

# Interrelations between Dynamical Properties and Structural Characteristics of Signal Transduction Networks

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## Abstract

We present a theoretical approach for understanding the interrelations between dynamics and structure of signal transduction pathways. We consider large sets of networks with a specific number of kinases and phosphatases. Our methods are based on nonlinear differential equations and pathway dynamics is characterised in terms of signal amplification and signal duration. We show that networks with a high number of kinases, high connectivities and low phosphatase activities tend to be unstable and run, therefore, the risk to display autoactivation. Analysis of signal transduction pathways retrieved from databases reveals that several structural characteristics required for pathway stability are fulfilled for networks of very large size.

**Keywords:** signal transduction, network design, stability, amplification, kinases, phosphatases, G-proteins

## 1 Introduction

In recent years several mathematical models of specific signalling processes have been proposed. Most elaborated are models of the MAPK-cascade which controls cell division processes [1, 3, 5, 13]. Another example is a model of the Wnt-pathway which is essential for cell differentiation [10]. These kinetic models describe the combined action of the participating reactions, such as stimulation of receptors, phosphorylations and dephosphorylations, binding to adaptor proteins, scaffolds or transcription factors. Some models are confined to stationary states [5] whereas others explore dynamic features [1, 3, 10, 13]. These and other models revealed interesting regulatory properties of signalling cascades. Examples are a switch-like response resulting from multiple phosphorylations [5], bistability resulting from positive feedback-mechanisms [2, 3, 6], signal amplification induced by protein turnover [10], and pathway modularisation by strong differences in the concentrations of scaffold proteins [10]. Although modelling of specific signalling pathways is rather advanced there is a lack of general theory which could provide a basis for deeper understanding the interrelation between dynamics and structural design of these processes. In the presents paper we present steps towards such a theory by studying the dynamical properties in terms of signal amplification, signal duration and stability [7]. The theory is applied to real networks by taking into account existing information on kinase interactions as documented in databases for signal transduction.

## 2 Results

### 2.1 Structural and Dynamical Properties of Kinase Networks

We analyse signalling networks consisting of kinases and phosphatases having the following properties: (1) each kinase may occur in an active and an inactive form; (2) activation of kinases takes place by

phosphorylation; (3) in their active forms kinases may phosphorylate other kinases; and (4) inactivation of the kinases takes by dephosphorylation catalyzed by phosphatases which are constitutively active. Effects of multiple phosphorylation are neglected and we do also not consider the action of adaptors or scaffolds. The analysis can be easily extended to networks containing G-proteins (see 2.6). We assume that initial activation of kinases occurs by its direct interaction with a stimulated receptor  $R$ . First we determine full sets of networks of a given size which fulfil these structural properties (up to a maximum of seven kinases). Second, we analyse the dynamic characteristics of all these networks in terms of signal amplification, signal dampening, and dynamic stability of the signalling off-state. Last, we investigate the design of a large signal transduction network retrieved from a database.

Figure 1 shows three examples of signalling networks. Network A represents the most simple arrangement of a receptor, and three pairs of kinases and phosphatases in form of a linear cascade resembling the classical MAPK pathway. The active and inactive forms of kinases  $K_i$  are denoted by  $X_i$  and  $\tilde{X}_i$ , respectively. Each dashed arrow indicates an activating effect of one kinase on another kinase. The other two networks in Figure 1 represent more complex structures which are also found in real cells [9]. In network B one of the kinases activates two kinases, and in network C one of the kinases receives two activating inputs. For the latter network these interconnections give rise to a cycle.

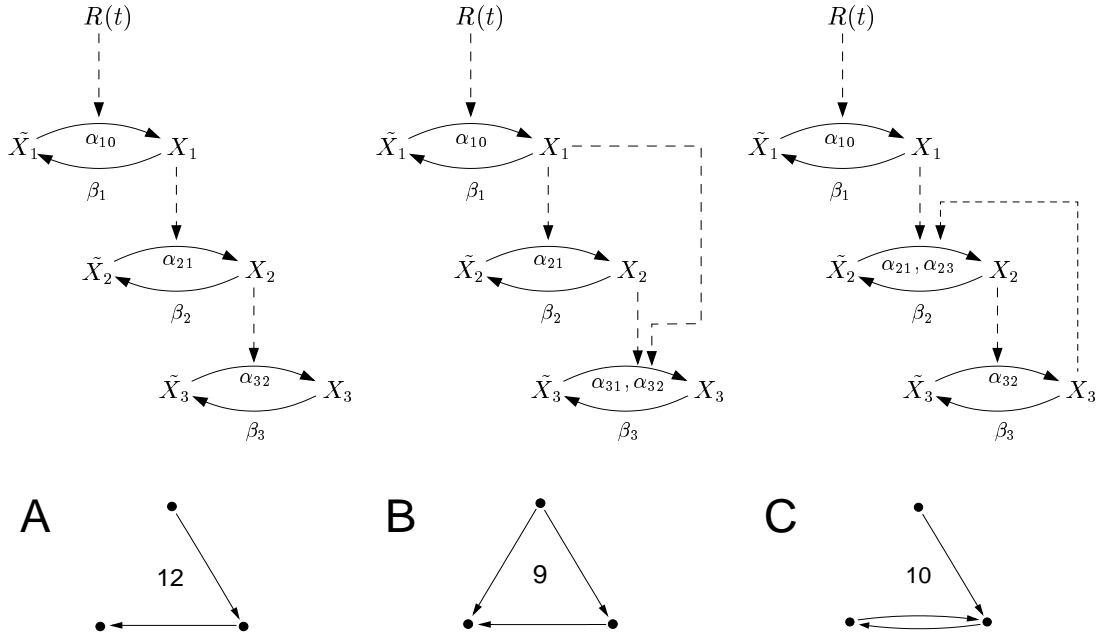


Figure 1: Different network structures with three kinases and three phosphatases. Upper panel: Interconversions of active and inactive forms of kinases catalyzed by other kinases and by phosphatases. Lower panel: graph representation of the same networks.

Our dynamic analysis is based on the following set of differential equations

$$\frac{dX_i}{dt} = \rho'_i R(t) \tilde{X}_i + \sum_{k \neq i}^n \alpha'_{ik} \tilde{X}_i X_k - \beta_i X_i. \quad (1)$$

The parameter  $n$  denotes the total number of kinases and phosphatases involved in a given pathway. Activation rates of kinases  $K_i$  by other kinases  $K_k$  are described by bimolecular terms  $\alpha'_{ik} \tilde{X}_i X_k$  where the factors  $\alpha'_{ik}$  are second order rate constants. The first order rate constants  $\beta_i$  characterise the dephosphorylation of the kinases by phosphatases. The first term in Eq. (1) describes kinase activation by the receptor. The corresponding rates are also written as bimolecular terms in which  $R(t)$  denotes the time dependent concentration of the stimulated receptor and  $\rho'_i$  the corresponding

second order rate constant. A transient stimulation of the receptor is described by an exponential decay, that is  $R(t) = R \cdot \exp(-\lambda t)$ . The case  $\lambda \rightarrow 0$  results in  $R = \text{const.}$  describing a sustained receptor activation. According to the reaction schemes in Figure 1 and the systems equations (1), only the concentrations of active and inactive forms contribute to the total concentrations of kinases leading the conservation relations

$$\tilde{X}_i + X_i = C_i = \text{const.} \quad (2)$$

This equation can be used to eliminate  $\tilde{X}_i$  from Eq.(1), resulting in

$$\frac{dX_i}{dt} = \left( \rho_i R(t) + \sum_{k \neq i}^n \alpha_{ik} X_k \right) \left( 1 - \frac{X_i}{C_i} \right) - \beta_i X_i \quad (3)$$

where  $\alpha_{ik} = C_i \alpha'_{ik}$  and  $\rho = C_i \rho'$  denote pseudo-first order rate constants. As shown in Figure 1 the networks can also be drawn as directed graphs. They consist of nodes representing the kinases  $K_i$  and of arrows representing activation of these kinases by other kinases. These graphs focus on the kinases whose interactions perform the signalling routes. However, the dynamical behaviour of the networks described by Eq. (3) also depends on the phosphatases acting on each kinase. In the following we only consider networks which cannot be decomposed into subnetworks, that is, each node is connected to at least one other node (weakly connected graphs). Any of these graphs can be characterised by the number  $n$  of nodes, the number  $e$  of edges for the activations, and the number  $c_L$  of cycles of length  $L$ . The outdegree and the indegree of a node are defined by the number of edges leaving this node or entering this node, respectively. The mean outdegree (which equals the mean indegree) and a related property, the network connectivity, are defined by

$$d = \frac{e}{n}, \quad (4a)$$

$$\kappa = \frac{e}{n(n-1)} = \frac{d}{n-1}, \quad (4b)$$

respectively.  $\kappa$  characterises on average the unspecificity of kinase activations in a given network and its values varies in the range  $1/n \leq \kappa \leq 1$ . In Table 1 the numbers of possible networks are listed depending on the number of kinases ( $n$ ) and on the number of activations ( $e$ ). Concerning primary activation of the kinases we consider a minimum set of receptors whose stimulation results in a subsequent activation of all kinases. For some networks a single input kinase may be sufficient. This is the case if the corresponding graph contains directed paths from this kinase to all other kinases. For other networks one might have to stimulate initially more than one kinases by receptors. There are also networks where each kinase is equally suited for a primary activation. The corresponding graphs are strongly connected.

## 2.2 Stationary States

For the time independent states for the concentrations  $X_i$  only solutions within the range  $0 \leq X_i \leq C_i$  are of interest. Such solutions always exist for permanent receptor activation ( $R \neq 0$ ), but can generally not be written in an explicit form due to the nonlinear character of Eq. (3). For  $R = 0$ , equation system (3) has always a time independent solution,  $X_i = 0$ , representing the signalling off-state. There may exist in this case also another stationary state with  $X_i \neq 0$  resulting from autoactivation of the pathway. We consider a network which contains a cycle of length  $L = 2$  between kinases  $K_1$  and  $K_2$  both of which activated a third kinase  $K_3$  (see network 3 in Fig. 3A). The steady state conditions

read in this case

$$\alpha_{12}X_2 \left(1 - \frac{X_1}{C_1}\right) - \beta_1X_1 = 0, \quad (5a)$$

$$\alpha_{21}X_1 \left(1 - \frac{X_2}{C_2}\right) - \beta_2X_2 = 0, \quad (5b)$$

$$\alpha_{32}X_2 \left(1 - \frac{X_3}{C_3}\right) + \alpha_{31}X_1 \left(1 - \frac{X_3}{C_3}\right) - \beta_3X_3 = 0. \quad (5c)$$

Besides the off-state,  $X_1 = X_2 = X_3 = 0$ , this equation system has the steady state solution

$$X_1 = \frac{C_1C_2(\alpha_{12}\alpha_{21} - \beta_1\beta_2)}{\alpha_{21}(\alpha_{12}C_2 + \beta_1C_1)}, \quad X_2 = \frac{C_1C_2(\alpha_{12}\alpha_{21} - \beta_1\beta_2)}{\alpha_{12}(\alpha_{21}C_1 + \beta_2C_2)} \quad (6)$$

where the corresponding  $X_3$ -value is obtained by introducing the solutions for  $X_1$  and  $X_2$  into Eq. (5c).

Eq. (6) describes an autoactivated state which only exists when the kinases are fast enough compared to the phosphatases, that is, for  $\alpha_{12}\alpha_{21} > \beta_1\beta_2$ . It can easily be proven that also for other networks autoactivated states may only occur if the corresponding graphs contain cycles.

Table 1: Numbers of networks depending on the number of nodes (kinases) and edges (activations).

$e$	$n = 2$	$n = 3$	$n = 4$	$n = 5$
0	0	0	0	0
1	1	0	0	0
2	1	3	0	0
3		4	8	0
4		4	22	27
5		1	37	108
6		1	47	326
7			38	667
8			27	1127
9			13	1477
10			5	1665
11			1	1489
12			1	1154
13				707
14				379
15				154
16				61
17				16
18				5
19				1
20				1
$\Sigma$	2	13	199	9364

### 2.3 Stability of the Signalling Off State

Proper functioning of a signal transduction pathway will generally require that the signalling off-state is dynamically stable. Stability means that the signalling network returns to this state after perturbations

such as spurious activation of the kinases. In the case of instability, auto-activation of the pathway may occur where the network will spontaneously leave the off-state approaching a stationary state with non-zero concentrations for the active forms of some kinases, as the state represented by Eq. (6). Stability analysis is performed by considering the spectrum of the eigenvalues of the Jacobian  $\mathbf{J} = (J_{ik})$  of the differential equation system (3). For the off-state the Jacobian reads

$$J_{ik} = \alpha_{ik} - \beta_i \delta_{ik} \quad (7)$$

where  $\delta_{ik}$  denotes the Kronecker symbol. Stability requires that the eigenvalues of this matrix have only negative real parts. For example, stability analysis of the signalling off-state for the network represented by graph 3 in Figure 3A reveals that the autoactivated state (Eq. (6)) is stable whereas the off-state is unstable. When condition  $\alpha_{12}\alpha_{21} > \beta_1\beta_2$  is not met, the autoactivated state does not exist and the off state is stable.

## 2.4 Signal Amplification

During transduction of a signal from a stimulated receptor to a target kinase, amplification or dampening may occur. As examples we consider the transient activation of the three pathways A, B, and C depicted in Figure 1. Receptor stimulation occurs for  $t = 0$ . Figure 2 shows the exponential decay of the concentration of the active receptor for  $t > 0$ , and the three other curves are the time dependent activation curves of the third kinase for the respective pathway.

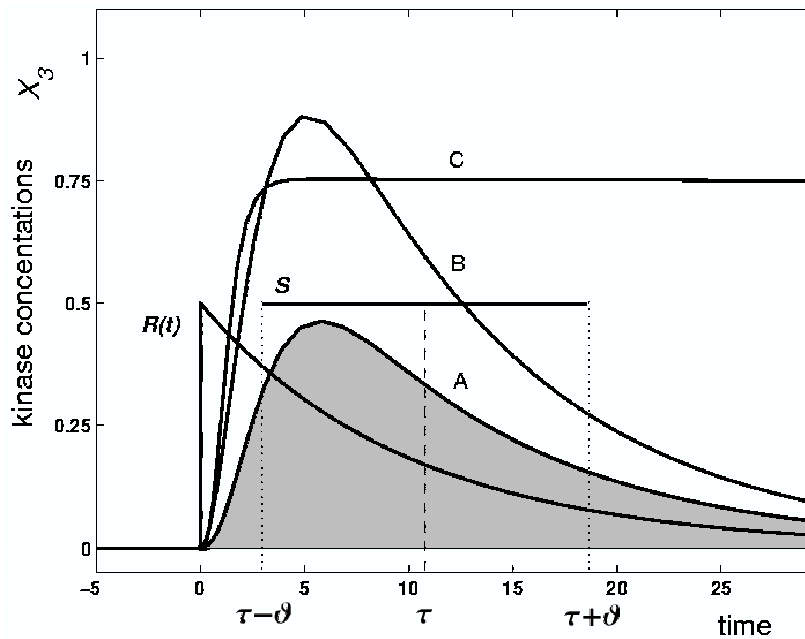


Figure 2: Time dependent changes in kinase networks.  $R(t)$ : decay of the active receptor; A, B, and C: concentrations of the active forms of the output kinases for the networks shown in Figure 1. Parameter values:  $\alpha_{ij} = \beta_i = 1$ ,  $\lambda = 0.1$ .

For pathways A and B the off states are stable. Accordingly, the target kinases are transiently activated and the networks return eventually to their off states. Compared to these responses, pathway C shows a completely different behaviour. The off-state is unstable and the network approaches a stable activated state which persists even after the decay of active receptor. We define a signal to be amplified at its transition from kinase  $K_i$  to  $K_{i+1}$  when during the time courses  $X_{i+1}$  attains a value larger than  $X_i$ . Otherwise dampening occurs. For signalling networks as depicted in Figures 1 or 3 it is convenient to define an overall amplification (or overall dampening) by comparing the highest

values of the concentrations of the active kinases with the initial concentration of the receptor  $R(0)$ . According to this definition pathway B amplifies the signal whereas in pathway A dampening occurs for the given parameter values. Amplification properties of the various networks can be analysed in principle by solving Eq.(3) numerically. However, determining the maxima of the concentrations of active kinases may render to be rather cumbersome. It is therefore more convenient, to analyse amplification in terms of average activation amplitudes  $S$ . For this we refer to the concept of averaged signalling amplitudes introduced in [7]. For a given kinase  $K_i$  these amplitudes are defined as follows:

$$S_i = \frac{I_i}{2\vartheta_i} \quad (8)$$

where the integrated response  $I_i$  and the signal duration  $\vartheta_i$  are defined in the following way:

$$I_i = \int_0^\infty X_i(t)dt, \quad (9a)$$

$$\vartheta_i^2 = \frac{1}{I_i} \int_0^\infty t^2 X_i(t)dt - \tau_i^2 \quad \text{with} \quad \tau_i = \frac{1}{I_i} \int_0^\infty t X_i(t)dt. \quad (9b)$$

The integral  $I_i$  corresponds to the area under the curve  $X_i(t)$ , and  $\tau_i$  denotes the average signal propagation time. We consider a signal to be amplified when  $S_i > R(0)$  or dampened when  $S_i < R(0)$ .

## 2.5 Dynamic Properties of Signal Transduction Networks Depending on Their Size, Connectivity, Number of Cycles and Kinetic Properties

Figure 3 gives an overview on the stability and amplification properties of the family of 13 networks consisting of only three kinases. Concerning amplification a proper choice has to be made for the output kinase to which the signal is transmitted. Therefore, we select eventually that kinase for which a maximum amplification is observed. Note that stability of off-states is independent of the choice of input and output kinases. The kinases as well as the phosphatases are assumed to have identical kinetic properties as characterised by their rate constants  $\alpha$  and  $\beta$ .

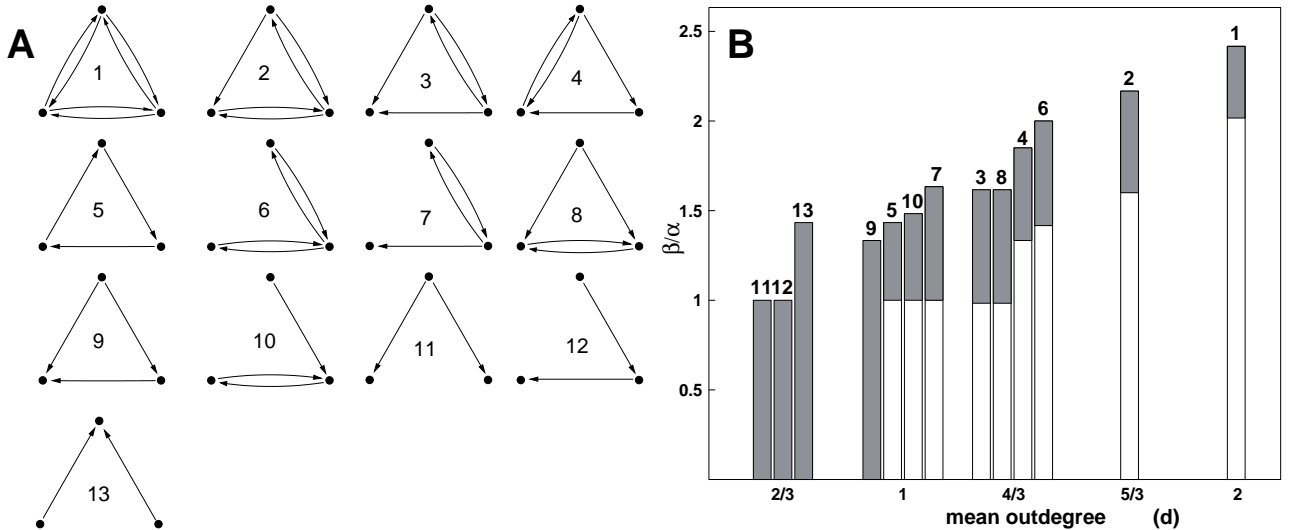


Figure 3: Stability and amplification of signalling networks with three kinases and three phosphatases. (A) The full set of 13 networks obtained for  $n = 3$ . The networks are represented as graphs similarly to Figure 1 (lower panel). (B) Regions of unstable off states (blank parts of the bars), signal amplification (shaded parts) depending on the activity of phosphatases and on the mean outdegree of the corresponding graphs, for the case  $\lambda = 0$ . For  $\beta$ -values above the bars the signal is dampened.

On the abscissa of the diagram the networks are grouped according to the mean outdegree of the corresponding graphs. The ordinate represents the values of the rate constant  $\beta$  for phosphatases normalised to the rate constant of kinases. The blank part of any bar characterises that region of  $\beta$ -values for which the corresponding network is unstable. The shadowed part is the region where the corresponding network is stable and displays amplification. In all cases,  $\beta$ -values above the shadowed parts of the bars result in stable networks with dampening properties. The results of this analysis can be summarized as follows:

1) The four graphs without any cycle (networks 9, 11 to 13) are always stable but they show slight differences in their amplification properties. For example, network 13 displays an amplification over a larger range of phosphatase activities than the other three graphs. This results from the fact that the output kinase of network 13 receives activation from two kinases which themselves are activated by the receptor. 2) All networks with cycles have a region of  $\beta$ -values where the off-states are unstable. The networks with only one cycle have the same critical  $\beta$ -value below which instability occurs. With increasing number of cycles the boundaries for instability are shifted toward higher  $\beta$ -values, with some slight differences in the effects of cycles of length 2 or 3. Network 1 which is represented by a totally connected graph, has a maximum number of 5 cycles and is therefore characterised by the largest region of instability. 3) There is the general tendency that networks with higher number of interactions are less stable, as it becomes evident by the increase of the instability region with increasing mean outdegree.

Figure 4 shows regions of stability and regions of amplification for networks consisting of more than three pairs of kinases and phosphatases. The abscissa represents the network connectivity  $\kappa$  which, according to Eq. (4) is directly related to the mean outdegree  $d$ . Due to normalization with respect the number of kinases,  $\kappa$  is better suited for comparing the properties of signalling networks of different size.

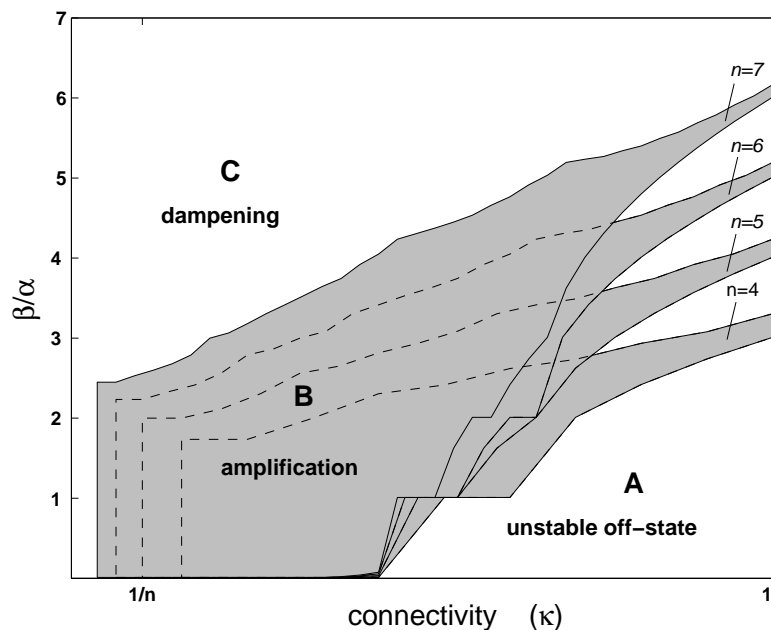


Figure 4: Effect of network connectivity and phosphatase activity on stability and signal amplification for networks of different size.

Figure 4 shows that for each family of networks having the same size  $n$  there are three distinct regions A, B, and C in the  $(\kappa, \beta/\alpha)$ -plane, characterizing parameter combinations leading to different dynamic properties of the networks. In region A all networks of a given family have an unstable signal off-state, in region B (shaded) stable networks exist showing amplification properties, and in region C all networks of the given family have a stable signal off-state and show a dampening in the

signalling amplitudes. The borders between regions A and B and between B and C are shifted towards higher  $\beta/\alpha$ -values when  $n$  increases, that is, networks with higher number of kinases tend to be more unstable. Keeping in mind that biological cells contain several hundreds different types of kinases, one may draw the conclusion that real kinase networks must exhibit only a low connectivity to avoid autoactivation. A similar conclusion can be drawn by considering the number of feedback cycles of a given network design. In figure 5 the  $(c, \beta/\alpha)$ -plane for the set of networks with  $n = 6$  is shown, where the abscissa displays the total number of cycles  $c$  within a network. The maximum number of cycles is determined as follows:

$$c_{max} = \sum_{i=2}^{n=6} \frac{n!}{i(n-i)!} = 409 \quad (10)$$

One identifies again three regions, A, B and C, representing parameter combinations with unstable, amplifying and dampening networks. The more cycles a network with a given  $\beta/\alpha$ -value contains the more unstable it gets. Moreover, a small number of cycles has already a high destabilising effect on the network. Indeed there are no records about positive feedback cycles found in protein kinase networks hitherto. Such feedbacks only exist via transcriptional and translational delay mechanisms, which bring about an additional stabilising effect [11].

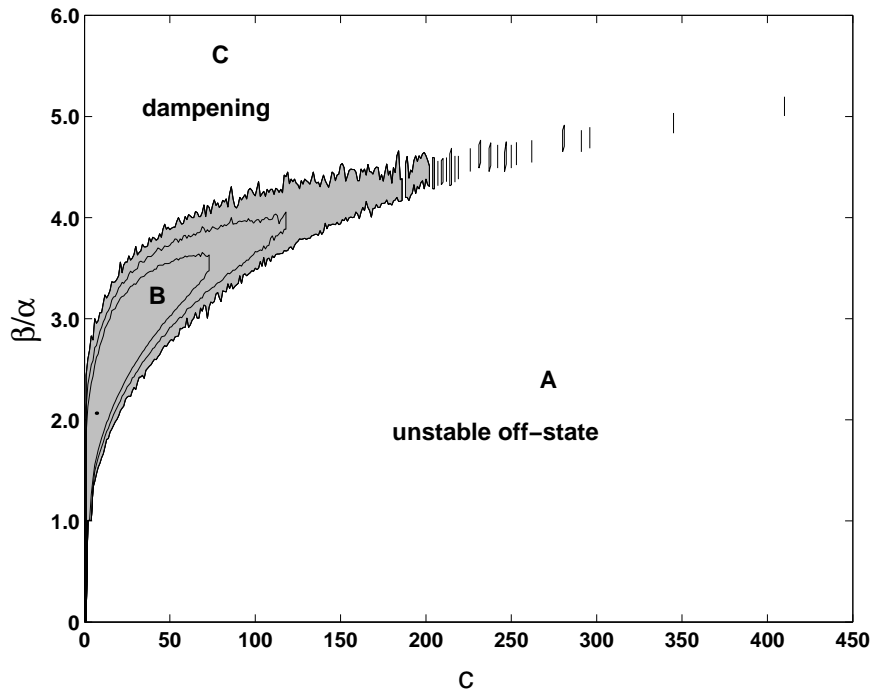


Figure 5: Effect of the total number of cycles within a network and phosphatase activity on stability and signal amplification for networks of size  $n = 6$ .

## 2.6 Analysis of Signal Transduction Networks Retrieved from Databases

In the previous sections we considered general relations between the structure and dynamics of a large class of signalling networks consisting of a relatively low number of kinases. Now we consider signalling networks as found in living cells. Particularly, we investigate the kinase network depicted in Figure 6A deduced from the database Transpath [15]. The network contains 86 kinases which, according to present knowledge, perform 171 mutual interactions. Closer inspection of this network reveals that it does not contain cycles of any length, a rather remarkable property in view of the high number of interconnections. We have performed an analysis of random networks having the same number of

kinases and activations leading to the result that these networks contain on average more than two cycles of length  $L = 2$  and more than two cycles of length  $L = 3$ . The database Transpath contains also informations on small G-proteins which may switch between an inactive GDP - and an active GTP - form. Adding these G-Proteins, and their activating effects, to the kinase network results in the network shown in Figure 6B. Compared to the network with only kinases the number of compounds is enlarged to 94 and the number of interactions to 199.

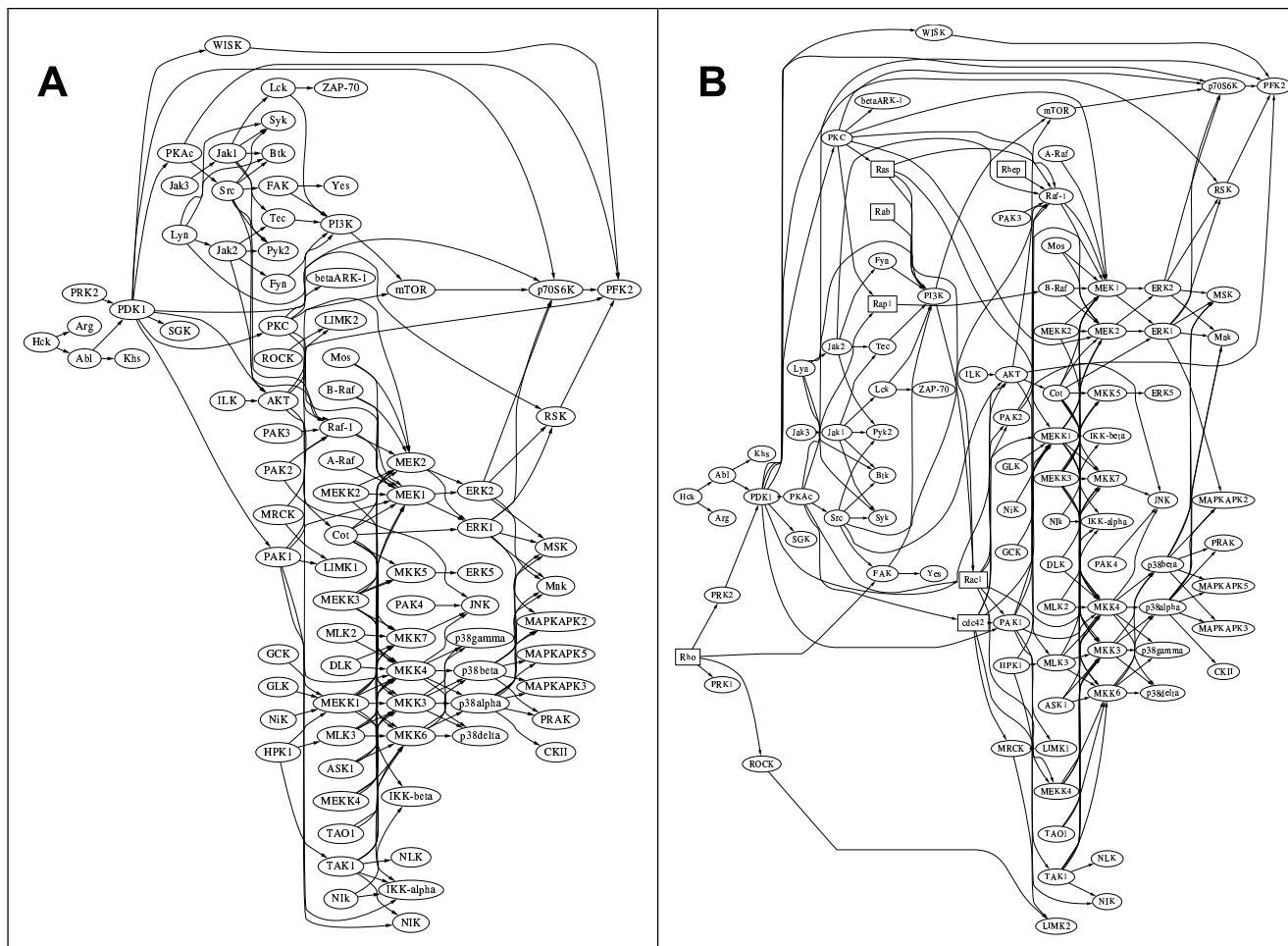


Figure 6: Signalling networks as deduced from the database Transpath [15]. (A): Network involving only kinases (encircled). (B): Network involving kinases (encircled) and small G-proteins (in boxes).

Inspection of this network shows that even this network still contains no cycles. For further structural characterization we calculated the connectivity of these two networks. According to formula (4b) we obtain  $\kappa = 0.0234$  (A) and  $\kappa = 0.0228$  (B). These low number support our hypothesis that realistic signalling networks must be characterised by a low connectivity for avoiding autoactivation in the absence of any receptor stimulus. A more detailed characteristics of the interconnections concerns the number of activations received or performed by any given kinase. For example PDK1 is activated by two upstream kinases and activates itself eight other kinases (see Figure 6A). MEK1 behaves in an opposite way. It has ten upstream and only two downstream kinases. The diagram in Figure 6 shows how many kinases have a given number of upstream and downstream interactions. There is a recognizable tendency of the kinases to divide into two groups. Some act predominantly as signal distributors having many downstream kinases as substrates but few activating kinases (located in the diagram of Figure 7 above the diagonal) whereas others act as signal receivers with many upstream kinases and few downstream kinases (located below the diagonal).

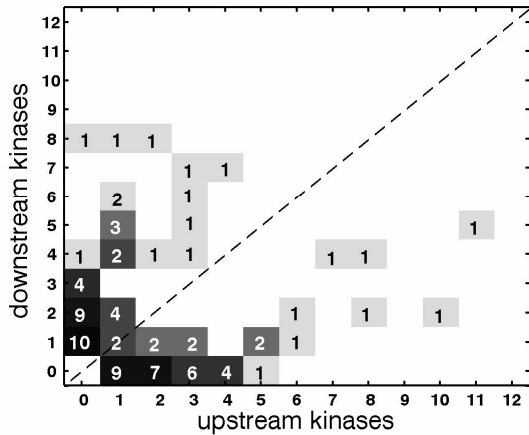


Figure 7: Unspecific interactions in the kinase network of Figure 6A. The entries give the numbers of kinases characterised by given numbers of input and output activations.

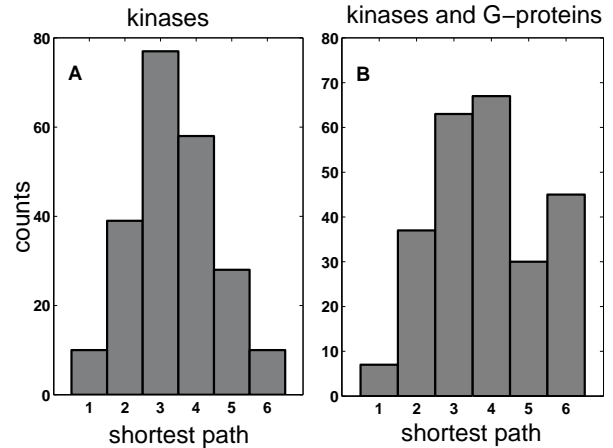


Figure 8: Lengths distribution of shortest pathways between input and output kinases. A) Kinase network (Figure 6A). B) Network of kinases and small G-proteins (Figure 6B).

Another structural characteristics of the network in Figure 6A is the length of the shortest routes from any input kinase to any output kinase. The lengths are counted as the number of activating steps along the routes. Figure 8 shows how many shortest routes of a given length exist. It is seen that no route is longer than six and there is a considerable number of routes consisting of only one step. Routes of length  $L = 3$  occur more often than shorter or longer routes. The typical lengths of routes in this network corresponds therefore to that of MAPK-pathways. In Figure 8B the lengths distributions is shown for the network depicted in Figure 6B which involves also G-proteins. Although the number of additional components is only slightly increased by the inclusion of G-proteins (from 86 to 94) there is a rather strong effect on the lengths distribution. Compared to the kinase network the number of the longest routes with six activations is increased by a factor of more than four. Moreover, the most frequent route length is shifted from three to four. This corresponds, for example, to the situation within the MAPK pathways whose first kinases are activated by a G-protein.

### 3 Discussion

In contrast to chemical reaction systems of inanimate nature biochemical reaction systems as metabolic networks, signal transduction or gene regulatory networks are the outcome of evolution. This means, that their design is not arbitrary but such that the networks may fulfil their specific functions for living cells in an optimal way [8, 14]. It is therefore of interest to analyse which kind of structures bring about certain advantageous dynamical features. Hitherto, studies of that kind have been performed only for metabolic networks (see [4, 14]) resulting in conclusions concerning an optimal ordering of reactions in pathways of energy metabolism as well as concerning their robustness against elimination or exchange of reactions. In the present paper we extended this kind of studies to signalling networks. As a starting point we have chosen networks consisting only of kinases and phosphatases. In contrast to simulation models which refer to specific pathways, we analysed very large sets of networks of different structure. This allowed us to identify groups of networks with specific dynamical properties such as signal amplification or dynamic stability. Some predictions could be made concerning an optimal design of signalling networks in terms of network connectivity and number of cycles. We plan to extend the theory and its applications to large scale networks by including multiple phosphorylation of kinases and by considering their interaction with scaffolds or transcription factors. This includes also a systematic search of subnetworks (e.g. [12]) with recurrent structural motifs.

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