Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
On going works

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

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26-11-2015

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency r...
On going works

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.

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Applications
Generally true Additivity Constraints and automatic consistency
On going works

Declative approach. Personnal wiew

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.

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency r
On going works

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

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
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:

$$a_0 : -a_1, \ldots, a_m, not \ a_{m+1}, \ldots, not \ a_n$$

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 ?).

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
On going works

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.
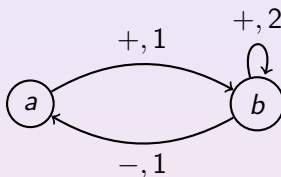
- From:
  ```
  flies(X) :- bird(X), not penguin(X).
  bird(tweety).
  ```
  one can prove flies(tweety).

- 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).

Inn our case, *additivity constraints* on gene interactions (see later) can be represented by such defaults.

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
On going works

Thomas GRNs. Interaction graph

Focal equations:

$$X_a = \begin{cases} K_a & \text{if } x_b < \theta_b^1 \\ K_a^b & \text{if } x_b \geq \theta_b^1 \end{cases}$$

$$X_b = \begin{cases} K_b & \text{if } x_a < \theta_a^1 \text{ and } x_b < \theta_b^2 \\ K_b^a & \text{if } x_a \geq \theta_a^1 \text{ and } x_b < \theta_b^2 \\ K_b^b & \text{if } x_a < \theta_a^1 \text{ and } x_b \geq \theta_b^2 \\ K_b^{ab} & \text{if } x_a \geq \theta_a^1 \text{ and } x_b \geq \theta_b^2 \end{cases}$$

x_a: (discrete) concentration of protein a. θ_a^1: threshold of a.

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Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
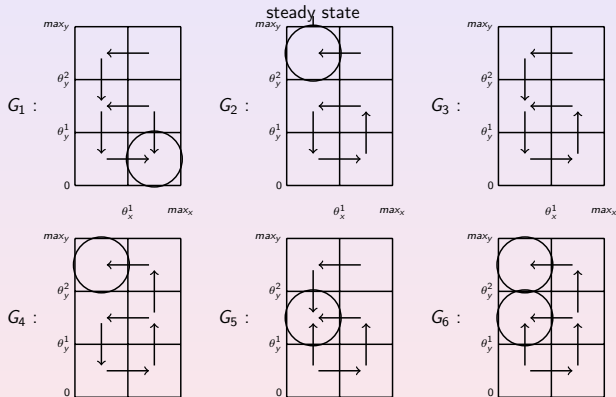On going works

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 $a \xrightarrow{+,1} b$:
  $(K_b < K_b^a) \vee (K_b^b < K_b^{ab})$

  Additivity constraint (**generally** true) for $a \xrightarrow{+,1} b$:
  $(K_b \leq K_b^a) \wedge (K_b^b \leq K_b^{ab})$
  i.e. no inhibition expected from a.

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Applications
Generally true Additivity Constraints and automatic consistency
On going works

Transition graph

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 $2^2 * 3^4 = 332$ possible set of parameters.

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Applications
Generally true Additivity Constraints and automatic consistency
On going works

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.

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Applications
Generally true Additivity Constraints and automatic consistency r
**On going works**

Other facilities

Include

- automatic inconsistency repairing.
- mutant specification.
- minimization (interactions and thresholds values): the ASP software provides para-logical operator like `#minimize{f_1,...,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, $(K_b < K_b^a) \vee \neg(K_b < K_b^{ab})$ true in all models.

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency r
On going works

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

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
On going works

Carbon Starvation in *E.Coli* [Ropers et al., Biosyst., 2006]
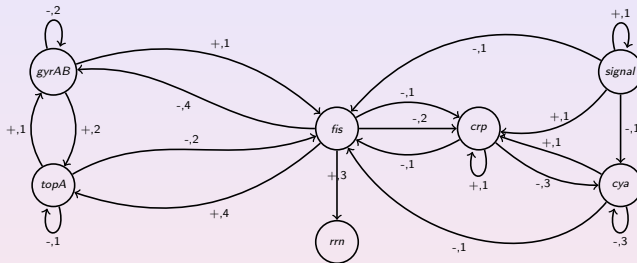
Two **steady states**: 1) with a high concentration of Fis and a high supercoiling (e.g. high ratio GyrAB / TopA), 2) after carbon deprivation, with a high concentration of Crp and a weaker supercoiling.

Two **response paths** to the two stresses: carbon deprivation, carbon-source availability.

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
On going works

Inconsistency repairing. [Corbin et al., Biosyst., 2009]

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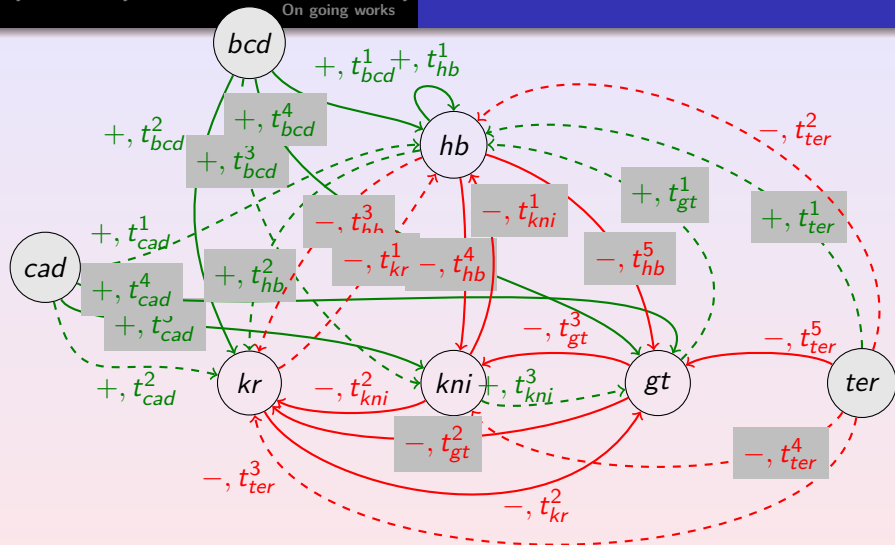constraints enforcing steady states and response paths.
Automatic repairing, by relaxing as few as possible additivity
constraints, offers two possibilities:

- Rejecting $K_{gyrAB} \geq K_{gyrAB}^{fis}$: 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).

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
On going works

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**.
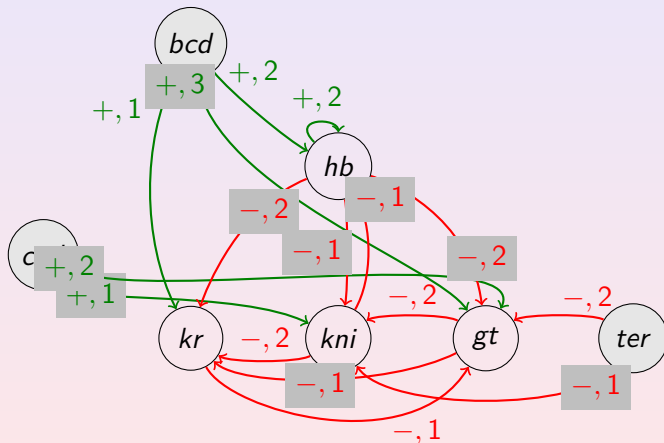
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Applications
Generally true Additivity Constraints and automatic consistency
On going works

Potential interactions are represented by dotted red arrows.

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Applications
Generally true Additivity Constraints and automatic consistency
On going works

Mutants and minimization. Results

- 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.

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
On going works

Minimized network, with only two potential interactions

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
On going works

IRMA network, from [G. Batt et al., Bioinformatics, 2010]
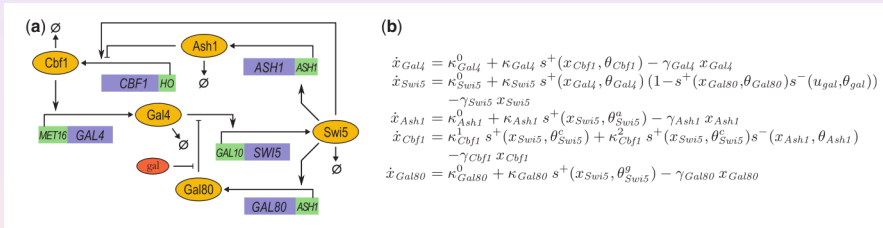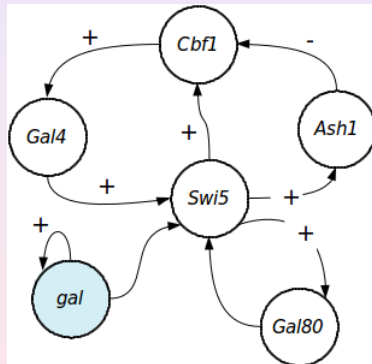

Figure : (a) The IRMA (In vivo benchmarking of Reverse-engineering and Modeling Approaches) network [I.Cantone et al., Cell., 2009], (b) the corresponding piecewise affine differential equations.

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
On going works

IRMA interaction network

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
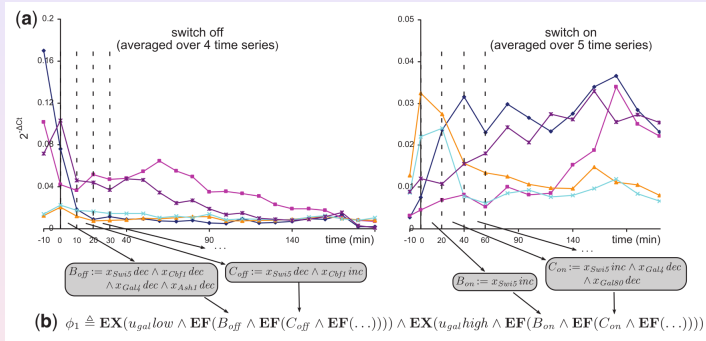On going works

Temporal profiles

Figure : (a)Temporal profiles encoding of averaged gene expression. "switch-on" ("swith-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 5×10^{-3} units are considered significant

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
On going works

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 $\varphi1$ (2 large EF formulas representing averaged "switch-off" and switch-on" experiments) and $\varphi2$ (9 large EF formulas representing all time series).

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
On going works

Results

- After discovering mistakes in Batt's work (and Batt in both our works...), we found the <u>same</u> results: 64 models for $\varphi 1$ and 4 for $\varphi 2$ (on 4860). No more precise results with singular states...
- <u>Better or equivalent</u> 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).

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
On going works

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).

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency r
On going works

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

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
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).

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency r
On going works

New solution (main lines). Efficient inconsistency repairing

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

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

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
On going works

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):
  ```
  p2 | u :- not p1.
  p1 | v :- not p2.
  ```
  Three Anwer Sets (ASs) : {u, v}, {p2} and {p1}.
  Challenge : transform these rules so that we get a conjunction of defaults with the only AS {u, v}, if no contraindication.

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
On going works

Construction of conjunction of defaults

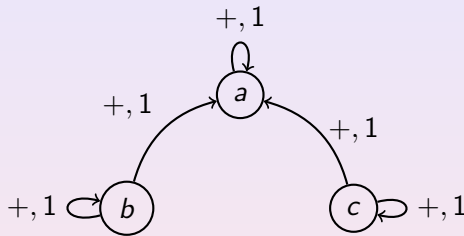Two steps :

- Defining the litteral c by :

  c :- p1.

  c :- p2.

  so that not 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 :- not p1, 1{c, not c}1.

  p1 | v :- not p2, 1{c, not c}1.

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
On going works

Possible inconsistency. Example

Observability constraints

$$(K_a < K_a^a \wedge K_a^a \geq 1) \vee (K_a^b < K_a^{ab} \wedge K_a^{ab} \geq 1)$$
$$\vee (K_a^c < K_a^{ac} \wedge K_a^{ac} \geq 1) \vee (K_a^{bc} < K_a^{abc} \wedge K_a^{abc} \geq 1)$$
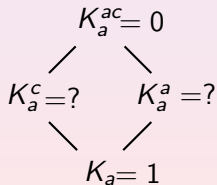
$$(K_a < K_a^b) \vee (K_a^a < K_a^{ab}) \vee (K_a^c < K_a^{bc}) \vee (K_a^{ac} < K_a^{abc})$$

$$(K_a < K_a^c) \vee (K_a^a < K_a^{ac}) \vee (K_a^b < K_a^{bc}) \vee (K_a^{ab} < K_a^{abc})$$

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
On going works

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 $a \to a$ and $c \to a$, 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:

$$K_a^{ac} = 0$$

$$K_a^c = ? \qquad K_a^a = ?$$

$$K_a = 1$$

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
On going works

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).

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency r
On going works

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

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
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) :- ap(Prop,S).
  eF(Prop,S) :- not ap(Prop,S),
                transition(S,Sp), eF(Prop,Sp).
- aF(Prop,S) :- ap(Prop,S).
  aF(Prop,S) :- not ap(Prop,S),
                aF(Prop,Sp): transition(S,Sp).

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
On going works

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).
  hypAFtrans(Prop,S,Sp) :- not transition(S,Sp).
  ```

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

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
On going works

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.

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
On going works

Multiplexes. Specification

For this purpose, we need a precise definition.

- **Syntax** of the language of the interactions targeting a species x:

  $I ::= Mul \mid Mul$ **or** I

  $Mul ::= Iu \mid Iu$ **and** Mul

  $Iu ::= Gene\_id \mid Sig \ Gene\_id$

  $Sig ::= + \mid -$

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

  $oc(+a) = K_x^a > K_x.$

  $oc(+a \text{ and } + b) = (K_x^{ab} > K_x) \wedge (K_x^a = K_x^b = K_x)$

  $oc(+a \text{ or } + b) = (K_x^{ab} > K_x^b \vee K_x^a > K_x) \wedge (K_x^{ab} > K_x^a \vee K_x^b > K_x)$

  $oc(-c \text{ and } + a \text{ and } + b) = (K_x^{ab} > K_x^c)$

  $$\wedge (K_x^{abc} = K_x^{ac} = K_x^{bc} = K_x^a = K_x^b = K_x = K_x^c)$$

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
On going works

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.

Basics: ASP and logical modeling of Thomas GRNs
Applications
Generally true Additivity Constraints and automatic consistency
On going works

Thanks

Thanks to Microsoft Research (scholarship for NM), ANR (Agence Nationale de la Recherche) projet CADMIA and the Postdam team (M. Gebser, T. Schaub) for the *clingo* ASP software.
And to Delphine, Emna, Claudine, ... Denis, Gilles, Gregory, Hans, Jean-Paul,...

Thank you for your attention

QUESTIONS ?

Declarative approach ...

Non monotonicity ...

Composition of defaults...

...