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Mathematical modeling deciphering balance between Cell Survival and Cell Death using Tumor Necrosis Factor-α

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ABSTRACT

In this paper we apply a deterministic numerical method to the analysis of a large, systematic dataset describing the dynamics of cell signalling downstream of, tumor necrosis factor-α (TNF) receptors in human colon carcinoma cells. Deterministic modeling is useful as a means to assemble and test what we know about proteins and networks. We extensively study the space of parameters to show that the model is structurally stable and robust over a broad range of parameter values. We have made the biological view of the main paths of the TNF showing cell survival and cell death. Than with the help of different parameters relating to that protein present in the model we have designed scheme of the biochemical paths/deterministic model. With those parameters equations were formed which vary with time i.e. differential equation. Thus, our model is suitable for implementation in multi-scale simulation programs that are presently under development to study the behavior of large tumor cell populations.

Keywords : TNF, Deterministic model, Cell survival, cell death

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INTRODUCTION

Large datasets for biological systems are becoming increasingly available due to ongoing improvements in high-throughput measurement methods. However, similarly improved computational methods are needed if we are to gain useful insights from these datasets. Among prominent examples of this current challenge in biology is in understanding how extra cellular cues influence highly interconnected and complex cell signalling pathways to yield cell behavioural responses [1]. In the case of programmed cell death, cytokines such as tumor necrosis factor-alpha (TNF-α) [2] function as a pro-death cue, whereas growth factor such as epidermal growth factor (EGF) and insulin [3] exert pro-survival effects. The magnitudes of the responses vary with cell type, but the pathways downstream of cytokine receptors are conserved and highly interconnected. It appears that the determination of whether a cell will live or die involves a balance between pro-death and pro-survival signals [4]. Thus, the intracellular signal transduction network stimulated by TNF, insulin, and other cytokines acts as a signal processor that converts opposing cues into a functional response that controls cell fate.

Although many of the components of cellular signalling systems have now been identified, it is not yet known how binary decisions, such as death–survival, are made. Moreover, the complexity of cell signalling networks precludes a simple protein-by-protein assignment of function. Increasingly, systematic methods are applied to the interpretation and computational analysis of cell signalling [1, 5]. These computational methods lie on a spectrum of approaches that vary in their level of abstraction and specificity [6]. The most abstract methods, such as multivariate analysis and clustering [7], are powerful because they can handle empirical data and prior knowledge about the underlying phenomena in a flexible fashion. At the other hand, differential equation-based models [8] are useful for encoding existing prior knowledge in pursuit of *in silico* predictions.

Current high-throughput experimental protocols allow us to measure an increasingly large number of variables describing cells or cellular populations. For example, large scale phosphorylation screens [9], protein–protein binding screens [10] and migration assays [11] have demonstrated that it is possible to simultaneously measure the activity of tens to thousands of variables in a biological system. Future advances in micro fluidics [12] and high throughput mass spectrometry [13], for example, promise even more measurements will be possible at a fraction of their current cost. Analysis of these data has generally focused on identifying a small number of pathway components involved in governing particular cell behaviour. Growing interest is focusing on how to use these multivariate data to determine integrated pathways in, for instance, signal transduction [14] and metabolic pathways [15].

In this paper, our purpose is to determine whether numerical model such as deterministic model can be used to uncover important aspects of cellular signals deciphering the fate of cell. Specifically, we examine the TNF mediated death-versus-survival response by deterministic approach. We wish to attract the interest of other experimental molecular cell biologists, so we emphasize the conceptual details of deterministic method as a practical

modelling technique. A noteworthy feature of deterministic model applied to biological data, is that biological measurements reflect tangible molecular entities with known, mechanistic roles in intracellular processes. Thus, these data-driven models of signalling are empirical, but not phenomenological, and suggest mechanistic dependencies. We show here that numerical and related methods can uncover key contributors to death–survival decisions. These contributions always involve multiple proteins working in concert, but the informative proteins consist of only a fraction of the original protein dataset. Thus, our results suggest that within the entire cellular signalling system lies a reduced set of information-rich protein measurements that together constitute an efficient model of the signalling network state and the relevant signal–response relationships.

THEORETICAL MODEL

Binding and internalization of TNF/TFN-R1 complexes

Current biochemical data show that the TNF-R1 receptors rapidly self-trimerized at the cell membrane because of the PLAD domains and interact with TNF homo trimers. Thus, the mechanism of TNF binding to TNF-R1 can in principle be viewed as the result of the monomeric interactions between one molecule of TNF and one molecule of receptor. This simplification is further supported by the following considerations:

1. The mechanism of receptor self-trimerization followed by ligand binding can be modeled by a set of 4 differential equations with 6 parameters. The model can fit experimental data of TNF binding, but one finds that the kinetics of receptor trimerization are much faster than the binding kinetics, and thus the trimerized receptor behaves as an effective monomer. In addition, experimental determinations of the parameter values for intermediate binding reactions are not available;
2. The model by Bajzer et al. (1989) was use to describe the early events of TNF interactions with cells. In particular, Bajzer et al. (1989) assumed that internalized ligand/receptor complexes could be recycled back at the cell surface. It is highly probable that TNF/TNF-R1 complexes do not recycle but are finally degraded into lysosomes. Therefore we modify the model by Bajzer et al (1989) and Chignola et al (2009) model as follows

$$\begin{aligned}
\frac{d[R]}{dt} &= V_r - k_d[R] - k_{on}[L][R] + k_{off}[N_c] \\
\frac{d[L]}{dt} &= -k_{on}[L][R] + k_{off}[N_c] \\
\frac{d[N_c]}{dt} &= k_{on}[L][R] - (k_{off} + k_{in})[N_c] \\
\frac{d[N_{in}]}{dt} &= k_{in}[N_c] - k_{\deg}[N_{in}]
\end{aligned} \qquad (1)$$

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Where square brackets denote molar concentrations of free TNF-R1 receptors (*R*), free TNF (*L*), TNF/TNF-R1 complexes bound at the cell membrane (N_C) and internalized complexes (N_{in}). Here k_{on} and k_{off} are the association and dissociation rate constants for TNF binding to TNF-R1, respectively, k_{in} is the internalization rate constant of TNF/TNF-R1 complexes and k_{deg} is the rate constant of lysosomal degradation of the complexes.

The two parameters V_r and k_d where introduced by Bajzer et al, although with a slightly different notation, to describe "the zero-order rate of insertion of receptors into the membrane and the turnover (internalization) rate constant of ligand-free receptors [16, 17, 18] respectively. This is an important aspect of the model, since in the absence of TNF, it reaches a steady concentration of receptors at the cell surface given by:

$$[R]_{[L]=0} = \frac{V_r}{k_d} \qquad (2)$$

In addition, for long times these terms prevent receptor loss (i.e. down modulation) from the cell surface, a process that would undesirably result in cell resistance to TNF independently of the dynamic interplay between the intracellular paths triggered by TNF.

Modeling the intracellular signaling pathways triggered by TNF

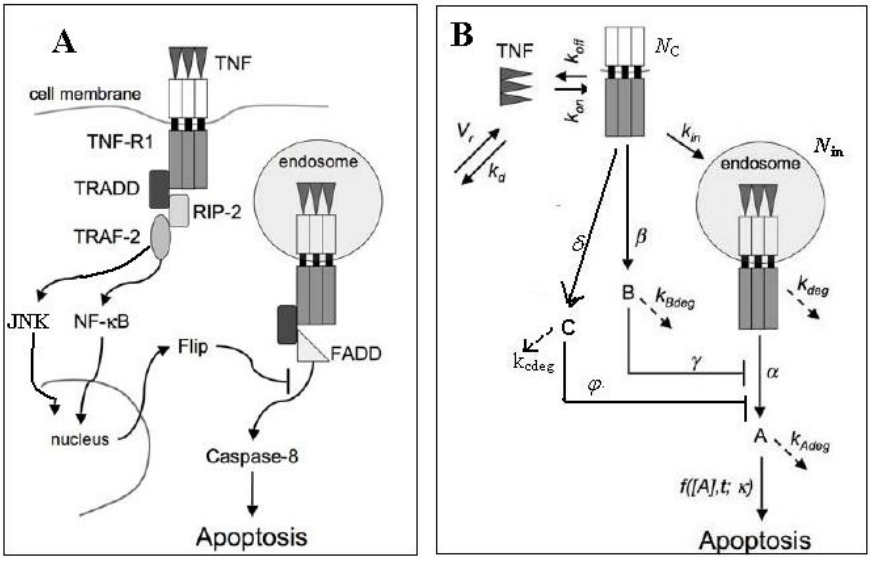

Fig.1 - Modeling TNF cytotoxicity. **A.** Biological view of the main TNF paths. Binding of TNF to TNF-R1 recruits a molecular complex formed by TRADD, RIP-2 and TRAF-2 which in turn activates NF-κB and JNK as well as expression of FLIP (which inhibit activity of caspase-8) leading to cell survival. Internalization of TRADD, RIP-2 and TRAF-2 within endosome leads to activation of caspase-8, leading to cell death.

B. Assume approximation model of the biochemical paths for the mathematical modeling. TNF binds to TNF-R1 with association and dissociation rate constants given by k_{on} and k_{off}. The complexes are internalized with a rate constant k_{in}. Binding activates signaling resulting in the formation of a molecule *B* with rate constant β. Molecule *B* inhibits the apoptotic signal which has been assumed to be proportional to concentration of molecule *A*, and this process occurs with rate constant *γ*. Activating signaling resulting in the formation of a molecule *C* with rate constant *δ*. Molecule *C* inhibits the apoptotic signal which has been assumed to be proportional to the concentration of molecule *A*, and this process occurs with rate constant *γ*. The apoptotic signal *A* is triggered by internalized TNF/TNF-R1 complexes with a rate constant α. Internalized receptors are finally degraded by lysosomes, and molecular signals *A*, *B* and *C* are cleared off by proteolytic cleavage with rate constants k_{deg} , k_{Adeg},

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k_{Bdeg} and k_{Cdeg} respectively. Equilibrium in the expression and down modulation of TNF receptors have been assumed to occur at the cell surface with rate constants V_r and k_d.

TNF binds to its receptors TNF-R1 and cascade cellular signals in both normal and tumor cells leading to cell death or cell survival as discussed in Figure 1a. For the cell to be survived, the signaling cascade leads to activation of proteins such as JNK and NF-κB. At the same time two other proteins (Flip and IAP) also expressed leading to inhibition of apoptotic pathway induced by TNF [19]. The cell to undergoes apoptotic, the activated protein complex that has formed due to binding of TNF with its receptor, is internalized (formed endosome) leading to activation of caspase-8 leading to cell death. Basically there are three chemical entities (C, B, A) which interact dynamically and regulate both the biochemical circuits of cell death and cell survival. The molecules *C*, B and *A* can be loosely identified as JNK/FLIP, NF-κB/FLIP and caspase-8, respectively with respect to Fig.1*a*. We assume that after the initial triggering of the pathway by TNF, it proceeds irreversibly to its endpoint. Thus we can neglect many details of pathways which involve a number of different molecular actors in between and thus we can obtain a more refined approximate model, represented as Fig. 1*b*. Mathematically we can write the deterministic equations for *A, B,* and *C* as:

$$\begin{aligned}
\frac{d[A]}{dt} &= \alpha[N_{in}] - \gamma[B][A] - \varphi[C][A] - k_{A\deg}[A] \\
\frac{d[B]}{dt} &= \beta[N_c] - k_{B\deg}[B] \\
\frac{d[C]}{dt} &= \delta[N_c] - k_{C\deg}[C]
\end{aligned} \qquad (3)$$

Where the variables N_C and N_{in} are the same as in the differential system (Eq.1). We see from the differential system (Eq 3), that the cell survival signal, modeled phenomenologically by means of the chemical species *B* and *C*, depends on the number of TNF-TNF-R1 complexes at the cell surface (e.g. N_C), with rate constant parameter β and δ. On the other hand, the apoptotic signal, modeled phenomenologically by means of the chemical species *A*, depends on the number of internalized ligand/receptor complexes (e.g. N_{in}) with rate constantα. The cell survival pathway inhibits the apoptotic one by destroying *A* with rate constant γ[*B*] and ϕ[*C*]. Finally, C, *B* and *A* can be degraded by means of ubiquitination and proteasome cleavage and/or irreversibly inhibited by other molecular species, and these processes are described by the rate constants k_{Cdeg} , k_{Bdeg} and k_{Adeg}, respectively.

NUMERICAL METHODS

Let us consider a differential equation as

$$\frac{dy}{dx} + Py = Q \qquad (4)$$

$\frac{d}{dx}$ can be written as D, replacing in Eq 4 we get

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$$D\,y + P\,y = Q \quad (5)$$

Now, we know the $\boxed{\text{Integarting factor} = e^{\int P\,dx}}$

Multiply Eq. 5 with Integrating factor both sides, we get

$$e^{\int P\,dx}.(D\,y + Py) = e^{\int P\,dx}.Q$$

$$D\,y\,e^{\int P\,dx} + P\,y\,e^{\int P\,dx} = e^{\int P\,dx}.Q$$

$$D\,y\,e^{\int P\,dx} = e^{\int P\,dx}.Q$$

Integrating Both Sides

$$y\,e^{\int P\,dx} = \int Q.e^{\int P\,dx}\,dx + c$$

Finally the solution of equation is

$$\boxed{\mathbf{y = \frac{\int Q.e^{\int P dx}\,dx + c}{e^{\int P dx}}}} \quad (6)$$

Now our main equation is $\frac{d[B]}{dt} + k_{B\deg}[B] = \beta[N_c]$ (7)

If we compare the above equation i.e Eq. 7 with Eq. 5 we get

$y = B; P = k_{B\deg}$; $Q = \beta[N_c]$

Putting all these values in Eq. 6 , we get

$$\boxed{B = \frac{\int \beta N_c.e^{\int k_{B\deg} dt}\,dt + c}{e^{\int k_{B\deg} dt}}} \quad (8)$$

$$B = e^{-k_{B\deg}t}\left[\left(\int \beta N_c.e^{k_{B\deg}t}\,dt\right) + c\right]$$

After solving the above equation we get

$$B = \frac{\beta N_c}{k_{B\deg}} + c.e^{-k_{B\deg}t} \quad (9)$$

Now applying the initial condition i.e. *t* = 0 and *B* = 0; in the Eq 9, we get

$$c = -\frac{\beta N_c}{k_{B\deg}}$$

Now putting the value of *c* in Eq 8, we get

$$\boxed{\mathbf{B = \frac{\beta N_c}{k_{B\deg}}\left(1 - e^{-k_{B\deg}t}\right)}} \quad (10)$$

Similarly, we can solve equations of different marker proteins involved in the cell survival and cell death pathway induced by TNF, EGF and Insulin [20].

Differential equation modeling activity of NF-κB

We have solved the differential equation of NF-κB as

$$\frac{d[B]}{dt}+k_{B\deg}[B]=\beta[N_c]$$

Solution comes out to be

$$[B]=\frac{\beta[N_c]}{k_{B\deg}}\left[1-e^{-k_{B\deg}t}\right] \quad (11)$$

By considering values of β= 0.33 min^{-1} and k_{Bdeg} = 0.033 min^{-1} [21], putting these values in Eq.10 we get

$$[B]=10\left[1-e^{-0.33t}\right] \quad (12)$$

Again by considering the different values of time [22] and solving Eq 12; the values of *B* have been obtained and get a plot between concentration of NF-κB and Time which comes out to be exponential shown in Fig 2.

Differential equation modeling activity of JNK

We have solved the differential equation of JNK as

$$\frac{d[C]}{dt}+k_{C\deg}[C]=\delta[N_c]$$

Solution comes out to be

$$[C]=\frac{\delta[N_c]}{k_{C\deg}}\left[1-e^{-k_{C\deg}t}\right] \quad (13)$$

By assuming the values of $\delta=0.33/\min$; $k_{C\deg}=0.018/\min$ and applying these values in Eq.13 we got

$$\boxed{[C]=18.33\left[1-e^{-0.018t}\right]} \quad (14)$$

Considering the different values of time [22] in Eq. 14 we calculated the corresponding values of C shown in Fig 3.

RESULTS AND DISCUSSIONS

Experimental findings

The cultures were stimulated 24 hr later by adding the stimulus diluted in 1/20 of the culture volume of serum-free medium, for final concentrations of 0, 0.2, 5, or 100 ng/ml TNF (Peprotech), 0, 1, or 100 ng/ml EGF (Peprotech), and 0, 1, 5, or 500 ng/ml insulin (Sigma). Triplicate plates were lysed at 0, 5, 15, 30, 60, and 90 min and 2, 4, 8, 12, 16, 20, and 24 hr or prepared for flow cytometry at 12, 24, and 48 hr. To explore systematically relationships between cytokine-receptor interaction, activation of intracellular signaling cascades, and apoptosis-survival cell-fate decisions, cells were exposed to a set of ten cytokine treatments shown in Table 1 and monitored over a 48-hr period. Each treatment consisted of a combination of TNF and either EGF or insulin Cells respond to TNF, EGF, and insulin in a dose-dependent manner and all three cytokines were therefore examined at sub saturating concentrations, designed to mimic physiological conditions, and at saturating concentrations, at which essentially all receptors were ligand-bound. At 13 time

points after cytokine addition, three replicate dishes of cells (six for the zero time point) were harvested to measure kinase activities, changes in protein phosphorylation, caspase cleavage, and changes in protein abundance. All together, AkT signals were examined. Kinases such as Akt and ERK were maximally active 5-15 min after cytokine addition whereas caspase cleavage was evident only after 4 hr time points were spaced closely from 0-2 hr (0, 5, 15, 30, 60, 90, 120 min) and then more sparsely from 4-24 hr (4, 8, 12, 16, 20, 24 hr), where t = 0 min is the time of cytokine addition taken from [22].

Table 1 : Ten cytokine treatments

	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)	(*f*)	(*g*)	(*h*)	(*i*)	(*j*)
TNF(ng/ml)	-	5	100	-	5	100	-	0.2	5	100
EGF(ng/ml)	-	-	-	100	1	100	-	-	-	-
Insulin(ng/ml)	-	-	-	-	-	-	500	1	5	500

Deterministic Finding

We have presented an integrated theoretical framework for describing the balance between cell survival and cell death regulated by TNF. The theoretical models consider here for mathematical intervention is minimal in the sense it takes into consideration only those reactions that are essential to describe the action of TNF on cell survival and cell death. The results illustrated the rate constants defined in the deterministic models are biologically relevant of key proteins in the signaling pathway which decide the fate of the cell.

Moreover, the rate constant κ parameterizes the triggering kinetics of the survival signal that initiates at the cell membrane level upon binding of TNF to TNF-R1. A complex of adaptor proteins (consisting of RIP-1 and TRAF-2) is formed at the cell surface transduces signal for activation of NF-κB and JNK leading to cell survival. However, the proteins TRADD, FADD and caspase-8 were not recruited; demonstrating the endocytosis and DISC formation are inseparable events. Thus it has been revealed that survival and death signals induced by TNF are temporarily separated and this is reflected in our model by the differences between the values of the parameters used. Simulations based on deterministic model recapitulate most features of the data and generate several predictions involving pathway crosstalk and regulation. We uncover a relationship between the key proteins involved in TNF cellular signalling pathways that might account for the cell survival and cell death decision of the cells. More generally, deterministic models are flexible, able to incorporate qualitative and noisy data, and powerful enough to produce quantitative predictions and new biological insights about the operation of signalling networks.

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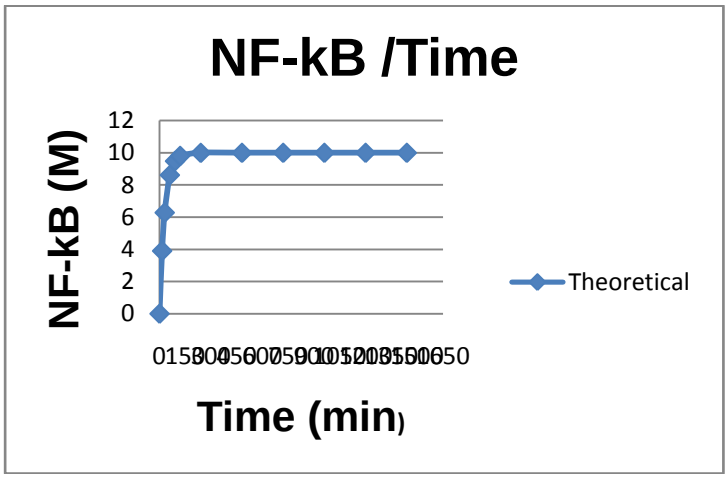

Fig 2: Theoretical result of NF-κB

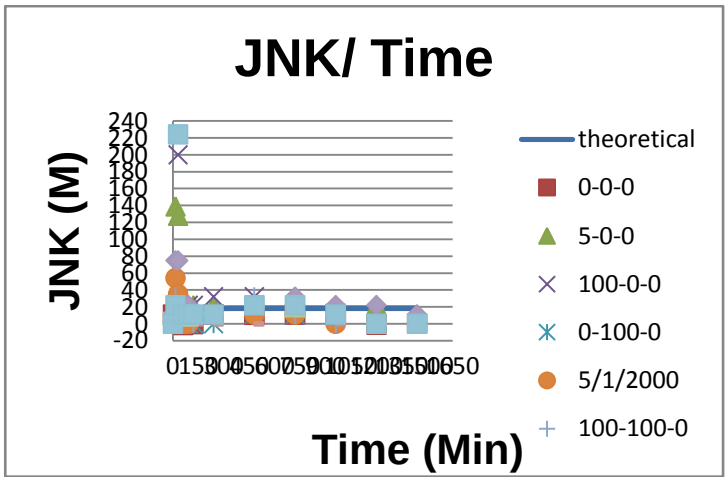

Fig 3: Theoretical and Practical results of JNK

The quality of fit between theoretical and experimental values (ten cytokine combinations) for the proteins such as NFκB and JNK revealed good accuracy of the model shown in Fig 2 and Fig 3.

CONCLUSIONS

We have made the biological view of the main paths of the TNF input showing cell survival and cell death. We have successfully made a deterministic model for TNF which verifies experimental and theoretical data. Thus, our model is suitable for implementation in multi-scale simulation programs that are presently under development to study the behavior of large tumor cell populations.

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REFERENCES

[1] Kitano H. Science 2002; 295, 1662–1664.
[2] Wajant H, Pfizenmaier K, and Scheurich P. Cell Death Differ 2003; 10: 45–65.
[3] Avruch J. Mol Cell Biochem 1998; 182: 31–48.
[4] Xia Z, Dickens M, Raingeaud J, Davis RJ, and Greenberg ME. Science 1995; 270: 1326–1331.
[5] Brent R. Cell 2000; 100: 169–183.
[6] Ideker T, and Lauffenburger D. Trends Biotechnol. 2003; 21: 255–262.
[7] Rives AW, and Galitski T. Proc Natl Acad Sci USA 2003; 100: 1128–1133.
[8] Schoeberl B, Eichler-Jonsson C, Gilles ED, and Muller G. Nat Biotechnol 2002; 20: 370–375.
[9] Lund-Johansen F, Davis K, Bishop J and de Waal Malefyt R. Cytometry 2000; 39: 250–259.
[10] Cagney G, Uetz P and Fields S. Methods Enzymol 2000; 328: 3–14.
[11] Maliakal JC. Methods Enzymol 2002; 352: 175–182.
[12] Burns MA, Johnson BN, Brahmasandra SN, Handique K, Webster JR, et al. Science 1998; 282: 484–487.
[13] Morand KL, Burt TM, Regg BT and Tirey DA. Curr Opin Drug Discov Devel 2001; 4: 729–735.
[14] Gardner TS, Di Bernardo D, Lorenz D and Collins JJ. Science 2003; 301: 102–105.
[15] Price ND, Papin JA, Schilling CH and Palsson BO. Trends Biotechnol 2003; 21:162–169.
[16] Bajzer Z, Myers AC, Vuk-Pavlovic S. J Biol Chem 1989; 264: 13623-13631
[17] Grell M, Wajant H, Zimmermann G, Scheurich P. Proc Natl Acad Sci USA 1998; 95: 570-575.
[18] Vuk-Pavlovic S, Kovach JS. FASEB J 1989; 3: 2633-2640
[19] Schneider-Brachert W, Tchikov V, Neumeyer J, Jakob M, Winoto-Morbach S, et al. Cell 2004; 21: 415-428.
[20] Jain S, Bhooshan SV, Naik PK. Network Biology 2011; 1(1): 46-58.
[21] Chignola Roberto, Farina Marcello , Liberati Diego, Fabbro Alessio Del, Milotti Eduardo. Biological Sciences, Biophysics and Computational Biology 2009; 1-3.
[22] Gaudet Suzanne, Janes Kevin A, Albeck John G, Pace Emily A, Lauffenburger Douglas A, and Sorger Peter K. A compendium of signals and responses trigerred by prodeath and prosurvival cytokines Manuscript 2005; M500158-MCP200.