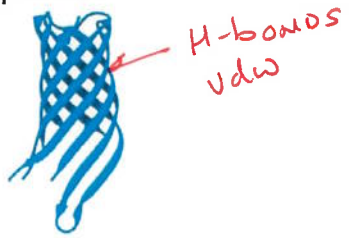
Lec 10: Supersecondary Structure, Fibrous Proteins, Domains, Disulfide Bonds, Immunoglobulins

- Super secondary structure
- Globular versus fibrous proteins.
- How disulfide bonds stabilize proteins
- Quaternary structure of antibodies
- Functional domains of antibodies
- Proteolytic domains of antibodies

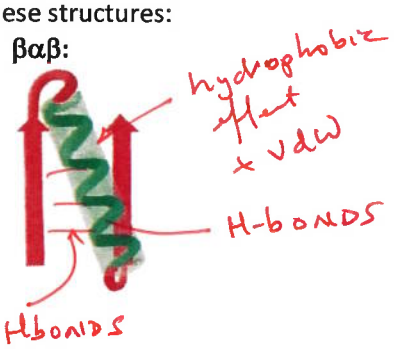
Supersecondary Structures: Motifs that make up larger proteins. You should be able to:

i. Sketch these structures (mainchain)
ii. Describe the energetics that stabilize these structures:

β-barrel:

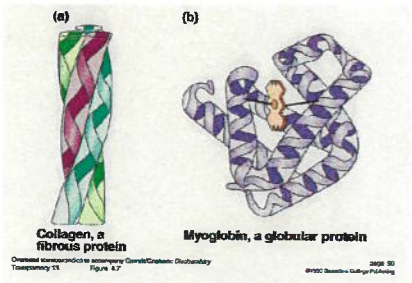


βαβ:



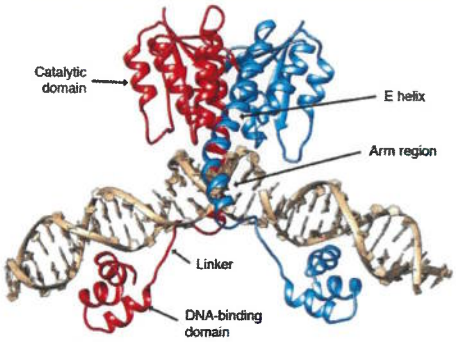
Non-globular proteins (Fibrous)

- Often stabilized by similar interactions as globular proteins
- Often play a structural role, e.g. collagen



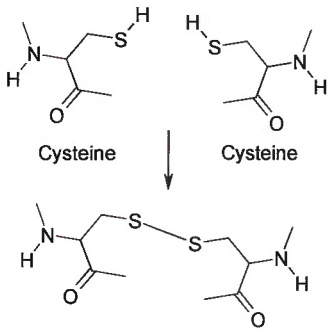
Domains (Motifs): Segments of proteins that generally fold as independent units. Each domain may have a specific function, e.g. binding to DNA – "DNA binding motif".

- cut between domain
→ still fold



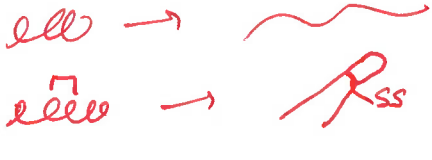
Disulfide Bonds: The formation of a covalent disulfide bond between two cysteine (Cys) residues can contribute to the stability of protein tertiary structure. The "S-S" bond covalently crosslinks two regions of the structure that may be distant in sequence, but close in the folded state.

Disulfide bonds are generally found in proteins that function outside of the cell, *e.g.* digestive enzymes, antibodies.



***Reflection**: Why would they be found on extracellular proteins?*

- stabilizing
- decrease the entropy of unfolded state



Overview of the Immune System

High diversity: *~ 10^8 different "things" (protein/carbohydrate) can be recognized*
High specificity: *Usually a thing is recognized by only one antibody*
Exclusion of self: ***You do not recognize your own tissues (self-tolerance)***

Some Terms:

Antigen: Foreign material that is recognized by the immune system. An antigen is usually a protein, or carbohydrate, but may be a small organic molecule, or a lipid, or nucleic acid.

Epitope: Region of an antigen to which the antibody binds, e.g. part of a protein.

Hapten: A small organic chemical that is recognized by an antibody but cannot generate an immune response by itself.

Cellular Immunity:

B-cells have an antibody as part of a cell surface receptor. After binding antigen, they are activated by **T-helper cells** (T_H) and the activated B-cell develops into **plasma cells** that produce soluble antibodies that destroy pathogens.

Antigen + B-cell + T-cell → Plasma cell (antibody secreting)

Antibodies – Y- shaped molecules that have:

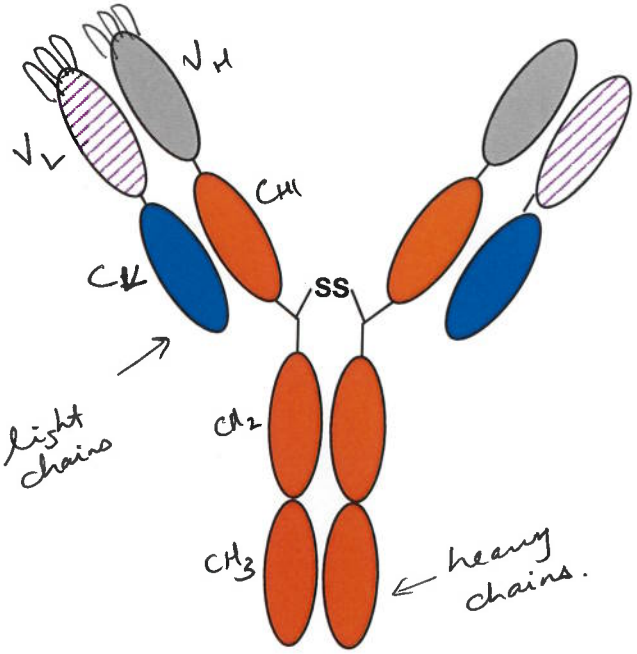
- two specific binding sites for antigens (e.g. surface protein on pathogen)
- domains (F_c) that lead to biological effects, such as the destruction of the pathogen.

Applications of Antibodies.

- **Immune system:** Antibodies bind to pathogens, leading to their inactivation/destruction.
- **Cancer Treatment:** Antibodies specific to cancer cells can cause their destruction.
- **Protein purification:** antibodies specific for a protein can be used to purify that protein.
- **Labeling components of a cell:** Antibodies with attached fluorescent groups.
- **Drug detoxification:** Antibodies that bind to harmful chemicals.

Quaternary structure:

- 2 Light + 2 heavy chains.
- Two binding sites/molecule.
- Chains held together by disulfide bonds and non-covalent forces.
- Light chains are identical on any given Ab.
- Heavy chains are identical on any given Ab. (There are four classes of heavy chain, IgM, IgG, IgE, IgA, each with a specific biological function.)
- The first 110 residues in each chain are highly variable. The variable region of both heavy and light are responsible for binding antigen. The sequence of these differ from one antibody to the next, resulting in a different specificity.
    - Estimated ~10^3 different light chain V_L sequences.
    - Estimated ~10^5 different V_H sequences
    - Any light chain can be paired with any heavy chain, = 10^8 different specificities.



Primary structure.

Light Chain Sequence – First 120 AA (identical residues indicated by "|", similar residues by "+")

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                1   5    10   15   20   25   30   35   40   45   50   55
Anti-flu     1  QSVLTQPPSVSGAPRQRVTISCSGGNSNIGN-NAVNWYQQLPGQAPKLLIHYDDLLPSGV  59  (res#)
                |||||||  ||||+|  | +|+|||+|  +|++||  |  |+|||  ||+  |||+|+  +   |||||
Anti-ebola   1  QSALTQPASVSGSPGQSITVSCTGTSSDVGNYNYVSWYQQHPGKVPKLMIYDVNNRPSGV  60  (res#)

                60   65   70   75   80   85   90   95  100  105  110
Anti-flu    60  SDRFSGSKSGTSASLAISGLQSEDEADYYCAAWDDSLNAQVFGGGTKLTVLGQPKAAPSV
                |+|||||||| +|||  |||||+||||  |||+++   |  +   |||  ||||||||||||||||
Anti-ebola  61  SNRFSGSKSGNTASLTISGLQAEDEAHYYCSSYTTS-DTWVFGEGTKLTVLGQPKAAPSV
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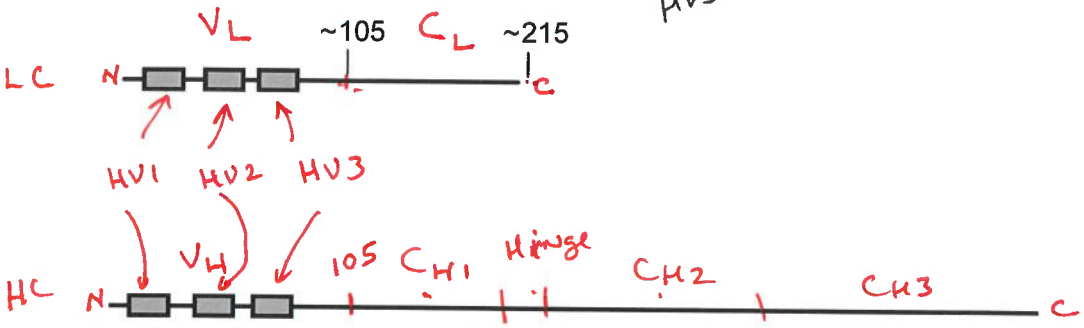
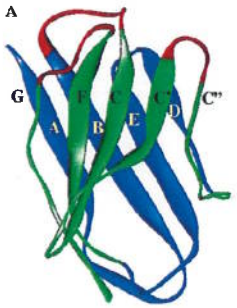
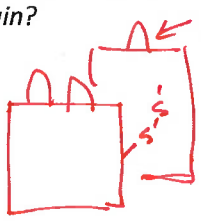


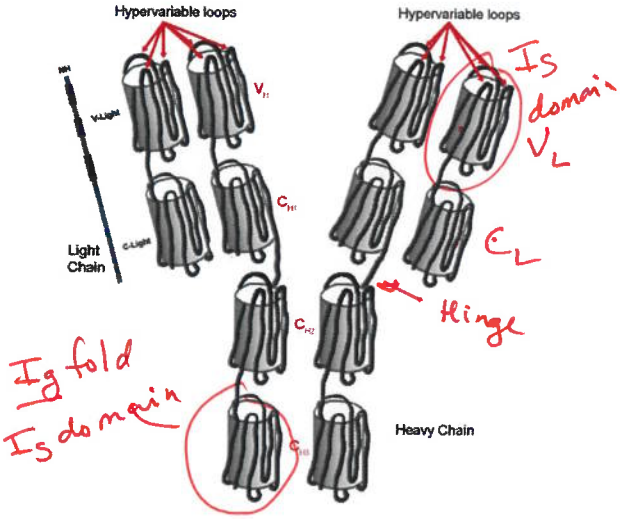
- V-region, ~105 amino acids, different sequence between antibodies with different specificities.
- Hypervariable regions. 3 per V region, highly different sequences between antibodies with different specificities. The hypervariable loops are also called complementary determining regions (CDRS).
- Constant region - conserved sequence on all antibodies. ~110 residues on L-chain, ~330 residues on H-chain.

Super-Secondary Structure: Antibodies consists of domains, called Ig folds or Ig motifs. These are two beta-sheets held together by a disulfide bond.



Where are the disulfide bonds in the light chain V_L domain?





Multiple Ig folds are linked together by peptide bonds to from the entire chain. Light chain = 2 Ig domains (V_L + C_L). Heavy chain = 4 Ig domains.

Antibody Fragments:

F_{AB} Fragment = light chain + ½ of heavy chain. Binds one antigen. Can be made by proteolysis using enzyme papain.

F_v Fragment Variable regions from the heavy and light chain. The F_v domain is the smallest unit that can bind antigen. Useful for cancer therapy due to better penetration into tumor. Usually made using recombinant DNA technology.

F_c Fragment = 2nd part of heavy chains.