

Reachability Analysis of Delta-Notch Lateral Inhibition using Predicate Abstraction

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Abstract. This paper examines the feasibility of predicate abstraction as a method for the reachability analysis of hybrid systems. A hybrid system can be abstracted into a purely discrete system by mapping the continuous state space into an equivalent finite discrete state space using a set of Boolean predicates and a decision procedure in the theory of real closed fields. It is then possible to find the feasible transitions between these states. In this paper, we propose new conditions for predicate abstraction which greatly reduce the number of transitions in the abstract discrete system. We also develop a computational technique for reachability analysis and apply it to a biological system of interest (the Delta-Notch lateral inhibition problem).

1 Introduction

A hybrid system has both discrete and continuous transitions, and so has an infinite number of state transitions over any continuous time interval. Although there exist methods to calculate the reachable sets of hybrid systems by solving the system equations, the infinite nature of the state space makes these methods computationally very expensive. It is appealing, therefore, to find a method by which we could extract equivalent finite state models of these systems and use them to find approximate reachable sets of the original systems.

Predicate abstraction has emerged as a powerful technique to extract such models from complex, infinite state models (Das et al[3], Graf et al[6]). Discrete abstraction and reachability analysis of hybrid systems based on predicate abstraction have been proposed in earlier work (Tiwari et al[12], Alur et al[1], Sokolsky et al[11], Lafferriere et al[8]). The extracted finite state model, called the Abstract Discrete System (ADS), is said to be an over-approximation of the original system if the original system satisfies any property of interest that is satisfied by the ADS.

In this paper, inspired by Tiwari et al[12][13], we return to our hybrid model of Delta-Notch lateral inhibition (Ghosh et al[5]), and attempt to use the above methods to perform reachability analysis. We propose new conditions for predicate abstraction, beyond those of Tiwari et al[12], that greatly reduce the number of transitions in the abstract discrete system given a set of polynomials. We then

develop a computational technique for the reachability analysis of hybrid systems. Finally, we analyze the Delta-Notch lateral inhibition problem using the technique thus developed.

This paper is organized as follows: the problem description, modeling, and analytical results of the Delta-Notch lateral inhibition problem are presented in Sections 2-3; the predicate abstraction algorithm and its implementation are described in Section 4 and Section 5 respectively. Conclusions are presented in Section 6.

2 Delta-Notch Signaling

The biological process under study, intercellular signaling, results in cellular differentiation in embryonic tissue, which is a complex control process regulated by a set of developmental genes, most of which are conserved in form and function across a wide spectrum of organisms. Found in almost all multicellular organisms from an early embryo stage, intercellular signaling is a feedback network which interrelates the fate of a single cell and its neighbors in a population of homogeneous cells. Among the various signaling channels, the Delta-Notch protein pathway in particular has gained wide acceptance as the arbiter of cell fate for an incredibly varied range of organisms (Artavanis-Tsakonas et al[2]).

Delta and Notch are both transmembrane proteins that are active only when cells are in direct contact, in a densely packed epidermal layer for example (Lewis[9]). Delta is a ligand that binds and activates its receptor Notch in neighboring cells. The activation of Notch in a cell affects the production of Notch ligands (i.e. Delta) both in itself and its neighbors, thus forming a feedback control loop. In the case of lateral inhibition, high Notch levels suppress ligand production in the cell and thus a cell producing more ligands forces its neighboring cells to produce less. The Delta-Notch signaling mechanism has been found to cause pattern formation in many different biological systems, like the South African claw-toed frog (*Xenopus laevis*) embryonic skin (Marnellos et al[10]) and the eye R3/R4 photoreceptor differentiation and planar polarity in the fruit fly *Drosophila melanogaster* (Fanto et al[4]). An example of the distinctive “salt-and-pepper” pattern formed due to lateral inhibition is the *Xenopus* epidermal layer where a regular set of ciliated cells form within a matrix of smooth epidermal cells as seen in Figure 1(a).

3 Model and Analytical Results

To model the regulation of intracellular Delta and Notch protein concentrations through the feedback network, experimentally observed rules governing the biological phenomenon have to be implemented. Firstly, since Delta and Notch are transmembrane proteins, cells have to be in direct contact for Delta-Notch signaling to occur. This implies that a cell is directly affected by, and directly affects in turn, only immediate neighbors. Secondly, Notch production is turned on by high Delta levels in the immediate neighborhood of the cell and Delta production

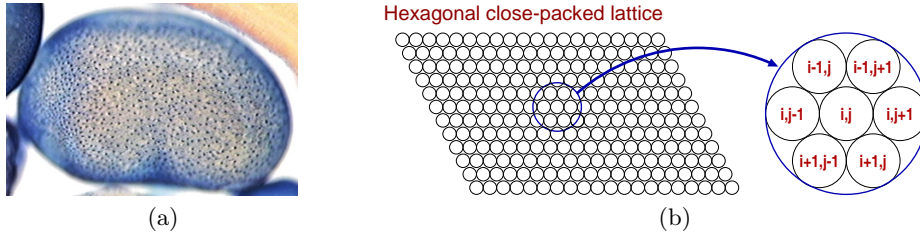


Fig. 1. (a) *Xenopus* embryo labeled by a marker for ciliated cell precursors seen as black dots. Photograph courtesy of P. D. Vize (*The Xenopus Molecular Marker Resource*, <http://vize222.zo.utexas.edu>). (b) Hexagonal close-packed layout scheme for cells in two dimensional arrays.

is switched on by low Notch concentrations in the same cell. Thirdly, at steady state, a cell with high Delta levels must have low Notch level and vice versa. This is essential for differentiation to occur, for cells with high Delta levels in steady state become ciliated, and cells with high Notch in steady state remain smooth. Finally, both Delta and Notch proteins decay exponentially through normal proteolysis. In the model, the cells are assumed to be hexagonal close packed, i. e. each cell has six neighbors in contact with it (Figure 1(b)). We will denote as “biologically consistent” our computational results which comply with these biological observations.

Each biological cell is modeled as a four state piecewise affine hybrid automaton. The four states capture the property that Notch and Delta protein production can be individually switched on or off at any given time. It is assumed that there is no command-actuation delay in the mode switching. The formal definition of the hybrid automaton is given by:

$$\begin{aligned}
 H_1 &= (Q_1, X_1, \Sigma_1, V_1, Init_1, f_1, Inv_1, R_1) \\
 Q_1 &= \{q_1, q_2, q_3, q_4\} \\
 X_1 &= (v_D, v_N)^T \in \mathbb{R}^2 \\
 \Sigma_1 &= \left\{ u_D, u_N : u_D = -v_N, u_N = \sum_{i=1}^6 v_D^i \right\} \\
 V_1 &= \emptyset \\
 Init_1 &= Q_1 \times \{X_1 \in \mathbb{R}^2 : v_D, v_N > 0\} \\
 f_1(q, x) &= \begin{cases} [-\lambda_D v_D; -\lambda_N v_N]^T & \text{if } q = q_1 \\ [R_D - \lambda_D v_D; -\lambda_N v_N]^T & \text{if } q = q_2 \\ [-\lambda_D v_D; R_N - \lambda_N v_N]^T & \text{if } q = q_3 \\ [R_D - \lambda_D v_D; R_N - \lambda_N v_N]^T & \text{if } q = q_4 \end{cases} \\
 Inv_1 &= \{q_1, \{u_D < h_D, u_N < h_N\}\} \cup \{q_2, \{u_D \geq h_D, u_N < h_N\}\} \\
 &\quad \cup \{q_3, \{u_D < h_D, u_N \geq h_N\}\} \cup \{q_4, \{u_D \geq h_D, u_N \geq h_N\}\}
 \end{aligned}$$

$$R_1 : \begin{bmatrix} R_1(q_1, \{u_D \geq h_D \wedge u_N < h_N\}) \in q_2 \times \mathbb{R}^2 \\ R_1(q_1, \{u_D < h_D \wedge u_N \geq h_N\}) \in q_3 \times \mathbb{R}^2 \\ \vdots \\ R_1(q_4, \{u_D < h_D \wedge u_N \geq h_N\}) \in q_3 \times \mathbb{R}^2 \end{bmatrix}$$

where, v_D and v_N : Delta and Notch protein concentrations, respectively, in a cell; v_D^i : Delta protein concentration in i^{th} neighboring cell; λ_D and λ_N : Delta and Notch protein decay constants respectively; R_D and R_N : constant Delta and Notch protein production rates, respectively; h_D and h_N : switching thresholds for Delta and Notch protein production, respectively. R_D , R_N , λ_D and λ_N are experimentally-determined constants. The switching thresholds h_D and h_N are unknown and possible ranges for them are derived in Ghosh et al[5], which are biologically consistent. In the single cell, $v_D^i = 0, \forall i \in \{1, \dots, 6\}$. The inputs u_D and u_N are the physical realization of the protein regulatory properties in the model outlined before.

The two cell hybrid automaton H_2 is the composition of two single cell automata, to form a model with four continuous states and 16 discrete modes. Here, $v_D^1 \neq 0$ for each of the two cells, and thus the Delta level of each cell is communicated to its neighbor to control Notch production. Modeling the full two dimensional layer of cells involves composing $N \times N$ single cell hybrid automata.

Both the single and two cell hybrid automata were analyzed in Ghosh et al[5], to obtain constraints on the range of the protein kinetic parameters and switching thresholds for biologically feasible equilibria to exist:

$$h_D, h_N : -\frac{R_N}{\lambda_N} < h_N \leq 0 \wedge 0 < h_N \leq \frac{R_D}{\lambda_D}$$

The two cell automaton was also shown to have a Zeno state with a particular Zeno execution that is an invariant: $v_{D_1} - v_{D_2} \wedge v_{N_1} - v_{N_2}$.

4 Predicate Abstraction

In this section, we review the predicate abstraction techniques proposed in Tiwari et al[12] and propose new conditions to refine transitions in the ADS. We then develop a method of finding approximate backward reachable sets of the equilibria. A flow-chart describing this procedure is shown in Figure 2.

Any trajectory of a hybrid system can be resolved into discrete and continuous transitions. We first consider the predicate abstraction of the continuous state space. A continuous dynamical system can be represented by a tuple $(X, InitX, f, Inv)$, where $X \in \mathbb{R}^n$ is the set of continuous states, $InitX$ is a set of initial states, $f : X \rightarrow TX$ is the continuous dynamics, and Inv is the invariant set. We assume $InitX$, f , and Inv are polynomials of continuous states.

4.1 Construction of the Abstract Discrete System

Given a continuous system, following the methods of Tiwari et al[12], we construct the ADS, $(Q, InitQ, \delta)$ where Q is a finite set of discrete states, $InitQ$ is

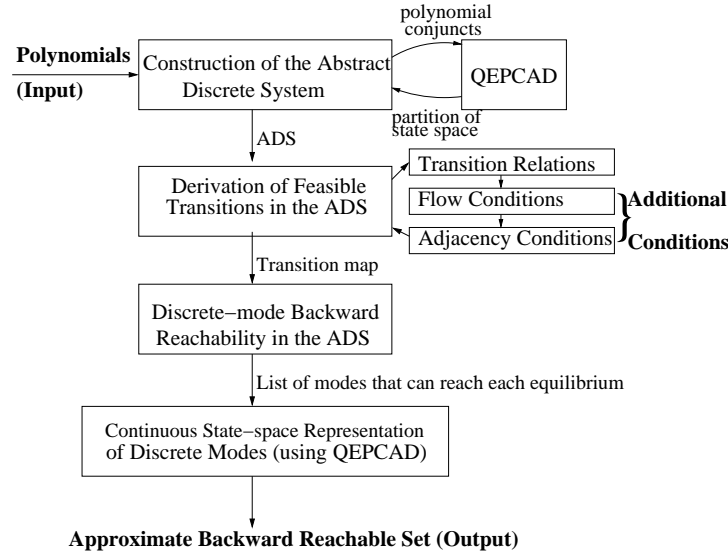


Fig. 2. Flow-chart describing the process of calculating approximate backward reachable sets using predicate abstraction techniques.

the set of discrete initial states, and δ is the set of transitions. We first construct an initial set P_0 which contains polynomials of interest (for example: state variables, governing equations, invariants, guards, or any properties we may wish to verify). We then construct a finite set P of polynomials following the inference rule: if a polynomial $p \in P$, then we add the derivative of p with respect to time, \dot{p} , to the set P , if \dot{p} is not a constant multiple of any existing polynomial in P . This process may be terminated either when the set P saturates (ie. taking derivatives of the elements in P adds no new polynomials) or at a convenient time. Given this finite set P of polynomials, the abstract discrete states (ψ_i) are described by conjunctions of all these polynomials evaluated over the domain $\{pos, neg, zero\}$ i.e. conjunctions of these polynomial inequalities. An abstract discrete state is therefore a truth-invariant region in \mathfrak{R}^n for all polynomial inequalities from the set P . The larger the number of polynomials in P , the larger the number of discrete states, and the finer the abstraction.

It is clear from the above that if we begin with n polynomials in our original system, we could have a total of 3^n discrete modes in our abstracted system. However, many (in fact, most) of these modes are physically infeasible regions, i.e. there is no solution to the conjuncts of these polynomial inequalities in real space. For example, consider the set $P = \{x, x - 1\}$. Although this would theoretically allow 2^3 discrete regions, regions represented by $[x < 0 \wedge (x - 1) > 0]$ and $[x = 0 \wedge (x - 1) > 0]$ can clearly not exist in real space. We use a decision making procedure in the theory of closed fields, QEPCAD (Hong[7]), to eliminate

these modes. Since QEPCAD is restricted to polynomial inputs, we can apply this procedure only to systems where the dynamics, invariants and initial states are polynomial functions of the state variables. We are similarly constrained to verify polynomial properties of the system.

4.2 Derivation of Feasible Transitions

The transition relations (transition map) in the ADS can be obtained using the three-step process outlined below. The first has been proposed in Tiwari et al[12]; we propose the other two.

1. **Transition relations:** *We add an abstract transition $(\psi_1, \psi_2) \in \delta$ if the signs of the derivatives are consistent with the transition.*
 For example, for any $p \in P$, If $p < 0$ is a conjunct in ψ_1 ,
 - (a) If $\dot{p} < 0$, then $p < 0$ is a conjunct in ψ_2 .
 - (b) If $\dot{p} = 0$, then $p < 0$ is a conjunct in ψ_2 .
 - (c) If $\dot{p} > 0$, then either $p < 0$ or $p = 0$ is a conjunct in ψ_2 .
 - (d) If the valuation of $\dot{p} < 0$ cannot be determined from ψ_1 , then either $p > 0$ or $p = 0$ is a conjunct in ψ_2 .

Similar conditions exist for the cases when $p > 0$ and $p = 0$ are conjuncts in ψ_1 (Tiwari et al.[12]). The state transitions in the ADS are thus determined by the signs of the derivatives of polynomials with respect to time *i.e. flow directions*. The abstract discrete transitions obtained by the above rules can be refined by eliminating abstract states and transitions which do not satisfy invariant conditions.

2. **Flow conditions:** *We include the signs of the derivatives of **all** the polynomials in the abstraction, and thus eliminate condition (d) in the above transition relations.*

In the abstraction procedure in Tiwari et al[12], we have no information about the flow direction of some polynomials in P unless it is saturated. As a result, we allow transitions which arise from the ambiguity in the sign of these derivatives. On the other hand, if we were to use the sign of these derivatives in the finding the abstract transitions, but not in forming the ADS, we could eliminate a large number of infeasible transitions without increasing computational time substantially. This problem was not specifically encountered in the examples used in Tiwari et al[12] because the system of polynomials saturated. For a more general system, however, this condition greatly refines the transition map.

3. **Adjacency conditions:** *We only allow abstract transitions between adjacent regions.*

The abstraction procedure maps the boundaries of different regions in the original system onto separate abstract states. We allow an abstraction of a continuous transition from a state only if it is either transitioning to itself or

to the abstraction of a boundary adjoining the original state. Even for a one-dimensional system, physically infeasible discrete transitions (discontinuous jumps) may occur in the ADS if the adjacency condition is not enforced.

With the two additional conditions, physically infeasible transitions in the ADS are eliminated and thus the complexity of the ADS may be dramatically reduced.

The predicate abstraction procedure for continuous systems can be easily extended to hybrid systems by considering discrete states in the original hybrid system as new abstract discrete states in the ADS as well. Then, discrete states in the ADS are pairs of the original discrete states and the abstract discrete states of the original continuous states. The transition relation in the ADS can be obtained by combining the discrete transitions in the original hybrid systems and the abstract transitions for continuous states. We find that an ADS is an over-approximation of the original system, *i.e.* any transitions that are possible in the original system are definitely manifested in the ADS.

4.3 Reachability

The reachability analysis is carried out in two phases.

1. **Backward reachability in the ADS:** Given the transition map, we compute the approximate backward reachable set of each equilibrium in the discrete state space, *i.e.* the set of modes that can reach a given equilibrium mode in the ADS.
2. **Continuous state-space representation of backward reachable set:** We combine the regions corresponding to the modes of the ADS that can reach a particular equilibrium point (mode) and find a continuous state-space representation of the backward reachable set of that equilibrium point. There are several ways to do this; we use QEPCAD for this in this paper.

5 Implementation

In this section we perform reachability analysis of the Delta-Notch model using the methods outlined above. We consider a model in which the protein kinetic parameters (λ 's and R 's) are set to unity. We assume that the switching thresholds are $h_D = -0.5$ and $h_N = 0.2$ which were found in Ghosh et al[5] to be in the range which produces sensible biological results. We compute reachable sets using predicate abstraction with the new conditions. In this reachability analysis, self-loops in the transition map are eliminated in all modes except those which correspond to the equilibria of the original system.

5.1 Single-cell Hybrid Automaton

The single-cell Delta-Notch lateral inhibition problem has two state variables (v_D and v_N) and 5 polynomials (the state variables, their derivatives, and a

switching line). We impose the additional condition that v_D and v_N (Delta and Notch concentrations) are positive. For the case where $u_N \geq h_N$, we find that the invariant boundaries correspond to sections of the switching lines themselves, and are polynomials in our original set, and is hence discrete modes of the ADS. The backward reachable set of the equilibrium ($v_D = 0, v_N = 1$) is simply the positive quadrant, as expected from Ghosh et al[5].

5.2 Two-cell Network

In the two-cell Delta-Notch lateral inhibition problem, we begin with 12 polynomials (state variables: $v_{D_1}, v_{N_1}, v_{D_2}, v_{N_2}$, their derivatives: $\dot{v}_{D_1}, \dot{v}_{N_1}, \dot{v}_{D_2}, \dot{v}_{N_2}$, and the switching conditions: $v_{N_1} + u_D, v_{D_1} - u_N, v_{N_2} + u_D, v_{D_2} - u_N$) in 4 dimensions. These polynomials are those that are derived from the dynamic equations and the switching conditions alone. We assume that the input threshold values satisfy the conditions proposed by Ghosh et al[5], i.e.,

$$h_D, h_N : -\frac{R_N}{\lambda_N} < h_N \leq 0 \wedge 0 < h_N \leq \frac{R_D}{\lambda_D}$$

As in Ghosh et al[5], we find that $u_D = -0.5$ and $u_N = 0.5$ satisfy these conditions. However, the abstraction in this form alone is not enough for us to find the invariant regions, since the boundaries of these regions do not correspond to any of the polynomials in the original set. From phase portrait analysis, we include projections of the invariant boundaries ($v_{D_1} - v_{D_2}$ and $v_{N_1} - v_{N_2}$) and their derivatives as predicates in our polynomial set. We now have 18 polynomials in 4 variables. The system has 2 equilibrium points, ($v_{D_1} = 1, v_{N_1} = 0, v_{D_2} = 0, v_{N_2} = 1$) and ($v_{D_1} = 0, v_{N_1} = 1, v_{D_2} = 1, v_{N_2} = 0$). Performing reachability analysis on this transition set, we find that we can divide the infinite state space into four sets: Set 1 is the backward reachable set of the first equilibrium, Set 2 is the backward reachable set of the other equilibrium, Set 3 is the invariant set and Set 4 is a region of ambiguity which is backwards reachable from either equilibrium. We also have an explicit way of mathematically describing the different sets in higher dimensions.

Set	Backward Reachable Set of	Equivalent region
1	$v_{D_1} = 1, v_{N_1} = 0, v_{D_2} = 0, v_{N_2} = 1$	$(v_{D_2} - v_{D_1} \leq 0) \wedge (v_{N_2} - v_{N_1} \geq 0) \wedge \{[(v_{D_1} \neq 1 \vee v_{N_1} > 0 \vee v_{D_2} > 0 \vee v_{N_2} - v_{N_1} \neq 1) \wedge v_{N_2} \neq v_{N_1}] \vee [v_{D_2} - v_{D_1} < 0 \wedge v_{N_2} = v_{N_1}]\}$
2	$v_{D_1} = 0, v_{N_1} = 1, v_{D_2} = 1, v_{N_2} = 0$	$(v_{D_2} - v_{D_1} \geq 0) \wedge (v_{N_2} - v_{N_1} \leq 0) \wedge \{[(v_{D_1} \neq 0 \vee v_{N_1} < 1 \vee v_{D_2} \neq 1 \vee v_{N_2} \neq 0) \wedge (\wedge v_{D_2} \neq v_{D_1})] \vee [(v_{N_1} > 1 \wedge (v_{N_1} > 0 \wedge v_{D_2} = v_{D_1})) \wedge v_{N_2} \neq v_{N_1}]\}$
3	Neither equilibrium (Invariant region)	$v_{D_2} - v_{D_1} = 0 \wedge v_{N_2} - v_{N_1} = 0$
4	Either equilibrium (Ambiguous region)	$[(v_{D_2} - v_{D_1} \geq 0) \wedge (v_{N_2} - v_{N_1} \geq 0)] \vee [(v_{D_2} - v_{D_1} \leq 0) \wedge (v_{N_2} - v_{N_1} \leq 0)]$

However, we find that when we take the projections on the $v_{N_1} = v_{N_2}$ or $v_{D_1} = v_{D_2}$ planes, this region of ambiguity (Set 4) disappears. Since it is difficult to visualize regions in 4 dimensions, we study projections along planes of interest. A comparison to the phase portraits analyzed in Ghosh et al[5] is shown in Figure 3. Since the system is deterministic, we know for certain that Set 4

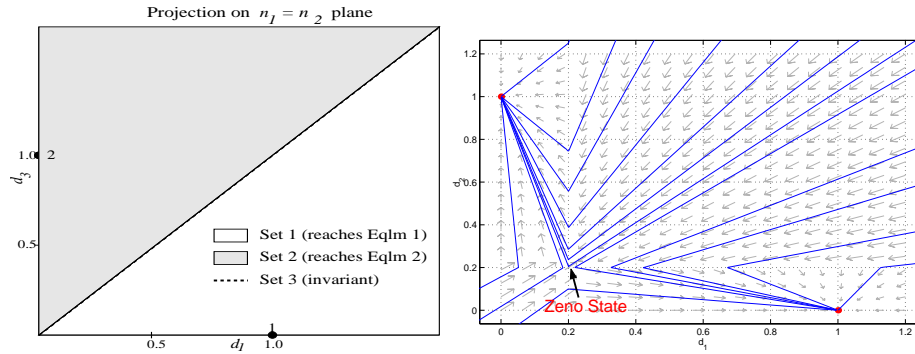


Fig. 3. Comparison of projections on the $v_{N_1} = v_{N_2}$ plane, from predicate abstraction (left) and simulations from [5] (right).

must be an empty set. However, with the chosen predicates, we can only reduce the region of ambiguity to one-eighth of the infinite continuous state space. We believe that adding more polynomials relevant to the dynamics of the system (obtained from further analysis and simulation) will help us further prune this ambiguous region. We are currently working on addressing this issue.

We must comment, at this point, on the restrictions imposed on the complexity of the problem by the use of QEPCAD for predicate abstraction. The time-complexity of the quantifier elimination procedure employed here is doubly exponential in the number of variables, and polynomial in the number of polynomials in our set. This restricts us to problems in a small number of dimensions. Also, since we are dealing with a large number of polynomials in each step of our elimination procedure, we employ a hierarchical application of QEPCAD while eliminating physically infeasible modes.

6 Conclusions

Predicate abstraction has been proposed as a means of finding approximate backward reachable sets of the equilibria of hybrid systems in this paper. Since the Abstract Discrete System (ADS) is an over-approximation of the original system, this reachability analysis is conservative. We have proposed new conditions in checking for feasible transitions in the ADS, which greatly reduces the number of transitions in the discrete transition map. We have also implemented this as a computational technique for the reachability analysis of the Delta-Notch lateral inhibition system. Approximate backward reachable sets for this system are computed efficiently and elegantly using this technique. However, we find the fact that the accuracy of reachability analysis using predicate abstraction greatly depends on the choice of polynomials for abstraction makes it important to have

information about a given system *a priori* (from analysis and simulations) to get good results in the reachability analysis.

References

1. R. Alur, T. Dang, and F. Ivančić. Reachability analysis of hybrid systems via predicate abstraction. In C. Tomlin and M. Greenstreet, editors, *5th International Workshop, Hybrid Systems: Computation and Control*, volume LNCS 2289, pages 35–48, Stanford, CA, USA, 2002. Springer.
2. Spyros Artavanis-Tsakonas, Matthew D. Rand, and Robert J. Lake. Notch signaling: cell fate control and signal integration in development. *Science*, 284:770–776, April 1999.
3. S. Das, D. Dill, and S. Park. Experience with predicate abstraction. In *Computer Aided Verification, 11th international Conference*, volume LNCS 1633, 1999.
4. Manolis Fanto and Marek Młodzik. Asymmetric notch activation specifies photoreceptors R3 and R4 and planar polarity in the *Drosophila* eye. *Nature*, 397:523–526, February 1999.
5. R. Ghosh and C. Tomlin. Lateral inhibition through delta-notch signaling: A piecewise affine hybrid model. In *4th International Workshop, Hybrid Systems: Computation and Control*, volume LNCS 2034, pages 232–246, Rome, Italy, 2001.
6. S. Graf and H. Saidi. Construction of abstract state graphs with PVS. In *Computer Aided Verification, 9th International Conference*, volume LNCS 1254, 1997.
7. H. Hong. An improvement of the projection operator in cylindrical algebraic decomposition. In *Proceedings of ISAAC 90*, pages 261–264, 1990.
8. G. Lafferriere, G.J. Pappas, and S. Yovine. Symbolic reachability computation for families of linear vector fields. *Journal of Symbolic Computation*, 32(3):231–253, September 2001.
9. Julian Lewis. Notch signalling and the control of cell fate choices in vertebrates. *Seminars in Cell & Developmental Biology*, 9:583–589, 1998.
10. G. Marnellos, G. A. Deblandre, E. Mjolsness, and C. Kintner. Delta-notch lateral inhibitory patterning in the emergence of ciliated cells in *Xenopus*: experimental observations and a gene network model. In *Pacific Symposium on Biocomputing*, pages 326–337, 2000.
11. O. Sokolsky and H. Hong. Qualitative modeling of hybrid systems. In *Proceedings of the Monterey Workshop*, June 2001.
12. A. Tiwari and G. Khanna. Series of abstractions for hybrid automata. In C. Tomlin and M. Greenstreet, editors, *5th International Workshop, Hybrid Systems: Computation and Control*, volume LNCS 2289, pages 465–478, Stanford, CA, USA, 2002.
13. A. Tiwari and P. Lincoln. Automated techniques for stability analysis of delta notch lateral inhibition mechanism. 2002.