On Continuous, Discrete and Timed Models in Systems Biology

Oded Maler

CNRS - VERIMAG
Grenoble, France

2009

Based on joint work with Gregory Batt, Thao Dang, Colas Le Guernic, Eugene Asarin, Marius Bozga, Ramzi Ben Salah and Antoine Girard

Dedicated to the memory of Moti Liscovitch

Systems Biology

- Systems Biology: the new gold rush for many mathematical and technical disciplines
- Biophysics, Biomatics, Bioinformatics, Biostatistics...
- The generic template:
  - I do X (for my pleasure, because I studied it, that's what I know) but... X can also be useful for Biology
- So Here I am, presenting my own X, a certain species of Informatics / Computer Science
- My X is based on **automata as dynamical systems** with excursions into **hybrid** dynamics (automata plus differential equations) and **timed** dynamics (automata plus quantitative time) as an intermediate level of abstraction
- When you have a hammer, everything looks like a nail

Summary

- Informatics is more than technology, it is also a kind of mathematics/physics
- Automata as dynamical systems
- Verification illustrated
- Between the discrete and the continuous
- Timed models and their applications
  - Adding time to discrete models of genetic regulatory networks
  - From continuous to timed systems (the technical contribution of the paper in the proceedings)
- Back to the big picture

Computer Technology

- Computers, Networks, Operating Systems, Data-Bases, Web, Search Engines, Graphics,
- Embedded Systems, Sensors, Programming Languages, Word Processing, Computer Control, Robotics, Security ..
- Influence on all domains of human activity, including Biology:
- String Processing for DNA, Statistical Computations, Simulation, Animation
- Date-Bases, Micro-Arrays, Ontologies and Description Languages
- Communication and Data Sharing, Lab Management

In all those activities the computer is a useful **material tool** in the **service** of others

A More Noble Role, Perhaps

- Biology seems to be trying to go through a kind of Newtonian revolution
- The essence of such revolution is to upgrade (as much as possible) descriptive "models" by **dynamic models** with stronger predictive power and refutability
- Classical models of dynamical systems are clearly not sufficient for effective modeling of biological phenomena
- Models, insights and computer-based analysis tools developed within Informatics can help

What Is Informatics ?

- Among other things informatics is: the (pure and applied) study of **discrete-event dynamical systems** (automata, transition systems)
- A natural point of view for the "reactive systems" parts of informatics (hardware, protocols, real-time, stream processing)
- Especially for people working on modeling and verification of such systems
- Sometimes obscured (intentionally or not) by fancy formalisms: Petri nets, process algebras, rewriting systems or temporal logics..
- All honorable topics with intrinsic importance, beauty, etc.
- But sometimes should be distilled to their **essence** in order to make sense for potential users from other disciplines (rather than frighten/impress them)

Dynamical System Models in General

- State variables whose set of valuations determine the state space
- Time domain along which these values evolve
- Dynamic law which says how state variables evolve over time, possibly under the influence of external factors
- System behaviors are progressions of states in time
- Having such a model, knowing an initial state $x(0)$ one can predict, to some extent, the value of $x(t)$
-
- Remark: Variables in Biology can be of various natures and granularities (concentrations, states of individual molecules, stages in processes, etc.)

Classical Dynamical Systems

- State variables: real numbers (location, velocity, energy, voltage, concentration)
- Time domain: the real time axis $\mathbb{R}$ or a discretization of it
- Dynamic law: differential equations

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

  or their discrete-time approximations

  $$x(t+1) = f(x(t), u(t))$$

- Behaviors: trajectories in the continuous state space
- Achievements: Apples, Stars, Missiles, Electricity, Heat, Chemical processes
- Theorems, Papers, Simulation tools

Automata as Dynamical Systems

- Abstract discrete state space, state variables need not have a numerical meaning
- Logical time domain defined by the events (order but not metric)
- Dynamics defined by transition tables: input event **a** takes the system from state **s** to state **s**′
- Behaviors are sequences of states and events
- Composition of large systems from small ones, hierarchical structuring
- Different modes of interaction: synchronous/asynchronous, state-based/event-based
- Sometime additional syntax may be required

Automata can Model many Phenomena and Devices

- Software, hardware,
- ATMs, user interfaces
- Administrative procedures
- Communication protocols
- Cooking recipes, Manufacturing instructions
- Any process that can be viewed as a sequence of steps

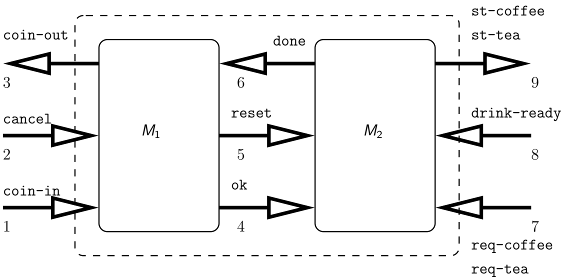
- But what can we do with these models?
- There are no analytical tools as in continuous systems
- We can simulate and sometimes do formal verification

What is Verification ?

- Given a complex discrete dynamical system with some uncontrolled inputs or unknown parameters
- Check whether ALL its behaviors satisfy some properties
- Properties:
    - Never reach some part of the state space
    - Always come eventually to some (equilibrium) state
    - Never exhibit some pattern of behavior
    - Quantitative versions of such properties..
- Existing tools can do this type of analysis for huge systems by sophisticated graph algorithms

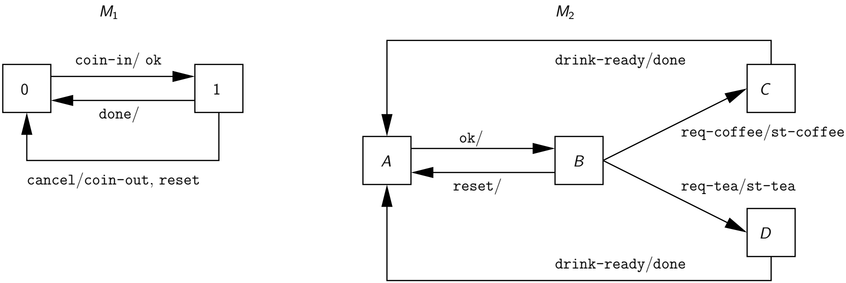
Illustration: The Coffee Machine

- Consider a machine that takes money and distributes drinks
- The system is built from two subsystems, one that takes care of financial matters, and one which handles choice and preparation of drinks
- They communicate by sending messages



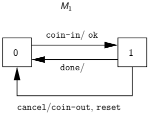
Automaton Models

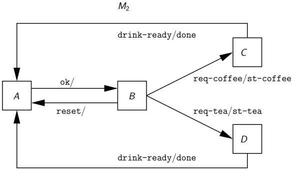
- The two systems are models as automata (state-transition systems)
- transitions are triggered by external events and events coming from the other subsystem

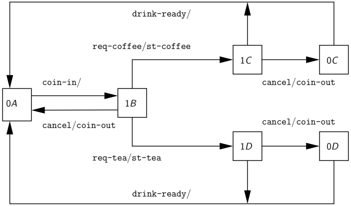


The Global Model

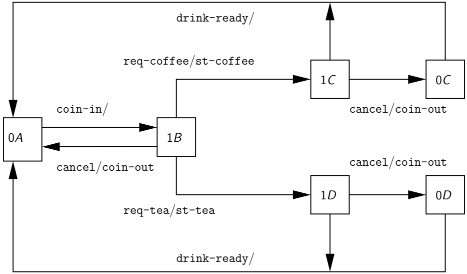
- The behavior of the whole system is captured by a composition (product) $M_1 \parallel M_2$ of the components
- States are elements of the Cartesian product of the respective sets of states, indicating the state of each component
- Some transitions are independent and some are synchronized, taken by the two components simultaneously
- Behaviors of the systems are paths in this transition graph







Normal Behaviors



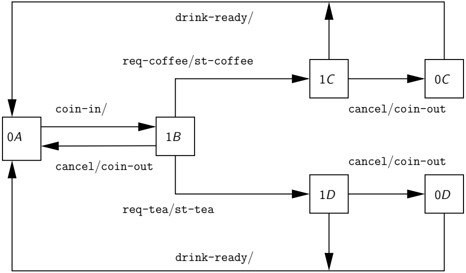
- Customer puts coin, then sees the bus arriving, cancels and gets the coin back

$$0A \; \texttt{coin-in} \; 1B \; \texttt{cancel} \; \texttt{coin-out} \; 0A$$

- Customer inserts coin, requests coffee, gets it and the systems returns to initial state

$$0A \; \texttt{coin-in} \; 1B \; \texttt{req-coffee} \; \texttt{st-coffee} \; 1C \; \texttt{drink-ready} \; 0A$$

An Abnormal Behavior



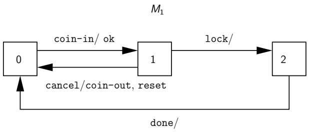
- Suppose the customer presses the cancel button *after* the coffee starts being prepared..

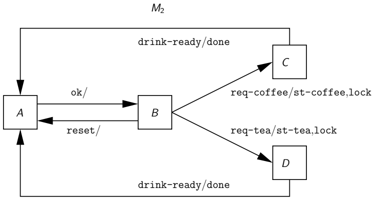
  0*A* `coin-in` 1*B* `req-coffee` `st-coffee` 1*C* `cancel` `coin-out` 0*C*
  `drink-ready` 0*A*

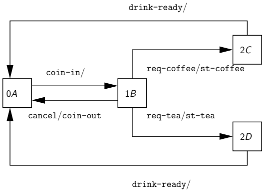
- Not so attractive for the owner of the machine

Fixing the Bug

- When M_2 starts preparing coffee it emits a `lock` signal
- When M_1 received this message it enters a new state where `cancel` is refused







The Moral of the Story I

- Many complex systems can be modeled as a composition of interacting automata
- Behaviors of the system correspond to paths in the global transition graph of the system
- The size of this graph is exponential in the number of components (state explosion, curse of dimensionality)
- These paths are labeled by input events representing influences of the outside environment
- Each input sequence may generate a different behavior
- We want to make sure that a system responds correctly to all conceivable inputs, that it behaves properly in any environment (robustness)

The Moral of the Story II

- How to ensure that a system behaves properly in the presence of all conceivable inputs and parameters?
- For every individual input sequence or parameter value we can **simulate** the reaction of the system. But we cannot do it exhaustively
- Verification is a collection of automatic and semi-automatic methods to analyze all the paths in the graph
- This is hard for humans to do and even for computers
- And this type of analysis and way of looking at phenomena is our **potential contribution** to Biology

Hybrid Systems: Motivation

- Hybrid systems combine the discrete dynamics of automata with continuous dynamics defined by differential equations
- Each state may correspond to a mode of a system (a gene is on, a valve/heater is closed, the car is in a second gear)

Hybrid Systems: Motivation

- Hybrid systems combine the discrete dynamics of automata with continuous dynamics defined by differential equations
- Each state may correspond to a mode of a system (a gene is on, a valve/heater is closed, the car is in a second gear)
- In each state there is a different continuous dynamics
- The system may switch between modes according to the values of the continuous variables
- For example, the heater is turned off when temperature is high, a valve is opened when the water level crosses a threshold

Hybrid Systems Analysis is Difficult

- Purely continuous systems (especially linear ones) admit a lot of mathematical analysis techniques
- Hybrid systems are much harder to analyze because switching breaks their nice mathematical properties

Hybrid Systems Analysis is Difficult

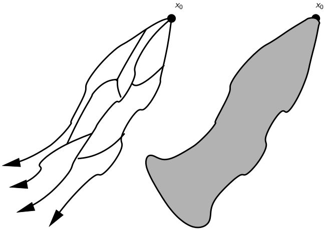
- Purely continuous systems (especially linear ones) admit a lot of mathematical analysis techniques
- Hybrid systems are much harder to analyze because switching breaks their nice mathematical properties
- New techniques inspired by discrete verification are being developed
- Combination of numerical analysis, graph algorithms and computational geometry

Verification for Continuous Systems

- The problem: a dynamical system $\dot{x} = f(x, p, u)$ where u is an external disturbance and p is a parameter
- Both u and p are not known exactly but are bounded

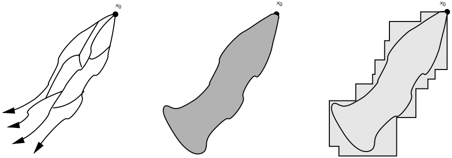
Verification for Continuous Systems

- The problem: a dynamical system $\dot{x} = f(x, p, u)$ where u is an external disturbance and p is a parameter
- Both u and p are not known exactly but are bounded
- Can something be said about all the possible behaviors of the system for all range of parameters and all external disturbances?



Verification for Continuous Systems

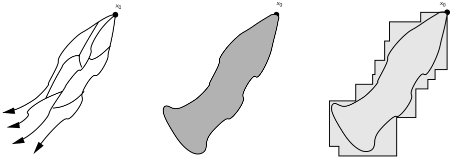
- A kind of set-based numerical integration to approximate the set of states reachable by all possible inputs and parameters



- Can replace an infinite number of simulations

Verification for Continuous Systems

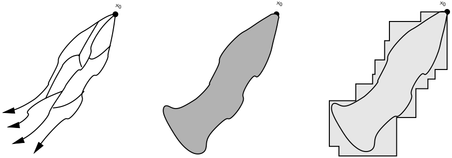
- A kind of set-based numerical integration to approximate the set of states reachable by all possible inputs and parameters



- Can replace an infinite number of simulations
- Useful for Biological models where exact parameters are hard or impossible to obtain

Verification for Continuous Systems

- A kind of set-based numerical integration to approximate the set of states reachable by all possible inputs and parameters



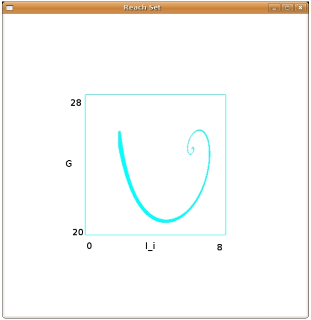
- Can replace an infinite number of simulations
- Useful for Biological models where exact parameters are hard or impossible to obtain
- State-of the-art: tools at various levels of sophistication and maturity can analyze linear systems with hundreds of state variables, as well as small nonlinear ones

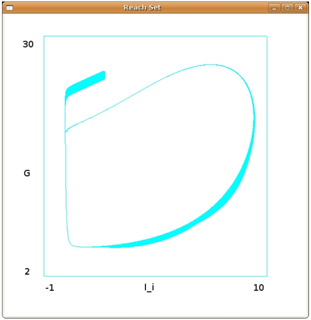
Reachability for Nonlinear Systems

- New algorithms for computing tubes of trajectories for systems defined by nonlinear differential equations
- Using new dynamic hybridization methods we can analyze nontrivial nonlinear systems
- Biological models: Lac operon (6 state variables) aging model (9 state variables)

Lac Operon

$$\begin{aligned}
\dot{R}_a &= \tau - \mu * R_a - k_2 R_a O_f + k_{-2}(\chi - O_f) - k_3 R_a I_i^2 + k_8 R_i G^2 \\
\dot{O}_f &= -k_2 r_a O_f + k_{-2}(\chi - O_f) \\
\dot{E} &= \nu k_4 O_f - k_7 E \\
\dot{M} &= \nu k_4 O_f - k_6 M \\
\dot{I}_i &= -2k_3 R_a I_i^2 + 2k_{-3} F_1 + k_5 I_r M - k_{-5} I_i M - k_9 I_i E \\
\dot{G} &= -2k_8 R_i G^2 + 2k_{-8} R_a + k_9 I_i E
\end{aligned}$$





On Levels of Abstraction

- A phenomenon can be described at different levels of abstraction and granularity
- Each level presents a trade-off in expressivity, accuracy and complexity of analysis
- When we consider processes inside the cell we encounter typically two major classes of models:
    - Evolution of protein concentrations (real numbers) following laws of mass action (continuous dynamical systems)
    - Discrete descriptions: the presence of A leads to the appearance of B which, eventually suppresses C
- I claim that not all the spectrum of possible model classes between these two has been explored

Timed Systems

- An extremely-important level of abstraction between the discrete and the continuous
- Continuous description: how the concentration of some product evolves over time
- Discrete description: the product level moves from low to high
- Timed description: the product level moves from low to high and this process takes between 3 and 5 hours to complete
- This is how we reason about our travel plans, workshop schedules and almost everything in daily life
- At this level the dynamical models are **timed automata**, automata with auxiliary clock variables

The Case for Timed Models

- Such timed discrete models will, perhaps, give a good complexity/informativeness trade-off
- This claim is illustrated (not demonstrated) using two meta-modeling case studies
  - Adding time to the purely-discrete models of genetic regulatory networks
  - Deriving timed models from continuous models (multi-affine differential equations)
- In both cases, some weaknesses of purely-discrete models are avoided
- These are proofs of concept and a lot of work remains to be done in order to improve accuracy and reduce complexity

Genetic Regulatory Networks for (and by) Dummies

- A set $G = \{g_1, \ldots, g_n\}$ of genes
- A set $P = \{p_1, \ldots, p_n\}$ of products (proteins)
- Each gene is responsible for the production of one product
- Genes activations are viewed as Boolean variables (On/Off)
- When $g_i = 1$ it will tend to increase the quantity of p_i
- When $g_i = 0$ the quantity of p_i will decrease (degradation)
- Feedback from products concentrations to genes: when the quantity of a product is below/above some threshold it may set one or more genes on or off

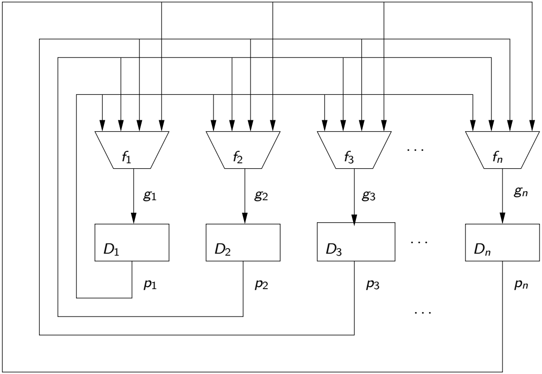
Continuous and Discrete Models of Genetic Networks

- Product quantities can be viewed as integer (quantity) or real (concentration) numbers
- The system can be viewed as a hybrid automaton with discrete states corresponding to combinations of gene activations states
- The evolution of product concentrations can be described using differential equations
- Alternatively, the domain of these concentrations can be discretized into a finite (and small) number of ranges
- The most extreme of these discretizations is to consider a Boolean domain $\{0, 1\}$ indicating **present** or **absent**

The Discrete Model of R. Thomas

- Gene activation is specified as a Boolean function over the presence/absence of products
- When a gene changes its value, its corresponding product will follow within some unspecified delay
- The resulting model is equivalent to an **asynchronous automaton**
- The relative speeds of producing different products are not modeled
- The model admits many behaviors which are not possible if these speeds are taken into account
- We add this timing information in a systematic manner, as we did in the past for asynchronous digital circuits [Maler and Pnueli 95]

Boolean Delay Networks



- A change in the activation of a gene is considered instantaneous once the value of f has changed
- This change is propagated to the product within a non-deterministic but bi-bounded delay specified by an interval

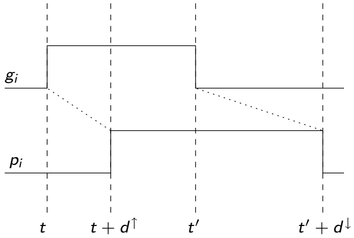
The Delay Operator

- For each i we define a delay operator D_i, a function from Boolean signals to Boolean signals characterized by 4 parameters

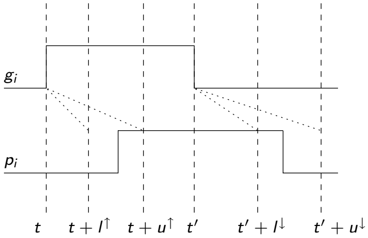
p_i	g_i	p'_i	Δ
0	0	0	$-$
0	1	1	$[l^{\uparrow}, u^{\uparrow}]$
1	0	0	$[l^{\downarrow}, u^{\downarrow}]$
1	1	1	$-$

- When $p_i \neq g_i$, p_i will catch up with g_i within $t \in [l^{\uparrow}, u^{\uparrow}]$ (rising) or $t \in [l^{\downarrow}, u^{\downarrow}]$ (falling)

The Delay Operator



Determinisitc



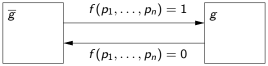
Nondeterministic

- The semantics of the network is the set of all Boolean signals satisfying the following set of signal inclusions

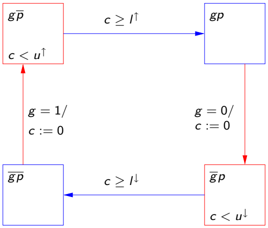
$$g_i = f_i(p_1, \ldots, p_n)$$
$$p_i \in D_i(g_i)$$

Modeling with Timed Automata

- For each equation $g_i = f_i(p_1, \ldots, p_n)$ we build the automaton



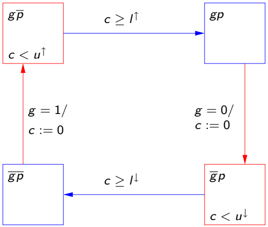
- For each delay inclusion $p_i \in D_i(g_i)$ we build the automaton



- Composing these automata we obtain a timed automaton whose semantics coincides with that of the system of signal inclusions

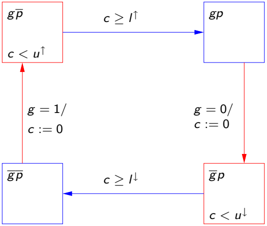
The Delay Automaton

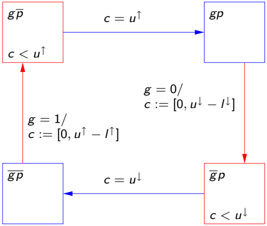
- The automaton has two stable states gp and $\overline{gp}$ where the gene and the product agree
- When g changes (excitation) the automaton moves to the unstable state and resets a clock to zero
- It can stay in an unstable state as long as $c < u$ and can stabilize as soon as $c > l$.



Expressing Temporal Uncertainty

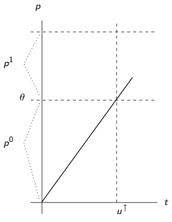
- In this automaton the uncertainty interval $[l, u]$ is expressed by the non-punctual intersection of the guard $c \geq l$ and the invariant $c < u$
- An alternative representation: making the stabilization transition deterministic and accompany the excitation transition with a non-deterministic reset



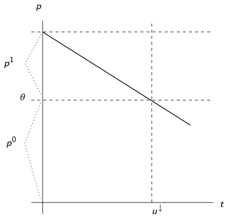


Where do Delay bounds Come From?

- These are abstractions of continuous growth and decay processes indicating the time it takes to move between points in domains $p^0 = [0, \theta]$ and $p^1 = [\theta, 1]$
- For example, for constant rates $k^{\uparrow}$ and $k^{\downarrow}$ the bounds will be $D^{\uparrow} = [0, \theta/k^{\uparrow}]$ and $D^{\downarrow} = [0, \theta/k^{\downarrow}]$



Production

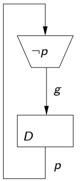


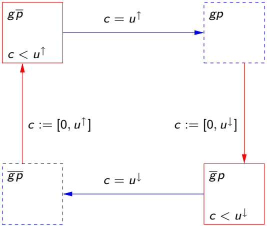
Decay

- In any case, if we want the abstraction to be conservative we should have a zero lower bound
- And this smells of Zenonism...

To Zeno or not to Zeno?

- Consider a negative feedback loop where the presence of p turns g off and its absence turns g on

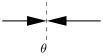




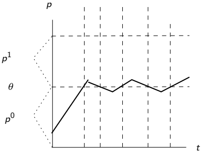
- Among the behaviors that the automaton may exhibit, if we allow a zero lower bound, is a zero time cycle
- Whether this is considered a bug or a feature depends on one's point of view
- This is related to the fundamental difference between the discrete and the continuous

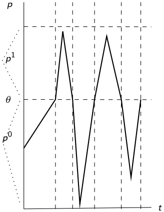
Zenonism from a Continuous Point of View

- The continuous model of the negative feedback loop is a one-dimensional vector field pointing to an equilibrium point θ



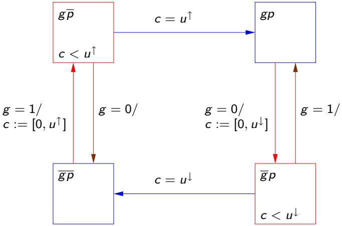
- In "reality" the value of p will have small oscillations around θ which is normal. Not much difference between θ, $\theta + \epsilon$, $\theta - \epsilon$
- Discrete abstraction amplifies this difference. The inverse image of the oscillating Boolean signal contains also large oscillations

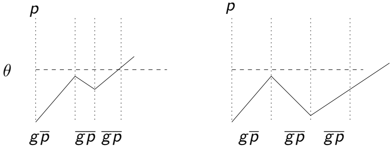




Regrets and Abortions

- Another point in favor of a zero lower bound:
- Suppose g changes, triggers a change in p and then switches back before p has stabilized, aborting the process

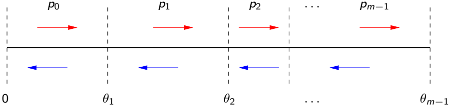




- In the “stable” state there is a decay process inside p^0
- Without additional clocks we do not now for how long
- Has the p level returned to the “nominal” low value or is still close to the threshold?

Multi-Valued Models

- The incompatibility between the discrete and the continuous is an eternal problem
- Its effect on modeling and analysis can be reduced significantly using multi-valued discrete models
- Instead of $\{0, 1\}$ we use $\{0, 1, \ldots, m-1\}$ which, via a set $0 < \theta_1 < \theta_2 < \ldots, < \theta_{m-1} < 1$ of thresholds, defines every discrete state as
$$p^i = [\theta_i, \theta_{i+1}]$$



- If you just entered p^i from p^{i-1}, you need to cross the whole p^i in order to reach p^{i+1}

Multi-Valued Delay Operator

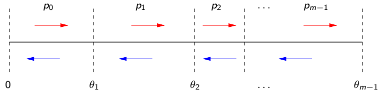
- The delay operator for multiple values will have $2(m-1)$ parameters in each direction.
- When $g = 1$, p will progress toward the next level and vice versa

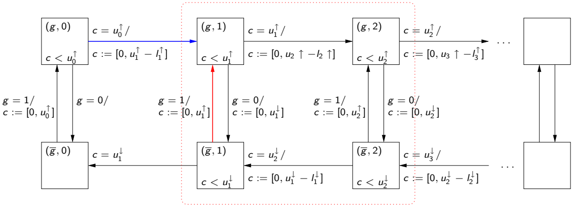
g	p	p'	Δ	g	p	p'	Δ
0	0	0	–	1	0	1	$[l_0^\uparrow, u_0^\uparrow]$
0	1	0	$[l_1^\downarrow, u_1^\downarrow]$	1	1	2	$[l_1^\uparrow, u_1^\uparrow]$
0	2	1	$[l_2^\downarrow, u_2^\downarrow]$	1	2	3	$[l_2^\uparrow, u_2^\uparrow]$
…	…	…	…	…	…	…	…
0	$m-1$	$m-2$	$[l_{m-1}^\downarrow, u_{m-1}^\downarrow]$	1	$m-1$	$m-1$	–

$$l_i^\uparrow = \min\{t : \theta_i \xrightarrow{t} \theta_{i+1}\} \qquad u_i^\uparrow = \max\{t : \theta_i \xrightarrow{t} \theta_{i+1}\}$$

$$l_i^\downarrow = \min\{t : \theta_i \xrightarrow{t} \theta_{i-1}\} \qquad u_i^\downarrow = \max\{t : \theta_i \xrightarrow{t} \theta_{i-1}\}$$

The Automaton for the Multi-Valued Model



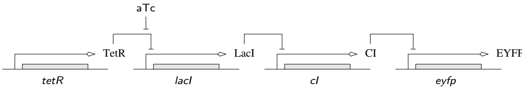


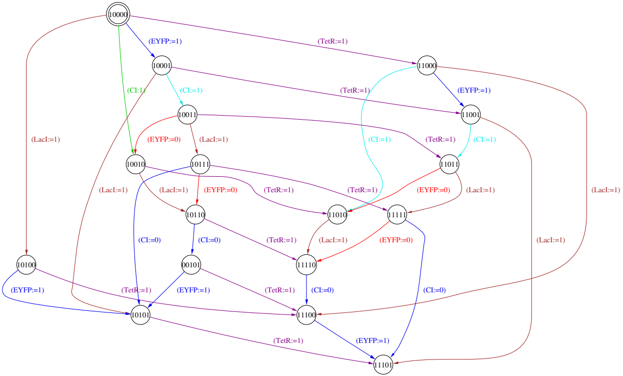
- The lower bound for moving from (g, i) to $(g, i+1)$ depends on the state from which (g, i) was entered
- If from $(g, i-1)$ (continuous evolution) then it is $l_i^{\uparrow}$
- If from $(\overline{g}, i)$ (change of direction) then it is 0
- Zero/Zeno cycles can happen only among neighbors i,$i+1$

The Global Automaton

- We then compose all these automata to obtain a global timed automaton with n clocks and roughly 2^n discrete states
- This automaton represents all the behaviors of the network while taking timing into account
- Existing tools can take a description of such a timed automaton and compute all the possible behaviors under **all** choices of delays
- We use our IF toolbox and demonstrate its capabilities on several examples
- Not much biological significance at this point (no experimental delay values available)

Example: Transcription Cascade for E. Coli

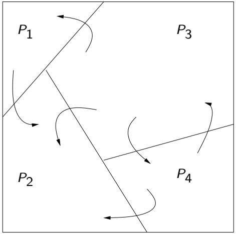


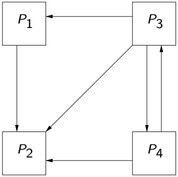


From Continuous Systems To Automata I

- Consider again a continuous dynamical system $\dot{x} = f(x)$ defined over $X \subseteq \mathbb{R}^n$
- A popular (and old) approach for analyzing such systems (qualitative physics, robotics motion planning, etc.) is to approximate it by a finite-state automaton as follows:
- Impose a finite partition $\Pi = \{P_1, \ldots P_k\}$ on X
- Define an automaton with state space Π and transition relation δ such that
- $(P, P') \in \delta$ iff P and P' are adjacent and there are points $x \in P$ and $x' \in P'$ and a trajectory leading from x to x'
- The latter fact can be sometimes determined easily by analyzing f on the boundary between P and P'

From Continuous Systems To Automata II

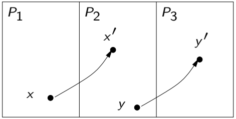


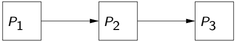


- Once you have a finite automaton you are happy because you can apply all the model-checking algorithms and tools that you already have
- But there is no free lunch

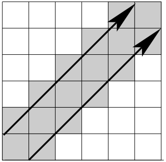
False Transitivity and Spurious Behaviors

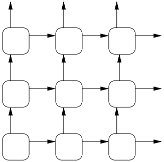
- Such abstract models often exhibit **spurious behaviors**, that are not possible in the concrete system
- You may go from $x \in P_1$ to $x' \in P_2$ and from $y \neq x' \in P_2$ to $y' \in P_3$ but not necessarily from P_1 to P_3





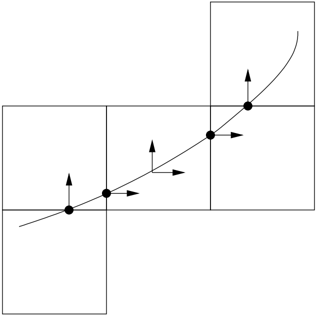
- Sometimes the approximation error renders the model useless





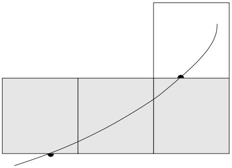
How to Reduce Spurious Behaviors

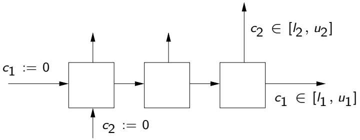
- One can see the evolution as a **competition** between state variables:
- Who will cross the next threshold in its direction
- A dimension that wins, starts the competition in the next block from an inferior position and is less likely to win again



Using Clocks

- We associate a clock c_i with each dimension which is reset whenever a boundary is crossed in direction i
- The next transition in **the same** direction is constrained to occur when $c_i \in [l_i, u_i]$





- The constants are inferred from the minimal and maximal value of f_i in the corresponding "slice" (slightly circular reasoning)
- It is easy to compute these min-max values for multi-affine systems

Current and Future Status

- Prototype implementation, does not work on the fly but generates the whole model in the IF format
- Not surprisingly, works rather well in monotone parts of the state space. In parts where some f_i admits a zero we need to be more careful
- Some examples, not yet convincing
- For the more general class of polynomial systems, extremal values of f_i should be computed numerically
- Future: a tighter tool integration, automatic choice of partition thresholds, model-checking against MITL

Back to the Big Picture

- Biology needs (among other things) more dynamic models to form verifiable predictions
- These models can benefit from the accumulated understanding of dynamical system within informatics and cannot rely only on 19th century mathematics
- The views of dynamical system developed within informatics are, sometimes, more adapted to the complexity and heterogeneity of Biological phenomena
- Biological modeling should be founded on various types of dynamical models: continuous, discrete, hybrid and timed
- These models should be strongly supported by computerized analysis tools offering a range of capabilities from simulation to verification and synthesis

Back to the Big Picture

- Systems Biology should combine insights from:
- Engineering disciplines: modeling and analysis of very complex man-made systems (chips, control systems, software, networks, cars, airplanes, chemical plants)
- Physics: experience in mathematical modeling of natural systems with measurement constraints
- Mathematics and Informatics as a unifying theoretical framework

Thank You