Identifying (un)controllable dynamical behavior with applications to biomolecular networks

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This manuscript was compiled on December 15, 2017

Detailed features of complex systems (e.g., precise variable values) are sometimes of secondary importance when considering system interventions. This is especially true in systems that are subject to a large degree of uncertainty. Instead, qualitative features (e.g., whether a set of variables is "high" or "low") often determine real-world control strategies, particularly in biological systems. This has inspired a great deal of work in qualitative modeling. Nevertheless, quantitative dynamical models can be required to accurately predict qualitative behavior. In these cases, a method for determining which qualitative features are robust to external controls and internal perturbations is desired. We present a new method to meet this need that generalizes a concept known as *stable motifs* from the analysis of Boolean networks to a large class of dynamical systems. This new method uses an auxiliary *expanded network* to help identify self-sustaining behavior and inform system control strategies. We demonstrate how to implement this approach on an example and illustrate its utility for two published biological network models: the segment polarity gene network of *Drosophila melanogaster*, and the T-cell signaling network.

Dynamical Systems | Network Control | Stable Module | Stable Motif | Expanded Network

A key goal in the study of complex dynamical systems is to extract important qualitative information from models of varying specificity (e.g., (1, 2)). This has been approached via the construction of qualitative models (e.g., discrete models (3–6)) and also by analytic techniques (e.g., enumerating qualitatively distinct regions of parameter space for generalized mass-action systems (7)). In this work, we describe a generalization of a technique for qualitative models that is applicable in any dynamical framework.

Many of the qualitative features one might wish to identify in the repertoire of a dynamical system arise from feedback loops in the associated regulatory network. Indeed, recent research has highlighted the importance of feedback loops in the control of dynamical systems (8–12). For example, control of any feedback vertex set (i.e., a set of variables whose removal eliminates all feedback) and of any source variables is sufficient to drive an ODE-described system into any of its natural attractors, provided these ODEs meet some relatively permissive criteria relating to continuity and boundedness (8, 10, 13).

Positive feedback loops in particular are associated with important qualitative information about a system, such as the presence of multistability (14–17). Multistability has been of particular interest in biomolecular systems because it is necessary for cell-fate branching and decision making (4, 18–21). Two approaches to identifying the effects of positive feedback loops are especially relevant here. The first of these is the methods put forth by Angeli and Sontag for studying monotone input-output systems (MIOS) (22). Their approach identifies steady states in sign-consistent monotone systems (those lacking negative cycles or incoherent feed-forward loops) and determines the stability of these states. The second is based on the concept of *stable motifs* of Boolean dynamical systems (11, 23). The method relies on the construction of an auxiliary network to topologically encode the regulatory logic. Within this auxiliary network, a strongly connected component (a set of nodes reachable by all its members, abbreviated SCC) obeying certain criteria corresponds to a self-sustained state of an associated subsystem (see SI Text or (5, 23) for details). Here, we propose an extension of this latter methodology that is applicable in any dynamical framework. Our framework allows one to identify dynamical modules that are self-sustaining and robust to disruption by variables not included in the module, even when these are subject to external control. We also show that MIOS techniques can be leveraged to specify the dynamical modules in systems with monotonic and bounded regulatory functions and linear decay, which are common in biology (see SI Text).

Stable Motifs in Expanded Networks

The core of our analysis strategy lies in the interpretation of an auxiliary network that is constructed from the dynamical system of interest. Following previous work in Boolean systems (3, 24), we call this auxiliary network an *expanded network*. In an expanded network, there are two types of nodes: virtual nodes and composite nodes. Virtual nodes are statements about the values of dynamic variables and can be assigned a

Significance Statement

Qualitative dynamical features of biological systems have been analyzed using Boolean models, which describe system elements as either "active" or "inactive". Existing techniques from Boolean modeling allow for the identification of self-sustaining subsystems. Here, we extend such methods beyond Boolean models and show how these new methods can be used to identify subsystem behaviors that are robust to outside interventions. This informs potential system control strategies (e.g., in identifying drug targets). We apply our methods to two quantitative biological models. This methodological framework enables development of new analysis techniques and system attractor control methods such as identification of control-robust attractor basins for oscillations.

JR performed the analysis under the advice and supervision of RA. Both authors prepared the manuscript.

The authors declare no conflicts of interest.

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definite truth value at any given time (e.g., the virtual node "$x > 0$" is true only when the value of the variable x is positive). Composite nodes also take Boolean values, and correspond to the composition of virtual nodes by "AND" ($\wedge$) rules. Each composite node receives directed edges from each of its factors in the network. As such, all factors of a composite node must be represented as virtual nodes in the expanded network. For example, the composite node $x > 0 \wedge y > 0$ is true only when x and y are positive, and there are directed edges from $x > 0$ and $y > 0$ to this composite node. In deterministic finite-level systems, it is possible to choose a finite number of statements that fully characterize the dynamics (25), but in general, the nodes of an expanded network embody partial information about the system.

If a virtual node, Y, cannot change from true to false while a (virtual or composite) node, X, is true, we say that X *maintains* Y and we draw a directed edge from X to Y in the expanded network. If we also require that an edge from X to Y implies that the truth of X implies the truth of Y in finite time, we can use the expanded network to explore causal relationships among system elements; this additional constraint is not necessary if we are only interested in self-sustaining behavior. In determining whether X maintains Y, we must consider all physically valid values of the regulators of Y (subject to the constraint that X and Y are true). The variable values we consider form a region in state-space that may not be fully accessible except when external control is applied. This region of state space is the region of validity for the expanded network analysis. We take this region to be as large as is reasonable because it provides stronger results and allows us to identify edges without detailed knowledge of the dynamical trajectories.

Any set of virtual and composite nodes with edges placed according to the conditions above constitutes an expanded network. A sub-network, S, of an expanded network, N, is a *stable module* if it satisfies three conditions: (i) all nodes X in S have a parent (regulator) node in S (possibly X itself if it has a self-loop), (ii) if a composite node $X = \bigwedge_{i=1}^{n} X_i$ is in S, then X_i is also in S for $i = 1..n$, and (iii) none of the nodes in the sub-network are logically exclusive (i.e., it is possible for all sub-network nodes to be simultaneously true). Our key result is the following: if all nodes in a stable module are simultaneously true, then they remain true under all state-space configurations in the region of validity.

To see why this is true, consider a virtual node, Y, in the stable module with the property that no node becomes false before it does. Because we have assumed that Y is part of a source-free component of true nodes, it must have a parent node, X, that is also true. By the conditions for edge placement (i.e., because X maintains Y), Y cannot become false unless X becomes false first, which is impossible if $X = Y$ and if $X \neq Y$, then this would contradict the assumption that no node becomes false before Y. Therefore, no node in the motif can become false if all are true at any given time.

If a stable module, S, is strongly connected and contains no strongly connected stable modules other than itself, then we say that S is a *stable motif*. Every variable represented by a node in a stable motif participates in feedback in the underlying dynamics. Variables represented in stable modules, however, might only be driven by feedback loops in which they do not participate. Stable modules can therefore be viewed as a union of stable motifs and downstream nodes that are maintained by one or more SCC. We remark that this definition of stable motifs encompasses the definition of stable motifs given in (23) in the case of Boolean systems (see SI Text for details). The reduction to the Boolean case follows immediately if for each Boolean variable x, we create a pair of virtual nodes $X \equiv (x = \text{True})$ and $\overline{X} \equiv (x = \text{False})$, along with any composite nodes that appear in the Boolean update functions written in disjunctive normal form. The edges in the resulting expanded network correspond exactly to the prime implicants of the Boolean update functions. Furthermore, the definition of stable motifs in Boolean dynamical systems ((23)) is identical to our definition. A similar specialization can be made to recover the multi-level stable motif results of (25). This correspondence allows us to generalize many results from Boolean modeling to general dynamical systems.

In particular, generalizing arguments in (11), we consider system control via expanded network topology. It is often of interest to identify variables to control such that a stable module (which may correspond, e.g., to a healthy cell state) is activated. This can be achieved by solving the topological problem of identifying stable motif *driver nodes*. A module *driver node set* D of module M in an expanded network is a set of virtual nodes D such that the truth of all nodes in D implies the truth of all nodes in M in finite time. Therefore, identification of a driver node set for a stable module prescribes a control strategy to trigger the module behavior. Conversely, if a stable module represents undesired behavior (e.g., a diseased cell state), one might seek to disrupt it. Because stable modules are self-sustaining, control of variables not represented in the undesired module can never achieve this goal. Disruption of a stable module requires direct control of at least one of its represented variables.

To illustrate the method, and some of its utility, we analyze a toy example (Figure 1, Equation 1).

$$\begin{aligned} \dot{u} &= \frac{1}{1+z} - u^3 \qquad \dot{w} = y - w/2 \qquad \dot{x} = \frac{w+z}{w+1/2} - x \\ \dot{y} &= \frac{x}{x+1/2} - y \qquad \dot{z} = x f(y) - z \qquad f(y) \geq f_{min} > 0 \end{aligned} \quad [1]$$

Here, we have very limited information about $f(y)$; perhaps it is stochastic or discontinuous. Nevertheless, we see that if x is positive, and greater than $\frac{1}{2}$ in particular, z will eventually become and remain positive. We can therefore conclude that there is an edge from $x > \frac{1}{2}$ to $z > 0$ in the expanded network. Similarly, we see that x will be maintained above $\frac{1}{2}$ if $w > \frac{1}{2}$ and $z > 0$ are both true. We therefore identify a composite node $\left(w > \frac{1}{2}\right) \wedge (z > 0)$ with incoming edges from $w > \frac{1}{2}$ and $z > 0$, and an outgoing edge to $x > \frac{1}{2}$. We continue to identify edges in the expanded network and search for stable modules. Some of the subgraphs of the expanded network that can be generated in this way are depicted in Figure 1 alongside the traditional network representation of the system. We have identified three stable modules, thereby proving, for example, that if the systems satisfies $x, y, w, z > 0$ at any time, it will always satisfy those conditions (as follows from the yellow module in the bottom left of Figure 1). The other two modules contain $x, y > \frac{1}{2}$ and $z > 0$ as well as either $w > 1$ or $w > \frac{1}{2}$. Thus, if the system satisfies the four conditions given by either module, it will continue to do so for all time. The

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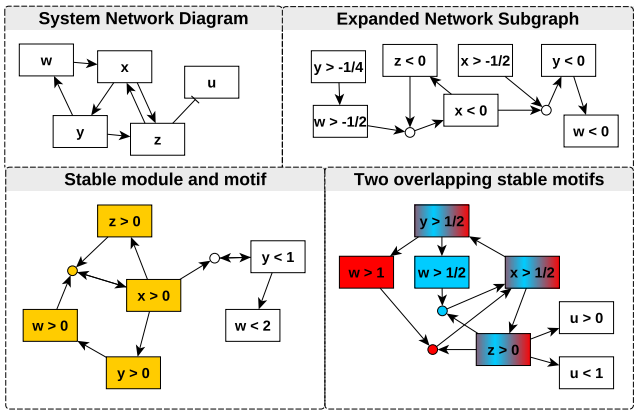

Fig. 1. A network representation of the system given in Equation 1 along with three subgraphs of the expanded network. Each circular node represents a composite node formed by composition of its parent nodes by an "AND" rule. Highlighted components are stable motifs (and therefore also stable modules). These represent conditions that, once satisfied, remain satisfied. In the overlapping motifs (marked in blue and red), we may choose to consider the motif containing $w > 1$ (red), which gives more information about the value of w when the motif is realized, or we may consider the $w > 1/2$ (blue) motif, which is more readily realized. By considering both motifs together, we see that the $w > 1/2$ (blue) motif drives the $w > 1$ (red) motif, i.e., states satisfying $w, x, y > \frac{1}{2}$ and $z > 0$ will eventually also satisfy $w > 1$. We remark also that the stable motifs shown could be expanded to incorporate the other nodes depicted in the components. Such structures are stable modules and are also self-sustaining, but include nodes that are not necessarily part of any feedback loop; they are instead driven by feedback elsewhere in the network.

arguments underlying the construction of the subgraphs of the expanded network hold for any $f(y) > f_{min} > 0$, and so we have extracted meaningful qualitative information despite large dynamical uncertainty. In addition to the subgraphs containing stable modules, many subgraphs that do not contain stable modules also exist. Such subgraphs contain information regarding the consequences of directly controlling particular nodes so that they satisfy virtual node statements (e.g., if we fix $y < 0$, we see that w will eventually become negative).

Choosing virtual nodes defined by inequalities, as is our main focus here, has important implications for how oscillations are observed. If a variable oscillates, but remains above or below some threshold, the statement indicating the variable value relative to that threshold can be part of a stable module. Oscillations can also manifest in the expanded network as SCCs that include contradictory virtual nodes.

The main difficulty in identifying stable modules is determining what statements are most useful for inclusion in the expanded network. If the statements are too general, then either the results will not provide much insight, or the network will be too sparse because the statements are not sufficiently restrictive to imply one another. If a statement is too restrictive, on the other hand, it may have an in-degree of zero in the expanded network, in which case it cannot be part of a stable motif. Despite these challenges, we have found a straightforward approach to analyzing threshold behavior of a large class of biologically relevant systems.

Application to Biological Systems

We consider a broad class of dynamical systems that take the form

$$\tau_i \dot{x}_i = F_i(\boldsymbol{x}) - x_i, \qquad [2]$$

where F_i is continuous, monotonic in each of its arguments, and bounded. This class of dynamical systems is well suited to describe biological systems (see SI Text). The special case in which F_i is independent of x_i is especially amenable to our approach. This is because when all other variables are held fixed x_i will approach the fixed value $F_i(\boldsymbol{x}_0)$. The monotonic property of F_i allows us to place bounds on x_i as follows (see SI Text for an example).

To each variable x_i, we assign a set of intervals $\{T_i^\alpha\}$ where T_i^α is of the form $(\inf F_i, a_i)$ or $(b_i, \sup F_i)$. At this stage, it is not necessary to specify the precise bounds of each T_i^α, i.e., these intervals may be parameterized. Furthermore, it is possible that the intervals T_i^α do not cover the full dynamical range of x_i. We consider virtual nodes $x_i \in T_i^\alpha$. We create composite nodes $\bigwedge_{k=1}^{m} \left(x_{i_k} \in T_{i_k}^{\alpha_k}\right)$ as needed. Next, we conjecture that particular edges exist in the expanded network for some (unspecified) choice of threshold parameters. For instance, when activity of one variable, x_1, is sufficient for activation of another, x_2, we would hypothesize the formation of an edge $x_1 \in (a, \sup F_1) \rightarrow x_2 \in (b, \sup F_2)$. This process generally requires intuition, experimentation, or separate analysis, but for monotonic regulation of positive quantities, it is usually not difficult to conjecture relationships between nodes of the given form. In the conjectured expanded network, consistent source-free components with all parents of member composite nodes are *potential* stable modules.

What remains is to choose bounds for the virtual nodes (if possible) to ensure that the potential stable modules are genuine. For this task, we employ the monotone input-output systems (MIOS) methods of Angeli and Sontag (22). Following Sontag (9), these techniques are applicable to systems in which we can assign $\sigma_i = \pm 1$ to each node of the associated regulatory network such that the product $\sigma_i \sigma_j$ is 1 whenever nodes i and j are connected by a positive edge, and -1 whenever they are connected by a negative edge. Such networks are called *sign-consistent* networks, and are networks representing systems for which the effect of each variable on any downstream variable is unambiguously monotonic. This implies that there are no negative feedback cycles and no incoherent feed-forward loops in the regulatory network. By identifying potential stable modules, we are able to apply these methods even when sign inconsistencies are present in the regulatory network. To do this, we consider a candidate module in the expanded network and the corresponding regulatory subnetwork, which consists of the nodes represented in the candidate module along with all incident edges. If edges not represented in the candidate module are removed, the resulting modified regulatory subnetwork is necessarily sign consistent; nodes represented by virtual nodes of the form $x_i \in (\inf F_i, a_i)$ can be assigned $\sigma_i = -1$ and all others can be assigned $\sigma_i = 1$. This is because the only sources of negative regulation in the modified subsystem arise from nodes this first type, which always contribute negative edges to the modified regulatory subnetwork in pairs.

To construct the sign-consistent modified subsystem for a candidate module, any regulation of x_i by x_j not represented in the module, is held fixed by replacing x_j with a "worst-case" value in F_i. The "worst-case" value is chosen such that x_i is as close as possible to a_i or b_i as determined by the motif node T_i^α; in the systems under consideration, this is either $x_j \equiv \inf F_j$ or $x_j \equiv \sup F_j$. For example, if x_j negatively

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regulates x_i and $x_i \in (\inf F_i, a_i)$ is in the module then we evaluate $F_i\big|_{x_j=\inf F_j}$.

In the resulting modified subsystem, we can follow the procedure of Angeli and Sontag (9, 22) to find the steady states. These steady states determine the thresholds a_i and b_i for each variable in the expanded network of the original system. The entire procedure is illustrated in the SI Text. It is worth noting that whenever regulation between module nodes is not represented in the module, the resulting thresholds are more strict than is necessary. The thresholds can be refined by using adjusted "worst-case" values for such regulation once initial thresholds are determined.

In the remainder of this paper, we use the above methodology to analyze two systems from the literature.

Single-Cell Drosophila Segment Polarity Network

The first example we consider is a simplified, one-cell model of the Drosophila segment polarity gene network. The original multi-cell model (26) uses coupled differential equations to model the concentrations of mRNAs and proteins of a family of genes that are important in the development of segments in *Drosophila melanogaster* embryos. This family of genes includes engrailed and cubitus interruptus, which encode transcription factors, as well as wingless and hedgehog, whose proteins are secreted and interact with proteins in the neighboring cells (26–28). We use a modified version of this model (equations 12-23 in (27)), which has incorporated more recent experimental results (e.g., on the *sloppy-paired* protein) and has been recast for a single cell while assuming steady-state values for neighboring cells. Because no measured values of the kinetic parameters in the model are available, and because our purpose here is illustrative, we have simply chosen parameter values for the model that qualitatively reproduce the four cell types observed experimentally.

For the parameter values we have chosen, and for fixed inputs from external cells, we are able to identify several stable modules of biological importance. When the neighbor cells have high levels of the *wingless* protein, we find two stable modules involving the products of the *sloppy-paired* and *engrailed* genes (see Figure 2). One of these stable modules (high *sloppy-paired*, low *engrailed*) persists for all values of the neighboring *wingless* protein, while the other (low *sloppy-paired*, high *engrailed*) vanishes below a certain threshold. For high concentrations of neighboring *hedgehog* protein, we find two stable modules involving the *wingless* sub-network.

By shading the nodes in the expanded network according to module membership (as in Figure 2) we can visually identify regions of state-space that correspond to different attractors of the system; specifically, these attractors distinguish the four cell-types observed in the development of *Drosophila melanogaster* segments (26, 28). By identifying driver node sets for these modules, we can prescribe control strategies to attain any of the four cell types. In general, the visual presentation afforded by the expanded network can highlight the causal relationship among overlapping regions of state-space, particularly if we only consider edges between expanded network nodes that manifest implication in finite time (indicated by solid lines in Figure 2; because the system is continuous, dotted lines indicate implication in infinite time, i.e., asymptotic approach).

T-Cell Receptor Signaling Network

The second biological example we consider here is a model describes the cascading activation of transcription factors when T-cell receptors are bound by external molecules (29). The model was constructed using the *Odefy* MATLAB toolbox ((30)) to transform a pre-existing Boolean model of T-cell activation ((31)) into an ODE model $\tau_i \dot{x}_i = F_i(\boldsymbol{x}) - x_i$, where each F_i is a polynomial of Hill functions with $F_i(\boldsymbol{x}) \in [0,1]$.

To simplify the example, we consider only the strongly connected core of the system, having taken the input signals to be fully saturated, though this is not generally necessary. The resulting network is depicted in Figure 3a (left), in which the edges are labeled according the Hill coefficient, n, and disassociation constant, k, of the function $H_i(x_i)$ for the corresponding regulatory effect. Small, circles in the diagram represent "AND" rules in the underlying Boolean model, while multiple incoming edges are combined using "OR" rules. In the ODE model we analyze, these Boolean rules determine the form of the regulatory functions F_i, as described above.

By considering when the activation or inhibition of a given node is sufficient to cause the activation or inhibition of other nodes, we have identified the cycle $TCRb \rightarrow Fyn \rightarrow PAG \rightarrow Lck \rightarrow ZAP \rightarrow cCbl \rightarrow TCRb$ as a candidate stable motif depicted in Figure 3a (right). This cycle is a positive feedback loop, but it is embedded in a sign-inconsistent network. As such, before we can implement the MIOS approach to determine valid thresholds for the motif, we must fix the effects of sign-inconsistent edges ((22)). For instance, in the motif, we expect $TCRb$ and PAG to achieve relatively high values, but there is a inhibitory effect between the two; indeed,

$$\tau_{PAG}\frac{dPAG}{dt} = (1 - H_1(TCRb))(1 - H_2(Fyn)) + H_2(Fyn) - PAG, \quad [3]$$

where H_1 and H_2 are Hill functions (of the form $\frac{x^n}{x^n+k^n}$ with $n \in \mathbb{Z}^+$ and $k \in (0,1)$). The inhibitory effect is maximized when $TCRb$ attains its maximum value, i.e., one (because all variables are normalized to their maximum values in this model). It is also possible to consider the possibility that $TCRb$ is delivered to the system via external control, in which case we would evaluate Equation 3 in the limit as $TCRb \rightarrow \infty$. For now, we shall only consider $TCRb = 1$ in this regulatory function. We therefore replace $H_1(TCRb)$ in Equation 3 with $H_1(1)$ and allow $TCRb$ to evolve according to its natural dynamics in this new network, in which the regulation of PAG is modified. Similar analysis is taken on any edge that either introduces a sign inconsistency, or does not connect two nodes of the stable motif. The resulting modified network is a single positive feedback loop with a single steady state that is easily identified using the MIOS approach (22). The steady state values of the nodes in the modified network serve as thresholds in the expanded network, and allow us to identify a stable motif (see Figure 3b). The thresholds this method generates are valid, but not maximally restrictive. That is, in most networks, it is possible to choose a smaller region of state-space to represent the stable motif. More restrictive bounds, however, are difficult to attain analytically and are often not of interest when the bounds already lie far from the transitions from "low" concentrations to "high" concentrations. Furthermore, the relatively permissive bounds allow for

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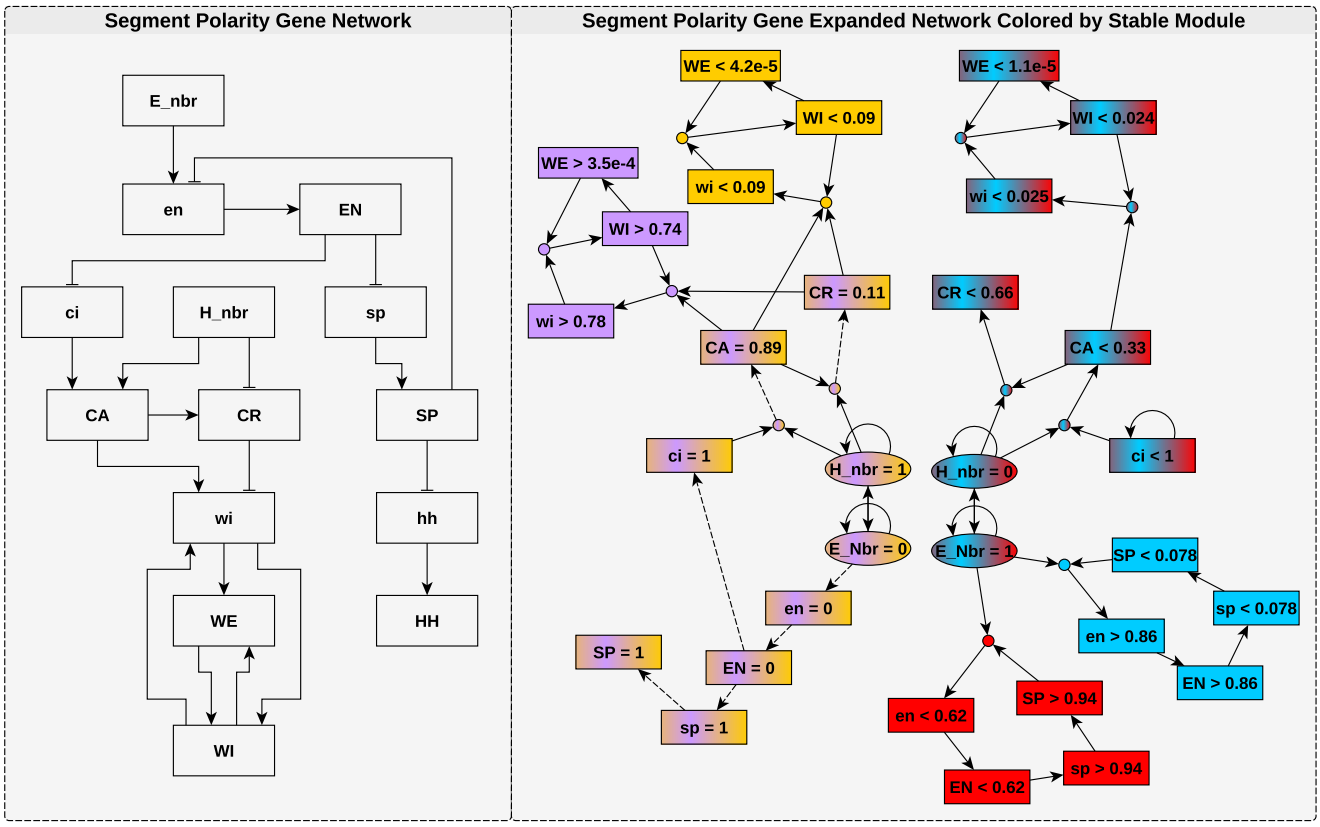

Fig. 2. The network schematic for the equations given in (27) (left) and expanded network, with nodes colored according to membership in each of four biologically meaningful stable modules (right). Edges represent the node maintenance relation. Solid lines also indicate implication in finite time, while dashed indicate only asymptotic approach. Thresholds in the expanded network are rounded and based upon parameters chosen to allow the system to exhibit the biologically relevant states. For two fixed values of the input variables (elliptical nodes), the expanded network highlights four regions of state-space corresponding to the four cell types enumerated in (26, 28). These are: high *engrailed*, low *wingless* (blue); low *engrailed*, high *wingless*, low neighboring cell *hedgehog* (red); low *engrailed*, high *wingless*, high neighboring *hedgehog* (purple); and low *engrailed*, low *wingless* (yellow).

robustness to variations in network variables due to external control as illustrated in Figure 3b. In the top left panel of Figure 3b, we show a natural evolution of the system for initial conditions satisfying the stable motif conditions. In the other panels, the value of the $TCRp$ node is subjected to one of three external controls (absence, saturation, and oscillation), and the motif variables continue to respect the stable motif conditions. These simulations illustrate an important conclusion we can draw from the existence of the stable motif: If one wishes to avoid states in which Fyn, PAG, and $TCRb$ are high while Lck, ZAP, and $cCbl$ are low, $TCRp$ is not a viable control target. Instead one must disrupt one of the six motif nodes directly, and furthermore, the motif bounds provide lower bounds on the magnitude of the required disruption.

Summary and Discussion

We have presented a new framework for identifying self-sustaining subsystems that cannot be controlled via the rest of the system. Full attractor control requires that control targets be chosen from these subsystems. The approach relies upon the construction of an auxiliary "expanded network" and is an extension of techniques first applied to Boolean models. In addition, we have showed that for ODE models that meet a set of conditions broadly applicable in biomolecular networks, our method can be refined to yield results in a more automatic way. We have demonstrated the implementation of this refined method on two systems of biological interest: the T-cell receptor signaling network and the *Drosophila melanogaster* segment polarity gene network.

The method of expanded networks can extract important qualitative features from quantitative or qualitative models of system behavior. We have emphasized the identification of stable modules, which correspond state-space regions that, once entered, cannot be exited without directly applying external control on the variables that define the region boundaries. We have also shown, for example in our analysis of the *Drosophila melanogaster* segment polarity gene network, how the consideration of expanded networks can elucidate meaningful partitioning of state-space. In these analyses, we have considered virtual nodes of the form $x_i \in (\inf F_i, a_i)$ or $x_i \in (b_i, \sup F_i)$, but other choices for virtual nodes are possible, and can be informative when x_i has inherently multi-level behavior.

Many existing results about the analysis of Boolean models via expanded networks remain valid in this more general framework and can therefore be applied to continuous systems. An example is the concept of a driver node set, which is a set of virtual nodes in the expanded network whose truth eventually implies the truth of a given stable module. Identification of driver nodes in the expanded network is related to finding

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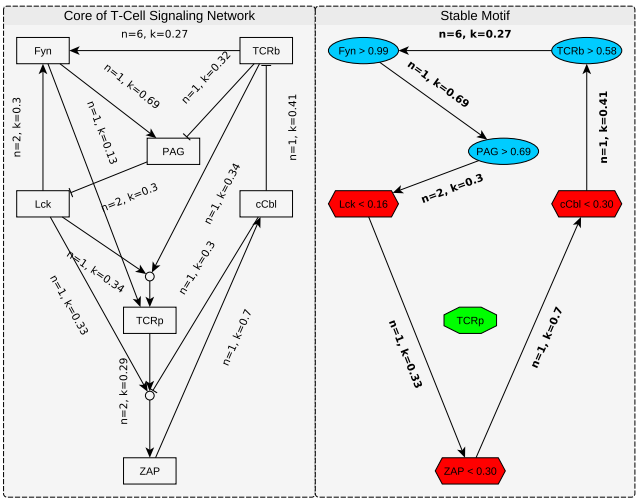
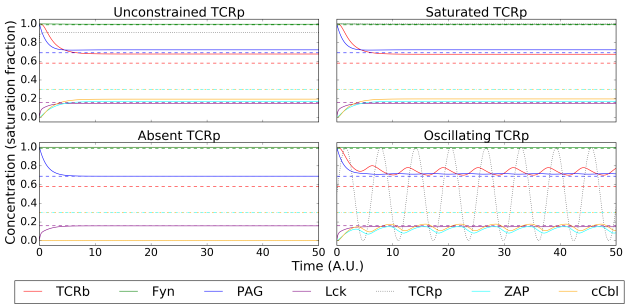


(a)



(b)

Fig. 3. (a) The network diagram and stable motif for the T-cell signaling model of (29) with all sources saturated. In the stable motif diagram, node shape and color indicate whether an upper or lower bound is specified (as indicated by the node labels). The variables constrained by the stable motif cannot leave the region of state-space specified by the stable motif once it has been entered. This remains true even when $TCRp$, which regulates ZAP, but is not included in the stable motif, is subjected to external control, provided it remains within the bounds considered when constructing the expanded network (between 0 and 1 in this case, though a similar result can be obtained for $0 \leq TCRp < \infty$). This robustness is illustrated in (b), in which solid colored lines indicate dynamic variable values that are constrained by stable motif thresholds (dashed lines). The black dotted line is the $TCRp$ value and is subject to different external controls in panel of sub-figure (b).

paths in logic hypergraphs (31). This identification problem has been partially addressed in (23, 32); developing a general and fast algorithm for driver node identification in arbitrary expanded networks is a promising direction for future research with applications for control target selection.

Some results do not generalize as easily because they rely upon completeness properties of discrete expanded networks; the oscillation analyses in ((23, 25)) are an example. Oscillations can manifest in the expanded network as source-free graph components that contain contradictory nodes. Such structures do not always indicate oscillatory behavior, and may instead indicate chaotic behavior or the existence of a steady state that violates all of the contradictory conditions. We are optimistic that results of this type might be recast in more general forms.

ACKNOWLEDGMENTS. This work was funded by NSF grants PHY 1205840 and PHY 1545832 to RA. We thank Jorge Zañudo for his helpful insights and conversations.

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