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From Physics to Pharmacology?

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Abstract

Over the last fifty years there has been an explosion of biological data, leading to the realization that to fully explain biological mechanisms it is necessary to interpret them as complex dynamical systems. The first stage of this interpretation is to determine which components (proteins, genes or metabolites) of the system interact. This is usually represented by a graph, or network. The behavior of this network can then be investigated using mathematical modeling. In vivo these biological networks show several remarkable (and seemingly paradoxical) properties including robustness, plasticity and sensitivity. Erroneous behavior of these networks is often associated with disease. Hence understanding the system-level properties can have important implications for the treatment of disease. Systems biology is an organized approach to quantitatively describe and elucidate the behavior of these complex networks. This review focuses on the progress and future challenges of a systems approach to biology.

1. Introduction

A biological system (which, in the context of systems biology, might be a cell, organ, organism or ecosystem) needs to be able detect and respond to environmental stimuli. Ultimately, regardless of the system or stimuli, it is individual cells which are the effectors of the response. Cells receive a signal (e.g. in the form of hormones, growth factors, nutrients or physical stresses) and process it in order to decide how to respond. For example, the response might be: cell cycle arrest, growth, differentiation, proliferation, migration or apoptosis. Signals are received and processed by intracellular signaling pathways. These pathways typically consist of proteins which process information via a complex series of interactions. For example, a protein might exist in an active or an inactive form, and cell fate might depend on the concentration of the active form. In this example, the concentration of the protein can be controlled by the signaling network: the network is initiated by a stimulus and processes this signal in order to control protein concentration. An example of such a process is the eponymously named p53 network. The p53 protein becomes activated by a signal which results from DNA damage and is a transcription factor that, when activated, can regulate the expression of many genes. It is suggested that cell fate is determined by p53 activity levels. At low p53 activity levels the cell operates in normal conditions, intermediate levels initiate a pathway that repairs the DNA damage and high p53 levels initiate an apoptotic pathway leading to cell death, figure 1 [1, 2].

Failure of these pathways to correctly carry out their task is known as dysregulation. If this dysregulation has abnormal consequences then we call it a disease. Disease inducing dysregulation can be due to: extreme environmental conditions, infection, or mutation in a

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gene. Mutations can occur at random, be inherited or arise as a result of exposure to some external factor. For example, cystic fibrosis is a result of mutations in the cystic fibrosis transmembrane regulator (CFTR) gene and sickle cell anaemia is a result of a single point mutation in the β-hemoglobin gene. Whereas cancer is caused by a series of mutations which lead to a change in cell phenotype [3]. On a heuristic level, dysregulation can be thought of as a perturbation of the network. Disease occurs when this perturbation is significant enough to cause symptoms. In the case of disease, drugs act to correct the dysregulation of a pathway or suppress the phenotypic effects of the pathway. Either of these effects could be temporary or permanent. For example, the dysregulation of a downstream pathway due to a vitamin deficiency can be remedied by a simple vitamin supplement, whereas chemotherapy aims to eradicate cells which carry the cancerous mutations.

Hence, understanding how information is received, transmitted and processed by cellular signaling pathways is of huge importance. A full understanding of how a cell executes a particular process should lead to quantitative predictions on how perturbations to pathways will effect the signaling network's response. Any such understanding of a cellular process must necessarily take into account that intracellular signaling networks represent complex systems. This complexity spans several orders of magnitude in space and time. For example, mammalian cells are typically tens of micrometers across yet proteins that control the cell are on the nanometer scale. The challenges of studying biological complexity across these scales has led to the emergence of a new field known as Systems Biology. Like all nascent terms *systems biology* means different things to different people. Generally any interdisciplinary and quantitative approach to describing properties of biological systems can be considered to be *systems biology*.

One of the most important goals of systems biology is to suggest novel therapeutic strategies (for example multiple drug targets) drawn from a systems level understanding of signaling and regulatory pathways. However, for now, this remains a future goal.

In the past cell biology has followed a reductionist approach with the goal of establishing the molecular components of the biological process under consideration. Unfortunately, signaling networks resist a reductionist approach to their study: the emergent properties of the network depend on the interactions of its components. New technology has led to an explosion of biological data describing genes, proteins, structures and phenotypes. As staggering as this wealth of information is, it has not led to a comparable explosion of knowledge for how cells are regulated, or how to treat disease. One of the goal of systems biology is to bridge this gap. Hence, whilst a reductionist approach can identify network components, systems biology's goal is to offer new tools to understand how the assembly of these components gives rise to system wide properties. Denis Noble notes:

> Systems biology is where we are moving to. Only, it requires a quite different mind-set. It is about putting together rather than taking apart, integration rather than reduction. It starts with what we have learned from the reductionist approach; and then it goes further. It requires that we develop new ways of thinking about integration that are as rigorous as our reductionist procedures, but different. [4]

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In practice systems approaches to biology do have a reductionist element. Practicalities, either computational or from lack of experimental data, force reductionism through necessity. One outstanding challenge of systems biology is how to systematically reduce a network to a minimal set of components that retain the system wide properties of the network.

In this review we focus on the systems biology approach to understanding cellular signaling. This signaling can be thought to have a hierarchical structure. Here, we focus on two levels of this hierarchy: global topology (section 2) and network motifs (section 3). Section 4 introduces spatial regulation of signaling. Section 5 briefly introduces the role of biological structures in cell signaling. This motivates the suggestion that an exciting current direction for systems biology is the coupling of biochemical and biophysical models.

2. Biological Networks

Trying to understand the inner-workings of a cell is analogous to trying to understand how an electric circuit works with little or no knowledge of the nature of the components. Taking this analogy a step further, we can compare a cell to a computer. Then, in this analogy, the computational modules of the computer (chips and circuit boards) are analogous to signaling networks in the cell: logic gates are analogous to reactions, or motifs of the network; and the physical components, transistors and diodes are analogous to proteins, protein-complexes, metabolites and membranes, figure 2. Hence, signaling networks can be thought of as the central processor of the cell. They are activated, by an external or internal signal, and process this signal into a response. This is exactly the case in the p53 example above, figure 1: the p53 network processes a signal into a response.

How a complicated circuit board functions can not, necessarily, be inferred from a visual inspection. However, there are well defined diagrammatical and mathematical tools to design, and infer, function. Similarly, the complexity of biological signaling networks requires quantitative approaches to understand their behavior.

Conceptually signaling networks can be represented by graphs where the nodes are proteins, genes or metabolites and the vertices represent some form of interaction (usually activation, deactivation or complex formation) between different nodes. The size of the the entire signaling network of a cell could be huge. For example, in humans it is estimated that there is ≈55,000 distinct proteins, [5]. Even if our knowledge of the network was complete (which, of course, it is far from) it is clear that there are huge computational, theoretical and practical difficulties in studying in detail at this level. A common approach is to assume the entire network is made up of modules of distinct signaling networks so that a sub-set of the signaling network can be investigated in relation to a particular problem, or a specific series of experiments.

When trying to establish the function of an electrical circuit we are aided by knowledge of principles which guide the organization of the circuit. Similarly, establishing organizing principles in biological networks may aid us in understanding the function of the network and how evolution has established these principles. The structure of biological networks seems to confer some properties that are universal across organisms.

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2.1. Properties of Biological Networks

It is on this 'processing' level that the computer analogy really fails for a cell. Not only must signaling networks operate at a high signal to noise ratio but they must be able to respond to wide variety of unpredictable stimuli. External or internal signals can induce gene transcription - which is an effective re-wiring of the signaling network. The most dramatic example of this rewiring is during cell differentiation, where a cell undergoes a transition (often via cell division) into a new cell type. Even though all cells contain the same genetic information it is expressed differently in different cells types. The wiring of the signaling network is determined by gene expression. In the computer analogy this plasticity would be the equivalent of a computer rewiring its hardware in response to some stimulus. It is perhaps for these reasons that signaling networks have evolved to have certain unique properties which would appear to arise, at least partly, from their structure.

In particular, signaling networks have been found to be robust, plastic and sensitive [6, 7, 8]. Kitano (2004) defines robustness of a biological network to mean the system maintains function in response to external and internal perturbations. Hence, robustness is a desirable trait of networks that need be responsive to their environment whilst maintaining core function. Plasticity we define as the ability of networks to self organize (rewire) in response to certain environmental conditions - for example altering translation and expression of protein constitutes a rewiring of the interaction network and potentially drastically different response in the network. Biological networks are also highly sensitive: phenotypic behavior can result from tiny stimuli (eg pN force, chemotropic gradients). Sensitivity is not always advantageous, for example single point genetic mutations can be highly deleterious. In this sense cells are fragile. However, biological networks can behave robustly to internal and external noise. That cells are simultaneously robust and and fragile initially seems paradoxical. However, for evolved complex networks there appears to be a direct correlation between fragility and robustness and there is a trade off between these two properties [6, 7]. It has been suggested that 'robustness + fragility' is in, some sense, a conserved quantity [9]. Hence robustness to some perturbations, must be balanced by fragility to others. If this truly is the case then it follows that biological networks are tuned by evolution to encourage robustness in certain functions and sensitivity, or fragility, in others.

Precisely how these system level properties emerge from the topology of the network, and how these networks can have apparently paradoxical properties (sensitivity and robustness), are open questions in the field of systems biology. However over the last few decades computational approaches have been hugely successful in elucidating biological processes. Primary to the success of this approach is the interpretation of any biological process as a complex system (or network, for example figure 3) that has emergent, and often unpredictable, properties - this is systems biology. The tools of systems biology are computational or analytical and depend on the scale and nature of the biological problem. Computational and analytical tools include: high level network approaches (such as bayesian network and graph theoretic approaches) and mechanistic modeling using ordinary or partial differential equations.

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2.2. Analysis of Biological Networks

Applying network measures to the global topologies of biological networks immediately gives rise to important observations. For example, the degree distribution of a network, $P(K)$, is defined as the probability that a node has K links. In biological networks nodes are usually proteins and links the interactions between proteins. Naively, it might be expected that the links between nodes occur independently of how many links the node already has, and that links are functionally specific but topologically random. In the case of a random network, $P(K)$ follows the Poisson distribution. However, it has been found that many biological networks follow a scale-free distribution, defined as $P(K)$ is proportional to K^{-a} for some $a > 0$ [10, 11]. So the majority of nodes have very few links, yet there are some nodes that are highly connected.

So heuristically, a scale free network is characterized by a small number highly connected ‘hubs’. It is suggested that scale-free networks emerge by constant addition of nodes coupled with preferential linking to existing, highly connected, nodes [12]. The scale-free property of biological networks may explain both their robustness and sensitivity to perturbations (for example, mutations), because they have been shown to be resistant to random attack but sensitive to attack directed at network hubs [13, 14]. It may be speculated that hubs give rise to sensitivity and the other components to robustness.

Intriguingly, it has been illustrated that robustness arises as a direct consequence of the scale-free topology of a signaling network, and robustness is a property independent of any specific biochemistry. Aldana *et. al*, [15], show that a homogenous network is only robust for certain parameter values, whereas scale-free networks can be robust for a wide variety of parameters. However, they also suggest that biological networks lie on a cusp between robust and chaotic behavior - giving an intriguing insight into how biological systems may be simultaneously robust to some perturbations but sensitive to others, [15].

This work by Aldana *et. al.* is an example of coarse graining a network. At the current time, detailed data on the kinetics of protein-protein interactions is, on the scale of large networks, unavailable. Hence, coarse graining approaches, which attempt to capture the system level behavior whilst approximating the detailed interactions, are invaluable. In the example above, Aldana *et. al* approximate biological networks with a boolean network - so that every element of the network can be in an active or an inactive state with switching between the two determined by the elements neighbors. Despite this simplification, boolean networks can exhibit a wide range of dynamic behavior observed in biological networks, [16, 17]. See [18] for an excellent introduction to boolean modeling of biological networks.

Robustness may also be conferred by a ‘bow-tie’ structure, figure 4. In this structure the network is separated into distinct components depending on how conserved (evolutionarily) they are. The biggest component, the core, is scale free and highly connected (typically, between any two nodes there are multiple routes, [6]). Kitano (2004) argues that robustness may arise from this structure, but in a manner that is adaptable. Clearly, a completely robust system, i.e. one in which any internal or external perturbation has no effect on the system, can never adapt to its environment. The bow-tie structure is a potential mechanism for striking this balance. The core, which is highly conserved by evolution because it is

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essential, is separated from the inputs and outputs by a weakly conserved interface which can interpret a wide variety of signals, figure 4. Hence, the essential functions are buffered from the inputs, or novel functions, by the interface. The robustness of the system arises from the core, which is robust due to its scale free architecture.

However, the emergent properties of signaling networks are not solely due to the topology of the network - the dynamics of the network are equally important. In the computer analogy, the topology of the network can be thought of as the wiring of the circuit and the dynamics the software. As exemplified in the boolean approach, network topology (in a sense) is a discrete approximation to the dynamics, with vertices between nodes existing if the interaction is strong enough. Although, clearly dynamics are crucial in inducing the emergent properties of signaling networks. Hence, the properties of a signaling network are due to both dynamics and network topology. For example, in metabolite networks it has been found that hubs of the network were not necessarily essential for survival [19], whereas for protein-protein networks deletion of ‘hubs’ often results in lethal phenotypes [20].

Furthermore, it is emerging that dynamics are crucial in order to define and ‘insulate’ separate modules within signaling networks [21, 22]. These modules can be functionally, spatially, kinetically or temporally separated. For example, functionally distinct cellular processes such as: the cell cycle, apoptosis, migration, endocytosis and adhesion can be thought of as distinct signaling modules with (in some cases) limited crosstalk [23, 24]. Modules can also be spatially distinct (for example, gene transcription and receptor activation occur in spatially distinct locations). Temporal separation occurs when two modules control processes that occur on time scales of differing orders of magnitude.

Hence, depending on the problem of interest, different components of the entire signaling network can be considered [23, 24]. In many computer simulations modularity does not evolve as an optimal solution to a fixed goal. However, it has been shown that evolving a network with varying goals leads to a modular structure [25]. Suggesting that in conditions where the environment is changing, networks can evolve a modular structure.

3. Characterizing Signaling Motifs

The global structure of signaling networks can, to some extent, be decomposed into functional modules. These modules themselves can be broken down into ‘signaling motifs’. Signaling motifs can be thought of as highly local properties of the network that are responsible for regulating particular aspects of cell behavior. Although, that is not to say that they act independently - but it will be illustrated that simple motifs can lead to the rich dynamics necessary for regulation of cellular function.

A network motif is usually thought of as a reoccurring pattern of interactions between a small number of genes, proteins or metabolites [26]. Figure 5 shows a typical example of a network motif, the coherent feed forward loop. In this example, protein A positively regulates protein C indirectly through protein B and directly (the feed forward branch). The term coherent is used to denote the fact that both branches of the loop affect C in the same way. Alon *et. al* (2007) describe how the coherent feed-forward architecture acts as a sign-sensitive delay element. The logic with which A and B activate C determines where the

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delay occurs. For AND logic, meaning both A and B are required for activation of C, the delay in the response of C is in activation step. Whereas for OR logic, meaning either A or B can activate C, the delay is in the deactivation step. Coherent feed forward loops have been observed in the arabinose and flagellum pathways of *E. Coli*.

3.1. Modeling Signaling Motifs with ODEs

Behavior of signaling motifs can rarely be deduced visually, hence mathematical modeling is an invaluable tool to quantitatively asses the dynamical behavior of signaling motifs.

For example, consider the system in figure 5. We shall assume that activation occurs enzymatically and that proteins A, B and C exist in active and inactive forms with A activated by a stimulus. If the number of proteins is suitably high we can study how the concentration of A, B and C varies deterministically. Furthermore, if (within our region of interest) the concentration is presumed, or observed, to be homogeneous it may be suitable to study the process using ordinary differential equations. In this case we represent figure 5 by a set of enzymatic reactions:

$$\begin{array}{c} S(t)+A_{k_r} \rightleftharpoons^{k_f} A^*+S(t) \\ A^*+B_{k_{-1}} \rightleftharpoons^{k_{+1}} [A^*-B] \\ \rightarrow^{k_2} A^*+B^* \\ A^*+C_{k_{-3}} \rightleftharpoons^{k_{+3}} [A^*-C] \\ \rightarrow^{k_4} A^*+C^* \\ B^*+C_{k_{-5}} \rightleftharpoons^{k_{+5}} [B^*-C] \\ \rightarrow^{k_6} B^*+C^* \end{array}$$

These eight reactions represent: activation of A by a time dependent stimulus S(t); enzymatic activation of B by A; and enzymatic activation of C by both A and B. The k_i's are reaction rates, for example k_{+1} is the rate of A**bindingto*B forming the complex [A*-B]. Systems of ODEs representing reactions can be written generally as d**R**/dt = **f**(**R**), where each element of **R** is a reactant in the system. For physically realistic systems there are restrictions on **f**. For example, any negative terms in f_i must have a factor of the *i*th reactant - so that the solutions remain positive [27].

Using a mass action law, we can write an ODE for each of the reactants. For example, for A*:

$$\frac{\mathrm{dA}^*}{dt} = k_f \mathrm{AS}(t) - k_r A^* + \mathrm{AB} + (k_{-1}+k_2)[A^*-B] - k_{+1} A^* B + (k_{-3}+k_4)[A^*-C] - k_{+3} A^* C \quad (1)$$

where the positive terms correspond to either conversion of A to A* (activation) or the restoration of A* from a complex. The negative terms correspond to conversion of A* to A (inactivation) or sequestration of A* in the [A*-B] complex. In the case of larger systems it is desirable to make simplifications to reduce the dimensions of the system. For example, if the concentration of the enzyme-substrate complexes ([A*-B] and [A*-C] in this case) remain approximately constant and if the total concentration of enzyme is approximately constant (for example if A^* reaches a steady state very quickly), then Michaelis-Menten

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kinetics can be assumed. In which case, in this example, B gets enzymatically activated by A^*, giving the product B^* at a rate:

$$\frac{v_m B}{K_m + B} \quad (2)$$

where v_m and K_m depend on the reaction rates and the total concentration of enzyme (A^*). Because the concentrations of the complexes are assumed to be constant we can simplify our system. The system could be further simplified by assuming other properties, for example conservation of mass. When Michaelis-Menten kinetics are not valid there are alternatives for modeling enzymatic reactions which may give better results. For example, a total quasi-steady state approximation [28].

Terms of the form of equation 2 give rise to the saturating dynamics often seen in biological systems. Similar saturation terms can give rich dynamics and, along with feedback loops, can regulate temporal and spatial activation.

As a brief example of how Michaelis-Menten saturation terms can give rise to rich dynamics, consider the motif in figure 6. In this motif, a protein *A* becomes activated due to a stimulus and in turn activates proteins B which activates protein C. A is deactivated by C, which also deactivates B. Lastly, C is directly inhibited by A. Essentially, this is negative feedback with delay. Figure 6 is highly similar to a motif that is central to cell cycle regulation [29].

Here we represent this mathematically, using saturating terms, as three ODEs and three conservation equations:

$$A + A' = 1,\ B + B' = 1,\ C + C' = 1 \quad (3)$$

$$\frac{dA}{dt} = k_1(1 - A) - \frac{k_2 C A}{k_3 + A} \quad (4)$$

$$\frac{dB}{dt} = \frac{k_4 A(1 - B)}{k_5 + 1 - B} - \frac{k_6 C B}{k_7 + B} \quad (5)$$

$$\frac{dC}{dt} = \frac{k_8 B(1 - C)}{k_9 + 1 - C} - \frac{k_{10} A C}{k_{11} + C} \quad (6)$$

here the primed terms refer to an inactive form of each protein *A*, *B* and *C*. Using the saturating Michaelis-Menten terms has simplified our system from 9 equations to 6 (otherwise ODEs for the complexes would be required).

For many parameter choices this system results in an oscillatory response, figure 7. Figure 8 illustrates that the oscillatory behavior of the system can be qualitatively captured using a single parameter bifurcation diagram.

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The system in figure 6 is an example of negative feedback with delay. In fact, it is well known that when coupled with delay (for example, activation of an intermediary protein) negative feedback can lead to oscillations. This has been observed in biological systems. For example, negative feedback with delay is believed to be used as a mechanism for oscillations of NF κB, which regulates the cell cycle, inflammation and apoptosis [30, 31]. Although this is not the only mechanism that can lead to oscillations. Hysteresis oscillations can be established by the time delay being introduced by positive feedback with a bistability [31].

As well as oscillations, negative feedback can also induce dose to duration encoding, so that the length (rather than amplitude) of the response is dependent on the dosage level. This has been shown to be a potential mechanism for encoding quantitative information about the pheromone level in yeast. On the basis of this information yeast cells will decide to mate or undergo chemotropic growth [32].

3.2. Modeling Robust, Sensitive and Adaptive Signaling Motifs

Feed-forward and feed-back loops also play a role in conferring robustness to a network. Some ‘modules’ of a network may be highly sensitive to external or internal signals. Such components of the signaling network are tuned to respond with exquisite sensitivity, yet the whole network is robust to the state of these components. Since biological networks are highly connected [33] it is interesting to consider whether there is an organizational principle of the network which confers robustness coupled with sensitivity. For example, endothelial cells (which line mammalian arteries) can respond to a $\approx$1 pN/μm^2 shear stress induced by fluid flow over their surface. In response to this force gene transcription is altered and they align and elongate in the direction of the fluid flow [34]. However, essential cellular functions are still maintained - they are robust and sensitive to the external signal. It would seem that biological networks have evolved to be highly tuned. So that, in the requisite scenario cells are ‘robustly sensitive’. A stark example of this is during cell differentiation. In response to particular environmental signals cells differentiate into a distinct phenotype, yet despite this fundamental shift in function the cell maintains essential functions. It may be that network structure can insulate connected signaling modules to confer robustness. It has been noted how global structure may confer robustness. Interestingly, robustness can also be conferred at the motif level.

For example. consider the network in figure 9. The signal, *A*, is connected to two components: *B*, which is sensitive to the signal and *C* which is robust to the signal. *B* and *C* could be individual proteins, or signaling modules in their own right. *A* could be an upstream signaling cue, or an external signal.

As a working example, consider this system expressed mathematically as

$$\dot{A} = k_1 e^{-k_1(t-100)} \quad (7)$$

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$$\dot{B} = k_2 \frac{A^2 B}{1+A^2} - k_3 B^2 \quad (8)$$

$$\dot{C} = k_4 AC - k_5 AC^2 \quad (9)$$

The signal A we take to be zero for *t* less than 100 units, and saturate for large *t*. *B* is activated by *A* with saturating kinetics, similar to the Michaelis-Menten terms discussed above. However, in this case the term is a function of A^2 indicating some form of cooperativity is required for the activation of B. The B^2 term represents auto-inhibition. The robustness of C is mathematically trivial in the sense that, for non-zero A, the steady state of *C* is independent of the signal. However, this may have biological relevance. If we consider $C = U + V$ where

$$\dot{U} = k_4 AV - k_5 AU^2 - \alpha U \quad (10)$$

$$\dot{V} = k_4 AU - k_5 AV^2 + \alpha U - 2k_5 AUV \quad (11)$$

Then the concentration of *C* can remain constant, whilst *U* and *V* vary. *U* and *V* could represent distinct chemical states of *C*. So, $\dot{C} = 0$ is actually a tread-milling case, where synthesis of *U* is balanced by degradation of *V*. However, in certain circumstances, despite the constant level of *C*, it may be the case that the the relative levels of *U* and *V* can play an important role in biological regulation. For example, *U* and *V* might have different diffusion rates. Therefore changing the balance between *U* and *V* could alter how signal is propagated spatially - see section 4. The result of integrating this system can be seen in figure 10.

Typically biological networks are highly connected. Yet signals specifically activate certain pathways, despite the interconnectivity of the network. So how are pathways insulated from the components of the network that are sensitive to the signal? For example, in figure 9 the outputs are not connected. So, that *B* is sensitive to the signal does not effect *C*. However, in a highly connected network output i) may well be indirectly (or directly) connected to *C* and output ii).

There are a couple of mechanisms that networks may employ as insulators. One such method is adaptivity to the signal - which means that the ‘output’ responds transiently to the signal before returning to basal levels. A robust adaptive system responds adaptively regardless of the parameter choice.

Figure 11 gives a simple (and, in fact, the simplest linear example) of a robust perfectly adaptive system [35]. In this system a protein has an active (*R*) and an inactive (R^*) state. The inactive state is synthesized and the active state is degraded. Conversion from the inactive to the active state is up-regulated by a signal *S*.

The ODEs for the system in figure 11 are

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$$\frac{dR^*}{dt} = k_a(S)R - k_d R^* - \delta R^* \quad (12)$$

$$\frac{dR}{dt} = -k_a(S)R + k_d R^* + \alpha \quad (13)$$

where $k_a(S)$ is the activation rate of R and is dependent on the signal, whereas the deactivation (k_d) of R^*, the synthesis (α) of R and the degradation (δ) of R^*, are all independent of the signal, S. Then the steady state value of R^* is easily found to be $R^{*\hat{}} = \alpha/\delta$ and hence, since this value is independent of S, this system is perfectly adaptive for any parameter set.

It has been shown that adaptive motifs can be classified into two groups - motifs that have negative feedback with a buffering node or motifs that have an incoherent feed forward loop with a 'proportioner' [36]. Incoherent loops are so called because the feed forward loop has an opposite sign to the linear pathway (so if the linear pathway up-regulates, the incoherent feed-forward loop down-regulates). In the linear example, figure 11, the feedback is more subtle. In fact, this is an example of linear integral feedback control, [35].

To illustrate how an incoherent feed forward loop can confer adaptivity, re-consider the network in figure 6. But now assume that there is crosstalk between B and C (figure 9) such that output i) activates C. Since B is sensitive to the signal, how can the robustness of C be maintained? A robustness in the response of C in this case can be obtained by the network orchestrating an adaptive mechanism. Figure 13 extends (in red) the network in figure 9 to give an adaptive response to the signal by C.

If the kinetics of this system are represented as

$$\dot{A} = k_1 e^{-k_1(t-100)} \quad (14)$$

$$\dot{B} = k_2 \frac{A^2 B}{1+A^2} - k_3 B^2 \quad (15)$$

$$\dot{C} = k_4 AC - k_5 AC^2 + k_{11} B - k_{12} CF \quad (16)$$

$$\dot{D} = k_6 A + k_7 E - K_8 BD \quad (17)$$

$$\dot{E} = k_8 BD - (k_9 + k_7) E \quad (18)$$

$$\dot{F} = k_9 E - k_{10} F \quad (19)$$

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then this system shows an adaptive response, figure 14. *E* represents the complex of *B* bound to *D*, which gets modified to a protein *F*, which, in turn, inhibits C. In this case the adaptation is near perfect - the activation returns to approximately basal levels after a transient response to the signal. This is an adaptive response because the signal remains, yet the activity is only transiently affected. But it is dependent on the parameter choices, so it is not robust adaptation.

This mechanism is dependent on an incoherent feed-forward loop. The other mechanism that Ma *et. al.* [36] identify is 'negative feedback loop with a buffering node', and they suggest that known biological examples of adaptivity use this method rather than the 'incoherent feed forward loop with a proportioner'. For example, *E. Coli* chemotaxis is believed to use a 'negative feedback loop with a buffering node' mechanism [37].

3.3. Pathway Specificity

Figures 9 and 13 can be thought of as mechanisms for achieving pathway specificity. Many signaling pathways share components yet generate distinct responses. This situation is analogous to sending multiple signals through a single cable. In theory this multiplexing can be accomplished through molecular motifs that act as high and low pass filters [22]. This mechanism of pathway specificity has been termed kinetic insulation. In kinetic insulation upstream signal processing encodes input from two distinct stimuli as sustained or transient responses. Downstream elements that only respond to sustained or transient signals decode the transmitted signal and effect the desired response.

Kinetic insulation may be one way in which separate motifs and modules integrate successfully. However, it appears to be the case that functional modules are topologically separated by low connectivity, which minimizes cross-talk and maintains specificity in signaling [38]. Furthermore, localized perturbations to biological networks on average, appear to remain localized [39]. Suggesting that, despite a highly connected topology functional modules can operate specifically and independently. However, the precise circumstances under which individual motifs or modules maintain their function upon coupling is an open research question. It may be that there is no *a priori* rationale for ensuring the successful integration of even weakly interacting signaling components. Hence, that cells do seem to have successfully accomplished this feat is a result of selective pressure to do so.

One open question is how to model entire signaling networks whilst maintaining resolution at the scale of the network motif. To do this requires new approaches to the deal with the dimensionality of the problem. Generally the problem is simplified to either consider boolean networks [18] or steady states [39, 40]. One successful steady-state, stoichiometric, method is metabolic flux analysis (MFA), which provides a systematic framework for analyzing metabolite networks [41]. Metabolite networks can also be analyzed using metabolic control analysis (MCA), another steady-state method. However, MCA has been extended to include dynamics and has been successfully applied to protein signaling pathways [42, 43], and represents a systematic methodology for analyzing large signaling networks. Both MCA and MFA are restricted to assuming spatial homogeneity (although

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separate reaction compartments can be considered). Section 4 introduces spatial regulation in more detail.

3.4. Model Fitting

Given the complexity of biological systems, a typical model will consist of many parameters. Hence, it is of crucial importance to determine parameter values systematically. It may be the case that experimental data exists for parameters, for example reaction rates. However, in many cases some (if not most) of the parameters of a model will need to be determined by some other methodology. If enough data is available then the model can be 'trained' by optimizing the parameters so that the model fits the data. Typically, this involves minimizing a function that defines the error of the model relative to the data - there are various well established methods and algorithms to do this efficiently [44].

For small parameter sets and simple models it may be possible to do a brute force search by defining a parameter range and dividing this range up into bins. However, due to the multiplicity of the search this method soon becomes cumbersome. A popular way to deal with this is simulated annealing, *SA*, which is a stochastic method based on the Metropolis Algorithm [45]. The suggestive name comes from analogy with the thermodynamics of annealing a metal: by increasing the temperature atoms are allowed to move out of their initial configuration. This allows the atoms to re-arrange into other, lower energy, configurations. As the metal cools they become trapped into a new configuration. SA is very similar in concept. However, in this case the cost (or energy) of being in particular configuration has to be defined.

SA is a global optimization method applied to minimize a given function. In this case this minimization is with respect to a parameter set, $\mathbf{p}$. Typically, the goal will be to fit a model output, $\mathbf{M}(\mathbf{p})$, to a time course of experimental data $\mathbf{E}$ (where each component of these vectors represents a single time-point). Hence, the target function is often taken to be $|\mathbf{F}|$ where $\mathbf{F} = \mathbf{M}(\mathbf{p}) - E$. One constraint of many parameter fitting methods is the choice of a model output to fit. For ODEs this is usually a natural choice. However, for PDEs (see below) the choice of output is clearly more complicated. Hence, how to fit parameters for PDEs is an ongoing area of research.

The SA algorithm is as follows. At each iteration a new parameter set $\mathbf{p}'$ is randomly generated from a distribution centered on the current parameter set, $\mathbf{p}$. This parameter set is accepted with probability $P(|\mathbf{F}(\mathbf{p})|, |\mathbf{F}(\mathbf{p}')|, T)$ where T is the 'temperature'. Generally, if $|\mathbf{F}(\mathbf{p})| > |\mathbf{F}(\mathbf{p}')|$ then $P = 1$ and the choice is automatically accepted. P is usually defined by the Boltzmann-Gibbs probability distribution:

$$P = Ce^{-\Delta F/k_B T}$$

where k_B is the Boltzmann constant and C is the reciprocal of the partition function, ensuring that the distribution sums to 1. The annealing occurs by decreasing T as the algorithm progresses. How this cooling is controlled can effect the efficiency of the fitting.

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However, for at least some cooling regimes true global minimum can be found given enough time [44].

Methods such as SA give systems biologists an array of tools to validate their models. It is of course by no means guaranteed that a particular model will be able to explain the experimental data. Furthermore, if a model can fit existing data then the model may be able to make novel predictions or suggest novel experiments the results of which may, or may not, support the model. It is at this interface where, as a field, systems biology thrives.

It is often the case that models (especially those with larger parameter sets) are left unconstrained due to the paucity of experimental data. However, such models may still be able to make quantitative predictions. Due to the typical architecture of biologically inspired models the model output may not be that sensitive to the parameters themselves. So called 'sloppy parameter sensitivity' suggests that even large amounts of experimental data will leave model parameters unconstrained (meaning that the data is explicable with a large spectrum of parameter sets) [46]. Importantly, Gutenkunst *et. al* showed that models that have weakly constrained parameters can make surprisingly strongly constrained predictions. This is actually what might be expected from a robust system in general. For if we view each parameter set as a perturbation away from an average parameter set then robustness implies that the response of the system will not be sensitive to these perturbations. However, given that there is a trade-off between robustness and fragility, as described in section 1, there should be a cost for the sloppy parameter property.

In some circumstances models are used to try and determine the structure of the network itself. As noted above, given that the data may not constrain the parameters or structure of the model, it may be that inverting the problem is more appropriate. That is to ask, what is the probability of a particular network structure given the data? Clearly this is a Bayesian problem: the prior probability for a structure S, $P(S)$, is updated by: the likelihood of the data given the structure, $P(D|S)$, the probability of the data independent of the structure $P(D)$. This results in the probability of the structure conditional on the data, $P(D|S)$. For more details on how to apply Bayesian approaches to network structure see [47, 48]

Applying this approach to a biological problem [48] showed that this approach could successfully reject hypotheses regarding how ERK1/2, a mitogen activate protein kinase (MAPK - MAPKs are a family of proteins that act as transducers of a wide variety of external stimuli) becomes activated. They also concluded with more data this methodology could distinguish between the two remaining hypotheses.

4. Examples of Spatial and Temporal Regulation in Biology

4.1. Modeling Signaling Motifs with PDEs

Spatial regulation is required for any process that involves spatially heterogeneous signaling. One method of describing this is by using partial differential equations (expressing the reactants as a function of space and time $\mathbf{R}(\mathbf{x}, t)$) and addition of a diffusion term, for example for the concentration, A, the diffusion term would be $D_A\nabla^2 A$ - where the diffusion coefficient D_A is usually a constant. For proteins the diffusion coefficient can vary over

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several orders of magnitude depending on whether the protein is soluble or not. Of course, for large systems it is desirable (computationally) to solve ODEs rather than PDEs.

As an example of how the feedback loops in figure 6 could also regulate spatial information we consider modifying the system into PDEs. Firstly, rewrite equations (4)–(6) as

$$\frac{dA}{dt}=f_1(A,B,C,A',B,C') \quad (20)$$

$$\frac{dB}{dt}=f_2(A,B,C,A',B',C') \quad (21)$$

$$\frac{dC}{dt}=f_3(A,B,C,A',B',C') \quad (22)$$

where the (1 − *A*) terms are replaced by *A*′. Similarly for *B*′ and *C*′. Then a spatial variant of equations (4)–(6) is:

$$\frac{\partial A}{\partial t}=f_1(A,B,C,A',B,C')+D_A\nabla^2 A \quad (23)$$

$$\frac{\partial B}{\partial t}=f_2(A,B,C,A',B,C')+D_B\nabla^2 B \quad (24)$$

$$\frac{\partial C}{\partial t}=f_3(A,B,C,A',B,C')+D_C\nabla^2 C \quad (25)$$

$$\frac{\partial A'}{\partial t}=-f_1(A,B,C,A',B,C')+D_{A'}\nabla^2 A' \quad (26)$$

$$\frac{\partial B'}{\partial t}=-f_2(A,B,C,A',B,C')+D_{B'}\nabla^2 B' \quad (27)$$

$$\frac{\partial C}{\partial t}=-f_3(A,B,C,A',B,C')+D_{C'}\nabla^2 C' \quad (28)$$

The diffusion coefficients are taken such that *D*′/*D* = 100 (the importance of different diffusion coefficients will be explained below). This model is implemented in a 40 μm one dimensional region with no-flux boundaries, figure 15. The results of this model are dependent on the initial conditions. For an initial condition of a linear gradient ranging between 0 and 1 for A, B and C and 1 to 0 for *A*′, *B*′ and *C*′ the cyclic behavior is retained (figure 15), however the dynamics are clearly complex.

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Biological systems repeatedly use negative and positive feedback (and feed-forward) motifs (figure 5 is example of a feed-forward loop) as spatial and temporal regulation mechanisms. [49] give an excellent review of the biological mathematical representations of these regulatory motifs.

As we have already seen, such feedback loops can regulate the duration and amplitude of response to a given signal, and they can also initiate spatial regulation of proteins. Spatial regulation in cells is crucial in many processes.

4.2. Spatial Regulation in Cell Migration

An important example of spatial regulation is cellular migration, which is a critical process in multicellular organisms. Cell migration is a crucial process during development, wound healing and immuno-surveillance. In response to external migratory signals cells establish distinct front and back signaling regions. This process is called polarization and is crucial in vascularization, epithelial development and morphogenesis. Polarization is a necessary precursor to migration. However, it can also occur spontaneously in the absence of external cues.

Intriguingly, cells polarize and migrate either in response to an external cue or at random. In the former case the cell needs to amplify a very small external signal - for example, cells migrate up (or down) a chemical gradient: the concentration difference across the cell length (typically tens of micrometers) is very small. This has led to the suggestion that the concentration gradient leads to a symmetry breaking. Similarly, in the case of random migration, random noise (of either external or internal origin) breaks the symmetry of the cell.

This raises an interesting question: How do cells break symmetry from a uniform state (no front and back) to localized distinct regions of signaling (front and back). This is usually framed as a mathematical problem. If the internal biochemistry of the cell is modelled by reaction diffusion equations (partial differential equations), can uniform steady state concentrations be unstable to non-uniform perturbations? If so, some modes of the perturbation can grow and eventually lead to a steady-state spatially heterogeneous distribution of reactants. Alan Turing ([50]), in the context of multiple-cells undergoing morphogenesis, showed that a two-component system (activator and inhibitor) have just this property: a homogeneous distribution that is stable to uniform perturbations but un-stable to non-uniform perturbations. Heuristically, the Turing instability requires local activation and global inhibition - which, in essence, is generated by slow diffusion of activators and fast diffusion of inhibitors.

In general local excitation and global inhibition (LEGI) signaling components have been widely used to model gradient sensing and polarity [51, 52, 53, 54, 55]. Under suitable conditions the PDEs describing these components can undergo a Turing bifurcation. The general idea of the LEGI mechanism in cell signaling is described in figure 16. However this mechanism can be subtly different, for example: mutual inhibition of spatial segregated signaling regions [56] or local activation along side substrate depletion (where the local excitation depletes substrate, which has a global effect) [57]. Polarity in yeast is believed to

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occur via a substrate depletion mechanism for a Turing instability [58]. Goryachev *et. al.* (2008) showed that yeast polarity could be initiated by a Turing-like mechanism, where the activator and substrate are the active and inactive forms of the Rho GTPase Cdc42. Initially their work focussed on a larger network, from which it is difficult to ascertain the root-mechanism. However, by using simplifying assumptions (for example, mass conservation and quasi-steady states, see section 3.1) they reduced the system to a two-variable Turing-like system involving only the active and inactive states of the Rho GTPase Cdc42. As will be discussed, Rho GTPases are proteins believed to be crucial for establishing polarity in eukaryotic cells.

In order for cells to be able to respond to the gradient of concentration independently of the magnitude of concentration [54] proposed an alternative scheme which coupled adaptive behavior with a Turing instability - so that the system is adaptive to uniform increases in stimulus concentration and persistent in response to stimulus gradient. This model was subsequently analyzed and extended, [53, 59]. Levine *et. al* proposed a mechanism based on 'balanced inactivation' which could mimic experimental results including: an initial global response of the cell to a gradient, followed by loss of response at the rear; reversal of polarity in response to a switching of the gradient and response over a wide range of concentrations and gradient strength. Polarity and directional sensing may also arise as a result of a bistability (the corresponding non-spatial ODEs have multiple steady states), [60, 61]. Although distinct from Turing instabilities, bistable systems also rely on different rates of diffusion for propagation of fronts, [62].

In terms of the internal biochemistry of the cell, these hypotheses requires that any signaling module that regulates polarity has at least two necessary components. Firstly at least some of the components of the module need different diffusion rates, for example some components could be soluble proteins and others membrane, or complex, bound (and hence diffuse slower). Secondly, there must be, in some sense, local positive feedback so that the perturbation modes are locally amplified.

Precisely how polarity is initiated is an open question, and may involve multiple parallel pathways [63]. However, proteins such as the Rho GTPases (Rac, Cdc42 and Rho), phosphoinositide-3-kinases (PI3Ks), protein tensin homolog (PTEN), PAR3, PAR6, aPKC, PIP_2, PIP_3, PAK, Ras and β-PIX are consistently found to play an important role in polarization and migration. [64, 65, 66]

Mathematical modeling and analysis has illustrated the required characteristics of any signaling module that regulates polarity. It is partly for this reason that the Rho GTPases are of central interest in the story of migration and polarity. However the main motivation for modeling the role of Rho GTPases in migration and polarity comes from experimental findings, which have found that the Rho GTPases play an important role in regulating cytoskeletal dynamics and polarity [67, 68, 69, 70].

Interestingly, the active forms of the Rho GTPases are membrane bound, whereas a proportion of the inactive form is sequestered away from the membrane by guanine dissociation inhibitors (GDIs) [71]. Furthermore it is suggested that Rac could be one

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component of a positive feedback loop. Hence, these make good candidates to fit the requirements of a local excitation global inhibition model (LEGI). However, Rho GTPases are not the only proteins associated with polarity, for example PTEN and PI3Ks are suggested to share similar characteristics [72, 73, 74].

Using Rho GTPase crosstalk as an example, it has been demonstrated analytically that mass-conserved reaction diffusion systems with diffusion driven instabilities give rise to the required conceptual properties of signaling modules that regulate polarity [75]. However, the constraining effect of mass-conservation in a reaction diffusion system is yet to be elucidated fully.

Modeling has shown that these properties of the Rho GTPases can, in theory at least, lead to polarization. For example, a one-dimensional model of inhibitory crosstalk (mediated by guanine nucleotide exchange factors) generated spatial segregation of the Rho GTPases into front and back regions [60].

This model was extended into a 2D model of cell migration, with a dynamic morphology modelled by a cellular Potts model [61]. Cellular Potts models are a biological application of the Potts model of statistical mechanics (itself a generalization of the Ising model). Just as in the Ising model, an energy is constructed which is, effectively, minimized by the Metropolis algorithm. This represents the effective energy of the cell, but it is not at all to be construed as physical. The best interpretation is that this 'energy', H, is simply some characteristic(s) that the cell seeks, for biological reasons, to minimize.

For example, if the plane is discretized into lattice sites (i), each of which is assigned a state $\sigma(i)$. The value of $\sigma(i)$ determines which cell the site is within (or whether it is empty space. Then the morphology of the m^{th} cell changes when grid sites change state to or from $\sigma = m$.

There are many ways to define H, but the standard definition of the Hamiltonian in 2-D is, [76]:

$$H=\sum_{(i,j)} J_{\sigma(i),\sigma(j)}(1-\delta(\sigma(i),\sigma(j)))+\lambda(a-A)^2. \quad (29)$$

The sum is over all (i,j) neighbor grid sites. The first term is the contribution to the effective energy of the cell boundary (where if $\sigma(i) \neq \sigma(j)$ then $J_{\sigma(i),\sigma(j)}$ is added to the Hamiltonian). The dependence on $\sigma(i)$ and $\sigma(j)$ means that in principle cell-cell boundaries and cell-ECM boundaries can confer different energy to the Hamiltonian. The second term, $\lambda(a - A)^2$ quantifies the energy arising from the difference in the cell's area (a) and its target area, (A). So the Hamiltonian is large if the cell is large or small relative to A.

Interestingly, Maree *et. al.* used the cellular Potts framework to couple models of different scales together [61]. In their model, the biochemistry regulates cell morphology, which can feedback to the biochemistry. This approach is interesting because it is an attempt to place biochemical signaling in a model cell - with the potential for feedback to the signaling arising from the morphology itself.

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5. Modelling Biological Structures

Usually modeling of biochemical signaling is discussed in the sense of the network abstraction, and the natural mathematical representation of this abstraction - namely differential equations with either mass action kinetics or an approximation thereof. However, these signaling networks are not complete abstractions - they are tightly integrated to the physical structure and morphology of their environment.

Since, in principle at least, signaling networks cannot be abstracted away from their physical environment it is crucial to their understanding that we understand how physical structure and rheology interact with signaling networks. Particularly those that have implications for disease. For example, the most damaging aspect of a malignant tumor is its potential to metastasize and invade surrounding tissues. These diseases have a significant biophysical aspect to their pathogenesis.

Many notable processes are initiated or regulated by mechanical forces. For example, stretch activated ion channels, adherence to the substrate or auditory responses. Furthermore, the actual shape of the cell could give rise to type of signal in itself. For example, a two component reaction-diffusion system u_1 and u_2 could undergo a Turing bifurcation, with bifurcation parameters D_1 and D_2 - the respective diffusion rates. However, the length scale units are determined by the units of the diffusion constants ($[D] = [L]^2/[T]$). If a characteristic length scale is used to non-dimensionalize then clearly D_1 and D_2 are scaled in proportion to $1/L^2$ - so changing the size of the domain is equivalent to changing diffusion rates - which recall are bifurcation parameters of the instability [77]. This effect can be realized by simply linearly scaling the domain, but more relevant biological effects can be observed with asymmetric perturbations to the domain. Interestingly, cell morphology itself can, in principle, act as a feedback to the signaling network [78]. For example, Meyers *et. al* (2006) showed that for membrane bound activators and cytoplasmic deactivators thin protrusions of the cell should be more active due to the activator being closer to the substrate. They verified this observation using experimental data.

The fundamental structure of the cell is conferred by the cytoskeleton. The cytoskeleton is a dynamic polymer network consisting of actin filaments (which are polymers about 8 nm in diameter), microtubules (cylinders of about 25 nm in diameter) and intermediate filaments (which are about 10 nm in diameter). The latter of which are the least understood, and have the wide variety in their composition. However, it is thought that they act as mediator between microtubules and actin filaments [79]. Actin plays an important role in determining cell morphology by regulating contractile and protrusive events. Firstly actin filaments provide an intra-cellular tensile force. This is generated by the shortening of bundles of actin filaments caused by the filaments sliding over one. The force for the sliding is generated by myosin II, a motor protein, which can walk along actin filaments.

In contrast, cell protrusions are believed to be generated by polymerization of actin. There are several different types of characteristic protrusions (for example, flat broad protrusions are called lamellipodia and thin ‘finger-like’ protrusions are called filopodia), and they are all thought to be generated by actin polymerization. Actin is an orientated polymer and,

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typically, preferentially polymerizes at the plus (or barbed) end and depolymerizes at the minus (or pointed) end. In both lamellipodia and filopodia the actin is, somehow (possibly by physical size constraints, or regulated by a localized signaling network) pre-orientated such that the barbed end is preferentially orientated to the membrane. Hence polymerization is directed out of the cell. Although it is not immediately apparent that this mechanism can generate sufficient force to initiate protrusion, careful consideration has led to a widely accepted biophysical model of force generation by actin polymerization[80, 81], figure 17. The difference in protrusion morphology between lamellipodia and filopodia is generated, it would seem, by the structure of the actin polymer network - which is itself governed by the local concentration of actin binding proteins which act as cross-links to the polymer mesh. In lamellipodia the mesh is a tree like structure, primarily due to the dominant actin binding protein, Arp2/3, cross-linking actin at a highly characteristic angle ($\approx \pi/6$) [82]. Whereas in filopodia the dominant binding protein is α-actinin, which links the actin into parallel bundles [83].

How the actin network is established, regulated and maintained has been investigated using a variety of mathematical methods. In the case of actin filaments aligning into parallel bundles the process may be considered to be a case of symmetry breaking - a transition between an isotropic to an anisotropic state. Although symmetry breaking is a conceptually aesthetic idea, it is not clear that this necessarily has to be the case. Nevertheless, using integro-differential equations, it has been shown that spontaneous filament alignment (symmetry breaking) can arise from a simple set of assumptions [84, 85, 86]. These models generally describe the concentration of actin as $f(\theta, t)$, where θ is in the orientation of the filament. f then includes a turning rate - represented by an integral over the θ space.

For example, [85], describe the rate of change of $f(\theta, t)$ as

$$\frac{\partial f(\theta,t)}{\partial t} = -f(\theta,t)\int_{-\pi}^{\pi}\eta(\theta-\theta_i)f(\theta_i,t)d\theta_i + \int_{-\pi}^{\pi}\int_{-\pi}^{\pi}\omega(\theta_0-\theta,\theta_0-\theta_i)\eta(\theta_0-\theta_i)f(\theta_0,t)f(\theta_i,t)d\theta_i d\theta_0. \quad (30)$$

where $\omega(\theta_0 - \theta, \theta_0 - \theta_i)$ is the probability of the a filament at θ_0 turning to θ as a result of interacting with a filament at angle θ_i. $\eta(\theta - \theta')$ is the rate of interaction of filaments at angles θ and θ_i. Equation 30 bears an interesting connection to the Boltzmann equation [85]. For simple choices for ω and η it is straightforward to show that the homogenous steady state is unstable. In fact, this is similar to the LEGI mechanism as described in section 4 - the local excitation occurs through positive feedback - higher concentration at a given angle recruit more filaments around that particular angle. The inhibition occurs is essentially via the substrate depletion mechanism (this system conserves mass). The difference here however, is that a diffusion term is not necessary for an instability. Heuristically speaking, this is replaced by the integral terms - which transition the filament concentration through the θ space. It might be surprising that a single equation can demonstrate this behavior (this is not possible with the typical LEGI mechanism), however equation (30) can be approximated by a large number of ODEs.

As interesting as this is, this approach is still an abstraction from the physical situation. A closer approximation lies in simulating the nucleation and polymerization of this network

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using Brownian dynamic simulations. Polymerization can be modelled using a coarse grained bead-spring approach [87]. In this Brownian dynamic methodology polymer sub units bind an unbind with a prescribed probability and remain attached due to a spring-like force. The position - **r**, of the sub units, or beads, is updated using a Langevin equation

$$\frac{d\mathbf{r}}{dt}=\sum_{i\neq j}\mathbf{f}_{ij}+\eta(t) \quad (31)$$

where η is a noise term and $\Sigma_{i\neq j}\,\mathbf{f}_{ij}$ is the sum of all forces on the beads. Generally this term consists of a repulsive force to keep the beads apart (for example, a truncated Lennard-Jones potential [88], spring forces to keep the polymer together and bending forces which confer lateral stiffness to the polymer.

[89] used a model of this type to show that the actin mesh in lamellipodia is reproduced provided if branching of the network happens preferentially in the membrane orientated direction (as in figure 17) or of polymerization is preferentially increased in this direction. [90] use a Brownian dynamic model to investigate the viscoelastic properties of a cross-linked actin network and showed that the stiffness of the network as a whole is highly mediated by the bending stiffness of the actin cross-linking proteins themselves.

Both biophysical and biochemical models require parameterization in order to make even qualitative predictions. Fortunately, there are systematic approaches to ascertaining parameters.

6. Conclusion

It has emerged that to truly understand and manipulate biological signaling networks a systems biology approach is a necessary and, perhaps, sufficient methodology. Systems biology offers the potential to understand biological systems at an unprecedented fidelity. This representation can, in principle, stretch across several orders of spatial and temporal magnitude - describing interactions at the picosecond-nanometer scale which have down stream effects on the micron-hour scale. However, true multi-scale approaches in systems biology are still the exception rather than the rule. Hence, to this extent, the integrative nature of systems biology still has a reductionist twist. However, a systems biologist will always recognize that some properties of biological networks are bestowed by their complex nature. For this reason, traditional tools developed in mathematics and physics are not completely satisfactory for investigating complex biological systems where the sum is more than the parts. For example, in physics we can calculate the field generated by a point mass and solve analytically the equations of motion for two bodies. We can also compute the orbits for any number of bodies, with the only restriction being computational. Newton postulated his law of gravitational attraction based on the force one mass exerts on another (and vice versa). So the problem of finding the equations of motion of system consisting of any number of bodies is reducible to a much simpler system. In the case of biological signaling networks this approach necessarily must fail because some functional aspects of the network are irreducible - for example, robustness is an irreducible facet of the network because it is bestowed by the structure of the network itself. Although, it is proper to say that

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this does not imply that robustness is not evolvable - robustness is a competitive advantage but not a necessity.

Robustness also presents a barrier to treat disease. Recall that robustness was defined as the ability of a biological network to maintain function in response to external and internal perturbations. For example, if a network has been subverted into a cancerous phenotype by mutation of a gene or genes, then the robustness of this network implies that it has a tendency to maintain its function despite perturbation by drugs, or other therapies. By generating quantitative predictions of suitable drug targets (targets which the network is sensitive to perturbations of, rather than robust), a systems level understanding of a network, or a disease, has the potential to revolutionize therapeutic approaches. Such targets evidently exist since it is known that many diseases are due to catastrophic single point mutations in genes.

Most successful drug targets have been discovered by perturbing single components of signaling networks. However, due to the robustness problem it is likely that pair-wise (and higher order) targets will yield more successful therapies (a network might be robust to any one knock-out but sensitive to a particular pair-wise interaction). Experimentally screening for successful pair-wise targets is multiplicatively more expensive in time and money. However, with a trusted model of a signaling network pair-wise targets may be more obvious, and in any case computational pair-wise screening is more plausible than the experimentally alternative.

Whilst systems biology is maturing as a tool for drug discovery its wider application in a clinical setting is still tens of years away. However, it is foreseeable that the research being actively pursued currently will have clinical applications in the future. If, as is widely predicted, medicine becomes personalized (for example, individual genome mapping) then for this information is to be at all useful then it must be interpreted at the phenotypic level - converting gene and protein expression in to their downstream and future effects is precisely what systems biology seeks to understand and manipulate.

Unlike mature quantitative sciences systems biology is lacking universally accepted laws and principles. One reason for this is the broad array of problems it has been applied to, which cover the whole gamut of biology and its associated breadth of length and time scales. However, great progress has been made in understanding the basic principles by which signaling networks are structured - and how this structure confers function. Consequently, new (and sometimes surprising) biological mechanisms have been elucidated. In order to coherently connect many disparate research efforts there is a concerted effort to standardize systems biology knowledge. For example, the systems biology mark-up language (SBML) is a language which codifies models - as an analogy consider the standard notation and diagrams used to describe electrical circuits: if every researcher used different notation then the sharing of knowledge would be severely impaired. Moreover, databases of both experimental data and computational models regarding, for example, signal transduction networks are available online (e.g. the Signal Transduction Knowledge Environment).

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In the future it may be possible to use the lessons learnt from systems biology to engineer *de novo* complicated phenotypes by, for example, modifying gene expression. Just as, compartmentalizing a signaling network into repeated motifs is reverse-engineering the biological system, constructing a desired phenotype is in an engineering problem. However, engineering a biological system is a fundamentally different proposition. For example, the components of a car have relatively simple inputs and outputs and have a single function. Whereas the components of a biological network are, potentially, highly connected and have context dependent function that may depend on the state of all the other components - it is not necessarily correct to consider a component of a biological system in an input and output sense.

It might be the case that the complexity and nature of systems biology requires drastic new tools, or even mathematics, to successfully capture the essence of complex biological mechanisms. However practically, the limiting factor for a systems biologist is the quality of the experimental collaboration - it is certainly the case that a surprising, yet surmountable, language barrier can exist between an life-science experimentalist and a systems biologist. The current and future re-structuring of education toward more inter-disciplinary approaches should mitigate this barrier. So, it is certainly not the case that ‘traditional’ tools such as ODEs, PDEs and SDEs are, at the moment, the limiting factor at the cutting edge of systems biology.

However, one hitherto unsolved aspect of applying different methodologies to investigate components of the same process is how to bridge the gaps between methodologies at the points of interaction of the methods. For example, it is not at all clear how to sensibly couple stochastic differential equations with deterministic ones or how to systematically couple models of different scales together. If we are to be able to simulate a biological network accurately, and in its entirety, then we must have systematic methods to integrate models not only described with different methodologies but that operate on different time and length scales. This challenge is particularly pressing when attempting to couple biochemical models with biophysical ones. In cellular processes the distinction between these two is not necessarily clearly delineated. In most modeling situations it is conceived that we are modeling a well mixed soluble environment. However, in reality reactions often occur on the surface of cellular structures and the process itself could regulate the nature of these structures. For example, reactions can occur on the cytoskeleton which is dynamically regulated. Furthermore, processes such as directed active transport (proteins can be transported along the cytoskeleton by biochemical motors) mean that some signaling components can advect as well as diffuse. In addition, biochemical signaling can dramatically change the biophysical environment (for example, morphogenesis). This complicated feedback between the biophysical and biochemical processes means that the two are tightly co-ordinated. How to systematically describe this coupling in computationally feasible framework is an open question.

As an emerging field systems biology has shown immense promise and has emerged as the only way to truly classify biological systems. At the root of systems biology is the approach of viewing any biological system as a complex network. Generally it appears that these networks have a structure that confers properties such as robustness, plasticity and

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sensitivity. That these properties are conferred simultaneously is remarkable, yet systems biology is uncovering just how network structure and network motifs cooperate for these properties to emerge. Biological networks regulate protein and gene regulation. This exquisite regulation is remarkable, yet not infallible in the case of disease. Computationally modeling signaling networks will elucidate exactly how genes proteins, and hence, phenotypes are regulated. This will, almost certainly, lead to new and sophisticated treatments that will seek to re-regulate networks back to the healthy state. Systems biology has huge potential. In the sense of being able to make testable quantitative predictions, it has, and will continue to do so, revolutionized biology into a truly quantitative science.

Acknowledgments

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Glossary of terms

Apoptosis	The process by which cells commit suicide. In certain circumstances, for example DNA damage, it is beneficial to the organism for a cell to die
Cell cycle	The process through which cells typically cycle. The cell cycle includes: cell growth, DNA synthesis and culminates in cell division
Cytoskeleton	A dynamic polymer network which confers structure and morphology to the cell
Cytosol	The internal solute of the cell
Differentiation	The process of a cell transforming into a more specialized cell type
Downstream and Upstream	In the context of a signaling pathway these terms correspond to the direction that information, or signal, is processed through the pathway. E.g. if *B* is downstream of *A* then the signal is modified by *A* before reaching *B*. In the case of more complicated pathways (including feedback loops and cross-talk) this terminology becomes less clear. However, it is still useful, conceptually, to describe how information is processed by the network
Eukaryote	An organism with cells that have nuclei
Gene	An inheritable genomic sequence
Genotype	The genetic constitution of an organism. Genotype, environment and inherited epigenetic factors contribute to the phenotype of an individual
Membrane	A lipid bilayer often thought as a two dimensional fluid which surrounds compartments of a cell, and encloses the cell itself
Migration	Cell locomotion. Migration can be directed by stimuli or undirected
Mutation	A change in the nucleotide sequence of a gene

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Phenotype	An observable characteristic of an organism or cell
Polarity	a polar cell is a cell which is asymmetric in structure, protein concentration or both
Proliferation	An increase in the number of cells via cell division
Protein	Compounds that fold into globular forms from linear chains of amino acids. Due to their morphology and biochemistry binding between proteins can be highly specific. Genes are transcribed into mRNA which is translated into proteins
Signaling network/pathway	A network of proteins and/or genes which (via interactions such as binding, modification, synthesis or degradation) interprets an input signal and organizes a response. *Network* and *pathway* are, in this context, synonymous. However, *network* is generally used to emphasize connectivity, whereas *pathway* emphasizes the spatio-temporal ordering of signaling events
Transcription Factor	A protein which controls expression of gene by binding to DNA and initiating gene transcription

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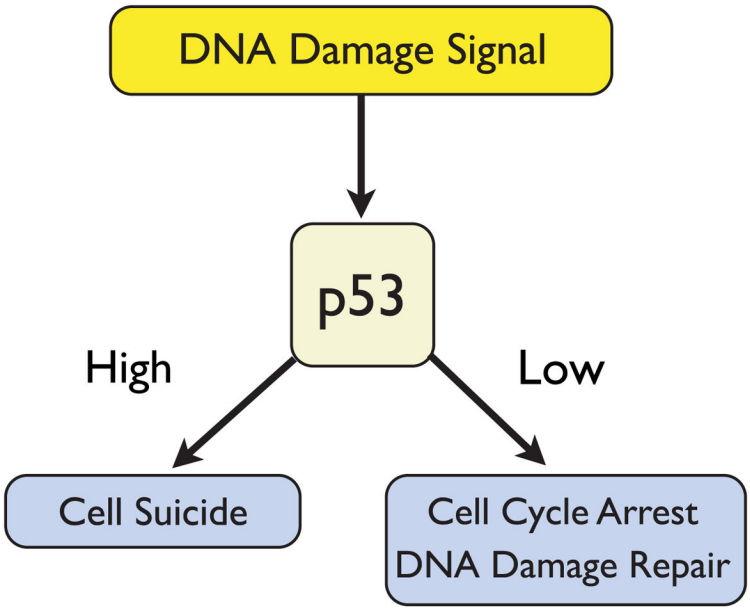

Figure 1.
The p53 network modulates a signal due to DNA damage into one of two responses: either a repair pathway is activated or, if the damage is significant enough, the cell is commanded to commit suicide [2].

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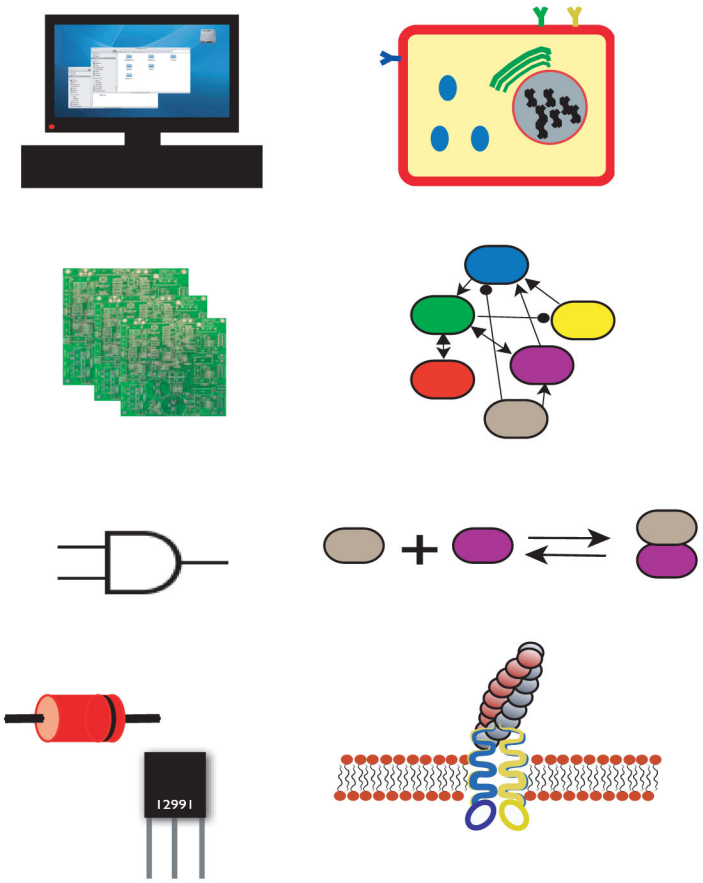

Figure 2.

The computer analogy of a cell. Cells are computational units - programmed to perform certain tasks, and to modulate a certain input into a certain response, or output. The analogy also holds with the components of the cell: computations are carried out by signaling networks, analogous to circuit boards; repeated motifs of the network are used to confer network function, analogous to logic gates; and motifs are made up of physical structures (proteins, complexes, metabolites and membranes, analogous to diodes and transistors etc.)

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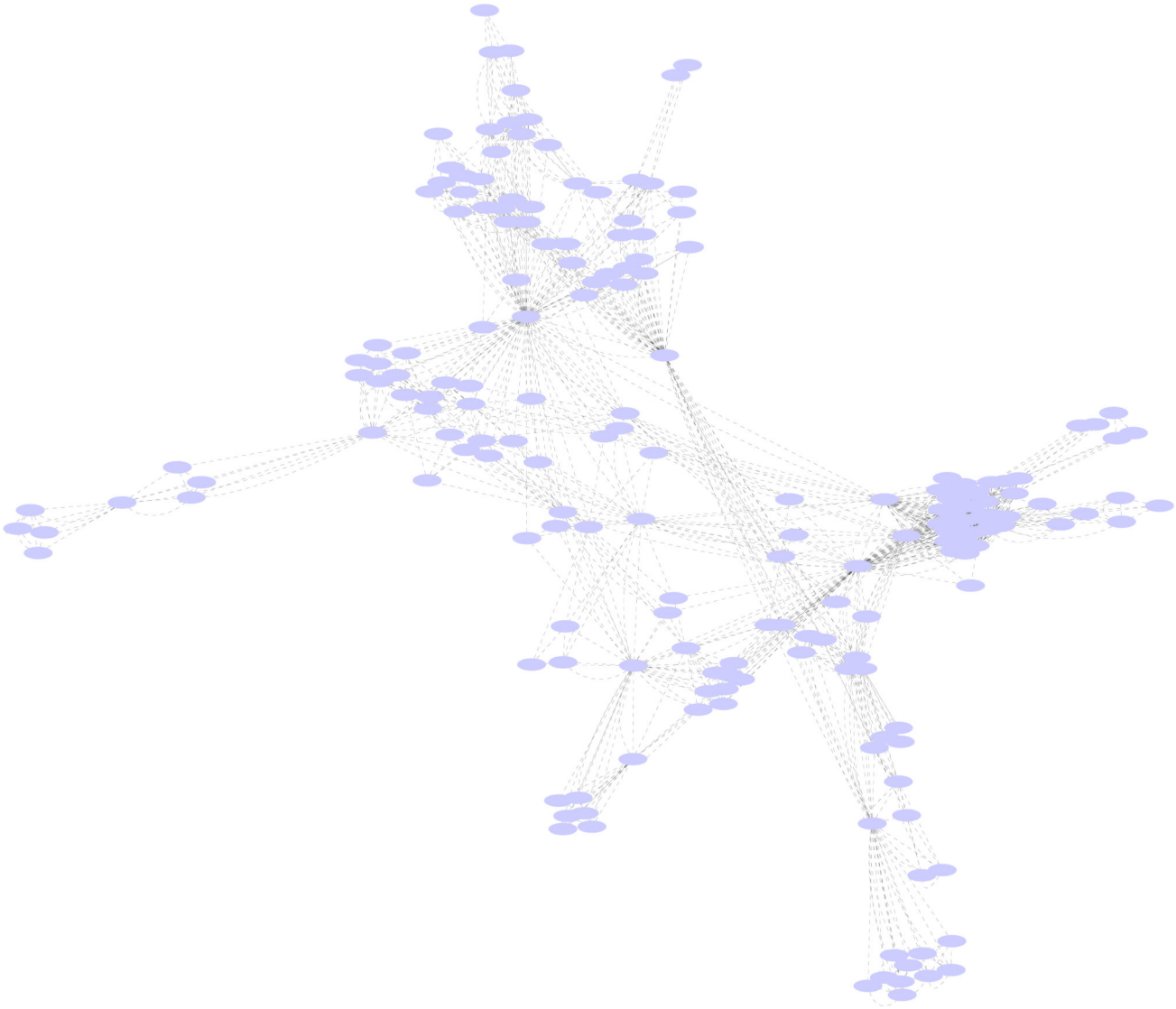

Figure 3.
Example of a biological network, integrin alpha-9 receptor http://www.pathwaycommons.org/pc/record2.do?id=83280

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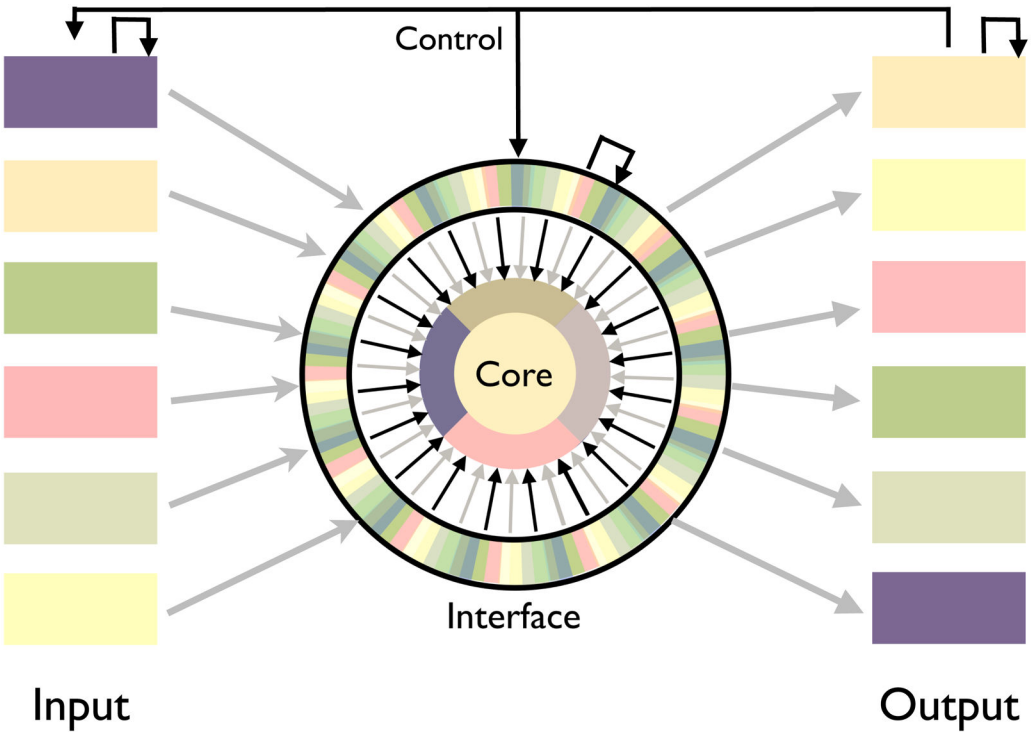

Figure 4.

Bow-tie structure of biological networks. The bow-tie structure is suggested to balance robustness with adaptivity [6]. A highly conserved core interfaces with a weakly conserved interface to the inputs and outputs of the networks. Robustness arises from the scale free nature of the core.

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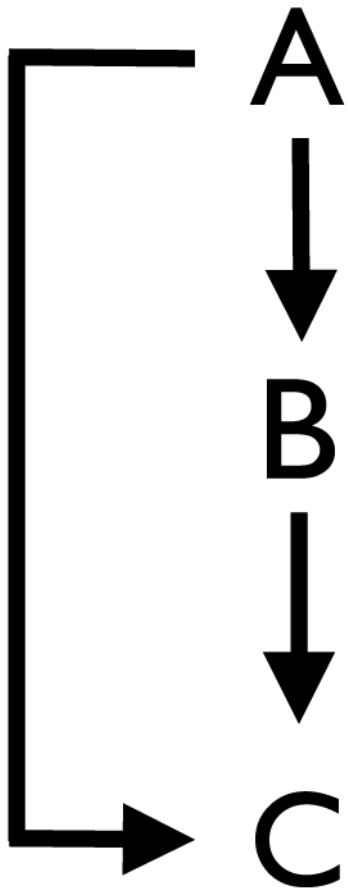

Figure 5.
An example of a network motif. In this motif A activates C directly and by activating an intermediary, B. This example is found in many biological networks, for example in the arabinose and flagellum pathways of *E. Coli*, where A, B and C would represent genes [26].

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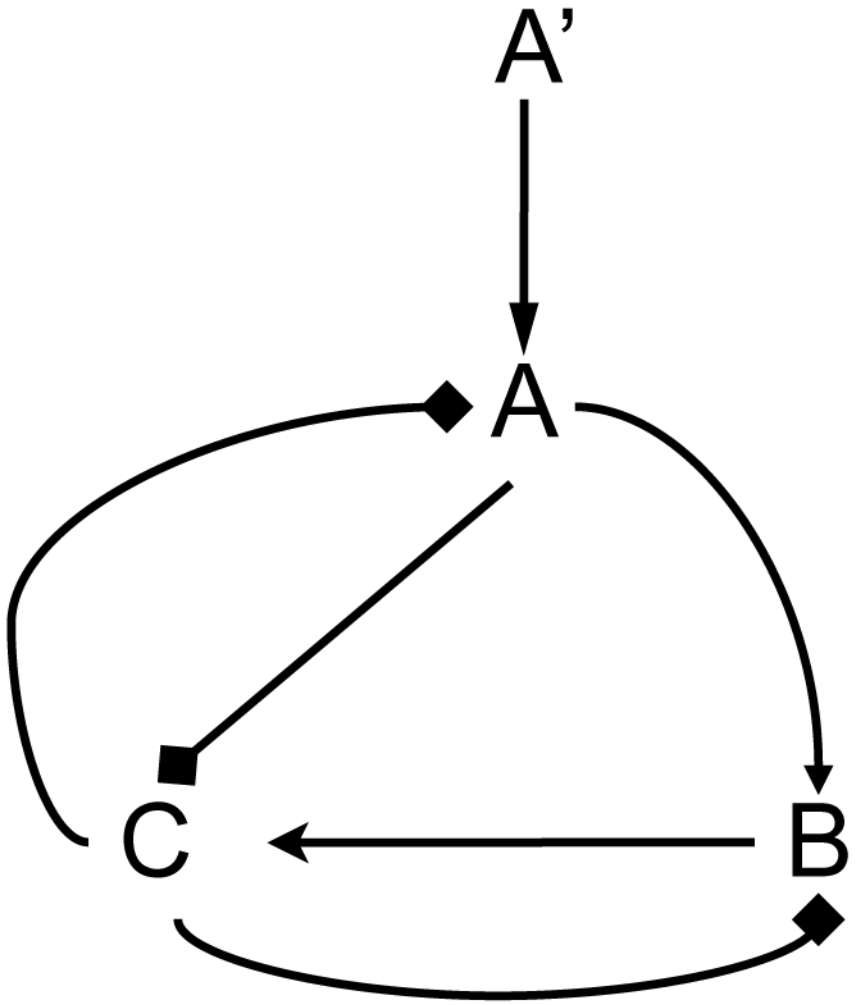

Figure 6.

An example of a network motif that can control temporal and spatial activation. This motif is highly similar to a motif that is central to cell cycle control [29]. A′, B′ and C′ are inactive forms. Arrows correspond to activation (e.g. $A' \rightarrow A$), and squares mean inhibition (e.g. $A \rightarrow A'$)

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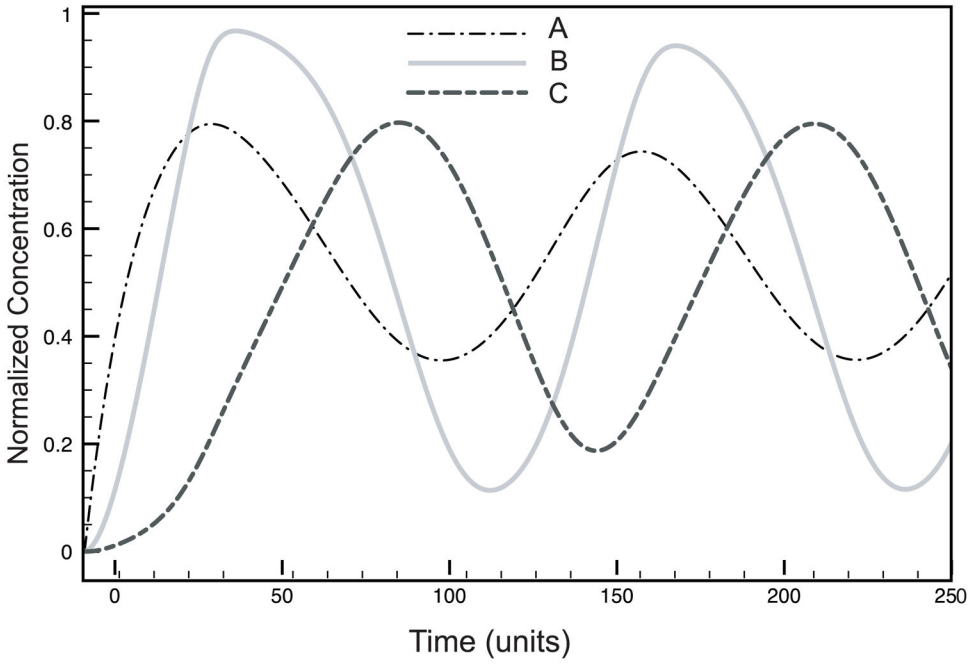

Figure 7.
Equations (4)–(6) integrated. $k_i = 0.05 \forall i$.

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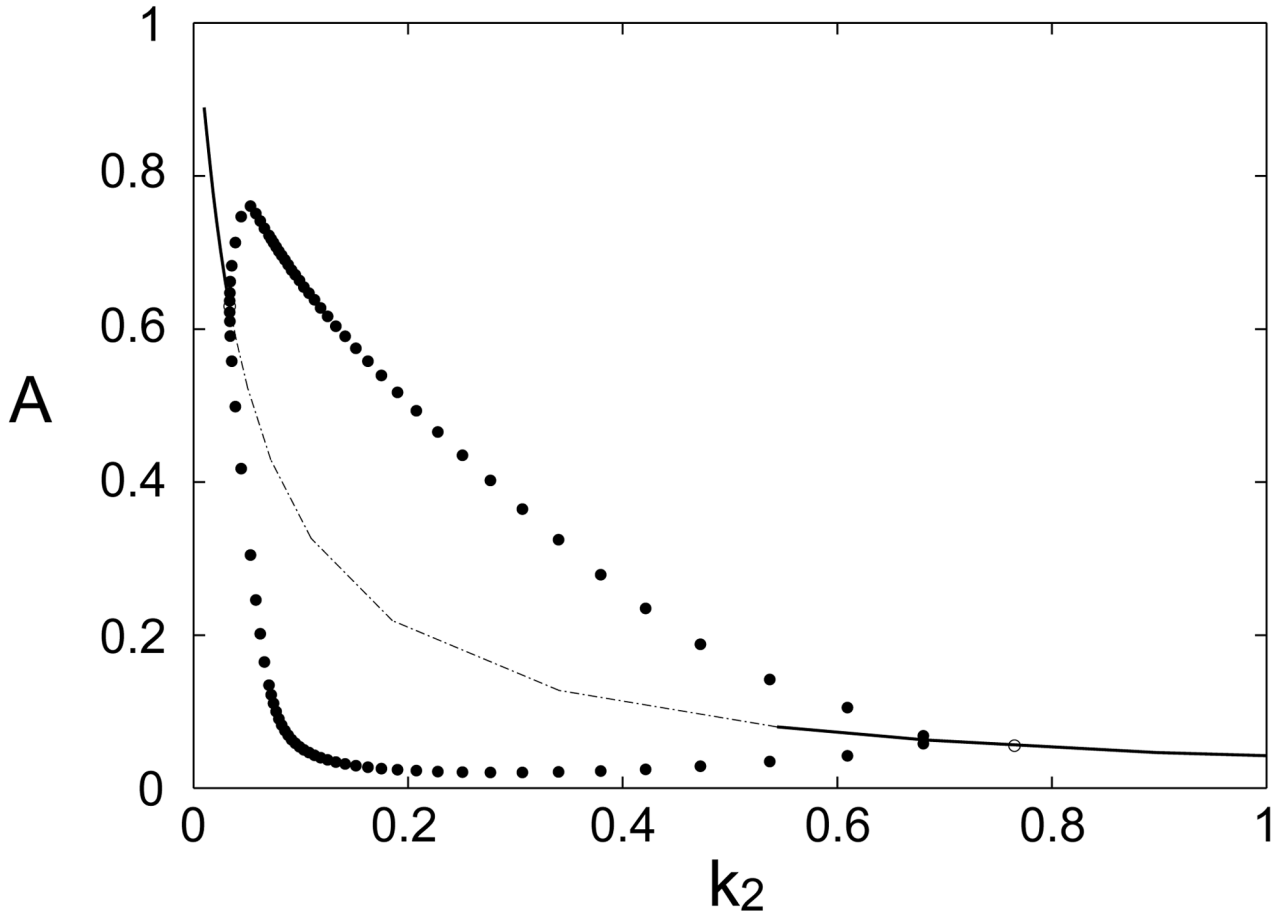

Figure 8.

Bifurcation Analysis by the freely available software xppAUT. Stable points are joined by solid lines, stable periodic solutions are solid dots, unstable fixed points are joined by a dotted line, and unstable periodic solutions are open dots

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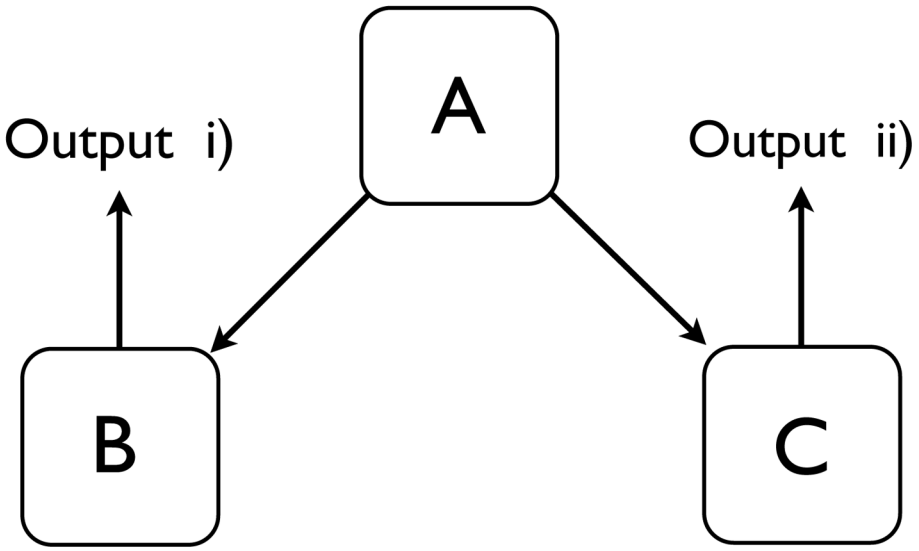

Figure 9.
Two pathways interpret the signal, *A*. *B* is sensitive to this signal and *C* is robust.

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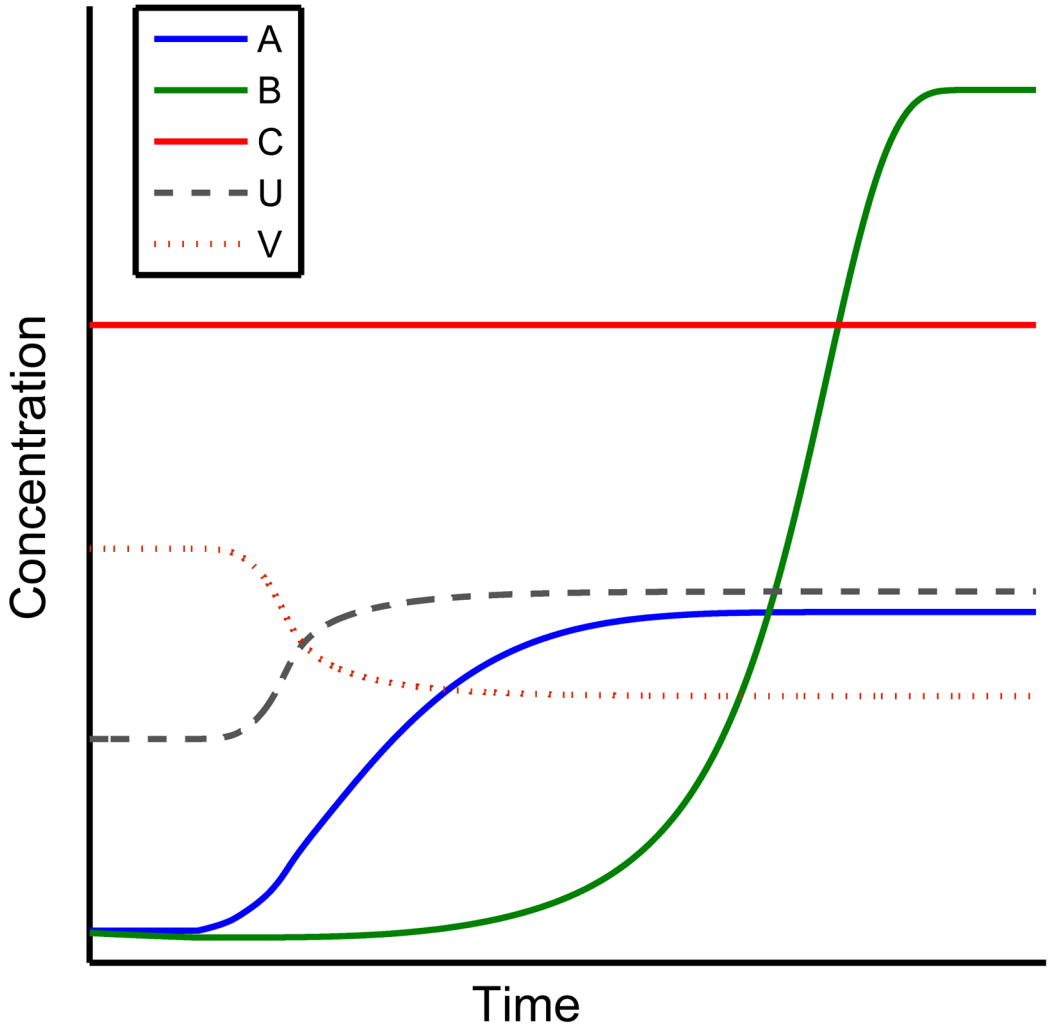

Figure 10.

B is sensitive to the signal *A*, whereas *C* is robust. The steady state of *C* is independent of *A* if $A \neq 0$. However, *U* and *V* (which could represent different states of *C*) can respond the signal whilst *C* remains constant.

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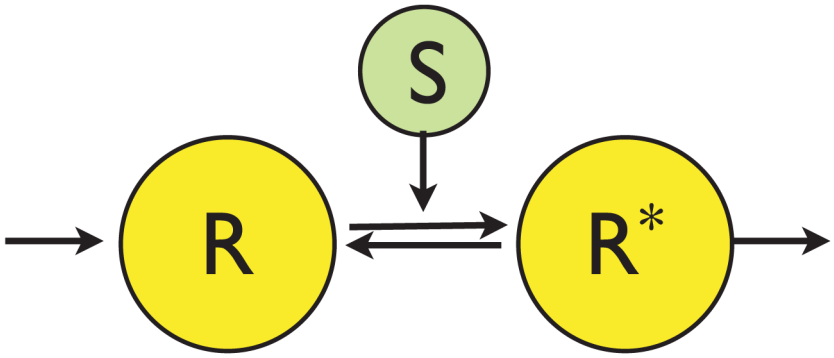

Figure 11.

A perfectly adaptive system. The protein is either inactive, *R*, or active R^*. The inactive form can be synthesized whilst the active form can be degraded. A signal, *S* increases the rate of conversion of *R* to R^*

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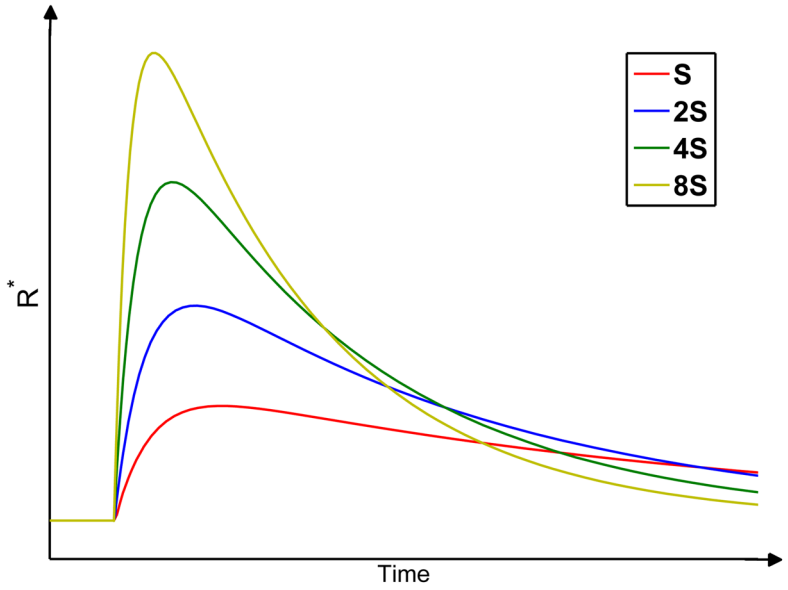

Figure 12.

Equation 12 integrated. R^* is perfectly adaptive to the signal (in this case taken to be kS, where k and S are constant). The adaptivity is perfect because the steady state of R^* is independent of the signal. R is not adaptive (not shown) -the response of R to the signal leads to the transient activation of R^*.

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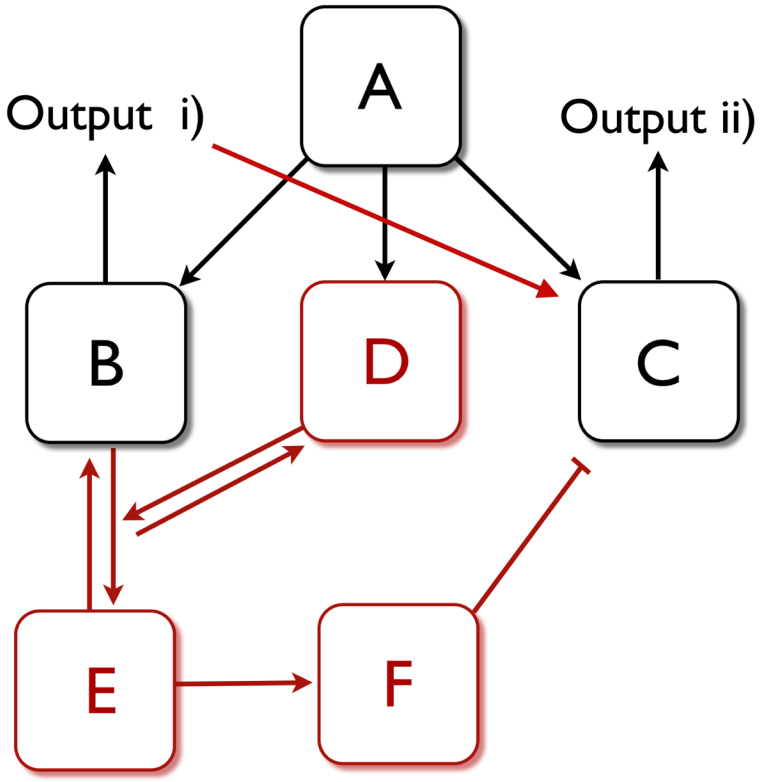

Figure 13.

Crosstalk from output i). activates C. Crosstalk and adaption mechanism in red. Crosstalk between pathways can be organized such that one output is insulated from the sensitivity of the other. Here the insulation is based on an adaptation mechanism which includes an incoherent feed-forward loop (*B*, *E*, *F*) and a proportioner *F*.

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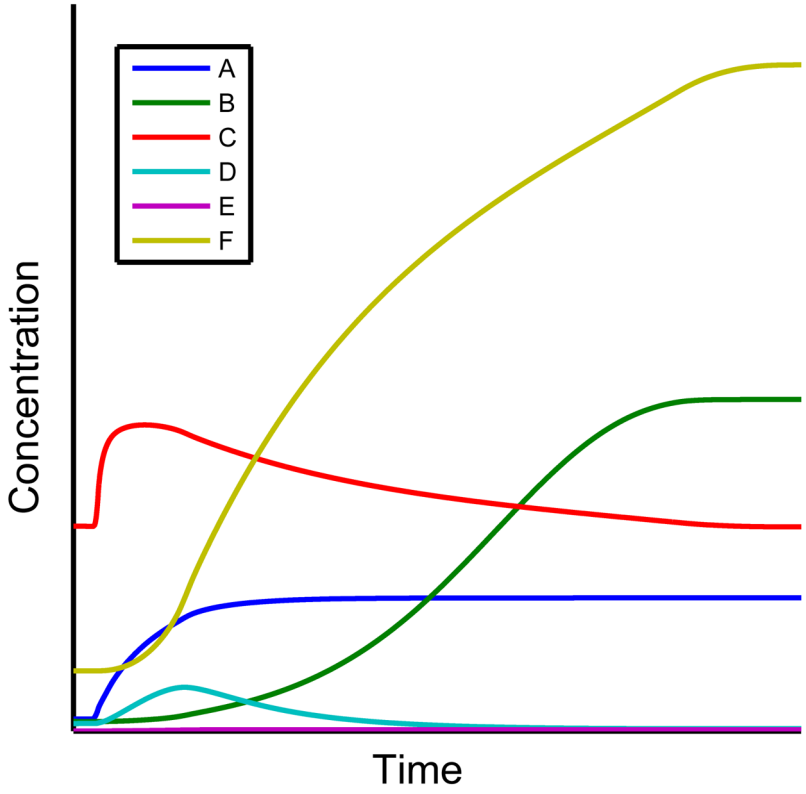

Figure 14.

System (14)–(19) integrated. *C* is adaptive to the signal *A*. Adaptivity is conferred by an incoherent feed forward loop and a proportioner, figure 13.

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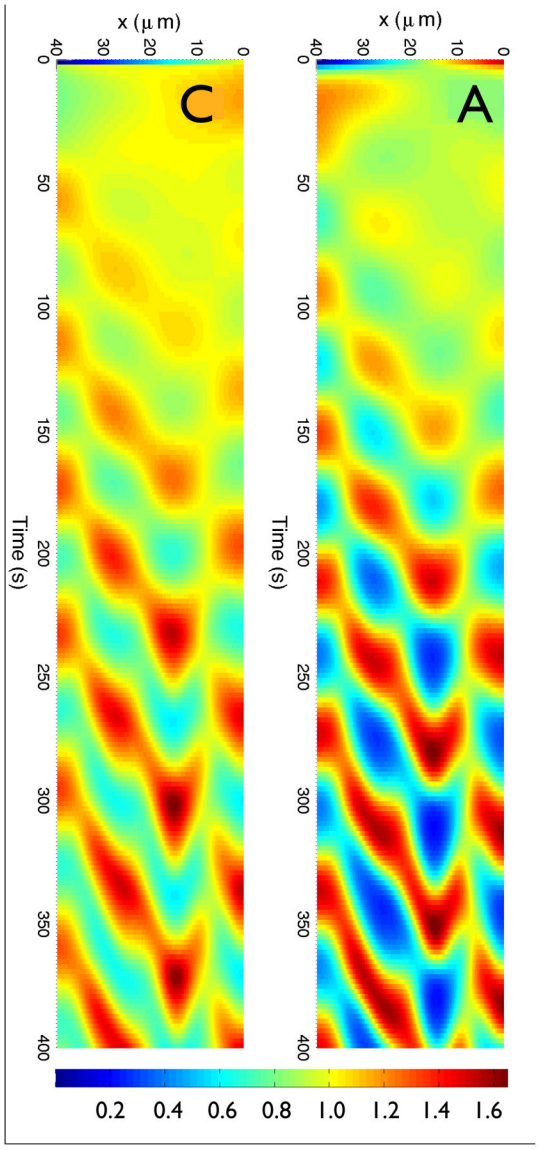

Figure 15.

Kymographs (Species A on the right, C on the left - activity of B is coincident with A) of equations 23 and 28. Time is increasing up the page. Reaction parameters as figure 7. The color values are in normalized units (to the maximum initial value of C. This is an example of spontaneous pattern formation.

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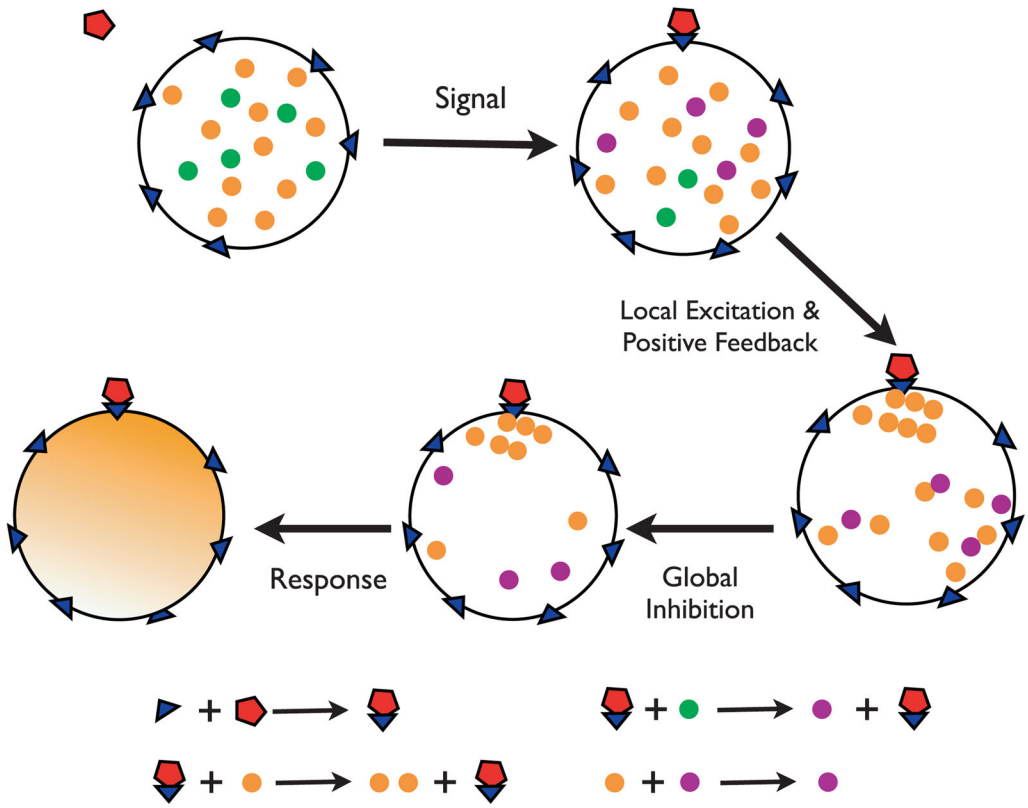

Figure 16.

The local excitation and global inhibition mechanism. Signal is received approximately uniformly, but small variations (or a shallow gradient) are amplified by local positive feedback. In this example the bound receptor (triangle and hexagon) activates a global inhibitor (inactive in green and active purple) as well as locally increasing (by positive feedback) the synthesis of orange dots. The inhibitor, when active (purple), degrades the orange dots globally. This, with the correct timing, can lead to a polarized response.

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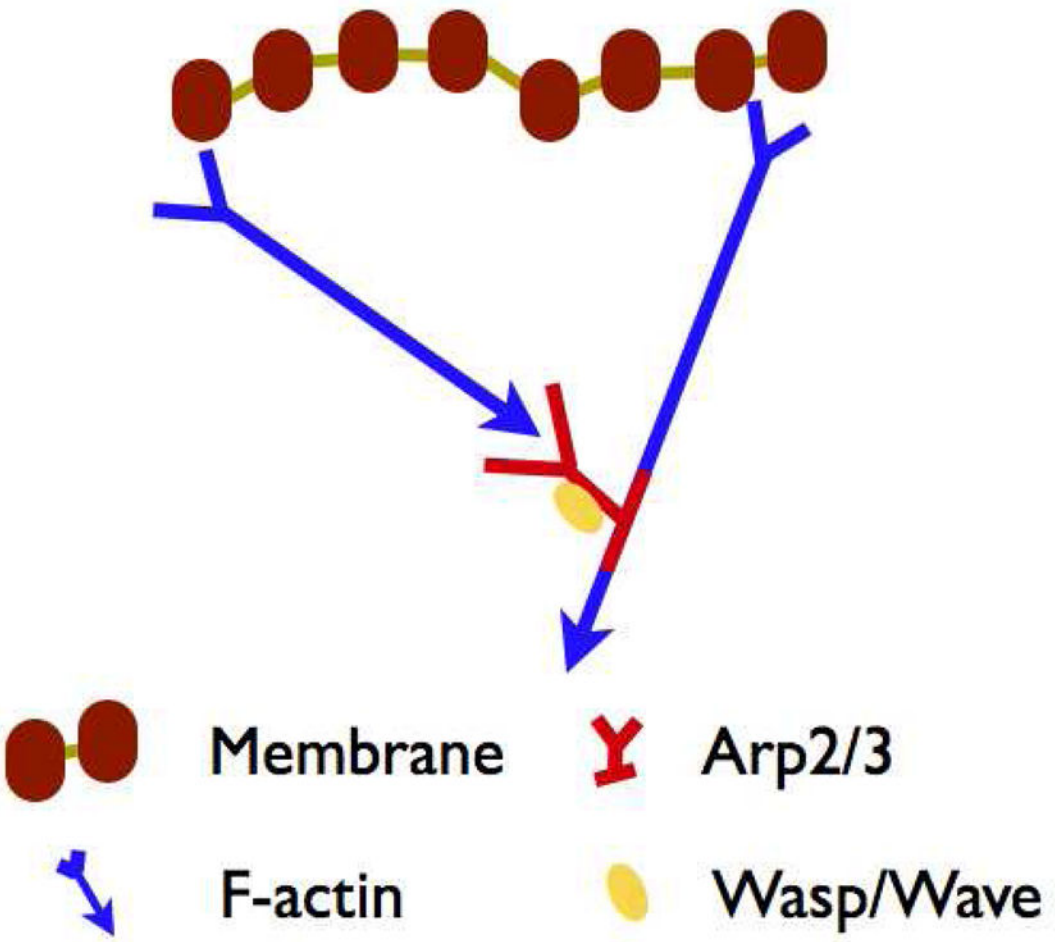

Figure 17.
Brownian ratchet force generation due to actin polymerization in lamellipodia. Polymerization occurs at the barbed ends of actin filaments orientated towards the membrane. Force on the membrane is generated from sustained polymerization and Brownian fluctuation in the relative position of the barbed end and the membrane.