SUPPLEMENTAL MATERIAL

1. Sequestration Model Used for Calculation of Figs. 2 and 3

The sequestration model which is depicted in Fig. 1D includes the previously described standard MAPK model (‘basic model’) [1], and additionally takes Mek sequestration by un-/monophosphorylated Erk into account. The impact of such Mek sequestration was investigated by altering the sequestration factor S, which enters the association rates of the Mek sequestration complexes (Mek~Erk, Mek~pErk, pMek~Erk and pMek~pErk). The sequestration factor was set to S = 0 in Fig. 2A (black line) in order to simulate the behaviour of the basic model devoid of Mek sequestration. S was set to unity in Figs. 2A (grey line), 2B and 3 in order to Mek sequestration into account.

i) Differential Equations

pRaf_Mek'=kon_Raf_Mek*(Mektot-pRaf_Mek-pMek-pRaf_pMek-ppMek-Mek_Erk-pMek_Erk-ppMek_Erk-ppMek_pErk-Mek_pErk-pMek_pErk)*(pRaftot-pRaf_Mek-pRaf_pMek)-koff_Raf_Mek*pRaf_Mek-kcat_Raf_Mek*pRaf_Mek

pMek'=kcat_Raf_Mek*pRaf_Mek-kon_Raf_Mek*pMek*(pRaftot-pRaf_Mek-pRaf_pMek)+koff_Raf_Mek*pRaf_pMek+(Vmax_PPase_Mek*ppMek/(KM_PPase_Mek+ppMek))-(Vmax_PPase_Mek*pMek/(KM_PPase_Mek+pMek))-kon_Mek_Erk*S*pMek*(Erktot-Mek_Erk-pMek_Erk-ppMek_Erk-pErk-ppMek_pErk-ppErk-Mek_pErk-pMek_pErk)+koff_Mek_Erk*pMek_Erk-kon_Mek_Erk*S*pMek*pErk+koff_Mek_Erk*pMek_pErk

pRaf_pMek'=kon_Raf_Mek*pMek*(pRaftot-pRaf_Mek-pRaf_pMek)-koff_Raf_Mek*pRaf_pMek-kcat_Raf_Mek*pRaf_pMek

ppMek'=kcat_Raf_Mek*pRaf_pMek-(Vmax_PPase_Mek*ppMek/(KM_PPase_Mek+ppMek))-kon_Mek_Erk*ppMek*(Erktot-Mek_Erk-pMek_Erk-ppMek_Erk-pErk-ppMek_pErk-ppErk-Mek_pErk-pMek_pErk)+koff_Mek_Erk*ppMek_Erk+ kcat_Mek_Erk*ppMek_Erk-kon_Mek_Erk*ppMek*pErk+koff_Mek_Erk*ppMek_pErk+kcat_Mek_Erk*ppMek_pErk

ppMek_Erk'=kon_Mek_Erk*ppMek*(Erktot-Mek_Erk-pMek_Erk-ppMek_Erk-pErk-ppMek_pErk-ppErk-Mek_pErk-pMek_pErk)-koff_Mek_Erk*ppMek_Erk-kcat_Mek_Erk*ppMek_Erk

pErk'=kcat_Mek_Erk*ppMek_Erk-kon_Mek_Erk*ppMek*pErk+koff_Mek_Erk*ppMek_pErk+(Vmax_PPase_Erk*ppErk/(KM_PPase_Erk+ppErk))-(Vmax_PPase_Erk*pErk/(KM_PPase_Erk+pErk))-kon_Mek_Erk*S*(Mektot-pRaf_Mek-pMek-pRaf_pMek-ppMek-Mek_Erk-pMek_Erk-ppMek_Erk-ppMek_pErk-Mek_pErk-pMek_pErk)*pErk+koff_Mek_Erk*Mek_pErk-kon_Mek_Erk*S*pMek*pErk+koff_Mek_Erk*pMek_pErk

ppMek_pErk'=kon_Mek_Erk*ppMek*pErk-koff_Mek_Erk*ppMek_pErk-kcat_Mek_Erk*ppMek_pErk

ppErk'=kcat_Mek_Erk*ppMek_pErk-(Vmax_PPase_Erk*ppErk/(KM_PPase_Erk+ppErk))

Mek_Erk'=kon_Mek_Erk*S*(Mektot-pRaf_Mek-pMek-pRaf_pMek-ppMek-Mek_Erk-pMek_Erk-ppMek_Erk-ppMek_pErk-Mek_pErk-pMek_pErk)*(Erktot-Mek_Erk-pMek_Erk-ppMek_Erk-pErk-ppMek_pErk-ppErk-Mek_pErk-pMek_pErk)-koff_Mek_Erk*Mek_Erk

pMek_Erk'=kon_Mek_Erk*S*pMek*(Erktot-Mek_Erk-pMek_Erk-ppMek_Erk-pErk-ppMek_pErk-ppErk-Mek_pErk-pMek_pErk)-koff_Mek_Erk*pMek_Erk

Mek_pErk'=kon_Mek_Erk*S*(Mektot-pRaf_Mek-pMek-pRaf_pMek-ppMek-Mek_Erk-pMek_Erk-ppMek_Erk-ppMek_pErk-Mek_pErk-pMek_pErk)*pErk-koff_Mek_Erk*Mek_pErk

pMek_pErk'=kon_Mek_Erk*S*pMek*pErk-koff_Mek_Erk*pMek_pErk

ii) Mass Conservation Relationships

pRaf=(pRaftot-pRaf_Mek-pRaf_pMek)

Mek=(Mektot-pRaf_Mek-pMek-pRaf_pMek-ppMek-Mek_Erk-pMek_Erk-ppMek_Erk-ppMek_pErk-Mek_pErk-pMek_pErk)

Erk=(Erktot-Mek_Erk-pMek_Erk-ppMek_Erk-pErk-ppMek_pErk-ppErk-Mek_pErk-pMek_pErk)

iii) Kinetic Parameters / Initial Concentrations

pRaftot: varied for calculation of stimulus-response
Mektot = 1 (in Fig. 2 and 3B) or Mektot varied in Fig. 3A
Erktot = 1 (in Fig. 2 and 3B) or Erktot varied in Fig. 3A
Vmax_PPase_Mek = 0.001 (in Fig. 2 and 3A) or Vmax_PPase_Mek varied in Fig. 3B
Vmax_PPase_Erk = 0.04 (in Fig. 2 and 3A) or Vmax_PPase_Erk varied in Fig. 3B
S = 0 (Fig. 2A, black line) or S = 1 (Figs. 2A (grey line), 2B and 3)

kon_Raf_Mek = 0.65;
koff_Raf_Mek = 0.065;
kcat_Raf_Mek = 0.18;
KM_PPase_Mek = 0.1;
kon_Mek_Erk = 0.88;
koff_Mek_Erk = 0.088;
kcat_Mek_Erk = 0.22;
KM_PPase_Erk = 0.5;

2. Extended Sequestration Model Used for Calculation of Fig. 4

Figure 4 shows that significant competition between Raf and Erk for binding to Mek is required for bistability to arise from the proposed mechanism. Additionally, bistability requires that bisphosphorylated Erk hardly binds to Erk, i.e., that product inhibition does *not* occur in Mek-mediated Erk phosphorylation.

Non-competitive binding of Raf and Erk to Mek was simulated by considering ternary complexes between Raf, Mek and Erk, and Raf-mediated catalysis on these ternary complexes (see Fig. S1). More specifically, pRaf~Mek complexes with Erk (Fig. S1A), with pErk (Fig. S1B) and with ppErk (Fig. S1C) were taken into account. The association rate constants of these ternary complexes equal those of the binary Raf~Mek complexes ($k_{on,Raf\sim Mek}$) of the Mek~Erk complexes ($k_{on,Mek\sim Erk}$) multiplied by the competition-factor c. The degree of competition was varied by changing c. A value of c = 0 means pure competitive binding, while a value of c = 1 implies pure non-competitive binding. The dissociation rate constants and the catalytic rates of the ternary complexes equal those of the binary complexes ($k_{off,Raf\sim Mek}$, $k_{off,Mek\sim Erk}$ and $k_{cat,Raf\sim Mek}$).

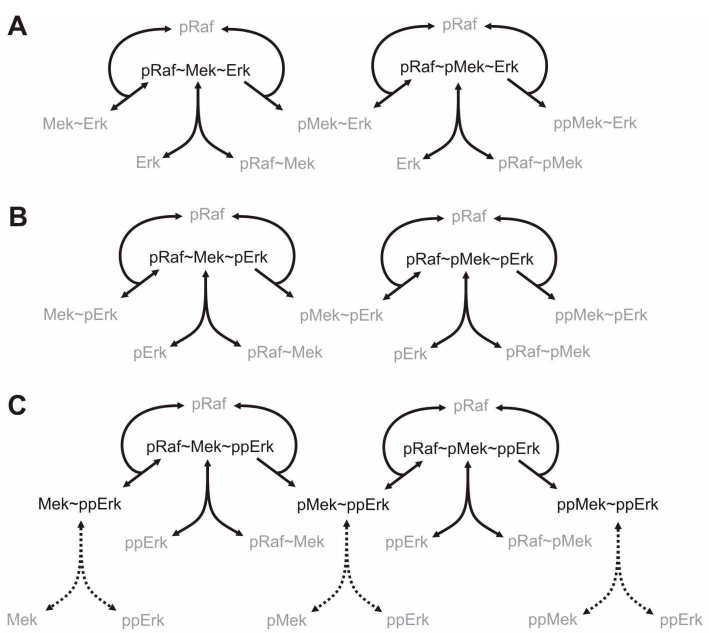

Figure S1

Product inhibition in Mek-mediated Erk phosphorylation was simulated by considering complexes of ppErk with Mek, pMek, and ppMek (see Fig. S1C). The association rates of these additional complexes equal those of the other Mek~Erk complexes ($k_{on,Mek\sim Erk}$) multiplied by the product-inhibition-factor p. The degree of product inhibition was varied by changing p. A value of p = 0 means no product inhibition, while a value of p = 1 implies strong product inhibition. The dissociation rate constants of the product inhibition complexes equal those of the other Mek~Erk complexes ($k_{off,Mek\sim Erk}$).

i) Differential Equations

pRaf_Mek'=+kon_Raf_Mek*(Mektot-pRaf_Mek-pMek-pRaf_pMek-ppMek-Mek_Erk-pMek_Erk-ppMek_Erk-ppMek_pErk-Mek_pErk-pMek_pErk-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk -ppMek_ppErk-pMek_ppErk-Mek_ppErk)*(pRaftot-pRaf_Mek-pRaf_pMek-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk)-koff_Raf_Mek*pRaf_Mek-kcat_Raf_Mek*pRaf_Mek-kon_Mek_Erk*C*pRaf_Mek*(Erktot-Mek_Erk-pMek_Erk-ppMek_Erk-pErk-ppMek_pErk-ppErk-Mek_pErk-pMek_pErk-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk-ppMek_ppErk-pMek_ppErk-Mek_ppErk)+koff_Mek_Erk*pRaf_Mek_Erk-kon_Mek_Erk*C*pRaf_Mek*pErk+koff_Mek_Erk*pRaf_Mek_pErk-kon_Mek_Erk*C*pRaf_Mek*ppErk+koff_Mek_Erk*pRaf_Mek_ppErk

pMek'=+kcat_Raf_Mek*pRaf_Mek-kon_Raf_Mek*pMek*(pRaftot-pRaf_Mek-pRaf_pMek-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk)+koff_Raf_Mek*pRaf_pMek+(Vmax_PPase_Mek*ppMek/(KM_PPase_Mek+ppMek))-(Vmax_PPase_Mek*pMek/(KM_PPase_Mek+pMek))-kon_Mek_Erk*S*pMek*(Erktot-Mek_Erk-pMek_Erk-ppMek_Erk-pErk-ppMek_pErk-ppErk-Mek_pErk-pMek_pErk-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk-ppMek_ppErk-pMek_ppErk-Mek_ppErk)+koff_Mek_Erk*pMek_Erk-kon_Mek_Erk*S*pMek*pErk+koff_Mek_Erk*pMek_pErk-kon_Mek_Erk*P*ppErk*pMek+koff_Mek_Erk*pMek_ppErk

pRaf_pMek'=+kon_Raf_Mek*pMek*(pRaftot-pRaf_Mek-pRaf_pMek-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk)-koff_Raf_Mek*pRaf_pMek-kcat_Raf_Mek*pRaf_pMek-kon_Mek_Erk*C*pRaf_pMek*(Erktot-Mek_Erk-pMek_Erk-ppMek_Erk-pErk-ppMek_pErk-ppErk-Mek_pErk-pMek_pErk-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk-ppMek_ppErk-pMek_ppErk-Mek_ppErk)+koff_Mek_Erk*pRaf_pMek_Erk-kon_Mek_Erk*C*pRaf_pMek*pErk+koff_Mek_Erk*pRaf_pMek_pErk-kon_Mek_Erk*C*pRaf_pMek*ppErk+koff_Mek_Erk*pRaf_pMek_ppErk

ppMek'=+kcat_Raf_Mek*pRaf_pMek-(Vmax_PPase_Mek*ppMek/(KM_PPase_Mek+ppMek))-kon_Mek_Erk*ppMek*(Erktot-Mek_Erk-pMek_Erk-ppMek_Erk-pErk-ppMek_pErk-ppErk-Mek_pErk-pMek_pErk-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk-ppMek_ppErk-pMek_ppErk-Mek_ppErk)+koff_Mek_Erk*ppMek_Erk+kcat_Mek_Erk*ppMek_Erk-kon_Mek_Erk*ppMek*pErk+koff_Mek_Erk*ppMek_pErk+kcat_Mek_Erk*ppMek_pErk-kon_Mek_Erk*P*ppErk*ppMek+koff_Mek_Erk*ppMek_ppErk

ppMek_Erk'=+kon_Mek_Erk*ppMek*(Erktot-Mek_Erk-pMek_Erk-ppMek_Erk-pErk-ppMek_pErk-ppErk-Mek_pErk-pMek_pErk-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk-ppMek_ppErk-pMek_ppErk-Mek_ppErk)-koff_Mek_Erk*ppMek_Erk-kcat_Mek_Erk*ppMek_Erk+kcat_Raf_Mek*pRaf_pMek_Erk

pErk'=+kcat_Mek_Erk*ppMek_Erk-kon_Mek_Erk*ppMek*pErk+koff_Mek_Erk*ppMek_pErk+(Vmax_PPase_Erk*ppErk/(KM_PPase_Erk+ppErk))-(Vmax_PPase_Erk*pErk/(KM_PPase_Erk+pErk))-kon_Mek_Erk*S*(Mektot-pRaf_Mek-pMek-pRaf_pMek-ppMek-Mek_Erk-pMek_Erk-ppMek_Erk-ppMek_pErk-Mek_pErk-pMek_pErk-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk-ppMek_ppErk-pMek_ppErk-Mek_ppErk)*pErk+koff_Mek_Erk*Mek_pErk-kon_Mek_Erk*S*pMek*pErk+ koff_Mek_Erk*pMek_pErk-kon_Mek_Erk*C*pRaf_Mek*pErk+koff_Mek_Erk*pRaf_Mek_pErk-kon_Mek_Erk*C*pRaf_pMek*pErk+koff_Mek_Erk*pRaf_pMek_pErk

ppMek_pErk'=+kon_Mek_Erk*ppMek*pErk-koff_Mek_Erk*ppMek_pErk-kcat_Mek_Erk*ppMek_pErk+kcat_Raf_Mek*pRaf_pMek_pErk

ppErk'=+kcat_Mek_Erk*ppMek_pErk-(Vmax_PPase_Erk*ppErk/(KM_PPase_Erk+ppErk))-kon_Mek_Erk*C*pRaf_Mek*ppErk+koff_Mek_Erk*pRaf_Mek_ppErk-kon_Mek_Erk*C*pRaf_pMek*ppErk+ koff_Mek_Erk*pRaf_pMek_ppErk-kon_Mek_Erk*P*ppErk*(Mektot-pRaf_Mek-pMek-pRaf_pMek-ppMek-Mek_Erk-pMek_Erk-ppMek_Erk-ppMek_pErk-Mek_pErk-pMek_pErk-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk-ppMek_ppErk-pMek_ppErk-Mek_ppErk)+koff_Mek_Erk*Mek_ppErk-kon_Mek_Erk*P*ppErk*pMek+koff_Mek_Erk*pMek_ppErk-kon_Mek_Erk*P*ppErk*ppMek+koff_Mek_Erk*ppMek_ppErk

Mek_Erk'=+kon_Mek_Erk*S*(Mektot-pRaf_Mek-pMek-pRaf_pMek-ppMek-Mek_Erk-pMek_Erk-ppMek_Erk-ppMek_pErk-Mek_pErk-pMek_pErk-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk-ppMek_ppErk-pMek_ppErk-Mek_ppErk)*(Erktot-Mek_Erk-pMek_Erk-ppMek_Erk-pErk-ppMek_pErk-ppErk-Mek_pErk-pMek_pErk-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk-ppMek_ppErk-pMek_ppErk-Mek_ppErk)-koff_Mek_Erk*Mek_Erk-kon_Raf_Mek*C*Mek_Erk*(pRaftot-pRaf_Mek-pRaf_pMek-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk)+koff_Raf_Mek*pRaf_Mek_Erk pMek_Erk'=+kon_Mek_Erk*S*pMek*(Erktot-Mek_Erk-pMek_Erk-ppMek_Erk-pErk-ppMek_pErk-ppErk-Mek_pErk-pMek_pErk-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk-ppMek_ppErk-pMek_ppErk-Mek_ppErk)-koff_Mek_Erk*pMek_Erk+kcat_Raf_Mek*pRaf_Mek_Erk-kon_Raf_Mek*C*pMek_Erk*(pRaftot-pRaf_Mek-pRaf_pMek-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk)+koff_Raf_Mek*pRaf_pMek_Erk

Mek_pErk'=+kon_Mek_Erk*S*(Mektot-pRaf_Mek-pMek-pRaf_pMek-ppMek-Mek_Erk-pMek_Erk-ppMek_Erk-ppMek_pErk-Mek_pErk-pMek_pErk-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk-ppMek_ppErk-pMek_ppErk-Mek_ppErk)*pErk-koff_Mek_Erk*Mek_pErk-kon_Raf_Mek*C*Mek_pErk*(pRaftot-pRaf_Mek-pRaf_pMek-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk)+koff_Raf_Mek*pRaf_Mek_pErk

pMek_pErk'=+kon_Mek_Erk*S*pMek*pErk-koff_Mek_Erk*pMek_pErk+kcat_Raf_Mek*pRaf_Mek_pErk-kon_Raf_Mek*C*pMek_pErk*(pRaftot-pRaf_Mek-pRaf_pMek-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk)+koff_Raf_Mek*pRaf_pMek_pErk

pRaf_Mek_Erk'=+kon_Raf_Mek*C*Mek_Erk*(pRaftot-pRaf_Mek-pRaf_pMek-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk)-koff_Raf_Mek*pRaf_Mek_Erk+kon_Mek_Erk*C*pRaf_Mek*(Erktot-Mek_Erk-pMek_Erk-ppMek_Erk-pErk-ppMek_pErk-ppErk-Mek_pErk-pMek_pErk-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk-ppMek_ppErk-pMek_ppErk-Mek_ppErk)-koff_Mek_Erk*pRaf_Mek_Erk-kcat_Raf_Mek*pRaf_Mek_Erk

pRaf_pMek_Erk'=+kon_Raf_Mek*C*pMek_Erk*(pRaftot-pRaf_Mek-pRaf_pMek-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk)-koff_Raf_Mek*pRaf_pMek_Erk+ kon_Mek_Erk*C*pRaf_pMek*(Erktot-Mek_Erk-pMek_Erk-ppMek_Erk-pErk-ppMek_pErk-ppErk-Mek_pErk-pMek_pErk-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk-ppMek_ppErk-pMek_ppErk-Mek_ppErk)-koff_Mek_Erk*pRaf_pMek_Erk-kcat_Raf_Mek*pRaf_pMek_Erk

pRaf_Mek_pErk'=+kon_Raf_Mek*C*Mek_pErk*(pRaftot-pRaf_Mek-pRaf_pMek-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk)-koff_Raf_Mek*pRaf_Mek_pErk+ kon_Mek_Erk*C*pRaf_Mek*pErk-koff_Mek_Erk*pRaf_Mek_pErk-kcat_Raf_Mek*pRaf_Mek_pErk

pRaf_pMek_pErk'=+kon_Raf_Mek*C*pMek_pErk*(pRaftot-pRaf_Mek-pRaf_pMek-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk)-koff_Raf_Mek*pRaf_pMek_pErk+ kon_Mek_Erk*C*pRaf_pMek*pErk-koff_Mek_Erk*pRaf_pMek_pErk-kcat_Raf_Mek*pRaf_pMek_pErk

pRaf_Mek_ppErk'=+kon_Raf_Mek*C*Mek_ppErk*(pRaftot-pRaf_Mek-pRaf_pMek-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk)-koff_Raf_Mek*pRaf_Mek_ppErk+ kon_Mek_Erk*C*pRaf_Mek*ppErk-koff_Mek_Erk*pRaf_Mek_ppErk-kcat_Raf_Mek*pRaf_Mek_ppErk

pRaf_pMek_ppErk'=+kon_Raf_Mek*C*pMek_ppErk*(pRaftot-pRaf_Mek-pRaf_pMek-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk)-koff_Raf_Mek*pRaf_pMek_ppErk+ kon_Mek_Erk*C*pRaf_pMek*ppErk-koff_Mek_Erk*pRaf_pMek_ppErk-kcat_Raf_Mek*pRaf_pMek_ppErk

Mek_ppErk'=-kon_Raf_Mek*C*Mek_ppErk*(pRaftot-pRaf_Mek-pRaf_pMek-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk)+koff_Raf_Mek*pRaf_Mek_ppErk+kon_Mek_Erk*P*ppErk*(Mektot-pRaf_Mek-pMek-pRaf_pMek-ppMek-Mek_Erk-pMek_Erk-ppMek_Erk-ppMek_pErk-Mek_pErk-pMek_pErk-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk-ppMek_ppErk-pMek_ppErk-Mek_ppErk)-koff_Mek_Erk*Mek_ppErk

pMek_ppErk'=+kcat_Raf_Mek*pRaf_Mek_ppErk-kon_Raf_Mek*C*pMek_ppErk*(pRaftot-pRaf_Mek-pRaf_pMek-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk)+koff_Raf_Mek*pRaf_pMek_ppErk+ kon_Mek_Erk*P*ppErk*pMek-koff_Mek_Erk*pMek_ppErk

ppMek_ppErk'=+kcat_Raf_Mek*pRaf_pMek_ppErk+kon_Mek_Erk*P*ppErk*ppMek-koff_Mek_Erk*ppMek_ppErk

ii) Mass Conservation Relationships

pRaf=(pRaftot-pRaf_Mek-pRaf_pMek-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk)

Mek=(Mektot-pRaf_Mek-pMek-pRaf_pMek-ppMek-Mek_Erk-pMek_Erk-ppMek_Erk-ppMek_pErk-Mek_pErk-pMek_pErk-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk-ppMek_ppErk-pMek_ppErk-Mek_ppErk)

Erk=(Erktot-Mek_Erk-pMek_Erk-ppMek_Erk-pErk-ppMek_pErk-ppErk-Mek_pErk-pMek_pErk-pRaf_Mek_Erk-pRaf_pMek_Erk-pRaf_Mek_pErk-pRaf_pMek_pErk-pRaf_Mek_ppErk-pRaf_pMek_ppErk-ppMek_ppErk-pMek_ppErk-Mek_ppErk)

iii) Kinetic Parameters / Initial Concentrations

pRaftot: varied for calculation of stimulus-response
C (= Competition factor) varied
P (= Product inhibition factor) varied
S = 1 (= Sequestration factor)
kon_Raf_Mek = 0.65
koff_Raf_Mek = 0.065
kcat_Raf_Mek = 0.18
Vmax_PPase_Mek = 0.001
KM_PPase_Mek = 0.1
kon_Mek_Erk = 0.66
koff_Mek_Erk = 0.066
kcat_Mek_Erk = 0.22
Vmax_PPase_Erk = 0.04
KM_PPase_Erk = 0.5

3. Model Used for Calculation of Fig. 5

Figure 5 shows that the bistability mechanism proposed in this paper synergizes with that proposed by Markevich et al. [2], which arises from enzyme depletion effects in Erk double phosphorylation. The models used for calculation of Fig. 5 exhibit the topology depicted in Fig. 1D.

The models used for the calculation of the blue and red lines in Fig. 5 exhibit positive cooperativity in Mek-mediated Erk phosphorylation, so that the (isolated) Erk cycle exhibits weak bistability [3]. More specifically, it was assumed that the first and the second phosphorylation steps of ppMek-mediated Erk phosphorylation proceed with different kinetic constants. More specifically, we assume relatively high affinity (i.e., a low $K_{M,M1}$), but slow catalysis (i.e., a low $k_{cat,M1}$) for the first phosphorylation step. By contrast, low affinity (i.e., a high $K_{M,M2}$), but very fast catalysis (i.e., a high $k_{cat,M2}$) was assumed for the second phosphorylation step. No phosphorylation-site-specific kinetic differences in phosphatase-mediated Erk dephosphorylation were assumed (see maximal velocities, $V_{m,PP1} = V_{m,PP2}$, and Michaelis-Menten constants, $K_{M,PP1} = K_{M,PP2}$ below), but dephosphorylation was modelled by a more complex Michaelis-Menten Mechanism, which takes enzyme depletion effects into account [2]

The blue line in Fig. 5 shows that relatively narrow range of bistability arises from the Markevich mechanism alone. In these simulations, we switched off the feedback mechanism proposed in this paper (i.e., Mek sequestration in Raf-inaccessible complexes) by abolishing the formation unproductive sequestration complexes (Mek~Erk, pMek~Erk, Mek~pErk and pMek~pErk). This was accomplished by setting $k_{onMek\sim Erk} = 0$.

The red line in Fig. 5 demonstrates that the feedback mechanism proposed in this paper brings about a broad range of bistability when combined with that proposed by Markevich et al. [2]. These simulations correspond to a system, which includes Mek sequestration into Raf-inaccessible complexes (black and grey arrows in Fig. 1D). The association rate constant of these complexes was set to the measured value $k_{onMek\sim Erk} = 0.88$ (Table 1). This value implies that the affinity of the ppMek~Erk-complex, which is the enzymatic intermediate on the first Erk phosphorylation site, equals that of the unproductive sequestration complexes (Mek~Erk, pMek~Erk, Mek~pErk and pMek~pErk).

The green line in Fig. 5 shows that relatively narrow range of bistability arises from the feedback mechanism proposed in this paper. Theses simulations correspond to a sequestration model (black and grey arrows in Fig. 1D), where positive cooperativity and enzyme depletion effects were eliminated from the Erk cycle. This was done by assuming the same catalytic rate constant for the first and the second steps of Mek-mediated Erk phosphorylation (kcatM1 = kcatM2 = 1).

i) Differential Equations

pRaf_Mek' = kon_Raf_Mek*(Mektot-pRaf_Mek-pMek-pRaf_pMek-ppMek-Mek_Erk-pMek_Erk-ppMek_Erk-ppMek_pErk-Mek_pErk-pMek_pErk)*(pRaftot-pRaf_Mek-pRaf_pMek)-koff_Raf_Mek*pRaf_Mek-kcat_Raf_Mek*pRaf_Mek

pMek' = kcat_Raf_Mek*pRaf_Mek-kon_Raf_Mek*pMek*(pRaftot-pRaf_Mek-pRaf_pMek)+koff_Raf_Mek*pRaf_pMek+(Vmax_PPase_Mek*ppMek/(KM_PPase_Mek+ppMek))-(Vmax_PPase_Mek*pMek/(KM_PPase_Mek+pMek))-kon_Mek_Erk*pMek*(Erktot-Mek_Erk-pMek_Erk-ppMek_Erk-pErk-ppMek_pErk-ppErk-Mek_pErk-pMek_pErk)+koff_Mek_Erk*pMek_Erk-kon_Mek_Erk*pMek*pErk+koff_Mek_Erk*pMek_pErk

pRaf_pMek'=kon_Raf_Mek*pMek*(pRaftot-pRaf_Mek-pRaf_pMek)-koff_Raf_Mek*pRaf_pMek-kcat_Raf_Mek*pRaf_pMek

ppMek'=+kcat_Raf_Mek*pRaf_pMek-(Vmax_PPase_Mek*ppMek/(KM_PPase_Mek+ppMek))-((koff_Mek_Erk+kcatM1)/KMM1*ppMek*(Erktot-Mek_Erk-pMek_Erk-ppMek_Erk-pErk-ppMek_pErk-ppErk-Mek_pErk-pMek_pErk))+koff_Mek_Erk*ppMek_Erk+kcatM1*ppMek_Erk-((koff_Mek_Erk+kcatM2)/KMM2*ppMek*pErk)+koff_Mek_Erk*ppMek_pErk+kcatM2*ppMek_pErk

ppMek_Erk'=((koff_Mek_Erk+kcatM1)/KMM1*ppMek*(Erktot-Mek_Erk-pMek_Erk-ppMek_Erk-pErk-ppMek_pErk-ppErk-Mek_pErk-pMek_pErk))-koff_Mek_Erk*ppMek_Erk-kcatM1*ppMek_Erk

pErk'=kcatM1*ppMek_Erk-((koff_Mek_Erk+kcatM2)/KMM2*ppMek*pErk)+koff_Mek_Erk*ppMek_pErk+(VmPP1*ppErk/KMPP1/(1+ppErk/KMPP1+pErk/KMPP2))-(VmPP2*pErk/KMPP2/(1+pErk/KMPP2+ppErk/KMPP1))-kon_Mek_Erk*(Mektot-pRaf_Mek-pMek-pRaf_pMek-ppMek-Mek_Erk-pMek_Erk-ppMek_Erk-ppMek_pErk-Mek_pErk-pMek_pErk)*pErk+koff_Mek_Erk*Mek_pErk-kon_Mek_Erk*pMek*pErk+koff_Mek_Erk*pMek_pErk

ppMek_pErk'=((koff_Mek_Erk+kcatM2)/KMM2*ppMek*pErk)-koff_Mek_Erk*ppMek_pErk-kcatM2*ppMek_pErk

ppErk'=+kcatM2*ppMek_pErk-(VmPP1*ppErk/KMPP1/(1+ppErk/KMPP1+pErk/KMPP2))

Mek_Erk'=kon_Mek_Erk*(Mektot-pRaf_Mek-pMek-pRaf_pMek-ppMek-Mek_Erk-pMek_Erk-ppMek_Erk-ppMek_pErk-Mek_pErk-pMek_pErk)*(Erktot-Mek_Erk-pMek_Erk-ppMek_Erk-pErk-ppMek_pErk-ppErk-Mek_pErk-pMek_pErk)-koff_Mek_Erk*Mek_Erk

pMek_Erk'=kon_Mek_Erk*pMek*(Erktot-Mek_Erk-pMek_Erk-ppMek_Erk-pErk-ppMek_pErk-ppErk-Mek_pErk-pMek_pErk)-koff_Mek_Erk*pMek_Erk

Mek_pErk'=kon_Mek_Erk*(Mektot-pRaf_Mek-pMek-pRaf_pMek-ppMek-Mek_Erk-pMek_Erk-ppMek_Erk-ppMek_pErk-Mek_pErk-pMek_pErk)*pErk-koff_Mek_Erk*Mek_pErk

pMek_pErk'=kon_Mek_Erk*pMek*pErk-koff_Mek_Erk*pMek_pErk

ii) Mass Conservation Relationships

pRaf=(pRaftot-pRaf_Mek-pRaf_pMek)
Mek=(Mektot-pRaf_Mek-pMek-pRaf_pMek-ppMek-Mek_Erk-pMek_Erk-ppMek_Erk-ppMek_pErk-Mek_pErk-pMek_pErk)
Erk=(Erktot-Mek_Erk-pMek_Erk-ppMek_Erk-pErk-ppMek_pErk-ppErk-Mek_pErk-pMek_pErk)

iii) Kinetic Parameters / Initial Concentrations

pRaftot: varied for calculation of stimulus-response
Mektot = 1
Erktot = 10
kon_Raf_Mek = 0.65
koff_Raf_Mek = 0.065
kcat_Raf_Mek = 0.18
Vmax_PPase_Mek = 0.001
KM_PPase_Mek = 0.01
kon_Mek_Erk = 0.88 (red and green lines) or kon_Mek_Erk = 0 (blue line)
koff_Mek_Erk = 0.1
kcatM1 = 0.2 (blue and red lines) or kcatM1 = 1 (green line)
KMM1 = 0.35
kcatM2 = 10 (blue and red lines) or kcatM2 = 1 (green line)
KMM2 = 1
VmPP1 = 0.4
VmPP2 = 0.4
KMPP1 = 0.5
KmPP2 = 0.5

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