Supplementary material for:

Oscillations induced by different timescales in signal transduction modules regulated by slowly evolving protein-protein interactions

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1 Parameter estimates

1.1 Bistability

To obtain analytic explicit expressions for the "high" and "low" steady states of the system

$$\dot{x} = \Phi(x) - \gamma_0 x = \left(k_1(w_0 + u) + V_1 \frac{x^n}{x^n + \theta_1^n} \right)(1 - x) - \gamma_0 x, \tag{1}$$

the function Φ in (1) is approximated as follows:

$$\Phi(x) \approx \begin{cases} \Phi_l(x) = \Phi_0(1 - x), & x < (1 - \Delta)\theta_1 \\ \Phi_m(x) = \left(\Phi_0 + \frac{V_1}{2\Delta\theta_1}(x - (1 - \Delta)\theta_1) \right)(1 - x), & (1 - \Delta)\theta_1 \le x \le (1 + \Delta)\theta_1 \\ \Phi_r(x) = (\Phi_0 + V_1)(1 - x), & x > (1 + \Delta)\theta_1, \end{cases} \tag{2}$$

where $\Delta = 2/n$ and $\Phi_0 = k_1(w_0 + u)$. Using this approximation, the steady states of the system as functions of the input u can be obtained by finding the solutions to:

$$\Phi_l(x) = \gamma_0 x, \quad \Phi_m(x) = \gamma_0 x, \quad \text{or} \quad \Phi_r(x) = \gamma_0 x.$$

The "low" steady state is always given by the intersection of the line $\gamma_0 x$ with the left branch $\Phi_l(x)$. The "high" steady state can be given by intersection of the line $\gamma_0 x$ with either the middle or the right branch, depending on the parameters. The two cases are illustrated in Fig. 1, blue and red lines.

To determine the beginning and end points of the bistability interval, $[u_{\min}, u_{\max}]$, note that the $u_{\max}$ is found by the last intersection possible between $\Phi_l(x)$ and $\gamma_0 x$. There are two possible cases for $u_{\min}$, shown in Fig. 1, depending on which branch intersects the line $\gamma_0 x$ first, as u decreases from large values. Thus

$$u = u_{\max} \Rightarrow \bar{x}_{\text{low}}(u_{\max}) = (1 - \Delta)\theta_1$$
$$u = u_{\min} \Rightarrow \bar{x}_{\text{high}}(u_{\min}) = \beta$$

where

$$\beta = \begin{cases} (1 + \Delta)\theta_1, & \text{if } \Phi_r(\beta; u_{\min}) - \gamma_0\beta = 0 \text{ (Fig. 1, right)} \\ -\frac{1}{2}\frac{c_1}{c_2}, & \text{if } \Phi_m(\beta; u_{\min}) - \gamma_0\beta = 0 \text{ (Fig. 1, left)} \end{cases}$$

with

$$c_0 = w_0 + u - \frac{1}{2\Delta\theta_1}\frac{V_1}{k_1}(1 - \Delta)\theta_1,$$

$$c_1 = \frac{1}{2\Delta\theta_1}\frac{V_1}{k_1}(1 + (1 - \Delta)\theta_1) - \left(w_0 + u + \frac{\gamma_0}{k_1} \right),$$

$$c_2 = -\frac{1}{2\Delta\theta_1}\frac{V_1}{k_1}.$$

For the optimal set of parameters, the function $\Phi(x; u)$ is of the form seen at left in Fig. 1.
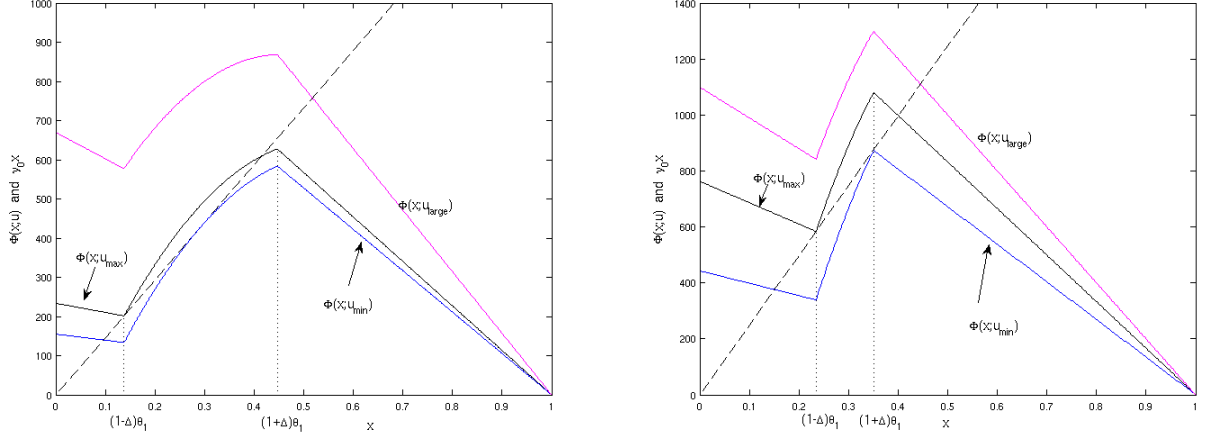
Figure 1: The two possible cases for calculating the expressions of the bistability interval, $[u_{\min}, u_{\max}]$. The value of $u_{\max}$ is always obtained from $\Phi_l((1 - \Delta)\theta_1; u_{\max}) = \gamma_0(1 - \Delta)\theta_1$ (black lines). A very large value of u lead to the existence of a unique steady state defined by the intersection with the right branch $\Phi_r(\bar{x}_{\text{high}}; u_{\text{large}}) = \gamma_0 \bar{x}_{\text{high}}$ (red lines). Left: $u_{\min}$ is obtained from $\Phi_m(\beta; u_{\min}) = \gamma_0\beta$, where β is such that the two roots of this quadratic expression are equal (see explicit expression in the Appendix of the article). Right: $u_{\min}$ is obtained from $\Phi_r((1 + \Delta)\theta_1; u_{\min}) = \gamma_0(1 + \Delta)\theta_1$ (blue line). The parameters used in each case were: $V_1/k_1 = 901$, $w_0 = 88.5$, $\theta_1 = 0.29$, and on the left $\gamma_0/k_1 = 1466$, $\Delta = 0.5$, while on the right $\gamma_0/k_1 = 2492$, $\Delta = 0.2$.

Since we assume that $n = 4$ is fixed from the start (to simplify the estimation algorithm), the vector of parameters is:

$$p(1) = \frac{V_1}{k_1}, \ p(2) = \theta_1, \ p(3) = \frac{\gamma_0}{k_1}, \ p(4) = w_0.$$

The function to minimize is

$$J(p) = 10 \sum_{i=\min,\max} \left| \frac{u_i - u_{obs,i}}{u_{obs,i}} \right|^2 + \sum_{u \in U} \left| \frac{\frac{\bar{x}(u)}{\bar{x}_{\text{high}}(100)} - \frac{w_u}{w_{100}^*}}{\frac{w_u}{w_{100}^*}} \right|^2$$

where $U = [0, 25, 40, 45, 50, 60, 75]$, w_u, $u_{obs,\min} = 42.5$, $u_{obs,\max} = 72.5$ denote the data points, $w_{100}^* = 160$, and $\bar{x}(u)$ denotes the corresponding "low" or "high" steady state expression. The factor 10 multiplying the first sum aims to increase the weight of the error related to the bistability region, since this was a very important part of the modelling. The computation of the cost $J(p)$ includes an algorithm to verify which of the forms of $\bar{x}_{\text{high}}(u)$ is the correct one.

To solve the optimization problem, we followed a Monte Carlo approach by randomly choosing 1000 initial conditions in the 4-dimensional set G, and optimizing the cost J with the Matlab function `lsqnonlin`. Numerical experiments showed that values of $\frac{V_1}{k_1}$, $\frac{\gamma_0}{k_1}$ lower than 100 yielded very high costs, and similarly for w_0 larger than 100. Recall that θ_1 is normalized to 1. Therefore we considered:

$$G = \left\{ p : \frac{V_1}{k_1}, \frac{\gamma_0}{k_1} \in [100, 2000], \theta_1 \in [0.1, 0.9], \ w_0 \in [1, 100] \right\}. \quad (3)$$

The parameter set p^* corresponding to the lower final cost was chosen as best fit. It satisfies:

$$p^*(1) = 430.86, \ p^*(2) = 0.2752, \ p^*(4) = 843.42, \ p^*(5) = 61.98,$$

$$J(p^*) = 0.9872.$$

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The sets of parameters satisfying to $J(p) \leq 0.99$ are shown in Fig. 2. They correspond to the 6% lower costs:

$$P = \{p \in G : J(p) \leq 1.06 J(p^*)\}.$$

Note that, for all $p \in P$, each parameter $p(i)$ falls on a reasonably small subinterval of its initial interval in G, with $V_1/k_1 \in [420, 445]$, $\gamma_0/k_1 \in [800, 900]$, $\theta_1 \in [0.27, 0.28]$, and $w_0 \in [58, 68]$.
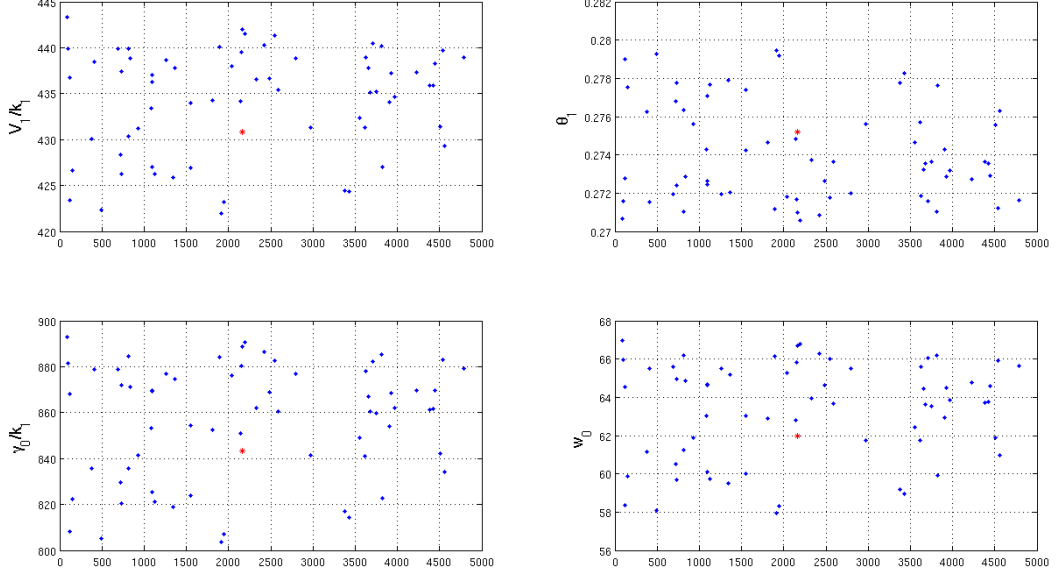

Figure 2: Sets of parameters satisfying $J(p) \leq 0.99$ (the top 6% best parameters). The optimal parameter is represented by the red star.

To access the quality of the parameter estimation, a classical method is the covariance matrix. For linear models this matrix provides the basis for computation of confidence intervals and correlation values [1]. However, our model is highly nonlinear and, in addition, involves some discontinuities in the functions to be fitted. For our case, the covariance matrix was ill-conditioned, and no useful confidence intervals could be deduced. Therefore, we used another classical method to compute a local *confidence region* for the parameters [1]. A $100(1 - \alpha)\%$ confidence region is given by:

$$P_{cr} = \{p : J(p) \leq J_{cr}\},$$

with

$$J_{cr} = J(p^*) \left(1 + \frac{N_p}{N_{data} - N_p} F(1 - \alpha; N_p, N_{data} - N_p)\right)$$

where $N_p = 5$ is the number of parameters, $N_{data} = 16$ is the number of data points and $F(1-\alpha; N_p, N_{data}-N_p)$ is the value at $1 - \alpha$ of the F-distribution with N_p and $N_{data} - N_p$ degrees of freedom. The diameter of this region can be computed by letting only one parameter vary at a time, as in a sensibility analysis (see Fig. 3). For $\alpha = 0.05$, the confidence region P_{cr} consists of the points p such that $J(p) \leq 1.22$. The approximate diameter of the 95% confidence region is given by:

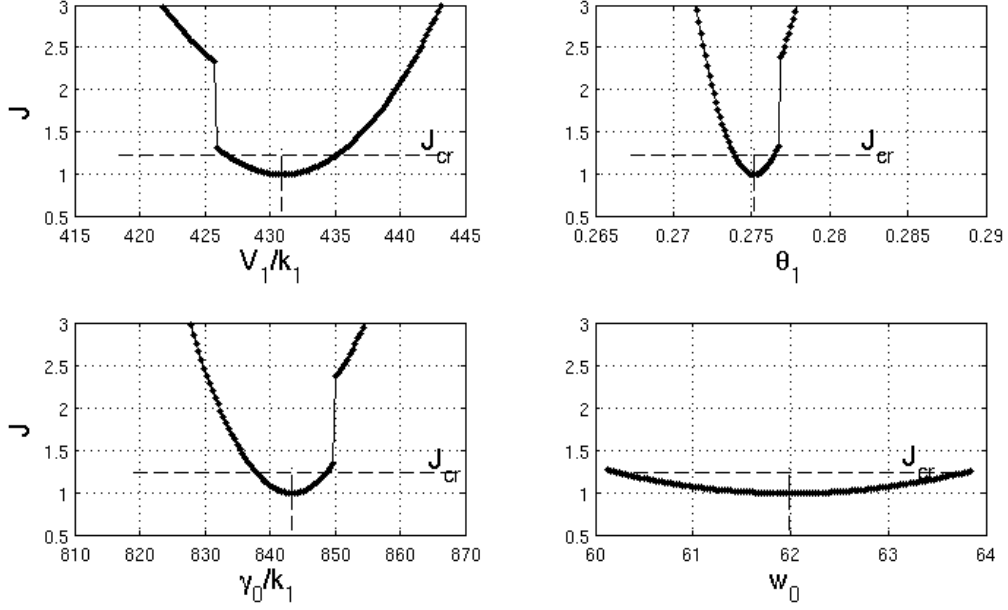| Parameter	Diameter of 95% conf. region
$p(1)$	$[426.77, 434.95]$
$p(2)$	$[0.2740, 0.2764]$
$p(3)$	$[838.36, 848.48]$
$p(4)$	$[60.28, 63.75]$

Figure 3: Estimating the diameter of a 95% confidence region (bistability data).

1.2 Oscillations

Using the time series data the following parameters were estimated:

$$p_o(1) = \gamma_1, p_o(2) = a_1, p_o(3) = \theta_2, p_o(4) = \frac{V_2}{\gamma_2}, p_o(5) = \gamma_2, p_o(6) = k_1, p_o(7) = w_0.$$

The cost function to be minimized was

$$J_o(p(1), \ldots, p(7)) = 5J_x + J_y,$$

where a larger weight was assigned to J_x because preliminary experiments showed that typically $J_x < J_y$.

$$J_x(p) = \sum_{t \in T} \left| \frac{x(t)}{\max_{t \in T} x(t)} - \frac{W^c(t)}{\max_{t \in T} W^c(t)} \right|^2 \bigg/ \left| \frac{W^c(t)}{\max_{t \in T} W^c(t)} \right|^2$$

$$J_y(p) = \sum_{t \in T} \left| \frac{1/y(t)}{\max_{t \in T} 1/y(t)} - \frac{W^B(t)}{\max_{t \in T} W^B(t)} \right|^2 \bigg/ \left| \frac{W^B(t)}{\max_{t \in T} W^B(t)} \right|^2$$

where $t \in \{22, 24, \ldots, 94\}$ (the data points used here start at $t = 22$, and are spaced 2mins apart), $W^c(t)$ denotes the concentration of cdc2-cyclin B, and $W^B(t)$ that of cyclin B.

As above, a Monte Carlo approach was used to find a good initial guess. The cost J_o was evaluated at randomly chosen points in the set G_o (some preliminary tests were performed to avoid a too large G_o):

$$G_o = \{p: \quad w_0 \in [1, 30], \ k_1 \in [10^{-4}, 10^{-3}], \ a_1 \in [0.8, 10], \ \gamma_1 \in [0.1, 1],$$
$$\frac{V_2}{\gamma_2} \in [2, 10], \ \theta_2 \in [0.5\theta_1, \theta_1], \ \gamma_2 \in [0.01, 0.1] \}$$

The initial conditions corresponding to the 1% lower costs were used to find an optimal set of parameters with the Matlab function `fminsearch`. The best fit parameter set is

$$p_o^*(1) = 0.358, p_o^*(2) = 1.504, p_o^*(3) = 0.269, p_o^*(4) = 9.438,$$
$$p_o^*(5) = 0.0266, p_o^*(6) = 3.769 \times 10^{-4}, p_o^*(7) = 19.72,$$
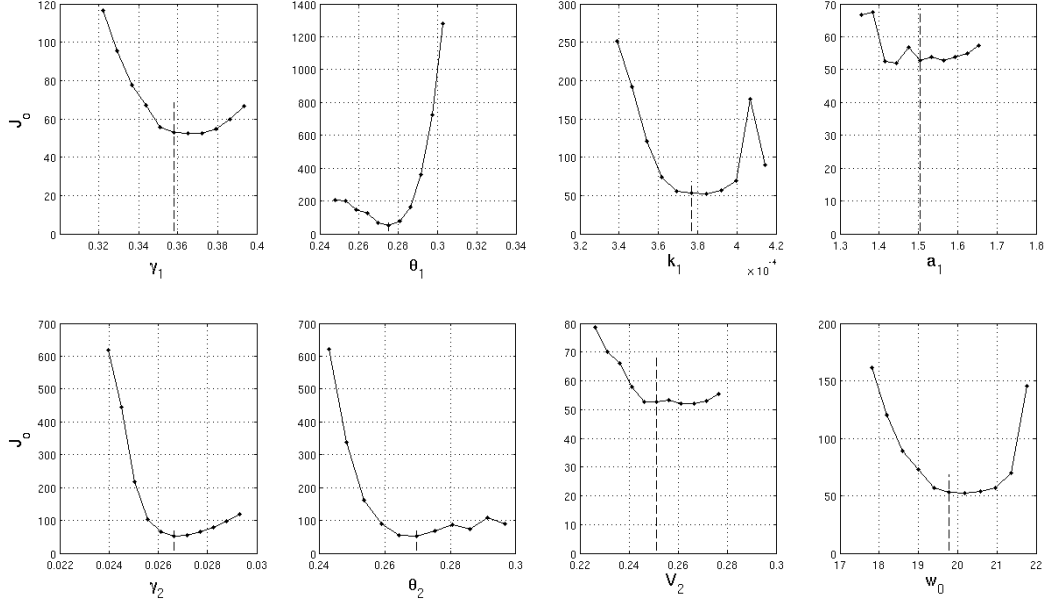
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Figure 4: Sensitivity of the cost function J_o with respect to each of the final parameters. Each parameter was varied between 90% and 110% of its original value (only one parameter is varied at a time).

with $J(p_o^*) = 52$. The covariance matrix (V) and confidence intervals for these parameters were then computed with `nlinfit` and `nlparci`, built-in functions in the Matlab Statistics toolbox. V is:

$$
\begin{bmatrix}
2.06 \times 10^{-3} & 3.21 \times 10^{-3} & 0.98 \times 10^{-3} & 10.5 \times 10^{-3} & 0.06 \times 10^{-3} & 4.81 \times 10^{-8} & -0.053 \\
3.21 \times 10^{-3} & 10.90 \times 10^{-3} & 1.95 \times 10^{-3} & -7.83 \times 10^{-3} & 0.26 \times 10^{-3} & -5.22 \times 10^{-8} & -0.187 \\
0.98 \times 10^{-3} & 1.95 \times 10^{-3} & 0.52 \times 10^{-3} & 3.31 \times 10^{-3} & 0.04 \times 10^{-3} & 1.40 \times 10^{-8} & -0.032 \\
10.5 \times 10^{-3} & -7.83 \times 10^{-3} & 3.31 \times 10^{-3} & 153.4 \times 10^{-3} & -0.38 \times 10^{-3} & 76.9 \times 10^{-8} & 0.163 \\
0.06 \times 10^{-3} & 0.26 \times 10^{-3} & 0.04 \times 10^{-3} & -0.38 \times 10^{-3} & 0.006 \times 10^{-3} & -0.22 \times 10^{-8} & -0.005 \\
4.81 \times 10^{-8} & -5.22 \times 10^{-8} & 1.40 \times 10^{-8} & 76.9 \times 10^{-8} & -0.22 \times 10^{-8} & 3.88 \times 10^{-12} & 103.5 \times 10^{-8} \\
-0.053 & -0.187 & -0.032 & 0.163 & -0.005 & 103.5 \times 10^{-8} & 3.24
\end{bmatrix}
$$

Parameter	95% conf. interval	75% conf. interval
$p_o(1)$	$[0.27, 0.44]$	$[0.31, 0.41]$
$p_o(2)$	$[1.28, 1.70]$	$[1.37, 1.61]$
$p_o(3)$	$[0.225, 0.315]$	$[0.244, 0.296]$
$p_o(4)$	$[8.66, 10.22]$	$[8.98, 9.89]$
$p_o(5)$	$[0.022, 0.032]$	$[0.024, 0.030]$
$p_o(6)$	$[3.73 \times 10^{-4}, 3.80 \times 10^{-4}]$	$[3.75 \times 10^{-4}, 3.79 \times 10^{-4}]$
$p_o(7)$	$[16.20, 23.37]$	$[17.70, 21.87]$

The error for $V_2 = \left[\frac{V_2}{\gamma_2}\right] \gamma_2 = p_o(4) \cdot p_o(5)$ was computed by error propagation:

$$ dV_2 = dp_o(4) \cdot p_0(5) + p_o(4) \cdot dp_0(5). $$

A similar formula was used to compute the error for $V_1 = p(1) \cdot p_o(6)$. Finally, for the optimal set of parameters, sensibility analysis was also performed by varying one parameter at a time, as shown in Fig. 4.

2 Period Estimates

As the difference between timescales becomes more marked, the sum $T_1 + T_2$ will provide better estimates, and their sum gives a reasonable estimate of the full cycle period. For instance, for the parameter set:

$$n = 3, \ m = 20, \ w_0 = a_1 = 1, \ k_1 = 0.1, \ V_1 = 2, \ V_2 = 0.1,$$
$$\gamma_1 = 0.14, \ \gamma_2 = 0.003, \ \theta_1 = 0.25, \ \theta_2 = 0.2, \tag{4}$$

the ratio $(T_1 + T_2)/\text{Period} \approx 404.6/434.3 = 0.93$, i.e., an error of 7%, as opposed to $(T_1 + T_2)/\text{Period} \approx 27.55/79.76 = 0.3$, an error of 66% for the optimized parameter set (see also Fig. 5).

The quality of the $T_1 + T_2$ estimate depends on how well the values $f_0(x_B) = f_0((1 - \Delta)\theta_1)$ and $f_0(x_A) = f_0(\theta_1)$ approximate the actual value of y as x jumps between low and high levels;
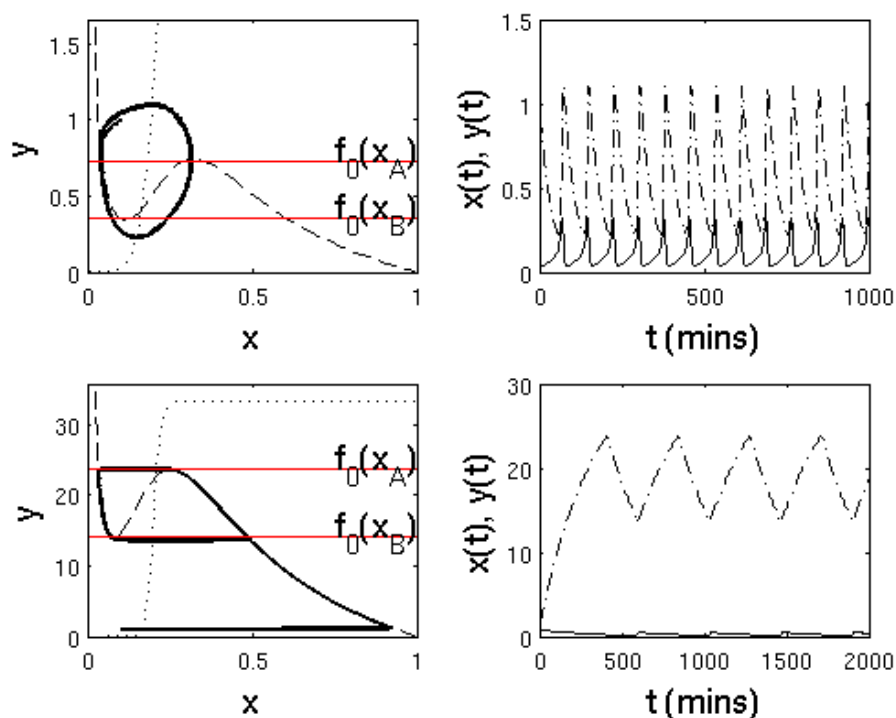

Figure 5: Periodic orbit and trajectories for system, with parameters as in Table 2 (top row), or with the parameters listed above (bottom row), which enhance the difference between timescales.

References

[1] D. Dochain and P.A. Vanrolleghen. *Dynamical modelling and estimation in wastewater treatment processes.* IWA Publishing, Alliance House, London, 2001.