- Metalloproteases → H_2O → OH^-
  - These enzymes use a metal-ion cofactor to activate water for direct attack on the substrate carbonyl
  - carboxypeptidase and thermolysin do not proceed through a stable intermediate
  - They bind a molecule of water to a metal ion, usually Zn^{2+}.
  - Binding H_2O to a transition metal ion lowers the pK_a of the bound water,
  - making it a better acid. It is thus much easier to turn metal-bound H_2O into OH^-
  - The metal-bound OH^- can attack the amide bond of a peptide substrate directly, hydrolyzing it without the need for a covalent enzyme intermediate.

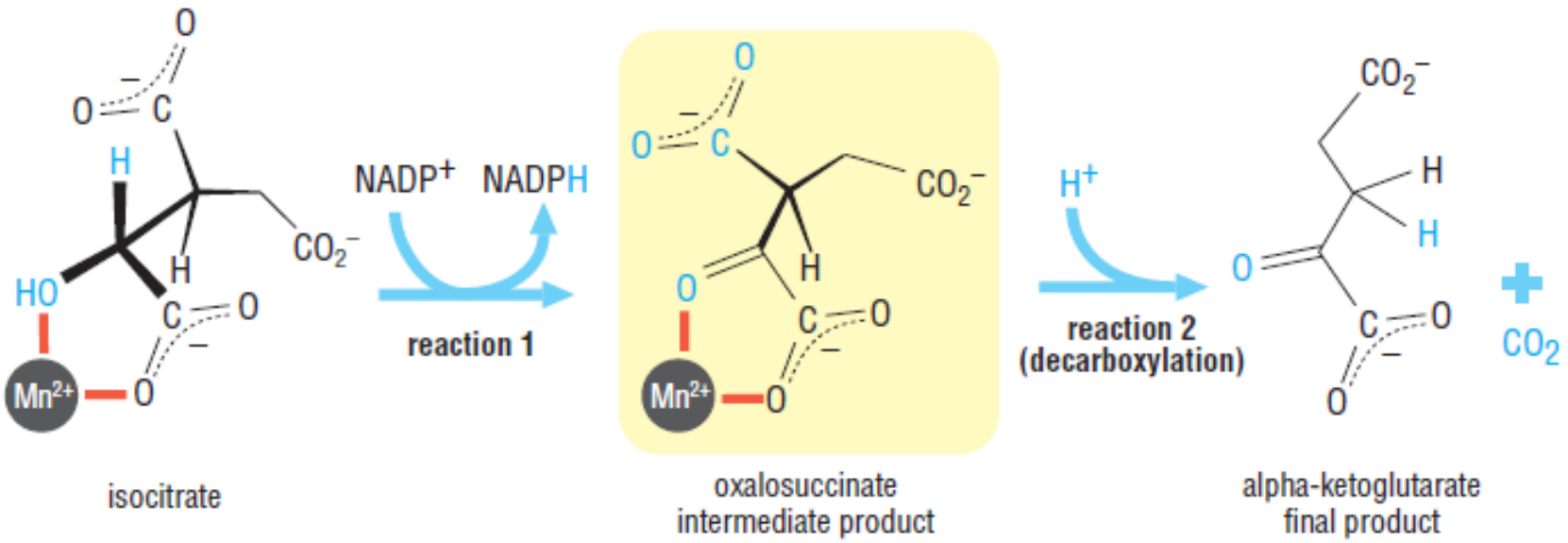
PHOSPHORYL-GROUP TRANSFER REACTIONS

- Two-step strategy
  - Catalyzed by kinases and phosphatases
  - Can occur with a wide variety of attacking groups Serine, threonine and tyrosine –OH groups, the carboxylate groups of aspartate and glutamate, cysteine –SH and the nitrogen of histidine
- Bacterial alkaline Phosphatase
  - which catalyzes the transfer of phosphate to water from many organophosphate substrates
  - a serine –OH on the enzyme first attacks the phosphorus atom
  - phosphoserine-enzyme intermediate
- Phosphoglucomutases
  - D-glucose 1-phosphate and D-glucose 6-phosphate
  - proceeds through a phosphoaspartyl anhydride intermediate
  - when the phosphate on the aspartyl group of the enzyme attacks the unphosphorylated position on the sugar and transfers the phosphate group to it.

Bifunctional or Multifunctional Enzymes

- Three classes
  - First class, the two reactions take place consecutively at the same active site
  - second, two separate chemical reactions are catalyzed by two distinct active sites, each located in a different domain some distance apart
  - In the third, two or more reactions are also catalyzed by two or more distinct active sites, but these are connected by internal channels in the protein

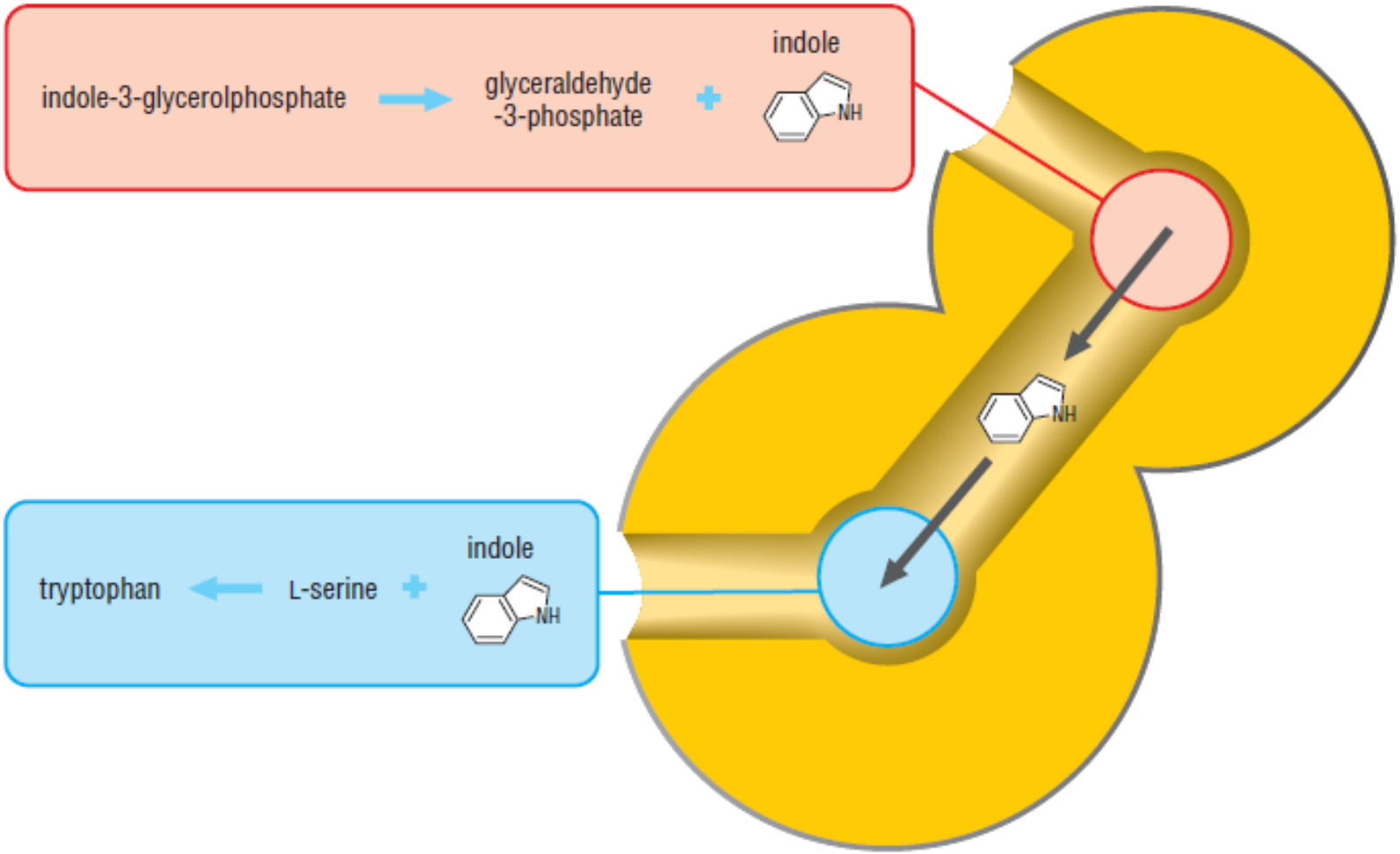
- In bifunctional enzymes that carry out two different reactions using the same active site
  - Isocitrate dehydrogenase (ICDH)
  - Isocitrate → oxalosuccinate (unstable) NADP
  - Oxalossuccinate → alpha ketoglutarate (same site) Mn



- Some bifunctional enzymes contain two active sites
- enzyme contains two independently folded domains
- First product → Dissociate → find other site
- Regulation, synthesis, two genes
- dihydrofolate reductase-thymidylate synthase
  - catalyzes two reactions in the biosynthesis of thymidine

BIFUNCTIONAL ENZYMES SHUTTLE

- Physical channel (or channels) used coz
  - first reaction product is an uncharged species
  - the reaction product is so unstable in free solution
- Tryptophan synthase
  - Tunnel is 25 Å long
  - Connects the active site of the alpha-subunit, in which indole, an uncharged molecule that might diffuse out of the cell, is generated from indole 3-glycerolphosphate, to the second active site, in which the indole is added to a molecule of acrylate, derived from serine, to produce tryptophan.


indole-3-glycerolphosphate
glyceraldehyde
-3-phosphate
indole
NH
NH
tryptophan
L-serine
indole
NH

Trifunctional enzymes

- carbamoyl phosphate synthetase
  - involved in the synthesis of 2`-deoxyUMP
  - three separate active sites connected by two tunnels through the interior of the protein
- first reaction produces Ammonia,
  - a neutral species, which travels along a tunnel to the second active site where
- it reacts with carboxyphosphate to give a carbamate intermediate
  - that would be too unstable to survive in aqueous solution. Therefore, it is transported through the interior of the protein to the third active site,
- where it is phosphorylated by ATP to give the final product.
- covers a distance of nearly 100 Å


glutamine
tunnel
ATP/HCO_3^-
carbamoyl phosphate
allosteric sites

ENZYMES ALSO HAVE NON-ENZYMATIC FUNCTIONS

- Regulatory function
  - some enzymes double as transcription factors;
  - others act as signaling proteins;
  - some are cofactors for essential reactions in protein synthesis; and
  - yet others are transported out of the cell to serve as cytokines or growth factors.
- Metabolic enzyme double as a repressor, for example, couples the expression of some genes to metabolism in a direct way
  - Folate-dependent enzyme thymidylate synthase
    - also functions as an RNA-binding protein.
    - interacts with its own mRNA to form a ribonucleoprotein complex
    - is also evidence that it can interact with a number of other cellular mRNAs, including transcripts of the *p53 tumor suppressor gene and the myc family of transcription factor genes*
    - It is also a target for several anti-cancer drugs