Systems Biology

Non-linear signal processing and hormetic signal-response rates in the TOR pathway ?

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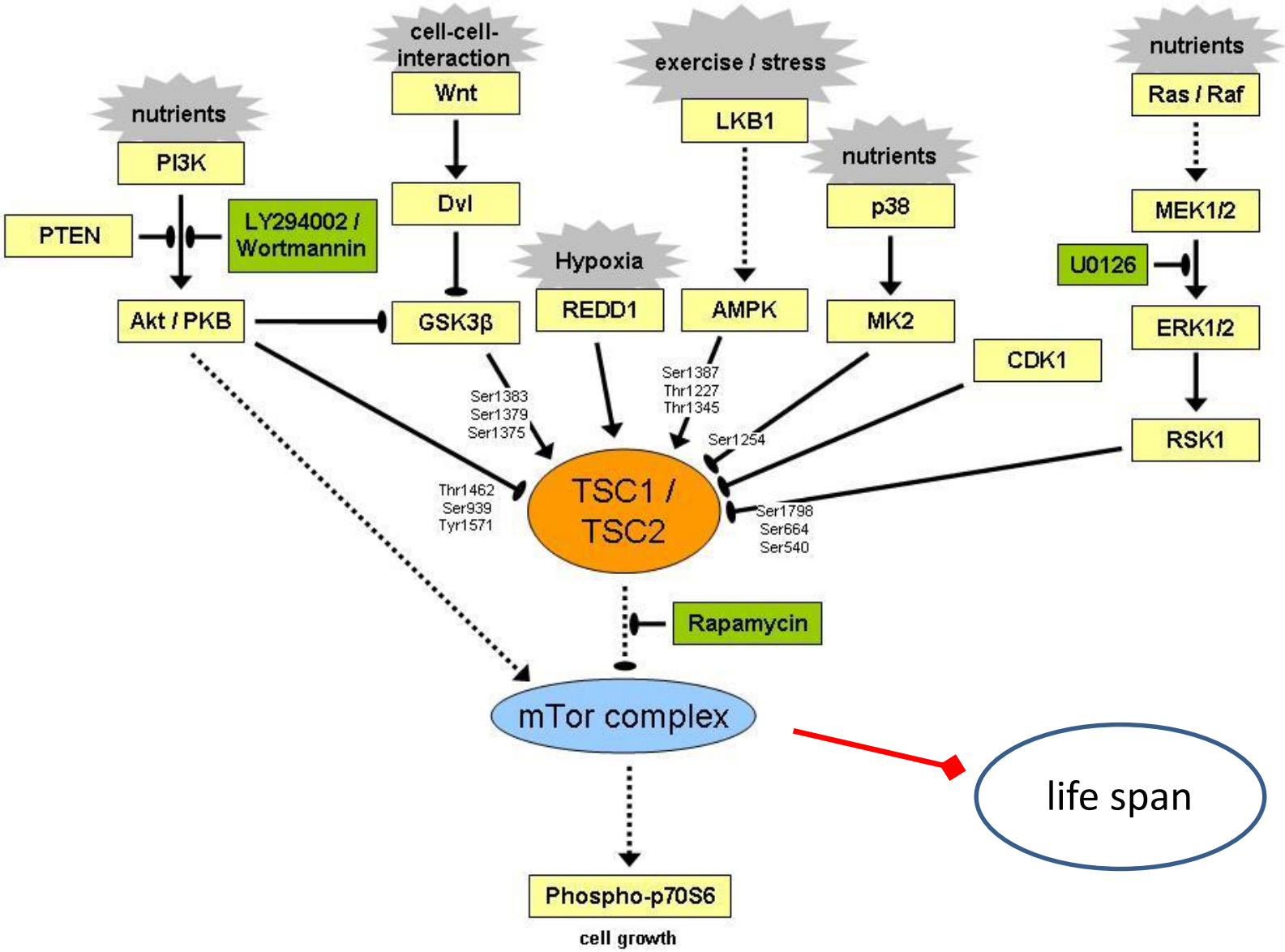
Target Of Rapamycin (TOR) pathway

- TOR plays a crucial role for a number of cellular processes such as ageing [Johnson 2013, Evans 2010, Lamming 2011, Bonawitz 2007], tumour formation [Populo 2012], adipogenesis, formation of insulin resistance, and activation of the immune response [Thomson 2009].
- Decreased TOR activity has been found to slow aging in S. cerevisiae, C. elegans, and D. melanogaster. The mTOR inhibitor rapamycin has been confirmed to increase lifespan in mice by independent groups ….
  - this can, for instance, be accomplished by activation of the proteins TSC1/2 (tuberous sclerosis complex 1+2)
- central role for regulation of cellular adaption and homeostasis
- responds to a large number of intracellular and extracellular signals
- regulates metabolism, growth, proliferation, …


nutrients
PI3K
PTEN
LY294002 / Wortmannin
Akt / PKB
cell-cell-interaction
Wnt
Dvl
GSK3β
Hypoxia
REDD1
exercise / stress
LKB1
AMPK
nutrients
p38
MK2
nutrients
Ras / Raf
MEK1/2
U0126
ERK1/2
RSK1
CDK1
Ser1383
Ser1379
Ser1375
Ser1387
Thr1227
Thr1345
Ser1254
Thr1462
Ser939
Tyr1571
TSC1 / TSC2
Ser1798
Ser664
Ser540
Rapamycin
mTor complex
life span
Phospho-p70S6
cell growth

Hormesis, Hormetic Response

- describes effects of an agent on a cell, an organism etc.
  - beneficial effect after exposure to low doses
  - toxic – or even lethal - effect for higher doses of the same agent.
- "For every substance, small doses stimulate, …, large doses kill." (Arndt–Schulz rule)
- **more general:** Hormesis is an adaptive response of biological systems to moderate (transient) levels of stress factors

Stress factor: Reactive Oxygen Species (ROS)

- ROS ("free radicals") is a main stress factor
- "free radical": atom, molecule, or ion with an unpaired valence electron
  - superoxide (O_2^-)
  - hydrogen peroxide (H_2O_2)
  - peroxynitrite ($OONO^-$)
- Consequences:
  - cancer
  - oxidation of LDL → plaque in arteries → heart disease, stroke
  - cross-linking between fat and protein molecules

“Traditional” free-radical theory of aging

- D. Harman, 1956: Free radical theory of aging
- “free radicals” produce cumulative damage of cells and shorten lifespan
- drugs (antioxydants) act against free radicals
  - β-carotene, superoxide dismutase, vitamines A, C, E, coenzyme Q [ubiquinol], glutathione, curcumin [E100]

New findings

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Review

How increased oxidative stress promotes longevity and metabolic health: The concept of mitochondrial hormesis (mitohormesis)

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[b] Dept. of Clinical Nutrition, German Institute of Human Nutrition, 114 Arthur-Scheunert-Allee, Nuthetal D-14558, Germany

New findings

Antioxidants prevent health-promoting effects of physical exercise in humans

Michael Ristow[a,b,1,2], Kim Zarse[a,2], Andreas Oberbach[c,2], Nora Klöting[c], Marc Birringer[a], Michael Kiehntopf[d], Michael Stumvoll[c], C. Ronald Kahn[e], and Matthias Blüher[c,2]

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Contributed by C. Ronald Kahn, March 31, 2009 (sent for review March 14, 2009)

Aging Cell (2006) **5**, pp463–471

High oxidative damage levels in the longest-living rodent, the naked mole-rat

Blazej Andziak,[1] Timothy P. O'Connor,[2] Wenbo Qi,[3]
Eric M. DeWaal,[4] Anson Pierce,[3,5]

We compare antioxidant defenses (reduced glutathione, GSH), redox status (GSH/GSSG), as well as lipid (malondialdehyde and isoprostanes), DNA (8-OHdG), and protein (carbonyls) oxidation levels in urine and various tissues from both mole-rats and similar-sized mice. Significantly lower GSH and GSH/GSSG in mole-rats indicate poorer antioxidant capacity and a surprisingly more pro-oxidative cellular environment, manifested by 10-fold higher levels of *in vivo* lipid peroxidation. Furthermore, mole-rats exhibit greater levels of accrued oxidative damage to lipids (twofold), DNA (~two to eight times) and proteins (1.5 to 2-fold) than physiologically age-matched mice, and equal to that of same-aged mice.

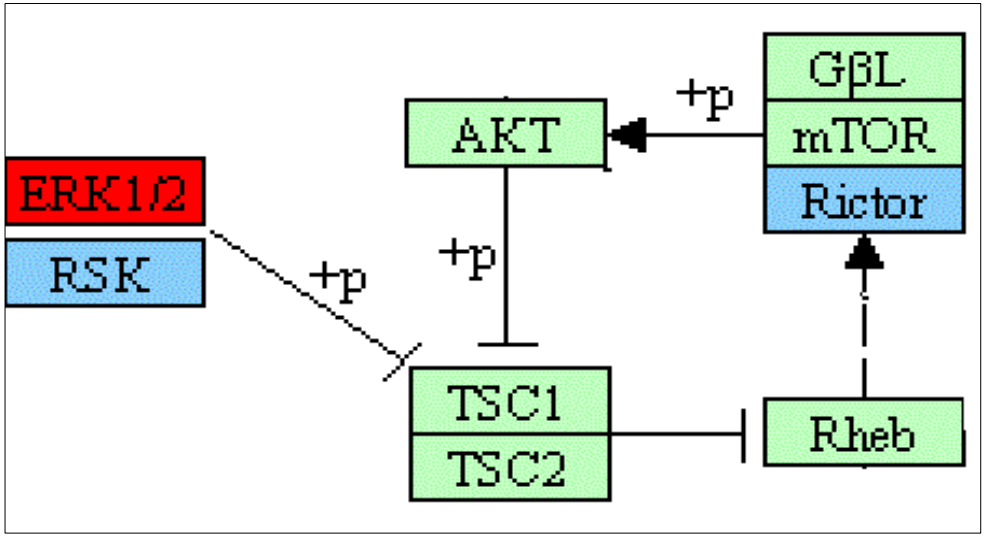
Mathematical model of hormesis in the TOR pathway

- **Goal:** model the following qualitative behavior mathematically:
  - short or low-intensity pulses of ROS → activation of a "defense enzyme" (against ROS)
  - long or high-intensity pulses → defense loop cannot be sustained, "destructive molecule" is released

"For every substance, small doses stimulate, moderate doses inhibit, large doses kill."

Arndt-Schulz rule

TOR contains a double-negative feedback loop (?)



TOR-C2 complex

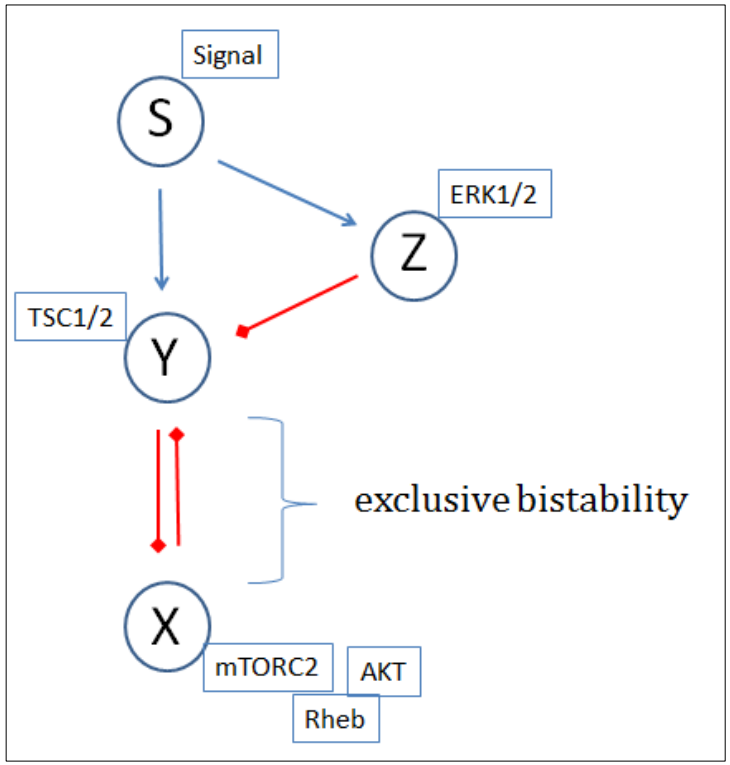
Arrow-headed: activation
T-shaped: inhibition

KEGG pathway

The double negative loop forms exclusive bistability between

- TOR-C2 complex (GβL, mTOR, Rictor) – "destructive"
- and TSC1/2 – "defense enzyme"

Note: It is not entirely sure if the KEGG pathway data are correct.



Schematic plot of the double-negative feedback loop

- AKT, TOR, and Rheb are merged into one variable **X**
- **X** suppresses the TSC1/2 complex (**Y**).
- TSC1/2 in turn suppresses **X** → exclusive bistability (red lines)
- Also ERK1/2 (**Z**) suppresses TSC1/2
- ERK1/2 and TSC1/2 are triggered by an external signal **S** (ROS)

Nonlinear system of ODE

$$\frac{dx}{dt} = \alpha_x - \beta_x \cdot x - \beta_{xy} \cdot h\left(y, K_{xy}\right) \cdot x$$ mTOR-C2

$$\frac{dy}{dt} = I_y \cdot S(t) + \alpha_y - \beta_y \cdot y - \beta_{xy} \cdot h\left(x, K_{yx}\right) \cdot y - \beta_{yz} \cdot h\left(z, K_{yz}\right) \cdot y$$ TSC1/2

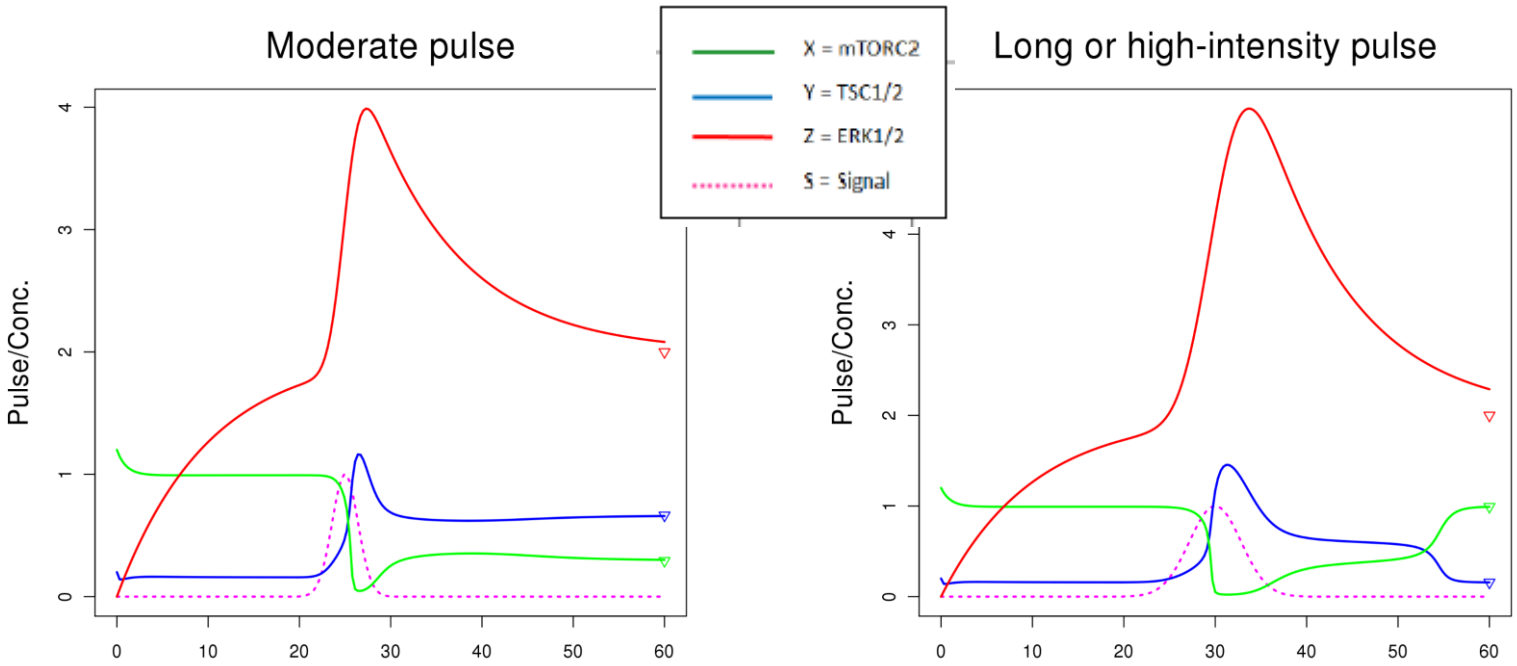
$$\frac{dz}{dt} = I_z \cdot S(t) + \alpha_z - \beta_z \cdot z$$ ERK1/2

$$h(x, K) = \frac{\left(\frac{x}{K}\right)^4}{1 + \left(\frac{x}{K}\right)^4}$$ Hill term

- α_i : basic time-independent synthesis terms
- β_i: spontaneous degradation of enzyme *i*

R: deSolve (LSODA), nleqslv, ...

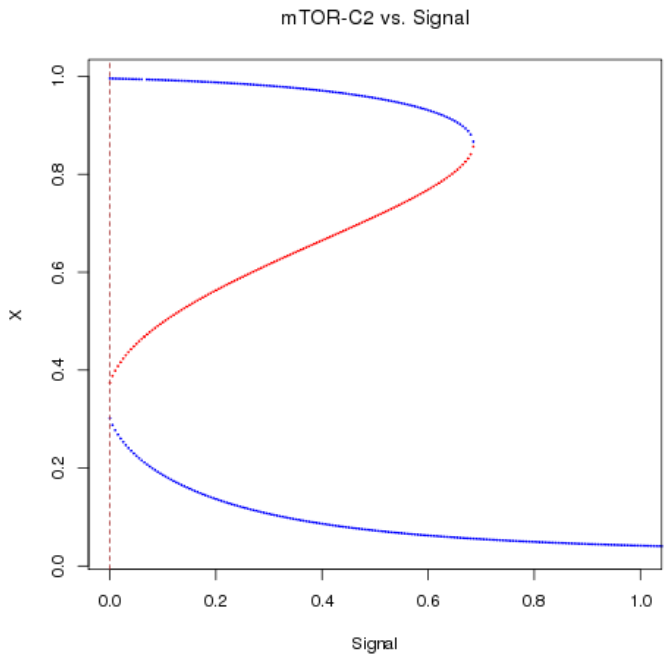
Results of the calculations

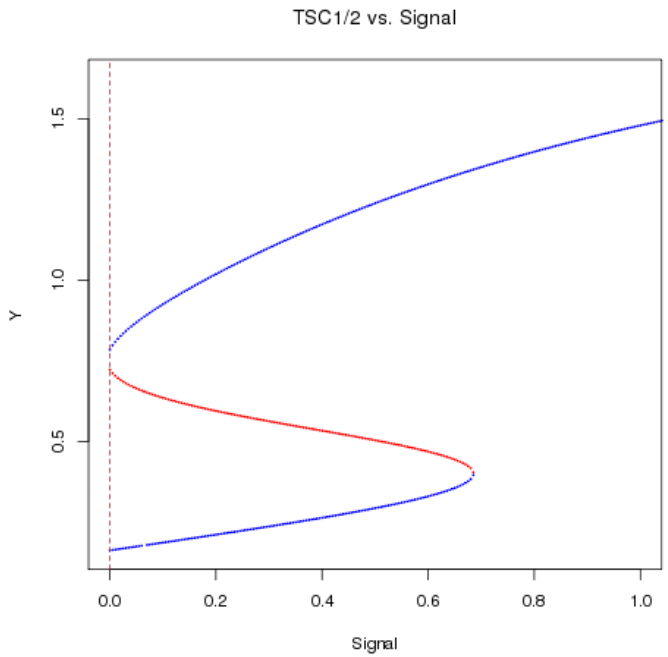


- external signal (dotted) , TOR-C2, TSC1/2, ERK1/2
- left: ROS signal with half-width 5
- right: ROS signal with half-width 10 (same amplitude)
- (stable) steady state values: triangles, same colours
- **moderate signal**: defense loop (TSC1/2) permanently upregulated
- **strong signal**: defense loop cannot be maintained
- → **characteristics of hormesis**

Bistability

- above: X, Y have 2 different stable states for signal $S = 0 \rightarrow$ bifurcation
- exhaustive search algorithm to find all solutions for different $S(t) = S$



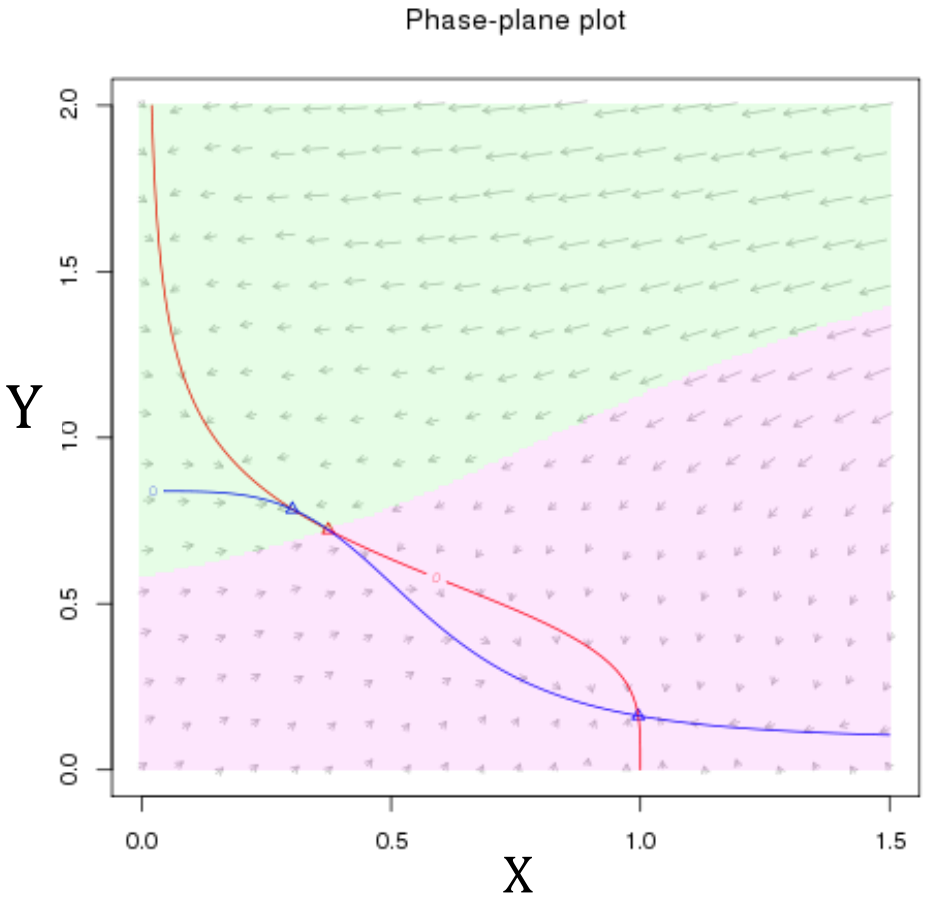


- TOR-C2 (left) and TSC1/2 (right) display irreversible hysteresis
- blue lines: stable states ; red lines: unstable states

Why does the defense state vanish for strong ROS signals ?

- irreversible hysteresis explains that ROS signal brings the system into defense state (TSC1/2 up, TOR-C2 down)
- … but cannot explain why strong pulses destroy the defense state
- the latter is a consequence of non-stationarity of ERK1/2 (variable Z)
- this can be understood by looking at the phase portraits for different values of Z

Phase portrait for equlibrium state of ERK1/2 (Z)



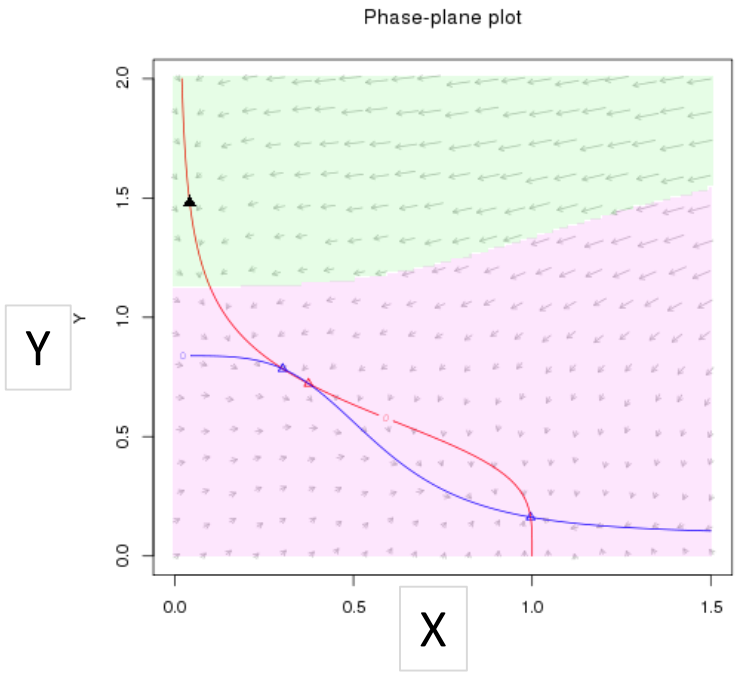
$$Z_0 = \frac{\alpha_z + I_z \cdot S_0}{\beta_z}$$

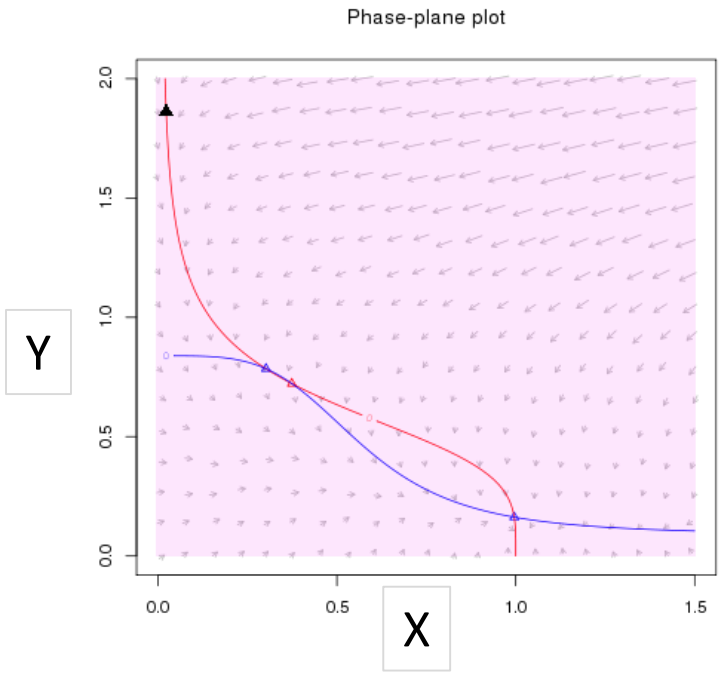
- steady state for ODE
- two stable solutions
- one instable solution
- nullclines
- attraction domains

Phase portraits for non-equlibrium states of ERK1/2 (Z)

- situation described above does not apply to times short after signal termination
- …. where ERK1/2 (Z) is **not** in equilibrium
- system "overheated" shortly after exposure to ROS

Phase portraits for non-equlibrium states of ERK1/2 (Z)





$(X, Y, Z) = (0.0416, 1.48, 2.0)$
(black triangle)

$(X, Y, Z) = (0.0227, 1.864, 3.99)$
(black triangle)

right plot: system state after signal in attractor region of lower equilibrium point → system returns to this unfavourable state

Response of the three-gene network motif to (ROS-) pulses of varying duration or intensity:

Pulse strength	Destructive mol.	Defense enzyme
moderate	Off	On
long	On	Off

Back to the hormesis model:

- On a so far simple level, the model meets our expectations:
  - moderate pulses bring up a "defense enzyme" (dismutase-associated?, peroxydase-associated?).
  - At long pulse durations, this enzyme can no longer be sustained
  - over a wide range of parameters, we find the same qualitative behavior (luckily …)
- Critics:
  - it can't be that simple
  - if just a third gene ("destructive molecule") would bring down the defense enzyme again, evolution would have knocked it out, **would it?**

Progress:

- Computations work (for practically arbitrary network motifs), large number of genes doesn't seem to be a (computational) problem
- Qualitative differences in molecule levels in response to varying signal durations modelled
- Qualitative results similar for a large range of parameter values
- Stable levels of a "defense enzyme" can be set up at appropiate pulse levels (modeling of varying pulse height yields similar behavior)
- can be expanded to more genes/equations and may lead to a model for a known pathway:

To think about:

- Detailed analysis of the reactions in the mTOR pathway
- Treat transcript- and protein concentrations as separate variables (e.g. Michaelis-Menten kinetics for phosphorylation) ?
- Include active/inactive states of transcription factors?
- How to compare with experimental results? (compare measured ↔ calculated ratios at steady state)
- Modeling of the source term in the equations
- Fit to RNA-Seq data (e.g. MC, Bayes, simulated annealing – seems feasable)

Appendix

Non-linear signal processing and hormetic signal-response rates in the TOR pathway ?

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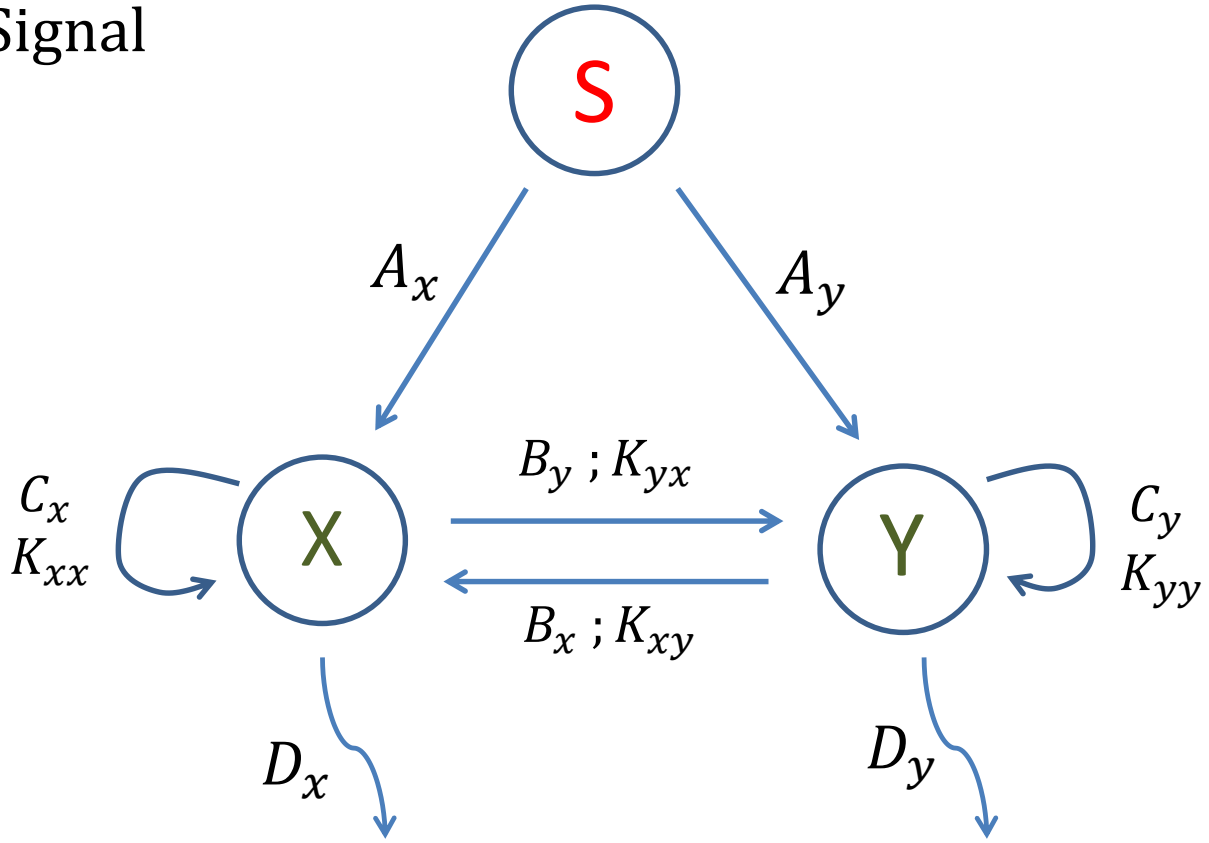
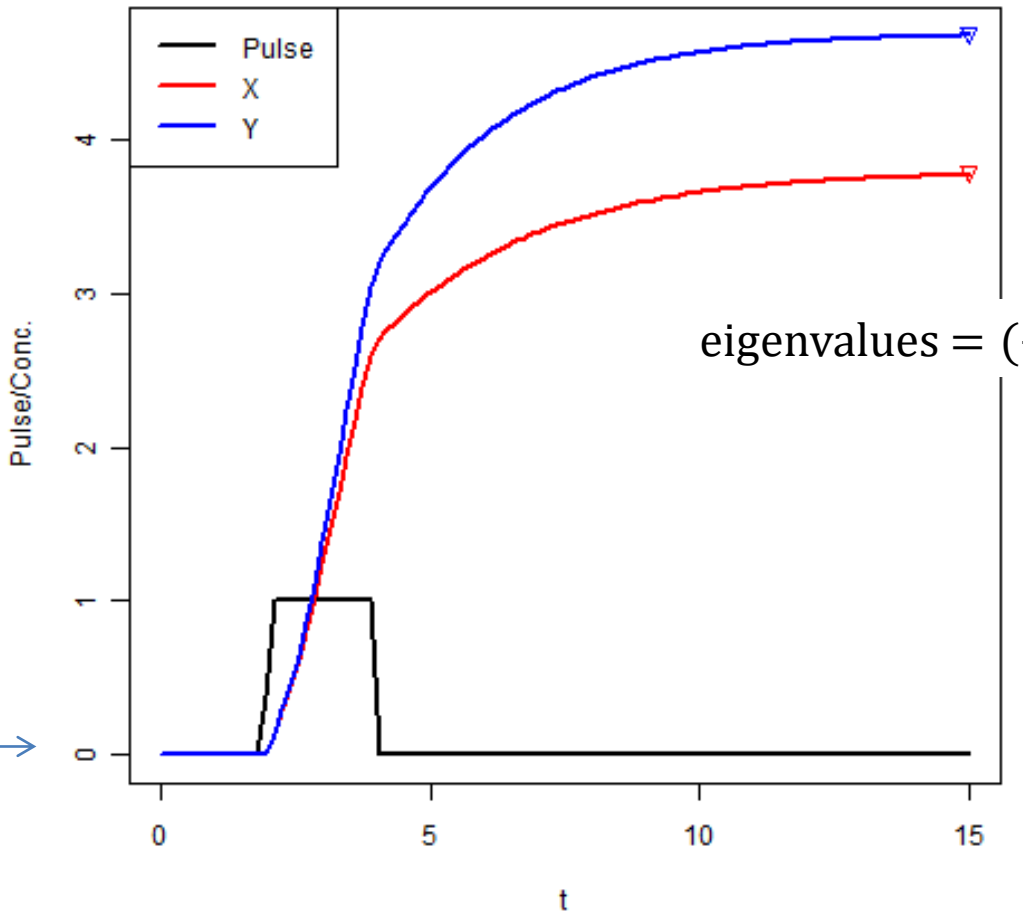
Simple motif: double-positive regulated

S: Signal



Short-pulse response

pulse:
2 units



steady-state sol.

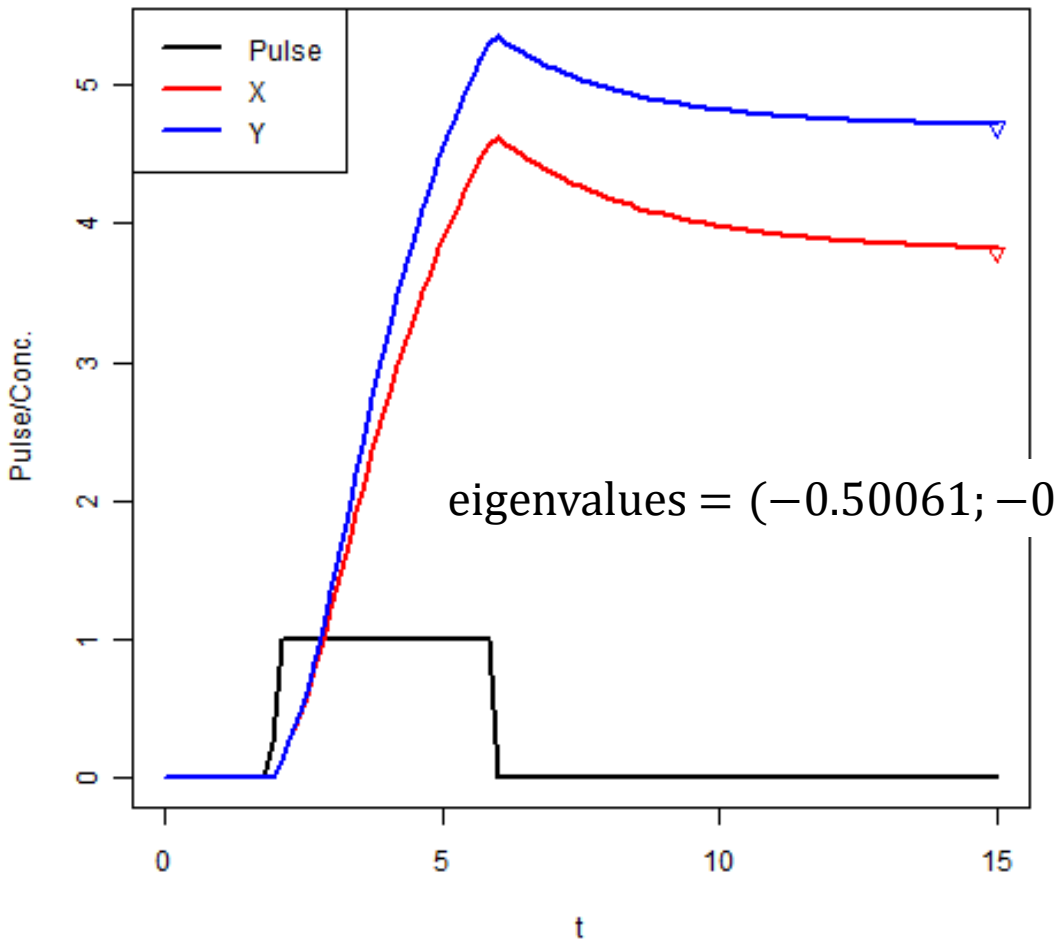
steady-state sol.

eigenvalues $= (-0.50\ ; -0.371)$

- both genes remain ON even after the pulse has terminated, i.e. defense enzyme (blue) is established
- concentrations increase even after pulse termination – approach "upper" steady state

Long-pulse response

pulse:
4 units



$$\text{eigenvalues} = (-0.50061; -0.37135)$$

- **longer pulse:** both concentrations obtain a higher level during the pulse but approach the **same** steady-state value after pulse termination

Least Squares Fit with Non-linear Optimization

$$0 = \alpha_x - \beta_x \cdot x - \beta_{xy} \cdot h\left(y, K_{xy}\right) \cdot x$$
$$0 = \alpha_y - \beta_y \cdot y - \beta_{xy} \cdot h\left(x, K_{yx}\right) \cdot y - \beta_{yz} \cdot h\left(z, K_{yz}\right) \cdot y$$
$$0 = \alpha_z - \beta_z \cdot z$$

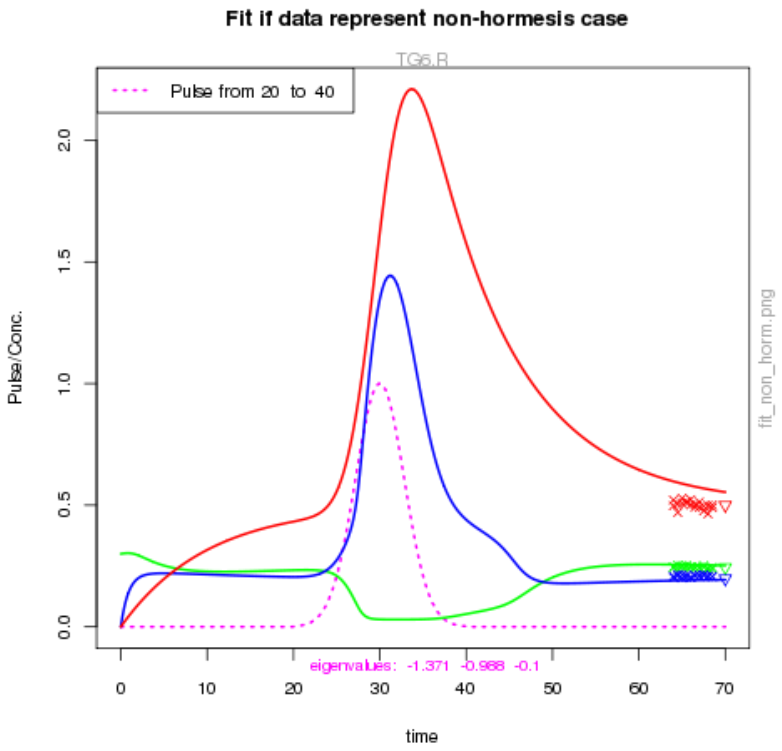
optim
constrOptim

Least squares: Minimize Sum of Squared Errors (SSE)

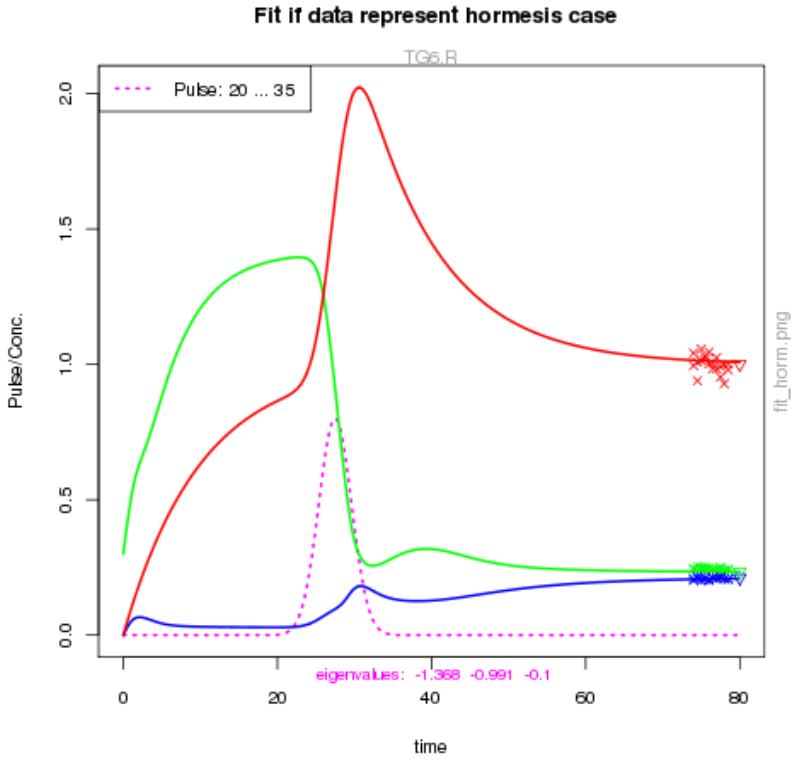
$$SSE\left(A_1, A_2, A_3\right) = \sum_{i=1}^{N} A_i^2 + B_i^2 + C_i^2 \quad \Longrightarrow \quad \text{Minimum}$$

$$A_i = \alpha_x - \beta_x \cdot x_i - \beta_{xy} \cdot h\left(y_i, K_{xy}\right) \cdot x_i$$
$$B_i = \alpha_y - \beta_y \cdot y_i - \beta_{xy} \cdot h\left(x_i, K_{yx}\right) \cdot y_i - \beta_{yz} \cdot h\left(z_i, K_{yz}\right) \cdot y_i$$
$$C_i = \alpha_z - \beta_z \cdot z_i$$

Fit to experimental data

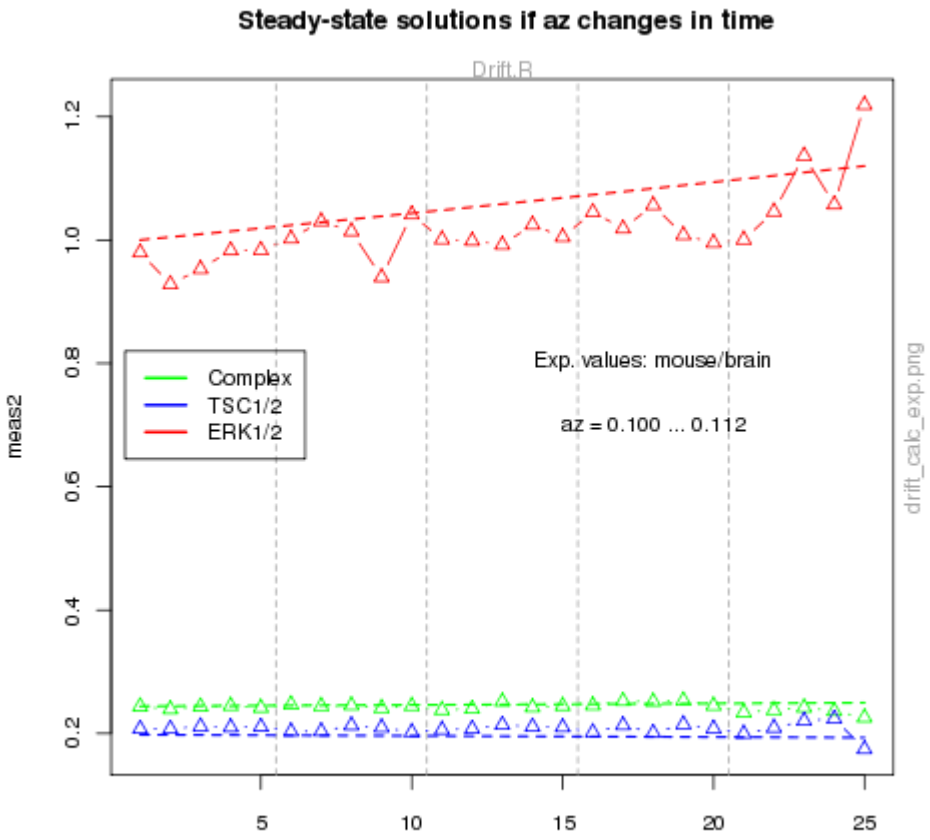


… if the data represent state 1



… if the data represent state 2 (“defense loop”)

Long-term behavior (ageing)



Increse of ERK1/2 and stationarity of both TSC1/2 and the "complex" can (only) be explained by an incresing intrinsic production rate of ERK1/2 (α_z).