

S5 Text: PhoPQ TCS models: Reactions, ODEs and Parameters

Model reactions: PhoPQ model with single bifunctional kinase

Note that for brevity in the figures and equations in all supplemental text files: We represent PhoQ by Q, MgrB by B and subscript P for phosphorylated state. For example, the complex PhoQ-P.MgrB is represented as QB_P .

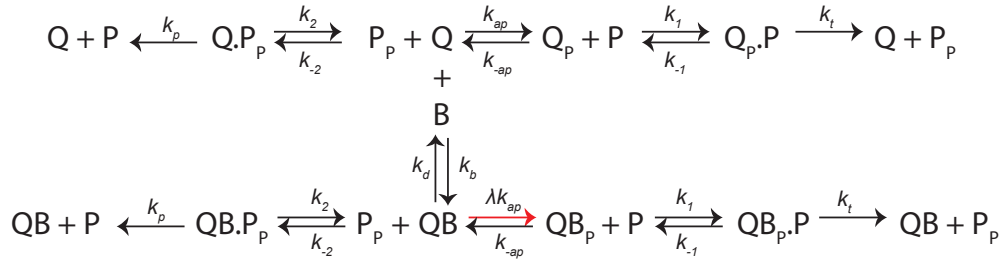
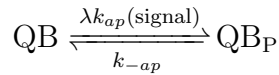
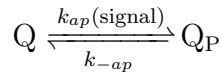


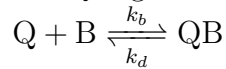
Figure 1: Figure shows all the reactions in PhoPQ TCS with associated rate constants. Reactions in the top row represent those in a typical TCS. This modified model includes Q binding B. QB undergoes all the same steps as Q (bottom row) with same rate constants, except autophosphorylation (shown by red arrow).

Post-translational reactions

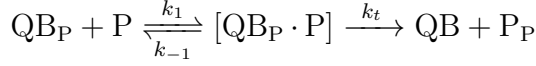
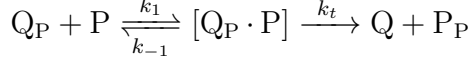
- Signal sensing i.e. autophosphorylation; $k_{ap} = k_{ap}^0 \frac{[Mg^{2+}]^n}{K_{mg}^n + [Mg^{2+}]^n}$



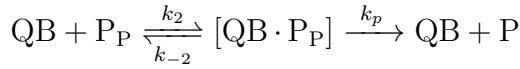
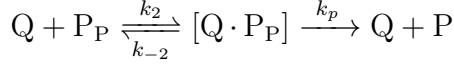
- PhoQ-MgrB binding



- Phosphotransfer

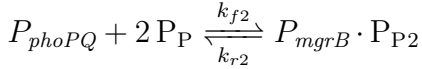
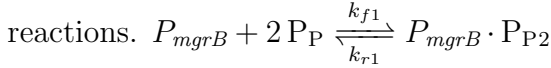


- Dephosphorylation



Transcription

Modeling of transcription and translation is the same for both models. PhoP-P binds promoter sites as a dimer, therefore we consider the following binding



We consider the binding reactions to be quasi-equilibrium state. Considering 1 copy of promoter per cell, the occupancy fraction of the promoter can be calculated as:

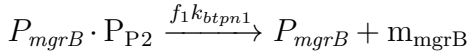
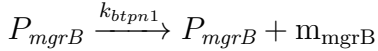
$$P_{mgrB} \cdot P_{P2} = P_{mgrB} \frac{[P_P]^2}{K_1^2}$$

Here $K_1^2 = \frac{k_{r1}}{k_{f1}}$.

$$P_{mgrB} + P_{mgrB} \cdot P_{P2} = 1$$

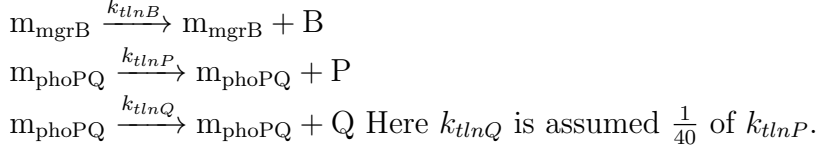
$$P_{mgrB} \cdot P_{P2} = \frac{\frac{[P_P]^2}{K_1}}{1 + \frac{[P_P]^2}{K_1}}$$

Transcription from a promoter occurs at two rates: basal rate and active rate (when promoter is bound by the transcription factor). All transcripts degrade at a constant rate k_{md}



The same calculation gives a model for transcription for any mRNA (PhoP, PhoQ, MgrB or fluorescent reporter) from any PhoP-P dependent promoter (P_{mgrB} or P_{phoPQ}).

Translation



Degradation

All proteins are assumed stable and dilute due to cell growth at a rate k_{pd} .

Model ODEs: PhoPQ model with single bifunctional kinase

$$\begin{aligned}
\frac{d[\text{P}]}{dt} &= k_{\text{tlnP}}m_{\text{phoPQ}} - k_1[\text{Q}_\text{P}][\text{P}] + k_{-1}[\text{Q}_\text{P}.\text{P}] + k_p[\text{Q}.\text{P}_\text{P}] \\
&\quad - k_1[\text{QB}_\text{P}][\text{P}] + k_{-1}[\text{QB}_\text{P}.\text{P}] + k_p[\text{QB}.\text{P}_\text{P}] - k_{pd}[\text{P}] \\
\frac{d[\text{P}_\text{P}]}{dt} &= k_t[\text{Q}_\text{P}.\text{P}] + k_t[\text{QB}_\text{P}.\text{P}] - k_2[\text{Q}][\text{P}_\text{P}] + k_{-2}[\text{Q}.\text{P}_\text{P}] \\
&\quad - k_2[\text{QB}][\text{P}_\text{P}] + k_{-2}[\text{QB}.\text{P}_\text{P}] - k_{pd}[\text{P}_\text{P}] \\
\frac{d[\text{Q}]}{dt} &= k_{\text{tlnQ}}m_{\text{phoPQ}} - k_{ap}[\text{Q}] + k_{-ap}[\text{Q}_\text{P}] - k_b[\text{B}][\text{Q}] + k_d[\text{QB}] \\
&\quad + k_t[\text{Q}_\text{P}.\text{P}] - k_2[\text{Q}][\text{P}_\text{P}] \\
&\quad + k_{-2}[\text{Q}.\text{P}_\text{P}] + k_p[\text{Q}.\text{P}_\text{P}] - k_{pd}[\text{Q}] \\
\frac{d[\text{Q}_\text{P}]}{dt} &= k_{ap}[\text{Q}] - k_{-ap}[\text{Q}_\text{P}] + k_{-1}[\text{Q}_\text{P}.\text{P}] - k_1[\text{Q}_\text{P}][\text{P}] - k_{pd}[\text{Q}_\text{P}] \\
\frac{d[\text{B}]}{dt} &= k_{\text{tlnB}}m_{\text{mgrB}} - k_b[\text{B}][\text{Q}] + k_d[\text{QB}] - k_{pd}[\text{B}] \\
\frac{d[\text{QB}]}{dt} &= k_b[\text{Q}][\text{B}] - k_d[\text{QB}] - \lambda k_{ap}[\text{QB}] + k_{-ap}[\text{QB}_\text{P}] + k_t[\text{QB}_\text{P}.\text{P}] \\
&\quad - k_2[\text{QB}][\text{P}_\text{P}] + k_{-2}[\text{QB}.\text{P}_\text{P}] + k_p[\text{QB}.\text{P}_\text{P}] - k_{pd}[\text{QB}] \\
\frac{d[\text{QB}_\text{P}]}{dt} &= \lambda k_{ap}[\text{QB}] - k_{-ap}[\text{QB}_\text{P}] + k_{-1}[\text{QB}_\text{P}.\text{P}] \\
&\quad - k_1[\text{QB}_\text{P}][\text{P}] - k_{pd}[\text{QB}_\text{P}] \\
\frac{d[\text{Q}.\text{P}_\text{P}]}{dt} &= k_2[\text{Q}][\text{P}_\text{P}] - k_{-2}[\text{Q}.\text{P}_\text{P}] - k_p[\text{Q}.\text{P}_\text{P}] - k_{pd}[\text{Q}.\text{P}_\text{P}] \\
\frac{d[\text{Q}_\text{P}.\text{P}]}{dt} &= k_1[\text{Q}_\text{P}][\text{P}] - k_{-1}[\text{Q}_\text{P}.\text{P}] - k_t[\text{Q}_\text{P}.\text{P}] - k_{pd}[\text{Q}_\text{P}.\text{P}] \\
\frac{d[\text{QB}.\text{P}_\text{P}]}{dt} &= k_2[\text{QB}][\text{P}_\text{P}] - k_{-2}[\text{QB}.\text{P}_\text{P}] \\
&\quad - k_p[\text{QB}.\text{P}_\text{P}] - k_{pd}[\text{QB}.\text{P}_\text{P}] \\
\frac{d[\text{QB}_\text{P}.\text{P}]}{dt} &= k_1[\text{QB}_\text{P}][\text{P}] - k_{-1}[\text{QB}_\text{P}.\text{P}] \\
&\quad - k_t[\text{QB}_\text{P}.\text{P}] - k_{pd}[\text{QB}_\text{P}.\text{P}]
\end{aligned}$$

$$\begin{aligned}
\frac{d[m_{phoPQ}]}{dt} &= k_{btpn1} \frac{1+f_2 \left(\frac{[P_P]}{K_2}\right)^2}{1+\left(\frac{[P_P]}{K_2}\right)^2} - k_{md}m_{phoPQ} \\
\frac{dm_{mgrB}}{dt} &= k_{btpn2} \frac{1+f_1 \left(\frac{[P_P]}{K_1}\right)^2}{1+\left(\frac{[P_P]}{K_1}\right)^2} - k_{md}m_{mgrB} \\
\frac{d[m_{YFP2}]}{dt} &= k_{btpn2} \frac{1+f_2 \left(\frac{[P_P]}{K_1}\right)^2}{1+\left(\frac{[P_P]}{K_1}\right)^2} - k_{md}[m_{YFP2}] \\
\frac{d[m_{YFP1}]}{dt} &= k_{btpn1} \frac{1+f_1 \left(\frac{[P_P]}{K_1}\right)^2}{1+\left(\frac{[P_P]}{K_1}\right)^2} - k_{md}[m_{YFP2}] \\
\frac{d[YFP_1]}{dt} &= k_{tlnY}[m_{YFP1}] - k_{pd}[YFP_1] \\
\frac{d[YFP_2]}{dt} &= k_{tlnY}[m_{YFP2}] - k_{pd}[YFP_2]
\end{aligned}$$

***in-silico* mutants**

In-silico mutants are modeled in the same way for both dynamical models.

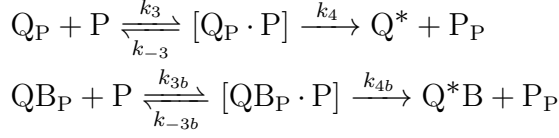
1. MgrB-deletion mutant, $\Delta mgrB$: Set $k_{btpn2} = 0$ in $\frac{d[m_{mgrB}]}{dt}$ only. This leads to zero MgrB but the reporter mRNA (mYFP2) and reporter protein (YFP) continue to report P_{mgrB} activity.
2. Autoregulation deletion: Set $f_1 = 1$ in $\frac{d[m_{phoPQ}]}{dt}$ only so that total PhoP, PhoQ remains constant but reporters mRNA (mYFP1) and protein (YFP1) levels continue to report P_{phoPQ} activity.
3. Constitutive $mgrB$: Set $f_2 = 1$ and vary k_{btpn2} in $\frac{d[m_{mgrB}]}{dt}$ only to set desired total MgrB expression at steady state, while mYFP2 and YFP2 continue to report on P_{mgrB} activity.
4. PhoQ-phosphatase activity mutant: Set all 3 rates associated with the Michaelis-Menten dephosphorylation reaction zero for Q and QB.
5. Double deletions will be combinations of individual *in-silico* mutants

Parameters: PhoPQ model with single bifunctional kinase

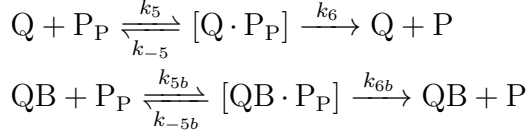
Parameter	Description	Units	Value
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k_{pd}	Protein dilution rate	s^{-1}	3.1×10^{-4}
k_{md}	mRNA decay rate	s^{-1}	1.7×10^{-3}
k_{tlnP}	PhoP translation rate	s^{-1}	5.0×10^{-2}
k_{tlnB}	MgrB translation rate	s^{-1}	4.0×10^{-3}
k_{btpn1}	basal rate of transcription from P_{phoPQ}	mRNA copies s^{-1}	4.1×10^{-6}
k_{btpn2}	basal rate of transcription from P_{mgrB}	mRNA copies s^{-1}	8.7×10^{-6}
f_1	Fold increase in transcription rate from P_{phoPQ}	-	23
f_2	Fold increase in transcription rate from P_{mgrB}	-	55
K_1	Affinity of PhoP-P to P_{phoPQ}	μM	0.47
K_2	Affinity of PhoP-P to P_{mgrB}	μM	0.45
k_2	PhoQ autophosphorylation rate	s^{-1}	3.1
k_{-2}	PhoQ-P autodephosphorylation rate	s^{-1}	0
k_3	PhoQ-P to PhoP association rate	$\mu M^{-1} s^{-1}$	0.8
k_{-3}	[PhoQ-P.PhoP] dissociation rate	s^{-1}	1.2×10^{-2}
k_4	[PhoQ-P.PhoP] phosphotransfer rate	s^{-1}	2.1×10^{-1}
k_5	PhoQ to PhoP-P association rate	$\mu M^{-1} s^{-1}$	1.13
k_{-5}	[PhoQ.PhoP-P] dissociation rate	s^{-1}	9.2×10^{-4}
k_6	[PhoQ.PhoP-P] dephosphorylation rate	s^{-1}	2.16
k_{b1}	PhoQ to MgrB association rate	$\mu M^{-1} s^{-1}$	0.039
k_{d1}	[PhoQ.MgrB] dissociation rate	s^{-1}	5.6×10^{-4}

Table 1: Parameters used for one state PhoPQ model simulation (S4 Fig)



- Dephosphorylation



Detailed balance condition constrains parameters as follows:

$$\frac{k_{b1}}{k_{b2}} = \frac{k_{1b}}{k_1} \times \frac{k_{d1}}{k_{d2}}$$

Model ODEs: PhoPQ model with two states of PhoQ

$$\begin{aligned} \frac{d[P]}{dt} &= k_{tlnP}[m_{phoPQ}] - k_3[Q_P][P] + k_{-3}[Q_P \cdot P] \\ &\quad + k_6[Q \cdot P_P] - k_{3b}[Q_{B_P}][P] + k_{-3b}[Q_{B_P} \cdot P] \\ &\quad + k_{6b}[Q_B \cdot P_P] - k_{pd}[P] \\ \frac{d[P_P]}{dt} &= k_4[Q_P \cdot P] - k_5[Q][P_P] + k_{-5}[Q \cdot P_P] \\ &\quad + k_{4b}[Q_{B_P} \cdot P] - k_{5b}[Q_B][P_P] \\ &\quad + k_{-5b}[Q_B \cdot P_P] - k_{pd}[P_P] \\ \frac{d[Q]}{dt} &= -k_1[Q] + k_{-1}[Q^*] - k_{b1}[Q][B] + k_{d1}[QB] - k_5[Q][P_P] \\ &\quad + k_{-5}[Q \cdot P_P] + k_6[Q \cdot P_P] - k_{pd}[Q] \\ \frac{d[Q^*]}{dt} &= k_{tlnQ}[m_{phoPQ}] + k_1[Q] - k_{-1}[Q^*] - k_2[Q^*] + k_{-2}[Q_P] \\ &\quad + k_4[Q_P \cdot P] - k_{b2}[Q^*][B] + k_{d2}[Q^*B] - k_{pd}[Q^*] \\ \frac{d[Q_P]}{dt} &= k_2[Q^*] - k_{-2}[Q_P] + k_{-3}[Q_P \cdot P] - k_3[Q_P][P] - k_{pd}[Q_P] \\ \frac{d[B]}{dt} &= k_{tlnB}[m_{mgrB}] - k_{b2}[Q^*][B] + k_{d2}[Q^*B] - k_{b1}[Q][B] + k_{d1}[QB] - k_{pd}[B] \\ \frac{d[QB]}{dt} &= -k_{1b}[QB] + k_{-1b}[Q^*B] + k_{b1}[Q][B] - k_{d1}[QB] - k_{5b}[QB][P_P] \\ &\quad + k_{-5b}[Q_B \cdot P_P] + k_{6b}[Q_B \cdot P_P] - k_{pd}[QB] \\ \frac{d[Q^*B]}{dt} &= +k_{1b}[QB] - k_{-1b}[Q^*B] - k_{2b}[Q^*B] + k_{-2b}[Q_{B_P}] + k_{4b}[Q_{B_P} \cdot P] \\ &\quad + k_{b2}[Q^*][B] - k_{d2}[Q^*B] - k_{pd}[Q^*B] \\ \frac{d[Q_{B_P}]}{dt} &= k_{2b}[Q^*B] - k_{-2b}[Q_{B_P}] \\ &\quad + k_{-3b}[Q_{B_P} \cdot P] - k_{3b}[Q_{B_P}][P] - k_{pd}[Q_{B_P}] \\ \frac{d[Q \cdot P_P]}{dt} &= k_5[Q][P_P] - k_{-5}[Q \cdot P_P] \\ &\quad - k_6[Q \cdot P_P] - k_{pd}[Q \cdot P_P] \end{aligned}$$

$$\begin{aligned}
\frac{d[\text{QB.PP}]}{dt} &= k_{5b}[\text{QB}][\text{PP}] - k_{-5b}[\text{QB.PP}] \\
&\quad - k_6b[\text{QB.PP}] - k_{pd}[\text{QB.PP}] \\
\frac{d[\text{QP.P}]}{dt} &= k_3[\text{QP}][\text{P}] - k_{-3}[\text{QP.P}] \\
&\quad - k_4[\text{QP.P}] - k_{pd}[\text{QP.P}] \\
\frac{d[\text{QB}_P.P]}{dt} &= k_{3b}[\text{QB}_P][\text{P}] - k_{-3b}[\text{QB}_P.P] \\
&\quad - k_{4b}[\text{QB}_P.P] - k_{pd}[\text{QB}_P.P] \\
\frac{d[m_{\text{phoPQ}}]}{dt} &= k_{btpn1} \frac{1+f_2 \left(\frac{[\text{PP}]}{K_2}\right)^2}{1+\left(\frac{[\text{PP}]}{K_2}\right)^2} - k_{md}m_{\text{phoPQ}} \\
\frac{dm_{\text{mgrB}}}{dt} &= k_{btpn2} \frac{1+f_1 \left(\frac{[\text{PP}]}{K_1}\right)^2}{1+\left(\frac{[\text{PP}]}{K_1}\right)^2} - k_{md}m_{\text{mgrB}} \\
\frac{d[m_{\text{YFP2}}]}{dt} &= k_{btpn2} \frac{1+f_2 \left(\frac{[\text{PP}]}{K_1}\right)^2}{1+\left(\frac{[\text{PP}]}{K_1}\right)^2} - k_{md}[m_{\text{YFP2}}] \\
\frac{d[m_{\text{YFP1}}]}{dt} &= k_{btpn1} \frac{1+f_1 \left(\frac{[\text{PP}]}{K_1}\right)^2}{1+\left(\frac{[\text{PP}]}{K_1}\right)^2} - k_{md}[m_{\text{YFP2}}] \\
\frac{d[\text{YFP}_1]}{dt} &= k_{tlnY}[m_{\text{YFP1}}] - k_{pd}[\text{YFP}_1] \\
\frac{d[\text{YFP}_2]}{dt} &= k_{tlnY}[m_{\text{YFP2}}] - k_{pd}[\text{YFP}_2]
\end{aligned}$$

Parameters: PhoPQ model with two states of PhoQ

Parameter	Description	Units	Value
k_{pd}	Protein dilution rate	s^{-1}	3.1×10^{-4}
k_{md}	mRNA decay rate	s^{-1}	4.1×10^{-3}
k_{tlnP}	PhoP translation rate	s^{-1}	3.7×10^{-2}
k_{tlnB}	MgrB translation rate	s^{-1}	2.3×10^{-2}
k_{btpn1}	basal rate of transcription from P_{phoPQ}	mRNA copies s^{-1}	4.97×10^{-6}
k_{btpn2}	basal rate of transcription from P_{mgrB}	mRNA copies s^{-1}	1.05×10^{-5}
f_1	Fold increase in transcription rate from P_{phoPQ}	-	26
f_2	Fold increase in transcription rate from P_{mgrB}	-	50
K_1	Affinity of PhoP-P to P_{phoPQ}	μM	0.62
K_2	Affinity of PhoP-P to P_{mgrB}	μM	0.19
k_1	PhoQ to PhoQ* activation rate	s^{-1}	3.2×10^{-3}

k_{-1}^0	PhoQ* to PhoQ deactivation rate (signal)	s^{-1}	8.7×10^{-2}
k_2	PhoQ* autophosphorylation rate	s^{-1}	12.5
k_{-2}	PhoQ-P autodephosphorylation rate	s^{-1}	0
k_3	PhoQ-P to PhoP association rate	$\mu M^{-1} s^{-1}$	5
k_{-3}	[PhoQ-P.PhoP] dissociation rate	s^{-1}	4.6×10^{-3}
k_4	[PhoQ-P.PhoP] phosphotransfer rate	s^{-1}	3.6×10^0
k_5	PhoQ to PhoP-P association rate	$\mu M^{-1} s^{-1}$	0.14
k_{-5}	[PhoQ.PhoP-P] dissociation rate	s^{-1}	3.6×10^{-3}
k_6	[PhoQ.PhoP-P] dephosphorylation rate	s^{-1}	1.56
k_{b2}	PhoQ to MgrB association rate	$\mu M^{-1} s^{-1}$	0.26
k_{d2}	[PhoQ.MgrB] dissociation rate	s^{-1}	1.0×10^{-4}

Table 2: Parameters used for two-state PhoPQ model (S6 Fig)