A Probabilistic Calculus for Molecular Systems

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Introduction

- Formal models for systems of interactive components can be easily adapted for the modeling of biochemical phenomena

- The modeling of biochemical reactions allows:
  1. the development of simulators
  2. the model checking of properties of biochemical systems
  3. (hopefully) the prediction of some unknown kinetic constants

- In this work:
  1. we introduce a probabilistic calculus for molecular reactions
  2. we use the calculus for modeling an example of real enzymatic activity
  3. we report some experimental results of simulation and model checking

A calculus of solutions (1)

- Let $\mathcal{E}$ be an infinite set of elementary particles:

(molecules) $m ::= X \mid m\text{-}m$

(solutions) $S ::= \mathbf{0} \mid m \mid S, S$

where X is any particle of $\mathcal{E}$ and 0 is the empty solution.

- The *structural congruence* for solutions $\equiv$ is the smallest equivalence relation such that:

$S_1, S_2 \equiv S_2, S_1 \qquad (S_1, S_2), S_3 \equiv S_1, (S_2, S_3) \qquad S, 0 \equiv S$

$m_1\text{-}m_2 \equiv m_2\text{-}m_1 \qquad (m_1\text{-}m_2)\text{-}m_3 \equiv m_1\text{-}(m_2\text{-}m_3)$

A calculus of solutions (2)

$$m ::= X \mid m\text{-}m$$

$$S ::= \mathbf{0} \mid m \mid S, S$$

$$S_1, S_2 \equiv S_2, S_1 \qquad S, 0 \equiv S$$

$$(S_1, S_2), S_3 \equiv S_1, (S_2, S_3)$$

$$m_1\text{-}m_2 \equiv m_2\text{-}m_1$$

$$(m_1\text{-}m_2)\text{-}m_3 \equiv m_1\text{-}(m_2\text{-}m_3)$$

- A solution can be considered as a multi-set of molecules
  - For instance, $\in$ can be defined as follows:

$$m \in S \iff \exists S' \ s.t. \ m, S' \equiv S$$

Probabilistic rules

- A *probabilistic rule* (or *reaction*) is a triple

  $$(S,P,S')$$

  where S and S' are solutions and P is a total function from the infinite set of molecules into $[0,1[$

- Examples (assume $P(m) = 0$ if not specified):

  $$R_1 = (\{a, b\}, \{a \mapsto \tfrac{2}{3}, b \mapsto \tfrac{2}{3}\}, \{a\text{-}b\})$$
  $$R_2 = (\{a\text{-}b\}, \{a\text{-}b \mapsto \tfrac{1}{2}\}, \{a, b\})$$

- A *system* is a pair $(S,\mathcal{R})$ where S is a solution and $\mathcal{R}$ is a finite set of probabilistic rules

- Example: $(\{a, b\}, \{R_1, R_2\})$

Well-formed set of rules

- A set of rules $\mathcal{R} = \{(S_1, P_1, S_1'), \ldots, (S_n, P_n, S_n')\}$ is well-formed if it satisfies the following:
  - for all $(S_i, P_i, S_i') \in \mathcal{R}$

    $S_i \cap S_i' = \emptyset$

    $\#(S_i) = \#(S_i')$

    $P_i(m) > 0$ if $m \in S_i$, $P_i(m) = 0$ otherwise

    if $m \equiv m'$ then $P_i(m) = P_i(m')$
  - for any molecule m

    $$0 < P^I(m) \stackrel{def}{=} (1\text{-} \sum_{i=0}^{n} P_i(m))$$
  - for all $i, j \in 1, \ldots, n$ if $i \neq j$ then either $S_i \not\equiv S_j$ or $S_i' \not\equiv S_j'$

Example of well-formed set of rules

$$\mathcal{R} = \{R_1, R_2\}$$

$$R_1 = (\{a, b\}, \{a \mapsto \tfrac{2}{3}, b \mapsto \tfrac{2}{3}\}, \{a\text{-}b\})$$

$$R_2 = (\{a\text{-}b\}, \{a\text{-}b \mapsto \tfrac{1}{2}\}, \{a, b\})$$

- for all $(S_i, P_i, S_i') \in \mathcal{R}$

  $S_i \cap S_i' = \emptyset$

  $\#(S_i) = \#(S_i')$

  $P_i(m) > 0$ if $m \in S_i$, $P_i(m) = 0$ otherwise

  if $m \equiv m'$ then $P_i(m) = P_i(m')$

- for any molecule m

  $$0 < P^I(m) \stackrel{def}{=} (1\text{-} \sum_{i=0}^{n} P_i(m))$$

- for all $i, j \in 1, \ldots, n$ if $i \neq j$ then either $S_i \not\equiv S_j$ or $S_i' \not\equiv S_j'$

Step semantics

- Given $\mathcal{R}$, we denote with $\mathcal{R}^I$ the (not well-formed) set of rules:

$$\mathcal{R}^I = \{(\{m\}, \{m \mapsto P^I(m)\}, \{m\}) \mid m \text{ is a molecule}\}$$

- The following recursive algorithm $Step(S)$ describes how a system $(S,\mathcal{R})$ executes a step (it also returns the solution for the next step):

1. **choose** m **in** S

   } RANDOMLY CHOOSE A MOLECULE m IN S

2. **let** $R_1, \ldots, R_n$ be the only rules in $\mathcal{R} \cup \mathcal{R}^I$ such that $R_i = (S_i, P_i, S_i')$, $m \in S_i$, $S_i \subseteq S$

   } CONSIDER ONLY THE RULES (IN $\mathcal{R} \cup \mathcal{R}^I$) THAT CAN BE APPLIED TO m IN S

3. **choose** $R = (S_R, P_R, S_R')$ **in** $R_1, \ldots, R_n$ **with probabilities** $\frac{P_1(m)}{\sum_{i=1}^n P_i(m)}, \ldots, \frac{P_n(m)}{\sum_{i=1}^n P_i(m)}$

   } RANDOMLY CHOOSE ONE OF THESE RULES AFTER NORMALIZING THEIR PROBABILITIES

4. **if** $S \setminus S_R = \emptyset$ **then return** S_R' **else return** $(S_R' \cup Step(S \setminus S_R))$

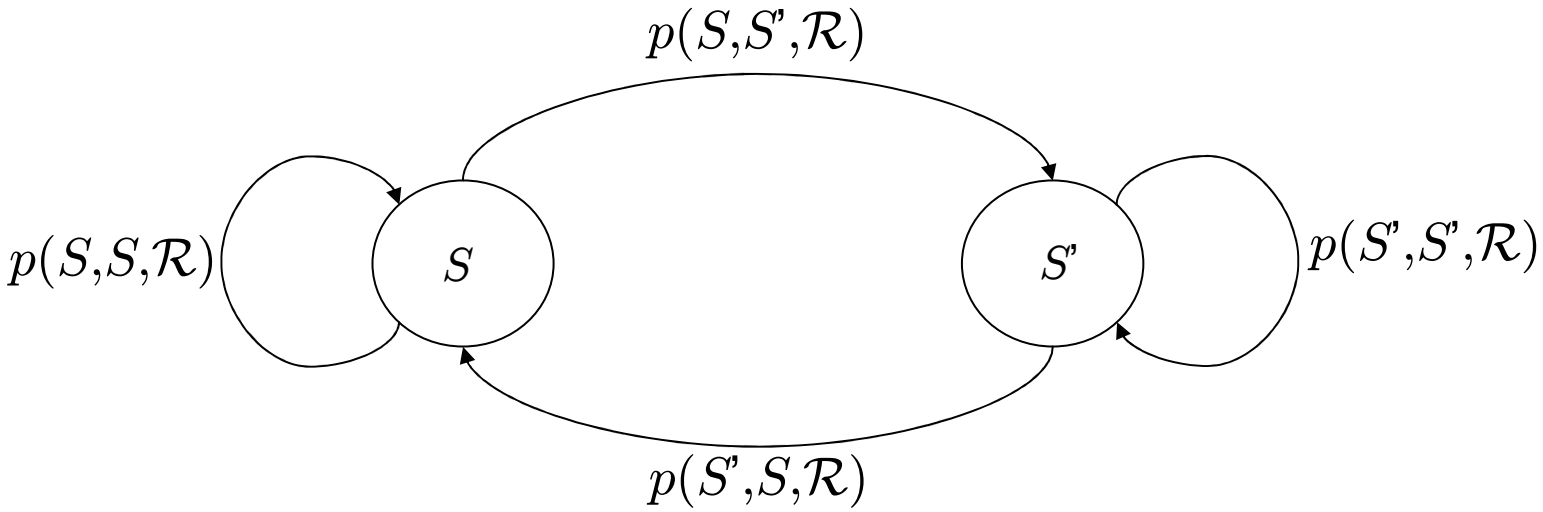
   } RECURSIVELY EXECUTE ON THE REST OF THE SOLUTION

Molecular Probabilistic Transition System (1)

- Given two solutions S,S' and a set of rules $\mathcal{R}$, it is possible to infer from the algorithm the probability of S to be transformed into S'

$$p(S,S',\mathcal{R})$$

- Moreover, it is possible to build a probabilistic transition system:



Molecular Probabilistic Transition System (2)

- Given a system $(S,\mathcal{R})$ the *Molecular Probabilistic Transition System (MPTS)* of the system is a tuple $M = (Q,S,\mathcal{R},\delta,\pi)$ where:
  - Q is a finite set of solutions of density equal to $\#(S)$
  - S is the initial solution (the solution of the system)
  - $\mathcal{R}$ is the set of rules of the system
  - $\delta \subseteq Q$ x Q is a finite set of transitions
  - $\pi : \delta \rightarrow [0,1]$ is a probability function such that if $e = (S_1,S_2) \in \delta$, then $\pi(e) = p(S_1,S_2,\mathcal{R})$

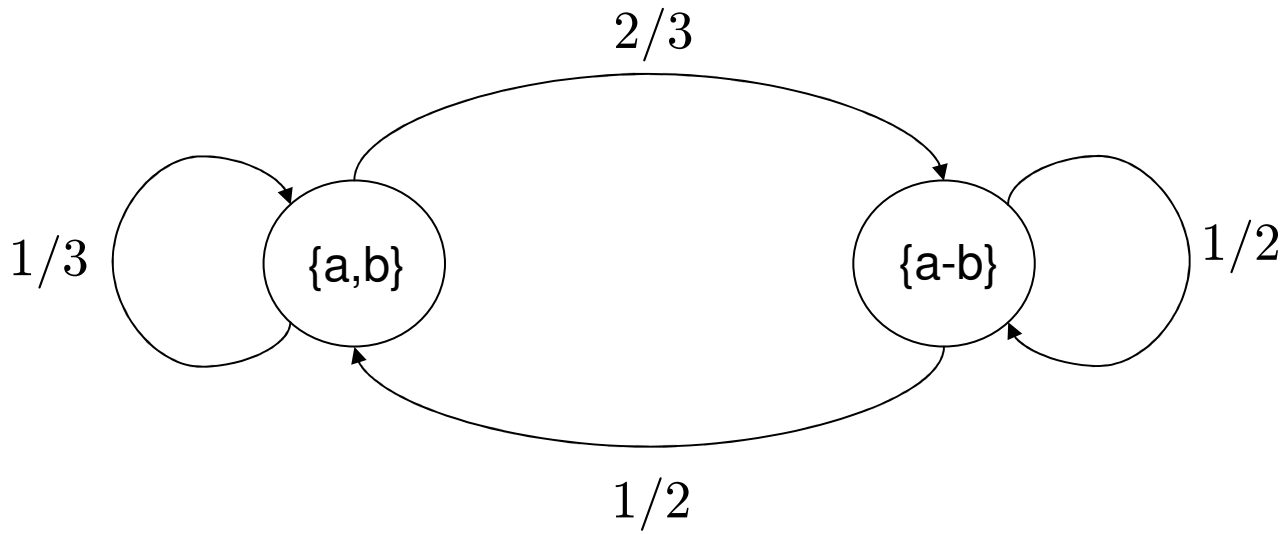
- (Correctness) Given a system $(S,\mathcal{R})$ and its MPTS $M=(Q,S,\mathcal{R},\delta,\pi)$, there is $e = (S_1,S_2) \in \delta$ with $\pi(e) = p(S_1,S_2,\mathcal{R})$ if and only if there is $S_2 = Step(S_1)$

Example of MPTS

System: $(S = \{a, b\}, \mathcal{R} = \{R_1, R_2\})$

where $R_1 = (\{a, b\}, \{a \mapsto \frac{2}{3}, b \mapsto \frac{2}{3}\}, \{a\text{-}b\})$

$R_2 = (\{a\text{-}b\}, \{a\text{-}b \mapsto \frac{1}{2}\}, \{a, b\})$



Application: Sorbitol dehydrogenase

$$E + NADH \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E\text{–}NADH$$

$$E\text{–}NADH + F \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E\text{–}NAD^+ + S \qquad E\text{–}NAD^+ \underset{k_6}{\overset{k_5}{\rightleftharpoons}} E + NAD^+$$

$\mathcal{R}$

1. ({E,NADH} , {(E,0.468),(NADH,0.862)} , {E-NADH})
2. ({E-NADH,F} , {(E-NADH,0.001),(F,0.002)} , {E-NAD⁺,S})
3. ({E-NAD⁺} , {(E-NAD⁺,0.774)} , {E,NAD⁺})
4. ({E,NAD⁺} , {(E,0.457),(NAD⁺,0.859)} , {E-NAD⁺})
5. ({E-NAD⁺,S} , {(E-NAD⁺,0.002),(S,0.008)} , {E-NADH,F})
6. ({E-NADH} , {(E-NADH,0.772)} , {E,NADH})

$\mathcal{R}^I$

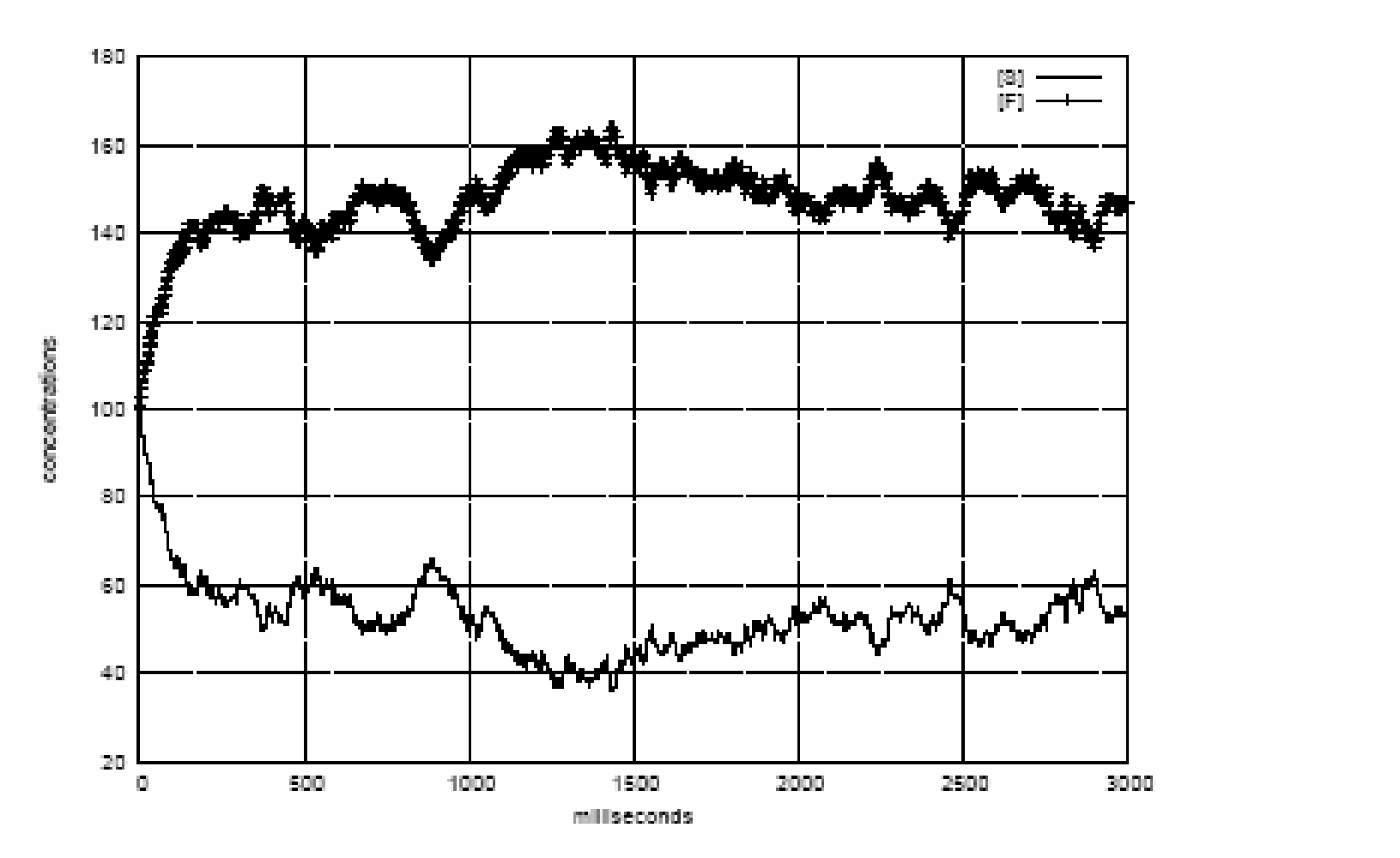
7. ({E} , {(E,0.075)} , {E})
8. ({S} , {(S,0.992)} , {S})
9. ({F} , {(F,0.998)} , {F})
10. ({NADH} , {(NADH,0.138)} , {NADH})
11. ({NAD⁺} , {(NAD⁺,0.141)} , {NAD⁺})
12. ({E-NADH} , {(E-NADH,0.227)} , {E-NADH})
13. ({E-NAD⁺} , {(E-NAD⁺,0.254)} , {E-NAD⁺})

Simulation (1)

- Simulator implemented in SICStus Prolog
- Given a system (S,R) the simulator
  - executes $S' = Step(S)$
  - gives the concentrations of the molecules in S' as output
  - iterates on $(S',\mathcal{R})$
- Sorbitol dehydrogenase: initial concentrations

E	S	F	NADH	NAD^+	E-NADH	E-NAD^+
10	100	100	100	100	0	0

Simulation (2)



Model checking (1)

- A probabilistic model checker can be easily used for verifying properties of a Molecular Probabilistic Transition System
- We used the PRISM model checker for verifying properties of the sorbitol dehydrogenase
- Properties have been specified in the CSL language:

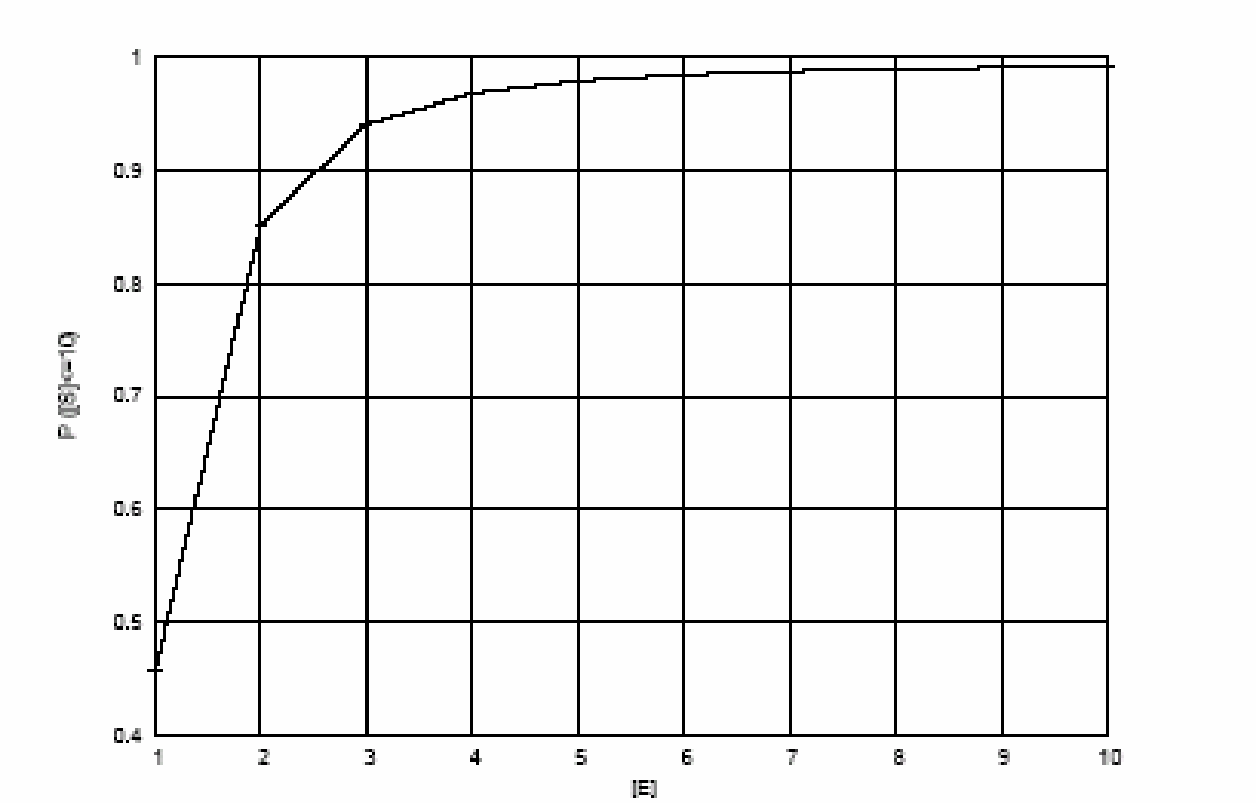
$$\phi ::= true \mid false \mid a \mid \phi \wedge \phi \mid \phi \vee \phi \mid \neg\phi \mid \mathcal{P}_{\sim p}[\psi] \mid \mathcal{S}_{\sim p}[\psi]$$

$$\psi ::= X\phi \mid \phi \mathcal{U}^{I} \phi \mid \phi \mathcal{U} \phi$$

- Sorbitol dehydrogenase: initial concentrations

S	F	NADH	NAD^+	E-NADH	E-NAD^+
25	25	25	25	0	0

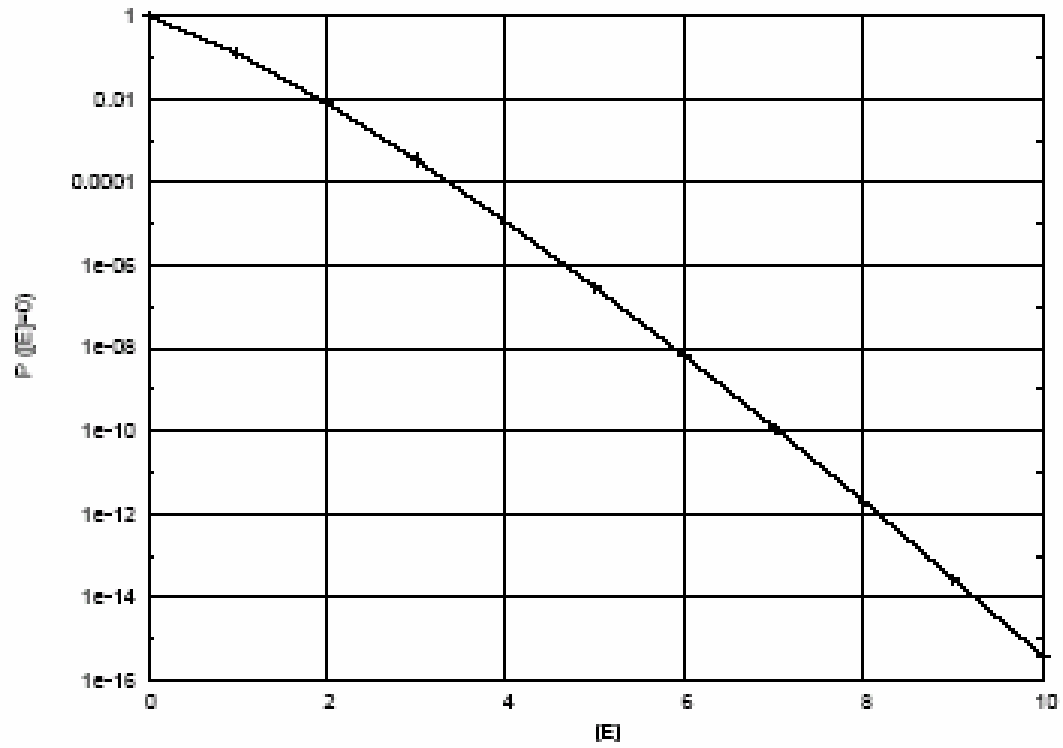
Model checking (2)



- Probability that the concentration of S becomes less than 10 in less then 5000 steps by varying the initial concentration of E

$$\mathcal{P}_{=?} \ [true \mathcal{U}^{<=5000} [S] <= 10]$$

Model checking (3)



- Probability that the concentration of E reaches 0 in less than 5000 steps by varying its initial value

$$\mathcal{P}_{=?} \; [true \mathcal{U}^{<=5000} [E] = 0]$$

Conclusions

- We have developed a framework to model biochemical reactions
  - we have implemented a simulator
  - we have used a model checker (PRISM) to verify properties of reactions
- The main idea is to approximate the speed of reactions by probabilities of rule applications
- The choice of the right probabilities is critical !!
- Experiments have suggested to revise the semantics of the calculus to have a better approximation

Future work

- Expected improvements (short term):
  - the model will be revised in order to obtain better quantitative results
  - the choice of the probabilities will not be done by the modeler but probabilities will be derived automatically by kinetic constants
  - the semantics will be given in SOS style
  - some results of biological correctness will be proved

- Expected improvements (mid/long term):
  - more complex examples of real biological systems will be simulated and verified
  - some additional operators will be considered
  - the problem of predicting unknown kinetic constants will be faced

THE END…

Computing probabilities…

$$p(m) = \frac{[m]_S}{|S|} \qquad p(R_{i,1<=i<=n}|m) = \frac{P_i(m)}{P_1(m) + \ldots + P_n(m)}$$

$$p(S, S', \mathcal{R}) = \begin{cases} 1 & \text{if } S = \emptyset = S' \\ 0 & \text{if } \#(S) \neq \#(S') \\ \sum_{i=1}^{k} p(m_i) \sum_{j=1}^{n_i} p(R_{i,1<=i<=n_i}|m_i) p(S \backslash S_j, S' \backslash S'_j, \mathcal{R}) & \text{otherwise} \end{cases}$$

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$$\psi ::= X\phi \mid \phi \mathcal{U}^{I} \phi \mid \phi \mathcal{U} \phi$$

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