

## Virtual Phosphorylation Site On-Rate Multipliers

Consider the scheme in Figure A1, where a receptor with two sites can be either fully phosphorylated (denoted (1,1)), fully unphosphorylated (denoted (0,0)), bound on the first site (denoted (2,1)) or bound on the second site (denoted (1,2)), and binding is fully competitive such that the (2,2) state does not exist. Assuming mass action kinetics, the differential equations governing the bound receptor dynamics are shown in Equations 1 and 2 below,

$$\frac{d(2,1)}{dt} = k_{f1}(1,1)[B_1] - k_{r1}(2,1) , \text{ and} \quad (1)$$

$$\frac{d(1,2)}{dt} = k_{f2}(1,1)[B_2] - k_{r2}(1,2) , \quad (2)$$

where  $k_{f1}$ ,  $k_{f2}$ ,  $k_{r1}$ , and  $k_{r2}$  are rate constants that describe the kinetics of binding, and  $B_1$  and  $B_2$  are proteins that bind to site 1 and 2, respectively. Consider now that  $B_1$  and  $B_2$  are the same binder (B), and that the rate constants of binding to the two sites are the same ( $k_{f1}=k_{f2}=k_f$ ;  $k_{r1}=k_{r2}=k_r$ ). By adding Equations 1 and 2, the differential equation of this lumped, experimentally observable state is

$$\begin{aligned} \frac{d(2,1)}{dt} + \frac{d(1,2)}{dt} &\equiv \frac{d(2_B)}{dt} = k_{f1}(1,1)[B] + k_{f2}(1,1)[B] - k_{r1}(2,1) - k_{r2}(1,2) . \\ &= 2 * k_f(1,1)[B] - k_r(2_B) \end{aligned}$$

Thus, under the above assumptions, when a protein can bind to two separate sites on a receptor (or scaffold), the apparent on-rate of the binder for the receptor is multiplied by two. In general, if a protein can bind  $n$  separate sites, then the on-rate is multiplied by  $n$ . It is important to note this implies nothing about free energy or binding energetics; this is strictly a kinetic phenomenon that results from the model reduction assumptions.

**Figure S1. Two-site phosphorylation and binding schematic.** The ordered pair corresponds to the state of sites; 0-unphosphorylated; 1-phosphorylated; 2-bound.  $B_1$  and  $B_2$  are binding proteins for site 1 and 2, respectively.

