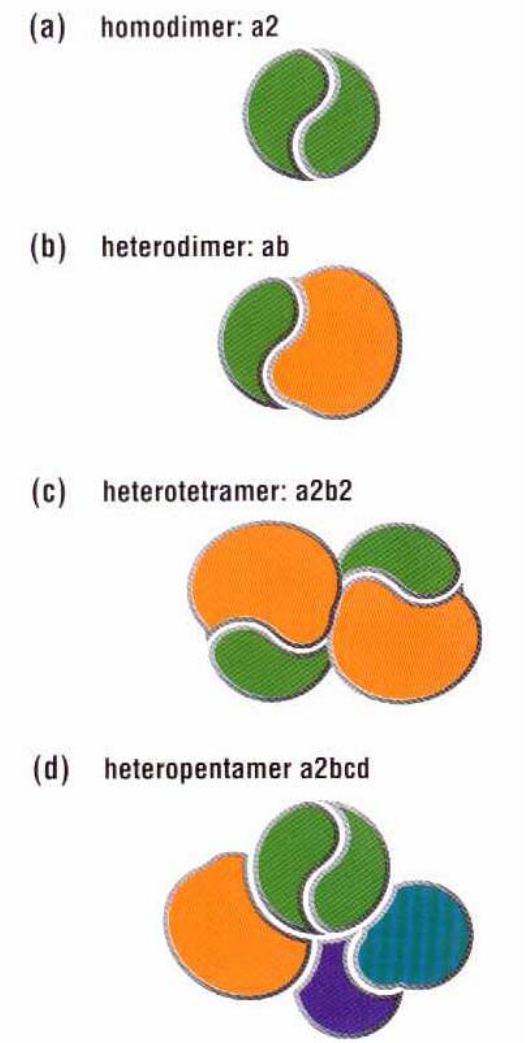
Quaternary structure

- Assembly of multiple polypeptide chains in one integral structure
- The arrangement of the subunits gives rise to a stable structure
- Subunits may be identical or different
- A common shorthand for describing such proteins is to use Greek letters for each type of subunit, and subscript numeral to specify numbers of units.
  - A protein designated $\alpha_2\beta\gamma$ consists of two α units and one each of β and γ

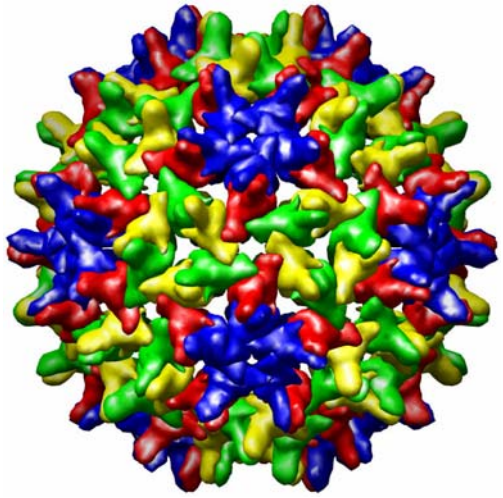


Petsko and Ringe

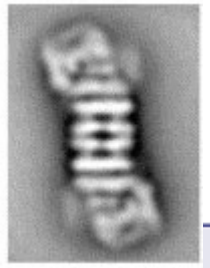
1 = monomer	7 = heptamer
2 = dimer	8 = octamer
3 = trimer	9 = nonamer
4 = tetramer	10 = decamer
5 = pentamer	11 = undecamer
6 = hexamer	12 = dodecamer

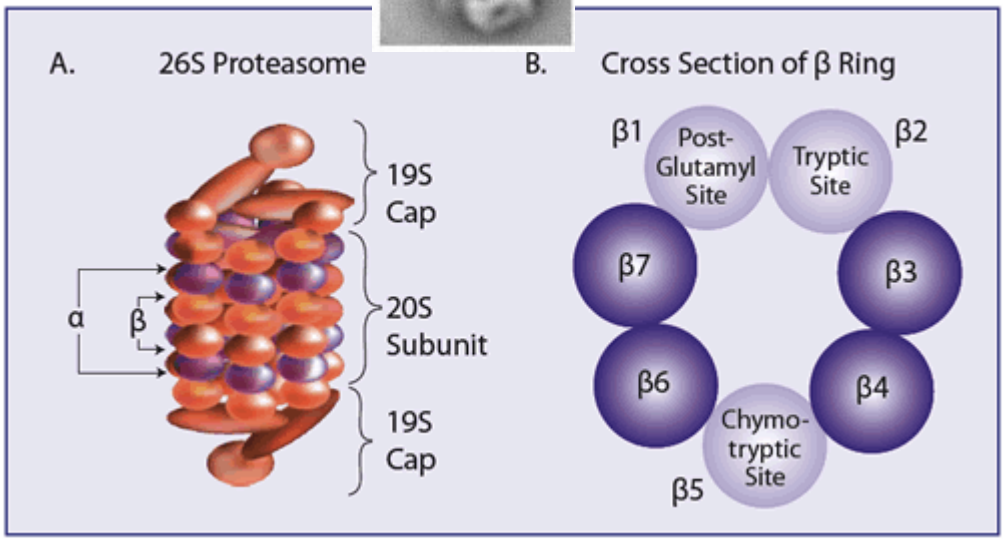
Escher

- Quaternary structure adds stability by decreasing the surface/volume ratio of smaller subunit
- Simplifies the construction of large complexes
  - viral capsids are often composed of multiples of 60 proteins
  - 20s subunit of proteosomes contain four heptameric rings (4 x 7 = 28 subunits)



Hepatitis B virus





proteosome degrades unfolded protein—cellular garbage disposal

Why are some enzymes so large

Small enzymes, e.g. hydrolases, may contain ~ 125 amino acids

Other enzymes, e.g. large dehydrogenase, may contain > 60,000 amino acids

- Large structure provides rigidity necessary to orient the substrate and key amino acids to enable catalysis
  - e.g. extremely small proteins require metals or disulfides for stability
- Large surface area can funnel small substrates to the active site—e.g. electric field gradient
- Substrates need to be shielded from the solvent
- Surface/volume ratio decreases with size, and reduces the tendency to aggregate

Klapper et al, Proteins 1, 47 (1986)

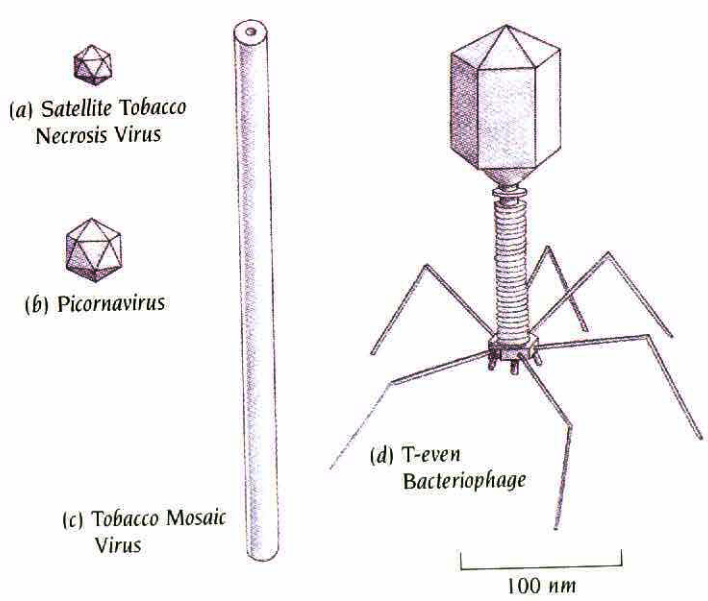
Viral capsid

Virus comprises a genome made of either DNA or RNA, a protein shell ("capsid") around it, and lipid bilayer outside in some cases

A nucleic acid cannot code for a single protein molecule large enough to enclose it. Therefore, many copies of short polypeptides must assemble to build the capsid

Structural information of viral capsid can lead to the rational design of antiviral drugs

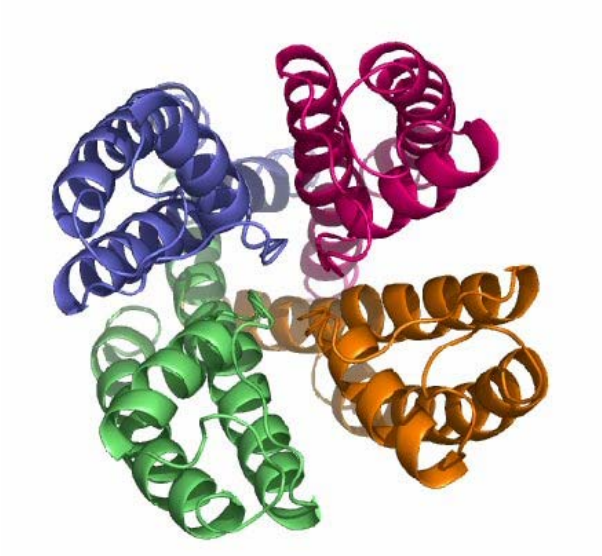
- prevent viral infection by blocking the interaction between viral protein and cellular target
- prevent viral assembly by disrupting quaternary association of viral capsid proteins

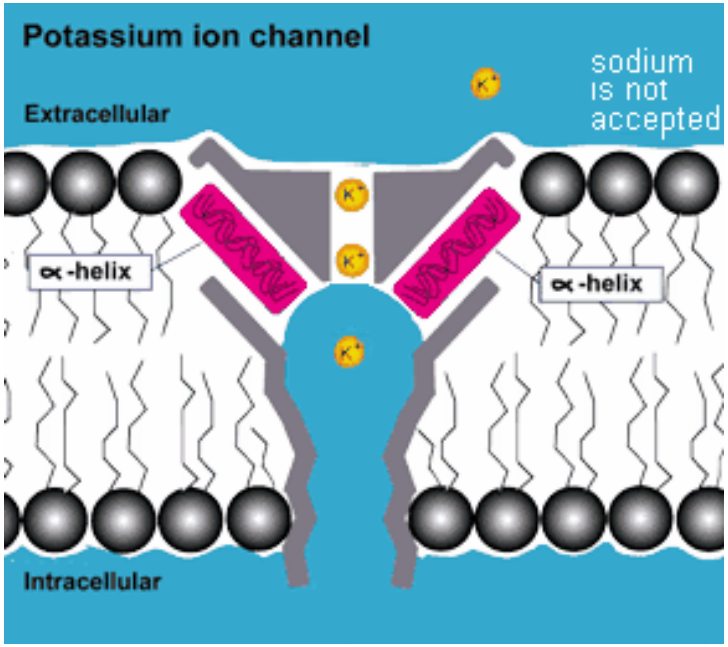


Branden & Tooze, Ch 16

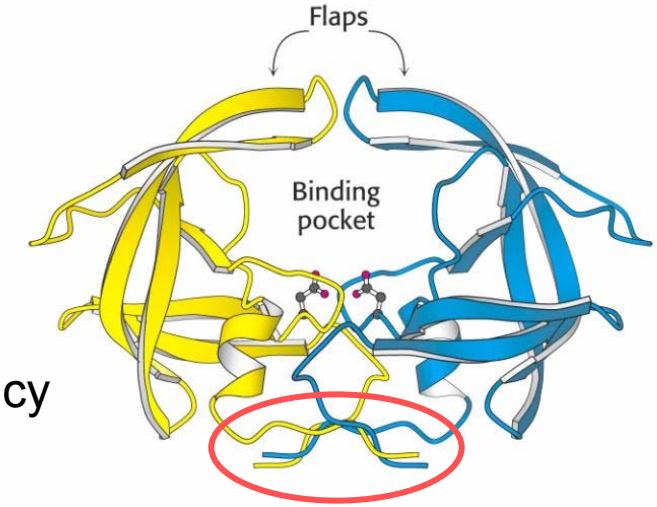
Arrangement of subunits

Most protein multimers have significant rotational symmetry in the placement of the subunits





Potassium channel
Chemistry Nobel prize, 2003



Human immunodeficiency virus aspartyl protease

Advantages of building protein complexes

- Easier to evolve—shorter genes
- Easier to transcribe and translate—quicker response
- Robust against error in transcription/translation (1 in 2000 amino acids)

- Additional layer of regulation
  - Allosteric properties not present in monomer
  - Many multiprotein complexes regulate their physiological function through conformational changes
  - Changes in quaternary structure can occur through **conformational** changes within individual subunits or through **reorientation** of the subunits relative to each other.

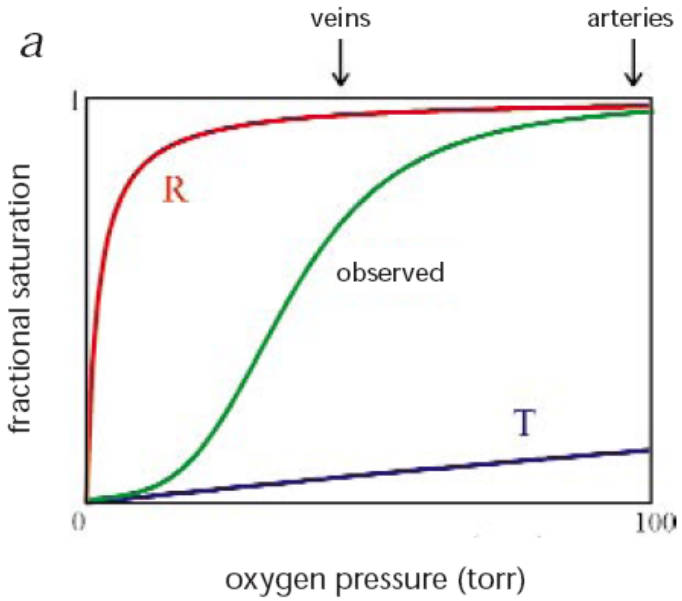
Hemoglobin v. myoglobin

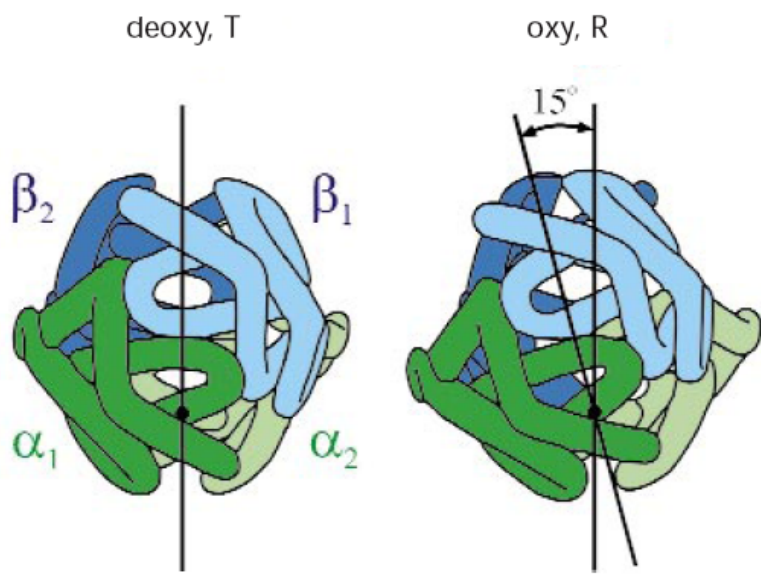
Hemoglobin: heterodimer of dimer ($\alpha 2 \beta 2$)

Oxygen binding in hemoglobin is highly cooperative

cooperativity is achieved through domain rotation

compare with myoglobin, which is a monomeric protein

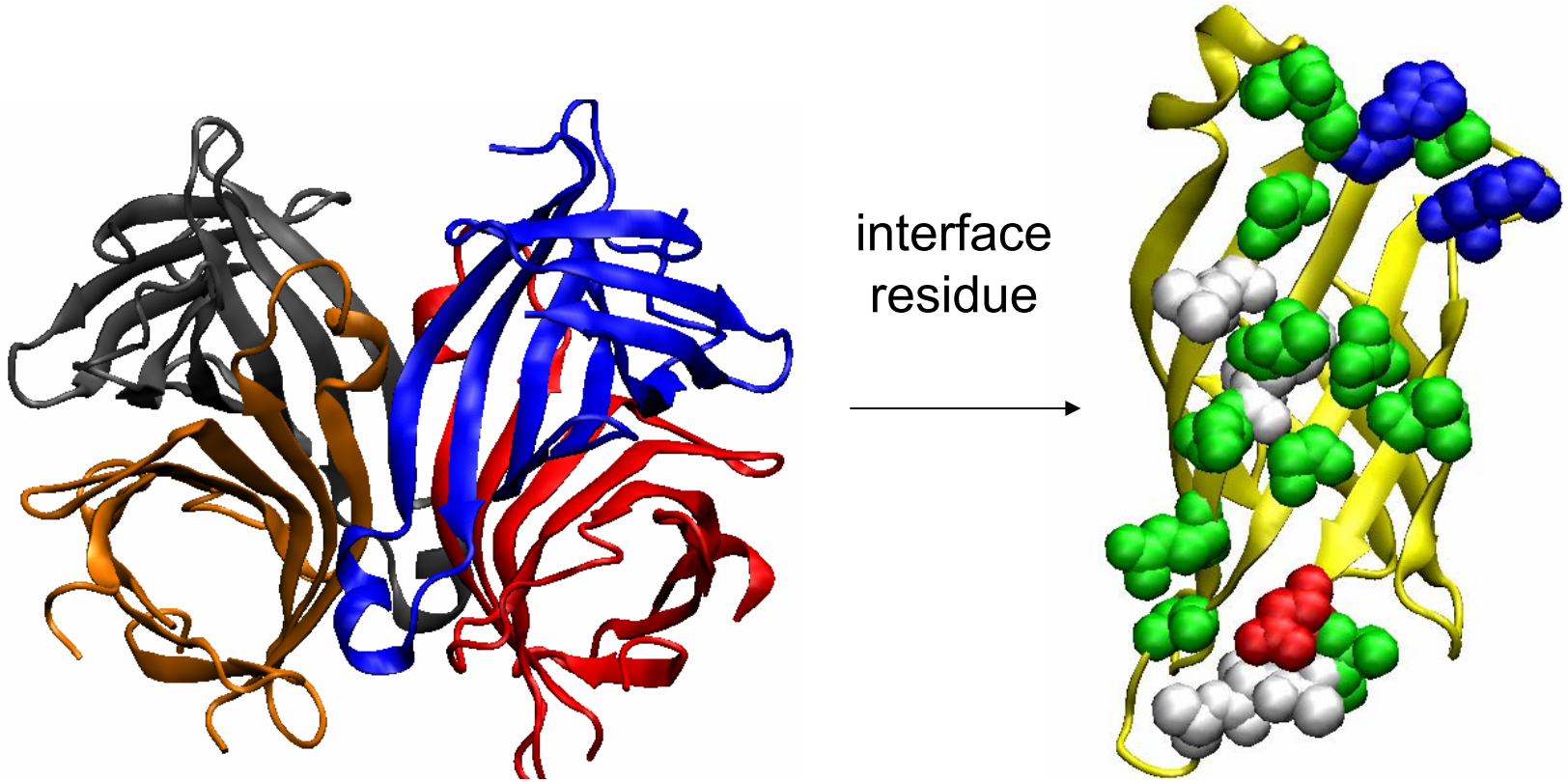




Eaton et al, NSB 6, 351 (1999)

Formation of quaternary structure

- The subunits are held together by both **hydrophobic interactions** and **ionic interactions** between polar/charged amino side chains
  - a quaternary structure may fall apart in high salt environment
- A monomer typically buries 600 – 5000 Å of surface
- Understanding protein-protein interaction is key to understanding and controlling the formation of protein complexes

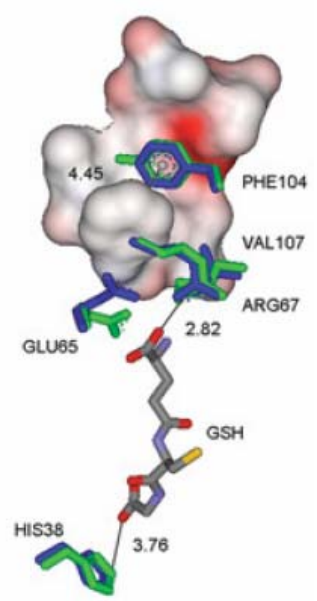


“Lock and key” at the dimer interface

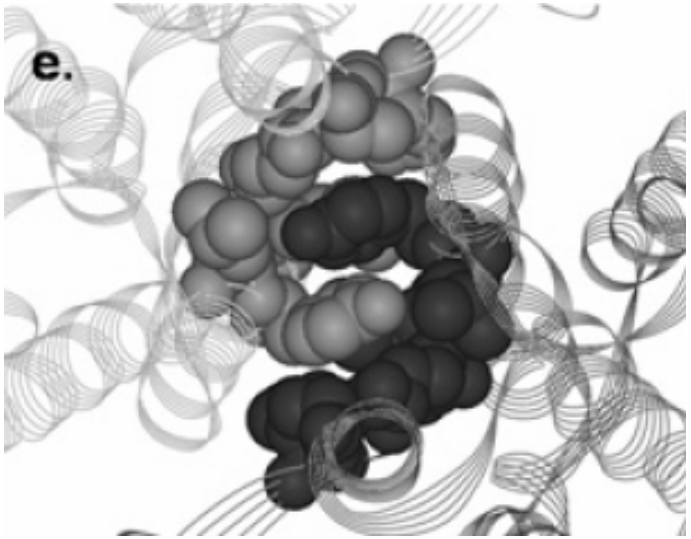
Glutathione S transferases (GST) are dimeric enzymes that neutralize nonpolar exogenous toxic compounds by nucleophilic addition of glutathione

A dimeric quaternary structure is essential for function and the active site is formed by amino acids from both subunits

The association relies on hydrophobic interaction in which an aromatic ‘key’ from one domain inserts into a hydrophobic pocket (‘lock’) of the other domain



	Key residue			Lock residue			
Enzyme*	104a†	65	67	68	103	104b†	107
adGSTD4	F	E	R	A	L	F	V
adGSTD1	F	E	R	A	L	F	M
adGSTD2	Y	E	R	A	L	Y	M

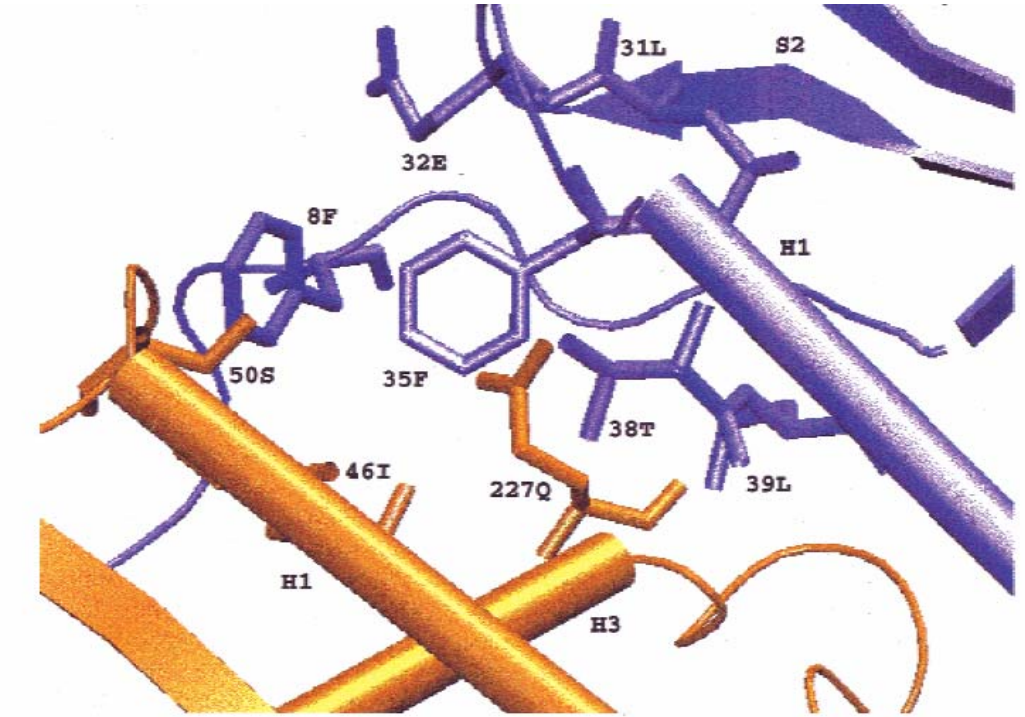


Wongsantichon & Ketterman, Biochem J 394, 135 (2006)

E. coli RNA polymerase dimer

The N terminal domain (NTD) is thought to be involved in dimer formation

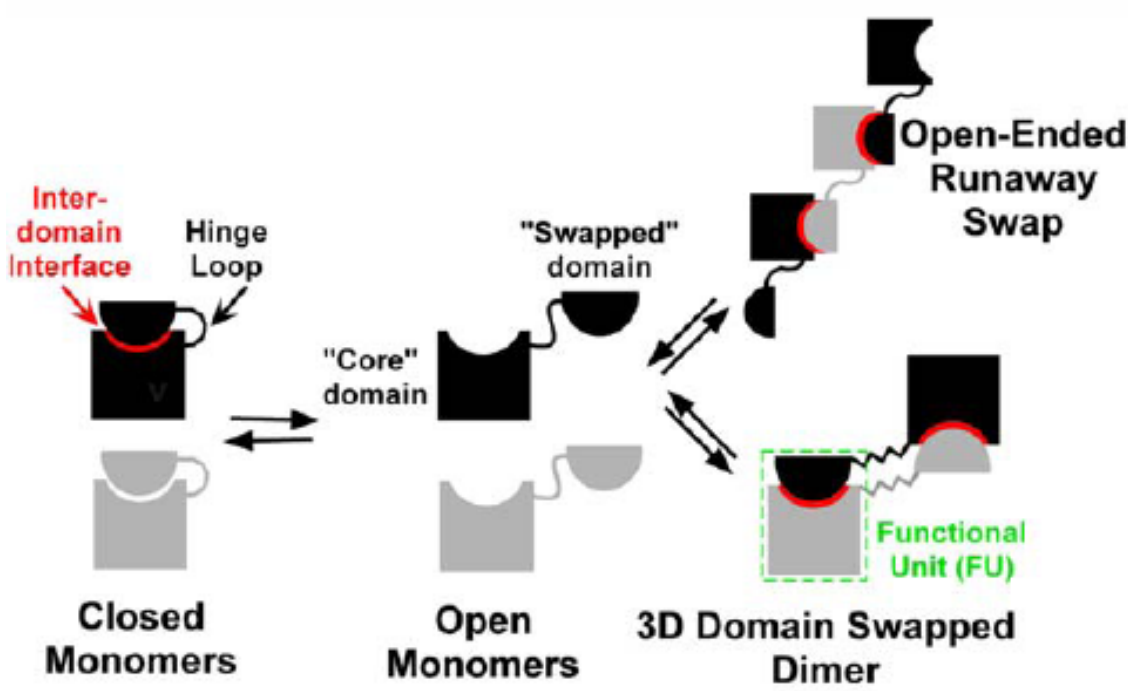
The interface consists of both polar (E32, T38, S50, and Q227) and hydrophobic (F35, F8, L31, L39, I46) residues, which together form a cluster that provides more stability than a single pair of polar–polar or hydrophobic–hydrophobic interaction



Kannan et al, Protein Sci 10, 46 (2001)

3D domain swapping

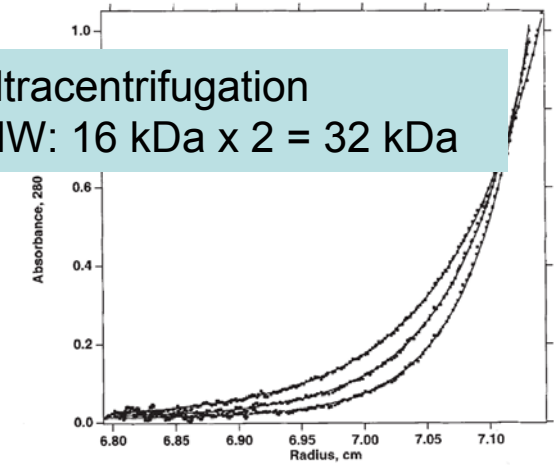
- A common mechanism for forming oligomeric proteins from monomers
- Creates an interface between different polypeptide chains which is identical to that seen within the monomer
- The interface for dimerization has already been evolved and optimized
- Monomers may exchange a secondary structural element (strand or helix) or an entire domain



Bennett et al. Structure 14, 811 (2006)

Altering quaternary structure

- Engineer monomeric staphylococcal nuclease into a dimer
- Deletion creates steric clash involving side chains and promotes dimerization



ultracentrifugation
MW: 16 kDa x 2 = 32 kDa

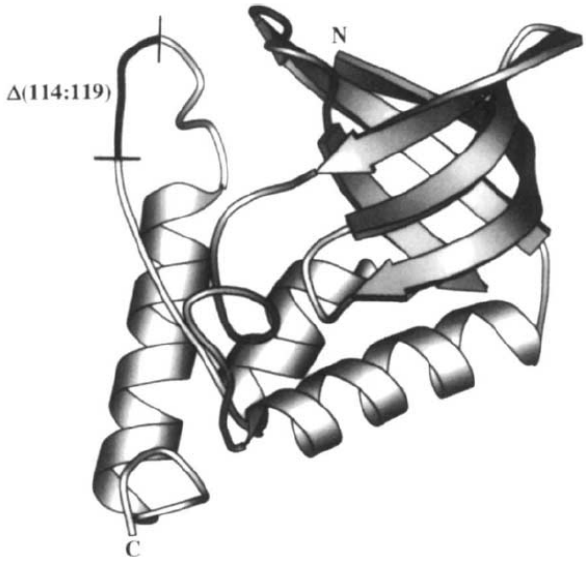
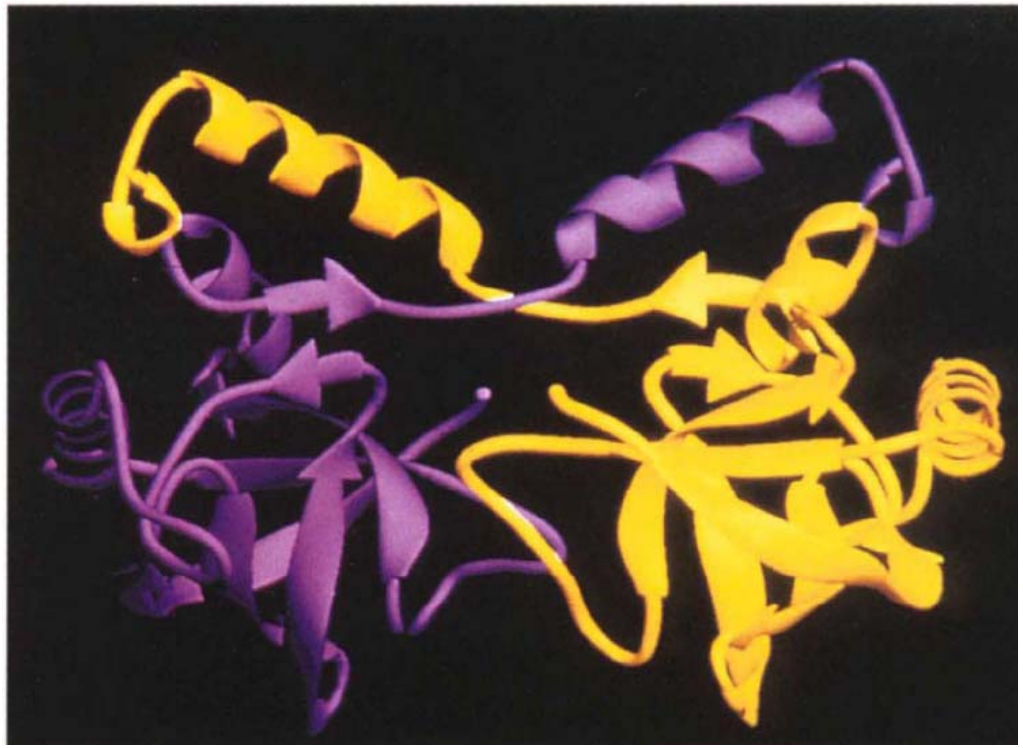
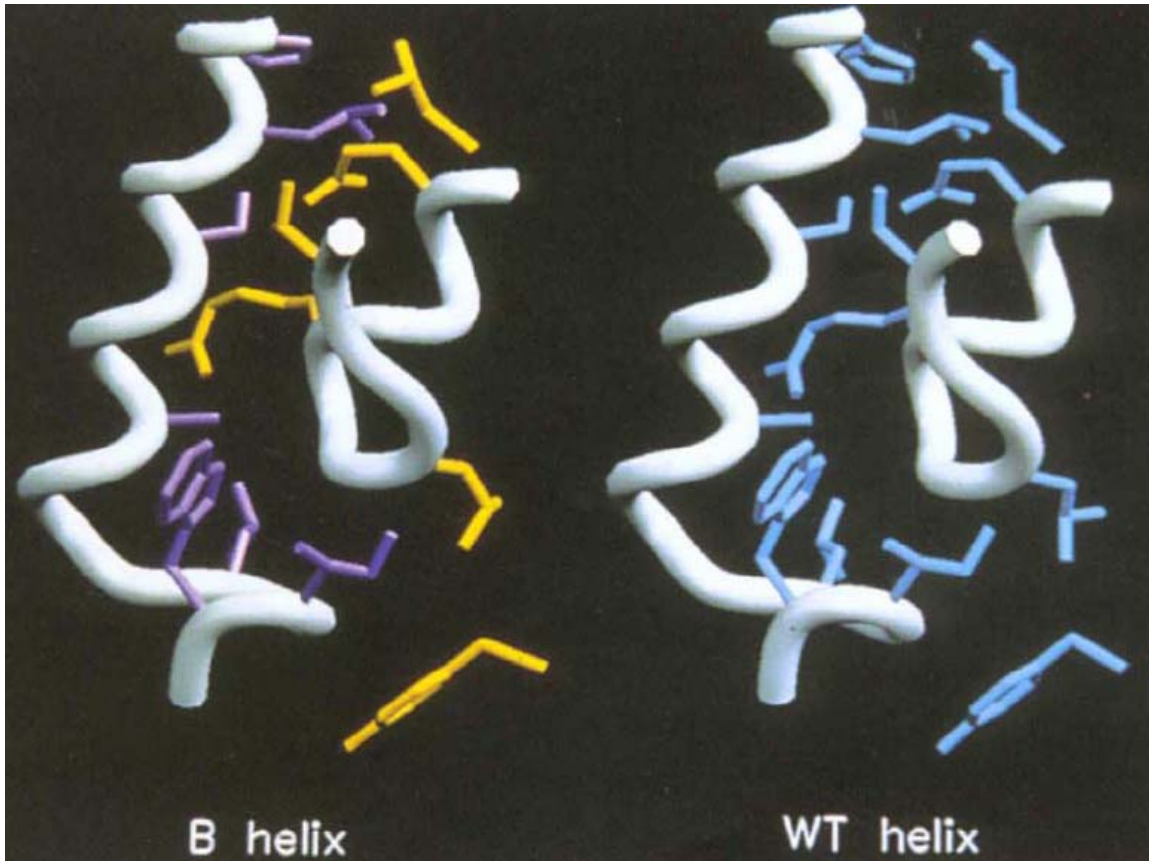


Fig. 1 Ribbon diagram showing the location of the mutation relative to the tertiary structure of staphylococcal nuclease. Residues 114–119 which are deleted in the mutant are highlighted in black. This figure was drawn using MOLSCRIPT[23] and the coordinates of Loll and Lattman[24].

Green et al, NSB 2, 746 (1995)

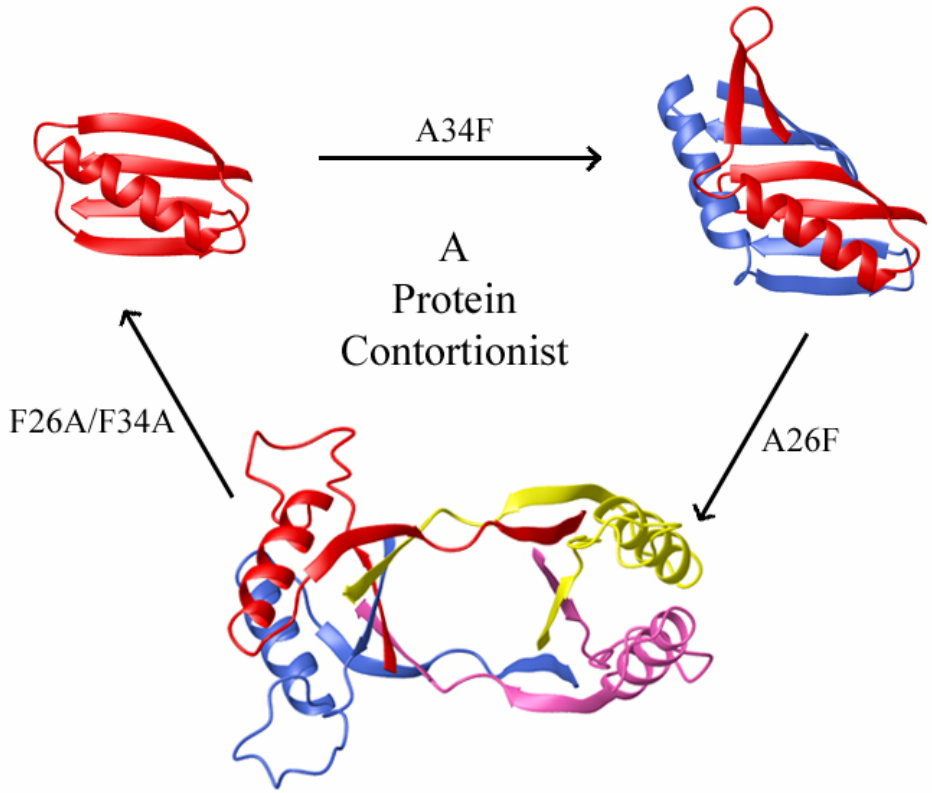




The details of the interface are maintained in the mutant

Protein G

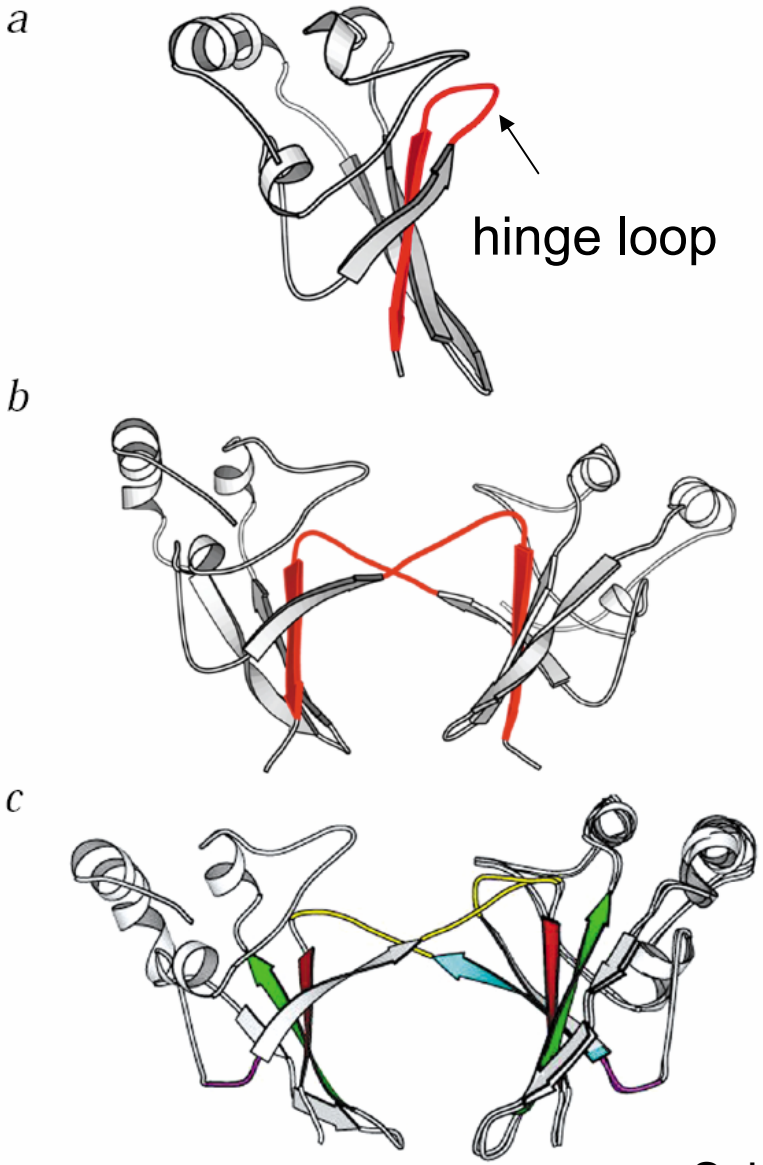
Highly stable and compact proteins (e.g. immunoglobulin binding domain of protein G) can change its **topology** and **quaternary structure** by a few changes in core residues



Byeon, et al JMB 333, (2003)

Strand exchange

p13suc1



Natural proteins often dimerize by exchanging a strand or a helix

Domain-swapped structures are biologically significant

In p13suc1, the hinge loop works as a loaded molecular spring

Mutation in the hinge loop shifts the monomer-dimer equilibrium

The binding of a ligand (e.g. phosphate and phosphopeptide) also shifts the equilibrium in wild type by altering the dynamic properties of the native state ensemble

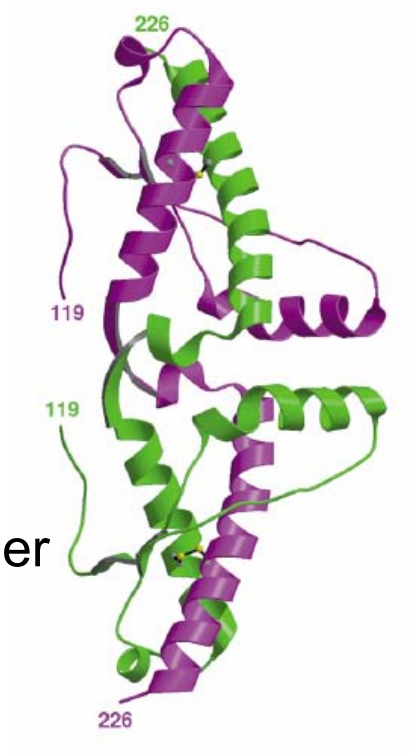
Schymkowitz et al, NSB 8, 888 (2001)

Domain swapped protein fibrils

may be involved in diseases

prion, hereditary hemorrhagic stroke disorder

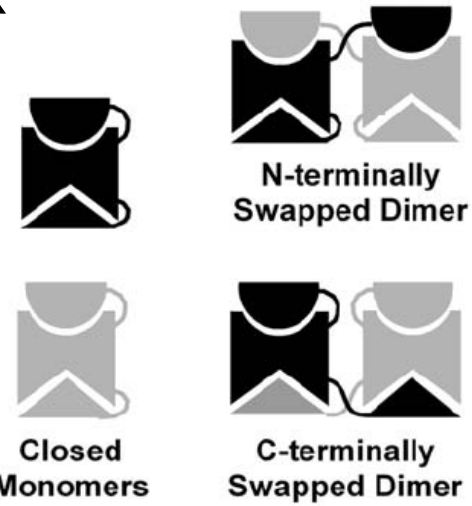
prion disease mutations map to domain swapping helix

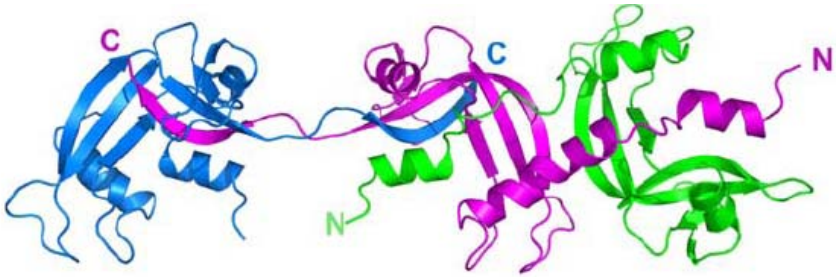


prion dimer

Knaus et al, NSB 8, 770 (2001)

consecutive domain swapping is also possible leading to fibril formation





Bennett et al. Structure 14, 811 (2006)

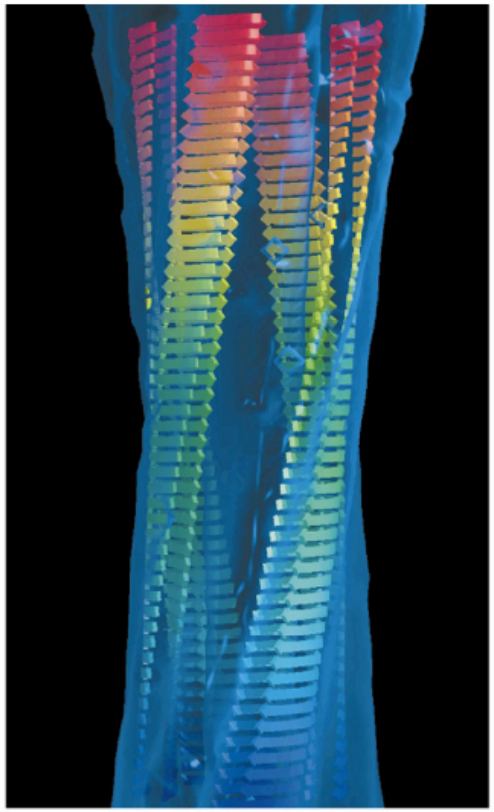
Deposition diseases

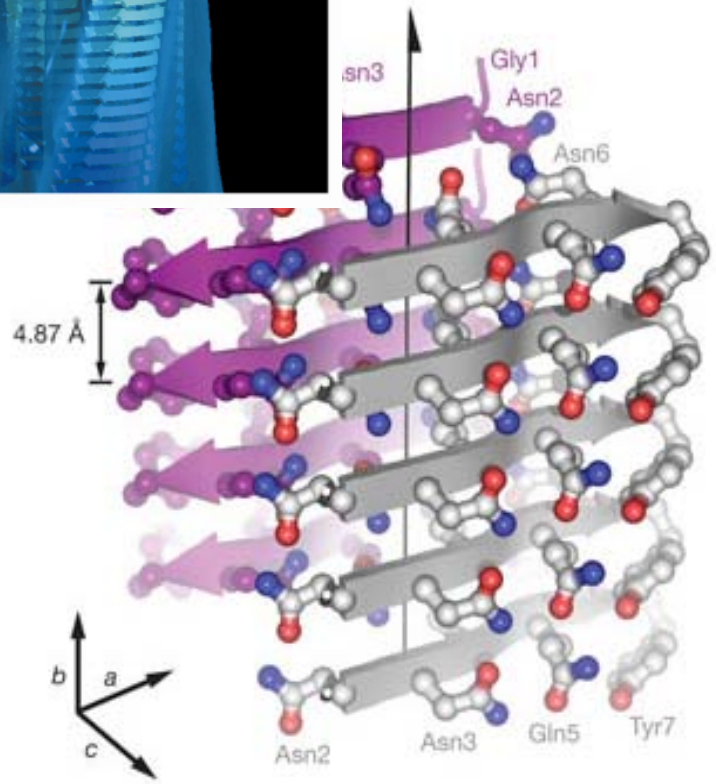
Deposition diseases are "**conformation**" diseases characterized by aggregation of native proteins

- –Amyloidic : ordered fibril like deposits of proteins
  - »Alzheimer's, Parkinson's, Huntington, Type II diabetes
  - »beta strands running perpendicular to the fibril axis
- –Non-amyloidic fibrils or aggregates
  - »sick cell anemia
  - »serpinopathies

The proteins implicated in these diseases usually perform well-characterized, essential biological functions under normal circumstances … until things fall apart

A change in environment or genetic predisposition exposes a protein for the growth of aggregates—unintended quaternary structure





Mechanism of fibril formation

3D domain swapping
End-to-end stacking
Cross-beta spine

Amyloids

Ordered fibrillar aggregations

Adopt a cross-β structure

- Fiber diffraction, EM, etc.
- 1.8 Å structure of yeast prion protein

Involve conformational changes

- (Partial) denaturation
- Proteolysis

All proteins may be induced to form amyloid fibrils

Nelson et al, Nature 435,773 (2005)

Etiology of amyloid disease

Amyoid diseases are **folding** diseases—a change in the primary and/or tertiary structure results in the formation of undesirable quaternary structure

This is an example of the breakdown in the principle of “negative design”

Negative design : structural elements that disfavor all non-native structures, including unfolded structure, oligomerization, alternate topologies

Q: How does a protein protect itself from forming unintended aggregates?

We need understand the sequence-structure relationship better to predict what sequences are amyloidic