

Visual DSD

User manual v0.14 beta: Matthew R. Lakin, Rasmus Petersen & Andrew Phillips

Introduction

Visual DSD is an implementation of a programming language for composable DNA circuits based on that described in (Phillips & Cardelli, 2009). The language includes basic elements of sequence domains, toeholds and branch migration, and assumes that strands do not possess any secondary structure. The Visual DSD tool compiles a collection of DNA molecules into a set of chemical reactions. It also includes a stochastic simulator which computes a possible trajectory of the system and graphs the populations of species over time and a deterministic simulator which forms and solves an ODE representation of the dynamics of the system. Furthermore the reachable state space of the system can be constructed as a continuous-time Markov chain.

This manual assumes that the user is familiar with the basics of DNA strand displacement and, in particular, the terminology and notation from (Phillips & Cardelli, 2009). The reader is referred to that paper for the technical details of the language semantics.

Installing Visual DSD

Visual DSD is available in two forms: a Silverlight-based graphical application and a text-based command-line tool.

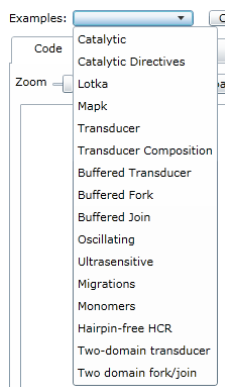
In order to run the Silverlight version you must install the correct Silverlight plug-in for your operating system and web browser from <http://www.microsoft.com/silverlight>. Silverlight compatibility has been tested on Windows and Mac OS X under various browsers, including Internet Explorer, Firefox, Safari and Chrome. With Silverlight installed, browse to <http://lepton.research.microsoft.com/webdna> and the user interface should load within a few seconds. In the top-right corner of the interface there is an “Install” button which you can click to install a local copy of the Silverlight program – this should work both on Windows and on Mac OS. We will describe the use of the Visual DSD Silverlight interface in the next section. Next to the “Install” button is a “License” button which brings up a copy of the Visual DSD license agreement. Once the software is installed, the “Install” button becomes an “Update” button which can be used to check for, and install, any newer releases of the software.

Note that when a new version of the software is released online, you may need to “reset” your web browser to delete the old version from the cache before your browser will load the new version.

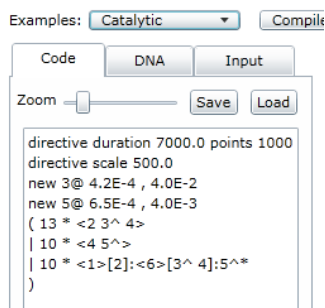
There is also a command-line version of Visual DSD which is included in the source distribution as pre-compiled binaries for Windows and Mac OS X 10.5. The command-line version can be compiled using the F# or Objective Caml compilers and contains build scripts to automate this process under Windows, Linux or Mac OS X. The command-line executable implements most of the functionality of the Silverlight version. We describe the use of the Visual DSD command-line interface at the end of this document.

Using Visual DSD under Silverlight

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.



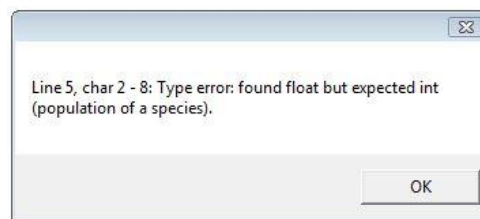
The Catalytic example is an implementation of the entropy-driven catalytic gate from (Zhang, Turberfield, Yurke, & Winfree, 2007). The Lotka example is the Lotka-Volterra predator-prey oscillator. The Mapk example models a mitogen-activated protein kinase (MAPK) signaling cascade (Huang & Ferrel, 1996) and the Migrations example serves to demonstrate the branch migration rate model (Zhang & Winfree, 2009). Most of the other examples are related to the implementation of chemical kinetics using DNA. We will use the catalytic gate as a running example. Selecting this populates the “Code” tab in the left-hand pane with the text of the example program – we can use the “Zoom” slider to adjust the text size. We will return to the DNA tab below.



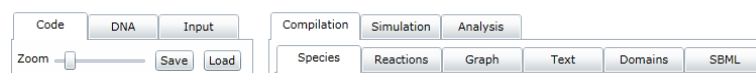
The text of this program begins with a directive to the simulator telling it the duration of the simulation run and how many sample data points to use. The next line specifies a “scaling factor” which the system uses to automatically scale up from molar concentrations to populations of individuals, for the stochastic simulation. (See the discussion of directives below for more details on this and other constants that can be specified within DSD programs.) 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. The syntax of the Visual DSD language is discussed in more detail below.

Now that we have a program to run, clicking on the “Compile” button performs the compilation into chemical reactions. The program is checked for errors in the syntax and also for “type errors”, such as when an integer is used where a floating point number would be expected. If an error is found it

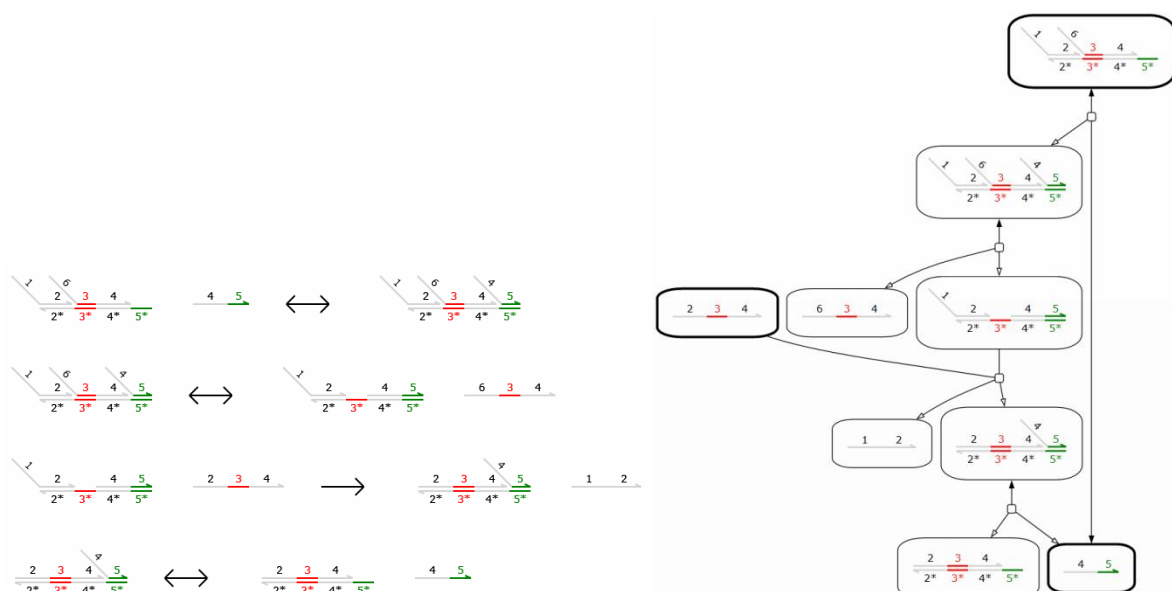
is reported in a message box together with the location of the offending section of the program, like the following example error message.



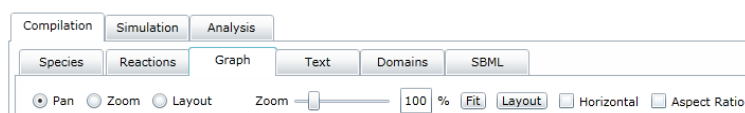
If the program is error-free then the compilation process proceeds as described in (Phillips & Cardelli, 2009). This produces output in the “Initial” tab and in the six sub-tabs of the “Compilation” tab on the right-hand side. This output persists until a modified program is compiled using the “Compile” button.



The “Input” tab visualises the initial DNA molecules in the system (exactly as they were entered in the code) using a common graphical notation from the DNA computing literature – see (Zhang, Turberfield, Yurke, & Winfree, 2007), for example. Note that the default is a different graphical notation from that used in (Phillips & Cardelli, 2009), although that notation can be selected as an alternative (see below). Looking 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 are particularly useful as they display the set of possible reactions between the various DNA species. The “Reactions” tab lists the reactions whereas the “Graph” tab visualizes them as a reaction network. The outputs of the graphical tabs for the catalytic gate example are as follows.



Each labelled node in the “Graph” tab denotes a species. The initial species are represented with a bold outline. Each unlabelled 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.



In the “Graph” tab you can adjust the zoom level of the network display using the slider or the text box, or use the “Fit” button to automatically select the zoom level which just fits in the entire graph. The graph viewer has three available modes: “Pan” (where the mouse can be used to drag the graph around), “Zoom” (where you can drag out a rectangle to zoom in on) and “Layout” (where nodes can be dragged around to modify the shape of the graph). The “Layout” button on the right recomputes the original layout of the graph if it has been edited. The “Horizontal” checkbox toggles whether the graph should be arranged with reactions going from top to bottom or from left to right, and the “Aspect Ratio” checkbox toggles whether the graph layout algorithm should attempt to match the aspect ratio of the window – in some cases this can improve the readability of the graph.

The “Text” tab gives an ASCII representation of the species and reactions in the system. The “SBML” tab contains a description of the species and the reactions in the XML-based Systems Biology Markup Language – for more information on SBML see the website at <http://sbml.org>. This output can be saved directly into an XML file for use with external tools which accept SBML input.

Visual DSD provides limited support for producing nucleotide sequences for *in vivo* implementation of the system described by the user. The “Domains” tab displays a mapping from domains to nucleotide sequences – see the “Implementing domains as nucleotide sequences” section below for details.

In addition to compiling DNA molecules into sets of chemical reactions, the Visual DSD tool includes a simulator which can produce stochastic or deterministic plots of a trajectory of the system of reactions. Once the program has been compiled (using the “Compile” button) it can be simulated using the “Simulate” button. This greys out the “Simulate” button and the simulation will run until it reaches its end point (or until no further reactions are possible) – in our example this is time $t = 7000$ (the first number in the line of code which begins “directive duration”). An ongoing simulation can be paused using the “Pause” button – the simulation can then be restarted from the beginning (using the ungreyed “Simulate” button) or unpaused (using the same button as for pausing, which will have been relabeled “Resume”). Running the simulator produces output in the four sub-tabs of the “Simulation” tab on the right-hand side.

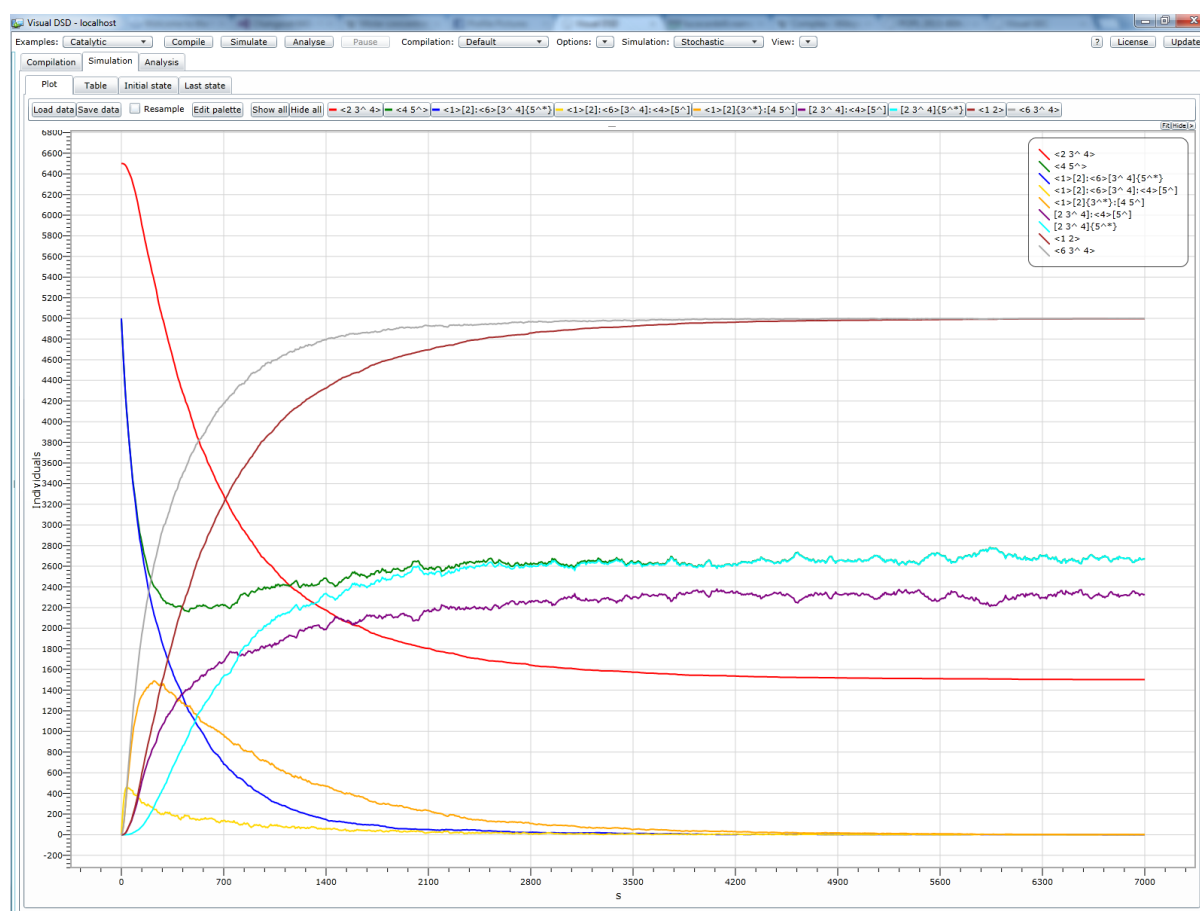


The output of the simulator is given as species populations at discrete points in simulation time. The number of sample time points is specified in the program (the second number in the line beginning

“directive duration”). By default (as in the catalytic gate example) the simulator samples the populations of all of the species in the system after every single reaction. It is possible programmatically to restrict this to a subset of the species, as discussed in the next section.

The “Table” tab contains a tabular representation of the simulation data as species populations over time. The data in this tab can be saved as a comma-separated (CSV) or tab-separated (TSV) file which can then be imported into a spreadsheet such as Microsoft Excel. Alternatively, the data can be copied and pasted directly into Excel as it stands.

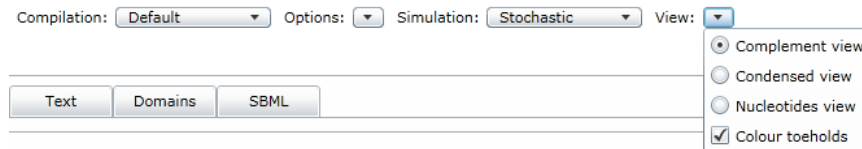
The “Plot” tab produces a real-time graph of the concentrations (or populations) of certain species. The species to plot can be specified in the program but our example gives no such directives – in this case the default behaviour is to plot the populations of *all* species. The chart window can be dragged using the mouse and zoomed in and out using the scroll wheel.



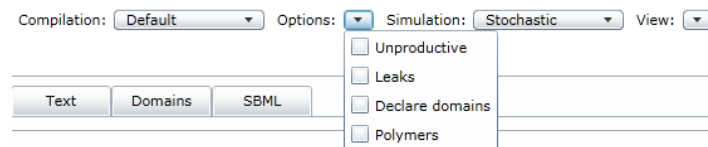
Along the top of the plot window is a collection of buttons which give more control over the plot. Clicking on the button for a particular species toggles the visibility of the relevant line in the plot. There are also buttons to show all plots and to hide all plots. This selection bar can itself be 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.

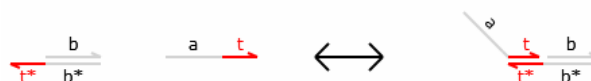
The remaining elements of the user interface allow more fine-grained control of the behaviour of the Visual DSD system. The “Compilation” menu allows the user to select between four different reaction models which range from simplistic and efficient to detailed and computationally expensive – see the “Alternative transition rules for Visual DSD” section below for a brief explanation. The “Simulation” menu allows the user to select which simulation algorithm to use for producing plots – see the “Simulation algorithms in Visual DSD” section below for more details.



The “View” menu contains a set of radio buttons which toggles between the default (“Complement view”) visualization mode which is used throughout this document, the “Condensed view” used in (Phillips & Cardelli, 2009) and a “Nucleotides view” which renders domains as actual nucleotide sequences (discussed in the “Implementing domains as nucleotide sequences” section below). Only one of these modes can be selected at a time. The “Colour toeholds” checkbox toggles whether toehold labels should be written in the same colour as their lines in the visualization (this is enabled by default).



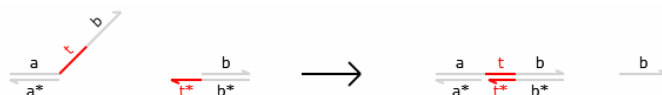
The “Options” menu allows further configuration of the semantics and behaviour of the Visual DSD language itself. The “Unproductive” checkbox toggles the inclusion of reactions of repeated toehold binding and unbinding. These reversible reactions go back and forth at a high rate and take up a lot of simulator time without affecting the overall result of the simulation greatly. An example of such a reaction is the following (*not* taken from the catalytic gate example):



This reaction is deemed unproductive because no other reactions can happen involving the molecule on the right-hand side (since none of the domains next to the toehold match up). Removing these spurious reactions slightly reduces the accuracy of the stochastic simulation but can make the simulation algorithm run considerably faster. Note that the “Unproductive” checkbox is greyed out when “Infinite” is selected from the “Compilation” menu because it has no effect under that semantic model.

The “Leaks” checkbox toggles the inclusion of leak reactions, which are a form of unwanted interference between strands and gates – see the “Leak model” section below for a brief explanation. The “Declare domains” checkbox allows the user to enable stricter syntax checking which produces an error if a domain is used without having first been declared using the “new” keyword. This can be helpful when debugging programs, as a mistyped domain can be hard to detect and can significantly alter the behaviour of the system.

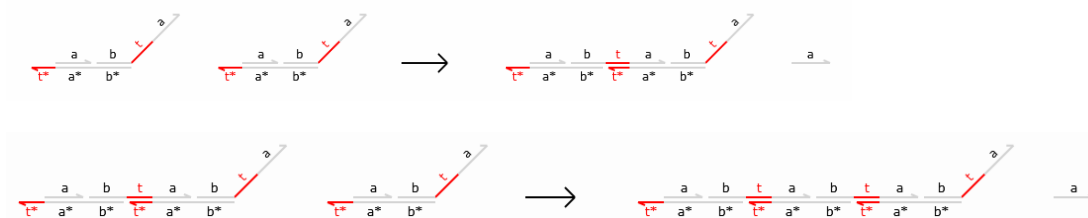
Finally, the “Polymers” checkbox enables additional reduction rules which allow DNA gates to bind together to form polymers of potentially unbounded length. This is a new feature that is not present in the language described in (Phillips & Cardelli, 2009). The following reaction is an example of DNA polymerization in which two gates bind together to form a stable complex.



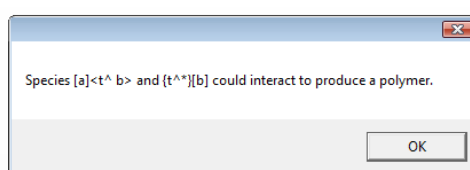
However, it is also possible to write DSD programs involving polymerization where the full reaction graph is infinite. For example, the following program

```
directive sample 10.0 100
( 10 * {t^*}[a]:[b]<t^ a> )
```

produces various reactions, including the following. Clearly this DNA polymer could grow indefinitely if we kept supplying it with monomers to join onto the chain.



Attempting to compile such a program to chemical reactions using the standard DSD compiler will cause the compiler to loop indefinitely as it attempts to discover the entire reaction graph, which is infinite. It is recommended to use the JIT simulator (described in the “Simulation algorithms in Visual DSD” section below) when dealing with programs that involve polymer reactions. To alert users to this problem, the system produces an error message if a polymerization reaction is detected when the “Polymers” checkbox is unchecked, as follows.



This error message also appears if an unproductive polymerization reaction is detected when the “Unproductive” checkbox is checked but the “Polymers” checkbox is not.

The “States” tab on the right-hand side (and its four sub-tabs, the “Graph”, “Text”, “PRISM” and “Visualise” tabs) are populated with output when the “Analyse” button is clicked. This computes the continuous time Markov chain of the system and displays it in various ways for analysis and output: see the “State space analysis” section below for more details.

Programming language syntax

Having learned how to use the Visual DSD tool on an example program, the next step is to write our own programs in the language. The system accepts user programs in the editor pane on the left-hand side (the font size can be adjusted using the “Zoom” slider).

The ASCII syntax of Visual DSD is an extension of that introduced in (Phillips & Cardelli, 2009). We will describe this in detail over the rest of this section – the collected grammar is also presented as an Appendix for reference purposes. In this section the terminal symbols of the language are written in `teletype` font and non-terminals are in *italics*.

Lexical conventions

The language uses the following lexical conventions. We write “digit” for a single character in the range 0-9, and “alphanumeric” for any character in the range A-Z or a-z.

- *Integer*: a non-empty sequence of digits.
- *Name*: the first character of a name must either be alphanumeric or the underscore character (`_`). This is followed by a possibly-empty sequence of characters which may be alphanumeric, digits, underscores or apostrophes (`'`).
- *String*: a possibly-empty sequence of characters enclosed by quotation marks (`"`). Any quotation marks appearing within the outer of quotation marks must be escaped by a preceding backslash (`\`).
- *Float*: there are three different ways to produce a float value:
 1. One or more digits followed by a decimal point (`.`), followed by zero or more digits. For example: `"3.141"`.
 2. One or more digits followed by an uppercase `'E'` or lowercase `'e'`, followed by a plus (`+`) or minus (`-`) sign, followed by one or more digits. For example: `"3e-5"`.
 3. One or more digits followed by a decimal point, followed by zero or more digits, followed by an uppercase `'E'` or lowercase `'e'`, followed by a plus or minus sign, followed by one or more digits. For example: `"1.4324e+2"`.
- *Char*: a single character enclosed by apostrophes. The character itself can be anything except for an apostrophe or a backslash.

Comments

Comments are opened with `(*` and closed with `*)`. They may be nested.

Reserved keywords

The following identifiers are reserved for use as keywords of the programming language.

```
directive  sample  plot  leak  tau  migrate  lengths
def  new  true  false  int_of_float  float_of_int  time  concentration
constant  tolerance  sum  scale  duration  points  toeholds
```

Programs

Programs written in the language may consist of three parts, in the following order:

1. **Directives** to the simulator and plotter.

2. **Declarations** of values, global domains and modules.
3. A **process** to run, which contains the species and their initial populations.

A program *must* contain a process and *may* contain directives and/or declarations.

```

Program ::= Directives Declarations Process
          | Directives Process
          | Declarations Process
          | Process

```

Directives and *Declarations* stand for possibly-empty sequences of the *Directive* and *Declaration* non-terminals, which are described below.

Directives

Directives are instructions to the Visual DSD simulator and data plotter.

```

Directive ::= directive duration Float
            | directive duration Float points Integer
            | directive sample Float
            | directive sample Float Integer
            | directive scale Float
            | directive concentration String
            | directive time String
            | directive plot Plots
            | directive leak Float
            | directive tau Float
            | directive migrate Float
            | directive lengths Integer Integer
            | directive tolerance Float
            | directive toeholds Float Float

```

“Duration” directives tell the simulator how long to run for (as a floating-point number). Optionally they may include a “Points” value with an integer value which specifies how many samples of the species populations to take during that time (if none is provided, the default is to sample species populations after every reaction, which can produce a large number of data points). The “Sample” directives are an alternative syntax which has the same behaviour and is included for backwards compatibility. Increasing the number of data points in the same period of simulation time produces more fine-grained results but the display may be less responsive. Similarly, if the number of data points stays constant but the simulation time is extended (or shortened), the resulting plot will be less (or more) detailed. If no such directives are provided, the default behaviour is to run the simulation for 1000 time units and take 10,000 samples of the species populations.

“Scale” directives allow the stochastic simulator to scale up from molar concentrations to populations of individuals. Concentrations are scaled by simply multiplying by the factor and the rates of binary reactions are modified following Section 4.2 of (Cardelli, 2008). Thus the user does not have to worry about the details involved in switching between continuous and discrete simulation methods (see below). The scale factor is 1.0 by default. The scale factor also modifies the tolerance parameter of the deterministic simulator, as described below. See the “Populations and concentrations” section below for further details.

“Concentration” directives allow one to specify the assumed unit of concentrations. It will be printed on the y-axis of simulation plots when the simulator is run in deterministic mode. It will also feature in the summary information in the “Text” tab. The default concentration units are nanomolar (nM).

“Time” directives allow one to specify the assumed unit of time. It will be printed on the x-axis of simulation plots. The default time units are seconds (s).

“Plot” directives tell the system which populations to sample at each time point. If no “Plot” directive is supplied, the system will sample the populations of all species at each time point.

```
Plots ::= String
        | Gate
        | Strand
        | sum ( Plots )
        | sub ( Plot ; Plot )
        | diff ( Plot ; Plot )
        | div ( Plot ; Plot )
        | Plots ; Plots
```

Plots is a semicolon-separated list of strand and gate species to plot. If any of the species contain the underscore character (`_`) this is interpreted as a wildcard which can match against a single domain, causing multiple species to be plotted. For example, the pattern `<_ s t>` will match against `<u s t>` and `<v s t>` but *not* against `<u v s t>`. “Plot” directives may also include quoted strings – in this case the decision of whether to plot is based on an exact string matching on the names of species. This can be useful when dealing with locally restricted domains, as these are automatically renamed by the system. It is also possible to plot simple arithmetic functions on species populations, such as the sum of the populations of multiple species, using the “sum” keyword followed by the species enclosed in brackets. For a given pair of plot species *P1* and *P2* one can also plot the population of *P1* minus the population of *P2* (using “sub”), the difference (using “diff”) or the ratio of *P1* to *P2* (using “div”).

The other directives allow the user to set the values of certain constants used by the system.

- **“Leak” directives** set the rate of a leak reaction (default is $10^{-9} \text{ nM}^{-1}\text{s}^{-1}$).
- **“Tau” directives** set the rate of a tau reaction in the Finite semantics (default is 0.1126 s^{-1}).
- **“Migrate” directives** set the rate of branch migration across a single nucleotide (default is 8000 s^{-1}). The branch migration rate for a domain of length *L* is given by r/L^2 , where *r* is the single nucleotide migration rate set by the directive.
- **“Lengths” directives** set default values for the lengths of toeholds and long domains. For the purpose of computing rate constants, all long domains are assumed to have the same length, which is set using the “lengths” directive. The code `“directive lengths 5 15”` assigns a length of 5nt to toeholds and 15nt to long domains (the default values are 6nt for toeholds and 20nt for long domains). The value provided for toeholds must be greater than that provided for specificity domains or the system will raise an error. Currently the assigned default length for toehold domains is not used to calculate rates. Instead the user may set toehold binding and unbinding rates directly on a per-toehold basis. Note that specific nucleotide sequences are not currently used when computing rate constants.

- **“Tolerance” directives** specify the tolerance parameter of the deterministic simulator, which provides a tradeoff between computational cost and smoothness of the resulting solution. The default value is 10^{-6} . It is crucial to choose a tolerance value which reflects the populations and reaction rates of the system in question, or the performance of the deterministic simulator may suffer. Note that the tolerance is multiplied by the scale factor in an attempt to maintain a reasonable value with respect to the species populations.
- **“Toeholds directives”** specify the default binding and unbinding rates (in that order) for domains which are declared without explicit rates. The default values are $3.0 \cdot 10^{-4} \text{ nM}^{-1} \text{ s}^{-1}$ for the binding rate and 0.1126 s^{-1} for the unbinding rate.

Declarations

These introduce new module definitions, globally-defined domains and value assignments.

```
Declaration ::=  def Name () = Process
                |  def Name ( Parameters ) = Process
                |  new Name @ Value , Value
                |  new Name
                |  def Name = Value
```

The first two lines are for module declarations, which are written with the “def” keyword. A module is simply a parameterised process. Here, *Parameters* stands for a non-empty, comma-separated list of *Names* which are the parameters of that particular module. The grammar also permits a module to have an empty parameter list. We will describe processes below. The name and parameters of a module are bound within the body of that module and the name of the module is bound in the remainder of the program.

A “new” declaration declares a new domain. This is global in the sense that the name of the domain is bound in the rest of the program. Optionally, the domain may be annotated with two floating-point values which explicitly state the binding and unbinding rates of the domain, respectively. If these are omitted, the default rates set by the “toeholds” directive are used (if no such directive is present in the program, the defaults are $3.0 \cdot 10^{-4} \text{ nM}^{-1} \text{ s}^{-1}$ for binding and 0.1126 s^{-1} for unbinding). It is worth pointing out that not all of the domains used in a program need to be declared globally in this way. If the system detects an undeclared domain in the program then it is assumed to have these default binding and unbinding rates. This allows programs to remain short while allowing the flexibility to modify the interaction rates of certain domains as needed. No units are supplied for the rate values – it is up to the programmer to ensure that all rates are given in the same (implicit) units. The unbinding rates are used in the Default semantic model (see below).

Value definitions (also written using “def”) assign a value to a name. Any subsequent uses of the name will refer to this value, unless there is an intervening binding occurrence of the same name (i.e. an instance of “def” or “new”). The system will raise an error if a name is used without having first been bound to a value (unless the name is used as a domain, as mentioned above).

Processes

The core of Visual DSD is a process calculus tailored to modeling DNA interactions. The grammar of processes is as follows.

```

Process ::= Value * Process
          | constant Process
          | constant Value * Process
          | Value * constant Process
          | Species
          | new Name @ Value , Value Process
          | new Name Process
          | ( Processes )

```

```

Processes ::= Process
              | Process | Processes

```

The first four lines of the grammar allow the populations of certain processes to be specified (in each case, the value should evaluate to an integer). The `constant` keyword specifies that the population of a particular species should never change, even if it participates in reactions which should consume that species. We will discuss the grammar of species themselves in detail below.

The “new” process declares a domain which has local scope, i.e. which can only be used within the body of that process. As with globally-declared domains, these can have optional binding and unbinding rates attached (otherwise, default rates are used as described above). This facility does not add expressive power to the language but makes it easier to organize larger programs by re-using the names of domains without mutual interference.

Finally, we can run multiple different kinds of processes in parallel using the vertical bar notation (`|`). This is essential because molecules must be in parallel in order to react with each other. If two sets of identical processes are placed in parallel, the system will notice and add up their populations to produce a single population value for each species.

Species

The Visual DSD language allows various kinds of DNA molecules to be expressed. We present a brief overview here – see (Phillips & Cardelli, 2009) for a more technical discussion of certain aspects, but note that Visual DSD expands on the syntax from that paper.

We start with the most basic elements and work our way up. The language does not work at the level of individual nucleotides – instead, the fundamental building blocks of species in Visual DSD are DNA sequences. A sequence represents some finite section of DNA, comprising many nucleotides (see below for a discussion on mapping domains to nucleotide sequences). We assume that distinct sequences are sufficiently different that they do not interact, and that they do not possess any secondary structure. The grammar for sequences in Visual DSD is as follows.

```

Sequence ::= Integer
              | Name
              | _

```

A *Sequence* is represented by a name, although this may actually be a number for convenience. Hence a species such as `<1 2 3>` is valid, where 1, 2 and 3 denote different DNA sequences. The underscore is permitted so that wildcards may be included in plotting directives (see above).

A *Domain* can either be a sequence or its Watson-Crick complement (obtained by reversing the directionality of the nucleotide sequence then swapping C ↔ G and T ↔ A throughout). We denote the complement of a sequence using an asterisk (*), so the grammar for domains is as follows.

$$\begin{array}{l} \text{Domain} ::= \text{Sequence} \\ \quad | \quad \text{Sequence}^* \end{array}$$

Toeholds are DNA domains which are sufficiently short that they can bind to (and unbind from) their complementary strands quickly and easily. The basic syntax of a toehold domain is simply a domain suffixed with a caret (^), but there is also the possibility of explicitly complementing the toehold domain, as described above.

$$\begin{array}{l} \text{Toehold} ::= \text{Sequence}^{\wedge} \\ \quad | \quad \text{Sequence}^{\wedge *} \end{array}$$

The explicit complementation operator is a new addition to the Visual DSD language. It was not included in the syntax of (Phillips & Cardelli, 2009) because there it was assumed that the complemented sequences were all on the lower strand of the DNA molecule. Our language provides for greater flexibility, hence explicit complementation is required in the syntax. To convert programs written in older versions of Visual DSD (v0.12 or before) to the new syntax, simply replace every exposed toehold t^{\wedge} by $t^{\wedge *}$.

We write *Domains* to stand for a non-empty sequence of *Domain* or *Toehold* domain elements, separated by a space.

$$\begin{array}{l} \text{Domains} ::= \text{Domain} \\ \quad | \quad \text{Toehold} \\ \quad | \quad \text{Domain Domains} \\ \quad | \quad \text{Toehold Domains} \end{array}$$

Domains are used to construct strands and more complicated DNA molecules. An *UpperStrand* represents a single “upper” strand of DNA sequences and a *LowerStrand* represents a single “lower” strand of DNA sequences. A *Double* represents an “upper” and a “lower” strand which are bound due to Watson-Crick complementarity, as described above. Since these are all just lists of *Domains* they must be distinguished by their ASCII syntax.

$$\text{Double} ::= [\text{Domains}]$$

$$\text{UpperStrand} ::= < \text{Domains} >$$

$$\text{LowerStrand} ::= \{ \text{Domains} \}$$

In a double strand, we assume that the domains listed between the square brackets are the domains on the upper strand, so a double strand written as $[D1 \ D2^* \ D3]$ would be visualized as follows, where the small arrows at the top left and bottom right denote the 3' ends of the DNA strands.

D1	D2*	D3
D1*	D2	D3*

DNA gates are a key aspect of the strand displacement computational mechanism. A *Gate* is simply a concatenation of one or more *Segments* of DNA.

```

Gate ::= Segment
      | Segment : Gate
      | Segment :: Gate

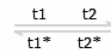
Overhangs ::= LowerStrand
             | UpperStrand
             | LowerStrand UpperStrand
             | UpperStrand LowerStrand

Segment ::= Toehold
           | LowerStrand
           | UpperStrand
           | Double
           | Double Overhangs
           | Overhangs Double
           | Overhangs Double Overhangs

```

A *Segment* can take various forms:

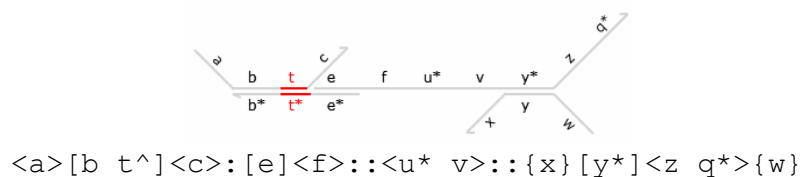
1. a single toehold domain on the lower strand t^* .
2. an upper strand with a non-empty list s of domains $\langle s \rangle$.
3. a lower strand with a non-empty list s of domains $\{s\}$.
4. a double strand $[t1 \ t2]$.



5. a double strand with overhanging upper and/or lower strands on the left and/or right $\{lb\}\langle lt\rangle[s]\{rb\}\langle rt\rangle$ (note that the order of the overhangs does not matter).

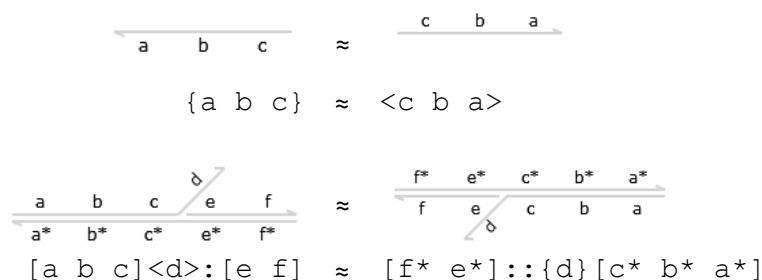


By concatenating multiple segments we can produce DNA gates with complex overhanging structures. The single colon (:) represents concatenation along the lower strand, while the double colon (::) denotes concatenation along the upper strand. This is a departure from the DSD syntax introduced in (Phillips & Cardelli, 2009) and allows us to represent DNA molecules which are not formed by interactions along a common lower strand and permits reactions where a strand binds onto the upper strand of a gate. This generalization seems necessary to allow DNA polymerization reactions. The following example uses the visual notation to represent a complex DNA gate which uses both upper and lower strand concatenation.



We impose a well-formedness criterion on Visual DSD programs that no long domain d and its complement d^* can be simultaneously exposed in the initial program. This suffices to ensure that no molecules can be produced which could interact on their long domains, which preserves our key assumption that all reactions are toehold-mediated.

Another important change in the latest version of Visual DSD is that strands and gates are considered to be equal up to rotation symmetry. This is necessary because the distinction between “upper” and “lower” strands is simply an artifact of their representation on the page. For example, the following are considered to be equivalent:



Note that when we rotate a gate, the domains in the double-stranded segments are complemented in the syntax. This is because we assume that the domain which appears in the syntax is the domain on the “upper” side of the double strand (as viewed on the page).

Species are the kinds of molecule which may be included in a process. As the following grammar shows, gates and strands are classed as species, as are instances of module definitions. A module instance may include a list of values as parameters – the number and type of parameters depends on the definition of the module. If the wrong number or type of parameters are supplied, the tool will signal an error.

```

Species ::= Name ( )
          | Name ( Values )
          | Gate
          | UpperStrand
          | LowerStrand

```

Values

The language of values in Visual DSD is fairly straightforward. *Values* stands for a non-empty, comma-separated list of *Value* elements.

```

Value ::= String
        | Integer
        | Char
        | Float
        | Name
        | true
        | false
        | Value + Value
        | Value - Value
        | Value * Value

```

```

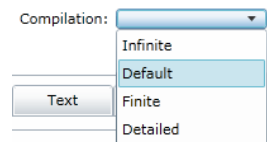
| Value / Value
| float_of_int Value
| int_of_float Value
| ( Value )

Values ::= Value
| Value , Values

```

The base types *String*, *Integer*, *Char*, *Float* and *Name* follow the lexical conventions described above, and the keywords `true` and `false` denote the usual Boolean constants. There are standard arithmetic operators for addition, subtraction, multiplication and division. Both arguments must be of the same type – either integers or floating point values. The `float_of_int` and `int_of_float` functions explicitly convert between the two types, thus offering a way round this restriction.

Alternative transition rules for Visual DSD



The tool offers a choice between four semantic models which are selected using the “Compilation” menu. These specify the reactions between DNA molecules which can take place. Note that all binary reactions are mediated by complementary toeholds. We summarise the choices as follows.

- **Infinite:** molecules are identified up to branch migration. Strand displacement, toehold covering and toehold unbinding are assumed to have infinite rate. Toehold binding is assumed to have a finite rate. Note that unproductive reactions do not appear in the Infinite semantics because the toeholds will unbind immediately if no displacement is immediately possible. This means that the “Unproductive” checkbox has no effect when the Infinite semantics is selected, so it is greyed out when Infinite is selected. This means that certain programming idioms such as cooperative strand displacement are not possible using the Infinite semantics.
- **Default:** molecules are identified up to branch migration. Strand displacement and toehold covering are assumed to have infinite rate. Toehold binding and unbinding are assumed to have a finite rate. This semantics was described in (Phillips & Cardelli, 2009) and it is the default selection when the program begins.
- **Finite:** molecules are identified up to branch migration. Strand displacement, toehold covering and toehold unbinding reactions have a finite rate τ (which can be adjusted using a directive as described above). Consecutive τ reactions are merged together into a single reaction which is also assigned the rate τ . Toehold binding has a finite rate as usual. This allows us to model cooperative displacement while satisfying the constraint that toehold unbinding and strand displacement have similar rates.
- **Detailed:** molecules are not identified up to branch migration. Strand displacement, branch migration, toehold covering, toehold binding and toehold unbinding reactions all have finite rates. The strand displacement and branch migration rates are calculated from the

elementary migration rate and the assumed values for domain lengths (which can all be set with directives) as described above.

Moving from the Infinite through to the Detailed semantic model the number of reactions in the system increases dramatically and the computational cost of the compilation and simulation processes increases also. The same is typically true when unproductive reactions are included.

The leak model

The “Leaks” checkbox enables the simulation of certain kinds of interference between gates and strands. These allow for a more realistic simulation of the actual behaviour of the system but may increase the computational cost of the compilation and simulation phases.

Leak reactions occur because the edges of double strands can sometimes fray up, exposing the nucleotides at the end of the lower strand. This allows a matching upper strand to bind onto the exposed nucleotides as if they were a toehold and displace the rest of the strand as normal. For example, in the reaction below the free upper strand and the bound upper strand are “swapped”. Note that leak reactions are distinguished from normal reactions by a grey reaction arrow.



If the bound strand has a toehold at one end a leak reaction can still take place, though the toehold must first flip up to allow the free upper strand to bind onto the frayed end of the normal strand, as in the following examples.

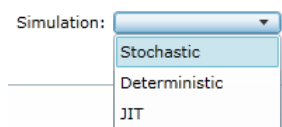


Note that the system disallows leak reactions where the bound upper strand has a toehold at both ends because in this situation both toeholds would need to flip up at the same time in order to allow the bound upper strand to leak. We assume that the rate of these reactions is sufficiently slow that we can disregard them entirely.

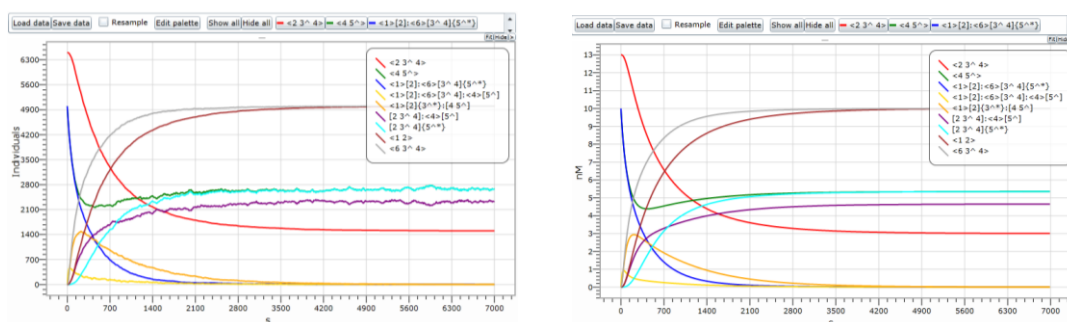
Enabling leak reactions can cause the number of reactions in the system to increase considerably. This is particularly apparent when the “Finite” or “Detailed” semantics are selected. In these cases the compilation stage can produce many thousands of reactions and take a long time to complete. To mitigate this effect the system automatically filters out duplicate leak reactions (i.e. those with the same reactants and the same products) and also leak reactions which are a duplicate of a non-leak reaction. This helps to reduce the number of reactions in some circumstances without affecting the behaviour of the system too much, but it is still possible to derived large numbers of leak reactions from a seemingly small system.

Note that Visual DSD does *not* currently support leak reactions which create DNA polymers by binding two gate molecules together.

Simulation algorithms in Visual DSD



Visual DSD provides a choice of three different simulation algorithms. The “Stochastic” simulator uses the Gillespie algorithm to generate a possible trajectory of the system over time. The “Deterministic” simulator uses a non-stiff ODE solver using a Runge-Kutta-Fehlberg method (Fehlberg, 1969) to produce a smooth, deterministic plot of concentrations evolving over time. In both the stochastic and deterministic simulations, mass action kinetics are assumed, such that the propensity of a reaction is proportional to the product of the reactants. The deterministic ODEs are equivalent to the stochastic dynamics in the limit where the population counts approach infinity. The difference between the plots can be seen by comparing the plots produced for the Catalytic example by the stochastic (left) and deterministic (right) simulators below.



The third option is the JIT (just-in-time) simulator. Selecting this option affects not only the simulation algorithm but also the compilation of DNA species into chemical reactions. The rationale behind the JIT simulator is that some systems can become very large, with many thousands of possible reactions. This means that the full compilation process can take a very long time. This is particularly true for the larger example programs when leaks are enabled. However, since leak reactions have a low probability of actually happening we can spend a large amount of time computing reactions that will probably never happen during a particular simulation run.

The JIT simulator alleviates this problem by compiling new reactions dynamically during the simulation run. When the “Compile” button is clicked in JIT mode, only the “Initial” tab is populated with the initial species. The other output tabs are populated when the simulation is paused or reaches the end of its run. When the JIT simulator is running, it checks after each reaction to see if the products of that reaction have been seen before – if not, a single compilation step occurs which augments the system with the new species and the new reactions which are made possible by the introduction of those species. Thus the reaction graph and list of species is gradually built up over the course of a simulation run. Running the same program multiple times in JIT mode may produce a different final reaction graph, depending on which species were produced during each run.

Using the JIT simulator offers significant practical advantages when handling large-scale systems such as those which include leak reactions. In many cases, simulating a system with leaks using the JIT simulator is comparable in speed to simulating that system without leaks using the stochastic simulator. Furthermore, the JIT simulator is essential when working with systems which have the potential to form DNA polymer molecules of unbounded size. In these cases the JIT simulator only compiles the subset of reactions which are reachable from a species that has been created during the simulation run, as opposed to the full (infinite) reaction graph. Thus the JIT simulator may be the only way to run DNA polymer programs without causing the compiler to loop forever.

Populations and concentrations

In Visual DSD, quantities of DNA complexes are specified as molar concentrations, which denote the number of moles per unit volume. The units of concentration can be set by the `concentration` directive. For example, `directive concentration "M"` sets the units of concentration to molar. The default units are nanomolar (nM), where $1\text{nM} = 10^{-9}\text{ mol/L}$.

In order to perform a stochastic simulation, concentrations must be converted to numbers of individuals. This can be achieved using the following equation:

$$n = \lceil c \cdot V \cdot N_A \rceil$$

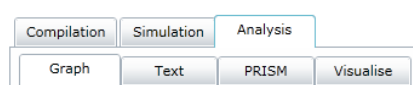
where n is the number of individuals, c is the molar concentration, V is the volume and N_A is Avogadro's constant, which denotes the number of individuals per mole of substance (approximately $6.02214 \times 10^{23}\text{ mol}^{-1}$). The function $\lceil x \rceil$ denotes the rounding up of x to its nearest natural number. Thus, in order to convert a concentration into a number of individuals, it is sufficient to multiply the concentration by a *scale factor* $s = V \cdot N_A$, which denotes the number of individuals per unit concentration. Essentially, this corresponds to choosing a volume V such that the number of individuals is equal to s for one unit of concentration. For example, a scale factor of 50 corresponds to choosing a volume that is 50 times the volume occupied by a single individual. The units of the scale factor are assumed to be the inverse of the units of concentration, and are given as nM^{-1} by default. Note that the conversion from concentrations to individuals is achieved using a scale factor s rather than specifying a volume V directly, since it is difficult to choose a volume such that the number of individuals is a natural number. The scale factor can be set by the `scale` directive, where the default scale factor is 1.0.

The choice of deterministic (continuous) or stochastic (discrete) simulation is also manifested in the units for the simulation plot. The vertical axis of the plot has units of individuals for stochastic simulation, and units of concentration for deterministic simulation. Note that the units for rate constants are assumed to be consistent with the units for time and concentration. For example, if the units for time are s and the units for concentration are nM, then the units for the bimolecular rate constants are assumed to be $\text{nM}^{-1}\text{s}^{-1}$, and the units for the unimolecular rate constants are assumed to be s^{-1} . Once a suitable scale factor has been selected, in order to perform a stochastic simulation the molar concentrations are multiplied by the scale factor, while the concentration-dependent rates are divided by the scale factor. For example, if the scale factor is 100 nM^{-1} then a concentration-dependent rate of $0.4\text{ nM}^{-1}\text{s}^{-1}$ is converted to a stochastic rate of 0.004 s^{-1} for

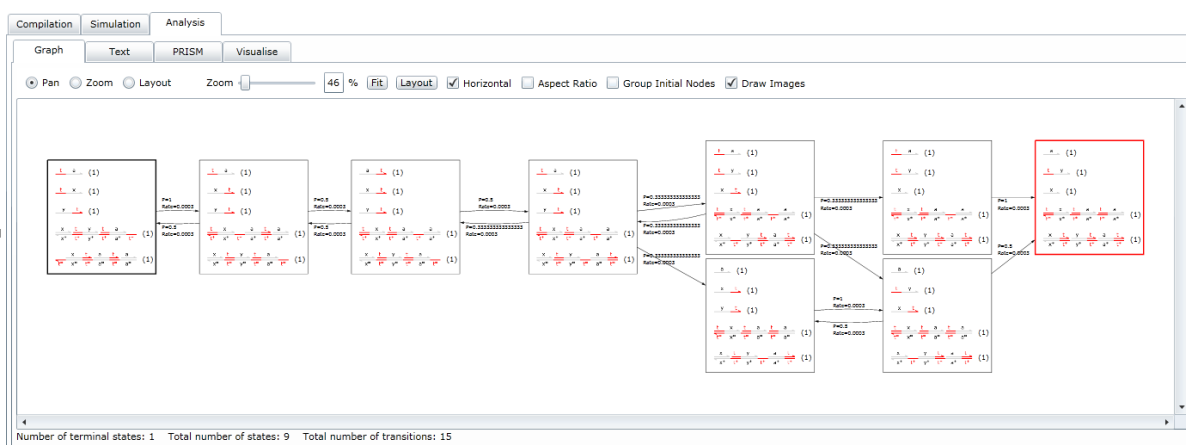
simulation. Additional details on converting between populations and concentrations can be found in Section 4.2 of (Cardelli, 2008), including specific conversion rules for homodimerization reactions.

State space analysis

Visual DSD can also compute the graph of all reachable states for the system from the initial molecules. This is useful for debugging individual components and for verifying larger completed designs. Note, however, that if there are many possible interleavings of the reactions the state graph can be very large and this can take a long time to compute – in such cases, the computation can be stopped using the “Pause” button. When the “Analysis” button is clicked the system computes the continuous time Markov chain and populates the four sub-tabs of the “States” tab on the right-hand side. Note that the state-space analyser always uses JIT compilation as it calculates the state space, regardless of what is selected in the “Simulation” menu.



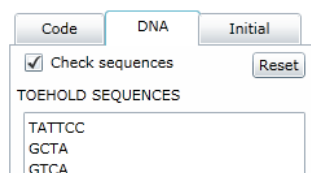
The “Graph” tab displays the set of reachable states as a graph, using a similar graph display to that used for the chemical reaction network. Each reachable state is represented as a node of the graph, and every possible reaction is represented as an edge. There are some additional statistics along the bottom on the size of the state space, and a checkbox to “Draw images” which toggles between graphical and textual views of the populations within each node. The initial state of the system is highlighted with a thick black border (on the left in the image below) and any terminal states (that is, states from which no reactions are possible) are highlighted with a thick red border (on the right in the image below).



The “Text” tab displays a textual summary of the state space, with statistics on the size of the state space (numbers of states, transitions and terminal states), the populations of species present in the initial state and any terminal states, and the minimum and maximum populations of each species in the state graph. The “PRISM” tab displays code generated for the PRISM model checker which corresponds to the state space of the system. This can be used to verify properties of the DNA circuit using probabilistic model checking. Finally, the “Visualise” tab presents a visual summary of the populations of species present in the initial state and any terminal states.

Implementing domains as nucleotide sequences

Visual DSD allows for domains to be compiled into nucleotide sequences, providing a template for implementation of systems in DNA in the laboratory. The “DNA” tab on the left-hand side of the interface allows the user to enter the sequences that are used to implement toehold and specificity domains.

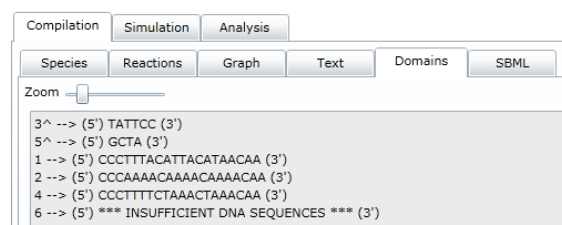


The windows for toehold and specificity sequences are preloaded with toehold domain sequences from the Appendix to (Zhang, Turberfield, Yurke, & Winfree, 2007) and specificity sequences which were generated by Qian for (Qian & Winfree, 2009). The user can edit these and enter their own additional DNA sequences but there are some constraints:

- The only characters allowed are upper case A, C, G and T.
- Only one sequence is permitted per line.
- The same sequence cannot be repeated.
- Toehold sequences cannot be longer than 9 nucleotides.
- Specificity sequences cannot be shorter than 10 nucleotides.

The tool does not perform any other sanity-checking than this, so further investigation of the sequences may be required to verify that they are appropriate for use in experiments. If a large number of DNA sequences is supplied, these sanity checks can slow down the compilation process. The “Check sequences” option allows the user to disable the checks for repeated sequences and for the lengths of toehold and specificity sequences, to speed up compilation. These checks can be safely disabled for the preloaded sequences that come with the Visual DSD tool.

The sequences are output (in order) into the “Domains” tab when the “Compile” button is clicked. If the program contains more domains than there are available sequences, the system indicates this in the “Domains” tab, as shown below. The “Domains” tab also shows explicitly which end of the DNA sequence is the 3’ end and which is the 5’ end.



If the “Show Nucleotides” checkbox is ticked, the mapping of domains to nucleotide sequences is used to display those sequences in the graphical representations of molecules in the “Initial” tab and in the “Species”, “Reactions” and “Graph” tabs. When the checkbox is clicked, these tabs are

automatically refreshed to reflect the new setting. As an example, the following image shows one of the reactions from the Catalytic gate example with the nucleotide view enabled.



Note that the mapping from domains to nucleotide sequences is primarily intended for demonstration purposes and does not currently affect the behaviour of systems in any way – reactions still place at the level of domains, not at the level of individual base-pairs.

Using Visual DSD from the command-line

In addition to the Silverlight interface, Visual DSD can be run from the command-line. The command-line tool contains a subset of the features of the Silverlight version except for the graphical visualizations and the compilation of domains into DNA sequences. The program is typechecked and compiled as in the Silverlight version, and text, DOT and SBML outputs are written to the filesystem so that they can be opened in other tools. It is also possible to run the simulator from the command-line, producing a CSV file which can be analysed in a spreadsheet application.

The command-line version of the tool is available from the Visual DSD webpage in binary format for Windows and Mac OS X 10.5, as well as a platform-independent Objective Caml bytecode file. The Objective Caml programming language (version 3.12.0) must be installed in order to run this version – see <http://caml.inria.fr/download.en.html> for downloads and further information. Binary distributions of Objective Caml are available for Windows, Linux and Mac OS X and the source code distribution can be run on a wide variety of machine architectures using the Objective Caml runtime.

Once the correct version of the Objective Caml runtime has been successfully installed, the command-line version of Visual DSD can be run with the following command:

```
ocamlrun dna.bytecode flags file1.dna ... filen.dna
```

The .dna files are a list of one or more files to be processed by the system. The *flags* consist of zero or more of the following optional commands, which mimic the behaviour of certain user interface elements from the Silverlight version of the tool.

- `-unproductive`: adding this flag includes unproductive reactions.
- `-leaks`: this turns on the leak model described above.
- `-polymers`: this enables polymer reactions, as described above.
- `-declare_domains`: enabling this option raises an error if there are undeclared domains in the program (for debugging purposes).
- `-profile`: produces additional profiling output such as the timings of various stages of the compilation process and the numbers of reactions produced.
- `-step <float>`: specify the percentage reporting step for the simulator.

- `-simulate <string>`: if this is enabled the system is simulated after it has been compiled, and the results of the simulation are written into a CSV file. As in the Silverlight interface there are three possible choices for the simulator mode: `Stochastic`, `Deterministic` and `JIT`.
- `-semantics <string>`: this modifies the semantic model used for computing the set of reactions. As in the Silverlight interface, the choices are `Infinite`, `Default`, `Finite` and `Detailed`. The default semantic model is `Default`.

The command-line arguments are the same in the command-line version compiled using F#, except that “`ocamlrun`” is not needed in the command-line invocation.

Bibliography

Cardelli, L. (2008). On process rate semantics. *Theoretical Computer Science* , 391 (3), 190-215.

Fehlberg, E. (1969). *Low-order classical Runge-Kutta formulas with stepsize control*. NASA Technical Report R-315.

Huang, C.-Y. F., & Ferrel, J. E. (1996). Ultrasensitivity of the mitogen-activated protein kinase cascade. *PNAS* , 93, 10078-10083.

Phillips, A., & Cardelli, L. (2009). A programming language for composable DNA circuits. *Journal of the Royal Society Interface* .

Qian, L., & Winfree, E. (2009). A simple DNA gate motif for synthesizing large-scale circuits. In G. A., F. C. Simmel, & P. Sosik (Ed.), *DNA 14. LNCS 5347*, pp. 70-89. Springer-Verlag.

Zhang, D. Y., & Winfree, E. (2009). Control of DNA strand displacement kinetics using toehold exchange. *J. Am. Chem. Soc.* , 131, 17303-17314.

Zhang, D. Y., Turberfield, A. J., Yurke, B., & Winfree, E. (2007). Engineering entropy-driven reactions and networks catalyzed by DNA. *Science* , 318, 1121-1125.

Appendix: Collected summary of the Visual DSD language

Grammar

The full grammar of Visual DSD programs is as follows. Terminal symbols of the language are written in teletype font and non-terminals are in *italics*.

<i>Program</i> ::=	<i>Directives Declarations Process</i> <i>Directives Process</i> <i>Declarations Process</i> <i>Process</i>	A program must contain a process to run and may optionally contain directives and/or declarations.
<i>Directive</i> ::=	<i>directive duration Float</i> <i>directive duration Float points Integer</i> <i>directive sample Float</i> <i>directive sample Float Integer</i> <i>directive scale Float</i> <i>directive concentration CU</i> <i>directive time TU</i> <i>directive plot Plots</i> <i>directive leak Float</i> <i>directive tau Float</i> <i>directive migrate Float</i> <i>directive lengths Integer Integer</i> <i>directive tolerance Float</i> <i>directive toeholds Float Float</i>	End time for simulation, with optional number of datapoints. End time for simulation, with optional number of datapoints. Specify a scaling factor. Specify concentration units. Specify time units. Which species to plot. Rate of leak reactions. Rate of tau reactions. Elementary migration rate. Default domain lengths. Specify ODE solver tolerance. Default toehold reaction rates.
<i>Directives</i> ::=	<i>Directive</i> <i>Directive Directives</i>	Single directive Multiple directives
<i>Plots</i> ::=	<i>String</i> <i>Gate</i> <i>Strand</i> <i>sum (Plots)</i> <i>sub (Plot ; Plot)</i> <i>diff (Plot ; Plot)</i> <i>div (Plot ; Plot)</i> <i>Plots ; Plots</i>	Plot exact string match only Gate species to plot Strand species to plot Plot sum of populations Plot population subtraction Plot population difference Plot population ratio Plot multiple populations
<i>Declaration</i> ::=	<i>def Name () = Process</i> <i>def Name (Parameters) = Process</i> <i>new Name @ Value , Value</i> <i>new Name</i> <i>def Name = Value</i>	Module definition Module definition Global channel with rates Global channel (default rates) Value assignment
<i>Declarations</i> ::=	<i>Declaration</i> <i>Declaration Declarations</i>	Single declaration Multiple declarations
<i>Parameters</i> ::=	<i>Name</i> <i>Name , Parameters</i>	Single parameter Comma-separated parameters

<i>Value</i> ::=	<i>String</i> <i>Integer</i> <i>Char</i> <i>Float</i> <i>Name</i> <i>true</i> <i>false</i> <i>Value</i> + <i>Value</i> <i>Value</i> - <i>Value</i> <i>Value</i> * <i>Value</i> <i>Value</i> / <i>Value</i> <i>float_of_int Value</i> <i>int_of_float Value</i> (<i>Value</i>)	String Integer Single character Floating point Name True False Addition Subtraction Multiplication Division Convert integer to float Convert float to integer Parenthesised value
<i>Values</i> ::=	<i>Value</i> <i>Value</i> , <i>Values</i>	Single value Comma-separated values
<i>Process</i> ::=	<i>Value</i> * <i>Process</i> <i>constant Process</i> <i>constant Value</i> * <i>Process</i> <i>Value</i> * <i>constant Process</i> <i>Species</i> <i>new Name</i> @ <i>Value</i> , <i>Value Process</i> <i>new Name Process</i> (<i>Processes</i>)	Repetition Constant population Constant repetition Repeated constant Species Restriction with rates Restriction (default rates) Parallel processes
<i>Processes</i> ::=	<i>Process</i> <i>Process</i> <i>Processes</i>	Single process Parallel processes
<i>Species</i> ::=	<i>Name</i> () <i>Name</i> (<i>Values</i>) <i>Gate</i> <i>UpperStrand</i> <i>LowerStrand</i>	Module instance Module instance Gate Upper strand Lower strand
<i>Gate</i> ::=	<i>Segment</i> <i>Segment</i> : <i>Gate</i> <i>Segment</i> : : <i>Gate</i>	Single segment Lower strand concatenation Upper strand concatenation
<i>Segment</i> ::=	<i>Toehold</i> <i>LowerStrand</i> <i>UpperStrand</i> <i>Double</i> <i>Double Overhangs</i> <i>Overhangs Double</i> <i>Overhangs Double Overhangs</i>	Lower toehold Lower strand segment Upper strand segment Double strand Double with right overhang(s) Double with left overhang(s) Double with both overhang(s)
<i>Overhangs</i> ::=	<i>LowerStrand</i> <i>UpperStrand</i>	Lower overhang Upper overhang

	<i>LowerStrand UpperStrand</i>	Lower and upper overhangs
	<i>UpperStrand LowerStrand</i>	Lower and upper overhangs
<i>Double</i> ::=	[<i>Domains</i>]	Double strand
<i>UpperStrand</i> ::=	< <i>Domains</i> >	Single upper strand
<i>LowerStrand</i> ::=	{ <i>Domains</i> }	Single lower strand
<i>Domains</i> ::=	<i>Domain</i>	Single domain
	<i>Toehold</i>	Toehold domain
	<i>Domain Domains</i>	Multiple domains
	<i>Toehold Domains</i>	Multiple domains
<i>Domain</i> ::=	<i>Sequence</i>	DNA sequence
	<i>Sequence</i> *	Complemented sequence
<i>Toehold</i> ::=	<i>Sequence</i> ^	Toehold sequence
	<i>Sequence</i> ^ *	Complemented toehold
<i>Sequence</i> ::=	<i>Integer</i>	DNA sequence
	<i>Name</i>	DNA sequence
	_	Wildcard
<i>TU</i> ::=	seconds s	Unit of time is seconds
	minutes m	Unit of time is minutes
	hours h	Unit of time is hours
<i>CU</i> ::=	molar M	1 mol·L ⁻¹
	millimolar mM	10 ⁻³ mol·L ⁻¹
	micromolar uM	10 ⁻⁶ mol·L ⁻¹
	nanomolar nM	10 ⁻⁹ mol·L ⁻¹
	picomolar pM	10 ⁻¹² mol·L ⁻¹
	femtomolar fM	10 ⁻¹⁵ mol·L ⁻¹
	attomolar aM	10 ⁻¹⁸ mol·L ⁻¹
	zeptomolar zM	10 ⁻²¹ mol·L ⁻¹
	yoctomolar yM	10 ⁻²⁴ mol·L ⁻¹

Lexical conventions

The language uses the following lexical conventions. We write “digit” for a single character in the range 0-9, and “alphanumeric” for any character in the range A-Z or a-z.

- *Integer*: a non-empty sequence of digits.
- *Name*: the first character of a name must either be alphanumeric or the underscore character (_). This is followed by a possibly-empty sequence of characters which may be alphanumeric, digits, underscores or apostrophes (').
- *String*: a possibly-empty sequence of characters enclosed by quotation marks ("). Any quotation marks appearing within the outer of quotation marks must be escaped by a preceding backslash (\).
- *Float*: there are three different ways to produce a float value:
 1. One or more digits followed by a decimal point (.), followed by zero or more digits. For example: 3.141.
 2. One or more digits followed by an uppercase 'E' or lowercase 'e', followed by a plus (+) or minus (-) sign, followed by one or more digits. For example: 3e-5.
 3. One or more digits followed by a decimal point, followed by zero or more digits, followed by an uppercase 'E' or lowercase 'e', followed by a plus or minus sign, followed by one or more digits. For example: 1.4324e+2.
- *Char*: a single character enclosed by apostrophes. The character itself can be anything except for an apostrophe or a backslash.

Comments

Comments are opened with (* and closed with *) . They may be nested.

Reserved keywords

The following identifiers are reserved for use as keywords of the programming language.

```
directive  sample  plot  leak  tau  migrate  lengths
def  new  true  false  int_of_float  float_of_int  time  concentration
constant  tolerance  sum  scale  duration  points  toeholds
```