

Lecture 9

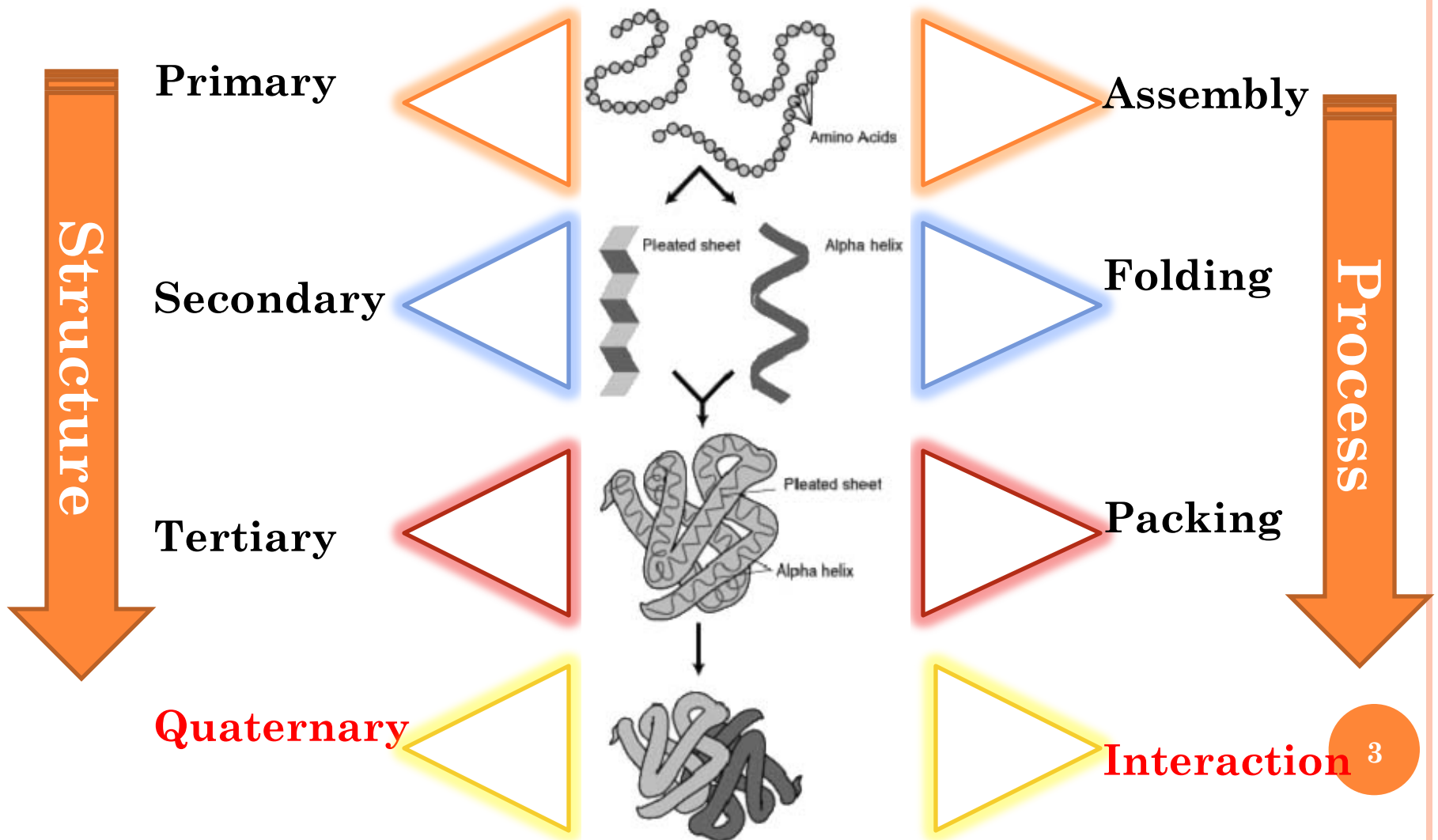
Levels of Organization in Proteins

# Quaternary Structures Stability

# LAST LECTURE

- Quaternary Structure
  - Structural and functional advantages
- Protein Stabilizing Interactions
- Protein Interface Properties
- Hydrogen-bonding potentials
- Inappropriate quaternary interactions
- Unstructured Proteins

# PROTEIN STRUCTURES



# Hemoglobin (Hb)

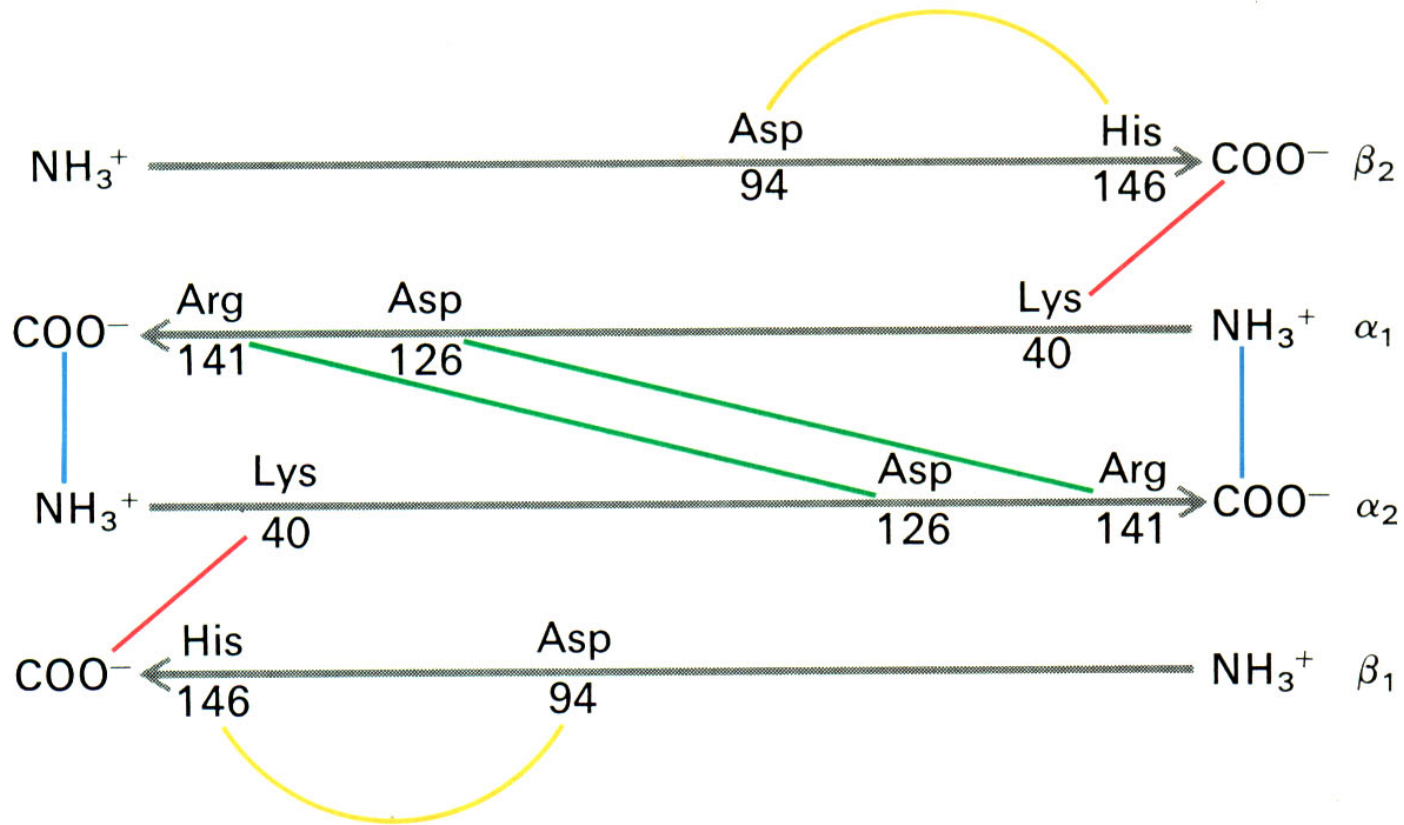
- O<sub>2</sub> transporter in erythrocyte
- 2  $\alpha$  subunits, 141 AAs
- 2  $\beta$  subunits, 146 AAs
- 4 subunits are maintained together by 8 pairs of ionic interactions.
- Each subunit contains one heme group.
- The conserved hydrophobic core stabilizes the 3D structure.



# STRUCTURE OF HEMOGLOBIN



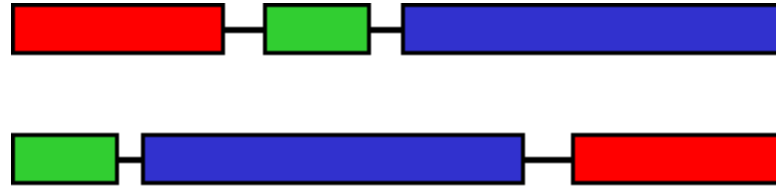
# IONIC FORCES AMONG HB SUBUNITS



- Asp<sup>-</sup> ... His<sup>+</sup>
- terminal-COO<sup>-</sup> ... Lys<sup>+</sup>
- Asp<sup>-</sup> ... Arg<sup>+</sup>
- terminal-COO<sup>-</sup> ... NH<sub>3</sub><sup>+</sup>-terminal



# PROTEIN DOMAINS



Pairwise sequence comparison of proteins led to strange results

- A domain is an independent folding unit
- A domain is the next step up in complexity from a motif
- There appear to be a limited number of folds (domains) that can be made from the 20 natural aa's
- Domain unit of evolution
- Mixing and matching can create new function and regulation
- Most proteins involved in cell signalling consist exclusively of small domains interspersed by linker regions. The linkers may be unstructured as described in the following section.

# PROTEIN DOMAIN CLASSIFICATION

- Five Classes
  - Based on the predominant secondary structure
- Alpha domains
  - comprised entirely of alpha helices.
- Beta domains
  - contain only beta sheet.
- Alpha/beta domains
  - contain beta strands with connecting helical segments.
- Alpha+beta domains
  - contain separate beta sheet and helical regions.
- Cross-linked domains
  - have little, if any, secondary structure but are stabilized by several disulfide bridges or metal ions.



# COMMON MOTIFS FOR ALPHA DOMAINS

## ○ **Four-Helix Bundle**

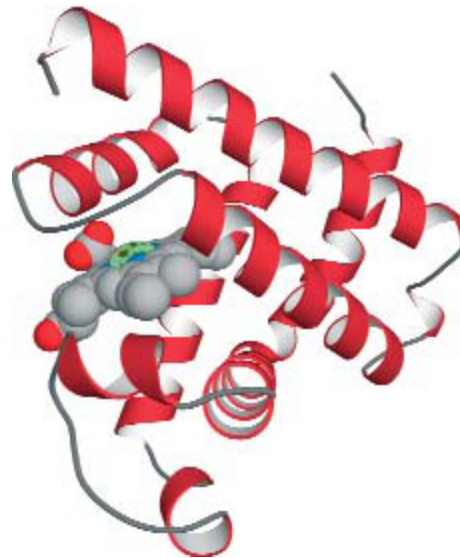
- A bundle of four antiparallel alpha helices, each crossing the next at an angle of about  $-20^\circ$ , so that the entire motif has a left-handed twist
- found in a wide variety of alpha domains
- where it serves in
  - Oxygen transport
  - Nucleic acid binding
  - Electron transport
- Examples include
  - Myohemerythrin, an oxygen-storage protein in marine worms
  - Human growth hormone, which helps promote normal body growth.



# COMMON MOTIFS FOR ALPHA DOMAINS

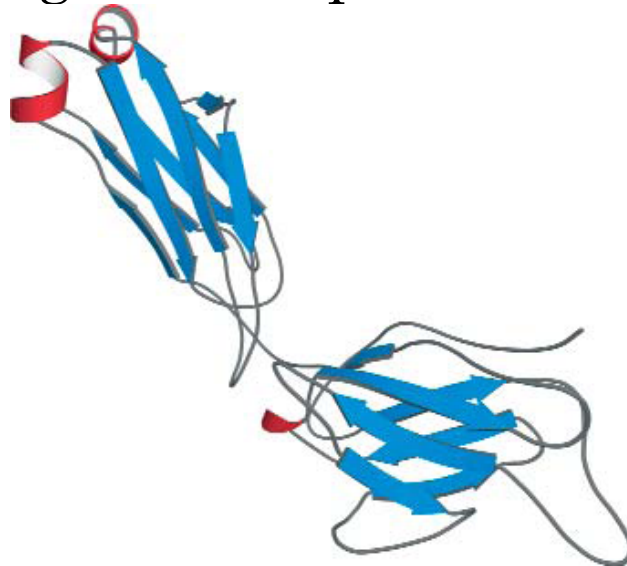
## ○ Globin fold

- consists of a bag of about eight alpha helices arranged at  $+90^\circ$  and  $+50^\circ$  angles with respect to each other.
- Forms a hydrophobic pocket in the domain interior
  - a large, hydrophobic organic and organometallic groups can bind to pocket
- Examples
  - Myoglobin
  - Cytochromes



# BETA DOMAINS

- contain only beta sheet, tight turns and irregular loop structures
  - Immunoglobulins,
  - Several enzymes such as superoxide dismutase,
  - Proteins that bind to sugars on the surfaces of cells.
- Essentially nothing but antiparallel beta structure.



# ALPHA/BETA DOMAINS

- composed of parallel or mixed strands
- connections are usually made by alpha helices
  - connecting parallel adjacent strands
  - give rise to beta-alpha-beta-alpha units.
- Two major families of alpha/beta domains:
  - Barrels
  - Twists

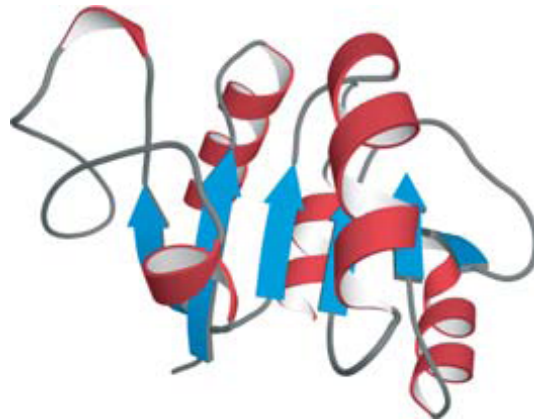
# ALPHA/BETA DOMAINS

- Two major families of alpha/beta domains:
  - Barrels
    - alpha/beta barrel
      - Most regular form
      - beta-alpha-beta-alpha motif is repeated four or more times.
      - stable when there are eight strands in the barrel (TIM Barrel)
      - occurring in 10% of all enzyme structures



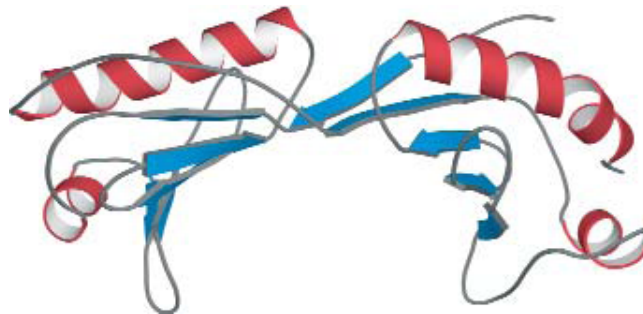
# ALPHA/BETA DOMAINS

- Families of alpha/beta domains:
  - Twists
    - alpha/beta twist
    - form an open sheet that is twisted into a saddle shaped structure.
    - strand order in the sheet is not consecutive
    - First beta strand in the primary sequence forms a strand in the middle of the sheet



# ALPHA+BETA DOMAINS (SEGREGATED)

- Are just clusters of interacting helices,
- Beta sheets tend to be antiparallel or mixed.
- Example
  - Saddle-shaped, antiparallel sheet with a layer of alpha helices covering one face
    - leaves the other face of the sheet exposed to the solvent
  - Peptide-binding groove layer in major histocompatibility proteins.
    - helices are used to form this recognition site.



# CROSS-LINKED IRREGULAR DOMAIN

- Found in small singledomain intra- and extracellular proteins.
- Two subclasses
  - Disulfide bond formation
  - Metal ions



# CROSS-LINKED IRREGULAR DOMAIN

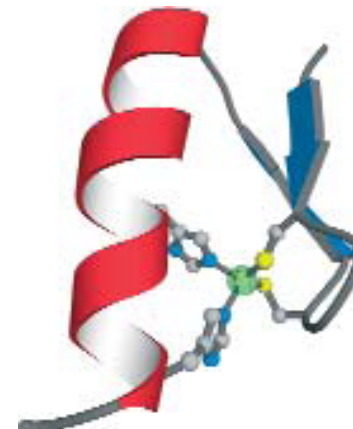


## ○ Disulfide bond formation

- In small irregular extracellular domains
- Involving a number of cysteine pairs
- Are often toxins that inhibit essential cellular proteins and prevent them from functioning
- Unusually stable to proteolytic digestion and heat denaturation
- Cobra venom neurotoxin, scorpion toxin, secreted protease inhibitors, and toxic proteins from marine snails

# CROSS-LINKED IRREGULAR DOMAIN

- Metal ions
  - In small irregular intracellular domains,
  - Connecting different parts of the domain through ligation by nucleophilic side chains.
- Examples
  - Zinc finger transcription factors
  - Iron–sulfur proteins called ferredoxins



# HOW PROTEINS ARE MADE FROM DOMAINS

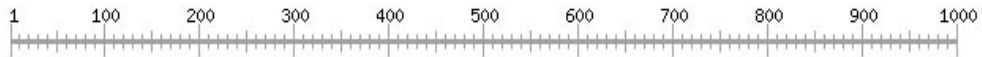
Some proteins consist only of domains that have no enzymatic activity. It is thought that they function as scaffolds for specific complex formation.



## PROSITE Domain View of selected proteins

This view ([Help](#)) shows all PROSITE profile matches on [Q92547] protein, together with rule-based predicted features inside matches (to obtain details about the predicted features for a protein of interest use the link to [scanprosite](#) tool on the right of its image).

ruler:



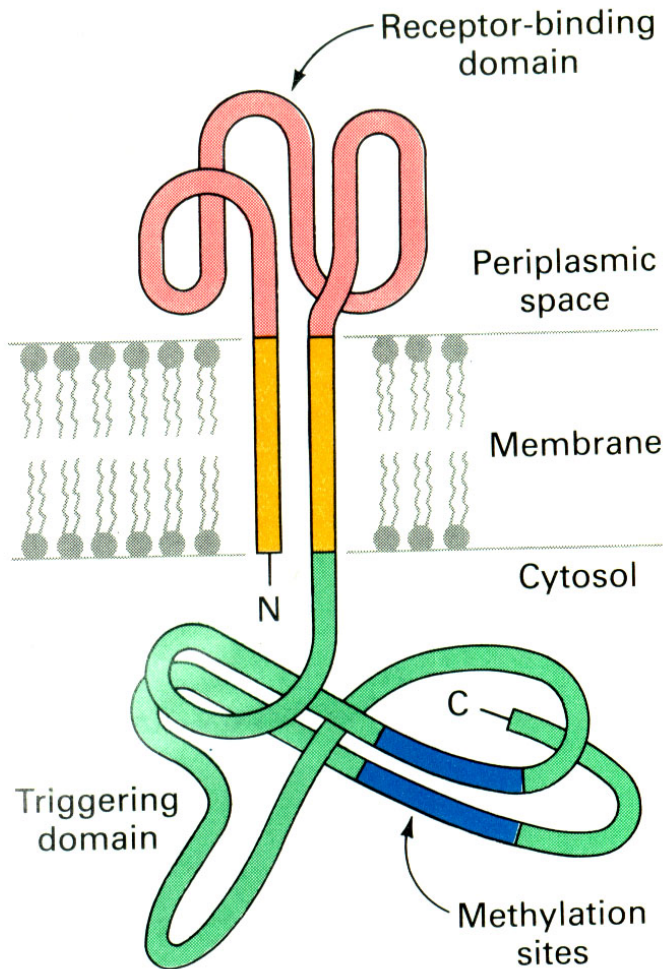
1 protein with a **BRCT** architecture:

[Q92547](#)  
(TOPB1\_HUMAN)



BRCT domains are a good example of divergent evolution. An ancient domain found in pro- and eukaryotes, it is characterised by a conserved fold despite significant sequence divergence. BRCTs are known to bind DNA and other proteins. Protein-protein interactions included self binding, binding BRCTs on other proteins, binding non-BRCT domains and binding to phosphoserine peptides.

# Methyl-accepting chemotaxis



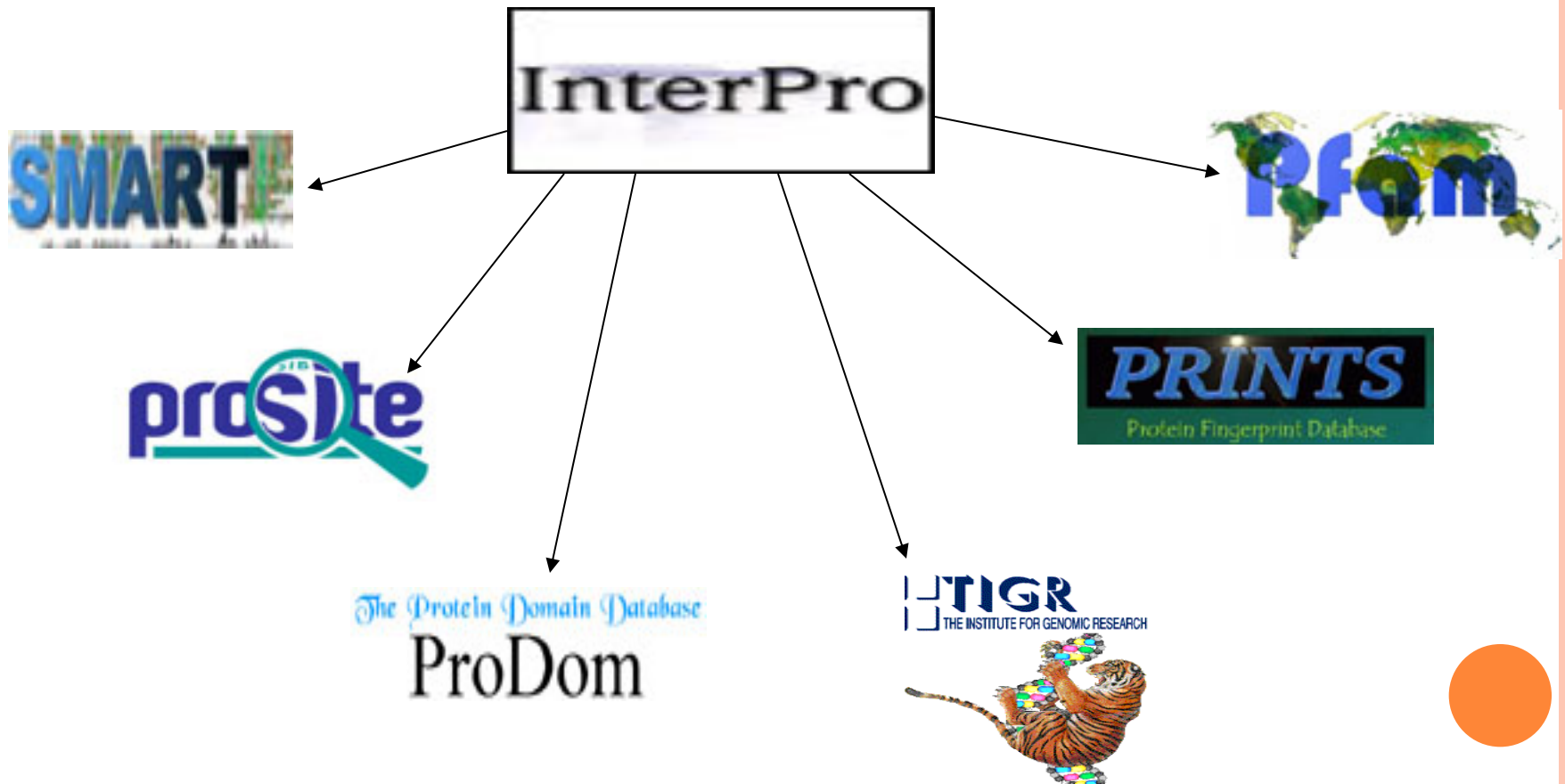
- Highly conservative cytosolic domain
- Divergent periplasmic domain serving as a chemosensor
- Transducing the external signals into the cell



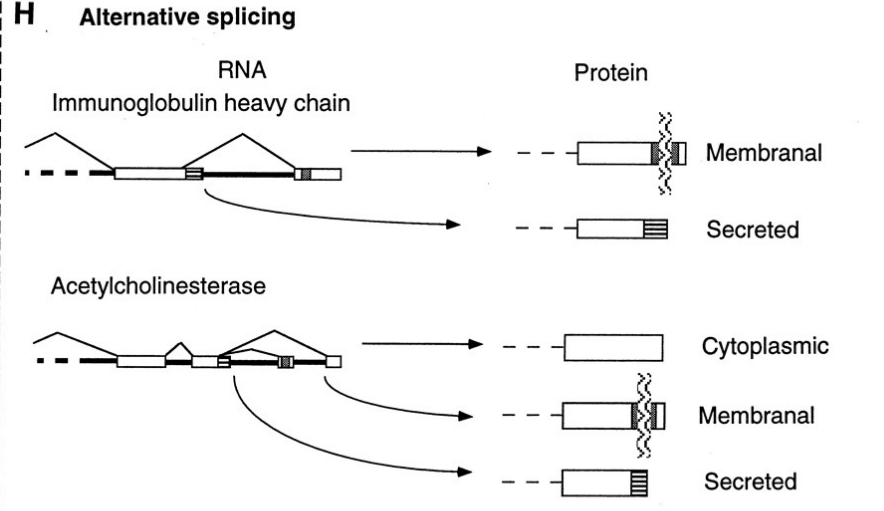
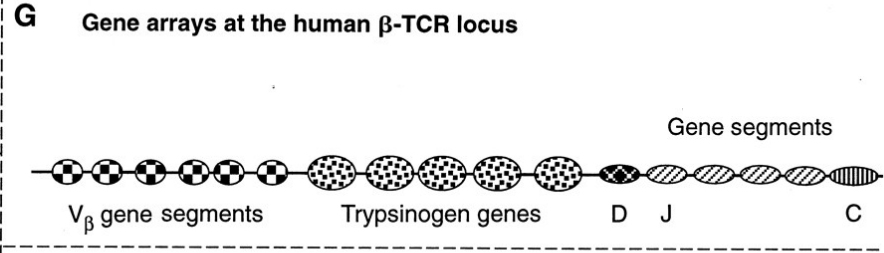
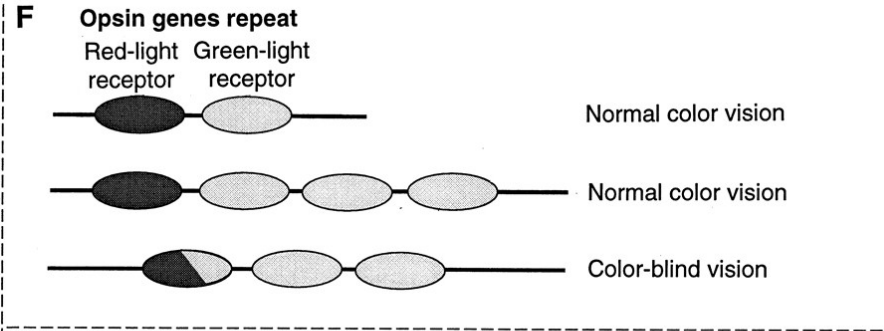
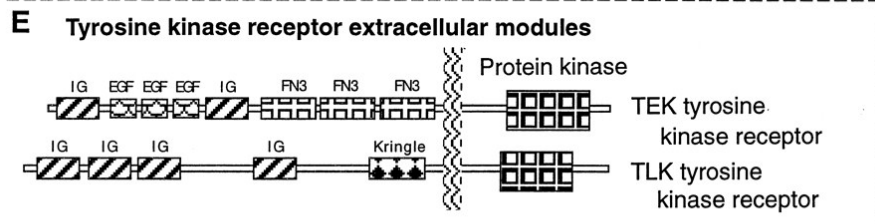
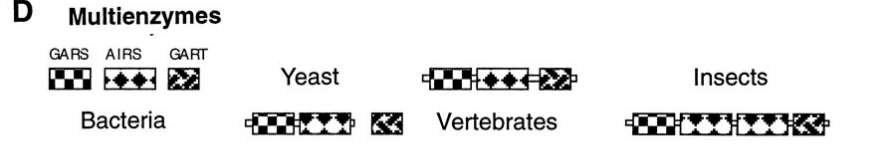
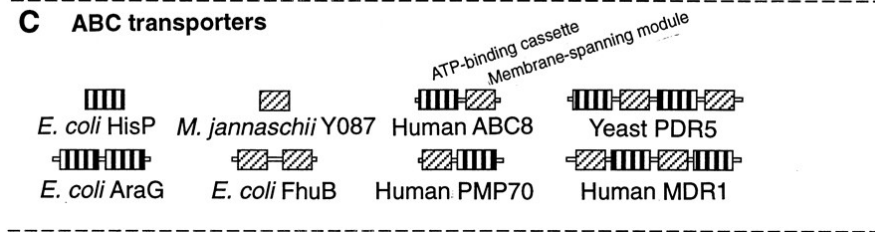
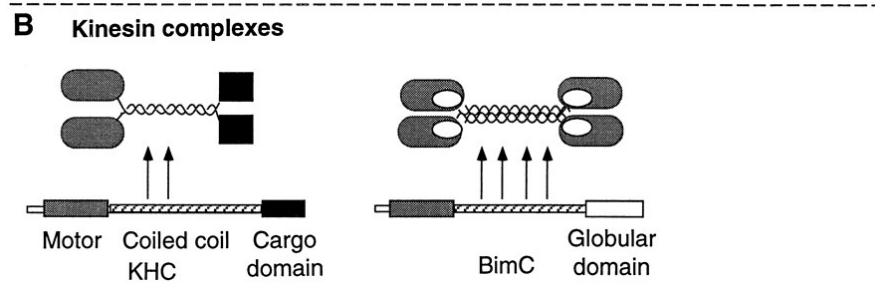
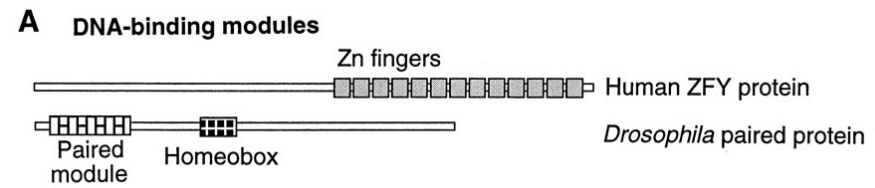
The good thing about standards is that there are so many of them  
to choose from...

# Interpro

An integrated resource of protein sites and functional domains



# The classification of proteins is made difficult by multidomained proteins



## Gene Families: The Taxonomy of Protein Paralogs and Chimeras

Steven Henikoff, Elizabeth A. Greene, Shmuel Pietrokovski, Peer Bork, Teresa K. Attwood, Leroy Hood  
*Science* 1997 October 24; 278: 609-614.