject a class of disturbances generated by a disturbance system, then your system has to contain the disturbance system as a subsystem."



$\Rightarrow$



In the particular case of step disturbances. the disturbance system Σ_w is an integral.

$\dot{w} = 0 \Longleftrightarrow w = \int_0^t 0 \, dt' = 0 \cdot t + c$

↑ constants for initial conditions.

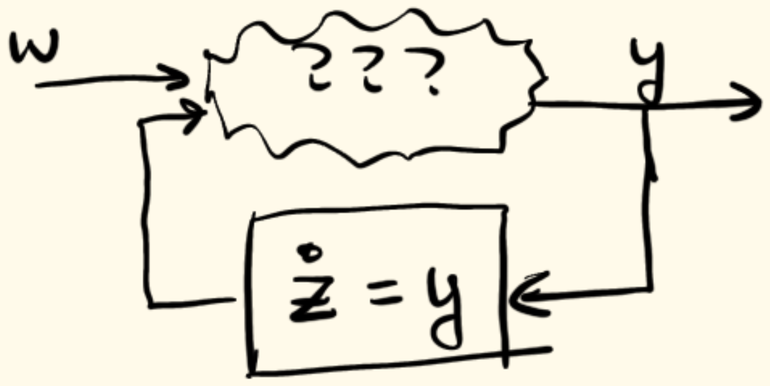
So. a system has perfect adaptation

$\Longleftrightarrow$ integral control, i.e. exists variable $z = g(x)$.

s.t. $\dot{z} = y$. and reaches steady state

($\Leftarrow$ is easy: at steady state. $\dot{z} = 0$).

This is powerful:



as long as the system can reach steady state.

you achieve perfect adaptation!

Pretty much independent of everything!

(Look back at the simple example with only integral feedback.)

④. Implementation of adaptation in engineered systems

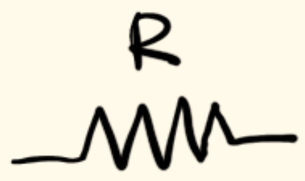
- Recall our goal: use machine control principles to understand biology. (And in biology.)
- Control theory tells us that Perfect adaptation $\Leftrightarrow$ integral feedback

But how to implement integral feedback?

i.e. physically make it happen.

- Electrical circuits: variables are voltages V, currents I.

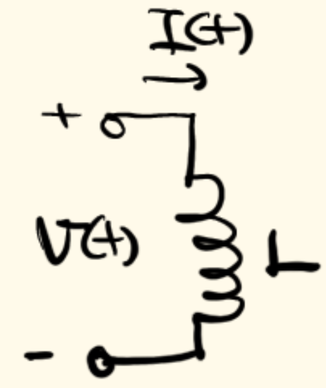
Resistors. 电阻 $V = RI$. $I = \frac{1}{R}V$.



This is proportional control.

Inductor. 电感. $V(t) = L\frac{dI(t)}{dt}$.

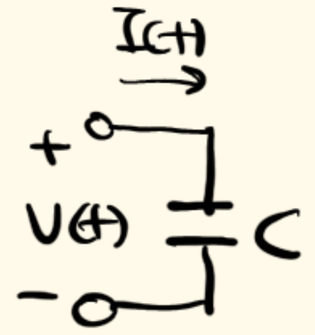
$$I(t) = I(0) + \frac{1}{L}\int_0^t V(\tau)d\tau$$



Integral or Derivative.

Capacitor. 电容. $V(t) = V(0) + \frac{1}{C}\int_0^t I(\tau)d\tau$

$$I(t) = C\frac{dV(t)}{dt}$$



Integral or Derivative.

So, PID control is very natural in electrical circuits!

- Exercise: show how PID can be done in mechanical systems.

(mass, spring, dampers...)

- But what about in biological reaction networks? (BRNs)

  — variables are concentrations, all positive.

  — dynamics are, by default, (in contrast to V, I. or position, velocity)
    constrained polynomials. (Rule CP).

$$\frac{dx}{dt} = \Gamma \Lambda_k x^{\alpha}.$$

Proportional : e.g. $u = k_p y = k_p (x_r - x)$.

often easy... $\frac{dx}{dt} = \underbrace{\mu - \nu x}_{u}$. $\mu = k_p x_r$. $\nu = k_p$.

in fact. any static regulation $u(y)$ works as proportional feedback... (upon linearization)

(another example: $y = \frac{x}{x_r}$ (more natural!)

$u = k_p y = \frac{k_p}{x_r} x$. $\Rightarrow \frac{dx}{dt} = \mu - \underbrace{\nu x}_{u}$. $\mu, \nu = \frac{k_p}{x_r}$)

Derivative : can often be done,
but not often used.
since biomolecular processes are noisy.
and derivative control amplifies noise...
(not used much in highly noisy scenarios...).

Integral — important for perfect adaptation. (if and only if).

but not naturally achieved!

because of rule CP.

$$z = \int y \, dt = \int x_r - x \, dt.$$

$\Rightarrow \dot{z} = x_r - x$. z can't be a species due to rule CP!

— To achieve integral control in biology.

needs further sophistication!

Strategy 1: virtual variable.

e.g. $\dot{z}_1 = \mu - C$

$\dot{z}_2 = x - C$

This is called antithetic integral controller. Cell systems 2016. Nature 2019. Mustafa Khammash group.

$z_1 + z_2 \rightleftharpoons C \to \emptyset$.

$\Rightarrow$. Let $z = z_1 - z_2$. then $\dot{z} = \mu - x$.

(Recall. this is like the dual rail strategy in last lecture to circumvent rule [CP].

but the problem is that rule [PU] makes it hard to directly use z in other variable's dynamics.

e.g. $\dot{x} = kz - \gamma x = kz_1 - kz_2 - \gamma x$

this requires kz_1 and kz_2 have perfectly matching parameters. violate rule [PU]!

So. the best we can do is $\dot{x} = kz_1 - \gamma x$

for example.

This doesn't hurt Perfect Adaptation.

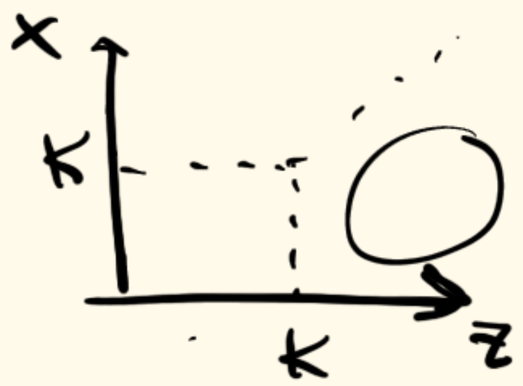
since the integral variable still exists.)

Strategy 2: constrain to certain regimes.

e.g. $\dot{z} = \mu - C$. $\quad x_f + z_f \rightleftharpoons C \to x_f$

In regime $C \sim x$.

we have $\dot{z} \approx \mu - x$.



– Summary: because of rule [CP] and positivity,

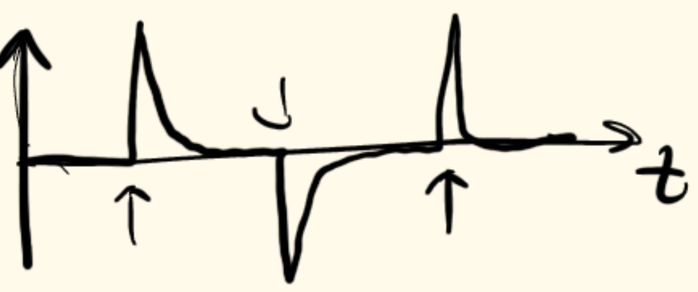
BRN doesn't naturally implement integral feedback.

To achieve this. BRN uses more sophisticated

strategies to indirectly achieve integral feedback.

e.g. bacterial chemotaxis achieves perfect adaptation

via sophisticated phosphorylation cascades!



HERE COMES THE TWIST..

(5). Incoherent Feedforward loops (IFFL)
reveals nonlinear structure in biology.

- Previously. we took the machine perspective on biological systems.

In particular. since adaptation is a hallmark behavior in biology. we figured as an adaptation machine, biology must achieve adaptation, following similar design principles as engineered adaptation machines.

Internal model principle in control theory

says. perfect adaptation ⇔ integral feedback.

And we figured due to physical constraints on biomolecular reactions, BRNs must achieve

Perfect adaptation by implementing integral feedback

by more sophisticated strategies.

- But This is NOT an accurate picture of what's happening in reality!

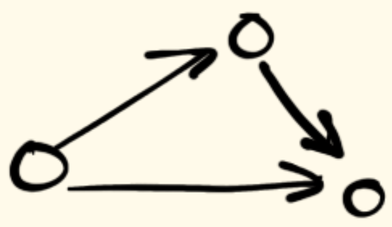
In 2002. Shen-Orr et.al. Nature. Genetics (Uri Alon) and Milo.et.al.2002.

looked at all 3-node network topologies

on transcription factors and operons in E.coli.
(115) (424)

And found network motif (i.e. more frequent than "random"...)

to be feedforward!



- But recall. perfect adaptation

can't be achieved by feedforward

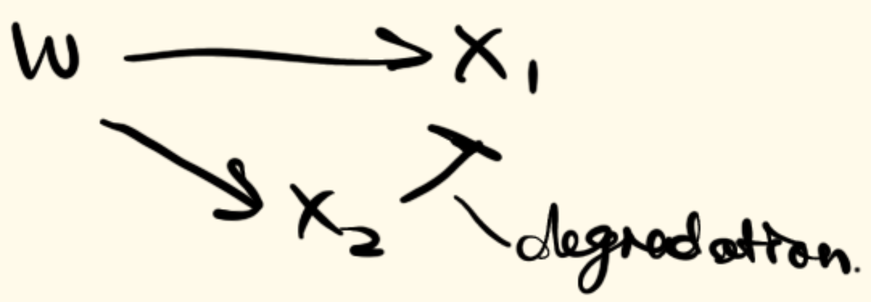
in engineered systems due to the requirement of

perfect matching of two processes' parameters!

How come biology is different?

what happened to rule PU?

- Example. (Snitker):



$$W \xrightarrow{k_1} W + X_1$$

$$W \xrightarrow{k_2} W + X_2$$

$$X_2 + X_1 \xrightarrow{\delta} X_2 \xrightarrow{\gamma} \phi$$

$$\frac{dx_1}{dt} = k_1 W - \delta x_1 x_2$$

$$\frac{dx_2}{dt} = k_2 W - \gamma x_2.$$

At steady state. $x_2^* = \frac{k_2}{\gamma} w$.

$$\Rightarrow x_1^* = \frac{k_1 w}{\delta x_2} = \frac{k_1 w}{\delta \frac{k_2}{\gamma} w} = \frac{k_1 \gamma}{k_2 \delta}.$$

So. x_1^* is invariant to w! (Perfect adaptation)

And this invariance is independent of the parameters! No perfect matching required!

- The key lies in the exponents.

  i.e. the reaction order. ($v = k x^{\alpha}$)

  (which is α in mass action.
  but could be more complicated..
  with time-scale separation.)

This is the nonlinear structure

that biological systems can utilize,

which is not present in engineered systems,

since they are often linearized etc...