SMT Solving for Vesicle Traffic Systems in Cells

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Abstract In biology, there are several questions that translate to combinatorial search. For example, vesicle traffic systems that move cargo within eukaryotic cells have been proposed to exhibit several graph properties such as three connectivity. These properties are consequences of underlying biophysical constraints. A natural question for biologists is: what are the possible networks for various combinations of those properties? In this paper, we present novel SMT based encodings of the properties over vesicle traffic systems and a tool that searches for the networks that satisfies the properties using SMT solvers. In our experiments, we show that our tool can search for networks of sizes that are considered to be relevant by biologists.

1 Introduction

The cytoplasm of eukaryotic cells is broken into membrane-bound compartments known as organelles. Cargo is moved between these compartments in small membrane-bound transporters known as vesicles\textsuperscript{1}. This organization resembles a transport logistics network (compartments are nodes, vesicles are directed edges) and is commonly known as the vesicle traffic system (VTS). Vesicle traffic underpins nearly every aspect of eukaryotic cellular physiology, including human cellular physiology. Understanding how this system functions is therefore one of the central challenges of cell biology.

Cell biologists have identified many molecules that drive vesicle traffic\textsuperscript{2}. The key steps are the creation of vesicles loaded with cargo from source compartments (budding), and the depositing of those vesicles and cargo into target compartments (fusion)\textsuperscript{3}. Budding is regulated by proteins known as coats and adaptors that select cargo. Fusion is regulated by proteins known as SNAREs and tethers, that ensure that vesicles fuse to the correct target\textsuperscript{4}. Abstractly, we think of SNARE proteins as labels that are collected from the source compartment by vesicles. There is a corresponding set of label molecules on potential target compartments. If the two sets of labels (on vesicles and targets) are compatible, then the vesicle will fuse to the target. Importantly, it is likely that SNARE proteins can be regulated by other molecules, thereby being in active or inactive states. However, SNARE regulation is not well understood.

There has been very little work done on how these molecular processes are integrated across scales to build the entire VTS. We have recently used ideas of
graph theory to address this question [4]. We have shown that SNARE regulation places constraints on the structure of the traffic network [5]. One informative constraint is graph connectivity. The nature of SNARE regulation determines whether graph topologies of particular levels of connectivity are biologically realizable. For example, if SNAREs are completely unregulated and thus always active, they cannot be used to generate a traffic network. If these SNAREs are regulated by relatively simple rules, then only highly connected traffic networks can be generated using them. In this way, biological inputs (about SNARE regulation) lead to clear testable predictions (about graph topologies).

The analysis of VTSs is a difficult computational problem because of the combinatorial scaling of graph topologies and regulatory rules. Many questions require us to check useful properties over all possible graphs and regulatory rules. Formal verification tools such as model checkers [6–10] and SAT solvers [11, 12] allow us to do this symbolically for graphs of finite size, without enumerating all instances. In a recent work, we employed the model checker CBMC [13, 14] meant to analyze ANSI C programs for studying this problem. VTSs and SNARE regulations were modeled as C code using arrays to represent graphs and Boolean tables. Queries about graph connectivity requirements for different variations of SNARE regulations were modeled as logical assertions to be checked by the model checker. CBMC uses its own built-in encodings to reduce the analysis problem to a Boolean satisfiability problem which is solved using SAT solvers. However, the scalability of our recent approach was limited (up to VTSs with 6 nodes) due to the encoding used to model VTSs was not completely fine-tuned to the structure of the problem. In particular, we had to encode conditional reachability between nodes (refer sec. 3, stability condition), and solved in CBMC using a combination of non-determinism and partial enumeration that is inefficient.

In this paper, we have developed a novel SMT based encoding for searching VTSs that satisfy the useful properties. We have improved the encoding of some of the conditions for VTSs. Especially, we have recursively defined the reachability condition such that that we avoid the inefficient enumeration. We have implemented the encoding using Z3 [15] python API and searched for VTS satisfying various properties up to size 14-18 nodes as compared to earlier tool that could only search graph up to size 6 nodes. Our tool is useful to cell biologists since VTSs of real cells have approximately 10 compartments.

The following are the contributions of this work:

– Novel encodings of reachability and 3-4 connectivity
– Direct encoding into the SMT solver
– A user friendly and scalable tool based on well known SMT solver Z3

4 The original experiments in [5] scaled up to 8. However, our current experiments only scale up to 6, because we are using less powerful machine.
2 Biological Problem

In this section, we will describe the basic constraints imposed by cell biology. These are all incorporated into an abstract model of a VTS, whose properties will then be explored using SMT solvers.

The cell as a transport graph: We consider a cell to be a collection of compartments (nodes) and vesicles (edges), thus defining a transport graph. Every compartment or vesicle has a set of molecular labels, such as SNARE proteins, associated with it.

Molecular flows and steady state: Each edge is associated with a flux of all the molecular types carried by the corresponding vesicle. The total amount of each molecular type on each compartment can therefore increase or decrease. We assume the cell is in a steady state where each compartment’s composition does not vary over short time scales. Therefore, incoming and outgoing fluxes are balanced for each molecular type at each compartment; it is the stability condition.

Vesicle targeting driven by molecular interactions: Once a vesicle has budded out of the source, the molecules it carries determine its properties. In particular, for any given pair of a vesicle and a compartment, the set of SNARE proteins that label the former and latter influence whether the vesicle will fuse to that compartment. Biophysically, fusion requires a direct physical interaction between at least one SNARE type on the vesicle and one SNARE type on the compartment. SNAREs fall into two classes (known as Q and R in the cell biology literature) and vesicle fusion requires a pairing of a Q-SNARE with an R-SNARE. The list of molecular pairs that can drive a fusion event is given in a pairing matrix between Q and R SNAREs. Without loss of generality we assume equal numbers of Q and R SNARE types.

Molecular regulation: We assume that for fusion to occur, the pair of SNARE types involved on the vesicle and compartment must both be in an active state. Whether these SNAREs are active or inactive depends on the other molecules found on the vesicle or compartment, respectively. We test many different versions of this kind of molecular regulation. Most generally, the activity state of a given SNARE can be a Boolean function of all the molecular types on a compartment or vesicle. We have also tested a particularly simple regulation mechanism in which two SNAREs that can pair to drive fusion inhibit one another; this is the pairing inhibition. This is motivated by the idea that pairing must generate an inactive bi-molecular complex.

Properties of a VTS that satisfies all cell-biological constraints: Suppose we are given a particular transport graph, a particular labeling of all the compartments and edges, a particular fusion pairing matrix, and a particular regulatory model. This information is then sufficient to check the following properties, which summarise the cell-biological constraints described above:

1. We can determine which molecules are active on every compartment or vesicle.
2. For every vesicle fusing to a compartment, we can determine whether there exists an active pair (one molecule on the vesicle, one on the compartment) which drives that fusion event.

3. For every vesicle-compartment pair where the vesicle does not fuse to the compartment, we can verify that there is no pairing of active molecules on the vesicle and compartment that could drive their fusion.

4. We can verify that every molecular type entering a compartment also leaves the compartment, and also that every molecular type entering a set of compartments also leaves that set. This is the steady state condition. In the biological literature this is often referred to as “homeostasis” and is a widespread and well-accepted assumption about cellular behaviour, at least over timescales of minutes to hours \[4\].

The biological problem often boils down to such an analysis. A cell biologist might determine which molecules flow between which set of compartments, and biochemical experiments could be used to see how these molecules activate one another. We can then ask: is this a complete and consistent description? That is, do all the required properties listed above hold, given what the experimentalists have told us? It is often the case that biological data is missing. For example, only a few of the dozens of molecules involved in real VTSs have been mapped out. Therefore, it is extremely likely that the description provided by the cell biologist is incomplete. We can use our model to find out which properties have failed to hold, and thus prescribe new experiments in order to fill in the missing information.

Can we find a simple test to see whether any information is missing, given the experimental data? We have shown that graph k-connectedness furnishes precisely such a test \[5\]. If the data provided by cell-biologists, suitably represented as a graph, does not have a certain degree of connectivity, this implies that some biological data has been missed. (The converse is not true: even if the required connectivity does hold, there might of course be more information missing.)

Our result about k-connectedness being a useful test of missing information \[5\] was obtained using SAT solvers for graphs up to a certain size, and a certain number of molecules. This was due to limitations in how we encoded the problem. Here we present a much more natural encoding that allows our result to be extended to graph sizes and molecule numbers that are typical of those found in real cells.

Since a VTS is a transport graph, it is but natural to formally model VTS as graphs (as used in computer science) with their nodes denoting compartments and labeled edges denoting transport vesicles with labels denoting the set of molecules being transported. The pairing mechanism can be represented as matching tables over sets of molecules. Given such a graph model of VTSs and their properties, such as stability condition and fusion rules, can be formally defined as constraints over graphs and uninterpreted Boolean functions.

A VTS is \(k\)-connected if every pair of compartments remain reachable after dropping \(k - 1\) vesicles. This property of VTSs has been considered informative
and studied by [5]. Here we have build an efficient tool that studies properties of VTSs that are not $k$-connected, from some $k$.

**Example 1.** In figure 1 we present a VTS that has 3 compartments and 8 molecules, and a corresponding pairing matrix. Molecules are numbered 0-7. In the VTS, labels are a string of molecule ids, and an overline over an id indicates that the molecule is active. Every molecule on the node is active. The activity of the molecules on an edge are controlled by presence of the other molecules on the edge. On the right side of the figure, we show the pairing matrix. An entry 1 represents pairing between molecules. $\times$ represents no pairing. Rows corresponds to the labels of edges, and columns corresponds to the labels of nodes. Every molecule flows on a cycle, ensuring steady state. This is a 3-connected graph.

3 The Problem Encoding

In this section, we will formally define VTSs as a labeled graph and the discussed conditions on the graphs. Subsequently, we will present the SMT encoding of the search problems for the graphs that satisfy the conditions.

3.1 Model

We model VTSs as labeled directed graphs. The graph labels both nodes and edges with sets of molecules to denote the set of molecules present in them. The graph is formally defined as follows.

**Definition 1** A VTS $G$ is a tuple $(N, M, E, L, P, g, f)$, where

- $N$ is a set of nodes representing compartments in the VTS,
- $M$ is the set of molecules flowing in the system,
We call $VTS$ has no self loops, and each edge carry only those molecules that are present constraints corresponding to the properties. Used to encode the $VTS$s and the properties. Afterwards, we will present the SMT solvers to find the satisfying $VTS$s. We will first present the variables

$3.2$ Boolean satisfiability of the search problem

Based upon earlier discussion, we need to answer the following search question among $VTS$s. For a given $k$, size $\nu$, and molecule number $\mu$, we are searching for well-structured, stable, and well-fused $VTS$ $G = (N, M, E, L, P, g, f)$ such that $|N| = \nu$, $|M| = \mu$, and $G$ is not $k$-connected.
Variables for VTS description

We assume that size of the graph is \( \nu \) and number of molecules is \( \mu \). Furthermore, we also limit the maximum number \( \pi \) of edges present between two nodes. Here, we list the vector of Boolean variables and uninterpreted function symbols that encode the VTSs.

1. Boolean variable \( n_{i,m} \) indicates if \( m \in L(i) \)
2. Boolean variable \( e_{i,j,q} \) indicates if \( q \)th edge exists between \( i \) and \( j \).
3. Boolean variable \( e_{i,j,q,m} \) indicates if \( q \)th edge between \( i \) and \( j \) contains \( m \).
4. Boolean variable \( p_{m,m'} \) indicates if \( (m, m') \in P \)
5. uninterpreted Boolean functions \( f_m : \mathbb{B}^\mu \rightarrow \mathbb{B} \) encoding \( f(m) \) map
6. uninterpreted Boolean functions \( g_m : \mathbb{B}^\mu \rightarrow \mathbb{B} \) encoding \( g(m) \) map

We also have auxiliary Boolean variables that will help us encode the well-fused and stability properties

1. \( a_{i,m} \) indicates that molecule \( m \) is active at node \( i \), i.e., \( f(m, L(i)) \) holds
2. \( b_{i,j,q,m} \) indicates that molecule \( m \) is active at \( q \)th edge \((i, M', j)\) between \( i \) and \( j \), i.e., \( g(m, M') \) holds
3. \( r_{i,j,m,p} \) indicates if there is an \( m \)-path from \( i \) to \( j \) of length less than or equal to \( p \)

For \( k \)-connected property, we also use the following auxiliary Boolean variables

1. \( d_{i,j,q} \) indicates \( q \)th edge between \( i \) and \( j \) is dropped
2. \( r'_{i,j} \) indicates if there is a path from \( i \) to \( j \) in the modified VTS

We will describe the Boolean constraints that encode VTSs in several categories. In the end, we will present in a table the constraints needed for the model variants. To avoid cumbersome notation, we will not explicitly write the ranges of the indexing in the constraints. \( i \) and \( j \) will range over nodes. \( m \) will range over molecules. \( q \) will range over edges between two nodes, i.e., from 1 to \( \pi \).

Constraints on presence, activity of the molecule, and pairing matrix

We need the following constraints \([V1]\) and \([V6]\) to encode the basic structure of VTSs. For an edge to exist it should have one molecule present.

\[
\bigwedge_{i,j,q,m} \left( e_{i,j,q,m} \right) \Rightarrow e_{i,j,q} \quad (V1)
\]

If a molecule is active on an edge, it should be present on the edge.

\[
\bigwedge_{i,j,q,m} b_{i,j,q,m} \Rightarrow e_{i,j,q,m} \quad (V2)
\]

A molecule should be present to be active on a node.

\[
\bigwedge_{i,m} a_{i,m} \Rightarrow n_{i,m} \quad (V3)
\]
The edge labels are subset of the node labels of the source and target nodes.

\[ \bigwedge_{i,j,q,m} e_{i,j,q,m} \Rightarrow (n_{i,m} \land n_{j,m}) \]  

(V4)

Self edges are not allowed.

\[ \bigwedge_{i,q} \neg e_{i,i,q} \]  

(V5)

We fix first half as Q-SNAREs and rest as R-SNAREs and set diagonal blocks in pairing matrix to be 0’s.

\[ \bigwedge (x<M/2 \land y<M/2) \lor (x>M/2 \land y>M/2) \neg p(x, y) \]  

(V6)

**Well-fused constraints** Constraint (V7) encodes that each edge must fuse with its destination node. Constraint (V8) encodes that each edge should not be able to potentially fuse with any node other than its destination node.

\[ \bigwedge_{i,j,q} e_{i,j,q} \Rightarrow \bigvee_{m,m'} (b_{i,j,q,m} \land a_{j,m'} \land p_{m,m'}) \]  

(V7)

\[ \bigwedge_{i,j,q,m} b_{i,j,q,m} \Rightarrow \neg \bigvee_{j \neq j',m''} (a_{j',m''} \land p_{m,m''}) \]  

(V8)

**Regulation on nodes and edges** We are considering several models that differ in constraints on the activity of molecules. We will present (Ann) - (Aep) that encodes the varying constraints. The following constraint encodes no conditions on activities on nodes, i.e., all the present molecules on the nodes are active.

\[ \bigwedge_{i,m} n_{i,m} = a_{i,m} \]  

(Ann)

The following constraint encodes that activity of a molecule \( m \) on the node is defined by a Boolean function \( f_m \) of presence of molecules present on that node.

\[ \bigwedge_{i,m} n_{i,m} \Rightarrow a_{i,m} = f_m(n_{i,1}, \ldots, n_{i,\mu}) \]  

(Amb)

The following constraint encodes no conditions on activities on edges, i.e., all the present molecules on the edges are active.

\[ \bigwedge_{i,j,q,m} e_{i,j,q,m} = b_{i,j,q,m} \]  

(Aen)

The following constraint encodes that activity of a molecule \( m \) on the edge is defined by a Boolean function \( g_m \) of presence of molecules present on that edge.

\[ \bigwedge_{i,j,q,m} e_{i,j,q,m} \Rightarrow b_{i,j,q,m} = g_k(e_{i,j,q,1}, \ldots, e_{i,j,q,\mu}) \]  

(Aeb)
The following constraint encodes that the activity of the molecules on edges is defined by inhibition by other molecules based on the pairing matrix.

\[ \bigwedge_{i,j,q,m} \left[ e_{i,j,q,m} \Rightarrow \bigwedge_{m' \neq m} \left( p_{m,m'} \Rightarrow e_{i,j,q,m'} \right) \right] \iff \left( \neg b_{i,j,q,m} \land \bigwedge_{m' \neq m} \neg b_{i,j,q,m'} \right) \text{ (Aep)} \]

**Constraints for stability condition** We use \( m \)-reachability to encode the stability condition in VTSs. The following constraint recursively encodes that node \( j \) is \( m \)-reachable from node \( i \) in less than \( p \) steps if either there is a direct edge between \( i \) and \( j \) with \( m \) present on the edge or there is an edge between \( i' \) and \( j \) with \( m \) present, and \( j \) is \( m \)-reachable from \( i' \) in less than \( p-1 \) steps.

\[ \bigwedge_{i,j,m,p} r_{i,j,m,p} \Rightarrow ( \bigvee_{q} e_{i,j,q,m} \lor \bigvee_{i' \neq i} ( \bigvee_{q} e_{i,i',q,m} \land r_{i',j,m,p-1} ) ) \text{ (R1)} \]

Now we can encode stability using the reachability variables, and say if there is an \( m \)-edge between \( i \) and \( j \), there is \( m \)-reachable path from \( j \) to \( i \).

\[ \bigwedge_{i,j,m} ( \bigvee_{q} e_{i,j,q,m} ) \Rightarrow r_{j,i,m} \text{ (R2)} \]

**\( k \)-connectivity constraints** To check whether \( k \)-connected is a necessary condition, we remove (drop) \( k-1 \) edges from the graph and if it disconnects the graph, and we get an assignment. We have a graph that is not \( k \)-connected. Constraints \( \text{D1-D4} \) encode the relevant constraints for reachability in the modified VTS. The following constraints encode that only existing edges can be dropped and exactly \( k-1 \) edges are dropped.

\[ \bigwedge_{i,j,q} d_{i,j,q} \Rightarrow e_{i,j,q} \text{ (D1)} \]

\[ \sum_{i,j,q} d_{i,j,q} = k - 1 \text{ (D2)} \]

We need to define reachability in the modified VTS, therefore we use a new variable \( r'_{i,j} \) to encode reachability from \( i \) to \( j \). In the following constraint, we encode \( r'_{i,j} \) is true if there is an edge \((i, j)\) and it is not dropped, or there is a node \( i' \) such that, there is an edge \((i', j)\) which is not dropped and \( r'_{i',j} \) is true.

\[ \bigwedge_{i,j} ( e_{i,j,q} \land \neg d_{i,j,q} ) \lor ( \bigvee_{i' \neq i} r'_{i',j} \land \bigvee_{q} ( e_{i,i',q} \land \neg d_{i,i',q} ) ) \Rightarrow r'_{i,j} \text{ (D3)} \]

Since we search for disconnected modified VTS, the following constraint encodes that there are unreachable pair of nodes in the underlying undirected graph.

\[ \bigvee_{i,j} \neg (r'_{i,j} \lor r'_{j,i}) \text{ (D4)} \]
The key improvement in our work over the earlier tool is the encoding of reachability, which was done using enumeration of paths. In the current work, we have encoded reachability in two different ways in constraints (R1) and (D3). The reachability is recursively defined in (D3) and has trivial solutions by making all \( r \)'s true. However, the trivial solutions are disallowed by constraint (D4) and we find only the solutions that captures the evidence of unreachability. On the other hand, we have added length of paths in our reachability encoding in constraint (R1), which needs relatively more auxiliary variables. This is because constraint (R2) has only positive occurrences of the reachability variables and if we had defined \( r \)'s in (R1) without paths, the circular dependencies in the recursive definitions of \( r \)'s may have resulted in spurious satisfying assignments that do not encode reachability. By adding the path length, we break the circular dependencies, the constraint remains polynomial in size, and satisfying assignments only correspond to correct reachability.

3.3 SMT problems for different variants

We have encoded the following variants of VTSs. The variants are due to different combinations of constraints on the activity of molecules on the nodes and edges.

A. Every present molecule is considered to be active.
B. Activity of molecules on the nodes is based on Boolean function of presence of other molecules.
C. Activity of molecules on the edges is based on Boolean function of presence of other molecules.
D. Activity of molecules both on the edges and nodes is based on Boolean function of presence of other molecules.
E. Activity of molecules on the edges is driven by pairing inhibition.
F. Activity of molecules on the nodes is based on Boolean function of presence of other molecules and on edge by pairing inhibition.

In the table we present the constraints involved in each version. The column two of the table shows that the constraints V1-V8, R1-R2, and D1-D4 are present for every variant. The column three of the table lists the constraints that are different among the variants. One of the restriction is where the activity of the present molecule is dependent on the presence of the other molecules. For example in version B, D, F activity of the molecules on the node is a boolean function of the presence of other molecules on that node; Anb. Similarly for the case of the edge in version C and D; Aeb. In the case of versions F the activity of the molecules on the edges is described by pairing matrix; Aep.

The constraints for the variants can be given to a SMT solver to find VTSs that belong to the variants.

4 Implementation and Experiments

We have implemented the encodings for each variants using the python interface of Z3 in a tool (MAA). Our tool allows the user to choose a model and the size
of the network besides other parameters like connectivity and number of parallel edges. It uses Z3 Python interface to build the needed constraints and applies Z3 solver on the constraints to find a model (a satisfying assignment that respects the constraints). This tool also translates the satisfying model found by Z3 into a VTS and presents a visual output to the user in form of annotated graph. We also visually report the dropped edges required to disconnect the graph, it gives information about the connectivity of the graph.

To illustrate usability of our tool in the last column of the table 1 we present the minimum connectedness needed for the different variants after applying our tool for sizes from 2 to 10. We found no graph for the variant A with constraints Ann and Aen. Replacing constraint on the node of Ann with Anb (variant B) does not affect the outcome. If we allow every present molecule to stay active (Ann) but constraint the edge by a boolean function (Aeb) the resultant VTS has to be at least 3-connected. Similarly, the results for the other cases are presented in the table.

In Table 2 we present the running times for the search of VTSs of sizes 2 to 10 that satisfy the variants, and compare with our old encoding (Old-e) from 5. For the comparison between both encodings we have fixed the total number of molecules to be $|M| = 2|N|$ for $|N| > 2$ and $|M| = 2|N| + 1$ for $|N| = 2$. For each variant, we fix maximum number of parallel edges to 2. In the

| Variant | Constraints | Graph connectivity |
|---------|-------------|---------------------|
| A.      | Ann Aen    | No graph            |
| B.      | V1 V8      | Ann Aen             |
| C.      | R1 R2      | Ann Aeb             |
| D.      | D1 D4      | Anb Aeb             |
| E.      | Ann Aep    | No graph            |
| F.      | Anb Aep    | 4-connected         |

Table 1: Activity regulation of molecules vs. graph connectivity.

| Size | Variant A | Variant C | Variant D | Variant F |
|------|-----------|-----------|-----------|-----------|
|      | 2-connected | 3-connected | 2-connected | 4-connected |
| MAA  | Old-e     | MAA | Old-e | MAA | Old-e | MAA | Old-e |
| 2    | 10.085 | 12.43 | 0.15 | 2.12 | 10.13 | 11.89 | 0.35 | 5.12 |
| 3    | 30.54  | 8.04  | 0.95 | 7.65 | 0.62  | 7.66  | 1.36 | 23.94 |
| 4    | 12.57  | 2297.93 | 2.33 | 22.74 | 2.85 | 48.35 | 4.81 | 123.34 |
| 5    | 7.17   | 3053.8 | 7.60 | 500.03 | 10.27 | 890.84 | 33.36 | 2482.71 |
| 6    | 122.98 | M/O   | 19.52 | M/O   | 30.81 | M/O   | 147.52 | M/O   |
| 7    | 57.07  | M/O   | 81.89 | M/O   | 82.94 | M/O   | 522.26 | M/O   |
| 8    | 164.14 | M/O   | 630.85 | M/O   | 303.19 | M/O   | 2142.76 | M/O   |
| 9    | 307.67 | M/O   | 2203.45 | M/O   | 971.01 | M/O   | 4243.34 | M/O   |
| 10   | 558.34 | M/O   | 7681.93 | M/O   | 2274.30 | M/O   | 7786.82 | M/O   |

Table 2: Run-times for searching for models (in secs).
table we have shown comparison for specific connectivity, for example variant A is checked against any graph with connectivity 2, variant B with connectivity 3 similarly for the rest of the Variants.

The experiments were done on a machine with Intel(R) Core(TM) i3-4030U CPU @ 1.90GHz processor and 4GB RAM. We have compared our performance with the performance of our earlier CBMC based implementation (old-encoding). For example, the formula for variation F, the total number of compartments ($|N|$) equals to 10, returns in 129.78 minutes (7786.8 secs) with a SAT result. In comparison, CBMC results in OUT OF MEMORY for $|N|$ greater than 5. “!” indicate that the constraints were unsatisfiable. Using this encoding in comparison to the old one, not only we got efficiency improved for finding a SAT model but also did better in the case of refutation that no model exist (Table 2 Variant A timing comparison and Variant D with N =2). Hence with the use of this novel encoding, we are able to scale the system to a much larger compartmentalized system, especially to eukaryotic cells with a total number of 10 compartments. Furthermore, we experimented with limits of our tools and found that Z3 was able to solve the constraints up to $\sim 14 – 18$ nodes.

5 Related Work

Modern day SAT/SMT solvers can handle millions of variables and due to the exhaustive nature of the searching, solving the combinatorial problem is a natural fit for the SAT/SMT solvers. There are many graphs related combinatorial work [16, 17] that have used SAT solvers. Thanks to the improvement in SAT solver and formal techniques, tools like model checkers and theorem provers scalability and combinatorial solving capability are now being used to model complex biological systems [18–20]. Many of these biological system uses these tools and techniques to reason about graphs and networks rules with possible exhaustive search [21, 22].

Recently these tools and techniques have found the use of modeling and understanding the gene regulatory networks (GNR) [23, 24]. GNR is a complex system driven by many complex rules. We extended this work and have applied model checkers to the more complex transport network (VTS) [44], but scaling it to cases which are interesting biologically is still a challenge. In this paper, we have achieved the scalability required for analyzing VTS for eukaryotic cells, of total ten compartments (N = 10).

6 Conclusion

In the experiments, we have demonstrated the power of SMT solvers and the value of careful encoding of the problems into SMT queries. We manage to scale the tool up to the size that makes tool biologically relevant. However, there are many further search problems or extensions that are of interest. For example, are there any k-connected graphs that can not be annotated into a VTS? This
problem induces a quantifier alternation in an encoding. Therefore, a simple call to SAT solver will not work. We are planning to use QBF (quantified Boolean formulas) solvers or develop iterative search algorithm for such queries.

One may be interested in counting the number of graphs that satisfy a given property. Exact counting of the graphs using SAT solvers may prove to be very difficult. We are also planning to employ some methods for approximate counting of solutions.

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