Mathematical modelling of the VEGF receptor

Tomás Alarcón1 and Karen M. Page2

1 Centre de Recerca Matemàtica, Campus de Bellaterra, Edifici C, 08193 Bellaterra, Barcelona, Spain tomasalarc@gmail.com
2 Department of Mathematics, University College London, Gower Street, London WC1E 6BT, UK kpage@math.ucl.ac.uk

Abstract This chapter is devoted to the formulation and analysis of several models of the VEGF receptor and the initial steps in the signalling cascade following receptor activation. Our models take into consideration different factors and processes such as receptor cross-linking, endocytosis, recycling, degradation and synthesis. The effect of each one of these factors is studied. In particular, we present an analysis of a stochastic model of the vascular endothelial growth factor (VEGF) receptor, which accounts for ligand binding-induced oligomerisation, activation of SH2 domain-carrying kinases, and receptor internalization. This is an analysis, based upon a generalisation of a WKB approximation of the solution of the corresponding Master Equation, of the role and contribution of each of these processes to the overall behaviour of the VEGF/VEGF receptor (VEGFR) system. The results of this analysis, in turn, allow us to formulate plausible mechanisms for tumour resistance to antiangiogenic therapy. We predict that tumour-mediated overexpression of VEGFRs in the endothelial cells (ECs) of tumour-engulfed vessels leads to an increased sensitivity of the ECs to low concentrations of VEGF, thus endowing the tumour with increased resistance to antiangiogenic treatment. We then show using a simplified version of the above model, that it exhibits different dynamical behaviours, which account for different cell responses to stimulation with growth factor, from perfect adaptation to sustained response thus providing a framework which attempts to understand how a single sensorial system can produce a variety of different responses.

1 Introduction

Angiogenesis is the process whereby new blood vessels are generated from the existing vasculature in response to substances secreted and released by the surrounding tissues. These substances are special types of cytokines called growth factors (GFs). Endothelial cells (ECs) possess surface receptors specific for each of these growth factors. There are many such growth factors
and cell surface receptors involved in angiogenesis, but there is a particularly important one, VEGF, as the VEGF receptor (VEGFR) is expressed only by ECs. Angiogenesis can occur in a variety of biological settings both normal and pathological, ranging from wound healing to cancer.

The onset of angiogenesis is controlled by the so-called angiogenic switch. The usual picture of the angiogenic switch is a scale measuring the levels of angiogenic factors and anti-angiogenic substances. When the former are found in excess of the latter, angiogenesis is triggered Berger & Benjamin (2003).

Here, we argue that this image of the angiogenic switch might be incomplete. The VEGFR is a receptor tyrosine kinase (RTK). Within their cytoplasmic domains, RTKs have regions which, upon phosphorylation, exhibit tyrosine kinase activity. Activation of these regions, however, occurs only upon receptor oligomerisation Alberts et al. (2002); Helmreich (2001). Most GF molecules are multivalent ligands, i.e. one molecule of GF has more than one receptor binding domain and therefore it can engage in binding with as many receptors as it possesses binding regions. Mathematical models of multivalent ligand/multivalent receptor systems have been formulated and analysed (see Posner et al. (1995); Woolf & Linderman (2004); Lauffenburger & Linderman (1993) and references therein). One remarkable property exhibited by these models concerns the behaviour of the response curve (which, roughly speaking, represents the probability of a cell within a population to respond to a given concentration of ligand). Whereas the response curve for receptors that do not depend on receptor oligomerisation for activation (e.g. G protein-linked receptors) is monotonic, saturating for high ligand concentration, the response curve for multivalent ligand/multivalent receptors is bell-shaped: cellular responses are inactivated at high concentrations of ligand. With the help of the models presented here, we aim to discuss the implications of this property of RTKs in relation to the onset of angiogenesis and antiangiogenic therapy.

In spite of the initial enthusiasm raised by antiangiogenic therapy, the actual results obtained on patients in clinical practice have been poor and its impact on the life expectancy of cancer patients has been very disappointing, in particular when the antiangiogenic drugs were used alone (see the review by Jain (2005) and references therein). This lack of results, especially in contrast with the success registered on laboratory animals, has been puzzling. A commonly adopted explanation for such a failure is that, whereas antiangiogenic drugs can kill many cancer cells, they do not eradicate the tumour completely and the remaining tumour cells will eventually trigger angiogenesis anew Hempton (2005). One of our aims is to use our models to try to produce plausible explanations of this failure.

Tumour vasculature, whether tumour vessels are the product of tumour-induced angiogenesis or they are native vessels of the host which have been engulfed by the growing tumour mass, presents many structural abnormalities in comparison to its normal counterpart Jain (2005). An example of such de-regulation is an overexpression of the VEGF surface receptor Cross et al. (2003); Ferrara (2002). Further evidence for this can be found in ex-
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Experiments carried out on retinal microvascular ECs under stimulation with estrogen Suzuma et al. (1999). Estrogen is known to promote proliferation of some types of breast cancer cells Amlal et al. (2006) and, therefore, it is plausible that the same mechanism upregulates VEGFR. Moreover, recent experiments by Zhang et al. (2005) show that the platelet-derived growth factor (PDGF) receptor, a system analogous to the VEGFR in every significant aspect, is upregulated in ECs of hepatocellular carcinoma. In this paper, we aim to study the effect of overexpression of surface VEGFR on antiangiogenic therapy. We consider two possible mechanisms for overexpression of surface VEGFR, namely increased rate of VEGFR synthesis Suzuma et al. (1999); Zhang et al. (2005) and downregulation of receptor endocytosis Polo et al. (2004).

The models presented here are formulated in terms of Markov processes and analysed by means of a Wenzel-Kramper-Brillouin (WKB) approximation of the Master Equation Kitahara (1973); Kubo et al. (1973). Our models include ligand/receptor binding, ligand-induced receptor dimerisation, receptor internalization and binding of enzymes carrying SH2 domains (e.g. members of the Src tyrosine kinase family) to activated (dimerised) receptors. This last process constitutes the earliest event in RTK-activation induced signalling. Our analysis allows us to discern the contribution of each of these processes to the overall behaviour of the VEGFR system as well as to assess plausible roles of each of them in resistance to antiangiogenic therapy in solid tumours.

There are several recent studies of models of GF/RTK ligation dynamics. Mac Gabham & Popel (2004) study a model of competitive binding of VEGF and placental growth factor (PIGF) to VEGFR. There is some evidence of synergy (i.e. enhancement of cell response) between PIGF and VEGF in pathological situations. The mathematical models presented by Mac Gabham & Popel (2004) help to elucidate the mechanisms of this synergy. Their models take into account receptor internalization but do not account for VEGFR dimerisation. Mac Gabham & Popel (2005a) have studied the system VEGF/VEGFR receptor 2/neuropilin-1. VEGF receptor 2 (VEGFR2) and neuropilin-1 (NRP1) are both found on the surface of endothelial cells. They do not interact directly but can be cross-linked by a VEGF isoform which has binding sites for both VEGFR2 and NRP1. This model considers cross-linking between VEGFR2 and NRP1 but does not account for either receptor internalization or VEGFR dimerisation.

A model of the PDGF/PDGFR receptor (PDGFR) has been proposed by Park et al. (2003). This model incorporates some early events in the signalling cascade triggered by PDGF/PDGFR binding, including phosphoinositide2 in Alarcón & Page (2007)-kinase-dependent activation of Akt. The authors also incorporate an alternative model for receptor dimerisation in which dimerisation is mediated by receptor domains which are only active or exposed when the receptors are bound, forming a sort of "pre-dimer". Ligand and receptor are supposed to associate and dissociate rapidly. The dissociation of one of the ligands from its receptor within a pre-dimer leads to the formation of a
stable dimerised complex. The model presented by Park et al. (2003) exhibits good agreement with experimental data.

Mac Gabhamn et al. (2005b) have proposed a stochastic analysis of VEGF binding to cell surface receptors. In physiological circumstances, VEGF is usually found in very low concentrations, typically of the order of the picomolar. These concentrations imply less than one ligand molecule in each cubic micron of fluid. Such low concentrations lead them to consider the validity of the excess of ligand assumption Sulzer et al. (1996) and the effects of the fluctuations in cellular response, especially in a scenario in which response is threshold-triggered. They find agreement between stochastic and deterministic models in the range of VEGF concentrations handled in in vitro experiments (of the order of the nanomolar), but argue than in in vivo situations the effects of fluctuations might be more important.

This paper is organised as follows. Section 2 is devoted to giving details of our model formulation and a brief summary of the necessary biological background. In Section 2 in Alarcón & Page (2007), the stochastic models formulated in Section 2 are analysed by means of an asymptotic analysis (a generalisation to arbitrary dimension of the work by Kubo et al. (1973)). This analysis produces a set of ordinary differential equations for the first and second moments which are then solved numerically. Section 2 in Alarcón & Page (2007) also contains details of estimation of parameter values. In Section 4, we present numerical simulations of the response of our models to antiangiogenic therapy, assuming both a physiological and a pathological scenario, the latter one characterised by overexpression of surface receptors by inhibition of endocytosis. Another possible source of overexpression of receptors is upregulation of receptor synthesis. This situation is analysed in Section 5. Section 6 presents an analysis of the fluctuations. Finally, in Section 7 we summarise and discuss our results.

2 Biological background and model formulation

Here we briefly summarise the biological background necessary to understand how our models are set up and then we discuss our model formulation.

2.1 Biological background

VEGF denotes a large family of dimeric glycoproteins which consists of five mammalian and one virus-encoded members. VEGF-A was the first member to be discovered and has been shown to be involved in a large number of processes, with both physiological and pathological functions. VEGF-A, in turn, is expressed as four isoforms of different lengths. The shortest of them (VEGF-$A_{121}$, 121 aminoacids long) differs from the other three in its lack of ability to bind to the extracellular matrix (ECM) and, therefore, it diffuses freely Hicklin & Ellis (2005).
Regarding the VEGF receptors, there are three different types VEGFR-1, -2 and -3. ECs in tumour blood vessels express mostly VEGFR-2, although VEGFR-1 and -3 might also be expressed. In physiological conditions, the vascular endothelium expresses VEGFR-1 and -2 whereas the lymphatic endothelium expresses VEGFR-2 and -3 Cross et al. (2003). Of the two receptors expressed on ECs, only VEGFR-2 seems to contribute to intracellular signalling, with the function of VEGFR-1 most likely being sequestering (excess) VEGF Cross et al. (2003).

In order to keep our model as simple as possible and stay focused on the study of how ligand/receptor binding dynamics affect the early events of the VEGF binding-induced signalling cascade, we concentrate on the effects of diffusible VEGF-A, VEGF-A_{121}, and its binding to VEGFR-2. This particular system appears to make a major contribution to tumour-induced angiogenesis. Thus, hereafter, for simplicity in the notation, the system VEGF-A_{121}/VEGFR-2 will be referred to simply as VEGF/VEGFR.

In the case of the VEGF/VEGFR system, the ligand (VEGF molecule) is bivalent and the receptor (VEGFR) is monovalent, meaning that one VEGF molecule binds two VEGFRs, while each VEGFR can bind a single VEGF molecule.

This property provides a mechanism for RTK activation, which is elusive from a purely structural perspective: receptors are oligomerised (in the particular case of the VEGFR, dimerised) upon ligand binding. The receptors within the oligomer are brought into close proximity which leads to receptor cross-phosphorylation Alberts et al. (2002). Cross-phosphorylation yields attachment of phosphate to the tyrosine kinase domains within the cytoplasmic tail of the RTKs, providing high-affinity docking sites for selected substrates to bind. These substrates, usually members of the Src family of tyrosine kinases, carry the src-homology 2 (SH2) domain, which has high specificity for the phosphorylated domains within the RTKs, and are themselves tyrosine kinases activated by binding to phosphorylated RTKs. These are the earlier events in the signalling cascade triggered by GF/RTK binding. Activated SH2-carrying kinases relay the signal on to other tyrosine kinases which lead to activation of the corresponding pathways and the alteration of cell behaviour.

Each VEGF receptor has two kinase domains. We consider that each of these has only one tyrosine residue that is cross-phosphorylated under ligand induced dimerisation, thus providing four high-affinity docking sites for SH2 domains Cross et al. (2003). Actually, dimerised receptors exhibit more than four possible docking sites (6 or more according to Cross et al. (2003)). We have made this approximation in order to keep the model as simple as possible. Below, we comment on the effects of this approximation.

According to Sawyer (1998), there are basically two types of SH2-bearing tyrosine kinase: those carrying only one SH2 domain, hereafter to be referred

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3 These three types of VEGFR are surface receptors. There is also a soluble form of VEGFR-1.
to as SH2 monomers, and those carrying two SH2 domains (e.g., ZAP70 or PI3K). In this paper, only the former ones are considered.

2.2 Model formulation

The stochastic models we analyse in this paper are specified in terms of three quantities, namely the state vector \( X \) whose components are the number of molecules of each of the species involved, the probability per unit time corresponding to each of the reactions involved in the process being modelled, \( W_i \), and the corresponding vector \( r_i \). The components of \( r_i \) are the increments in the number of molecules when the \( i \)th reaction occurs. To summarise, the occurrence of the \( i \)th reaction induces the change in the state vector \( X \rightarrow X + r_i \) and occurs with probability proportional to \( W_i \). The system is then described by the probability density of the system being in state \( X \) at time \( t \), \( \Psi(X, t) \), whose dynamics is given by the Master Equation:

\[
\frac{\partial \Psi(X, t)}{\partial t} = \sum_i \left( W(X - r, r, t)\Psi(X - r, t) - W(X, r, t)\Psi(X, t) \right).
\]

(1)

Next, we present a description of the three models to be analysed.

2.3 Receptor binding model

We use a version of the stochastic model for multivalent ligand-induced receptor oligomerisation developed in Alarcón & Page (2006). Here, the model corresponds to a bivalent ligand and a univalent receptor, which corresponds to the case of the VEGF/VEGFR system. The stochastic dynamics of this model is summarised in Table 1 in Alarcón & Page (2007), where the precise forms of the transition rates for the different events involved in the ligand-receptor binding model are given, and depicted in Fig. 1 in Alarcón & Page (2007), where the different reactions involved in the ligand/receptor binding are represented schematically. In Fig. 1 in Alarcón & Page (2007), \( U \) is the number of unbound receptors, \( B \) is the number of bound receptors and \( X \) is the number of dimers \( (U + B + 2X = N_R, \) with \( N_R \) is the number of surface receptors). In Table 1 in Alarcón & Page (2007), \( u \equiv U/N, b \equiv B/N \) and \( x \equiv X/N^4 \). Here \( N = N_S + N_R \) is the total number of molecules in our simulation. \( N_S \) is the number of SH2-carrying enzymes (see Table 1). \( L \) is the

\[ ^4 \text{Throughout the paper, we use the same convention: an upper-case letter represents numbers of molecules of a given type, whereas the corresponding lower-case letter represents the proportion of molecules of that particular kind with respect to the total number of molecules. An exception to this rule is } L, \text{ whose meaning is explained in the text.} \]
concentration (in moles/litre) of free ligand, which is assumed to be constant, i.e. ligand is supplied at a rate that matches its rate of binding at the surface of the cells.

The actual model (i.e. transition rates for each reaction and the corresponding vectors \( r_i \)) used for receptor binding is summarised in Table 1 in Alarcón & Page (2007) and Fig. 1 in Alarcón & Page (2007). This model will be hereafter referred to as Model 1. The transition rate corresponding to reaction \( r_3 \) \((k_{eff}^{s} \text{ in Fig. 1 in Alarcón & Page (2007)})\) needs further clarification Alarcón & Page (2006). The transition rate for this reaction, which corresponds to the formation of a dimer, is obtained as the product of two factors: the rate of binding between an unbound receptor and a ligand-receptor heterodimer and the probability of finding another receptor within a characteristic distance \( \Delta \) of the ligand-receptor heterodimer. The latter is given by \( \pi \Delta^2 \rho \), with \( \rho = N/4\pi R^2 \) being the surface density of receptors on the cell surface and \( R \), the average radius of an EC.

### 2.4 SH2 binding to dimerised receptors

We consider that each VEGF receptor provides two high affinity docking sites for tyrosine kinases carrying SH2 domains upon ligand-induced dimerisation, thus providing four high-affinity docking sites for SH2 domains.

In Fig. 2 in Alarcón & Page (2007) and Table in Alarcón & Page (2007), \( S_F \equiv N_S - S \) stands for the number of free SH2 domains, i.e those that are not bound to dimerised receptor. \( S \) is the number of bound SH2 domains. \( X \) is the total number of dimers, \( X_1 \) is the number of dimers bound to a single SH2 domain, \( X_2 \) is the number of dimers bound to two SH2 domains, \( X_3 \) is the number of dimers bound to three SH2 domains, \( X_4 \) is the number of dimers bound to four. \( X_0 \) is defined as the number of “free” (i.e. not bound to SH2) receptor dimers. Taking into account these definitions, it is straightforward to see that \( S \equiv X_1 + 2X_2 + 3X_3 + 4X_4 \) and \( X_0 \equiv X - X_1 - X_2 - X_3 - X_4 \).

The rate constants \( k_{eff}^{s} \), \( i = 1, \ldots, 4 \) that appear in Fig. 2 in Alarcón & Page (2007) need further clarification. According to Table in Alarcón & Page (2007), \( k_{eff}^{s} = (4 - (i - 1)) \frac{k_{on}^{s}}{V_c N_A} \). \( k_{on}^{s} \) is the binding rate of a SH2 domain to a phosphoryl-tyrosine residue on a receptor dimer (see Table 1). Because this constant is given in \( M^{-1}sec^{-1} \), we need to use the factor \( V_c N_A \), where \( V_c \) is the volume of an EC and \( N_A \) is Avogadro’s number, to convert to appropriate units. The factor \( 4 - (i - 1) \) corresponds to the number of free docking sites that are left on the receptor dimer.

The model summarised in Table in Alarcón & Page (2007) will be hereafter referred to as Model 2.

### 2.5 Endocytosis of surface receptors

We introduce a third element in our model, namely, receptor internalization Teis & Huber (2003). Receptor tyrosine kinases undergo clearance from the
surface upon ligand-mediated activation (i.e. dimerisation) in a very efficient manner. Inactivated receptors (i.e. unbound and non-dimerised bound receptors) also undergo internalization.

Receptor endocytosis is a complex process involving a sophisticated network of protein interactions of which not all the details are known. As we intend to produce a simple model of receptor endocytosis capturing its essential features, we only give a very brief and incomplete summary of the biology of receptor internalization. The reader is referred to Helmreich (2001) and Teis & Huber (2003) for more detailed reviews.

Both inactive (i.e. non-dimerised) RTKs and RTK dimers undergo internalization by essentially the same mechanism\(^5\). The first step is the formation of a structure called a clathrin-coated pit around the RTK dimer. This pit eventually pinches off the membrane forming a vesicle containing the RTK dimer. Once these vesicles are formed, the RTKs enter the so-called early endosome. Although the mechanism of early RTK internalization is the same for both non-dimerised and dimerised receptors, the rate of endocytosis of the latter is much in excess of the former. This indicates that a protein network regulating endocytosis is upregulated upon RTK dimerisation Teis & Huber (2003).

After entering the early endosome, dimer and non-dimer RTKs follow different pathways. Non-dimer RTKs are rapidly recycled to the membrane (we will assume that they do so as unbound receptors). However, most of the dimerised RTKs are transported to the so-called late endosomes, from where they pass into the lysosomes, where they undergo degradation Teis & Huber (2003).

As the total number of cell surface receptors seems to stay constant, parallel to endocytosis, RTK production must be sustained by the cell at some given rate Lauffenburger & Linderman (1993).

Further to these assumptions, we make the hypothesis that somewhere down the endocytic pathway, the SH2-carrying enzymes potentially attached to RTK dimers are detached and released into the cytoplasm. This assumption allows us to ensure that the total number of SH2 domains stays constant. A further simplifying assumption will be that the rate of internalization and degradation for dimerised receptors is independent of the number of SH2 domains bound to their active sites. We also assume that unbound RTKs and non-dimerised ligand/receptor complexes are internalized and recycled back to the membrane at the same rate.

Our stochastic model is inspired by a model by Lauffenburger & Linderman (1993). Our model, however, contains some simplifications with respect to Lauffenburger & Linderman (1993). We will assume that all the internalized

\(^5\) Teis & Huber (2003) distinguish between active and inactive RTKs. We will assume that “active” refers to dimerised receptors, which seems to be pretty clear from the context, and that “inactive” refers to both unbound receptors and non-dimerised ligand/receptor complexes.
RTK dimers go to degradation in the lysosomes (without considering the two intermediate compartments described above) and that only unbound RTKs and non-dimerised ligand/receptor complexes undergo recycling. We further assume that none of these pass into the lysosome and that they are not degraded. Moreover, we assume that the rate at which receptors are synthesized is such that receptor degradation is exactly balanced by receptor synthesis. This assumption is introduced in order to have a constant number of “particles” in our model. This assumption could be relaxed by considering $N_R$ as a random variable included in the model rather than as a model parameter.

In Table 2, the variables bearing the superindex “$i$” are the internalized counterparts of the surface variables, which bear no index. The physical meaning of the “surface” variables is the same as in Model 2. For example, $X_1$ is the number of surface dimers bound to a single SH2 domain, whereas $X^i_1$ is the number of internalized dimers bound to a single SH2 domain. The total number of bound SH2 dimers is now $S \equiv X_1 + X^i_1 + 2(X_2 + X^i_2) + 3(X_3 + X^i_3) + 4(X_4 + X^i_4)$.

The model summarised in Table 2 will be hereafter referred to as Model 3. Models 1 and 2 are considered as sub-models of Model 3.

3 Model analysis: WKB approximation

The methodology we use to analyse the models presented in Sec. 2, originally proposed within the field of chemical physics, is due to Van Kampen (1992) and Kubo et al. (1973). This technique essentially consists of extending the form of the equilibrium probability density to a non-equilibrium setting. In thermodynamic systems, the equilibrium probability density is given by:

$$\Psi_e(X) = C \exp(-\Phi_e(X)),$$

where $C$ is the normalization constant, $X$ is a set of extensive variables, whose values determine the state of the system, and the function $\Phi_e(X)$ has the properties of a thermodynamic potential, i.e. is a homogeneous function:

$$\Phi_e(X) = N\phi_e(x); \quad x = \frac{X}{N},$$

where $N$ is the size of the system which, for example, can correspond to the number of particles. From these two equations, we can see that $\Psi_e(X)$ is the probability density for fluctuations of the macroscopic extensive variables $X$ with respect to the equilibrium state, as $\Psi_e(X)$ is a Boltzmann-like function, i.e. the exponential of an homogeneous function which plays the role of thermodynamic potential.

6 Within the statistical physics community, this approximation is often referred to as the eikonal approximation.
Kubo et al. (1973) have proved that, under the appropriate scaling substitution, the time-dependent solution of the ME can be approximated by a function of the same form as its equilibrium solution (Eq. 2), namely, the exponential of a homogeneous function, which we call $S$, of $X$:

$$
\Psi(X, t) = C \exp(-S(X, t)) = C \exp(-Ns(x, t)).
$$

(4)

Intuitively, the accuracy of this approximation can be assessed in terms of the comparison between the characteristic time scale associated with the disturbance that drives the system out of equilibrium and the time scale of the phenomena occurring locally in the system. If the former is much shorter than the latter (which usually happens in weak noise limits), the system may be considered locally in equilibrium. From a more rigorous point of view, Van Kampen (1992) have shown that the extensive variables exhibit the following asymptotic behaviour

$$
X(t) = \langle X(t) \rangle + \xi(t)N^{1/2} \text{ for } N \gg 1,
$$

(5)

where $\langle X(t) \rangle$ is the solution of the macroscopic equations, i.e. the average value of $X$ and $\xi$ is a Gaussian random variable. This equation reveals that out of equilibrium the fluctuations of an extensive Markovian variable around its mean value depend on the volume in the same way as in equilibrium. Thus, this fact justifies the substitution of Eq. (4).

Let us consider a system whose stochastic dynamics is described by the ME:

$$
\frac{\partial \Psi(X, t)}{\partial t} = \sum_r (W(X - r, r, t)\Psi(X - r, t) - W(X, r, t)\Psi(X, t)).
$$

(6)

Kubo et al. (1973) have shown that the transition rates $W(X, r, t)$ must be homogeneous functions of $X$ to obtain a solution of the ME of the form of Eq. (4):

$$
W(X, r, t) = Na(x, r, t).
$$

(7)

Accordingly, the probability of a given reaction to occur within an infinitesimal interval of time is proportional to the size of the system and is determined only by the state of the system, represented by the set of intensive variables $x$. The following definition:

$$
\psi(x, t) = N\Psi(X, t),
$$

(8)

together with Eq. (7), enables us to write the ME (6) in WKB form:

$$
\frac{1}{N} \frac{\partial \psi(x, t)}{\partial t} = \sum_r (e^{-\frac{\psi}{N}} \cdot \frac{\partial}{\partial x} - 1)a(x, r, t)\psi(x, t).
$$

(9)
where we have used that $e^{-r \cdot \partial \over \partial x}$ is the generator of the translations in the space of states of the system.

For arbitrary $n$, the scaling substitution for the cumulants of the probability distribution (for example: $(q_1)_i = \langle x_i \rangle; (q_2)_{ij} = \langle x_i x_j \rangle - \langle x_i \rangle \langle x_j \rangle$ and so on) $q_n(t) = e^{n-1} q_{n1}(t) + e^n q_{n2}(t) + O(e^{n+1})$ yields a consistent expansion leading to balanced equations for the cumulants $q_n(t)$. Eventually, this leads to the following equation for the leading order term for $q_1(t)$:

$$\dot{q}_{11}(t) = \sum_r r (2\pi)^d \int_{-\infty}^{\infty} d v e^{-i v \cdot q_{11}} w(v, r, t) = c(q_{11}, t) \quad (10)$$

where $w(v, r, t)$ is the Fourier transform of $a(x, r, t)$ and the quantity $c(q_{11}, t)$ is defined by:

$$c(q_{11}, t) = \sum_r r a(q_{11}(t), r, t). \quad (11)$$

Note that this is the result predicted by the Law of Mass Action. Likewise, the equation for the cumulants of order 2 is given by:

$$\dot{Q}_{ij}(t) = \sum_k \left( Q_{ik} \frac{\partial c_i(q_{11}, t)}{\partial q_{11k}} + \frac{\partial c_i(q_{11}, t)}{\partial q_{11k}} Q_{kj} \right) + \sum_r r_i r_j a(q_{11}, r, t) \quad (12)$$

where $Q_{ij} \equiv (q_{21})_{ij}$ and the first term on the right hand side has been symmetrised ($q_{21}$ is a symmetrical matrix). Eqs. (10) and (12) are our final result and constitute the generalisation to arbitrary dimension of the results obtained by Kubo et al. (1973). A detailed proof of these results is given in the supplementary material that accompanies this paper. This supplementary material includes also an alternative derivation using stochastic calculus rather than asymptotic methods.

### 3.1 Evolution equations for the VEGFR model

The results obtained in Section 3 are valid in general, as long as the transition rates in the ME fulfil the homogeneity condition stated by Eq. (7). In this Section we apply these results to the particular case of Model 3 described in Section 2, i.e. we use Eqs. (10) and (12) to formulate the systems of ODEs for the leading order contributions to the first and second cumulants (i.e. the first and second moments, respectively). Model 3 is the most general of the three described in Section 2. Models 1 and 2 can be obtained as particular cases of Model 3 as detailed below.

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7 Kubo et al. (1973) states the multidimensional result without a proof.
The conservation laws \( u + u' + b + b' + 2 \sum_j (x_j + x_j') = n_R \) and \( s_F = n_s - s_B \) (\( s_F \) standing for the fraction of unbound SH2 domains) are used and the quantities \( N = N_R + N_S \), \( n_R = N_R/N \) and \( n_s = N_s/N \), \( s_B = x_1 + 2x_2 + 3x_3 + 4x_4 \) have been defined. \( L \) stands for the concentration of ligand, in this particular case VEGF. We assume the so-called excess of ligand regime, namely, the concentration of (free) ligand is not affected by binding Sulzer et al. (1996). \( N(t = 0) = 2.3 \cdot 10^5 \) (see Table 1). The evolution equations corresponding to Model 2 can be obtained from Eqs. (13)-(26) by setting \( k_{in}^{nd} = 0 \), \( k_{in}^{d} = 0 \), \( k_{re} = 0 \), \( k_d = 0 \), and \( k_s = 0 \). The equations for Model 1 are Eqs. (13)-(14) with \( k_{in}^{nd} = 0 \), \( k_{re} = 0 \) and \( k_s = 0 \).

By substituting the corresponding values of \( a(x, r, t) \) and \( r \) from Model 3 Table 1 in Alarcón & Page (2007) into Eq. (10) we obtain:

\[
\frac{du}{dt} = k_{off}b - k_{on}Lu + k_{off}x_0 - k_{on}^{x}\pi\Delta^2\rho_{ub}b - k_{in}^{nd}u
\]

\[
+ k_{r}c(u^i + b^i) + 2k_d(x_0^i + x_1^i + x_2^i + x_3^i + x_4^i)
\]

(13)

\[
\frac{db}{dt} = k_{on}Lu + k_{off}^{x}x_0 - k_{off}b - k_{on}^{x}\pi\Delta^2\rho_{ub}b - k_{in}^{nd}b
\]

(14)

\[
\frac{dx_0}{dt} = -k_{off}x_0 + k_{on}^{x}\pi\Delta^2\rho_{ub}b + k_{off}x_1 - k_{in}^{d}x_0 - \frac{4k_{on}^{x}}{V_N^{x}}x_0s_F
\]

(15)

\[
\frac{dx_1}{dt} = \frac{4k_{on}^{x}}{V_N^{x}}x_0s_F - k_{off}x_1 + 2k_{off}^{x}x_2 - k_{in}^{d}x_1 - \frac{3k_{on}^{x}}{V_N^{x}}x_1s_f
\]

(16)

\[
\frac{dx_2}{dt} = \frac{3k_{on}^{x}}{V_N^{x}}x_1s_f - 2k_{off}^{x}x_2 + 3k_{off}^{x}x_3 - k_{in}^{d}x_2 - \frac{2k_{on}^{x}}{V_N^{x}}x_2s_F
\]

(17)

\[
\frac{dx_3}{dt} = \frac{2k_{on}^{x}}{V_N^{x}}x_2s_F - 3k_{off}^{x}x_3 + 4k_{off}^{x}x_4 - k_{in}^{d}x_3 - \frac{k_{on}^{x}}{V_N^{x}}x_3s_F
\]

(18)

\[
\frac{dx_4}{dt} = \frac{k_{on}^{x}}{V_N^{x}}x_3s_F - 4k_{off}^{x}x_4 - k_{in}^{d}x_4
\]

(19)

\[
\frac{du'}{dt} = k_{in}^{nd}u - k_{re}u^i
\]

(20)

\[
\frac{db^i}{dt} = k_{in}^{nd}b - k_{re}b^i
\]

(21)

\[
\frac{dx_0^i}{dt} = k_{in}^{d}x_0 - k_{d}x_0^i
\]

(22)

\[
\frac{dx_1^i}{dt} = k_{in}^{d}x_1 - k_{d}x_1^i
\]

(23)

\[
\frac{dx_2^i}{dt} = k_{in}^{d}x_2 - k_{d}x_2^i
\]

(24)

\[
\frac{dx_3^i}{dt} = k_{in}^{d}x_3 - k_{d}x_3^i
\]

(25)

\[
\frac{dx_4^i}{dt} = k_{in}^{d}x_4 - k_{d}x_4^i
\]

(26)
Likewise, a system of ODEs can be written for $q_{21}(t)$. This quantity is a symmetric $13 \times 13$ matrix and, therefore, has 91 independent components. Hence the corresponding ODE system has 91 equations. Furthermore this system of 91 ODEs is coupled to Eqs. (13)-(26). As a result, a full analysis of the fluctuations for the proposed model implies a system of 104 ODEs.

In general, for a system with dimension $d$, the system of ODEs determining the dynamics of the first and second order cumulants at leading order has $d(d + 3)/2$ equations. In fact, this is the most serious shortcoming of the method presented here: the size of the resulting system of ODEs makes the analysis painstaking, even for modestly complex models like the one given in Table in Alarcón & Page (2007). With some degree of uncertainty in the parameter values and 104 equations, further simplification seems necessary.

Consequently, only the behaviour of the mean value of the full model of Table in Alarcón & Page (2007) (Eqs. (13)-(26)) is analysed. However, if we restrict ourselves to the receptor model described in Table 1 in Alarcón & Page (2007), we obtain a system that we can easily handle.

Using Eq. (12) and Table 1 in Alarcón & Page (2007), the equations for the fluctuations of $u$ and $b$ corresponding to the receptor model read:

\[
\frac{dQ_{11}}{dt} = - (k_{on}L + k_{off} + k_{x} \Delta^2 \rho b) Q_{11} + (k_{off} - k_{on} \Delta^2 \rho u) Q_{12} + (k_{on} Lu + k_{off} b + k_{x} \Delta^2 \rho ub + k_{off} (n_r - u - b))
\]

\[
\frac{dQ_{22}}{dt} = - (k_{off} + k_{x} \Delta^2 \rho u) Q_{22} + (k_{on} L - k_{off} - k_{x} \Delta^2 \rho b) Q_{12} + (k_{on} Lu + k_{off} b + k_{x} \Delta^2 \rho ub + k_{off} (n_r - u - b))
\]

\[
\frac{dQ_{12}}{dt} = - (k_{on} L + k_{off} + k_{x} \Delta^2 \rho b) Q_{11} - (k_{off} + k_{x} \Delta^2 \rho u) Q_{22} - (AL + k_{off} + k_{x} \rho (u + b)) Q_{12} + k_{on} \Delta^2 \rho (n_r - u - b)
\]

\[8\] The system Eqs. (13)-(26) has 14 equations but the conservation law $u + u' + b + b' + 2 \sum (x_j + x'_j) = n_R$ allows us to reduce the dimensionality of the system by one unit

\[9\] In fact, this method produces a hierarchy of “kinetic” equations where the cumulants of order $n$ depend on the all the cumulants of order up to $n - 1$. 


where $Q_{ij} = Q_{ji}$. $Q_{11}$ and $Q_{22}$ correspond to the variance of $u$ and $b$ respectively. These equations are to be solved together with Eqs. (13) and (14) with $k_{in}^{nd} = 0$, $k_{re} = 0$ and $k_s = 0$. Using the conservation law $x = (n_R - u - b)/2$, we obtain the following expression for the variance of $x$, $Q_{33}$, in terms of the dependent variables of Eqs. (27)-(29): $Q_{33} = (Q_{11} + Q_{22} + 2Q_{12})/4$.

It is important to bear in mind that, whilst the dynamics of the mean value of the variables corresponding to the receptor model ($u$, $b$ and $x$) is unaffected by the dynamics of the intracellular SH2 domains, the dynamics of the fluctuations ($q_{21}$) of the receptor variables depends on fluctuations and mean values of SH2 variables. Therefore, whereas our restricted analysis may provide useful insights, its conclusions should not be applied to the dynamics of the fluctuations of the whole system.

4 Perfect and imperfect adaptation

Growth factors (GFs) are extracellular signalling molecules that bind a type of surface receptors called receptor tyrosine kinases (RTKs). Upon receptor binding, these molecules stimulate cell growth and differentiation. Most growth factors, however, can also induce a variety of other cellular responses depending on the particular context and the cell type they are acting on Alberts et al. (2002); Helmreich (2001). The wide range of cellular responses in addition to growth and differentiation goes from cell division to chemotactic behaviour. Growth factor signalling is also instrumental for activation of survival pathways in many types of cells Alberts et al. (2002).

Many GFs have been shown to have chemotactic effects on different types of cells. Some examples are the vascular endothelial growth factor (VEGF) Terranova et al. (1985); Lash et al. (2003), platelet-derived growth factor (PDGF) Grotendorst et al. (1982); Klominek et al. (1998); Shneider & Haugh (2005), hepatocyte growth factor Ebens et al. (1996); Ohshima et al. (2001), nerve growth factor Sawada et al. (2000) and transforming growth factor $\beta 1$ Reibman et al. (1991) among others.

A common feature of chemotactic (or, more precisely, chemokinetic) systems is their ability to, upon stimulation with a chemotactic ligand, adapt or desensitise after a short excitation transient. This means that the cell response terminates regardless of the presence of the ligand: after the initial excitation in response to the ligand the system relaxes to a “background” state which is independent of the concentration of ligand. Only a change in ligand concentration will induce a new cellular response (resensitation), which will eventually decay again Knox et al. (1986); Tyson et al. (2003). This behaviour has been demonstrated experimentally in chemotactic bacteria Alon et al. (1999) and several theoretical models have been proposed Spiro et al. (1997); Yi et al. (2000). A model that appears to reproduce the chemotactic behaviour of some eukaryotic cells (amoebae and neutrophils) has been
recently proposed which also accounts for excitation and perfect adaptation Levchenko & Iglesias (2002). Although perfect adaptation is regarded as an important component in gradient-sensing systems, recent experiments combined with mathematical modelling Shneider & Haugh (2005) have shown that the chemotactic response of fibroblasts to gradients of PDGF does not involve perfect adaptation. Shneider & Haugh (2005) have rather shown that fibroblasts exhibit transient activation of the PDGF receptors (i.e. an activation peak in response to stimulation with PDGF followed by a relaxation to a steady state value) but not perfect adaptation.

On the other hand, other cell responses such as differentiation are better described in terms of a switch-like behaviour in which the response is activated and sustained over time Marshall (1995). Furthermore, GF-induced proliferation seems to be triggered by a transient activation without perfect adaptation Marshall (1995).

The question arises as to how a single system (i.e. the GF/RTK system) is able to produce such different cell responses as chemotaxis (perfect adaptation), proliferation (transient response without perfect adaptation) and differentiation (sustained activation), as there seems to be no specific pathway for each of these responses. Our aim in this paper is to formulate a simple mathematical model that accounts for the basic mechanisms involved in the onset of GF-induced signalling (GF-induced dimerisation, receptor endocytosis and receptor synthesis and degradation) and can explain the three different cell behaviours described above. We will show that downregulation of internalised receptor degradation leads to perfect adaptation whereas upregulation of either receptor synthesis or internalised receptor degradation produces a sustained response. Similar results have been reported by Vilar et al. Vilar et al. (2006) in a different context.

4.1 The model

The model (Model 4) we consider to study how different cellular responses can be elicited by the same sensory system is a sub-model of Model 3, where we consider ligand binding and receptor endocytosis and recycling.

We further assume that all the internalised RTK dimers, $x_i$, are degraded in the lysosomes (without considering the two intermediate compartments described in the Introduction). We will also assume that no internalised dimers are recycled back to the surface. We also assume that a fraction of inactive receptors, $f_u$ and $f_b$ for unbound and GF/RTK complexes, respectively, passes into the lysosome for degradation, whereas the rest is recycled and sent back to the cell surface, $1 - f_u$ and $1 - f_b$ for unbound and GF/RTK complexes, respectively Lauffenburger & Linderman (1993). All the receptors sent to the lysosomes are assumed to be degraded at the same rate, $k_d$.

Receptor synthesis is assumed to produce receptors that are incorporated in the surface, increasing $u$ at a rate $K_s(x)$. Different models for $K_s(x)$ are considered and discussed in Section 4.2.
The corresponding ODE system for the evolution of our model is given by:

\[ \frac{du}{dt} = k_{off} b - k_{on} L u + k_{off} x - K_{cl}(y_s) u b - k_{in}^d u + k_{re}^u u_{re} + k_{re} b_{re} + K_s(x) \]  
\[ \frac{db}{dt} = k_{on} L u + k_{off} x - k_{off} b - K_{cl}(y_s) u b - k_{in}^d b \]  
\[ \frac{dx}{dt} = -k_{off} x + K_{cl}(y_s) u b - k_{in}^d x \]  
\[ \frac{du_{re}}{dt} = k_{in}^d (1 - f_u) u - k_{in}^a u_{re} \]  
\[ \frac{du_{md}}{dt} = k_{in}^d f_u u - k_{in}^a u_{md} \]  
\[ \frac{db_{re}}{dt} = k_{in}^d (1 - f_b) b - k_{in}^a b_{re} \]  
\[ \frac{db_{md}}{dt} = k_{in}^d f_b b - k_{in}^a b_{md} \]  
\[ \frac{dx_i}{dt} = k_{in}^d x - k_{in}^a x_i \]  
\[ y(t) = u(t) + b(t) + 2x(t) + u_{re}(t) + u_{md}(t) + b_{re}(t) + b_{md} + 2x_i(t) \]

where \( y_s = u + b + 2x \) is the proportion of surface receptors.

4.2 Models of dimer-formation rate and receptor synthesis

We consider a number of different models for the rate of formation of receptor dimers out of an unbound RTK and a GF/RTK complex. In particular, we consider three different models: density-limited model Alarcón & Page (2007), diffusion-limited Lauffenburger & Linderman (1993) and, for comparison, \( K_{cl}(y_s) = \) constant. The models we use and the corresponding expressions for \( K_{cl}(y_s) \) are summarised in Table 3.

All these models have been considered in detail somewhere else and, consequently, we only give here a general summary.

The density-limited model considers that the intrinsic cross-linking rate needs to be corrected by a factor which depends on the probability of finding an unbound RTK within a distance \( \Delta \) of a GTK/RTK complex, which is proportional to the surface density of RTK and the surface of a region of radius \( \Delta \). The expression given in Table 3 follows immediately (see Alarcón & Page (2007) for more details).

The diffusion-limited model is the result of assuming that the unbound receptors diffuse with respect to a given bound receptor and that when they are close enough, at a distance \( r = \Delta \), they bound to each other at a rate \( k_{on}^x \). This condition is mathematically imposed as a boundary condition on the
stationary diffusion problem. To close the problem properly, a second boundary condition that prescribes a bulk the concentration of unbound receptors at \( r = r_0 \) is imposed. The parameter \( r_0 \) is one half of the average distance between receptors and is given by \( r_0 = (S_c/\pi N_{R_0})^{1/2} \). The reader is referred to Lauffenburger & Linderman (1993) for a detailed account.

4.3 Parameter values

The parameter values used in our simulations are summarised in Table 1. Estimation of the parameter values corresponding to the ligand binding and receptor dimerisation has been described in detail in Alarcón & Page (2007). Regarding the parameter values of the part of the model corresponding to receptor internalisation and synthesis, a detailed discussion follows.

The processes of endocytosis, recycling and degradation of surface receptors have been analysed in order to elucidate whether these processes depend on receptor occupancy, in other words whether they are affected by the receptors being free or bound to a GF molecule, or kinase activity Mitchell et al. (2004); Wiley (1991).

Endocytosis appears to be dependent on kinase activity, rather than purely on receptor occupancy. Wiley (1991) found that active receptors are internalised at a rate about 10 times higher than free or bound but inactive receptors. Hence, in our model, the internalisation rates of non-dimerised receptors, i.e. unbound and GF/RTK complexes, are assumed to be equal, \( k_{in}^{nd} \). According to the values given in Table 1, the internalisation rate of dimerised (active) receptors, \( k_{in}^d \), is 10 times bigger than \( k_{in}^{nd} \).

Recycling of internalised receptors is thought to be independent of receptor status and, therefore, we assume that all the receptors are recycled at the same rate, \( k_u^{re} = k_b^{re} = k_{re} \), regardless of receptor type Mitchell et al. (2004); Wiley (1991).

The situation with respect to receptor degradation rates seems to be less clear. Whereas there is some consensus with respect the two previous points, the behaviour of receptor degradation has been observed to differ in different experimental situations. For example, whilst Mitchell et al. (2004) find that receptors in the TGF-\( \beta \)/TIR1/TIR2 system are degraded at the same rate regardless of receptor occupation, Wiley (1991) report that receptor degradation depends on receptor occupation but not on kinase activity: degradation is accelerated upon receptor binding in a kinase-activity independent way. Here, we assume that \( k_x^{x} = k_x^{a} = k_x^{b} = k_x^d \) (see Table 1).

Regarding the rate of receptor synthesis, we have followed the same procedure as in Alarcón & Page (2007), namely, we fix all the other parameter values and then fit the value of \( k_s \) to obtain a sensible total receptor number (of the order of \( 10^4 \)) for experimentally achievable GF concentrations (between \( L = 0.01\text{nM} \) and \( L = 10 \text{nM} \)). The result is the value given in Table 1.
5 Results: Anti-VEGF therapy

We now proceed to summarise our main results for the models presented in the previous sections. We start by presenting our modelling results for the analysis regarding anti-angiogenic therapy using Models 1, 2, and 3 in Alarcón & Page (2007). We then move on to present our results regarding perfect adaptation behaviour of the VEGFR system.

5.1 Receptor dimerisation induces non-monotonic response functions

Response functions characterise the cellular response to stimulation with a given concentration of ligand, VEGF in our case. Depending on the model, they can be given in terms of the steady state of dimerised receptors Sulzer et al. (1996) or in terms of the peak values of receptor activation Park et al. (2003). A property that Models 2 and 2 in Alarcón & Page (2007) inherit from Model 1 is that fact that the corresponding response functions are not monotonic. The leading order equations in the WKB approximation for Model 1 are given in terms of dimensionless quantities (see Alarcón & Page (2007)) read:

\[
\frac{du}{dt} = b - ALu + \frac{k_{\text{eff}}}{2} (n_R - u - b) - k_{\text{on}} \pi \Delta^2 \rho ub
\]
\[
\frac{db}{dt} = ALu + \frac{k_{\text{eff}}}{2} (n_R - u - b) - b - k_{\text{on}} \pi \Delta^2 \rho ub
\]

which in steady state correspond to:

\[
b_* - ALu_* = 0 \tag{40}
\]
\[
n_R - (1 + AL)u_* - 2AxAL\pi \Delta^2 \rho u_*^2 = 0. \tag{41}
\]

We can see that if \( AL \gg 1 \) then \( u_* = O(\epsilon) \) and \( b_* = n_R - O(\epsilon) \) with \( \epsilon = (AL)^{-1} \). If \( AL \ll 1 \), \( u_* = n_R - O(\epsilon) \) and \( b_* = O(\epsilon) \) with \( \epsilon = AL \). In both cases the number of dimerised receptors is so small that it is impossible for the cell to produce a response. Eqs. (40) and (41) yield the following solution for the steady state of the receptor model:

\[
u_* = -\frac{(1 + AL) + \sqrt{(1 + AL)^2 + 8n_RA_xAL\pi \Delta^2 \rho}}{4A_xAL\pi \Delta^2 \rho}
\]
\[
b_* = ALu_* \tag{42}
\]
\[
x_* = \frac{1}{2} (n_R - (1 + AL)u_*) \tag{44}
\]
The corresponding response curve $x_*(AL)$ Sulzer et al. (1996) is shown in Fig. 1(a), where we can appreciate that it is bell-shaped (in log(AL)) rather monotonously growing.

Models 2 and 2 in Alarcón & Page (2007), of which Model 1 is a sub-model, have a much more complex dynamics than the latter and they are not amenable to such straightforward analysis. However, when we look at their characteristic response curves in terms of the height of corresponding activation peaks as a function of $AL$ (Fig. 1(b)), we observe that it exhibits a non-monotonic behaviour inherited from Model 1. In other words, in either model large concentrations of VEGF inhibit cellular response. Although the experimental evidence for such behaviour may not be extensive, the work by Cai et al. (2006) appears to point in that direction. Cai et al. (see Fig. 5(c) of Cai et al. (2006)) have found that the proliferative activity induced by VEGF on retinal microvascular ECs is a bimodal function of the concentration of VEGF. Thus, both experiments and theory point to an inhibition of the cellular response for high ligand concentration, although our models do not predict the bimodal response curve found by Cai et al. (2006).

This property of the function response of the VEGF receptor has obvious consequences on anti-VEGF therapies: reducing the (effective) concentration of VEGF may not yield the expected result, i.e. a reduction on EC response, as this appears not to exhibit a non-monotous dependence on ligand concentration.

5.2 The dynamical behaviour of the VEGFR strongly depends on ligand concentration

Another factor that may affect the effectiveness of anti-VEGF therapies is related to how the relaxation time, $\tau$, i.e. the time the system takes to settle down to a close-to-equilibrium state, depends on log(AL), the relaxation process being faster for larger values of log(AL). Two illustrative examples of this are shown in Figs. 8 in Alarcón & Page (2007)(a) and (b) for log(AL) = −3 and log(AL) = 1, respectively.

From these numerical results and from the model equations we can see that $\tau(L) \approx O(AL)^{-1}$. This implies that, in the pathological situation this model is aimed to reproduce, the stationary response of the system for $L = 10^{-7}$ M has the same intensity as that for $L = 10^{-13}$ M, but whereas in the former case this response built up in a time of the order of $\tau(L = 10^{-7}) \approx 10^{-3}$, in the later the time required is $\tau(L = 10^{-13}) \approx 10^3$, which in dimensional terms corresponds to 0.0167 min and 16667 min, respectively. The reader should note that values of $L = 10^{-7}$ M (100 nM) for the VEGF concentration are possibly unrealistc as such high concentrations are unlikely to be found in either physiological or pathological circumstances. We are only using these extreme values here to illustrate our point.
5.3 Upregulation of VEGFR expression contributes to resistance to anti-VEGF therapy

An observation with respect to the physiological situation described by Model 3 is that blocking receptor endocytosis appears to induce an important change in the dynamical behaviour of the system. Whilst Model 3 supports a scenario in which there is a fast, transient (a peak in receptor) activation followed by a decay to a stationary state, Model 2 suggests that, in the pathological setting, the response is slow and sustained.

This observation may have deep therapeutic significance. We have run simulations of anti-VEGF therapy to compare the responses of the physiological (Model 3) with the pathological (Model 2) situations. As in Section 4.1 we have performed simulations in which at some point during the evolution of the system the concentration of VEGF has been reduced from \( L = 10^{-8} \text{ M} \) (10 nM) to \( L = 10^{-11} \) (0.01 nM) as shown in Fig. 9 in Alarcón & Page (2007). From the results shown in Alarcón & Page (2007), we observe that this therapeutic intervention, in spite of reducing the concentration of VEGF by three orders of magnitude, has had virtually no effect on the peak or steady state of the surface dimers or bound SH2 in the pathological system. Moreover, we find that the anti-VEGF therapy does much worse in the pathological than in the physiological case. A far more efficient clearance of active VEGF by the anti-VEGF drug is needed in order to produce better outcomes.

Furthermore, as the dynamics of the system has been slowed down by three orders of magnitude (from time scales of the order of magnitude of minutes to hours or days) by the reduction of ligand concentration, even if a decrease in the angiogenic activity is initially observed, angiogenic activity could resume simply because VEGF clearance by the drug was not effective enough due to inefficient VEGF clearance by the anti-VEGF agent.

From this discussion we can infer that the therapeutic outcome of an anti-VEGF drug could be improved by some strategy aimed to narrow the width of the bell-shaped stationary response curve. Results shown in Alarcón & Page (2007) show that decreasing the value of the dimerisation rate \( k_{on} \) yields such an effect. Simulation results of simultaneously reducing \( L \) and \( k_{on} \) are presented in Alarcón & Page (2007) where a substantial improvement in anti-VEGF performance when \( k_{on} \) is reduced.

6 Results: Perfect and imperfect adaptation

6.1 Downregulation of inactivated receptor degradation yields perfect adaptation

In a previous work, we have shown that absence of internalisation (and net receptor production) leads to a situation in which the stationary value of surface dimers, \( x_* \), has a bell-shaped dependence on \( \log(AL) \) Alarcón & Page
(2007), and, consequently, the corresponding system does not exhibit perfect adaptation. On the other hand, removal of the ligand-induced cross-linking from the model leads to a linear system that lacks the necessary feed-backs to produce perfect adaptation behaviour Tyson et al. (2003). In such cases, additional elements need to be introduced in the model pathway to reproduce such behaviour Levchenko & Iglesias (2002).

These results indicate that up- or down-regulation of the different processes involved in our model for RTK activation, i.e. receptor dimerisation, synthesis, internalisation and degradation, may lead to different dynamical behaviours corresponding to different cellular responses.

In fact, our model for RTK activation exhibits different dynamical behaviour depending on the strength of the degradation of inactive receptors. Fig. 2 shows that upon downregulation of the degradation rate of the inactive receptors the system exhibits perfect adaptation. Otherwise, the system exhibits transient activation, i.e. a peak of activity followed by a relaxation to a steady state activation, but not perfect adaptation: the steady state value is now a function of $L$ (see Figs. 2(a) and (b)).

Furthermore, the perfect adaptation behaviour observed when degradation of the inactive receptors is negligible compared to the degradation rate of active receptors is a fairly robust feature of the model with respect to model details and parameter values. To assess this issue, we analyse the steady state behaviour of Eqs. (30)-(36) with $\dot{u} = \dot{b} = 0$:

\begin{align}
  k_{off} b - k_{on} L u + k_{off} x - K_{cl}(y) u b - k_{in}^{nd} u + k_{re}^{u} u_{re} + k_{re}^{b} b_{re} + K_s(x) &= 0
  \tag{45} \\
  k_{on} L u + k_{off} x - k_{off} b - K_{cl}(y) u b - k_{in}^{nd} b &= 0
  \tag{46} \\
  K_{cl}(y) u b - k_{off} x - k_{in}^{nd} x &= 0
  \tag{47} \\
  k_{in}^{nd} u - k_{re}^{u} u_{re} &= 0
  \tag{48} \\
  k_{in}^{nd} b - k_{re}^{b} b_{re} &= 0
  \tag{49} \\
  k_{d}^{x} x - k_{d}^{x} x_{i} &= 0
  \tag{50} \\
  y &= u + b + 2x + u_{re} + b_{re} + 2x_{i}
  \tag{51}
\end{align}

We have use that for $\dot{u} = \dot{b} = 0$ $u_{md} = b_{md} = 0$. Adding up Eqs. (45) and (46), we obtain:

\begin{align}
  2k_{off} x - 2K_{cl}(y) u b - k_{in}^{nd} (u + b) + k_{re}^{u} u_{re} + k_{re}^{b} b_{re} + K_s(x) &= 0.
  \tag{52}
\end{align}

Likewise, adding up Eqs. (48) and (49) leads to:

\begin{align}
  k_{in}^{nd} (u + b) - k_{re}^{u} u_{re} - k_{re}^{b} b_{re} &= 0
  \tag{53}
\end{align}

which, in combination with Eq. (50), leads to:
2k_{off}^x x - 2K_{cl}(y) ub + K_s(x) = 0. \quad (54)

Eqs. (47) and (54) lead to:

\[ x = \frac{K_s(x)}{2k_{in}^d}, \quad (55) \]

Thus, we conclude that, as long as the function \( K_s(x) \) is such that Eq. (54) has a positive root, the steady state value of \( x \) will not depend on \( L \) providing the system with perfect adaptation behaviour. A feature of the system that this analysis puts forward is the independence of the perfect adaptation behaviour with respect to \( K_{cl} \), as illustrated in the numerical simulations presented in Fig. 3(c) and (d), where we have solved our model equations for constant degradation rate and constant and diffusion-limited dimerisation rate.

These analytical results are illustrated by the simulations shown in Fig. 3, which shows results corresponding to numerical solution of eqs (30)-(37) with \( K_{cl}(y) = k_{on}^x \pi \Delta^2 N_R y / S_c \) and different assumptions for receptor synthesis. We observe that both excitation and perfect adaptation are robust to changes in the model for receptor synthesis. There are however some features of the dynamics of the system that are sensitive to the value of \( x_h \) (see Table 3). If this parameter is chosen to be \( x_h > 0.05 \), receptor synthesis is not able to sustain a reasonable number of receptors on the cell surface, i.e. \( y(t) \) decays towards zero (results not shown).

6.2 Upregulation of receptor synthesis leads to sustained cellular response

In the previous section we have shown that our model predicts that down-regulation of degradation of inactivated receptors leads to perfect adaptation in the RTK system. Perfect adaptation involves, in addition to a relaxation to a steady state condition which is independent of the stimulus, a transient peak of activation whose height is a function of the concentration of signalling molecule. This leads to an scenario in which the response is triggered by the activation reaching a threshold level. The response is switched off when the activation level falls below threshold.

However, there are other situations in which the cellular response is triggered only when activation by the signalling molecule is sustained over time rather than by a transient excitation Tyson et al. (2003); Marshall (1995). The results shown in Fig. 3 indicate that upregulation of receptor synthesis leads to a sustained RTK activation in response to stimulation with growth factor. We can see from Figs. 3(b), (d) and (f) that as the rate of receptor synthesis grows the transient response is substituted by a sustained one.
6.3 Comparison to other models incorporating receptor dimerisation

In Section 6.1 we have proved that our results regarding the emergence of perfect adaptation upon downregulation of degradation of inactive RTKs is robust with respect to most of the model details. In this section we take this one step forward and proof that the mentioned results are, in fact, robust to the actual mechanism for receptor dimerisation and activation.

Park et al. (2003) have proposed an alternative model of receptor dimerisation which has been adapted by Shneider & Haugh (2005) to study the chemotactic response of fibroblasts to PDGF gradients. This model assumes a different mechanism for receptor dimerisation. They assume that the rate-limiting process is the association of two PDGF/PDGFR complexes. When this complex dimer has formed one of them releases its PDGF molecule, thus forming an active receptor dimer. This last process is assumed to be very fast. We refer the reader to the on-line supplementary material of Park et al. (2003) and Shneider & Haugh (2005) for a thorough derivation of their model.

The corresponding model, in the notation used by Shneider & Haugh (2005), is given by:

\[
\begin{align*}
\frac{dR}{dt} &= k_r C_1 - k_f L R - k_t R + k_x C_2 + V_s \quad (56) \\
\frac{dC_1}{dt} &= k_f L R - k_t C_1 - k_t C_1 + k_x C_2 - 2k_x C_1^2 \quad (57) \\
\frac{dC_2}{dt} &= k_x C_1^2 - (k_x + k_e)C_2 \quad (58)
\end{align*}
\]

where \(R\) is the concentration of free receptors, \(C_1\) the concentration of PDGF/PDGFR complexes, \(C_2\) the concentration of active receptor dimers, \(k_f\) and \(k_r\) are the binding and unbinding rates, respectively, \(k_t\) the rate of degradation of free and PDGF-bound receptors, and \(k_x\) and \(k_{-x}\) are the dimer formation and dissociation rates, respectively.

Fig. 4 shows simulation results corresponding to Eqs. (56)-(58) with \((k_t \neq 0)\) and without \((k_t = 0)\) inactive receptor degradation. The remaining parameter values are given by Shneider & Haugh (2005). We see that whereas for \(k_t \neq 0\), the system exhibits a transient activation with relaxation to a steady state that depends on \(L\), when \(k_t = 0\) the system exhibits perfect adaptation. In fact, a steady state analysis with \(k_t = 0\) reveals that \(C_2 = V_s / 2k_e\) which is independent of the concentration of growth factor.

Thus, in spite of incorporating a totally different model of activation by dimerisation, the behaviour of this model upon downregulation of inactive receptor degradation is the same as the behaviour of our model. This property, therefore, appears to be a general feature of receptor models incorporating activation by dimerisation, regardless of the particular activation mechanism involved.
7 Discussion

We have proposed a model for the ligation of the VEGF receptor by VEGF ligands which includes receptor dimerisation, endocytosis of surface VEGFR and the early events in the corresponding signalling cascade following cross-phosphorylation of dimerised receptors. The model is formulated as continuous-time Markov process and analysed using a WKB approximation on the corresponding Master Equation. Our aim is to address two different issues, namely how the dynamics of the VEGFR response may affect the outcome of anti-VEGF therapy and what mechanisms are likely to be involved in the triggering of different cellular responses by a single sensory system (in this case, the VEGF receptor).

Concerning the former, our analysis shows that factors such as the bell-shaped curve response induced by receptor dimerisation upon ligand binding and the increased sensitivity to low VEGF concentrations due to up-regulation of surface VEGFR have a negative effect on the efficiency of anti-VEGF therapies. Bell-shaped response curves or, more generally, non-monotonic response curves (see Fig. 1) have the implication that, rather paradoxically, a decrease in the concentration of available ligand (VEGF) may lead to an increase in the potency of the cellular response triggered by the VEGFR reaction to the remaining ligand. On the other hand, over-expression of surface VEGFR, as observed in the endothelial cells of tumour vessels, increases their sensitivity to low levels of VEGF, thus compromising the efficiency of anti-VEGF.

We have analysed the effects of overexpression of surface receptors, including its potential effects on response to anti-VEGF treatment. We have first studied the effect of receptor overexpression by inhibition of endocytosis (Model 3).

A second way of achieving overexpression of surface receptors is by up-regulation of receptor synthesis. This scenario cannot be studied within the framework of our stochastic models, as our model formulation requires a system with a constant number of particles. Instead, we have formulated a deterministic model based on the Law of Mass Action that allows us to study this situation (Model 4). The physiological scenario, characterised by the value of the rate of receptor synthesis corresponding to this model, yields a dynamical behaviour similar to the one exhibited by Model 3: an initial transient activation followed by a relaxation to a steady state. However, there is an important difference with respect to Model 3, namely, Model 4 shows perfect adaptation to the VEGF concentration. Within the framework of Model 4, the pathological case is characterised by an increased rate of receptor synthesis.

The main result of our analysis is that both mechanisms of overexpression of surface receptors lead to a substantially increased resistance to treatment with an anti-VEGF drug. In both cases the dynamical mechanism appears to be similar: the transient activation exhibited by the physiological case is replaced by a slower and more sustained response. Moreover, in both cases there is an increased sensitivity to low values of the concentration of VEGF.
Model 2 exhibits close-to-full activation for concentrations as low as $10^{-5}$ nM (see Fig. 7 in Alarcón & Page (2007)), whereas physiological activation occurs in the proximity of 1 nM (see Fig. 4(b) in Alarcón & Page (2007) and Park et al. (2003)).

In the case of Model 4, increasing the rate of receptor synthesis to pathological levels leads to a larger steady-state activation value than the one observed for physiological receptor synthesis, which means that the angiogenic response may not be shut down after the initial transient is over.

A feature that Models 2, 3 and 4 inherit from Model 1 is the inhibition of the cell response for high ligand concentrations. Although the experimental evidence for such behaviour may not be extensive, the work by Cai et al. (2006) appears to point in that direction. Fig. 18(b) in Alarcón & Page (2007) shows their experimental results (data extracted from Fig. 5(c) of Cai et al. (2006)), in which the proliferative activity induced by VEGF on retinal microvascular ECs is measured with respect to the activity of unstimulated cells. Fig. 1(b) shows the peak of activated (dimerised) surface receptors as a function of $L$ for Models 3 and 4 (in physiological conditions). We can see that both experiments and theory point to an inhibition of the cellular response for high ligand concentration. However, our models do not predict the bimodal response curve found by Cai et al. (2006).

Regarding the eliciting of different cell responses by the VEGFR, our model suggests that regulation of the rates receptor synthesis and degradation are involved in switching from transient cellular response to sustained cellular response to VEGF ligation by VEGF ligand molecules. Since these two types of response curves are usually associated with different cellular responses, we argue the regulation of these processes is involved in switching from one type of response to the other. We also show that the VEGFR system is capable of perfect adaptation, a behaviour typically associated with chemotactic response. Similar results have been obtained by Vilar et al. Vilar et al. (2006) in the context of the TGF-β receptor.

The aim of this paper is to formulate a model which helps to understand a fundamental question in cell biology, namely, how stimulation with growth factor molecules leads to substantially different cell responses without the existence of response-specific pathways Marshall (1995).

We have proposed a simple model of a receptor tyrosine kinase such as the VEGFR, in which the basic ingredients of its dynamics (ligand binding, growth factor-induced dimerisation (activation), internalisation and synthesis) have been included. We have shown that this simple model accounts for different patterns of receptor tyrosine kinase patterns, namely, sustained activation, transient activation and perfect adaptation.

A paradigmatic and much studied system is the cell line PC12 and its response to NGF and EGF (see Marshall (1995); Vaudry et al. (2000) and references therein). Upon stimulation with NGF, PC12 cells undergo differentiation. In contrast, stimulation of PC12 cells with EGF leads to proliferation. Moreover, activation of both NGFR and EGFR converge to and are mediated
by activation of the extracellular-signal-regulated kinase (ERK) Sasagawa et al. (2005). Although, as pointed out by Marshall (1995), there are no great qualitative difference between the transduction events that lead to proliferation and differentiation, there are important quantitative difference between them: whilst NGF induces sustained ERK activation, EGF produces transient ERK activation. These results are further confirmed by experiments in which mutant PC12 cells unable of differentiating exhibit transient ERK activation upon NGF stimulation Yaka et al. (1998).

In the context of our model results, the results reported by Traverse et al. (1994) and Schelessinger & Bar-Sagi (1995) are particularly interesting. Traverse et al. (1994) present results according to which overexpression of EGFR leads to sustained ERK activation, producing differentiation. In turn, in PC12 lines that do not respond to NGF as a differentiation signal, NGFR is downregulated, ERK activation is transient and the response is to stimulation with NEGF is proliferation Schelessinger & Bar-Sagi (1995).

Our model results are in agreement with these experimental results, as we predict that overexpression of RTKs leads to sustained activation and, on the contrary, downregulation of RTK synthesis yields transient activation.

Another main result presented here concerns the possibility of inducing perfect adaptation by downregulation of inactive receptor degradation. There is extensive evidence for the chemotactic response many growth factors induce in a wide variety of cells. However, recent studies carried out by Shneider & Haugh (2005) appear to indicate that perfect adaptation is not necessary for chemotactic behaviour. Shneider & Haugh (2005) have carried out experiments with fibroblasts exposed to a gradient of PDGF, showing that, whilst fibroblasts migrate up the PDGF concentration gradient, they do not exhibit perfect adaptation. Shneider & Haugh (2005) highlight some differences between fibroblasts and other eukaryotic cells, such as neutrophils, which exhibit both chemotactic and perfect adaptation behaviour. They found that fibroblasts exhibit a much narrower range of chemoattractant concentrations to which they respond efficiently. Fibroblasts also need much steeper gradients than neutrophils. Hence, it seems that perfect adaptation is necessary for increasing efficiency of the chemotactic response although this response can be induced without perfect adaptation. If this is actually the case, this provides a way to verify our predictions: downregulating inactive receptor degradation should lead to a more efficient chemotactic response in fibroblasts, as this would induce a perfectly adapted response.

Our model provides a framework in which we can study the different cellular responses induced by a single sensorial system (i.e. GF/RTK). Our model predicts that pathways controlling receptor synthesis and degradation may be instrumental for controlling the cellular response, through either transient or sustained signals, to a given signalling cue in a particular cellular context.

The scope of the models presented here may be considered as limited since they do not include some of the complexities involved in the VEGFR sensory system. For example, we have considered only one ligand and one receptor
type, which is likely to constitute an over-simplification as it has been shown that different tyrosine kinases can be phosphorylated upon receptor activation, each of them capable of initiating different signalling pathways Shibuya & Claesson-Welsh (2006). Heterodimerisation, i.e. dimerisation of receptors of different types, contributes to increase the complexity of signalling since the phosphorylation profile on each receptor depends upon its partner receptor in the dimer Dixelsius et al. (2003), as does multiplicity of ligands: different ligands activate different sets of tyrosine sites within the receptors Autiero et al. (2003). All these different patterns of receptor activation affect and modify cellular response. However, since this scenario implies that the regulation of the level of each receptor is a critical parameter to control receptor signalling, mechanisms and behaviours such as those shown in Section 6 will still be useful to understand the different patterns of cell response. Additionally, our models focus on general mechanisms which allow us to identify generic biophysical mechanisms which will apply to models including a more detailed description of the patterns of phosphorylation of the tyrosine sites in active receptors.

References


Vascular endothelial growth factor induced cells proliferation is regulated by interaction between VEGFR-s, SH-PTP1 and eNOS. Microvasc. Res. 71, 20-31.


Fig. 1. Panel (a) shows the steady-state value of dimerised receptor, $x^*$, for Model 1 as a function of the dimensionless quantity $AL$ for different values of the dimensionless quantity $k_{on}$. Parameter values have been taken from Table 1. Key: squares $k_{on} = 4.6 \times 10^3 \text{ sec}^{-1}$, triangles $k_{on} = 4.6 \times 10^2 \text{ sec}^{-1}$, circles $k_{on} = 4.6 \times 10^1 \text{ sec}^{-1}$. Panel (b) shows simulation results corresponding to Models 2 in Alarcón & Page (2007) and 4 (with $k_s = 9 \times 10^{-5} \text{ sec}^{-1}$). The squares (Model 3) and triangles (Model 4) in this plot show the maximum values achieved by the proportion of surface dimers as a function of ligand concentration when the models were simulated until $t = 1.2$, i.e. 20 minutes in dimensional terms. Other parameter values are taken from Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>value (units)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{on}$</td>
<td>$10^7 (\text{M}^{-1} \times \text{sec}^{-1})$</td>
<td>Mac Gabham &amp; Popel (2004)</td>
</tr>
<tr>
<td>$k_{off}$</td>
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<td>Cross et al. (2003)</td>
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<tr>
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<td>Alarcón &amp; Page (2007)</td>
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<tr>
<td>$k_{off}^d$</td>
<td>$10^{-3} (\text{1/sec})$</td>
<td>Alarcón &amp; Page (2007)</td>
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<td>$\Delta$</td>
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<td>Alarcón &amp; Page (2007)</td>
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<td>$1000 (\mu\text{m}^2)$</td>
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<td>$V_c$</td>
<td>$2974 (\mu\text{m}^3)$</td>
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<td>$k_{off}^d$</td>
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<td>Mac Gabham &amp; Popel (2004)</td>
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<tr>
<td>$k_{re}$</td>
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<td>Lauffenburger &amp; Linderman (1993)</td>
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<td>$k_d$</td>
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<td>$k_s$</td>
<td>$9 \cdot 10^{-5} (\text{min}^{-1})$</td>
<td>Alarcón &amp; Page (2007)</td>
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Table 1. Parameter values for the VEGF receptor models.
Fig. 2. Simulation results corresponding to Eqs (30)-(37). This plot shows the time course of the proportion of surface dimers, $x(t)$. Solid lines correspond to $L = 10$ nM and dashed lines to $L = 0.01$ nM. $K_{cl}(y) = k_{on}^{cl} \pi \Delta x N_R \frac{y}{y + 1}$. $K_s(x) = k_s$. (a) $k_s = 4.5 \times 10^{-4}$ sec$^{-1}$ and $f_u = f_b = 0$. (b) $k_s = 4.5 \times 10^{-4}$ sec$^{-1}$ and $f_u = f_b = 1$. (c) $k_s = 4.5 \times 10^{-3}$ sec$^{-1}$ and $f_u = f_b = 0$. (d) $k_s = 4.5 \times 10^{-3}$ sec$^{-1}$ and $f_u = f_b = 1$. Other parameter values taken from Table 1.
Fig. 3. Analysis of the robustness of the perfect adaptation behaviour. Plots (a) and (b) show simulation results corresponding to Eqs (30)-(37) with $f_u = f_b = 0$. These plots show the time course of the proportion of surface dimers, $x(t)$, with $K_{cl}(y) = k_{xon}$ for different models of receptor synthesis. (a) $K_s(x) = k_s$. (b) $K_s(x) = k_s \frac{x}{x_h + x}$ with $x_h = 0.02$. Plots (c) and (d) show simulation results corresponding to Eqs (30)-(37) with $f_u = f_b = 0$. These plots show the time course of the proportion of surface dimers, $x(t)$, with $K_s(y) = k_s$ for different models of receptor dimerisation. (a) $K_{cl}(y_s) = F_{on} = 4.6 \cdot 10^3$. (b) $K_{cl}(y) = \left( \frac{\log(2/\Delta)}{2 \pi D} + \frac{1}{k_{xon}} \right)^{-1}$. According to Lauffenburger & Linderman (1993), $D = 10^{-9} \text{ cm}^2/\text{seg}$. For all the panels shown in this figure $k_d^* = k_d$. Key: solid line corresponds to $L = 10 \text{ nM}$ and dashed line to $L = 0.01 \text{ nM}$. Parameter values taken from Table 1.
Fig. 4. Simulation results corresponding to Eqs (55)-(57) Shneider & Haugh (2005). This plot shows the time course of the proportion of surface dimers, $C_2(t)$. Key: solid line corresponds to $L = 10$ nM, dotted line to $L = 1$ nM, dot-dashed line to $L = 0.1$ nM, and dashed line to $L = 0.01$ nM. Plot (a) corresponds to $k_t = 0.02$ min$^{-1}$ Shneider & Haugh (2005), whereas plot (b) corresponds to $k_t = 0$. Other parameter values are taken from Shneider & Haugh (2005): $k_f/k_r = 1.5$ min$^{-1}$nM$^{-1}$, $k_r = 1$ min$^{-1}$, $k_x = 0.3$ min$^{-1}$, $k_{x-x} = 0.07$ min$^{-1}$, $k_e = 0.3$ min$^{-1}$, $V_s = 0.02$ min$^{-1}$.
Reaction probability p.u.t $r_i$ Reaction

$W_1 = k_{on} L N u$ $(-1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)$ Receptor binding

$W_2 = k_{off} N b$ $(1, -1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)$ Receptor dissociation

$W_3 = k_{in} \pi N \Delta^2 p u$ $(-1, -1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)$ Dimer formation

$W_4 = k_{off} N x_0$ $(1, -1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)$ Dimer dissociation

$W_5 = 4 \left( \frac{k_{in} \pi}{N_A} \right) N x_0 \left( \frac{N_A}{N} - s \right)$ $(0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)$ SH2 binding

$W_6 = 3 \left( \frac{k_{in} \pi}{N_A} \right) N x_1 \left( \frac{N_A}{N} - s \right)$ $(0, 0, 0, -1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)$ SH2 binding

$W_7 = 2 \left( \frac{k_{in} \pi}{N_A} \right) N x_2 \left( \frac{N_A}{N} - s \right)$ $(0, 0, 0, 0, -1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)$ SH2 binding

$W_8 = \left( \frac{k_{in} \pi}{N_A} \right) N x_3 \left( \frac{N_A}{N} - s \right)$ $(0, 0, 0, 0, 0, -1, 0, 0, 0, 0, 0, 0, 0, 0, 0)$ SH2 binding

$W_9 = k_{off} N x_1$ $(0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)$ SH2 dissociation

$W_{10} = 2 k_{off} N x_2$ $(0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)$ SH2 dissociation

$W_{11} = 3 k_{off} N x_3$ $(0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)$ SH2 dissociation

$W_{12} = 4 k_{off} N x_4$ $(0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)$ SH2 dissociation

$W_{15} = k_{in} \pi N u$ $(-1, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0)$ internalization

$W_{14} = k_{in} \pi N b$ $(0, -1, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0)$ internalization

$W_{16} = k_{in} \pi N x$ $(0, 0, -1, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0)$ internalization

$W_{17} = k_{in} \pi N x_1$ $(0, 0, 0, -1, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0)$ internalization

$W_{18} = k_{in} \pi N x_2$ $(0, 0, 0, 0, -1, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0)$ internalization

$W_{19} = k_{in} \pi N x_3$ $(0, 0, 0, 0, 0, -1, 0, 0, 0, 0, 0, 1, 0, 0, 0)$ internalization

$W_{20} = k_{on} N u'$ $(1, 0, 0, 0, 0, 0, -1, 0, 0, 0, 0, 0, 0, 0, 0)$ Non-dimer recycling

$W_{21} = k_{on} N b'$ $(1, 0, 0, 0, 0, 0, 0, -1, 0, 0, 0, 0, 0, 0, 0)$ Non-dimer recycling

$W_{22} = k_{d} N x'$ $(2, 0, 0, 0, 0, 0, 0, 0, -1, 0, 0, 0, 0, 0, 0)$ Degradation

$W_{23} = k_{d} N x_1'$ $(2, 0, 0, 0, 0, 0, 0, 0, 0, -1, 0, 0, 0, 0, 0)$ Degradation

$W_{24} = k_{d} N x_2'$ $(2, 0, 0, 0, 0, 0, 0, 0, 0, 0, -1, 0, 0, 0, 0)$ Degradation

$W_{25} = k_{d} N x_3'$ $(2, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, -1, 0, 0, 0)$ Degradation

$W_{26} = k_{d} N x_4'$ $(2, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, -1, 0, 0, 0)$ Degradation

**Table 2.** Reaction probability per unit time, $W_i = W(X, r_i, t)$, $i = 1, \ldots, 12$. $r_i = (r_{i,1}, r_{i,2}, r_{i,3}, r_{i,4}, r_{i,5}, r_{i,6}, r_{i,7}, r_{i,8}, r_{i,9}, r_{i,10}, r_{i,11}, r_{i,12})$. In this Table $N$ refers to the number of receptors plus the number of proteins carrying a SH2 domain. $N_A$ is Avogadro’s number. We assume that each receptor dimer carries four phosphorylated tyrosines to which two SH2 domains can bind. See Table 1 for a summary of parameter values.

**Models for $K_{ij}(y)$**

| Density-limited model $K_{ij}(y_e) = \frac{k_{on} \pi \Delta^2 N R}{S}$ | Alarcón & Page (2007) |
| Diffusion-limited model $K_{ij}(y_e) = \left( \frac{\ln(r_0/2 \Delta)}{2 \pi D} + \frac{1}{\pi \Delta} \right)^{-1}$ | Lauffenburger & Linderman (1993) |

**Models for $K_s(x)$**

| Constant $K_{ij}(y_e) = k_{on}$ | – |

**Table 3.** Different scenarios considered in the present work. In this Table $r_0 = (S_i/\pi N K_{ij})^{1/2}$ (see Lauffenburger & Linderman (1993) for a full account) and $y_e = u + b + 2x$, i.e. the proportion of receptors on the surface of the cell.