

# Chapter 5

## Kinetic Model that Describes the Cold Shock Adaptation in *B.* *subtilis*

### 5.1 Motivation

### 5.2 Toy model: Adaptation to Low Temperatures in *B. subtilis*

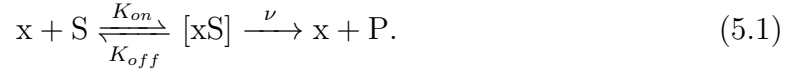
#### *Response of the Histidine Kinase to Temperature*

After reviewing each step in the response to low temperatures by the bacterium *B. subtilis*, the initial process in the model is the union and subsequent phosphorylation of the Response Regulator (RR) DesR by the Histidine Kinase (HK) DesK triggered by a decrease in temperature. This process is coupled to the the signal transduction shut down, which is achieved by the dephosphorylation of DesR; therefore, these two reactions working in opposite directions are the initial building blocks of the model. First, I shall define the notation for the concentrations of each of the enzymes pertaining to this system that will be used throughout the document.

- Enzyme DesK in kinase form  $:= x$

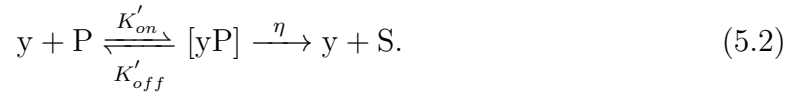
- Enzyme DesK in phosphatase form  $:= y$
- Total quantity of enzyme DesK  $:= \pi$
- DesR dimer  $:= S$
- Phosphorylated DesR dimer  $:= P$
- Total quantity of DesR dimers  $:= \pi$
- Enzyme  $\Delta$ -5 des  $:= \Delta$

First we write the reaction that describes the union of the enzyme DesK in the HK form,  $x$ , to its substrate DesR,  $S$ , followed by the conversion of the substrate to the phosphorylated form,  $P$ , as:



The symbol  $[xS]$  represents the complex formed by the union of  $x$  and  $S$ , which then dissociates into the phosphorylated form of the substrate,  $P$ , and the enzyme molecule. We assume that the formation of  $[xS]$  is reversible while its dissociation is not. The symbols  $\nu$ ,  $K_{on}$ , and  $K_{off}$  are rate constants.

On the other hand, the reaction that works towards the deactivation of the system is:



Similarly, in this reaction the phosphatase form of the enzyme DesK,  $y$ , reverses the effect of the first reaction by dephosphorylating the substrate  $P$  through the formation of another intermediate complex,  $[yP]$ . The symbols  $\eta$ ,  $K'_{on}$ , and  $K'_{off}$  are rate constants.

In order to track the time evolution of the complexes, enzymes and substrates, we use the information contained in the canonical enzymatic reactions (5.1, 5.2). Such a task can be fulfilled by means of the law of mass action, to which the present study is

restricted. The mass action approximation states that the rate of a reaction is equal to a constant multiplied by the product of the concentrations of the reactants. This holds for a well-mixed reaction in which the number of molecules present is great enough in order to assume continuous distributions, and that reaction dynamics are deterministic. Mass action kinetics are an approximation of a discrete and stochastic treatment based on the Chemical Master Equation (CME). However, most physiological processes can be accurately described using deterministic, continuous models because the magnitude of stochastic fluctuations for a single reaction scales with  $1/\sqrt{N}$ , where  $N$  is the number of molecules in the compartment. Thus, by assuming a number of molecules per reactant greater than  $10^2 - 10^3$ , we can safely apply the Law of Mass Action [2]. Having in mind the previous considerations, the dynamical system that describes the two-step enzymatic reactions consists of six coupled ODEs

$$\begin{aligned} \frac{dx}{dt} &= -xSK_{on} + [xS](K_{off} + \nu) & \frac{dy}{dt} &= -yPK'_{on} + [yP](K'_{off} + \eta), \\ \frac{d[xS]}{dt} &= xSK_{on} - [xS](K_{off} + \nu), & \frac{d[yP]}{dt} &= yPK_{on}' - (K'_{off} + \eta)[yP], \\ \frac{dS}{dt} &= -xSK_{on} + [xS]K_{off} + [yP]\eta, & \frac{dP}{dt} &= -yPK'_{on} + [yP]K'_{off} + [xS]\nu. \end{aligned} \quad (5.3)$$

This set of differential equations can be reduced if we use the information provided in the form of conservation or mass or mass balance conditions:

$$\begin{aligned} x(t) + [xS](t) &\equiv x_0, \\ y(t) + [yP](t) &\equiv y_0, \\ S(t) + P(t) + [xS](t) + [yP](t) &\equiv S_0. \end{aligned} \quad (5.4)$$

From these constraints, follows that  $S(t) = S_0 - P(t) - [xS](t) - [yP](t)$ ,  $x(t) =$

$x_0 - [xS](t)$  and,  $y(t) = y_0 - [yP](t)$ , making it possible to describe our system with three of the six ODEs proposed in 5.3. Therefore, we obtain:

$$\begin{aligned}\frac{dP}{dt} &= -K'_{on}Py_0 + (PK'_{on} + K'_{off})[yP] + [xS]\nu \\ \frac{d[xS]}{dt} &= K_{on}(S_0 - P - [xS] - [yP])(x_0 - [xS]) - [xS](K_{off} + \nu) \\ \frac{d[yP]}{dt} &= y_0PK'_{on} - [yP](PK'_{on} + K'_{off} + \eta).\end{aligned}\tag{5.5}$$

For the system under study, we will separate the dynamics into fast and slow. Thus, for this type of dynamical system, we can assume that fast processes evolve on time scales over which slow processes can be assumed to be constant (Quasistatic state: QSSA). Analogously, when slower processes predominate, the fast processes are assumed to be in quasiequilibrium [2]. Employing these ideas, we will assume that the complexes  $[xS]$  and  $[yP]$  change fast and reach equilibrium before  $P$  changes considerably. Hence, we can simplify 5.5 as:

$$\frac{dP}{dt} = -K'_{on}Py_0 + (PK'_{on} + K'_{off})[yP] + [xS]\nu,\tag{5.6}$$

$$0 = K_{on}(S_0 - P - [xS] - [yP])(x_0 - [xS]) - [xS](K_{off} + \nu),\tag{5.7}$$

$$0 = y_0PK'_{on} - [yP](PK'_{on} + K'_{off} + \eta).\tag{5.8}$$

Assuming that the levels of substrate and phosphorylated substrate are much greater than the levels of the two complexes,  $S \approx S_0 - P$ , we can approximate equation 5.7 as:

$$0 \approx (S_0 - P)(x_0) - [xS] \left( \frac{\nu + K_{off}}{K_{on}} + S_0 - P \right)\tag{5.9}$$

Using equations 5.8 and 5.9 we can find expressions for the complexes  $[xS]$  and  $[yP]$  in terms of  $P$ :

$$[xS] = \frac{x_0(S_0 - P)}{K_{m1} + S_0 - P}, \quad (5.10)$$

$$[xS] = \frac{y_0 P}{K_{m2} + P}; \quad (5.11)$$

where  $K_{m1} := (K_{off} + \nu)/K_{on}$  and  $K_{m2} := (K'_{off} + \eta)/K'_{on}$ . Finally, we arrive to an expression for the time evolution of the phosphorylated substrate in the Quasi-Steady-State Approximations:

$$\frac{dP}{dt} = \frac{\nu x_0(S_0 - P)}{K_{m1} + S_0 - P} - \frac{\eta y_0 P}{K_{m2} + P}. \quad (5.12)$$

In the following mathematical expressions we shall work with first order catalytic constants; namely,  $\alpha = \nu x_0$  and  $\beta = \eta y_0$ . Therefore, equation 5.12 can be simplified to:

$$\frac{dP}{dt} = \frac{\alpha(S_0 - P)}{K_{m1} + S_0 - P} - \frac{\beta P}{K_{m2} + P}. \quad (5.13)$$

### *Membrane Fluidity Control by DesR*

The signal transduction is followed by the union of phosphorylated RR to regulatory regions upstream of *Pdes* which, subsequently, recruit the RNA polymerase leading to the transcription of the desaturase that regulates membrane fluidity. There are two adjacent, non-identical regulatory regions upstream of the promoter and, binding of the phosphorylated RR occurs in a hierarchical fashion. Hence, I shall represent the union to the first region with reaction 5.14



where  $D_I$  and  $C_I$  represent the first regulatory region and the complex formed by the union of  $P$  to  $D_I$ , respectively. The time evolution of  $P$  was derived above, so

the remaining equations that describe reaction 5.14 are:

$$\begin{aligned}\frac{dD_I}{dt} &= -K_{I+}PD_I + K_{I-}C_I \\ \frac{dD_I}{dt} &= -\frac{dC_I}{dt}\end{aligned}\tag{5.15}$$

Once again, we are able to use the Quasi-Steady State approximation in order to simplify the ODE in 5.15. Additionally, this equation can be further simplified if we use the mass balance condition:  $C_I + D_I = D_{Itotal}$ , where  $D_{Itotal}$  is the number of available sites for binding in the first regulatory region. Having provided this information, equation 5.15 can be expressed as:

$$\begin{aligned}0 &= -K_{I+}PD_I + K_{I-}C_I \\ \frac{C_I}{PD_I} &= K_I \\ \frac{C_I}{P(D_{Itotal} - C_I)} &= K_I.\end{aligned}\tag{5.16}$$

Here,  $K_I = K_{I+}/K_{I-}$ . Equation 5.16 can also be written in terms of proportion of complex formed if we divide by  $D_{Itotal}$ :

$$\tilde{C}_I = \frac{\tilde{K}_I P}{1 + \tilde{K}_I P},\tag{5.17}$$

where,  $\tilde{C}_I = C_I/D_{Itotal}$  and  $\tilde{K}_I = K_I/D_{Itotal}$ .

The final step in the cold temperature signal transduction described by this model is the binding to the second regulatory region by the phosphorylated RR. This step can be better represented by the following reaction:



In this reaction,  $D_{II}$  and  $C_{II}$  represent the second regulatory region and the complex formed by the union of  $P$  and  $C_{II}$ , respectively. The equations that describe the time

evolution of the reagents are:

$$\begin{aligned}\frac{dD_{II}}{dt} &= -K_{II+}PD_{II} + K_{II-}C_{II} \\ \frac{dD_{II}}{dt} &= -\frac{dC_{II}}{dt}.\end{aligned}\tag{5.19}$$

In this case, the QSSA remains valid. Consequently, we obtain expressions similar to 5.16 and 5.17:

$$K_{II} = \frac{C_{II}}{P(D_{IItotal} - C_{II})}\tag{5.20}$$

$$\tilde{C}_{II} = \frac{K_{II}P}{1 + \tilde{K}_{II}P}.\tag{5.21}$$

Here, the variables introduced are:  $K_{II} = K_{II+}/K_{II-}$ ,  $\tilde{K}_{II} = K_{II}/D_{IItotal}$  and  $\tilde{C}_{II} = C_{II}/D_{IItotal}$ .

The final objective is to find the dynamic equation that describes the transcription of the desaturase *des*. In order to do so, first we need to find the average proportion of response regulators bound to the regulatory regions. With the information obtained above, such a expression can be easily found:

$$\begin{aligned}\bar{P} &= \frac{1}{2}(\tilde{C}_I + \tilde{C}_{II}) \\ \bar{P} &= \frac{1}{2}\left(\frac{K_I P}{1 + \tilde{K}_I P} + \frac{K_{II} P}{1 + \tilde{K}_{II} P}\right)\end{aligned}\tag{5.22}$$

Finally, the dynamic equation that describes the time evolution of *des* transcription is:

$$\frac{d\text{des}}{dt} = \nu_2 \bar{P}' - \gamma \text{Des}.\tag{5.23}$$

In this equation, the parameters are: the something ( $\nu$ ), the average number of  $P$

bound ( $\bar{P}'$ ) and the dilution coefficient ( $\gamma$ ).

## **5.3 Computational Approach**

## **5.4 Results**