Quasi-thermodynamic Push-Pull Models

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I. ONE-WAY PUSH MODEL

In this model, we have a substrate S^u and a writer (kinase) W. The writer is able to phosporylate the substrate from S^u to S^p. In this model, we do not explicitly include an eraser (phosphatase), but there is a dephosphoralation background rate (and a phosphoralation background rate).

Reactions:

$$W + S^u \leftrightarrows WS^u \to WS^p \leftrightarrows W + S^p \tag{1}$$

Background Reactions:

$$S^p \leftrightarrows S^u \tag{2}$$

$$WS^p \leftrightarrows WS^u \tag{3}$$

Total Concentrations: We define total concentrations for each species and also separately for unphosporylated and phosporylated states.

- Total writer: $[W_T] = [W] + [WS^u] + [WS^p]$
- Total substrate: $[S_T] = [S_T^u] + [S_T^p]$
- Total unphosporylated substrate: $[S_T^u] = [S^u] + [WS^u]$
- Total phosporylated substrate: $[S_T^p] = [S^p] + [WS^p]$

If we know the

Binding Energies: We assume the binding energies are the same for the unphosporylated and phosporylated states.

- Writer + substrate: $\Delta\epsilon_{WS}$

One-way Reaction Rates: These are the reaction rates that cannot be described by binding energies.

- Phosphorylation of substrate by writer: k_{WS}^p
- Background phosphorylation of substrate (independent of binding state): k_S^p
- Background dephosphorylation of substrate (independent of binding state): k_S^u

Partition Functions and Probabilities: We write down partition functions for each species based on the states listed in the total concentrations above. Partition function cannot mix unphosporylated and phosporylated states for the same species, so we do not write down a partition function for the total S. We assume the unbound state of each species is zero energy (although this choice doesn't matter).

- Writer: $Z_W = 1 + e^{-\beta(\Delta\epsilon_{WS} - k_B T \log[S^u])} + e^{-\beta(\Delta\epsilon_{WS} - k_B T \log[S^p])}$
- Unphosphorylated substrate: $Z_{S^u} = 1 + e^{-\beta(\Delta\epsilon_{WS} - k_B T \log[W])}$
- Phosphorylated substrate: $Z_{S^p} = 1 + e^{-\beta(\Delta\epsilon_{WS} - k_B T \log[W])}$

We define the reaction velocity

$$\alpha_{WS} = e^{\beta\Delta\epsilon_{WS}} \tag{4}$$

The associated conditional probabilities are then

$$p(W|S^u,S^p) = \frac{[W]}{[W_T]} = \frac{1}{Z_W} = \frac{1}{1+\frac{[S^u]+[S^p]}{\alpha_{WS}}} \tag{5}$$

$$p(WS^u|S^u,S^p) = \frac{[WS^u]}{[W_T]} = \frac{e^{-\beta(\Delta\epsilon_{WS}-k_BT\log[S^u])}}{Z_W} = \frac{\frac{[S^u]}{\alpha_{WS}}}{1+\frac{[S^u]+[S^p]}{\alpha_{WS}}} \tag{6}$$

$$p(WS^p|S^u,S^p) = \frac{[WS^p]}{[W_T]} = \frac{e^{-\beta(\Delta\epsilon_{WS}-k_BT\log[S^p])}}{Z_W} = \frac{\frac{[S^p]}{\alpha_{WS}}}{1+\frac{[S^u]+[S^p]}{\alpha_{WS}}} \tag{7}$$

$$p(S^u|W) = \frac{[S^u]}{[S^u_T]} = \frac{1}{Z_{S^u}} = \frac{1}{1+\frac{[W]}{\alpha_{WS}}} \tag{8}$$

$$p(WS^u|W) = \frac{[WS^u]}{[S^u_T]} = \frac{e^{-\beta(\Delta\epsilon_{WS}-k_BT\log[W])}}{Z_{S^u}} = \frac{\frac{[W]}{\alpha_{WS}}}{1+\frac{[W]}{\alpha_{WS}}} \tag{9}$$

$$p(S^p|W) = \frac{[S^p]}{[S^p_T]} = \frac{1}{Z_{S^p}} = \frac{1}{1+\frac{[W]}{\alpha_{WS}}} \tag{10}$$

$$p(WS^p|W) = \frac{[WS^p]}{[S^p_T]} = \frac{e^{-\beta(\Delta\epsilon_{WS}-k_BT\log[W])}}{Z_{S^p}} = \frac{\frac{[W]}{\alpha_{WS}}}{1+\frac{[W]}{\alpha_{WS}}} \tag{11}$$

Reactions in Detailed Balance: From the probabilities, we focus on the equations for the concentration of each species in its unbound state. These correspond to the kinetic equations for reactions not involving (de)phosphorylation.

$$\frac{[W]}{[W_T]} = \frac{1}{1+\frac{[S^u]+[S^p]}{\alpha_{WS}}} \tag{12}$$

$$\frac{[S^u]}{[S^u_T]} = \frac{1}{1+\frac{[W]}{\alpha_{WS}}} \tag{13}$$

$$\frac{[S^p]}{[S^p_T]} = \frac{1}{1+\frac{[W]}{\alpha_{WS}}} \tag{14}$$

Reactions not in Detailed Balance: This is the kinetic equation for reactions involving (de)phosphorylation. It is possible for either S^u or WS^u to undergo this process, so we have two equations

$$\frac{\mathrm{d}[S^p]}{\mathrm{d}t} = k^-_{WS}[WS^p] - k^+_{WS}[W][S^p] + k^p_S[S^u] - k^u_S[S^p] \tag{15}$$

$$\frac{\mathrm{d}[WS^p]}{\mathrm{d}t} = k^+_{WS}[W][S^p] - k^-_{WS}[WS^p] + k^p_{WS}[WS^u] + k^p_S[WS^u] - k^u_S[WS^p] \tag{16}$$

$$\frac{\mathrm{d}[S^u]}{\mathrm{d}t} = k^-_{WS}[WS^u] - k^+_{WS}[W][S^u] + k^u_S[S^p] - k^p_S[S^u] \tag{17}$$

$$\frac{\mathrm{d}[WS^u]}{\mathrm{d}t} = k^+_{WS}[W][S^u] - k^-_{WS}[WS^u] - k^p_{WS}[WS^u] + k^u_S[WS^p] - k^p_S[WS^u] \tag{18}$$

where we have defined the forward and backward dissociation rates k^+_{WS} and k^-_{WS} such that

$$\frac{1}{\alpha_{WS}} = \frac{k^+_{WS}}{k^-_{WS}} \tag{19}$$

Instead of writing these four equations, we can instead write the kinetic equations so that they only describe reactions that are not in detailed balance and do not depend on k^+_{WS} and k^-_{WS}. To see what these equations are, we can simple write down the reaction rates for the total phosphorylated and unphosphorylated substrate. These will just be related by a negative sign since they sum to a constant, so we can write

$$\frac{\mathrm{d}[S_T^p]}{\mathrm{d}t} = k_{WS}^p[WS^u] + k_S^p[S_T^u] - k_S^u[S_T^p] \tag{20}$$
$$= k_{WS}^p[S_T^u]p(WS^u|W) + k_S^p[S_T^u] - k_S^u[S_T^p] \tag{21}$$
$$= k_{WS}^p([S_T] - [S_T^p])p(WS^u|W) + k_S^p([S_T] - [S_T^p]) - k_S^u[S_T^p] \tag{22}$$

We note that this equation can also be obtained by summing together the first two kinetic equations. Similarly, the equation for $[S_T^u]$ can be obtained by summing the last two kinetic equations.

Setting this equation to zero, we get

$$\frac{[S_T^p]}{[S_T]} = \frac{k_{WS}^p p(WS^u|W) + k_S^p}{k_{WS}^p p(WS^u|W) + k_S^p + k_S^u} \tag{23}$$
$$= \frac{\alpha_{WS}^p p(WS^u|W) + v_S^p}{\alpha_{WS}^p p(WS^u|W) + v_S^p + 1} \tag{24}$$

where we have defined the reaction velocities relative to the background dephosphorylation rate,

$$v_{WS}^p = \frac{k_{WS}^p}{k_S^u} \tag{25}$$
$$v_S^p = \frac{k_S^p}{k_S^u} \tag{26}$$

Numerical Strategy:

We assume that $[W_T]$ and $[S_T]$ are known. Add together Eqs. (13)and(14) and defining $[S_f] = [S^p] + [S^u]$, we find a pair of equations that can be solved for $[W]$ and $[S_f]$,

$$\frac{[W]}{[W_T]} = \frac{1}{1 + \frac{[S_f]}{\alpha_{WS}}} \tag{27}$$
$$\frac{[S^f]}{[S_T]} = \frac{1}{1 + \frac{[W]}{\alpha_{WS}}} \tag{28}$$

Combining these equations, we find a quadratic equation in terms of S_f,

$$0 = \left(\frac{[S_f]}{\alpha_{WS}}\right)^2 + \left(1 + \frac{[W_T] - [S_T]}{\alpha_{WS}}\right)\frac{[S_f]}{\alpha_{WS}} - \frac{[S_T]}{\alpha_{WS}} \tag{29}$$

Generally, we find that this has one positive solution for $[S_f]$. Using this solution, we solve for $[W]$ and then use the kinetic equation so solve for $[S_T^p]$. The rest of the concentrations are then straightforward to calculate without any further need to solve any equations.

A. Gradient

$$0 = \frac{\partial}{\partial \alpha_{WS}}\left[\left(\frac{[S_f]}{\alpha_{WS}}\right)^2 + \left(1 + \frac{[W_T] - [S_T]}{\alpha_{WS}}\right)\frac{[S_f]}{\alpha_{WS}} - \frac{[S_T]}{\alpha_{WS}}\right] \tag{30}$$
$$= -\frac{1}{\alpha_{WS}^2}\left[([W_T] - [S_T])\frac{[S_f]}{\alpha_{WS}} - [S_T]\right] + \left[2\frac{[S_f]}{\alpha_{WS}} + 1 + \frac{[W_T] - [S_T]}{\alpha_{WS}}\right]\left(\frac{1}{\alpha_{WS}}\frac{\partial [S_f]}{\partial \alpha_{WS}} - \frac{[S_f]}{\alpha_{WS}^2}\right) \tag{31}$$
$$= -\frac{1}{\alpha_{WS}}\left[2\left(\frac{[S_f]}{\alpha_{WS}}\right)^2 + \left(1 + 2\frac{[W_T] - [S_T]}{\alpha_{WS}}\right)\frac{[S_f]}{\alpha_{WS}} - \frac{[S_T]}{\alpha_{WS}}\right] + \left[2\frac{[S_f]}{\alpha_{WS}} + 1 + \frac{[W_T] - [S_T]}{\alpha_{WS}}\right]\frac{1}{\alpha_{WS}}\frac{\partial [S_f]}{\partial \alpha_{WS}} \tag{32}$$
$$\frac{\partial [S_f]}{\partial \alpha_{WS}} = \frac{2\left(\frac{[S_f]}{\alpha_{WS}}\right)^2 + \left(1 + 2\frac{[W_T]-[S_T]}{\alpha_{WS}}\right)\frac{[S_f]}{\alpha_{WS}} - \frac{[S_T]}{\alpha_{WS}}}{2\frac{[S_f]}{\alpha_{WS}} + 1 + \frac{[W_T]-[S_T]}{\alpha_{WS}}} \tag{33}$$
$$= \tag{34}$$

$$0 = \frac{\partial}{\partial \alpha_{WS}} \left(\alpha_{WS}[S_f]^2 + (1 + \alpha_{WS}([W_T] - [S_T]))[S_f] - [S_T] \right) \tag{35}$$

$$= [S_f]^2 + ([W_T] - [S_T])[S_f] + [2\alpha_{WS}[S_f] + 1 + \alpha_{WS}([W_T] - [S_T])] \frac{\partial [S_f]}{\partial \alpha_{WS}} \tag{36}$$

$$\frac{\partial [S_f]}{\partial \alpha_{WS}} = -\frac{[S_f]([S_f] + [W_T] - [S_T])}{[2\alpha_{WS}[S_f] + 1 + \alpha_{WS}([W_T] - [S_T])]} \tag{37}$$

$$\frac{\partial [W]}{\partial \alpha_{WS}} = -[W_T] \frac{[S_f]}{(1 + \alpha_{WS}[S_f])^2} - [W_T] \frac{\alpha_{WS}}{(1 + \alpha_{WS}[S_f])^2} \frac{\partial [S_f]}{\partial \alpha_{WS}} \tag{38}$$

$$= -[W_T] \frac{1}{(1 + \alpha_{WS}[S_f])^2} \left([S_f] + \alpha_{WS} \frac{\partial [S_f]}{\partial \alpha_{WS}} \right) \tag{39}$$

$$\frac{\partial p(WS^u|W)}{\partial \alpha_{WS}} = \frac{[W]}{1 + \alpha_{WS}[W]} - \frac{\alpha_{WS}[W]^2}{(1 + \alpha_{WS}[W])^2} + \left[\frac{\alpha_{WS}}{1 + \alpha_{WS}[W]} - \frac{\alpha_{WS}^2[W]}{(1 + \alpha_{WS}[W])^2} \right] \frac{\partial [W]}{\partial \alpha_{WS}} \tag{40}$$

$$= \frac{[W]}{(1 + \alpha_{WS}[W])^2} + \frac{\alpha_{WS}}{(1 + \alpha_{WS}[W])^2} \frac{\partial [W]}{\partial \alpha_{WS}} \tag{41}$$

$$= \frac{1}{(1 + \alpha_{WS}[W])^2} \left([W] + \alpha_{WS} \frac{\partial [W]}{\partial \alpha_{WS}} \right) \tag{42}$$

$$\frac{\partial [S_T^p]}{\partial \alpha_{WS}} = [S_T] \left[\frac{\alpha_{WS}^p}{\alpha_{WS}^p p(WS^u|W) + v_S^p + 1} - \frac{\alpha_{WS}^p(\alpha_{WS}^p p(WS^u|W) + v_S^p)}{\left(\alpha_{WS}^p p(WS^u|W) + v_S^p + 1\right)^2} \right] \frac{\partial p(WS^u|W)}{\partial \alpha_{WS}} \tag{43}$$

$$= [S_T] \frac{\alpha_{WS}^p}{\left(\alpha_{WS}^p p(WS^u|W) + v_S^p + 1\right)^2} \frac{\partial p(WS^u|W)}{\partial \alpha_{WS}} \tag{44}$$

$$\frac{\partial [S_T^p]}{\partial \alpha_{WS}^p} = [S_T] \frac{p(WS^u|W)}{\alpha_{WS}^p p(WS^u|W) + v_S^p + 1} - [S_T] \frac{p(WS^u|W)(\alpha_{WS}^p p(WS^u|W) + v_S^p)}{\left(\alpha_{WS}^p p(WS^u|W) + v_S^p + 1\right)^2} \tag{45}$$

$$= [S_T] \frac{p(WS^u|W)}{\left(\alpha_{WS}^p p(WS^u|W) + v_S^p + 1\right)^2} \tag{46}$$

$$\frac{\partial [S_T^p]}{\partial v_S^p} = [S_T] \frac{1}{\alpha_{WS}^p p(WS^u|W) + v_S^p + 1} - [S_T] \frac{\alpha_{WS}^p p(WS^u|W) + v_S^p}{\left(\alpha_{WS}^p p(WS^u|W) + v_S^p + 1\right)^2} \tag{47}$$

$$= [S_T] \frac{1}{\left(\alpha_{WS}^p p(WS^u|W) + v_S^p + 1\right)^2} \tag{48}$$

II. GOLDBETER PUSH-PULL AMPLIFIER

In the Goldbeter model, we have a push-pull amplifier with a substrate S, a writer (kinase) W and an eraser (phosphatase) E with no background (de)phosphorylation [1]. In addition, we lack the intermediate states directly after phosphorylation WS^p and dephosphorylation ES^u. These two states are assumed to be very short-lived.

Reactions:

$$W + S^u \leftrightarrows WS^u \rightarrow W + S^p \tag{49}$$

$$E + S^p \leftrightarrows ES^p \rightarrow E + S^u \tag{50}$$

Total Concentrations:

- Total writer: $[W_T] = [W] + [WS^u]$

- Total Eraser: $[E_T] = [E] + [ES^p]$
- Total substrate: $[S_T] = [S_T^u] + [S_T^p]$
- Total unphosporylated substrate: $[S_T^u] = [S^u] + [WS^u]$
- Total phosporylated substrate: $[S_T^p] = [S^p] + [ES^p]$

Binding Energies:

- Writer + substrate: $\Delta\epsilon_{WS}$
- Eraser + substrate: $\Delta\epsilon_{ES}$

One-way Reaction Rates:

- Phosphorylation of substrate by writer: k_{WS}^p
- Dephosphorylation of substrate by eraser: k_{ES}^u

Partition Functions and Probabilities:

- Writer: $Z_W = 1 + e^{-\beta(\Delta\epsilon_{WS} - k_B T \log[S^u])}$
- Eraser: $Z_E = 1 + e^{-\beta(\Delta\epsilon_{ES} - k_B T \log[S^p])}$
- Unphosphorylated substrate: $Z_{S^u} = 1 + e^{-\beta(\Delta\epsilon_{WS} - k_B T \log[W])}$
- Phosphorylated substrate: $Z_{S^p} = 1 + e^{-\beta(\Delta\epsilon_{ES} - k_B T \log[E])}$

We define the reaction velocities

$$\alpha_{WS} = e^{-\beta\Delta\epsilon_{WS}} \tag{51}$$

$$\alpha_{ES} = e^{-\beta\Delta\epsilon_{ES}} \tag{52}$$

The associated conditional probabilities are then

$$p(W|S^u, S^p, E) = \frac{[W]}{[W_T]} = \frac{1}{Z_W} = \frac{1}{1 + \alpha_{WS}[S^u]} \tag{53}$$

$$p(WS^u|S^u, S^p, E) = \frac{[WS^u]}{[W_T]} = \frac{e^{-\beta(\Delta\epsilon_{WS} - k_B T \log[S^u])}}{Z_W} = \frac{\alpha_{WS}[S^u]}{1 + \alpha_{WS}[S^u]} \tag{54}$$

$$p(E|S^u, S^p, W) = \frac{[E]}{[E_T]} = \frac{1}{Z_E} = \frac{1}{1 + \alpha_{ES}[S^p]} \tag{55}$$

$$p(ES^p|S^u, S^p, W) = \frac{[ES^p]}{[E_T]} = \frac{e^{-\beta(\Delta\epsilon_{ES} - k_B T \log[S^p])}}{Z_E} = \frac{\alpha_{ES}[S^p]}{1 + \alpha_{ES}[S^p]} \tag{56}$$

$$p(S^u|E, W) = \frac{[S^u]}{[S_T^u]} = \frac{1}{Z_{S^u}} = \frac{1}{1 + \alpha_{WS}[W]} \tag{57}$$

$$p(WS^u|E, W) = \frac{[WS^u]}{[S_T^u]} = \frac{e^{-\beta(\Delta\epsilon_{WS} - k_B T \log[W])}}{Z_{S^u}} = \frac{\alpha_{WS}[W]}{1 + \alpha_{WS}[W]} \tag{58}$$

$$p(S^p|E, W) = \frac{[S^p]}{[S_T^p]} = \frac{1}{Z_{S^p}} = \frac{1}{1 + \alpha_{ES}[E]} \tag{59}$$

$$p(ES^p|E, W) = \frac{[ES^p]}{[S_T^p]} = \frac{e^{-\beta(\Delta\epsilon_{ES} - k_B T \log[E])}}{Z_{S^p}} = \frac{\alpha_{ES}[E]}{1 + \alpha_{ES}[E]} \tag{60}$$

Reactions in Detailed Balance:

$$\frac{[W]}{[W_T]} = \frac{1}{1+\alpha_{WS}[S^u]} \tag{61}$$
$$\frac{[E]}{[E_T]} = \frac{1}{1+\alpha_{ES}[S^p]} \tag{62}$$
$$\frac{[S^u]}{[S^u_T]} = \frac{1}{1+\alpha_{WS}[W]} \tag{63}$$
$$\frac{[S^p]}{[S^p_T]} = \frac{1}{1+\alpha_{ES}[E]} \tag{64}$$

Reactions not in Detailed Balance: We only focus on the equations for the total amounts of phosphorylated and unphosphorylated substrate, allowing us to ignore any of the reactions in detailed balance.

$$\frac{\mathrm{d}[S^p_T]}{\mathrm{d}t} = k^p_{WS}[WS^u] - k^u_{ES}[ES^p] \tag{65}$$
$$= k^p_{WS}[S^u_T]p(WS^u|E,W) - k^u_{ES}[S^p_T]p(ES^p|E,W) \tag{66}$$
$$= k^p_{WS}([S_T] - [S^p_T])p(WS^u|E,W) - k^u_{ES}[S^p_T]p(ES^p|E,W) \tag{67}$$

Setting this to zero, we get

$$\frac{[S^p_T]}{[S_T]} = \frac{k^p_{WS}p(WS^u|E,W)}{k^p_{WS}p(WS^u|E,W) + k^u_{ES}p(ES^p|E,W)} \tag{68}$$
$$= \frac{\alpha^p_{WS}p(WS^u|E,W)}{\alpha^p_{WS}p(WS^u|E,W) + p(ES^p|E,W)} \tag{69}$$
$$\tag{70}$$

where we have defined the reaction velocity

$$\alpha^p_{WS} = \frac{k^p_{WS}}{k^u_{ES}} \tag{71}$$

III. COMPLETE PUSH-PULL AMPLIFIER

This model generalizes the Goldbeter model to include the intermediate states and background (de)phosphorylation.
Reactions:

$$W + S^u \leftrightarrows WS^u \rightarrow WS^p \leftrightarrows W + S^p \tag{72}$$
$$E + S^p \leftrightarrows ES^p \rightarrow ES^u \leftrightarrows E + S^u \tag{73}$$

Background:

$$S^p \leftrightarrows S^u \tag{74}$$
$$WS^p \leftrightarrows WS^u \tag{75}$$
$$ES^p \leftrightarrows ES^u \tag{76}$$

Total Concentrations:

- Total writer: $[W_T] = [W] + [WS^p] + [WS^u]$
- Total Eraser: $[E_T] = [E] + [ES^p] + [ES^u]$
- Total substrate: $[S_T] = [S^u_T] + [S^p_T]$
- Total unphosporylated substrate: $[S^u_T] = [S^u] + [WS^u] + [ES^u]$
- Total phosporylated substrate: $[S^p_T] = [S^p] + [WS^p] + [ES^p]$

Binding Energies:

- Writer + substrate: $\Delta\epsilon_{WS}$
- Eraser + substrate: $\Delta\epsilon_{ES}$

One-way Reaction Rates:

- Phosphorylation of substrate by writer: k_{WS}^p
- Dephosphorylation of substrate by eraser: k_{ES}^u
- Background phosphorylation of substrate (independent of binding state): k_S^p
- Background dephosphorylation of substrate (independent of binding state): k_S^u

Partition Functions and Probabilities:

- Writer: $Z_W = 1 + e^{-\beta(\Delta\epsilon_{WS} - k_B T \log[S^p])} + e^{-\beta(\Delta\epsilon_{WS} - k_B T \log[S^u])}$
- Eraser: $Z_E = 1 + e^{-\beta(\Delta\epsilon_{ES} - k_B T \log[S^p])} + e^{-\beta(\Delta\epsilon_{ES} - k_B T \log[S^u])}$
- Unphosphorylated substrate: $Z_{S^u} = 1 + e^{-\beta(\Delta\epsilon_{WS} - k_B T \log[W])} + e^{-\beta(\Delta\epsilon_{ES} - k_B T \log[E])}$
- Phosphorylated substrate: $Z_{S^p} = 1 + e^{-\beta(\Delta\epsilon_{WS} - k_B T \log[W])} + e^{-\beta(\Delta\epsilon_{ES} - k_B T \log[E])}$

We define the reaction velocities

$$\alpha_{WS} = e^{-\beta\Delta\epsilon_{WS}} \tag{77}$$

$$\alpha_{ES} = e^{-\beta\Delta\epsilon_{ES}} \tag{78}$$

The associated conditional probabilities are then

$$p(W|S^u, S^p, E) = \frac{[W]}{[W_T]} = \frac{1}{Z_W} = \frac{1}{1 + \alpha_{WS}([S^p] + [S^u])} \tag{79}$$

$$p(WS^u|S^u, S^p, E) = \frac{[WS^u]}{[W_T]} = \frac{e^{-\beta(\Delta\epsilon_{WS} - k_B T \log[S^u])}}{Z_W} = \frac{\alpha_{WS}[S^u]}{1 + \alpha_{WS}([S^p] + [S^u])} \tag{80}$$

$$p(WS^p|S^u, S^p, E) = \frac{[WS^p]}{[W_T]} = \frac{e^{-\beta(\Delta\epsilon_{WS} - k_B T \log[S^p])}}{Z_W} = \frac{\alpha_{WS}[S^p]}{1 + \alpha_{WS}([S^p] + [S^u])} \tag{81}$$

$$p(E|S^u, S^p, W) = \frac{[E]}{[E_T]} = \frac{1}{Z_E} = \frac{1}{1 + \alpha_{ES}([S^p] + [S^u])} \tag{82}$$

$$p(ES^u|S^u, S^p, W) = \frac{[ES^u]}{[E_T]} = \frac{e^{-\beta(\Delta\epsilon_{ES} - k_B T \log[S^u])}}{Z_E} = \frac{\alpha_{ES}[S^u]}{1 + \alpha_{ES}([S^p] + [S^u])} \tag{83}$$

$$p(ES^p|S^u, S^p, W) = \frac{[ES^p]}{[E_T]} = \frac{e^{-\beta(\Delta\epsilon_{ES} - k_B T \log[S^p])}}{Z_E} = \frac{\alpha_{ES}[S^p]}{1 + \alpha_{ES}([S^p] + [S^u])} \tag{84}$$

$$p(S^u|E, W) = \frac{[S^u]}{[S_T^u]} = \frac{1}{Z_{S^u}} = \frac{1}{1 + \alpha_{WS}[W] + \alpha_{ES}[E]} \tag{85}$$

$$p(WS^u|E, W) = \frac{[WS^u]}{[S_T^u]} = \frac{e^{-\beta(\Delta\epsilon_{WS} - k_B T \log[W])}}{Z_{S^u}} = \frac{\alpha_{WS}[W]}{1 + \alpha_{WS}[W] + \alpha_{ES}[E]} \tag{86}$$

$$p(ES^u|E, W) = \frac{[ES^u]}{[S_T^u]} = \frac{e^{-\beta(\Delta\epsilon_{ES} - k_B T \log[E])}}{Z_{S^u}} = \frac{\alpha_{ES}[E]}{1 + \alpha_{WS}[W] + \alpha_{ES}[E]} \tag{87}$$

$$p(S^p|E, W) = \frac{[S^p]}{[S_T^p]} = \frac{1}{Z_{S^p}} = \frac{1}{1 + \alpha_{WS}[W] + \alpha_{ES}[E]} \tag{88}$$

$$p(WS^p|E, W) = \frac{[WS^p]}{[S_T^p]} = \frac{e^{-\beta(\Delta\epsilon_{WS} - k_B T \log[W])}}{Z_{S^p}} = \frac{\alpha_{WS}[W]}{1 + \alpha_{WS}[W] + \alpha_{ES}[E]} \tag{89}$$

$$p(ES^p|E, W) = \frac{[ES^p]}{[S_T^p]} = \frac{e^{-\beta(\Delta\epsilon_{ES} - k_B T \log[E])}}{Z_{S^p}} = \frac{\alpha_{ES}[E]}{1 + \alpha_{WS}[W] + \alpha_{ES}[E]} \tag{90}$$

Reactions in Detailed Balance:

$$\frac{[W]}{[W_T]} = \frac{1}{1+\alpha_{WS}([S^p]+[S^u])} \tag{91}$$
$$\frac{[E]}{[E_T]} = \frac{1}{1+\alpha_{ES}([S^p]+[S^u])} \tag{92}$$
$$\frac{[S^u]}{[S^u_T]} = \frac{1}{1+\alpha_{WS}[W]+\alpha_{ES}[E]} \tag{93}$$
$$\frac{[S^p]}{[S^p_T]} = \frac{1}{1+\alpha_{WS}[W]+\alpha_{ES}[E]} \tag{94}$$

Reactions not in Detailed Balance:

$$\frac{\mathrm{d}[S^p_T]}{\mathrm{d}t} = k^p_{WS}[WS^u] - k^u_{ES}[ES^p] + k^p_S[S^u_T] - k^u_S[S^p_T] \tag{95}$$
$$= k^p_{WS}[S^u_T]p(WS^u|E,W) - k^u_{ES}[S^p_T]p(ES^p|E,W) + k^p_S[S^u_T] - k^u_S[S^p_T] \tag{96}$$
$$= k^p_{WS}([S_T]-[S^p_T])p(WS^u|E,W) - k^u_{ES}[S^p_T]p(ES^p|E,W) + k^p_S([S_T]-[S^p_T]) - k^u_S[S^p_T] \tag{97}$$

Setting this to zero, we get

$$\frac{[S^p_T]}{[S_T]} = \frac{k^p_{WS}p(WS^u|E,W)+k^p_S}{k^p_{WS}p(WS^u|E,W)+k^u_{ES}p(ES^p|E,W)+k^p_S+k^u_S} \tag{98}$$
$$= \frac{\alpha^p_{WS}p(WS^u|E,W)+v^p_S}{\alpha^p_{WS}p(WS^u|E,W)+\alpha^u_{ES}p(ES^p|E,W)+v^p_S+1} \tag{99}$$
$$\tag{100}$$

where we have defined the reaction velocities

$$\alpha^p_{WS} = \frac{k^p_{WS}}{k^u_S} \tag{101}$$
$$\alpha^u_{ES} = \frac{k^u_{ES}}{k^u_S} \tag{102}$$
$$v^p_S = \frac{k^p_S}{k^u_S} \tag{103}$$

Numerical Strategy:
We assume that $[W_T]$, $[E_T]$, and $[S_T]$ are known. Add together Eqs. (13) and (14) and defining $[S_f]=[S^p]+[S^u]$, we find three equations that can be solved for $[W]$, $[E]$, and $[S_f]$,

$$\frac{[W]}{[W_T]} = \frac{1}{1+\alpha_{WS}[S_f]} \tag{104}$$
$$\frac{[E]}{[E_T]} = \frac{1}{1+\alpha_{ES}[S_f]} \tag{105}$$
$$\frac{[S_f]}{[S_T]} = \frac{1}{1+\alpha_{WS}[W]+\alpha_{ES}[E]} \tag{106}$$

Combining these equations, we find a cubic equation in terms of S_f,

$$0 = \alpha_{WS}\alpha_{ES}[S_f]^3 + (\alpha_{WS}+\alpha_{ES}+\alpha_{WS}\alpha_{ES}([W_T]+[E_T]-[S_T]))[S_f]^2 \tag{107}$$
$$+ (1+\alpha_{WS}([W_T]-[S_T])+\alpha_{ES}([E_T]-[S_T]))[S_f] - [S_T] \tag{108}$$

Generally, we find that this has one positive solution for $[S_f]$. Using this solution, we solve for $[W]$ and $[E]$ and then use the kinetic equation to solve for $[S^p_T]$. The rest of the concentrations are then straightforward to calculate without any further need to solve any equations.

IV. TWO-STEP AMPLIFIER

Reactions:

$$R + W^u \leftrightarrows RW^u \rightarrow RW^p \leftrightarrows R + W^p \tag{109}$$
$$E_W + W^p \leftrightarrows E_W W^p \rightarrow E_W W^u \leftrightarrows E_W + W^u \tag{110}$$
$$W^p + S^u \leftrightarrows W^p S^u \rightarrow W^p S^p \leftrightarrows W^p + S^p \tag{111}$$
$$E_S + S^p \leftrightarrows E_S S^p \rightarrow E_S S^u \leftrightarrows E_S + S^u \tag{112}$$

Are these viable?

$$W^u + S^u \leftrightarrows W^u S^u \tag{113}$$
$$W^u + S^p \leftrightarrows W^u S^p \tag{114}$$

Background:

$$\text{any } W^p \leftrightarrows \text{any } W^u \tag{115}$$
$$\text{any } S^p \leftrightarrows \text{any } S^u \tag{116}$$
$$\tag{117}$$

Total Concentrations:

- Total writer: $[W_T] = [W_T^u] + [W_T^p]$
- Total unphosporylated writer: $[W_T^u] = [W^u] + [RW^u] + [EW^u]$
- Total phosporylated writer: $[W_T^p] = [W^p] + [W^p S^p] + [W^p S^u] + [RW^p] + [EW^p]$
- Total Writer-Eraser: $[E_{WT}] = [E_W] + [E_W W^p] + [E_W W^u]$
- Total Substrate-Eraser: $[E_{ST}] = [E_S] + [E_S S^p] + [E_S S^u]$
- Total substrate: $[S_T] = [S_T^u] + [S_T^p]$
- Total unphosporylated substrate: $[S_T^u] = [S^u] + [W^p S^u] + [W^u S^u] + [ES^u]$
- Total phosporylated substrate: $[S_T^p] = [S^p] + [W^p S^p] + [W^u S^p] + [ES^p]$

Binding Energies:

- Receptor + Writer: $\Delta\epsilon_{RW}$
- Writer-Eraser + Writer: $\Delta\epsilon_{E_W W}$
- Writer + substrate: $\Delta\epsilon_{WS}$
- Substrate-Eraser + substrate: $\Delta\epsilon_{E_S S}$

One-way Reaction Rates:

- Phosphorylation of writer by receptor: k_{RW}^p
- Dephosphorylation of writer by eraser: $k_{E_W W}^u$
- Phosphorylation of substrate by writer: k_{WS}^p
- Dephosphorylation of substrate by eraser: $k_{E_S S}^u$
- Background phosphorylation of writer (independent of binding state): k_W^p
- Background dephosphorylation of writer (independent of binding state): k_W^u
- Background phosphorylation of substrate (independent of binding state): k_S^p
- Background dephosphorylation of substrate (independent of binding state): k_S^u

Partition Functions and Probabilities:

- Unphosphorylated writer: $Z_{W^u} = 1 + e^{-\beta(\Delta\epsilon_{RW} - k_BT\log[R])+e^{-\beta\left(\Delta\epsilon_{E_W W} - k_BT\log[E_W]\right)}}$
- Phosphorylated writer: $Z_{W^p} = 1 + e^{-\beta(\Delta\epsilon_{RW} - k_BT\log[R])+e^{-\beta\left(\Delta\epsilon_{E_W W} - k_BT\log[E_W]\right)}} + e^{-\beta(\Delta\epsilon_{WS} - k_BT\log[S^p])} + e^{-\beta(\Delta\epsilon_{WS} - k_BT\log[S^u])}$
- Eraser: $Z_E = 1 + e^{-\beta(\Delta\epsilon_{ES} - k_BT\log[S^p])} + e^{-\beta(\Delta\epsilon_{ES} - k_BT\log[S^u])}$
- Unphosphorylated substrate: $Z_{S^u} = 1 + e^{-\beta(\Delta\epsilon_{WS} - k_BT\log[W])} + e^{-\beta(\Delta\epsilon_{ES} - k_BT\log[E])}$
- Phosphorylated substrate: $Z_{S^p} = 1 + e^{-\beta(\Delta\epsilon_{WS} - k_BT\log[W])} + e^{-\beta(\Delta\epsilon_{ES} - k_BT\log[E])}$

We define the reaction velocities

$$\alpha_{WS} = e^{-\beta\Delta\epsilon_{WS}} \tag{118}$$
$$\alpha_{ES} = e^{-\beta\Delta\epsilon_{ES}} \tag{119}$$

The associated conditional probabilities are then

$$p(W^u|S^u, S^p, R, E_W, E_S) = \frac{[W^u]}{[W_T^u]} = \frac{1}{Z_{W^u}} = \frac{1}{1 + v_{RW}[R] + v_{E_W W}[E_W]} \tag{120}$$
$$p(RW^u|S^u, S^p, R, E_W, E_S) = \frac{[RW^u]}{[W_T^u]} = \frac{e^{-\beta(\Delta\epsilon_{RW} - k_BT\log[R])}}{Z_{W^u}} = \frac{v_{RW}[R]}{1 + v_{RW}[R] + v_{E_W W}[E_W]} \tag{121}$$
$$p(E_W W^u|S^u, S^p, R, E_W, E_S) = \frac{[E_W W^u]}{[W_T^u]} = \frac{e^{-\beta\left(\Delta\epsilon_{E_W W} - k_BT\log[E_W]\right)}}{Z_{W^u}} = \frac{v_{E_W W}[E_W]}{1 + v_{RW}[R] + v_{E_W W}[E_W]} \tag{122}$$

Continue Here

$$p(W^p|S^u, S^p, R, E_W, E_S) = \frac{[W^p]}{[W_T^p]} = \frac{1}{Z_W} = \frac{1}{1 + \alpha_{WS}([S^p] + [S^u])} \tag{123}$$
$$p(RW^p|S^u, S^p, R, E_W, E_S) = \frac{[RW^p]}{[W_T^p]} = \frac{e^{-\beta(\Delta\epsilon_{RW} - k_BT\log[R])}}{Z_W} = \frac{v_{RW}[R]}{1 + v_{RW}[R] + v_{E_W W}[E_W]} \tag{124}$$
$$p(E_W W^p|S^u, S^p, R, E_W, E_S) = \frac{[E_W W^p]}{[W_T^p]} = \frac{e^{-\beta\left(\Delta\epsilon_{E_W W} - k_BT\log[E_W]\right)}}{Z_W} = \frac{v_{E_W W}[E_W]}{1 + v_{RW}[R] + v_{E_W W}[E_W]} \tag{125}$$
$$p(W^pS^u|S^u, S^p, R, E_W, E_S) = \frac{[W^pS^u]}{[W_T^p]} = \frac{e^{-\beta(\Delta\epsilon_{WS} - k_BT\log[S^u])}}{Z_W} = \frac{\alpha_{WS}[S^u]}{1 + \alpha_{WS}([S^p] + [S^u])} \tag{126}$$
$$p(W^pS^p|S^u, S^p, R, E_W, E_S) = \frac{[W^pS^p]}{[W_T^p]} = \frac{e^{-\beta(\Delta\epsilon_{WS} - k_BT\log[S^p])}}{Z_W} = \frac{\alpha_{WS}[S^p]}{1 + \alpha_{WS}([S^p] + [S^u])} \tag{127}$$

$$p(E|S^u, S^p, W) = \frac{[E]}{[E_T]} = \frac{1}{Z_E} = \frac{1}{1 + \alpha_{ES}([S^p] + [S^u])} \tag{128}$$
$$p(ES^u|S^u, S^p, W) = \frac{[ES^u]}{[E_T]} = \frac{e^{-\beta(\Delta\epsilon_{ES} - k_BT\log[S^u])}}{Z_E} = \frac{\alpha_{ES}[S^u]}{1 + \alpha_{ES}([S^p] + [S^u])} \tag{129}$$
$$p(ES^p|S^u, S^p, W) = \frac{[ES^p]}{[E_T]} = \frac{e^{-\beta(\Delta\epsilon_{ES} - k_BT\log[S^p])}}{Z_E} = \frac{\alpha_{ES}[S^p]}{1 + \alpha_{ES}([S^p] + [S^u])} \tag{130}$$

$$p(S^u|E, W) = \frac{[S^u]}{[S_T^u]} = \frac{1}{Z_{S^u}} = \frac{1}{1 + \alpha_{WS}[W] + \alpha_{ES}[E]} \tag{131}$$
$$p(WS^u|E, W) = \frac{[WS^u]}{[S_T^u]} = \frac{e^{-\beta(\Delta\epsilon_{WS} - k_BT\log[W])}}{Z_{S^u}} = \frac{\alpha_{WS}[W]}{1 + \alpha_{WS}[W] + \alpha_{ES}[E]} \tag{132}$$
$$p(ES^u|E, W) = \frac{[ES^u]}{[S_T^u]} = \frac{e^{-\beta(\Delta\epsilon_{ES} - k_BT\log[E])}}{Z_{S^u}} = \frac{\alpha_{ES}[E]}{1 + \alpha_{WS}[W] + \alpha_{ES}[E]} \tag{133}$$

$$p(S^p|E,W) = \frac{[S^p]}{[S_T^p]} = \frac{1}{Z_{S^p}} = \frac{1}{1+\alpha_{WS}[W]+\alpha_{ES}[E]} \tag{134}$$

$$p(WS^p|E,W) = \frac{[WS^p]}{[S_T^p]} = \frac{e^{-\beta(\Delta\epsilon_{WS}-k_BT\log[W])}}{Z_{S^p}} = \frac{\alpha_{WS}[W]}{1+\alpha_{WS}[W]+\alpha_{ES}[E]} \tag{135}$$

$$p(ES^p|E,W) = \frac{[ES^p]}{[S_T^p]} = \frac{e^{-\beta(\Delta\epsilon_{ES}-k_BT\log[E])}}{Z_{S^p}} = \frac{\alpha_{ES}[E]}{1+\alpha_{WS}[W]+\alpha_{ES}[E]} \tag{136}$$

textbfReactions not in Detailed Balance:

$$\frac{\mathrm{d}[S_T^p]}{\mathrm{d}t} = k_{WS}^p[WS^u] - k_{E_SS}^u[ES^p] + k_S^p[S_T^u] - k_S^u[S_T^p] \tag{137}$$

$$\frac{\mathrm{d}[W_T^p]}{\mathrm{d}t} = k_{RW}^p[RW^u] - k_{E_WW}^u[E_WW^p] + k_W^p[W_T^u] - k_W^u[w_T^p] \tag{138}$$

[1] A. Goldbeter and D. E. Koshland, Proc. Natl. Acad. Sci. **78**, 6840 (1981).