Text S1

Mathematical modeling

Our mathematical model consists of a system of coupled ordinary differential equations (ODEs) which describe the dynamic concentrations of the phosphotransfer proteins throughout the *R. sphaeroides* cell. We applied the law of mass action to the phosphotransfer reactions in Table 1 and obtained the following system of non-linear ODEs.

$$\frac{dA_2}{dt} = -k_1 A_2 + k_3 A_{2P} Y_3 - k_{-3} A_2 Y_{3P} + k_4 A_{2P} Y_4 - k_{-4} A_2 Y_{4P} + k_5 A_{2P} Y_6 - k_{-5} A_2 Y_{6P} + k_6 A_{2P} B_1 - k_{-6} A_2 B_{1P} + k_7 A_{2P} B_2 - k_{-7} A_2 B_{2P} \quad (1)$$

$$\frac{dA_{2P}}{dt} = k_1 A_2 - k_3 A_{2P} Y_3 + k_{-3} A_2 Y_{3P} - k_4 A_{2P} Y_4 + k_{-4} A_2 Y_{4P} - k_5 A_{2P} Y_6 + k_{-5} A_2 Y_{6P} - k_6 A_{2P} B_1 + k_{-6} A_2 B_{1P} - k_7 A_{2P} B_2 + k_{-7} A_2 B_{2P} \quad (2)$$

$$\frac{dA_3}{dt} = -k_2 A_3 + k_8 A_{3P} Y_6 - k_{-8} A_3 Y_{6P} + k_9 A_{3P} B_2 - k_{-9} A_3 B_{2P} \quad (3)$$

$$\frac{dA_{3P}}{dt} = k_2 A_3 - k_8 A_{3P} Y_6 + k_{-8} A_3 Y_{6P} - k_9 A_{3P} B_2 + k_{-9} A_3 B_{2P} \quad (4)$$

$$\frac{dY_3}{dt} = -k_3 A_{2P} Y_3 + k_{-3} A_2 Y_{3P} + k_{10} Y_{3P} \quad (5)$$

$$\frac{dY_{3P}}{dt} = k_3 A_{2P} Y_3 - k_{-3} A_2 Y_{3P} - k_{10} Y_{3P} \quad (6)$$

$$\frac{dY_4}{dt} = -k_4 A_{2P} Y_4 + k_{-4} A_2 Y_{4P} + k_{11} Y_{4P} \quad (7)$$

$$\frac{dY_{4P}}{dt} = k_4 A_{2P} Y_4 - k_{-4} A_2 Y_{4P} - k_{11} Y_{4P} \quad (8)$$

$$\frac{dY_6}{dt} = -k_5 A_{2P} Y_6 + k_{-5} A_2 Y_{6P} - k_8 A_{3P} Y_6 + k_{-8} A_3 Y_{6P} + k_{12} Y_{6P} + k_{15a} Y_{6P} A_3 + k_{15b} Y_{6P} A_{3P} \quad (9)$$

$$\frac{dY_{6P}}{dt} = k_5 A_{2P} Y_6 - k_{-5} A_2 Y_{6P} + k_8 A_{3P} Y_6 - k_{-8} A_3 Y_{6P} - k_{12} Y_{6P} - k_{15a} Y_{6P} A_3 - k_{15b} Y_{6P} A_{3P} \quad (10)$$

$$\frac{dB_1}{dt} = -k_6 A_{2P} B_1 + k_{-6} A_2 B_{1P} + k_{13} B_{1P} \quad (11)$$

$$\frac{dB_{1P}}{dt} = k_6 A_{2P} B_1 - k_{-6} A_2 B_{1P} - k_{13} B_{1P} \quad (12)$$

$$\frac{dB_2}{dt} = -k_7 A_{2P} B_2 + k_{-7} A_2 B_{2P} - k_9 A_{3P} B_2 + k_{-9} A_3 B_{2P} + k_{14} B_{2P} \quad (13)$$

$$\frac{dB_{2P}}{dt} = k_7 A_{2P} B_2 - k_{-7} A_2 B_{2P} + k_9 A_{3P} B_2 - k_{-9} A_3 B_{2P} - k_{14} B_{2P} \quad (14)$$

Here A_i=[$CheA_i$], A_{iP}=[$CheA_{iP}$], Y_j=[$CheY_j$], Y_{jP}=[$CheY_{jP}$] , B_k=[$CheB_k$] and Y_{kP}=[$CheY_{kP}$] where *i=[2,3], j=[3,4,6]* and *k=[1,2]* with the reaction rates as given in Table S2.

In order to close the system of equations we defined a set of initial conditions:

$$\begin{gathered} A_i(\mathbf{x},0) = A_{i0}, \; A_{iP}(\mathbf{x},0) = 0, \; Y_j(\mathbf{x},0) = Y_{j0}, \; Y_{jP}(\mathbf{x},0) = 0, \\ B_k(\mathbf{x},0) = B_{k0} \; \text{ and } \; B_{kP}(\mathbf{x},0) = 0 \end{gathered} \tag{15}$$

Here A_{i0} is the initial concentration of $CheA_i$, Y_{j0} the initial concentration of $CheY_j$ and B_{k0} the initial concentration of $CheB_k$. The model was populated with the experimental data in Table S2 and equations (1)-(14), along with the respective initial conditions in equation (15), were solved using an adaptive time stepping ODE solver in Matlab (ode15s) due to the stiffness of the problem.