Two Step Amplifier System

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(Dated: October 29, 2020)

Abstract

These are notes on modeling the stop step amplifier system

Let us start with the basic set-up. We have a receptor R which here we will assume refers to the dimerized, active form of the receptor. This receptor can bind and phosphorylate a writer (i.e. kinase) W. The kinase K can also bind an eraser (i.e phosphatase) E. Importantly, the R and P cannot be bound to the kinase at the same time. When the K is phosphorylated, it can bind a substrate S and phosphorylate S. The substrate can also bind a second eraser E_2.

I. KINASE EQUATIONS

Let us start at viewing this from the view point of the phosphorylated kinase $W - P$. This can be found in one fo the following configurations

- $[W - P]_f$ – concentration of unbound phophorylated kinase
- $[WR{-}P]$ – concentration of phophorylated kinase bound to active (dimerized) receptor
- $[WE - P]$ – concentration of phophorylated kinase bound to eraser
- $[WS - P]$ – concentration of phophorylated kinase bound to substrate

We can also consider the states of the unphorphorylated kinase (note this cannot bind substrate as is SH3 domain)

- $[W]_f$ – concentration of unbound phophorylated kinase
- $[WR]$ – concentration of phophorylated kinase bound to active (dimerized) receptor
- $[WE]$ – concentration of phophorylated kinase bound to active (dimerized) receptor

Since all the interactions in each of the lists is thermodynamic, we can use thermodynamic models to write separate partition functions for each of these. Let us denote the binding energies of the receptor to writer, phosphatase/substrate as $\Delta\epsilon_{WR}$, $\Delta\epsilon_{WE}$, $\Delta\epsilon_{WS}$. We then can write the probabilities of being in each of these states as (where the subscript f depicts the concentration of free, unbound species)

$$
\begin{aligned}
p^P_{[W-P]_f} &= \frac{1}{Z_{WP}} && (1)\\
p^P_{[WR-P]} &= \frac{e^{-\beta(\Delta\epsilon_{WR}-k_BT\log[R]_f}}{Z_{WP}} \equiv \frac{\frac{[R]_f}{\alpha_{WR}}}{Z_{WP}} && (2)\\
p^P_{[WE-P]} &= \frac{e^{-\beta(\Delta\epsilon_{WE}-k_BT\log[E]_f)}}{Z_{WP}} \equiv \frac{\frac{[E]_f}{\alpha_{WE}}}{Z_{WP}} && (3)\\
p^P_{[SE-P]} &= \frac{e^{-\beta(\Delta\epsilon_{SE}-k_BT\log[S]_f)}}{Z_{WP}} \equiv \frac{\frac{[S]_f}{\alpha_{WE}}}{Z_{WP}} && (4)\\
Z_{WP} &= 1 + \frac{[R]_f}{\alpha_{WR}} + \frac{[E]_f}{\alpha_{WE}} + \frac{[S]_f}{\alpha_{WE}} && (5)
\end{aligned}
$$

Similarly, we can do this for unphosphorylated stuff and since binding energies (except to the substrate) don't depend on the phosphorylation state we have

$$
\begin{aligned}
p_{[W]_f} &= \frac{1}{Z_W} && (6)\\
p_{[WR]} &= \frac{e^{-\beta(\Delta\epsilon_{WR}-k_BT\log[R]_f}}{Z} \equiv \frac{\frac{[R]_f}{\alpha_{WR}}}{Z_W} && (7)\\
p_{[WE]} &= \frac{e^{-\beta(\Delta\epsilon_{WE}-k_BT\log[E]_f)}}{Z} \equiv \frac{\frac{[E]_f}{\alpha_{WE}}}{Z_W} && (8)\\
Z_W &= 1 + \frac{[R]_f}{\alpha_{WR}} + \frac{[E]_f}{\alpha_{WE}} && (9)
\end{aligned}
$$

So now let us denote the total amount of writer (all, phosphorylated, and unphosphorylated) by $[W_{tot}]$, $[W-P]$, and $[W]$ respectively. Then, we know by definition we have that

$$[W_{tot}] = [W-P] + [W]. \tag{10}$$

Furthermore, the equations governing kinetics is just given by

$$
\begin{aligned}
\frac{d[W-P]}{dt} &= k^a_R[W]p_{[WR]} + k^a_{bg}[W] - k^p_E[W-P]p^P_{[WE-P]} - k^p_{bg}[W-P] && (11)\\
&= k^a_R([W_{tot}]-[W-P])p_{[WR]} + k^a_{bg}([W_{tot}]-[W-P]) - k^p_E[W-P]p^P_{[WE-P]} - k^p_{bg}[W-P] && (12)
\end{aligned}
$$

At steady-state this gives

$$\frac{[W-P]}{[W_{tot}]} = \frac{v^a_R p_{[WR]} + v^a_{bg}}{v^a_R p_{[WR]} + v^a_{bg} + v^p_E p^P_{[WE-P]} + 1} \tag{13}$$

where we have defined the velocities $v_R^a = k_R^a/k_{bg}^p$, $v_{bg}^a = k_{bg}^a/k_{bg}^p$,$v_E^p = k_E^p/k_{bg}^p$. This also gives combining equations above

$$\frac{[W]}{[W_{tot}]} = 1 - \frac{[W-P]}{[W_{tot}]} = \frac{v_E^p p_{[WE-P]}^P + 1}{v_R^a p_{[WR]} + v_{bg}^a + v_E^p p_{[WE-P]}^P + 1} \tag{14}$$

Note that now we can use the probabilities above in terms of the free/unbound concentration of the receptor $[R_f]$, the free/unbound concentration of the eraser $[E_f]$, and free substrate concentration, and the total amount of kinase, $[W_{tot}]$.

II. RECEPTOR EQUATIONS

Let us start with treating the receptor. To do this, we will just use the fact that dimerized receptor can be in three states, bound to the phosphorylated kinase, bound to unphosphorylated kinase. So we have the law

$$[R_{tot}] = [R_f] + [WR] + [WR-P] \tag{15}$$

Furthermore, we can write the reader in three states and once again write the probability of these

$$q_{[R]_f} = \frac{1}{Z_R} \tag{16}$$

$$q_{[WR-P]} = \frac{e^{-\beta(\Delta\epsilon_{WR} - k_B T \log [W-P]_f)}}{Z_R} = \frac{\frac{[W-P]_f}{\alpha_{WR}}}{Z_R} = \frac{p_{[W-P]_f^P} \frac{[W-P]}{\alpha_{WR}}}{Z_R} \tag{17}$$

$$q_{[WR]} = \frac{e^{-\beta(\Delta\epsilon_{WR} - k_B T \log [W]_f)}}{Z_R} = \frac{\frac{[W]_f}{\alpha_{WR}}}{Z_R} = \frac{p_{[W]_f} \frac{[W]}{\alpha_{WR}}}{Z_R} \tag{18}$$

$$Z_R = 1 + \frac{[W]_f}{\alpha_{WR}} + \frac{[W-P]_f}{\alpha_{WR}} = 1 + p_{[W-P]_f}^P \frac{[W-P]}{\alpha_{WR}} + p_{[W]_f} \frac{[W]}{\alpha_{WR}} \tag{19}$$

This of course gives us the equation

$$\frac{[R_f]}{[R_{tot}]} = q_{[R]_f} = \frac{1}{1 + p_{[W]_f} \frac{[W]}{\alpha_{WR}} + p_{[W-P]_f}^P \frac{[W-P]}{\alpha_{WR}}} \tag{20}$$

III. PHOSPHATASE EQUATION

We can also write the phosphatase equations which are essentially identical. We have

$$[E_{tot}] = [E_f] + [WE] + [WE-P] \tag{21}$$

Furthermore, we can write the reader in three states and once again write the probability of these

$$h_{[E]_f} = \frac{1}{Z_E} \tag{22}$$

$$h_{[WE-P]} = \frac{e^{-\beta(\Delta\epsilon_{WE} - k_B T \log{[W-P]_f})}}{Z_E} = \frac{\frac{[W-P]_f}{\alpha_{WE}}}{Z_E} = \frac{p_{[W-P]_f^P}\frac{[W-P]}{\alpha_{WE}}}{Z_E} \tag{23}$$

$$h_{[WE]} = \frac{e^{-\beta(\Delta\epsilon_{WE} - k_B T \log{[W]_f})}}{Z_E} = \frac{\frac{[W]_f}{\alpha_{WE}}}{Z_E} = \frac{p_{[W]_f}\frac{[W]}{\alpha_{WE}}}{Z_R} \tag{24}$$

$$Z_R = 1 + \frac{[W]_f}{\alpha_{WE}} + \frac{[W-P]_f}{\alpha_{WE}} = 1 + p^P_{[W-P]_f}\frac{[W-P]}{\alpha_{WE}} + p_{[W]_f}\frac{[W]}{\alpha_{WE}} \tag{25}$$

This of course gives us the equation

$$\frac{[E_f]}{[E_{tot}]} = h_{[E]_f} = \frac{1}{1 + p_{[W]_f}\frac{[W]}{\alpha_{WE}} + p^P_{[W-P]_f}\frac{[W-P]}{\alpha_{WE}}} \tag{26}$$

IV. SUBSTRATE EQUATION

We now consider the substrate $[S]$ (we will not distinguish between phosphorylated or unphosphorylated substrate here).

- $[S]_f$ – concentration of unbound/free substrate
- $[WS-P]$ – concentration of substrate bound to phosphorylated writer
- $[SE_2]$ – concentration of substrate bound to it's own eraser/phosphatase (E_2).

As before we have that total number is conserved

$$[S_{tot}] = [S]_f + [WS-P] + [SE_2 - P] \tag{27}$$

Therefore, we can write usual partition function

$$l_{[S]_f} = \frac{1}{Z_S} \tag{28}$$

$$l_{[WS-P]} = \frac{e^{-\beta(\Delta\epsilon_{WS} - k_B T \log{[W-P]_f})}}{Z_S} = \frac{\frac{[W-P]_f}{\alpha_{WS}}}{Z_S} = \frac{p_{[W-P]_f^P}\frac{[W-P]}{\alpha_{WS}}}{Z_S} \tag{29}$$

$$l_{[SE2]} = \frac{e^{-\beta(\Delta\epsilon_{SE_2} - k_B T \log{[E2]_f})}}{Z_S} = \frac{\frac{[E2]_f}{\alpha_{SE2}}}{Z_S} = \frac{d_{[SE2]}\frac{[E2_{tot}]}{\alpha_{SE2}}}{Z_s} \tag{30}$$

$$Z_S = 1 + \frac{[W-P]_f}{\alpha_{WE}} + \frac{[E2]_f}{\alpha_{SE2}} \tag{31}$$

Now we just need the usual kinetic equation

$$\frac{d[S-P]}{dt} = k^a_W[W]l_{[WS-P]} + k^a_{bg}[S] - k^p_{E2}[S-P]l_{[SE2]} - k^p_{bg}[S-P] \tag{32}$$

$$= k^a_W([S_{tot}] - [S-P])l_{[WS-P]} + k^a_{bg}([S_{tot}] - [S-P]) - k^p_{E2}[S-P]l_{[SE2]} - k^p_{bg}[S-P] \tag{33}$$

Setting this to zero gives ratio of phosphorylated substrate

$$\frac{[S-P]}{[S_{tot}]} = \frac{v^a_W l_{[WS-P]} + v^a_{bg}}{v^a_W l_{[WS-P]} + v^p_{E2} l_{[SE2]} + +v^a_{bg} + 1} \tag{34}$$

and unphosphorylated substrate

$$\frac{[S]}{[S_{tot}]} = \frac{v^p_{E2} l_{[WS-P]} + 1}{v^a_W l_{[WS-P]} + v^p_{E2} l_{[SE2]} + +v^a_{bg} + 1} \tag{35}$$

V. SECOND PHOSPHATASE/ERASER

The final equation we will need is the final phosphatase equation $[E2]$. This will essentially be the same derivation as above

$$[E2_{tot}] = [E2_f] + [SE2] \tag{36}$$

Furthermore, we can write the reader in three states and once again write the probability of these

$$d_{[E2]_f} = \frac{1}{Z_{E2}} \tag{37}$$

$$d_{[SE2]} = \frac{e^{-\beta(\Delta\epsilon_{SE2} - k_B T \log [S]_f)}}{Z_{E2}} = \frac{\frac{[S_f]}{\alpha_{SE2}}}{Z_{E2}} \tag{38}$$

$$Z_{E2} = 1 + \frac{[S_f]}{\alpha_{SE2}} \tag{39}$$

This of course gives us the equation

$$\frac{[E2_f]}{[E2_{tot}]} = d_{[E2]_f} = \frac{1}{1 + \frac{[S_f]}{\alpha_{SE2}}} \tag{40}$$