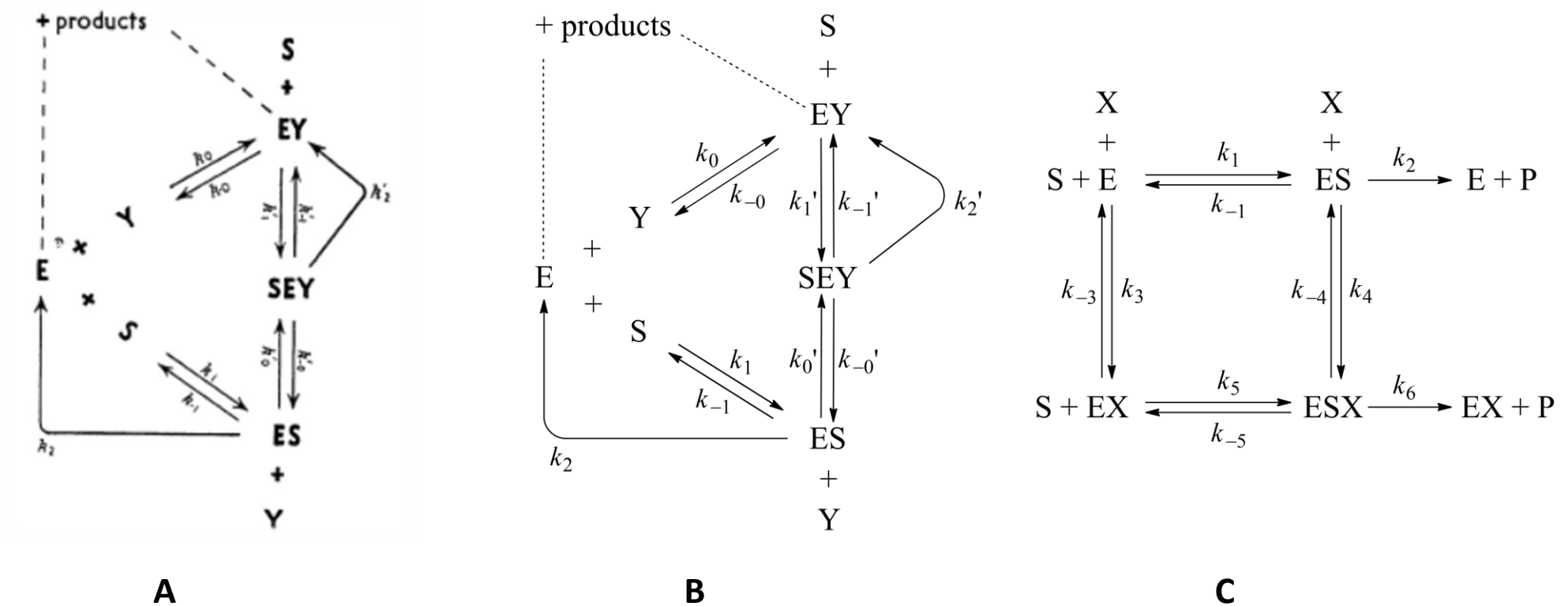


Reaction scheme and rate equation of the general modifier mechanism by Botts and Morales



Scheme 1. Reaction paths of the general modifier mechanism. **A**, captured from the original of Botts and Morales [1]. **B**, the scheme in A redrawn for more clarity. **C**, reaction scheme using the symbols in [2]. In A and B, Y denotes the modifier (the same as X in the variant C); S = substrate, E = enzyme, P = Product.

Rate equation captured from the original paper [1]

$$v = \frac{\left[k_2 \bar{K}_1 [S] + k_2' K_0' \bar{K}_1 [S] [Y] + \frac{k_{-0} k'_{-1} (r - r') \{k_2 k'_{-0} + k_2' \{k_0' [Y] + k_{-1} (1 + r)\}\} K_0' \bar{K}_1 [S] [Y]}{k_{-0} k'_{-1} (1 + r') \{k_0' [Y] + k_{-1} (1 + r)\} + k_{-0}' k_{-1} (1 + r) \{k_1' [S] + k_{-0}\}} \right] [E_0]}{1 + K_0 [Y] + \bar{K}_1 [S] + K_0' \bar{K}_1 [S] [Y] + \frac{k'_{-1} (r - r') \{(k_{-0} - k'_{-0}) k_{-1} (1 + r) + k_{-0} k'_{-0} (1 + K_0' [Y])\} K_0' \bar{K}_1 [S] [Y]}{k_{-0} k'_{-1} (1 + r') \{k_0' [Y] + k_{-1} (1 + r)\} + k'_{-0} k_{-1} (1 + r) \{k_1' [S] + k_{-0}\}}},$$

Original rate equation rewritten for more clarity

$$v = \frac{\left[k_2 \bar{K}_1 [S] + k_2' K_0' \bar{K}_1 [S] [Y] + \frac{k_{-0} k'_{-1} (r - r') \{k_2 k'_{-0} + k_2' \{k_0' [Y] + k_{-1} (1 + r)\}\} K_0' \bar{K}_1 [S] [Y]}{k_{-0} k'_{-1} (1 + r') \{k_0' [Y] + k_{-1} (1 + r)\} + k'_{-0} k_{-1} (1 + r) \{k_1' [S] + k_{-0}\}} \right] [E_0]}{1 + K_0 [Y] + \bar{K}_1 [S] + K_0' \bar{K}_1 [S] [Y] + \frac{k'_{-1} (r - r') \{(k_{-0} - k'_{-0}) k_{-1} (1 + r) + k_{-0} k'_{-0} (1 + K_0' [Y])\} K_0' \bar{K}_1 [S] [Y]}{k_{-0} k'_{-1} (1 + r') \{k_0' [Y] + k_{-1} (1 + r)\} + k'_{-0} k_{-1} (1 + r) \{k_1' [S] + k_{-0}\}}}$$

Rate equation using the symbols in Scheme 1C, [2, Chapter 2]

$$v = \frac{\left\{ \frac{k_2[S]}{K_m^0} + \frac{k_6[S][X]}{K_m^0 K_4} + \frac{k_{-3}k_{-5} \left(\frac{k_2}{k_{-1}} - \frac{k_6}{k_{-5}} \right) \left\{ k_2k_{-4} + k_6 \left[k_4[X] + k_{-1} \left(1 + \frac{k_2}{k_{-1}} \right) \right] \right\} \frac{[S][X]}{K_m^0 K_4}}{k_{-3}k_{-5} \left(1 + \frac{k_6}{k_{-5}} \right) \left[k_4[X] + k_{-1} \left(1 + \frac{k_2}{k_{-1}} \right) \right] + k_{-1}k_{-4} \left(1 + \frac{k_2}{k_{-1}} \right) (k_5[S] + k_{-3})} \right\} [E]_t}{1 + \frac{[X]}{K_3} + \frac{[S]}{K_m^0} + \frac{[S][X]}{K_m^0 K_4} + \frac{k_{-5} \left(\frac{k_2}{k_{-1}} - \frac{k_6}{k_{-5}} \right) \left\{ (k_{-3} - k_{-4})k_{-1} \left(1 + \frac{k_2}{k_{-1}} \right) + k_{-3}k_{-4} \left(1 + \frac{[X]}{K_4} \right) \right\} \frac{[S][X]}{K_m^0 K_4}}{k_{-3}k_{-5} \left(1 + \frac{k_6}{k_{-5}} \right) \left[k_4[X] + k_{-1} \left(1 + \frac{k_2}{k_{-1}} \right) \right] + k_{-1}k_{-4} \left(1 + \frac{k_2}{k_{-1}} \right) (k_5[S] + k_{-3})}}$$

Here we can appreciate the strategy of Botts and Morales in constructing the rate equation for their general modifier mechanism. First step: write the equation under the assumption that all species are in equilibrium. These are the terms in red that represent the condition of generalized microscopic reversibility, i.e. $k_2/k_{-1} = k_6/k_{-5}$. Second step: add perturbation terms, in blue, when the system operates at steady-state and equilibrium does not prevail. If $k_2/k_{-1} = k_6/k_{-5}$, all terms in blue become zero (verify yourself) reducing the equation to equilibrium conditions (red). In the same manner, the assumption of quasi equilibrium can be derived by simplification of the complete equation. Full details are given in [2, Chapter 2].

- [1] Botts J, Morales M (1953) Analytical description of the effects of modifiers and of enzyme multivalency upon the steady state catalyzed reaction rate. *Trans Faraday Soc* 49: 696-707.
- [2] Baici A (2015) *Kinetics of Enzyme-Modifier Interactions - Selected Topics in the Theory and Diagnosis of Inhibition and Activation Mechanisms*. Springer, Vienna