RuleDSD: A Rule-based Modelling and Simulation Tool for DNA Strand Displacement Systems

Vinay Gautam[a], Shiting Long and Pekka Orponen[b]
Department of Computer Science, Aalto University, 00076 Aalto, Finland

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Abstract: RuleDSD is a tool to support the rule-based modelling and simulation of DNA Strand Displacement (DSD) systems. It constitutes a software pipeline programmed in Python and integrated with PySB, a standard framework for rule-based modelling of biochemical systems. The input to RuleDSD is a domain-level model of a DSD system, where each initial DNA complex is described at the level of named pairing domains. The RuleDSD pipeline converts these domain-level descriptions into a canonical graph representation, and based on this performs a full state-space enumeration of DNA species reachable by applying the basic rules of DNA strand displacement reactions to the ensemble of initial species. The resulting chemical reaction network is then converted into a BioNetGen model and imported into the PySB framework for deterministic or stochastic simulation and analysis. Altogether, RuleDSD thus provides a customised front-end for rule-based modelling and simulation of DNA Strand Displacement systems using the BioNetGen simulation engine, and opens up further possibilities for harnessing the well-established rule-based modelling methods and tools that can easily be utilised through the PySB wrapper.

1 INTRODUCTION

DNA molecules have proven to be a versatile substrate for engineering synthetic biochemical systems with programmable dynamic behaviours (Zhang and Seelig, 2011). Over two decades, a variety of DNA Strand Displacement (DSD) systems, such as molecular motors (Yurke et al., 2000), walkers (Shin and Pierce, 2004), DNA logic circuits (Seelig et al., 2006; Qian and Winfree, 2011), enzyme-free catalytic systems (Zhang et al., 2007; Yin et al., 2008), and systems implementing Chemical Reaction Network (CRN) dynamics (Chen et al., 2013; Srinivas et al., 2017) have been constructed. It has been demonstrated theoretically that any CRN can be converted into a DSD system of approximately equivalent behaviour (Soloveichik et al., 2010; Cardelli, 2013).

The DNA-based reaction mechanism of Toehold-mediated DNA Strand Displacement (Yurke and Mills, 2003; Zhang et al., 2007) provides an expedient reaction toolbox (illustrated in Section 2) for designing DSD systems. Starting with an initial set of domain-level DNA molecular species and the set of DNA interactions based on the DSD reaction toolbox, one can formally derive a reaction network of the DSD system by enumerating all possible reactions between DNA species (Phillips and Cardelli, 2009; Kawamata et al., 2011; Grun et al., 2015a). The derived network provides a basis for both qualitative and quantitative modelling and analysis of the DSD system. The network information can, e.g., be used for computing the state space of the DSD system, verifying its input/output behaviour, and checking that the derived behaviour of a DSD system corresponds to the designer's intentions.

Although small DSD systems can be modelled by hand (Seelig et al., 2006; Zhang et al., 2007), automated design and modelling frameworks are needed for large, complex DSD systems (Yin et al., 2008; Qian and Winfree, 2011; Chen et al., 2013; Srinivas et al., 2017). Recently, several modelling methods have been proposed and implemented as software tools: Visual DSD (Lakin et al., 2011), KinDA (Berleant et al., 2018), and DyNAMiC Workbench (Grun et al., 2015b). Visual DSD originally allowed the modelling of only very simple DSD systems based on single-stranded DNA and partially double-stranded DNA structures. However, over the years, the tool has gone through several changes to

[a] https://orcid.org/0000-0003-1506-2071
[b] https://orcid.org/0000-0002-0417-2104

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enable the modelling of DSD systems consisting of DNA loops, multi-branch DNA structures (Petersen et al., 2016; Spaccasassi et al., 2018), and tethered structures designed to interact locally (Lakin et al., 2014). The other two DSD system design software tools, KinDA and DyNAMiC Workbench, also provide integrated frameworks for the design, modelling and analysis of a DSD system at the domain and sequence levels. Both these tools use an alternative enumeration method based on time-scale separation of the reactions, which reduces the state-space of DSD systems by condensing reactions leading to combinatorial DNA structures (Grun et al., 2015a)

Domain-level modelling of DSD system behaviour is currently facing two main problems. First, the increasingly large number of reactions considered between DNA species makes full enumeration of a system's state-space computationally infeasible. To address this issue, (Kawamata et al., 2011) and (Grun et al., 2015a) have proposed alternative methods to enumerate state spaces of DSD systems. Second, as the complexity of DSD systems continues to increase, new DNA structures with loops (Yin et al., 2008), multiple branches (Kotani and Hughes, 2017) and even pseudoknots (Bui et al., 2017) are used in the implementation of DSD systems. Although the modelling of DSD systems with such DNA structures was previously not supported by any of the existing DSD modelling tools, new implementation methods (Petersen et al., 2016; Spaccasassi et al., 2018) have recently begun to address the issue.

In the field of biochemical system modelling, rule-based models, such as BioNetGen (BNGL, in short) (Faeder et al., 2009) and Kappa (Danos and Laneve, 2004) have successfully employed methods to address similar issues. For example, rule-based models use a concise representation of combinatorial structures and compact sets of rules to encode a large number of biochemical interactions. In the present work, we model DSD systems using a rule-based approach (Faeder et al., 2009). First, domain-level DNA species are described using a representation scheme inspired by rule-based models, as described in (Petersen et al., 2016). These textual descriptions are then abstracted into a graph representation, which reduces structurally equivalent species representations to a canonical format. Next, new DNA species are created by applying graph rewriting rules that are based on a set of generic DSD reaction types. This graph processing produces a list of DNA species and their DSD reaction network, which can be further translated into the BNGL format and simulated using the BNGL simulation engine. We provide a full software pipeline for this rule-based DSD modelling approach, integrated with the PySB framework (Lopez et al., 2013). The main contributions of this work are twofold. First, the modelling approach discussed here is general. Second, the work connects the task of DSD modelling directly to the broader rule-based modelling frameworks. Therefore, DSD models can easily be imported to any of the engines developed so far, or to be developed in future, for rule-based modelling without need to develop own simulators.

In the following, Section 2 reviews the background on DSD systems, the mechanism of toehold-mediated strand displacement, the DSD reaction toolbox, domain-level representation of DSD systems, and provides an overview of the rule-based modelling approach. Section 3 outlines our RuleDSD pipeline for modelling and simulation of domain-level DSD systems and describes some implementation details of the pipeline. Section 4 demonstrates simulation results on a few example DSD systems. Section 5 concludes with some general observations and future work.

2 BACKGROUND

We start our discussion by describing the terminology and conventions used in this paper. We then present the concept of domain-level design of DSD systems and illustrate an established toolbox of DSD reactions. We also outline a domain-level description language for DSD models. At the end of the section, the rule-based modelling approach and the BioNetGen framework are briefly introduced.

2.1 DNA Strand Displacement Systems

In the domain-level design of Figure 1 and other designs presented in the paper, each DNA strand is denoted by a line segment, with the 3' end marked by a half-arrowhead. Lowercase letters and numbers are used as domain names, and complementary domains are indicated by asterisks. Short "toehold" domains are indicated by appending a caret (^) to their name, for example a toehold domain x would be represented as $x^{\wedge}$ and its complement as $x^{\wedge *}$. Double-stranded DNA regions appear as two anti-parallel line segments. We use the terms *DNA species* and *DNA complex* interchangeably in the case of multi-stranded DNA molecules, but DNA species containing only single DNA strands are explicitly mentioned as single-stranded DNA.

Over the years, theoretical and experimental studies have established a toolbox of elementary DNA reactions comprising both inter- and intramolecular

DNA interactions, as shown in Figure 1. The first two mechanisms in the toolbox, the *binding reaction (RB)* and the *unbinding reaction (UR)*, are illustrated by the domain-level schema in Figure 1a. In the RB reaction, two single-stranded DNA molecules $x^\wedge y$ and $x^{\wedge *}z$ which contain matching complementary domains $x^\wedge$ and $x^{\wedge *}$ undergo a bimolecular reaction that produces a duplex combining the domains. The RU process, which is the reverse of RB, is a unimolecular reaction in which the duplex dissociates into its constituent DNA strands.

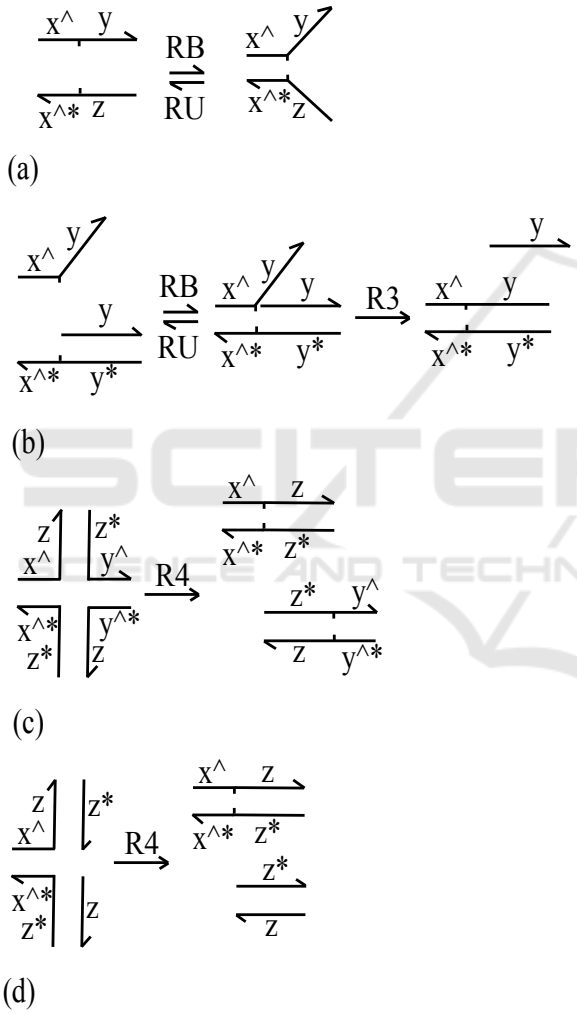


Figure 1: A toolbox of DSD reactions. (a) Binding and unbinding reactions between two separate DNA strands. (b) The toehold-mediated strand displacement process between a partially double-stranded DNA complex and a DNA strand: an initiating toehold binding/unbinding is followed by a 3-way branch migration. (c) A 4-way branch migration reaction between two DNA complexes each having two DNA strands with mutually complementary domains. (d) A variant of 4-way branch migration, where the two DNA complexes are bound by a single toehold domain.

Figure 1b illustrates the key DSD process of Toehold-mediated Strand Displacement (TSD) (Yurke and Mills, 2003; Zhang et al., 2007) that introduces dynamic control over the interactions of DNA strands and consists of two reaction steps: (1) toehold binding, and (2) branch migration. In the TSD process, a short single-stranded *toehold domain* $x^{\wedge *}$ serves as a binding site within a partially double-stranded DNA molecular complex. The toehold in the DNA complex co-localizes another single-stranded DNA molecule, the *invader* or *signal* strand $x^\wedge y$. The toehold binding catalyzes a unimolecular 3-way *branch migration* reaction (R3) (Zhang et al., 2007), which releases the previously bound DNA strand y. Another type of branch migration process, a 4-way migration (Panyutin and Hsieh, 1994; Kotani and Hughes, 2017) (R4), occurs when two double-stranded DNA molecules that have mutually complementary DNA strands exchange their strands (Figure 1c, d).

Toehold domains play a major role in the design of programmable DSD systems. First, their length and sequence composition have a significant influence over the kinetic rate of strand displacement (Zhang et al., 2007): the rate varies million-fold over a toehold length of six bases or less, and saturates for longer toeholds. Second, toeholds also serve as recognition domains for input signals (Zhang et al., 2007). While the first feature provides a design mechanism for programmable kinetic control based on competing DNA strand displacement reactions (Zhang and Seelig, 2011), the second feature enables the design of DNA strand displacement cascades using DNA complexes with inactivated toeholds that are conditionally activated as the reaction proceeds (Seelig et al., 2006). In principle, any mechanism that sequesters the toehold domain and inhibits its hybridization can be used for the inactivation. For example, toeholds can be buried within double-stranded regions (Seelig et al., 2006) or inside hairpin loops (Dirks et al., 2004; Yin et al., 2008) to make them inactive.

2.2 Domain-level Description of DSD Systems

DSD system modelling typically starts with an abstract domain-level description in terms of a set of DNA molecular species, each consisting of multiple interacting DNA strands. Each DNA strand is composed of a set of *domains* (Phillips and Cardelli, 2009), where each domain is functionally distinct and corresponds to a contiguous sequence of nucleotides. Domains can repeat within the same DNA strand, or can appear in different DNA strands, but any two non-

complementary domains are assumed to be mutually orthogonal, i.e. the DNA sequences representing different domains are presumed to be designed for minimal interference as enforced by the Watson-Crick base-pairing rules.

A domain-level description language called the *DSD language* was introduced in (Phillips and Cardelli, 2009) for formally describing DSD systems so as to model and analyse their behaviour. Here we illustrate the syntax of the DSD language briefly with the examples of DNA molecules shown in Figure 1. A typical DSD system comprises two types of DNA species: single-stranded DNA and multi-stranded DNA complexes. A single-stranded DNA species with domain sequence $d_1, d_2....d_n$ is represented as $< d_1 d_2....d_n >$ or $\{d_1 d_2....d_n\}$ in 5'-to-3' or 3'-to-5' orientation, respectively. For example, the two single-stranded DNA species in Figure 1a (left) would be represented as $< x\hat{}\ y >$ (upper strand) and $\{x\hat{}^*\ z\}$ (lower strand). Note that since DNA strands have a rotational symmetry, the two representations in this case can be used interchangeably. A multi-stranded DNA complex in the DSD language is represented as a sequence of segments consisting of either single-stranded or double-stranded regions. Single-stranded segments are represented as discussed above, while the double-stranded segments are represented as $[d_i]$, where d_i is the domain in the upper strand, oriented in the 5'-to-3' direction. A nick in the upper or lower strand is represented by ':' or '::', respectively. For example, the double-stranded DNA complexes in Figure 1b would be represented as $\{x\hat{}^*\}[y]$ (left) and $[x\hat{}] < y >: [y]$ (middle).

The syntax of the original DSD language is limited to describing only simple DNA molecules without multi-branch structures and hairpins. Therefore, the four-arm DNA structures shown in Figure 1c,d, and others having hairpins (Yin et al., 2008) could not be modelled in the original framework. Recently, (Petersen et al., 2016) introduced a modified DSD language following the bond notation used in the formal languages of rule-based models, such as BNGL (Faeder et al., 2009) and Kappa (Danos and Laneve, 2004). In the modified DSD language a bond between two domains (d_1 and d_2) is represented by a bond label ($d_1!i$ and $d_2!i$, where i is a bond label and ! is a positional operator) that is local to the DNA species. Note that the two domains could be within a single DNA strand or in two different DNA strands. The other notations, such as the representations for toehold domains and complementary domains remain the same in the modified language as in the original language. However, DSD models are represented by processes, where a process $\mathcal{P}$ is a set of DNA strands represented as $\mathcal{P} =< S_1|S_2....|S_n >$. In this framework, DSD systems having DNA species with arbitrary, complex DNA structures, such as multi-branches, hairpin loops and pseudoknots can be represented.

2.3 Rule-based Modelling and BNGL

Rule-based modelling has recently emerged as a powerful method for modelling and analysis of biochemical systems with complex reaction networks (Chylek et al., 2014). Rule-based modelling frameworks, such as BNGL (Faeder et al., 2009) and Kappa (Danos and Laneve, 2004), are used to develop compact models of biochemical systems using a finite set of rules that can encode a large number of biochemical interactions. A rule-based model can be used to study stochastic and deterministic behaviours of a given biochemical system. For biochemical system with relatively small state-spaces, a system of equations or an entire reaction network can be extracted that can further be simulated deterministically using ODE solvers or stochastically using the Gillespie algorithm (Gillespie, 2007). For the modelling of biochemical systems with large state-spaces, such as polymerization reactions, network-free simulation methods (Danos et al., 2007; Sneddon et al., 2011) have been developed.

Here, we briefly describe the syntax of the BNGL language. This language describes biochemical systems in terms of molecules or agents with sites, where a site can either interact with other sites or manifest different states. For example, a molecule M_1 with a binding site b and another site s that can be in three different states is represented as $M_1(b, s \sim 0 \sim 1 \sim 2)$. If there is another molecule M_2 with a matching binding site b (represented as $M_2(b)$), a binding rule between these two can be written as:

$$M_1(b, s \sim 0 \sim 1 \sim 2) + M_2(b) \rightarrow M_1(b!1).M_2(b!1)\ k_b,$$

where the LHS represent reactant molecules (separated by a '+'), the RHS represents a complex of two molecules (bound by '.'), and the k_b is rate constant associated with the rule. The bond between two molecules is represented by '!1', where 1 is the bond label and '!' is a positional operator. The expressive power in this rule comes from the language's ability that enables the rule to be applied to any molecule M_1 that is not bound at site b regardless of the state of s and whether or not s is bound to another molecule.

Although it seems quite intuitive to apply rule-based modelling to DSD systems, a direct translation of DSD models into rule-based models using BNGL

is challenging due to two reasons (Petersen et al., 2016). First, binding domains in DSD systems have a spatial ordering, i.e. a binding at one domain enables a configuration at another domain that may be bound or free; however, rule-based models so far do not allow to encode such spatial aspects into the rule definitions. Second, such translation would require writing a large number of rules even for small systems due to the many possibilities of pattern matching between the sets of domains of two DNA complexes.

3 THE RuleDSD PIPELINE

Here, we introduce RuleDSD, a tool for rule-based modelling of DSD systems that converts domain-level DSD models into BNGL models and simulates them using the PySB framework. The software pipeline, as illustrated in Figure 3, is developed as a Python package DSDPy and integrated with the PySB framework.

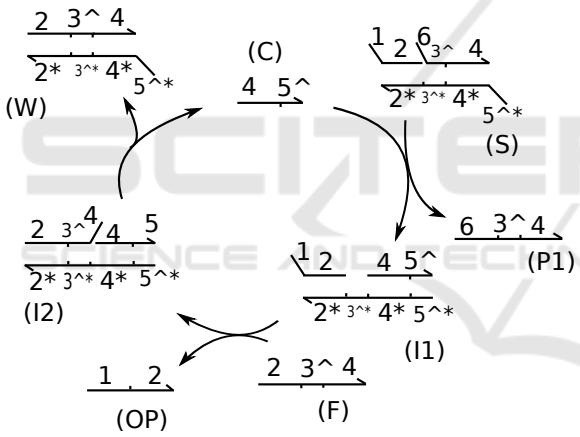


Figure 2: The catalytic DSD system described by (Zhang et al., 2007), showing the domain-level network derived by hand. There are three initial DNA species: Catalyst (*C*), Substrate (*S*), and Fuel (*F*). As the substrate and catalyst react, the displacement reaction produces intermediate DNA species (I1) and a signal (P1). The DSD system further generates intermediate species (*I2*) and output (*OP*), as Fuel (F) is consumed by intermediate species I1 to cause second displacement reaction. The intermediate DNA species I2 further turns into a non-reactive waste product (W) and releases catalyst C back into the cycle.

In this section we discuss the pipeline and its input and output with a representative example of a catalytic DSD system shown in Figure 2. The input to the pipeline, shown in Figure 4, is a simple text file that consists of domain-level descriptions of the initial DNA species, their initial counts/concentrations and kinetic rates of the DSD reactions. At the other end of the pipeline, the user can run a script to simulate the deterministic or stochastic dynamics of the described DSD system.

The translation/simulation pipeline, as shown in Figure 3, comprises three modules. The first module, DNA species graphs generation, converts the initial DNA species in a domain-level DSD model to a canonical graphs representation. The second module, DSD network generation, reads these input graphs and transforms them using rules from the DSD reaction toolbox to produce a netlist (a list of DNA species and a corresponding reaction network) that represents the full state-space of the DSD system. The output from this module is a text file consisting of a list of domain-level DNA species produced from graph processing and chemical reactions between these species with their kinetic rates. The final module, BNGL-based simulation, reads the DSD netlist and generates a PySB model in Python that can be simulated using ODE-based or BNGL-based simulators in the PySB framework.

3.1 The Strand Graph and the Bond Graph

The first module in the RuleDSD pipeline, shown in Figure 3, is a converter that reads the domain-level DSD descriptions of the initial DNA species of our representative example of a catalytic DSD system and creates two types of abstract representations called the *Strand Graph (SG)* and *Bond Graph (BG)* and displayed in Figure 5a and b, respectively. For the domain-level description of DSD systems, we use a modified representation syntax based on article (Petersen et al., 2016). A DSD system $\mathcal{D}$ that has n DNA species $P_1, P_2, \ldots, P_n$, is represented as:

$$\mathcal{D} = P_1 // P_2 // \ldots // P_n$$

where species P_i is an N-stranded DNA molecule, represented as:

$$\mathcal{P}_i = < S_{i1} > < S_{i2} > \ldots < S_{iN} >$$

The Strand Graph is a special type of hybrid graph that is based on the representation used in (Petersen et al., 2016). The *SG* of the representative catalytic DSD system is shown in Figure 5a. The *SG* is specified by a pair of sets (V, E), where sets V and E represent vertices and edges, respectively. Each element in set V consists of a directed graph whose vertices are domains in the DNA strand and directed edges are covalent bonds between two domains, where edges are directed in the 5'-to-3' orientation. Each element in set E consists of edges representing both existing and admissible bonds between domains in the given set

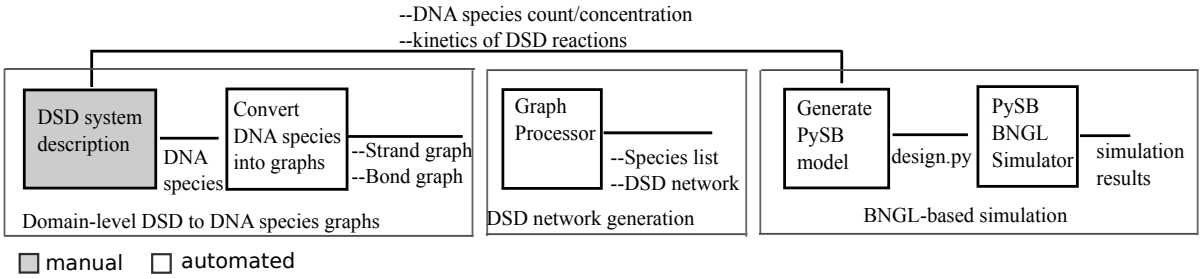


Figure 3: The RuleDSD pipeline for modelling and simulation of domain-level DSD systems.

catalytic_dsd_input.txt

```
<2 3^ 4>
//
<4 5^>
//
<1 2!1>
<6 3^!2 4!3>
<5^* 4*!3 3^*!2 2*!1>
--
ss1 6500
ss2 5000
ss3 5000
--
RB 0.0003
RU 0.1126
R3 20
R4 20
```

Figure 4: Input to the RuleDSD pipeline: a domain-level DSD model of the catalytic system (catalytic_dsd_input.txt).

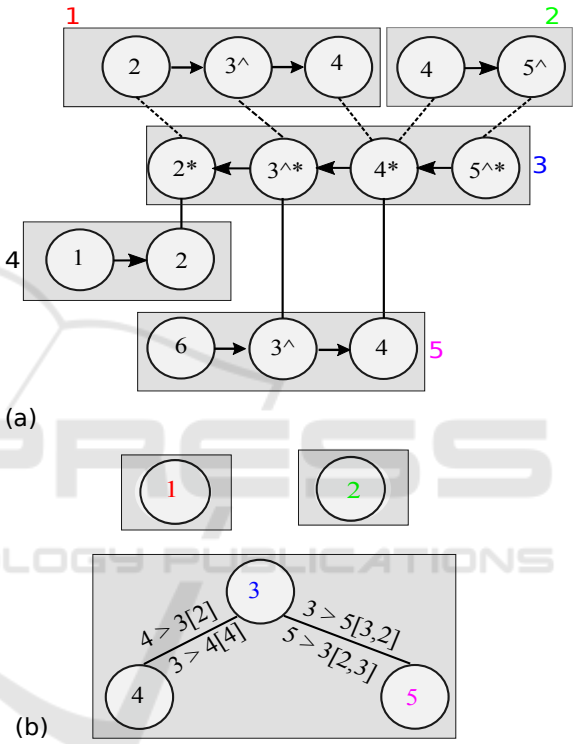


Figure 5: Graph representations of the initial DNA species of the catalytic DSD system example. (a) Strand Graph: the five DNA strands, each inside a gray rectangular outline represents a vertex of the graph. The vertices are enumerated as 1 (red),2 (green), 3 (blue), 4 (black) and 5 (pink). Each vertex is again a directed graph having number of vertices equal to the number of domains in the corresponding DNA strand. There are three types of connecting lines between the domains (shown inside circles): lines with arrowhead represent domain-domain covalent bond within a DNA strand; solid and dotted lines respectively represent existing and admissible bonds between the domains of the DNA strands. (b) Bond Graph has five vertices each representing a single DNA strand. The three vertices (black, blue and pink), constituting a partially double-stranded DNA complex, are connected while the other two (red and green) that represent the single-stranded DNA species are disconnected.

of DNA strands. The set E consists of two subsets representing existing edges and admissible edges, respectively.

The Bond Graph, which is derived from the Strand Graph, is an undirected graph that may also include cycles. The set of vertices V in a BG consists of an enumerated list of DNA strands in the SG. The edge set E represents existing domain-domain bonds between any two DNA strands or within a DNA strand (cycle), and therefore it is equivalent to the subset of edges that represent existing bonds in its corresponding SG. The BG of the catalytic DSD example is shown in Figure 5b. The edges are shown with the domain-domain bond information ($m > n$[i]) that represents an existing bond between i^{th} domain of enumerated DNA strand #m and a domain in #n. A symmetric bond information with respect to the other DNA strand is given by $n > m$[j]. There can be multiple edges between a set of vertices in a BG, for example, the vertices *3* and *5* in the figure have two edges represented by $3 > 5$[3,2], and symmetrically, $5 > 3$[2,3].

3.2 Graph Processing and DSD Network Generation

The central role of the RuleDSD pipeline is to generate the DSD network given the initial species, and this role is played by the graph processor in Figure 3. There is an outer phase and an inner phase in the graph processor to generate the DSD network: *generate new species* and *map species*. In the outer phase, the processor steps into a loop where it performs checks on currently available species to obtain all possible valid rules (as described in Section 2.1) that can be applied to those species, resulting in new species. The processor first derives a list of initial species from the *Strand Graph* input, then executes Algorithm 1.

Algorithm 1: Generate New Species.

Input: *InitSL*: List of Initial species
Output: *SL*: List of all possible species

function GENERATION(*InitSL*)
  $SL \leftarrow InitSL$
  $visited[i] \leftarrow$ **False for** $i = 0$ **to** $len(SL) - 1$
  $cursor \leftarrow 0$
  while not $visited[cursor]$ **do**
    $oldlen \leftarrow len(SL)$
    for $i = cursor$ **to** $oldlen - 1$ **do**
      $SL \leftarrow SL + CheckMonoReaction(i)$
      $visited[i] \leftarrow$ **True**
    end for
    $comb \leftarrow$ combinations of old species (whose index i in SL satisfies $i < oldlen$) and new species (whose index $i \geq oldlen$)
    for $j \in comb$ **do**
      $SL \leftarrow SL + CheckBiReaction(j)$
    end for
    if $oldlen \neq len(SL)$ **then**
      $cursor \leftarrow oldlen$
    else
      $cursor \leftarrow oldlen - 1$
    end if
    $newlen \leftarrow len(SL)$
    for $i = oldlen$ **to** $newlen - 1$ **do**
      $visited \leftarrow visited +$ **False**
    end for
  end while
  return SL
end function

There are two types of checks on species: `CheckMonoReaction()`, which checks possible monomolecular reactions (i.e., RB, RU, R3 and R4), and `CheckBiReaction()`, which checks possible bimolecular reactions (i.e., RB). These functions convert the input species in text representation to its *SG*, perform the checks on its *BG* to speed up the calculations, and translate the results from *BG* to text using *SG*.

The inner phase *map species* happens within both checks above. Note that a species generated at a later point might coincide with a species that has occurred at an earlier point. Therefore, a mapping scheme that creates a relation between an *SG* and its unique representation (*id*) must be addressed. In this paper, we use the unique canonical form of an *SG* as the id of a particular species. Such canonical form can be derived by using an alphabetic sort and a Breadth-first search (BFS) on the *SG*. When the outer phase terminates, the graph processor has information on the entire list of DNA species and chemical reaction network, as shown in Table 1 and Figure 6, respectively, for our representative example of catalytic DSD system.

Table 1: The list of DNA species representing state-space of the catalytic DSD system. Three species (ss1, ss2, and ss3) are the initial species of the DSD system, and the other eleven species (sp_4 - sp_14) are automatically generated by RuleDSD.

ss1 < 2 3^ 4 >	**ss2** < 4 5^>
ss3 <1 2!1 > < 5^* 4*!2 3^*!3 2*!1> <6 3^!3 4!2 >	**sp_4** <4 5^!1> <5^*!1 4*!2 3^*!3 2*!4 > <6 3^!3 4!2> <1 2!4 >
sp_5 <4!1 5^!2 > <5^*!2 4*!1 3^*!3 2*!4 > < 6 3^!3 4>	**sp_6** < 4!1 5^!2> <5^*!2 4*!1 3^* 2*!3 > <1 2!3 > < 1 2!4>
sp_7 <6 3^ 4 >	**sp_8** <2 3^!1 4 > <5^*!2 4*!3 3^*!1 2*!4 > <4!3 5^!2 > <1 2!4 >
sp_9 <2!1 3^!2 4 > < 5^*!3 4*!4 3^*!2 2*!1> < 4!4 5^!3>	**sp_10** < 1 2>
sp_11 <2 3^!1 4!2 > < 5^*!3 4*!2 3^*!1 2*!4> <4 5^!3 > < 1 2!4>	**sp_12** <2!1 3^!2 4!3 > <5^*!4 4*!3 3^*!2 2*!1 > <4 5^!4 >
sp_13 < 2 3^!1 4!2> <5^* 4*!2 3^*!1 2*!3 > < 1 2!3>	**sp_14** <2!1 3^!2 4!3 > <5^* 4*!3 3^*!2 2*!1 >

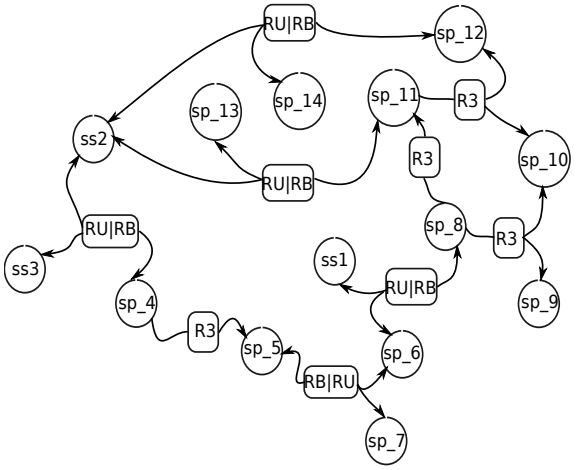
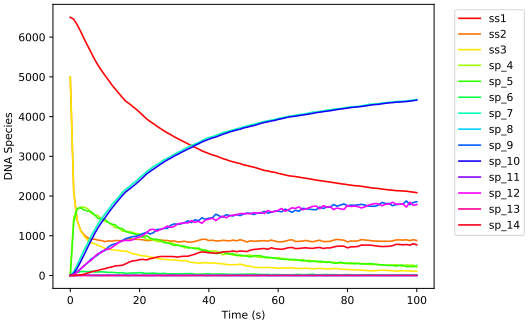
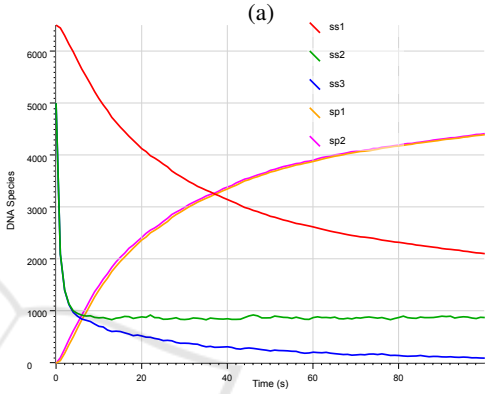


Figure 6: The RuleDSD generated chemical reaction network of our representative example of catalytic DSD system. DNA species and reactions between them have circular and rectangular outlines, respectively. Reaction transitions between DNA species are shown in curved lines, where an arrowhead marks the direction of the reaction transition. For example, ss2 and ss3 react (shown by reaction rate, RB) to produce sp_4, and this is a reversible reaction (reverse reaction is shown by rate, RU).



(a)



(b)

Figure 7: Time-course data from stochastic simulations of the catalytic DSD system using RuleDSD (a), and Visual DSD (b).

4 RESULTS AND ANALYSIS

In this section, we present simulation results of two DSD systems that have been modelled using RuleDSD. We compare the results to the simulations based on Visual DSD (Lakin et al., 2011). While the deterministic simulations from both the tools are identical, stochastic simulations have some stochastically indistinguishable variance.

4.1 Example 1

As a first example, we demonstrate a RuleDSD-based simulation of the catalytic DSD system illustrated in Section 3, Figure 2. The RuleDSD-generated full list of DNA species of the catalytic DSD system is presented in Table 1. Note that the species are named differently in the hand-derived reaction network (Figure 2) and the automatically generated RuleDSD list.

Figure 7 displays time-course plots of data from a stochastic simulation of our catalytic DSD system using RuleDSD and Visual DSD. The RuleDSD input for this simulation is as in Figure 4, where initial DNA species, ss1, ss2, and ss3 are initialised with population counts of 6500, 5000, and 5000, respectively. The same simulation settings were also used for the Visual DSD simulation.

While the dynamics of all DNA species generated by graph processing are plotted in the RuleDSD simulation, only the initial, signal and output species are plotted in the Visual DSD version. Note that the species sp_10 and sp_7 in Figure 7a are identical to the species sp1 and sp2 in Figure 7b, respectively. The time-course data from the two stochastic simulations reveal statistically equivalent simulation trajectories.

4.2 Example 2

As a second example, we present RuleDSD-based simulation results of a DSD system consisting of surface-tethered DNA complexes whose displacement process involves both three-way and four-way branch migration reactions (Qian and Winfree, 2014). The initial DNA complexes, main reactions and intermediate DNA complexes are shown in Figure 8.

Time-course data from the stochastic simulation of surface-tethered DSD system using RuleDSD is shown in Figure 9. The two initial DNA species, ss1 (blue) and ss2 (orange), undergo toehold binding, as shown by a swift decrease in their respective initial populations, and produce an unstable intermedi-

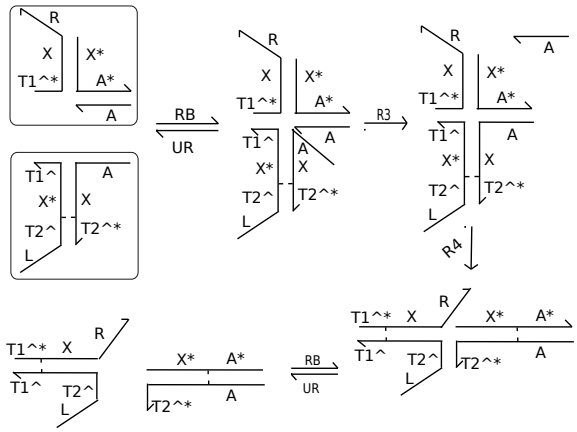


Figure 8: A surface-tethered DSD System involving both 3-way and 4-way branch migration reactions. Initial DNA complexes are highlighted with outlines.

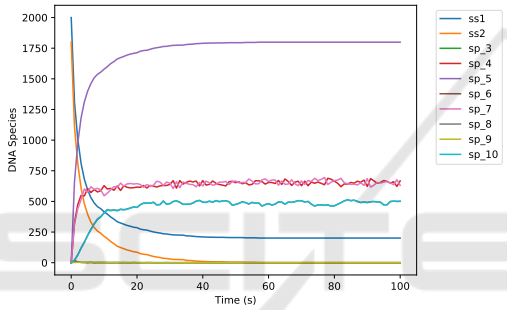


Figure 9: Stochastic simulation of a DSD system involving both three-way and four-way branch migrations. The initial species, ss1 and ss2, have starting species counts of 2000 and 1800, respectively. Eight new DNA species (sp_3 – sp_10) were generated by the RuleDSD tool.

ate DNA complex, sp_3 (green that is covered by sp_6 (light green)).

The unstable DNA species, sp_3, undergoes a 3-way branch migration that produces a sharp rise in the populations of species sp_4 (red) and sp_5 (purple). The DNA species sp_5 is another transitory DNA structure that further reconfigures via a 4-way branch migration producing sp_7 (pink), which further undergoes a toehold dissociation to produce species sp_8 and sp_10. The dynamics of DNA species sp_4 and sp_7 enter into a state of stochastic equilibrium due to an effective reversible process that drives the transition back and forth between the two species. We also simulated this DSD system using Visual DSD and found identical time-course data, also reported in (Petersen et al., 2016, Figure 7).

5 CONCLUSION AND FUTURE WORK

In the context of domain-level DSD system modelling, we have used a rule-based modelling approach to represent DNA structures, converted them into canonical graph representations, and applied rules from the DSD reaction toolbox to generate a reaction network that is simulated using the BNGL simulator in the PySB framework. The RuleDSD pipeline is publicly available for download as a Python package at DSDPy.

We have studied several DSD systems using RuleDSD and compared the results, such as the generated reaction networks and dynamics with the simulations from Visual DSD. Due to space constraints only two examples are reported here in Section 4; others are available online at DSDPy. Both deterministic and stochastic simulations of the studied systems show compliance with Visual DSD (Lakin et al., 2011) simulations.

The RuleDSD implementation currently assumes that there is more than one copy of each of the initial DNA species in a given DSD system, thus the reaction networks often grow very large or even infinite, as both the generated DNA species and initial DNA species can react to produce larger DNA structures. However, the user can set a threshold value to limit the size of the generated reaction networks in such cases to prevent infinite growth. We aim to further develop the RuleDSD tool to incorporate network-free simulation methods, where species are generated by graph processing only on demand, so that the entire network does not need to be generated beforehand and hence also DSD systems with very large state-spaces can be modelled.

RuleDSD currently implements four basic types of DSD reactions with generic kinetic rates. Although a variety of DSD systems can quantitatively be modelled using these generic reaction rates, we need more types of reactions and variants of kinetic rates to be included for qualitative analysis and accurate modelling, respectively. For example, modelling localised reactions (Bui et al., 2017), hairpin-based reactions (Yin et al., 2008), and tethered DSD systems (Lakin et al., 2014) need specific kinetic rates to be defined. Another feature currently lacking in the RuleDSD that we are working on is a graphical user interface for the visualization of DNA species and reaction networks that are presently produced only in text format.

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