Visual DSD: a design and analysis tool for DNA strand displacement systems

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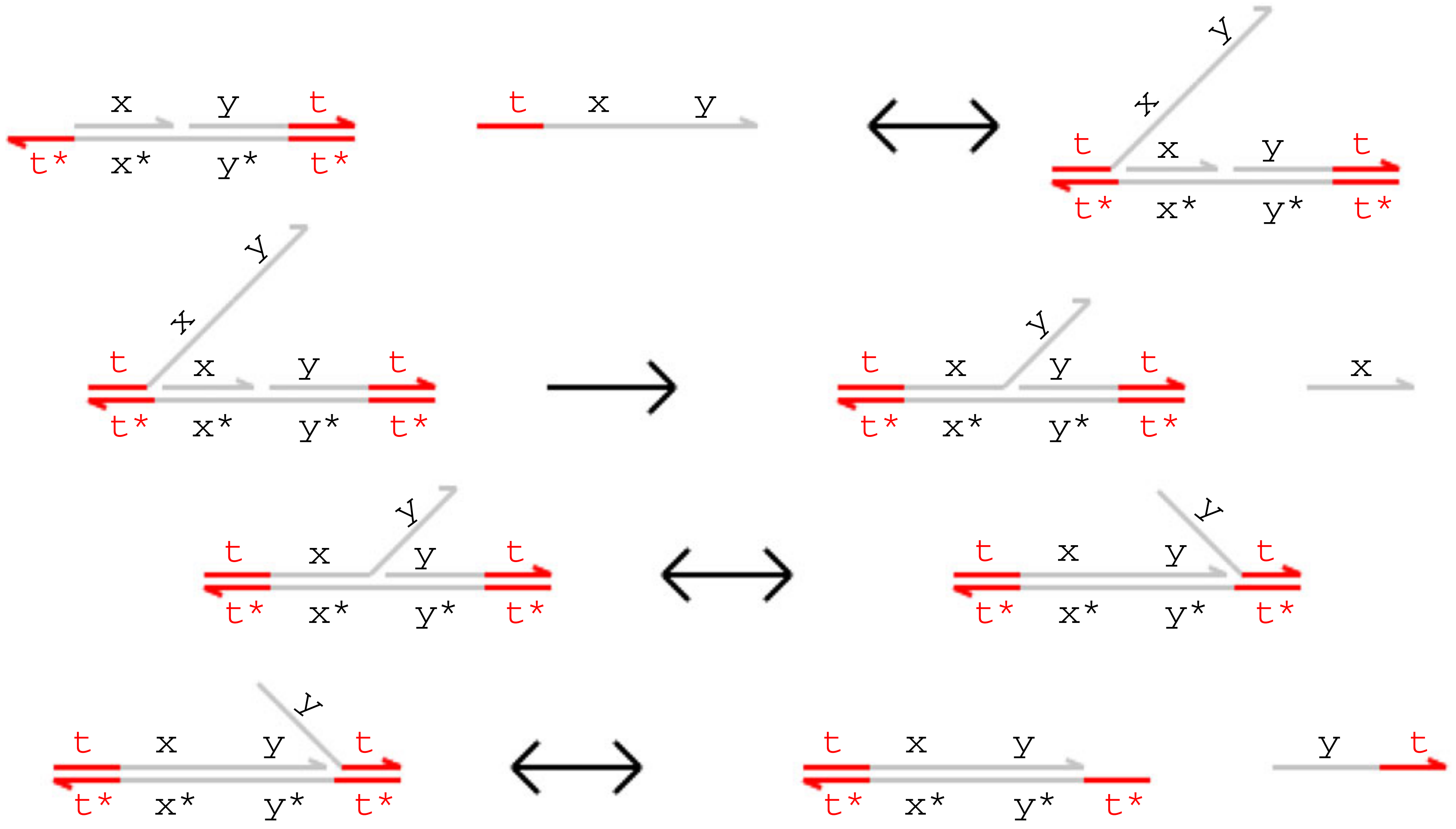
Matthew R. Lakin David Parker, Luca Cardelli, Marta Kwiatkowska and Andrew Phillips, Design and analysis of DNA strand displacement devices using probabilistic model checking, J. R. Soc. Interface. doi:10.1098/rsif.2011.0800 http://rsif.royalsocietypublishing.org/content/early/2012/01/03/rsif.2011.0800

Visual DSD (DNA Strand Displacement) software tool:

- allows rapid prototyping and analysis of computational devices implemented using DNA strand displacement
web- based graphical interface.

- implementation of DSD programming language described by :
Lakin,M.R. et al. (2011) Abstractions for DNA circuit design. J. R. Soc. Interface, doi:10.1098/rsif.2011.0343, July 20, 2011

DSD Provides:
- stochastic and deterministic simulation,
- construction of continuous-time Markov chains
- various export formats (allowing models to be analyzed using third-party tools)



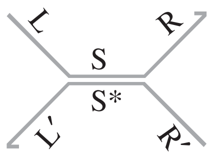
Example:

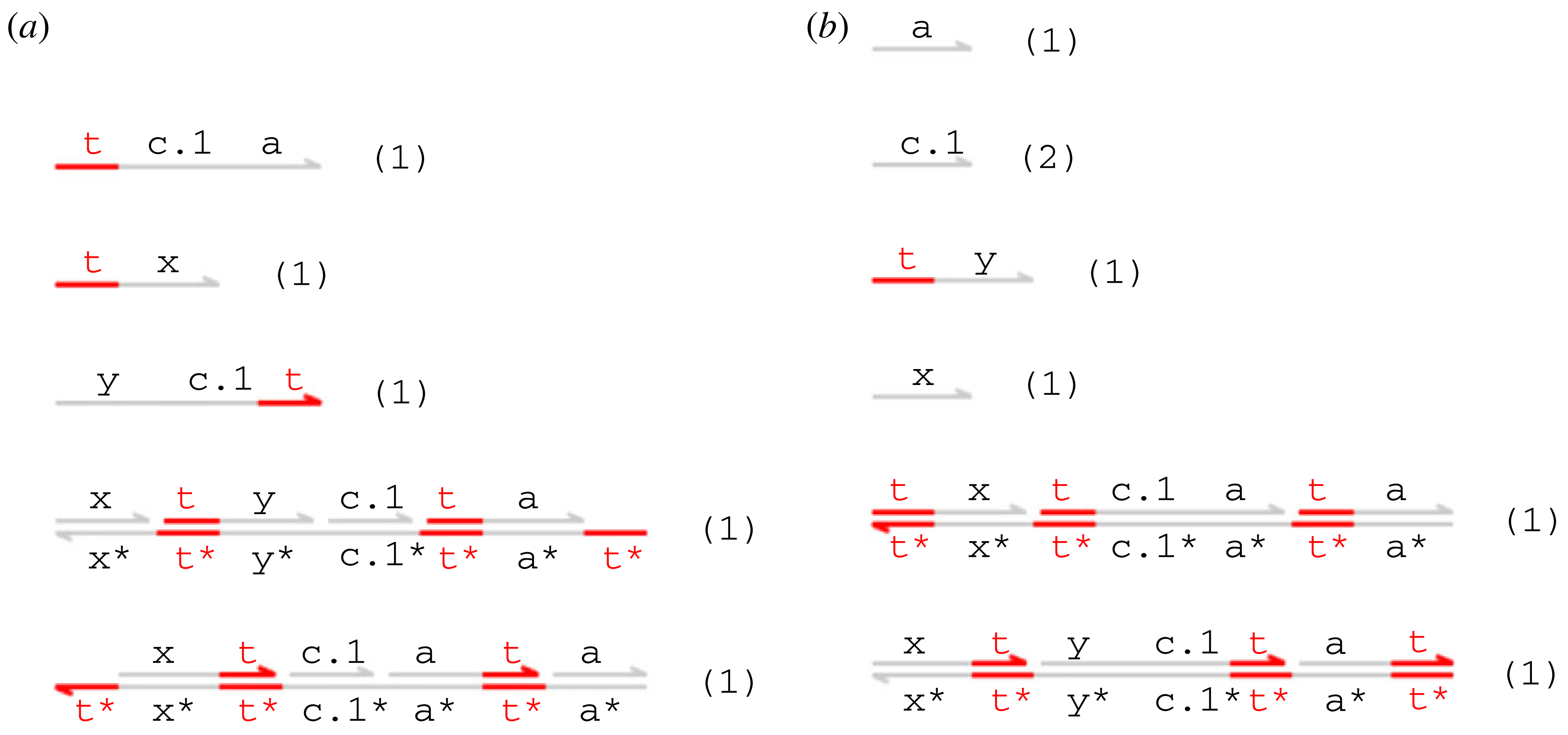
Toehold-mediated DNA branch migration and strand displacement.

Syntax of the DNA strand displacement (DSD) language:

- **Using strands A, gates G and systems D.**

- **Where present, the graphical representation below is equivalent to the program code.**

strand	syntax	description
A	`⟨S⟩`	upper strand with sequence `S`
	`{S}`	lower strand with sequence `S`
G	`{L'}⟨L⟩[S]⟨R⟩{R'}`	double stranded complex `[S]` with overhanging single strands `⟨L⟩`, `⟨R⟩` and `{L'}`, `{R'}`
		
	`G1:G2`	gates joined along a lower strand
	`G1::G2`	gates joined along an upper strand
D	`A`	strand `A`
	`G`	gate `G`
	`D1 | D2`	parallel systems `D1`, `D2`
	`new N D`	system `D` with private domain `N`
	`X(ñ)`	module `X` with parameters `(ñ)`



Example of initial transducer gate:
(a) Initial species and
(b) expected final species for the transducer gate.

```
(* Signal strand *)
def S(N, x) = N * <t^ x>

(* Transducer gate *)
def T(N, x, y) = new c
  ( N * {t^*}:[x t^]:[c]:[a t^]:[a]
  | N * [x]:[t^ y]:[c]:[t^ a]:{t^*}
  | N * <t^ c a>
  | N * <y c t^> )
```

Example of initial transducer gate code,

with additional definition for signal strands.

Model Checking:

Is an automated formal verification technique, based on the exhaustive construction and analysis of a finite-state model of the system being verified.

- The model is usually a labeled state-transition system, in which each state represents a possible configuration of the system and each transition between states represents a possible evolution from one configuration to another.

- The desired correctness properties of the system are typically expressed in temporal logics, such as computation tree logic (CTL) or linear-time temporal logic.

Example typical CTL formulae (along with their corresponding informal meanings):

— A [G !(“access1” & “access2”): ‘processes 1 and 2 never simultaneously access a shared resource’.

— A [F “end”]: ‘the algorithm always eventually terminates’.

— E [!“fail” U “end”]: ‘it is possible for the algorithm to terminate without any failures occurring’.

Action of Model Checker:

- **Once the desired correctness properties of the system have been formally expressed in this way, they can then be verified using a model checker.**
- **This performs an exhaustive analysis of the system model, for each property either concluding that it is satisfied or, if not, providing a counterexample illustrating why it is violated.**

Probabilistic model checking

This is a generalization of model checking for the verification of systems that exhibit stochastic behavior.

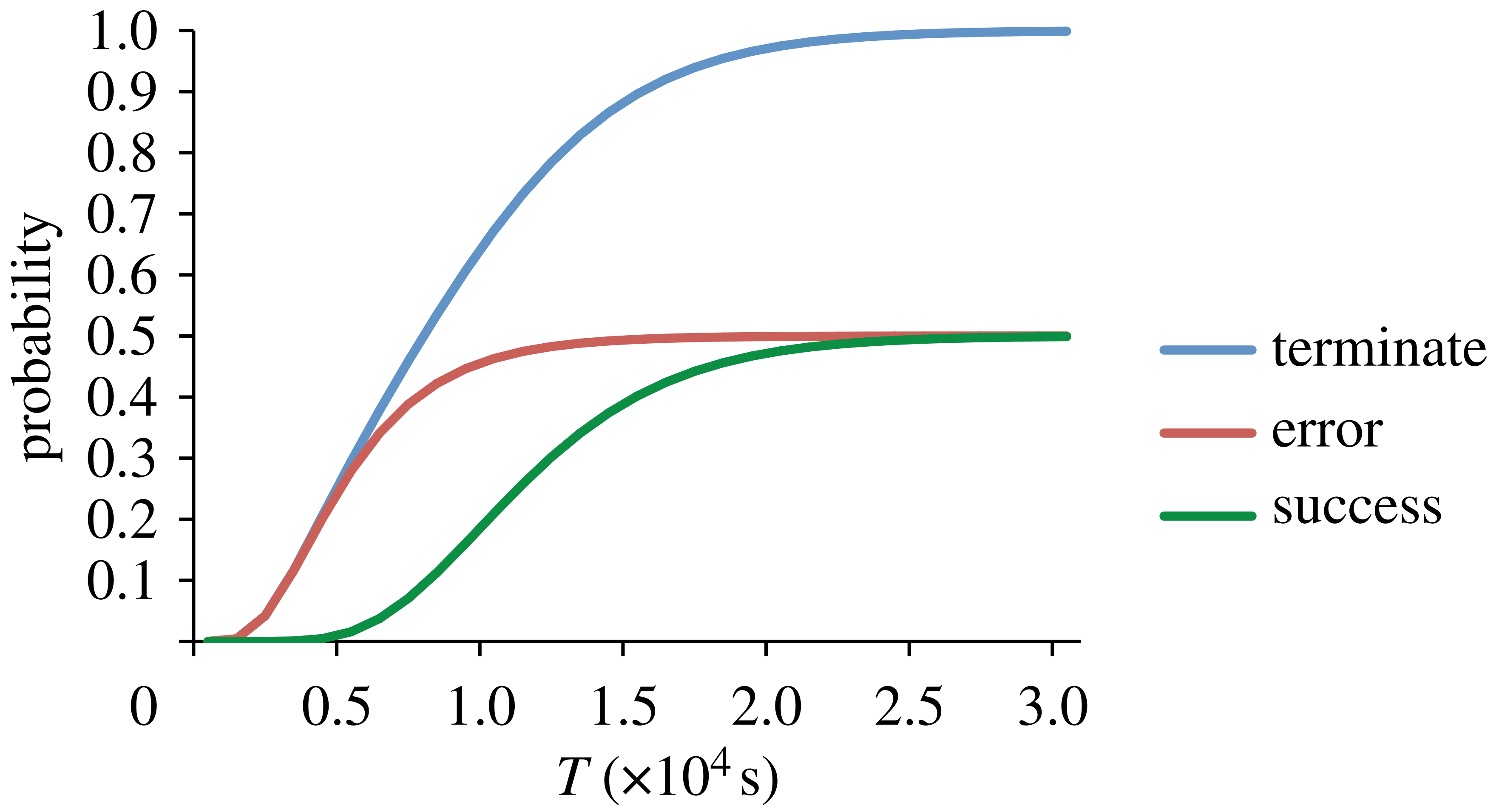
- **The models that are constructed and analyzed are augmented with quantitative information regarding the likelihood that transitions occur and the times at which they do so.**
- **- In practice, these models are typically Markov chains or Markov decision processes.**

Continuous-time Markov chains (CTMCs): **Used to model**

systems of reactions at a molecular level, the appropriate model is in which transitions between states are assigned (positive, real- valued) rates. These values are interpreted as the rates of negative exponential distributions.

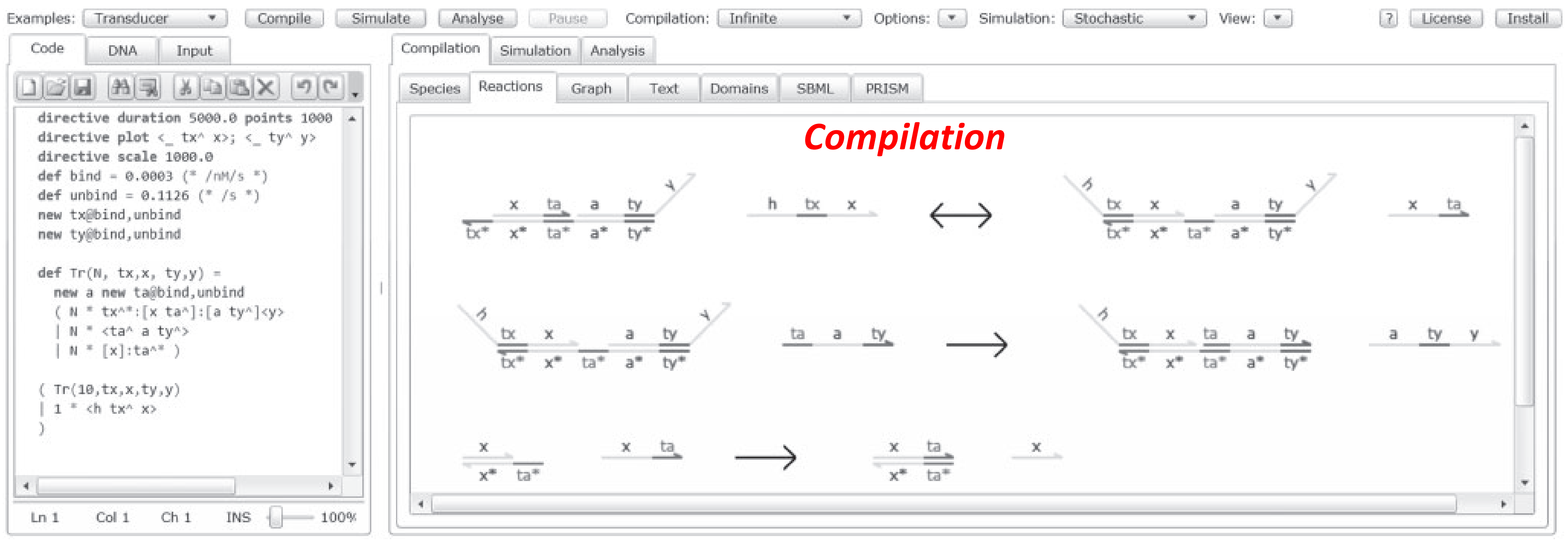
Properties of CTMCs (like in non-probabilistic model checking) are expressed in temporal logic, but are now expressed quantitative in nature.

- **For this, one uses probabilistic temporal logics such as continuous stochastic logic (CSL.**
- **Examples: rather than verifying that ‘the protein always eventually degrades’, using CSL allows us to ask**
  - **‘what is the probability that the protein eventually degrades?’ or**
  - **‘what is the probability that the protein degrades within t hours?’**



Plot showing the probability for each possible outcome of the faulty transducer pair, after T seconds.

Compilation tab: *Simulation* tab: *Analysis* tab:



Examples: Transducer Compilation: Infinite Simulation: Stochastic

```
directive duration 5000.0 points 1000
directive plot <_ tx^ x>; <_ ty^ y>
directive scale 1000.0
def bind = 0.0003 (* /nM/s *)
def unbind = 0.1126 (* /s *)
new tx@bind,unbind
new ty@bind,unbind

def Tr(N, tx,x, ty,y) =
  new a new ta@bind,unbind
  ( N * tx^*:[x ta^]:[a ty^]<y>
  | N * <ta^ a ty^>
  | N * [x]:ta^* )

( Tr(10,tx,x,ty,y)
| 1 * <h tx^ x>
)
```

Ln 1 Col 1 Ch 1 INS 100%

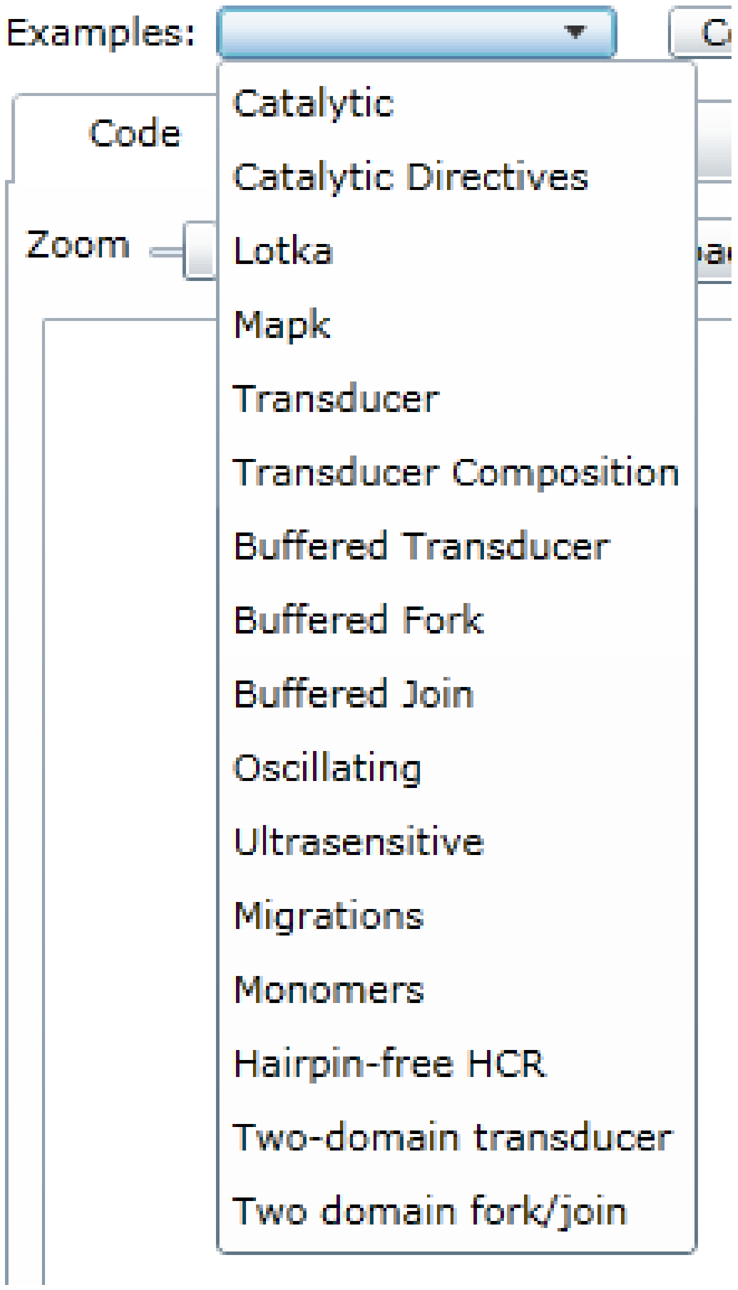
Screenshot of the Visual DSD tool:

- Code entry box on the left and
- Output tabs on the right.

Along the top of the screen are options to select example programs, adjust the semantics and control the simulator.

The example shown implements a simple transducer gate:

- The *Compilation* tab: on the right-hand side displays output from the compiler, in this case a visualization of all the individual reactions.
- The *Simulation* tab: shows time-course plots and data tables from stochastic and deterministic simulations.
- The *Analysis* tab: shows various representations of the continuous-time Markov chain.



The Visual DSD tool comes with a number of example systems implemented using DNA molecules. These are accessible from the drop-down menu labeled "Examples" in the top-left corner of the Silverlight user interface.

Built in Visual DSD Examples:

The Catalytic example: is an implementation of the entropy-driven catalytic gate from (Zhang, Turberfield, Yurke, & Winfree, 2007).

The Lotka example: is the Lotka-Volterra predator-prey oscillator.

The Mapk example: models a mitogen-activated protein kinase (MAPK) signaling cascade (Huang & Ferrel, 1996)

The Migrations example: serves to demonstrate the branch migration rate model (Zhang & Winfree, 2009).

Using the catalytic gate as an example:

- Selecting this populates the “Code” tab in the left-hand pane with the text of the example program

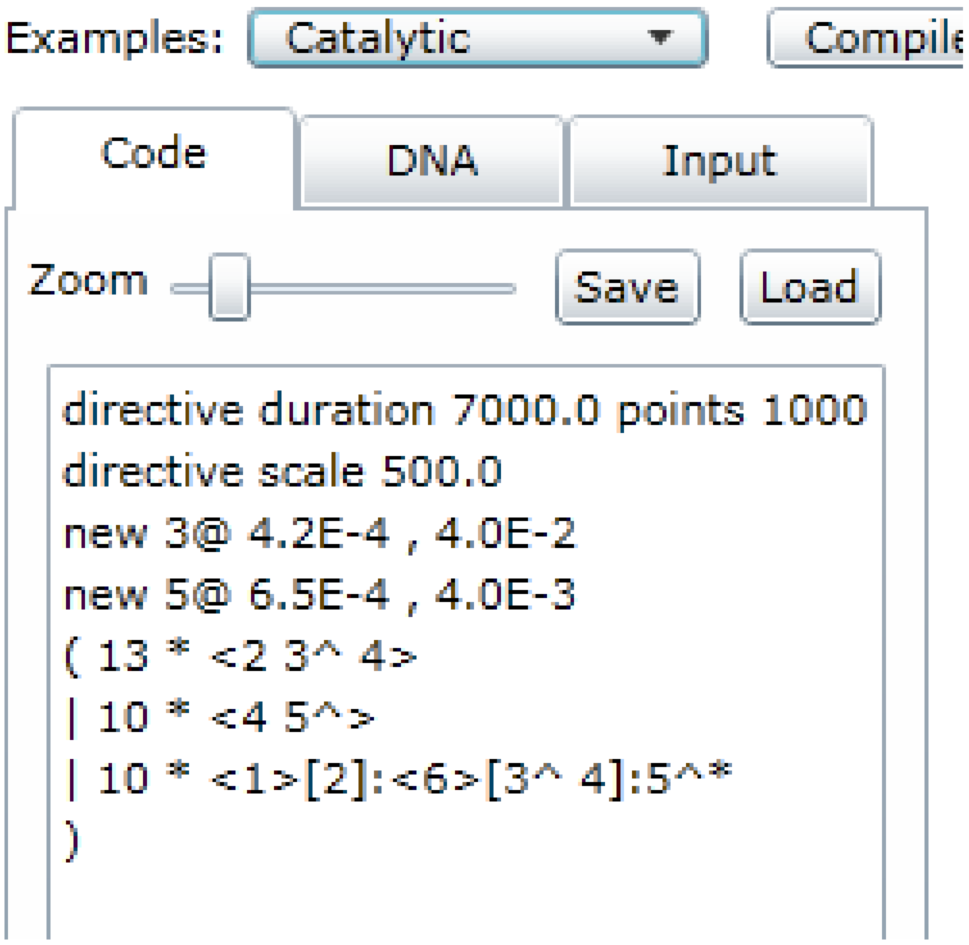
- The text of this program begins with a directive to the simulator telling it the duration of the simulation run and how many sample data points to use.

- The next line specifies a “scaling factor” which the system uses to automatically scale up from molar concentrations to populations of individuals, for the stochastic simulation.

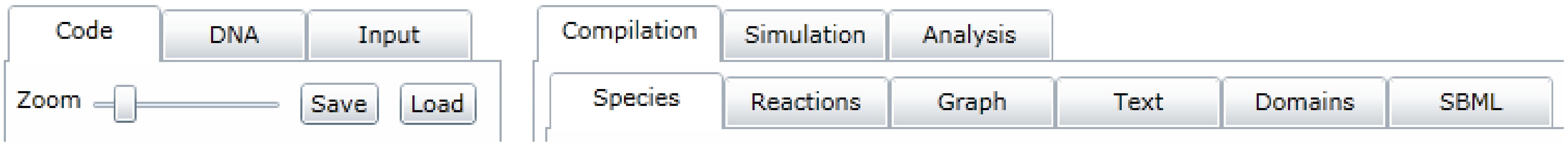
- The third and fourth lines declare two domains with specified binding and unbinding rates.

- The final element of the program is a collection of DNA molecules with their respective concentrations.

- Now that we have a program to run, clicking on the “Compile” button performs the compilation into chemical reactions.



```
directive duration 7000.0 points 1000
directive scale 500.0
new 3@ 4.2E-4 , 4.0E-2
new 5@ 6.5E-4 , 4.0E-3
( 13 * <2 3^ 4>
| 10 * <4 5^>
| 10 * <1>[2]:<6>[3^ 4]:5^*
)
```



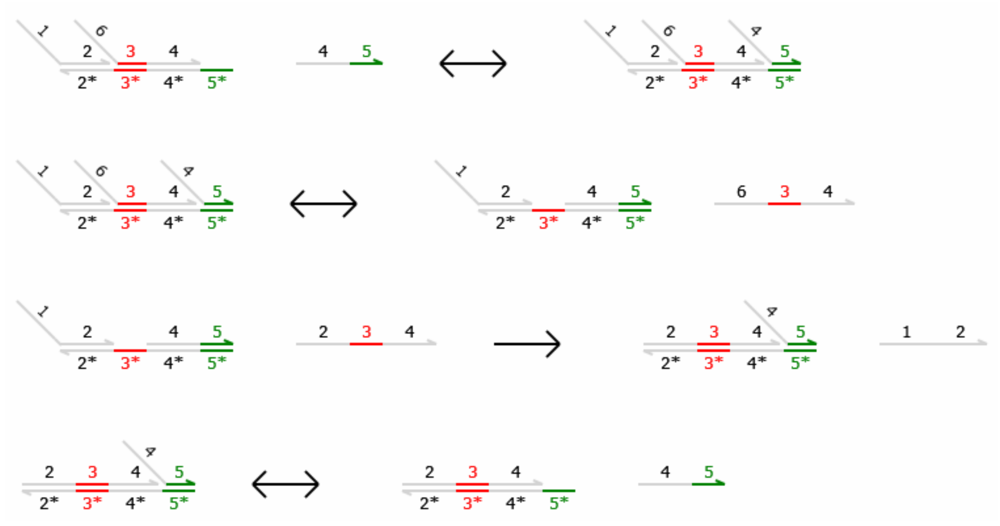
Using the catalytic gate as an example, Cont:

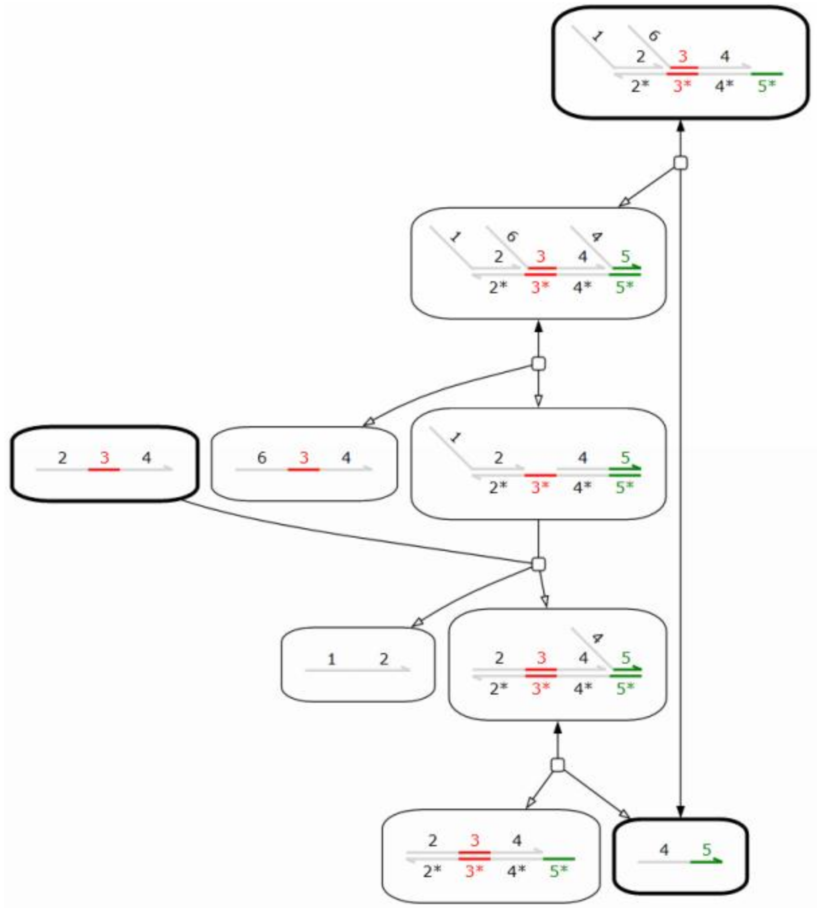
The "Input" tab: visualizes the initial DNA molecules in the system (exactly as they were entered in the code) using a common graphical notation.

Within the "Compilation" tab: the "Species" tab: uses the same graphical notation but provides a list of all of the species which could possibly be produced by reactions from the initial species presented in the input program.

The "Reactions" and "Graph" tabs: display the set of possible reactions between the various DNA species.

- **The "Reactions" tab:** lists the reactions.
- **The "Graph" tab:** visualizes them as a reaction network.



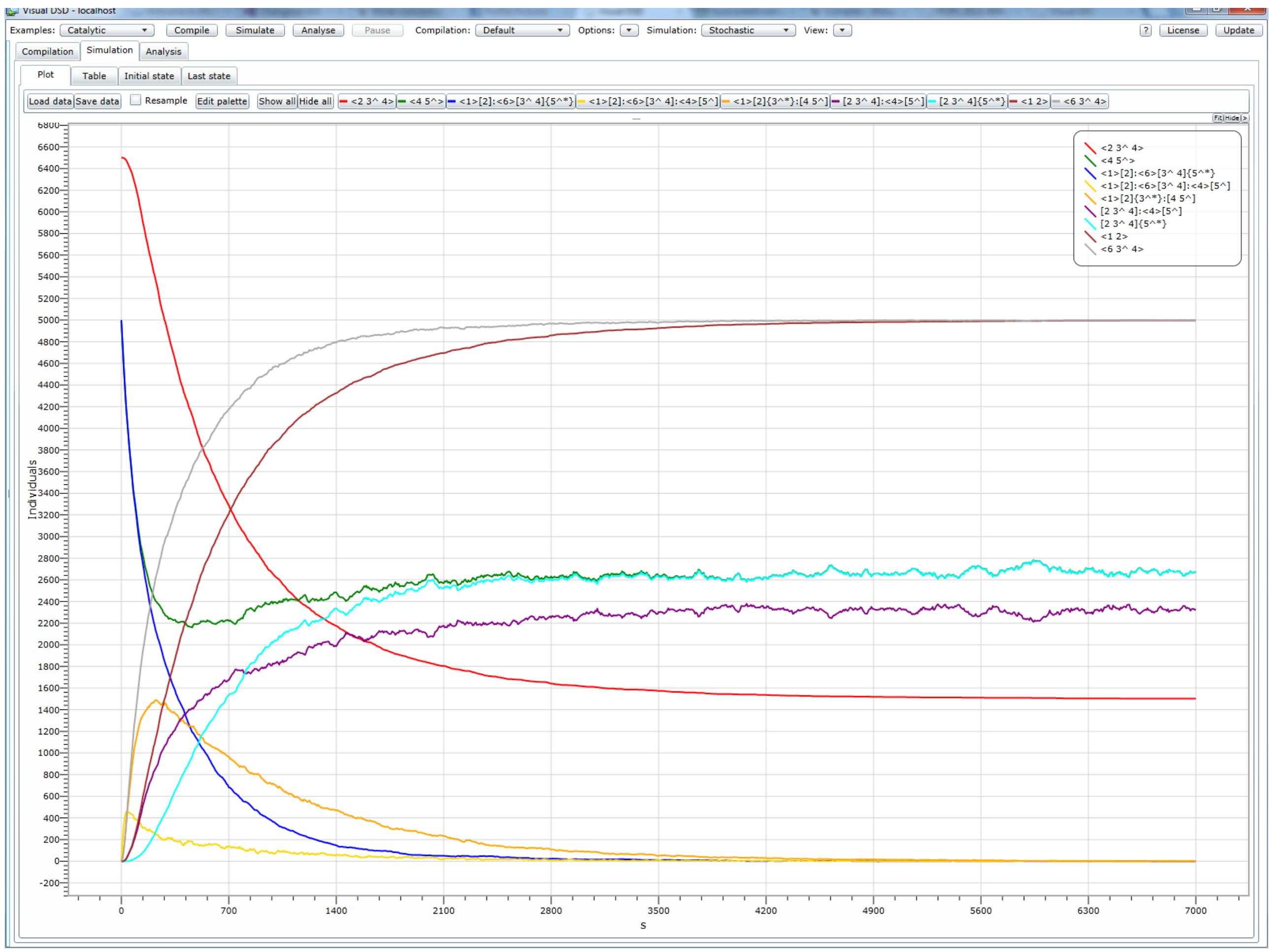


Outputs of the graphical tabs for the catalytic gate example:

Labeled Nodes: Each labelled node in the "Graph" tab denotes a species. (The initial species are represented with a bold outline.)

Unlabled Nodes: Each unlabeled node represents a reaction, which may or may not be reversible, with edges connected to reactant and product species.:

- **For irreversible reactions:** edges with no arrows denote reactants, while edges with hollow arrows denote products.
- **For reversible reactions**: hollow and solid arrows are used to distinguish between the products of the forward and reverse reactions, respectively.



The “Plot” tab produces a real-time graph of the concentrations (or populations) of certain species:

- The species to plot can be specified in the program but our example gives no such directives – in this case the default behaviour is to plot the populations of all species.
- The chart window can be dragged using the mouse and zoomed in and out using the scroll wheel.
- Along the top of the plot window is a collection of buttons which give more control over the plot.
- Clicking on the button for a particular species toggles the visibility of the relevant line in the plot.
- There are also buttons to show all plots and to hide all plots. This selection bar can itself be hidden or moved to dock at the right-hand side of the screen instead of at the top.

When the simulation terminates or is paused:

- The “Initial state” and “Last state” tabs are populated with a visualization of the initial and final states of the simulation run, respectively.
This includes a graphical visualization of each molecular species, along with their populations.