



# Exercise 1: Solution

Bound enzyme species:

$$K_s = \frac{e \cdot s}{es}; \therefore es = \frac{e \cdot s}{K_s} \quad K_p = \frac{e \cdot p}{ep}; \therefore ep = \frac{e \cdot p}{K_p}$$

$$K_i = \frac{es \cdot i}{esi}; \therefore esi = \frac{es \cdot i}{K_i} = \frac{e \cdot s \cdot i}{K_s K_i}$$

Fractional saturation:

$$Y_{ES} = \frac{es}{e_T} = \frac{es}{e + es + ep + esi} = \frac{\frac{s}{K_s}}{1 + \frac{s}{K_s} + \frac{p}{K_p} + \frac{s \cdot i}{K_s K_i}}$$

$$Y_{EP} = \frac{ep}{e_T} = \frac{ep}{e + es + ep + esi} = \frac{\frac{p}{K_p}}{1 + \frac{s}{K_s} + \frac{p}{K_p} + \frac{s \cdot i}{K_s K_i}}$$

Rate equation:

$$\begin{aligned}v &= k_2 e s - k_{-2} e p = \frac{k_2 e_T \frac{s}{K_s} - k_{-2} e_T \frac{p}{K_p}}{1 + \frac{s}{K_s} + \frac{p}{K_p} + \frac{s \cdot i}{K_s K_i}} \\&= \frac{V_f \frac{s}{K_s} - V_r \frac{p}{K_p}}{1 + \frac{s}{K_s} \left(1 + \frac{i}{K_i}\right) + \frac{p}{K_p}} \\&= \frac{V_f \frac{s}{K_s} \left(1 - \frac{\Gamma}{K_{eq}}\right)}{1 + \frac{s}{K_s} \left(1 + \frac{i}{K_i}\right) + \frac{p}{K_p}}\end{aligned}$$

# Exercise 2

$$v = \frac{V_{mf} \cdot \frac{s}{K_S} \left(1 - \frac{\Gamma}{K_{eq}}\right) \left(\frac{s}{K_S} + \frac{p}{K_P}\right)^{h-1}}{\left(\frac{s}{K_S} + \frac{p}{K_P}\right)^h + \frac{1 + (x/K_x)^h}{1 + \alpha(x/K_x)^h}}$$

Reduce the above equation by substituting:  $\alpha=1$ ,  $h=1$ ,  $p=0$ .

$$v = \frac{V_{mf} \cdot \frac{s}{K_S}}{1 + \frac{s}{K_S}} = \frac{V_{mf} \cdot s}{K_S + s}$$

Reduces to the irreversible Michaelis-Menten equation!!

# Exercise 3: Analysis of experimental data

For an enzyme catalyzed, chemical reaction,  
 $A \leftrightarrow B$ , the following initial rates were obtained  
( $a$  and  $b$  given at  $t = 0$ ):

$v$	$a$	$b$
(mM/s)	(mM)	(mM)
9.9	10	0
7.86	10	5
9.8	5	0
6.01	10	10
-30.3	0	10
-32.68	0	50

Assume a random order mechanism and rapid equilibrium binding of substrate and product.

Calculate:

- $V_{mf}$
- $V_{mr}$
- $K_a$
- $K_b$
- $K_{eq}$

# The calculations shown here are examples; there are several correct ways to come to the answer.

Random order mechanism, rapid equilibrium binding; the reversible MM equation can be used:

$$v = \frac{V_m \cdot \frac{a}{K_a} \cdot \left(1 - \frac{\Gamma}{K_{eq}}\right)}{1 + \frac{a}{K_a} + \frac{b}{K_b}}$$

## $V_{max,f}$ and $K_a$

Both parameters can be calculated from the 2 incubations without b added at  $t=0$ . In absence of b the above equation reduces to the irreversible MM equation. Using the irreversible MM equations the 2 incubations lead to 2 equations with 2 unknowns:

$$v = \frac{V_m \cdot \frac{a}{K_a}}{1 + \frac{a}{K_a}}; 9.9 = \frac{V_m \cdot \frac{10}{K_a}}{1 + \frac{10}{K_a}}; 9.8 = \frac{V_m \cdot \frac{5}{K_a}}{1 + \frac{5}{K_a}}$$

Which can be solved (e.g. express  $V_m$  in terms of  $K_a$  using the first equation and substitute this expression in the second equation):  $V_{max,f}=10$  mM/s,  $K_a=0.1$  mM

**$V_{max,r}$  and  $K_b$**  Same as for  $V_{max,f}$  and  $K_a$  but now using the 2 incubations without a:

$$v = -\frac{V_{mr} \cdot \frac{b}{K_b}}{1 + \frac{b}{K_b}}; -30.30 = -\frac{V_{mr} \cdot \frac{10}{K_b}}{1 + \frac{10}{K_b}}; -32.68 = -\frac{V_{mr} \cdot \frac{50}{K_b}}{1 + \frac{50}{K_b}}$$

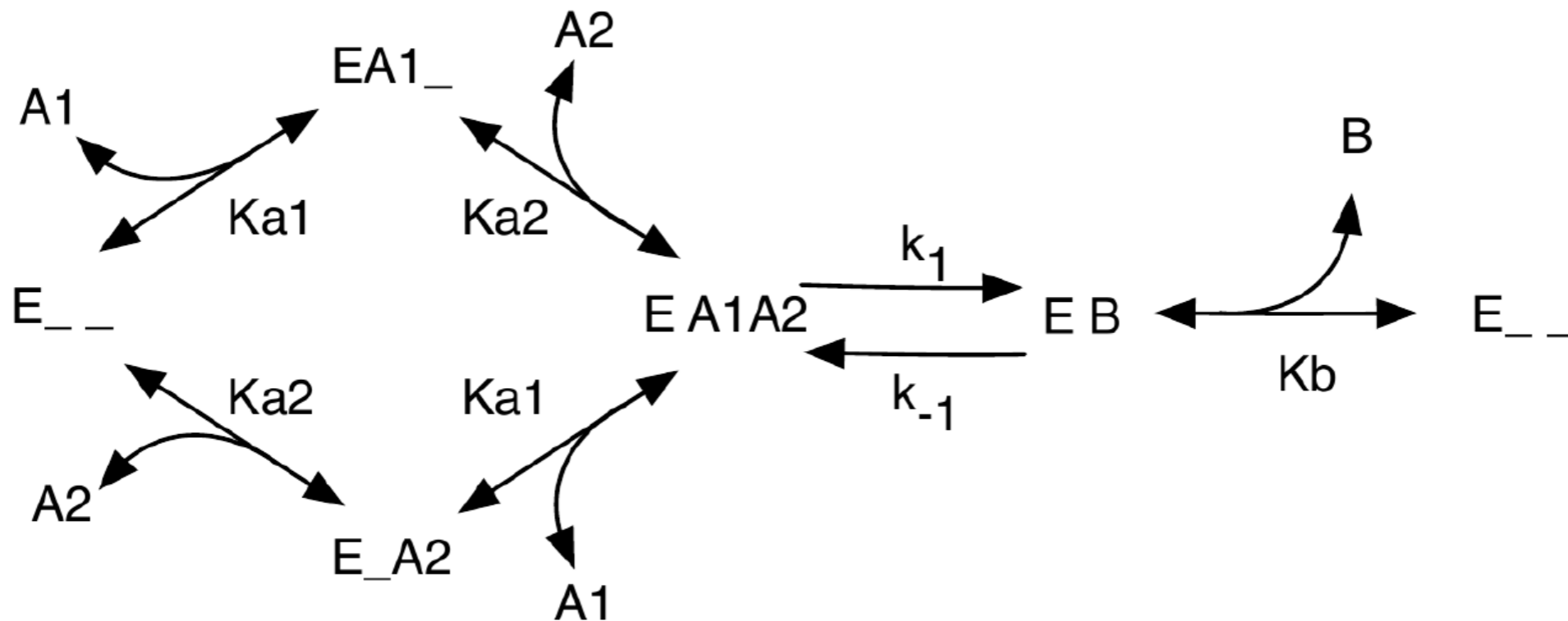
$V_{max,r}=33.33$  mM/s,  $K_b=1$  mM

## Haldane relation

$$K_{eq} = \frac{V_{mf} \cdot K_b}{V_{mr} \cdot K_a}; 3 = \frac{10 \cdot 1}{33.33 \cdot 0.1}$$

# Exercise 4

Derive a rate equation for the following reversible, two-substrate, one-product, random order mechanism, using the rapid equilibrium binding assumption.



# Exercise 4: Solution

Bound enzyme species:

$$K_{a1} = \frac{e \cdot a1}{ea1}; \therefore ea1 = \frac{e \cdot a1}{K_{a1}} \quad K_{a2} = \frac{e \cdot a2}{ea2}; \therefore ea2 = \frac{e \cdot a2}{K_{a2}}$$

$$K_{a1} = \frac{ea2 \cdot a1}{ea1a2}; \therefore ea1a2 = \frac{ea2 \cdot a1}{K_{a1}} = \frac{e \cdot a1 \cdot a2}{K_{a1}K_{a2}} \quad K_b = \frac{e \cdot b}{eb}; \therefore eb = \frac{e \cdot b}{K_b}$$

Fractional saturation:

$$Y_{EA1} = \frac{ea1}{e_T} = \frac{ea1}{e + ea1 + ea2 + ea1a2 + eb} = \frac{\frac{a1}{K_{a1}}}{1 + \frac{a1}{K_{a1}} + \frac{a2}{K_{a2}} + \frac{a1 \cdot a2}{K_{a1}K_{a2}} + \frac{b}{K_b}}$$

Let  $D = 1 + \frac{a1}{K_{a1}} + \frac{a2}{K_{a2}} + \frac{a1 \cdot a2}{K_{a1}K_{a2}} + \frac{b}{K_b}$ ; then

$$Y_{EA2} = \frac{\frac{a2}{K_{a2}}}{D}; \quad Y_{EA1A2} = \frac{\frac{a1 \cdot a2}{K_{a1}K_{a2}}}{D}; \quad Y_{EB} = \frac{\frac{b}{K_b}}{D}$$



Rate equation:

$$\begin{aligned} v = k_1 e a_1 a_2 - k_{-1} e b &= \frac{k_1 e_T \frac{a_1 \cdot a_2}{K_{a_1 a_2}} - k_{-1} e_T \frac{b}{K_b}}{1 + \frac{a_1}{K_{a_1}} + \frac{a_2}{K_{a_2}} + \frac{a_1 \cdot a_2}{K_{a_1} K_{a_2}} + \frac{b}{K_b}} \\ &= \frac{V_f \frac{a_1 \cdot a_2}{K_{a_1 a_2}} - V_r \frac{b}{K_b}}{1 + \frac{a_1}{K_{a_1}} + \frac{a_2}{K_{a_2}} + \frac{a_1 \cdot a_2}{K_{a_1} K_{a_2}} + \frac{b}{K_b}} \\ &= \frac{V_f \frac{a_1 \cdot a_2}{K_{a_1 a_2}} \left( 1 - \frac{\Gamma}{K_{eq}} \right)}{1 + \frac{a_1}{K_{a_1}} + \frac{a_2}{K_{a_2}} + \frac{a_1 \cdot a_2}{K_{a_1} K_{a_2}} + \frac{b}{K_b}} \end{aligned}$$