Design and Analysis of DNA Strand Displacement Devices using Probabilistic Model Checking

Dave Parker

School of Computer Science, University of Birmingham

Joint work with:

Matthew Lakin, Luca Cardelli, Marta Kwiatkowska and Andrew Phillips

Centre for Systems Biology, Birmingham, June 2012

Overview

- Quantitative verification
  - probabilistic model checking and PRISM

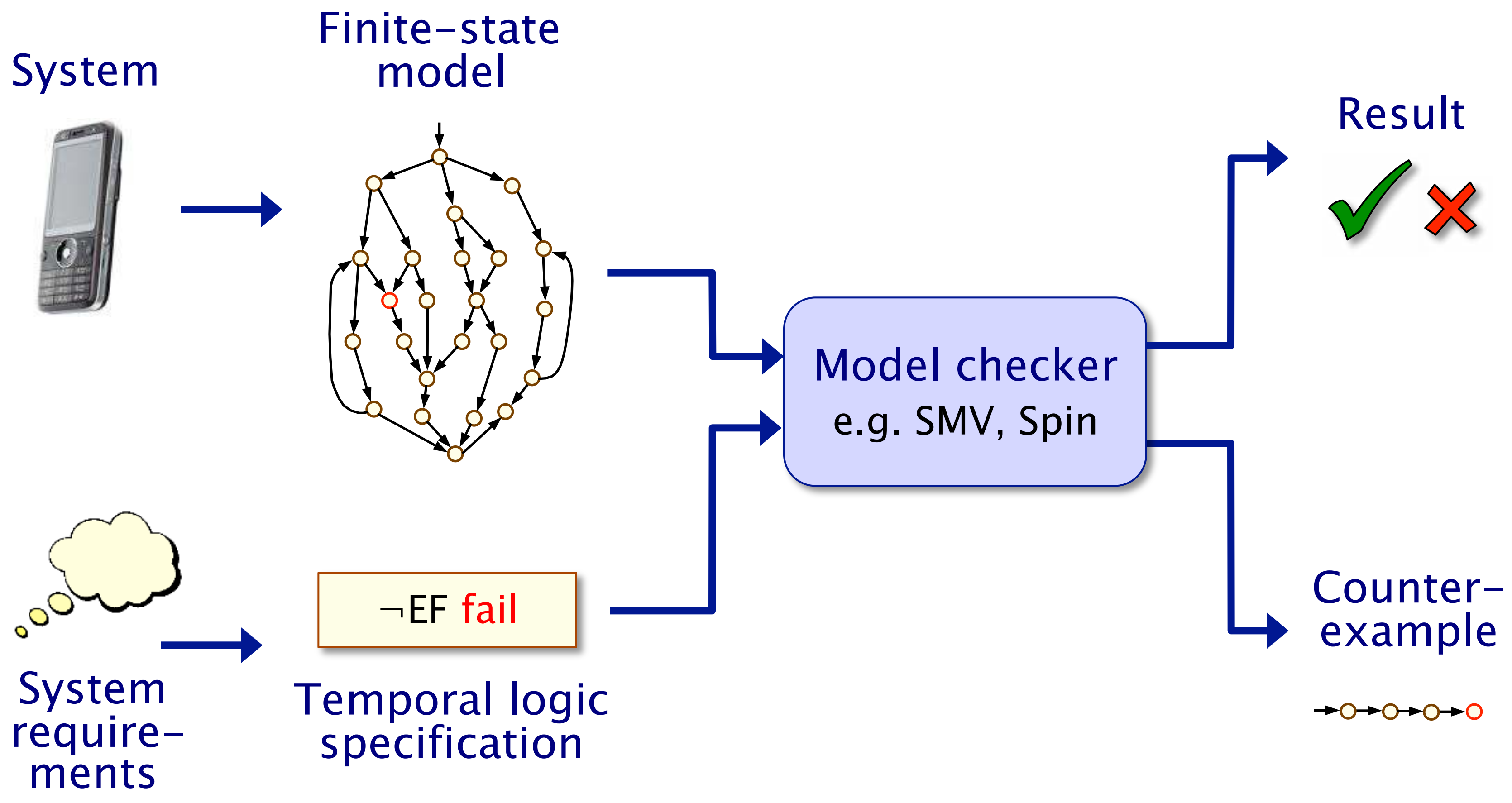
- Modelling and analysis of biological systems
  - a discrete stochastic approach
  - probabilistic model checking: “in-silico” experiments

- Two-domain DNA strand displacement
  - gate correctness, reliability and performance
  - design optimisation: garbage collection
  - a larger example: approximate majority
  - see: [Lakin/Parker/..., Royal Society Interface, 2012]

- Summary, challenges & directions

Verification via model checking

Model checking: Automatic formal verification of correctness properties of computerised systems



Probabilistic model checking

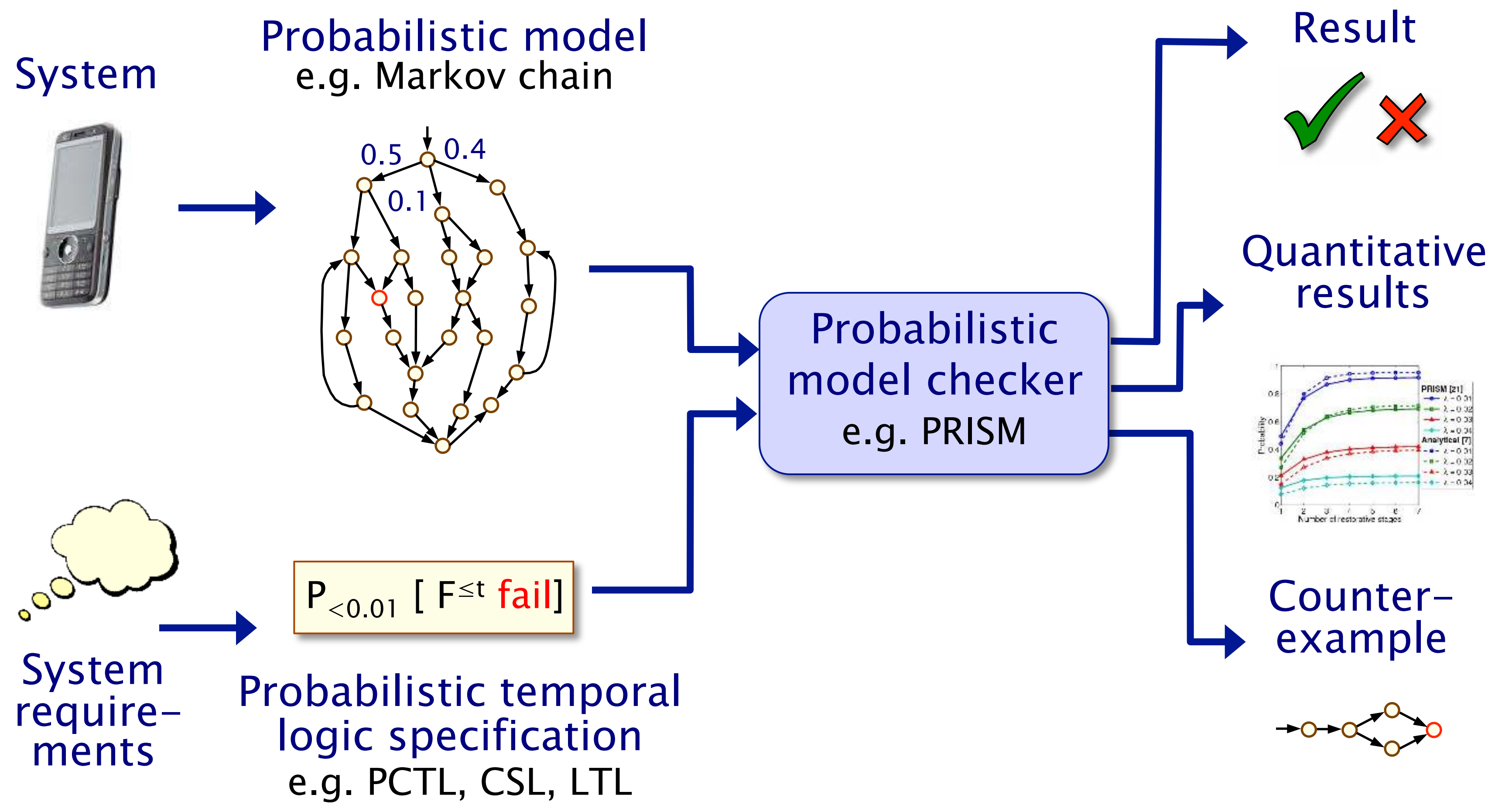
- Why and what?

- Why probability?
  - unreliability (e.g. component failures)
  - uncertainty (e.g. message losses/delays over wireless)
  - randomisation (e.g. in protocols such as Bluetooth, ZigBee)
  - stochasticity (e.g. biological/chemical reaction rates)

- Quantitative properties
  - reliability, performance, quality of service, …
  - “the probability of an airbag failing to deploy within 0.02s”
  - “the expected power usage of a sensor network over 1 hour”
  - “the expected time for a cell signalling pathway to complete”

Probabilistic model checking

Probabilistic model checking: Automatic verification of quantitative properties of systems with stochastic behaviour



Probabilistic model checking

- Construction and analysis of finite probabilistic models
  - e.g. Markov chains, Markov decision processes, …
  - specified in high-level modelling formalisms
  - exhaustive model exploration (all possible states/executions)

- Automated analysis of wide range of quantitative properties
  - properties specified using temporal logic
  - “exact” results obtained via numerical computation
  - linear equation systems, iterative methods, uniformisation, …
  - as opposed to, for example, Monte Carlo simulations
  - efficient techniques from verification + performance analysis
  - mature tool support available

The PRISM tool

- PRISM: Probabilistic symbolic model checker
  - developed at Birmingham/Oxford University, since 1999
  - free, open source software (GPL), runs on all major OSs
- Support for:
  - models: Markov chains, Markov decision processes, …
  - properties: PCTL, CSL, LTL, PCTL*, costs/rewards, …
- Features:
  - simple but flexible high-level modelling language
  - user interface: editors, simulator, experiments, graph plotting
  - multiple efficient model checking engines (e.g. symbolic)
- Many import/export options, tool connections
  - in: (Bio)PEPA, stochastic π-calculus, DSD, SBML, Petri nets, …
  - out: Matlab, MRMC, INFAMY, PARAM, …
- See: http://www.prismmodelchecker.org/

PRISM – Case studies

- Randomised communication protocols
  - Bluetooth, FireWire, Zeroconf, 802.11, Zigbee, gossiping, …
- Randomised distributed algorithms
  - consensus, leader election, self-stabilisation, …
- Security protocols/systems
  - pin cracking, anonymity, quantum crypto, contract signing, …
- Planning & controller synthesis
  - robotics, dynamic power management, …
- Performance & reliability
  - nanotechnology, cloud computing, manufacturing systems, …
- Biological systems
  - cell signalling pathways, DNA computation, …

- See: www.prismmodelchecker.org/casestudies

Overview

- Quantitative verification
  - probabilistic model checking and PRISM
- Modelling and analysis of biological systems
  - a discrete stochastic approach
  - probabilistic model checking: “in-silico” experiments
- Two-domain DNA strand displacement
  - gate correctness, reliability and performance
  - design optimisation: garbage collection
  - a larger example: approximate majority
- Summary, challenges & directions

Modelling biological systems

- Aim: model a mixture of interacting molecules
  - multiple molecular species, interacting through reactions
  - cell signalling pathway, gene regulatory network, …
  - fixed volume (spatially uniform), pressure and temperature
- Simple example:
  - 3 species A, B and AB; 3 reactions:
  - reversible binding of A and B to form AB; degradation of A

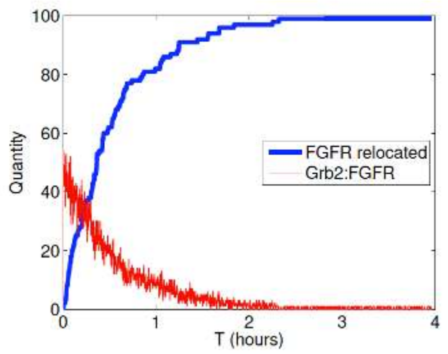
$$A + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} AB \qquad A \xrightarrow{k_3}$$

- Two approaches to modelling
  - discrete, stochastic
  - continuous, deterministic

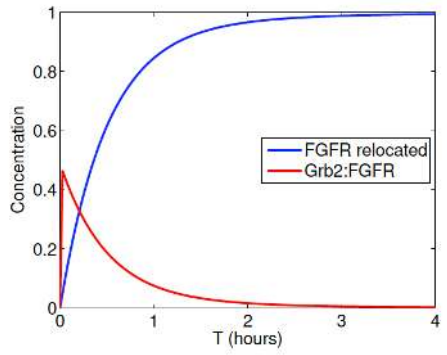
Modelling biological systems

- Discrete, stochastic approach
  - (integer) counts of number of each molecule: $\mathbf{x}=(x_A,x_B,x_{AB})$
  - inherently stochastic process [McQuarrie, Gillespie]
  - continuous-time Markov chain with states $\mathbf{x}$
  - stochastic simulation, numerical soln., probabilistic model checking, …

$$A + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} AB \qquad A \xrightarrow{k_3}$$



- Continuous, deterministic approach
  - (real-valued) concentrations: [A], [B], [AB]
  - solution of system of coupled ordinary differential equations
  - good approximation of E[$\mathbf{x}$] for very large num.s of molecules



Discrete stochastic approach

- Chemical master equation
  - state vector $\mathbf{x}=(x_A,x_B,x_{AB})$
  - probability $P(\mathbf{x},t)$ that at time t there will be x_Z of species Z

$$A + B \underset{k_2}{\overset{k_1}{\rightleftarrows}} AB \qquad A \xrightarrow{k_3}$$

$$\frac{\delta P(\mathbf{x},t)}{\delta t} = \sum_{i=1}^{3} a_i(\mathbf{x}-\mathbf{v}_i)P(\mathbf{x}-\mathbf{v}_i,t) - a_i(\mathbf{x})P(\mathbf{x},t)$$

  - stoichiometric vectors: $\mathbf{v}_1=(-1,-1,1)$, $\mathbf{v}_2=(1,1,-1)$, $\mathbf{v}_3=(-1,0,0)$
  - $a_i(\mathbf{x})$ are time-independent propensity functions
  - mass-action: proportional to reactant combinations
    - e.g. $a_1(x_A,x_B,x_{AB}) = k_1 \cdot x_A \cdot x_B$

- Stochastic process: continuous-time Markov chain (CTMC)
  - transition rates (of exponential delays) derived from a_i

Continuous-time Markov chain (CTMC)

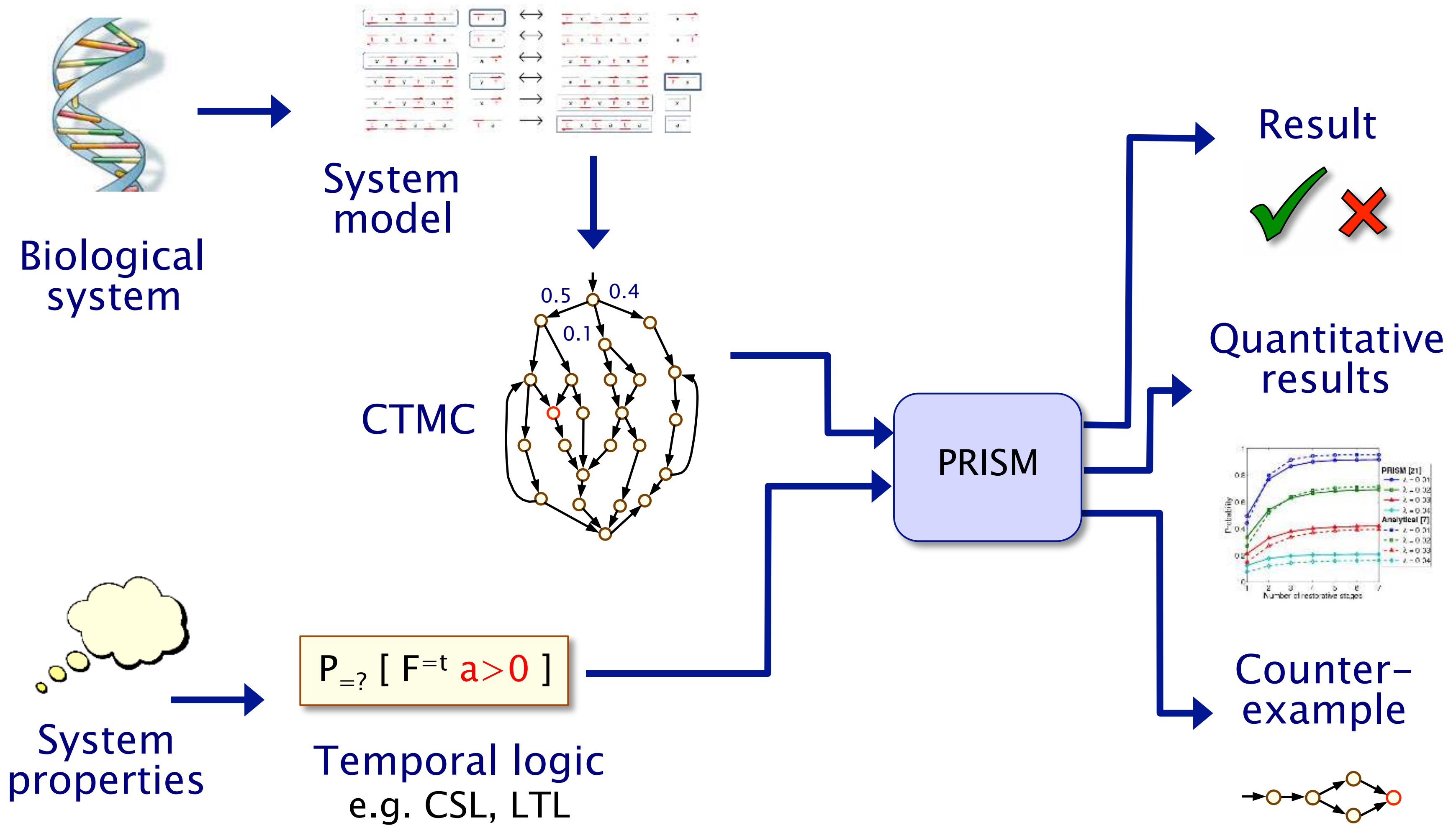
- CTMC $C = (S, s_i, \mathbf{R})$
  - states S, initial state $s_i \in S$
  - rate matrix $\mathbf{R} : S \times S \rightarrow \mathbb{R}_{\geq 0}$
  - $\mathbf{R}(s,s')$: rate of exponential delay before moving $s \rightarrow s'$
  - probability $s \rightarrow s'$ triggered before time $t = 1 - e^{-\mathbf{R}(s,s')\cdot t}$
- Example: CTMC with:
  - states $(x_A, x_B, x_{AB}) \in S = \{0,1,2\}^3$
  - initial state $(2,2,0)$
- Rates for reactions
  - r_1 (binding): rate $= x_A \cdot x_B \cdot k_1$
  - r_2 (unbinding) rate $= x_{AB} \cdot k_2$
  - r_3 (degradation): rate $= x_A \cdot k_3$

$$A + B \underset{k_2}{\overset{k_1}{\rightleftarrows}} AB \qquad A \xrightarrow{k_3}$$

$$\begin{tikzcd}
{} \arrow[r] & 2,2,0 \arrow[r, bend left, "4k_1"] \arrow[d, "2k_3"'] & 1,1,1 \arrow[l, bend left, "k_2"] \arrow[r, bend left, "k_1"] \arrow[d, "k_3"] & 0,0,2 \arrow[l, bend left, "2k_2"] \\
 & 1,2,0 \arrow[r, bend left, "2k_1"] \arrow[d, "k_3"'] & 0,1,1 \arrow[l, bend left, "k_2"] & \\
 & 0,2,0 & &
\end{tikzcd}$$

Probabilistic model checking

Probabilistic model checking for systems biology…



PRISM modelling language

- Simple, textual, state-based modelling language
  - for Markov chains (and other models)

- Language basics
  - networks formed from interacting modules
  - state of each module given by finite-ranging variables
  - behaviour of each module specified by guarded commands
  - interactions between modules through synchronisation
  - interactions are associated with state-dependent rates

$$\underbrace{[r_1]}_{\text{action}} \quad \underbrace{(a>0)}_{\text{guard}} \quad \rightarrow \quad \underbrace{k_1*a}_{\text{rate}} \; : \; \underbrace{(a'=a-1)\&(ab'=ab+1);}_{\text{update}}$$

PRISM language – example

```
module A
    a : [0..N] init N;
    ab : [0..N] init 0;
    [r1] a>0 → k1*a : (a'=a-1)&(ab'=ab+1);
    [r2] ab>0 → k2*ab : (a'=a+1)&(ab'=ab-1);
    [r3] a>0 → k3*a : (a'=a-1);
endmodule
```

```
module B
    b : [0..N] init N;
    [r1] b>0 → b : (b'=b-1);
    [r2] b<N → b : (b'=b+1);
endmodule
```

Reactions r_1/r_2 :

$$A + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} AB$$

Reaction r_3 :

$$A \xrightarrow{k_3}$$

Example (r_1):

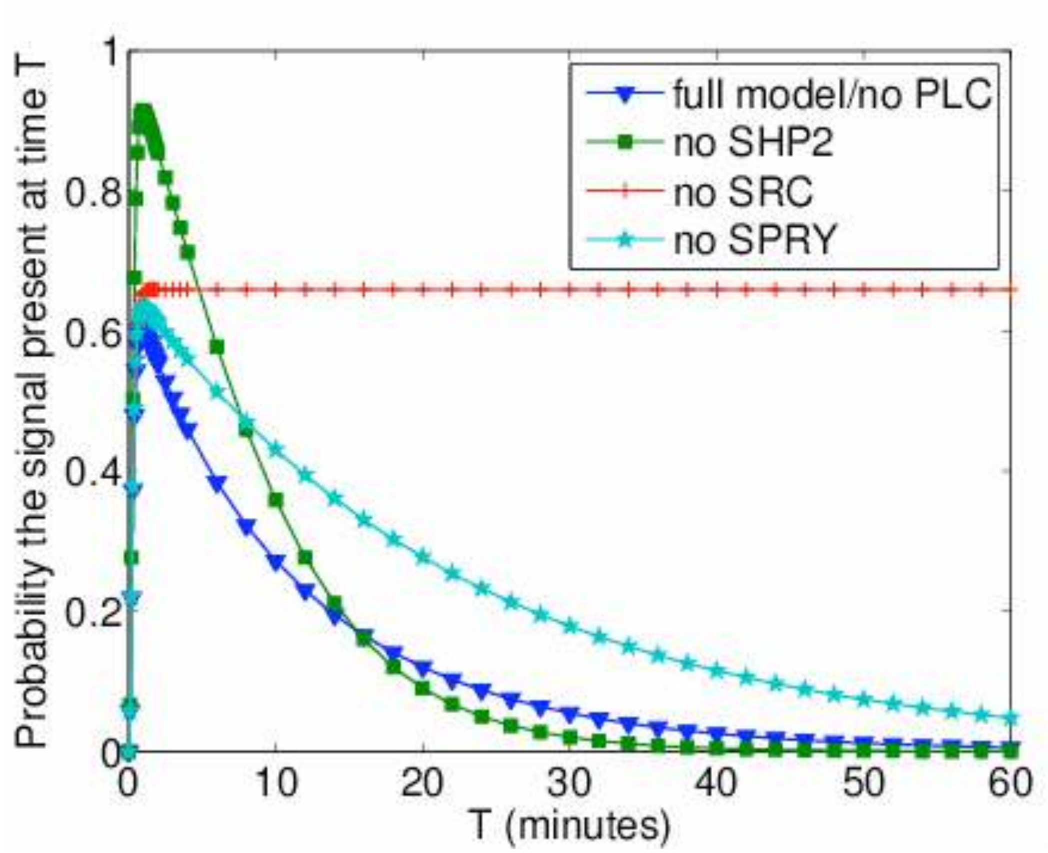
$$\begin{tikzcd}(a,ab,b) \arrow[d, "k_1 \cdot a \cdot b"] \\ (a-1,ab+1,b-1)\end{tikzcd}$$

Property specifications

- Property specifications are based on temporal logic
  - PRISM uses continuous stochastic logic (CSL) + extensions
  - also supports linear temporal logic (LTL)
  - flexible, compact, unambiguous definition
  - small subset of patterns/templates in common use
  - can express properties about the probability of occurrence of an event or the expected value of some cost/reward measure
- CSL example: $P_{>0.9}$ [$F^{\leq T}$ kpp>0]
  - “with probability greater than 0.9, at least some MAPK is activated within the first T seconds”
- Usually focus on “quantitative” CSL: $P_{=?}$ [$F^{\leq T}$ kpp>0]
  - “what is the probability that at least some MAPK is activated within the first T seconds?”
  - typically compute/plot for a range of parameter values

Example (FGF)

- Probability that a signal is present at time T
  - $P_{=?}$ [$F^{=T}$ (FRS2_GRB>0 & relocFRS2=0 & degFRS2=0)]



More examples of (extended) CSL

- $P_{=?}$ [$F^{[t,t]}$ a=i]
  - "the probability that there are exactly i A after t seconds"
- $P_{=?}$ [F a=0]
  - "probability that all A proteins are eventually degraded"
- $S_{=?}$ [c+d>M]
  - "long-run probability that the total number of Cs and Ds activated is above M"
- $P_{=?}$ [c=0 $U^{>t}$ c>0 {c=0}{"max"}]
  - "highest probability of it taking more than t seconds for C to become activated, from any state where there are none"
- $P_{=?}$ [F c=N] / $P_{=?}$ [F c>0]
  - "the (conditional) probability that all C proteins are eventually activated, given that at least some of them are"
- $R_{\{\text{"active\_d"}\}=?}$ [$I^{=t}$]
  - "the expected number of activated D at time instant t"

Case studies

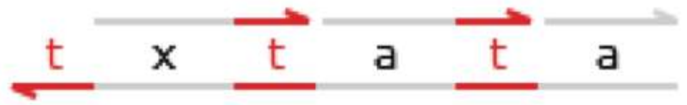
- Fibroblast Growth Factor (FGF) pathway
  - [Heath/Kwiatkowska/Norman/Parker/Tymchyshyn/Gaffney]
  - 12 species, 14 sets of reaction rules
  - model checking (PRISM)+ simulation (stochastic π-calculus)
  - “in-silico” experiments: systematic removal of components
  - results validated by subsequent lab experiments
- RKIP-inhibited ERK pathway [Calder/Vyshemirsky/Gilbert/Orton]
  - model checking using PEPA and PRISM models
  - formal analysis highlighted errors in existing models
  - corrected models then validated against experimental data
- And more: Codon bias, Ribosome kinetics, Sorbitol dehydrogenase, T Cell Signalling Events, …
  - www.prismmodelchecker.org/casestudies/index.php#biology

Overview

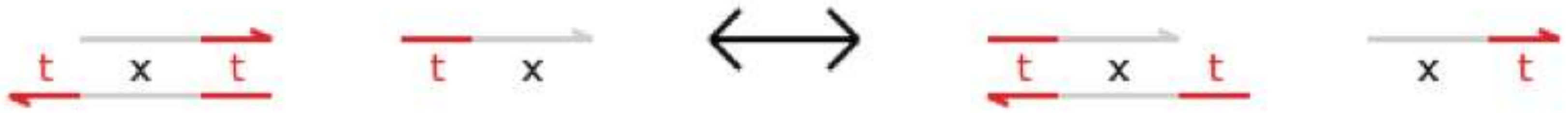
- Quantitative verification
  - probabilistic model checking and PRISM
- Modelling and analysis of biological systems
  - a discrete stochastic approach
  - probabilistic model checking: "in-silico" experiments
- Two-domain DNA strand displacement
  - gate correctness, reliability and performance
  - design optimisation: garbage collection
  - a larger example: approximate majority
- Summary, challenges & directions

Two-Domain DNA Strand Displacement

- DNA computing with a restricted class of DNA strand displacement structures
  - double strands with nicks (interruptions) in the top strand



  - and two-domain single strands consisting of one (short) toehold domain and one recognition domain

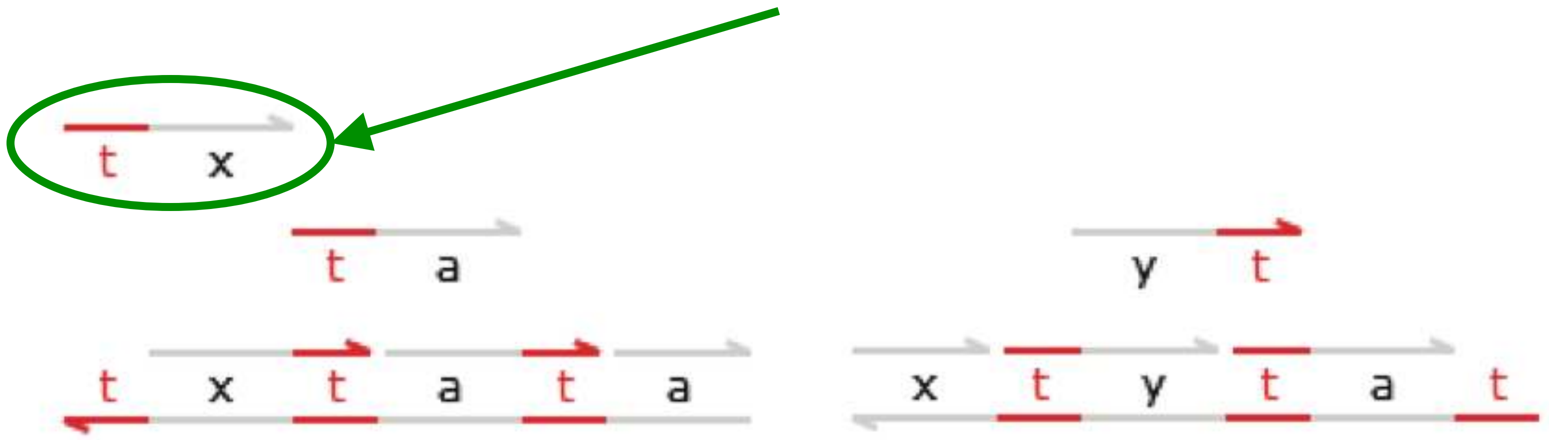


  - "toehold exchange": branch migration of strand <t^ x> leading to displacement of strand <x t^>
- Used to construct transducers, fork/join gates
  - which can emulate Petri net transitions

[Cardelli'10] Luca Cardelli. Two-Domain DNA Strand Displacement. Proc. *Development of Computational Models* (DCM'10)

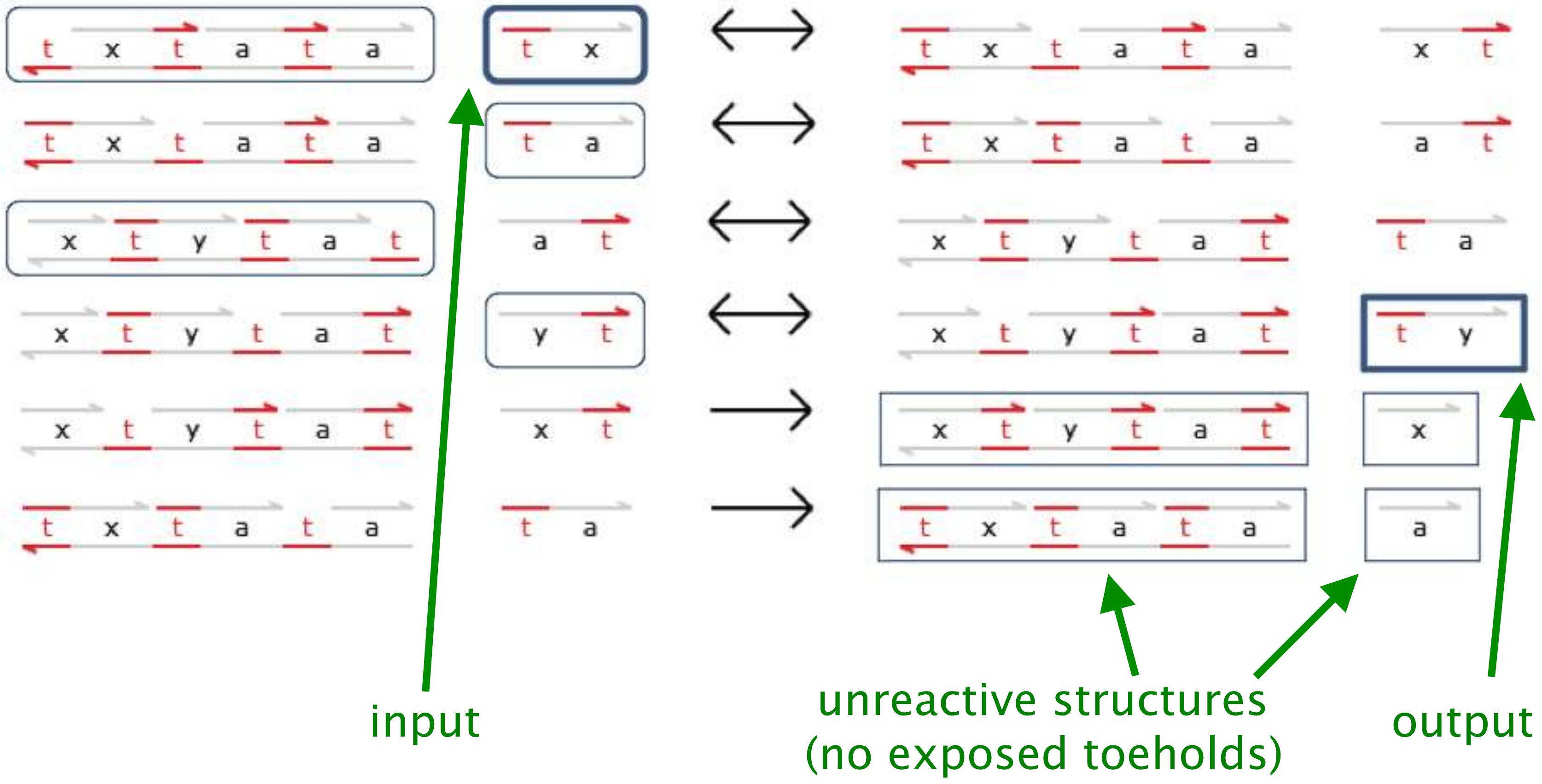
Example: Transducer

- Transducer: converts input <t^ x> into output <t^ y>



Example: Transducer

- Transducer: full reaction list



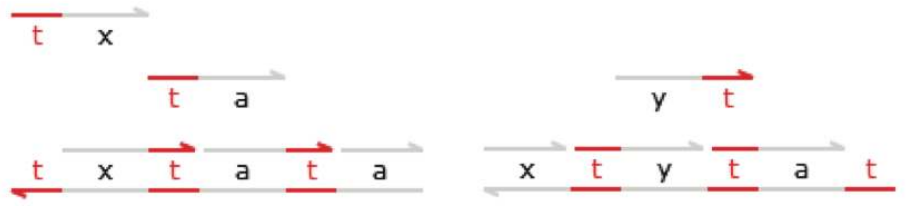
DNA programming

- Challenge: correct, reliable designs; avoid interference

- [Cardelli'10] proposes a "nick algebra" to formalise the definition and behaviour of these two-domain DNA strands
  - syntax, algebraic equivalence relation, reduction rules

- Strict subset of DSD (DNA Strand Displacement) language
  - [Cardelli, Phillips, et al.]
  - accompanying software Visual DSD for analysis/simulation
  - now extended to include auto-generation of PRISM models

- Example:



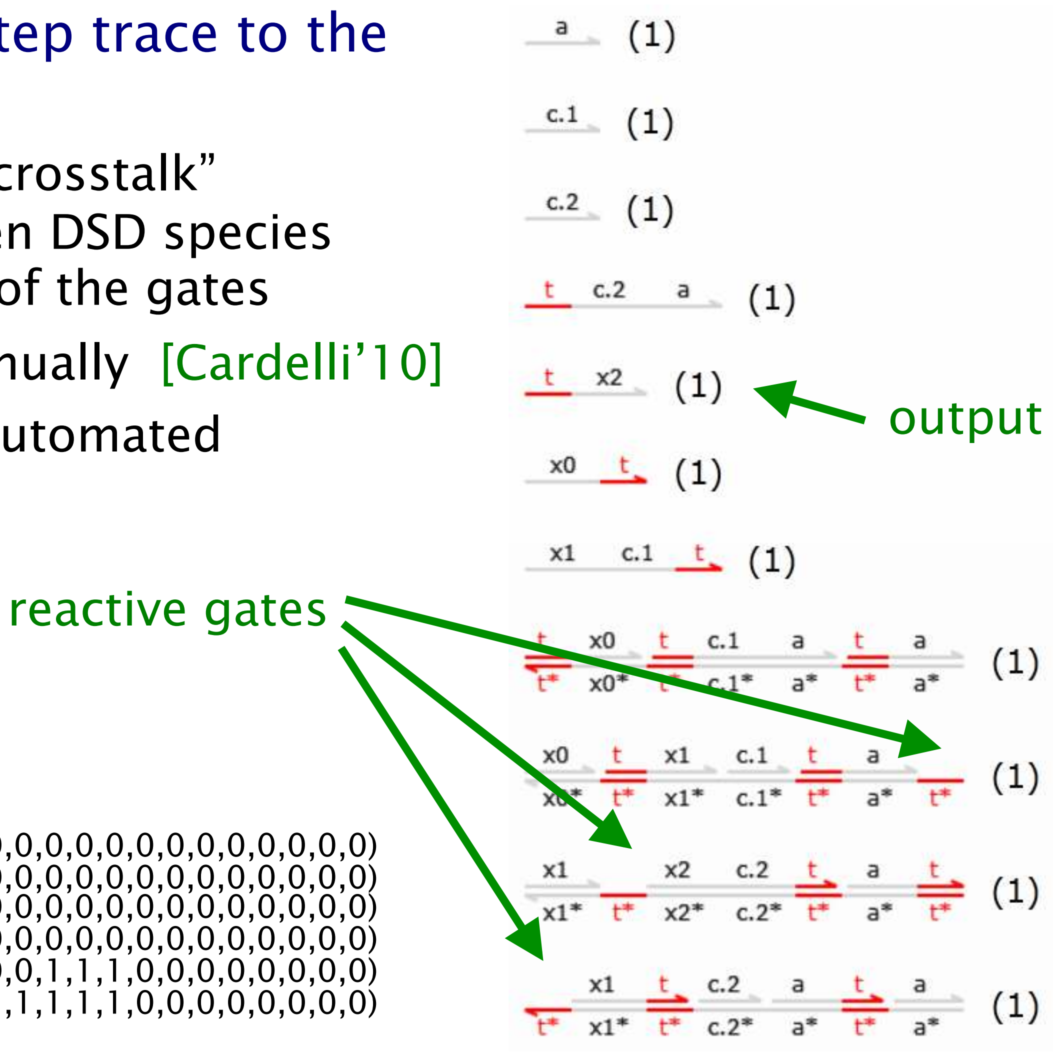
```
new t@0.0003,0.1126
def T(N, x, y) =
( N* <t^ a> | N* <y t^>| N* t^:[x t^]:[a t^]:[a] | N* [x]:[t^ y]:[t^ a]:t^ )
( T(1, x, y) | 1 * <t^ x> )
```

Transducers: Correctness

- Formalising correctness…
  - identify states where gate has terminated correctly: "all_done"
  - (correct number of outputs, no reactive gates left)
- Check:
  - (i) any possible deadlock state that can be reached must satisfy "all_done"
    (ii) there is at least one path through the system that reaches a state satisfying "all_done"
- In temporal logic (CTL):
  - A [G "deadlock" => "all_done"]
  - E [F "all_done"]
- Verify using PRISM…
  - for one transducer: both properties true
  - for two transducers in series: (ii) is true, but (i) is false

Transducer flaw

- PRISM identifies a 5-step trace to the “bad” deadlock state
  - problem caused by “crosstalk” (interference) between DSD species from the two copies of the gates
  - previously found manually [Cardelli’10]
  - detection now fully automated
- Bug is easily fixed
  - (and verified)

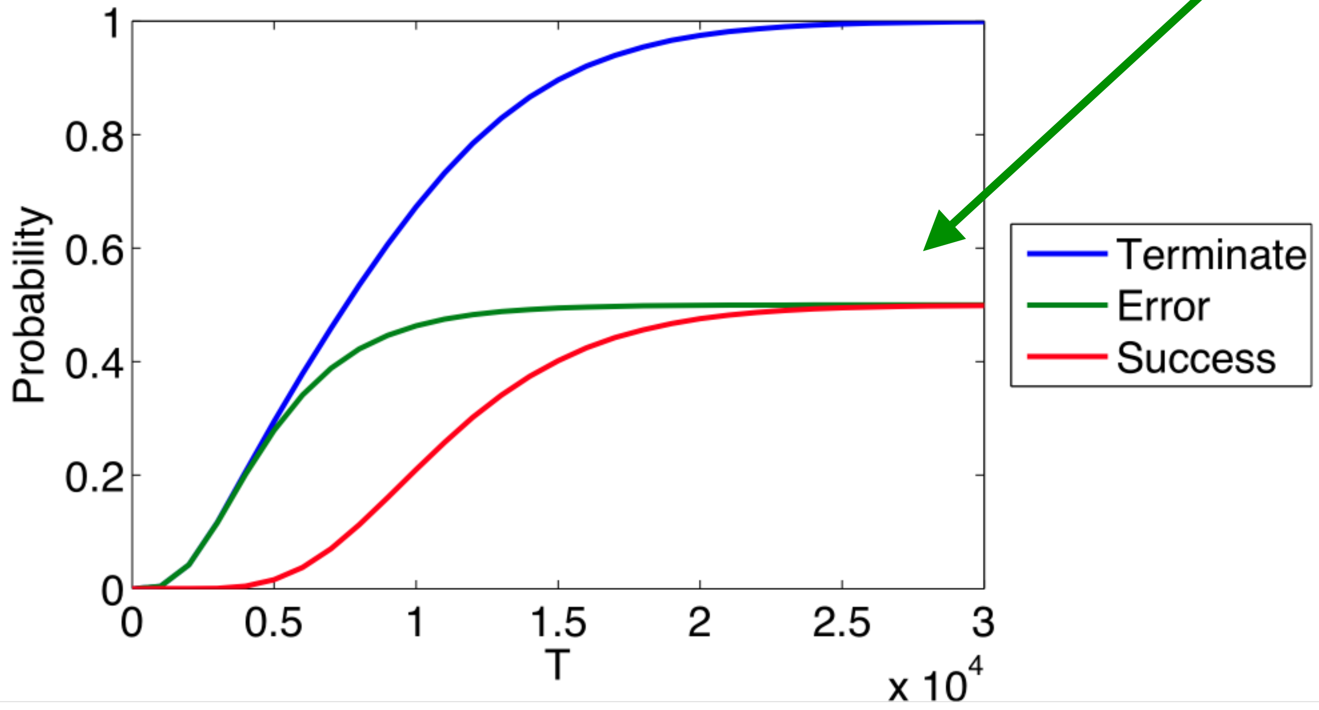

a (1)
c.1 (1)
c.2 (1)
t c.2 a (1)
t x2 (1)
output
x0 t (1)
x1 c.1 t (1)
reactive gates
t x0 t c.1 a t a t* x0* t* c.1* a* t* a* (1)
x0 t x1 c.1 t a x0* t* x1* c.1* t* a* t* (1)
x1 x2 c.2 t a t x1* t* x2* c.2* t* a* t* (1)
x1 t c.2 a t a t* x1* t* c.2* a* t* a* (1)

Counterexample:
```
(1,1,1,1,1,1,1,1,1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0)
(0,1,1,0,1,1,1,1,1,1,1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0)
(0,0,1,0,1,1,1,1,1,0,1,1,1,1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0)
(0,0,1,0,1,1,1,1,0,0,1,1,1,0,0,1,1,0,0,0,0,0,0,0,0,0,0,0,0,0,0)
(0,0,1,0,1,1,0,1,0,0,1,1,1,0,0,0,1,0,0,0,0,1,1,1,0,0,0,0,0,0,0)
(0,0,1,0,1,1,0,1,0,0,1,0,1,0,0,0,0,0,0,1,1,1,1,1,0,0,0,0,0,0,0)
```

Transducers: Quantitative properties

- We can also use PRISM to study the kinetics of the pair of (faulty) transducers:
  - $P_{=?}$ [$F^{[T,T]}$ "deadlock"]
  - $P_{=?}$ [$F^{[T,T]}$ "deadlock" & !"all_done"]
  - $P_{=?}$ [$F^{[T,T]}$ "deadlock" & "all_done"]

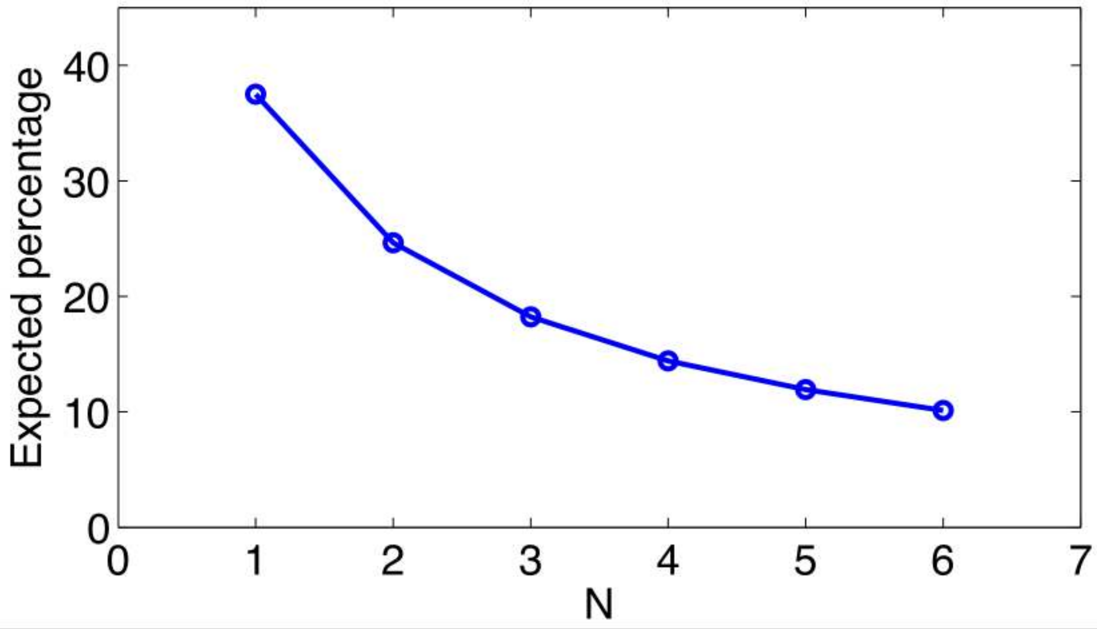
success/error equally likely



Transducers: Reliability

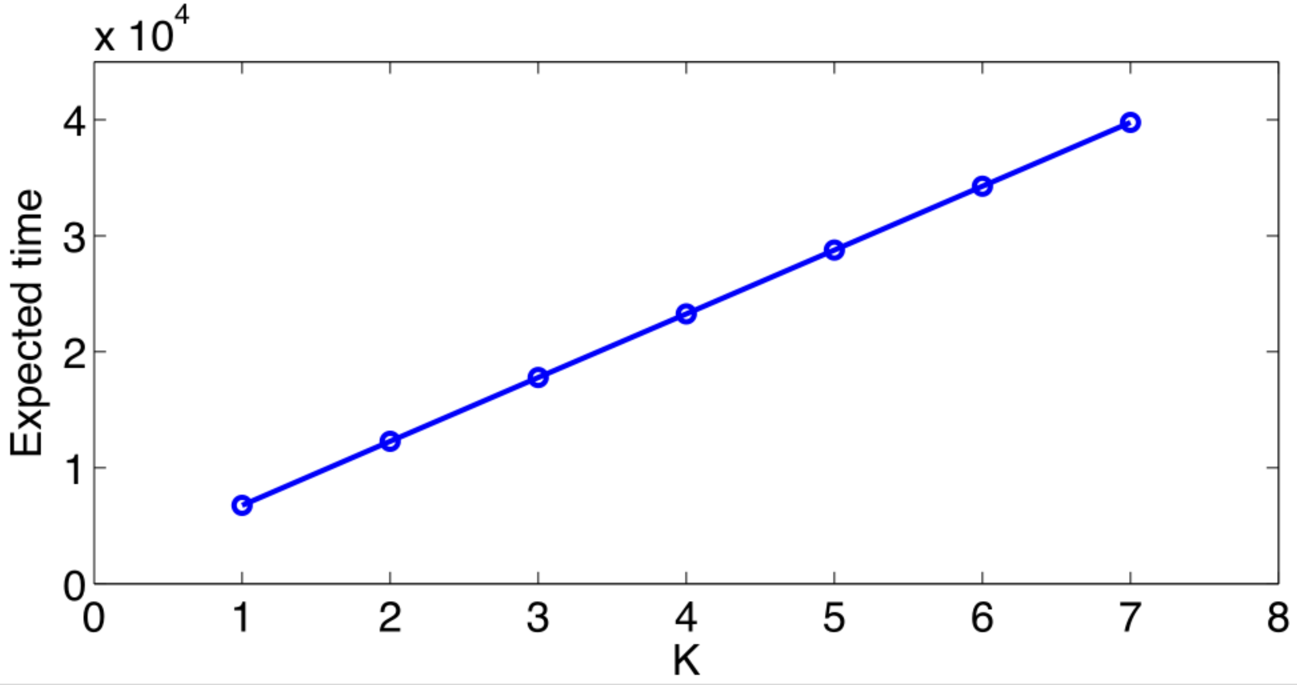
- Even without fixing the flaw in the transducer design…
  - we can improve reliability by using larger numbers of copies

- Plot: Expected number of reactive gates in the final state
  - for N copies of the faulty transducer pair



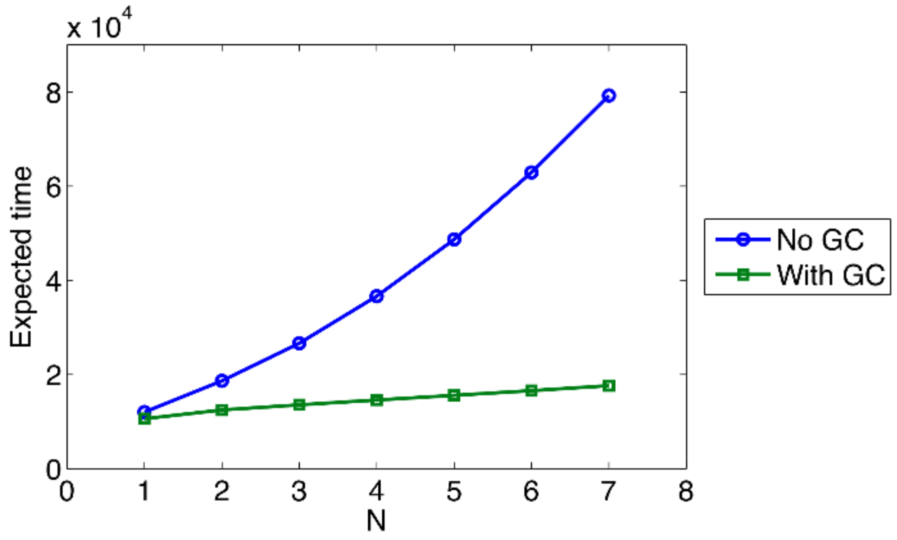
Transducers: Performance

- We analyse the performance of the (corrected) transducers
  - circuit composed of chain of K transducers
  - Seelig/Soloveichik showed execution time linear in depth
- Analysed for DSD model in PRISM:
  - $R_{\{\text{"time"}\}=?}$ [F "all_done"]



Catalysts in DSD

- Slightly more complex DSD gate design
  - extension of the transducer gate design
- Chemical reaction $X \rightarrow Z$ catalysed by 3rd species Y
  - i.e. $X + Y \rightarrow Y + Z$
- Design decision:
  - can/should we implement garbage collection (GC)?
  - i.e. tidying up of intermediate species into inert structures
  - omitting GC makes design simpler and cheaper
  - but is it still correct?
    and what about efficiency?
- PRISM analysis:
  - both designs correct
  - GC speeds up gate execution significantly
  - due to extra reactions



Approximate Majority

- Approximate majority population protocol [Angluin et al.]
  - two populations X, Y and an auxiliary species B
  - aim is to converge to a consensus: either X or Y
  - should converge to population with initial majority

- Reactions:

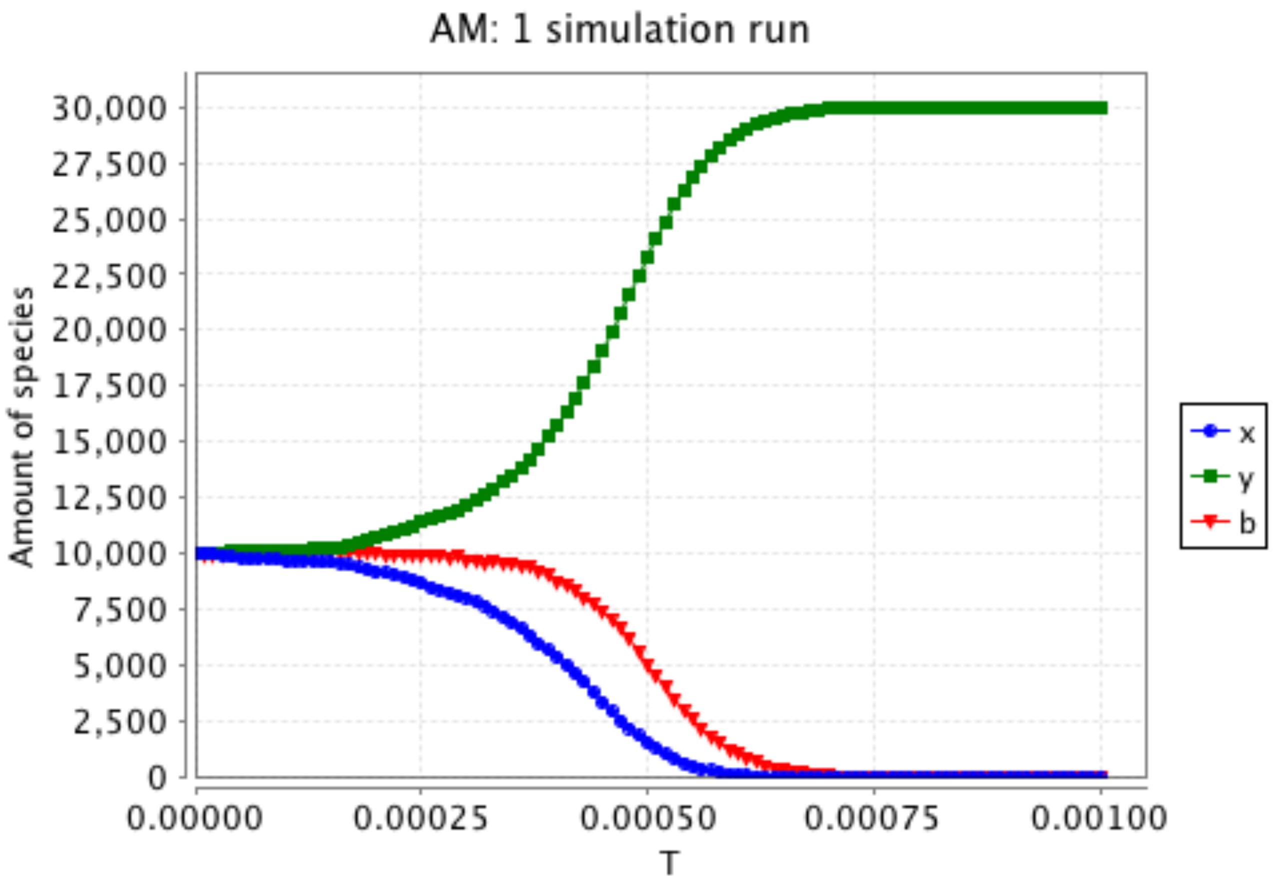
$$X + Y \xrightarrow{k_1} Y + B \qquad B + X \xrightarrow{k_3} X + X$$

$$Y + X \xrightarrow{k_2} X + B \qquad B + Y \xrightarrow{k_4} Y + Y$$

- We implement the approximate majority protocol in DSD
  - using the catalyst reactions shown earlier
  - and then analyse its correctness

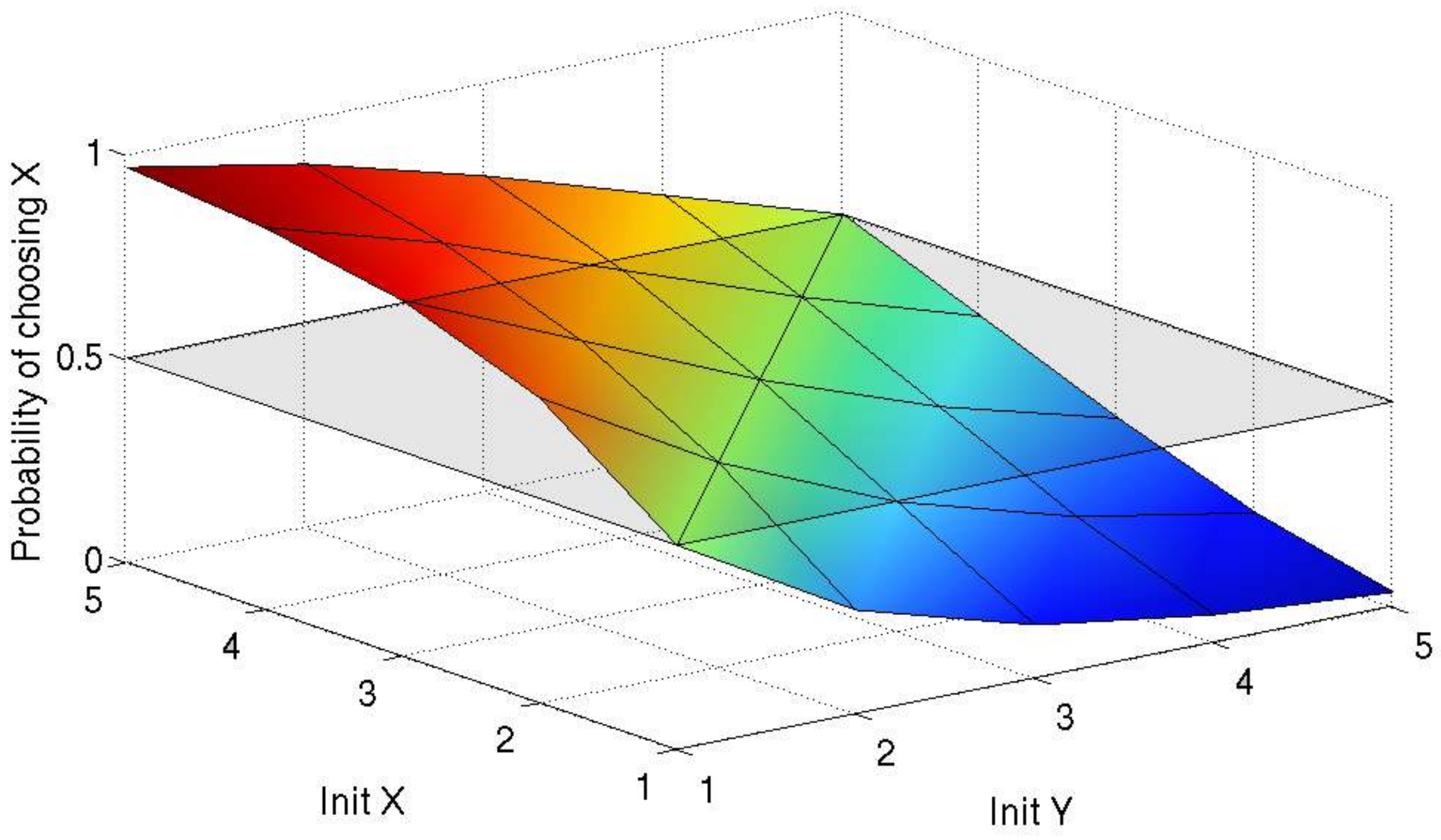
Approximate majority: Simulation

- Typical simulation run:
  - in this instance, the protocol chooses Y



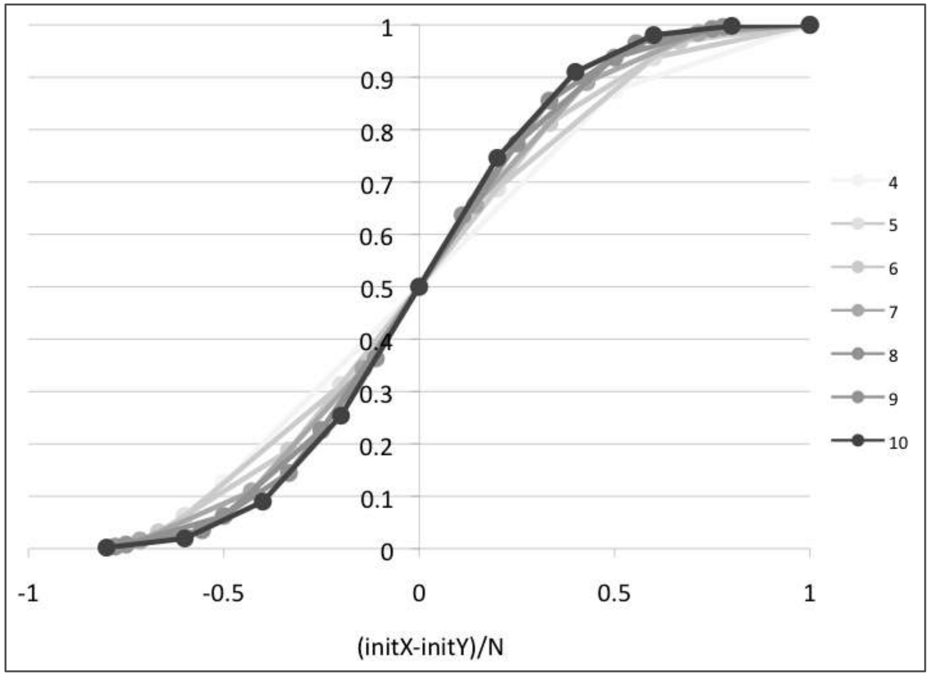
Approximate majority: Analysis

- Plot probability of choosing X for varying initial X/Y
  - 0.5 for equal initX and initY
  - rapidly approaches 1 as majority increases



Approximate majority: Analysis

- [Angluin et al.] prove correct consensus obtained with high probability if the initX–initY margin is above ω(√N log N)
  - re-plot same data against (relative) initX–initY margin
  - for various total initial population sizes N (=4,…,10)
  - note increasingly clear threshold for larger N



Model checking DNA: Limitations

- Key challenge (as always): state space explosion
  - CTMCs solved for this work up to approx. 2m states
- Already using various methods to reduce state space:
  - careful gate design to reduce number of asynchronous steps
  - highest level of abstraction for reactions in DSD tool
  - for approximate majority, fuels modelled as “constant species”
- Some positive results:
  - bugs found in small systems, which also exist in bigger ones
  - we illustrated useful design trade-offs with small populations
  - earlier work (FGF): successful expt. validation for small sizes
- On the other hand:
  - transducer bug only arises for a transducer pair, not when studied in isolation; can we explore all possible interfaces?
  - how can we formally relate results obtained from smaller models to larger ones?

Overview

- Quantitative verification
  - probabilistic model checking and PRISM
- Modelling and analysis of biological systems
  - a discrete stochastic approach
  - probabilistic model checking: “in-silico” experiments
- Two-domain DNA strand displacement
  - gate correctness, reliability and performance
  - design optimisation: garbage collection
  - a larger example: approximate majority
- Summary, challenges & directions

Summary

- Probabilistic model checking
  - automatic, exhaustive construction of probabilistic models
  - analysis of formally specified quantitative properties
  - efficient techniques, tools available
- Probabilistic model checking for systems biology
  - discrete, stochastic model: chemical master equation
  - solution of continuous-time Markov chains
  - quantitative properties expressed in temporal logic
- DNA strand displacement
  - two-domain DSD designs analysed with Visual DSD, PRISM
  - correctness, reliability, performance, design decisions

Challenges and Directions

- Challenges
  - scalability, infinite-state systems
  - correct level of abstraction for formal languages?
  - appropriate (and testable) model checking queries?
  - closer integration of model checking tools, engines

- Directions
  - model abstractions (and automatic construction of)
  - infinite state systems: truncation for time-bounded properties
  - model reduction techniques: bisimulation, symmetry, …
  - approximate/statistical model checking (simulation-based)
  - stochastic hybrid systems: discrete + continuous populations
  - compositional probabilistic model checking