Recent Developments in Non-Monotonic Logical Modeling of Regulatory Genetic Networks

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Motivations

To show the interest of a **declarative** approach based on **Answer Set Programming** (ASP)

- for modeling Thomas’ logical discrete **Genetic Regulatory Networks** (GRNs)
- for inducing GRNs a priori consistent with experiments (**reverse engineering**).
- for taking into account both automatic inconsistency repairing and gene interaction properties which are only **generally true**, by using **default rules** provided by ASP.
Declative approach. Personnal view

Four steps methodology.

- Formalization (network structure, behaviors, ...) with constraints.
- Consistency test (see below).
- Extraction of properties (theorems).
- Choice of experiments / Knowledge addition. Return to 2.
Content

1 Basics: ASP and logical modeling of Thomas GRNs

2 Applications

3 Generally true Additivity Constraints and automatic consistency repairing

4 On going works
Belief revision with ASP (Answer Set Programming).

ASP is a logic programming technology based on a non monotonic logic with models said "stable" which are minimal. Rules are:

\[ a_0 : \neg a_1, \ldots, a_m, \text{not } a_{m+1}, \ldots, \text{not } a_n \]

The typical example for introducing to non monotonic logics:

- From the axioms in ordinary (monotonic) logic:
  \( flies(X) \leftarrow bird(X) \). and \( bird(tweety) \).
  one deduces \( flies(tweety) \).

- The problem is with penguins. Taking them into account demands:
  - completing the 1st axiom by \( \neg penguin(X) \) as a premise,
  - qualifying **by hand** every bird (is it or not a penguin ?).
Advantages of ASP. Belief revision (cont.)

With ASP, these manual revisions may be avoided by using **defaults**. Unless the contrary is **proved**, a bird is not a penguin.

- From:
  
  flies(X) :- bird(X), not penguin(X).
  
  bird(tweety).

  one can prove flies(tweety).

- But if later on, by addition of new knowledge (belief revision, e.g. result of experimentation), penguin(tweety) can been proved then flies(tweety) is no more deducible (non monotony).

In our case, *additivity constraints* on gene interactions (see later) can be represented by such defaults.
Thomas GRNs. Interaction graph

\[ X_a = \begin{cases} 
K_a & \text{if } x_b < \theta_b^1 \\
K_b & \text{if } x_b \geq \theta_b^1 
\end{cases} \]

\[ X_b = \begin{cases} 
K_a & \text{if } x_a < \theta_a^1 \text{ and } x_b < \theta_b^2 \\
K_b & \text{if } x_a \geq \theta_a^1 \text{ and } x_b < \theta_b^2 \\
K_b & \text{if } x_a < \theta_a^1 \text{ and } x_b \geq \theta_b^2 \\
K_{ab} & \text{if } x_a \geq \theta_a^1 \text{ and } x_b \geq \theta_b^2 
\end{cases} \]

\( x_a \): (discrete) concentration of protein \( a \). \( \theta_a^1 \): threshold of \( a \).
Thomas GRNs. Dynamics

- **Focal** equations relate a state \([x_a, x_b]\) and its focal state \([X_a, X_b]\) indicating in which direction are its neighboring successors, thanks to parameters \(K\).

- Semantics of signs:

  - **Observability** constraint (always true) for \(a \xrightarrow{+1} b\):
    \[
    (K_b < K_a^a) \lor (K_b^b < K_{ab}^b)
    \]

  - **Additivity** constraint (generally true) for \(a \xrightarrow{+1} b\):
    \[
    (K_b \leq K_a^a) \land (K_b^b \leq K_{ab}^b)
    \]

  i.e. no inhibition expected from \(a\).
Transition graphs satisfying observability and additivity constraints.

One equilibrium for $G_1$, $G_3$, $G_5$. **Mutistationarity** for $G_2$, $G_4$, $G_6$.

There are $2^2 \times 3^4 = 332$ possible set of parameters.
Experiments (behaviors) represented as paths

- Enforcing the existence of a path of two successive identical states (steady state) gives all transition graphs except $G_3$.
- Enforcing the existence of a path beginning with the state $[0, 0]$ and reaching the state $w$ $[0, 2]$ leads to the models $G_4$ and $G_6$.

Several functionalities are available including automatic inconsistency repairing, mutant specification, minimization (interactions and thresholds values), deduction of properties on domains specified by biologists.
Other facilities

- automatic inconsistency repairing.
- mutant specification.
- minimization (interactions and thresholds values): the ASP software provides para-logical operator like #minimize\{f_1,\ldots,f_n\} that produces only models with the lowest number of literals f_i true.
- deduction of properties on domains specified by biologists: for example, \((K_b < K^a_b) \lor \neg (K_b < K^{ab}_b)\) true in all models.
Content

1. Basics: ASP and logical modeling of Thomas GRNs

2. Applications

3. Generally true Additivity Constraints and automatic consistency repairing

4. On going works
Two steady states: 1) with a high concentration of Fis and a high supercoiling (e.g. high ratio GyrAB / TopA), 2) after carbon deprivation, with a high concentration of Crp and a weaker supercoiling.

Two response paths to the two stresses: carbon deprivation, carbon-source availability.
Inconsistency repairing. [Corbin et al., Biosyst., 2009]

**Inconsistency** with: observability and additivity constraints + constraints enforcing steady states and response paths. **Automatic repairing**, by relaxing as few as possible additivity constraints, offers two possibilities:

- Rejecting $K_{gyrAB} \geq K_{fis}^{gyrAB}$: means that *Fis* does not inhibit *GyrAB* when the bacteria are not stressed. **Disagrees** experimental data from [Schneider et al., Mol. Microbiol., 1999].

- Rejecting $K_{topA}^{fis} \geq K_{topA}$: would imply that TopA synthesis is possible even if the concentration of *Fis* is low. **Supported** by [Westein Fischer et al., Mol. Microbiol., 2007] for a stress due to hydrogen.

By relaxing this last constraint, we get only 3 different instantiated models (on the 279,936 possible instantiated models due to the possible values of the 22 logical parameters).
Drosophila embryo gap genes net. [Corblin et al. IPCAT 2012]

- Three maternal genes (cad, bcd, ter) and four gap genes (kr, hb, kni, gt). Well-established or potential interactions.
- Spatio-temporal expression profile of the main genes along the antero-posterior axis, giving seven regions (stable states).
- Expressions of genes also available from the seven mutants.

Objective: networks with the number of potential interactions and the number of thresholds minimized.
Potential interactions are represented by dotted red arrows.
The set of constraints is found **consistent** (3338 s., on a PC, 2 proc. 2.4 GHz, 2.9GB memory).

A **unique** minimal regulatory graph is then obtained (1016 s.) which includes only **two** potential interactions. Finally we get a unique minimal instantiation of the thresholds (368 s.).

Deduced properties on parameters: 52 fixed parameters (over 72), 48 inequalities on the remaining ones: 12 between one parameter and one threshold, 36 between two parameters.

The story is not finished... Enforcing CTL AF-like formulas is now required.
Minimized network, with only two potential interactions

N. Mobilia, A. Rocca, S. Chorlton, E. Fanchon, L. Trilling
IRMA network, from [G. Batt et al., Bioinformatics, 2010]

Figure: (a) The IRMA (In vivo benchmarking of Reverse-engineering and Modeling Approaches) network [I.Cantone et al., Cell., 2009], (b) the corresponding piecewise affine differential equations.

\[ \begin{align*}
\dot{x}_{\text{Gal4}} &= k_{\text{Gal4}}^{0} + k_{\text{Gal4}} s^{+}(x_{\text{CBF1}}, \theta_{\text{CBF1}}) - \gamma_{\text{Gal4}} x_{\text{Gal4}} \\
\dot{x}_{\text{Swi5}} &= k_{\text{Swi5}}^{0} + k_{\text{Swi5}} s^{+}(x_{\text{Gal4}}, \theta_{\text{Gal4}}) (1 - s^{+}(x_{\text{Gal80}}, \theta_{\text{Gal80}}) s^{-}(u_{\text{Gal4}}, \theta_{\text{Gal4}})) \\
\dot{x}_{\text{Ash1}} &= k_{\text{Ash1}}^{0} + k_{\text{Ash1}} s^{+}(x_{\text{Swi5}}, \theta_{\text{Swi5}}) - \gamma_{\text{Ash1}} x_{\text{Ash1}} \\
\dot{x}_{\text{CBF1}} &= k_{\text{CBF1}}^{0} s^{+}(x_{\text{Swi5}}, \theta_{\text{CBF1}}) + k_{\text{CBF1}}^{1} s^{+}(x_{\text{Swi5}}, \theta_{\text{Swi5}}) s^{-}(x_{\text{Ash1}}, \theta_{\text{Ash1}}) \\
\dot{x}_{\text{GAL80}} &= k_{\text{GAL80}}^{0} + k_{\text{GAL80}} s^{+}(x_{\text{Swi5}}, \theta_{\text{Swi5}}) - \gamma_{\text{GAL80}} x_{\text{GAL80}} 
\end{align*} \]
IRMA interaction network
**Temporal profiles**

Figure: (a) Temporal profiles encoding of averaged gene expression. "switch-on" ("switch-off") refers to the activation (inhibition) of Swi5 during growth of galactose (glucose). (b) Temporal encoding of the switch-on and switch-off behaviors. Only changes greater than $5 \times 10^{-3}$ units are considered significant.
Two approaches

- Batt’s approach (with singular states): a new modeling with more regular states, such that each species has a unique derivative sign in them + the model checking tool NuSMV. He claims, when comparing its work, that it applies to "incompletely instantiated models" and provides "more precise results" and "efficient coding".

- Our approach for replying: Thomas initial model (no singular states), with adequate constraints expressing that a path satisfies a temporal series.

- Programming experiments for inferring parameters and thresholds: which models satisfy formulas $\varphi_1$ (2 large EF formulas representing averaged "switch-off" and switch-on" experiments) and $\varphi_2$ (9 large EF formulas representing all time series).
Results

- After discovering mistakes in Batt’s work (and Batt in both our works...), we found the same results: 64 models for $\varphi_1$ and 4 for $\varphi_2$ (on 4860). No more precise results with singular states...
- Better or equivalent performances: 139 s. vs 885 s. for $\varphi_1$, 2002 s. vs 2021 s. for $\varphi_2$, with a regular ASP solver (no incremental solving).
Constraints vs model checking

- Model checking is based on CTL with a weak expression power compared to Prolog like ASP language, e.g. enforcing the existence of at least two steady states is not possible.

- Model checking is oriented toward verification of transition systems, Logic programming toward programming with logic, e.g. Batt performs out of NuSMV by using a counter-example delivery facility and a para-logic help for producing models by extension (not appropriate to deduce properties).
Content

1. Basics: ASP and logical modeling of Thomas GRNs
2. Applications
3. Generally true Additivity Constraints and automatic consistency repairing
4. On going works
Two modeling issues and a first solution

Issues:

- Escaping inconsistency if some additivity constraints are not satisfied.
- Accepting only models with as many as "possible" additivity constraints.

A first solution:

- Enumerating all models, i.e. all possible atoms $kparam(K, Ik)$ where $K$ is the value of the parameter named $Ik$. **Costly.**
- Maximizing, with a para-logical process (Max-SAT like), the number of satisfied additivity constraints. **Costly** too and debatable (logical minimization versus para-logic global criterion).
New solution (main lines). Efficient inconsistency repairing

- Restricted **firm** production of $k_{\text{param}}$ atoms to:
  - rules specifying the paths.
  - rules specifying the observability constraints.
  **Important**: these constraints are disjunctions, like
  $(K_b < K_a^b) \lor (K_b^b < K_{ab}^b)$. Non minimal models of them should
  be rejected (unless imposed by a behavior), e.g. the rules must
  reject $(K_b < K_a^b) \land (K_b^b < K_{ab}^b)$ (if no contraindication).
  Naturally expressible in ASP.

- Conditional production of $k_{\text{param}}$ atoms due to the additivity
  constraints, by **default** rules like:
  
  $$\text{addit}(+, a, b) :- \text{obs}(+, a, b), \text{not obs}(-, a, b).$$
  
  % (applicable only if a negative observability is not proved)
  where $\text{addit}(+, a, b)$ implies $k_{\text{param}}$ atoms satisfying
  $(K_b \leq K_a^b) \land (K_b^b \leq K_{ab}^b)$. 

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New solution. Retaining only appropriate models

- **Aim**: retaining models with maximum allowed additivity constraints.
- **Issue**: avoiding, if no contraindication, that additivity on one edge infers non-additivity on another one (interaction between defaults).

The following rules mimic influences between two edges targeting the same species (\(|\) is for minimal disjunction):

- \(p_2 \mid u \leftarrow \neg p_1\).
- \(p_1 \mid v \leftarrow \neg p_2\).

Three Answer Sets (ASs): \(\{u, v\}, \{p_2\}\) and \(\{p_1\}\).

**Challenge**: transform these rules so that we get a conjunction of defaults with the only AS \(\{u, v\}\), if no contraindication.
Construction of conjunction of defaults

Two steps:

- Defining the literal \( c \) by:
  \[
  c : - \ p1. \\
  c : - \ p2. \\
  \]
  so that \( \neg c \) represents the case where both \( p1 \) and \( p2 \) are unknown or false,

- Adding to each rule a new and curious tautological term serving as a **guard**:
  \[
  p2 \ | \ u : - \ \neg p1, 1\{c, \neg c\}1. \\
  p1 \ | \ v : - \ \neg p2, 1\{c, \neg c\}1. \\
  \]
Possible inconsistency. Example

\[ (K_a < K_a^{a} \wedge K_a^{a} \geq 1) \vee (K_a^{b} < K_a^{ab} \wedge K_a^{ab} \geq 1) \]
\[ \vee (K_a^{c} < K_a^{ac} \wedge K_a^{ac} \geq 1) \vee (K_a^{bc} < K_a^{abc} \wedge K_a^{abc} \geq 1) \]
\[ (K_a < K_a^{b}) \vee (K_a^{a} < K_a^{ab}) \vee (K_a^{c} < K_a^{bc}) \vee (K_a^{ac} < K_a^{abc}) \]
\[ (K_a < K_a^{c}) \vee (K_a^{a} < K_a^{ac}) \vee (K_a^{b} < K_a^{bc}) \vee (K_a^{ab} < K_a^{abc}) \]
Possible inconsistency (cont.)

The instantiations $K_a = 1$, $K_a^b = 0$, $K_a^{ab} = 0$, $K_a^{ac} = 0$, $K_a^{bc} = 0$ and $K_a^{abc} = 1$ ensure the observability constraints.

But for respecting both additivity constraints related to the edges $a \rightarrow a$ and $c \rightarrow a$, $K_a^a$ and $K_a^c$, not a priori known, should be higher (resp. lower) than or equal to $K_a = 1$ (resp. $K_a^{ac} = 0$). See the lattice:

\[ K_a^{ac} = 0 \]
\[ K_a^c = ? \]
\[ K_a^a = ? \]
\[ K_a = 1 \]
Consistency prevention

In short

- For each couple of edges targeting a species N, constructing a guard guarantying the conjunction of their additivities only in case of absence of lattices like above (in a generalized form).
- Expressing the conjunction of all additivities on N as the logical conjunction of the above guards for each couple of edges.
- There are possibly 101 completely instantiated ASs. With defaults rules producing additivity, only 51. With $K_a = 0, K_a^a = 0, K_a^b = 0, K_a^c = 1, K_a^{ab} = 1$, 8 ASs with only unitary defaults, 1 AS having all additivity constraints with the global guard as defined above. If adding $K_a^{ac} = 0$, that forbids the conjunction of defaults with $a \rightarrow a$, one AS is fortunately obtained (non monotonic effect).
Content

1. Basics: ASP and logical modeling of Thomas GRNs

2. Applications

3. Generally true Additivity Constraints and automatic consistency repairing

4. On going works
Applications

Generally true Additivity Constraints and automatic consistency repairing

On going works

On going work. Ensuring CTL formulas in ASP

EF formulas, already available, for **analysis** purpose.
AF formulas necessary for **synthesis** purpose.

Trivial definitions (apparently):

- \[ eF(Prop,S) :- \neg ap(Prop,S). \]
  \[ eF(Prop,S) :- \neg ap(Prop,S), \]
  \[ transition(S,Sp) \land eF(Prop,Sp). \]

- \[ aF(Prop,S) :- ap(Prop,S). \]
  \[ aF(Prop,S) :- \neg ap(Prop,S), \]
  \[ aF(Prop,Sp) : transition(S,Sp). \]
Ensuring CTL formulas in ASP. Three Issues

- Loops, like:
  
  ```
  eF(Prop,s1) :- eF(Prop,s2), transition(s1,s2).
  eF(Prop,s2) :- eF(Prop,s1), transition(s2,s1).
  ```

  No need for checking loops, due to the minimality of stable models.

- AF formulas in case of not known transitions: grounding not possible. In the recursive definition \( aF \) replaced by \( hypAFtrans \) such that:

  ```
  hypAFtrans(Prop,S,Sp) :- transition(S,Sp), aF(Prop,Sp).
  ```

- Limitation on the number of states necessary for tractability (limitation on the length of paths non longer adequate).
On going work. Multiplexes

When working with a PhD student (lab. IRcyn, Nantes) for comparison with another approach, we had to implement networks where multiplexes were present: ERBB receptor-regulated G1/S transition network (Sahin et al., 18 species), tail resorption during the metamorphosis of tadpole (Khalis et al., 8 species) and the T-cell Signaling network (Klamt et al., 40 species).

Then, for efficiency and learning purpose, we consider multiplexes similar to R. Thomas’ SOP (Sum Of Products, disjunctive normal form) and formalize them in terms of kinetic parameters.
Multiplexes. Specification

For this purpose, we need a precise definition.

- **Syntax** of the language of the interactions targeting a species x:
  
  \[ I ::= \text{Mul} \mid \text{Mul or } I \]
  
  \[ \text{Mul ::= Lu} \mid \text{Lu and Mul} \]
  
  \[ \text{Lu ::= Gene\_id} \mid \text{Sig Gene\_id} \]
  
  \[ \text{Sig ::= +} \mid - \]

- **Semantics**: the value of \( oc(I) \), a logical function of the parameters, constructed following the *composition principle*.

Examples:

\[ oc(+a) = K^a_x > K_x. \]

\[ oc(+a \text{ and } + b) = (K^{ab}_x > K_x) \land (K^a_x = K^b_x = K_x) \]

\[ oc(+a \text{ or } + b) = (K^{ab}_x > K^b_x \lor K^a_x > K_x) \land (K^{ab}_x > K^a_x \lor K^{b}_x > K_x) \]

\[ oc(-c \text{ and } + a \text{ and } + b) = (K^{ab}_x > K^c_x) \land (K^{abc}_x = K^{ac}_x = K^{bc}_x = K^a_x = K^b_x = K_x = K^c_x) \]
On going work. Mamalian circadian cycle

Knowing the existence of three such cycles (equinox, winter, summer), find models and delays. With a very reduced network (3 species including light).

Delay modeling is rather simple, but demands at least large integers for expressing ratios of clocks. Linear equations on integers, provided by the ASP solver that we use, are helpful for this purpose.
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Thank you for your attention

QUESTIONS?

Declarative approach ...
Non monotonicity ...
Composition of defaults...
...

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