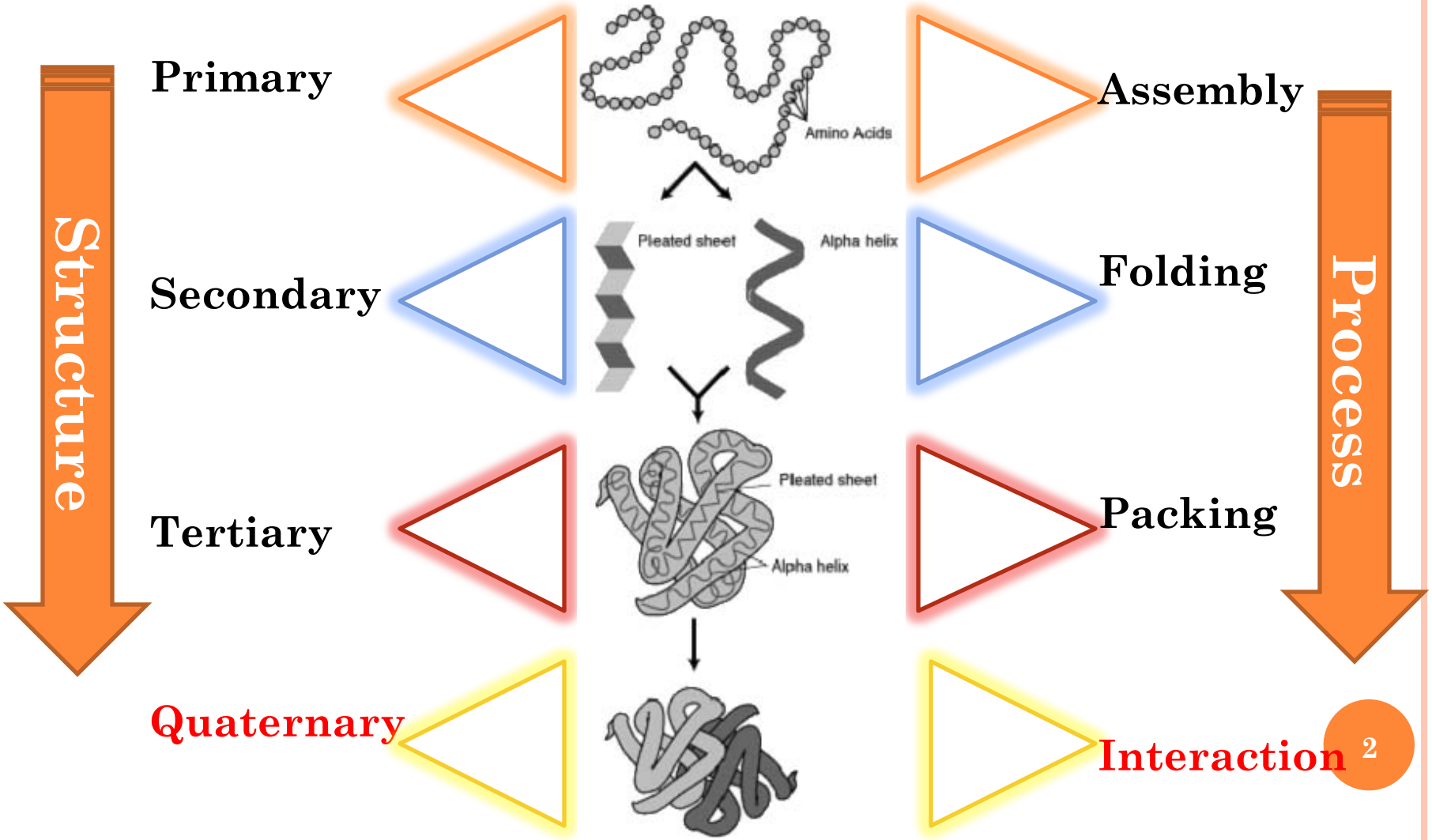
Last Lecture

- Stability of Protein Tertiary Structure
  - Stabilizing Forces
    - Non-Covalent
    - Covalent
      - Disulphide Bond
      - Metal Ions
      - Organometalic Cofactor
- Post-translational modification
- Protein Denaturation
  - Causes
  - Denaturant
- Protein Renaturation

Protein Structures



Quaternary Structure

- Organization of subunits (2 to 6 in common) in a protein with multiple subunits
- Subunits may be identical or different
- Subunits have a defined stoichiometry and arrangement
- Subunits held together by weak, noncovalent interactions (hydrophobic, electrostatic)
- Associate to form dimers, trimers, tetramers etc. (oligomer)

Quaternary Structure

(a) homodimer: a2

(b) heterodimer: ab

(c) heterotetramer: a2b2

(d) heteropentamer a2bcd

Quaternary Structure

- Homotrimer
  - Three identical chains, for example, keratin
- Heterotetramer
  - Built from two homodimers for example, hemoglobin
- Macromolecular Assemblies
- Specific intermolecular interactions
  - Protein Surface
  - Protein Shape
  - Weak bonds that hold complexes together
  - Hydrogen-bond donors are opposite acceptors
  - Non-polar groups are opposite other nonpolar groups
  - Positive charges are opposite negative charges
  - strength of binding must be greater than about 15–20 kJ/mole

Structural and functional advantages of quaternary structure

- Stability: reduction of surface to volume ratio
- Genetic economy and efficiency
- Bringing catalytic sites together
- Cooperativity (allostery)

There are a number of advantages in forming oligomers

- ❑ Size without loss of stability
- ❑ Modular construction – one gene – big protein
- ❑ Complex catalytic sites in enzymes
- ❑ Regulation

- Complementarity ensures
  - All possible van der Waals contacts are made
  - Hydrogen bond donors and acceptors at the interface between the two molecules pair with each other instead of making hydrogen bonds to water.
- coiled-coil structures
  - Dimers of alpha helices formed through the ridges and grooves arrangement
  - Lucines Heptad Repeat
    - Hydrophobic side chains, often those of leucines, are repeated at intervals of seven amino acids in the chain, forming the “ridge” of hydrophobic side chains that fit into spaces on the interacting helix.

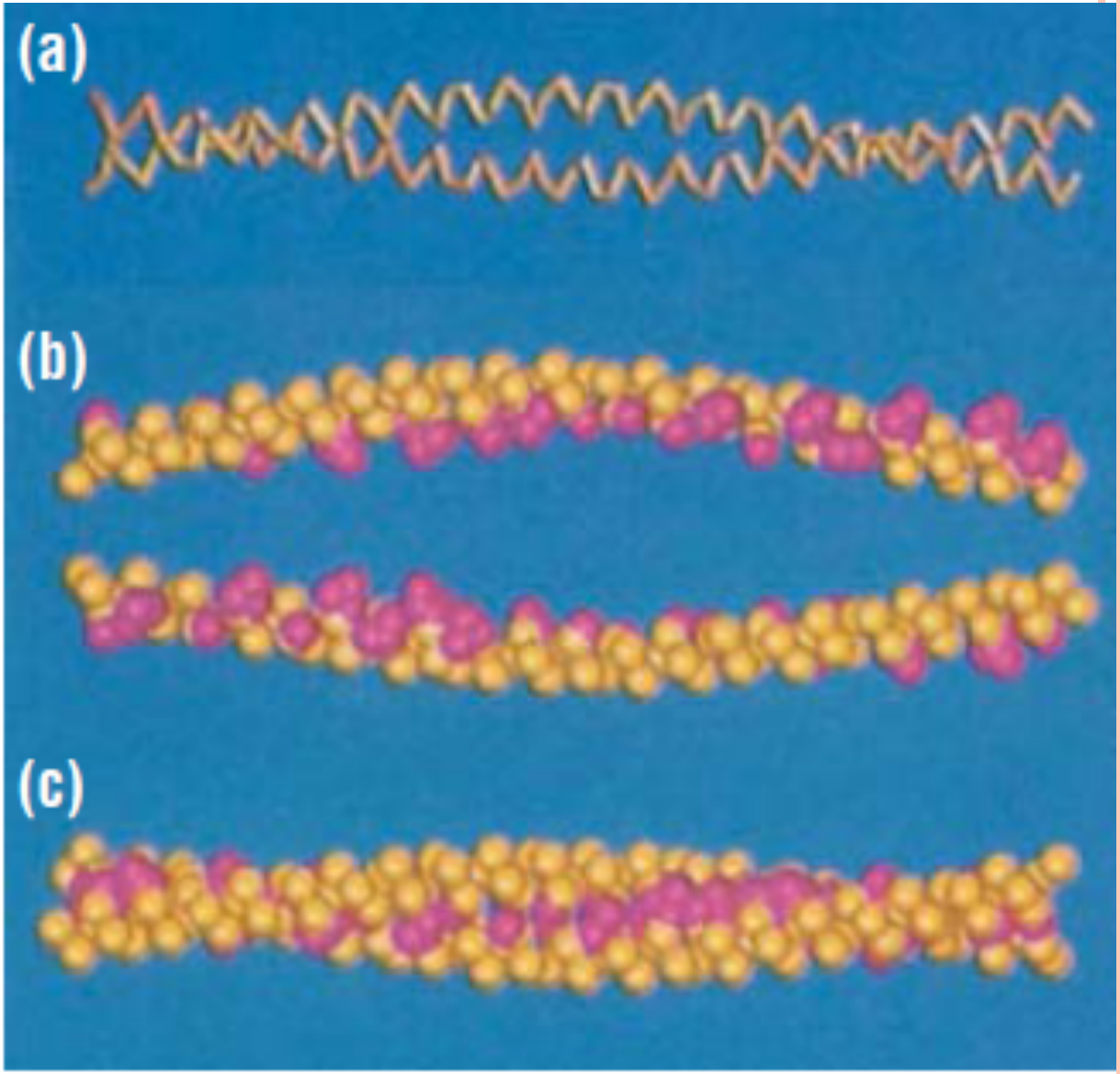


Figure 1-67 Coiled-coil alpha-helical interactions **(a)** Two interacting alpha helices of tropomyosin shown in a chain representation; **(b)** a space-filling representation of the separate alpha helices of tropomyosin with the hydrophobic side chains shown as dark protrusions; **(c)** the tropomyosin dimer showing how the hydrophobic side chains interdigitate in the coiled coil in a knobs in holes arrangement.

Protein Stabilizing Interactions At Protein Intramolecular Interface

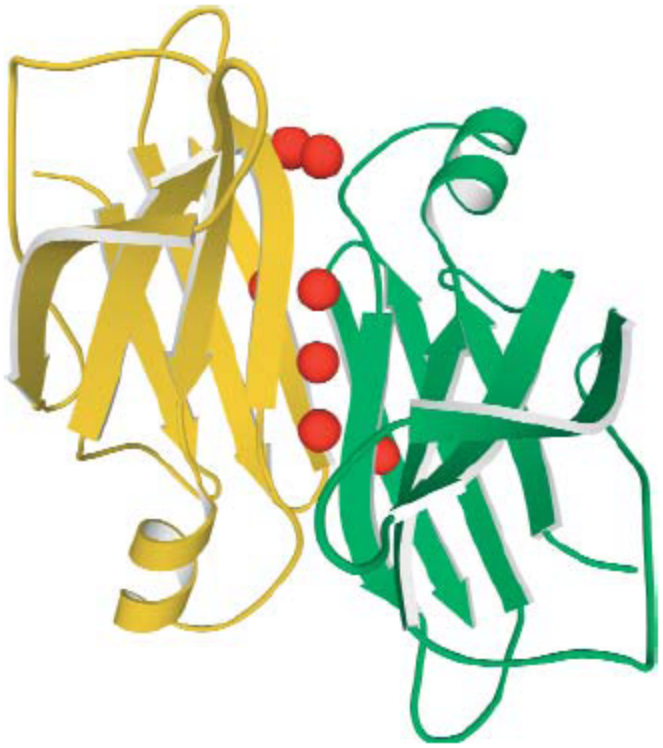
- All Weak bonds
  - Hydrophobic interactions
  - hydrogen bonds and
  - salt bridges
- Cross-linking interactions
  - Disulfide interactions and
  - metal-ion ligation

Protein Interface Properties

- Hydrophobic effect
  - Deserves special mention
  - Amount of surface area that is actually buried at an interface varies greatly
- Salt Bridges
  - Which would usually be found at the exposed surface as they involve charged residues, are surprisingly common at interfaces.
  - Hemoglobin has several intersubunit salt bridges
- Correlation exists between the stability of the oligomer and the type of interaction thatpredominates at the interface.
- Very stable oligomers tend to bury a large hydrophobic surface area between subunits

Protein Interface Properties

- Protein interface atom packaging
- Water Trap
  - Subunits of many oligomeric proteins are only partially folded before oligomerization
  - Their neighbors do they assume their final, correct, tertiary structure
  - Water molecules found at interfaces are essential for preserving the structures of the partially folded monomer units

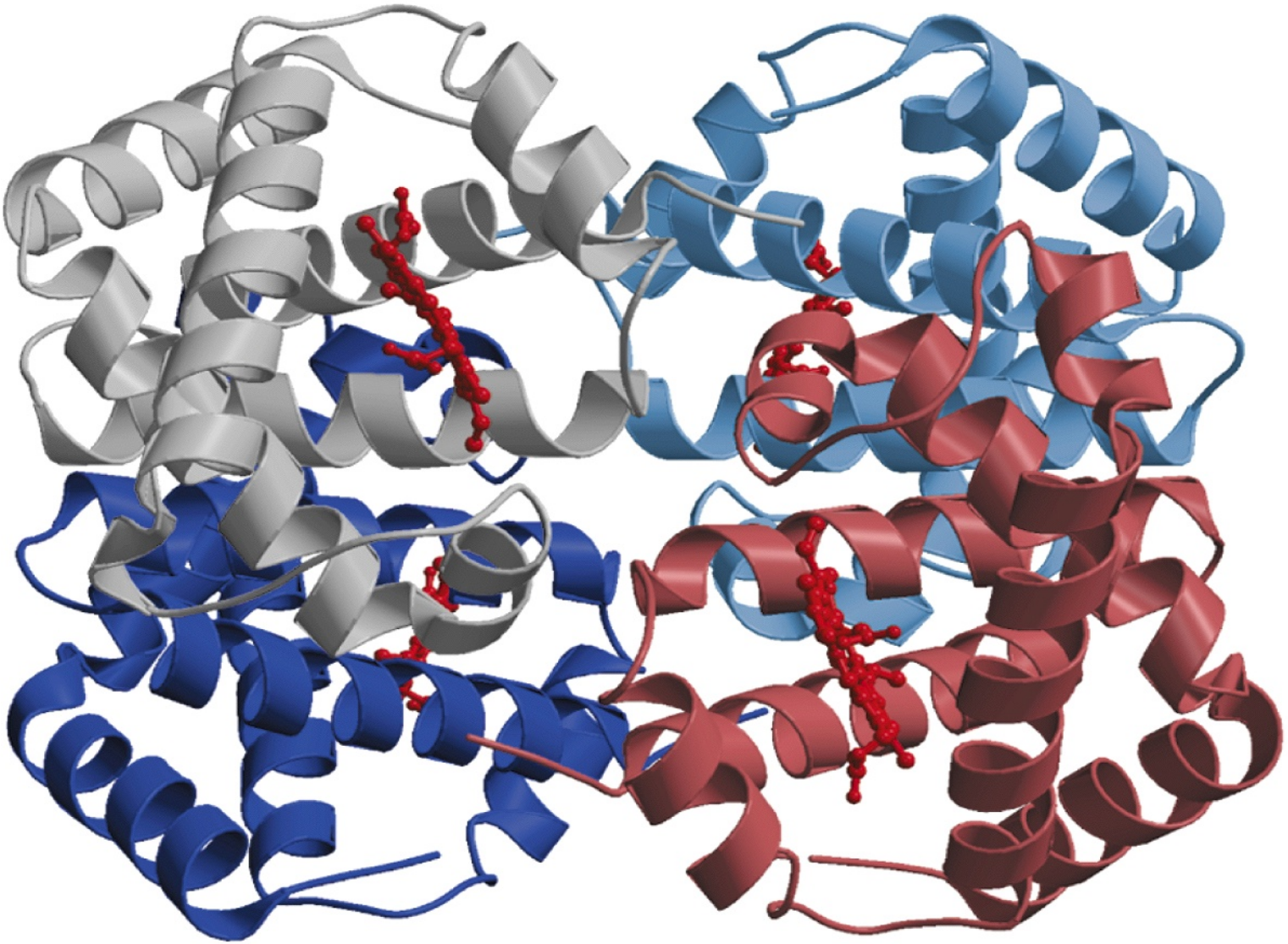


Hydrogen-bonding potentials at the interface

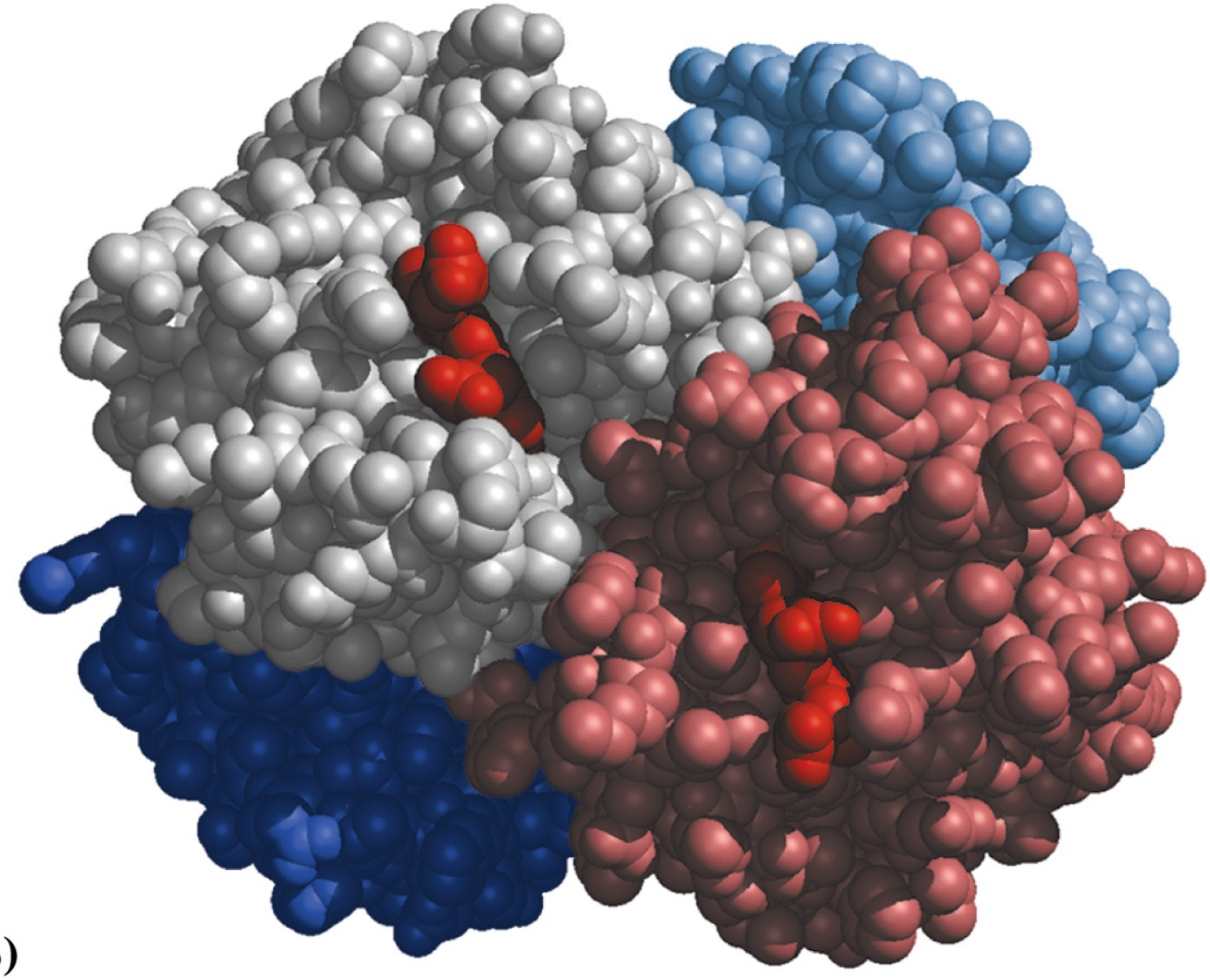
- Many of the hydrogen bonds are between subunits, and the rest are with interfacial waters.
- orient interactions between subunits, and provide much of the specificity for complex oligomerizations.

Inappropriate quaternary interactions

- Genetic diseases, or striking phenotypes, originate from hydrophobic surfaces that are inappropriately created as a result of mutation.
  - Sickle-cell anemia
    - hydrophobic patch that causes hemoglobin tetramers to polymerize into long fibrils
    - abnormal polymerization occurs through the generation of an inappropriate hydrophobic interaction.
- In oligomeric proteins (dominant-negative effect)
  - Mutant subunits produced by one copy of the gene may disrupt the function of normal subunits produced by the other, unmutated, copy so that the effect of the mutation is seen even in the presence of one normal gene: that is, when the individual is heterozygous.



Haemoglobin-An example of quaternary structure i.e. complex formation by multiple subunits.



Haemoglobin-An example of quaternary structure i.e. complex formation by multiple subunits.

Not all proteins are structured: Intrinsically Unstructured Proteins

What are unstructured proteins? Proteins (segments of proteins) that are lacking well-structured 3-dimentional fold. They are referred as "natively denatured/unfolded", "intrinsically unstructured/unfolded".

Why are they relatively obscure? Our view of protein universe was strongly determined by the tools we had: X-ray crystallography will not "see" such proteins, as they difficult to crystallize.

How prevalent are unstructured proteins? About 35-51% of the proteins have unstructured regions that are longer than 50 residues; 6-17% of proteins in the Swiss-Prot are probably fully disordered.

Determined by neural networks predictors (based on the protein sequence).

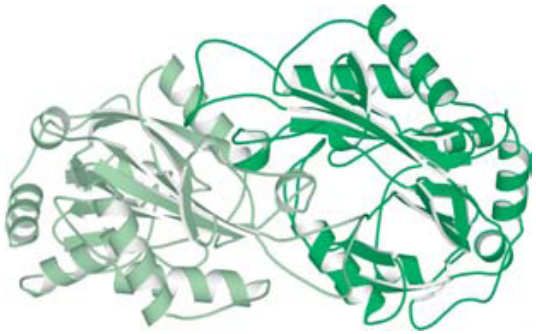
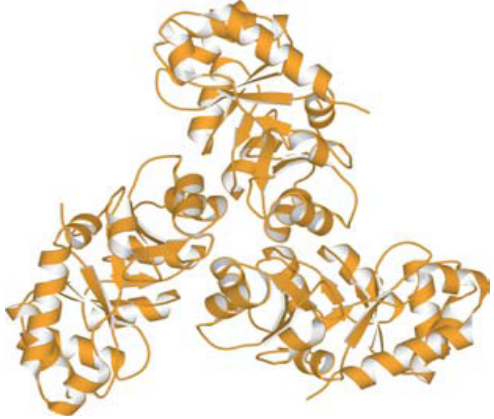
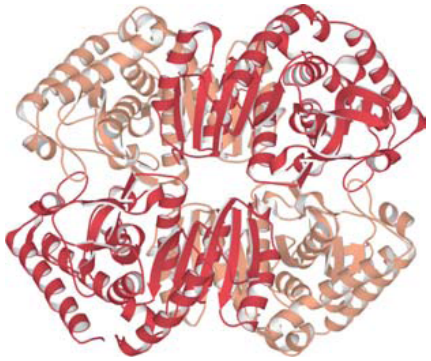
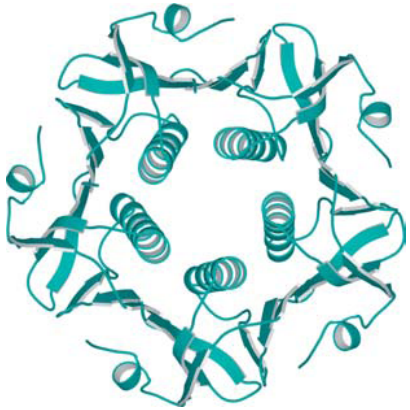
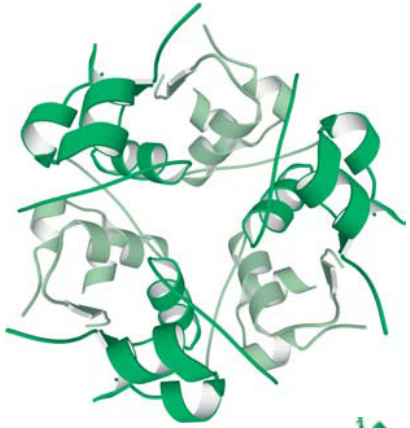
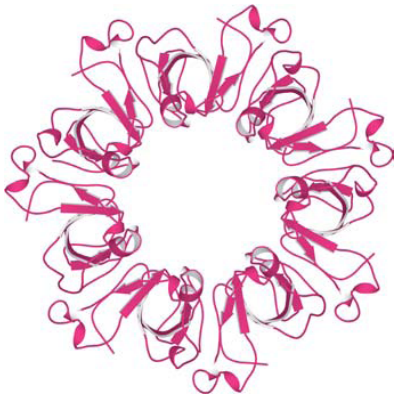
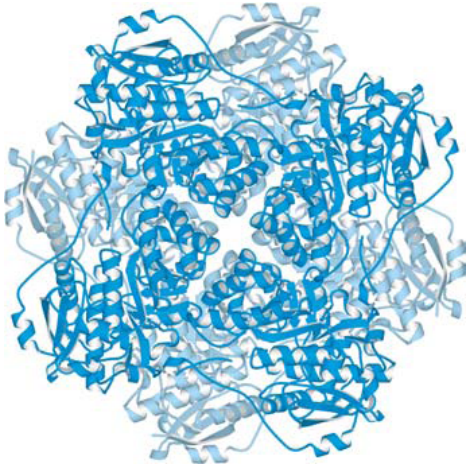
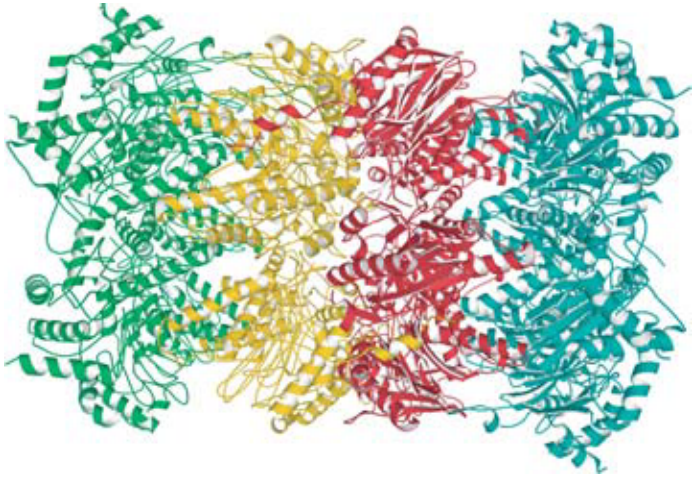
What determines if the protein will be folded or unfolded? There is a sequence signature that describes unfolded regions.

Signature:

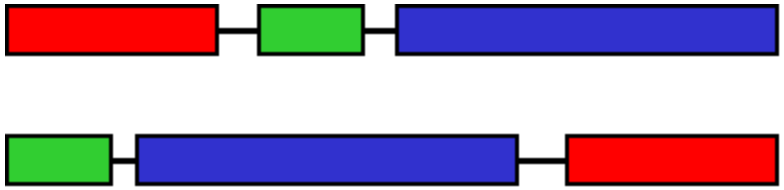
- low sequence complexity
- bias toward polar and charged amino acids (Gln, Ser, Pro, Glu, Lys, and occasionally Ala and Pro)
- bias away from bulky hydrophobic residues (Val, Leu, Met, Phe, Trp, Tyr)

An array of programs are available now to predict disordered regions:

PONDR (Dunker's group)
FoldIndex (Uversky's group)
DisEMBL (Gibson's group)

Protein domains



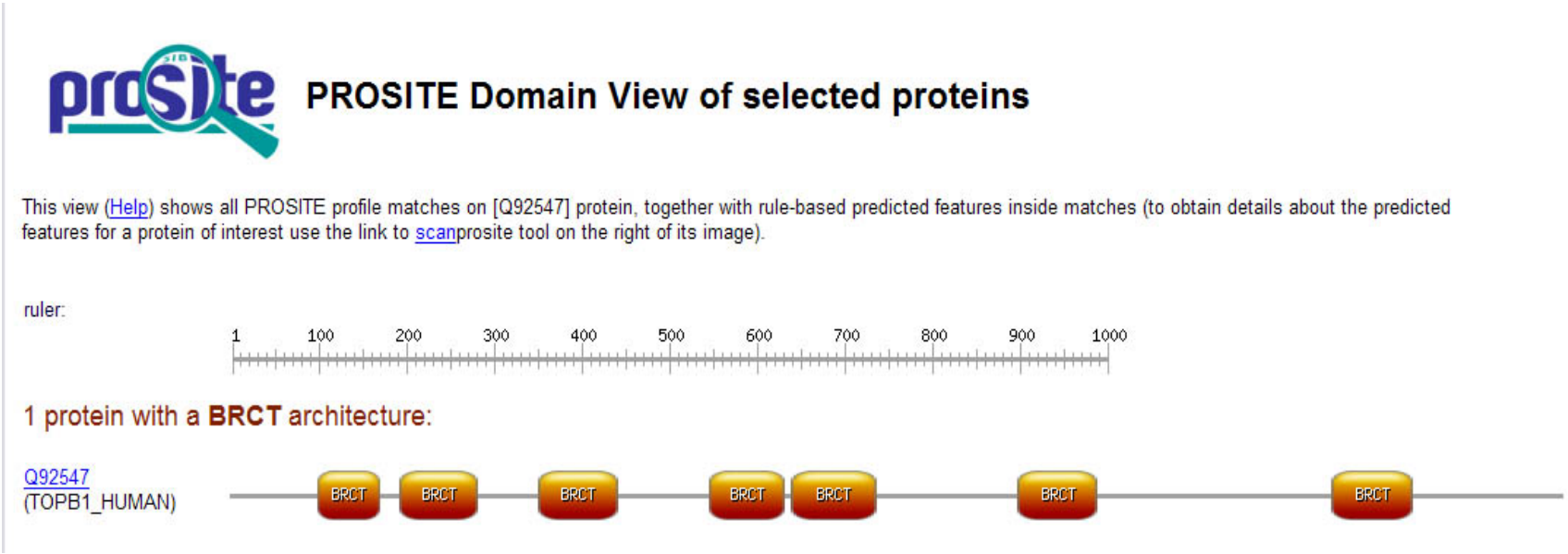
Pairwise sequence comparison of proteins led to strange results

- 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.

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.

GRB2



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.