LEVELS OF PROTEIN FUNCTION

- function of a protein requires a description at several different levels
- if it is an enzyme
  - unction refers to the reaction catalyzed
- it is a signaling protein or a transport
  - interactions of the protein with other molecules
- cellular roles of the protein

FOUR FUNDAMENTAL BIOCHEMICAL FUNCTIONS

- Binding
  - Enzymes must bind substrates, as well as cofactors
- Catalysis
- Switching
  - GTPases
- Structural Elements
  - Structural proteins are, at their simplest, assemblages of a single type of protein molecule
    - bound together for strength or toughness
  - bind to other types of molecules to form specialized structures
    - such as the actin-based intestinal microvilli or
    - the spectrin-based mesh that underlies the red blood cell membrane and helps maintain its integrity

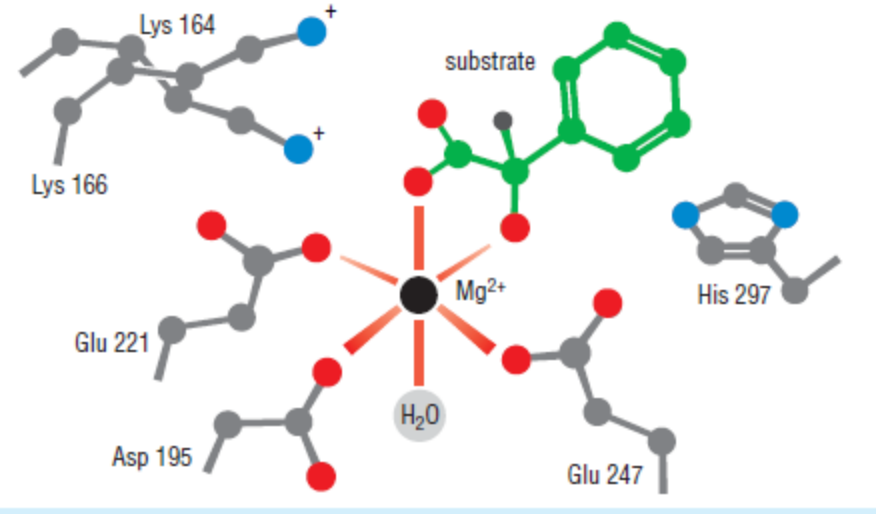
MOLECULAR RECOGNITION AND CATALYSIS

- Depend on the ability to bind other molecules, or ligands.
- Ligand binding involves the formation of noncovalent interactions between ligand and protein surface
- Specificity
  - complementarity of shape and charge distribution between the ligand and its binding site on the protein surface
  - the distribution of donors and acceptors of hydrogen bonds

Molecular recognition and Binding

- ligand-binding sites → molecular recognition
- active sites → promote chemical catalysis
- Microscopic Environment
  - Hydrophobic or Hydrophilic Ligands
- Specialized microenvironments at binding sites
  - *general acid-base catalysis*
  - Proton transfer can be promoted in two ways
    - a strong electrostatic field is produced in which acids and bases are close to each other but cannot react with each other.
      - K-E → carboxylate–ammonium ion charge pair.
      - enzyme active sites → aspartate aminotransferase, K-D

- other type of environment is one in which the affinity of a functional group for protons has been altered dramatically.
  - Placing two lysine side chains close to one another will lower the proton affinity of both of them, producing a stronger acid
  - effect is the enzyme pyruvate decarboxylase



EFFECT IS THE ENZYME PYRUVATE DECARBOXYLASE

- Ligand binding
  - a protein must have, or be able to form, a binding site whose stereochemistry, charge configuration, and potential hydrogen-bond-forming groups are complementaryto those of the ligand.
- key fitting into a lock.
  - holds for many proteins and ligand
- model of **induced fit**
  - conformational changes are allowed because of the inherent flexibility of proteins

Protein flexibility is essential for biochemical function

- chemical transformation of the ligand (catalysis),
- a conformational change in the protein
- translocation of the protein to another part of the cell
- Transport of the ligand
- alteration of the properties of the ligand
- net free energy released by binding and/or chemical transformation of the ligand can drive the required changes in the protein
  - binding can range from weak (dissociation constant Kd ~ 10–3 M) to extremely strong (Kd ~ 10–12 M or even tighter structure or properties
- if the protein is too flexible, neither specific recognition nor specific action can occur.
  - Adding SDS to protein

Protein Flexibility

- mutant enzymes
  - that are stable at higher temperatures than normal
  - more rigid, have shown that, at least for some such proteins, rigidifying the structure abolishes function.
  - the thermophilic enzymes are less active than the normal ones at lower temperatures
  - stability at higher temperatures has been achieved at the expense of flexibility at lower ones
- Protein flexibility is a natural consequence of the weak forces that hold the tertiary structure

The degree of flexibility varies in proteins

- Not all proteins are equally flexible.
- some very small rearrangements in atomic positions always occur on ligand binding
- Many of these are extracellular proteins, and their rigidity may help them survive in the more hostile environment outside the cell
- Other proteins undergo very large shape changes when the correct ligand binds

Binding sites for macromolecules on a protein's surface

- can be concave, convex, or flat
- specific recognition of a macromolecule by a protein usually involves interactions
  - over a large contiguous surface area
  - over several discrete binding regions
- A macromolecule will make many points of contact with the protein's surface;
- these add up to provide a great deal of binding energy, so a binding site for a macromolecule can occur, in theory, anywhere on a protein's surface.
- Sites are protruding loops or large cavities because these provide specific shape complementarity, but relatively flat binding sites are also found
- Many binding sites for RNA or DNA on proteins are protruding loops or alpha helices

Binding sites for small ligands are clefts, pockets or cavities

- Many important biological ligands are small molecules:
  - substrates for enzyme catalysis;
  - cofactors that bind to the active sites of enzymes and contribute to catalysis; and
  - allosteric effectors, which bind at sites remote from the active site yet modulate enzyme activity.
- Such small-molecule ligands bind at depressions on the protein surface.
- Clefts or cavities can easily provide unusual microenvironments.
- Clefts or cavities can easily provide unusual microenvironments.

Catalytic sites often occur at domain and subunit interfaces

- Binding in an interior cavity requires
  - ligand diffuses through the protein structure within a reasonable time frame.
- Enzyme active sites and most receptor binding sites for small ligands are found at generally predictable locations on protein surfaces.