Multi-substrate Reactions Kinetics

•Multi-substrate reactions follow complex rate equations that describe how the substrates bind and in what sequence.

•The analysis of these reactions is much simpler if the concentration of substrate A is kept constant and substrate B varied.

•Under these conditions, the enzyme behaves just like a single-substrate enzyme and a plot of v by [S] gives apparent K_m and V_{max} constants for substrate B.

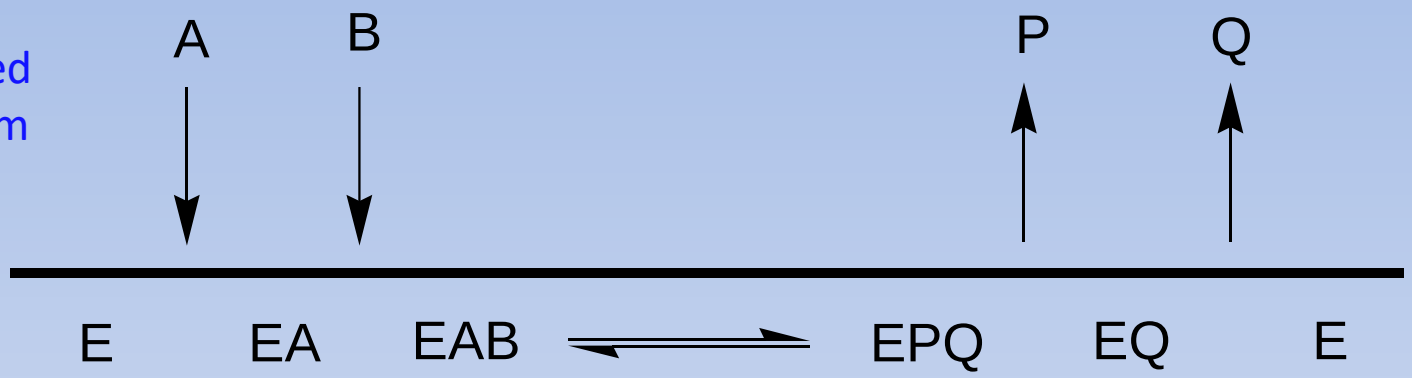
•If a set of these measurements is performed at different fixed concentrations of A, these data can be used to work out what the mechanism of the reaction is.

•For an enzyme that takes two substrates A and B and turns them into two products P and Q, there are two types of mechanism: ternary complex and ping–pong.

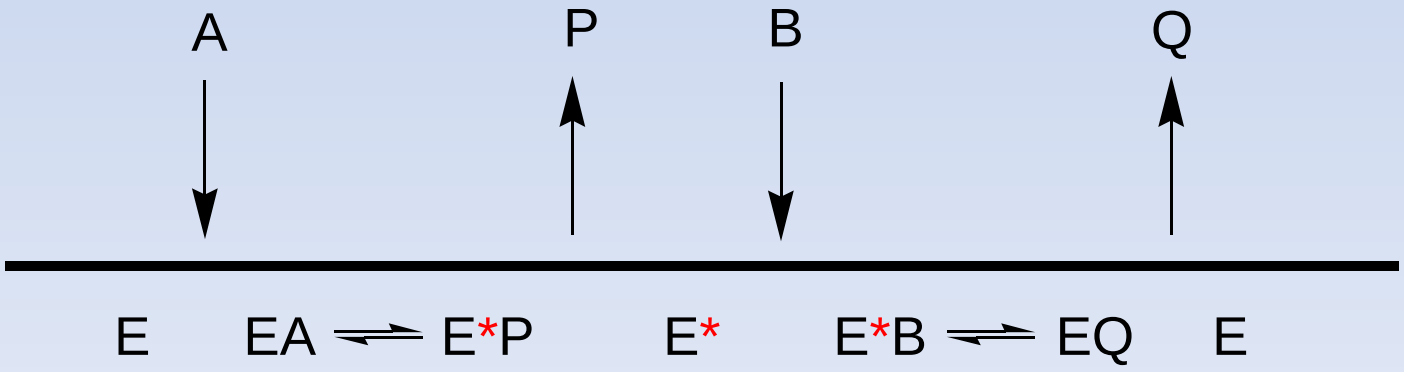
Bi-substrate Enzyme Kinetics

Sequential

1. ordered
2. random



Ping-pong



Ping–pong mechanisms:

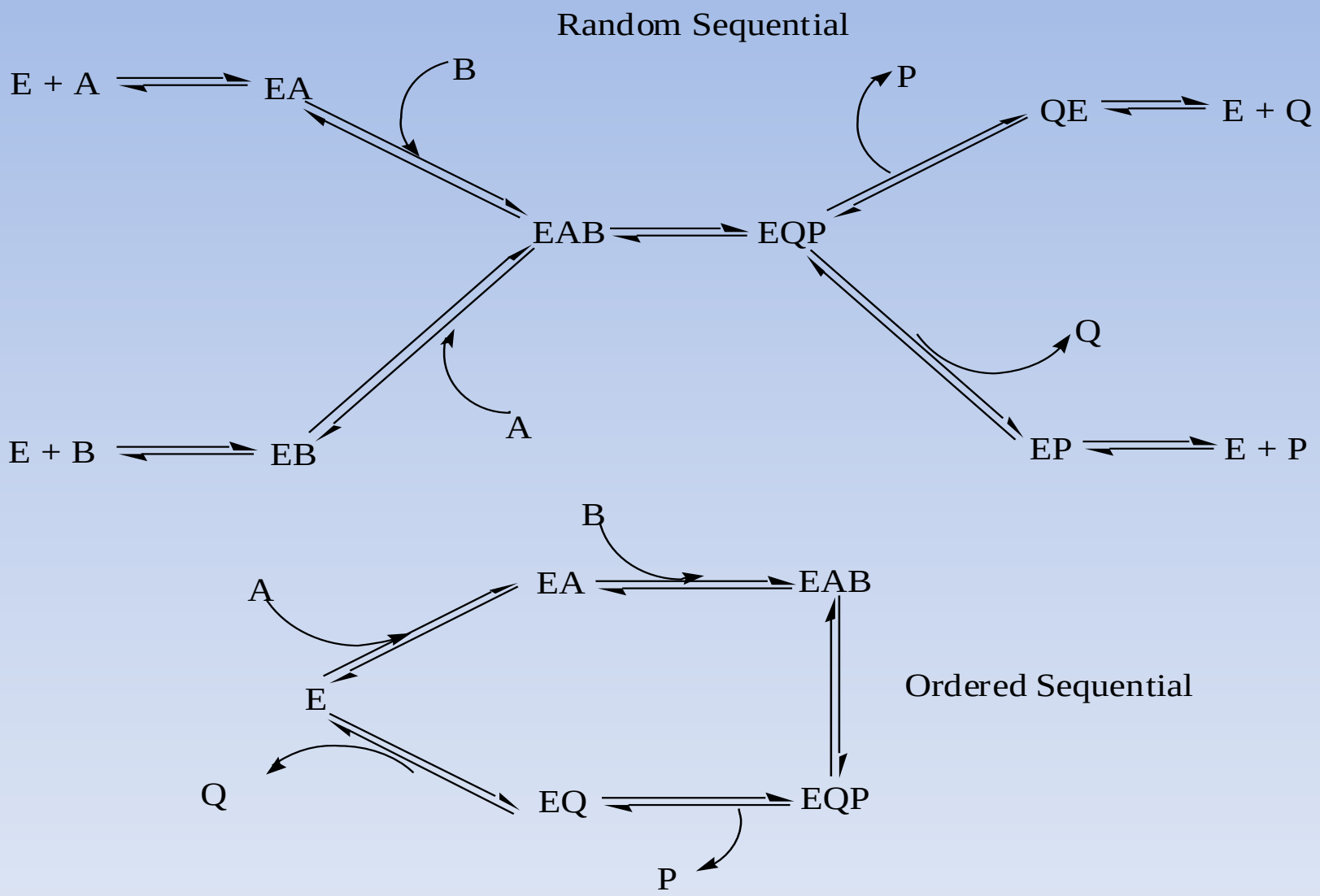
•Enzymes with a ping-pong mechanism can exist in two states, E and a chemically modified form of the enzyme E*.

•This modified enzyme is known as an intermediate.

•In such mechanisms, substrate A binds, changes the enzyme to E* by, for example, transferring a chemical group to the active site, and is then released.

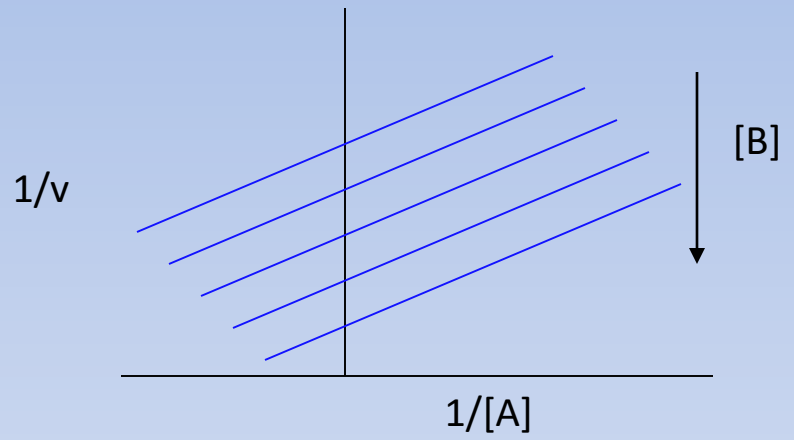
• Only after the first substrate is released can substrate B bind and react with the modified enzyme, regenerating the unmodified E form.

•When a set of *v* by [S] curves (fixed A, varying B) from an enzyme with a ping–pong mechanism are plotted in a Lineweaver–Burk plot, a set of parallel lines will be produced.

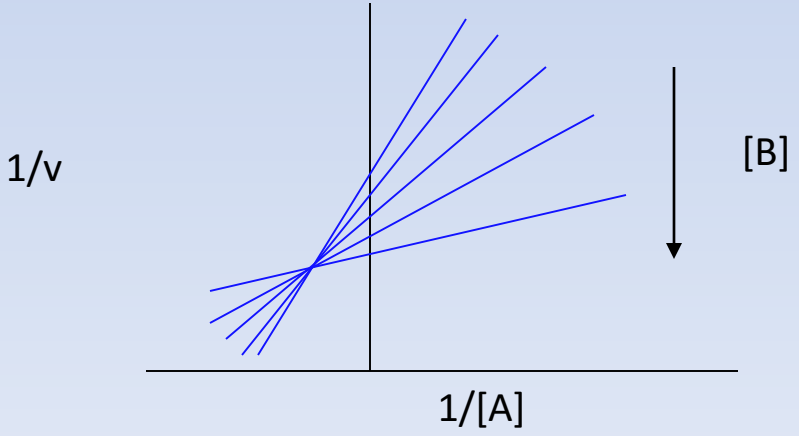

Random Sequential
E + A
EA
B
P
QE
E + Q
EAB
EQP
Q
A
E + B
EB
EP
E + P
B
EA
EAB
A
E
Ordered Sequential
Q
EQ
EQP
P

Equations for Bi-substrate Kinetics

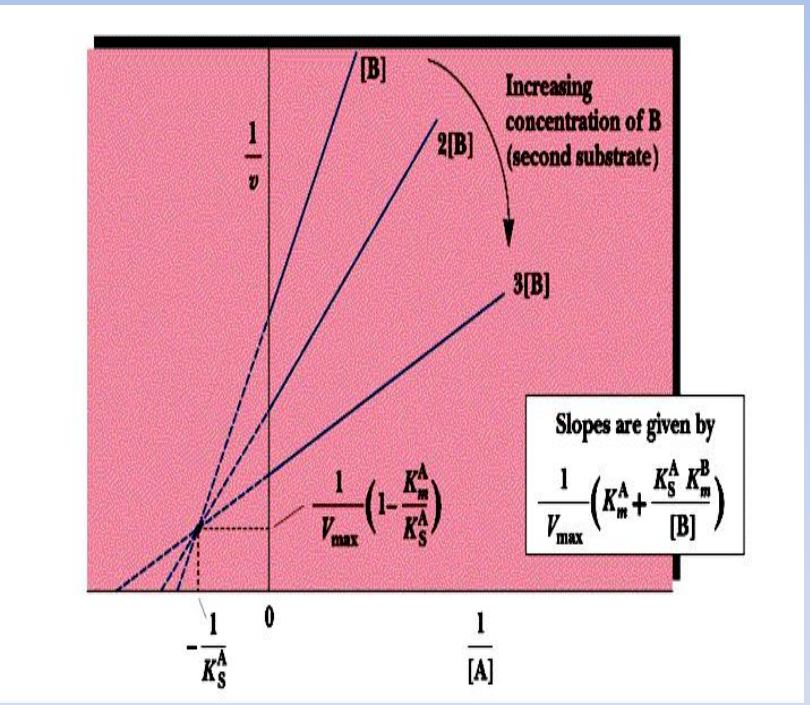
$$v = \frac{V_{max}[A][B]}{K_a[B] + K_b[A] + [A][B]}$$



$$v = \frac{V_{max}[A][B]}{[A][B] + K_a[B] + K_b[A] + K_aK_b}$$



Sequential Kinetics



- Sequential kinetics can be distinguished from ping-pong kinetics by initial rate studies.

In practice, measure initial rates as a function of the concentration of one substrate while holding the concentration of the second constant. Next, vary the concentration of the second substrate and repeat.

Lineweaver-Burk (double-reciprocal) analysis should yield a family of lines that intersect at the left of the y-axis of the graph.

Within the realm of sequential reactions lies ordered sequential and random sequential at the extreme ends. The equations for the two are identical; therefore, simple initial rate studies cannot differentiate between the two.

In ordered sequential reactions, one substrate is obligated to bind to the enzyme before a second substrate. In random sequential mechanisms there is no preference. In practice, there is usually some degree of order in binding.

•Enzymes with ping–pong mechanisms include some oxidoreductases such as thioredoxin peroxidase, transferases such as acylneuraminate cytydilyltransferase and serine proteases such as trypsin and chymotrypsin.

•Serine proteases are a very common and diverse family of enzymes, including digestive enzymes (trypsin, chymotrypsin, and elastase), several enzymes of the blood clotting cascade and many others.