# CELL SIGNALLING DYNAMICS IN TIME AND SPACE

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## Supplementary Information

**Supplementary Table S1. EGFR docking sites and binding partners.**

| Tyrosine residue | | Surrounding Sequence | Kinase(s) | Binding Partner(s) | Reference(s) |
|---|---|---|---|---|---|
| Precursor Index* | Processed Index* | | | | |
| 869 | 845 | AEEKEYHAEGG | Src[1,2,3] | STAT1/3[&] | [1], [2], [3] |
| 915 | 891 | FGSKPYDGIPA | Src[4] | p85[4], Src[4] | [4] |
| 944 | 920 | CTIDVYMIMVK | Src[4], Insulin Receptor Kinase[#] | p85[4,#], Src[4] | [4] |
| 978 | 954 | RDPQRYLVIQG | ND[$] | PLCγ | |
| 998 | 974 | TDSNFYRALMD | NA[$,%] | AP-2[5,%] | [5] |
| 1016 | 992 | VDADEYLIPQQ | ND[$] | PLCγ[6,#], PTP-1B[7], RasGAP[8],SHP2[8] | [6], [7], [8] |
| 1069 | 1045 | SFLQRYSSDPT | ND[$] | Eps15[&&], Cbl[9] | [9] |
| 1092 | 1068 | LPVPEYINQSV | EGFR[10,#] | Grb2[11,#] | [10], [11] |
| 1110 | 1086 | VQNPVYHNQPL | EGFR[12,#] | Grb2[11,#], Dok-R[13], Abl[14], Shc[#] | [11], [12], [13], [14] |
| 1125 | 1101 | SRDPHYQDPHS | Src[15] | Abl[#] | [15] |
| 1138 | 1114 | VGNPEYLNTVQ | EGFR[#] | Shc[#] | |
| 1172 | 1148 | LDNPDYQQDFF | EGFR[10,#] | Shc[16], Dok-R[13], PTP-1B[7] | [7], [10], [13], [16] |
| 1197 | 1173 | AENAEYLRVAP | EGFR[10,#] | Shc[16,#], PLCγ[6,#], SHP1[17] | [6], [10], [15], [17] |

*Swiss-Prot entry EGFR_HUMAN (P00533) includes a 24 amino acid N-terminal plasma membrane targeting motif that is subsequently cleaved. Residue indices based on the Swiss-Prot entry are referred to as "Precursor index", while indices based on the sequence without the targeting motif are referred to as "Processed index"

[#]Predicted by Scansite (scansite.mit.edu)

[$]Not Determined (ND) or Not Applicable (NA)

[%]Tyrosine 974 does not require phosphorylation to associate with AP-2[5]

[&]Activation of this site is implicated in STAT1/3 activation, but there is no direct evidence of STAT1/3 binding to this site.

[&&]Ubiquitin moieties appended to activated EGFR complexes may act as docking sites for Eps15[18].

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Protein Abbreviations

Abl, Abelson's protein tyrosine kinase; AP-2, Adaptor protein 2; Cbl, Casitas b-lineage lymphoma; Dok-R, Downstream of kinase related protein; Eps15, Epidermal growth factor receptor pathway substrate 15; Grb2, Growth factor receptor binding protein 2; PI3K, Phosphatidylinositol-3-kinase; p85, 85 kDa regulator subunit of PI3K; PLCγ, Phospholipase C gamma; PTB-1B, Protein tyrosine phosphatase 1B; RasGAP, Ras GTPase activating protein; Shc, Src homology and collagen-domain protein; SHP1, Src homology 2 domain containing tyrosine phosphatase 1; SHP2, Src homology 2 domain containing tyrosine phosphatase 2; Src, Sarcoma protein.

## References


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**Supplementary Table S2. Designations of molecules in Figure, Part A, BOX 1.**

| Designation | Name |
|---|---|
| R | EGFR monomer |
| $R_a$ | EGFR-EGF monomer |
| $R_2$ | $(R_a)_2$, EGFR-EGF dimer |
| RP | Tyrosine phosphorylated EGFR-EGF dimer |
| GAP | RasGAP, Ras GTPase activating protein |
| GAPP | Tyrosine phosphorylated RasGAP, RasGAPP |
| R-GAP | Complex of tyrosine phosphorylated EGFR and RasGAP, RP-RasGAP |
| R-GAPP | Complex of tyrosine phosphorylated EGFR and tyrosine phosphorylated RasGAP, RP-RasGAPP |
| R-PL | Complex of tyrosine phosphorylated EGFR and PLCγ, RP-PLCγ |
| R-PLP | RP-PLCγP |
| R-G | Complex of tyrosine phosphorylated EGFR and Grb2, RP-Grb2 |
| R-G-S | Complex of tyrosine phosphorylated EGFR and Grb2-SOS, RP-Grb2-SOS |
| G-S | Complex of Grb2 and SOS, Grb2-SOS |
| R-Sh | Complex of tyrosine phosphorylated EGFR and Shc, RP-Shc |
| R-ShP | RP-ShcP |
| ShP | Tyrosine phosphorylated Shc, ShcP |
| R-Sh-G | RP-ShcP-Grb2 |
| Sh-G | ShcP-Grb2 |
| R-Sh-G-S | RP-ShcP-Grb2-SOS |
| Sh-G-S | ShcP-Grb2-SOS |
| RasD | RasGDP |
| RasT | RasGTP |
| R-G-S-rD | RP-Grb2-SOS-RasGDP |
| R-G-S-r | RP-Grb2-SOS-Ras |
| R-G-S-rT | RP-Grb2-SOS-RasGTP |
| R-GP | Complexes of tyrosine phosphorylated EGFR with RasGAP or tyrosine phosphorylated RasGAP, R-GAP and R-GAPP |
| R-GP-rT | RP-GP-RasGTP |
| R-Sh-G-S-rD | RP-ShcP-Grb2-SOS-RasGDP |
| R-Sh-G-S-r | RP-ShcP-Grb2-SOS-Ras |
| R-Sh-G-S-rT | RP-ShcP-Grb2-SOS-RasGTP |

**Feedback designs that turn a universal covalent-modification cycle into bistable switch and relaxation oscillator.**

A universal motif of cellular networks is the cycle formed by two forms of a signalling protein, which is modified by two opposing enzymes, such as a kinase and phosphatase, or a guanosine nucleotide exchange factor (GEF) and GTPase–activating protein (GAP). Assuming that the total concentration of these two forms ($M$ and $M_p$) of the protein remains constant $M_p + M = M_{tot} = const$, the temporal dynamics of this system is described by a single differential equation,

$$\frac{dM_p}{dt} = v_{kin} - v_{phos} \qquad \text{(S1)}$$

This simple system can turn bistable in four distinct ways; either $M_p$ activates $v_{\text{kin}}$ or inhibits $v_{\text{phos}}$, or $M$ activates $v_{\text{phos}}$ or inhibits $v_{\text{kin}}$. All these feedback designs are equivalent to positive (autocatalytic) feedback in Eq. S1, by which $M_p$ or $M$ activate their own production rates. The figure, part A in BOX 2 of the main text shows hysteresis of the dependence of $M_p$ on the kinase concentration $E_{kin}$, where the values of kinetic parameters (Eq. 1) are the following, $k_{kin}^{cat} = 1\,s^{-1}$; $A = 100$; $K_a = 500\,nM$; $K_{m1} = 500\,nM$; $E_{phos} = 200$ nM, $k_{phos}^{cat} = 1\,s^{-1}$; $K_{m2} = 10\,nM$.

For each of the four bistable switches, eight different forms of negative feedback, where $M_p$ inhibits its production rate through the negative or positive influence of either form ($M$ and $M_p$) on the abundance of the kinase protein or phosphatase protein, give rise to 32 different feedback designs that may exhibit relaxation oscillations (FIG. 2 of the main text and FIG. S1). The temporal dynamics of each of these simple positive-negative feedback systems can be described by two differential equations only (although in FIG. 2 and FIG. S1 each diagram formally corresponds to a system of three differential equations, the protein concentration that is not controlled by feedback from the M cycle can be considered as a constant parameter, leaving only two differential equations in each case). When the abundance of the kinase protein is subject to feedback regulation by $M$ or $M_p$, these equations are (see Eq. 2 in BOX 2 and Table S3),

$$\frac{dM_p}{dt} = v_{kin} - v_{phos},$$

$$\frac{dE_{kin}}{dt} = v_{kin}^{synth} - v_{kin}^{\deg} \qquad \text{(S2)}.$$

If the abundance of the phosphatase protein is controlled by feedback (see Eq. 3 in BOX 2 and Table S3), this phosphorylation cycle is described by the two following equations,

$$\frac{dM_p}{dt} = v_{kin} - v_{phos},$$

$$\frac{dE_{phos}}{dt} = v_{phos}^{synth} - v_{phos}^{\deg} \qquad \text{(S3)}.$$

Table S3 gives the rate expressions and kinetic parameters that generate relaxation oscillations in the universal signalling cycle for a subset of sixteen positive-negative feedback designs shown in FIG. 1 of the main text (using these rate expressions, Eqs S2 and S3 can be readily analyzed analytically and the emergence of oscillations can be shown). The remaining sixteen relaxation oscillation designs shown in FIG. S1 may require some degree of cooperativity of feedback regulations.

**Supplementary Table S3. Feedback loops render the universal signalling cycle into bistable switch and relaxation oscillator.**

This table provides the rate expressions and kinetic parameters that correspond to the 16 feedback designs (from **A** to **H***) shown in FIG. 2 of the main text.

**A-D.** Bistability in the M phosphorylation/dephosphorylation cycle arises from positive feedback (activation of the kinase rate by its product $M_p$) that operates on the time scale of seconds to minutes (Eq. 1). $M_{tot} = M_p + M = 300$. The rate expressions and kinetic parameters of this bistable M cycle are given below.

| **Reaction** | **Rate expression** | **Kinetic constants** |
|---|---|---|
| Kinase: $M \rightarrow M_p$ | $v_{kin} = \frac{k_{kin}^{cat} E_{kin} M}{(K_{m1} + M)} \cdot \frac{(1 + AM_p / K_a)}{(1 + M_p / K_a)}$ | $k_{kin}^{cat} = 1 s^{-1}; A = 100; K_a = 500 nM;$ $K_{m1} = 500 nM;\ E_{kin} = 80 nM$ |
| Phosphatase $M_p \rightarrow$ M | $v_{phos} = \frac{k_{phos}^{cat} E_{phos} M_p}{(K_{m2} + M_p)}$ | $k_{phos}^{cat} = 1 s^{-1}; K_{m2} = 10 nM;$ $E_{phos} = 200 nM$ |

**A.** Relaxation oscillations arise from additional negative feedback brought about by inhibition of the kinase protein synthesis rate by the phosphorylated form $M_p$ (see Eqs. 1 and 2 of the main text), which can occur at a slower time scale (tens of minutes for immediate early genes and hours for gene expression circuits).

| **Reaction** | **Rate expression** | **Kinetic constants** |
|---|---|---|
| Kinase: $M \rightarrow M_p$ | $v_{kin} = \frac{k_{kin}^{cat} E_{kin} M}{(K_{m1} + M)} \cdot \frac{(1 + AM_p / K_a)}{(1 + M_p / K_a)}$ | $k_{kin}^{cat} = 1 s^{-1}; A = 100; K_a = 500 nM;$ $K_{m1} = 500 nM;$ |
| Phosphatase $M_p \rightarrow$ M | $v_{phos} = \frac{k_{phos}^{cat} E_{phos} M_p}{(K_{m2} + M_p)}$ | $k_{phos}^{cat} = 1 s^{-1}; K_{m2} = 10 nM;$ $E_{phos} = 200 nM$ |
| Kinase synthesis $\rightarrow E_{kin}$ | $v_{kin}^{synth} = V_{kin}^0 \frac{(1 + M_p / K_I)}{(1 + I \cdot M_p / K_I)}$ | $V_{kin}^0 = 150 nM \cdot hr^{-1}; K_I = 100 nM;$ $I = 7.5$ |
| Kinase degradation $E_{kin} \rightarrow$ | $v_{kin}^{deg} = k_{kin}^{deg} E_{kin}$ | $k_{kin}^{deg} = 1 hr^{-1}$ |

**B.** Relaxation oscillations arise from extra negative feedback brought about by activation of the kinase protein degradation rate by $M_p$.

| **Reaction** | **Rate expression** | **Kinetic constants** |
|---|---|---|
| Kinase: $M \rightarrow M_p$ | $v_{kin} = \frac{k_{kin}^{cat} E_{kin} M}{(K_{m1} + M)} \cdot \frac{(1 + AM_p / K_a)}{(1 + M_p / K_a)}$ | $k_{kin}^{cat} = 1 s^{-1}; A = 100; K_a = 500\, nM;$ $K_{m1} = 500\, nM;$ |
| Phosphatase $M_p \rightarrow$ M | $v_{phos} = \frac{k_{phos}^{cat} E_{phos} M_p}{(K_{m2} + M_p)}$ | $k_{phos}^{cat} = 1 s^{-1}; K_{m2} = 10\, nM;$ $E_{phos} = 200\, nM$ |
| Kinase synthesis $\rightarrow E_{kin}$ | $v_{kin}^{synth} = V_{kin}^0$ | $V_{kin}^0 = 150\, nM \cdot hr^{-1}$ |
| Kinase degradation $E_{kin} \rightarrow$ | $v_{kin}^{\deg} = k_{kin}^{\deg} \frac{(1 + A_{dk} M_p / K_d)}{(1 + M_p / K_d)} E_{kin}$ | $k_{kin}^{\deg} = 1 hr^{-1}; K_d = 100\, nM; A_{dk} = 7.5$ |

**C.** Relaxation oscillations arise from extra negative feedback brought about by activation of the phosphatase protein synthesis rate by the active form $M_p$ (Eqs. 1 and 3 of the main text).

| **Reaction** | **Rate expression** | **Kinetic constants** |
|---|---|---|
| Kinase: $M \rightarrow M_p$ | $v_{kin} = \frac{k_{kin}^{cat} E_{kin} M}{(K_{m1} + M)} \cdot \frac{(1 + AM_p / K_a)}{(1 + M_p / K_a)}$ | $k_{kin}^{cat} = 1 s^{-1}; A = 100; K_a = 500\, nM;$ $K_{m1} = 500\, nM; E_{kin} = 150\, nM$ |
| Phosphatase $M_p \rightarrow$ M | $v_{phos} = \frac{k_{phos}^{cat} E_{phos} M_p}{(K_{m2} + M_p)}$ | $k_{phos}^{cat} = 1 s^{-1}; K_{m2} = 10\, nM$ |
| Phosphatase synthesis $\rightarrow E_{phos}$ | $v_{phos}^{synth} = V_{phos}^0 \frac{(1 + A_p M_p / K_d)}{(1 + M_p / K_d)}$ | $V_{phos}^0 = 200\, nM \cdot hr^{-1}; K_d = 100\, nM;$ $A_p = 7.5$ |
| Phosphatase degradation $E_{phos} \rightarrow$ | $v_{phos}^{\deg} = k_{phos}^{\deg} E_{phos}$ | $k_{phos}^{\deg} = 1 hr^{-1}$ |

**D.** Relaxation oscillations arise from extra negative feedback brought about by inhibition of the phosphatase protein degradation rate by the active form $M_p$.

| **Reaction** | **Rate expression** | **Kinetic constants** |
|---|---|---|
| Kinase: $M \rightarrow M_p$ | $v_{kin} = \frac{k_{kin}^{cat} E_{kin} M}{(K_{m1} + M)} \cdot \frac{(1 + AM_p / K_a)}{(1 + M_p / K_a)}$ | $k_{kin}^{cat} = 1 s^{-1}; A = 100; K_a = 500\, nM;$ $K_{m1} = 500\, nM; E_{kin} = 150\, nM$ |
| Phosphatase $M_p \rightarrow$ M | $v_{phos} = \frac{k_{phos}^{cat} E_{phos} M_p}{(K_{m2} + M_p)}$ | $k_{phos}^{cat} = 1 s^{-1}; K_{m2} = 10\, nM$ |
| Phosphatase synthesis $\rightarrow E_{phos}$ | $v_{phos}^{synth} = V_{phos}^0$ | $V_{phos}^0 = 200\, nM \cdot hr^{-1};$ |
| Phosphatase degradation $E_{phos} \rightarrow$ | $v_{phos}^{\deg} = k_{phos}^{\deg} \frac{(1 + M_p / K_I)}{(1 + I \cdot M_p / K_I)} E_{phos}$ | $k_{phos}^{\deg} = 1 hr^{-1}; K_I = 100\, nM;$ $I = 7.5$ |

**E-H**. Bistability in the M phosphorylation/dephosphorylation cycle is brought about by positive feedback in the $M_p$ production cycle. This feedback is the result of inhibition of the phosphatase rate by its substrate $M_p$ and usually operates on the time scale of seconds to minutes. The rate expressions and kinetic parameters of the M cycle with this feedback are given below. $M_{tot} = M_p + M = 300$.

| **Reaction** | **Rate expression** | **Kinetic constants** |
|---|---|---|
| Kinase:<br>$M \rightarrow M_p$ | $v_{kin} = \frac{k_{kin}^{cat} E_{kin} M}{(K_{m1} + M)}$ | $k_{kin}^{cat} = 1 s^{-1}; K_{m1} = 500 nM$<br>$E_{kin} = 80 nM$ |
| Phosphatase:<br>$M_p \rightarrow M$ | $v_{phos} = \frac{k_{phos}^{cat} E_{phos} M_p}{(K_{m2} + M_p)} \cdot \frac{(1 + M_p / K_s)}{(1 + I_s \cdot M_p / K_s)}$ | $k_{phos}^{cat} = 1 s^{-1}; K_{m2} = 10 nM$ ;<br>$I_s = 100; K_s = 500 nM$ ;<br>$E_{phos} = 200 nM$ |

For **E-H** designs, relaxation oscillations arise from extra negative feedback, brought about by the influence of the phosphorylated form $M_p$ on the rates of protein synthesis or degradation of the kinase or phosphatases. If the effect is on the kinase synthesis or degradation rates, $E_{phos}$ = 200 nM, and if it is on the phosphatases synthesis or degradation rates, $E_{kin}$ = 150 nM. These reactions might occur at a slower time scale (tens of minutes for immediate early genes and hours for gene expression circuits. For feedback designs **E, F, G,** and **H**, the rate expressions and kinetic parameters of the kinase/phosphatase synthesis and degradation rates are the same as for feedback designs **A, B, C,** and **D**, respectively, which are given above.

Designs **A*-H* (**with the superscript *) are mirror images of designs **A-H** and have exactly the same equation structure and parameter values. The differences are that the variables $M_p$ and $E_{kin}$ and their respective parameters are replaced by $M$ and $E_{phos}$ and their respective parameters, and vice versa. For instance, the rate expressions and parameters for design **A*** are the following:

| **Reaction** | **Rate expression** | **Kinetic constants** |
|---|---|---|
| Kinase: $M \rightarrow M_p$ | $v_{kin} = \frac{k_{kin}^{cat} E_{kin} M}{(K_{m2} + M)}$ | $k_{kin}^{cat} = 1 s^{-1}; K_{m2} = 10 nM$ ;<br>$E_{kin} = 200 nM$ |
| Phosphatase $M_p \rightarrow M$ | $v_{phos} = \frac{k_{phos}^{cat} E_{phos} M_p}{(K_{m1} + M_p)} \cdot \frac{(1 + AM / K_a)}{(1 + M / K_a)}$ | $k_{phos}^{cat} = 1 s^{-1}; K_{m1} = 500 nM$ ; $A = 100$ ;<br>$K_a = 500 nM$ |
| Phosphatase synthesis<br>$\rightarrow E_{phos}$ | $v_{phos}^{synth} = V_{phos}^{0} \frac{(1 + M / K_I)}{(1 + I \cdot M / K_I)}$ | $V_{phos}^{0} = 150 nM \cdot hr^{-1}; K_I = 100 nM$ ;<br>$I = 7.5$ |
| Phosphatase degradation<br>$E_{phos} \rightarrow$ | $v_{phos}^{deg} = k_{phos}^{deg} E_{phos}$ | $k_{phos}^{deg} = 1 hr^{-1}$ |

**Supplementary Figure S1.**

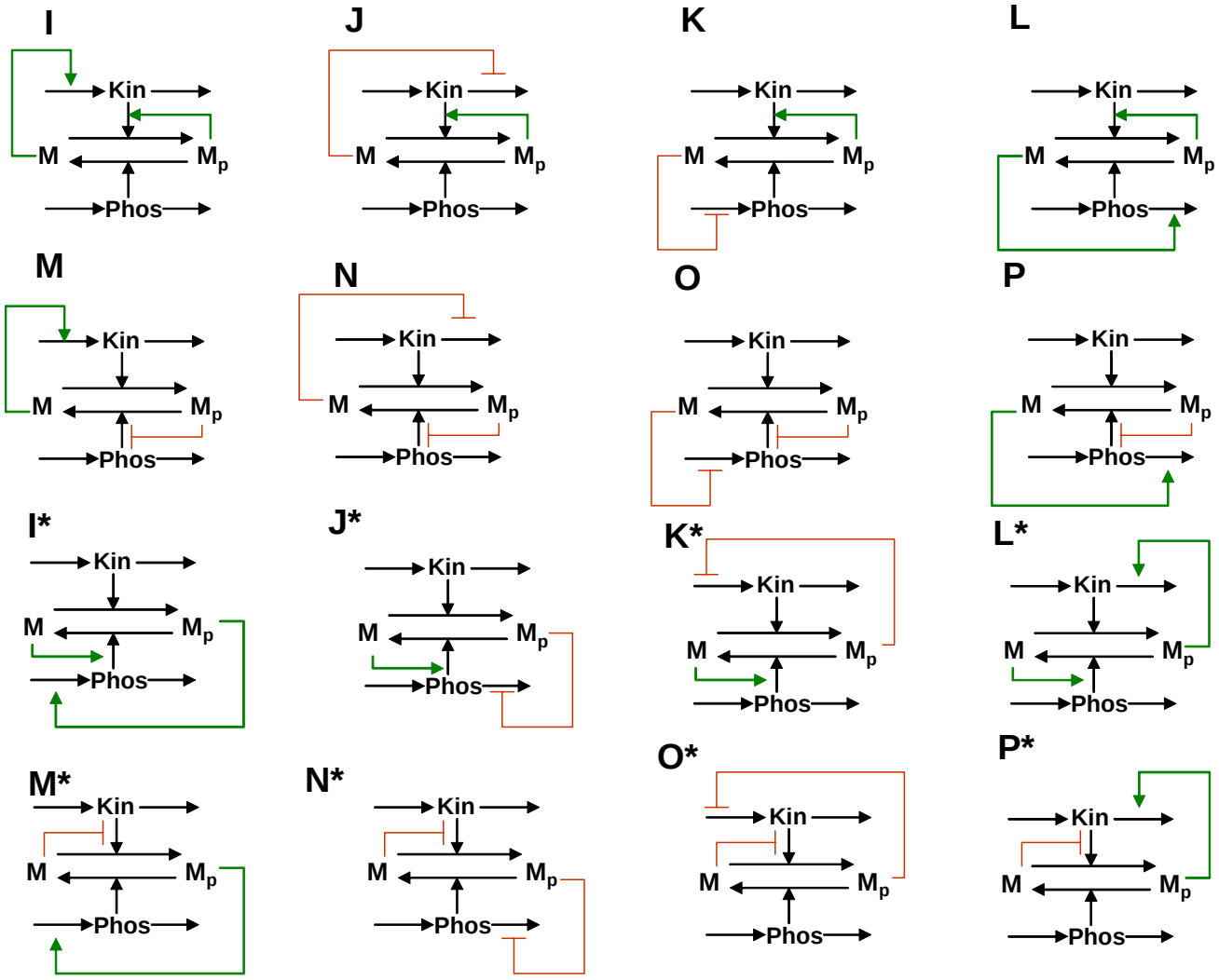


**Fig. S1. Sixteen additional feedback designs that give rise to relaxation oscillations.** Although the equation structure is similar to equations present in Table S3, these designs usually require some degree of cooperativity of feedback regulations. Exploration of possible rate expression and parameter ranges (bifurcation diagrams) that bring about relaxation oscillations for these feedback designs is an instructive exercise for Systems Biology students.

**Supplementary Figure S2.**

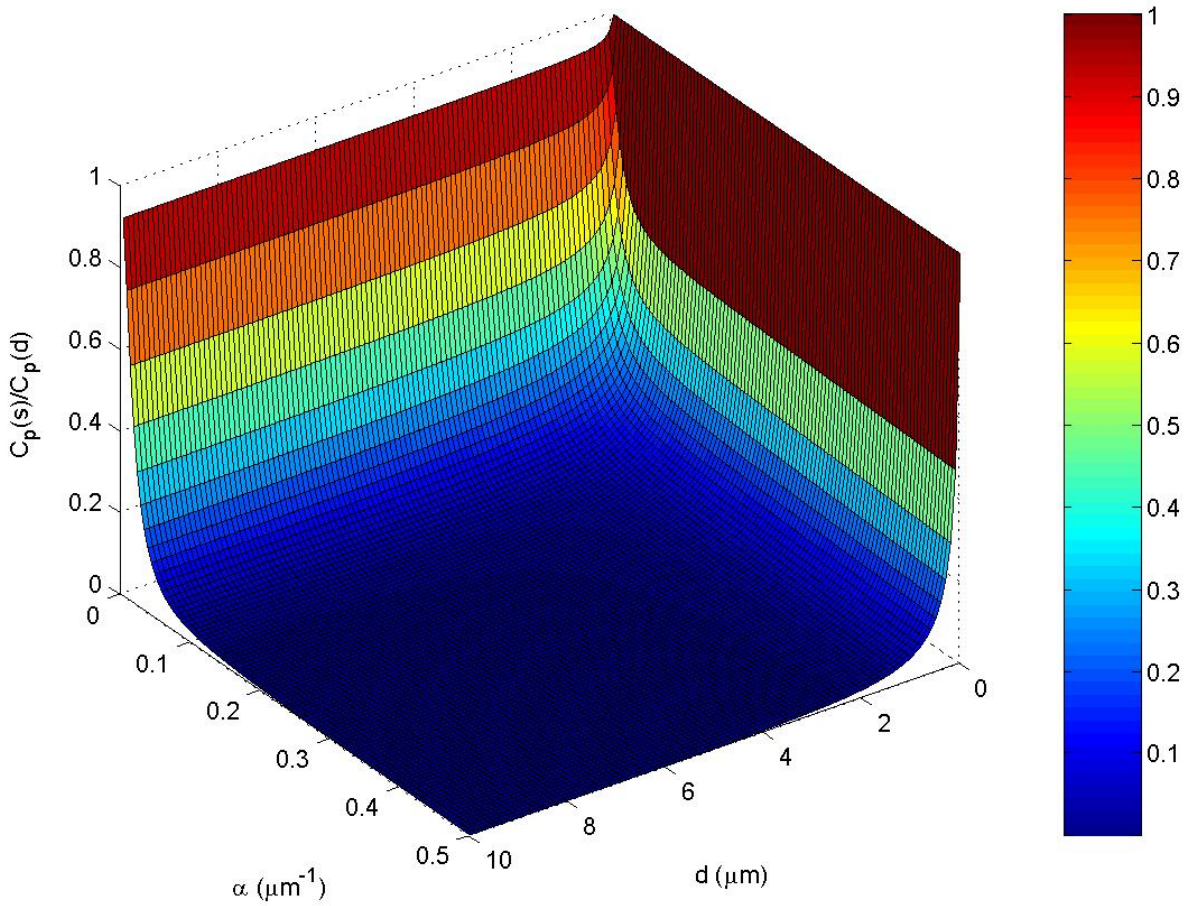


**Fig. S2. Stationary profile of a phospho-protein phosphorylated by a kinase localized to a supra-molecular structure and dephosphorylated by a homogeneously distributed phosphatase.** The relative phosphorylated fraction $c_p(s)/c_p(d)$ declines with the distance $d$ from the kinase that is confined to the spherical structural element of radius $s$. The steepness of the gradient (reciprocal of the characteristic length) is determined by the parameter $\alpha$ ($\alpha^2 = k_p/D$ is the ratio of the phosphatase activity $k_p$ and the protein diffusivity $D$).

**Supplementary Table S4. Kinetic description of a three-kinase cascade.**

The spatio-temporal dynamics is described by the reaction-diffusion system (Eq. 1 in BOX 4 of the main text). Both phosphorylated and unphosphorylated forms of kinases $M_1$, $M_2$ and $M_3$ diffuse in the cytoplasm. The cell radius $L$ = 10 μm, the diffusion coefficient $D$ is assumed to be equal for all kinase forms, $D$ = 5 μm$^2$/sec. Total concentrations of the unphosphorylated ($M_i$) form and phosphorylated form ($M_iP$) are assumed constant on the time scale considered, $M_i + M_iP = M_{tot}$. Eq. 1 can be solved for dimensionless variables $M_iP/M_{tot}$, therefore, in the table the Michaelis constants ($K_{mi}$ and $K_{mpi}$, $i$ =1,2,3) and the maximal activites ($V^i_{phos}$) are normalized by $M_{tot}$.

| Reaction | Rate expression | Kinetic constants |
|---|---|---|
| Membrane kinase<br>$M_1 \rightarrow M_1P$ | $v^{mem}_{kin} = \frac{V^{mem}_{kin} M_1}{(K_{m1} + M_1)}$ | $V^{mem}_{kin} / M_{tot} = (7.5 \cdot L/3)$ μm s$^{-1}$<br>$K_{m1}/M_{tot} = 0.5$ |
| Cytoplasmic phosphatase-1<br>$M_1P \rightarrow M_1$ | $v^1_{phos} = \frac{V^1_{phos} M_1P}{(K_{mp1} + M_1P)}$ | $V^1_{phos} / M_{tot} = 3$ s$^{-1}$<br>$K_{mp1}/M_{tot} = 0.7$ |
| Kinase $M_1P$<br>$M_2 \rightarrow M_2P$ | $v^1_{kin} = \frac{k^{cat}_2 M_1P \cdot M_2}{(K_{m2} + K_2)}$ | $k^{cat}_2 = 9$ s$^{-1}$<br>$K_{m2}/M_{tot} = 0.7$ |
| Cytoplasmic phosphatase-2<br>$M_2P \rightarrow M_2$ | $v^2_{phos} = V^2_{phos} \frac{M_2P}{(K_{mp2} + M_2P)}$ | $V^2_{phos} / M_{tot} = 3$ s$^{-1}$<br>$K_{mp2}/M_{tot} = 0.7$ |
| Kinase $M_2P$<br>$M_3 \rightarrow M_3P$ | $v^2_{kin} = \frac{k^{cat}_3 M_2P \cdot M_3}{(K_{m3} + M_3)}$ | $k^{cat}_3 = 9$ s$^{-1}$<br>$K_{m3}/M_{tot} = 0.5$ |
| Cytoplasmic phosphatase-3<br>$M_3P \rightarrow M_3$ | $v^3_{phos} = V^3_{phos} \frac{M_3P}{(K_{mp3} + M_3P)}$ | $V^3_{phos} / M_{tot} = 3$ s$^{-1}$<br>$K_{mp3}/M_{tot} = 0.7$ |

Steady-state spatial profiles shown in BOX 4 of the main text (see figure, Part A) are calculated using the reaction diffusion equations with the rate expressions and parameters given in Table S4.

**Propagation of phospho-protein waves through kinase cascades.**

Bistability in protein kinase/phosphatase cascades might give rise to travelling waves of phosphorylated kinase that transmit signals to distant targets[1]. FIG. S3 shows one-dimensional bifurcation diagram of the dependence of the active form $M_3P$ on the activity of the membrane-bound kinase, and Table S4 provides the rate expressions and kinetic parameters for a bistable cytoplasmic cascade.

**References.**

1. Kholodenko, B. N. Four-dimensional organization of protein kinase signaling cascades: the roles of diffusion, endocytosis and molecular motors. *J Exp Biol* **206**, 2073-82 (2003).

**Supplementary Figure S3.**

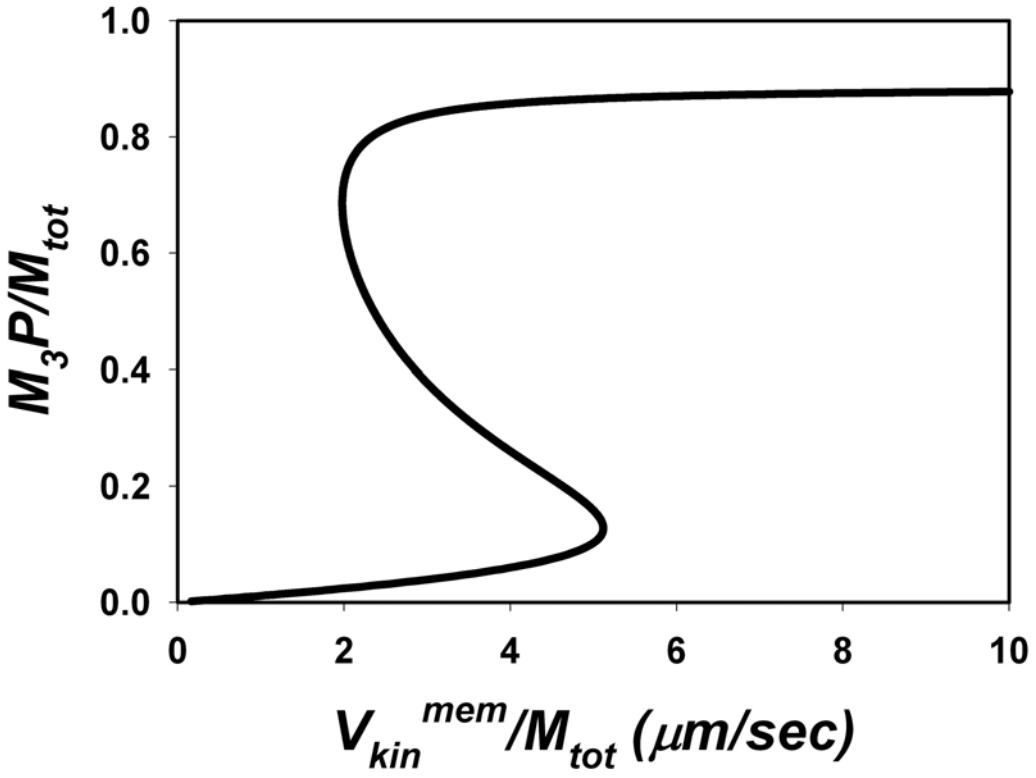


**FIG. S3. Hysteresis and bistability in a three level phosphorylation cascade.** The dependence of the phosphorylation kinase fraction at the terminal level on the input activity of the membrane-bound kinase. The rate expressions and kinetic parameters are given in Table S4.

**Supplementary Table S5. Kinetic description of a bistable three-kinase cascade.**

Bistability (FIG. S3) emerges from inhibition of the rate of cytoplasmic phosphatase-2 by the active form $M_3P$. The spatio-temporal dynamics of the cascade is described by the reaction-diffusion system (Eq. 1 in BOX 4 of the main text). Both phosphorylated and unphosphorylated forms of kinases $M_1$, $M_2$ and $M_3$ diffuse in the cytoplasm.The cell radius $L$ = 25 μm, the diffusion coefficient $D$ is assumed to be equal for all kinase forms, $D$ = 5 μm$^2$/sec. Total concentrations of the unphosphorylated ($M_i$) form and phosphorylated form ($M_iP$) are assumed constant on the time scale considered, $M_i + M_iP = M_{tot}$. Eq. 1 can be solved for dimensionless variables $M_iP/M_{tot}$, therefore, in the table the Michaelis constants ($K_{mi}$ and $K_{mpi}$, $i$ =1,2,3) and the maximal activites ($V^i_{phos}$) are normalized by $M_{tot}$.

| Reaction | Rate expression | Kinetic constants |
|---|---|---|
| Membrane kinase<br>$M_1 \rightarrow M_1P$ | $v^{mem}_{kin} = \frac{V^{mem}_{kin} M_1}{(K_{m1} + M_1)}$ | $V^{mem}_{kin} / M_{tot} = (1.5 \cdot L/3)$ μm s$^{-1}$<br>$K_{m1}/M_{tot} = 0.5$ |
| Cytoplasmic phosphatase-1<br>$M_1P \rightarrow M_1$ | $v^1_{phos} = \frac{V^1_{phos} M_1 P}{(K_{mp1} + M_1 P)}$ | $V^1_{phos} / M_{tot} = 3$ s$^{-1}$<br>$K_{mp1}/M_{tot} = 0.7$ |
| Kinase $M_1P$<br>$M_2 \rightarrow M_2P$ | $v^1_{kin} = \frac{k^{cat}_2 M_1 P \cdot M_2}{(K_{m2} + K_2)}$ | $k^{cat}_2 = 9$ s$^{-1}$<br>$K_{m2}/M_{tot} = 0.7$ |
| Cytoplasmic phosphatase-2<br>$M_2P \rightarrow M_2$ | $v^2_{phos} = V^2_{phos} \frac{M_2 P}{(K_{mp2} + M_2 P)(1 + (M_3 P / K_I)^2)}$ | $V^2_{phos} / M_{tot} = 9$ s$^{-1}$<br>$K_{mp2}/M_{tot} = 0.7$, $K_I / M_{tot} =$ 0.15 |
| Kinase $M_2P$<br>$M_3 \rightarrow M_3P$ | $v^2_{kin} = \frac{k^{cat}_3 M_2 P \cdot M_3}{(K_{m3} + M_3)}$ | $k^{cat}_3 = 9$ s$^{-1}$<br>$K_{m3}/M_{tot} = 0.5$ |
| Cytoplasmic phosphatase-3<br>$M_3P \rightarrow M_3$ | $v^3_{phos} = V^3_{phos} \frac{M_3 P}{(K_{mp3} + M_3 P)}$ | $V^3_{phos} / M_{tot} = 3$ s$^{-1}$<br>$K_{mp3}/M_{tot} = 0.7$ |

The traveling wave shown in BOX 4 of the main text (see figure, part B) was calculated for the input impulse of $V^{mem}_{kin} / M_{tot} = (7.5 \cdot L/3)$ μm s$^{-1}$ in the time range from 0 to 30 sec, and $V^{mem}_{kin} / M_{tot} = 0$ at t > 30 s, using the reaction diffusion equations (Eq. 1 in BOX 4) with the rate expressions and parameters given in Table S5. Note that in the absence of bistability, the phosphorylation signal cannot reach the distance of 20 μm, since it will be terminated by cytoplasmic phosphatases.