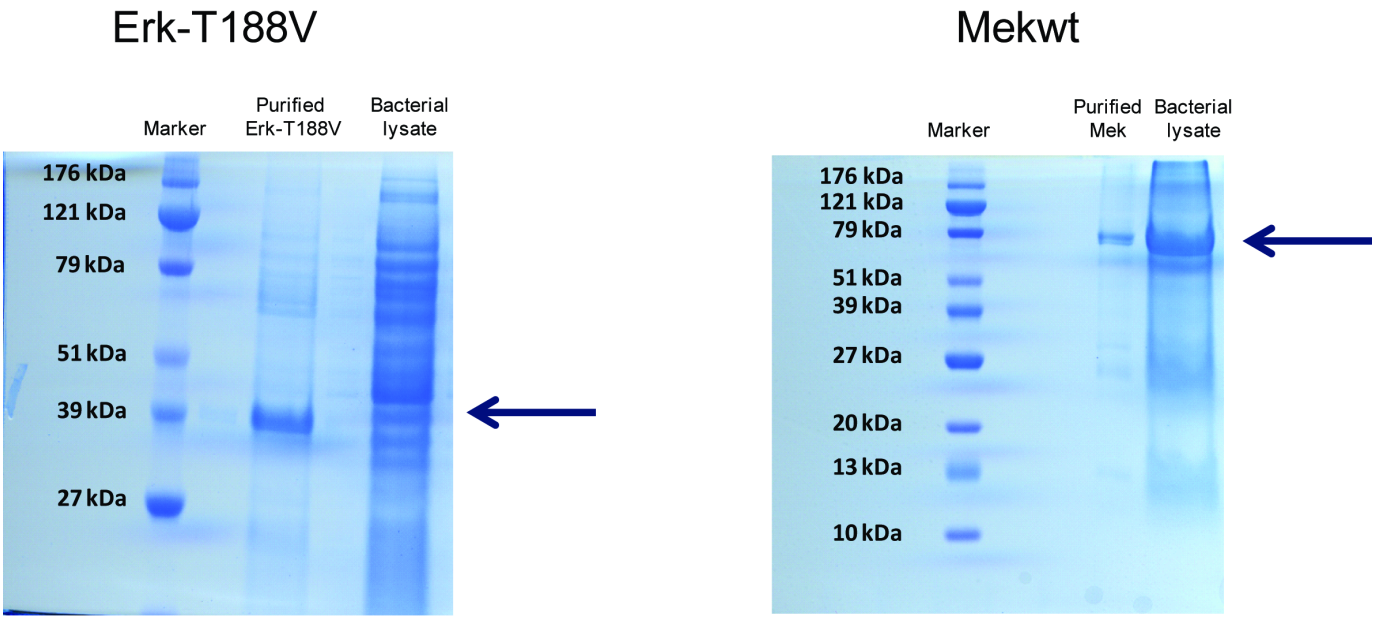
Supplementary Materials

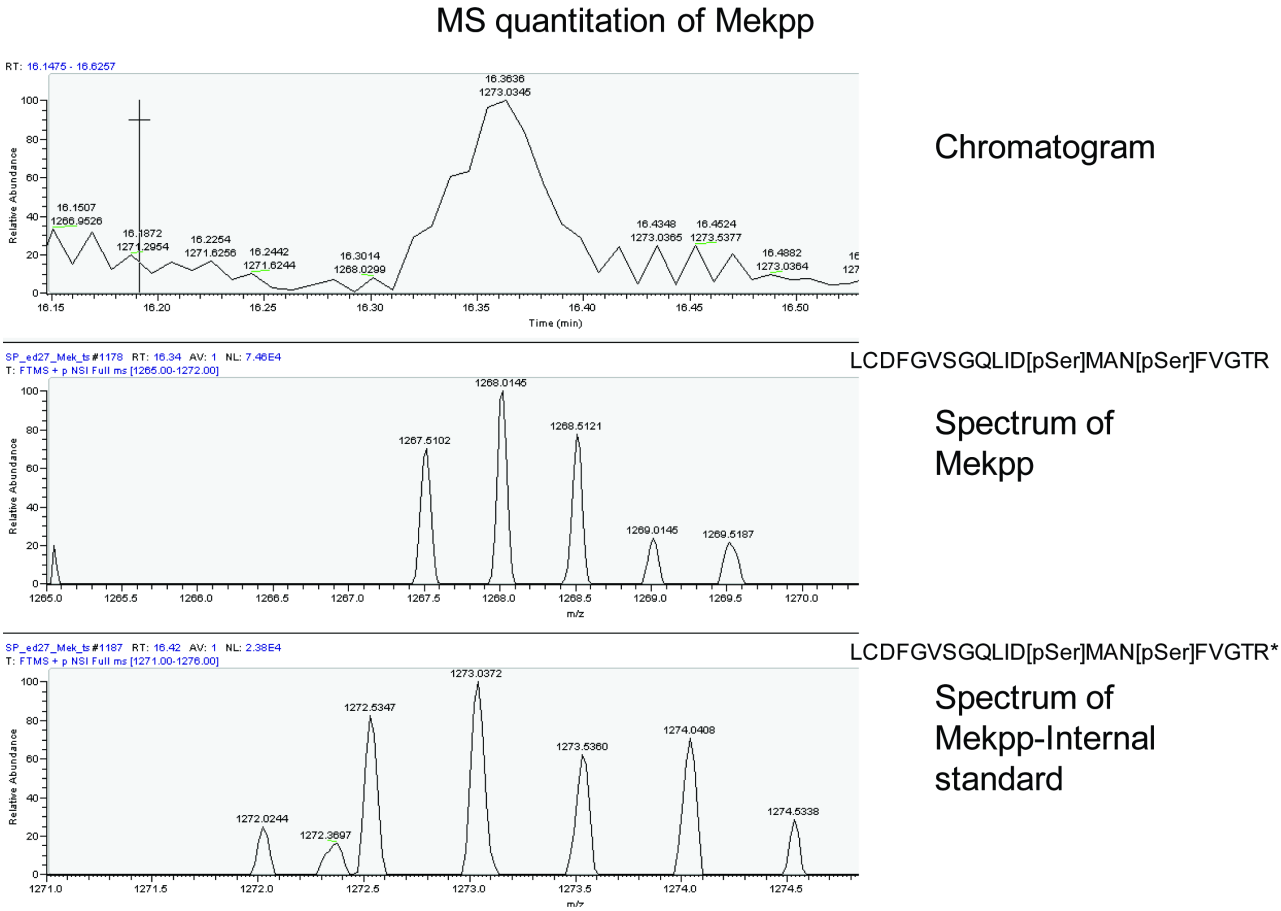
Paradoxical results in perturbation-based signaling network reconstruction

Sudhakaran Prabakaran, Jeremy Gunawardena, and Eduardo D. Sontag

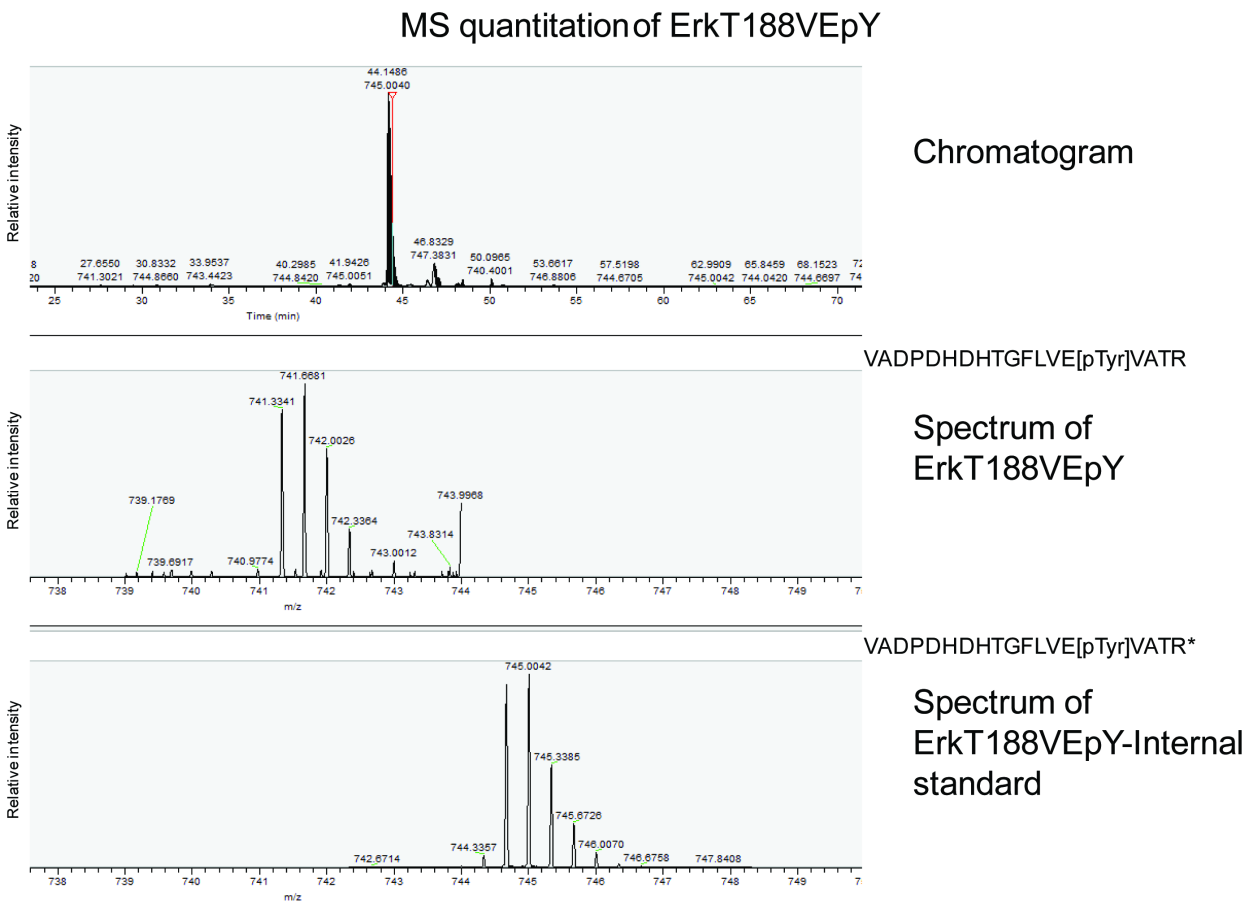
Additional experimental data



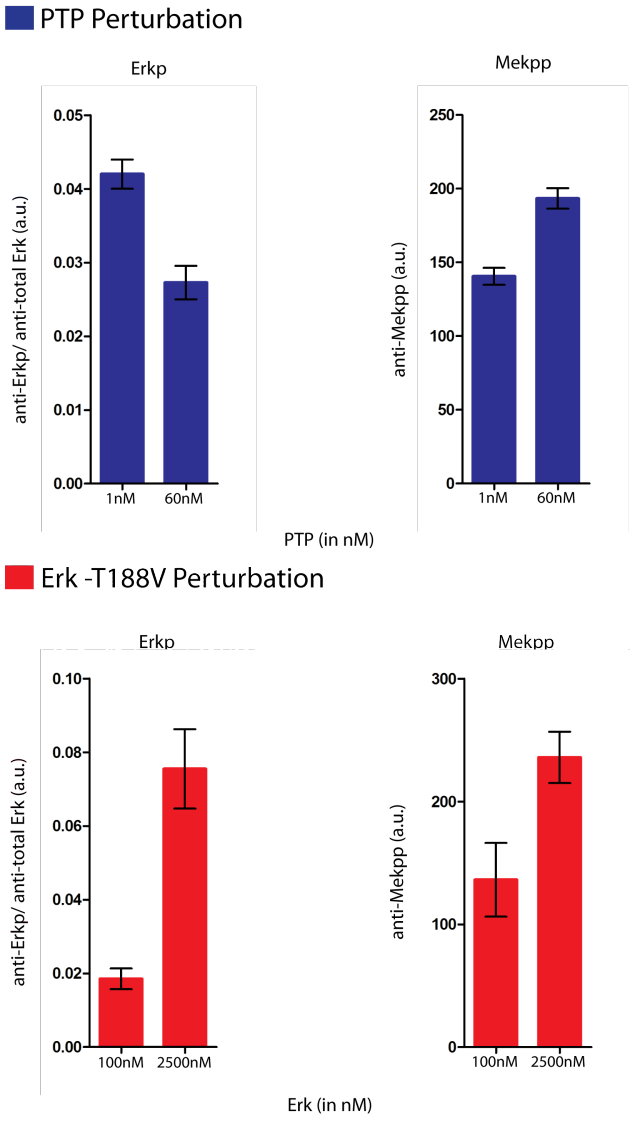
Supplementary Figure 1: The figure shows coomassie stained gels of purified Erk and Mek. Mutated Erk (ErkT88V) was expressed in bacteria and purified using Ni/NTA column (molecular weight ∼43 kDa). It is purified to almost 95% purity. Mek1 was also expressed and purified to 95% purity.



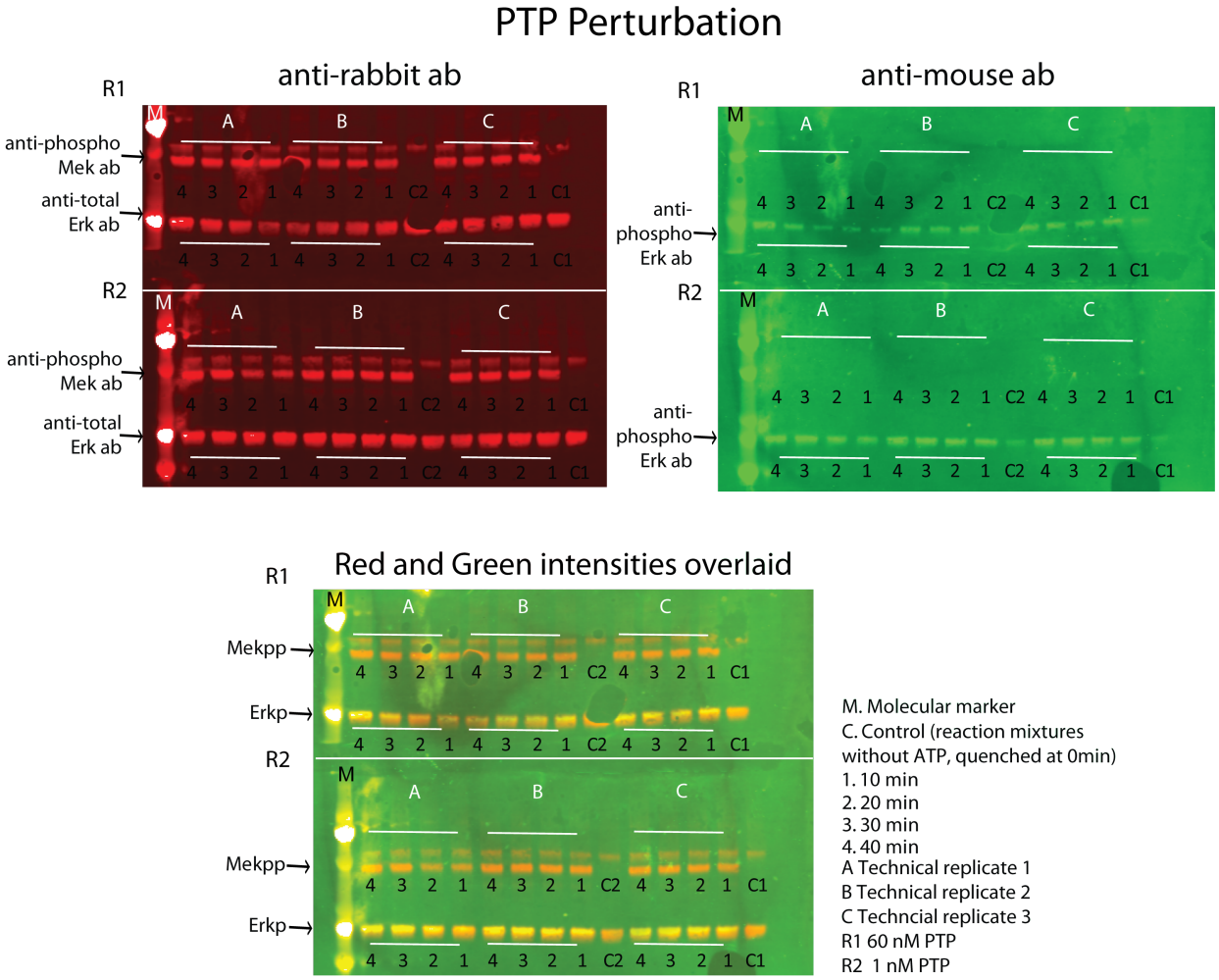
Supplementary Figure 2: Representative chromatogram, representative spectrum of the analyte Mekpp peptide (doubly charged) along with the representative spectrum of its corresponding internal standard peptide is shown in the figure. Relative area under the curve of the analyte Mekpp peptide to its internal standard is used for Mekpp quantitation.



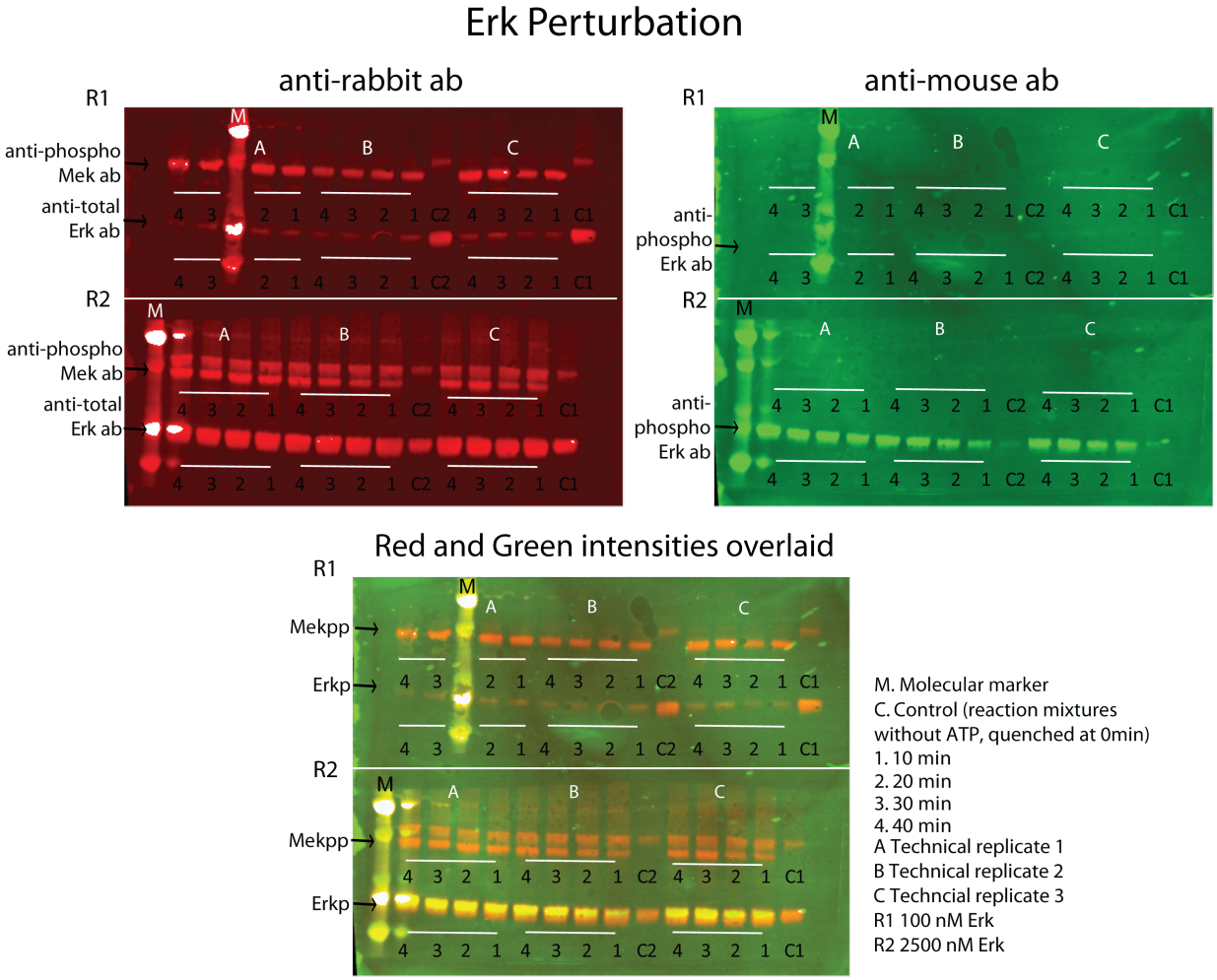
Supplementary Figure 3: Representative chromatogram, representative spectrum of the analyte Erkp peptide (triply charged) along with the representative spectrum of its corresponding internal standard peptide is shown in the figure. Relative area under the curve of the analyte Erkptide to its internal standard is used for Erkp quantitation.



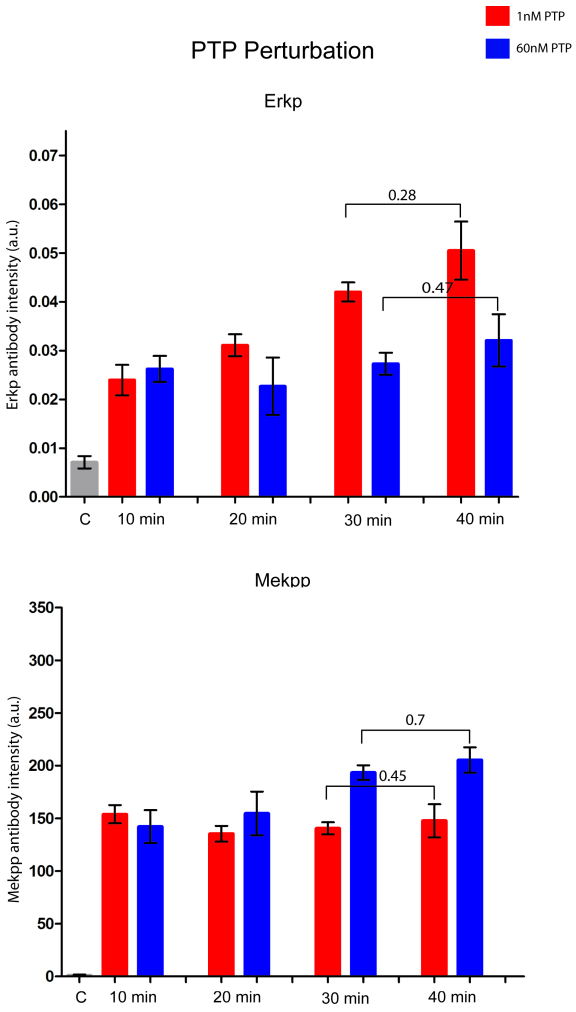
Supplementary Figure 4: PTP perturbation and Erk perturbation were further done for pairs of new values and analysed with western blots. The results are consistent with the previous results (Figure 2). Briefly, amounts of Erkp and Mekpp, quantified as described in Materials and Methods, are plotted at the concentrations of the perturbed enzymes after 30 minutes of reaction time. Error bars indicate standard errors of the mean of three technical replicates (same sample, three different reaction tubes).



Supplementary Figure 5: Representative western blots for experiment in Supplementary Figure 4 along with time-course data are shown in the Figure. These blots were done for PTP pertubation experiments. Reaction aliquots containing Erk (∼43kDa) and Mek (∼71kDa), collected at 0min, 10min, 20min, 30min and 40min of reaction times were snap frozen and run on the same SDS-PAGE, transferred and probed with mouse, anti-phospho Erk ab, rabbit, anti-total Erk ab and rabbit anti-phospho-Mek ab. Secondary anti- mouse (green pseudo color) and anti-rabbit (red pseudo color) were used to probe the primary antibodies. The relative intensities of green and red channels were used for Erkp quantitation (overlaid image), the red channel intensity was used for Mekpp quantitation. Error bars show the standard error of the mean of three technical replicates (same sample, three different reaction tubes).

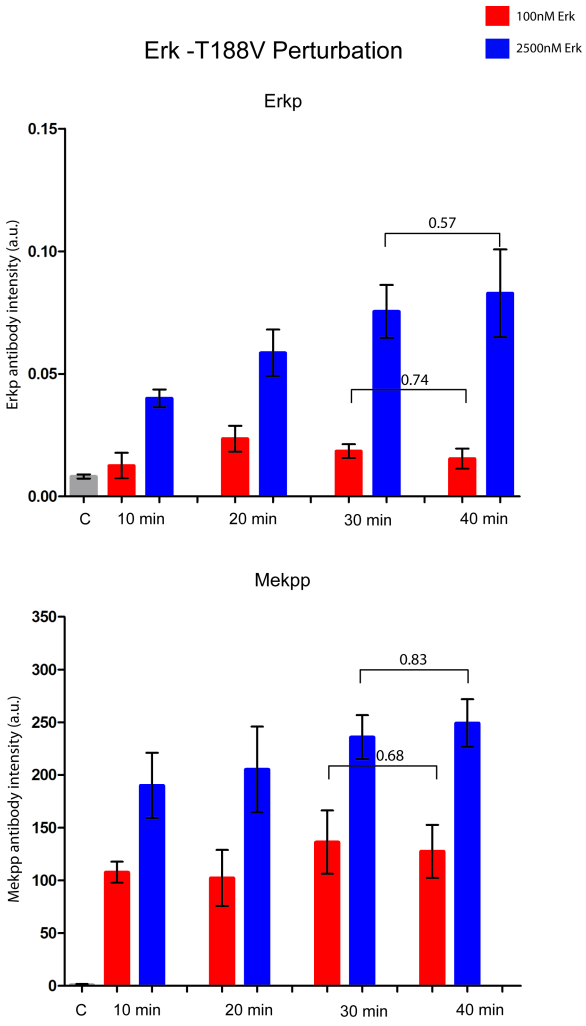


Supplementary Figure 6: Representative western blots for experiment in Supplementary Figure 4 along with time-course data are shown in the Figure. These blots were done for Erk pertubation ex- periments. Reaction aliquots containing Erk (∼43kDa) and Mek (∼71kDa), collected at 0min, 10min, 20min, 30min and 40min of reaction times were snap frozen and run on the same SDS-PAGE, transferred and probed with mouse, anti-phospho Erk ab, rabbit, anti-total Erk ab and rabbit anti-phospho-Mek ab. Secondary anti- mouse (green pseudo color) and anti-rabbit (red pseudo color) were used to probe the primary antibodies. The relative intensities of green and red channels were used for Erkp quantitation (overlaid image), the red channel intensity was used for Mekpp quantitation. Error bars show the standard error of the mean of three technical replicates (same sample, three different reaction tubes).



Supplementary Figure 7: Quantitative values of reactions in Supplementary Figure 5. Mekpp and Erkp quantitative values do not show significant differences between 30 and 40 min, as shown by p-values computed using Student's t-test, 2-tailed, unequal variance.

On the other hand, the discovered effects are significant, at both 30 and 40 minute time-points. For Erkp under PTP Pertubation, one has at 30 min, p-value = 0.01 and at 40 min, p-value = 0.08. For Mekpp under PTP Pertubation, one has at 30 min, p-value = 0.004 and at 40 min, p-value = 0.047



Supplementary Figure 8: Quantitative values of reactions in Supplementary Figure 6. Mekpp and Erkp quantitative values do not show significant differences between 30 and 40 min, as shown by p-values computed using Student's t-test, 2-tailed, unequal variance.

On the other hand, the discovered effects are significant, at both 30 and 40 minute time-points. For Erkp under Erk Pertubations, one has at 30 min, p-value = 0.027 and at 40 min, p-value = 0.057. For Mekpp under Erk Pertubation, one has at 30 min, p-value = 0.059 and at 40 min, p-value = 0.02

Simulations

We provide here some simulations that confirm the predictions of the theorem. We take initially the following values for the kinetic constants:

$$\alpha = 0.1,\ \beta = 0.1,\ \gamma = 0.01,\ \delta = 1,\ \xi = 1,\ \varphi = 0.01,\ \eta = 1, \psi = 1,$$

and conserved quantities:

$$m_T = 100,\ p_T = 100,\ e_T = 1000$$

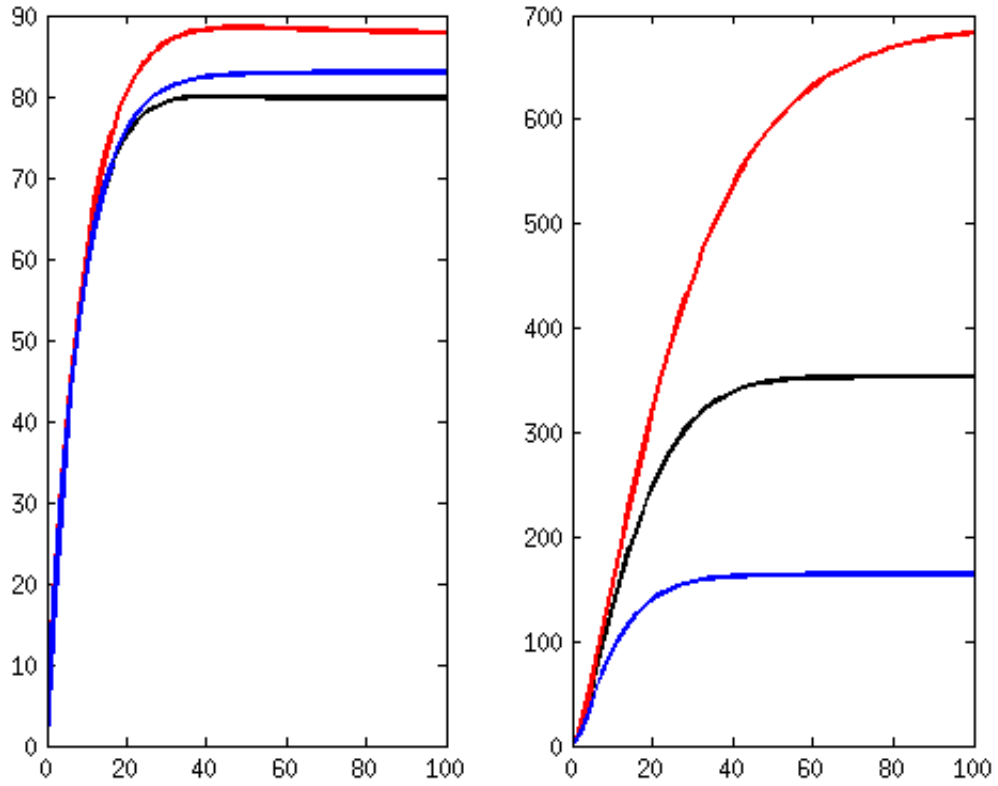
(units: s^{-1} for constants in monomolecular and $nM^{-1}s^{-1}$ for bimolecular reactions, and nM for conserved quantities). As precise values of kinetic constants are not known, we picked these numbers as a rough order of magnitude approximation of the different Raf/Mek/Erk constants in the reactions given in the Online Supplementary Materials for the paper (1). (The values of α and β are obtained from multi-step reactions as $k_{\text{cat}}e_T/K_m$, assuming a first-order linear regime in a Michaelis-Menten approximation.) The conserved quantity numbers represent a rough range of the concentrations of enzymes used in our experimental work. Later, we also show simulations where parameters and enzyme concentrations are taken to have very different values.

As initial states in the first set of simulations, we take:

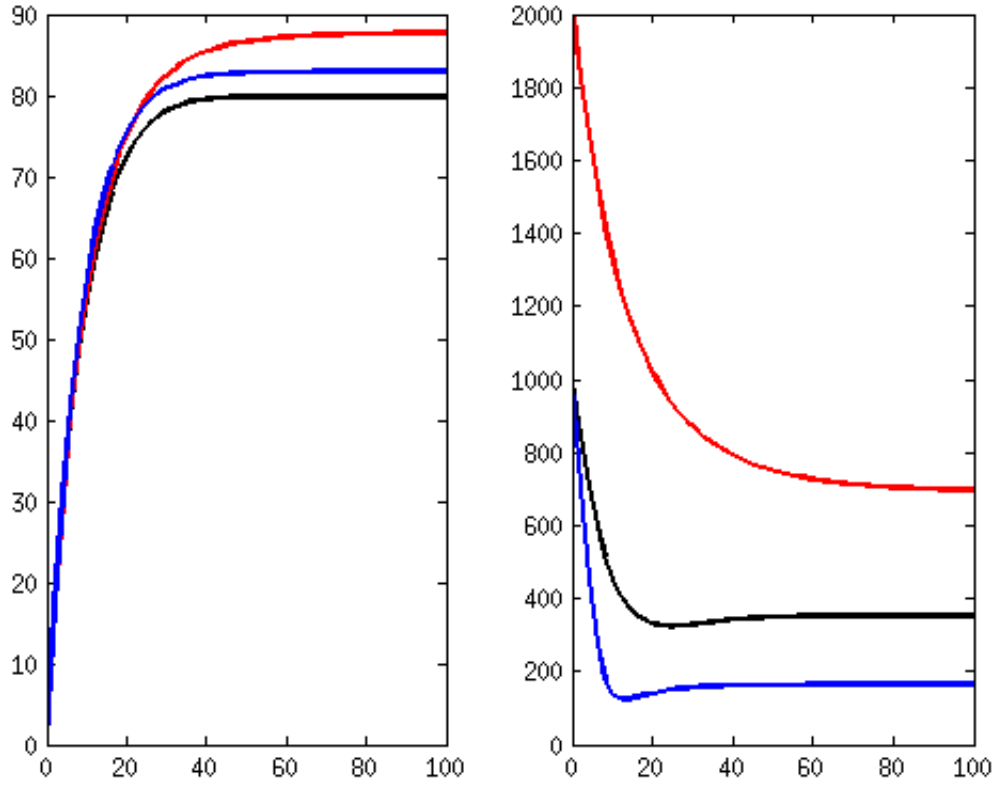
$$m(0) = m_T,\ p(0) = p_T,\ e(0) = e_T,\ m^*(0) = 0,\ c_{m^*e}(0) = 0,\ e^*(0) = 0,\ c_{pe^*}(0) = 0.$$

The simulations in Fig. 9 compare the solutions for the above values of p_T and e_T and the solutions obtained when perturbing each of these by doubling them one at a time. Shown are plots of total active enzyme (left) and total active substrate (right). Observe that not only the predicted changes in steady states are as claimed, but in addition the dynamic behavior at intermediate states, even under non-infinitesimal perturbations, is consistent with the predictions.

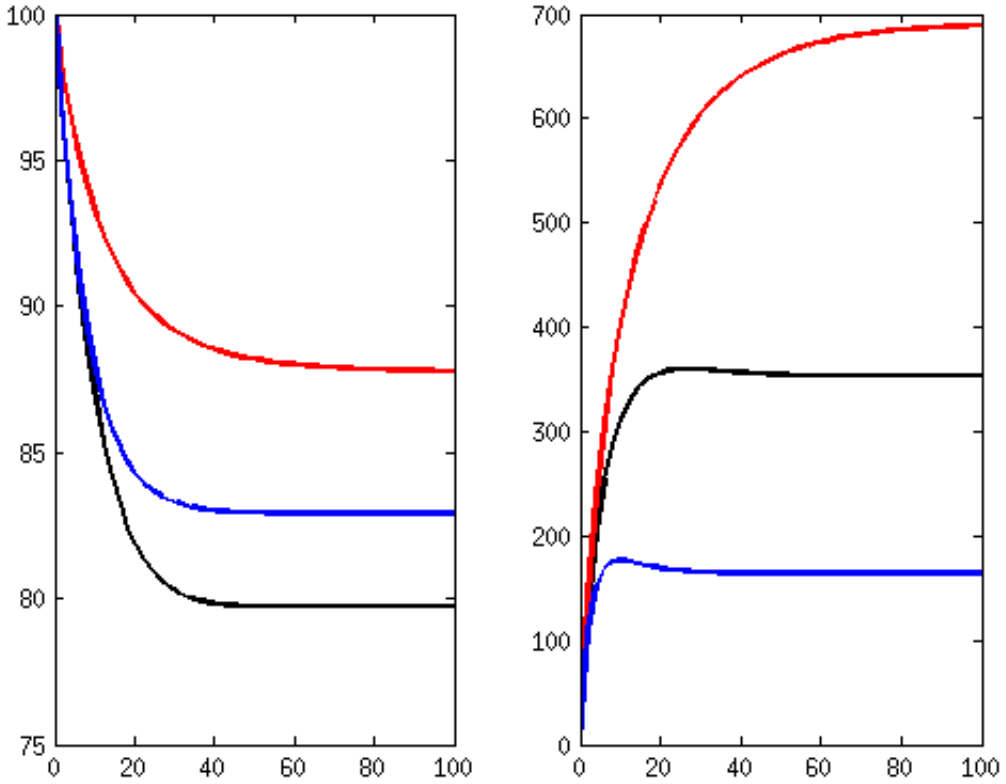
Keeping our parameters unchanged, we investigate next the effect of starting at fully activated substrate, enzyme, or both, see Figs. 10, 11, and 12. At steady state, the same values obtain, but transient behaviors are different. Nonetheless, the conclusions regarding the effect of perturbations stay unchanged: For the active enzyme ($x(t)$) all values are higher, at steady state, for both types of perturbations. For active substrate ($y(t)$), all values are higher both transiently and at steady state for substrate perturbations, while all values are lower both transiently and at steady state for phosphatase perturbations.



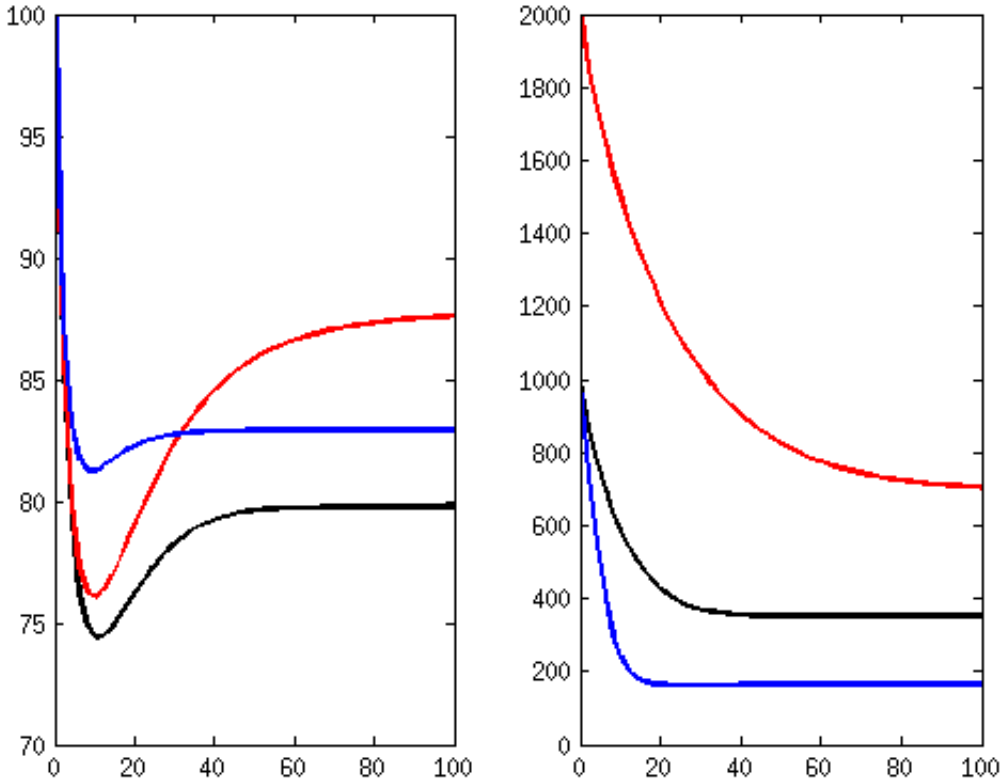
Supplemental Figure 9: Left: active enzyme ($x(t)$) as function of time, using $m_T = 100$, $p_T = 100$, $e_T = 1000$, $\alpha = \beta = 0.1$, $m(0) = m_T$, $e(0) = e_T$, $m^*(0) = 0$, $e^*(0) = 0$. Observe that all values are higher, both transiently and at steady state, for both types of perturbations. Right: active substrate ($y(t)$) as function of time, using same parameters. Observe that all values are higher, respectively lower, both transiently and at steady state, for substrate (Erk_T, red), respectively phosphatase (PTP_T, blue), perturbations. Black plot is reference (unperturbed).



Supplemental Figure 10: Left: active enzyme ($x(t)$) as function of time, using $p_T = 100$, $m_T = 100$, $e_T = 1000$, $\alpha = \beta = 0.1$, $m(0) = m_T$, $e(0) = 0$, $m^*(0) = 0$, $e^*(0) = e_T$. Right: active substrate ($y(t)$) as function of time, using same parameters. Colors are as in Fig. 9.



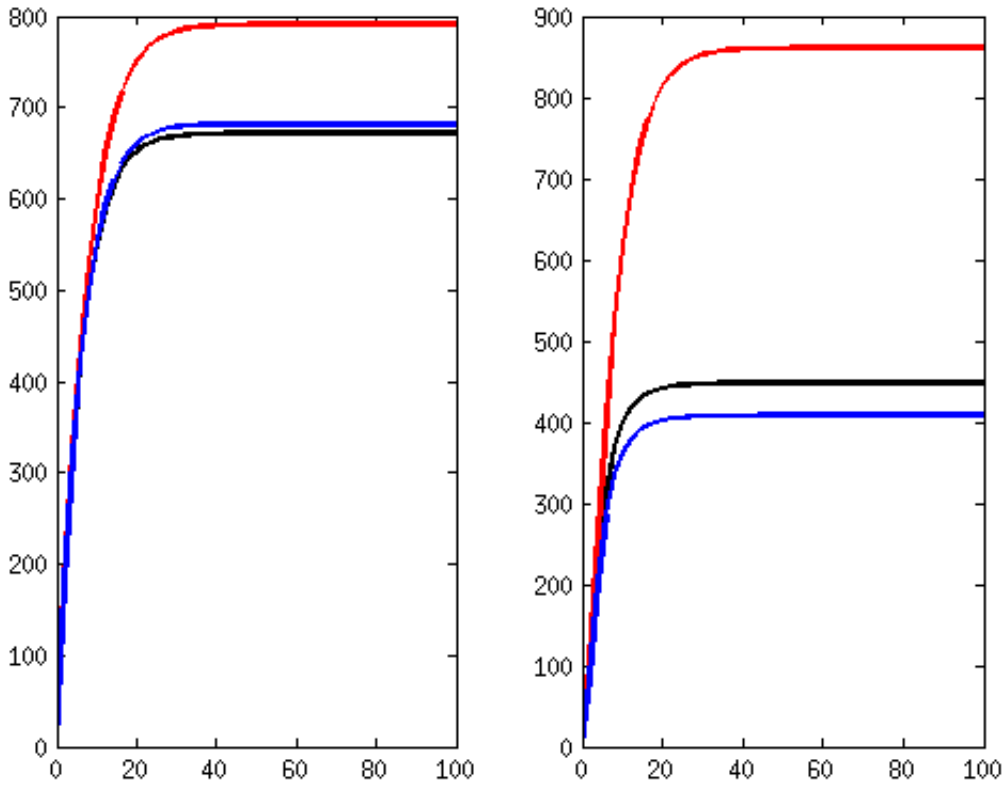
Supplemental Figure 11: Left: active enzyme ($x(t)$) as function of time, using $p_T = 100$, $m_T = 100$, $e_T = 1000$, $\alpha = \beta = 0.1$, $m(0) = 0$, $e(0) = e_T$, $m^*(0) = m_T$, $e^*(0) = 0$. Right: active substrate ($y(t)$) as function of time, using same parameters. Colors are as in Fig. 9.



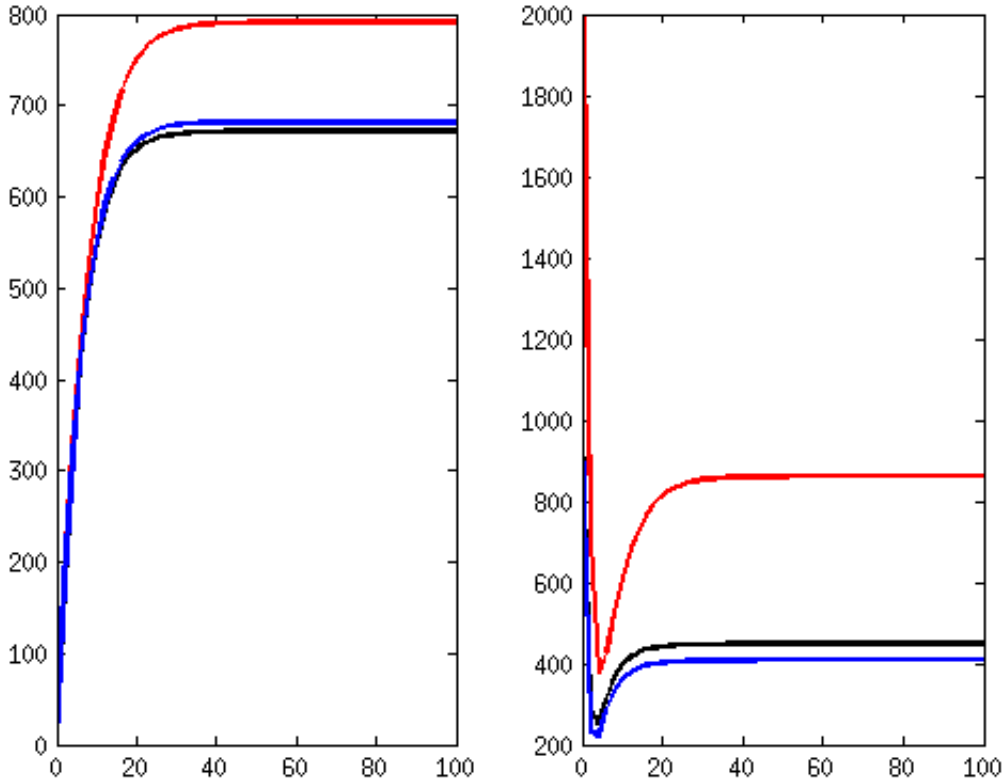
Supplemental Figure 12: Left: active enzyme ($x(t)$) as function of time, using $p_T = 100$, $m_T = 100$, $e_T = 1000$, $\alpha = \beta = 0.1$, $m(0) = 0$, $e(0) = 0$, $m^*(0) = m_T$, $e^*(0) = e_T$. Right: active substrate ($y(t)$) as function of time, using same parameters. Colors are as in Fig. 9.

Different nominal kinase and phosphotase amounts

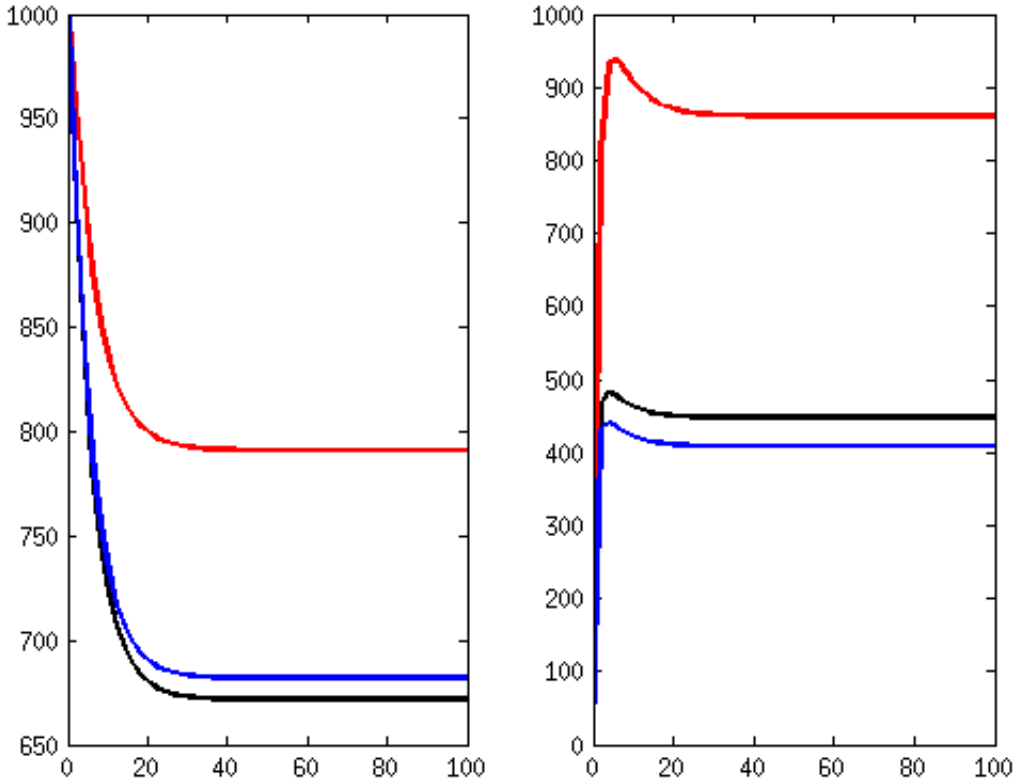
We study here the effect of increasing enzyme amounts by an order of magnitude. Once again, conclusions are unchanged. See Figs. 13, 14, 15, and 16.



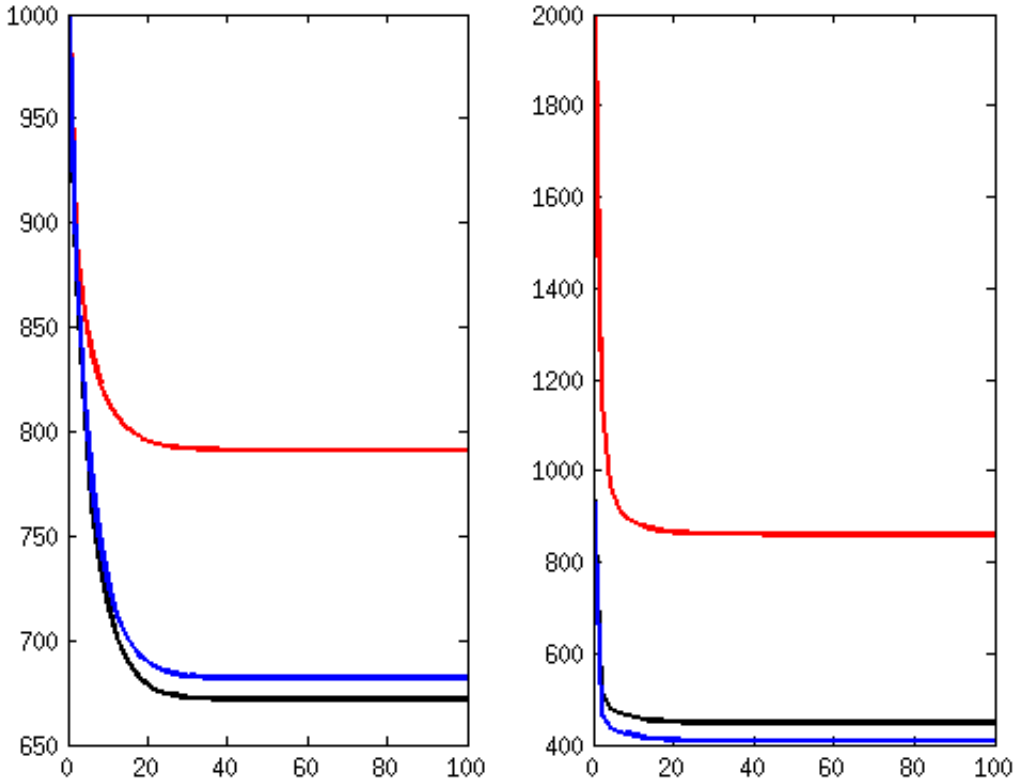
Supplemental Figure 13: Left: active enzyme ($x(t)$) as function of time, using $p_T = 1000$, $m_T = 1000$, $e_T = 1000$, $\alpha = \beta = 0.1$, $m(0) = m_T$, $e(0) = e_T$, $m^*(0) = 0$, $e^*(0) = 0$. Right: active substrate ($y(t)$) as function of time, using same parameters. Colors are as in Fig. 9.



Supplemental Figure 14: Left: active enzyme ($x(t)$) as function of time, using $p_T = 1000$, $m_T = 1000$, $e_T = 1000$, $\alpha = \beta = 0.1$, $m(0) = m_T$, $e(0) = 0$, $m^*(0) = 0$, $e^*(0) = e_T$. Right: active substrate ($y(t)$) as function of time, using same parameters. Colors are as in Fig. 9.



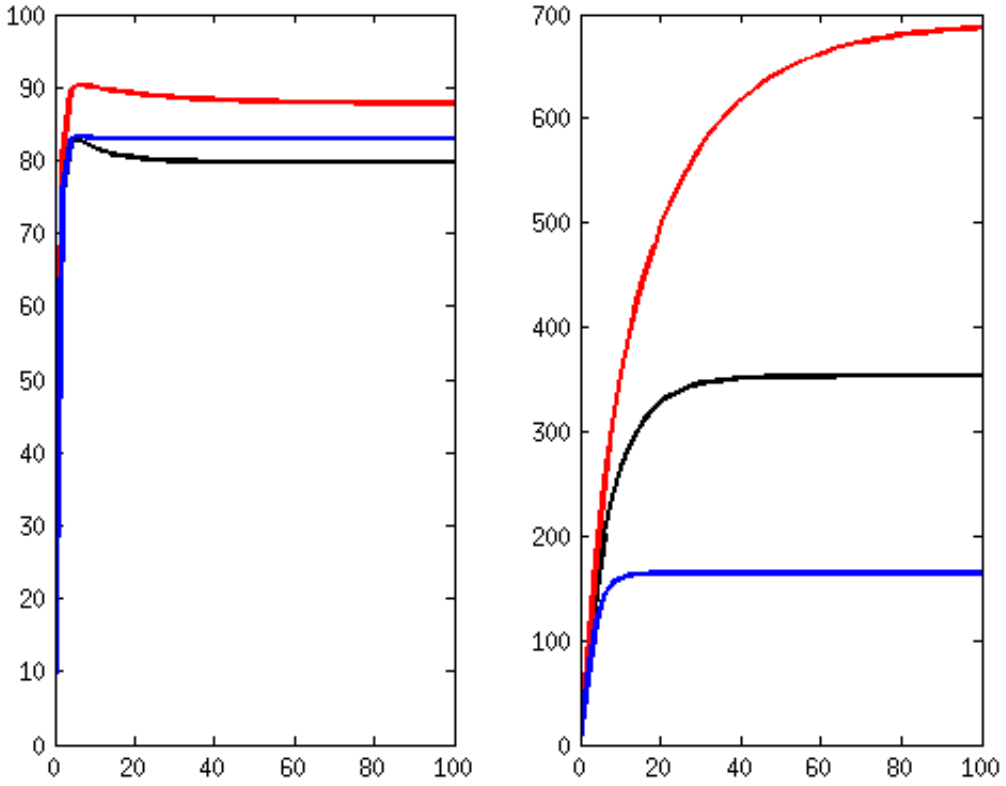
Supplemental Figure 15: Left: active enzyme ($x(t)$) as function of time, using $p_T = 1000$, $m_T = 1000$, $e_T = 1000$, $\alpha = \beta = 0.1$, $m(0) = 0$, $e(0) = e_T$, $m^*(0) = m_T$, $e^*(0) = 0$. Right: active substrate ($y(t)$) as function of time, using same parameters. Colors are as in Fig. 9.



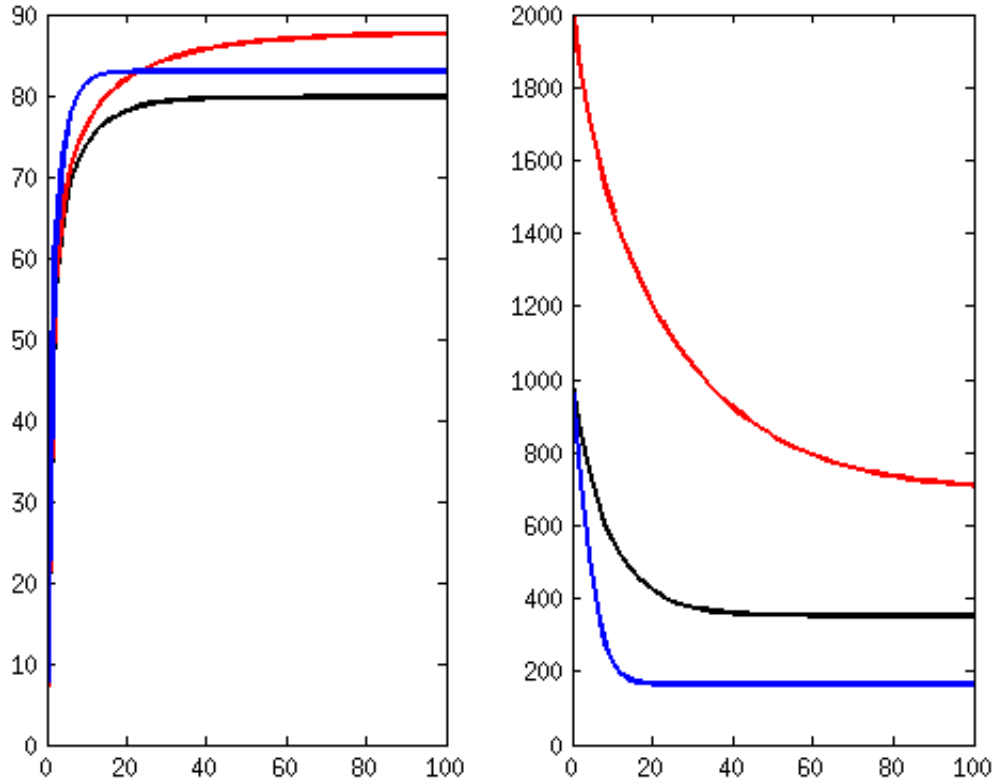
Supplemental Figure 16: Left: active enzyme ($x(t)$) as function of time, using $p_T = 1000$, $m_T = 1000$, $e_T = 1000$, $\alpha = \beta = 0.1$, $m(0) = 0$, $e(0) = 0$, $m^*(0) = m_T$, $e^*(0) = e_T$. Right: active substrate ($y(t)$) as function of time, using same parameters. Colors are as in Fig. 9.

Different kinetic constants for kinase modifications

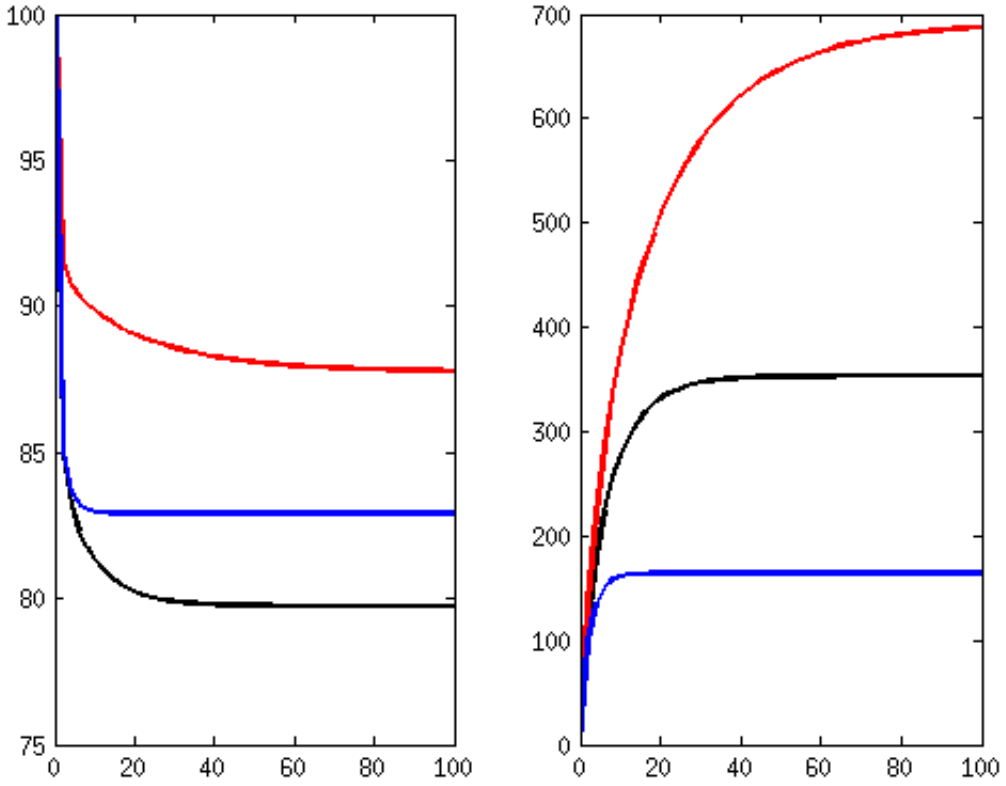
We study here the effect of increasing by a factor the rates α and β at which the enzyme E may be phosphorylated or dephosphorylated. Conclusions remain unchanged. See Figs. 17, 18, 19, and 20.



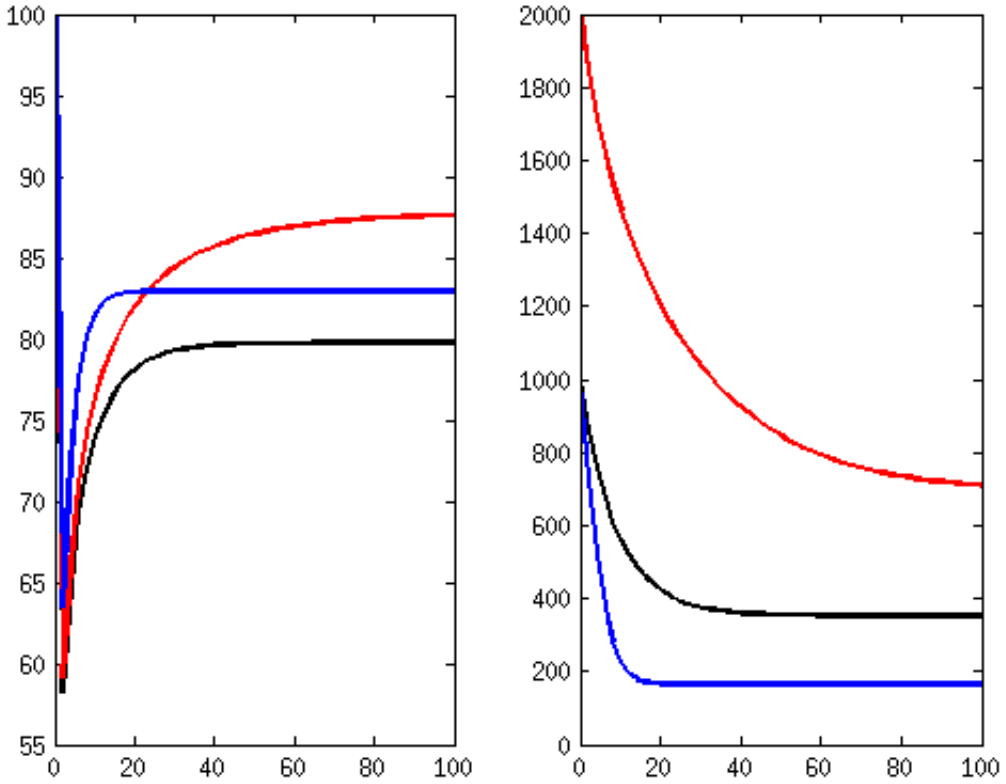
Supplemental Figure 17: Left: active enzyme ($x(t)$) as function of time, using $p_T = 100$, $m_T = 100$, $e_T = 1000$, $\alpha = \beta = 1$, $m(0) = m_T$, $e(0) = e_T$, $m^*(0) = 0$, $e^*(0) = 0$. Right: active substrate ($y(t)$) as function of time, using same parameters. Colors are as in Fig. 9.



Supplemental Figure 18: Left: active enzyme ($x(t)$) as function of time, using $p_T = 100$, $m_T = 100$, $e_T = 1000$, $\alpha = \beta = 1$, $m(0) = m_T$, $e(0) = 0$, $m^*(0) = 0$, $e^*(0) = e_T$. Right: active substrate ($y(t)$) as function of time, using same parameters. Colors are as in Fig. 9.



Supplemental Figure 19: Left: active enzyme ($x(t)$) as function of time, using $p_T = 100$, $m_T = 100$, $e_T = 1000$, $\alpha = \beta = 1$, $m(0) = 0$, $e(0) = e_T$, $m^*(0) = m_T$, $e^*(0) = 0$. Right: active substrate ($y(t)$) as function of time, using same parameters. Colors are as in Fig. 9.



Supplemental Figure 20: Left: active enzyme ($x(t)$) as function of time, using $p_T = 100$, $m_T = 100$, $e_T = 1000$, $\alpha = \beta = 1$, $m(0) = 0$, $e(0) = 0$, $m^*(0) = m_T$, $e^*(0) = e_T$. Right: active substrate ($y(t)$) as function of time, using same parameters. Colors are as in Fig. 9.

References

1. Markevich, N. I., J. B. Hoek, and B. N. Kholodenko, 2004. Signaling switches and bistability arising from multisite phosphorylation in protein kinase cascades. *J. Cell Biol.* 164:353–359.