Statistical Computing

Fuzzy Logic Modelling of Aging

Uwe Menzel, 2011

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Contents

1. [GerontoSys project: Motives and goals]
2. Why using fuzzy models?
3. How does a fuzzy model work?
4. The Bionet software for fuzzy modelling
5. Example of a fuzzy calculation
6. Improving the model with machine learning
7. Things to include into a model of ageing

1. GerontoSys: Motives and goals

- How/why do organisms get older (and die)?
- **Hypothesis**: generic (molecular) model of aging exists
- Influence of oxydative stress on ageing
  - Reactive Oxygen Species (ROS) [“free radicals”] O_2^-
  - ROS are unavoidable (mitochondria)
  - ROS degrade cells (proteins, nuclear DNA, mtDNA, …)
  - **BUT**: mild oxydative stress activates maintenance/repair networks working **against** accumulation of cell damage
  - Can mild oxydative stress promote healthy aging?

“Traditional” assumptions

- D. Harman, 1956: Free radical theory of aging
- “free radicals” produce cumulative damage of cells and shorten lifespan
- drugs (antioxydants) 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)

Michael Ristow [a,b,*], Kim Zarse [a]

[a] Dept. of Human Nutrition, Institute of Nutrition, University of Jena, 29 Dornburger Str., Jena D-07743, Germany
[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]

[a]Department of Human Nutrition, Institute of Nutrition, University of Jena, Jena D-07743, Germany; [b]German Institute of Human Nutrition, Potsdam-Rehbrücke D-14558, Germany; [c]Department of Medicine, University of Leipzig, Leipzig D-04103, Germany; [d]Institute of Clinical Chemistry and Laboratory Medicine, University of Jena, Jena D-07743, Germany; and [e]Research Division, Joslin Diabetes Center, Harvard Medical School, Boston, MA 02215

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. DeWeal[4] Aaron 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.

New findings

Revisiting the free radical theory using next-generation sequencing technology

William C. Burhans and Martin Weinberger

Departmentof Molecular and Cellular Biology, Roswell Park Cancer Institute, Buffalo NY 14263, USA

Commentary on: *Timmermann B. et al. A new dominant peroxidredoxin allele identified by whole-genome re-sequencing of random mutagenized yeast causes oxidant-resistance and premature aging. Aging 2010; 2: this issue.*

E-mail: *wburhans@buffalo.edu*

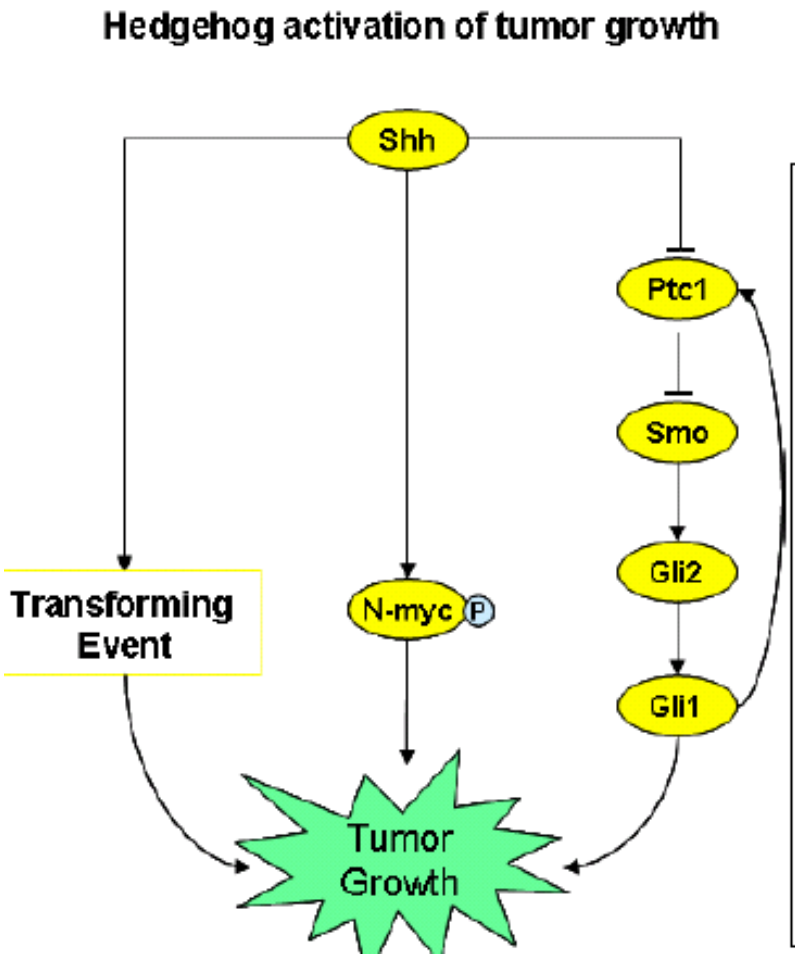
New findings

- “some” evidence that a certain amount of free radicals leads to an improvement of the orgamism’s antioxidant defense
- protective against diseases and age-related processes associated with oxidative stress
- [“vaccination”]

GerontoSys

- Role of oxidative stress (ROS) in ageing
- Animal models (worm, fish, mouse) and human – generic model?
- RNA-Seq data
- discover regulatory networks that describe ROS-induced ageing
- identify potential biomarkers for ROS-associated ageing
- support personalized strategies for healthy ageing

2. Why using fuzzy models?



Rules for Hedgehog model

1. If Shh is medium or higher, Transforming Event is on.
2. NmycP expression is proportional to the level of Shh.
3. Ptc1 expression rate is inversely proportional to Shh level; Gli1 must be low or higher for Ptc1 expression. Gli1 partially counters the repression of Shh: the effects of each on the reaction are averaged.
4. Smo expression level is very high if Ptc1 level is zero, very low if Ptc1 is very low to medium, and is zero if Ptc1 is high or very high.
5. Gli2 expression level is proportional to Smo level.
6. Gli1 expression level is proportional to Gli2 level.
7. Tumor Growth: (1) is proportional to the level of NmycP, (2) requires the transforming event level to be high, (3) is zero unless Gli1 is medium high or higher.
8. The proteins NmycP, Ptc1, Smo, Gli1 and Gli2 decay at a constant rate proportional to their concentrations.

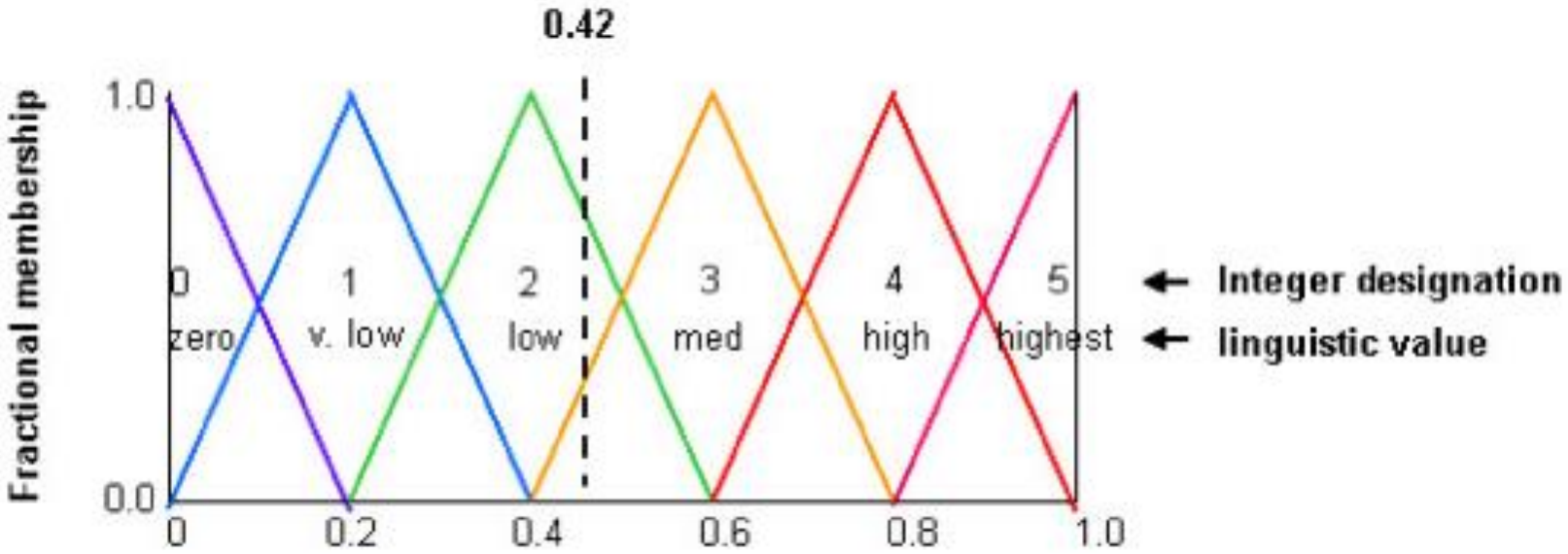
Source: W. Bosl. Systems biology by the rules: hybrid intelligent systems for pathway modeling and discovery. BMC Systems Biology 2007

What Fuzzy models can do:

- "translate" linguistic information to numbers and vice versa!
- start with little data …
- … but refine models more and more when additional data is available
- incorporate expert knowledge (JenAge, literature, databases, …)
- draw logical conclusions about functionality of networks even if input data is imprecise

3. How does a fuzzy model work?

"Fuzzyfication":

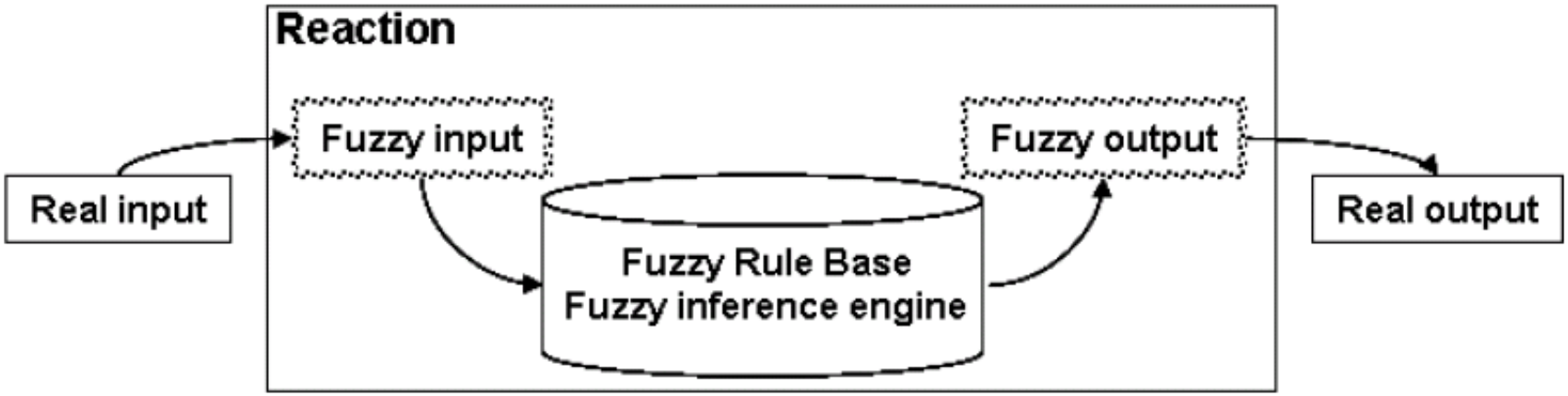


Source: W. Bosl. Systems biology by the rules: hybrid intelligent systems for pathway modeling and discovery. BMC Systems Biology 2007

$$x = 0.42 = 0.75 \cdot low + 0.25 \cdot medium$$

Can work with fuzzy rules now !!

The Fuzzy Engine



Source: W. Bosl. Systems biology by the rules: hybrid intelligent systems for pathway modeling and discovery. BMC Systems Biology 2007

$x = 0.4 \rightarrow x = \text{low} \rightarrow$

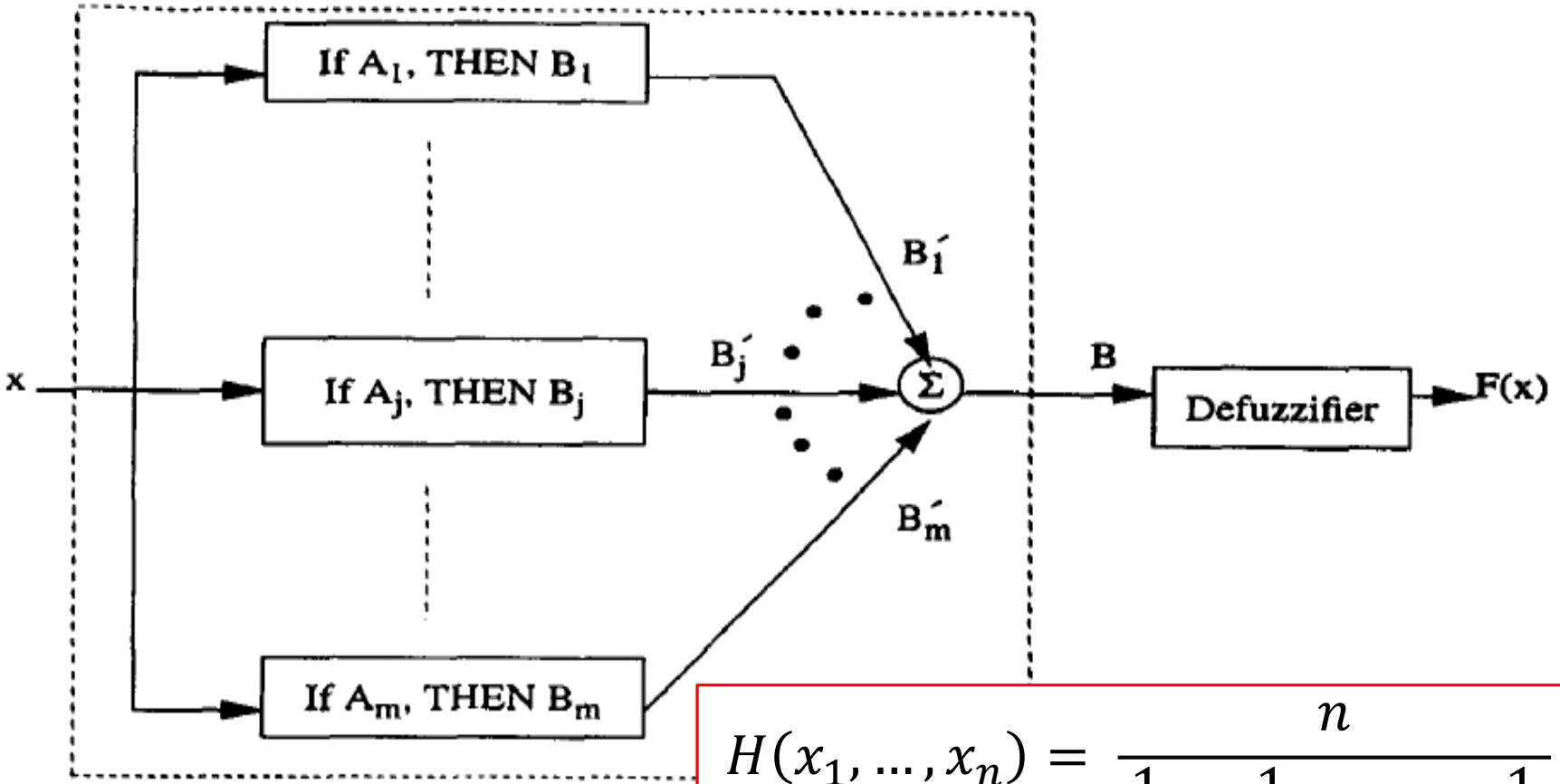
$\text{if } (x = \text{low}) \text{ then } y = \text{medium} \rightarrow$

$y = \text{medium} \rightarrow y = 0.6$

large networks can be simulated!

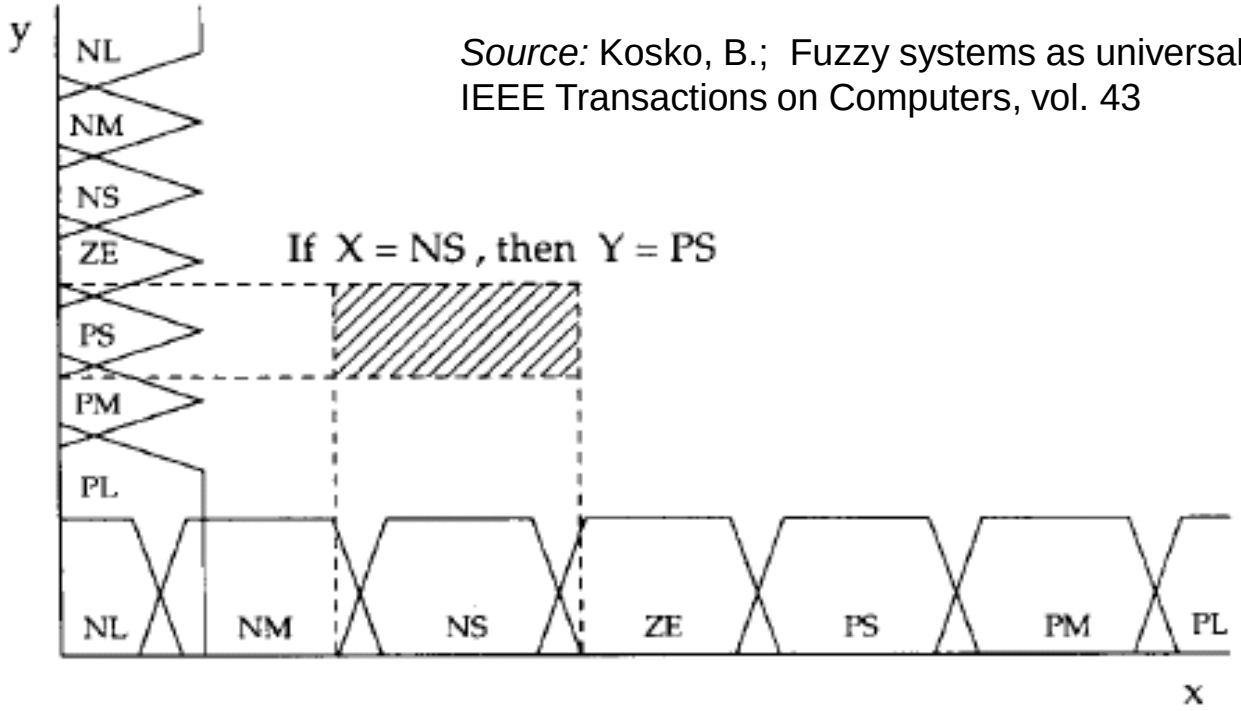
Averaging

Many reactions can contribute to the reaction rate of a given substrate $\rightarrow$ averaging



$$H(x_1, \ldots, x_n) = \frac{n}{\frac{1}{x_1} + \frac{1}{x_2} + \ldots + \frac{1}{x_n}}$$

Approximation of real functions by a fuzzy model



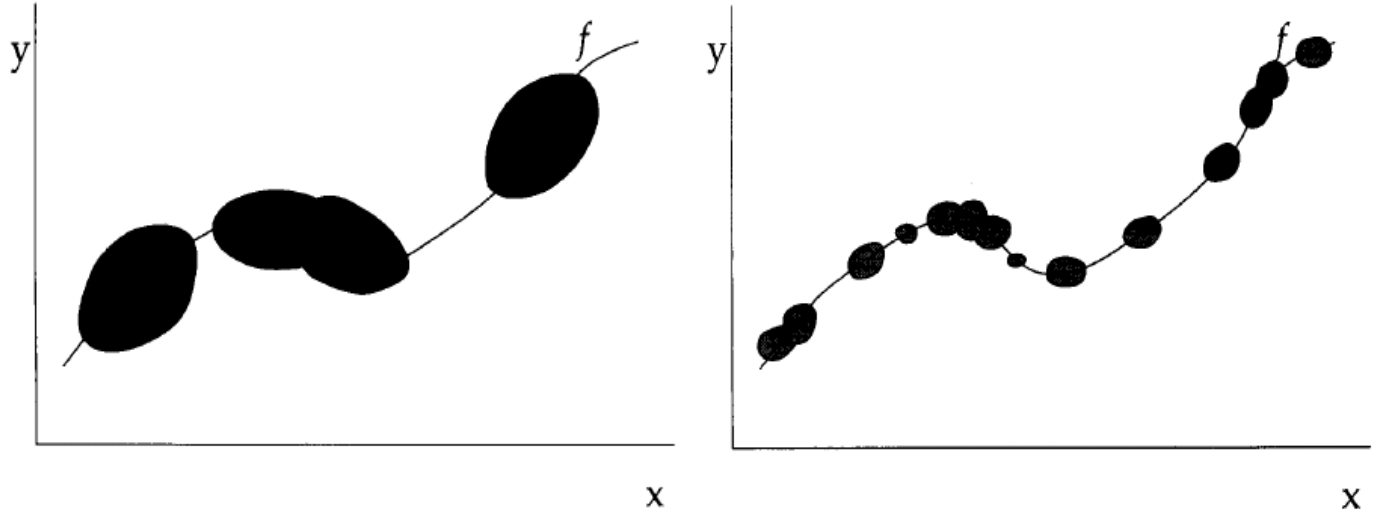
Source: Kosko, B.; Fuzzy systems as universal approximators, IEEE Transactions on Computers, vol. 43

Real function: if $x = 3$ then $y = 9$ (for the function $y = x^2$)

Fuzzy patch: if x is between 2 and 4, then y is between 8 and 10

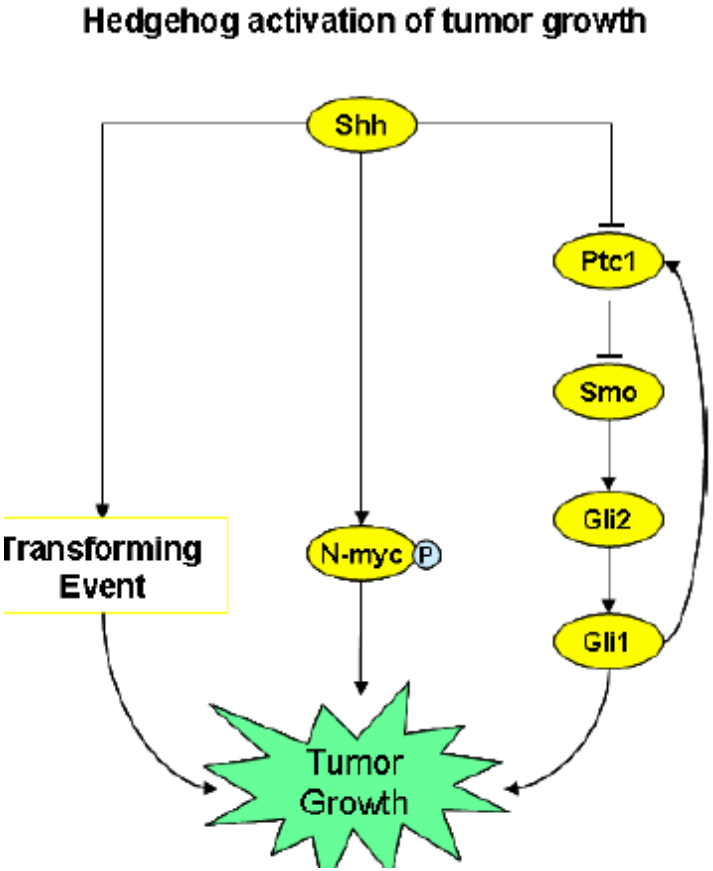
Refinement of the fuzzy model

Source: Kosko, B.; ibidem



- **Left**: Four large patches cover part of the graph of the unknown function (rough model). Fewer patches decrease computation effort but decrease accuracy.
- **Right**: Smaller patches cover the function better but at greater computational cost. A large number of fuzzy rules covers the graph with arbitrary accuracy.

4. The Bionet software for fuzzy modelling



Shh – sonic hedgehog, protein; **Ptc1** –Patched-1, protein, PTCH1 gene;
SMO – Smoothened, protein, SMO gene; Gli – protein (Glioblastoma);
Nmyc – protein, transcription factor (?)

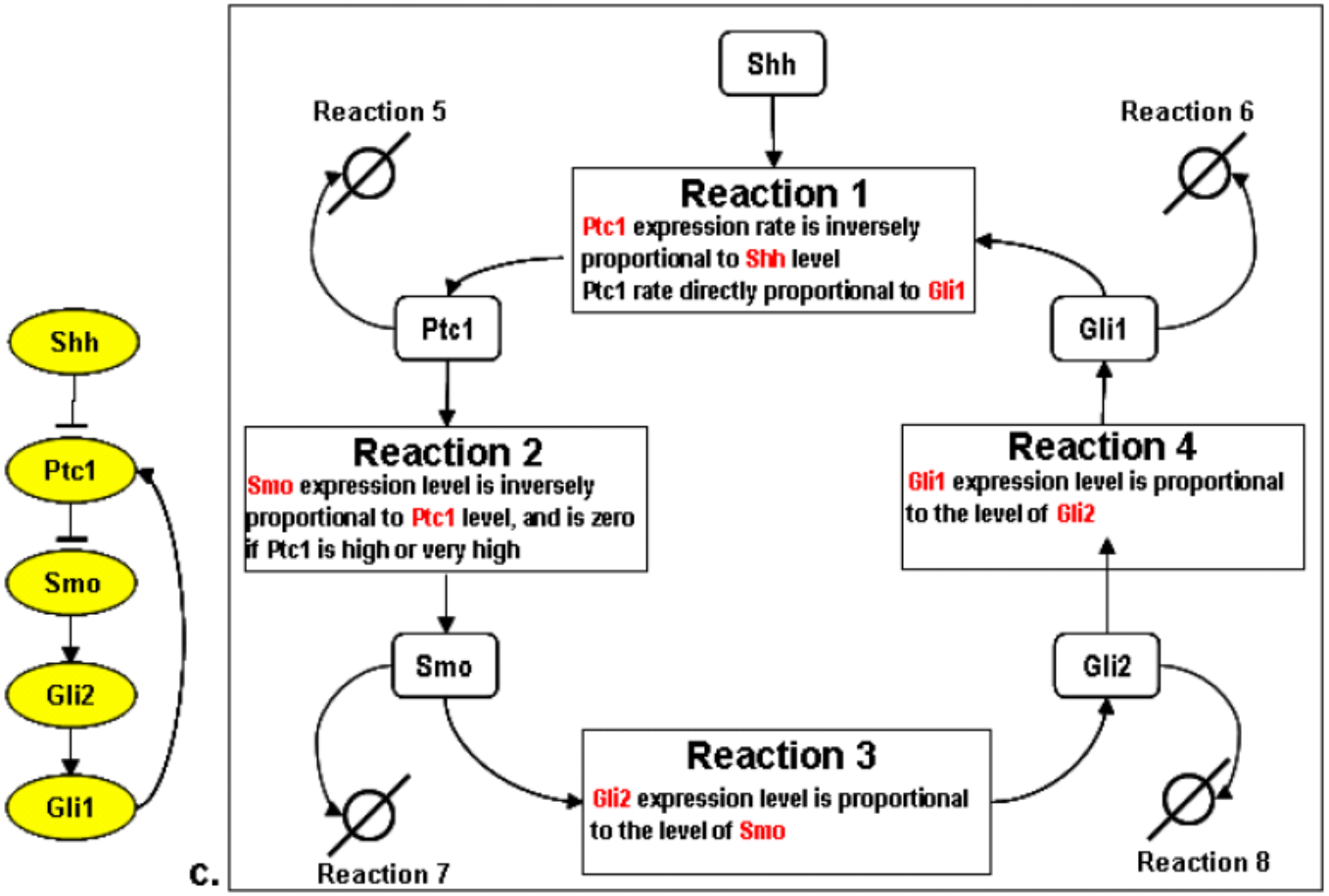
The Bionet software for fuzzy modelling

W. Bosl. Systems biology by the rules: hybrid intelligent systems for pathway modeling and discovery. BMC Systems Biology 2007

A. Kriete et al. Rule-Based Cell Systems Modelof Aging using Feedback Loop Motifs Mediated by Stress Response. PloS Computational Biology, vol. 6 (20100)

- https://simtk.org/home/bionet
- java -jar ~/Adrenal/Bionet.jar inputfile.net
- nodes and edges of the network have to be defined with a special syntax (*.net file)

Bionet syntax



Bionet syntax

```
starttime 0
endtime 10
ntimesteps 1000

Node Shh  0.1    0.001   0.1      #  initial value and range
Node Ptc1  0.5
Node Smo   0                      # default initial value
Node Gli1  0

plot time
plot Shh
plot Smo
plot Gli1
plot Ptc1
```

Reaction syntax

Reaction AAA-Production 0.002 # low rate

pro AAA 5 5 5 5 5 0 # default

sub BBB 0 1 2 3 4 5

inh CCC 5 2 1 1 0 0

act DDD 0 1 2 3 4 5

"**inh CCC 5 2 1 1 0 0**"
yields the following contributions to the AAA-reaction rate:

CCC	zero	very low	low	medium	high	highest
CCC	0	1	2	3	4	5
Δr	5	2	1	1	0	0

Bionet syntax

```
Reaction  Smo_production  0.002                    # Reaction 2
   pro Smo
   inh Ptc1 5 2 1 1 0 0
# “Smo expression is inversely proportional to Ptc1 level,
#  and is zero if  Ptc1 is high or very high”
Reaction  Smo_decay   0.002                        # Reaction 7
   sub Smo
# Smo decays (spontaneously) with a rate of 0.002
Reaction Gli1_production                           # Reaction 4
   pro Gli1
   act Gli2 0 1 2 3 4 5
# Gli1 expression level is proportional to the level of Gli2
```

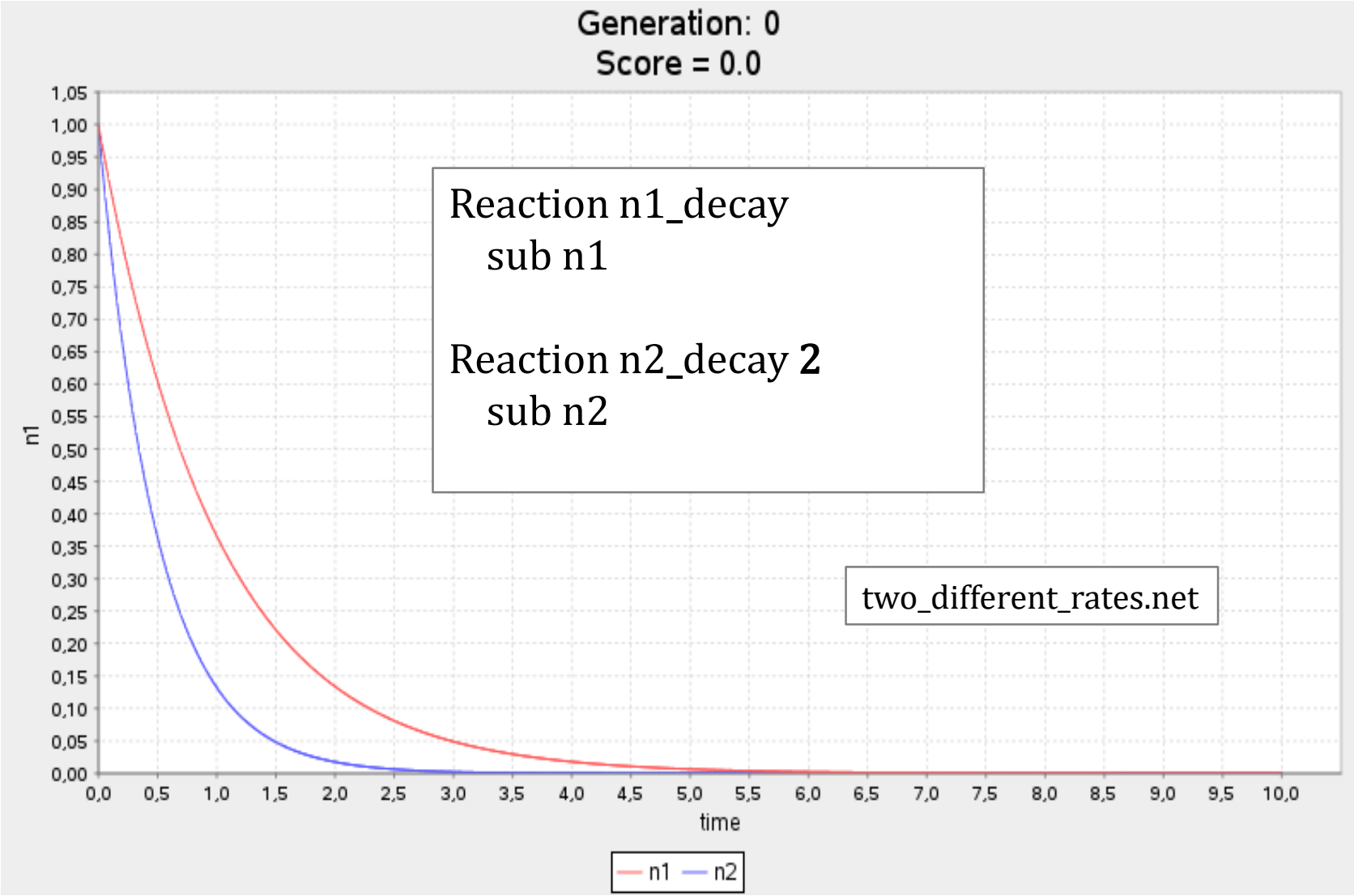
Bionet syntax

- Initial values and range can be redefined
  - default:
  - node Smo 0 0.0 1.0
- Stoichiometry and centroids can be redefined
  - default:
  - sub Smo 0 1 2 3 4 5 stoi -1 0.0 0.2 0.4 0.6 0.8 1.0

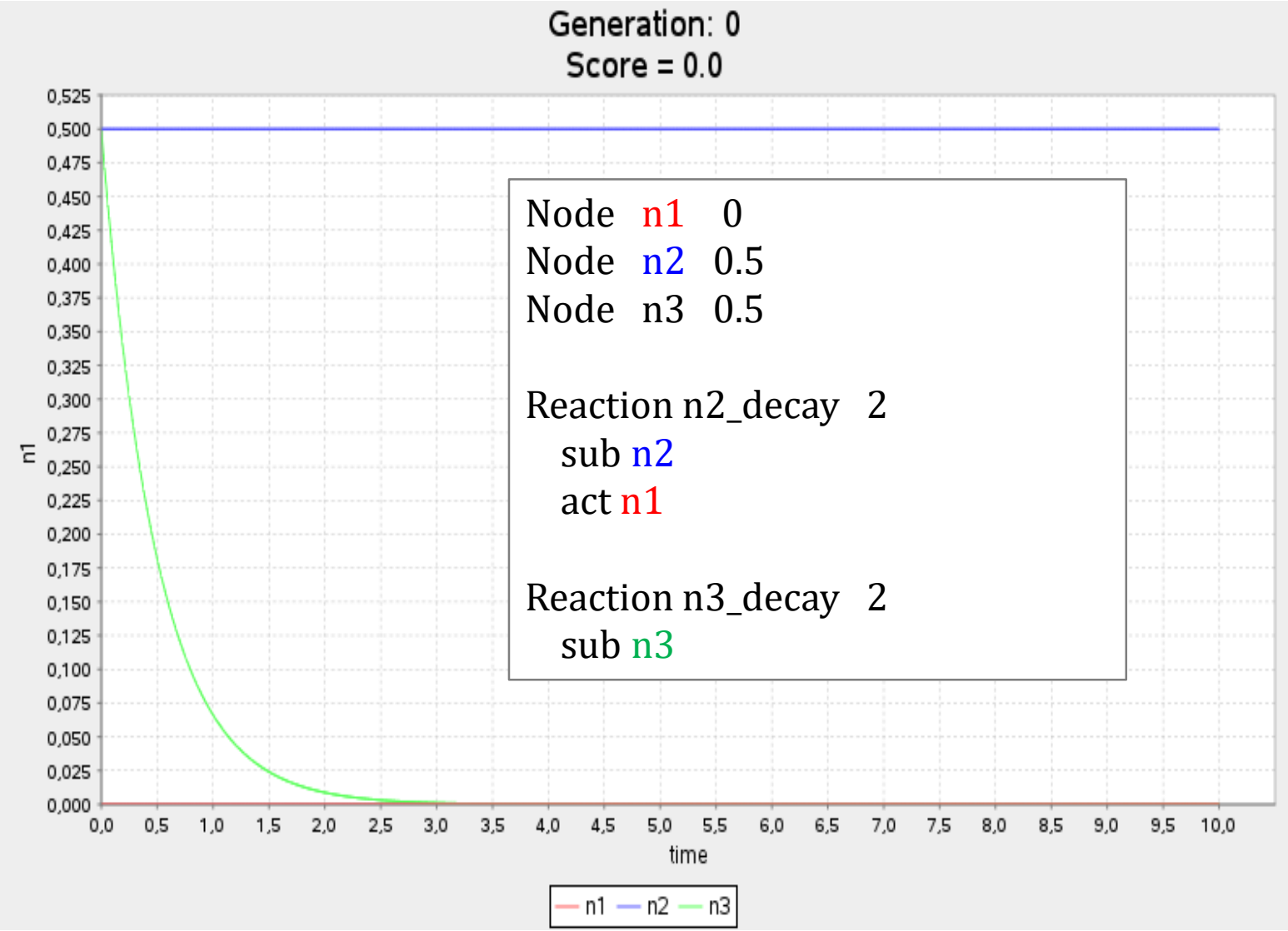
Δr

centroids

Reaction rate

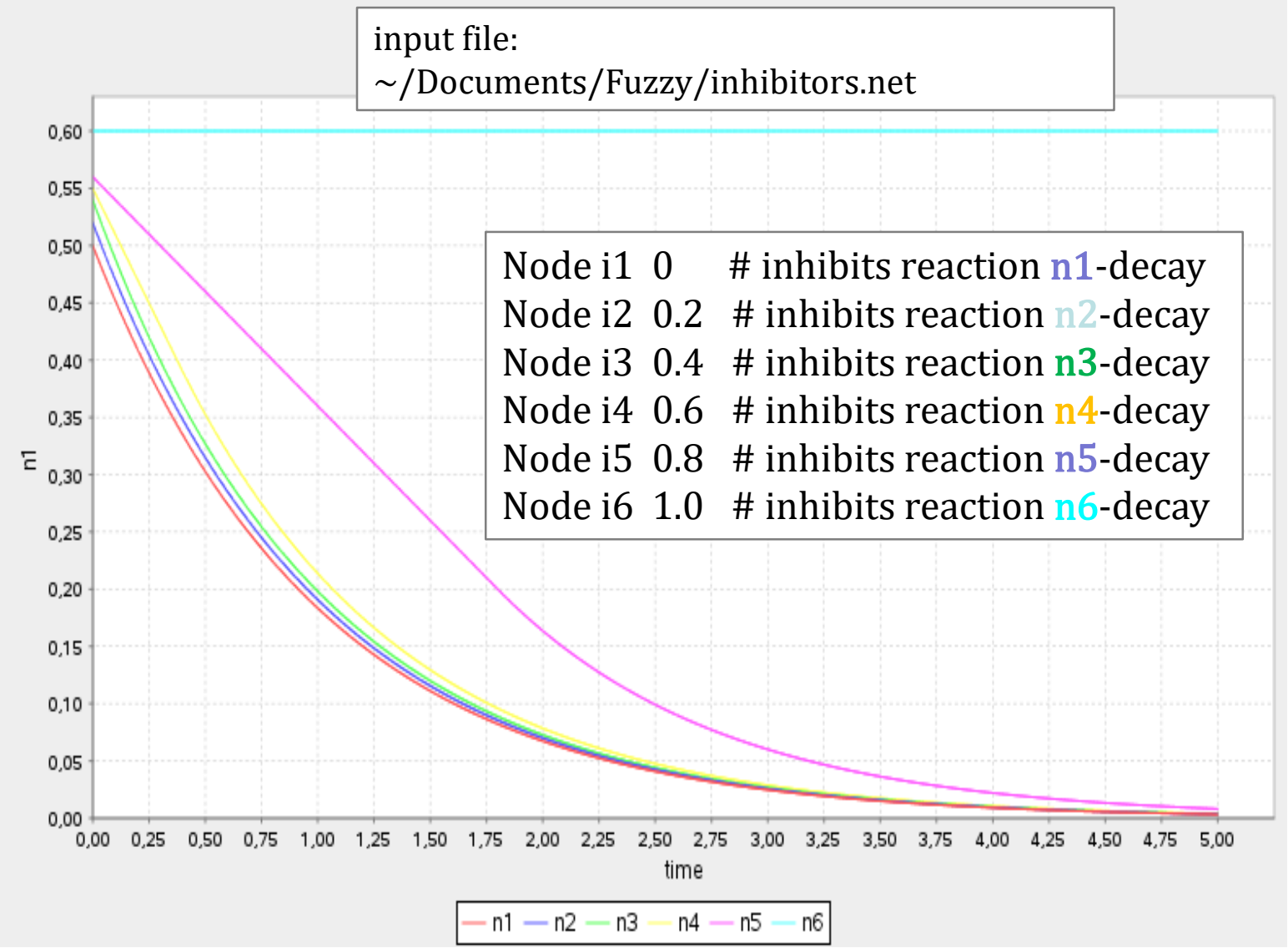


Activator

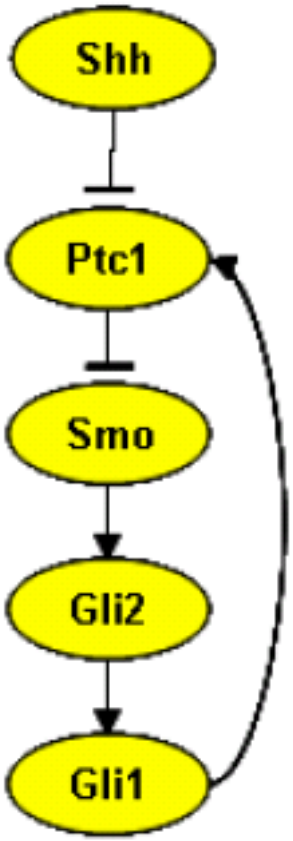


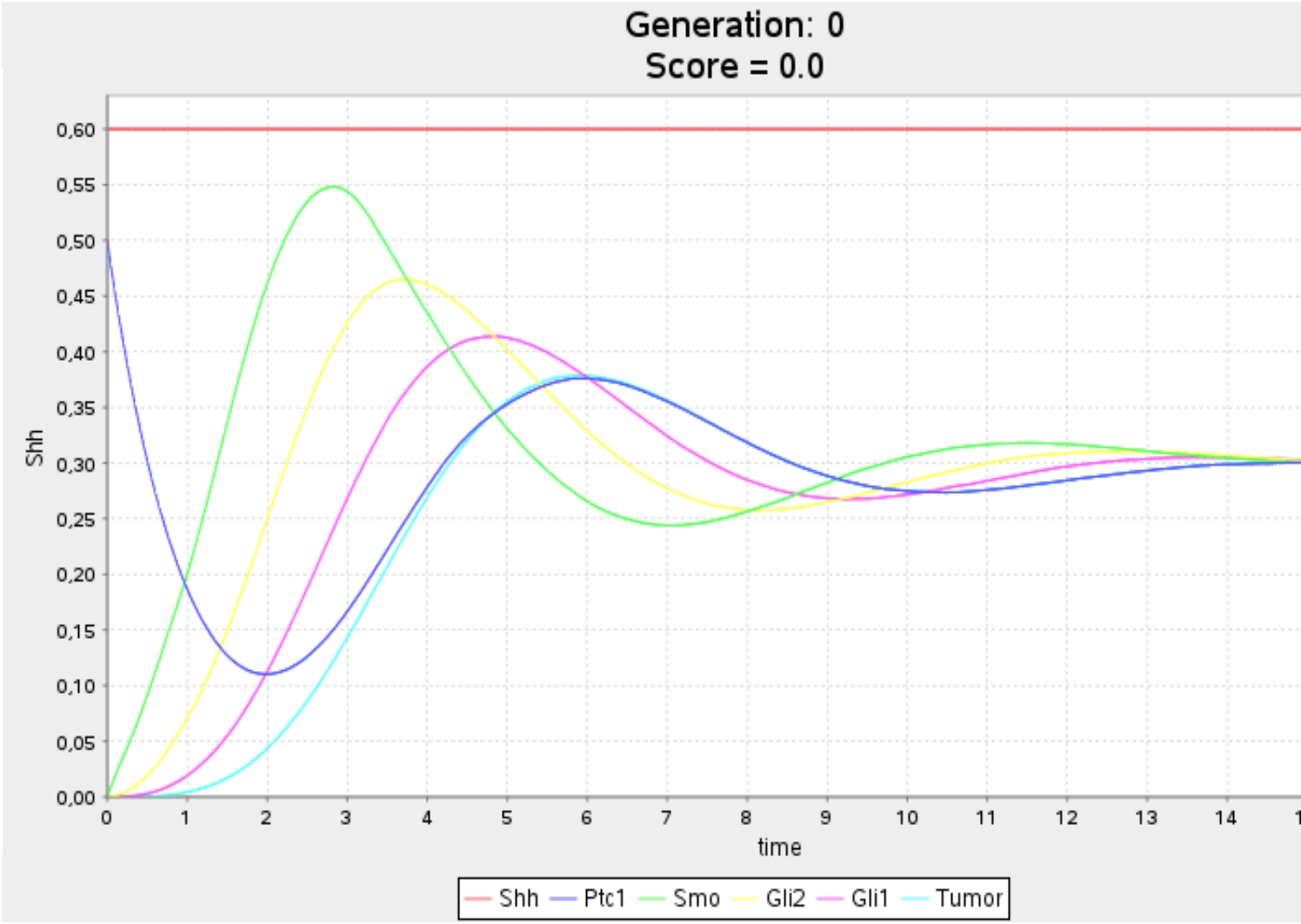
If the activator n1 (red) is not present, the node n2 (blue) cannot decay

Inhibitor

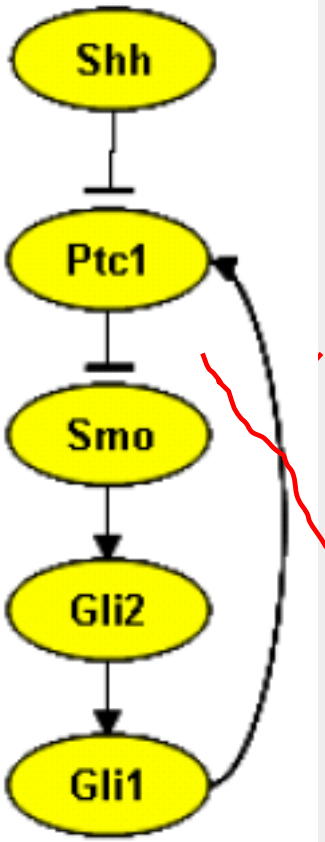

input file:
~/Documents/Fuzzy/inhibitors.net
Node i1 0 # inhibits reaction n1-decay
Node i2 0.2 # inhibits reaction n2-decay
Node i3 0.4 # inhibits reaction n3-decay
Node i4 0.6 # inhibits reaction n4-decay
Node i5 0.8 # inhibits reaction n5-decay
Node i6 1.0 # inhibits reaction n6-decay
n1
0,60
0,55
0,50
0,45
0,40
0,35
0,30
0,25
0,20
0,15
0,10
0,05
0,00
0,00 0,25 0,50 0,75 1,00 1,25 1,50 1,75 2,00 2,25 2,50 2,75 3,00 3,25 3,50 3,75 4,00 4,25 4,50 4,75 5,00
time
n1 n2 n3 n4 n5 n6

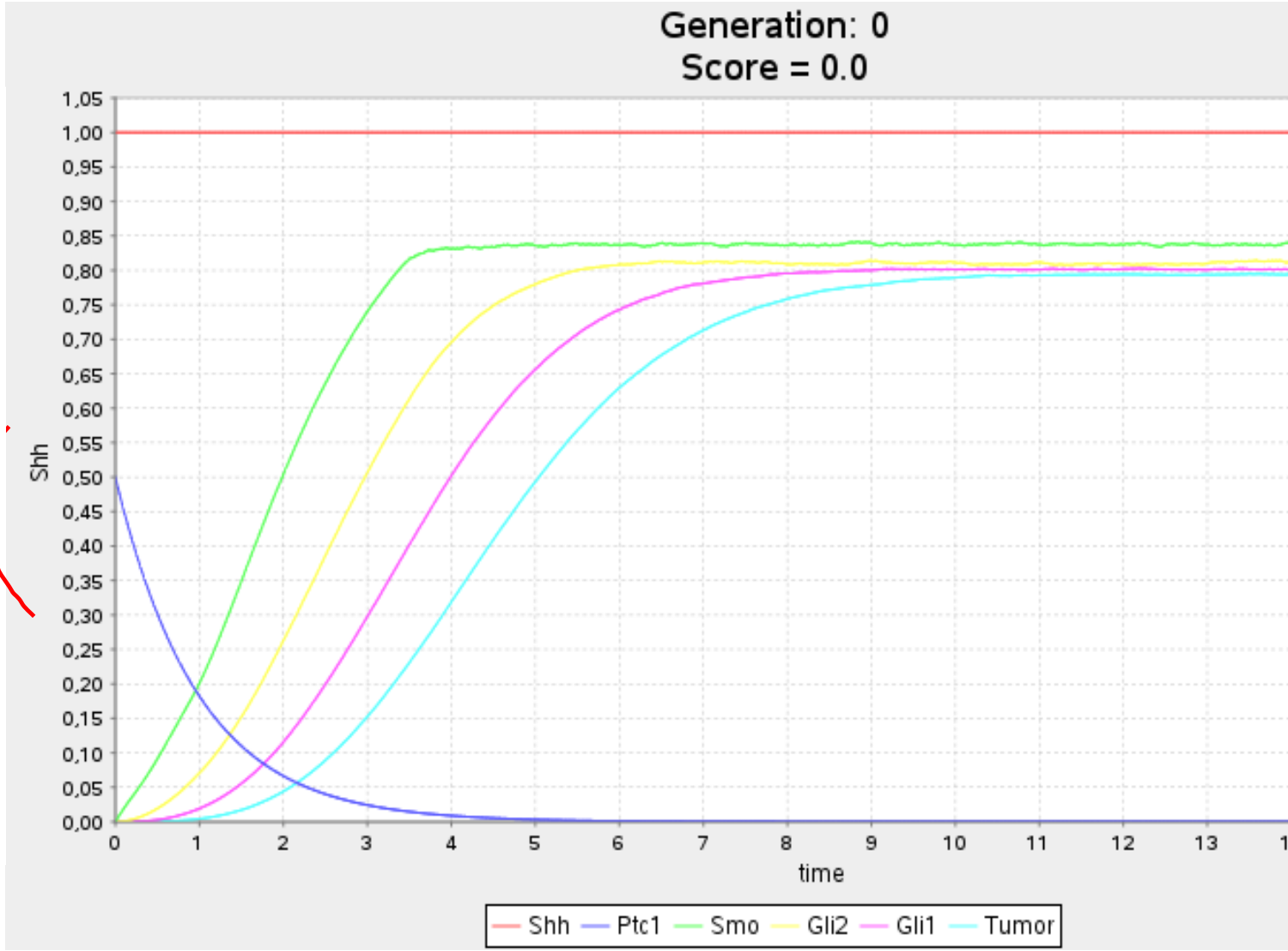
Feedback - oszillation





No feedback - growth



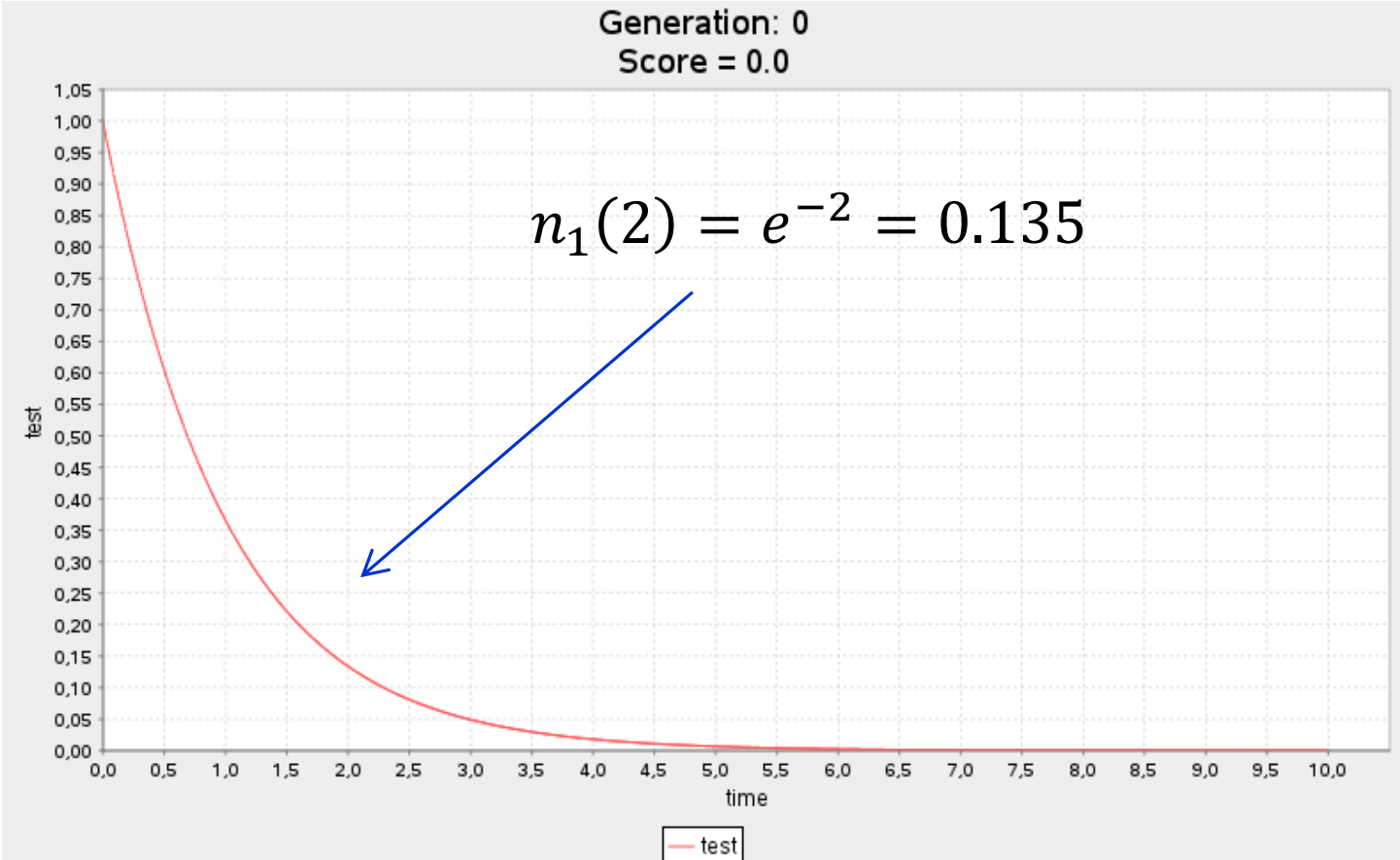


Can the Fuzzy-model solve a differential equation?

$$\frac{dn_1}{dt} = -1 \cdot n_1$$

?

Reaction n1- decay 1
sub n1



Can the Fuzzy-model solve a differential equation?

$$\frac{dn_1}{dt} = -1 \cdot n_1 + 1 \cdot n_2$$

$$\frac{dn_2}{dt} = 3 \cdot n_1 + -2 \cdot n_2$$

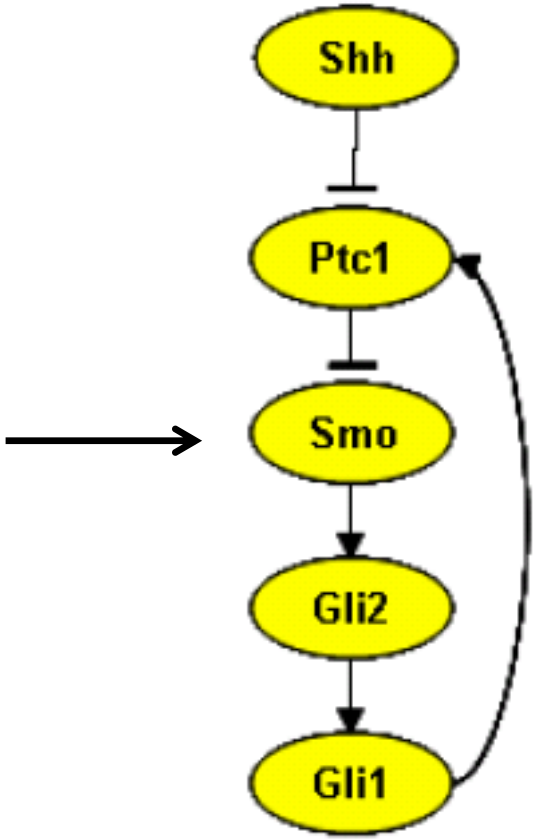
Reaction n1-change 1
sub n1
pro n2
Reaction n2-change 2
sub n2
pro n1

uses deSolve

```
source("linODE.R")
a <- matrix(c(-1, 1, 2, -2), nrow = 2, byrow = TRUE)
init <- c(y1 = 0.5, y2 = 0.5)
tmax = 10
nrsteps = 200
res ← linODE(a, init, tmax, nrsteps)
```

5. Example of a fuzzy calculation

Example 1




```
Reaction Smo_production    0.002
    pro Smo  5  5 5 5 5 0
    inh Ptc1   5 2 1 1 0 0
    #  Ptc1 inhibits expression of Smo
```

Example 1

Reaction Smo_production 0.002
pro Smo 5 5 5 5 5 0
inh Ptc1 5 2 1 1 0 0

Contributions to the Smo-reaction rate:

Smo	zero	very low	low	medium	high	highest
Smo	0	1	2	3	4	5
Δr	5	5	5	5	5	0

Ptc1	zero	very low	low	medium	high	highest
Ptc1	0	1	2	3	4	5
Δr	5	2	1	1	0	0

Initial values: ⇒ Fuzzification:

$t = t_0$

$Smo = 0.85;\ \ Ptc1 = 0.75$

$Smo = 0.25 \cdot high + 0.75 \cdot highest$

$Ptc1 = 0.4 \cdot med + 0.6 \cdot high$

Example 1

$$\text{Smo} = 0.25 \cdot \text{high} + 0.75 \cdot \text{highest}$$

$$\text{Ptc1} = 0.4 \cdot \text{med} + 0.6 \cdot \text{high}$$

Smo	*Ptc1*
$0.25 \cdot high$	$0.4 \cdot med$
$0.75 \cdot highest$	$0.6 \cdot high$

Standard Additive Model (SAM):

combine all Smo-components with all Ptc1-components and calculate the corresponding contributions to the reaction rate

1. $\text{Smo} = 0.25 \cdot \text{high}$ with $\text{Ptc1} = 0.4 \cdot \text{med}$
2. $\text{Smo} = 0.25 \cdot \text{high}$ with $\text{Ptc1} = 0.6 \cdot \text{high}$
3. $\text{Smo} = 0.75 \cdot \text{highest}$ with $\text{Ptc1} = 0.4 \cdot \text{med}$
4. $\text{Smo} = 0.75 \cdot \text{highest}$ with $\text{Ptc1} = 0.6 \cdot \text{high}$

$\text{Smo} = \text{high}\ (0.25) \rightarrow \Delta r = 5$
$\text{Smo} = \text{highest}\ (0.75) \rightarrow \Delta r = 0$
$\text{Ptc1} = \text{med}\ (0.4) \rightarrow \Delta r = 1$
$\text{Ptc1} = \text{high}\ (0.6) \rightarrow \Delta r = 0$

Smo	high	highest
Smo	4	5
Δr	5	0

Ptc1	mediu m	high
Ptc1	3	4
Δr	1	0

Example 1

1. $\text{Smo} = 0.25 \cdot \text{high}$ with $\text{Ptc1} = 0.4 \cdot \text{med}$
2. $\text{Smo} = 0.25 \cdot \text{high}$ with $\text{Ptc1} = 0.6 \cdot \text{high}$
3. $\text{Smo} = 0.75 \cdot \text{highest}$ with $\text{Ptc1} = 0.4 \cdot \text{med}$
4. $\text{Smo} = 0.75 \cdot \text{highest}$ with $\text{Ptc1} = 0.6 \cdot \text{high}$

$\text{Smo} = \text{high}\ (0.25) \rightarrow \Delta r = 5$
$\text{Smo} = \text{highest}\ (0.75) \rightarrow \Delta r = 0$
$\text{Ptc1} = \text{med}\ (0.4) \rightarrow \Delta r = 1$
$\text{Ptc1} = \text{high}\ (0.6) \rightarrow \Delta r = 0$

$$\Delta r = 0.25 \cdot 0.4 \cdot H(5,1) + 0.25 \cdot 0.6 \cdot H(5,0) + 0.75 \cdot 0.4 \cdot H(0,1) + 0.75 \cdot 0.6 \cdot H(0,0)$$
$$= 0.1 \cdot H(5,1) = 0.1 \cdot \frac{2}{\frac{1}{5} + \frac{1}{1}} = \frac{1}{6} \approx \underline{\underline{0.17}}$$

```
Reaction Smo_production  0.002
   pro Smo  5 5 5 5 5 0
   inh Ptc1   5 2 1 1 0 0
```

Example 1

- The reaction rate was 0.002 which is used as a scaling factor here:

$$\Delta r = 0.17 \cdot 0.002 = 0.00034$$

- Calculation of the variables on the next time-step:

$$Smo(t + \Delta t) = Smo(t) + \Delta r = 0.85 + 0.00034 = 0.85034$$

Ptc1 is not changed in this reaction because it acts as an inhibitor (catalyzer)

- The same procedure is repeated using the new value 0.85034 as input.

6. Improving the model with machine learning methods

- „We have implemented a simple genetic algorithm that manipulates the rules and centroids.“ (W. Bosl: Systems biology by the rules: hybrid intelligent systems for pathway modeling and discovery. BMC Systems Biology 2007)
- Source code available

Genetic algorithm

- Solving optimization and search problems by simulating natural evolution
- **Goal**: better model performance (better fit to experiment)
- Create a population of models ("generation") by randomly introducing small changes to the parent model
- Recombination, Mutation → Selection → new generation
- Selection:
  - proportion of the existing population is selected to breed a new generation
  - "individuals" are selected based on their fitness ("score"), fitter solutions are more likely to be selected
- https://en.wikipedia.org/wiki/Evolutionary_algorithm

https://en.wikipedia.org/wiki/Evolutionary_algorithm

Task: find integer numbers a, b, c, d, e that minimize the function:

$$f(a, b, c, d, e) = |a - b| + |b - c| + |c - d| + |d - e| + e - a|$$

- **Genome:** variables of **f**, i.e. the list (a,b,c,d,e).
  - examples: (1, 2, 5, 7, 0); (3, 3, -46, 36, 8); (3, -3, 19, 14, 8).
- **Recombination**: crossover of two randomly choosen parent-genomes:
  - first p alleles of the "mother" and the last 5-p alleles of the "father"
  - p is also randomly chosen between 0 and 5, e.g $p = 2$
  - "child" of M = (**1, 2**, 5, 7, 0) and F = (3, -3, **6, 6, 8**) → C = (**1, 2, 6, 6, 8**)
- **Mutations:** add -1, 0, or 1 at every position in an individual genome
  - example: (1, 2, 6, 6, 8) → (1, 3, 6, 6, 7).
  - probability of a mutation: ≈ 1% (per generation, at each position).
- **Selection:** choose primarily the fittest individuals of a generation
- **Start population:** 50 individuals, with randomly chosen genomes.
  - Every "gene" is a number between -50 and 50, e.g. (33, -3, 6, 16, -48)
- **Termination:** if average fitness does not change for 10 generations
- **Output:** genome of the single fittest individual after termination

Genetic algorithm in Bionet

nGenerations 100
populationSize 50
mutationRate 0.025

„Bionet Primer.doc“

https://simtk.org/websvn/wsvn/bionet/tags/release-3.0a/bionet/Fitness.java

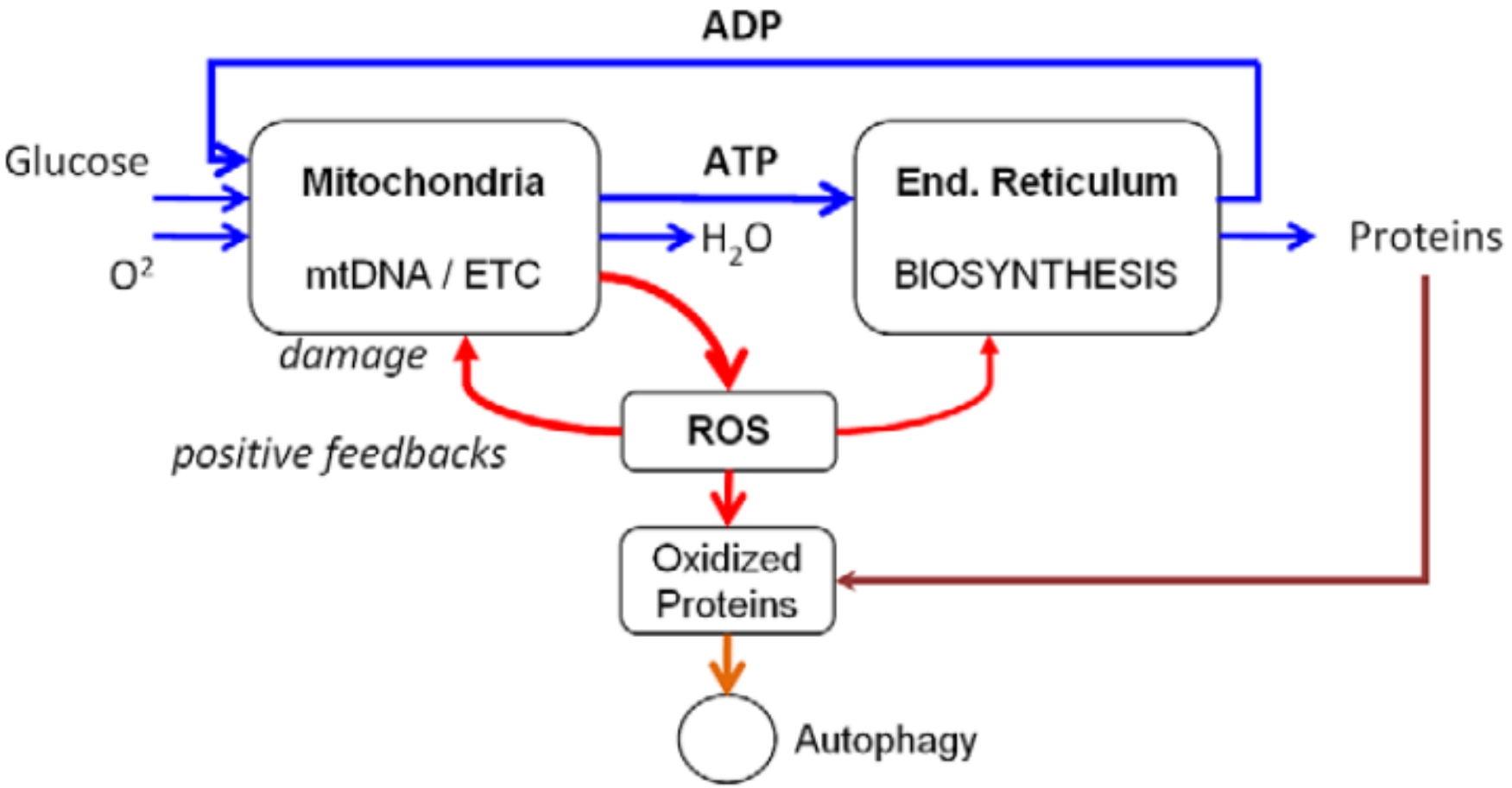
Code-snippet:

```
//obtain the fitness of the Bionet by comparison to user-defined points
 public static double leastSquare(Bionet bn) {
    bn.fitness = 0.0;
```

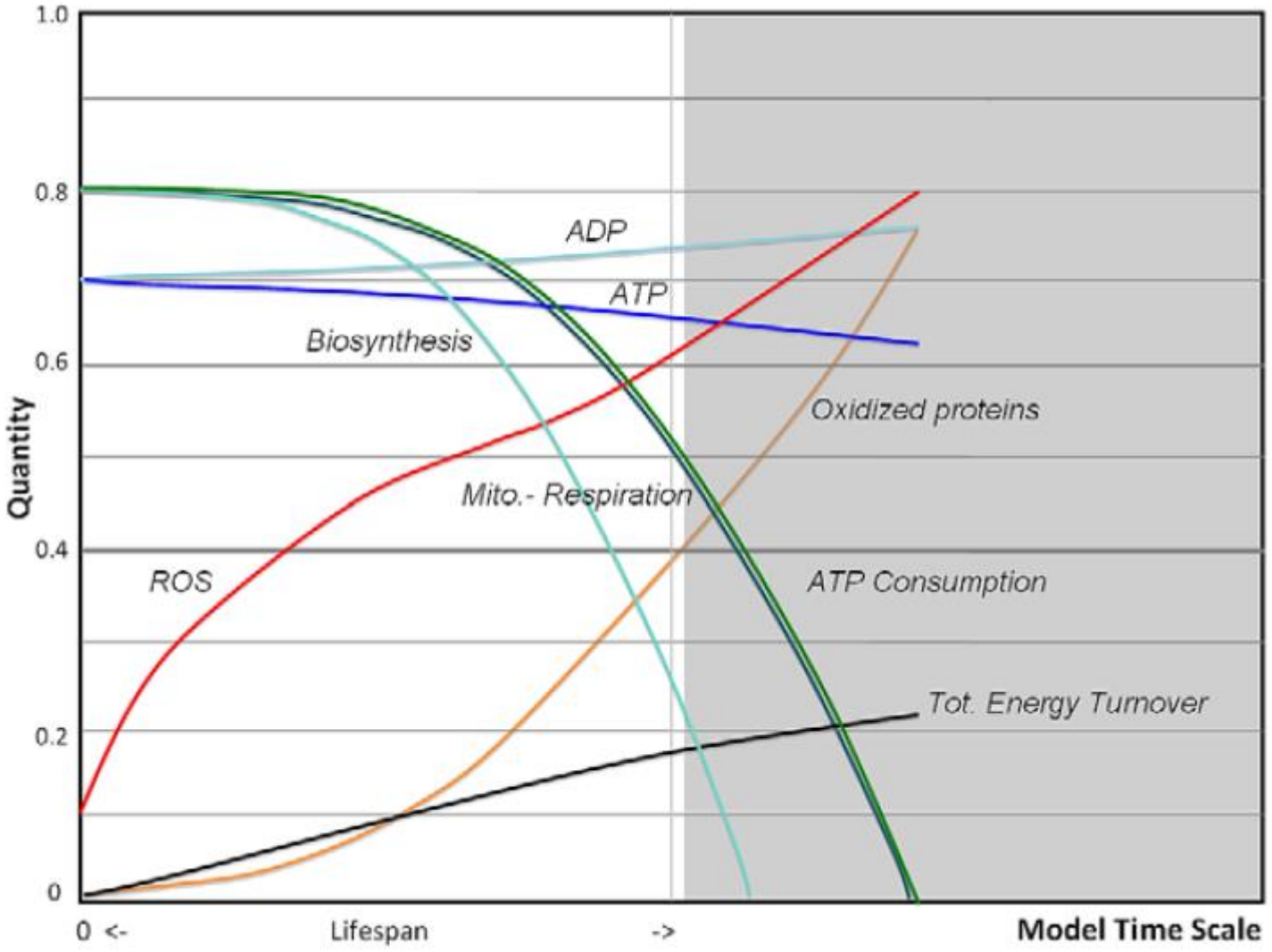
7. Things to include into an ageing model

- Combine different motifs:
  - negative and positive feedback loops, feed-forward motifs
- Short, modest ROS dosage:
  - not (much) irreversible damage to the cell but defense system activation
- The “good” guys:
  - (little)ROS, dismutase, peroxydase
- The “bad” guys:
  - (too much) ROS, AGEs, mTOR, ….

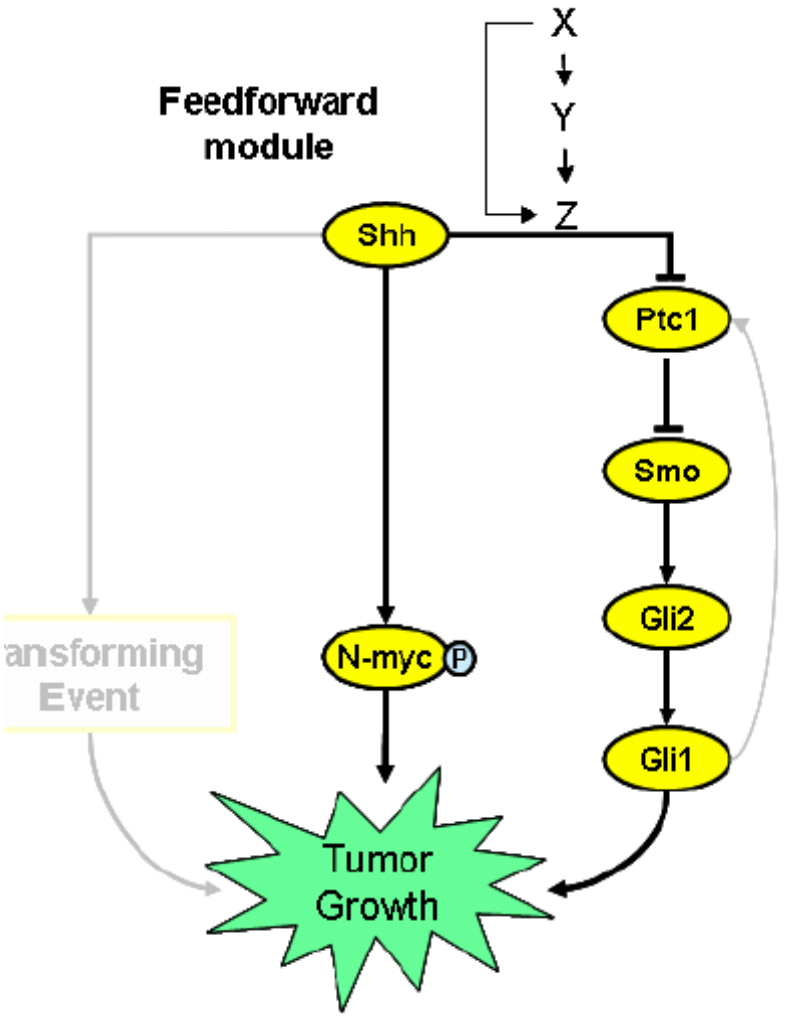
Action of ROS

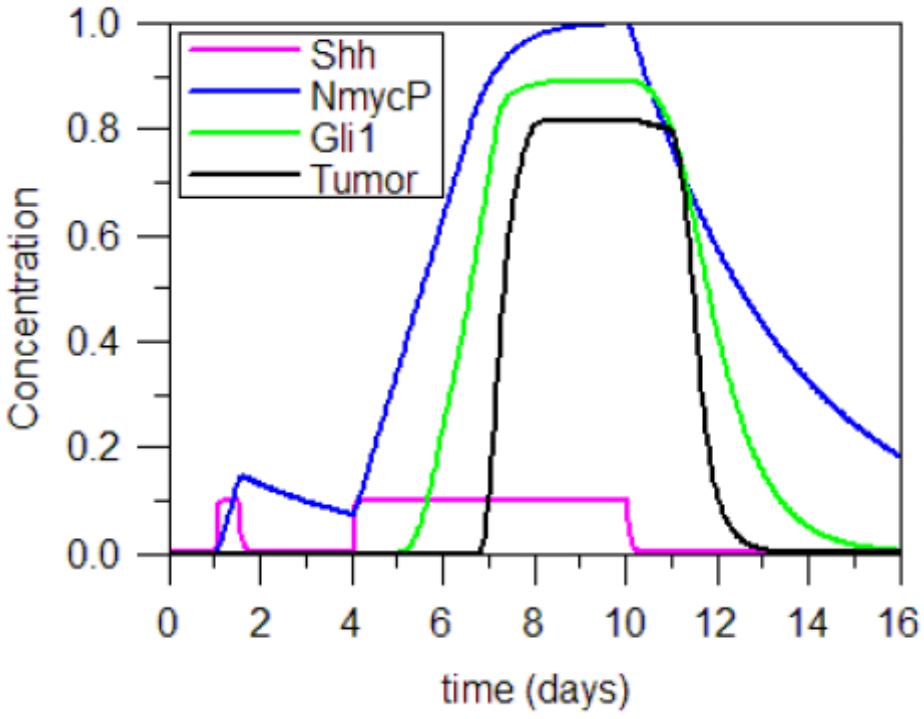


Action of ROS



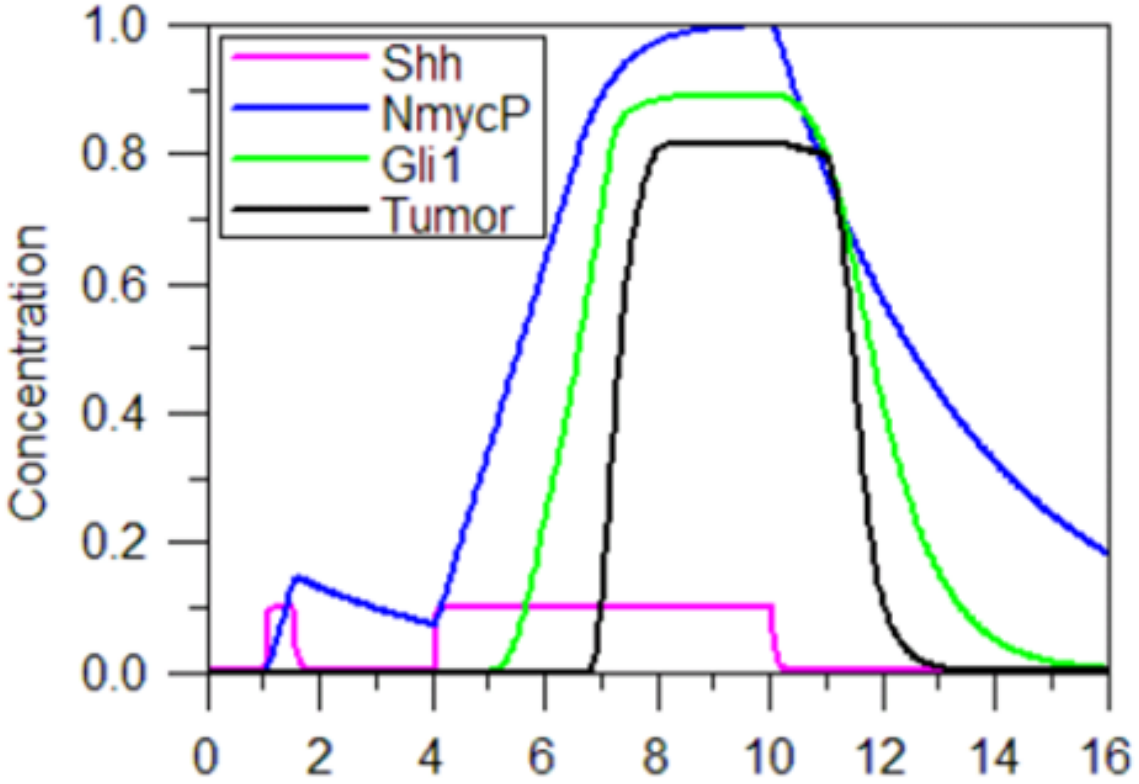
Feed-forward loop





b.

Shh is triggered from "outside". The N-mycP path is fast but the other "arm" is slow. A short Shh-pulse cannot produce tumor growth but a longer can!



Assumptions:

- Short, mild ROS pulse cannot produce AGEs and therewith age but can trigger a dismutase and/or peroxydase loop.
- However, strong and long-lasting oxydative stress can also trigger the glycation process (producing AGE) which would result in ageing.

Thanks!

TODO

- create own network using expert knowledge
- start with little data -> refine
  - runPCA, runGO
- SBML-wrapper
- implicit scheme (similar to Runge-Kutta)?
- new software (in R)?

2nd Law of Thermodynamics

- Entropy change of a system at temperature T absorbing an infinitesimal amount of thermal energy δQ in a reversible way is $\delta S = {}^{\delta Q}/_{T}$
- Live is a "mystery", seems to be a violation of the 2nd law (but isn't: does not go on in a closed system)
  - egg → chicken: just provide thermal energy
- energy required to maintain a living organism
- one day it isn't worth the energy any more …

Fuzzy Systems Are Universal Approximators

- If properly constructed, fuzzy systems can perform very complex operations. Actually, many fuzzy systems are known to satisfy the "universal approximation property".
- For any real continuous function $\psi(u)$ defined on a closed and bounded set and an arbitrary $\varepsilon > 0$, there exists a fuzzy system $f(u)$ such that $\sup_u |f(u) - \psi(u)| < \varepsilon$.
- Note, however, that all this "universal approximation property" does is guarantee that there **exists** a way to define the fuzzy system f(u) (e.g., by picking the membership function parameters). It does **not** say **how** to find the fuzzy system, which can, in general, be very difficult. Furthermore, for arbitrary accuracy, you may need an arbitrarily large number of rules.
- The value of the universal approximation property for fuzzy systems is simply: if you work hard enough at tuning, you should be able to make the fuzzy system do what you are trying to do.

Advanced Glycation Endproducts (AGEs)

- http://en.wikipedia.org/wiki/Glycation
- http://en.wikipedia.org/wiki/Advanced_Glycation_Endproduct

- Glycation (non-enzymatic glycosylation) is the result of the bonding of a protein or lipid molecule with a sugar molecule, such as fructose or glucose, without the controlling action of an enzyme
- glycation is a haphazard process that impairs the functioning of biomolecules
- glycosylation (with enzymes) occurs at defined sites on the target molecule and is required for molecule function
- AGE's are the result of a chain of chemical reactions after an initial glycation reaction
- Glycation → Amadori → Schiff base → Maillard → AGE's slow!
  - Side product: hydrogen peroxide (H_2O_2) - vicious cycle?