Supporting Information for:

A mechanism for Src kinase-dependent signaling by non-catalytic receptors

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This file contains:

Three Excel spreadsheets are available as separate files.

Table 2. Cellular concentrations and reaction rates for SFKs and SDRs

Parameter	Description	Value	Explanation	Citation[a]
Q	Eqm. between closed vs open conformation of SFK	200 - 500 or 0	Inferred from activity of fully active SFK relative to activity of fully inhibited SFK For mutant or C-term. dephos. SFK	(*1-4*)
K_3	Association constant for binding of E to R^*	$(0.1 - 1)\times10^7$ M^{-1}	Association constant for binding of SFK SH2 domain to phosphopeptide	(*5, 6*)
E_t	Total concentration of SFK in open conformation	$\sim 0.12\times10^{-7}$ M	Biosynthetic labeling: 5×10^5 viral Src molecules/cell and 50x lower level of Src relative to viral Src	(*7, 8*)
		1.6×10^{-7} M	Quantitative Western blot: 5×10^4 Fyn molecules per T cell	(*9*)
R_t	Total concentration of R	1.3×10^{-7} M	Quantitative flow cytometry: 3×10^5 Fc receptors per RBL cell	(*10*)
		$(0.6 - 1.2)\times10^{-7}$ M	$(2 - 4)\times10^4$ TCRs per T cell	(*11*)
k_{cat}	Turnover number	40 min^{-1}	Phosphorylation of peptide substrate by Hck, in presence of activator	(*1, 12*)
		40 - 200 min^{-1}	Phosphorylation of peptide substrates by activated Src	(*13, 14*)
K_M	Michaelis constant, K_M for E	10^{-4} - 10^{-3} M	Phosphorylation assays	(*1, 13-15*)
k_1	R phos. by E	$(4 - 200)\times10^4$ M^{-1} min^{-1}	k_{cat}/K_M	
k_4	R phos. in RR^*-E complex	$\sim k_{cat}$	Receptor tails occupy a hemisphere of radius ~3 nm (5×10^{-23} L), so receptor and kinase are both ~10 mM, well above K_M.	(*10*)
σ	Receptor trans-phos. effect, $\frac{k_4 K_3}{k_1}$	20-5000	Derived parameter (Appendix 2)	

ϕ	Increase in SFK activity due to activation loop phosphorylation	~ 4 - 20	(2.5 - 3)-fold lower kinase activity of autophosphorylation site mutant of Src or Lck, compared with wildtype Src or Lck, corrected for 20-30% autophosphorylation stoichiometry.	(*8, 16-18*)
			4-fold higher kinase activity of fully dephosphorylated Hck after autophosphorylation	(*1*)
			20-times higher activity of C-terminally phosphorylated Hck after autophosphorylation	(*1, 12, 19*)
q_1	Autophos. of SFK	10^6 $M^{-1}min^{-1}$	~10 min lag time for autophosphorylation of a solution of 10^{-7} M dephosphorylated Src or Hck	(*1, 14*)
q_3	Trans-phos. of SFK in E-R*-R*-E complex	~ k_{cat}	Receptor tails occupy a hemisphere of radius ~3 nm ($5x10^{-23}$L), so effective E_t and R_t are both ~10 mM, well above K_M.	(*10*)
ξ	SFK trans-phos. effect $\frac{q_3 K_3^2 R_t}{q_1}$	2 - 800	Derived parameter (Appendix 3)	

General assumptions: RBL cell is sphere of radius 7 μm, volume 1.4 pL (*10*). Fibroblast cell contains 0.3 ng protein, volume 4.5 pL (*8*). T cell sphere of radius 5 μm, volume 0.5 pL. Note that nuclear volume may be as much as 50% of cell volume.

[a] REFERENCES for Table 2:

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Appendix 1: Effect of positive and negative feedback on phosphorylation of monomeric receptor

For the monomeric receptor, phosphorylation by *E* creates *R** according to:

$$\begin{array}{c} a \\ \boldsymbol{R} \Leftrightarrow \boldsymbol{R^*} \\ b \\ 1\text{-}y \qquad y \end{array} \tag{1}$$

in which ***R**** includes *R** and *R**-*E*. Because only *R** can be dephosphorylated, and because only unbound *E* can phosphorylate *R*, the effective rate constants *a* and *b* are:

$$a = k_1[E] = k_1 E_t x, \quad b = \frac{k_2}{1 + K_3[E]} = \frac{k_2}{1 + K_3 E_t x}, \text{ where } x = \frac{[E]}{E_t}. \tag{2}$$

Let R_t be the total concentration of receptor, and

$$y = \frac{[R^*] + [R^* - E]}{R_t}, \tag{3}$$

$$= \frac{\frac{a}{b}}{1 + \frac{a}{b}} = \frac{\frac{k_1 E_t x}{k_2}(1 + K_3 E_t x)}{1 + \frac{k_1 E_t x}{k_2}(1 + K_3 E_t x)} \tag{4}$$

x is not know *a priori*, but it depends on *y* as follows:

$$x = \frac{[E]}{[E\dagger] + [E] + [R^* - E]} \tag{5}$$

$$= \frac{1}{Q + 1 + K_3[R^*]}$$

$$= \frac{1}{Q + 1 + \frac{K_3 R_t y [R^*]}{[R^*] + [R^* - E]}}$$

$$= \frac{1}{Q + 1 + \frac{K_3 R_t y}{1 + K_3 R_t x}} \tag{6}$$

Solving *x* and *y* simultaneously gives us *y* as a function of all the parameters.

Introducing:

$$\boxed{\theta_R = \frac{k_1 E_t}{k_2},} \tag{7}$$

then we have, from Eq. (4):

$$y = \frac{\frac{k_1 E_t x}{k_2}(1 + K_3 E_t x)}{1 + \frac{k_1 E_t x}{k_2}(1 + K_3 E_t x)} = \frac{x\theta_R (1 + K_3 E_t x)}{1 + x\theta_R (1 + K_3 E_t)} \tag{8}$$

The fraction of phosphorylated receptor molecules is $f_R = y$, so:

$$\boxed{f_R = \frac{x\theta_R (1 + K_3 E_t x)}{1 + x\theta_R (1 + K_3 E_t x)},} \tag{9}$$

and rearranging:

$$\boxed{\theta_R = \frac{f_R}{x(1 - f_R)(1 + K_3 E_t x)}.} \tag{10}$$

And from Eq. (6):

$$x = \frac{1}{1 + Q + \frac{K_3 R_t y}{1 + K_3 R_t x}} = \frac{1 + K_3 R_t x}{(1 + K_3 R_t x)(1 + Q) + K_3 R_t f_R}$$

$$x K_3 R_t f_R = 1 + K_3 R_t x - x(1 + K_3 R_t x)(1 + Q)$$

$$f_R = \frac{1 + K_3 R_t x - x(1 + Q + K_3 R_t x(1 + Q))}{x K_3 R_t}$$

Introducing:

$$\boxed{R_t' = \frac{R_t}{1 + Q},\ z = x(1 + Q),} \tag{11}$$

$$\boxed{f_R = \frac{1 + K_3 R_t' z - z - K_3 R_t' z^2}{K_3 R_t' z} = \frac{(1 - z)(1 + K_3 R_t' z)}{K_3 R_t' z}} \tag{12}$$

Hence, given *z*, we can compute f_R from Eq. (12), and with $z = x(1 + Q)$ and f_R we can compute θ_R from Eq. (10). Thus we have θ_R and f_R as functions of *z*. These were used to plot the graphs in Fig. 2b and for Spreadsheet 1.

Note that if $Q = 0$, Eq. (12) becomes:

$$f_R = \frac{(1 - x)(1 + K_3 R_t x)}{K_3 R_t x}. \tag{13}$$

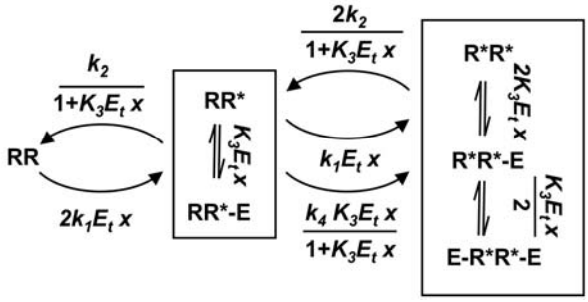
Also, regardless of *Q*, if $K_3 E_t << 0$, then Eq. (10) gives $\theta_R = \frac{f_R}{x(1 - f_R)}$ and $x \approx 1$. Hence,

$$f_R = \frac{x\theta_R}{1 + x\theta_R} \approx \frac{\theta_R}{1 + \theta_R}\ . \tag{14}$$

This is the canonical situation in the absence of feedback, if *E* does not bind *R**.

Appendix 2: Trans-phosphorylation of receptor dimers, allowing for feedback

For receptor dimers, we can group the rapid association-dissociation reactions and link them by phosphorylation-dephosphorylation reactions, as shown in the Figure (below). The rate constants need to be modified, as in Appendix 1, to give apparent rate constants that allow for reduced concentrations of reactants:-



where x, the fraction of the total enzyme in the free form, will be determined below.

Re-writing:

$$\underset{1\text{-}y_1\text{-}y_2}{\boldsymbol{RR}} \underset{b}{\overset{2a}{\Leftrightarrow}} \underset{y_1}{\boldsymbol{RR^*}} \underset{2b}{\overset{a+a'}{\Leftrightarrow}} \underset{y_2}{\boldsymbol{R^*R^*}} \qquad (1)$$

in which ***RR**** and ***R*R**** include the respective dimers with associated E. Then, from the figure:

$$a = k_1E_t x,\ b = \frac{k_2}{1+K_3E_t x},\ a' = \frac{k_4K_3E_t x}{1+K_3E_t x}, \text{ where } x = \frac{[E]}{E_t}. \qquad (2)$$

Let R_t be the total concentration of dimers, and

$$y_1 = \frac{[RR^*]+[RR^*-E]}{R_t},\ y_2 = \frac{[R^*R^*]+[R^*R^*-E]+[E-R^*R^*-E]}{R_t} \qquad (3)$$

then

$$y_1 = \frac{\frac{2a}{b}}{1+\frac{2a}{b}+\frac{a(a+a')}{b^2}} \qquad (4)$$

$$= \frac{\frac{2k_1E_t x}{k_2}(1+K_3E_t x)}{1+\frac{2k_1E_t x}{k_2}(1+K_3E_t x)+\frac{2k_1(E_t x)^2}{2k_2^{\,2}}(1+K_3E_t x)(k_4K_3+k_1+k_1K_3E_t x)} \qquad (5)$$

$$y_2 = \frac{\frac{a(a+a')}{b^2}}{1+\frac{2a}{b}+\frac{a(a+a'))}{b^2}} \tag{6}$$

$$= \frac{\frac{2k_1(E_t x)^2}{2k_2{}^2}(1+K_3E_t x)(k_4K_3+k_1+k_1K_3E_t x)}{1+\frac{2k_1E_t x}{k_2}(1+K_3E_t x)+\frac{2k_1(E_t x)^2}{2k_2{}^2}(1+K_3E_t x)(k_4K_3+k_1+k_1K_3E_t x)} \tag{7}$$

x in the scheme is not know *a priori*, but it depends on y_1 and y_2, as follows:

$$x = \frac{[E]}{[E\dagger]+[E]+[RR*-E]+[R*R*-E]+2[E-R*R*-E]}$$
$$= \frac{1}{Q+1+K_3[RR*]+2K_3[R*R*]+2K_3{}^2E_t x[R*R*]}$$

Introducing y_1R_t and y_1R_t:

$$x = \frac{1}{Q+1+\frac{K_3R_t y_1[RR*]}{[RR*]+[RR*-E]}+\frac{2K_3(1+K_3E_t x)R_t y_2[R*R*]}{[R*R*]+[R*R*-E]+[E-R*R*-E]}}$$

Now, $\frac{[RR*]+[RR*-E]}{[RR*]} = 1+K_3R_t x$, and

$\frac{[R*R*]+[R*R*-E]+[E-R*R*-E]}{[R*R*]} = (1+K_3R_t x)^2$, so

$$x = \frac{1}{Q+1+\frac{K_3R_t y_1}{1+K_3R_t x}+\frac{2K_3R_t y_2}{1+K_3R_t x}}$$
$$= \frac{1}{Q+1+\frac{K_3R_t}{1+K_3R_t x}(y_1+2y_2)} \tag{8}$$

Solving for x, y_1 and y_2 simultaneously gives us what we want.

Introducing:

$$\sigma = \frac{k_4K_3}{k_1},\ E_t' = \frac{E_t}{1+Q},\ R_t' = \frac{R_t}{1+Q},\ \theta' = \frac{\theta_R}{(1+Q)} = \frac{k_1E_t}{k_2(1+Q)} = \frac{k_1E_t'}{k_2},\ z = x(1+Q), \tag{9}$$

then we have $E_t x = E_t' z$, and:

$$y_1 = \frac{2z\theta'(1+K_3E_t' z)}{1+2z\theta'(1+K_3E_t' z)+z^2\theta'^2(1+K_3E_t' z)(\sigma+1+K_3E_t' z)} \tag{10}$$

$$y_2 = \frac{z^2\theta'^2\,(1+K_3E_t'z)(\sigma+1+K_3E_t'z)}{1+2z\theta'(1+K_3E_t'z)+z^2\theta'^2\,(1+K_3E_t'z)(\sigma+1+K_3E_t'z)} \tag{11}$$

$$z = \frac{1}{1+\dfrac{K_3R_t'(y_1+2y_2)}{1+K_3R_t'z}} \tag{12}$$

The fraction of phosphorylated receptor molecules is $f_R = \dfrac{y_1+2y_2}{2}$.

Combining Eqs. (10) and (11), we have:

$$f_R = \frac{z\theta'(1+K_3E_t'z)+z^2\theta'^2\,(1+K_3E_t'z)(\sigma+1+K_3E_t'z)}{1+2z\theta'(1+K_3E_t'z)+z^2\theta'^2\,(1+K_3E_t'z)(\sigma+1+K_3E_t'z)}, \tag{13}$$

and Eq. (12) can be written as

$$z = \frac{1+K_3R_t'z}{1+K_3R_t'z+2K_3R_t'f_R}. \tag{14}$$

We can solve θ' as a function of f_R and z from Eq. (13):

$$A\theta'^2+B\theta'+C=0', \tag{15}$$

that is,

$$\theta' = \frac{-B+\sqrt{B^2-4AC}}{2A}\quad, \tag{16}$$

in which

$$A = z^2(1+K_3E_t'z)(\sigma+1+K_3E_t'z)(1-f_R)$$

$$B = z(1+K_3E_t'z)(1-2f_R)$$

$$C = -f_R$$

We can also solve f_R as a function of z from Eq. (14)

$$f_R = \frac{(1-z)(1+K_3R_t'z)}{2zK_3R_t'}. \tag{17}$$

Hence, given z, we can compute f_R from Eq. (17), and with z and f_R we can compute $\theta_R = \theta'(1+Q)$ from Eq. (16). Thus we have θ_R and f_R as functions of z. These were used to plot the graphs in Fig. 3 and S1 and for Spreadsheet 2.

Note that if $k_4 = 0$, $\sigma = 0$, Eq. (13) becomes:

$$f_R = \frac{z\theta'(1+K_3E_t'z)+z^2\theta'^2\,(1+K_3E_t'z)^2}{1+2z\theta'(1+K_3E_t'z)+z^2\theta'^2\,(1+K_3E_t'z)^2}$$

$$= \frac{(1+\zeta)\zeta}{(1+\zeta)^2}$$

$$= \frac{\zeta}{1+\zeta}, \text{ where } \zeta = z\theta'(1+K_3E_t'z) = x\theta_R(1+K_3E_t x). \quad (18)$$

In other words, if there is no receptor trans-phosphorylation ($k_4 = 0$), then Q does not enter into the equation. This curve is the same as the monomer curve in Appendix 1.

If, in addition, we ignore binding of E to R^*, then $x = 1$ and

$$f_R = \frac{\zeta}{1+\zeta}, \text{ where } \zeta = \theta_R(1+K_3E_t), \text{ and if } K_3E_t << 1, \; f_R \approx \frac{\theta_R}{1+\theta_R}, \quad (19)$$

as expected (Appendix 1, Eq. 14).

Appendix 3: The effect of SFK trans-phosphorylation, independent of receptor trans-phosphorylation

The additional reactions in which open conformation SFK, *E*, is phosphorylated to *E** are shown in Fig. 4. We assume complete independence of phosphorylation/dephosphorylation of *E*, phosphorylation/dephosphorylation of *R*, and SH2-mediated binding. We do not include the effects of *E* binding to *R** on either the reduced rate of *R* phosphorylation or on protecting *R** from phosphatases (Appendix 1) or the receptor trans-phosphorylation (Appendix 2).

For receptor monomers, the reaction for phosphorylating *E* in solution or in complexes with *R** is:

$$E \xrightarrow{q_1 E'_t} E^* \qquad (1)$$

where E'_t is the total of open-conformation SFK, $\boxed{E'_t = E + E^* = \frac{E_t}{1+Q}}$.

Since $E'_t << K_M$ (Table 2), q_1 is approximately the ratio of the Michaelis-Menten parameters for phosphorylation of *E* (or various *R**-*E* complexes) by *E*. So, $q_1 \sim k_{cat}/K_M$.

For receptor dimers, which can form *R***R**-*E* or *R***R**-*E** complexes, there are two parallel reactions to phosphorylate an *E*. One *E* can directly phosphorylate the other:

$$E + R^*R^*\text{-}E \xrightarrow{q_1} E^* + R^*R^*\text{-}E \text{ or } E + R^*R^*\text{-}E^* \qquad (2a)$$

or, *R***R** can act as a scaffold:

$$E + R^*R^*\text{-}E \underset{u}{\overset{K_3/2}{\Leftrightarrow}} E\text{-}R^*R^*\text{-}E \underset{w}{\overset{2q_3}{\rightarrow}} E^*\text{-}R^*R^*\text{-}E \underset{x}{\overset{1/K_3}{\Leftrightarrow}} E^* + R^*R^*\text{-}E \qquad (2b)$$

u v w x y v

Then $K_3 = \frac{2w}{uv} = \frac{x}{yv}$, so $\frac{y}{x+y} = \frac{1}{1+K_3 v}$ and $w = \frac{K_3 uv}{2}$. The rate of production of *E** by this pathway is

$$\frac{dy}{dt} = \frac{1}{1+K_3 v}\frac{d(x+y)}{dt} = \frac{2q_3 w}{1+K_3 v} = \frac{q_3 K_3 v}{1+K_3 v}u$$

The concentration of doubly phosphorylated receptor dimers, *[R*R*]*, is $R_t f_R^2$, and

$v = 2[R^*R^*]E'_t K_3$ (Fig. 2h), so

$$v = 2R_t f_R^2 E'_t K_3 \text{ , and}$$

$$\frac{dy}{dt} = \frac{2q_3 K_3^2 R_t f_R^2 E'_t}{1+2K_3^2 R_t f_R^2 E'_t}u \approx q_3 K_3^2 R_t f_R^2 E'_t u \text{, provided that } K_3^2 R_t f_R^2 E'_t << 1.$$

If we set $\boxed{\xi = \frac{q_3 K_3^2 R_t}{q_1}}$, then:

$$\frac{dy}{dt} \approx q_1 \xi E'_t f_R^2 u,$$

and the effective rate constant $\frac{1}{u}.\frac{dy}{dt}$ for the second route to *E** is $q_1 \xi E'_t f_R^2$.

Combining reactions (2a) and (2b):

$$E \underset{q_2}{\overset{(1+\xi f_R^2)q_1 E'_t}{\Leftrightarrow}} E^* \qquad (3)$$

Define f_E as the fraction of open-conformation *E*, bound and unbound, in the *E** state:

$f_E = \frac{E^*}{E'_t} = \frac{E^*}{E_t}(1+Q)$. Then:

$$\frac{1}{f_E} = 1 + \frac{E}{E^*} = 1 + \frac{q_2}{(1+\xi f_R^2)q_1 E'_t}, \text{ so } f_E = \frac{(1+\xi f_R^2)q_1 E'_t}{(1+\xi f_R^2)q_1 E'_t + q_2}.$$

If we define the control parameter for SFK phosphorylation,

$$\boxed{\theta_E = \frac{q_1 E'_t}{q_2} = \frac{q_1 E_t}{q_2(1+Q)}}, \qquad (4)$$

then

$$f_E = \frac{(1+\xi f_R^{\,2})\theta_E}{(1+\xi f_R^{\,2})\theta_E + 1} \qquad (5)$$

Now we need to take account of the increased SFK activity in calculating the level of receptor phosphorylation. The activity of SFK driving receptor phosphorylation is increased from $k_1 E'_t$ to $k_1(1 - f_E + \phi f_E)E'_t$ and the control parameter $\theta_R = \frac{k_1 E_t}{k_2}$ for receptor phosphorylation needs to be modified to $\hat{\theta}_R = \theta_R(1 - f_E + \phi f_E)$ for the increased SFK activity. The new fraction of phosphorylated receptor monomers is:

$$\boxed{f_R = \frac{\hat{\theta}_R}{1+\hat{\theta}_R} = \frac{\theta_R(1 - f_E + \phi f_E)}{1+\theta_R(1 - f_E + \phi f_E)}} \qquad (6)$$

Combining Eqs. (5) and (6) and eliminating f_E, we have:

$$\boxed{\theta_R = \frac{f_R\left[1+\theta_E(1+\xi f_R^{\,2})\right]}{(1-f_R)\left[1+\phi\theta_E(1+\xi f_R^{\,2})\right]}} \qquad (7)$$

This relationship between f_R and θ_R for various ξ, θ_E, and ϕ (Eq. 7) was used to plot Fig. 4c and d (black lines). Eq. (5) was used to plot f_E against θ_R for various ξ, θ_E, and ϕ in Fig. 4b (blue lines). These calculations are in Spreadsheet 3.

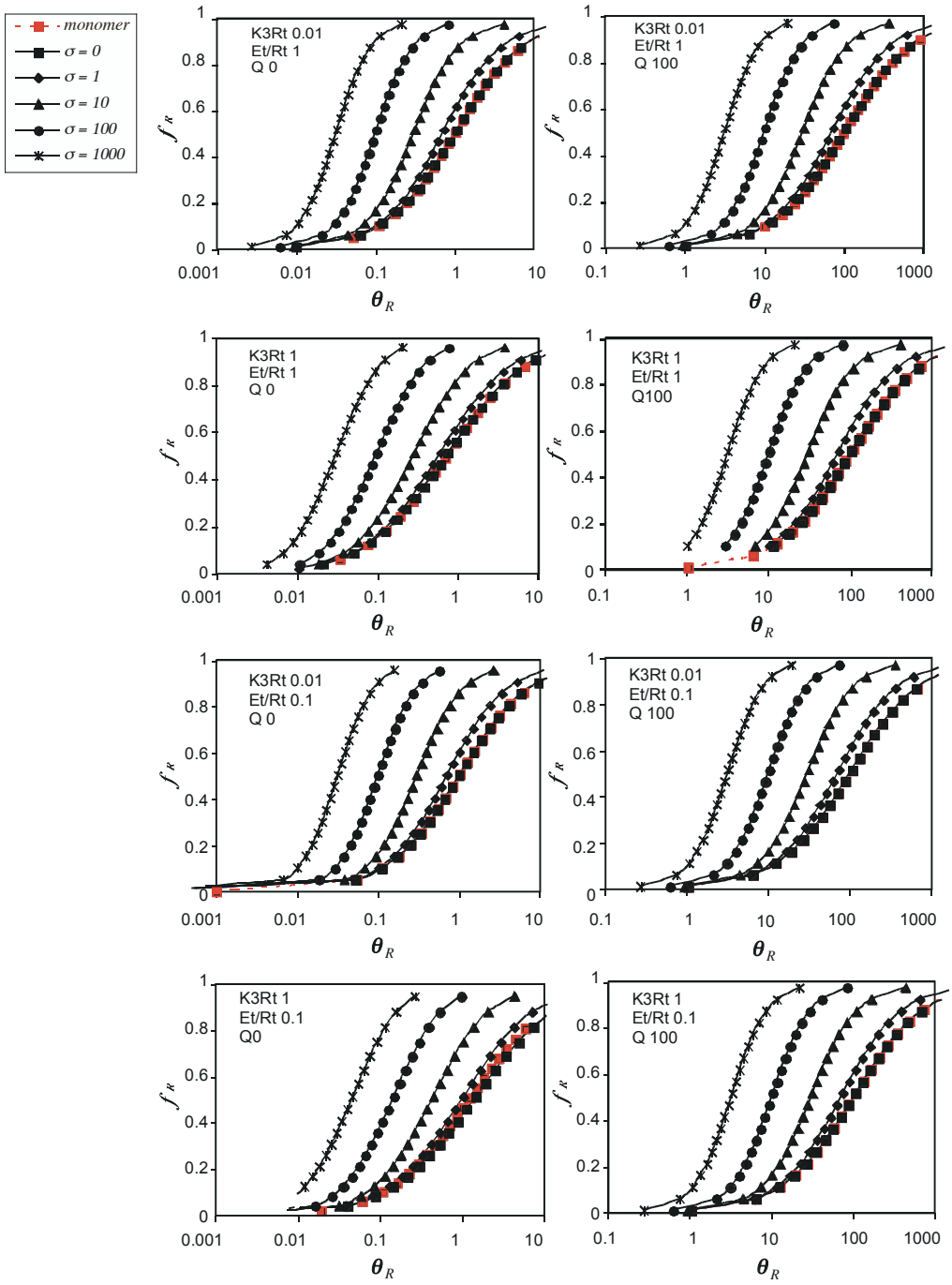


Fig. S1. Effect of E binding to R^* on phosphorylation of monomeric R and of dimeric RR on receptor transphosphorylation.

The levels of phosphorylation (f_R) of a receptor dimer (black lines) and monomer (red) are plotted for a variety of values of σ, K_3R_t, E_t/R_t, Q and θ_R that may be found in nature (see Table 2). For each set of conditions, there are values of θ_R for which the receptor dimer is phosphorylated at a greater than >2-fold level compared with receptor monomer. Note that curves on the left are for SFK that is completely in the active conformation ($Q = 0$), while curves on the right are for SFK that is 99% in the inactive conformation ($Q = 100$), and that the values of θ_R on the abscissa are different for the left and right graphs.

Calculations of monomer were performed according to Appendix 1 and dimer according to Appendix 2. Note that $\sigma = 0$ for the dimer is equivalent to the monomer result, as expected.

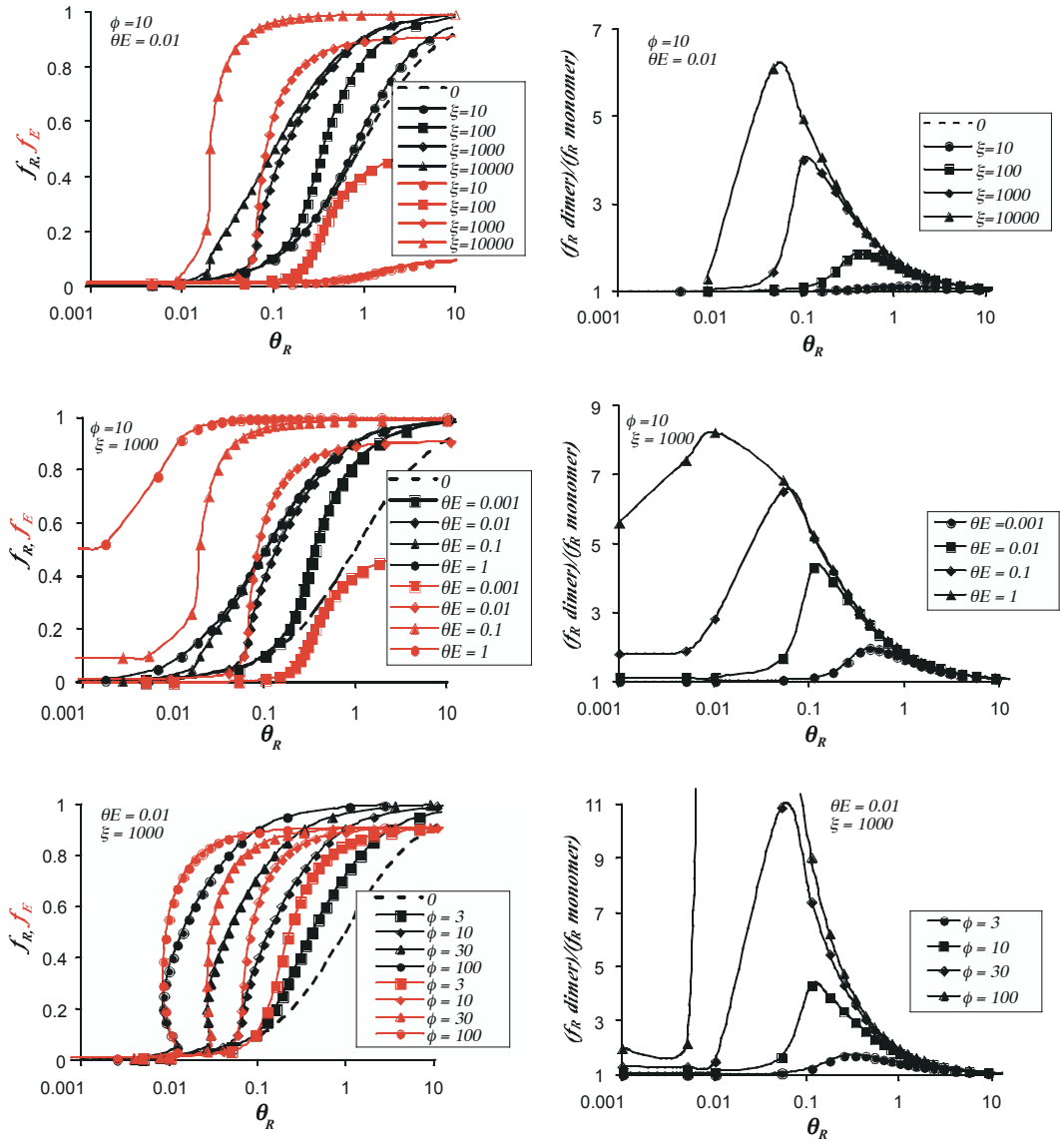


Fig. S2. Effect of varying ξ, ϕ, and θ_E on f_E and f_R (left panels) and the fold-stimulation in f_R due to dimerization (right panels).

Calculations according to Appendix 3. Note the > 10-fold stimulation of f_E and f_R that can occur at low θ_E and high ξ.