1. Mathematical model

The equations for a two-member scaffold are given below (the case of a three-member scaffold is modeled similarly, with the number of equations describing scaffold complexes tripled because of increased combinatorial possibilities for complex formation. Intermediate molecular complexes are indicated by parentheses. Asterisks denote activation (MAPKKK) and phosphorylation (MAPKK and MAPK).

Activation of MAPKK (designated as RAF for convenience):

RAF is assumed to be activated by an enzyme upstream in the pathway (designated as RAF-K) and deactivated by a phosphatase RAF-P.

$$d/dt\ RAF[t] = -a_1\ RAF[t]\ (RAF\text{-}K_{tot} — (RAF\ RAF\text{-}K)[t]) + d_1(RAF\ RAF\text{-}K)[t] + k_2\ (RAF^*\ RAF\text{-}P)[t],$$

$$d/dt\ (RAF\ RAF\text{-}K\text{-}GTP)[t] = a_1\ RAF[t]\ (RAF\text{-}\ K_{tot} — (RAF\ RAF\text{-}K\ [t]) - (d_1 + k_1)\ (RAF\ RAF\text{-}K)[t],$$

$$d/dt\ RAF^*[t] = -\ a_2\ RAF^*[t]\ (RAF\text{-}P_{tot} - (RAF^*\ RAF\text{-}P)[t]) + d_2\ (RAF^*\ RAF\text{-}P)[t] + k_1\ (RAF\ RAF\text{-}K)[t] + (k_3 + d_3)\ (MEK\ RAF^*)[t] - a_3\ RAF^*[t]\ MEK[t] + (k_5 + d_5)\ (MEK^*RAF^*)[t] - a_5\ MEK^*[t]\ RAF^*[t],$$

$$d/dt\ (RAF^*\ RAF\text{-}K)[t] = a_2\ RAF^*[t]\ (RAF\text{-}P_{tot} - (RAF^*\ RAF\text{-}P)[t]) - (d_2 + k_2)\ (RAF^*\ RAF\text{-}P)[t]$$

Activation of MAPKK (designated as MEK for convenience):

MEK is assumed to be phosphorylated by RAF* and dephosphorylated by MEK-P. Only the inactive form of MEK is assumed to associate with the scaffold.

$$d/dt\ MEK[t] = -\ a_3\ MEK[t]\ RAF^*[t] + d_3\ (MEK\ RAF^*)[t] + k_4\ (MEK^*\ MEK\text{-}P)[t] + of_1\ (C_2[t] + C_6[t] + C_9[t]) - on_1\ MEK[t]\ (C_1[t] + C_4[t] + C_5[t]),$$

$$d/dt\ (MEK\ RAF^*)[t] = a_3\ MEK[t]\ RAF^*[t] - (d_3 + k_3)\ (MEK\ RAF^*)[t],$$

$$d/dt\ MEK^*[t] = -\ a_4\ MEK^*[t]\ ]\ (MEK\text{-}P_{tot} - (MEK^*\ MEK\text{-}P)[t] — (MEK^{**}\ MEK\text{-}P)[t]) + d_4\ (MEK^*\ MEK\text{-}P)[t] + k_3\ (MEK\ RAF^*)[t] + k_6\ (MEK^{**}\ MEK\text{-}P)[t] + d_5\ (MEK^*RAF^*)[t] - a_5\ MEK^*[t]\ RAF^*[t],$$

$$d/dt\ (MEK^*\ MEK\text{-}P)[t] = a_4\ MEK^*[t]\ (MEK\text{-}P_{tot} - (MEK^*\ MEK\text{-}P)[t] — (MEK^{**}\ MEK\text{-}P)[t]) — (d_4 + k_4)\ (MEK^*\ MEK\text{-}P)[t],$$

$$d/dt\ (MEK^*RAF^*)[t] = a_5\ MEK^*[t]\ RAF^*[t] - (d_5 + k_5)\ (MEK^*RAF^*)[t],$$

$$d/dt\ MEK^{**}[t] = k_5\ (MEK^*RAF^*)[t] - a_6\ MEK^{**}[t]\ (MEK\text{-}P_{tot} - (MEK^*\ MEK\text{-}P)[t] — (MEK^{**}\ MEK\text{-}P)[t]) + d_6\ (MEK^{**}\ MEK\text{-}P)[t] - a_7\ MEK^{**}[t]\ MAPK[t] + (d_7 + k_7)\ (MAPK\ MEK^{**})[t] + (d_9 + k_9)\ (MAPK^*\ MEK^{**})[t] - a_9\ MAPK^*[t]\ MEK^{**}[t] + of_3\ (C_3[t] + C_7[t] + C_8[t]),$$

$$d/dt\ (MEK^{**}\ MEK\text{-}P)[t] = a_6\ MEK^{**}[t]\ (MEK\text{-}P_{tot} - (MEK^*\ MEK\text{-}P)[t] — (MEK^{**}\ MEK\text{-}P)[t]) — (d_6 + k_6)\ (MEK^{**}\ MEK\text{-}P)[t]$$

Activation of MAPK:

MAPK is assumed to be phosphorylated by MEK** and dephosphorylated by MAPK-P. Only the inactive form of MAPK is assumed to associate with the scaffold.

$$d/dt\ MAPK[t] = -\ a_7\ MAPK[t]\ MEK^{**}[t] + d_7\ (MAPK\ MEK^{**})[t] + k_8\ (MAPK^*\ MAPK\ Pase)[t] + of_2\ (C_4[t] + C_6[t] + C_7[t]) - on_2\ MAPK[t]\ (C_1[t] + C_2[t] + C_3[t]),$$

d/dt (MAPK MEK**) [t] = a_7 MAPK[t] MEK** [t] - (d_7 + k_7) (MAPK MEK**) [t],

d/dt MAPK*[t] = k7 (MAPK MEK**) [t] - a_8 MAPK*[t] (MAPK-P_{tot} - (MAPK* MAPK-P)[t] — (MAPK** MAPK-P)[t]) + d_8 (MAPK* MAPK-P) [t] - a_9 MAPK*[t] MEK**[t] + d_9 (MAPK*MEK**)[t] + k_{10} (MAPK** MAPK-P)[t],

d/dt (MAPK*MEK**)[t] = a_9 MAPK*[t] MEK**[t] - (d_9 + k_9) (MAPK*MEK**)[t],

d/dt MAPK**[t] = - a_{10} MAPK**[(MAPK-P_{tot} - (MAPK* MAPK-P)[t] - (MAPK** MAPK-P)[t]) + d_{10} (MAPK** MAPK-P)[t] + k_9 (MAPK*MEK**)[t] + of_4 (C_5[t] + C_8[t] + C_9[t]),

d/dt (MAPK* MAPK-P)[t] = a_8 MAPK*[t] (MAPK-P_{tot} - (MAPK* MAPK-P)[t] — (MAPK** MAPK-P)[t]) - (d_8 + k_8) (MAPK* MAPK-P)[t],

d/dt (MAPK** MAPK-P)[t] = a_{10} MAPK**[(MAPK-P_{tot} - (MAPK* MAPK-P)[t] — (MAPK** MAPK-P)[t]) - (d_{10} + k_{10}) (MAPK** MAPK-P)[t]

Scaffold complexes:

It is assumed that MEK can be activated by RAF* when bound to the scaffold with the reaction constant kr_1 = k_5. It is also assumed that MAPK can be activated within the scaffold complex by MEK** with the reaction constant kr_2 = k_9. Association of MEK*, MEK**, MAPK* and MAPK** with the scaffold is treated as negligible. This assumption was relaxed in control simulations as explained in the paper.

d/dt C_1[t] = -C_1[t] (on_1 MEK[t] + on_2 MAPK[t]) + of_1 C_2 [t] + of_2 C_4[t] + of_3 C_3[t] + of_4 C_5[t],

d/dt C_2[t] = on_1 C_1[t] MEK[t] + of_2 C_6[t] + of_4 C_9[t] - (of_1 + on_2 MAPK[t]) C_2[t] - kr_1 C_2[t] RAF*[t],

d/dt C_3[t] = - on_2 C_3[t] MAPK[t] + of_2 C_7[t] - of_3 C_3[t] + of_4 C_8[t] + kr_1 C_2[t] RAF[t],

d/dt C_4[t] = of_1 C_6[t] + on_2 MAPK[t] C_1[t] + of_3 C_7[t] - (of_2 + on_1 MEK[t]) C_4[t],

d/dt C_5[t] = of_1 C_9[t] + of_3 C_8[t] - (on_1 MEK[t] + of_4) C_5[t],

d/dt C_6[t] = on_1 MEK[t] C_4[t] + on_2 MAPK[t] C_2[t] - (of_1 + of_2) C_6 [t] - kr_1 C_6[t] RAF[t],

d/dt C_7[t] = - of_3 C_7[t] + on_2 MAPK[t] C_3[t] - kr_2 C_7[t] + kr_1 C_6[t] RAF[t] - of_2 C_7[t],

d/dt C_8[t] = kr_2 C_7[t] - (of_3 + of_4) C_8[t] + kr_1 RAF[t] C_9[t],

d/dt C_9[t] = on_1 MEK[t] C_5[t] - (of_1 + of_4) C_9[t] - kr_1 RAF[t] C_9[t].

2. Sensitivity to parameters of scaffold-kinase binding

In our simulations, we assumed certain values for binding constants of scaffold-kinase interaction. Since presently no experimental estimates of these parameters exist, we wanted to check the sensitivity of our results to variation in values of these parameters. The results of sensitivity to MAPK affinity to the scaffold can be found in the paper. Sensitivity to MAPKK affinity is shown in supplementary Fig. 5. Here again the K_d values are allowed to vary 0-100 nM, whereas the value assumed in the modeling is 5 nM. It is evident that there is no significant shift in the position of the optimum in either MAPK activation or formation of C_6.

3. Hill coefficient is reduced even if the assumption of processive MAPK activation is relaxed

The data illustrated in supplementary Fig. 6 provide additional data to Section 2 of *Results* in the text. Here we examine how the Hill coefficients of fully and partially processive MAPK activation vary with the scaffold concentration. It is evident that in both cases the Hill coefficient decreases significantly with the scaffold concentration implying diminishing sigmoidness of MAPK activation curve.

4. MAPK activation at high scaffold concentrations varies as 1/[scaffold]$^{m-1}$, where m the scaffold membership

At high scaffold concentration, the fraction f_i of scaffold molecules that have a particular kinase attached to them can easily be shown as:

$f_i = \frac{[Kinase]_i}{K_{Di} + [Scaffold]}$, where K_{Di} is the corresponding dissociation constant.

If several kinases can bind to the scaffold, the fraction f of the scaffold molecules with all possible kinases attached to it is the product of f_i:

$$f = \bigsqcup_i f_i$$

Thus the concentration C of the functional complexes (having all kinases present) is:

$$C = [Scaffold] \bigsqcup_i \frac{[Kinase]_i}{K_{Di} + [Scaffold]}.$$

As the scaffold concentration becomes much greater than the largest of K_{Di}, the concentration of the functional complexes approaches the following value:

$C \blacklozenge \frac{\bigsqcup_{i=1}^{m} [Kinase]_i}{[Scaffold]^{m-1}}$, where m is the total number of kinases binding to a scaffold molecule, i.e., the scaffold membership. Of course, the result holds for any scaffold-binding molecule, not necessarily a kinase.

Parameter values used in the model (unless otherwise stated)

Parameter	Value assumed[1]
Concentrations (μM):	
[MAPKKK]	0.3
[MAPKK]	0.2
[MAPK]	0.4
[MAPKKK K-ase]	0.2
[MAPKKK P-ase]	0.3
[MAPKK P-ase]	0.2
[MAPK P-ase]	0.3
Association rate constants ($\mu M^{-1} sec^{-1}$):	
a_1	1
a_2	0.5
a_3	3.3
a_4	10
a_5	3.3
a_6	10
a_7	20
a_8	5
a_9	20
a_{10}	5
on_1	10
on_2	10
Dissociation rate constants (sec^{-1}):	
d_1	0.4
d_2	0.5
d_3	0.42
d_4	0.8
d_5	0.4
d_6	0.8
d_7	0.6
d_8	0.4
d_9	0.6
d_{10}	0.4
off_1	0.05
off_2	0.05
off_3	0.05
off_4	0.5
Reaction rate constants (sec^{-1}):	
k_1	0.1
k_2	0.1
k_3	0.1
k_4	0.1
k_5	0.1
k_6	0.1
k_7	0.1
k_8	0.1
k_9	0.1
k_{10}	0.1

[1] The concentrations and individual *a*, *d* and *k* values correspond to estimates in reports (refs. 26,28).

FIGURE LEGENDS:

Supplementary Fig. 5. Sensitivity of the signaling to variation of binding constant of MAPKK interaction with the scaffold. Dependence of the signaling output and C_6 on the dissociation constants of MAPKK is shown. The graphs presented are contour plots with lighter areas corresponding to higher levels of activation.

Supplementary Fig. 6. Dependence of the Hill coefficient of the input-output relationships for the cases of monophosphorylated (dashed line) and biphosphorylated (solid line) MAPK dissociation from the scaffold.