The Cell Cycle Switch Computes Approximate Majority

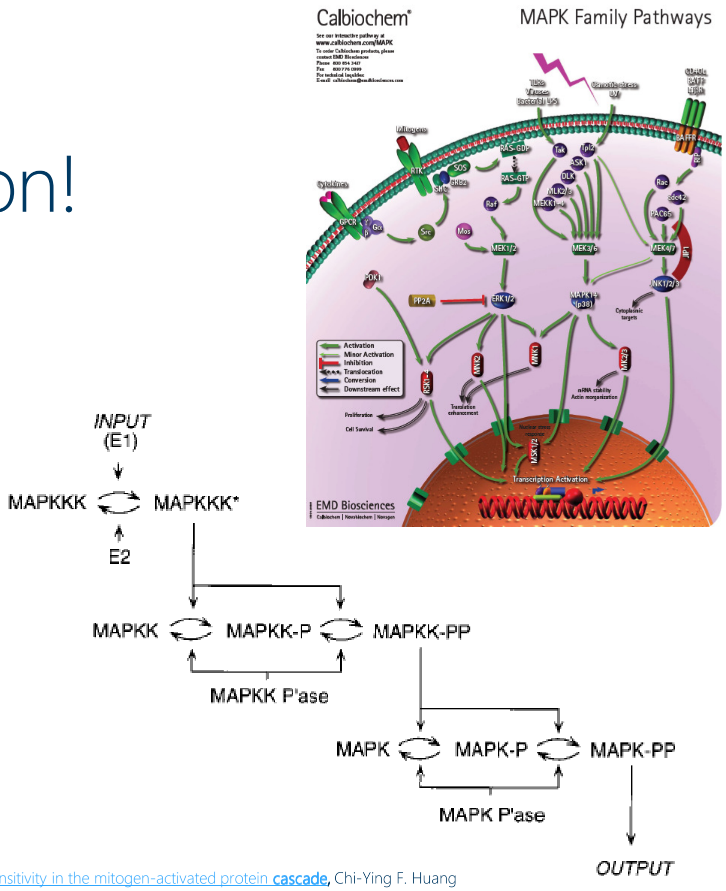
Luca Cardelli, Microsoft Research

Joint work with Attila Csikász-Nagy, CoSBi & King's College London

Aalborg, 2013-08-07

Cells Compute

- No survival without computation!
  - Finding food
  - Avoiding predators
- How do they compute?
  - Clearly doing “information processing”
  - Based on complex, higher-order interactions
    - MAPKKK = MAP Kinase Kinase Kinase = *that which operates on that which operates on that which operates on protein.*
  - How ‘sophisticated’ are natural algorithms?



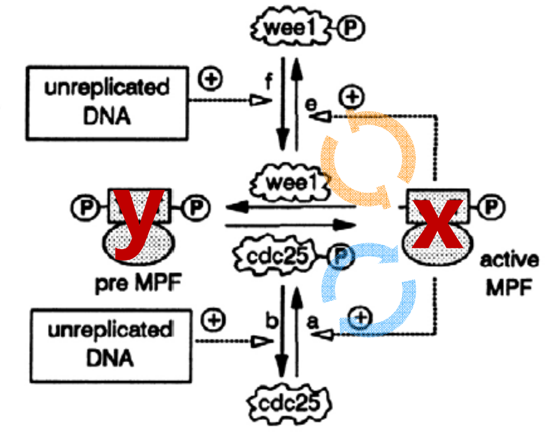
Ultrasensitivity in the mitogen-activated protein **cascade**, Chi-Ying F. Huang and James E. Ferrell, Jr., 1996, *Proc. Natl. Acad. Sci. USA*, 93, 10078-10083.

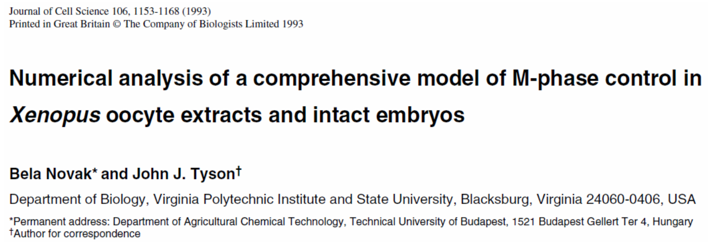
Outline

- Analyzing biomolecular networks
  - Try do understand the function of a network
  - But also try to understand its *structure*, and what determines it
- The Cell-Cycle Switches
  - Some of the best studied molecular networks
  - Important because of their fundamental function (cell division) and the stability of the network across evolution
- We ask:
  - What does the cell cycles switch compute?
  - How does it compute it?

The Cell Cycle Switch

- This network is universal in all Eukaryotes [P. Nurse]
  - I.e., the *network* at the core of cell division is *the same* from yeast to us
  - *Not the components* of the network, *nor the rates*





Journal of Cell Science 106, 1153-1168 (1993)
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Numerical analysis of a comprehensive model of M-phase control in *Xenopus* oocyte extracts and intact embryos

Bela Novak* and John J. Tyson†

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†Author for correspondence

Double positive feedback on x
Double negative feedback on x
No feedback on y
What on earth … ???

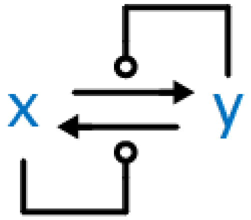
- The function is very well-studied. But why this structure?
- I.e., why this algorithm?

How to Build a Good Switch

- What is a “good” switch?
  - We need first a bistable system: one that has two *distinct* and *stable* states. I.e., given any initial state the system must settle into one of two states
  - The settling must be fast (not get stuck in the middle for too long) and robust (must not spontaneously switch back)
  - Finally, we need to be able to flip the switch by external inputs
- “Population” Switches
  - Populations of identical agents (molecules) with the whole population switching from one state to another as a whole
  - Highly concurrent (stochastic)

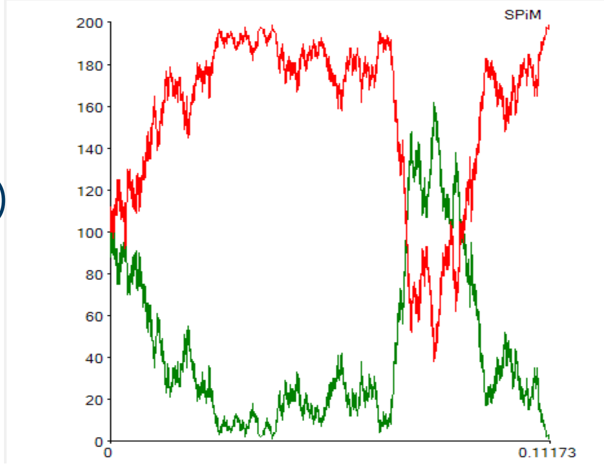
A Bad Algorithm

- Direct Competition
  - x catalyzes the transformation of y into x
  - y catalyzes the transformation of x into y
  - when all-x or all-y, it stops
- This system has two end states, but
  - Convergence to an end state is slow (a random walk)
  - Any perturbation of an end state can start a random walk to the other end state (hence not really *bistable*)



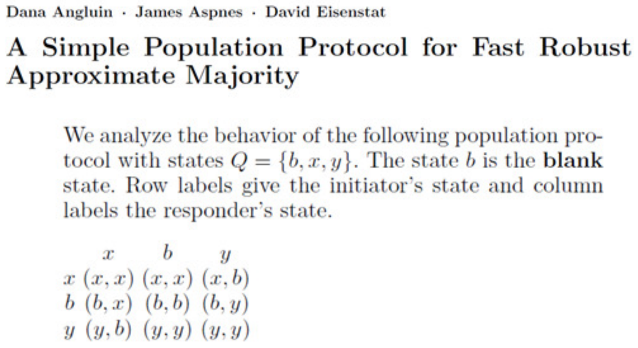
$y + x \rightarrow x + x$

$x + y \rightarrow y + y$



A Very Good Algorithm

- Approximate Majority (AM)
  - Decide which of two populations is in majority
- A fundamental 'population protocol'
  - Agents in a population start in state x or state y
  - A pair of agents is chosen randomly at each step, they interact ('collide') and change state
  - The whole population must eventually agree on a majority value (all-x or all-y) with probability 1

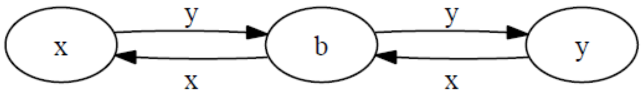


Dana Angluin · James Aspnes · David Eisenstat

A Simple Population Protocol for Fast Robust Approximate Majority

We analyze the behavior of the following population protocol with states $Q = \{b, x, y\}$. The state b is the **blank** state. Row labels give the initiator's state and column labels the responder's state.

	x	b	y
x	(x, x)	(x, x)	(x, b)
b	(b, x)	(b, b)	(b, y)
y	(y, b)	(y, y)	(y, y)



Third 'undecided' state

1) Disagreements cause agents to become undecided
2) Undecided agents believe any non-undecided agent they meet

Properties

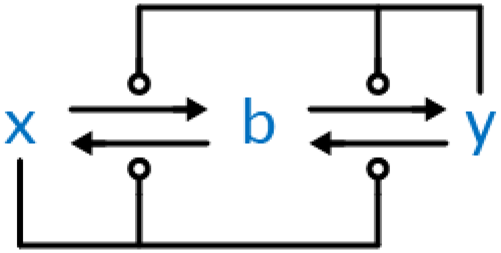
[Angluin et al., http://www.cs.yale.edu/homes/aspnes/papers/disc2007-eisenstat-slides.pdf]

- With high probability, for *n* agents
  - The total number of interactions before converging is *O*(*n log n*)
    ⇒ fast
  - The final outcome is correct if the initial disparity is *ω(sqrt*(*n*) *log n*)
    ⇒ solution states are robust to perturbations
- Logarithmic time bound in parallel time
  - *Parallel time* is the number of steps divided by the number of agents
  - In parallel time the algorithm converges with high probability in *O*(*log n*)

Chemical Implementation

Chemistry as a programming language for population algorithms!

$x + y \to y + b$
$y + x \to x + b$
$b + x \to x + x$
$b + y \to y + y$

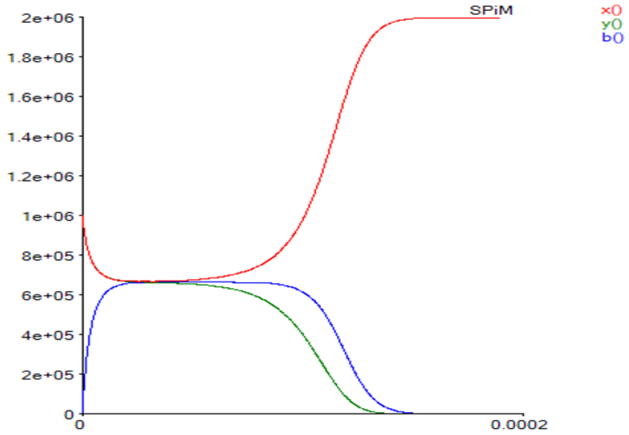


Bistable
  Even when x=y! (stochastically)

Fast
  $O(\log n)$ convergence time

Robust to perturbation
  above a threshold, initial majority wins *whp*

Worse-case scenario example, starting with x=y, b=0:



Correspondence PP ↔ (normalized)CRN

[Soloveichik., http://arxiv.org/abs/0803.1030 Appendix A.4 + personal communication]

- Suppose we have a Chemical Reaction Network with:
  - All the reactions are unit-rate, bimolecular, with two products: A + B $\to^1$ C + D
  - At most one reaction with the same reactants.
  - "Saturated" with null reactions E + F $\to^1$ E + F and G + G $\to^1$ G + G for all the other possible reactants among existing species (these null reactions have no effect on the kinetics).
  - So there is a one-to-one reaction/interaction correspondence with a Population Protocol (which also has null interactions).
- Consider the sum λ of the Gillespie propensities of all reactions in any state
  - It is always the <u>same</u> (everything interacts with everything else at rate 1): for n molecules in volume v, λ = n(n-1)/2v *
  - By Gillespie, the time to the next (possibly null) reaction is an exponential random variable with rate λ.
  - There are on average 1/λ (possibly null) reactions per unit time. And since n/v ("concentration") is assumed constant, λ is O(n).
- Transferring Population Protocols Results
  - PPs measure time complexity in expected number of interactions. E.g.: AM converges in O(m log m) interactions WHP.
  - But there is now a correspondence with CNR time: let E(m) be the expected number of interactions of the PP, then E(m/λ) = E(m)/λ is the expected running time of the CRN. This can be bounded tightly with Chernoff bounds.
  - For AM, E(m) is O(m log m), and λ is O(n), hence the expected running time of its CRN, E(m/λ), is logarithmic in the system size.

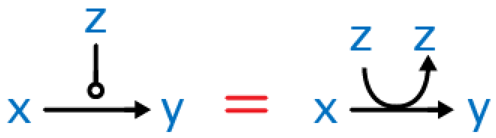
* Just to confirm, splitting the reactions between the same species and between different species, the sum of the propensities is $\sum_i \frac{x_i(x_i-1)}{2V} + \sum_{i<i'} \frac{x_i x_{i'}}{V} = \frac{1}{2V}(\sum_i x_i x_i - \sum_i x_i + 2\sum_{i<i'} x_i x_{i'}) = \frac{1}{2V}(\sum_{i,i'} x_i x_{i'} - \sum_i x_i) = \frac{n(n-1)}{2V}$ using the fact that $2\sum_{i<i'} x_i x_{i'} = \sum_{i\neq i'} x_i x_{i'}$ and $\sum_i x_i x_i + \sum_{i\neq i'} x_i x_{i'} = \sum_{i,i'} x_i x_{i'}$.

Back to the Cell Cycle

- The AM algorithm has ideal properties for settling a population into one of two states
- But that is not what the cell cycle uses
- Or is it?

Influence Network Notation

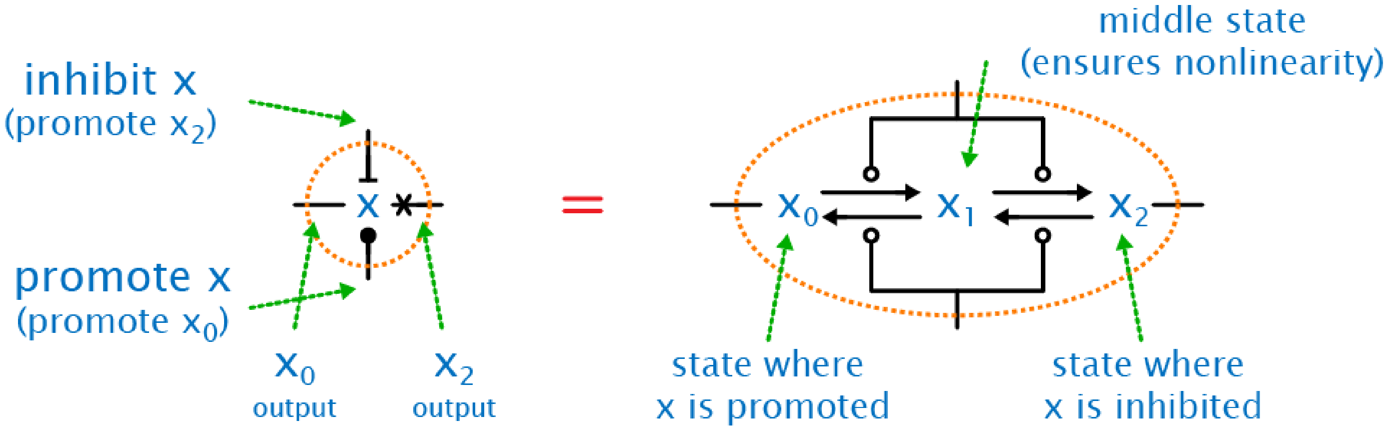
- Catalytic reaction



z is the catalyst

$x + z \rightarrow z + y$

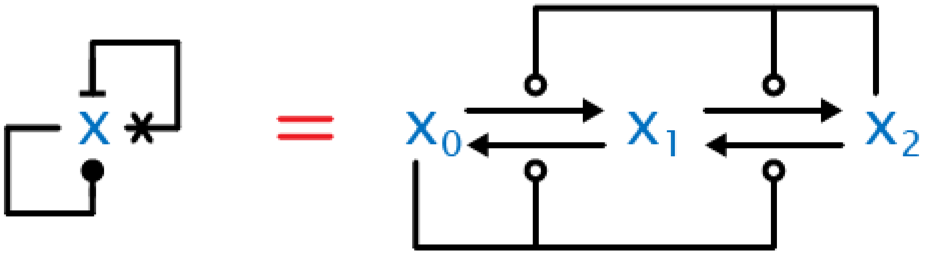
- 'Double kinase-phosphatase' motif



influence node

catalytic node

Step 1: the AM Network



(x_2 promotes x_2)

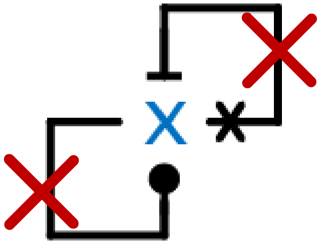
(x_0 promotes x_0)

- … not biochemically plausible

Natural Constraint #1

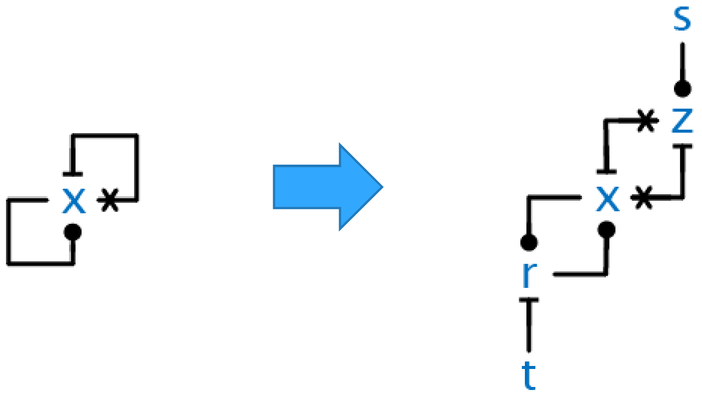
- Direct autocatalysis is not commonly seen in nature

$$x_1 + x_0 \rightarrow x_0 + x_0$$
$$x_1 + x_2 \rightarrow x_2 + x_2$$



Step 2: remove auto-catalysis

- Replace autocatalysis
  - By *mutual* (simple) catalysis, introducing intermediate species z and r
  - z and r need to ‘relax back’ when they are not being promoted: s and t provide the back pressure for such relaxation



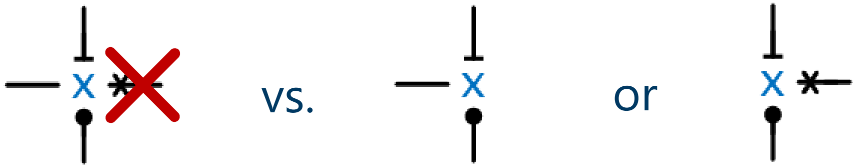
(x_2 promotes z_2, promotes x_2)

(x_0 promotes r_0, promotes x_0)

- … still not biochemically plausible.

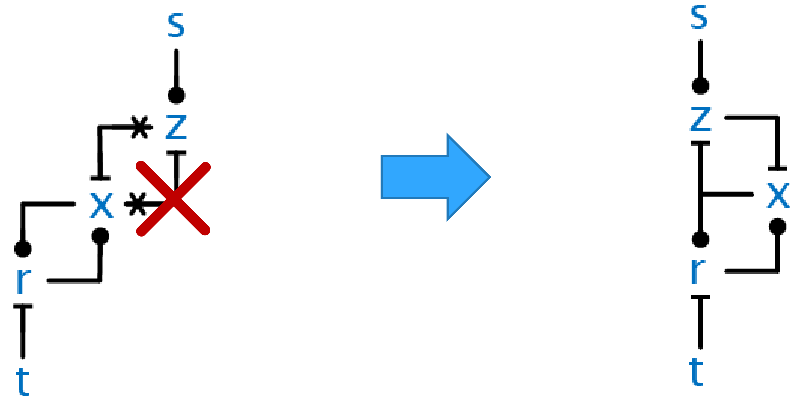
Natural Constraint #2

- x_0 and x_2 (usually two states of the same molecule) are both active catalysts in that network
- That is not commonly seen in nature



Step 3: only one active state per species

- Remove the catalytic activity of x_2
  - By “flipping the z feedback to the other side”



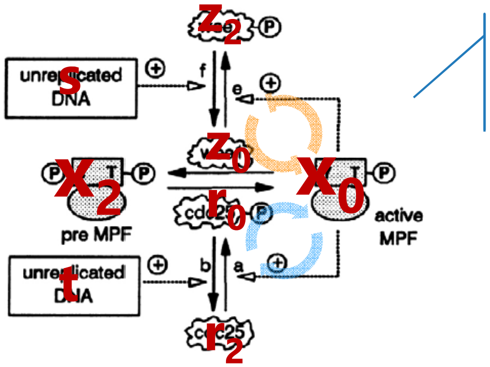
(x_2 promotes z_0 via s bias,
z_0 promotes x_2 via inhibiting x_0)

(x_0 promotes r_0, promotes x_0)

- All species now have one active (x_0,z_0,r_0) and one inactive (x_2,z_2,r_2) form
- This is ‘biochmically plausible’

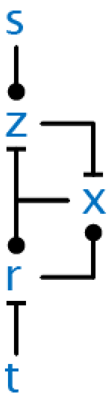
Network Structure

- … and that is the cell-cycle switch!



Nobel-prize winning network

Variation on a distributed algorithm

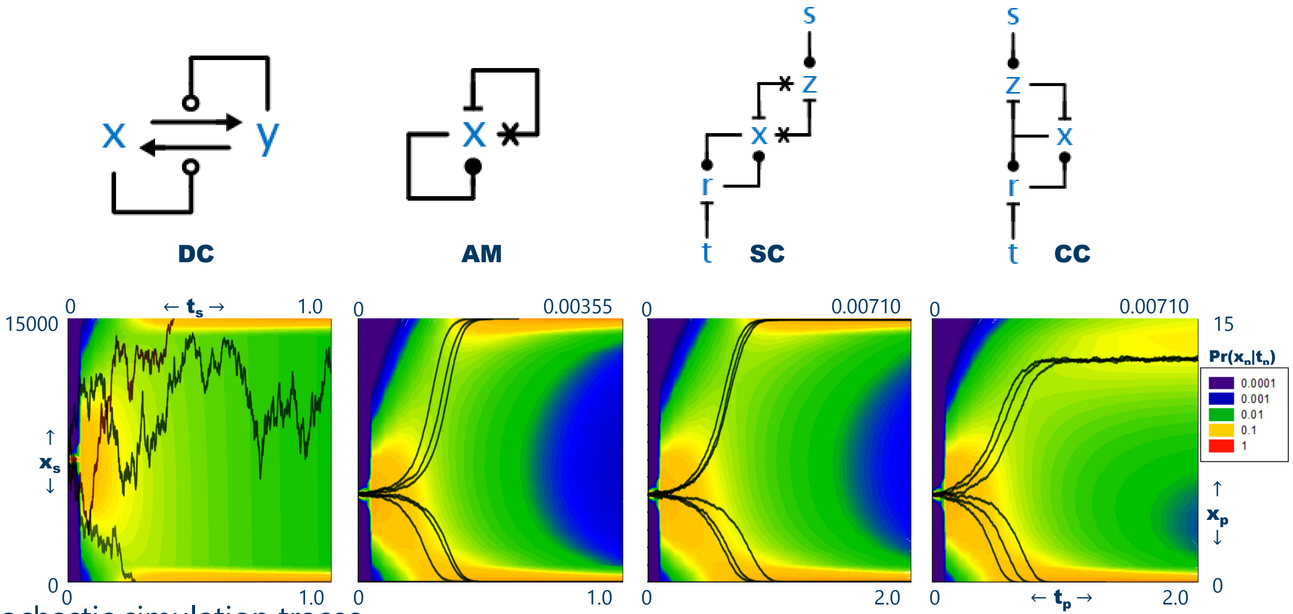


- But did we preserve the AM function through our network transformations?
- Ideally: prove either that the networks are ‘contextually equivalent’ or that the transformations are ‘correct’
- Practically: compare their ‘typical’ behavior

Convergence Analysis

- Switches as computational systems

Start symmetrical
(x_0=x_1=x_2 etc.)

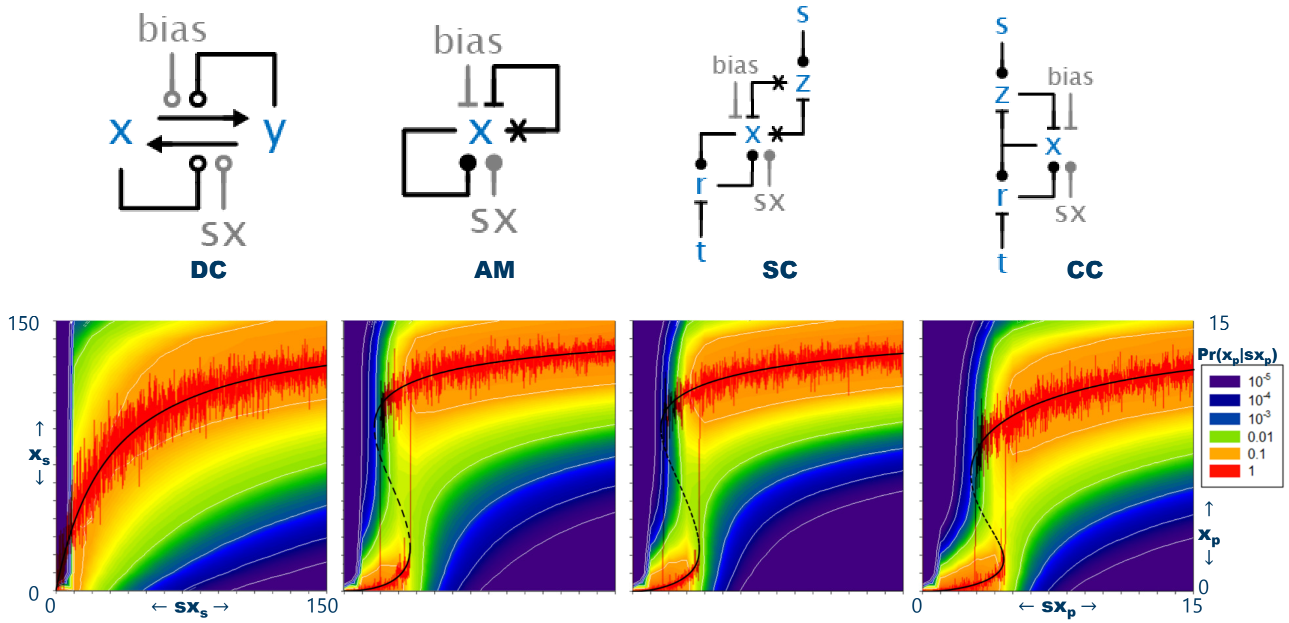


Black lines: several stochastic simulation traces
Color: full probability distribution of small-size system

NEW!
CC appears to converge in log time

Steady State Analysis

- Switches as dynamical systems

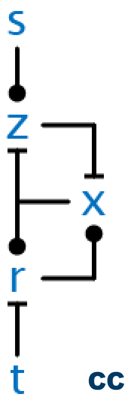


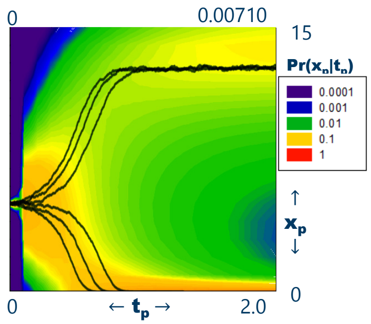
Black lines: deterministic ODE bifurcation diagrams
Red lines: noisy stochastic simulations
Color: full probability distribution of small-size system

NEW!
AM shows hysteresis

Evidence that CC is ‘similar’ to AM

- But there was a difference
  - The output of CC does not go ‘fully on’ like AM:

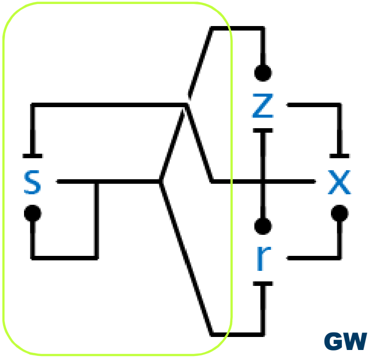


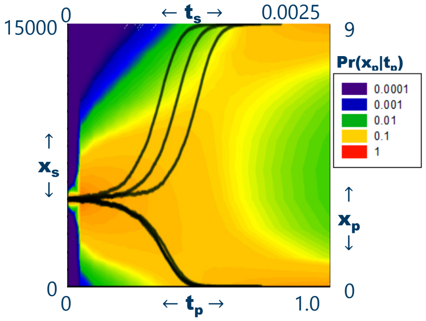


  - Because s continuously inhibits x through z, so that x cannot fully express
  - Q: Why didn’t nature do better than that?

Nature fixed it!

- There is another known feedback loop
  - By which x suppresses s “in retaliation” via the so-called Greatwall loop
  - Also, s and t happen to be the same molecule





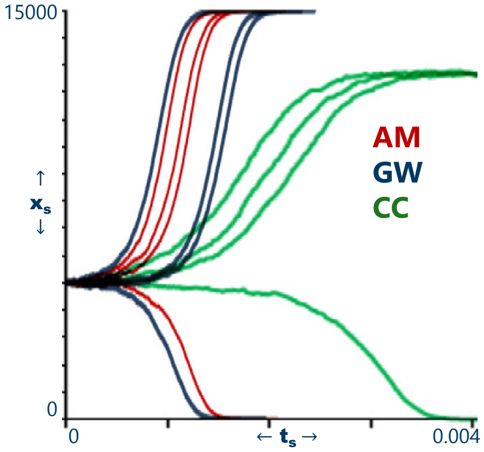
Full activation!

- (As usual, there are many more details in real biological networks; this is one of the many details people knew about without fully understanding its function)

More surprisingly

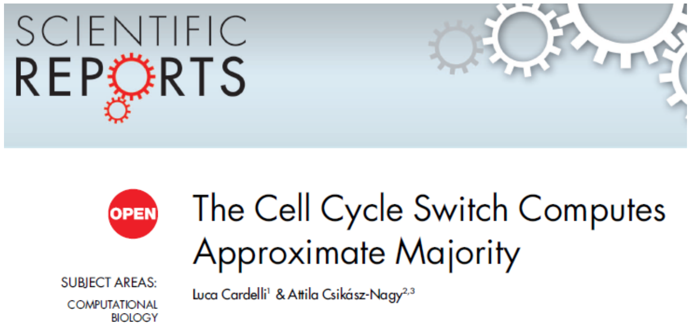
- Made it faster too!
  - The extra feedback also speeds up the decision time of the switch, making it about as good as the 'optimal' AM switch:

Conclusion (in our published paper):
Nature is trying as hard as it can to implement an AM-class algorithm!



The Greatwall Kinase

- Our paper appeared:
  - Suggesting GW is a better switch than CC, also in the context of oscillators



SCIENTIFIC REPORTS

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SUBJECT AREAS:
COMPUTATIONAL BIOLOGY

The Cell Cycle Switch Computes Approximate Majority

Luca Cardelli[1] & Attila Csikász-Nagy[2,3]

- Another paper the same week:
  - Showing experimentally that the Greatwall loop is a necessary component of the switch, i.e. the not-as-good-as-AM network has been ‘refuted’

nature COMMUNICATIONS

OPEN

ARTICLE

Received 6 Jul 2012 | Accepted 14 Aug 2012 | Published 11 Sep 2012

DOI:10.1038/ncomms2062

Greatwall kinase and cyclin B-Cdk1 are both critical constituents of M-phase-promoting factor

Masatoshi Hara[1,†], Yusuke Abe[1,†], Toshiaki Tanaka[2], Takayoshi Yamamoto[1,†], Eiichi Okumura[1] & Takeo Kishimoto[1]

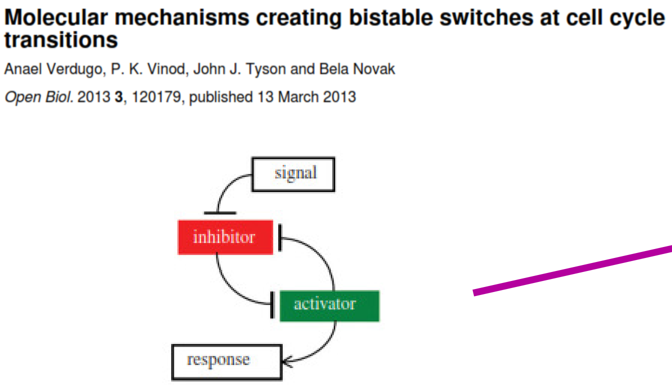
But what about network equivalence?

- Our evidence is empirical
  - Although quantitative and covering both kinetic and steady state behavior
  - Also, contextual equivalence holds in the context of oscillators (see paper)

- Analytical evidence is harder to obtain
  - The proof techniques for the AM algorithm are hard and do not generalize easily to more complex networks
  - Quantitative theories of behavioral equivalence and behavioral approximation, e.g. in process algebra, are still lacking (although rich qualitative theories exist)

Mutual Inhibition

- A new paper suggests that all cellular switches in all phases of the cell cycle follow (abstractly) a mutual inhibition pattern:

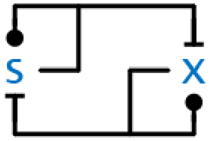


Molecular mechanisms creating bistable switches at cell cycle transitions

Anael Verdugo, P. K. Vinod, John J. Tyson and Bela Novak

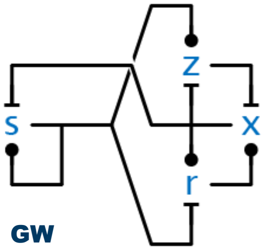
Open Biol. 2013 **3**, 120179, published 13 March 2013

In our notation:



MI

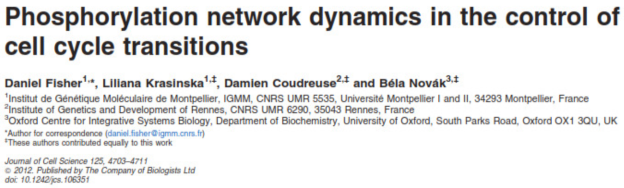
cf.:



GW

New Cell Cycle Network

- A new paper presents a more complete view of the cell cycle switch
- N.B. “phosphorylation network dynamics” is the same as our x_0-x_1-x_2 motif



Phosphorylation network dynamics in the control of cell cycle transitions

Daniel Fisher[1,*], Liliana Krasinska[1,‡], Damien Coudreuse[2,‡] and Béla Novák[3,‡]
[1]Institut de Génétique Moléculaire de Montpellier, IGMM, CNRS UMR 5535, Université Montpellier I and II, 34293 Montpellier, France
[2]Institute of Genetics and Development of Rennes, CNRS UMR 6290, 35043 Rennes, France
[3]Oxford Centre for Integrative Systems Biology, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK
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‡These authors contributed equally to this work

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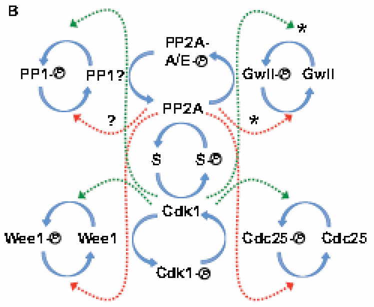
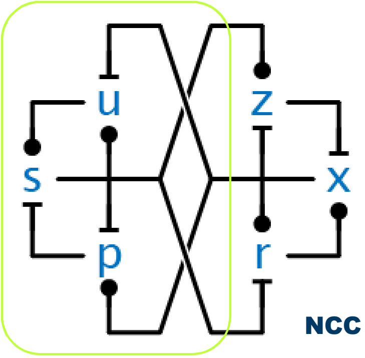
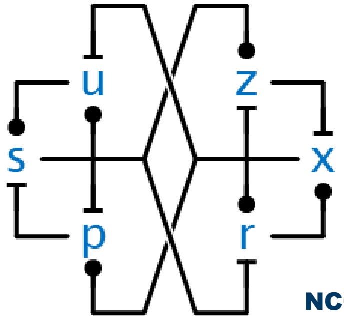


In our notation:



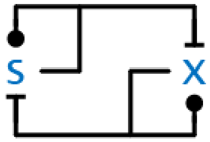
Network Emulation

- For chosen (uniform) initial conditions, the ODEs (and hence trajectories) of **NCC** collapse to those of **MI** (thanks to David Soloveichik):

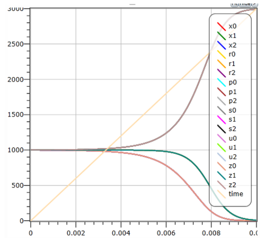


NCC

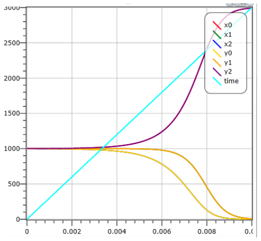
x,r,p ⇢ x
s,u,z ⇢ s



MI



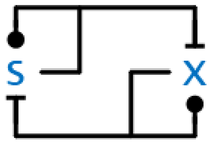
(18 species on 3 trajectories)



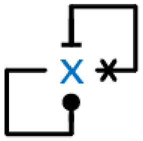
(6 species on 3 trajectories)

Network Emulation

- For chosen (uniform) initial conditions, the ODEs (and hence trajectories) of **MI** collapse to those of **AM**:

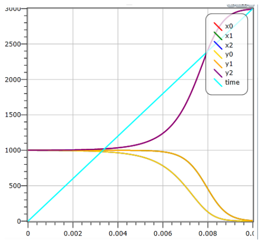


$s,x \dashrightarrow x$

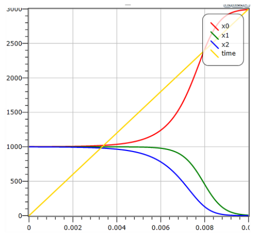


MI

AM



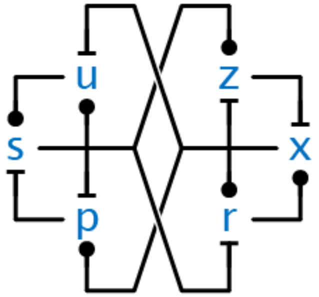
(6 species on 3 trajectories)



(3 species on 3 trajectories)

Conclusions

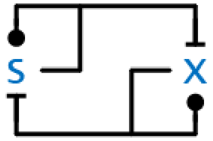
- The cell cycle switch *can* exactly emulate AM



NCC

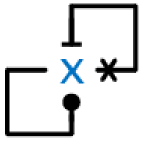
(New) cell cycle switch

emulates:



MI

emulates:



AM

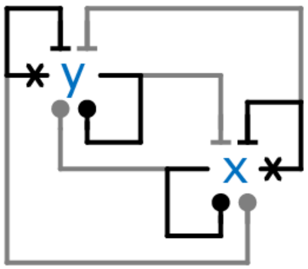
Approximate majority algorithm

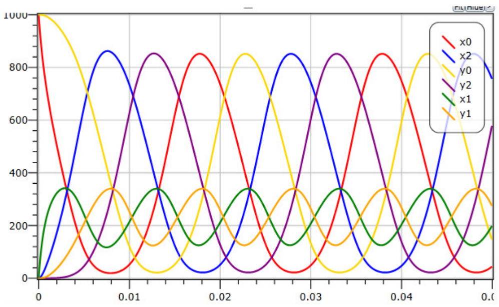
- Nature likes a good algorithm!

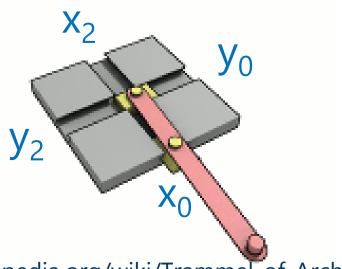
Extra

Cell Cycle Oscillator

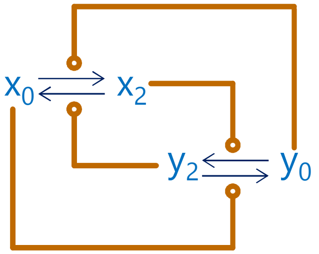
- The cell cycle switch is part of an oscillator network
  - The cell cycle oscillation: grow-divide-grow-divide…
- The principle of the oscillator
  - Two interconnected switches yield a limit-cycle oscillator; e.g. two AM switches
  - In a Trammel of Archimedes configuration (gray rates < black rates)
  - (The biological network lacks some of these links and still oscillates)





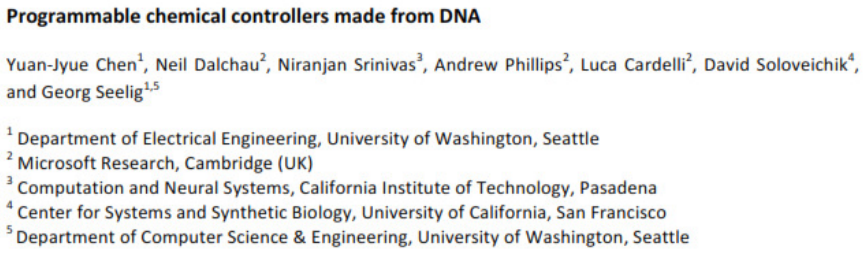


en.wikipedia.org/wiki/Trammel_of_Archimedes



In separate work…

- We have a chemical implementation of AM using DNA gates
- I.e., a ‘synthetic reimplementation’ of the central cell-cycle switch.



Programmable chemical controllers made from DNA

Yuan-Jyue Chen[1], Neil Dalchau[2], Niranjan Srinivas[3], Andrew Phillips[2], Luca Cardelli[2], David Soloveichik[4], and Georg Seelig[1,5]

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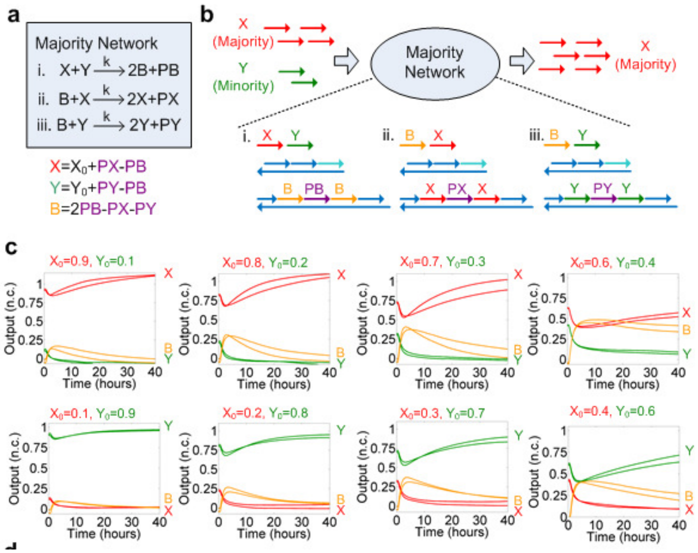
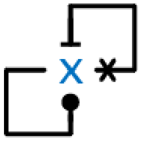
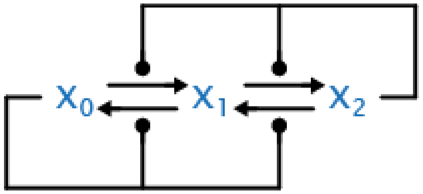
[2] Microsoft Research, Cambridge (UK)
[3] Computation and Neural Systems, California Institute of Technology, Pasadena
[4] Center for Systems and Synthetic Biology, University of California, San Francisco
[5] Department of Computer Science & Engineering, University of Washington, Seattle



Collapse in detail

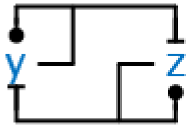


AM

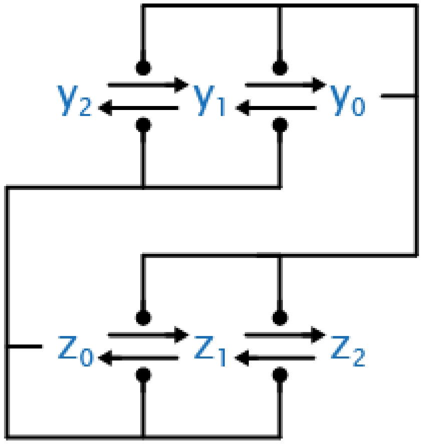


$dx_0/dt = x_0*x_1 - x_0*x_2$
$dx_1/dt = x_0*x_2 + x_2*x_0 - x_1*x_0 - x_1*x_2$
$dx_2/dt = x_1*x_2 - x_2*x_0$

$x_{tot} = x_0+x_1+x_2$



MI

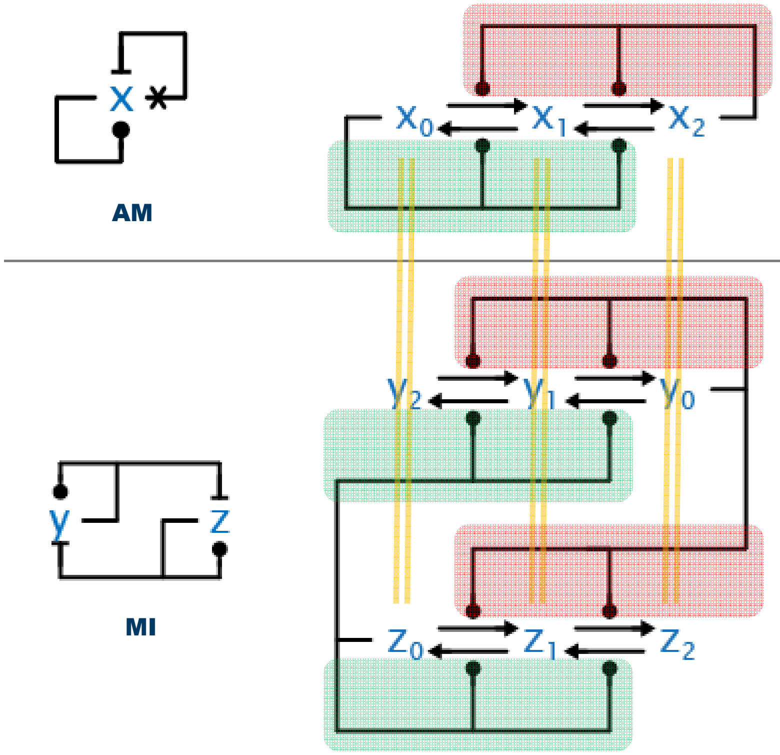


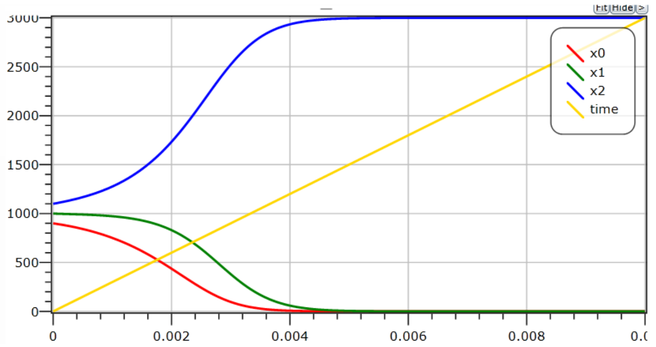
$dz_0/dt = z_1*z_0 - z_0*y_0$
$dz_1/dt = z_2*z_0 + z_0*y_0 - z_1*z_0 - z_1*y_0$
$dz_2/dt = z_1*y_0 - z_2*z_0$

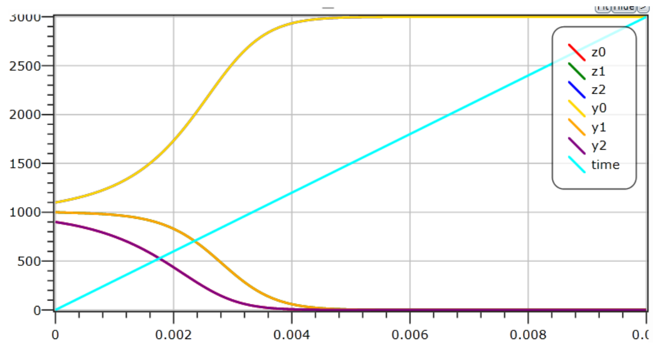
$dy_0/dt = y_1*y_0 - y_0*z_0$
$dy_1/dt = y_0*z_0 + y_2*y_0 - y_1*z_0 - y_1*y_0$
$dy_2/dt = y_1*z_0 - y_2*y_0$

$y_{tot} = y_0+y_1+y_2$
$z_{tot} = z_0+z_1+z_2$

Collapse in detail







$x_0(0) = y_2(0) = z_0(0)$
$x_1(0) = y_1(0) = z_1(0)$
$x_2(0) = y_0(0) = z_2(0)$

(at time 0)

Collapse in detail

[David Soloveichik]
Assume that at some time t, in **MI**:

$y_2(t) = z_0(t)$
$y_1(t) = z_1(t)$ (at time t)
$y_0(t) = z_2(t)$

then, e.g.:

$(dy_2/dt)(t)$
$= y_1(t)*z_0(t) - y_2(t)*y_0(t)$
$= z_1(t)*z_0(t) - z_0(t)*y_0(t)$
$= (dz_0/dt)(t)$

this implies that $y_2(t+dt) = z_0(t+dt)$ and so on at any future time; i.e. $y_2 = z_0$.

Similarly $y_1 = z_1$ and $y_0 = z_2$. So the trajectories of **MI** overlap in pairs.

Now assume at some time t in **AM** and **MI**:

$x_0(t) = y_2(t) = z_0(t)$
$x_1(t) = y_1(t) = z_1(t)$ (at time t)
$\mathbf{x_2(t) = y_0(t) = z_2(t)}$

we again have that, e.g.:

$(dx_0/dt)(t)$
$= x_0(t)*x_1(t) - x_0(t)*x_2(t)$
$= z_0(t)*z_1(t) - z_0(t)*y_0(t)$
$= (dz_0/dt)(t)$

so $x_0 = z_0$ (= y_2) at any future time,
and similarly $x_1 = z_1$ (= y_1) and $x_2 = z_2$ (= y_0)

And if we start with initial conditions satisfying:

$x_0(0) = y_2(0) = z_0(0)$
$x_1(0) = y_1(0) = z_1(0)$ (at time 0)
$x_2(0) = y_0(0) = z_2(0)$

then we have the same time evolution for **AM** and **MI**.

Question (Cris Moore)

- Is it true that any trajectory of the 'bigger' system converges to a trajectory of the 'smaller' system?
  - This is more than ability to simulate or approximate the smaller system. (We already know from the ODEs that the bigger system can in fact simulate the small one *exactly*.)
  - If the above is true, it further means that the bigger system, even though it has a richer state space and many more trajectories, cannot in fact "stay away" from the behavior of the smaller systems, even if it starts in a state that is not representable in the smaller system.

  - "Hi Luca. I have been trying to wrestle with the 18-dimensional (actually 12-dimensional) system all at once. Establishing linear stability of the manifold equivalent to AM seems fairly easy, but I want to show it's globally stable, at least over a large range of initial conditions. Just wanted to let you know. - Cris" [Last I heard.]

Microsoft