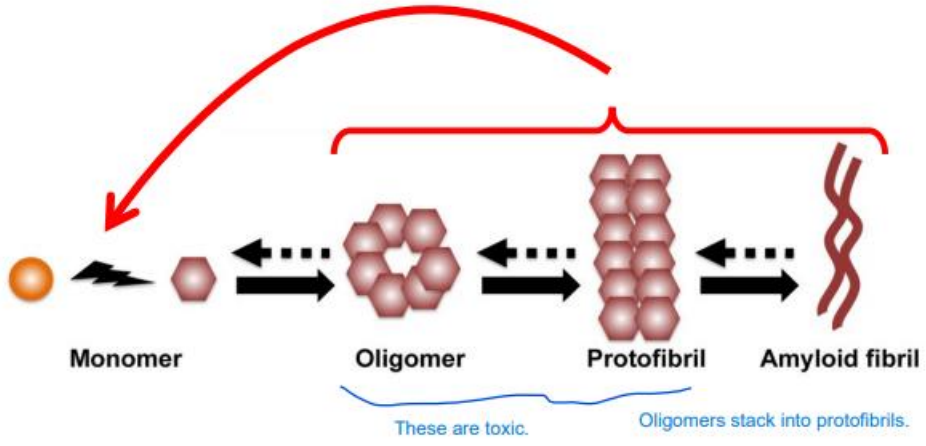
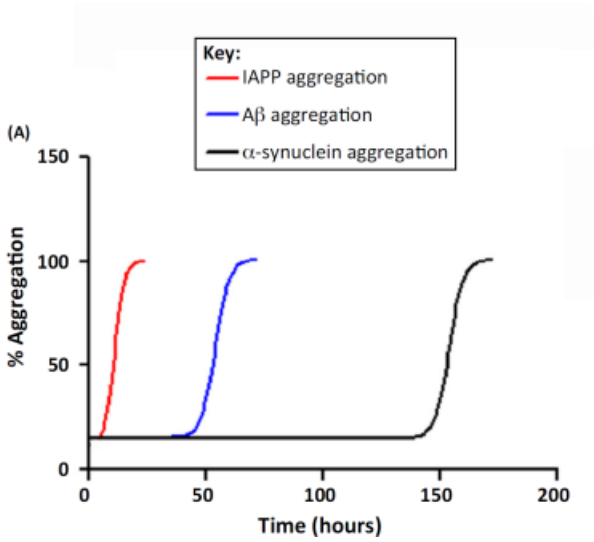
TEMA 1: Protein Aggregation and Protein Misfolding Disorders

- **Protein misfolding diseases (definition)**: Diseases caused by proteins that become structurally abnormal, that then aggregate and disrupt the funcions of cells, tissues, organs. Examples:
    - Alzheimers
    - Huntington
    - Parkinsons
    - ALS
    - Even diabetes type 2.
- **Prion:** A misfolded protein that can induce misfolding of normal variants of the same protein and then trigger cellular death
- **.**
- **How**
    - General biological principle: DNA gets transcribed into mRNA, and then aminoacids come in and for every codon, they create an aminoacid. That way, the mRNA is converted into an aminoacid chain. Some aminoacids are hydrophobic and some are hydrophylic.
    - Folding: For the protein to work, this chain has to fold into the right shape. The folding can happen because the hydrophobic aminoacids group together in the inside of the protein to avoid the water.
    - Misfolding: Sometimes, the proteins are misfolded, so that they have a hydrophobic aminoacid exposed by accident. If two such misfolded proeins come together, they can group by joining those hydrophobic areas. Then, they become very hard to separate and they stack
- **Process of aggregation:**
    - 

- Process: Monomer misfolds, then a collection of misfolded monomers forms an oligomer, then a protofibril and then a Amyloid fibril
- The middle steps are toxic. But the ends might not be, even the amyloid. So, to reduce toxicity, a solution might be increasing the rate from the middle section to outside.
- **Propagation:** Also, those three species at the right can act as enzymes for misfolding monomers, creating a positive feedback and causing propagation.
- **How are they toxic?** They may kill something, may be non functional and therefore eliminate the function they were supposed to do. However, they might even have positive functions.
- **Place:** The monomer and oligomer usually only act intracellular, so they might only kill the cell. The other two can propagate extracellularly and kill other cells.

- **Assemble**
  - Different kinds of proteins assemble at different rates.
  - 

  - We start with monomers misfolded and wait to see how long they take to aggregate until all of them have aggregated somewhere.
  - There is some time it doesn´t grow, and then they all start growing in pretty much the same way.
    - Slow step (nucleation): One explanation as to why some take longer may be due to some species requiring more monomers to build the nucleation (the black line for example).
    - Fast step (Growth): Then, they all grow proportionally to the surface area, so the growing phase is the same in all.
- **Experimental techniques**
  - Using light we can measure how much aggregate we have, because aggregates fluoresce at a certain wavelength.
  - Another way is to shine light and see how the scattered light correlates to the incoming. Small molecules move very fast and they rapidly cause the light intensity to become uncorrelated because of this fast motion. Big molecules move slower and take longer to

create this uncorrelation. So, how fast we loose correlation tells us about the proportion of big molecules (aggregates).

- With those things, and tracking polymers, we can find the size distribution of polymers.
- **Labeling:** There are some proteins that fluoresce, such as GFP (green fluorescent protein) and we can use them to see our proteins of interest.
    - We join our proteins of interest with GFT and see how the GFT moves around by following its fluorescence.
    - We can put different fluorescent proteins of different colors connected to different interesting proteins to see the number of each. If blue and yellow are connected and we send something that fluorseces the blue one, the energy will move to the yellow one and emit in yellow. If they are not connected, blue will only emit. Therefore, we can see how many agregates there are by seeing how much yellow we get.
    - Instead of putting GFP to the tau proteins, we only connect a piece of GFP and also throw around the other piece of GFP. Then, GFP will join in monomers, but not in aggregates, since there will be no space for the second part. Then, fluorecense indicates monomers.
    - Opposite to the last one pretty much. Monomers have separate parts of yellow fluoresence protein. If they join, they emit light.

SUMMARY

- What are protein misfolding diseases?
- How misfolding can happen
- Process of aggregation: Toxic parts, rates of change.
- Aggregation steps: Nucleation and growth.
- Experimental techniques.

Physical model

We now describe it mathematically. For that we define:

- f(t,j) = number of polymers of size j at time t.
- P(t) = number of polymers at time t.
- M(t) = total mass of polymers at time t.
- n_c = nucleation size (smallest possible polymer)

$P(t) = \sum_{j=n_c}^{\infty} f(t,j)$

$M(t) = \sum_{j=n_c}^{\infty} jf(t,j)$ (The first moment of the distribution) $\forall j < n_c, f(t,j) = 0$, n_c nucleation size.

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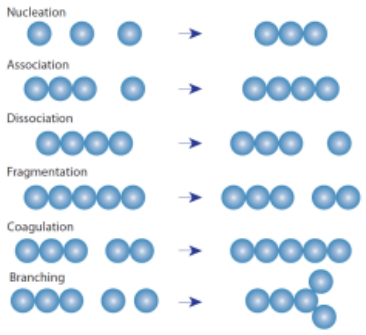
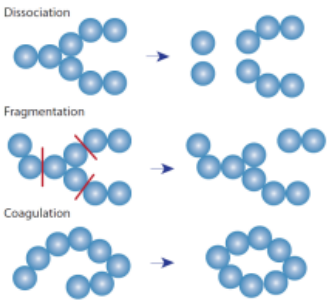
- There are a couple of processes that change the polymers:

These are markovian. The probabilities of events don't depend on history, only on the last state.



(a) The possible polymer aggregation processes.



(b) Situations that cannot easily be described as a Markov process due to general coagulation or branching.

These are non Markovian, because the probabilities depend on the history (because they depend on the shape of the polymer).

- Figure 2: Possible polymer aggregation processes

Oosawa model

- The change of f(j,t) is due to nucleation, association, dissociation and fragmentation. For this part, we care only about nucleation and association.
- We then get the following equations:

Master equation with nucleation and association

Oosawa model: Nucleation + Association

$\frac{df(t,j)}{dt} = k_a m(t)[f(t,j-1) - f(t,j)], j > n_c$

For j > n_c, there is only association.

Boundary, no polymers smaller than n_c:

$\frac{df(t,n_c)}{dt} = k_n m(t)^{n_c} - k_a m(t) f(n_c, t)$

For j=n_c, we have the nucleation event and we can loose poly if they become bigger by association.

Principle moment equations: We can also get the differential equations for P(t) and M(t)

$\frac{dP(t)}{dt} = \sum_{j=n_c}^{\infty} \frac{df(t,j)}{dt} = k_n m(t)^{n_c}$ $\quad$ $\frac{dM(t)}{dt} = \sum_{j=n_c}^{\infty} j\frac{df(t,j)}{dt} = n_c k_n m(t)^{n_c} + k_a m(t) P(t)$

OBS: Used $\sum_{j=n_c}^{\infty} f(t,j) = P(t)$ + Telescoping sums + a few resonable assumptions about the possible lengths of the polymers.

Interpretation?:

Association doesn't contribute to P(t), since it doesn't change the number of aggregates. P(t) is only controll nucleation.

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- As time goes to inifinity, M reaches 100% and it is always increasing, so there is no steady state.

Beyond Oosawa 1 Dissociation

- We can add a dissociation term, which doesn't change the derivative of P(t), but it does the M(t).
- This change can allow for steady states other than M=100%.

2 Fragmentation

- We add a fragmentation term to the master equation. This finally does change the derivative of P(t) and makes it proportional to M(t).
- Fragmentation can introduce the time delay we saw in the graphs of M(t), because it can reduce significantly the mass of aggregates. Also, fragmentation creates a lot of ends, which can then be associated by a monomer. Therefore, this term can explain the sharp increase of M(t) after some waiting time that we see in experimental results.
- Also dM/dt depends on P(t), so we have a positive feedback, which makes it so that once M(t) starts growing it does so very fast.

- Theory says that not having fragmentation leads to not having a peak of f(j,t) in the middle (it is at the end or beginning). However, experimental data has a peak in the middle, so it seems to be necessary to add fragmentation.

Finke Watzky: Propose a different model, in which the process is separated into two steps (as we know from the graphs of M(t)). The model is very simple, assuing a first step in which monomers aggregate into polymers and a second one in which polymeras can associate with a monomer. The model is very simple, with only two parameters, but it still works very well. The possible problem is that it doesn't explain mechanistically what is happening.

Summary

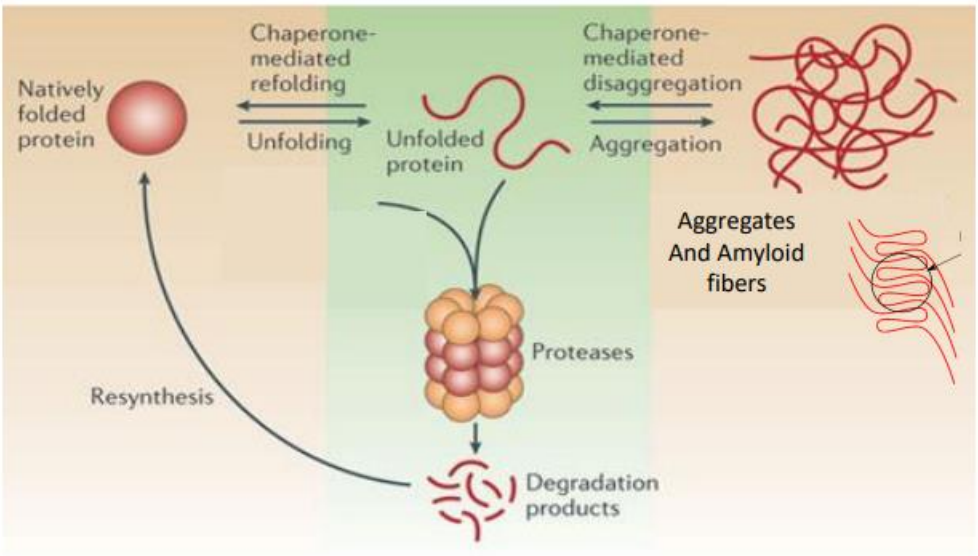
- We can do the master equation describing the how the number of polymers changes with time due to nucleation, association, dissociation, fragmentation and others we don't consider.
- M(t) is the total mass of polymers and P(t) the total number
- When we only have nucleation and association, M(t) reaches 100% and always grows.
- If we include dissociation, we can have steady states of M(t) other than 100%.
- If we also include fragmentation, we can better replicate the experimental results, M(t) grows after a waiting period and then grows very fast.
- Finke Watzky propose a simpler model but not mechanistic.

Exercise

1. Simulate Oosawa using Gillespie
    a. Gillespie jumps to the next event randomly.
    b. Start with only nucleation and association: M goes to 100%
    c. Add dissociation: M stabilizes before 100%
    d. Add fragmentation: M has a delay and then increases to something below 100%
2. Fanky Watzky:
    a. Solve it using euler method
    b. Fit it to experiments.

Topic 2: Protein Quality Control

- Remember: DNA is converted to mRNA, then mRNA into aminoacids, which chained together form a protein. Once we have the chain of aminoacids, to have a functional protein, we need to fold it into shape.
- Some proteins fold into shape (hydrophobic inside) by themselves.
- PQC (Protein quality control)
    - **Chaperons**: Chaperons are proteins that assist in **folding** proteins during or after synthesis. They also help **disaggregating** aggregates.
    - **Proteases**: Enzymes that can **cut** misfolded proteins into pieces, so that we can recycle them into functioning proteins.



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    - **Autophagy:** Envelope the problematic thing (failing organelles or an aggregate) into a vesicle. Then it can degrade what is inside into its original components. Then, it can recycle those aminoacids or throw them out.
    - **UPR:** Unfolded protein response is a type of PQC inside the endoplasmic reticulum, which is used to simulate the environment outside the cell.
    - **Transportation:** Transporting misfolding monomers outside the cell. See more in next section.
- **Aggregates inhibit PQC:** Having aggregates such as oligomers, protofibrils, amyloid fibrils can inhibit PQC. Two reasons:
    - PQC is used up in the aggregates and can't be used for something else.
    - PQC get stuck inside the aggregate and can't get out.
- **PQC inhibit aggregates:** This is just what they do.

- Therefore, there is a positive feedback in the creation of aggregates.
- **Negative feedback:** Cells are smart. They can detect when you have an increase amount of aggregates or misfolded proteins and it can then increase the amount of PQC. This is a negative feedback and helps maintain homeostasis.
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- How does PQC affect M(t)? We saw the Finky-Watzy model, but that didn't include PQC because it was in vitro.

Examples of stuff?

- **Type 2 Diabetes:** Insulin is a hormone produced by pancreas. When glucose level rise, insulin should be produced so that it signals cells to take glucose up and avoid having too much glucose. Insulin has to be produced in the right amount to not have too much or too little glucose.
  Insulin has to go outside of the cell, so it has to resist a different type of environment.
  To simulate it, it is folded in the Endoplasmic Reticulum.
  How type 2 diabetes progresses: Insulin resistance causes chronic high glucose
- **Example UPR: IRE1 pathway:** IRE1 sits on the membrane of the ER, with tail on the cytoplasm and head on ER. If there is unfolded protein in the ER, this happens:
  - 1. It activates the IRE1, which then sends the signal to create more chaperones.
  - 2. The chaperones deal with the unfolded proteins.
  - 3. The chaperones stop the IRE1 from still signaling, so as to not produce more chaperones.
- **Example UPR: PERK pathway:** Similar drawing to IRE1, but now the tail of this molecule, called PERK, is different. If Perk senses an unfolded protein in ER:
  - It tells the protein eIF2a-P to stop translation, and therefore produce less new peptides.
  - Therefore, the chaperones that were normally occupied in folding new proteins are now free to go to ER to deal with the problem.
- Therefore, we have two solutions for UPR:
  - Ire1: create more chaperones (slow process).
  - Perk: Stop the production of new peptides to free up chaperones (fast process).

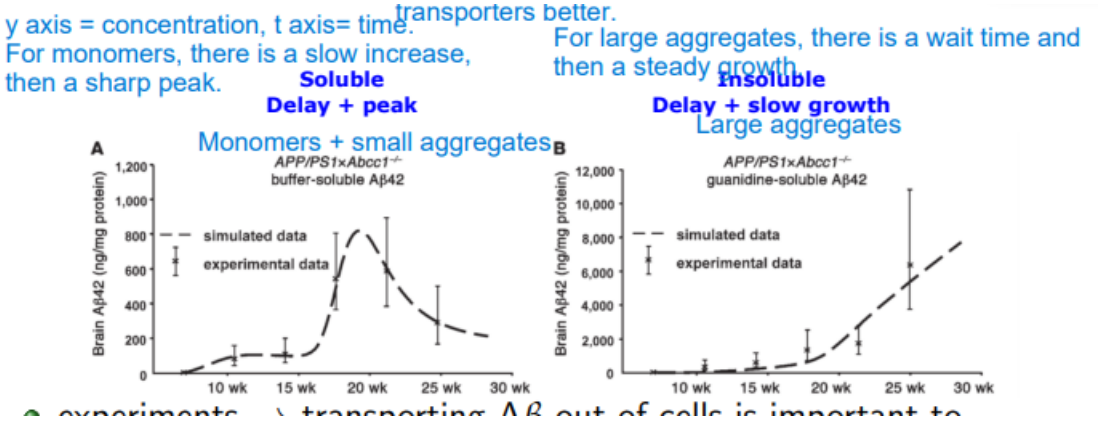
Summary

- How do cells deal with the problem of aggregation:
    - Unfolded/misfolded proteins upregulate PQC → increase folding and degradation
    - chaperones, proteases to deal with small aggregates
    - Autophagy removes large aggregates
    - Slow down translation
    - If can't manage the stress, commit suicide (e.g. PERK branch of UPR).
- However, aggregates can escape cellular control because of a run-away positive feedback loop caused by the mutual inhibition of PQC and Aggs.

TOPIC 2 Physical model (Krohn):

Question

- **Question**: Does the mutual inhibition of PQC and Aggs (which causes a positive feedback) explain the long delay in diseases onset?
- **Model motivation:** We know that transportation (transporting misfolded monomers out of the cell) can control the production of aggregates.
- Transporting Abeta out of cells is important to prevent aggregation. One of the important transporters is ABCC1.
- We divide the aggregates into
    - Soluble: Includes monomers and small aggregates up to size s_s
    - Insoluble: Includes big aggregates, from size s_s onwards.



- experiments → transporting Aβ out of cells is important to
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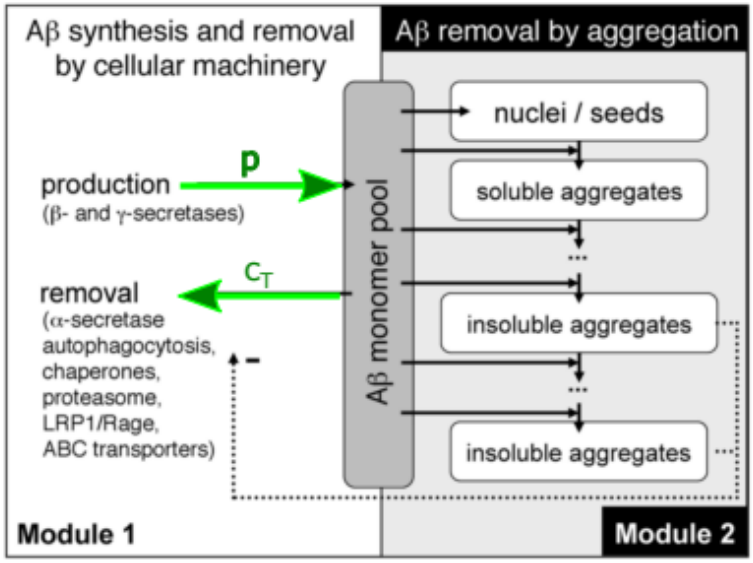
- Can a model explain the shape of Sol.Agg(t). Why the delay and then sharp increase?

Model



- Right side: What we already know. We have nucleation, growth of size by association to reach soluble aggregates, then more growth to larger (insoluble) aggregates. That is, we have nucleation and association.
- The left side tells us what we are putting into or out of the system. We are producing monomers with rate p and removing monomers with rate c_T (rate of transport). So is like last week, but with active bringing and taking out monomers.

Change in monomers:

$$\frac{dM}{dt} = p - c_T M$$

So this equation tells us how the number of monomers of this protein Abeta changes with time.

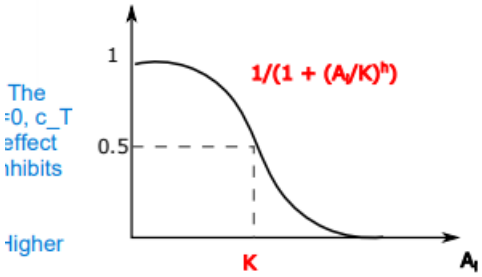
- M: Aβ monomers
- p: constant production rate
- c_T clearance capacity ($_{\text{efficiency of PQC}}$)

- We change the factor cT by

$$\frac{dM}{dt} = p - \frac{c_T}{1+(A_i/K)^h} M$$

However, the insoluble aggregates, can inhibit the removal (clog the transportation let's say). Therefore, c_T is reduced and converted in what is shown there

- A_i: insoluble aggs.
- K: inhibition threshold. (Often referred to as EC50)
- h: hill coefftient



The
=0, c_T
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- This is because the insoluble aggregates, can inhibit the removal (clog the transportation let's say). Therefore, c_T is reduced and converted in what is shown there.
- The more aggregates we have, the lower cT becomes. When A=0, c_T = 1, and the constant K is the threshold when the inhibition effect has reached 50% and as A_i gets to infinitiy, it completely inhibits transport.
- Hill coefficient: Controlls how fast is the fall of the function. Higher h, the sharper the decrease.

Oosawa + Dissociation

- As before, the nucleation term is $T_n = k_n n_n M^{n_n}$, with n_n the nucleation size. Then, we can define the total number of soluble and insoluble aggregates:

  Soluble: $A_s(t) = f(t, n_n) + \sum_{j=n_n+1}^{j=n_s} f(t,j)$

  Total number of soluble things.

  Insoluble: $A_i(t) = \sum_{j=n_s+1}^{j=n_i} f(t,j)$

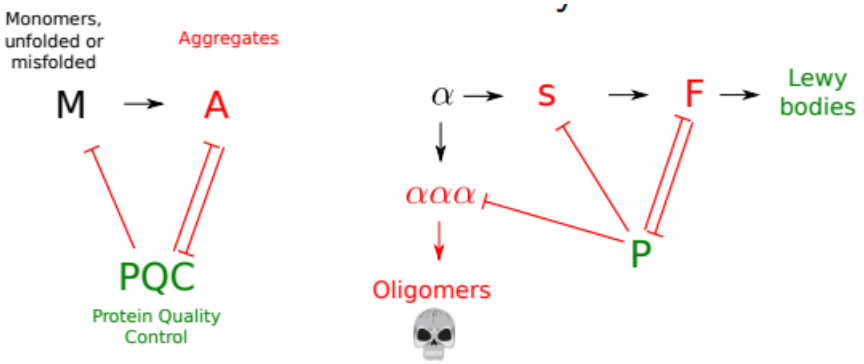
- Experimentally and with the model, we see that if we increase c_T, then the time delay increases. **Therefore, time delay is set by PQC.**
- The sharp increase is due to A_i positive feedback. Basically, A_i inhibits PQC, which inhibits A_i, so there is a positive feedback.

Krohn model takeaway:

- It is Oosawa model plus production and removal of monomers.
- The rate of removal of monomers becomes smaller the more big aggregates we have. So we have a feedback. Transporters inhibit the creation of aggregates by disposing of monomers, but aggregates inhibit transporters by making Ct smaller.
- Question to answer: How to understand the delay of creation of aggregates?
- They find that increasing the transport rate by a bit, greatly increases the time delay (because of non-linearity).

Model of the proteasome and alpha-synuclein

- Now we have a model for proteasome. On the left is the general shape of the model, on the right the specific for alpha.



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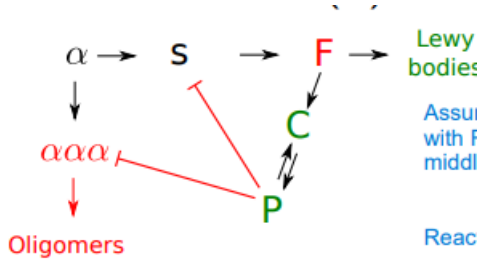
    - M = monomers (unfolded or misfolded)
    - A = Aggregates.
    - P = Proteasome
    - alpha = A type of protein species.
    - s = protofilaments
    - F = mature fibrils
    - Then they become Lewy bodies, which we assume are not dangerous.
    - P = proteasome.
    - Alpha alpha alpha = oligomer seeds, which are toxic.

- F and P connection: Assumption, protase can quickly deal with S and alpha^3, but not so fast with Fibrils because of how big they are. C is the complex*, like the middle step for degrading the Fibril
- Reaction: Fibril + Protase <- -> Complex -> Protase + nothing (degraded)



Equations

$$\begin{aligned} \frac{dF}{dt} &= \omega \cdot s - \gamma \cdot F \cdot P \\ \frac{ds}{dt} &= m - \gamma_s \cdot s \cdot P - \omega \cdot s = 0 \\ \Rightarrow s &= \frac{m/\omega}{1 + \frac{P}{\omega/\gamma_s}} \end{aligned}$$

- Since s changes so much faster (m = rate of production of s, we assume it is constant) , we assume it reached equilibrium, set it equal to 0, and therefore the equation for F is:

$$\frac{dF}{dt} = \frac{m}{1+\frac{P}{\omega/\gamma_s}} - \gamma \cdot F \cdot P$$

- So the total set of equations is:

$$\begin{aligned} \frac{dF}{dt} &= \frac{m}{1 + \frac{P}{\omega/\gamma_s}} - \gamma \cdot F \cdot P \\ \frac{dC}{dt} &= \gamma F \cdot P - \nu C \\ \frac{dP}{dt} &= \Sigma - \frac{P}{\tau} - \gamma F \cdot P + \nu C \end{aligned}$$

P/tau because protase has a given stability (half life) and it degrades by itself.

- eta is the time spent chewing up a fibril, to get back the protease.

- dP/dt has a creation term sigma, a decay rate tau, it diminishes because it gets occupied with fibrils and it increases once it finishes eating a fibril and complex C breaks.
- To reduce the number of parameters, we rescale
  - t' = t/tau
  - P' = P / (omega / gamma_s)
  - sigma = Sigma * tau * gamma_s / omega.
- Therefore, the equations are:

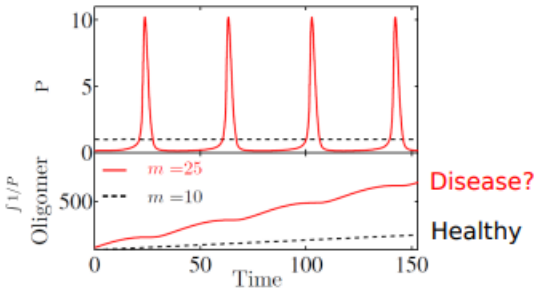
$$\frac{dF}{dt} = \frac{m}{1+P} - \gamma \cdot F \cdot P$$
$$\frac{dC}{dt} = \gamma F \cdot P - \nu C$$
$$\frac{dP}{dt} = \sigma - P - \gamma F \cdot P + \nu C$$

Results

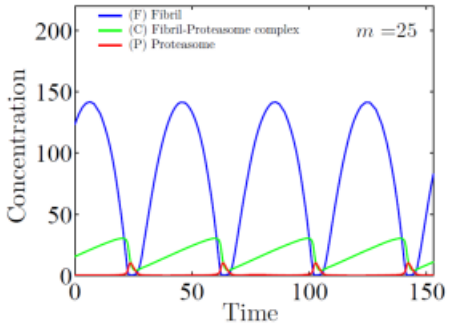
- We can solve the equations. Some of the parameters are found experimentally, some are at least contrained by things that make sense.
- To understand the results, we find that the number of oligomers is proportional to 1/P. Proof:
  - Oligomers can be created from monomers or can fall apart or can be degraded by Protase.:
  - $\frac{d[\alpha\alpha\alpha]}{dt} = k_1\alpha - k_2[\alpha\alpha\alpha] - b\,P\,[\alpha\alpha\alpha]$
  - In steady state, this is equal to 0. And we find then that [alpha^3] is proportional to c2 / (c1 + P) , where c1, c2 are consants. Therefore, [alpha^3] is proportional to 1/P (under the assumption that P is larger than c1.
  - Then, the cumulative quantity of oligomers, is the integral of 1/P.

- We can also vary the parameters and see what happens. In general, we vary m, which is the rate of forming protofibrils. It is like how sticky the monomers are. Depending on m we have two cases:
  - If m < m_T = (1+eta)(1+sigma)^2:
    - Not all protases are getting inactivated by fibrils and they can keep check on oligomers, so that they don't accumulate very much.
    - The number of protases stays fairly constant, number of oligomers increases very slowly.
    - Healthy curves in the first graph.

- If m> m_T = (1+eta)(1+sigma)^2:
  - From time to time, all free protase are absorbed and occupied by fibrils.
  - Then, at some point enough protases can be created to reduce F again (we reach the peak of F) and we have a lot of free protases.
  - Then, again fibrils start growing and they occupy all free protases. (see second graph). The lack of free protases, lets oligomers grow.
  - This is the disease state in the following graph. Protase jumps in very clear peaks (image is a zoom in version of the second image).
  - There are long periods where P is very small, and therefore oligomers are accumulated and there is disease.



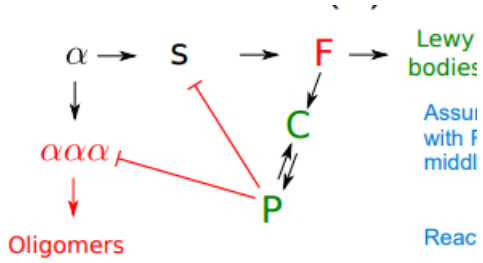
Lower graph is the accumulated number of oligomers. The production of oligomers is proportional to 1/P



P is number of free protase.

Takeaway Sneppen Parrkinsons.

- Question: See the effect of protease on Parkinsons disease.



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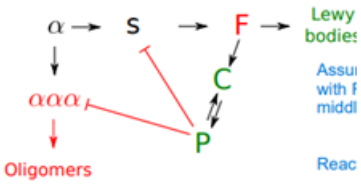
- Monomers (alpha) can wake two paths: They can become oligomers or can follow the top path, becoming eventually fibrils. The oligomers are the toxic thing.
- Protease dissolves monomers and fibrils. But if protease is too occupied taking care of fibrils, it cannot control oligomers, which therefore increase.
- m is the propensity with which monomers becomes protofibrils. There are two cases:
    - if m < mT = (1+eta)(1+sigma)^2: In this case, we have a steady state, the number of P stays pretty much constant, there is very slow disease onset (proportional to integral of 1/P).
    - if m > mT = (1+eta)(1+sigma)^2: See notes on top.
- For example, increasing sigma (the creation rate of P), greatly increase mT, so that there won't be disease. Increasing eta (how fast protase devours fibrils) also increases mT.

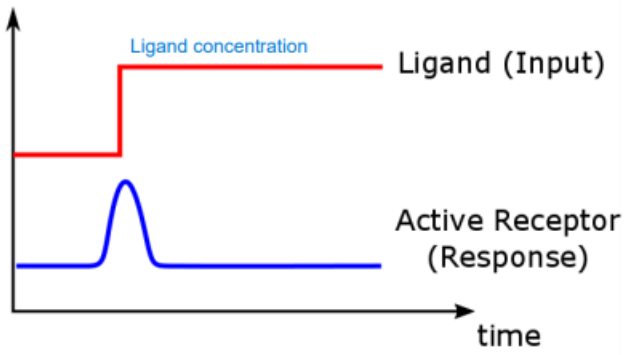
Summary

- Model 1: It is like an Oosawa model but with monomers coming in and going out.
    - This model shows that the time delay in large aggregates is in part caused by PQC. Making better transportation, increases the decay.
- Model 2: Protase and alpha:
    - A model that looks like 

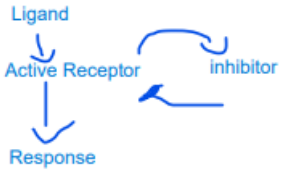
    - We set up the equation for F, s, P,C.
    - We assume s is very fast, so that we can set its derivative to cero, since it is always in steady state.
    - The number of oligomers (toxic) is the integral of 1/P
    - When we run the model we see two results depending on parameters:
        - Steady state, with a steady number of P, such that oligomers grow very slowly and we are healthy.

- Unsteady state, where there is a pull and push between F and P, which depletes free P and causes the growth of oligomers. Unhealthy.

Topic 3: Receptor Desensitization

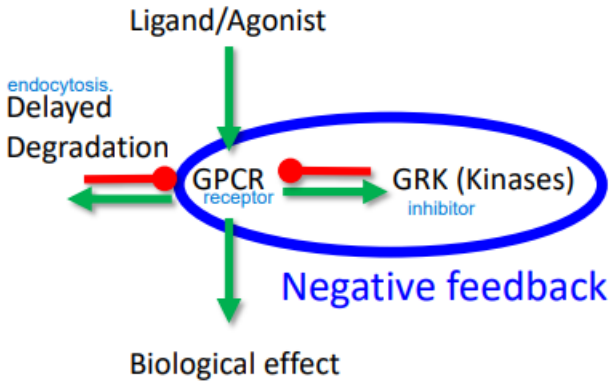
- Ligand is a signal molecule that comes from outside the cell. It binds to a receptor (sitting in the membrane) and then the cell responds accordingly.
- The response to ligand concentration looks like this:
- 

    - The response only happens for a short time during the change in ligand concentration
    - After a while, even though the concentration is still high, the receptor stops responding.
    - That is, they respond to changes, not to absolute concentrations.
- **How it works:** The receptor itself activates an inhibitor, which inhibits the receptor, and that is how the response goes back to the initial value (in the perfect adaptaation case).



- **Circuit:**
    - The ligand activates the receptor, which makes the response but at the same time activates the inhibitor. The inhibitor inhibits the response, so that it goes back to initial values.

- More detailed circuit for a specific case



  - The ligand activates the receptor. The receptor activates an inhibitor that creates a negative feedback. It also can activate a secondary negative feedback that takes longer time: **Endocytosis**: At the end of the process, the receptor + ligand are encircled by a vesicle, which then eats the receptor + ligand. (lysosome) or only eats the ligand (dephosphorylation) and takes the receptor back.
  -
- **Why do desensitation instead of increasing response accordingly to ligand concentration.** Note: in that case, the receptor response would look like a sigmoid, with a linear response and then a saturation. Reasons:
  - **1.** It reports changes in concentration rather than absolute concentration. If we had a sigmoidal response, there would be an upper bound in responses, if we keep increasing ligands, we will reach the saturation level of response and we won´t respond very well to further changes.
  - **2.** The response is equally good at low and high initial concentrations: After the relaxation, the response goes back to zero, so it can again respond to ligand change. With the sigmoid model, it would only increase very slightly when you change the ligand concentration at high values, because you are saturated.
  - **3.** Filters out noise: If the ligand change is a short pulse, the receptor will also generate a short bump, which will not lead to a response (has to cross some threshold or something for that). Therefore, we filter out short pulses.
- **Examples:**
  - Insulin resistance:
    - Insulin is the ligand, which has the objective of promoting the absorption of glucose from the blood into liver, fat and skeletal muscle cells. In these tissues the absorbed glucose is converted into glycogen (which works as a way to store extra glucose, and then we can transform it back to glucose

if needed when fasting) or fats. It is important for glucose to not be too low or too high.

- Beta cells are sensitive to blood sugar levels so that they secrete insulin into the blood in response to high level of glucose, and inhibit secretion of insulin when glucose levels are low.
- In diabetes, the inhibitor is always high, so that it always inhibits the effect of insulin and doesn´t allow there to be a response to high insulin levels. Therefore, glucose is not converted to glycogen and there is high blood sugar.
- Causes:
    - High glucose: If you have consistent high glucose levels, your body secretes insulin all the time, which activates the response (glucose transport) and the inhibition. Since you are inhibiting all the time, the body eventually becomes resistant to insulin and stops responding to it. In general, chronically high insulin can lead to desentization.
    - Inflammation (TNF): Can work as an insulin receptor desensitizer.
        - Even more, Diabetes can be linked to protein misfolding diseases: Toxic oligomers cause inflammation, which inhibits Insulin receptors; this reduces the sugar uptake of cells, so that they don't have enough energy and can't do PQC and control the amount of aggregates. So we have a bad positive feedback.

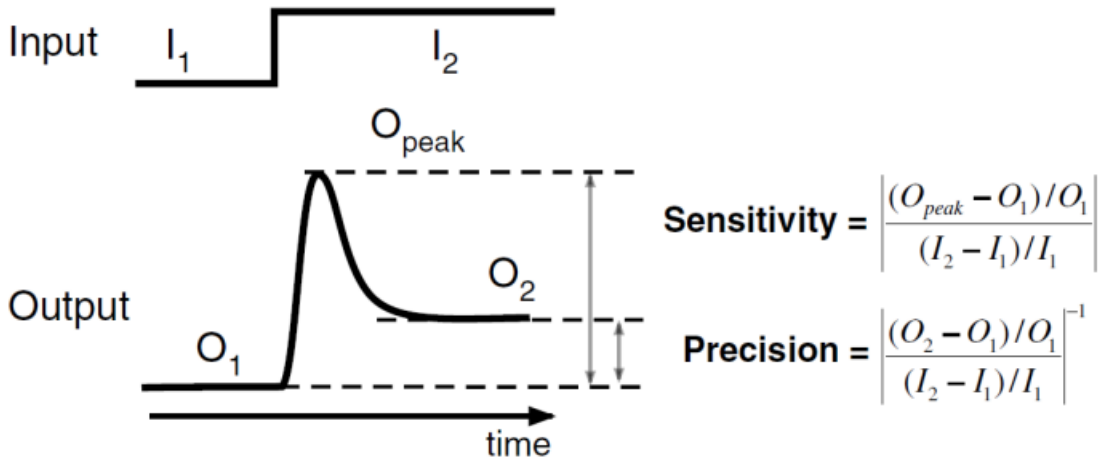
- Ligands happen in pulses instead of continuous, since that way we can always have changes and be detectable. Because a continuous value wouldn't be detectable4

PHYSICS model to encode adaptation and find topologies

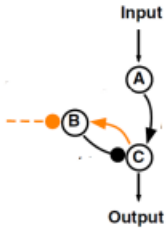
- Enzymes: Proteins that activate substrates (which can be other protein).
- **Michaelis Menten reactions:** We have an enzyme E, an active substrate S, which becomes P when activated by the enzyme $\mathrm{E} + \mathrm{S} \underset{k_2}{\overset{k_1}{\rightleftarrows}} \mathrm{ES} \xrightarrow{k_3} \mathrm{E} + \mathrm{P}$ (converts to).
    - The equation for P production is (see exercise): $dP/dt = \underset{\text{Vmax}}{k_3 * E_t} \frac{S}{S+K_M}$.
      Where KM = (k2+k3)/k1 is the substrate concentration when dP/dt is half

- There is an assumption that the total amount of substrate is very high with respect to enzyme, so Sfree = Stotal.

- **Quantifying adaptation:** We can define sensitivity (how high is the relative response with respect to the change in input) and precision (how good it goes back to initial value).



- **Equation for integral negative feedback**



$$\frac{dA}{dt} = Ik_{IA}\frac{(1-A)}{(1-A)+K_{IA}} - F_A k'_{F_AA}\frac{A}{A+K'_{F_AA}}$$

$$\frac{dB}{dt} = Ck_{CB}\frac{(1-B)}{(1-B)+K_{CB}} - F_B k'_{F_BB}\frac{B}{B+K'_{F_BB}}$$

$$\frac{dC}{dt} = Ak_{AC}\frac{(1-C)}{(1-C)+K_{AC}} - Bk'_{BC}\frac{C}{C+K'_{BC}}$$

  - They can work as enzymes and substrates. We have an input I that activates protein A, which can activates C. . C activates B, which inhibits back C, while C also gives an output. We can find the equations using the MM equation and using the active form and inactive form of each protein (A and 1-A for example). For B and C, we consider outside deactivation.

- **Regimes:** For a sigmoidal f(B) = B/(B+K) we have:
  - B>>K: Then f(B) is constant at the saturated value
  - B<<K: Then f(B) =~ B, linear regime
  - If B =~ K, then we have the full equation

- **When is C* independent of input (perfectly adapting):** We want C to go up and back down. For this, we want C* (C steady state) not depending on input, or any time depending thing.

- To see the steady state of C, we can set dB/dt = 0 and find C*. If we have the conditions that (1-B) >> KCB and also B >> KFB, then both fractions are equal to 1 and we get that C* = F_BK_{FB}/K_{CB}.
- Then, the equation can be written as dB/dt = k_{CB} * (C-C*). So the rate of change of B is dependent on how far from steady state level we are.
- If we integrate, we get that B(t)-B(0) = \int_0^t k_{CB} (C-C*) dt. Therefore, the value of B is the integral of the difference of C with respect to steady state.

- **Can we analytically derive conditions for adapting networks?**
  - We consider all possible relations between A,B ,C (inhibitions, activations, self activation or inhibition, etc) for all possible pairs and create the equations. We consider

general case

X_i:Activating enzymes

Y_i:Inhibiting/deactivating enzymes

$$\begin{cases} \dfrac{dA}{dt} = \mathrm{f}_A = k_{IA} I \dfrac{1-A}{1-A+K_{IA}} + \sum_i k_{X_iA} X_i \dfrac{1-A}{1-A+K_{X_iA}} - \sum_i k'_{Y_iA} Y_i \dfrac{A}{A+K'_{Y_iA}} \\ \dfrac{dB}{dt} = \mathrm{f}_B = \sum_i k_{X_iB} X_i \dfrac{1-B}{1-B+K_{X_iA}} - \sum_i k'_{Y_iB} Y_i \dfrac{B}{B+K'_{Y_iB}} \\ \dfrac{dC}{dt} = \mathrm{f}_C = \sum_i k_{X_iC} X_i \dfrac{1-C}{1-C+K_{X_iA}} - \sum_i k'_{Y_iC} Y_i \dfrac{C}{C+K'_{Y_iC}} \end{cases}$$

  -
  - We then linearize the equations as responses to a small change in input. So we expand f_A with a linear taylor series in terms of Delta A, delta B, delta C, delta I and similarly for f_B, f_C. The last part takes into account that A depends on input too.
    -

$$\begin{bmatrix} \frac{d\Delta A}{dt} \\ \frac{d\Delta B}{dt} \\ \frac{d\Delta C}{dt} \end{bmatrix} = \begin{bmatrix} \frac{\partial f_A}{\partial A} & \frac{\partial f_A}{\partial B} & \frac{\partial f_A}{\partial C} \\ \frac{\partial f_B}{\partial A} & \frac{\partial f_B}{\partial B} & \frac{\partial f_B}{\partial C} \\ \frac{\partial f_C}{\partial A} & \frac{\partial f_C}{\partial B} & \frac{\partial f_C}{\partial C} \end{bmatrix} \begin{bmatrix} \Delta A \\ \Delta B \\ \Delta C \end{bmatrix} + \begin{bmatrix} \frac{\partial f_A}{\partial I} \\ 0 \\ 0 \end{bmatrix} \Delta I$$

$$\equiv \begin{bmatrix} \alpha_{AA} & \beta_{BA} & \beta_{CA} \\ \beta_{AB} & \alpha_{BB} & \beta_{CB} \\ \beta_{AC} & \beta_{BC} & \alpha_{CC} \end{bmatrix} \begin{bmatrix} \Delta A \\ \Delta B \\ \Delta C \end{bmatrix} + \begin{bmatrix} \frac{\partial f_A}{\partial I} \\ 0 \\ 0 \end{bmatrix} \Delta I$$

- The matrix represents a matrix of links. They can be positive, negative or cero, representing a positive link, a negative link or no link.
- In steady state, we set the equation equal to 0 and isolate Delta A*, delta B*, Delta C* and we can find the steady state values.
- From that set to 0 and isolating Delta C, we can find that precision is equal to

$$\frac{\Delta C^* / C^*}{\Delta I / I} = \frac{I}{C^*} \frac{\partial f_A}{\partial I} \frac{|N|}{|J|}$$

, where N is the matrix minor for (C,C).

- We want Delta C* = 0 for best precision, so that the minor |N| has to be 0.
- Then, we impose two conditions:
  - 1. Delta C* = 0 so that we return to the initial state

- Return to the same steady state, $\Delta C^* = 0 \Rightarrow |N| = 0$.

$$|N| = \underbrace{\beta_{AB}\beta_{BC}}_{I} - \underbrace{\alpha_{BB}\beta_{AC}}_{I}$$

a Either I=II=0 $\Rightarrow \beta_{AB}\beta_{BC} = 0$ and $\alpha_{BB}\beta_{AC} = 0$

b or I=II≠0 $\Rightarrow \beta_{AB}\beta_{BC} = \alpha_{BB}\beta_{AC}$

- In case a) We cant go through A->B->C, because of the first thing being 0 (either AB or BC has no connection) . Therefore, beta_AC has to be nonzero (so that we connect A and C) and therefore alpha BB has to be 0 (to fulfill the condition).

  - 2. |J| < 0 so that there is stability

Continuing from case a), we know alpha BB = 0 and let's take beta AB = 0 too (its either that ot beta BC). Then, the jacobian is simply

Let's continue with option (1) for |N|

$$|J| = \underbrace{\beta_{AC}\beta_{CB}\beta_{BA}}_{i} - \underbrace{\alpha_{AA}\beta_{BC}\beta_{CB}}_{ii},$$

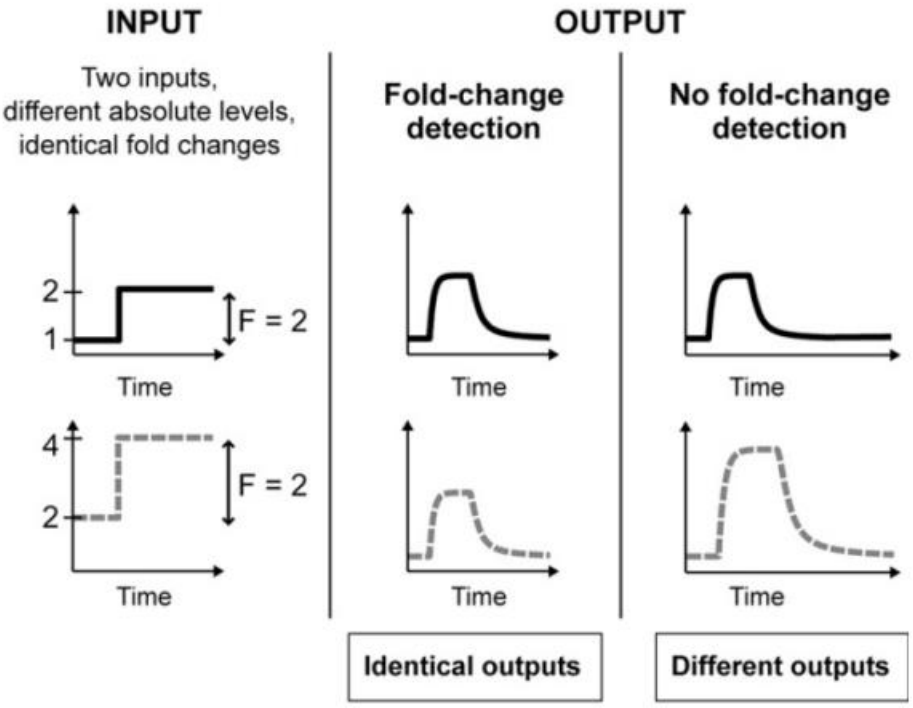
For it to be negative, we can have:

- i<0 and ii=0, which would imply A-> C -> B -> A is a negative feedback
- or ii>0 and i=0, which implies that B->C->B is a negative feedback.
- Or i<0 and ii>0 ……

- ,At the end, we find two possible topologies for adaptation circuit:
  - **Integral negative feedback:** A activates C, C activates B, but B inhibits C.
  - **Incoherent feedforward:** A activates C but A activates also B, which inhibits C.

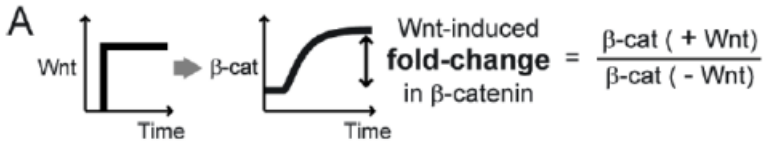
FOLD CHANGE DETECTION AND WEBERS LAW

- **Fold change detection:** Instead of measuring changes at absolute values, we measure them as Delta O_F = log O2/O1. That is the fold change.

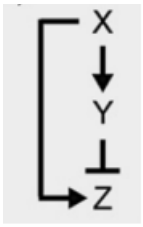


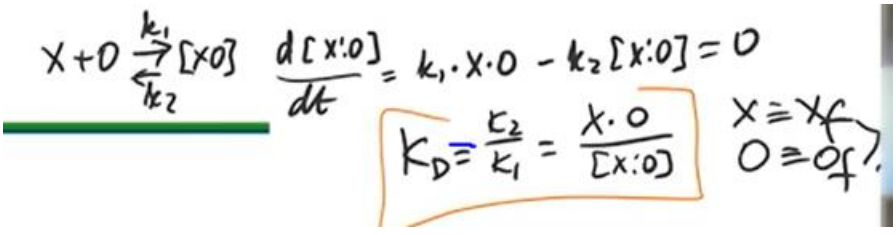
- These two inputs have different absolute changes, but have the same fold changes. And they show what the results would look like for fold-change detection systems and for no fold-change detection.
- Exact mathematical definition from the paper.
- Definition of Adaptation and how it is a consequence of FCD.
- **Where does it appear in organisms:**
    - WNT: Is a very important ligand for embryo development. It determines the axis of the embrio (in what direction it elongates after being a blob). It also controls cell divisions and tells them when to stop dividing, which is what we will care about.
        - Pathway: WNT is a ligand outside the cell, which can be bound to a receptor, then the receptor activates something called beta-catenin, which tells the cell to do some transcription or something.

- They found in a paper that the beta catenin change is fold change, not absolute.



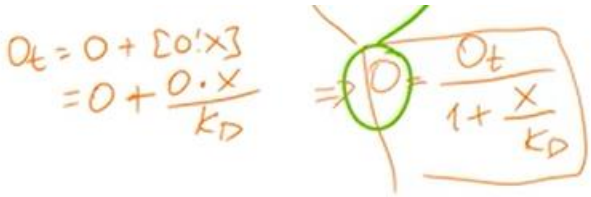
- They measure the levels of Beta –cat as functions of time with and without WNT (even without WNT, beta changes with time). They find that beta-cat(+Wnt) / beta/cat(- WNT) looks like in the figure and shows how WND creates a fold-change.

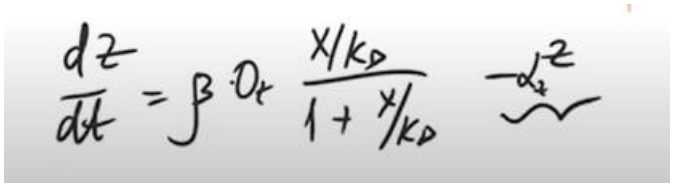
- **Biological model for the circuit**
  - We have a gene Z that is transcribed to produce protein Z. this transcription happens with help of RNAp. There is a chunk of DNA (Operator site) that can bind to recruiters or inhibitors. When we have recruiters (X) bound to the operator site (So we have the complex XO), they work by recruiting RNAp to transcribe Z. So X activates Z (when it is bound to O)
  - But we also have a protein Y which can bind to the operator site and when it does so, it doesn't allow the RNAp to fit, so it inhibits the creation of Z.
  - (therefore, X activates Z, so dZ/dt = beta * [X:O], where [X:O] means X joins to the Operator site).
  - 

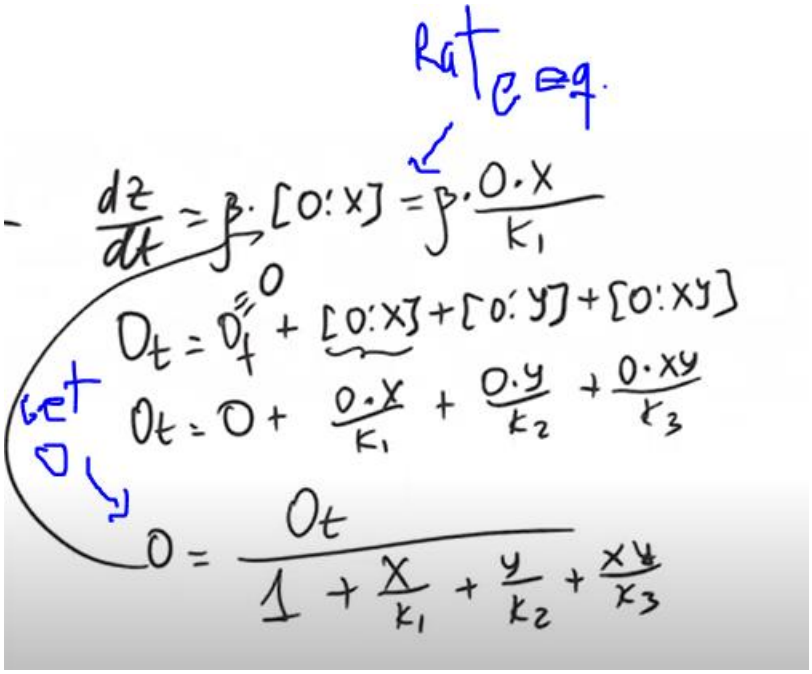
- **Finding the equations**
  - From the process regarding only X, we know that $\frac{dz}{dt} = \beta \cdot [X:O]$
  - We wish to substitute the concentration [X:O] for which we use:
  - 

  - Then, dz/dt = beta * X*O / k_D

- Finally, we want to find O (which is Ofree). For that, we can use that Ot = O + [OX] and then substitute the expression for [X:O] to find



- Finally, we get: 

where alpha is a dissociation term.
- If we assume X/kD << 1 and some renaming of constnats, we finally get that dz/dt = beta x – alpha Z
- This is however not complete. If we want to consider also Y, we need.
- 

- Where [O:X], [O:Y], [O:XY] are obtained as before, with k1 instead of kD. Then, we can isolate O to write it in terms of Ot.
- Finally, we assume that y/k2 >> than the other terms in the denominator, so we get dz/dt = beta0 x/y – alpha2 z.
- The same thing (but considering x activates y) leads to dy/dt = beta1 x – alpha1 y.

- $\frac{dY}{dt} = \beta_1 X - \alpha_1 Y$ (3)

  $\frac{dZ}{dt} = \frac{\beta_2 X}{Y} - \alpha_2 Z$ (4)

- **We want to prove that it has fold change detection**. That means that if we define F = X/X0 (X0 is the prestimulated value), we want to show that the dynamics of Z depend only on F, so only on fold changes.

- How to show that incoherent feedforward loop detects $FC(X/X_0)$?

  Let us define the following dimensionless variables,

  $y = \frac{Y}{\beta_1 X_0/\alpha_1}$ $z = \frac{Z}{\beta_2 \alpha_1 / \beta_1 \alpha_2}$ (5)

  $F = \frac{X}{X_0}$ $\tau = \alpha_1 t$ (6)

  and rescale equations 3-4,

  $\frac{dy}{d\tau} = F - y$ (7)

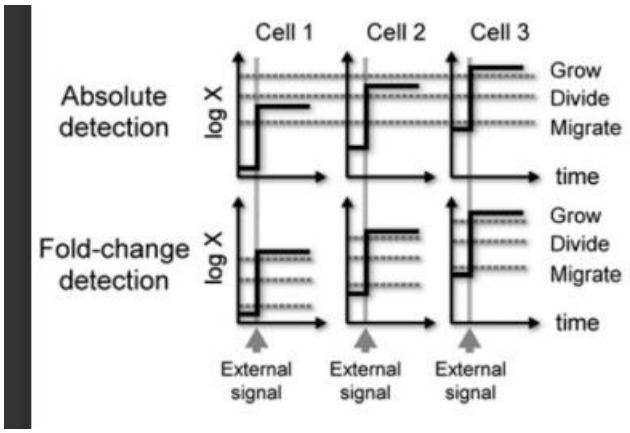
  $r\frac{dz}{d\tau} = \frac{F}{y} - z$ (8)

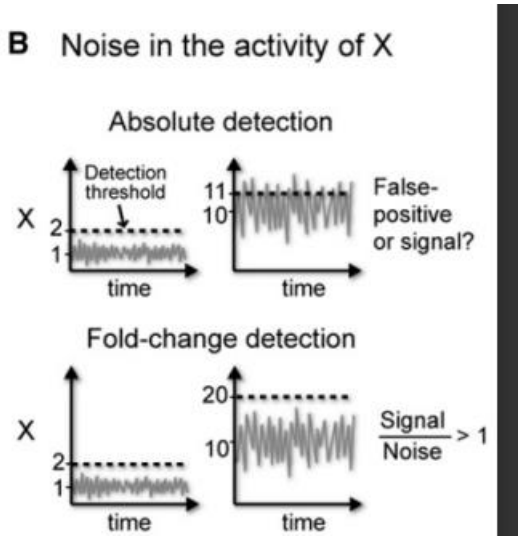
  - Basically, they first define F and tau and then the others are kind of natural.
  - We see that the equations only depend on F, not on X

- **Why is it good to have fold change detection and not absolute?**
  - Because cells may have different initial values of beta catenin. And we want all of them to respond equally to changes in WNT.

  - Despite of the starting levels of beta, which are different for different cells, the decisions of different cells will be the same given a change in WND

- 

- Also, fluctuations have less possibility of giving false positives.
- 

Topic 4: Excitable media (Heart arrhythmia and Pulsatile insulin secretion)

- **What is excitable media:** An abstraction. A system consisting of excitable units, which can be in 3 states:
    a. Not excited.
    b. Excited.
    c. Refractory: Phase when it is recovering, and cannot be excited again.
- **Examples:** Forest fires. The trees can be non-excited, excited (on fire) or refractory (there is no trees).
- **Time scales and feedbacks:**
    a. Positive: Excited parts excite other parts, so that excitation has a positive feedback (fires spread, heart signals spread). This one is fast.
    b. Negative: Excited parts create refractory parts, which cannot extend the excitation anymore (the fire burns out, the ion channels close and cells cannot get excited again for a while). This one is slower than the positive one, that way, the excitability expands
    c. The system sparks fast but takes long time to die out.
- **Heart:**
    a. Heart tissue: Muscle cells work by contraction. They need to coordinate to push the blood.
    b. Single cell: Contraction needs an electrical/chemical stimulation. Inside the cell, the signal propagates and creates a Calcuium induced calcium release.
    c. Propagation: Excited cells produce electrical pulses that stimulate neighbors to have CICR and contract themselves.
    d. Refractory: Contraction of a cell is followed by refractory period.
    e. How contraction works: We have fibers of actin (cables) inside the cells and we have myosin (little heads).
        i. If the cell is inactive, the myosin doesn't bind to the actin. There is a layer of tropomyosin in the actin which doesn't allow the myosin to bind to the actin.
        ii. When calcium comes into the cell, myosin binds to the acting and in the process burns energy (converts ATP to ADP). Then, the myosin can pull on the cable.

iii. Then the myosin unbinds, the heads cock back and then they bind again and so they keep on moving the acting.

iv. When we run out of calcium, tropomysin comes back and doesn't allow the mysing to bind, so we are back in inactive.

f. Process in the cell:

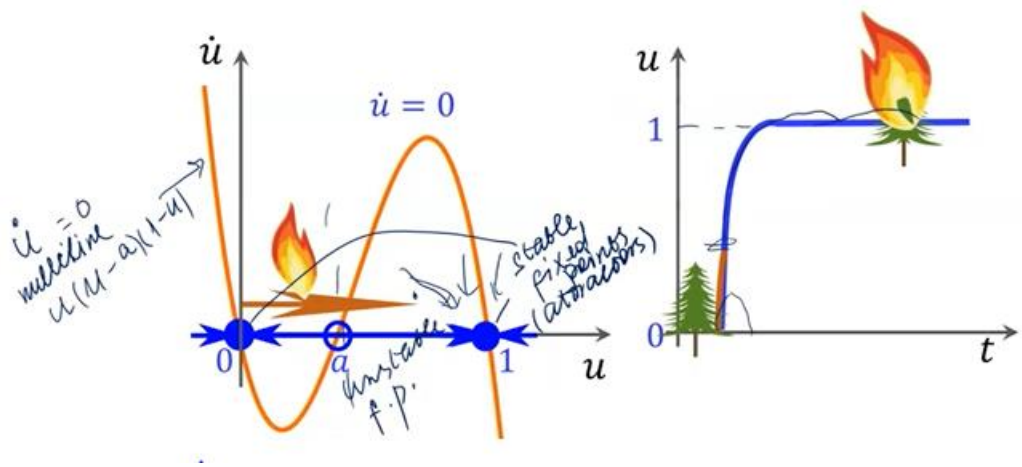
i. Once calcium comes in the cell from outside because of an electrical stimulation, the little bit of calcium releases the calcium that the cell had stored in the ER. So a little calcium from outside creates a big release of Calcium in the cell. This calcium allows the myosin to act and all that

ii. However, then there is a pump activated that brings calcium back to the endoplasmic reticulum which creates a negative feedback.. While these pumps are working, cell is in refractory phase, because even if we put calcium, it will not activate the cell back again, since the pumps will push the calcium to the ER.

iii. Then the pumps stop working, the Calcium is in the ER and we are again in non excited state.

- **Heart arrhythmia:**
  a. Healthy heart: There is a pace maker in the heart that excites a certain region and then waves move in planar waves through the heart.
  b. Spiral waves: We can get spiral waves in the heart, which makes the heart not effective in pumping blood and we get an arrhythmia. The period of the oscillations becomes very small, but still periodic and we have a tachycardia.
  c. Ventricular fibrillation: If we have a lot of spiral waves, and they collide and come together and etc. we get aperiodic behavior and that is a ventricular fibrillation.

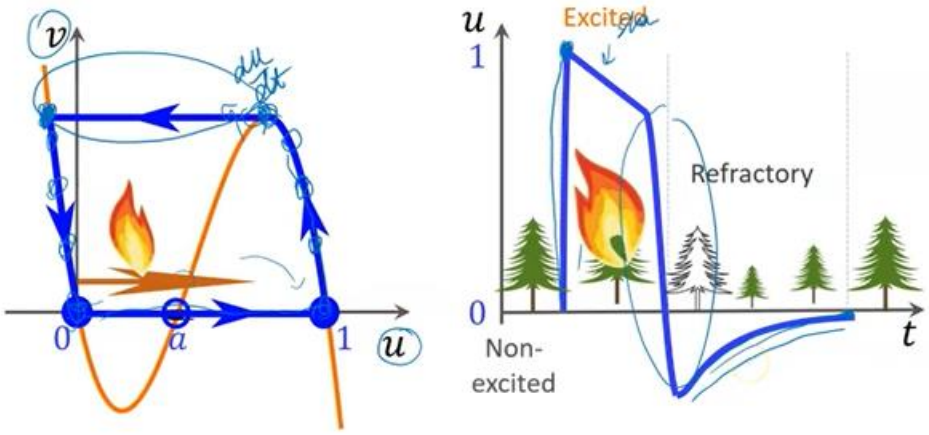
- ## Fitz Hugh Nagumo model for excitable media
  a. Let's say u is our calcium concentration. Then, the model is:
  $u' = u(u-a)(1-u)$, where $0<a<0.5$ and $0<u<1$. This gives us a cubic function.
  b. We have three steady states: 0,a,1. 0 and 1 are stable and a is unstable.
    i. A small excitation in u will not be able to cross the fixed point at a.
    ii. A large excitation above some region will cross the fixed point at a and can move all the way to 1.



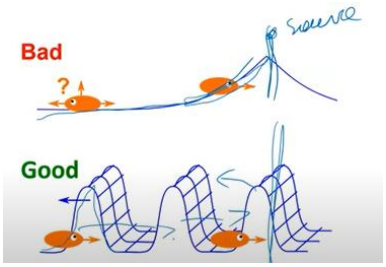
c. We can add an inhibitor to the system by putting u' = u(u-a)(1-u)-v. Then, we can see how the stable fixed points change as we change v. When v is big enough, we have a bifurcation and the stable states at a and 1 anhillate and we have only the low stable fixed point, so the system is not excitable.

d. We assume that v changes with time, following v' = eps * u, where eps is very small, since it corresponds to the negative feedback that brings us back to the origin. That way, we can have a cycle:

- **i.** First u is at 0 and we have an excitation which brings it to 1. (excited state)
- **ii.** Then, since v starts increasing because of v' = eps*u, the state of u=1 starts getting smaller and eventually disappears when it collides with the state at a.
- **iii.** Then, the system jumps back to the low state, which is now negative. Therefore, now v decreases with time and eventually goes back to 0 and resets. During this time, we are in refractive state.

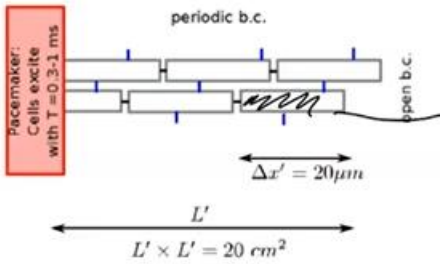


e. Finally, to have a complete model, we add a diffusion term to u, so that it looks like u' = u(u-a)(1-u) – v + Du * nabla^2 u. So that it can propragate in space.

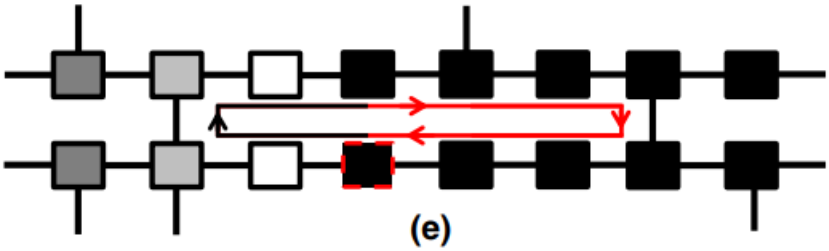
- **Why waves:**
  a. Because it allows us to define directions for cells to move to If they are following the gradient. 

Physical model heart

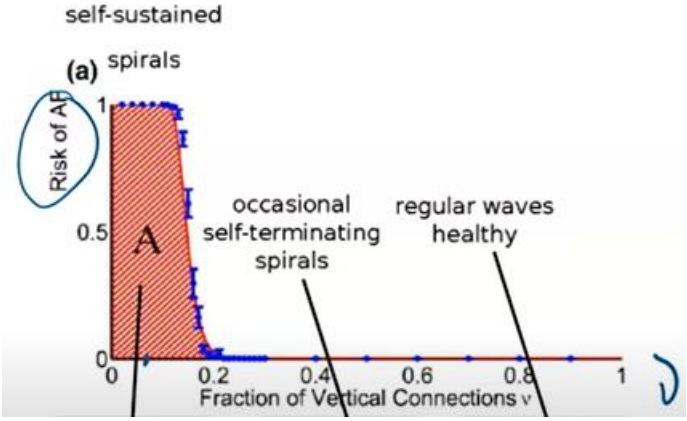
- Healthy hearts have one wavefront so that they contract orderly.
- A heart with arrhythmia is caused by many wavefronts in random directions.
  - Treatment: Drugs to extend refractory period.
  -
- Model: It is an agent based model. The agents are the cell, which can be intactive, can be excited (which lasts for some short time) and then they are in refractive period for a while (considerably longer than refractory time).
  - Muscle fiber is anisotropic, so that cells are like cylinders, which couple in fibers longitudinally, but don't couple as much transversally.
  - So we couple the cells like that, in fibers and every once in a while we couple fibers transversally. The parameter defining transversal couplings is nu. It is 1 for total transversal coupling and 0 for no transversal coupling. Healthy hearts have nu =1 and as we get old, nu gets smaller.
  - We put a pacemaker in the left part of the tissue and we put open boundary conditions in the x direction but periodic conditions in the y direction.
  - A proportion delta of the cells are dysfunctional. Which means that they have a probability epsilon to fail to get excited in a given excitation moment.



- We rescale parameters. We join up b cells into one, so that distances are rescales and we rescale time too so that speed stays the same.
- **Results:**
  - When nu = 1, we get a plane wavefront that moves nicely. Transversal couplings can solve problems of terminations.
  - When nu < 1, we can get spiral waves. Zooming in, this is caused by **reentrant circuit** which are caused by small nu and a dysfunctional cell**:**



    - The length between transverse bounds has to be bigger than tau/2 (tau is the refractory period).
  - For a fixed delta and epsilon, this is the probability of fibrilation:



  - Analytically: We can calculate the probability analytically.
    - The risk of AF is proportional to the probability of having a reentrant circuit.

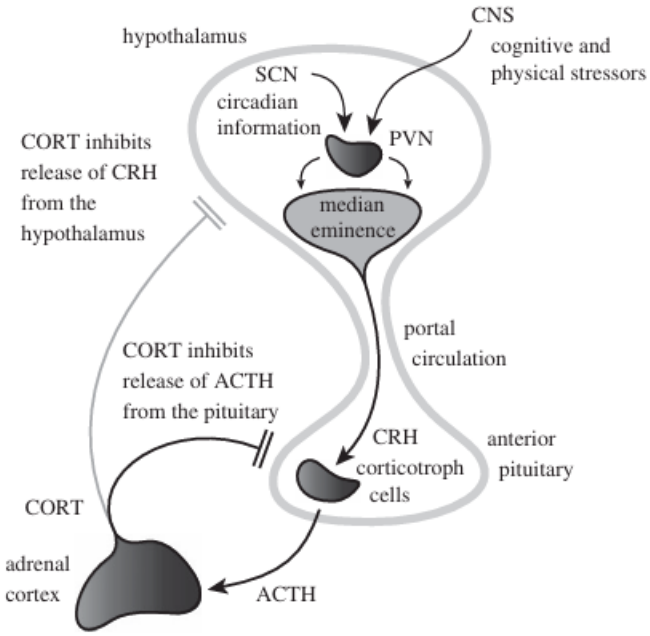
- The probability of having at least one transversal coupling is p_v = 1-(1-v)^2 (there are 2 possible coupling with prob v each.
- The reentrant circuit should be long enough, Starting from a cell with transversal, the probability that the length without a transversal coupling is of l_i is (1-p_v)^{l_i} p_v.
  Therefore, the probability that we have a circuit of length l_i smaller than tau/2 (so no reentrant circuit) is
  - $P(l_i < \tau/2) = \sum_{l_i=0}^{\tau/2-1}(1-p_v)^{l_i}p_v = 1-(1-v)^{\tau}$

  Therefore, the probability of having a reentrant circuit is 1-that.
- If we consider that delta*L^2 is the number of dysfunctional cells, the probability of risk is:
  Thus $P_{risk} = 1-[P(l_i < \tau/2)]^{\delta L^2} = 1-[1-(1-v)^{\tau}]^{\delta L^2}$

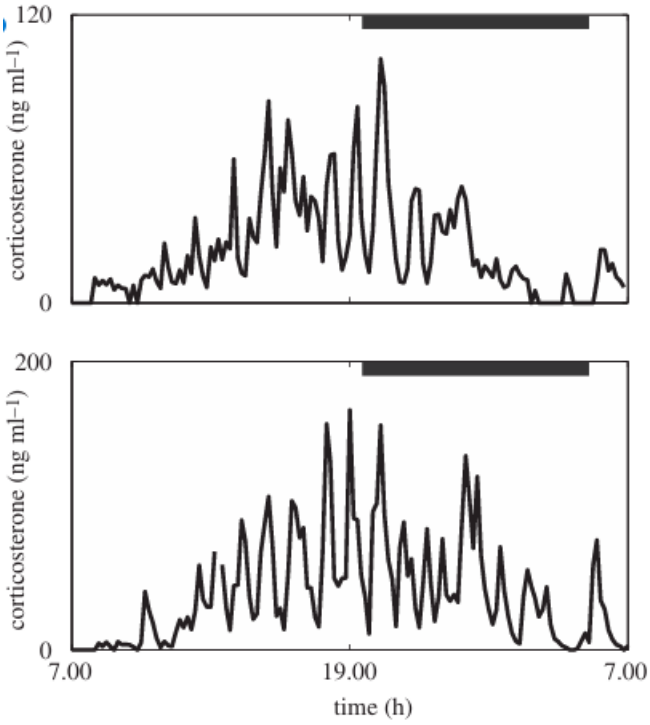
- Why bother:
  - It explains how prolonging refractory period lowers risk.
  - It allows us to do ablation in a more directed way, without shooting in the dark.

Topic 5: HPA Axis

1. **Frequency encoding:** Means that the information of a signal is totally encoded in its frequency, not in its amplitude. So two signals with the same frequency but different amplitudes give the same results.
    a. Example: Slime mould only aggregates in response to external pulses of cyclic AMP with periodicity of 5 minutes, and not to greater than 2 minutes or to constant.
    b. Example: The endocrine system uses it. For example, in insulin and in the one we will study.
2. **Rythms:**
    a. Cyrcadian: is a natural oscillation that repeats roughly every 24 hours. Circadian rhythms can refer to any process that originates within an organism (i.e., endogenous) and responds to the environment (is entrained by the environment).
    b. Ultradian: A cycle repeated throughout a 24 hour day. They complete many cycles daily.
3. **System we will study (HPA Axis):** Hypothalamic-pituitary-adrenal axis, which regulates the circulating levels of glucocorticoid hormones (CORT), which upregulate the expression of anti-inflammatory proteins, stimulate gluconeogenesis and are a response to stress. Their production goes like this:
    a. The hypothalamic PVN receives circadian inputs from the SCN (which coordinates circadian rythms)
    b. The PVN releases CRH to the corticotroph cells in the anterior pituitary.
    c. The corticotrophs release ACTH, which travel to the adrenal cortex (delayed effect).
    d. The adrenal cortex release CORT. They are released in discrete pulses
    e. CORT inhibits the release of CRH (but not very much, it was proven experimentally that taking away this inhibition doesn't change the oscillations we will se)
    f. Cort inhibits the relsease of ACTH. (mediated by the glucocorticoid receptor GR)



4. **Results**



Experimental data of CORT for two mice over 24h periods. Blood samples collected every 10 minutes. We can see that there is an underlying circadian rythom which on top has the ultradian rhythm of release of CORT. Also, the scale for the two mice is different, but what Is important is the frequency, not amplitude, so its ok.

5. **Question:** We don't know what is the mechanism responsible for the regulation of ultradian rhythm.
    a. We hypothesize that the pituitary-adrenal system, which has a delayed feedforward connection between ACTH and CORT and a negative feedback of CORT on ACTH (mediated by the glucocorticoid receptor GR) is what supports the ultradian oscillations.
    b. To address this hypothesis, we meke the model.
6. **Model:** The differential equations are the following:
    a. **Full model:**

$$\begin{aligned}
\frac{dC}{dT} &= \frac{K_c + F}{1 + O/k_{i1}} - K_{cd}C, \\
\frac{dA}{dT} &= \frac{K_a C}{1 + OR/k_{i2}} - K_{ad}A, \\
\frac{dR}{dT} &= \frac{K_r (OR)^2}{K + (OR)^2} + K_{cr} - K_{rd}R, \\
\frac{dO}{dT} &= K_o A - K_{od}O,
\end{aligned}$$

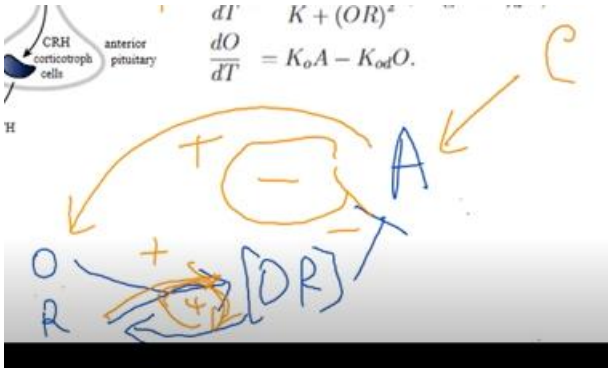
Productio
proportior
reduces p
CRH.

        i. C = CRH, A = ACTH, O = CORT, R = GR
        ii. First equation: Production of CRH due to stresser F (cte) and inversely proportional to CORT (faded link in fig 1). So more CORT reduces production speed of C. Second term is decay of CRH.
        iii. Second equation: Same as last one. Now the production of ACTH is inhibited by Cort pairing with a receptor.
        iv. Third equation: In this one, the production term is a sigmoidal of OR, so more OR increases production of R, instead of reducing it as was for C. The squared term makes it nonlinear and implies that we need cooperativity, we need two OR for some reason. There is also a constant creation of R given by Kcr.
        v. Fourth equation: Cort is produces by ACTH and decays also.
    b. Simplification: The inhibition of CRH by CORT is not important, so C is no longer a variable, but a system parameter.
    c. Delay: The production of CORT has a delay with respect to ACTH.
    d. Redefining constants:

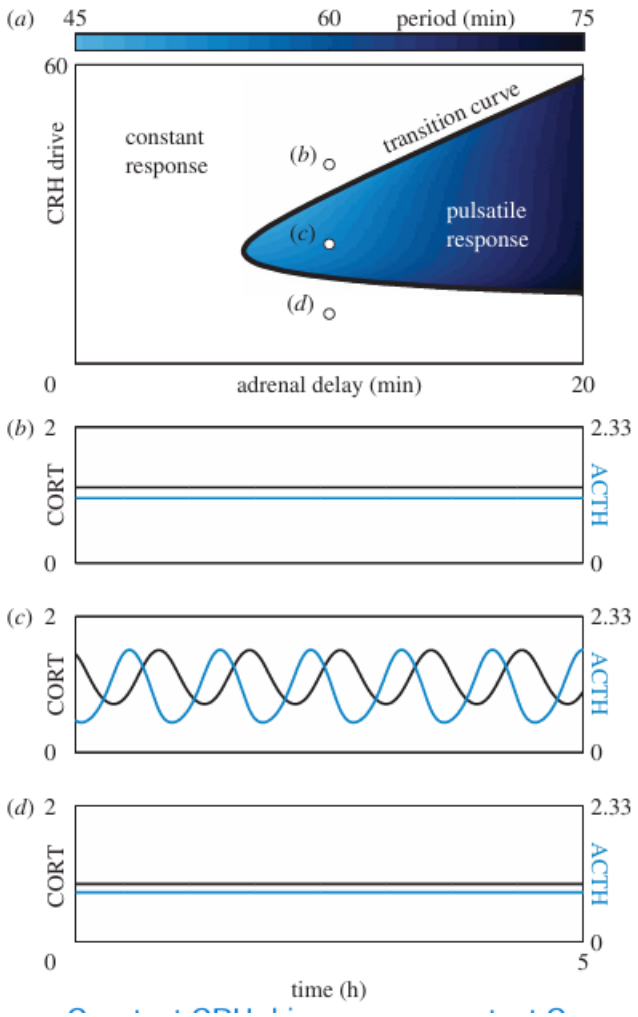
$$\begin{aligned}
\frac{da}{dt} &= \frac{p_1}{1+p_2 ro} - p_3 a, \\
\frac{dr}{dt} &= \frac{(or)^2}{p_4 + (or)^2} + p_5 - p_6 r, \\
\frac{do}{dt} &= a\,(t-\tau) - o.
\end{aligned}$$

e. P1 is the CRH drive and tau is the delay.

7. **Results 1: constant P1**
   a. Final shape of the model:



   b. AIM: Does the system support ultradian CORT oscillation?
   c. They found a range of values in drive and delay that lead to two different responses:
      i. The white part has a response with constant levels in ACTH and CORT.
      ii. The blue part has a fluctuating result, despite the fact that the CRH drive p1 is constant. The frequency of the response is indicated by the colorbar

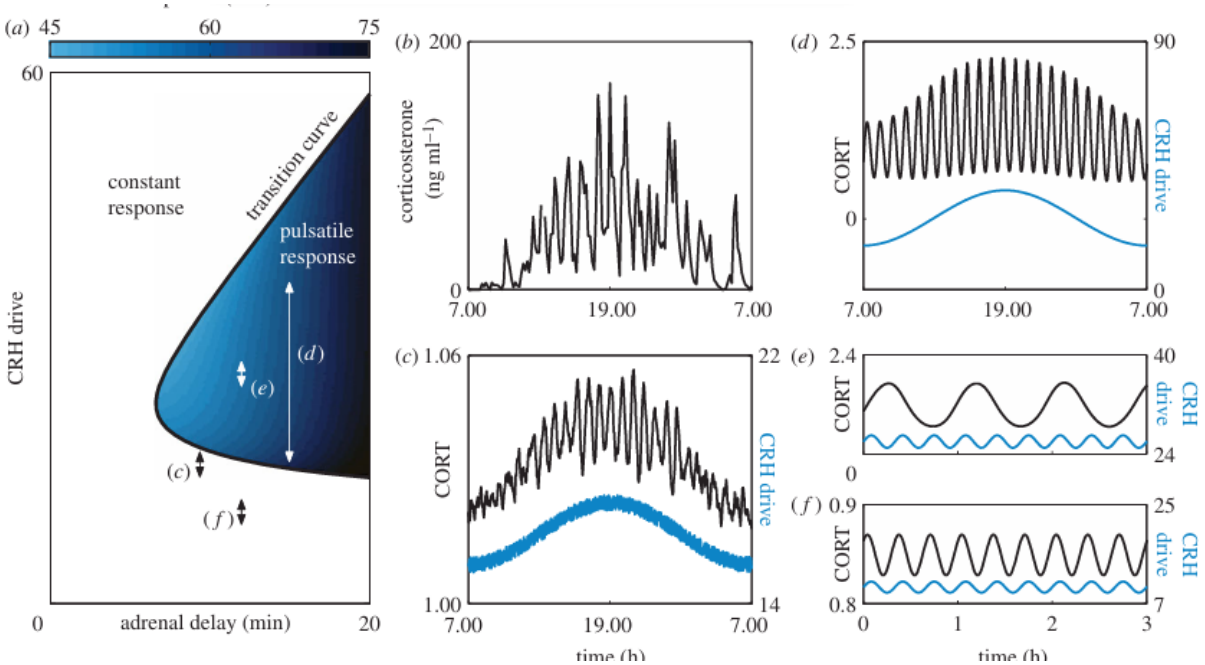


iii.

iv. The result is consisten with previous studies, which reported an interpulse range of around 50minutes.

8. **Results 2: Circadian p1.**
    a. Now they make the p1 oscillate with a circadian rhythm.

b.



i. Figure a) is the same as figure, it is the response for the case of constant p1. They just put on top of that the oscillations they are using.

ii. Figure b) is the experimental result.

iii. Figure c) Is the result for a noisy circadian CRH drive close to the pulsatile region, which shows NICOs during the peak of the circadian drive:

   1. NICO: Noise induced coherent oscillations: They look similar to the experimental data, providing evidence for the hypothesis that feed-forward and feedback interactions within the pitu itary–adrenal system are the foundation of ultradian activity observed experimentally.

iv. Figure d) The response to oscillating p1 for a circadian drive that is inside the pulsatile region. It shows increased pulse amplitude during the peak CRH drive.

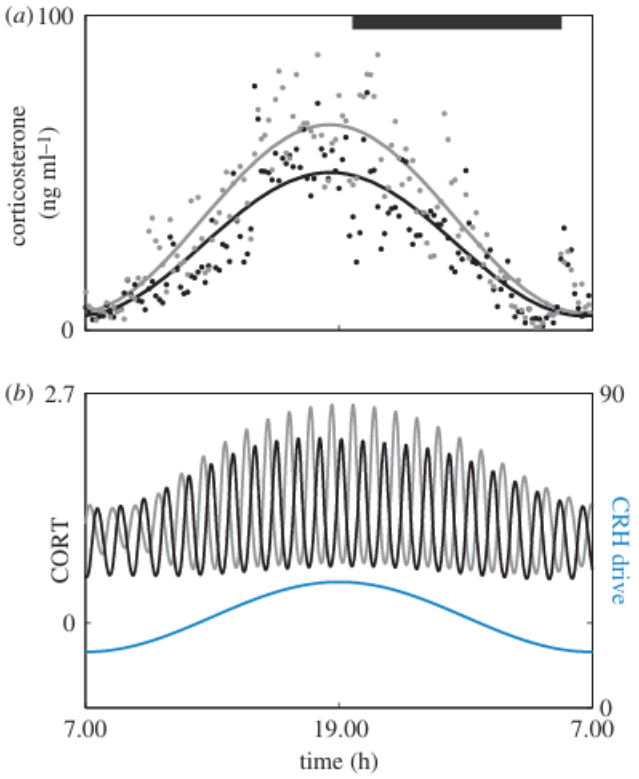
Now for ultradian drives:

v. Figure e) is for an ultradian oscillating p1, which shows a response that is not governed by the frequency of the CRH forcing.
Figure f) is as the one for e) but outside the pulsatile region. Now the response is governed by the freq of CRH forcing.

So inside the blue zone, CORT doesn't respond with the same frequency as the CRH ultradian drive, but outside it does.

9. **Applications / discussion:**

a. Depression has been consistently associated with elevations of HPA activity. This increased activity is associated with a diminution of sensitivity to the negative feedback by endogenous glucocorticoids.
    i. According to experiments on mime, it seems like GR plays a major role on that.
b. The model we employ is the first to incorporate the dynamics of GR, so it can be used to investigate the effects of GR antagonist or agonists.
    i. Infusion of a GR antagonist increases the amplitude of the ultradian glucocorticoid rhythm during the peak of the circadian CRH drive (fig 6 b), while having minor effect in ultradian frequency. This is consistent with rats.



    ii. The mechanisms underlying many of these rhythms have been very unclear, and in this paper we have been able to show that relatively simple feed-forward and feedback interactions between the pituitary and adre nal cortex are sufficient to account for the glucocorticoid rhythms we observe experimentally.
    iii. This theoretical approach, which simply depends upon systems having delayed feed-forward and feedback pathways, could also provide the basis for understanding ultradian rhythmicity in many other biological systems.