

Signal transduction networks: Topology, response and biochemical processes

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Abstract

Conventionally, biological signal transduction networks are analysed using experimental and theoretical methods to describe specific protein components, interactions, and biochemical processes and to model network behavior under various conditions. While these studies provide crucial information on specific networks, this information is not easily converted to a broader understanding of signal transduction systems. Here, using a specific model of protein interaction we analyse small network topologies to understand their response and general properties. In particular, we catalogue the response for all possible topologies of a given network size to generate a response distribution, analyse the effects of specific biochemical processes on this distribution, and analyse the robustness and diversity of responses with respect to internal fluctuations or mutations in the network. The results show that even three- and four-protein networks are capable of creating diverse and biologically relevant responses, that the distribution of response types changes drastically as a function of biochemical processes at protein level, and that certain topologies strongly predispose a specific response type while others allow for diverse types of responses. This study sheds light on the response types and properties that could be expected from signal transduction networks, provides possible explanations for the role of certain biochemical processes in signal transduction and suggests novel approaches to interfere with signaling pathways at the molecular level. Furthermore it shows that network topology plays a key role on determining response type and properties and that proper representation of network topology is crucial to discover and understand so-called building blocks of large networks.

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1. Introduction

Biological signal transduction allows a cell or organism to sense its environment and react accordingly. This is achieved through cascades of proteins that interact via activation and inhibition to convert an external signal into a physiological response. In order to understand such a cascade (or network) one first needs to define its protein components and their interactions. Given the appropriate experimental data, a signal transduction network can be described by a mathematical model in order to obtain a quantitative understanding of its

behavior (see for example, Bray et al., 1993; Morton-Firth and Bray, 1998; Bhalla and Iyengar, 1999; Bower and Bolouri, 2001; Shimizu et al., 2003). Typically this understanding is limited to the specific network under investigation and cannot be easily used to extrapolate the behavior of other types of networks. Combined with the fact that the experimental work needed to gather enough quantitative information to develop accurate mathematical models is highly labor-intensive, the modeling of specific networks may be limited in developing a broad understanding of the general properties of biological signaling networks.

Such a broad understanding of biological signal transduction would provide answers to questions such as: What are the possible responses a given biological

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network can generate? What is the role of certain biochemical processes for generating such responses? How is response robustness or diversity related to response type or network structure? The answers to such general questions could help us deduce conclusions on the response and properties of a specific biological network from its topology (i.e. the protein interactions making up the network), or deduce certain topological features just by observing a biological response. One approach that attempts to address some of the above questions is that of motif search (Milo et al., 2002, 2004; Sporns and Kotter, 2004). Based on the idea of modularity in biological systems (Hartwell et al., 1999), the main aim of this approach is to identify so-called building blocks of networks. While there is some evidence for the existence of motifs that are significantly overrepresented in biological networks, their role in signal transduction is unclear, as the currently used motif representation does not contain any information on the nature of interaction among nodes (i.e. inhibition vs. activation) and on the direction of information flow over the nodes (i.e. which node in the motif acts as a receptor and which acts as an effector). As we demonstrate here, including such information is crucial to understand building blocks and their role in signal transduction.

Here we present a novel approach to answer broad questions on signal transduction systems. In particular, we want to understand what types of responses a network of given size can generate, how their distribution relates to biochemical processes at protein level, and how network topology affects response robustness and diversity. Answers to these questions would complement information gained from other approaches (see for example, Barkai and Leibler, 1997; von Dassow et al., 2000; Tyson et al., 2003). The presented approach concentrates on the analysis of network topology, which is the complete list of interactions between all proteins in the network and the nature and strength of these interactions. Using a specific model of network dynamics and protein interaction, we analyse all possible topologies of a network with a given number of proteins and classify their response to an incoming signal. We then extend this analysis with several assumptions regarding various biochemical processes. Finally we quantify response robustness and diversity under internal fluctuations and mutations. The strength of this approach is that it does not require knowledge of exact parameter values defining the system and that it provides exhaustive results over the topology space. Here, we analyse three to five protein networks, which are of biologically relevant size as manifested in the abundance of so-called two-component signaling systems (Stock et al., 2000). We find that even three-protein networks are capable of generating diverse response types and that the distribution of specific responses

changes drastically as a function of the incorporated biochemical processes. The results also indicate that response robustness and diversity of a network is dictated mainly by network topology and is affected by the incorporated biochemical processes. We discuss these findings and their implications on both our understanding of signal transduction systems and our way of interfering with them through drug treatments.

2. Results

We first analyse the response of all possible three-protein network topologies with a path from the receptor to the effector and classify these into one of the five broad response categories given a specific model (see Methods). Fig. 1 shows a sample topology for each of these response categories. Given the indicated model and interaction coefficients, these topologies generate shown responses to a bell-shaped signal (see Methods). Responses for all topologies are summarized in Fig. 2A as a distribution over response classes. The first set of distributions is for the “null model”, where no relaxation of proteins into their active or inactive forms is allowed (i.e. $sa = sd = 0$). Then, with each new model we incorporate these biochemical processes and regenerate the response distribution (i.e. $sa = 0.01$, $sd = 0.01$ and $sa = sd = 0.01$ for the models labeled as SA, SD, and SA + SD, respectively).

The results for the null model show that networks of three proteins can have switch, Gauss-, or derivative-like responses but fail to generate an oscillatory response. These three response types are generated by 13.6%, 9.6%, and 0.2% of all topologies, respectively. The remaining topologies are incapable of giving any response to an incoming signal. For these topologies it is typically the case that the steady-state concentration of the active effector or receptor is already at its maximal level so that the signal has no effect on the system.

Distribution of response types changes clearly as we introduce relaxation type processes into the model (see Fig. 2A). Such processes are commonly found in proteins, with probably the best known example being the intrinsic GTPase activity of G-Proteins resulting in constant but slow deactivation of G-Proteins (Hamm and Gilchrist, 1996). Inclusion of intrinsic protein activity in the model increases the number of responsive topologies. For the model that allows relaxation of proteins both to their active and inactive forms (model SA + SD), only 24.9% of all topologies are non-responsive compared to 76.6% for the null model. This effect is mostly due to the role of such processes in keeping the steady-state concentrations of proteins at intermediate levels so that the system can respond to an incoming signal. More than 90% of topologies, that

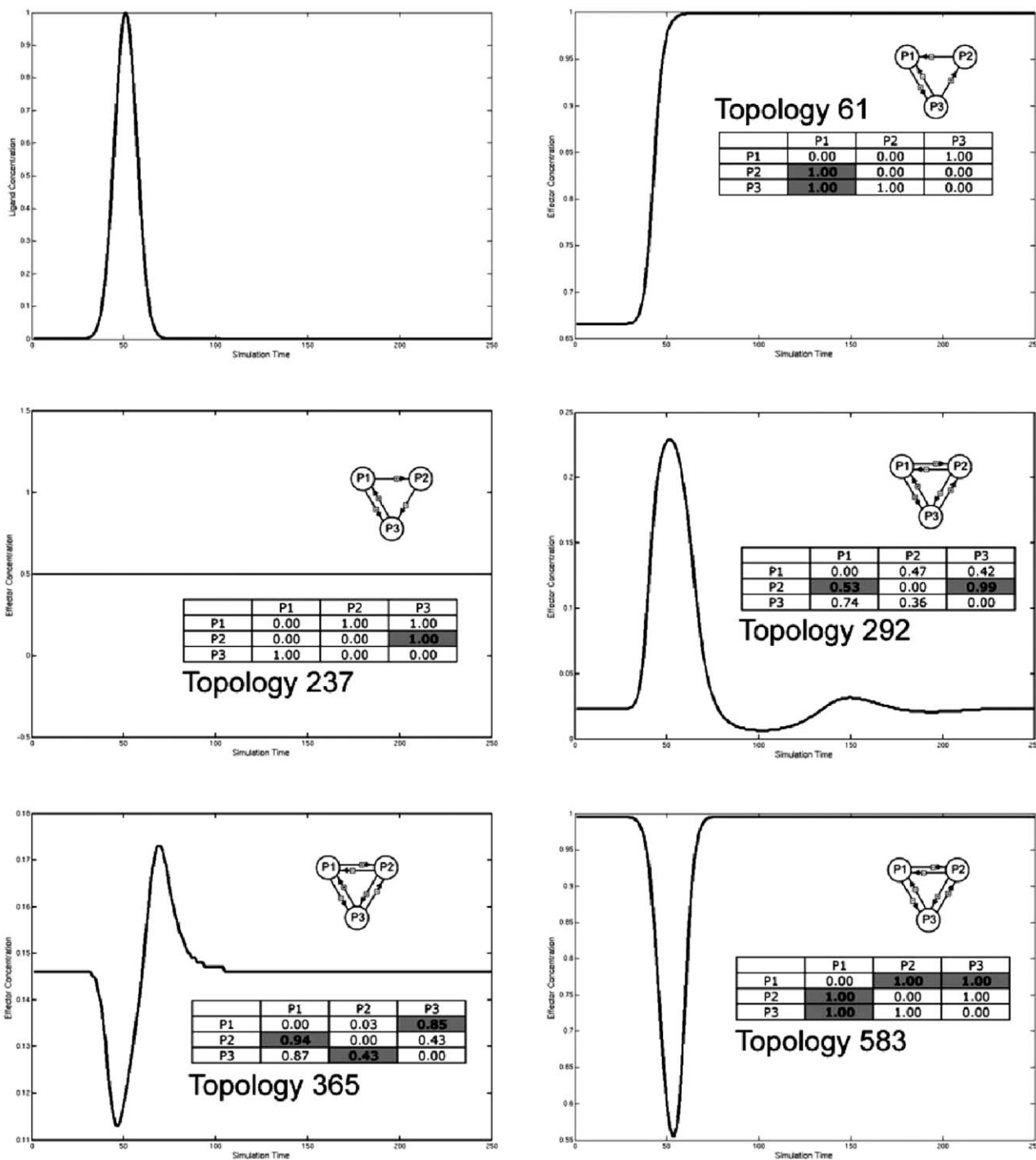


Fig. 1. Sample topologies for the three-protein network resulting in a response from each response class. Top left panel shows the bell-shaped signal used throughout the analysis (see Methods), while the remaining panels show the response of selected topologies to this signal. These topologies are shown in matrix and cartoon representation as an inlet on each graph. The matrix notation gives the coefficients for activating and deactivating (in bold) interactions among different proteins in the system, with row *i* listing the actions of protein *i* on other proteins. Nodes labeled as P1 and P3 represent receptor and effector, respectively. Responses are generated using one of the four models discussed in the text; null model ($sa = sd = 0.0$) for topologies 61 and 237, “SD” model ($sa = 0.0, sd = 0.01$) for topologies 365 and 292, and “SA” model ($sa = 0.01, sd = 0.0$) for topology 583.

become capable of a response with the introduction of a one or both of the relaxation processes, give Gauss-like responses. The remaining of these topologies produces derivative-like responses with SA and SD models and

both derivative-like and oscillatory responses with SA + SD model. Here it is important to note that such oscillatory responses were not observed with the null model. Finally, we find that the introduction of

relaxation processes either in one (model SA or SD) or both directions (model SA + SD) results in diminishing of switch responses. An intuitive explanation for this observation would be that these intrinsic processes make it difficult for the active effector concentration to be maintained at two different steady-state levels.

Next, we address how the response distributions change with varying signal strength. Changes in signal strength could result both through external and internal means such as regulation of receptor sensitivity. The latter is a commonly observed theme in signal transduction networks. In chemotaxis for example, the activation of receptors is regulated through methylation (Falke et al., 1997; Sourjik and Berg, 2002), while in case of G-protein coupled receptors (GPCRs) it might be dimerization that plays a similar role. This large class of receptors are shown to form both homo- and heterodimers (Zawarynski et al., 1998; Zeng and Wess, 1999; Angers et al., 2000; Franco et al., 2000; Fotiadis et al., 2003) and recent experimental evidence suggests that this regulates their ligand affinity or even specificity (Rios et al., 2001; Angers et al., 2002). In order to assess the possible effects of ligand concentration on the response distributions we repeat the above analysis with a 10-fold decrease in ligand concentration. We find that the general distribution of responses remains unaffected (data not shown), while some topologies change their behavior to generate a new response type. These correspond to 2%, 1%, 6% and 6% of all topologies in case of the null, SA, SD, and SA+SD models, respectively. This observation indicates that regulating receptor sensitivity or signal strength could have important biological consequences depending on the topology of the signal transduction network.

Finally, we analyse the effects of autocatalytic activity. Here, we refer to autocatalytic activity as the interaction between active proteins and their inactive form and assume that this could be either an activation or inhibition (see Methods). Allowing this process in the model does not affect the response distributions significantly except for the models that include relaxation of proteins (see Fig. 2B). Combined existence of autocatalytic activity with relaxation of proteins towards their active form (model SA) results in oscillatory responses (in 0.04% of all topologies) and with relaxation of proteins towards their inactive form (model SD) results in both oscillatory and switch responses (in 0.4% and 1% of all topologies, respectively). Note that these response types are not observed with these models in absence of autocatalytic activity (see Fig. 2A).

So far, we have considered only 0 or 1 as an interaction coefficient in the above analyses. In reality, protein interactions can be of different strengths, which could affect the response of a given topology and the

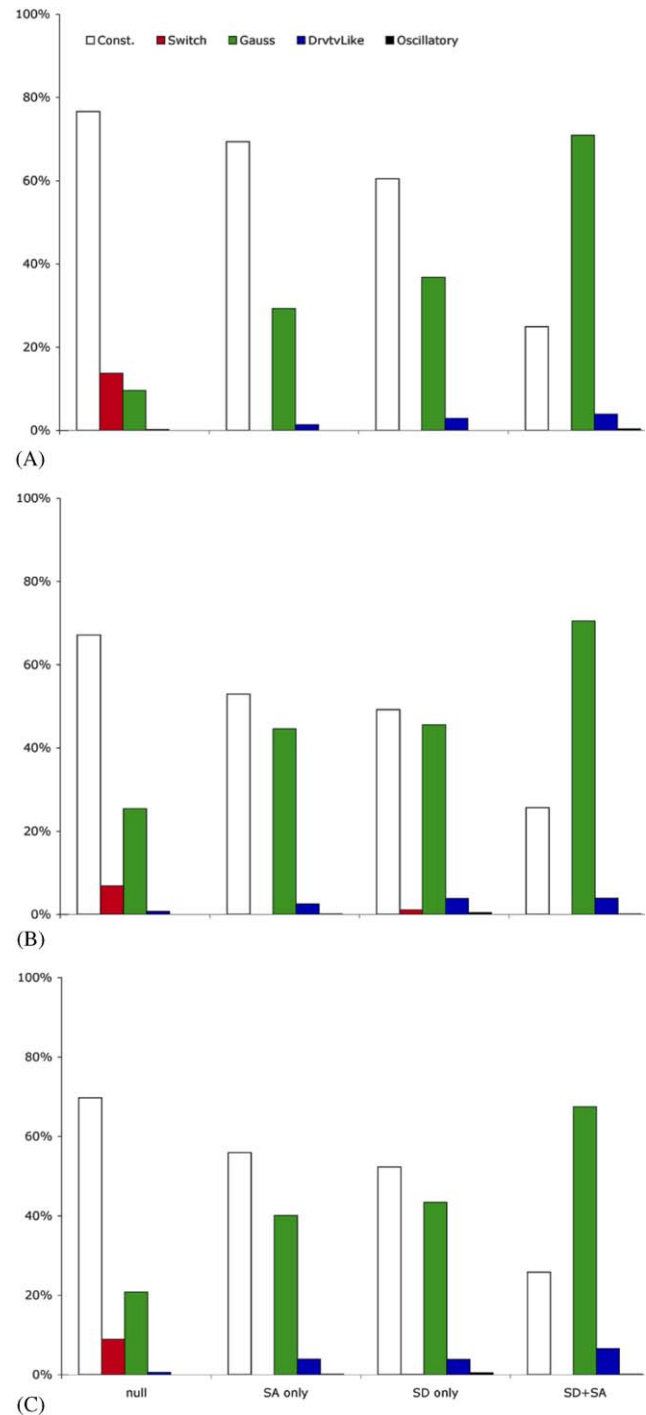


Fig. 2. Response distributions for three- and four-protein networks. Distribution of network responses from all possible three-protein topologies created using binary interaction coefficients with (panel B) and without autocatalytic activity (panel A). Panel C shows response distribution for all possible four-protein topologies without autocatalytic activity and using binary coefficients. Notations “null”, “SA”, and “SD” indicate the use of the null model, and models allowing relaxation of proteins towards their inactive and active forms, respectively. Model “SA + SD” allows relaxation both towards active and inactive forms. See text for detailed discussion of models and response classification.

distribution of responses over all topologies. In order to address this issue, we generate 1000 networks using random interaction coefficients between 0 and 1 for each three-protein topology without considering autocatalytic activity and analyse their response. As shown in Fig. 4A, this analysis reveals a similar distribution as before across all network topologies (compare Fig. 2A with Fig. 4A), indicating that interaction strength has a limited effect on the overall distribution of response types. However, we find that varying interaction coefficients can have a strong effect for specific topologies and can lead to a change in their response type. For the null, SA, SD, and SA+SD models respectively, 62%, 34%, 34%, and 86% of all topologies generate more than one response type when the interaction coefficients are allowed to vary randomly. More interestingly, we find that certain response types are now possible with a given model. For example oscillatory responses, which were not observed with the null, SA, and SD models when using binary values for interaction coefficients, are now observed for 2%, 13%, and 5% of all topologies, respectively. Similarly, 2% and 0.5% of all topologies now produce a switch response with the SA and SD models, respectively. In order to assess whether there are specific parameter ranges that facilitate the generation of these response types, we repeated the same analysis by restricting the values available for random coefficients. In three different analyses we created random networks using coefficients from the range 0–0.1, from 0.9–1 and from both (i.e. parameters are selected randomly from range 0–0.1 or 0.9–1) using the null, SA, and SD models. We find that the number of oscillatory responses is not significantly affected by such restriction of interaction coefficients to specific parameter ranges. On the other hand, switch responses are more frequently observed with the SD model when using parameters from the range 0–0.1, and only observed with the SA model when we allowed parameters from both ranges 0–0.1 and 0.9–1. This analysis suggests that slow interactions and a combination of slow and fast interactions facilitate switch responses in SA and SD models, respectively, in three-protein topologies.

The above results indicate that the effect of changes in interaction strength on the response type depends on network topology. To address this further we tried to quantify the response diversity and robustness for each topology using the created sample networks. Robustness is defined here as the capacity of a topology to maintain its response type despite quantitative changes in interaction coefficients. In biological terms, this would translate into robustness of a response type against internal perturbations, either due to mutations that affect protein interactions or due to stochastic fluctuations in gene expression. For each response type we calculate robustness as the percentage of networks—created from a given topology—generating this response type. Response diversity measures the capacity of a topology to produce a diverse set of response types upon quantitative changes in interaction coefficients among proteins and is calculated using an information theoretic approach (see legend of Table 1). Note that both these measures are qualitative in nature (i.e. they provide a measure of qualitative rather than quantitative changes in network response). As with the previous analyses, we perform these calculations with the different models discussed above.

Described measures of robustness and diversity, averaged over all topologies, are shown in Table 1. These average values show that in general topologies with a Gauss-like response are robust while topologies with other response types are highly sensitive to changes in the interaction coefficients among proteins. We find that relaxation type reactions increase the average robustness of topologies with oscillatory, Gauss-, and derivative-like responses, while decreasing that of those with switch responses. The latter observation may be due to the fact that these processes make it very difficult for a network to generate switch-like responses as discussed above. Furthermore, these relaxation type reactions increase the capacity of a topology to generate diverse responses. Robustness and diversity values from specific topologies clearly indicate the important role of the network topology on its response. Some topologies have a very high robustness for a specific response type showing that they strongly pre-dispose a certain

Table 1
Robustness and response diversity averaged over all possible three-protein topologies

Model	Avg. robustness				Avg. diversity
	Gauss-like	Derivative-like	Oscillatory	Switch	
Model 0	64.79	6.63	0.19	26.16	0.13
SA	83.21	15.89	0.37	0.27	0.14
SD	78.07	10.39	15.38	0.10	0.18
SD+SA	77.99	16.45	0.16	—	0.29

Robustness for each specific response is calculated as the percentage of mutant networks generating that response. Response diversity is calculated using $Diversity = \sum_i -f_i \log_2(f_i)$, where f_i stands for the percentage of response type i .

Topology Nr. 19



Model	Response Summary of 1000 Mutants					Response Diversity
	Constant	Gauss-like	Derivative-like	Oscillatory	Bistable Switch	
Null	0	0	0	0	1000	0.034
SD	0	1000	0	0	0	0.034
SA	1000	0	0	0	0	0.039
SD+SA	2	998	0	0	0	0.036

Topology Nr. 72



Model	Response Summary of 1000 Mutants					Response Diversity
	Constant	Gauss-like	Derivative-like	Oscillatory	Bistable Switch	
Null	0	996	0	0	4	0.067
SD	0	1000	0	0	0	0.034
SA	0	1000	0	0	0	0.034
SD+SA	0	1000	0	0	0	0.034

Topology Nr. 260



Model	Response Summary of 1000 Mutants					Response Diversity
	Constant	Gauss-like	Derivative-like	Oscillatory	Bistable Switch	
Null	554	0	0	167	279	0.549
SD	23	460	103	413	0	1.391
SA	1000	0	0	0	0	0.039
SD+SA	12	979	2	0	0	0.078

Topology Nr. 366



Model	Response Summary of 1000 Mutants					Response Diversity
	Constant	Gauss-like	Derivative-like	Oscillatory	Bistable Switch	
Null	2	998	0	0	0	0.036
SD	0	698	302	0	0	0.905
SA	1	999	0	0	0	0.035
SD+SA	0	29	971	0	0	0.216

Topology Nr. 291



Model	Response Summary of 1000 Mutants					Response Diversity
	Constant	Gauss-like	Derivative-like	Oscillatory	Bistable Switch	
Null	4	996	0	0	0	0.039
SD	0	306	305	388	0	1.585
SA	2	998	0	0	0	0.036
SD+SA	1	348	651	0	0	0.954

Topology Nr. 362



Model	Response Summary of 1000 Mutants					Response Diversity
	Constant	Gauss-like	Derivative-like	Oscillatory	Bistable Switch	
Null	0	351	530	11	99	1.439
SD	0	635	365	0	0	0.967
SA	0	521	470	9	0	1.079
SD+SA	0	320	671	9	0	0.989

Topology Nr. 526



Model	Response Summary of 1000 Mutants					Response Diversity
	Constant	Gauss-like	Derivative-like	Oscillatory	Bistable Switch	
Null	1000	0	0	0	0	0.039
SD	8	468	153	359	0	1.468
SA	1000	0	0	0	0	0.039
SD+SA	6	991	5	0	0	0.081

Topology Nr. 592



Model	Response Summary of 1000 Mutants					Response Diversity
	Constant	Gauss-like	Derivative-like	Oscillatory	Bistable Switch	
Null	998	0	0	0	1	0.047
SD	1000	0	0	0	0	0.039
SA	2	328	668	2	0	0.952
SD+SA	0	292	707	1	0	0.901

Fig. 3. Sample networks with special properties. Top and bottom panels show sample three-protein topologies with high robustness and response diversity, respectively. The tables summarize the response distribution of mutant networks created from these topologies. Information for all topologies is available as supplementary material. See text and legend of Table 1 for details on the calculation of diversity and robustness.

response type on the network. Others allow for various response types as a function of the strength of various interactions in the network. Fig. 3 lists some example topologies that are highly robust or that can produce a high diversity of responses (information on all topologies is available at <http://www.eco.ethz.ch/tbdata/topo3/html/networks2.html>). Given their special properties, it is plausible to expect that such topologies would be abundant in natural systems depending on the requirements imposed by the environment or on the mechanisms involved in network evolution. Recently, the *sin* operon in *Bacillus subtilis* is found to make up such a network that allows diverse set of responses depending on internal parameters of the system (Voigt et al., 2005).

All above-discussed analyses were conducted on three-protein topologies with or without autocatalytic activity. While such small signal transduction networks are commonly found in biological systems, there are also many larger networks. To understand the effects of network size on possible responses and their distribution, we analyse all possible four-protein topologies using binary coefficients and 500 000 randomly selected four- and five-protein topologies using random coefficients. Even though the latter analyses are not exhaustive over topology space, their results indicate that the general pattern in the variation of response distribution with various models is similar to that observed with three-protein networks (see Figs. 2C and 4B and C). This indicates that for the broad classification of responses that is employed in this analysis, network size has limited effects on the distribution of responses over all topologies and that the observations we make regarding the effects of biological processes may be generalized to signal transduction networks of larger

size. However, we find that the capacity of a network to achieve certain response types may change with network size. For example, under the same model assumptions we observe oscillatory responses with four-protein networks but not with three-protein networks (compare Fig. 2A with C).

In order to better relate this analysis to real signal transduction networks we analysed one of the best-known two-component signal transduction pathways: the chemotaxis pathway in *Escherichia coli*. As a result of two decades of intense research, there is almost complete knowledge on the protein components involved in this pathway and their interactions (see Blair, 1995; Bren and Eisenbach, 2000 for a good review) and also the response they generate to a stimuli (Block et al., 1982, 1983; Segall et al., 1986; Sourjik and Berg, 2002). This pathway acts as a derivative sensor and allows bacteria to respond to changes in nutrient concentrations in its environment (Spiro et al., 1997). Both experimental and mathematical studies indicate that a “chemotactic” response follows the derivative of the signal (Segall et al., 1986; Bray et al., 1993; Spiro et al., 1997; Rao et al., 2004). We have implemented the topology of the chemotaxis pathway (as shown in Falke et al., 1997) in terms of the generic model used in this analysis and analysed its behavior by creating 1000 sample networks using random interaction coefficients. Allowing relaxation type reactions both towards active and inactive proteins in the model (model SA + SD) we find that 35% of these give a derivative-like response to an incoming signal. Hence this topology allows “chemotactic” behavior in a significant part of the vast space of interaction coefficients (i.e. kinetic parameters for phosphorylation and methylation reactions in this

pathway), despite the simplicity of the generic model used here. The observation on the robustness of the natural chemotaxis topology is already confirmed by more extensive theoretical and experimental studies (Barkai and Leibler, 1997; Alon et al., 1999). We find that allowing relaxation of proteins towards their active and inactive forms in the model is crucial for a derivative-like response; repeating the same analysis with other models (null, SA, and SD) we were not able to find interaction coefficient sets that give derivative-like responses.

3. Discussion

We performed an extensive analysis of signal processing in small signal transduction networks using a simple and generic model of protein interaction that captures the essential features of signal transduction systems. This analysis is the first step towards classifying response types that a small signal transduction network can generate and to analyse the relation between response types and network topologies. It is exhaustive in the sense that we considered all possible topologies of small networks but was specific in the sense of the employed model and classification of responses. The generic model used in the analysis accounted only for one type of protein interaction dynamics (i.e. first-order interactions) and the classification scheme accounted only for a broad classification of responses. In the future, this type of analyses could be extended to explore the effects of various dynamics of protein interaction and their combinations on the behavior of small signal transduction networks.

For small networks, we analysed the distribution of their response types over all possible topologies in absence and presence of specific biological processes such as relaxation and autocatalysis. The results show that even three-protein networks can generate complex and diverse responses, which underlie many biological processes. For example, a derivative-like response that traces the derivative of the incoming signal allows adaptive behavior and is believed to be employed in chemotaxis (Spiro et al., 1997; Stock et al., 2002), while a switch underlines many physiological responses. The distribution of these responses changes drastically with biochemical processes at protein level. Most particularly, the presence of relaxation type reactions, allowing proteins to switch between active and inactive states at slow rates, increases the number of topologies that responds to an incoming signal. Autocatalytic activity enhances oscillatory responses irrespective of the specific model assumed, and enhances switch behavior when coupled with relaxation of proteins to their inactive form. Furthermore, we find that the relaxation processes

have a high impact on response robustness and diversity of a given topology.

There are several implications of these observations for our understanding of signal transduction networks. First, they could explain the existence and abundance of intrinsic relaxation type reactions and autocatalytic activity of proteins involved in signal transduction. Such processes increase the chances to evolve a responsive network and more importantly to evolve certain types of responses. Examples of the latter case include the association between relaxation type processes and derivative-like responses and between autocatalysis and oscillatory responses. Second, these observations give insights on the topology–response relation and the effect of biological processes on this. We find that some topologies are highly robust for producing certain response types, while others are capable of producing a diverse set of response types. The latter would be favored by evolution if for example the modularity concept is an important feature for network evolution (Hartwell et al., 1999), while the former would be selected for if the network is required to have a robust response. Hence, we expect topologies that impose such properties on the network to be more common in systems where there is a need for the associated property. This hypothesis could be tested by looking at the abundance of topologies such as those shown in Fig. 3 in known biological signal transduction networks. Unlike the current motif search approaches, such an analysis would require including at least the binary nature of interactions (inhibition versus activation) and direction of information flow in the motif definition.

Finally, these observations provide new suggestions for interfering with signal transduction pathways. The drug treatments that interfere with signal transduction pathways most commonly involve agonists and antagonists for receptors. This study shows that targeting other biochemical processes such as relaxation or receptor dimerization and autocatalytic activity of downstream proteins may also be an efficient strategy. Such treatment approaches could be implemented both for changing the response generated by a given pathway or to turn it off completely.

4. Methods

Here, we use a generic model of protein interaction to describe the dynamics of a signal transduction network. The signal transduction network is modeled as a set of proteins, each of which can exist either in an active (P_i^*) (e.g. phosphorylated) or inactive (P_i) state. The equilibrium between the two states of a protein depends on the interactions between this protein and other active proteins in the system, where each interaction is

characterized by a specific interaction coefficient (e.g. rate constant). The dynamics of the model are given by the following reaction:



The energy required for this reaction is assumed to be provided from outside sources such as high-energy molecules. In this reaction scheme, P_i^* and P_i represent the active and inactive forms of protein i . The reaction kinetics are determined by the interactions between proteins (where k_{ij} and l_{ij} represent the strength of the interaction between protein i and j) and by relaxation type processes (where the global parameter sa (sd) defines the rate for relaxation of an inactive (active) protein to the active (inactive) form).

In order for the system to be able to respond to an incoming signal we arbitrarily define protein one as the receptor and allow its dynamics to be influenced by the ligand (i.e. the signal) concentration $[L]$;



Finally, we arbitrarily choose another protein as the effector and assume that the concentration of its active form dictates the behavior produced by the network. Given the interaction coefficients for a protein network, we can monitor the changes in the active effector concentration in response to a signal and evaluate the signal processing capacity of the network. Active and inactive protein concentrations can be calculated by solving the set of differential equations resulting from Eqs. (1) and (2):

$$\frac{d[P_i]}{dt} = \left[[P_i^*](sd + \sum_j l_{ij}[P_j^*]) \right] - \left[[P_i](sa + \delta_{i1}[L] + \sum_j k_{ij}[P_j^*]) \right], \quad (3)$$

where $\delta_{i1} = 1$ for $i = 1$ and $\delta_{i1} = 0$ for $i \neq 1$. Note, that the total concentration of each protein $[P_i^{tot}]$ is constant and set to one. For ease of computation, all parameters and the ligand concentration are also set between zero and one. All presented results for models with relaxation type processes use the same value for the global parameters sa and sd (namely 0.01). Analyses of three-protein networks using the null model and binary coefficients with different values for these parameters (namely 0.001, 0.005, 0.05, 0.1, and 0.5) give similar results as shown in Fig. 2A, indicating that qualitative conclusions discussed in the text are not sensitive to exact values of these parameters.

In this model, we can define any network topology by the number of proteins it contains and the set of coefficients k_{ij} and l_{ij} defining their interactions (see Fig. 1). Assuming that proteins can only activate or deactivate each other (i.e. $k_{ij} \cdot l_{ij} = 0$), that the interaction coefficients are binary in nature (i.e. interaction vs. no-interaction), and that proteins do not react with themselves, we can calculate the number of all possible topologies for a network of i proteins as 3^n where $n = i^2 - i$ is the number of interactions in the system. The final assumption regarding interactions can be relaxed to allow active proteins to activate or deactivate themselves. Biologically, this would mean that proteins could have autocatalytic activity (Fig. 4). Including autocatalytic activity in the system increases the number of interactions to $n = i^2$ and consequently the number of possible topologies. Hence, a network of three proteins would have 729 or 19 683 possible topologies depending on the assumption regarding autocatalytic activity. This number increases rapidly with network size: with five proteins and assuming no autocatalytic activity there are already over 3×10^9 possible topologies. Here we limit the analysis of all possible topologies of a given network to three and four proteins to keep it computationally tractable and analyse five-protein networks only through random sampling of topologies.

We create models for all possible topologies that have a path leading from the receptor to the effector (594, 16 038, and 482 598 topologies for three proteins with and without autocatalytic activity, and for four proteins, respectively). The system defined by each topology is initiated with equal amounts of active and inactive proteins, and is allowed to equilibrate by integrating it until steady state or maximally 10 000 time steps. Then, a simulation is started where we input a bell-shaped signal into the system with a peak concentration of one at time step 50 (see Fig. 1). The simulation is continued until steady state or maximally 10 000 time steps. In order to assure proper classification, the stability of steady states is determined with an eigenvalue analysis. Responses that result in different pre- and post-signal steady states are classified as a switch, which are then further classified as an integrator-like or bistable switch depending on the eigenvalues and eigenvectors of the system at these states. Responses that result in the same pre- and post-signal steady-state levels are classified using the number of sign changes in the response derivative as; constant (zero-sign changes), Gauss-like (two-sign changes), derivative-like (three- or four-sign changes) and oscillatory (more than four-sign changes). Any response that is indicated as unstable according to the eigenvalue analysis of steady states is discarded from the analysis. Such unstable responses never make up more than 0.1% of all analysed topologies in any of the discussed cases.

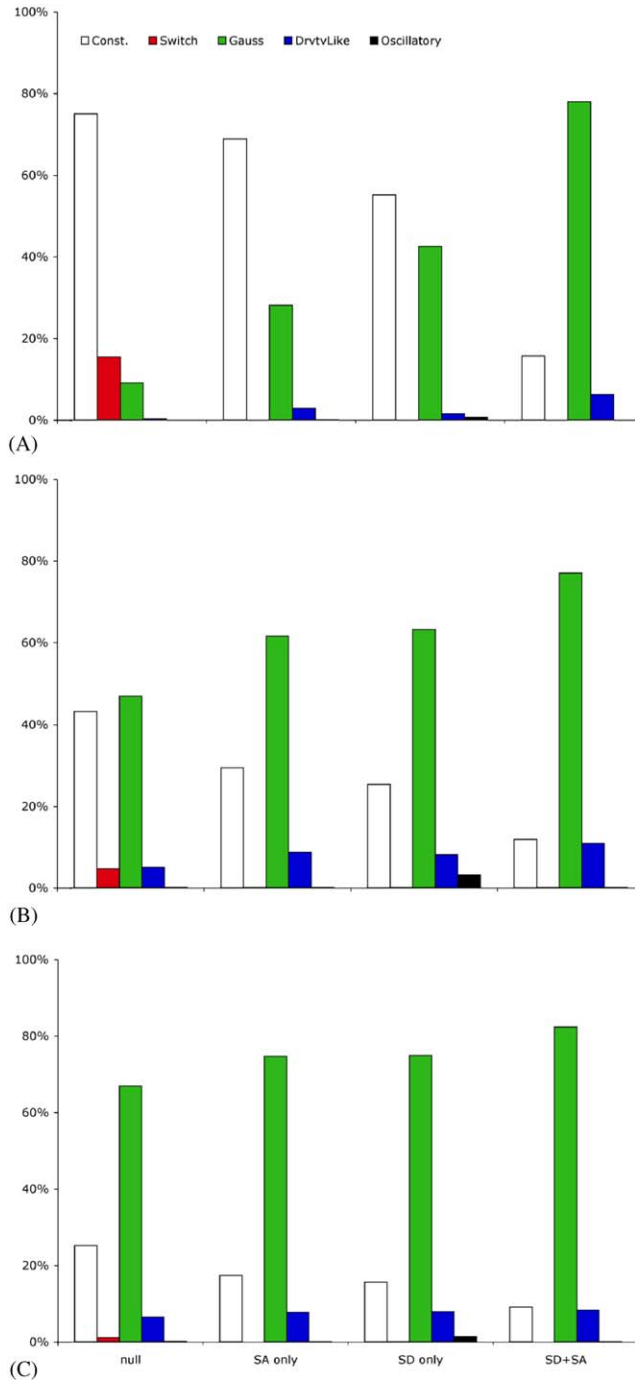


Fig. 4. Response distributions for three-, four- and five-protein networks. Distribution of network responses from all possible three-protein topologies created using random interaction coefficients (panel A). Panels B and C shows response distribution for randomly selected four- and five-protein topologies using random coefficients. None of the topologies include autocatalytic activity of proteins. See legend of Fig. 2 for model notations.

To minimize numerical artifacts on response classification, we only consider changes in the response level that are larger than 0.001. A response classified as a switch (bistable or integrator-like) indicates that the network attains two distinct concentration levels for the

effector before and after the signal. Gauss-, and derivative-like responses trace the signal and its derivative, respectively. Classification results are summarized to create a response distribution for the network of given size. This analysis is repeated with different biochemical processes allowed at protein level in order to evaluate the effects of these on the response distribution. The set of differential equations describing each system is solved using the Adams method implemented in NAG library Mark 7. Computer code is written in C++ and is available from authors upon request. A user-friendly version of the protein interaction model and a web-based tool for the analysis of signal transduction networks can be found at: www.eco.ethz.ch/SITNA.

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References

- Alon, U., Surette, M.G., et al., 1999. Robustness in bacterial chemotaxis. *Nature* 397 (6715), 168–171.
- Angers, S., Salahpour, A., et al., 2000. Detection of beta 2-adrenergic receptor dimerization in living cells using bioluminescence resonance energy transfer (BRET). *Proc. Natl Acad. Sci. USA* 97 (7), 3684–3689.
- Angers, S., Salahpour, A., et al., 2002. Dimerization: an emerging concept for G protein-coupled receptor ontogeny and function. *Annu. Rev. Pharmacol. Toxicol.* 42, 409–435.
- Barkai, N., Leibler, S., 1997. Robustness in simple biochemical networks. *Nature* 387 (6636), 913–917.
- Bhalla, U.S., Iyengar, R., 1999. Emergent properties of networks of biological signaling pathways. *Science* 283 (5400), 381–387.
- Blair, D.F., 1995. How bacteria sense and swim. *Annu. Rev. Microbiol.* 49, 489–522.
- Block, S.M., Segall, J.E., et al., 1982. Impulse responses in bacterial chemotaxis. *Cell* 31 (1), 215–226.
- Block, S.M., Segall, J.E., et al., 1983. Adaptation kinetics in bacterial chemotaxis. *J. Bacteriol.* 154 (1), 312–323.
- Bower, J.M., Bolouri, H. (Eds.), 2001. *Computational Modeling of Genetic and Biochemical Networks*. The MIT Press, Cambridge, MA.
- Bray, D., Bourret, R.B., et al., 1993. Computer simulation of the phosphorylation cascade controlling bacterial chemotaxis. *Mol. Biol. Cell* 4 (5), 469–482.
- Bren, A., Eisenbach, M., 2000. How signals are heard during bacterial chemotaxis: protein-protein interactions in sensory signal propagation. *J. Bacteriol.* 182 (24), 6865–6873.
- Falke, J.J., Bass, R.B., et al., 1997. The two-component signaling pathway of bacterial chemotaxis: a molecular view of signal transduction by receptors, kinases, and adaptation enzymes. *Annu. Rev. Cell Dev. Biol.* 13, 457–512.

- Fotiadis, D., Liang, Y., et al., 2003. Atomic-force microscopy: Rhodopsin dimers in native disc membranes. *Nature* 421 (6919), 127–128.
- Franco, R., Ferre, S., et al., 2000. Evidence for adenosine/dopamine receptor interactions: indications for heteromerization. *Neuropsychopharmacology* 23 (4 Suppl.), S50–S59.
- Hamm, H.E., Gilchrist, A., 1996. Heterotrimeric G proteins. *Curr. Opin. Cell Biol.* 8 (2), 189–196.
- Hartwell, L.H., Hopfield, J.J., et al., 1999. From molecular to modular cell biology. *Nature* 402 (6761 Suppl.), C47–C52.
- Milo, R., Shen-Orr, S., et al., 2002. Network motifs: simple building blocks of complex networks. *Science* 298 (5594), 824–827.
- Milo, R., Itzkovitz, S., et al., 2004. Superfamilies of evolved and designed networks. *Science* 303 (5663), 1538–1542.
- Morton-Firth, C.J., Bray, D., 1998. Predicting temporal fluctuations in an intracellular signalling pathway. *J. Theor. Biol.* 192 (1), 117–128.
- Rao, C.V., Kirby, J.R., et al., 2004. Design and diversity in bacterial chemotaxis: a comparative study in *E. coli* and *Bacillus subtilis*. *PLoS Biol.* 2 (2), E49.
- Rios, C.D., Jordan, B.A., et al., 2001. G-protein-coupled receptor dimerization: modulation of receptor function. *Pharmacol. Ther.* 92 (2–3), 71–87.
- Segall, J.E., Block, S.M., et al., 1986. Temporal comparisons in bacterial chemotaxis. *Proc. Natl Acad. Sci. USA* 83 (23), 8987–8991.
- Shimizu, T.S., Aksenov, S.V., et al., 2003. A spatially extended stochastic model of the bacterial chemotaxis signalling pathway. *J. Mol. Biol.* 329 (2), 291–309.
- Sourjik, V., Berg, H.C., 2002. Receptor sensitivity in bacterial chemotaxis. *Proc. Natl Acad. Sci. USA* 99 (1), 123–127.
- Spiro, P.A., Parkinson, J.S., et al., 1997. A model of excitation and adaptation in bacterial chemotaxis. *Proc. Natl Acad. Sci. USA* 94 (14), 7263–7268.
- Sporns, O., Kotter, R., 2004. Motifs in brain networks. *PLoS Biol.* 2 (11), e369.
- Stock, A.M., Robinson, V.L., et al., 2000. Two-component signal transduction. *Annu. Rev. Biochem.* 69, 183–215.
- Stock, J.B., Levit, M.N., et al., 2002. Information processing in bacterial chemotaxis. *Sci. STKE* 2002 (132), PE25.
- Tyson, J.J., Chen, K.C., et al., 2003. Sniffers, buzzers, toggles and blinkers: dynamics of regulatory and signaling pathways in the cell. *Curr. Opin. Cell Biol.* 15 (2), 221–231.
- Voigt, C.A., Wolf, D.M., et al., 2005. The *Bacillus subtilis* *sin* Operon: an evolvable network motif. *Genetics* 169 (3), 1187–1202.
- von Dassow, G., Meir, E., et al., 2000. The segment polarity network is a robust developmental module. *Nature* 406 (6792), 188–192.
- Zawarynski, P., Tallero, T., et al., 1998. Dopamine D2 receptor dimers in human and rat brain. *FEBS Lett.* 441 (3), 383–386.
- Zeng, F.Y., Wess, J., 1999. Identification and molecular characterization of m3 muscarinic receptor dimers. *J. Biol. Chem.* 274 (27), 19487–19497.