Complexity of the proteins and structure determination
Protein plays many important roles in the body

- Helping facilitate muscular contraction
- Promoting satiety and appetite control
How Much Protein Do You Need?

- Healthy, nonpregnant adults
  - Should consume enough to replace what is used every day
  - The goal is nitrogen balance
- Pregnant woman, people recovering from surgery or injury, and growing children should consume enough to build new tissue
Nitrogen Balance and Imbalance

- **Protein intake**
  - **Positive nitrogen balance**
    - Pregnant women, growing children and adolescents, and some athletes tend to be in positive nitrogen balance.

- **Protein intake**
  - **Equilibrium**
    - A healthy adult is typically in nitrogen balance.

- **Protein intake**
  - **Negative nitrogen balance**
    - An individual who is experiencing a medical trauma or not eating a healthy diet is in negative nitrogen balance.

- **Nitrogen excretion**
Protein Needs

- Protein intake recommendations
- 10–35% of total daily kilocalories
- Adults over 18 approx. 0.8 g/kg daily
Not All Protein Is Created Equal

- High quality protein
- Is digestible
- Contains all essential amino acids
- Provides sufficient protein to synthesize nonessential amino acids

- It helps to be aware of:
  - Amino acid score
  - Biological value of protein rates absorption and retention of protein for use
Protein Quality

• Complete proteins
  • Contain all essential amino acids
  • Usually animal source are complete proteins
  • Are considered higher quality

• Incomplete proteins
  • Low in one or more essential amino acid
  • Usually plant sources are incomplete
Best Sources of Protein

- Proteins are abundant in
  - Dairy foods
  - Meats
  - Poultry
  - Meat alternatives such as dried beans, peanut butter, nuts, and soy

- 3 oz serving of cooked meat, poultry, or fish
  - Provides 21–25 grams of protein
  - Adequate amount for one meal
• A well-balanced diet can meet daily protein needs
• Best source of protein are animal products
  • Eggs
  • Lean meats
  • Low-fat or fat-free dairy products
• Plant proteins such as soy, grains, and vegetables supply substantial proteins
• Most people consume adequate protein from their diet and do not need protein supplements
Eating Too Much Protein

- Risk of heart disease
- Risk of kidney stones
- Risk of calcium loss from bones
- Risk of colon cancer
- Displacement of other nutrient-rich, disease preventing foods
Eating Too less Protein

- Protein-energy malnutrition (PEM)
- Protein is used for energy rather than its other functions in the body
- Other important nutrients are in short supply
- More prevalent in infants and children

Approx. 17,000 children die each day as a result
• Without adequate protein
• Cells lining the GI tract are not sufficiently replaced as they slough off
• Digestive function is inhibited
• Absorption of food is reduced
• Intestinal bacteria gets into the blood and causes septicemia
• Immune system is compromised due to malnutrition and cannot fight infection
Types of PEM: Kwashiorkor

- Severe protein deficiency
- Generally result of a diet high in grains and deficient in protein
- Symptoms range from
  - Edema in legs, feet, and stomach
  - Muscle tone and strength diminish
  - Hair is brittle and easy to pull out
  - Appear pale, sad, and apathetic
  - Prone to infection, rapid heart rate, excess fluid in lungs, pneumonia, septicemia, and water and electrolyte imbalances

(Image from http://www.thachers.org/pediatrics.htm)
Types of PEM: Marasmus

- Results from a severe deficiency in kilocalories
- Frail, emaciated appearance
- Weakened and appear apathetic
- Many cannot stand without support
- Look old
- Hair is thin, dry, and lacks sheen
- Body temperature and blood pressure are low
- Prone to dehydration, infections, and unnecessary blood clotting
Types of PEM: Marasmic Kwashiorkor

- Chronic deficiency in kilocalories and protein
- Have edema in legs and arms
- Have a “skin and bones” appearance
- With treatment the edema subsides and appearance becomes more like someone with marasmus
Treatment for PEM

• Medical and nutritional treatment can dramatically reduce mortality rate
• Should be carefully and slowly implemented
  • Step 1 – Address life-threatening factors
    ✤ Severe dehydration
    ✤ Fluid and nutrient imbalances
  • Step 2 – Restore depleted tissue
    ✤ Gradually provide nutritionally dense kilocalories and high-quality protein
  • Step 3 – Transition to foods and introduce physical activity
Vegetarian Diet

• People choose vegetarian diets for a variety of reasons
  • Ethical
  • Religious
  • Environmental
  • Health

• Vegetarians must consume adequate amounts of a variety of food and should plan meals well
<table>
<thead>
<tr>
<th>Type</th>
<th>Eats</th>
<th>Avoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lacto-vegetarian</td>
<td>Grains, vegetables, fruits, legumes, nuts, dairy foods</td>
<td>Meat, fish, poultry, and eggs</td>
</tr>
<tr>
<td>Lacto-ovo-vegetarian</td>
<td>Grains, vegetables, fruits, legumes, seeds, nuts, dairy foods, eggs</td>
<td>Meat, fish, and poultry</td>
</tr>
<tr>
<td>Ovo-vegetarian</td>
<td>Grains, vegetables, fruits, legumes, seeds, nuts, eggs</td>
<td>Meat, fish, poultry, dairy foods</td>
</tr>
<tr>
<td>Vegan</td>
<td>Grains, vegetables, fruits, legumes, seeds, nuts</td>
<td>Any animal foods, meat, fish, poultry, dairy foods, eggs</td>
</tr>
<tr>
<td>Semivegetarian</td>
<td>A vegetarian diet that occasionally includes meat, fish, and poultry</td>
<td>Meat, fish, and poultry on occasion</td>
</tr>
</tbody>
</table>
Potential Benefits and Risks of a Vegetarian Diet

• Benefits of a healthy vegetarian diet
  • Reduced risk of
    ◆ Heart disease
    ◆ High blood pressure
    ◆ Diabetes

• Potential risks of a vegetarian diet
  • Under consumption of certain nutrients
    ◆ Protein
    ◆ Vitamin B12
Many proteins contain intrinsic metal atoms

- Excellent ligands: His, Cys, Asp, Glu, H2O
- Common metals: iron, zinc, magnesium, calcium
Many proteins contain metal atoms

<table>
<thead>
<tr>
<th>Metal</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>Charge carrier; osmotic balance</td>
</tr>
<tr>
<td>Potassium</td>
<td>Charge carrier; osmotic balance</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Structure; hydrolase; isomerase</td>
</tr>
<tr>
<td>Calcium</td>
<td>Structure; trigger; charge carrier</td>
</tr>
<tr>
<td>Vanadium</td>
<td>Nitrogen fixation; oxidase</td>
</tr>
<tr>
<td>Chromium</td>
<td>Unknown, possible involvement in glucose tolerance</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>Nitrogen fixation; oxidase; oxo transfer</td>
</tr>
<tr>
<td>Tungsten</td>
<td>Dehydrogenase</td>
</tr>
<tr>
<td>Manganese</td>
<td>Photosynthesis; oxidase; structure</td>
</tr>
<tr>
<td>Iron</td>
<td>Oxidase; dioxygen transport and storage; electron transfer; nitrogen fixation</td>
</tr>
<tr>
<td>Cobalt</td>
<td>Oxidase; alkyl group transfer</td>
</tr>
<tr>
<td>Nickel</td>
<td>Hydrogenase; hydrolase</td>
</tr>
<tr>
<td>Copper</td>
<td>Oxidase; dioxygen transport; electron transfer</td>
</tr>
<tr>
<td>Zinc</td>
<td>Structure; hydrolase</td>
</tr>
</tbody>
</table>
Cys and His coordinate metal atoms

Zn-finger protein (Xenopus Xfin)

His coordinating Fe in Heme-binding protein
Hemoglobin (Hb)

• Protein molecule that transports respiratory gases

• Normal hemoglobin (adult male):
  • -14–18 g/dL whole blood

• Normal hemoglobin (adult female):
  • -12–16 g/dL whole blood
• Hemoglobin Function
• Carries oxygen
• With low oxygen (peripheral capillaries):
  • Hemoglobin releases oxygen
  • Binds carbon dioxide and carries it to the lungs
Sickle-Cell Disease: A Change in Primary Structure

- A slight change in primary structure can affect a protein’s structure and ability to function.
- Sickle-cell disease, an inherited blood disorder, results from a single amino acid (valine) substitution (for glutamic acid) in the protein hemoglobin.
Primary structure

Hemoglobin A

Secondary and tertiary structures

α subunit

β subunit

Quaternary structure

Hemoglobin A

Function

Molecules do not associate with one another, each carries oxygen.

Red blood cell shape

Normal cells are full of individual hemoglobin molecules, each carrying oxygen.

Sickle-cell hemoglobin

Primary structure

Hemoglobin S

Secondary and tertiary structures

α subunit

β subunit

Quaternary structure

Hemoglobin S

Function

Molecules interact with one another to crystallize into a fiber, capacity to carry oxygen is greatly reduced.

Red blood cell shape

Figure 5.21
What Determines Protein Conformation?

- Protein conformation depends on the physical and chemical conditions of the protein’s environment!
- pH, salts, temperature have impact on conformation
- Altering the agents lead to loss of natural conformation = denaturation
• **Denaturation**
  
  • Is when a protein unravels and loses its native conformation
Denaturation

- Transfer from aqueous environment to organic solvents
- Chemicals that disrupt chemical bonds
- Excessive heat (high fevers can be fatal because proteins in the blood become denatured!)
- But **sometimes** it can return its functional shape by removing of denaturing agents
The Protein-Folding Problem

- Protein folding problem is not simple!
- Most proteins probably go through several intermediate states on their way to a stable conformation
- Tracking the protein through the intermediate stages by chaperonins (or chaperon proteins)
- Assist the proper folding by keeping the new polypeptide from “bad influences” in the cytoplasmic environment while it folds spontaneously
• Chaperonins
• *E. coli*

**Chaperonin**
(fully assembled)

Steps of Chaperonin Action:
1. An unfolded polypeptide enters the cylinder from one end.
2. The cap attaches, causing the cylinder to change shape in such a way that it creates a hydrophilic environment for the folding of the polypeptide.
3. The cap comes off, and the properly folded protein is released.

Figure 5.23
Structure determination

- From primary sequence to the three-dimensional structure
cis-trans isomers

- double-bond character of the peptide bond, the C(alpha)-carbons can be either in a *trans* or *cis* position
- trans-position is much more stable
- Cis peptide group is rare in proteins
- only exception: peptide bonds with a proline

In this X-Pro peptide, the cis-peptide is only slightly destabilized and approx. 20% of all X-Pro peptides occur in this isomeric form.
Flexibility in the C(alpha)-C’ and N-C(alpha)-bond

- peptide bond is rigid
- only degree of flexibility in the polypeptide are rotations around the C(alpha)-C’ and N-C(alpha)-bond
- Each unit can rotate around these two bonds
- the angle of rotation around the N-C(alpha)-bond is called **phi**
- Angle around the C(alpha)-C’-bond from the same C(alpha)-atom **psi**
- each amino acid residue is associated with two conformational angles
- the conformation of the whole main chain of the polypeptide is completely determined when the angles for each amino acid is defined
Proteins can be crystallized!
Determination of 3D structure

• Not simple!
• X-ray crystallography (crystals)
• NMR (nuclear magnetic resonance)
• Cryo-EM
• Structures stored in PDB Database (!)
Protein crystals diffract x-rays
The surprising complexity of protein structures

- first protein crystal structure was reported in 1958
- myoglobin structure revealed a complex topology
- impossible to draw any general conclusions from it
• majority of the amino acids in the core have hydrophobic side chains
• Main driving force for the folding of the protein - packing of hydrophobic side chains into the interior of the protein
• Hydrophobic core
• The hydrophilic side chains, on the other hand, are at the water-exposed surface of the protein
• **Problem**: C=O and N-H of the peptide bond are polar hydrogen-bonding groups - require a polar environment
• solved in the two dominant so-called secondary structure elements: \(\alpha\)-helix and \(\beta\)-sheet
• C=O and N-H groups are engaged in hydrogen-bond interactions.
The α-helix

• Predicted in 1951 by Linus Pauling

• 3.6 amino acids per turn

• Hydrogen bonds between C=O of residue n and N-H of residue n+4

• α-helices vary considerably in length:
  4-40 residues with an average of 10 residues (average length 15Å)

• Most α-helices are right-handed

• The α-helix has a significant dipole
Amino acids in α-helices

- Some amino acids are good α-helix „formers“: Ala, Glu, Leu and Met
- poor α-helix „formers“: Pro, Gly, Tyr and Ser
- Pro – ring structure; very often found at the beginning of an α-helix and causes a bend when it is located in an α-helix
- preferences are not strong enough to yield accurate structure predictions
- The position of an amino acid can be depicted in a helica wheel, where an amino acid is plotted every 100° in a spiral
β-sheets

- is not build by a continuous region but rather different regions of the protein
- approx. 5-10 residues long and have an almost fully extended conformation
- C=O and N-H groups of adjacent strands can form hydrogen bonds
Formation of a parallel β-sheet

- the edges of two strands must be adjacent to each other and hence the polypeptide chain has to cross over to this end
- crossing over - made by α-helices
- motif is called beta-alpha-beta motif
Visualization of protein topology

- $\alpha$-helices can be shown as cylinders or ribbons
- $\beta$-strands are often depicted as arrows, with the arrowhead indicating the C-terminus
Topology diagrams

- left, five-stranded parallel $\beta$ sheet of the bacterial redox protein flavodoxin

- right, eight-stranded closed antiparallel barrel in the electron carrier plastocyanin
Loop regions are at the surface of proteins

- secondary structure elements are connected by short so-called loop regions
- vary in length and have an irregular structure
- exposed to a polar aqueous environment - hydrophilic and charged amino acid residues are commonly found in loop regions.
Loops

- loops also participate very often in formation of:
  - Binding sites (antigen binding sites of antibodies)
  - Enzyme active sites

- An arrangement of secondary structure elements - motif
β-hairpin motifs in proteins

(a) one β-hairpin motif

(b) two β-hairpin motifs

bovine trypsin inhibitor
inhibits the activity of the protease trypsin

erabutoxin
toxin from a sea snake that inhibits the acetylcholin receptor in nerve cells
The Greek key motif

- Four adjacent antiparallel β-strands are arranged
- similar to the repeating unit of one of the ornamental patterns used in ancient Greece – the Greek key
Large proteins - domains

- Large polypeptide chains very often encompass separate units of function which fold independently
- These units are called *domains*
Summary

- Complexity of the proteins
- Sources of proteins
- Conformation and folding
- Determination of the structures
- Visualization and topology