

Relative Effectiveness of Inhibition Strategies for Control of Biological Signaling Pathways

by
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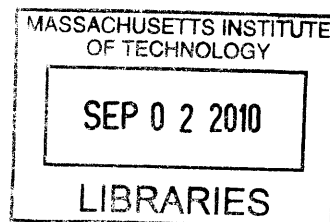
Submitted to the School of Engineering
in partial fulfillment of the requirements for the degree of
Master of Science in Computation for Design and Optimization

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Abstract

Many therapeutically useful and critically important drugs are inhibitors of particular enzymes in a signaling pathway. The efficiency with which an inhibitor inactivates its target can be characterized by the binding kinetics and thermodynamics. However, the overall efficiency with which the inhibitor shuts down a pathway or process, arrests a signal, or interferes with an outcome can be quite different and is often measured in cell-based assays. Because of non-linear effects, particularly in signaling pathways but elsewhere as well, non-obvious and possibly useful relationships may exist such that much greater inhibition is needed at some points in a pathway as compared to others to achieve the same overall effect. To investigate the relationship between inhibiting a signaling molecule and interrupting the result of that signal, we used pathway simulations to study the effects of inhibition for different pathway components and the effect on pathway output. We use two biological characteristics to assess the inhibitory effects: the peak and integrated pathway response. In the epidermal growth factor receptor (EGFR) pathway, 50% inhibition of most enzymes in the pathway yields less than 50% reduction of the final activated ERK output. Inhibiting MEK was found most effective in EGFR pathway and it is more effective than directly inhibiting ERK itself. Inhibiting two signaling molecules at the same time yields an effect similar to the linear superposition of effects of inhibiting them separately. In the extrinsic apoptosis pathway, 50% inhibition of most signaling molecules in the pathway yields less than 50% reduction of the final caspase-3 output. The most effective inhibitor found is XIAP which is already included in the extrinsic apoptosis pathway.

Thesis Supervisor: Bruce Tidor
Professor of Biological Engineering and Computer Science

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Introduction

Many diseases are associated with abnormal regulation of signaling pathways. For abnormally up-regulated pathways, one therapeutic approach is to develop chemical entities that block receptors or inhibit particular enzymes. Here we make a distinction between the efficiency with which a potential therapeutic molecule binds its target and the efficiency with which it modulates the overall process in which the target is involved. We introduce specific terminology as terms are used to mean different things in the literature. Here we define TBE to mean the target binding efficiency and the specific term TBE_{50} to refer to the concentration of the binding molecule that leads to 50% occupancy of the target molecule binding sites. This definition is similar to the IC_{50} for an enzyme when defined as the concentration of a molecule required to inhibit the targeted enzyme by half (I), but this term has also come to be used for the very different concept of shutting down a pathway to a level of 50% of some previous conditions. Additionally, we use the term TBE_{50} whether or not the target molecule is an enzyme, as the occupancy and blocking of a binding site for a signaling molecule can have important pathway effects whether or not the target is an enzyme and whether or not any enzyme activity is affected. The TBE_{50} (IC_{50}) values for clinically approved therapeutics vary greatly, but a typical value would be in the nanomolar (nM) range.

Therapeutic effectiveness, however, is generally measured using an assay that measures a functional outcome, often downstream from the inhibitory event. For example, an antibiotic may inhibit an enzyme target with some binding efficiency, but its effectiveness as a therapeutic might be measured in the reduction of the doubling time of

the target bacteria or the efficiency with which it kills bacterial cells. Here we introduce the term PME to refer to the pathway modulating efficiency of a potential therapeutic molecule and the specific term PME_{50} to refer to the concentration of binding molecule that leads to a 50% change in the pathway output under some set of assay conditions. For cell-based assays this value spans a wide range, but a typical value is in the micromolar (μM) range, which is 1000 times as high as a typical TBE_{50} .

Any difference in effectiveness as measured by the PME_{50} compared to the TBE_{50} could be due to many factors, such as bioavailability; partitioning between plasma, membrane, and cytoplasm; and distribution to the affected tissue. While all of these effects are true to some extent, here we explore the importance of a different mechanism due to the nonlinear properties of signaling pathways and other biochemical networks. Signals are filtered, combined, split, amplified, attenuated, thresholded, and saturated during transduction through a pathway. Thus 50% inhibition of the final output may require more or less than 50% inhibition of a particular intermediate signal. In this work we use pathway simulations to study this possibility and find significant effects due to nonlinearity in signal transduction. Moreover, we compare the effectiveness of inhibiting different enzymes in pathways and find significant differences as to their suitability as drug targets as measured by the ratio of PME_{50} to TBE_{50} .

EGFR pathway

Epidermal growth factor (EGF) was first discovered by Stanley Cohen in 1962 (2) while its receptor, EGFR was purified two decades later (3). EGFR belongs to the tyrosine kinase family of receptors. It plays an important role in the progression of

tumors. Aberrant EGFR signaling is an important characteristic of many types of cancer, including lung (4), breast (5), and head and neck (6).

This signaling pathway is initiated by binding of EGF to the extracellular domain of EGFR, which induces EGFR dimerization and autophosphorylation. A group of adapter proteins is then recruited to activated receptor. The complex of signaling molecules and receptor activates the mitogen activated protein kinase (MAPK) cascade, and the final output of this pathway is activated ERK (Figure 1). Activated ERK regulates expression of several cellular proteins and transcription factors (7). As a mechanism of signal attenuation, active EGFR complexes are internalized and degraded, with internalized but non-degraded complexes continuing to signal in the endosome (8).

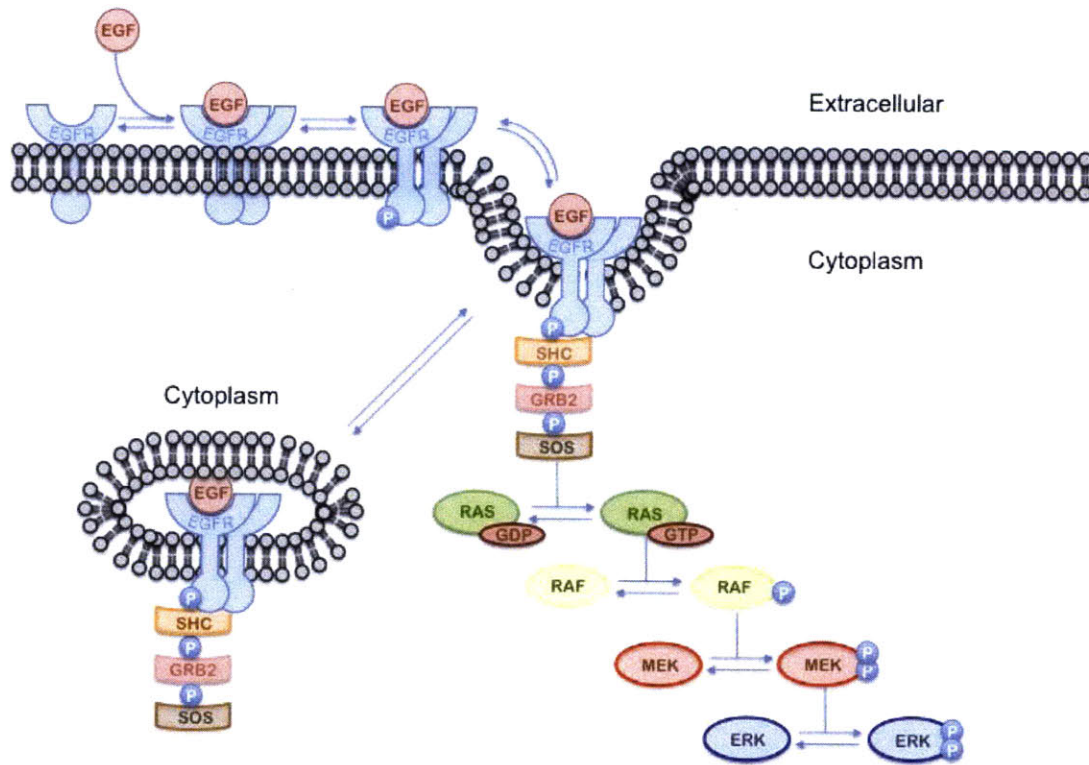


Figure 1. Schematic of EGF-Induced Signaling pathway.

Extrinsic apoptosis pathway

Apoptosis, the process of programmed cell death, is regulated by a variety of cell signals, including extracellular (extrinsic) and intracellular (intrinsic) pathways. Toxins and hormones are common types of extracellular signals (9). The apoptosis pathway model used in the current work is an extrinsic one based on the work of Albeck et al. (10). The pathway begins with assembly of death-inducing signaling complexes (DISCs) on TNF-related apoptosis-inducing ligand (TRAIL) receptor. Assembly activates initiator caspase-8, which cleaves effector procaspase-3, creating active effector caspase-3, which

we treat as the final output of this signaling pathway. Additional regulators of active effector caspase-3 include other factors such as X-linked inhibitor protein (XIAP). XIAP inhibits caspase-3 activity by binding to its active site (11). XIAP also induces ubiquitination and degradation of caspase-3 by an E3 ubiquitin ligase (12). XIAP itself is regulated by a pathway involving membrane permeability changes of the mitochondria, called MOMP (mitochondrial outer membrane permeabilization) (13). MOMP is regulated by the Bcl-2 family of proteins, which are either pro-apoptotic, like Bid and Bax, or anti-apoptotic, like Bcl-2. In this pathway the initiator caspase-8 cleaves BH3 interacting domain death agonist (Bid), which then activates Bcl-2-associated X protein (Bax). Bcl-2 acts as an inhibitor of Bax. The activated Bax translocates to the mitochondria and changes the outer membrane permeability by forming pores in it, which results in the translocation of cytochrome c and second mitochondria-derived activator of caspases (Smac) from mitochondria to cytosol. Smac blocks the caspase-3 inhibitory activity of XIAP by tightly binding to it. Cytochrome c binds Apaf-1 and caspase-9 to form the apoptosome, which leads to apoptosis. In addition, there is a positive feedback loop in this pathway. Activated effector caspase-3 cleaves procaspase-6, which further activates initiator caspase-8 (Figure 2).

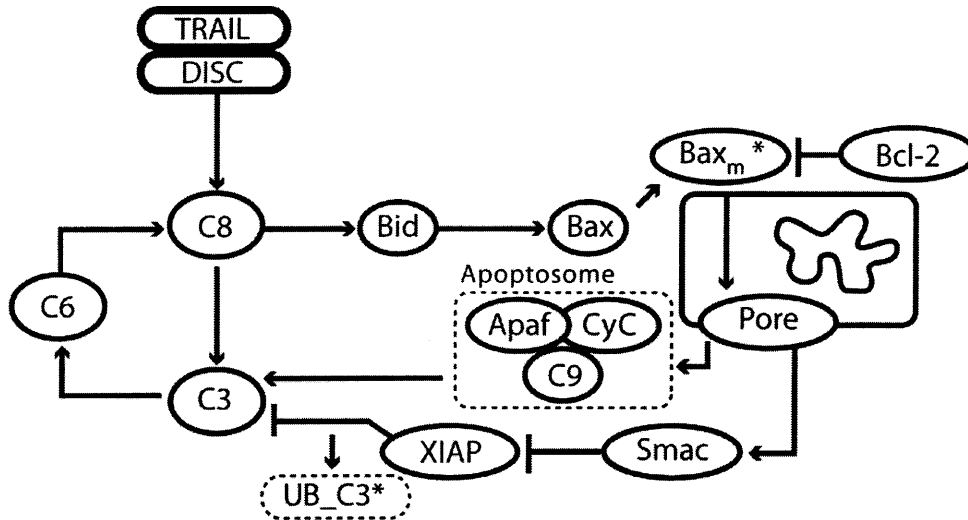


Figure 2. Schematic of extrinsic apoptosis pathway.

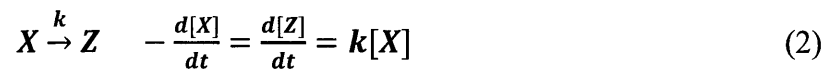
Methods

In this project we use a previously constructed mass-action version of the ordinary differential equation signaling models consisting of zeroth-, first-, and second-order reactions described by ordinary differential equations. In the following equations, X , Y and Z represent species. k is the rate constant and \emptyset is empty set or nothing. All these reactions can be reversible.

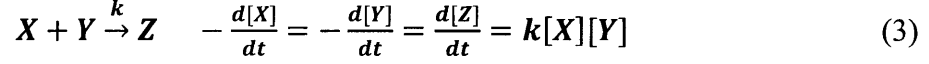
Zeroth-order reaction:



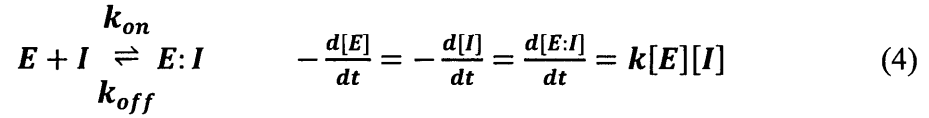
First-order reaction:



Second-order reaction:



We augmented the model through the addition of a competitive binder to the pathway; in separate experiments, the binder interfered with essentially each protein in the pathway, one at a time, in systematic fashion. The reversible binding reaction is of the following form, which is expressed for an inhibitor binding to an enzyme, although the target need not be an enzyme and the binder need not be an inhibitor. The effect of each binder was to titrate away its target so that while in complex the target participated in no further reactions except dissociation from the binder.



E , I and $E:I$ represent the target, the binder, and their one-to-one complex, respectively. k_{on} is the association rate and k_{off} the dissociation rate. The ratio of k_{off} to k_{on} is the equilibrium dissociation constant for the binding molecule and was set to the TBE_{50} .

$$TBE_{50} = K_i = \frac{k_{off}}{k_{on}} \quad (5)$$

In trial simulations, values of k_{on} and k_{off} were varied while the ratio was fixed to identify sufficiently rapid kinetics that signaling results became independent of k_{on} and k_{off} and depended only on their ratio. Production simulations were carried out with these rapid kinetics.

To investigate the relationship between inhibition of a target signaling molecule (TBE) and interference with the output (PME), we studied pathway behavior as a function of inhibitor concentration. We tabulated both a measure of TBE (% binding of

target molecule) and a measure of PME (% reduction in output or other signaling molecules). Our definition implies,

$$\begin{aligned} & \% \text{ reduction in active target signaling molecules} \\ &= \left(1 - \frac{[\text{active signaling molecules}]}{[\text{total signaling molecules}]} \right) \times 100\% \end{aligned} \quad (6)$$

Because simulations were run with sufficiently fast inhibition kinetics so that the inhibitor was always in steady state,

$$\% \text{ reduction in active molecules} = 1 - \frac{[Raf^*]}{[Raf^*:I] + [Raf^*]} = 1 - \frac{1}{\frac{[I]}{K_i} + 1} \quad (7)$$

which indicates that inhibitor concentration $[I]$ and binding affinity K_i do not affect binding independently but rather only as their ratio. The typical values of k_{on} and k_{off} used are 1.66×10^{-2} cell molecule $^{-1}$ s $^{-1}$ and 10 s $^{-1}$.

To assess the effects of binding individual pathway components on pathway output, two characteristics of the output response curve were used, the peak and the integrated response. The peak is defined as the maximum of the response curve and the integrated response is defined as the integrated area under the curve until end of the simulation which is 1 hour for EGFR signaling pathway and 100 hours for the intrinsic apoptosis pathway (Figure 3). At the end of simulation, concentrations of key signaling molecules are restored to initial conditions.

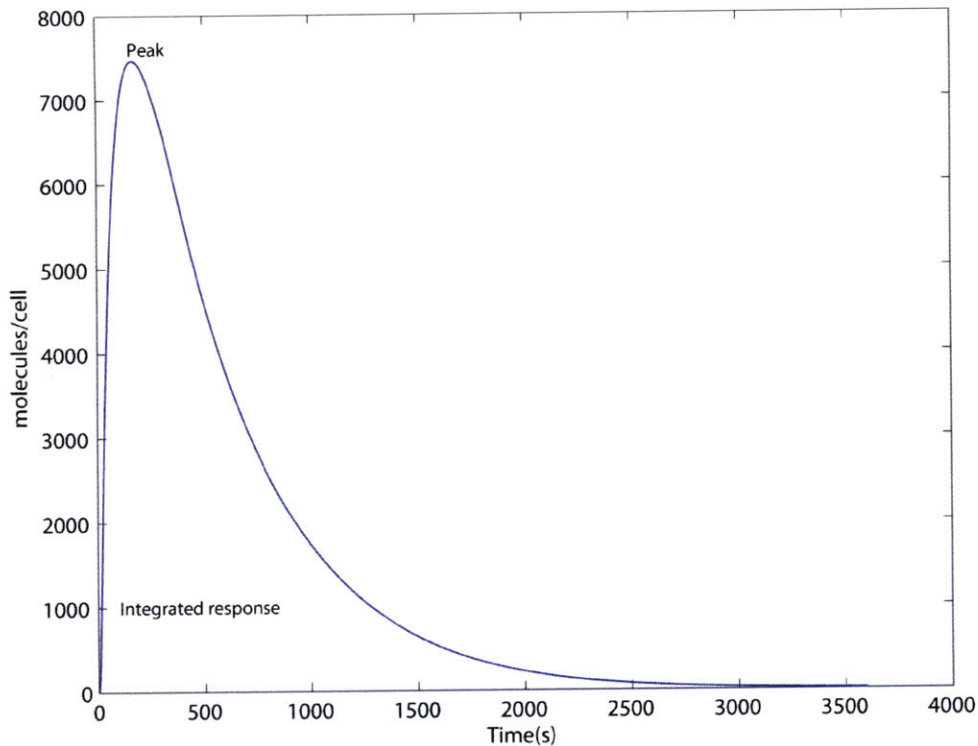


Figure 3. A general response curve and the two characteristics we use. X axis is time in second and Y axis is concentration of a specific species.

EGFR signaling model we use in this project is based upon the work of Schoeberl et al. and Hornberg et al. (7, 14, 15). The model contains 103 chemical species and 148 elementary reactions. Parameters of this model include 97 distinct reaction rates and 103 initial conditions. There are three compartments taken into consideration in this model: the extracellular medium ($1\text{ml}/10^6$ cells), the cytoplasm, and the endosome. The cytoplasmic volume of a cell is taken as 1×10^{-12} liters, and the volume of an endosome is taken as 4.2×10^{-18} liters (7). The unit of species concentration is molecules/compartiment, which is readily converted into nM using volume of the compartment.

The extrinsic apoptosis signaling model used in this project is that of Albeck et al. (10). It contains 58 chemical species, 43 elementary reactions, and 60 reaction rate constants. Two compartments are taken into account in this model: the cell and the mitochondrion with volumes of 1 liter and 0.07 liters, respectively. Unlike the EGFR pathway, the extrinsic apoptosis pathway contains inhibitors itself. Bid and Bax are downstream signaling molecules of caspase-8 and they are inhibited by Bcl-2. XIAP is anti-apoptosis. It inhibits effector caspase-3 and apoptosome.

Results

EGFR signaling pathway

Studies were carried out of the EGFR signaling pathway, with EGF treated as the sole input to the signaling model. EGF was applied as a rectangular input pulse with a height of 1 nM and duration of 5 minutes. EGFR2* (activated EGFR dimer), Shc*, Ras-GTP, Raf*, MEK-PP, and ERK-PP are key activated forms of signaling molecules in this pathway, and their responses to the input pulse are shown in Figure 4. It can be seen that the signal is amplified along the pathway from the increasing range of concentrations as one progresses deeper in the pathway. The peak value of the upstream signaling molecule EGFR2* is 2.24×10^4 molecules cell⁻¹, and the peak value of the output ERK-PP (activated ERK) is 1.13×10^7 molecules cell⁻¹. There is thus a 500-fold increase in the magnitude of the peak signal value.

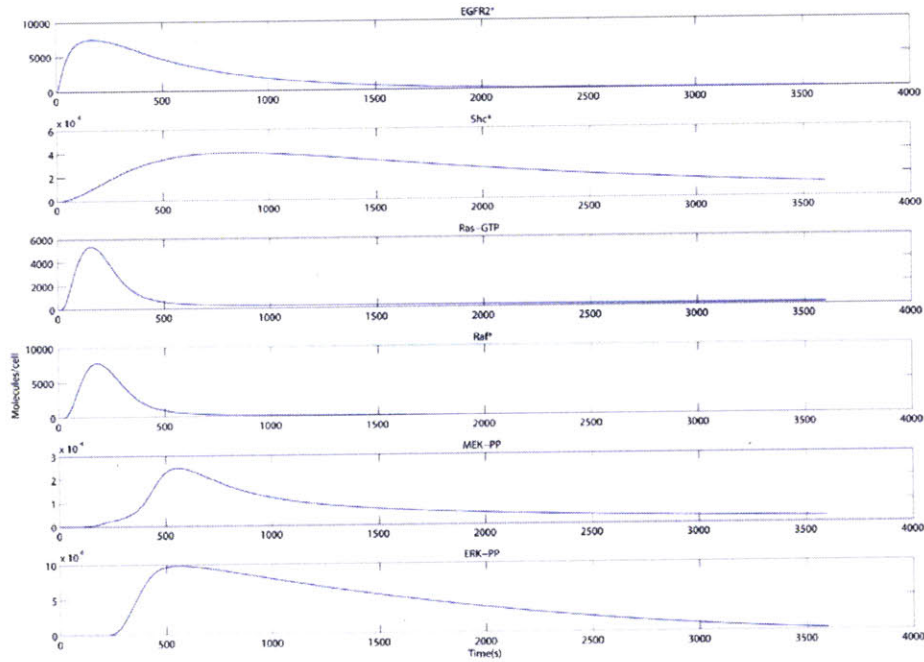


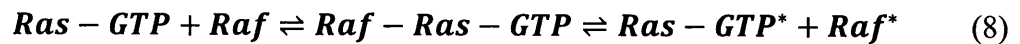
Figure 4. Computational simulation of EGFR signaling model without any inhibitor. EGFR2*, Shc*, Ras-GTP, Raf*, MEK-PP, ERK-PP are key activated forms of signaling molecules in this pathway. Rectangular pulse input of EGF was applied with a concentration of 1 nM and duration of 5 minutes. Peak values of the six signaling molecules are 7.462×10^3 , 4.022×10^4 , 5.381×10^3 , 7.817×10^3 , 2.463×10^4 , 9.764×10^6 molecules cell⁻¹, respectively.

Raf kinase inhibitor

We first investigate an inhibitor incorporated into the EGFR pathway model that binds to and inactivates Raf. We consider the behavior of a 1-nM competitive inhibitor of Raf, its activated form Raf*, and the internalized Raf* (Rafⁱ*). One might think of this as a simplified model for sorafenib, a multikinase inhibitor that has demonstrated antitumor activity (16). Sorafenib targets Raf kinase isoforms (16).

With increasing concentration of Raf inhibitor, there are significant decreases in the peak concentration of Raf* and subsequent signaling molecules such as MEK-PP and ERK-PP. Peaks of Raf*, MEK-PP and ERK-PP are delayed with increasing Raf kinase inhibitor concentration.

Interestingly, the immediately upstream signal, Ras-GTP, shows increased concentration when Raf* is titrated out of the system through addition of an exogenous binding partner. There are 8 forms counted as Ras-GTP in this model: free form of Ras-GTP (denoted as Ras-GTP+), complex formed with Raf (Raf-Ras-GTP), complex formed with GAP (GAP-Ras-GTP), and activated form of GAP-Ras-GTP (GAP-Ras-GTP*) and internalized version of the above four molecules. The concentration of Ras-GTP is mainly contributed by Ras-GTP+ (Figure 6). Production rate of Ras-GTP+ is not affected by Raf kinase inhibitor while the consumption rate is reduced because less Raf is available to Ras-GTP due to competition of the inhibitor. Thus there is an accumulation of Ras-GTP.



Signaling molecules further upstream, such as EGFR2* and Shc*, do not show significant change (Figure 5).

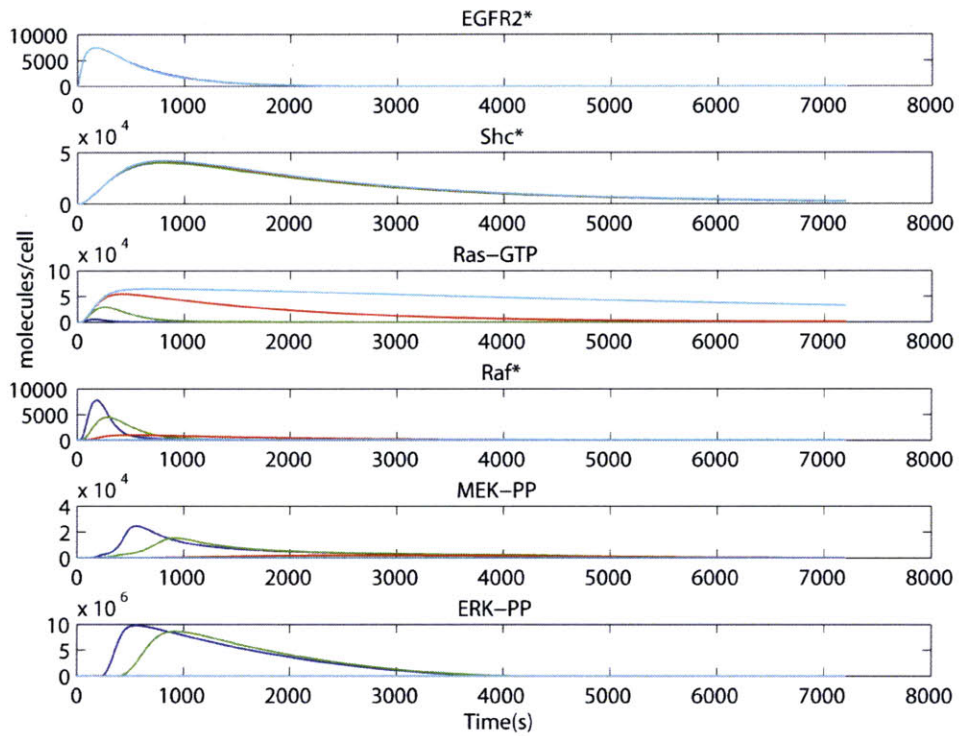


Figure 5. Computational simulation of EGFR signaling model at different concentrations of Raf kinase inhibitor: 0 (blue line), 1 nM (green line), 10 nM (red line), and 100 nM (cyan line). Input of the inhibitor is constant during the simulation. k_{on} and k_{off} of the inhibitor are $0.0166 \text{ cell molecules}^{-1} \text{ s}^{-1}$ and 10 s^{-1} , respectively, giving a K_i of $602 \text{ molecules cell}^{-1}$ (1 nM). Red and cyan lines of MEK-PP and ERK-PP responses are barely seen because of small values compared to blue and green line.

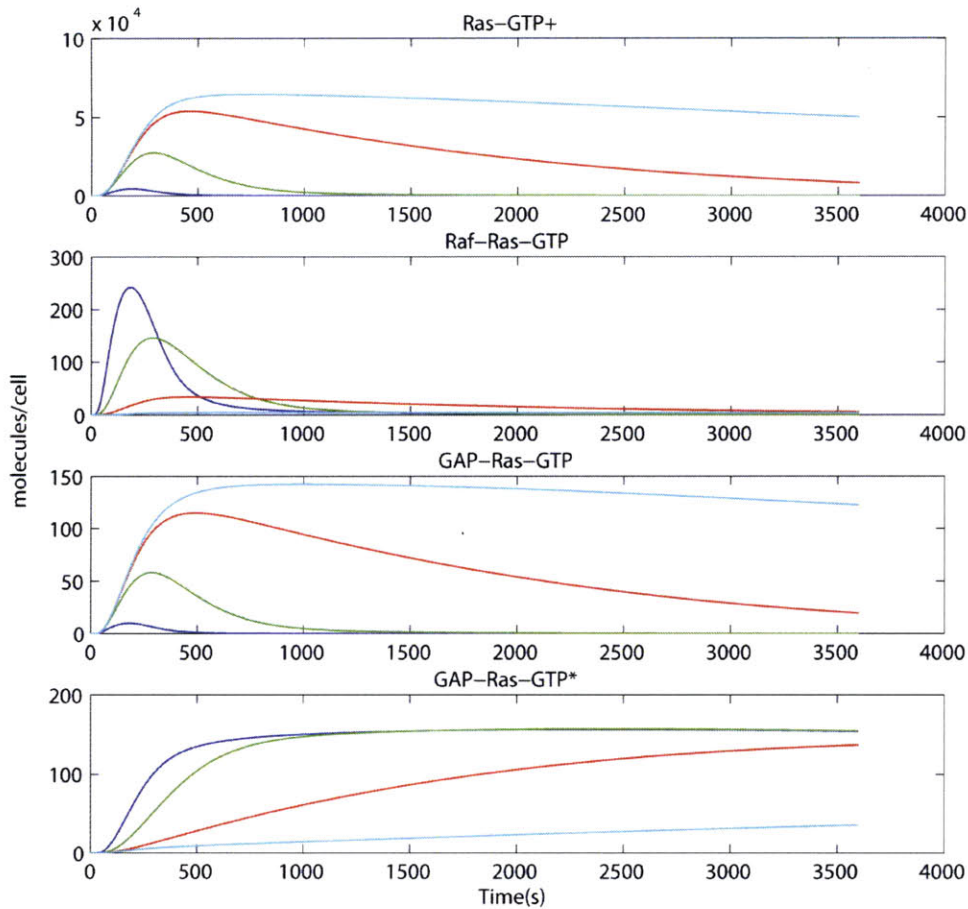


Figure 6. Computational simulation of each form of Ras-GTP at different concentrations of Raf kinase inhibitor: 0 (blue line), 1 nM (green line), 10 nM (red line), and 100 nM (cyan line). Each form includes its internalized version.

Figure 7 illustrates continuous change in each species with an increasing concentration of Raf kinase inhibitor. First two key signaling molecules of this pathway, EGFR2* and Shc* have no significant change regarding increasing concentration of Raf inhibitor while peak concentrations and integrated responses of Raf* and subsequent signaling molecules MEK-PP and ERK-PP show nonlinear decrease at different speeds. Peak concentrations of Raf* and MEK-PP decrease faster than that of ERK-PP, while their integrated responses drop more slowly than ERK-PP.

In drug development, chemical optimization of binding interactions has effects on the TBE of a drug candidate; the resulting effects on pathway behavior are generally more important for overall potency, and PME is one measure of how much of a drug candidate is necessary to modulate a pathway under some given set of conditions. The relationship between PME and TBE is shown in Figure 7C, and gives important information about molecular binding and pathway control.

The relationship between inhibiting Raf kinase and modulating ERK-PP output is highly nonlinear. Inhibitor concentrations leading to a decrease to 80% in the peak value of active Raf concentration lead to barely any change in ERK-PP signaling. Significant inhibitory effects on ERK-PP appear only when more than 80% of Raf kinase molecules are inactive and there is a rapid increase in the effect when more than 90% of Raf molecules are inhibited (Figure 7C).

Detailed information of activity of Raf kinase inhibitor with intermediate signaling molecules is shown in Figure 8. MEK-PP signal is saturated at current Raf* concentration without inhibitor. There is no significant decrease in peak or integrated response of MEK-PP until more than 80% of Raf is inhibited. Saturation at MEK-PP could be the reason of highly nonlinear relationship between inhibiting Raf kinase and modulating ERK-PP output.

PME₅₀ for Raf kinase inhibitor is clearly larger than TBE₅₀. From Equation 7 we can derive Equation 9, where $[I]$ is the concentration of the inhibitor, r is reduction in the targeted molecules in percentage and K_i is the disassociation constant of the inhibitor.

$$[I] = \frac{rK_i}{100-r} \quad (9)$$

From simulation results, 96.44% of active Raf molecules have to be inhibited to achieve a 50% reduction in the peak of ERK-PP. With $r = 96.44$, we get $PME_{50} = 27.13K_i$, which is 27 times larger than TBE_{50} . For the integrated response 96.84% inhibition of active Raf molecules is required to inhibit ERK-PP by half, resulting in an PME_{50} 30 times larger than TBE_{50} .

This suggests that Raf kinase may be a less than ideal candidate drug target for two reasons. Firstly its PME_{50} is much larger than TBE_{50} . A large dose is required to achieve a given therapeutic effect. The steep slope near 100% reduction in active signaling molecules also leads to difficulty; the steep slope implies that a tiny change in drug concentration could induce dramatic change in the overall effect. This makes the drug hard to control and could result in it being potentially dangerous.

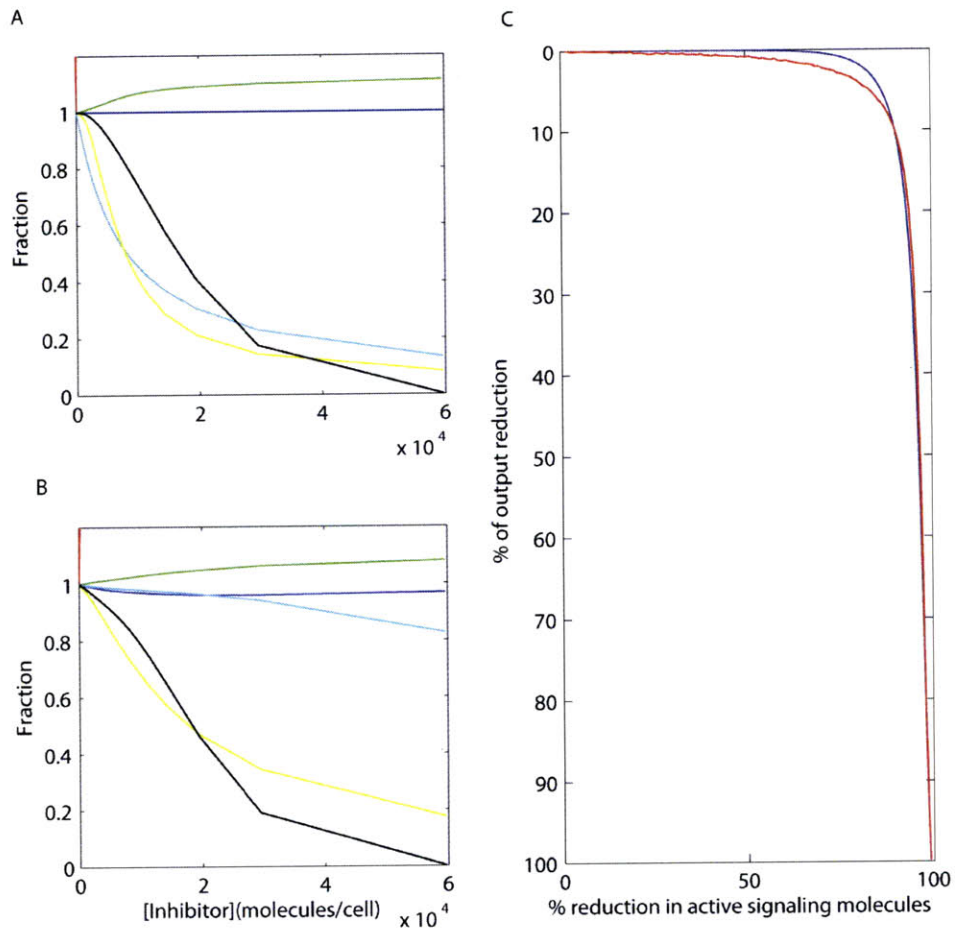


Figure 7. (A) Peak and (B) integrated responses of key signaling molecules EGFR2* (blue line), Shc* (green line), Ras-GTP (red line), Raf* (cyan line), MEK-PP (yellow line), and ERK-PP (black line) in EGFR pathway with an increasing concentration of Raf kinase inhibitor. (C) Plot of the percentage of output reduction vs. percentage reduction in active signaling molecules which is Raf in this plot. Peak (blue line) and integrated response (red line) are used as the two measures.

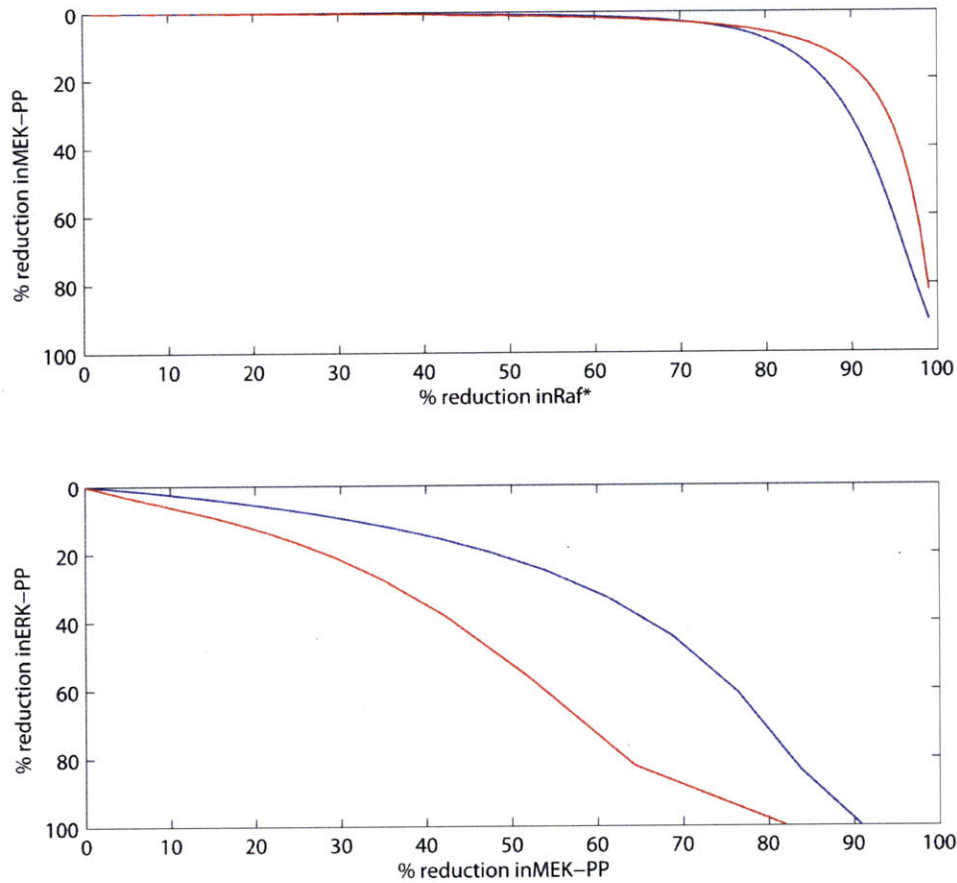


Figure 8. Step by step information of inhibitory activity of Raf kinase inhibitor with intermediate signaling molecules. Peak (blue line) and integrated response (red line) are used as the two measures.

Inhibitors of other signaling molecules in EGFR pathway

Competitive inhibitors of other signaling molecules were added into the model, one at a time. Figure 9 shows a plot of percentage reduction in active molecules vs. reduction in the output (TBE vs. PME) for inhibition at a variety of different locations throughout the pathway.

Most inhibitors (EGFR, Ras-GTP, MEK-PP, and ERK inhibitors) have their TBE vs. PME curves above that of ERK-PP, which implies their PME_{50} is larger than their TBE_{50} . When the target molecule is inhibited by 50%, there is a less than 50% reduction of the original pathway output.

Inhibition of MEK is unique in the pathway, in that its PME_{50} less than its TBE_{50} . The ratio of PME_{50} to TBE_{50} is 0.364 for peak and 0.333 for integrated response. There is no significant change in the slope of the curves, which implies the therapeutic effect changes smoothly with changes in drug dose. These properties suggest MEK as a candidate drug target for down-modulating the EGFR signaling pathway.

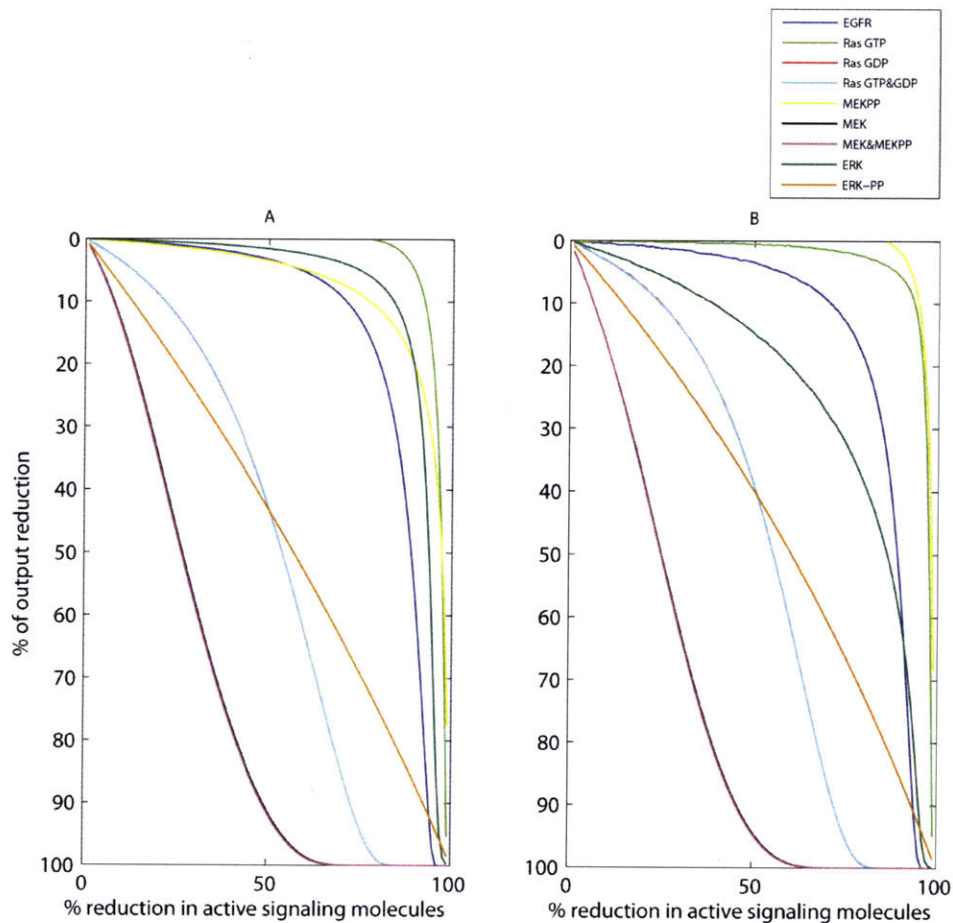


Figure 9. Plot of the percentage of output reduction vs. percentage reduction in active signaling molecules in EGFR pathway other than Raf kinase. (A) Peak (B) Integrated response

Inhibition of two species simultaneously

Inhibiting multiple signaling molecules in the same pathway may be a useful strategy for pathway down regulation. Here we consider a binder to MEK simultaneously with one to Ras-GDP. Concentrations of the two inhibitors were the same during these simulations but could be different depending on their relative efficacies.

The result is extremely similar to the linear superposition of curves due to inhibiting each of the two species separately, but not exactly. When less than 30% of MEK and Ras-GDP are inhibited, inhibiting them simultaneously produces a larger percentage reduction in ERK-PP than the sum of inhibiting them separately. But when 30–50% of active signaling molecules are inhibited, the opposite case is true (Figure 11).

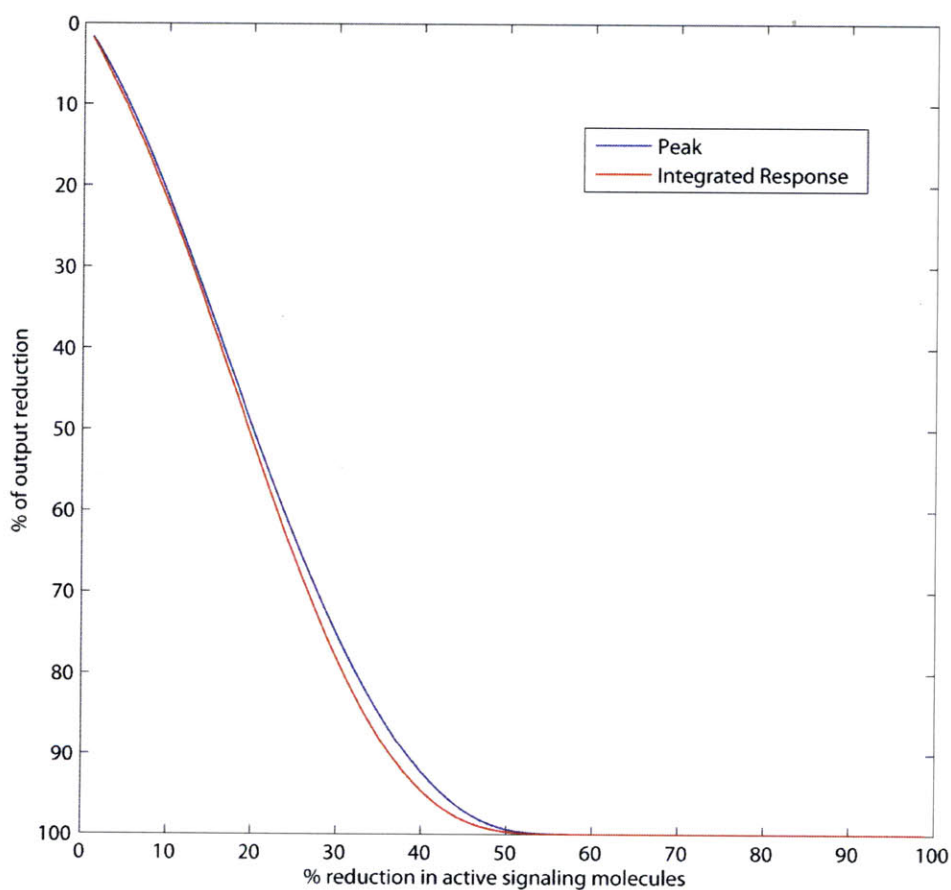


Figure 10. Plot of the percentage of output reduction vs. percentage reduction in active MEK and Ras-GDP molecules when they are inhibited simultaneously. k_{on} and k_{off} of the inhibitor are $0.0166 \text{ cell molecules}^{-1} \text{ s}^{-1}$ and 10 s^{-1} , respectively, giving a K_i of $602 \text{ molecules cell}^{-1}$ (1 nM).

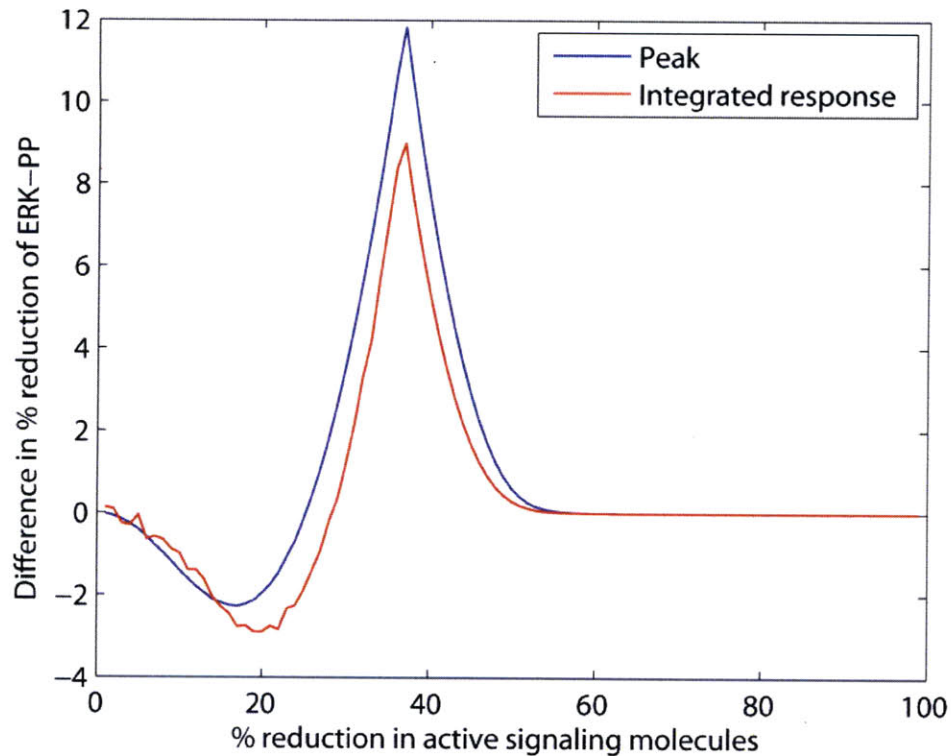


Figure 11. Difference in final output inhibition between inhibiting MEK and Ras-GDP simultaneously and linear superposition of inhibiting the two separately. A negative value means inhibiting the two simultaneously produces a greater inhibition on the output than the linear superposition. Since the output cannot be reduced by more than 100%, the linear superposition is capped at 100%.

Table 1 summarizes the $PME_{50}:TBE_{50}$ values of inhibitors of signaling molecules in the EGFR pathway. Six out of seven independent inhibitors have a PME_{50} to TBE_{50} ratio larger than 1. Nonlinearity of this pathway is a contributing factor causing the PME_{50} of inhibitors to be larger than TBE_{50} due to saturation during signal transduction.

| Inhibitor of | EGFR | Ras-GDP | Ras-GTP | Ras-GTP & Ras-GDP | Raf kinase |
|---|--------------|-------------------|-----------------------------|----------------------------------|-------------------------------|
| PME ₅₀ :TBE ₅₀ (peak, integrated response) | 8.045, 8.165 | 1.174, 1.242 | 35.818, 42.302 | 1.174, 1.242 | 26.309, 30.205 |
| Inhibitor of | MEK | MEK-PP | MEK & MEK-PP | ERK | MEK & Ras- GDP |
| PME ₅₀ :TBE ₅₀ (peak, integrated response) | 0.364, 0.333 | 36.221, 61.001 | 0.360, 0.332 | 15.131, 6.271 | 0.269, 0.258 |

Table 1. Ratio of PME₅₀ to TBE₅₀ of different inhibitors of EGFR signaling pathway.

It was found that inhibiting the inactive form of a signaling molecule (e.g. Ras-GDP and MEK) is more effective than inhibiting the active form (e.g. Ras-GTP and MEK-PP). But this is not true for ERK. The most effective inhibitor is MEK inhibitor with PME₅₀ to TBE₅₀ ratio of 0.364 for peak and 0.333 for integrated response.

Inhibiting two molecules simultaneously produces effects roughly equal to sum of effects of inhibiting them separately. Thus inhibiting MEK and Ras-GDP at the same time is the most effective way to cut the signal.

In this project an inhibitor is simplified as a competitive binder which binds an enzyme and makes them inactive. But in real cases binding can be more complex. The inhibitor need not be competitive and the binding need not be one-to-one. A signaling molecule can have more than one function. For example, EGFR binds EGF, forms dimer and activates. The inhibitor can have more elaborate behavior by inhibiting one but not

all functions of the target. Making the inhibitors more realistic is the future work of this project.

Extrinsic apoptosis pathway

We applied the same procedures to the extrinsic apoptosis pathway. PME_{50} of most inhibitors are larger than their TBE_{50} . Some inhibitors (e.g., inhibitors of the receptor, M, and caspase 6) do not even have a PME_{50} to TBE_{50} ratio because they show no inhibitory effects on the final output. For M and caspase-6 inhibitor this can be rationalized by the non-sequential structure of this pathway. Inhibiting signal of one branch may be compensated by signals of other branches and the overall effect on the output level can be negligible. In this case we can say PME_{50} to TBE_{50} ratios of these inhibitors approach positive infinity. Thus nonlinearity of a signaling pathway is possible to cause a 1000-fold difference between TBE_{50} and PME_{50} .

XIAP is the only inhibitor with PME_{50} less than TBE_{50} . It is an inhibitor included in the original model. If we use peak to measure effect of inhibition, XIAP is a good candidate for a drug target because of its small PME_{50} to TBE_{50} ratio (0.42) and almost uniform slope in Figure 12A. On the contrary if we use integrated response as the measure, XIAP is less than ideal despite the small value of PME_{50} to TBE_{50} ratio (0.01), mainly because the extreme steep slope between 0–10% reduction in active signaling molecules in Figure 12B, which implies it is hard to control.

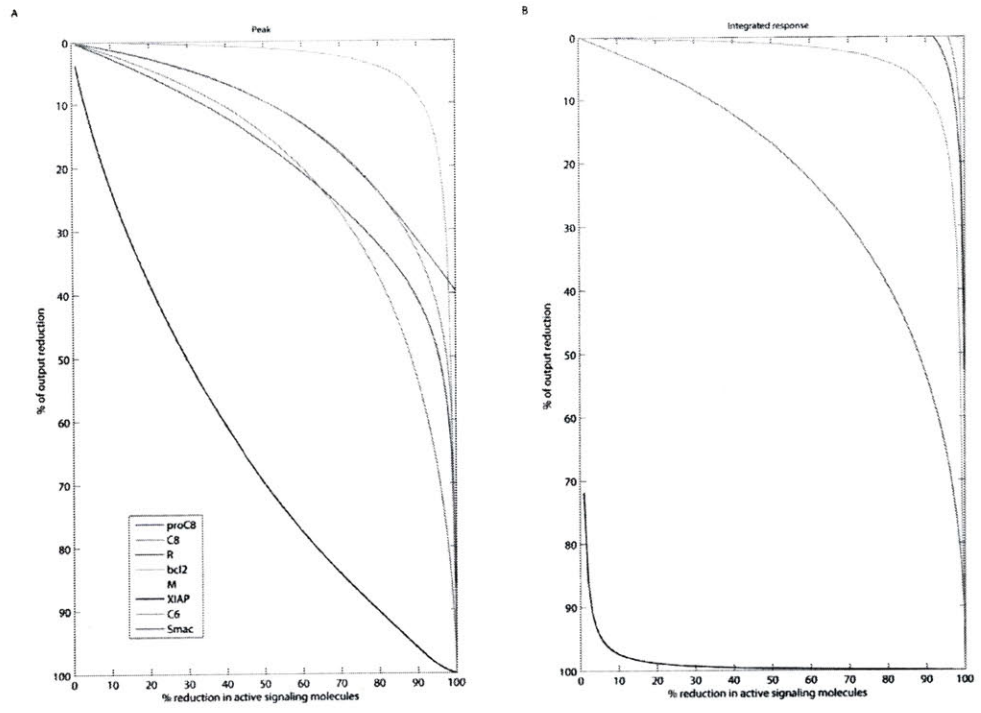


Figure 12. Plot of the percentage of output reduction vs. percentage reduction in active signaling molecules in extrinsic signaling pathway.

| Inhibitor (of) | Pro-caspase 8 | Caspase 8 | Receptor | Bcl2 |
|---|---------------|---------------|-----------|--------------|
| PME ₅₀ :TBE ₅₀ (peak, integrated response) | 21.96, 544.02 | 41.03, 247.41 | Na, Na | 75.31, 78.48 |
| Inhibitor (of) | M | XIAP | Caspase 6 | Smac |
| PME ₅₀ :TBE ₅₀ (peak, integrated response) | Na, Na | 0.42, 0.01 | Na, Na | 7.59, 7.33 |

Table 2. Ratio of EC₅₀ to IC₅₀ of different inhibitors of extrinsic apoptosis signaling pathway. Values of EC₅₀ to IC₅₀ ratio of some inhibitors are not available because they have no or extreme small inhibitory effect on the final output caspase 3.

Conclusion

In this project we augmented inhibitors to previously constructed signaling pathway models to investigate between inhibiting a signaling molecule and interrupting the result of that signal systematically. In both EGFR and extrinsic apoptosis pathway, 50% inhibition of most enzymes yields less than 50% reduction of the final output. Nonlinearity of the pathway is a possible reason of the large discrepancy between TBE₅₀ and PME₅₀ due to saturation during signal transduction.

Nevertheless nonlinear property of the pathway may also make inhibitors more effective. Inhibitors with PME₅₀ to TBE₅₀ ratio less than 1 were found in both signaling pathways (MEK inhibitor in EGFR pathway and XIAP in extrinsic apoptosis pathway). In addition, we found that inhibiting two signaling molecules simultaneously leads to an

effect roughly equals to the linear superposition of effects of inhibiting them separately.

This approach may be useful in the selection of targets for therapeutic intervention.

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Appendix

Effect of k_{on} and k_{off}

Because some of the inhibitors added to the model are not real, and for the actual inhibitors, rate constants are not available; k_{on} and k_{off} are set to values which we consider as reasonable. So we want to investigate what will happen when we change values while keep the ratio of them (The ratio K_i is kept at 602 molecules cell⁻¹ which equals to the typical value of TBE₅₀). Here we use Raf kinase inhibitor to investigate the effects.

When k_{on} and k_{off} are too small (e.g. 1.66×10^{-9} cell molecules⁻¹ s⁻¹ and 1×10^{-6} s⁻¹), the result does not make sense because no inhibition is observed. When k_{off} is greater than 0.001 s⁻¹, the curves in Figure 13 do not change any more. Thus unless k_{on} and k_{off} have extremely small values, the simulation results are insensitive to values of k_{on} and k_{off} while the ratio is a constant. In the previous simulations, k_{off} set to 10 s⁻¹ which is a reasonably good choice.

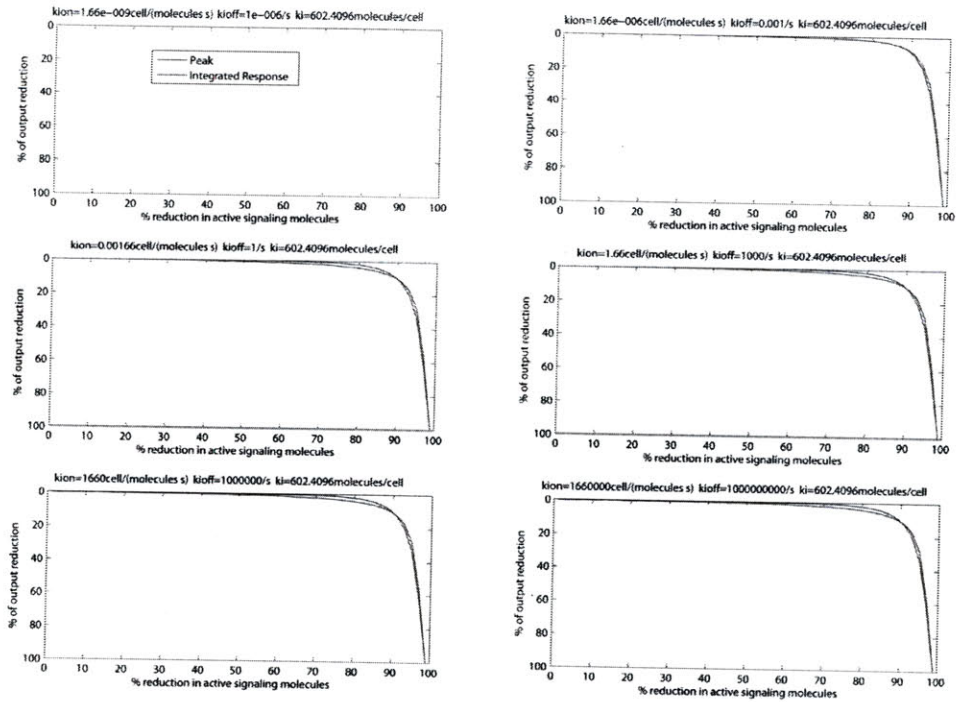


Figure 13. Simulation results of Raf inhibitor with different k_{on} and k_{off} values while the ratio is kept at 1nM.