Exercise 1

Given an open system consisting of two enzymes that catalyze the conversion of substrate S (fixed at 10 mM) to product P (fixed at 1 mM), with common intermediate X.

The enzymes obey reversible Michaelis-Menten kinetics with identical parameter values:
V_{mf} = 1 mM/s, K_{eq} = 10, $K_{m,substrate}$ = 1mM,
$K_{m,product}$ = 10 mM

Calculate the steady-state flux and the steady-state concentration of the intermediate X.

Exercise 1: Solution

Reaction 1: S ↔ X (S is substrate, X is product)

$$v_1 = \frac{V_f \frac{s}{K_s}\left(1 - \frac{x/s}{K_{eq1}}\right)}{1 + \frac{s}{K_s} + \frac{x}{K_x}} = \frac{1 \cdot \frac{10}{1}\left(1 - \frac{x/10}{10}\right)}{1 + \frac{10}{1} + \frac{x}{10}} = \frac{10 - \frac{x}{10}}{11 + \frac{x}{10}}$$

Reaction 2: X ↔ P (X is substrate, P is product)

$$v_2 = \frac{V_f \frac{x}{K_x}\left(1 - \frac{p/x}{K_{eq2}}\right)}{1 + \frac{x}{K_x} + \frac{p}{K_p}} = \frac{1 \cdot \frac{x}{1}\left(1 - \frac{1/x}{10}\right)}{1 + \frac{x}{1} + \frac{1}{10}} = \frac{x - \frac{1}{10}}{1.1 + x}$$

Steady state: $v_1 = v_2$

$$\frac{10 - \frac{x}{10}}{11 + \frac{x}{10}} = \frac{x - \frac{1}{10}}{1.1 + x} \qquad \therefore \left(10 - \frac{x}{10}\right)(1.1 + x) = \left(11 + \frac{x}{10}\right)\left(x - \frac{1}{10}\right)$$

$$\therefore 0.2x^2 + 1.1x - 12.1 = 0$$

Solve for x: $x = -11$ (discard negative answer) or $x = 5.5$

Steady-state conc. of x is 5.5 mM

Substitute in v_1 and v_2:

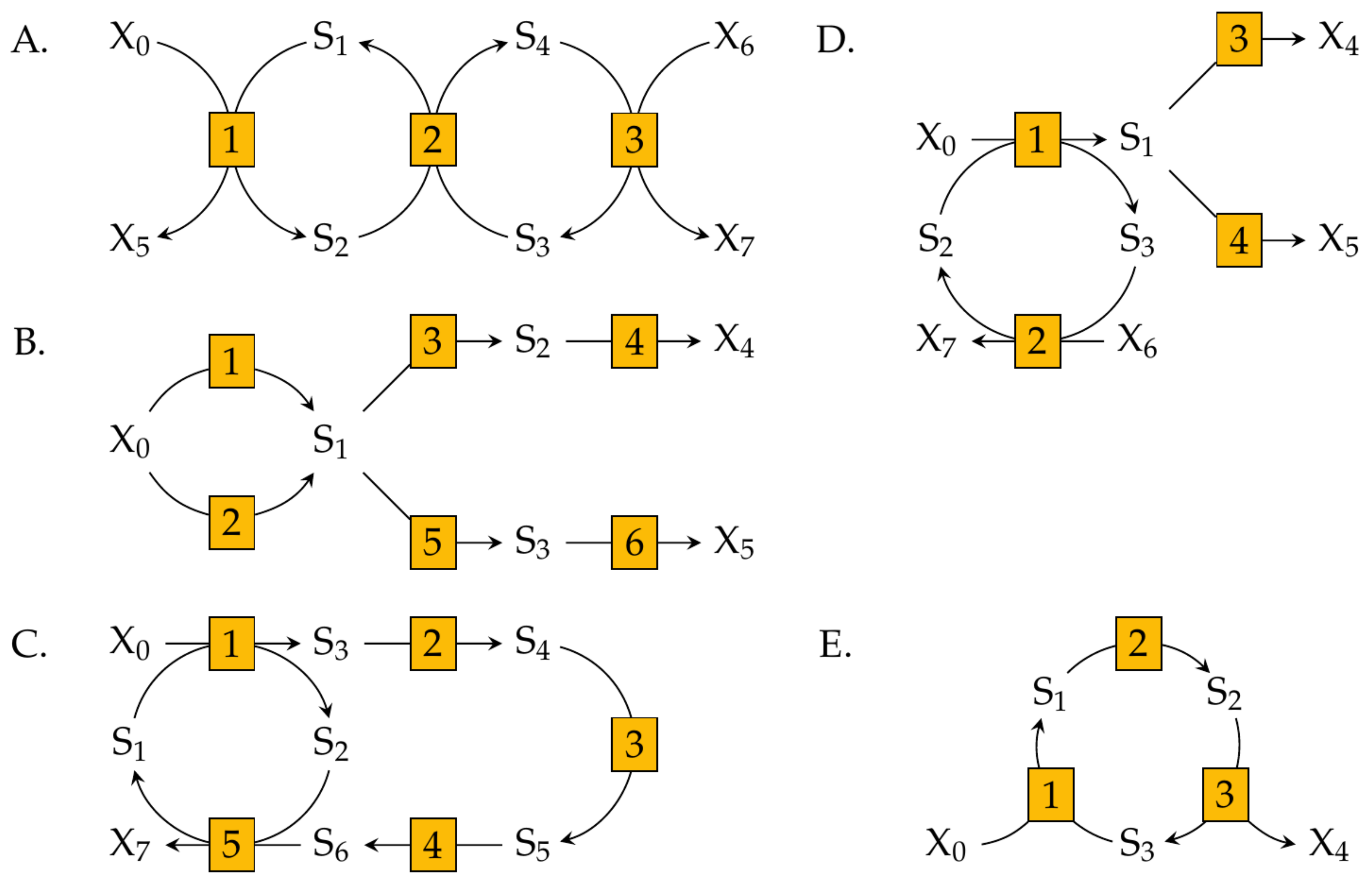
$$v_1 = \frac{10 - \frac{5.5}{10}}{11 + \frac{5.5}{10}} = 0.8182 \qquad v_2 = \frac{5.5 - \frac{1}{10}}{1.1 + 5.5} = 0.8182$$

Steady-state flux is 0.8182 mM/s

Exercise 2

For each of the following pathways, write down the

- Balance equations
- Steady-state flux relationships
- Moiety-conservation relationships (if present)



A.

- Balance equations

  $ds_1/dt = J_1 - J_2 = 0$
  $ds_2/dt = J_2 - J_1 = 0$
  $ds_3/dt = J_3 - J_2 = 0$
  $ds_4/dt = J_2 - J_3 = 0$

- Steady-state flux relationships

  $J_1 = J_2 = J_3$

- Moiety-conservation relationships (if present)

  $ds_1/dt + ds_2/dt = d/dt(s_1 + s_2) = 0$
  $s_1 + s_2 = \text{constant}$
  $ds_3/dt + ds_4/dt = d/dt(s_3 + s_4) = 0$
  $s_3 + s_4 = \text{constant}$

B.

- Balance equations

$ds_1/dt = J_1 + J_2 - J_3 - J_5 = 0$

$ds_2/dt = J_3 - J_4 = 0$

$ds_3/dt = J_5 - J_6 = 0$

- Steady-state flux relationships

$J_1 + J_2 = J_3 + J_5$

$J_3 = J_4$

$J_5 = J_6$

- Moiety-conservation relationships (if present)

None.

C.

- Balance equations

$$ds_1/dt = J_5 - J_1 = 0$$
$$ds_2/dt = J_1 - J_5 = 0$$
$$ds_3/dt = J_1 - J_2 = 0$$
$$ds_4/dt = J_2 - J_3 = 0$$
$$ds_5/dt = J_3 - J_4 = 0$$
$$ds_6/dt = J_4 - J_5 = 0$$

- Steady-state flux relationships

$$J_1 = J_2 = J_3 = J_4 = J_5$$

- Moiety-conservation relationships (if present)

$$ds_1/dt + ds_2/dt = d/dt(s_1 + s_2) = 0$$
$$s_1 + s_2 = \text{constant}$$
$$ds_1/dt + ds_3/dt + ds_4/dt + ds_5/dt + ds_6/dt$$
$$= d/dt(s_1 + s_3 + s_4 + s_5 + s_6) = 0$$
$$s_1 + s_3 + s_4 + s_5 + s_6 = \text{constant}$$

D.

- Balance equations

$ds_1/dt = J_1 - J_3 - J_4 = 0$

$ds_2/dt = J_2 - J_1 = 0$

$ds_3/dt = J_1 - J_2 = 0$

- Steady-state flux relationships

$J_1 = J_2 = J_3 + J_4$

- Moiety-conservation relationships (if present)

$ds_2/dt + ds_3/dt = d/dt(s_2 + s_3) = 0$

$s_2 + s_3 = \text{constant}$

E.

- Balance equations

$$ds_1/dt = J_1 - J_2 = 0$$
$$ds_2/dt = J_2 - J_3 = 0$$
$$ds_3/dt = J_3 - J_1 = 0$$

- Steady-state flux relationships

$$J_1 = J_2 = J_3$$

- Moiety-conservation relationships (if present)

$$ds_1/dt + ds_2/dt + ds_3/dt = d/dt(s_1 + s_2 + s_3) = 0$$
$$s_1 + s_2 + s_3 = \text{constant}$$