**3.1 Cellular Functions**

Structural Proteins

* Compose the cytoskeleton, anchoring proteins, and much of the extracellular matrix
  + E.g. collagen, elastin, keratin, actin and tubulin
* Generally **fibrous with repeating domains**

Motor Proteins

* Have one or more heads capable of force generation through conformational change
* Have catalytic activity, acting as **ATPases** to power movement
  + E.g. muscle contraction, vesicle movement within cells, and cell motility

1. Myosin (interacts with actin)
2. Dynein (+) and kinesin (-) (associated with microtubules)

Binding Proteins

* Bind to a specific substrate, either to sequester it in the body or hold its concentration at steady state
  + E.g. hemoglobin, calcium-binding proteins, DNA-binding proteins (often transcription factors)

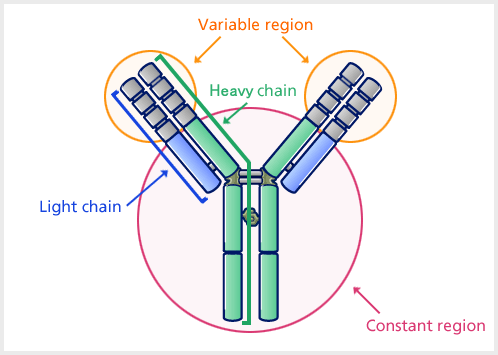
Cell Adhesion Molecules (CAM)

* Allow cells to bind to other cells or surfaces

1. Cadherins
   1. **Calcium-dependent** glycoproteins that hold **similar cells** together
      1. I.e. Two cells of the same or similar type using calcium
2. Integrins
   1. Have **two membrane-spanning chains** and permit cells to adhere to **proteins in the extracellular matrix**
      1. I.e. One cell to proteins in the extracellular matrix
   2. Some also have signaling capabilities
3. Selectins
   1. Allow cells to adhere to **carbohydrates on the surfaces** of other cells, and are most commonly used in the immune system
      1. I.e. One cell to carbohydrates, usually on the surface of other cells
   2. Have the weakest bond among all CAMs

Immunoglobulins

* Ig = Antibodies
* Used by the immune system to target a specific antigen, which may be a protein on the surface of a pathogen (invading organism) or a toxin
* Contain a constant region and a variable region
  + The variable region is responsible for antigen binding
* Two identical heavy chains and two identical light chains form a single antibody
  + They are held together by disulfide linkages and non-covalent interactions
* Three possible outcomes from antigen binding:
  + Neutralization
  + Opsonization
  + Agglutination



**3.2 Biosignaling**

Ion Channels

* Can be used for regulating ion flow into or out of a cell

1. Ungated channels
   1. Always open
2. Voltage-gated channels
   1. Open within a range of membrane potentials
3. Ligand-gated channels
   1. Open in the presence of a specific binding substance, usually a hormone or neurotransmitter

Enzyme-Linked Receptors

* Display catalytic activity in response to ligand binding
* Three primary protein domains:
  1. Membrane spanning domain
     1. Anchors the receptor in the cell membrane
  2. Ligand-binding domain
     1. Stimulated by the appropriate ligand → induces conformational change that activates the catalytic domain
  3. Catalytic domain
     1. Results in the initiation of a second messenger cascade

1. Receptor Tyrosine Kinase (RTK)
   1. Composed of monomer that dimerizes upon ligand binding
   2. Dimer is an active that phosphorylates additional cellular enzymes

G Protein-Coupled Receptors

* Have a membrane-bound protein associated with a trimeric G protein
* Also initiate second messenger systems
  + Ligand binding engages the G protein
  + GDP is replaced with GTP; the α subunit dissociates from the β and γ subunits
  + The activated α subunit alters the activity of **adenylate cyclase** (affects cAMP levels) or **phospholipase C** (which cleaves to form PIP2 → DAG and IP3 (affects calcium levels))
  + GTP is dephosphorylated to GDP; the α subunit rebinds to the β and γ subunits

**3.3 Protein Isolation\***

Electrophoresis

* Uses a gel matrix to observe the migration of proteins in response to an electric field
* Uses an electrolytic cell (ΔG > 0, Ecell < 0)

1. Native PAGE
   1. Maintains the protein’s shape (can be recovered later)
   2. But results are difficult to compare because the mass-to-charge ratio differs for each protein
2. SDS-PAGE
   1. Denatures the proteins and masks the native charge so that comparison of size is more accurate
   2. But functional protein cannot be recaptured from the gel
3. Isoelectric focusing
   1. Separates proteins by their isoelectric point (pI)
   2. The protein migrates toward an electrode until it reaches a region of the gel where pH = pI of the protein
      1. E.g. a protein with pI = 9 when placed onto a gel at pH = 7, protein will become positively charged (as environment is more acidic → more H+) and will migrate to the negatively charged cathode

Chromatography

* Separates protein mixtures on the basis of their affinity for a **stationary phase** or a **mobile phase**
* Refer to Organic Chemistry: Chapter 12 Separations and Purifications

1. Column chromatography
2. Ion-exchange chromatography
3. Size-exclusion chromatography
4. Affinity chromatography

**3.4 Protein Analysis**

Protein Structure

* Primarily determined through X-ray crystallography after the protein is isolated
* Although NMR can also be used

Amino Acid Composition

1. Edman degradation (for small proteins)
   1. Selectively and sequentially removes the N-terminal amino acid of the protein, which can be analyzed via mass spectrometry
2. Chymotrypsin, trypsin, and cyanogen bromide (for large proteins)
   1. Selectively cleaves proteins at specific amino acid residues, creating smaller fragments that can then be analyzed by electrophoresis or Edman degradation

Activity Analysis

* Activity levels for enzymatic samples are determined by following the process of a known reaction, often accompanied by a color change

Concentration Determination

* Protein concentration is determined colorimetrically, either by:
  + UV spectroscopy
  + Color change reaction

1. Bradford Protein Assay
   1. Working principle
      1. Protein + Coomassie Brilliant Blue Dye (protonated and green-brown in color)
      2. Dye, upon binding to amino acid groups, gives up protons → become blue
      3. More protein concentration → more blue dye
   2. Accurate when only one type of protein is present in the solution
   3. Inaccurate when more than one type of protein is present in the solution because of variable binding of the dye with different amino acids
2. Bicinchoninic (BCA) assay
3. Lowry reagent assay