Bioinformation by Biomedical Informatics Publishing Group *open access*

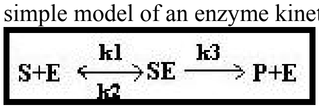
www.bioinformation.net **Hypothesis**

Supplementary material

Methodology:

Mathematical model:

The temporal dynamics of signaling cascades is described by ordinary differential equations, which are known as chemical-kinetics equations. The derivation begins by listing all chemical transformations (Figure S1), thereby providing a kinetic scheme of a kinase cascade. A basic assumption of this approach is that the cell presents a well-stirred biochemical reactor. The following simple model of an enzyme kinetic reaction serves as a subsystem for signaling cascade **[8]**.



Where S refers to the substrate,

E denotes the enzyme, SE is the substrate - enzyme complex and P is the product. Referring to the law of mass action, one arrives at the state – space model

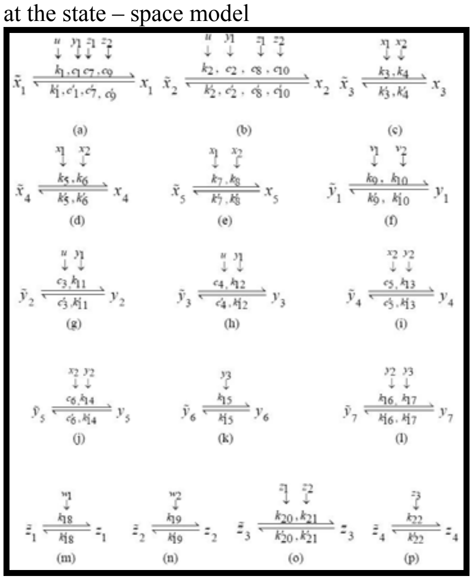
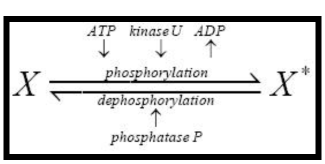


Figure S1: (a) to (p): Reaction schemes of activation (phosphorylation) and inactivation (de-phosphorylation) of all the proteins in ERK, JNK and P38 kinase cascades with cross–talks.

$$\left.\begin{aligned}
\frac{d}{dt}E(t) &= -k_1E(t)S(t)+k_2S(t)E(t)+k_3S(t)E(t)\\
\frac{d}{dt}S(t) &= -k_1E(t)S(t)+k_2S(t)E(t)\\
\frac{d}{dt}S(t)E(t) &= k_1E(t)S(t)-k_2S(t)E(t)-k_3S(t)E(t)\\
\frac{d}{dt}P(t) &= k_3S(t)E(t).
\end{aligned}\right\} \qquad (1)$$

Where k_1, k_2 *and* k_3 are reaction rate constants.



ISSN 0973-2063 (online) 0973-8894 (print)
Bioinformation 3(6): 244-249 (2009)

Bioinformation, an open access forum

Figure S2: Biochemical reaction

Modeling system of ERK5, JNK and P38 kinase cascades with cross-talks

In signaling, activation/inactivation of proteins corresponds to phosphorylation/ dephosphorylation. Denoting the kinase as U, the phosphatase as P and the protein as X, and assuming a constant phosphatase, (**Figure S2**) illustrates a simple biochemical model of a signaling step is given by $\dot{x} = v_1(u,\tilde{x}) - v_2(x),$ where $v_1(.)\, and\, v_2(.)$ are mappings, describing the reaction rates for phophorylation and dephophorylation respectively **[8]**. We write $\tilde{x}$ for the non – phosphorylated form of the protein X, u for the kinase U, and x corresponds to the activated protein X^*. Referring to a power-law representation, one would have $v_1(u,\tilde{x}) = k_1 u^a \tilde{x}^b, v_2(x) = k_2 x^c$ where we choose $a = b = c = 1$, such that $\dot{x} = k_1 u(t)\tilde{x}(t) - k_2 x(t)$. If we assume that the total $\bar{x} = \tilde{x}(t) + x(t)$ is constant for all time t, we require only this one differential equation $\dot{x} = k_1 u(t)(\bar{x}(t) - x(t)) - k_2 x(t)$ to model a signaling step **[9]**. In our model we have assumed Michaelis–Menten kinetics **[10]**.

$$\dot{x} = \underbrace{\frac{k_1 u(t)(\bar{x}(t) - x(t))}{Km1 + (\bar{x}(t) - x(t))}}_{phosphorylation} - \underbrace{\frac{k_2 x(t)}{Km2 + x(t)}}_{dephosphorylation} \quad (2)$$

To model the entire ERK5, JNK and P38 Kinase cascades with cross-talks **(Figure 1)**, we have the following state variables representing the concentration of each protein involved in the system **(Figure 1)**. Let u = ASK1, v_1 = TAB1 (TAK1 (Transforming growth factor-beta-activated kinase 1)binding protein 1), v_2 = TAB2 (TAK1 binding protein 2), w_1 =GAB1(Grb2-associated binder 1), w_2 =LAD(Lck-associated adapter), $x_1(t)$ =MKK7, $x_2(t)$ =MKK4, $x_3(t)$ =JNK1, $x_4(t)$ =JNK2, $x_5(t)$ =JNK3, $y_1(t)$ =TAK1, $y_2(t)$ =MKK3, y_3 (t)=MKK6, $y_4(t)$ = P38 α, $y_5(t)$ = P38 β, $y_6(t)$ = P38 γ, $y_7(t)$ = P38 δ, $z_1(t)$ =MEKK3, $z_2(t)$ =MEKK2, $z_3(t)$ =MKK5, and $z_4(t)$ =ERK5. Also, let $\{x_i(t)\}_{i=1to5}$, $\{y_j(t)\}_{j=1to7}$ and $\{z_k(t)\}_{k=1to4}$ are the activated (phosphorylated) form of the proteins and the tilted ones are corresponds to the inactivated (non–phosphorylated) in JNK and P38 and ERK5 Kinase cascades respectively. We assume that the total concentrations $\{\bar{x}_i = \tilde{x}_i(t) + x_i(t)\}_{i=1to5}, \{\bar{y}_j = \tilde{y}_j(t) + y_j(t)\}_{j=1to7}\ and\ \{\bar{z}_k = \tilde{z}_k(t) + z_k(t)\}_{k=1to4}$ are constant for all t.

Based on the reaction schemes described in (Figure 3(a) - 3(p)), a set of differential equations (3a)–(3p) have been developed to form the dynamic system, to analyse the impact of cross-talks between the ERK5, JNK and P38 Kinase cascades **(Figure 1).**

$$\frac{dx_1(t)}{dt} = \frac{k_1 u(\bar{x}_1 - x_1(t))}{Km_1 + (\bar{x}_1 - x_1(t))} + \frac{c_1 y_1(t)(\bar{x}_1 - x_1(t))}{Cm_1 + (\bar{x}_1 - x_1(t))} + \frac{c_7 z_1(t)(\bar{x}_1 - x_1(t))}{Cm_7 + (\bar{x}_1 - x_1(t))} + \frac{c_9 z_2(t)(\bar{x}_1 - x_1(t))}{Cm_9 + (\bar{x}_1 - x_1(t))} - \frac{k_1' x_1(t)}{K'm_1 + x_1(t)} - \frac{c_1' x_1(t)}{C'm_1 + x_1(t)} - \frac{c_7' x_1(t)}{C'm_7 + x_1(t)} - \frac{c_9' x_1(t)}{C'm_9 + x_1(t)} \quad (3a)$$

$$\frac{dx_2(t)}{dt} = \frac{k_2 u(\bar{x}_2 - x_2(t))}{Km_2 + (\bar{x}_2 - x_2(t))} + \frac{c_2 y_1(t)(\bar{x}_2 - x_2(t))}{Cm_2 + (\bar{x}_2 - x_2(t))} + \frac{c_8 z_1(t)(\bar{x}_2 - x_2(t))}{Cm_8 + (\bar{x}_2 - x_2(t))} + \frac{c_{10} z_2(t)(\bar{x}_2 - x_2(t))}{Cm_{10} + (\bar{x}_2 - x_2(t))} - \frac{k_2' x_2(t)}{K'm_2 + x_2(t)} - \frac{c_2' x_2(t)}{C'm_2 + x_2(t)} - \frac{c_8' x_2(t)}{C'm_8 + x_2(t)} - \frac{c_{10}' x_2(t)}{C'm_{10} + x_2(t)} \quad (3b)$$

$$\frac{dx_3(t)}{dt} = \frac{k_3 x_1(t)(\bar{x}_3 - x_3(t))}{Km_3 + (\bar{x}_3 - x_3(t))} + \frac{k_4 x_2(t)(\bar{x}_3 - x_3(t))}{Km_4 + (\bar{x}_3 - x_3(t))} - \frac{k_3' x_3(t)}{K'm_3 + x_3(t)} - \frac{k_4' x_3(t)}{K'm_4 + x_3(t)} \quad (3c)$$

$$\frac{dx_4(t)}{dt} = \frac{k_5 x_1(t)(\bar{x}_4 - x_4(t))}{Km_5 + (\bar{x}_4 - x_4(t))} + \frac{k_6 x_2(t)(\bar{x}_4 - x_4(t))}{Km_6 + (\bar{x}_4 - x_4(t))} - \frac{k_5' x_4(t)}{K'm_5 + x_4(t)} - \frac{k_6' x_4(t)}{K'm_6 + x_4(t)} \quad (3d)$$

$$\frac{dx_5(t)}{dt} = \frac{k_7 x_1(t)(\bar{x}_5 - x_5(t))}{Km_7 + (\bar{x}_5 - x_5(t))} + \frac{k_8 x_2(t)(\bar{x}_5 - x_5(t))}{Km_8 + (\bar{x}_5 - x_5(t))} - \frac{k_7' x_5(t)}{K'm_7 + x_5(t)} - \frac{k_8' x_5(t)}{K'm_8 + x_5(t)} \quad (3e)$$

ISSN 0973-2063 (online) 0973-8894 (print)
Bioinformation 3(6): 244-249 (2009)

$$\frac{dy_1(t)}{dt} = \frac{k_9 v_1(\bar{y}_1 - y_1(t))}{Km_9 + (\bar{y}_1 - y_1(t))} + \frac{k_{10} v_2(\bar{y}_1 - y_1(t))}{Km_{10} + (\bar{y}_1 - y_1(t))} - \frac{k'_9 y_1(t)}{K'm_9 + y_1(t)} - \frac{k'_{10} y_1(t)}{K'm_{10} + y_1(t)} \quad (3f)$$

$$\frac{dy_2(t)}{dt} = \frac{c_3 u(\bar{y}_2 - y_2(t))}{Cm_3 + (\bar{y}_2 - y_2(t))} + \frac{k_{11} y_1(t)(\bar{y}_2 - y_2(t))}{Km_{11} + (\bar{y}_2 - y_2(t))} - \frac{c'_3 y_2(t)}{C'm_3 + y_2(t)} - \frac{k'_{11} y_2(t)}{K'm_{11} + y_2(t)} \quad (3g)$$

$$\frac{dy_3(t)}{dt} = \frac{c_4 u(\bar{y}_3 - y_3(t))}{Cm_4 + (\bar{y}_3 - y_3(t))} + \frac{k_{12} y_1(t)(\bar{y}_3 - y_3(t))}{Km_{12} + (\bar{y}_3 - y_3(t))} - \frac{c'_4 y_3(t)}{C'm_4 + y_3(t)} - \frac{k'_{12} y_3(t)}{K'm_{12} + y_3(t)} \quad (3h)$$

$$\frac{dy_4(t)}{dt} = \frac{c_5 x_2(t)(\bar{y}_4 - y_4(t))}{Cm_5 + (\bar{y}_4 - y_4(t))} + \frac{k_{13} y_2(t)(\bar{y}_4 - y_4(t))}{Km_{13} + (\bar{y}_4 - y_4(t))} - \frac{c'_5 y_4(t)}{C'm_5 + y_4(t)} - \frac{k'_{13} y_4(t)}{K'm_{13} + y_4(t)} \quad (3i)$$

$$\frac{dy_5(t)}{dt} = \frac{c_6 x_2(t)(\bar{y}_5 - y_5(t))}{Cm_6 + (\bar{y}_5 - y_5(t))} + \frac{k_{14} y_2(t)(\bar{y}_5 - y_5(t))}{Km_{14} + (\bar{y}_5 - y_5(t))} - \frac{c'_6 y_5(t)}{C'm_6 + y_5(t)} - \frac{k'_{14} y_5(t)}{K'm_{14} + y_5(t)} \quad (3j)$$

$$\frac{dy_6(t)}{dt} = \frac{k_{15} y_3(t)(\bar{y}_6 - y_6(t))}{Km_{15} + (\bar{y}_6 - y_6(t))} - \frac{k'_{15} y_6(t)}{K'm_{15} + y_6(t)} \quad (3k)$$

$$\frac{dy_7(t)}{dt} = \frac{k_{16} y_2(t)(\bar{y}_7 - y_7(t))}{Km_{16} + (\bar{y}_7 - y_7(t))} + \frac{k_{17} y_3(t)(\bar{y}_7 - y_7(t))}{Km_{17} + (\bar{y}_7 - y_7(t))} - \frac{k'_{16} y_7(t)}{K'm_{16} + y_7(t)} - \frac{k'_{17} y_7(t)}{K'm_{17} + y_7(t)} \quad (3l)$$

$$\frac{dz_1(t)}{dt} = \frac{k_{18} w_1(\bar{z}_1 - z_1(t))}{Km_{18} + (\bar{z}_1 - z_1(t))} - \frac{k'_{18} z_1(t)}{K'm_{18} + z_1(t)} \quad (3m)$$

$$\frac{dz_2(t)}{dt} = \frac{k_{19} w_2(\bar{z}_2 - z_2(t))}{Km_{19} + (\bar{z}_2 - z_2(t))} - \frac{k'_{19} z_2(t)}{K'm_{19} + z_2(t)} \quad (3n)$$

$$\frac{dz_3(t)}{dt} = \frac{k_{20} z_1(t)(\bar{z}_3 - z_3(t))}{Km_{20} + (\bar{z}_3 - z_3(t))} + \frac{k_{21} z_2(t)(\bar{z}_3 - z_3(t))}{Km_{21} + (\bar{z}_3 - z_3(t))} - \frac{k'_{20} z_3(t)}{K'm_{20} + z_3(t)} - \frac{k'_{21} z_3(t)}{K'm_{21} + z_3(t)} \quad (3o)$$

$$\frac{dz_4(t)}{dt} = \frac{k_{22} z_3(t)(\bar{z}_4 - z_4(t))}{Km_{22} + (\bar{z}_4 - z_4(t))} - \frac{k'_{22} z_4(t)}{K'm_{22} + z_4(t)} \quad (3p)$$

Where $k_i, k'_i; i = 1 to 22 \; and \; c_j, c'_j; j = 1 to 10$ are reaction rate constants and $Km_i, K'm_i; i = 1 to 22 \; and \; Cm_j, C'm_j; j = 1 to 10$ are Michaelis-Menten's constants. The parameter values are given in (Table 1 under supplementary material).

Equation no: Parameter values	Equation no: Parameter values

(3a)
k1 =2.5; rk1 =0.025; km1=15 ;rkm1 = 15;c1 = 1.05;rc1 = .05;
cm1 = 15;rcm1 = 15; c7 = 2.05;rc7 = 1.05;cm7 =15;rcm7 =15;
c9 = 0.07 ;rc9 = 1.15; cm9 = 15;rcm9 = 15;u =100 ;$\bar{x}_1$ =300;
(3b)
k2 =0.025; rk2 =0.75;km2 = 15; rkm2 = 15; c2 = 0.03; rc2 = 2.05;
cm2 = 15;rcm2 = 15; c8 = 1.03; rc8 = 2.05; cm8 = 15; rcm8 = 15;
c10 = 2.9; rc10 = 1.05; cm10 = 15; rcm10 = 15; $\bar{x}_2$ =300;
(3c)
k3 =0.025; rk3 = 0.5; km3 = 15; rkm3 = 15;k4 = 0.5; rk4 = 0.5;
km4 = 15; rkm4 = 15; $\bar{x}_3$ = 300;
(3d)
k5 = 1.25; rk5 = 0.015; km5 = 15; rkm5 = 15; k6 = 2.25; rk6 = 0.019;
km6 = 15; rkm6 = 15; $\bar{x}_4$ = 300;
(3e)
k7 = 2.25; rk7= 0.15;km7 = 15; rkm7 = 15;k8 = 0.025; rk8 = 1.05;
km8 = 15; rkm8 = 15;$\bar{x}_5$ = 300;
(3f)
	(3i)
	k13 = 0.06; rk13 = 2.6; km13 =15; rkm13 = 15; c5 = 0.05; rc5 = 1.5;
	cm5 = 15; rcm5 = 15; $\bar{y}_4$=300;
	(3j)
	k14 = 0.02; rk14 = 2.01;km14 = 15; rkm14=15; c6 = 2.5; rc6 = 1.05;
	cm6 = 15; rcm6 = 15; $\bar{y}_5$=300;
	(3k)
	k15 = 1.03; rk15 =0.09; km15 = 15; rkm15 = 15; $\bar{y}_6$=300;
	(3l)
	k16 = 2.03; rk16 =0.05; rkm16 = 15; cm6 = 15; k17 = 1.03; rk17 = 0.03;
	km17 = 15 ;rkm17 = 15;$\bar{y}_7$=300;
	(3m)
	k18 =1.75 ;rk18 =1.025 ;km18=10;rkm18 =8;w1=1;$\bar{z}_1$=100;
	(3n)
	k19 =2.025 ;rk19 =0.75 ;km19 = 10; rkm19 = 8; w2=1;$\bar{z}_2$=100;
	(3o)
	k20 = 1.025; rk20 =1.5; km20 = 15; rkm20 = 15; k21 = 1.5;rk21 = 0.5;
	km21 = 15; rkm21 =15; $\bar{z}_3$=300;

ISSN 0973-2063 (online) 0973-8894 (print)
Bioinformation 3(6): 244-249 (2009)

k9 =2.5; rk9 =0.05; km9 =10; rkm9 = 8; k10 = 0.05; rk10 = 1.5; km10 = 10; rkm10 = 8; v1=1; v2 = 1; $\overline{y}_1$ =100; **(3g)** k11 =2.5; rk11 =0.075; km11 = 15; rkm11 = 15; c3 = 1.9; rc3 = 1.05; cm3 = 15; rcm3 = 15; $\overline{y}_2$ = 300; **(3h)** k12 = 0.095; rk12 = 1.9; km12 = 15; rkm12 = 15; c4 = 2.5; rc4 = 1.5; cm4 = 15; rcm4 = 15; $\overline{y}_3$=300;	**(3p)** k22 = 0.25; rk22 = 1.015; km22 =15; rkm22 = 15; $\overline{z}_4$=300;

Table 1: Parameter values, molar concentrations are in nM and the reaction rate constants are in nM / s.

ISSN 0973-2063 (online) 0973-8894 (print)
Bioinformation 3(6): 244-249 (2009)