MAPK Cascade

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MAPK Cascade - Huang&Ferrell

Ultrasensitivity in the mitogen-activated protein **cascade**, Chi-Ying F. Huang and James E. Ferrell, Jr., 1996, *Proc. Natl. Acad. Sci. USA*, 93, 10078-10083.

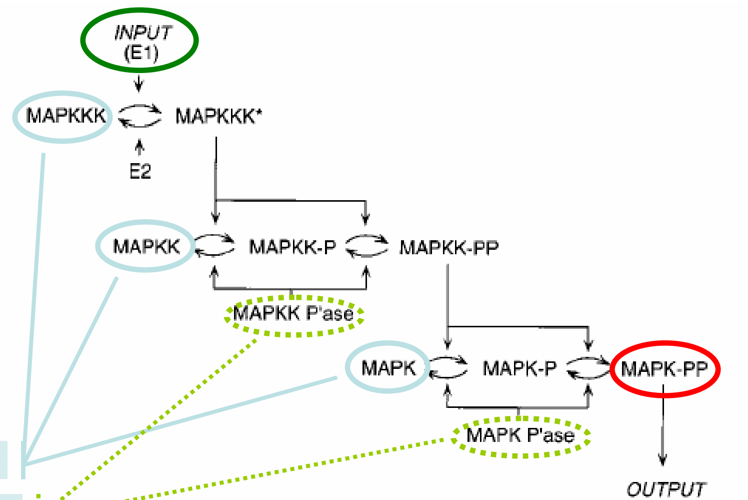
Biochemistry: Huang and Ferrell *Proc. Natl. Acad. Sci. USA 93 (1996)*

10 chemical reactions

Table 2. Predicted Hill coefficients for MAP kinase cascade components: Varying the assumed K_m values

Reaction	Range of assumed K_m values	Range of effective Hill coefficients (nH) predicted for MAPKKK	MAPKK	MAPK
1. MAPKKK → MAPKKK*	60–1500 nM	1.0	1.7	4.9
2. MAPKKK* → MAPKKK	60–1500 nM	1.0	1.7	4.9
3. MAPKK → MAPKK-P	60–1500 nM	1.0	1.3–2.3	4.0–5.1
4. MAPKK-P → MAPKK	60–1500 nM	1.0	1.5–1.9	3.6–6.7
5. MAPKK-P → MAPKK-PP	60–1500 nM	1.0	1.3–2.4	3.8–5.2
6. MAPKK-PP → MAPKK-P	60–1500 nM	1.0	1.7–1.8	4.1–6.4
7. MAPK → MAPK-P	60–1500 nM (300 nM†)	1.0	1.7	3.7–6.2
8. MAPK-P → MAPK	60–1500 nM	1.0	1.7	4.3–5.2
9. MAPK-P → MAPK-PP	60–1500 nM	1.0	1.7	3.4–6.1
10. MAPK-PP → MAPK-P	60–1500 nM	1.0	1.7	4.7–5.1

The assumed K_m values for each reaction were individually varied over the ranges shown, with the assumed K_m values for the other nine reactions held constant. The effective Hill coefficients were calculated from the steepness of the predicted stimulus/response curves, as described in the text.
†The K_m value for reaction 7 has been measured to be 300 nM for the phosphorylation of a mammalian MAPK by a MAPKK (N. Ahn, personal communication). All of the other K_m values were initially assumed to be 300 nM as well.



Reservoirs

Back Enzymes

FIG. 1. Schematic view of the MAPK cascade. Activation of MAPK depends upon the phosphorylation of two conserved sites [Thr-183 and Tyr-185 in rat p42 MAPK/Erk2 (4, 5)]. Full activation of MAPKK also requires phosphorylation of two sites [Ser-218 and Ser-222 in mouse Mek-1/MKK1 (6–10)]. Detailed mechanisms for the activation of various MAPKKKs (e.g., Raf-1, B-Raf, Mos) are not yet established; here we assume that MAPKKKs are activated and inactivated by enzymes we denote E1 and E2. MAPKKK* denotes activated MAPKKK. MAPKK-P and MAPKK-PP denote singly and doubly phosphorylated MAPKK, respectively. MAPK-P and MAPK-PP denote singly and doubly phosphorylated MAPK. P'ase denotes phosphatase.

Calculations. Eqs. **1-10** represent the reactions of the MAPK cascade, which are shown schematically in Fig. 1. We have used Goldbeter and Koshland's nomenclature for the rate constants—the letter a denotes association, d denotes dissociation without catalysis, and k denotes product formation (11). KKK denotes MAPKKK; KK denotes MAPKK; and K denotes MAPK.

$$\mathrm{KKK + E1} \underset{d_1}{\overset{a_1}{\rightleftharpoons}} \mathrm{KKK{\cdot}E1} \overset{k_1}{\longrightarrow} \mathrm{KKK^* + E1} \quad [1]$$

$$\mathrm{KKK^* + E2} \underset{d_2}{\overset{a_2}{\rightleftharpoons}} \mathrm{KKK{\cdot}E2} \overset{k_2}{\longrightarrow} \mathrm{KKK + E2} \quad [2]$$

$$\mathrm{KK + KKK^*} \underset{d_3}{\overset{a_3}{\rightleftharpoons}} \mathrm{KK{\cdot}KKK^*} \overset{k_3}{\longrightarrow} \mathrm{KK\text{-}P + KKK^*} \quad [3]$$

$$\mathrm{KK\text{-}P + KK\ P'ase} \underset{d_4}{\overset{a_4}{\rightleftharpoons}} \mathrm{KK\text{-}P{\cdot}KK\ P'ase} \overset{k_4}{\longrightarrow} \mathrm{KK + KK\ P'ase} \quad [4]$$

$$\mathrm{KK\text{-}P + KKK^*} \underset{d_5}{\overset{a_5}{\rightleftharpoons}} \mathrm{KK\text{-}P{\cdot}KKK^*} \overset{k_5}{\longrightarrow} \mathrm{KK\text{-}PP + KKK^*} \quad [5]$$

$$\mathrm{KK\text{-}PP + KK\ P'ase} \underset{d_6}{\overset{a_6}{\rightleftharpoons}} \mathrm{KK\text{-}PP{\cdot}KK\ P'ase} \overset{k_6}{\longrightarrow} \mathrm{KK\text{-}P + KK\ P'ase} \quad [6]$$

$$\mathrm{KK\text{-}PP + K} \underset{d_7}{\overset{a_7}{\rightleftharpoons}} \mathrm{KK\text{-}PP{\cdot}K} \overset{k_7}{\longrightarrow} \mathrm{KK\text{-}PP + K\text{-}P} \quad [7]$$

$$\mathrm{K\text{-}P + K\ P'ase} \underset{d_8}{\overset{a_8}{\rightleftharpoons}} \mathrm{K\text{-}P{\cdot}K\ P'ase} \overset{k_8}{\longrightarrow} \mathrm{K + K\ P'ase} \quad [8]$$

$$\mathrm{K\text{-}P + KK\text{-}PP} \underset{d_9}{\overset{a_9}{\rightleftharpoons}} \mathrm{K\text{-}P{\cdot}KK\text{-}PP} \overset{k_9}{\longrightarrow} \mathrm{K\text{-}PP + KK\text{-}PP} \quad [9]$$

$$\mathrm{K\text{-}PP + K\ P'ase} \underset{d_{10}}{\overset{a_{10}}{\rightleftharpoons}} \mathrm{K\text{-}PP{\cdot}K\ P'ase} \overset{k_{10}}{\longrightarrow} \mathrm{K\text{-}P + K\ P'ase} \quad [10]$$

As 18 Ordinary Differential Equations
Plus 7 conservation equations

$$\frac{d}{dt}[\text{KKK}] = -a_1[\text{KKK}][\text{E1}] + d_1[\text{KKK}\cdot\text{E1}] + k_2[KKK^*\cdot E2] \quad [11]$$

$$\frac{d}{dt}[\text{KKK}\cdot\text{E1}] = a_1[\text{KKK}][\text{E1}] - (d_1 + k_1)[\text{KKK}\cdot\text{E1}] \quad [12]$$

$$\frac{d}{dt}[\text{KKK}^*] = -a_2[\text{KKK}^*][\text{E2}] + d_2[\text{KKK}^*\cdot\text{E2}] + k_1[KKK\cdot E1] + (k_3 + d_3)[KK\cdot KKK^*] - a_3[KKK^*][KK] + (k_5 + d_5)[KK\text{-}P\cdot KKK^*] - a_5[KK\text{-}P][KKK^*] \quad [13]$$

$$\frac{d}{dt}[\text{KKK}^*\cdot\text{E2}] = a_2[\text{KKK}^*][\text{E2}] - (d_2 + k_2)[\text{KKK}^*\cdot\text{E2}] \quad [14]$$

$$\frac{d}{dt}[\text{KK}] = -a_3[\text{KK}][\text{KKK}^*] + d_3[\text{KK}\cdot\text{KKK}^*] + k_4[KK\text{-}P\cdot KK\ P'ase] \quad [15]$$

$$\frac{d}{dt}[\text{KK}\cdot\text{KKK}^*] = a_3[\text{KK}][\text{KKK}^*] - (d_3 + k_3)[KK\cdot KKK^*] \quad [16]$$

$$\frac{d}{dt}[\text{KK-P}] = -a_4[\text{KK-P}][\text{KK P}'\text{ase}] + d_4[\text{KK-P}\cdot\text{KK P}'\text{ase}] + k_3[KK\cdot KKK^*] + k_6[KK\text{-}PP\cdot KK\ P'ase] + d_5[KK\text{-}P\cdot KKK^*] - a_5[KK\text{-}P][KKK^*] \quad [17]$$

$$+ d_5[KK\text{-}P\cdot KKK^*] - a_5[KK\text{-}P][KKK^*] \quad [17]$$

$$\frac{d}{dt}[\text{KK-P}\cdot\text{KK P}'\text{ase}] = a_4[\text{KK-P}][\text{KK P}'\text{ase}] - (d_4 + k_4)[KK\text{-}P\cdot KK\ P'ase] \quad [18]$$

$$\frac{d}{dt}[\text{KK-P}\cdot\text{KKK}^*] = a_5[\text{KK-P}][\text{KKK}^*] - (d_5 + k_5)[KK\text{-}P\cdot KKK^*] \quad [19]$$

$$\frac{d}{dt}[\text{KK-PP}] = k_5[\text{KK-P}\cdot\text{KKK}^*] - a_6[\text{KK-PP}][\text{KK P}'\text{ase}] + d_6[KK\text{-}PP\cdot KK\ P'ase] - a_7[KK\text{-}PP][K] + (d_7 + k_7)[K\cdot KK\text{-}PP] + (d_9 + k_9)[K\text{-}P\cdot KK\text{-}PP] - a_9[K\text{-}P][KK\text{-}PP] \quad [20]$$

$$\frac{d}{dt}[\text{KK-PP}\cdot\text{KK P}'\text{ase}] = a_6[\text{KK-PP}][\text{KK P}'\text{ase}] - (d_6 + K_6)[\text{KK-PP}\cdot\text{KK P}'\text{ase}] \quad [21]$$

$$\frac{d}{dt}[\text{K}] = -a_7[\text{K}][\text{KK-PP}] + d_7[\text{K}\cdot\text{KK-PP}] + k_8[K\text{-}P\cdot K\ P'ase] \quad [22]$$

$$\frac{d}{dt}[\text{K}\cdot\text{KK-PP}] = a_7[\text{K}][\text{KK-PP}] - (d_7 + k_7)[\text{K}\cdot\text{KK-PP}] \quad [23]$$

$$\frac{d}{dt}[\text{K-P}] = k_7[\text{K}\cdot\text{KK-PP}] - a_8[\text{K-P}][\text{K P}'\text{ase}] + d_8[K\text{-}P\cdot K\ P'ase] - a_9[K\text{-}P][KK\text{-}PP] + d_9[K\text{-}P\cdot KK\text{-}PP] + k_{10}[K\text{-}PP\cdot K\ P'ase] \quad [24]$$

$$\frac{d}{dt}[\text{K-P}\cdot\text{K P}'\text{ase}] = a_8[\text{K-P}][\text{K P}'\text{ase}] - (d_8 + k_8)[K\text{-}P\cdot K\ P'ase] \quad [25]$$

$$\frac{d}{dt}[\text{K-P}\cdot\text{KK-PP}] = a_9[\text{K-P}][\text{KK-PP}] - (d_9 + k_9)[K\text{-}P\cdot KK\text{-}PP] \quad [26]$$

$$\frac{d}{dt}[\text{K-PP}] = -a_{10}[\text{K-PP}][\text{K P}'\text{ase}] + d_{10}[K\text{-}PP\cdot K\ P'ase] + k_9[K\text{-}P\cdot KK\text{-}PP] \quad [27]$$

$$\frac{d}{dt}[\text{K-PP}\cdot\text{K P}'\text{ase}] = a_{10}[\text{K-PP}][\text{K P}'\text{ase}] - (d_{10} + k_{10})[K\text{-}PP\cdot K\ P'ase] \quad [28]$$

The 10 reactions described above give rise to 18 rate equations.

One equation for each species (8) and complex (10), but not for constant concentration enzymes (4)

$$[\text{E1}_{tot}] = [\text{E1}] + [\text{KKK}\cdot\text{E1}] \quad [30]$$

$$[\text{E2}_{tot}] = [\text{E2}] + [\text{KKK}^*\cdot\text{E2}] \quad [31]$$

$$[\text{KK}_{tot}] = [\text{KK}] + [\text{KK-P}] + [\text{KK-PP}] + [\text{KK}\cdot\text{KKK}^*] + [KK\text{-}P\cdot KKK^*] + [KK\text{-}P\cdot KK\ P'ase] + [KK\text{-}PP\cdot KK\ P'ase] + [KK\text{-}PP\cdot K] + [KK\text{-}PP\cdot K\text{-}P] \quad [32]$$

$$[\text{KK P}'\text{ase}_{tot}] = [\text{KK P}'\text{ase}] + [\text{KK P}'\text{ase}\cdot\text{KK-P}] + [KK\ P'ase\cdot KK\text{-}PP] \quad [33]$$

$$[\text{K}_{tot}] = [\text{K}] + [\text{K-P}] + [\text{K-PP}] + [\text{KK-PP}\cdot\text{K}] + KK\text{-}PP\cdot K\text{-}P] + [K\text{-}P\cdot K\ P'ase] + [K\text{-}PP\cdot K\ P'ase] \quad [34]$$

$$[\text{K P}'\text{ase}_{tot}] = [\text{K P}'\text{ase}] + [\text{K-P}\cdot\text{K P}'\text{ase}] + [K\text{-}PP\cdot K\ P'ase] \quad [35]$$

These equations were solved numerically using the Runge–Kutta-based NDSolve algorithm in Mathematica (Wolfram Research, Champaign, IL). An annotated copy of the Mathematica code for the MAPK cascade rate equations can be obtained from J.E.F.

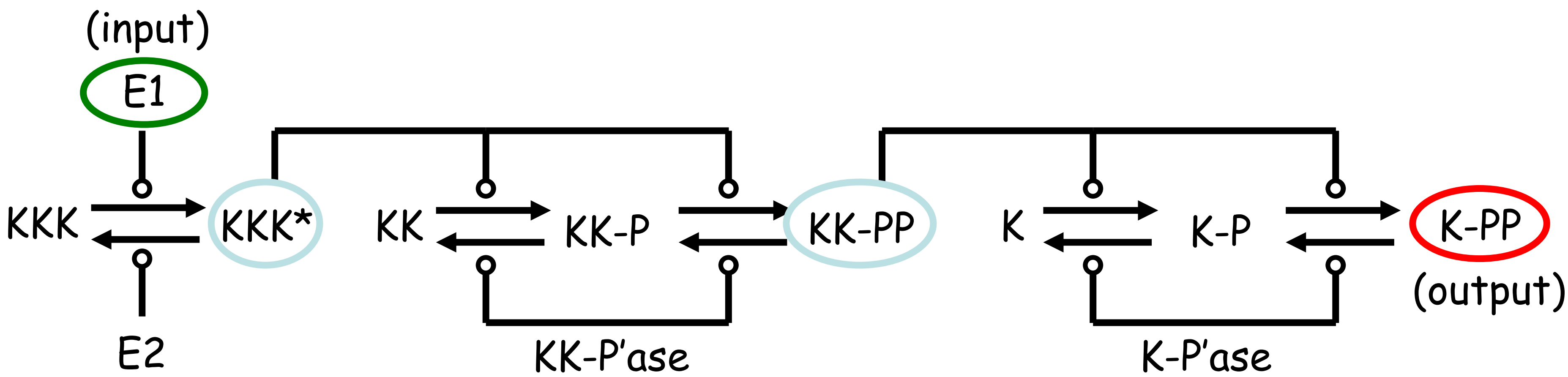
In addition, there are seven conservation equations (Eqs. 29-35).

$$[\text{KKK}_{tot}] = [\text{KKK}] + [\text{KKK}^*] + [\text{KKK}\cdot\text{E1}] + [KKK^*\cdot E2] + [KKK^*\cdot K] + [KKK^*\cdot K\text{-}P] \quad [29]$$

in exactly one state

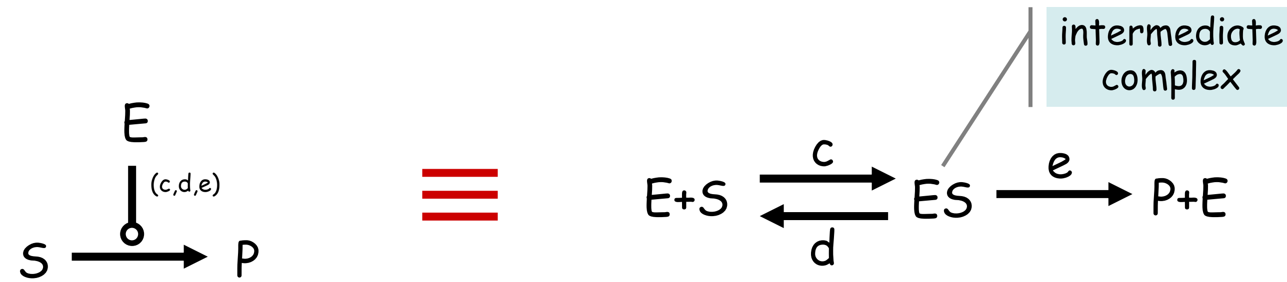
Each molecule

The Circuit

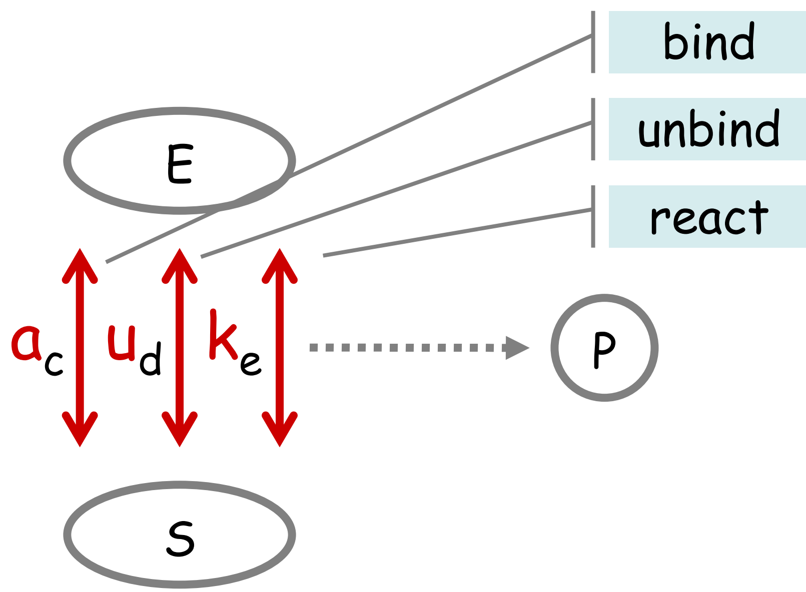


Enzymatic Reactions

Reaction View



Interaction View



private bindings between one S and one E molecule

$S() \triangleq$ new u@d new k@e
$!a_c(u,k);\ (!u_d;\ S() + !k_e;\ P())$

bind unbind react

$E() \triangleq\ ?a_c(u,k);\ (?u_d;\ E() + ?k_e;\ E())$

$P() \triangleq\ \ldots$

MAPK Cascade in SPiM

```
let KKK() =
   (new u1@d1:Release new k1@r1:React
    !a1(u1,k1); (do !u1;KKK() or !k1;KKKst()))            [1]substrate
```

KKK:E1 complex

```
and KKKst() =
   (new u2@d2:Release new k2@r2:React
    do !a2(u2,k2); (do !u2;KKKst() or !k2;KKK())          [2]substrate
    or ?a3(u3,k3); (do ?u3;KKKst() or ?k3;KKKst())        [3]kinase
    or ?a5(u5,k5); (do ?u5;KKKst() or ?k5;KKKst()))       [5]kinase

let E1() =
   ?a1(u1,k1); (do ?u1;E1() or ?k1;E1())                  [1]enzyme
```

E1:KKK complex

```
let E2() =
   ?a2(u2,k2); (do ?u2;E2() or ?k2;E2())                  [2]enzyme

let KK() =
   (new u3@d3:Release new k3@r3:React
    !a3(u3,k3); (do !u3;KK() or !k3;KK_P()))              [3]substrate

and KK_P() =
   (new u4@d4:Release new k4@r4:React
    new u5@d5:Release new k5@r5:React
    do !a4(u4,k4); (do !u4;KK_P() or !k4;KK())            [4]substrate
    or !a5(u5,k5); (do !u5;KK_P() or !k5;KK_PP()))        [5]substrate

and KK_PP() =
   (new u6@d6:Release new k6@r6:React
    do !a6(u6,k6); (do !u6;KK_PP() or !k6;KK_P())         [6]substrate
    or ?a7(u7,k7);                                        [7]kinase
    or ?a9(u9,k9);                                   ))   [9]kinase

and KKPse() =
   do ?a4(u4,k4);                                         [4]phtase
   or ?a6(u6,k6); (do ?u6;KKPse() or ?k6;KKPse())         [6]phtase

let K() =
   (new u7@
    !a7(u7,k7)                                            [7]substrate

and K_P() =
   (new u8@d8:Release new k8@r8:React
    new u9@d9:Release new k9@r9:React
    do !a8(u8,k8); (do !u8;K_P() or !k8;K())              [8]substrate
    or !a9(u9,k9); (do !u9;K_P() or !k9;K_PP()))          [9]substrate

and K_PP() =
   (new u10@d10:Release new k10@r10:React
    !a10(u10,k10); (do !u10;K_PP() or !k10;K_P()))        [10]substrate

and KPse() =
   do ?a8(u8,k8); (do ?u8;KPse() or ?k8;KPse())           [8]phtase
   or ?a10(u10,k10); (do ?u10;KPse() or ?k10;KPse())      [10]phtase
```

One process for each component (12) including enzymes, but not for complexes.

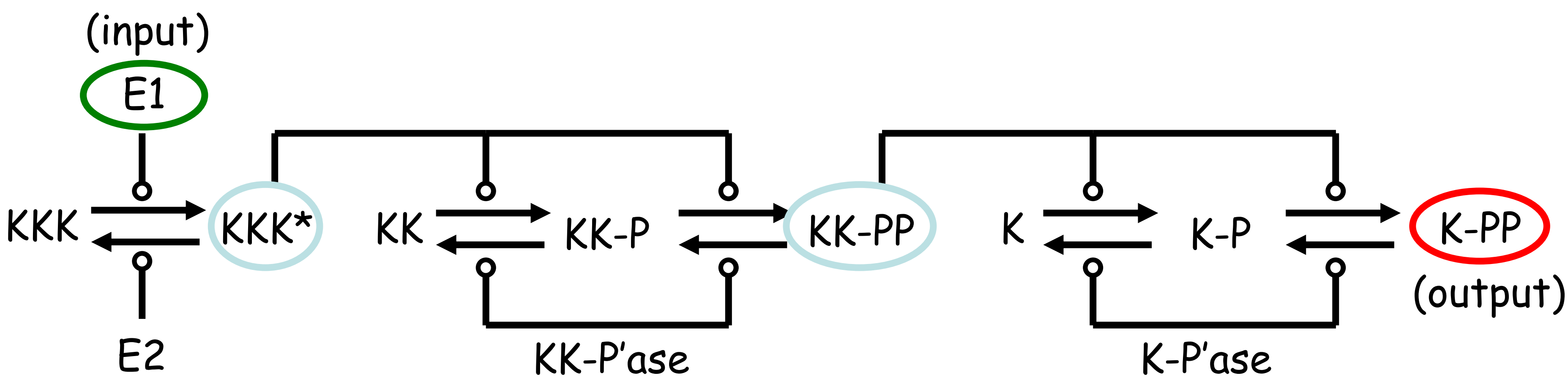
No need for conservation equations: implicit in “choice” operator in the calculus.

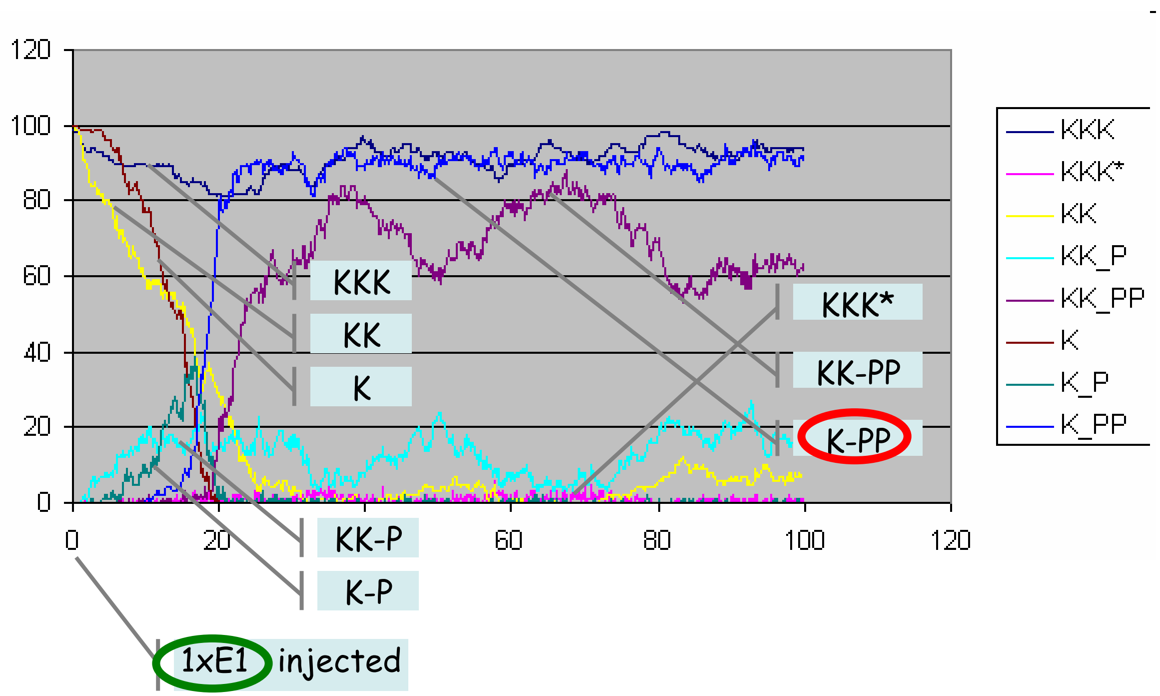
… globals

```
type Release = chan()
type React = chan()
type Bond = chan(Release,React)

new a1@1.0:Bond val d1=1.0 val r1=1.0
new a2@1.0:Bond val d2=1.0 val r2=1.0
new a3@1.0:Bond val d3=1.0 val r3=1.0
new a4@1.0:Bond val d4=1.0 val r4=1.0
new a5@1.0:Bond val d5=1.0 val r5=1.0
new a6@1.0:Bond val d6=1.0 val r6=1.0
new a7@1.0:Bond val d7=1.0 val r7=1.0
new a8@1.0:Bond val d8=1.0 val r8=1.0
new a9@1.0:Bond val d9=1.0 val r9=1.0
new a10@1.0:Bond val d10=1.0 val r10=1.0

…

run 100 of KKK()  run 100 of KK()   run 100 of K()
run 1 of E2()  run 1 of KKPse()  run 1 of KPse()
run 1 of E1()
```

$a_i(u_i,k_i)$: release (u_i@d_i) and react (k_i@r_i) channels passed over bond (a_i) channel. (No behavior attached to channels except interaction rate.)

MAPK Cascade Simulation in SPiM





1[st] stage:
 KKK* barely rises

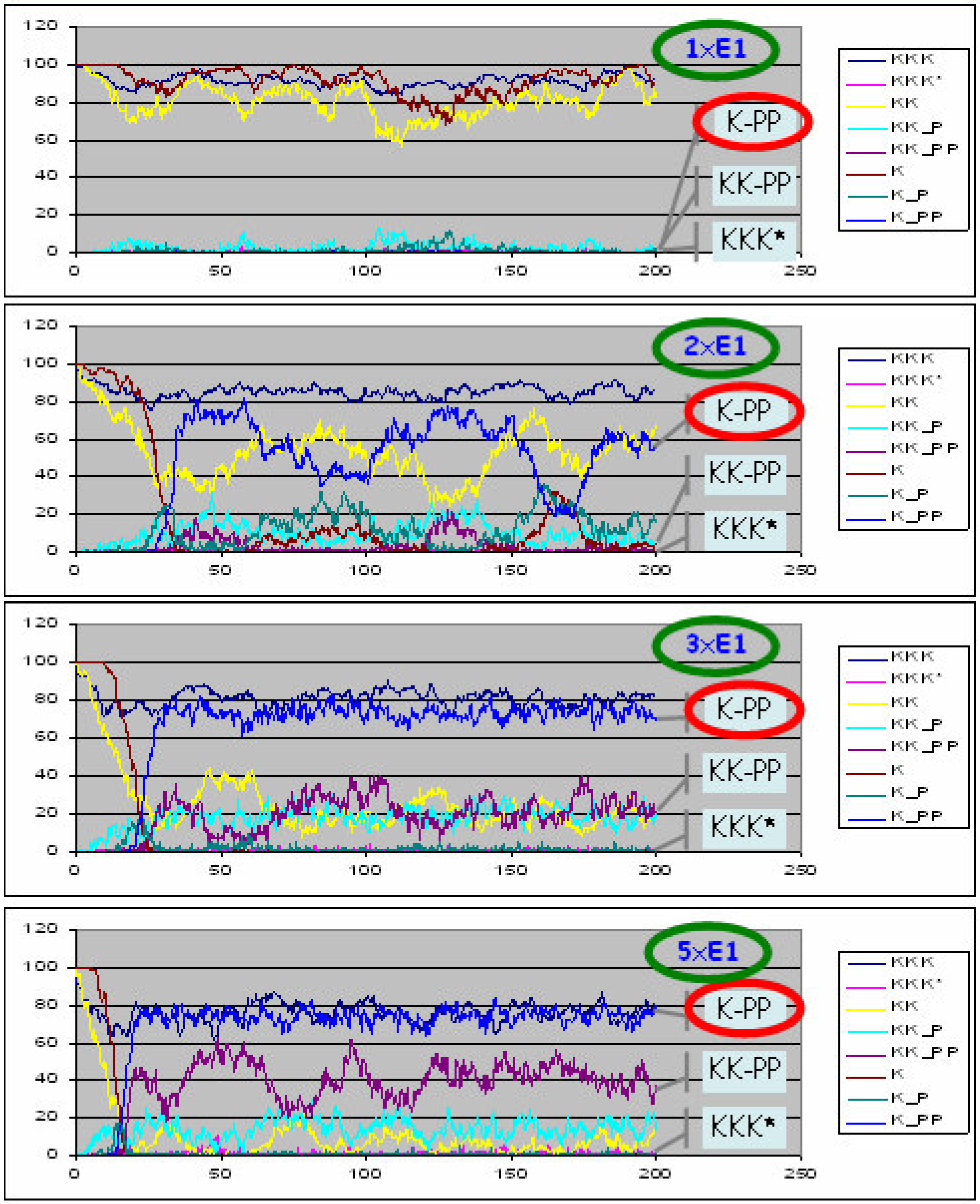
2[nd] stage:
 KK-PP rises, but is not stable

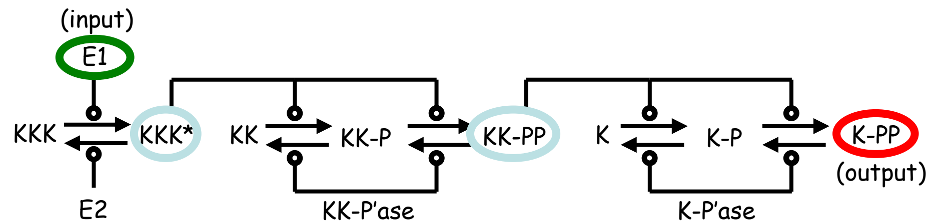
3[rd] stage:
 K-PP flips up to max
 even anticipating 2[nd] stage

All coefficients 1.0 !!!
100xKKK, 100xKK, 100xK,
5xE2, 5xKKPse, 5xKPse.

Input is 1x**E1**.
Output is 90x**K-PP** (ultrasensitivity).

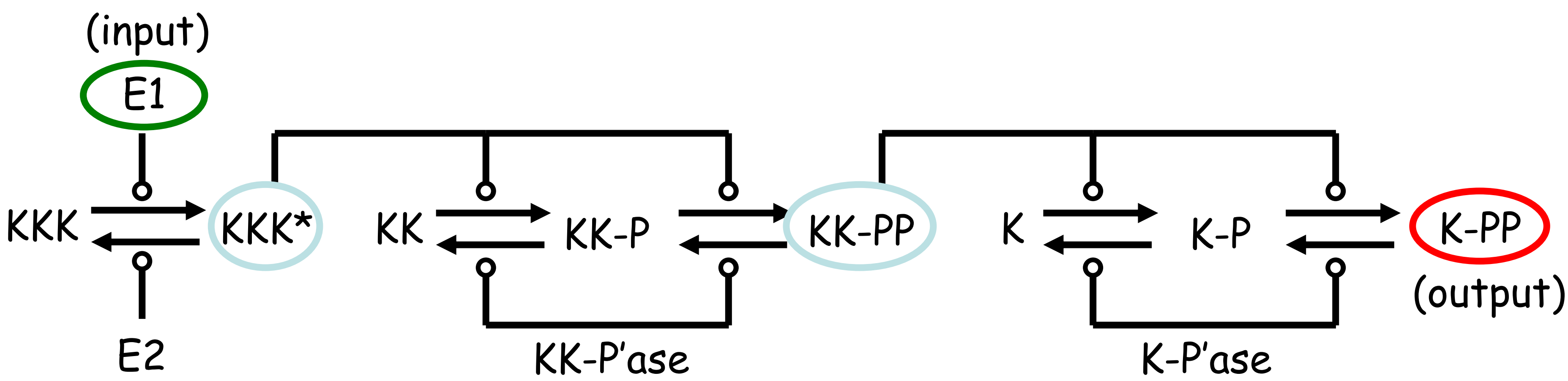
MAPK Cascade Simulation in SPiM

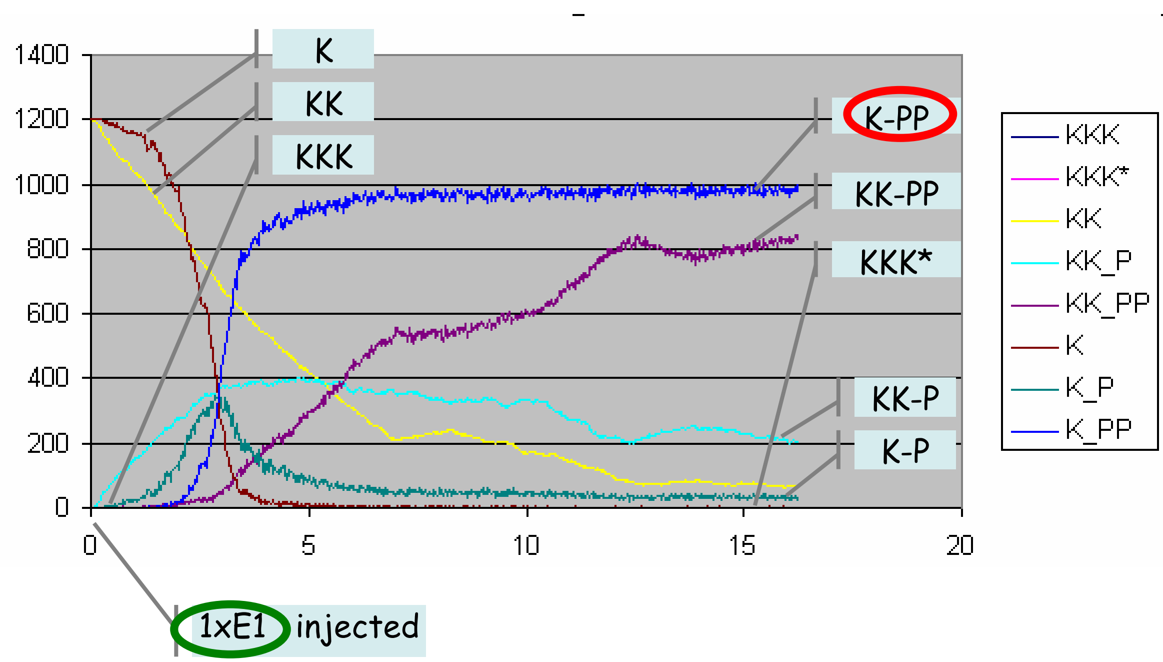




All coefficients 1.0 !!!
100xKKK, 100xKK, 100xK,
13xE2, 13xKKPse, 13xKPse.
nxE1 as indicated
(1xE1 is not sufficient to produce an output)

MAPK Cascade Simulation in SPiM





Rates and concentrations from paper:

1xE2 (0.3 nM)
1xKKPase (0.3 nM)
120xKPase (120 nM)
3xKKK (3 nM)
1200xKK (1.2 uM)
1200xK (1.2 uM)

dx = rx = 150, ax = 1
(Kmx = (dx + rx) / ax, Km = 300 nM)

1xE1