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Supplemental Material to:

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How MAP kinase modules function as robust, yet adaptable, circuits

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Supplementary Information

This supplementary information will give detailed description of the assumptions of the mathematical models, chemical reactions and systems of differential equations regarding different numbers of the tiered structure and different mechanisms of kinase activation deactivation.

Section 1. The 3T P-P-D MAP kinase module

In this MAPK module, it is assumed that the upstream signal (Ras-GTP loading) activates MAP kinase kinase kinase (M3K). The activated M3K then subsequently activates MAP kinase kinase (M2K) which in return activates MAP kinase (M1K). The activated M1K is the signal output of the MAPK module. Simultaneously the phosphatases of different kinases, namely PM3K for M3K, PM2K for M2K, and PM1K for M1K, deactivate the activated kinases by dephosphorylation reactions.

Chemical reactions in the 3-tiered MAPK module include: the upstream input signal (UIS) activates M3K in a single step (reaction 1.1); activated M3K (denoted as M3Kp) is dephosphorylated by phosphatase PM3K (reaction 1.2); Although the M2K is activated in two steps, the activation is very quick and recent study has established that the activation of M2K is processive (reaction 1.3). However, the deactivation of activated M2Kpp by phosphatase PM2K is a process of two steps (reactions 1.4 and 1.5). The activation of M1K is distributive via reaction (1.6) and (1.7) and the activated M1Kpp is dephosphorylated by phosphatase PM1K in two steps (reactions 1.8 and 1.9).

According to the mechanisms of kinase phosphorylation, this module is termed as 3T P-P-D module.

$$\text{UIS} + \text{M3K} \underset{d1}{\overset{a1}{\rightleftarrows}} \text{UIS-M3K} \xrightarrow{k1} \text{UIS} + \text{M3Kp} \tag{1.1}$$

$$\text{M3Kp} + \text{PM3K} \underset{d2}{\overset{a2}{\rightleftarrows}} \text{M3Kp-PM3K} \xrightarrow{k2} \text{M3K} + \text{PM3K} \tag{1.2}$$

$$\text{M3Kp} + \text{M2K} \underset{d3}{\overset{a3}{\rightleftarrows}} \text{M3Kp-M2K} \xrightarrow{k3} \text{M3Kp} + \text{M2Kpp} \tag{1.3}$$

$$\text{M2Kp} + \text{PM2K} \underset{d4}{\overset{a4}{\rightleftarrows}} \text{M2Kp-PM2K} \xrightarrow{k4} \text{M2K} + \text{PM2K} \tag{1.4}$$

$$\text{M2Kpp} + \text{PM2K} \underset{d5}{\overset{a5}{\rightleftarrows}} \text{M2Kpp-PM2K} \xrightarrow{k5} \text{M2Kp} + \text{PM2K} \tag{1.5}$$

$$\text{M2Kpp} + \text{M1K} \underset{d6}{\overset{a6}{\rightleftarrows}} \text{M2Kpp-M1K} \xrightarrow{k6} \text{M2Kpp} + \text{M1Kp} \tag{1.6}$$

$$\text{M2Kpp} + \text{M1Kp} \underset{d7}{\overset{a7}{\rightleftarrows}} \text{M2Kpp-M1Kp} \xrightarrow{k7} \text{M2Kpp} + \text{M1Kpp} \tag{1.7}$$

$$\text{M1Kp} + \text{PM1K} \underset{d8}{\overset{a8}{\rightleftarrows}} \text{M1Kp-PM1K} \xrightarrow{k8} \text{M1K} + \text{PM1K} \tag{1.8}$$

$$\text{M1Kpp} + \text{PM1K} \underset{d9}{\overset{a9}{\rightleftarrows}} \text{M1Kpp-PM1K} \xrightarrow{k9} \text{M1Kp} + \text{PM1K} \tag{1.9}$$

A system of 21 differential equations was developed based on the chemical reaction (1.1~1.9), given below

$$\frac{d[UIS]}{dt} = -a_1[UIS][M3K] + (d_1 + k_1)[UIS - M3K]$$

$$\frac{d[M3K]}{dt} = -a_1[UIS][M3K] + d_1[UIS - M3K] + k_2[M3Kp - PM3K]$$

$$\frac{d[UIS - M3K]}{dt} = a_1[UIS][M3K] - (d_1 + k_1)[UIS - M3K]$$

$$\frac{d[M3Kp]}{dt} = -a_2[M3Kp][PM3K] + d_2[M3Kp - PM3K] + k_1[UIS - M3K]$$
$$- a_3[M3Kp][M2K] + (d_3 + k_3)[M3Kp - M2K]$$

$$\frac{d[PM3K]}{dt} = -a_2[M3Kp][PM3K] + (d_2 + k_2)[M3Kp - PM3K]$$

$$\frac{d[M3Kp - PM3K]}{dt} = a_2[M3Kp][PM3K] - (d_2 + k_2)[M3Kp - PM3K]$$

$$\frac{d[M2K]}{dt} = -a_3[M3Kp][M2K] + d_3[M3Kp - M2K] + k_4[M2Kp - PM2K]$$

$$\frac{d[M3Kp - M2K]}{dt} = a_3[M3Kp][M2K] - d_3[M3Kp - M2K]$$

$$\frac{d[M2Kp]}{dt} = -a_4[M2Kp][PM2K] + d_4[M2Kp - PM2K] + k_5[M2Kpp - PM2K]$$

$$\frac{d[M2Kpp]}{dt} = -a_5[M2Kpp][PM2K] + d_5[M2Kpp - PM2K] + k_3[M3Kp - M2K]$$
$$- a_6[M2Kpp][M1K] + (d_6 + k_6)[M2Kpp - M1K] - a_7[M2Kpp][M1Kp] + (d_7 + k_7)[M2Kpp - M1Kp]$$

$$\frac{d[PM2K]}{dt} = -a_4[M2Kp][PM2K] + (d_4 + k_4)[M2Kp - PM2K]$$
$$- a_5[M2Kpp][PM2K] + (d_5 + k_5)[M2Kpp - PM2K]$$

$$\frac{d[M2Kp - PM2K]}{dt} = a_4[M2Kp][PM2K] - (d_4 + k_4)[M2Kp - PM2K]$$

$$\frac{d[M2Kpp - PM2K]}{dt} = -a_5[M2Kpp][PM2K] + (d_5 + k_5)[M2Kpp - PM2K]$$

$$\frac{d[M1K]}{dt} = -a_6[M2Kpp][M1K] + d_6[M2Kpp - M1K] + k_8[M1Kp - PM1K]$$

$$\frac{d[M2Kpp - M1K]}{dt} = a_6[M2Kpp][M1K] - (d_6 + k_6)[M2Kpp - M1K]$$

$$\frac{d[M1Kp]}{dt} = -a_7[M2Kpp][M1Kp] + d_7[M2Kpp - M1Kp] + k_6[M2Kpp - M1K]$$
$$- a_8[M1Kp][PM1K] + d_8[M1Kp - PM1K] + k_7[M1Kpp - PM1K]$$

$$\frac{d[M2Kpp - M1Kp]}{dt} = a_7[M2Kpp][M1Kp] - (d_7 + k_7)[M2Kpp - M1Kp]$$

$$\frac{d[M1Kpp]}{dt} = -a_9[M1Kpp][PM1K] + d_9[M1Kpp - PM1K] + k_7[M2Kpp - M1Kp]$$

$$\frac{d[PM1K]}{dt} = -a_8[M1Kp][PM1K] + (d_8 + k_8)[M1Kp - PM1K] - a_9[M1Kpp][PM1K] + (d_9 + k_9)[M1Kpp - PM1K]$$

$$\frac{d[M1Kp - PM1K]}{dt} = a_8[M1Kp][PM1K] - (d_8 + k_8)[M1Kp - PM1K]$$

$$\frac{d[M1Kpp - PM1K]}{dt} = -a_9[M1Kpp][PM1K] + (d_9 + k_9)[M1Kpp - PM1K]$$

The development of the above kinetic model from chemical reactions (1.1~1.9) is straightforward. Thus in the following sections, we will only list all the chemical reactions and do not provide the systems of differential equations.

We used the experimentally determined kinetic rates and protein concentrations {Huang and Ferrell PNAS} to simulate the proposed mathematical model. Here $a_i = 1000,\ d_i = 150,\ k_i = 150,$ $(i = 1,...,9).$ The total protein concentrations are [UIS] (Upstream input signal, Ras)=0.13 μM,

[M3K]=0.003 μM, [M2K]=1.2 μM, [M1K]=1.2 μM, [PM3K]=0.0003 μM, [PM2K]=0.0003 μM, [PM1K]=0.12 μM. The rate constants and concentration of each kinase or phosphatase remain the same in the following models of other MAPK modules.

Figure 1 gives the simulated signal output stimulated by different strengths of the upstream signal input. The signal output was approximated by a Hill function

$$[Output] = a\frac{[UIS]^H}{K^H + [UIS]^H}$$

where H is the Hill coefficient and K is the disassociate constant. We use the Hill coefficient to characterize the relationship between the signal input and output. The Hill coefficient measures the speed of a system from the basal signal output to reach the maximal signal output; while the disassociate constant is an indicator of the systems sensitivity to the upstream input signal. When [UIS]=K, the signal output is a half of the maximal signal output of the module.

For the 3T P-P-D MAPK module, Figure 2 suggested that the Hill coefficient of the signal output is 2.1.

$$[Output] = 1.04932\frac{[USI]^{2.1}}{0.0000015^{2.1} + [USI]^{2.1}}$$

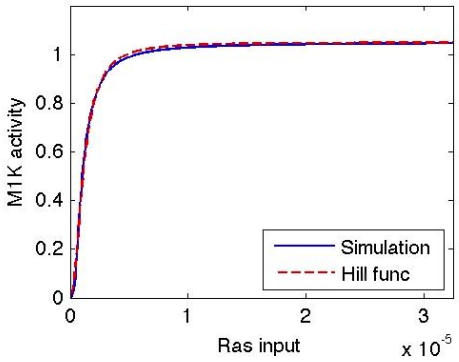

Fig. 1 Simulated MIK signal output of the 3T P-P-D MAPK module based on different strength of the upstream signal input. The signal output can be approximated by a Hill function with coefficient H=2.1.

Section 2. The 3T P-D-D MAP kinase module

Chemical reactions in the 3-tiered MAPK module include: the upstream input signal (UIS) activates M3K in a single step (reaction 2.1); activated M3K (denoted as M3Kp) is dephosphorylated by phosphatase PM3K (reaction 2.2); The M2K is activated distributively in two steps (reaction 2.3 and 2.4). In addition, the deactivation of activated M2Kpp by phosphatase PM2K is a process of two steps (reactions 2.5 and 2.6). The activation of M1K is distributive via reaction (2.7) and (2.8) and the activated M1Kpp is dephosphorylated by phosphatase PM1K in two steps (reactions 2.9 and 2.10).

According to the mechanisms of kinase phosphorylation, this module is termed as 3T P-P-D module.

$$\text{UIS} + \text{M3K} \underset{d1}{\overset{a1}{\rightleftarrows}} \text{UIS-M3K} \xrightarrow{k1} \text{UIS} + \text{M3Kp} \qquad (2.1)$$

$$\text{M3Kp} + \text{PM3K} \underset{d2}{\overset{a2}{\rightleftarrows}} \text{M3Kp-PM3K} \xrightarrow{k2} \text{M3K} + \text{PM3K} \qquad (2.2)$$

$$\text{M3Kp} + \text{M2K} \underset{d3}{\overset{a3}{\rightleftarrows}} \text{M3Kp-M2K} \xrightarrow{k3} \text{M3Kp} + \text{M2Kp} \qquad (2.3)$$

$$\text{M3Kp} + \text{M2Kp} \underset{d4}{\overset{a4}{\rightleftarrows}} \text{M3Kp-M2Kp} \xrightarrow{k4} \text{M3Kp} + \text{M2Kpp} \qquad (2.4)$$

$$\text{M2Kp + PM2K} \underset{d5}{\overset{a5}{\rightleftarrows}} \text{M2Kp-PM2K} \xrightarrow{k5} \text{M2K + PM2K} \quad (2.5)$$

$$\text{M2Kpp + PM2K} \underset{d6}{\overset{a6}{\rightleftarrows}} \text{M2Kpp-PM2K} \xrightarrow{k6} \text{M2Kp + PM2K} \quad (2.6)$$

$$\text{M2Kpp + M1K} \underset{d7}{\overset{a7}{\rightleftarrows}} \text{M2Kpp-M1K} \xrightarrow{k7} \text{M2Kpp + M1Kp} \quad (2.7)$$

$$\text{M2Kpp + M1Kp} \underset{d8}{\overset{a8}{\rightleftarrows}} \text{M2Kpp-M1Kp} \xrightarrow{k8} \text{M2Kpp + M1Kpp} \quad (2.8)$$

$$\text{M1Kp + PM1K} \underset{d9}{\overset{a9}{\rightleftarrows}} \text{M1Kp-PM1K} \xrightarrow{k9} \text{M1K + PM1K} \quad (2.9)$$

$$\text{M1Kpp + PM1K} \underset{d10}{\overset{a10}{\rightleftarrows}} \text{M1Kpp-PM1K} \xrightarrow{k10} \text{M1Kp + PM1K} \quad (2.10)$$

We used the experimentally determined kinetic rates and protein concentrations {Huang and Ferrell PNAS} to simulate the proposed mathematical model. Here $a_i = 1000,\ d_i = 150,\ k_i = 150,$ $(i = 1,...,9)$. The total protein concentrations are [UIS] (Upstream input signal, Ras)=0.13 μM, [M3K]=0.003 μM, [M2K]=1.2 μM, [M1K]=1.2 μM, [PM3K]=0.0003 μM, [PM2K]=0.0003 μM, [PM1K]=0.12 μM. The rate constants and concentration of each kinase or phosphatase remain the same in the following models of other MAPK modules.

For the 3T P-D-D MAPK module, Figure 2 suggested that the Hill coefficient of the signal output is 4.1.

$$[Output] = 1.07598 \frac{[USI]^{4.1}}{0.0000042^{4.1} + [USI]^{4.1}}$$

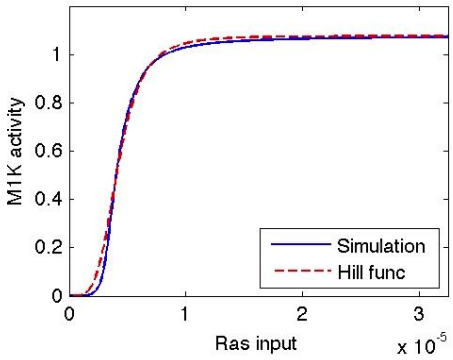

Fig. 2 Simulated MIK signal output of the 3T P-D-D MAPK module based on different strength of the upstream signal input. The signal output can be approximated by a Hill function with coefficient H=4.1.

Section 3. The 2-tiered MAPK modules

In the proposed 2-tiered MAPK module, it is assumed that the upstream signal will activate MAP kinase kinase (M2K) which in return activates MAP kinase (M1K). The activated M1K is the signal output of the MAPK module. Simultaneously the phosphatases of different kinases, namely PM2K for M2K, and PM1K for M1K, will deactivate the activated kinases by dephosphorylation reactions.

Based on the activation processes of kinase M2K and M1K, we will discuss three different modules. They are the 2T P-P module in which both M2K and M1K are activated processively; the 2T P-D module in which M2K is activated processively while M1K is activated distributively; and the 2T D-D module in which both M2K and M1K are activated distributively. In any one of these

modules, the activated kinases, namely M2Kpp and M1Kpp, are dephosphorylated in a process of two steps.

3.1 Reactions in the 2T P-P module

$$\text{UIS} + \text{M2K} \underset{d1}{\overset{a1}{\rightleftarrows}} \text{UIS-M2K} \xrightarrow{k1} \text{UIS} + \text{M2Kpp} \tag{3.1}$$

$$\text{M2Kp} + \text{PM2K} \underset{d2}{\overset{a2}{\rightleftarrows}} \text{M2Kp-PM2K} \xrightarrow{k2} \text{M2K} + \text{PM2K} \tag{3.2}$$

$$\text{M2Kpp} + \text{PM2K} \underset{d3}{\overset{a3}{\rightleftarrows}} \text{M2Kpp-PM2K} \xrightarrow{k3} \text{M2Kp} + \text{PM2K} \tag{3.3}$$

$$\text{M2Kpp} + \text{M1K} \underset{d4}{\overset{a4}{\rightleftarrows}} \text{M2Kpp-M1K} \xrightarrow{k4} \text{M2Kpp} + \text{M1Kpp} \tag{3.4}$$

$$\text{M1Kp} + \text{PM1K} \underset{d5}{\overset{a5}{\rightleftarrows}} \text{M1Kp-PM1K} \xrightarrow{k5} \text{M1K} + \text{PM1K} \tag{3.5}$$

$$\text{M1Kpp} + \text{PM1K} \underset{d6}{\overset{a6}{\rightleftarrows}} \text{M1Kpp-PM1K} \xrightarrow{k6} \text{M1Kp} + \text{PM1K} \tag{3.6}$$

3.2 Reactions in the 2T P-D module

$$\text{UIS} + \text{M2K} \underset{d1}{\overset{a1}{\rightleftarrows}} \text{UIS-M2K} \xrightarrow{k1} \text{UIS} + \text{M2Kpp} \tag{3.7}$$

$$\text{M2Kp} + \text{PM2K} \underset{d2}{\overset{a2}{\rightleftarrows}} \text{M2Kp-PM2K} \xrightarrow{k2} \text{M2K} + \text{PM2K} \tag{3.8}$$

$$\text{M2Kpp} + \text{PM2K} \underset{d3}{\overset{a3}{\rightleftarrows}} \text{M2Kpp-PM2K} \xrightarrow{k3} \text{M2Kp} + \text{PM2K} \tag{3.9}$$

$$\text{M2Kpp} + \text{M1K} \underset{d4}{\overset{a4}{\rightleftarrows}} \text{M2Kpp-M1K} \xrightarrow{k4} \text{M2Kpp} + \text{M1Kp} \tag{3.10}$$

$$\text{M2Kpp} + \text{M1Kp} \underset{d5}{\overset{a5}{\rightleftarrows}} \text{M2Kpp-M1Kp} \xrightarrow{k5} \text{M2Kpp} + \text{M1Kpp} \tag{3.11}$$

$$\text{M1Kp} + \text{PM1K} \underset{d6}{\overset{a6}{\rightleftarrows}} \text{M1Kp-PM1K} \xrightarrow{k6} \text{M1K} + \text{PM1K} \tag{3.13}$$

$$\text{M1Kpp} + \text{PM1K} \underset{d7}{\overset{a7}{\rightleftarrows}} \text{M1Kpp-PM1K} \xrightarrow{k7} \text{M1Kp} + \text{PM1K} \tag{3.14}$$

3.3 Reactions in the 2T D-D module

$$\text{UIS} + \text{M2K} \underset{d1}{\overset{a1}{\rightleftarrows}} \text{UIS-M2K} \xrightarrow{k1} \text{UIS} + \text{M2Kpp} \tag{3.15}$$

$$\text{UIS} + \text{M2K} \underset{d2}{\overset{a2}{\rightleftarrows}} \text{UIS-M2K} \xrightarrow{k2} \text{UIS} + \text{M2Kpp} \tag{3.16}$$

$$\text{M2Kp} + \text{PM2K} \underset{d3}{\overset{a3}{\rightleftarrows}} \text{M2Kp-PM2K} \xrightarrow{k3} \text{M2K} + \text{PM2K} \tag{3.17}$$

$$\text{M2Kpp} + \text{PM2K} \underset{d4}{\overset{a4}{\rightleftarrows}} \text{M2Kpp-PM2K} \xrightarrow{k4} \text{M2Kp} + \text{PM2K} \tag{3.18}$$

$$\text{M2Kpp + M1K} \underset{d5}{\overset{a5}{\rightleftarrows}} \text{M2Kpp-M1K} \xrightarrow{k5} \text{M2Kpp + M1Kp} \quad (3.19)$$

$$\text{M2Kpp + M1Kp} \underset{d6}{\overset{a6}{\rightleftarrows}} \text{M2Kpp-M1Kp} \xrightarrow{k6} \text{M2Kpp + M1Kpp} \quad (3.20)$$

$$\text{M1Kp + PM1K} \underset{d7}{\overset{a7}{\rightleftarrows}} \text{M1Kp-PM1K} \xrightarrow{k7} \text{M1K + PM1K} \quad (3.21)$$

$$\text{M1Kpp + PM1K} \underset{d8}{\overset{a8}{\rightleftarrows}} \text{M1Kpp-PM1K} \xrightarrow{k8} \text{M1Kp + PM1K} \quad (3.22)$$

The kinetic rates and protein concentrations are the same as the corresponding values in the 3-tiered MAPK module in Section 1.

Figure 3 gives the simulated signal output stimulated by different strengths of the upstream signal input for the three 2-tiered modules. The signal output of each module can be approximated by a Hill function, given by

2T P-P module: $[Output] = 0.5645 \dfrac{[USI]^{2.02}}{0.0000231^{2.02} + [USI]^{2.02}}$

2T P-D module: $[Output] = 1.0523 \dfrac{[USI]^{2.5}}{0.0000231^{2.5} + [USI]^{2.5}}$

2T D-D module: $[Output] = 1.0794 \dfrac{[USI]^{5.4}}{0.000175^{5.4} + [USI]^{5.4}}$

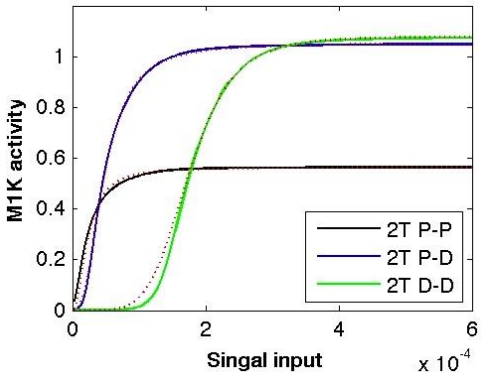

Fig. 3 Simulated MIK signal output of the 2-tiered MAPK modules based on different strength of the upstream signal input. The signal output of the 2T P-P module, 2T P-D module and 2T D-D module can be approximated by a Hill function with coefficient H=2.02, H=2.5, H=5.4, respectively.

Section 4. The 4-tiered MAP kinase model

Next we consider the MAPK module with 4 tiers by adding a kinase protein into the system. The system thus includes 4 kinase proteins, namely M4K, M3K, M2K and M1K, and the corresponding 4 phosphatase proteins, namely PM4K, PM3K, PM2K and PM1K. There are a large number of different combinations of the phosphorylation/dephosphorylation mechanisms of these 4 kinase proteins. For simplicity we only consider the possible modules that were expanded from the 3-tiered MAPK module in Sections 1 and 2. Kinases M4K, M3K and M1K in the 4-tiered modules are kinases M3K, M2K and M1K in the 3-tiered modules, respectively. Kinase M2K is an inserted kinase to the existing 3T MAPK modules. Based on different inserted kinase and different phosphorylation mechanism, this work discussed the following 4 modules

4.1 The 4T P-P-P-D module. In this module the added kinase M2K is the M2K kinase in the 3T MAPK module. It was also assumed that both M3K and M2K kinases in the 4-tiered module were activated processively. Reactions in this module are

$$\text{USI} + \text{M4K} \underset{d1}{\overset{a1}{\rightleftarrows}} \text{USI-M4K} \xrightarrow{k1} \text{UIS} + \text{M4Kp} \quad (4.1)$$

$$\text{M4Kp} + \text{PM4K} \underset{d2}{\overset{a2}{\rightleftarrows}} \text{M4Kp-PM4K} \xrightarrow{k2} \text{M4K} + \text{PM4K} \quad (4.2)$$

$$\text{M4Kp} + \text{M3K} \underset{d3}{\overset{a3}{\rightleftarrows}} \text{M4Kp-M3K} \xrightarrow{k3} \text{M4Kp} + \text{M3Kpp} \quad (4.3)$$

$$\text{M3Kp} + \text{PM3K} \underset{d4}{\overset{a4}{\rightleftarrows}} \text{M3Kp-PM3K} \xrightarrow{k4} \text{M3K} + \text{PM3K} \quad (4.4)$$

$$\text{M3Kpp} + \text{PM3K} \underset{d5}{\overset{a5}{\rightleftarrows}} \text{M3Kpp-PM3K} \xrightarrow{k5} \text{M3Kp} + \text{PM3K} \quad (4.5)$$

$$\text{M3Kpp} + \text{M2K} \underset{d6}{\overset{a6}{\rightleftarrows}} \text{M3Kpp-M2K} \xrightarrow{k6} \text{M3Kpp} + \text{M2Kpp} \quad (4.6)$$

$$\text{M2Kp} + \text{PM2K} \underset{d7}{\overset{a7}{\rightleftarrows}} \text{M2Kp-PM2K} \xrightarrow{k7} \text{M2K} + \text{PM2K} \quad (4.7)$$

$$\text{M2Kpp} + \text{PM2K} \underset{d8}{\overset{a8}{\rightleftarrows}} \text{M2Kpp-PM2K} \xrightarrow{k8} \text{M2Kp} + \text{PM2K} \quad (4.8)$$

$$\text{M2Kpp} + \text{M1K} \underset{d9}{\overset{a9}{\rightleftarrows}} \text{M2Kpp-M1K} \xrightarrow{k9} \text{M2Kpp} + \text{M1Kp} \quad (4.9)$$

$$\text{M2Kpp} + \text{M1Kp} \underset{d10}{\overset{a10}{\rightleftarrows}} \text{M2Kpp-M1Kp} \xrightarrow{k10} \text{M2Kpp} + \text{M1Kpp} \quad (4.10)$$

$$\text{M1Kp} + \text{PM1K} \underset{d11}{\overset{a11}{\rightleftarrows}} \text{M1Kp-PM1K} \xrightarrow{k11} \text{M1K} + \text{PM1K} \quad (4.11)$$

$$\text{M1Kpp} + \text{PM1K} \underset{d12}{\overset{a12}{\rightleftarrows}} \text{M1Kpp-PM1K} \xrightarrow{k12} \text{M1Kp} + \text{PM1K} \quad (4.12)$$

The total protein concentrations are [UIS] (Upstream input signal, Ras)=0.13 μM, [M4K]=0.003 μM, [M3K]=1.2 μM, [M2K]=1.2 μM, [M1K]=1.2 μM, [PM4K]=0.0003 μM, [PM3K]=0.0003 μM, [PM2K]=0.0003 μM, [PM1K]=0.12 μM.

4.2. The 4T P-P-D-D module. In this module the added kinase M2K is the M1K kinase in the 3T MAPK module. It was also assumed that M3K kinase in the 4-tiered module was activated processively. But the inserted M2K kinase in this 4-tiered module was activated distributively. Reactions in this module are

$$\text{USI} + \text{M4K} \underset{d1}{\overset{a1}{\rightleftarrows}} \text{USI-M4K} \xrightarrow{k1} \text{UIS} + \text{M4Kp} \quad (4.13)$$

$$\text{M4Kp} + \text{PM4K} \underset{d2}{\overset{a2}{\rightleftarrows}} \text{M4Kp-PM4K} \xrightarrow{k2} \text{M4K} + \text{PM4K} \quad (4.14)$$

$$\text{M4Kp} + \text{M3K} \underset{d3}{\overset{a3}{\rightleftarrows}} \text{M4Kp-M3K} \xrightarrow{k3} \text{M4Kp} + \text{M3Kpp} \quad (4.15)$$

$$\text{M3Kp} + \text{PM3K} \underset{d4}{\overset{a4}{\rightleftarrows}} \text{M3Kp-PM3K} \xrightarrow{k4} \text{M3K} + \text{PM3K} \quad (4.16)$$

$$\text{M3Kpp} + \text{PM3K} \underset{d5}{\overset{a5}{\rightleftarrows}} \text{M3Kpp-PM3K} \xrightarrow{k5} \text{M3Kp} + \text{PM3K} \quad (4.17)$$

$$\text{M3Kpp + M2K} \underset{d6}{\overset{a6}{\rightleftarrows}} \text{M3Kpp-M2K} \xrightarrow{k6} \text{M3Kpp + M2Kp} \quad (4.18)$$

$$\text{M3Kpp + M2Kp} \underset{d6}{\overset{a6}{\rightleftarrows}} \text{M3Kpp-M2Kp} \xrightarrow{k6} \text{M3Kpp + M2Kpp} \quad (4.19)$$

$$\text{M2Kp + PM2K} \underset{d7}{\overset{a7}{\rightleftarrows}} \text{M2Kp-PM2K} \xrightarrow{k7} \text{M2K + PM2K} \quad (4.20)$$

$$\text{M2Kpp + PM2K} \underset{d8}{\overset{a8}{\rightleftarrows}} \text{M2Kpp-PM2K} \xrightarrow{k8} \text{M2Kp + PM2K} \quad (4.21)$$

$$\text{M2Kpp + M1K} \underset{d9}{\overset{a9}{\rightleftarrows}} \text{M2Kpp-M1K} \xrightarrow{k9} \text{M2Kpp + M1Kp} \quad (4.22)$$

$$\text{M2Kpp + M1Kp} \underset{d10}{\overset{a10}{\rightleftarrows}} \text{M2Kpp-M1Kp} \xrightarrow{k10} \text{M2Kpp + M1Kpp} \quad (4.23)$$

$$\text{M1Kp + PM1K} \underset{d11}{\overset{a11}{\rightleftarrows}} \text{M1Kp-PM1K} \xrightarrow{k11} \text{M1K + PM1K} \quad (4.24)$$

$$\text{M1Kpp + PM1K} \underset{d12}{\overset{a12}{\rightleftarrows}} \text{M1Kpp-PM1K} \xrightarrow{k12} \text{M1Kp + PM1K} \quad (4.25)$$

The total protein concentrations are [UIS] (Upstream input signal, Ras)=0.13 μM, [M4K]=0.003 μM, [M3K]=1.2 μM, [M2K]=1.2 μM, [M1K]=1.2 μM, [PM4K]=0.0003 μM, [PM3K]=0.0003 μM, [PM2K]=0.12 μM, [PM1K]=0.12 μM. Note that the total concentration of [PM2K] in the 4T P-P-P-D and P-P-D-D modules is different.

4.2. The 4T P-P-D-D module. In this module the added kinase M2K is the M1K kinase in the 3T MAPK module. It was also assumed that M3K and M2K kinases in the 4-tiered module were activated distributively. Reactions in this module are

$$\text{USI + M4K} \underset{d1}{\overset{a1}{\rightleftarrows}} \text{USI-M4K} \xrightarrow{k1} \text{UIS + M4Kp} \quad (4.26)$$

$$\text{M4Kp + PM4K} \underset{d2}{\overset{a2}{\rightleftarrows}} \text{M4Kp-PM4K} \xrightarrow{k2} \text{M4K + PM4K} \quad (4.27)$$

$$\text{M4Kp + M3K} \underset{d3}{\overset{a3}{\rightleftarrows}} \text{M4Kp-M3K} \xrightarrow{k3} \text{M4Kp + M3Kpp} \quad (4.28)$$

$$\text{M3Kp + PM3K} \underset{d4}{\overset{a4}{\rightleftarrows}} \text{M3Kp-PM3K} \xrightarrow{k4} \text{M3K + PM3K} \quad (3.29)$$

$$\text{M3Kpp + PM3K} \underset{d5}{\overset{a5}{\rightleftarrows}} \text{M3Kpp-PM3K} \xrightarrow{k5} \text{M3Kp + PM3K} \quad (3.30)$$

$$\text{M3Kpp + M2K} \underset{d6}{\overset{a6}{\rightleftarrows}} \text{M3Kpp-M2K} \xrightarrow{k6} \text{M3Kpp + M2Kp} \quad (3.31)$$

$$\text{M3Kpp + M2Kp} \underset{d7}{\overset{a7}{\rightleftarrows}} \text{M3Kpp-M2Kp} \xrightarrow{k7} \text{M3Kpp + M2Kpp} \quad (3.32)$$

$$\text{M2Kp + PM2K} \underset{d8}{\overset{a8}{\rightleftarrows}} \text{M2Kp-PM2K} \xrightarrow{k8} \text{M2K + PM2K} \quad (3.33)$$

$$\text{M2Kpp + PM2K} \underset{d9}{\overset{a9}{\rightleftarrows}} \text{M2Kpp-PM2K} \xrightarrow{k9} \text{M2Kp + PM2K} \quad (3.34)$$

$$\text{M2Kpp + M1K} \underset{d10}{\overset{a10}{\rightleftarrows}} \text{M2Kpp-M1K} \xrightarrow{k10} \text{M2Kpp + M1Kp} \quad (3.35)$$

$$\text{M2Kpp + M1Kp} \underset{d11}{\overset{a11}{\rightleftarrows}} \text{M2Kpp-M1Kp} \xrightarrow{k11} \text{M2Kpp + M1Kpp} \quad (3.36)$$

$$\text{M1Kp + PM1K} \underset{d12}{\overset{a12}{\rightleftarrows}} \text{M1Kp-PM1K} \xrightarrow{k12} \text{M1K + PM1K} \quad (3.37)$$

$$\text{M1Kpp + PM1K} \underset{d13}{\overset{a13}{\rightleftarrows}} \text{M1Kpp-PM1K} \xrightarrow{k13} \text{M1Kp + PM1K} \quad (3.38)$$

The total protein concentrations are [UIS] (Upstream input signal, Ras)=0.13 μM, [M4K]=0.003 μM, [M3K]=1.2 μM, [M2K]=1.2 μM, [M1K]=1.2 μM, [PM4K]=0.0003 μM, [PM3K]=0.0003 μM, [PM2K]=0.12 μM, [PM1K]=0.12 μM. Note that the total concentrations of kinases and phosphatases in the 4T P-P-D-D and P-D-D-D modules are the same.

Figure 3 gives the simulated signal output stimulated for these 4-tiered modules based on different strengths of the upstream signal input. The signal output can be approximated by a Hill function, given by

4T P-P-P-D module: $[Output] = 1.0527 \dfrac{[USI]^{6.0}}{0.00000000000487^{6.0} + [USI]^{6.0}}$

4T P-P-D-D module: $[Output] = 1.0745 \dfrac{[USI]^{8.5}}{0.000000000145^{8.5} + [USI]^{8.5}}$

4T P-D-D-D module: $[Output] = 1.0728 \dfrac{[USI]^{11.0}}{0.0000028^{11.0} + [USI]^{11.0}}$

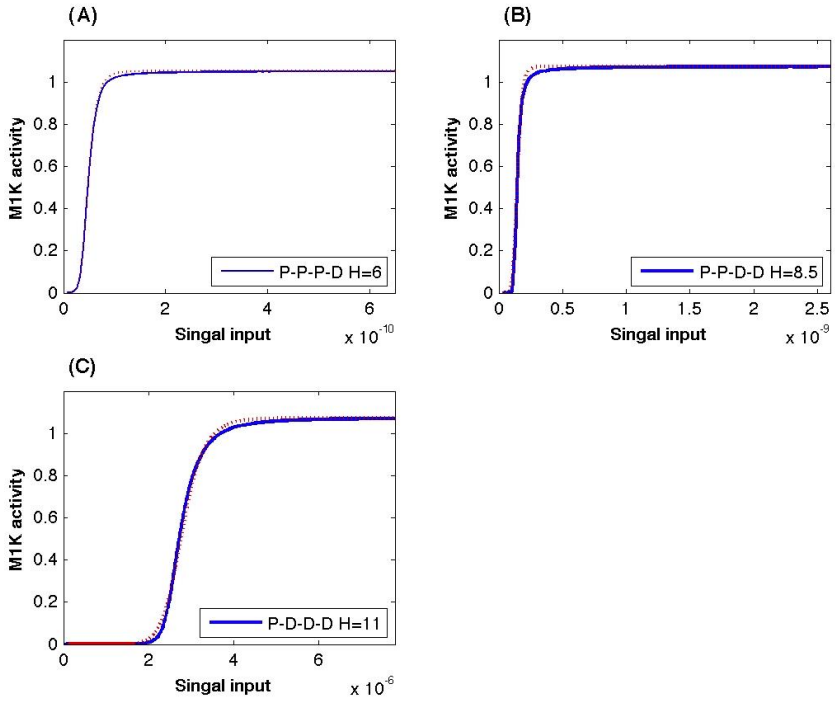

Fig. 4 Simulated MIK signal output of the 4-tiered MAPK modules based on different strength of the upstream signal input. The signal output of the 4T P-P-P-D module, 4T P-P-D-D module and 4T P-D-D-D module can be approximated by a Hill function with coefficient H=6.0, H=8.5, H=11.0, respectively.

Section 5. The 1-tiered MAPK module.

For completeness we also studied the 1-tiered module. In this simple module, it was assumed that the upstream signal input will activate M1K kinase either processively or distributively. In addition, it was assumed that the activated M1Kpp was dephosphorylated by PM1K in a process of two steps. Thus there are two 1-tiered modules.

5.1. 1T processive module

$$\text{USI} + \text{M1K} \underset{d1}{\overset{a1}{\rightleftarrows}} \text{USI-M1K} \xrightarrow{k1} \text{UIS} + \text{M1Kpp} \quad (5.1)$$

$$\text{M1Kp} + \text{PM1K} \underset{d2}{\overset{a2}{\rightleftarrows}} \text{M1Kp-PM1K} \xrightarrow{k2} \text{M1K} + \text{PM1K} \quad (5.2)$$

$$\text{M1Kpp} + \text{PM1K} \underset{d2}{\overset{a2}{\rightleftarrows}} \text{M1Kpp-PM1K} \xrightarrow{k2} \text{M1Kp} + \text{PM1K} \quad (5.3)$$

5.2 1T distributive module

$$\text{USI} + \text{M1K} \underset{d1}{\overset{a1}{\rightleftarrows}} \text{USI-M1K} \xrightarrow{k1} \text{UIS} + \text{M1Kp} \quad (5.4)$$

$$\text{USI} + \text{M1Kp} \underset{d1}{\overset{a1}{\rightleftarrows}} \text{USI-M1Kp} \xrightarrow{k1} \text{UIS} + \text{M1Kpp} \quad (5.5)$$

$$\text{M1Kp} + \text{PM1K} \underset{d2}{\overset{a2}{\rightleftarrows}} \text{M1Kp-PM1K} \xrightarrow{k2} \text{M1K} + \text{PM1K} \quad (5.6)$$

$$\text{M1Kpp} + \text{PM1K} \underset{d2}{\overset{a2}{\rightleftarrows}} \text{M1Kpp-PM1K} \xrightarrow{k2} \text{M1Kp} + \text{PM1K} \quad (5.7)$$

Figure 5 gives the simulated signal output stimulated for these 1-tiered modules based on different strengths of the upstream signal input. The signal output can be approximated by a Hill function, given by

1T processive module: $[Output] = 0.5653 \frac{[USI]^{1.2}}{0.0441^{1.2} + [USI]^{1.2}}$

1T distributive module: $[Output] = 1.0700 \frac{[USI]^{2.5}}{0.1233^{2.5} + [USI]^{2.5}}$

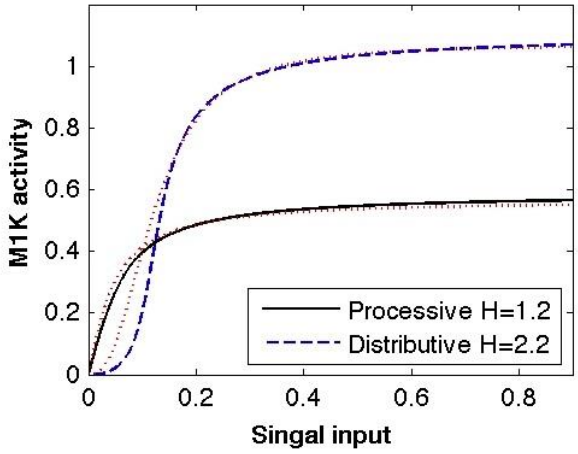

Fig. 4 Simulated MIK signal output of the 1-tiered MAPK modules based on different strength of the upstream signal input. The signal output of the 1T processive module and 1T distributive module can be approximated by a Hill function with coefficient H=1.2, and H=2.2, respectively.

Section 6. Robustness analysis

We used the concept defined by {Kitano, 2007 #1443} to measure the robustness property of the proposed model. The robustness property of a mathematical model with respect to a set of perturbations P is defined as the average of an evaluation function $D^s_{a,P}$ of the system over all perturbations $p \in P$, weighted by the perturbation probabilities $prob(p)$, given by

$$R^s_{a,P} = \int_{p \in P} prob(p) D^s_{a,P} \, dp \quad (6.1)$$

Here we use the Hill coefficient of the signal output as the evaluation function of the MAPK module, because the Hill coefficient provides a single value that describes different types of signal module outputs, and accurately describes both the graded and digital outputs.

There are three types of kinetic rates in the mathematical model, namely the binding rates a_i, disassociation rate d_i, and (de-)activation rate k_i. For each type of kinetic rate, the rate constants can be further classified as forward rates for activation and backward rates for deactivation. Thus for each MAPK module, we consider nine cases of rate constant perturbation, that is, for each of the three types of rate constant, we consider the forward rates only, backward rate only and both forward and backward rates. In each case of rate perturbation, we considered the perturbation that is within the range of 50% of the original values. For example, for the classic 3-tiered MAPK module, we considered the following nine cases

Case 1: $a_i^P = a_i(1+j*0.1),\ i=1,3,6,7,\ j=0,1,2,3,4,5$

Case 2: $a_i^P = a_i(1+j*0.1),\ i=2,4,5,8,9,\ j=0,1,2,3,4,5$

Case 3: $a_i^P = a_i(1+j*0.1),\ i=1,\ldots,9,\ j=0,1,2,3,4,5$

Case 4: $d_i^P = d_i(1+j*0.1),\ i=1,3,6,7,\ j=0,1,2,3,4,5$

Case 5: $d_i^P = d_i(1+j*0.1),\ i=2,4,5,8,9,\ j=0,1,2,3,4,5$

Case 6: $d_i^P = d_i(1+j*0.1),\ i=1,\ldots,9,\ j=0,1,2,3,4,5$

Case 7: $k_i^P = k_i(1+j*0.1),\ i=1,3,6,7,\ j=0,1,2,3,4,5$

Case 8: $k_i^P = k_i(1+j*0.1),\ i=2,4,5,8,9,\ j=0,1,2,3,4,5$

Case 9: $k_i^P = k_i(1+j*0.1),\ i=1,\ldots,9,\ j=0,1,2,3,4,5$

where a_i^P is the perturbed parameter of the original rate constant a_i.

Here we propose to use the following measures to evaluate the average behavior

$$R_{a,P}^M = \sum_{i,j}\left[\int_{p\in P} prob(p)x_{ij}(p)\,dp\right] \tag{6.2}$$

and the impact of perturbations on nominal behavior, given by

$$R_{a,P}^N = \sum_{i,j}\left[\int_{p\in P} prob(p)(\overline{x_{ij}(p)} - x_{ij}(p))^2\,dp\right] \tag{6.3}$$

where $x_{ij}(p)$ and x_{ij} is the simulated activity of kinase x_i at time point t_j with and without perturbed kinetic rate, respectively, and $\overline{x_{ij}(p)}$ is the mean of $x_{ij}(p)$ over all the perturbed kinetic rates.

Figure 6 gives the perturbation analysis results of all the MAPK modules. Fig 6A suggested that the 2T P-D module has the best average behaviour. If the dynamics in a single cell is regarded as a simulation with perturbed rate constants, the average behaviour is the ability of the system to maintain its underlying property at the population level. Fig 6B shows that the 2T P-D module also has very good nominal behaviour, though it was suggested that the 1T processive module, 2T P-P module and 3T P-P-D module also have good nominal behaviour. In our following discussion, we will concentrate on the average behaviour because the difference of nominal behaviour between these modules is not significant.

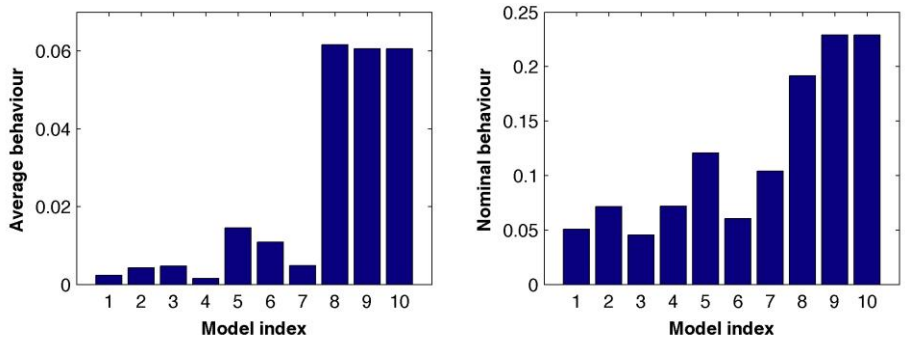

Fig 6. Simulated robustness properties of the MAPK modules. (A) The average behaviour. (B) The nominal behaviour (model index 1: 1T processive module; 2: 1T distributive module; 3: 2T P-P module; 4: 2T P-D module; 5: 2T D-D module; 6: 3T P-P-D module; 7: 3T P-D-D module; 8: 4T P-P-P-D module; 9: 4T P-P-D-D module; 10: 4T P-D-D-D module).

Section 7: Positive feedback regulation

We next tested the effects of positive feedback regulation on the signal output and robustness property. It was assumed that the activated M1Kpp can enhance the binding and activation activities of the input signal as well as increase the amount of the signal input. Using the 2-tiered processive-distributive module as the test system (3.7~3.14), the positive feedback regulation is realized by

$$a_1 = a_{10} + \eta_1 a_{10} \frac{[M1Kpp]}{[M1K]_0 + [M1Kpp]},$$

$$k_1 = k_{10} + \eta_1 k_{10} \frac{[M1Kpp]}{[M1K]_0 + [M1Kpp]},$$

$$[Singal\ Input] = [Signal\ Input]_0 + \eta_2 [Signal\ Input]_0 \frac{[M1Kpp]}{[M1K]_0}$$

where a_{10} and k_{10} are the basal rate constants without any feedback regulation, η_1 and η_2 are the feedback strengths, $[M1K]_0$ is the total concentration of [M1K], and $[Signal\ Input]_0$ is the basal signal input without any feedback regulation.

For the 2-tiered P-D module, Figure 7 suggested that the positive feedback regulation increased the values of the Hill coefficient. The feedback on the signal input has more influence on the Hill coefficient that the feedback on the rate constant. In addition, we further studied the robustness property of the 2-tiered P-D module with positive feedback regulation. Similar to the discussion in Section 4, we considered the nine cases of the model parameter perturbation. Figure 7 suggested that the positive feedback regulation decreased the robustness property of the MAPK module. Positive feedback on the signal input makes the system robustness property worse than the feedback on the rate constants.

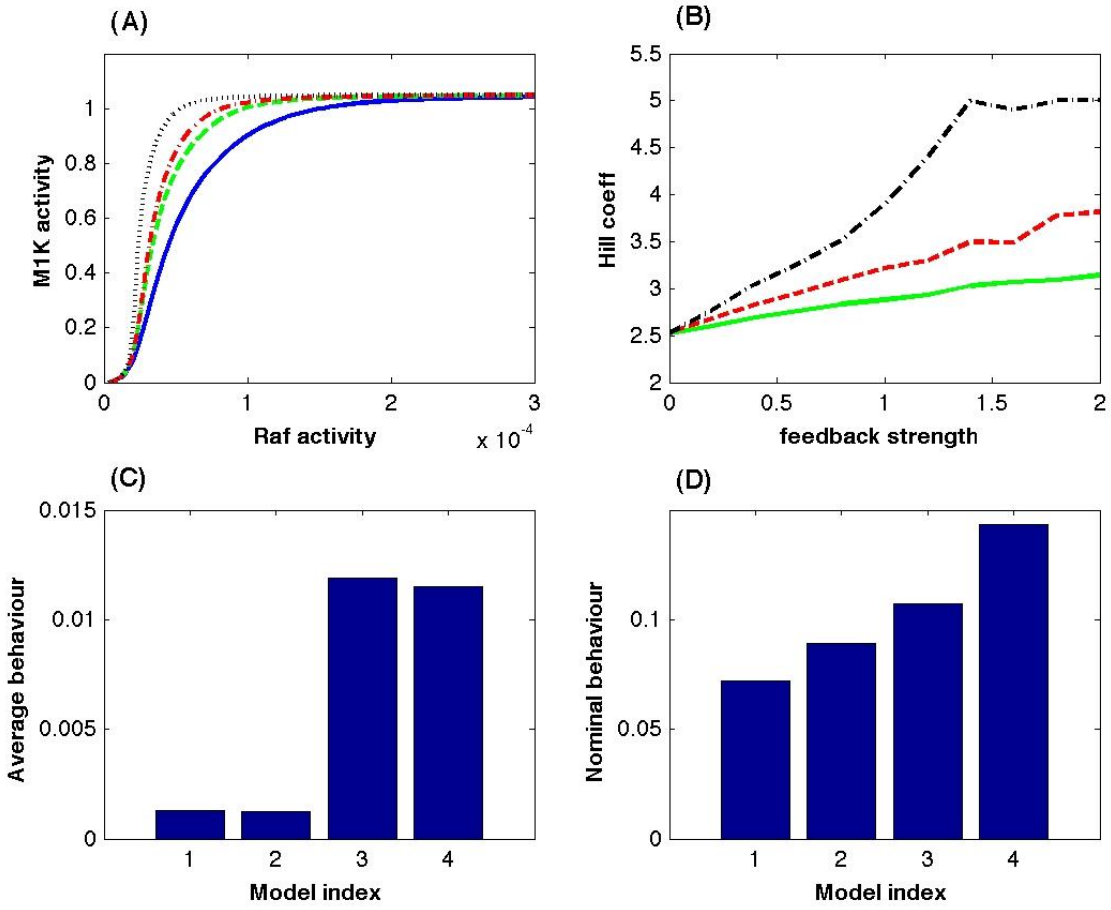

Figure 7. Signal output and robustness property of the 2-tiered P-D module.

(A) Simulated signal output over different signal input Raf activities (solid-blue-line: no feedback $m_1 = m_2 = 0$; dash-green-line: feedback to rate constants ($m_1 = 1, m_2 = 0$); dash-dot-red-line: feedback to signal input ($m_1 = 0, m_2 = 1$); dot-black-line: positive feedback to both rate constants and signal input $m_1 = 1, m_2 = 1$).

(B) The Hill coefficients over different feedback strength (solid-green-line: feedback to rate constants $m_1 = 0 \sim 2, m_2 = 0$; dash-red-line: feedback to signal input $m_1 = 0, m_2 = 0 \sim 2$; dash-dot-black-line: feedback to $m_1 = m_2 = 0 \sim 2$).

(C) **Averaged behaviour (robustness analysis) of the 2-tiered P-D module (model index 1: no feedback regulation ($m_1 = m_2 = 0$); index 2: feedback to rate ($m_1 = 1, m_2 = 0$); index 3: feedback to signal input ($m_1 = 0, m_2 = 1$); index 4: feedback to both coefficients and input ($m_1 = 1, m_2 = 1$).**

(D) **Nominal behaviour (robustness analysis) of the 2-tiered P-D module (model index 1: no feedback regulation ($m_1 = m_2 = 0$); index 2: feedback to rate ($m_1 = 1, m_2 = 0$); index 3: feedback to signal input ($m_1 = 0, m_2 = 1$); index 4: feedback to both coefficients and input ($m_1 = 1, m_2 = 1$).**

Section 8: Negative feedback regulation

Next we studied the effect of negative feedback regulation on the robustness properties of the MAPK module. It was assumed that the negative feedback regulation would enhance the binding and activation activities of phosphatases as well as increase the concentrations of the phosphatases PM2K and PM1K. Again, we used the 2-tiered P- module as the test system. The negative feedback regulation is realized by

$$a_i = a_{i0} + m_1 a_{i0} \frac{[M1Kpp]}{[M1K]_0 + [M1Kpp]},$$

$$k_i = k_{i0} + \eta_1 k_{i0} \frac{[M1Kpp]}{[M1K]_0 + [M1Kpp]}, \qquad i = 2,3,6,7,$$

$$[PM2K] = [PM2K]_0 + \eta_2 [PM2K]_0 \frac{[M1Kpp]}{[M1K]_0}$$

$$[PM1K] = [PM1K]_0 + \eta_2 [PM1K]_0 \frac{[M1Kpp]}{[M1K]_0}$$

where a_{i0} and k_{i0} are the basal rate constants of dephosphorylation without any feedback regulation, η_1 and η_2 are feedback strengths, $[M1K]_0$ is the total concentration of [M1K], and $[PM2K]_0$ and $[PM1K]_0$ are the basal concentrations of phosphatase PM2K and PM1K respectively without any feedback regulation.

Figure 8 suggested that, when the negative feedback regulation is stronger, the signal output of the MAPK module has a smaller Hill coefficient. In addition, we further studied the robustness property of the 2T P-D module with negative feedback regulation. Similar to the discussion in Section 4, we considered the nine cases of the model parameter perturbation. Results in Figure 8 suggested that the negative feedback regulation increased the robustness property of the MAPK module. With more strength of feedback regulation, the more robust the system becomes.

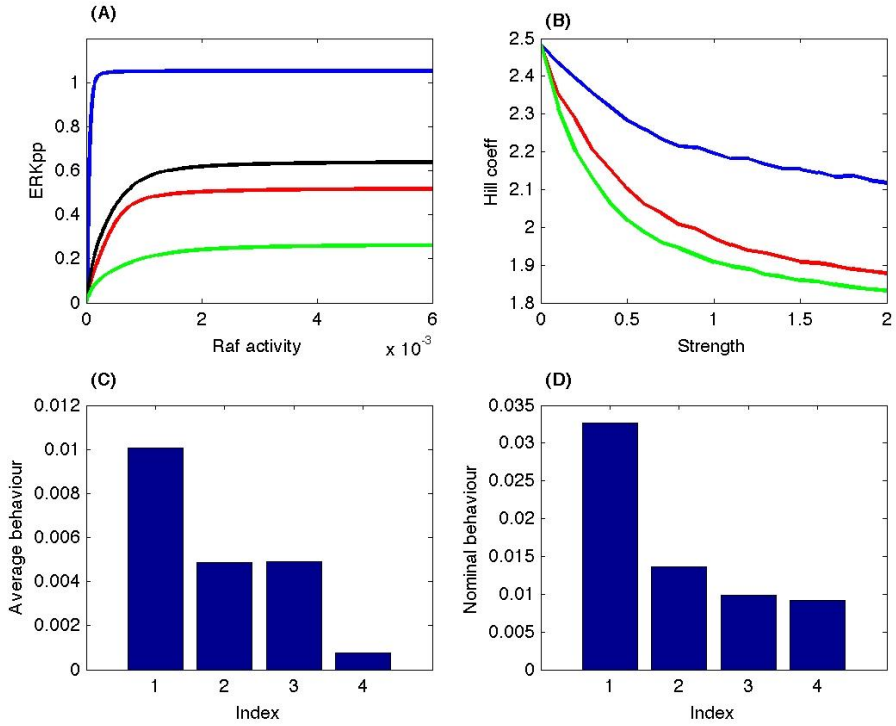

Figure 8: Simulation results of the @T P-D module with negative feedback regulation.

(A) Simulated signal output (blue-line: no feedback regulation, $\eta_1 = \eta_2 = 0$; red-line: feedback to coefficient only, $\eta_1 = 2, \eta_2 = 0$; black-line: feedback to the phosphatase concentration only, $\eta_1 = 0$, $\eta_2 = 2$; green-line: feedback to both coefficients and phosphatase $\eta_1 = 2, \eta_2 = 2$).

(B) Hill coefficient of the signal output (blue-line: feedback to coefficient only, $\eta_1 = 0 \sim 2, \eta_2 = 0$; red-line: feedback to the phosphatase concentration only, $\eta_1 = 0, \eta_2 = 0 \sim 2$; green-line: feedback to both coefficients and phosphatase $\eta_1 = \eta_2 = 0 \sim 2$).

(C) And (D) The average and nominal robustness property of the 2T P-D module (index 1: no feedback regulation, $\eta_1 = \eta_2 = 0$; index 2: feedback to coefficient only, $\eta_1 = 2, \eta_2 = 0$; index 3: feedback to the phosphatase concentration only, $\eta_1 = 0, \eta_2 = 2$; index 4: feedback to both coefficients and phosphatase $\eta_1 = 2, \eta_2 = 2$).

Section 9. Function of scaffold proteins

We next examine the function of scaffold protein in determining the robustness property of the MAPK module. The major function of scaffold protein is to accelerate the process of kinase phosphorylation via the processive activation of both M2K and M1K in the scaffold complex. Since the kinase activation is already processive in the 2T P-P module, we only considered the 2T P-D and 2T D-D modules. In addition to the chemical reactions in Section 2, the following reactions were added to the mathematical model for studying the function of scaffold proteins (SCD).

$$\text{SCD} + \text{M2K} \underset{v1}{\overset{u1}{\rightleftarrows}} \text{SCD-M2K} \tag{9.1}$$

$$\text{SCD} + \text{M1K} \underset{v2}{\overset{u2}{\rightleftarrows}} \text{SCD-M1K} \tag{9.2}$$

$$\text{SCD-M2K} + \text{M1K} \underset{v3}{\overset{u3}{\rightleftarrows}} \text{SCD-M2K-M1K} \tag{9.3}$$

$$\text{SCD-M1K} + \text{M2K} \underset{v4}{\overset{u4}{\rightleftarrows}} \text{SCD-M2K-M1K} \tag{9.4}$$

$$\text{UIS} + \text{SCD-M2K} \underset{v5}{\overset{u5}{\rightleftarrows}} \text{UIS-SCD-M2K} \tag{9.5}$$

$$\text{SCD} + \text{KSR-M2K-M1K} \underset{v6}{\overset{u6}{\rightleftarrows}} \text{SCD-KSR-M2K-M1K} \tag{9.6}$$

$$\text{SCD-KSR-M2K} + \text{M1K} \underset{v7}{\overset{u7}{\rightleftarrows}} \text{SCD-KSR-M2K-M1K} \tag{9.7}$$

$$\text{UIS-SCD-M2K} \xrightarrow{w1} \text{UIS-SCD-M2Kpp} \tag{9.8}$$

$$\text{UIS-SCD-M2K-M1K} \xrightarrow{w2} \text{UIS-SCD-M2Kpp-M1K} \tag{9.9}$$

$$\text{UIS-SCD-M2Kpp-M1K} \xrightarrow{w3} \text{UIS-SCD-M2Kpp-M1Kpp} \tag{9.10}$$

$$\text{UIS-SCD-M2Kpp} + \text{M1K} \underset{v8}{\overset{u8}{\rightleftarrows}} \text{UIS-SCD-M2Kpp-M1Kpp} \tag{9.11}$$

$$\text{UIS-SCD-M2Kpp-M1Kpp} \xrightarrow{w4} \text{UIS-SCD-M2Kpp} + \text{M1Kpp} \tag{9.12}$$

$$\text{UIS-SCD-M2Kpp} \xrightarrow{w5} \text{UIS} + \text{SCD} + \text{M2Kpp} \tag{9.13}$$

Fig. 9 gives the simulated signal output and corresponding Hill coefficient of the 2-tiered processive-distributive modules. Similar to the established results of the 3-tiered classic MAPK module {Levchenko, 2000 #1467}, which was also confirmed by our simulations, the addition of scaffold protein will increase the values of the Hill coefficient. However, when the amount of scaffold protein is large, the Hill coefficient value decreased, because the excessive scaffold proteins separated M2K and M1K kinases. Thus the signal output in Fig 9A and C decreased when the scaffold protein has large concentrations. Using the conclusions derived from Section 7 and 8, we claimed that the addition of scaffold protein would decrease the robustness property (because the Hill coefficient increased), whereas scaffold proteins with a large concentration increase the robustness properties of the MAPK module.

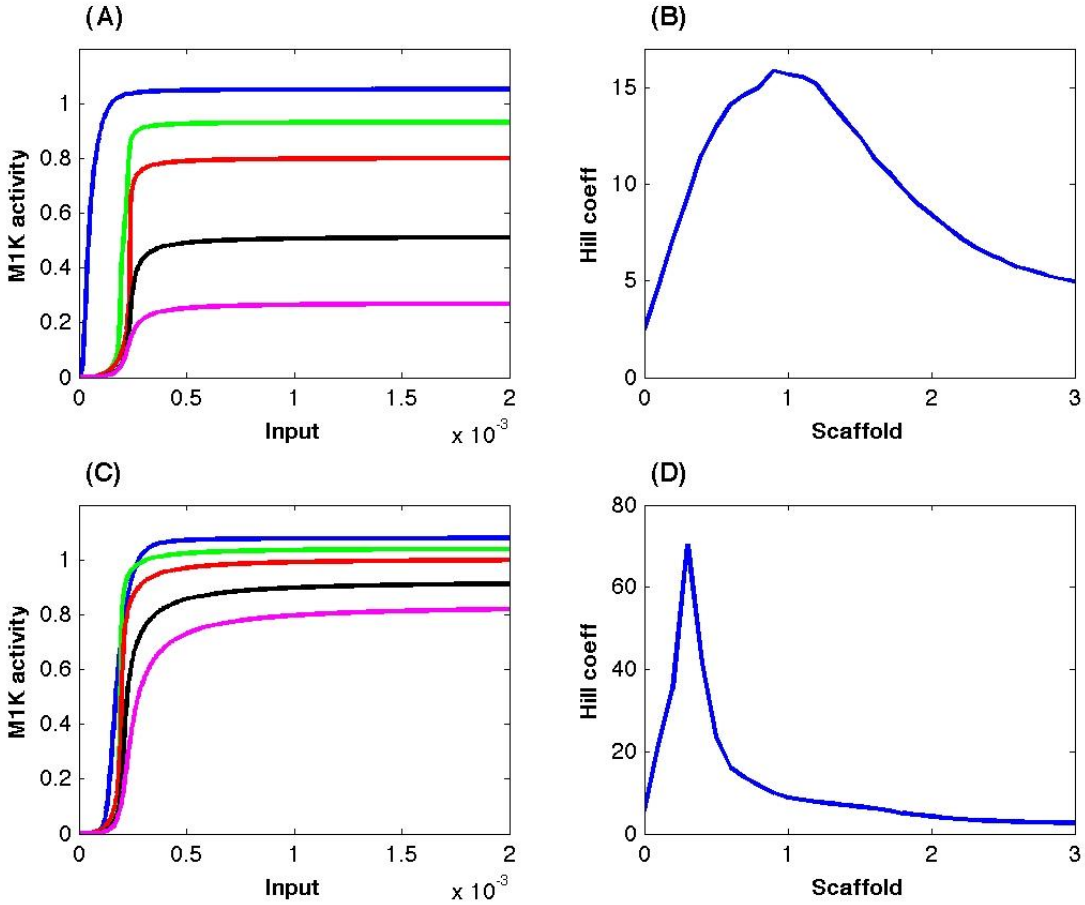

Fig 9. Function of scaffold proteins.
(A) The simulated signal output with different scaffold concentrations of the 2T P-D module (blue-line: SCD=0; green-line: SCD=0.5; red-line: SCD=1.0; black-line: SCD=1.5; magenta-line: SCD=2.0);
(B) Hill coefficient values of the 2T P-D module based different concentrations of scaffold proteins;
(C) The simulated signal output with different scaffold concentrations of the 2T D-D module (blue-line: SCD=0; green-line: SCD=0.5; red-line: SCD=1.0; black-line: SCD=1.5; magenta-line: SCD=2.0);
(D) Hill coefficient values of the 2T D-D module based different concentrations of scaffold proteins

Section 10. Flexibility property of the MAPK module

We have shown that the 2T P-D module has the best robustness property among the remaining modules, an important question is whether this module has adequate flexibility property to realize a versatile of signal output.

Experimental studies have shown that, under various experimental conditions, the MAPK pathway can produce either the graded signal output or digital signal output (Tian 2007 NCB).

We have shown in Sections 7 and 9 that both the strong positive feedback regulation and addition of an appropriate amount of scaffold protein will produce digital signal output with a large Hill coefficient.

Therefore the next question is whether the 2T P-D module can produce the graded signal output by using the feedback regulation and/or scaffold protein. We have tested the signal output by using negative feedback regulation with large regulation strength and/or a large amount of scaffold proteins.

Simulations in Fig. 10(A) suggested that when the concentration of scaffold protein is large, we realized linear signal output by a properly selected strength of negative feedback regulations. In

addition, for a given scaffold concentration, there is a wide range of negative feedback regulation strength that can produce graded signal output.
However, the 3T P-P-D module does not have such ability to realize graded signal output by testing different scaffold concentrations and negative feedback strength. To compare the results of the 2T P-D module in Fig 10(A), we gave simulations of the 3T P-P-D module in Fig 10(B) based on the same conditions.

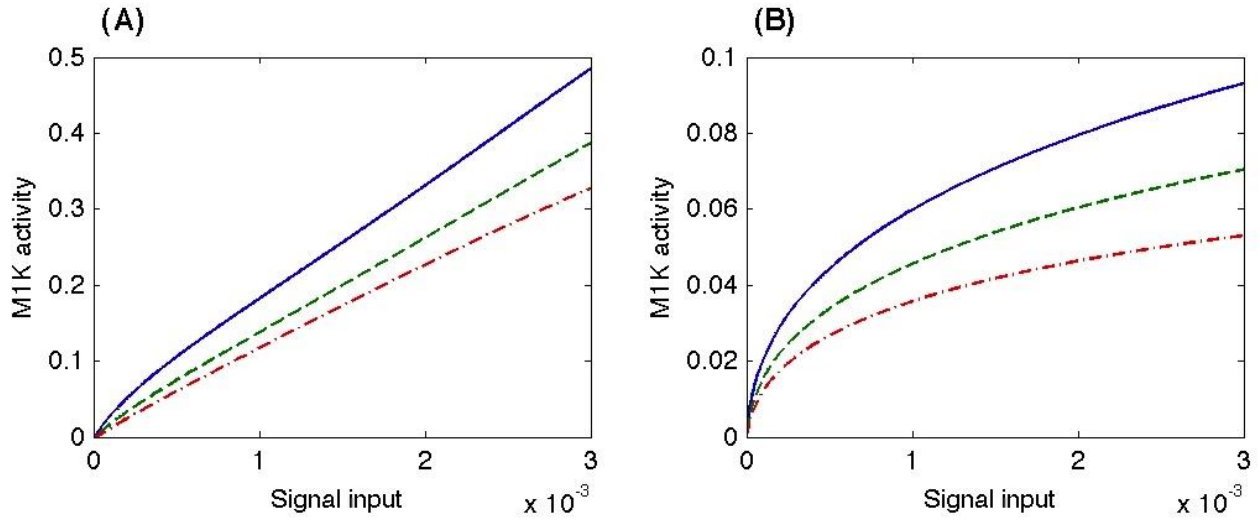

Fig 10. Simulated signal output using different scaffold protein concentrations and negative feedback strengths. A. Signal output of the 2T P-D module (Solid-blue-line: scaffold concentration=1.0, negative feedback to PM2K concentration $\eta_2 = 30$; Dash-green-line: scaffold concentration=1.2, negative feedback to PM2K concentration $\eta_2 = 37$; Dash-dot-red-line: scaffold concentration=1.2, negative feedback to PM2K concentration $\eta_2 = 41$) B. Signal output of the 3T P-P-D module (Solid-blue-line: scaffold concentration=1.0, negative feedback to PM2K concentration $\eta_2 = 30$; Dash-green-line: scaffold concentration=1.2, negative feedback to PM2K concentration $\eta_2 = 37$; Dash-dot-red-line: scaffold concentration=1.2, negative feedback to PM2K concentration $\eta_2 = 41$)

We also tested the adaptability of the 1-tier distributive module for realizing the graded signal output using negative feedback regulations. In this case the rate constants related to dephosphorylation will increase proportionally to the activated M1Kpp. In addition, the concentration of phosphatase will also increase proportionally to the activated M1Kpp. These mechanisms were realized by

$$a_i = a_{i0} + \eta a_{i0} \frac{[M1Kpp]}{[M1K]_0 + [M1Kpp]},$$

$$k_i = k_{i0} + \eta k_{i0} \frac{[M1Kpp]}{[M1K]_0 + [M1Kpp]}, \qquad i = 3, 4$$

$$[PM1K] = [PM1K]_0 + \eta [PM1K]_0 \frac{[M1Kpp]}{[M1K]_0}$$

We simulated the system with different strength of negative feedback regulation, which was realized by different values of parameter η. In our simulations imulations the 1-tier module failed to show the adaptability even when the large feedback strength $\eta = 5$ was used.

Section 11. Bistability property

Finally we examine whether the 2T P-D module can realize the hysteresis that is the ability of a system to remains on while reducing the activating stimulus. For the 2T P-D module, we considered two simulations. In one simulation, the signal input (M3K activity) gradually increases from zero to the maximum from time 200 to 400, namely

$$[Input]=\begin{cases}0 & t<200\\ [Max\_Input]\dfrac{t-200}{200} & 200<t<400\\ [Max\_input] & t>400\end{cases} \tag{11.1}$$

Simultaneously, this module also includes the positive feedback regulation (i=1, 4, 5):

$$a_i = a_{i0} + \eta_1 a_{i0}\frac{[M1Kpp]}{[M1K]_0+[M1Kpp]},$$

$$k_i = k_{i0} + \eta_1 k_{i0}\frac{[M1Kpp]}{[M1K]_0+[M1Kpp]}, \tag{11.2}$$

$$[Singal\ Input]=[Signal\ Input]_0+\eta_1[Signal\ Input]_0\frac{[M1Kpp]}{[M1K]_0}$$

In another simulation, the signal input was the maximal at the beginning. Then the signal input decreases gradually to zero from 200 to 400, namely

$$[Input]=\begin{cases}[Max\_input] & t<200\\ [Max\_Input](1-\dfrac{t-200}{200}) & 200<t<400\\ 0 & t>400\end{cases} \tag{11.3}$$

At the same time, the positive feedback regulation (11.2) was also applied. Figure 11(A) gives the simulations of the 2T P-D module. To compare the simulations, the second simulation with (11.3) and (11.2) was plotted by using time period (600-t). Simulations show that the 2T P-D module can realize hysteresis.

We also realized the hysteresis of the 3T P-P-D module by using the signal input in (11.1) and (11.3). For the positive regulation, the coefficients that were influenced by (11.2) include i=1, 3, 6, 7. Since more coefficients were influenced by the positive feedback regulation in the 3T P-P-D module, the area of hysteresis in Fig 11(B) of the 3T P-P-D module is larger than that in Fig. 11(A).

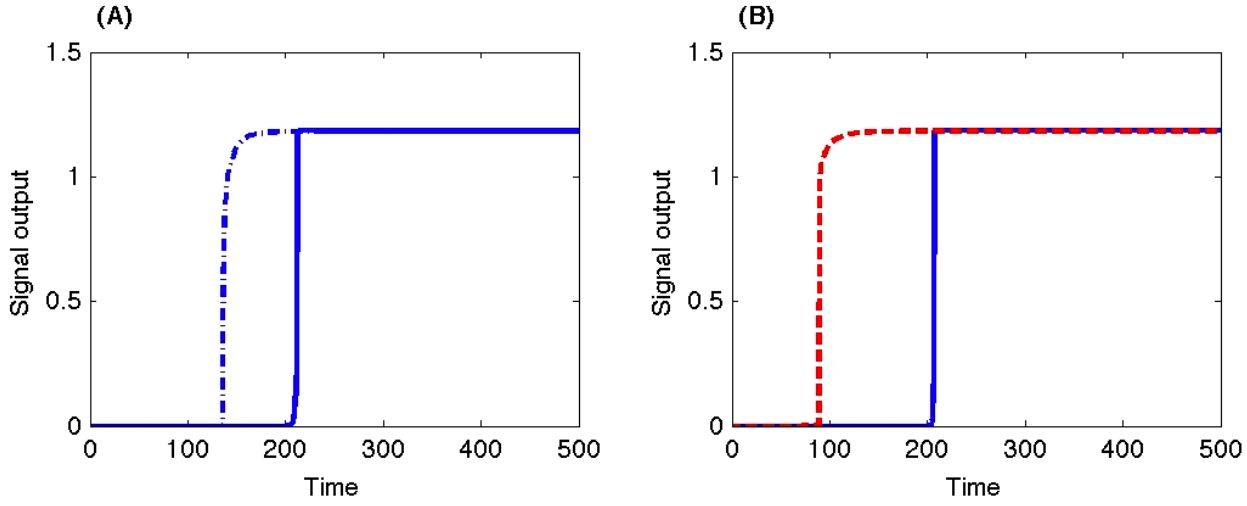

Fig 11. Hysteresis of the 2T P-D module (A) and 3T P-P-D module (B). The positive feedback coefficient $\eta_1=15$ for both modules. The solid-line is the solution based on the system when the input increases from zero to maximum is increasing (i.e. switching on), while the dash-line is the system when the input is decreased from maximum down to zero (i.e. switching the system off).