# Reaction networks with delays applied to toxicity analysis

## Joint work with Hanna Klaudel and Franck Delaplace

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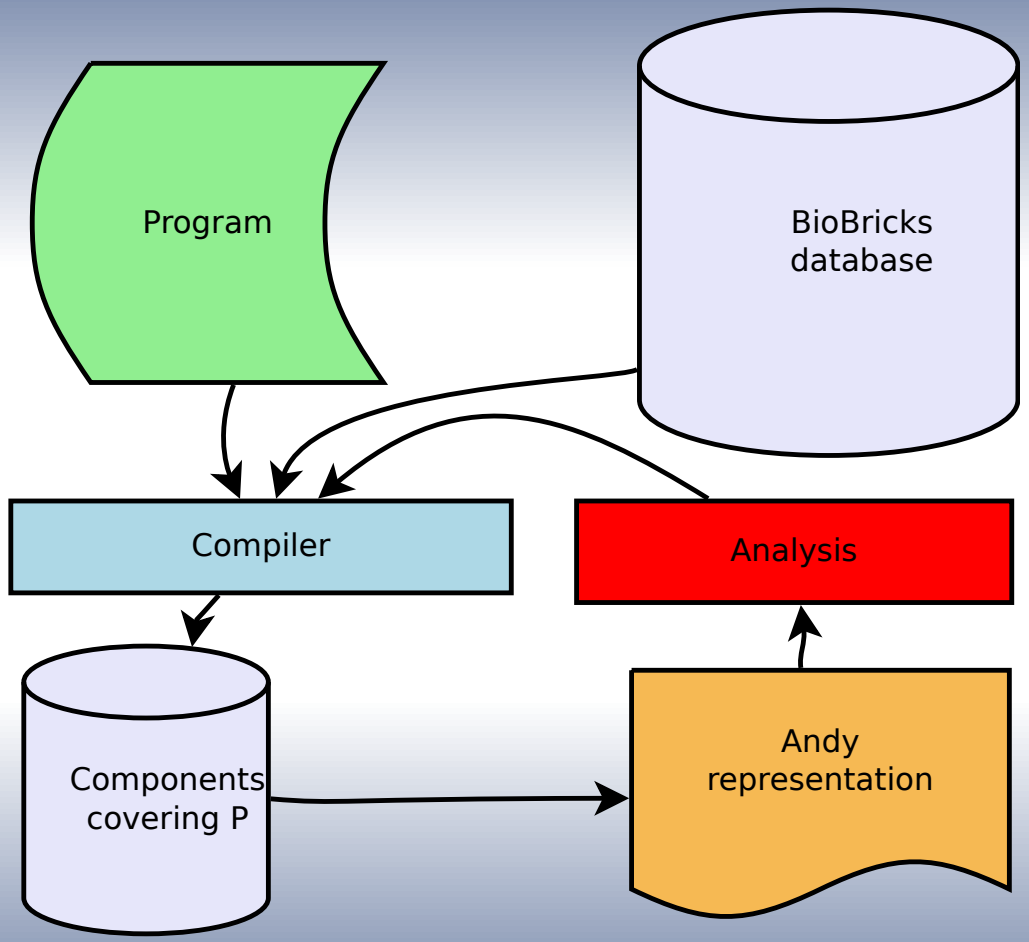
# The SYNBIOTIC project




# Motivation

- **Main goal**: design of artificial bio-systems
- **How**: development of computer-aided tools
- **What**: specification and analysis of cellular regulation networks (i.e., genetic and signalization networks and metabolic pathways)

# Requirements

We want to build a model where:

- different regulatory networks can be expressed
- safety properties can be guaranteed

**Safety**

- in general ⇒ nothing bad can happen
- in a bio-framework ⇒ the system do not exhibit toxic behaviors

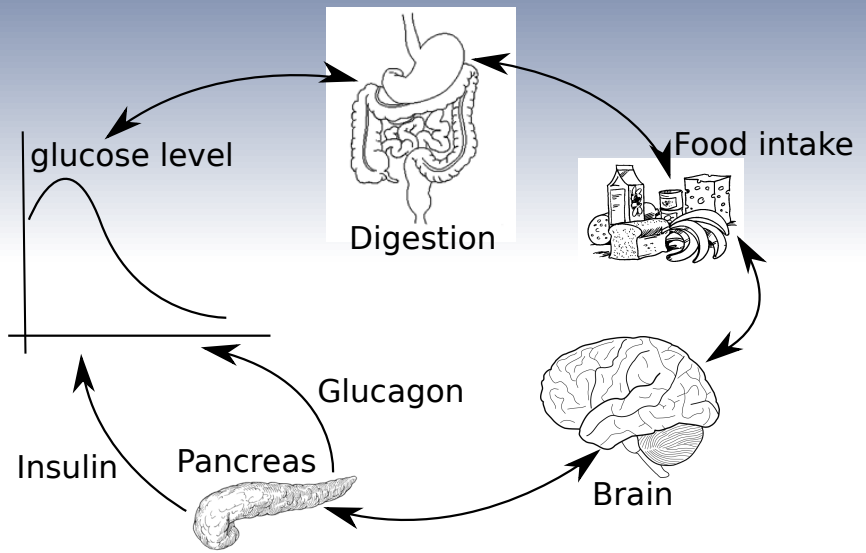
# Toxicology

- The **toxicity process** is a sequence of physiological events that causes the abnormal behavior of a living organism with respect to its healthy state.
- Healthy physiological states generally correspond to **homeostasis**.
- Toxicity highly depends on the exposure time and the thresholds dosage delimiting the ranges of safe and hazardous effects.

**Definition (Toxicity)**

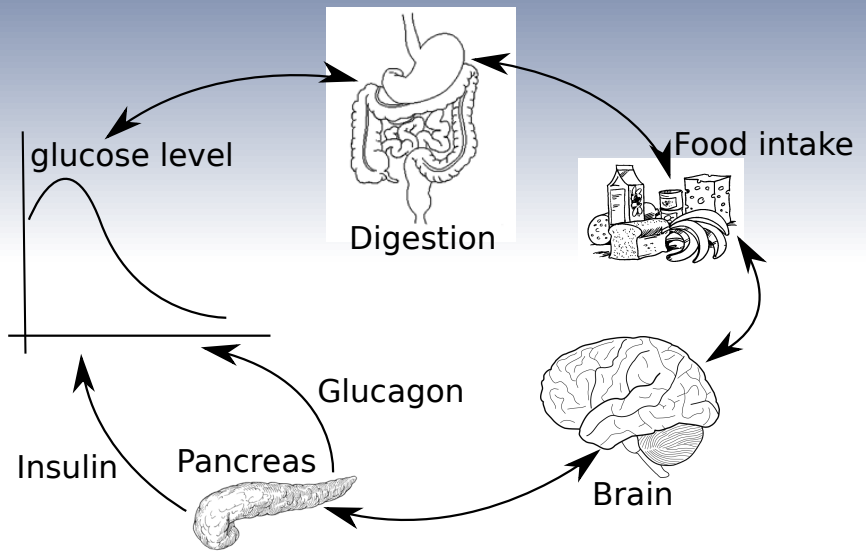
Toxicity is the deregulation of the homeostasis processes


# Blood glucose regulation




- Glucose regulation is a homeostatic process.
- Glycemia is regulated by insulin and glucagon.
- Assimilation of sugars vs aspartame.


# Blood glucose regulation

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**Toxic!**

Assimilation of food (even if it contains aspartame) should calm hunger and induce satiety, not the opposite!

# Features

Our model features

- An explicit notion of discrete time
- Species with expression levels and decay
- Reactions with duration

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**ANDy**

An ANDy network is a set of species $\mathcal{S}$ governed by a set of reactions $\mathcal{R}$

# Species

- Species have a finite number $\mathcal{L}_s$ of **expression** levels.
- Each species $s$ is initialized at level $\eta_s$ and it decays gradually as time passes by.
- Duration of decay vary among levels:

$$\delta_s : [0..\mathcal{L}_s - 1] \to \mathbb{N}^+ \cup \{\omega\}.$$

$\delta_s(0) = \omega$.

# Reactions

- Reactions govern evolution of species

$$\rho ::= A_\rho \ ; \ I_\rho \xrightarrow{\Delta} R_\rho$$

- $A_\rho$, $I_\rho$ are sets of pairs $(s, \eta_s)$
- $R_\rho$ is a set of pairs $(s, \pm n)$
- Each reaction has a **response time**

$$\Delta : \mathcal{R} \to \mathbb{N}^+$$

Time required for yielding increase (+) and/or decrease (-) of levels of results.

# Dynamics

A reaction of response time $\Delta$ can take place if

- each activator/reactant stays at least at a given level
- each involved inhibitor is at most at a given level

during the whole reaction time.

- **Outcome:** the level of results of the reaction can be increased or decreased.

# Formalization

The dynamics of ANDy is formalized using high-level Petri nets.

- Time is explicitly represented.
- Places: Species + 1 place for time
- Transition: Reaction + 1 transition for time

# Places

- We assume a unique discrete global clock that starts at zero and always shows the current date (timestamp).
- Each species is represented by a place
- The state of a species $s$ is a tuple $\langle l_s, u_s, \lambda_s \rangle$
  - $l_s$ stores the current level;
  - $u_s$ is a timestamp recording the last date when the level has been updated;
  - $\lambda_s$ is a tuple of timestamps with $\mathcal{L}_s$ fields;

# Transitions – 1

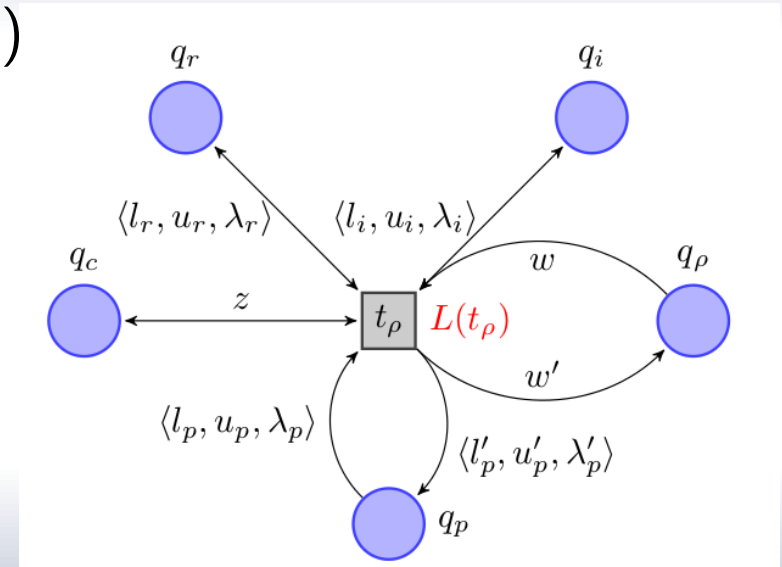
ANDy networks can evolve in two ways:

1. as effect of an enabled reaction $\rho$
2. as an effect of the clock:

# Transition: reaction

Transition guard:

$$w < z \wedge w' = z \quad \wedge$$
$$\bigwedge_{(a,\eta_a)\in A_\rho}(l_a \geq \eta_a \wedge z - \lambda[\eta_a] \geq \Delta(\rho)$$
$$\bigwedge_{(i,\eta_i)\in I_\rho}(l_i < \eta_i \wedge z - \lambda[\eta_i] \geq \Delta(\rho))$$



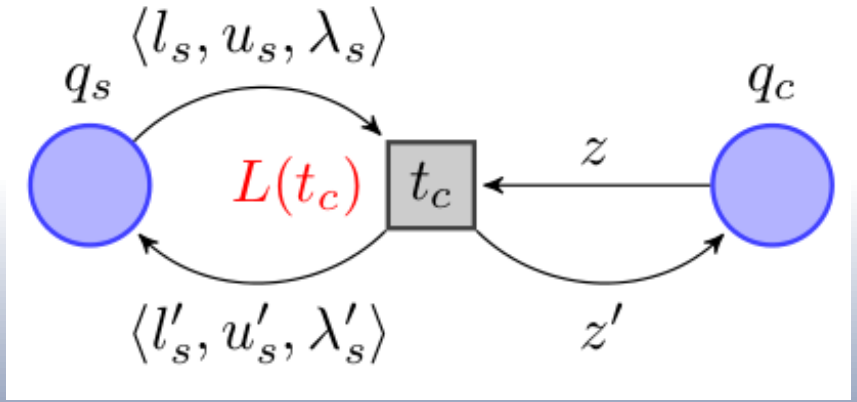

Result: a result $r$ at level $l_r$ and the clock at time $t$

$$(r, +1)$$
$$\langle l_r, u_r, \lambda_r\rangle \rightarrow \langle l_r + 1, t, \lambda_r\{^t/_{l_r+1}\}\rangle$$
$$(r, -1)$$
$$\langle l_r, u_r, \lambda_r\rangle \rightarrow \langle l_r - 1, t, \lambda_r\{^t/_{l_r}\}\rangle$$

# Transitions – Clock

2. as an effect of the clock:
   - The timestamp $t$ stored in the clock is incremented by one $(t + 1)$.
   - A species may stay at level $l$ for $\delta(l)$ time units. Decay happens as soon as the interval $\delta(l)$ is elapsed ,

$$\langle l, u, \lambda \rangle \rightarrow \langle l - 1, t + 1, \lambda\{^{t+1}/_{l}\} \rangle$$

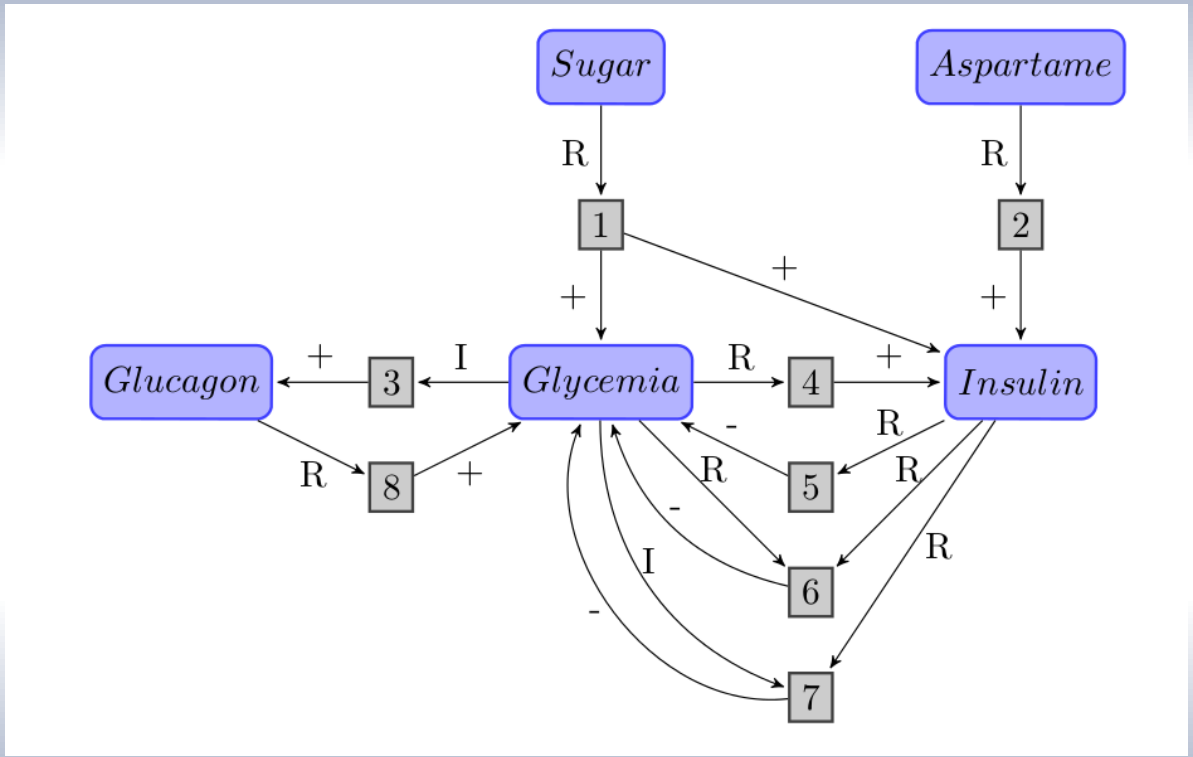


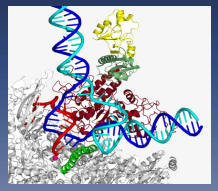
# Glucose regulation – 1

The set of species involved:

| | | |
|---|---|---|
| *Sugar* | $\mathcal{L}_{sugar} = \{0, 1\}$ | $\delta_{sugar}(1) = 2$ |
| *Aspartame* | $\mathcal{L}_{aspartame} = \{0, 1\}$ | $\delta_{aspartame}(1) = 2$ |
| *Glycemia* | $\mathcal{L}_{glycemia} = \{0, 1, 2, 3\}$ | $\delta_{glycemia}(1) = 8$ |
| | | $\delta_{glycemia}(2) = 8$ |
| | | $\delta_{glycemia}(3) = 8$ |
| *Glucagon* | $\mathcal{L}_{glucagon} = \{0, 1\}$ | $\delta_{glucagon}(1) = 3$ |
| *Insulin* | $\mathcal{L}_{insulin} = \{0, 1, 2\}$ | $\delta_{insulin}(1) = 3$ |
| | | $\delta_{insulin}(2) = 3$ |

# Glucose regulation – 2





# Glucose regulation – 3

The set of reactions:

| $\rho_k$ | *Activators* $A_k$ | *Inhibitors* $I_k$ | *Results* $R_k$ | $\Delta_k$ |
|---|---|---|---|---|
| $\rho_1$ | $\{(Sugar, 1)\}$ | $\emptyset$ | $\{(Insulin, +), (Glycemia, +)\}$ | 1 |
| $\rho_2$ | $\{(Aspartame, 1)\}$ | $\emptyset$ | $\{(Insulin, +)\}$ | 1 |
| $\rho_3$ | $\emptyset$ | $\{(Glycemia, 1)\}$ | $\{(Glucagon, +)\}$ | 1 |
| $\rho_4$ | $\{(Glycemia, 3)\}$ | $\emptyset$ | $\{(Insulin, +)\}$ | 1 |
| $\rho_5$ | $\{(Insulin, 2)\}$ | $\emptyset$ | $\{(Glycemia, -)\}$ | 2 |
| $\rho_6$ | $\{(Insulin, 1), (Glycemia, 3)\}$ | $\emptyset$ | $\{(Glycemia, -)\}$ | 2 |
| $\rho_7$ | $\{(Insulin, 1)\}$ | $\{(Glycemia, 2)\}$ | $\{(Glycemia, -)\}$ | 2 |
| $\rho_8$ | $\{(Glucagon, 1)\}$ | $\emptyset$ | $\{(Glycemia, +)\}$ | 2 |

# Observation

- Decay and reactions are different types of behaviors
- Decay is synchronous
  it corresponds to an abstraction of the action of the environment
- Reactions are asynchronous
  their duration corresponds to the time required to observe an effect
- Execution time vs Simulation time
  More reactions are enabled less probable is the execution of time

# Toxicity analysis

- ANDy can be used to detect and predict toxic behaviors related to the dynamics of bio-molecular networks.
- We resort to temporal logics and model checking techniques.
- We use computation tree logic (CTL)
- We provide an abstraction of ANDy into Kripke structures

# Examples of questions

We are interested in checking whether the inner equilibrium of an organism is maintained when administrating drugs or applying stressors.

Toxicology properties can be classified into:

1. properties checking for the appearance of **symptoms**,
2. properties characterizing **causal relations** between events.

# Glucose regulation

## Causality:

Does assimilation of sweeteners cause hypoglycemia?

$$\mathbf{EF}[((\mathit{Sugar}, 1) \vee (\mathit{Aspartame}, 1)) \wedge (\mathit{Glycemia}, 1)] \rightarrow \mathbf{AF}(\mathit{Glycemia}, 2)$$

# Paths for glucose regulation

$$\mathbf{EF}[((\mathit{Sugar},1) \vee (\mathit{Aspartame},1)) \wedge (\mathit{Glycemia},1)] \rightarrow \mathbf{AF}(\mathit{Glycemia},2)$$

- Path that satisfies

$$(\mathit{Sugar},1),(\mathit{Aspartame},0),(\mathit{Glycemia},1),(\mathit{Insulin},0),(\mathit{Glucagon},0) \xrightarrow{\rho_1}$$
$$(\mathit{Sugar},1),(\mathit{Aspartame},0),(\mathbf{Glycemia},\mathbf{2}),(\mathit{Insulin},1),(\mathit{Glucagon},0)$$

- Path that contradicts

$$(\mathit{Sugar},0),(\mathit{Aspartame},1),(\mathit{Glycemia},1),(\mathit{Insulin},0),(\mathit{Glucagon},0) \xrightarrow{\rho_2}$$
$$(\mathit{Sugar},0),(\mathit{Aspartame},1),(\mathit{Glycemia},1),(\mathit{Insulin},1),(\mathit{Glucagon},0) \xrightarrow{\rho_7}$$
$$(\mathit{Sugar},0),(\mathit{Aspartame},0),(\mathbf{Glycemia},\mathbf{0}),(\mathit{Insulin},1),(\mathit{Glucagon},0)$$

# Sound and completeness

**Theorem**

*Given an* ANDy *network* $(\mathcal{S}, \mathcal{R})$*, its encoding into*

- *Kripke structures*
- *Timed Automata*

*is sound and complete.*

# Summing up

- ANDy, a high-level Petri net framework for cellular regulation networks.
- Species that can degrade as time passes by governed by a set of reactions.
- Toxicity properties can be expressed via a temporal logic.
- Properties can be verified thanks to a sound and complete abstraction.

# Final remarks

- Comparison with stochastic models à la Gillespie
- Refinement of the abstraction
- Implementation: Snakes, Snoopy + Marcie

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