Protein folding

How does a protein fold to its native conformation?
Protein folding is not a random process

Protein folding pathways: a one-way process

Takes less than a few seconds, because via directed pathways
As protein folds, conformational stability increases sharply
(free energy decrease)

A hypothetical protein folding pathway →

Efficient folding is also important

Proteins have evolved to have efficient folding pathways as well as stable native conformations

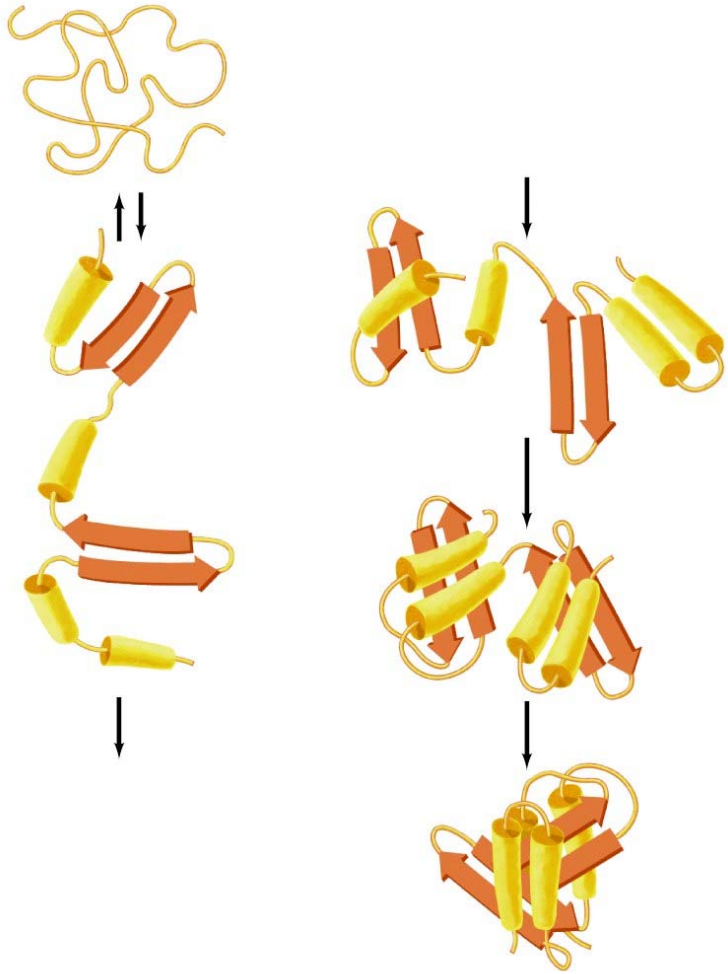
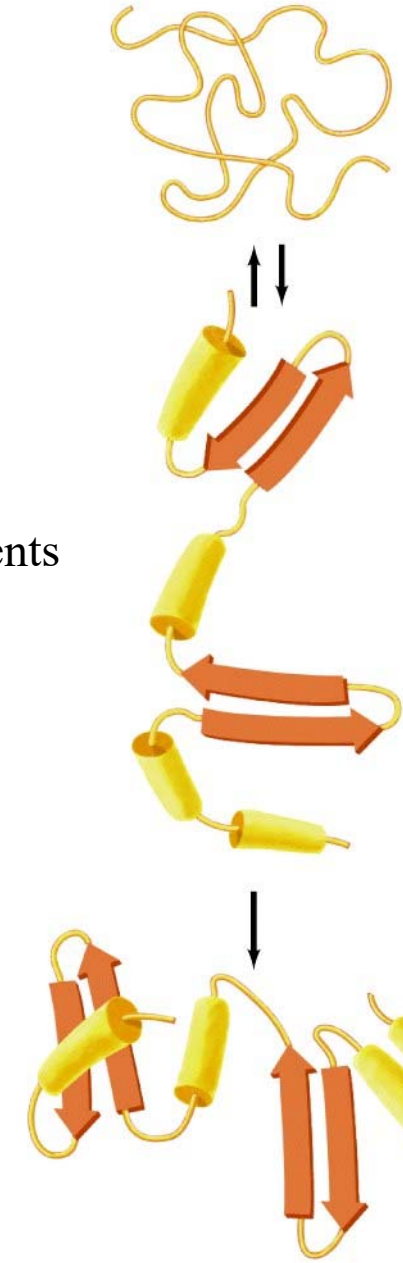


Figure 6-40 Fundamentals of Biochemistry, 2/e



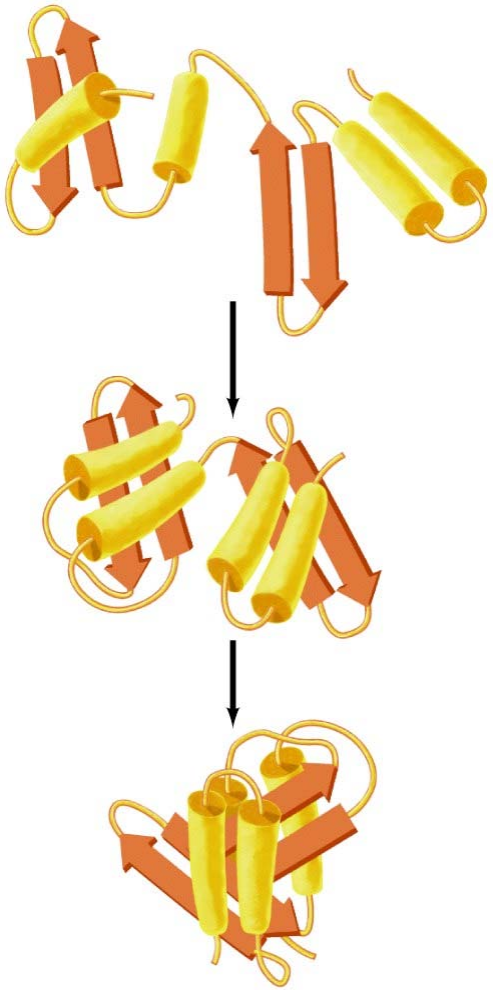
5 ms

Formation of local segments of secondary structure

Hydrophobic collapse

5-1000 ms

Figure 6-40 part 1 Fundamentals of Biochemistry, 2/e



Molten globule
(protein folding intermediates)

A few seconds

Side chain packing

Figure 6-40 part 2 Fundamentals of Biochemistry, 2/e

Protein appear to fold in a hierarchical manner,
with small local elements of structure forming
and then coalescing to yield larger elements,
which coalesce with other such elements to form yet larger elements, etc.

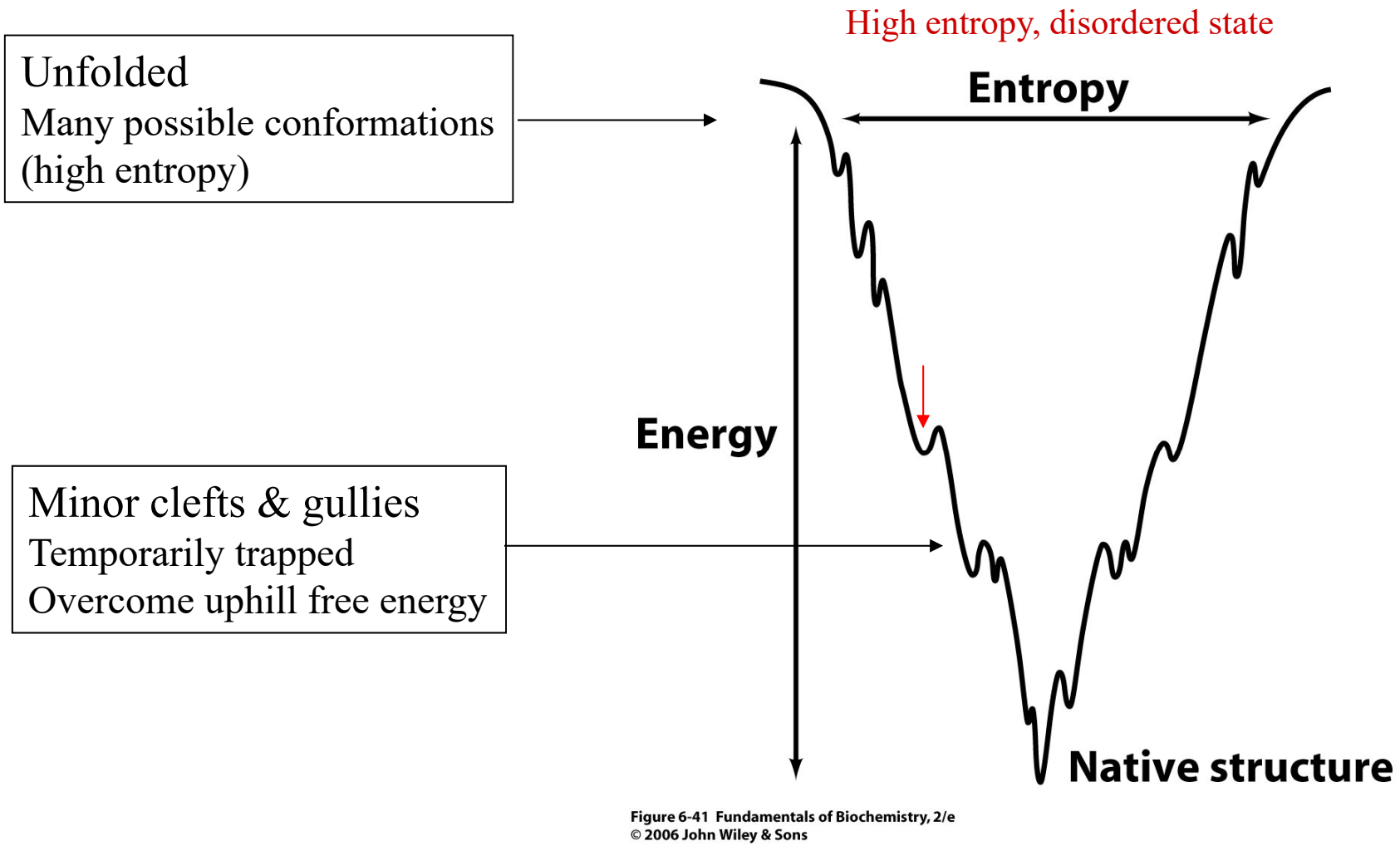


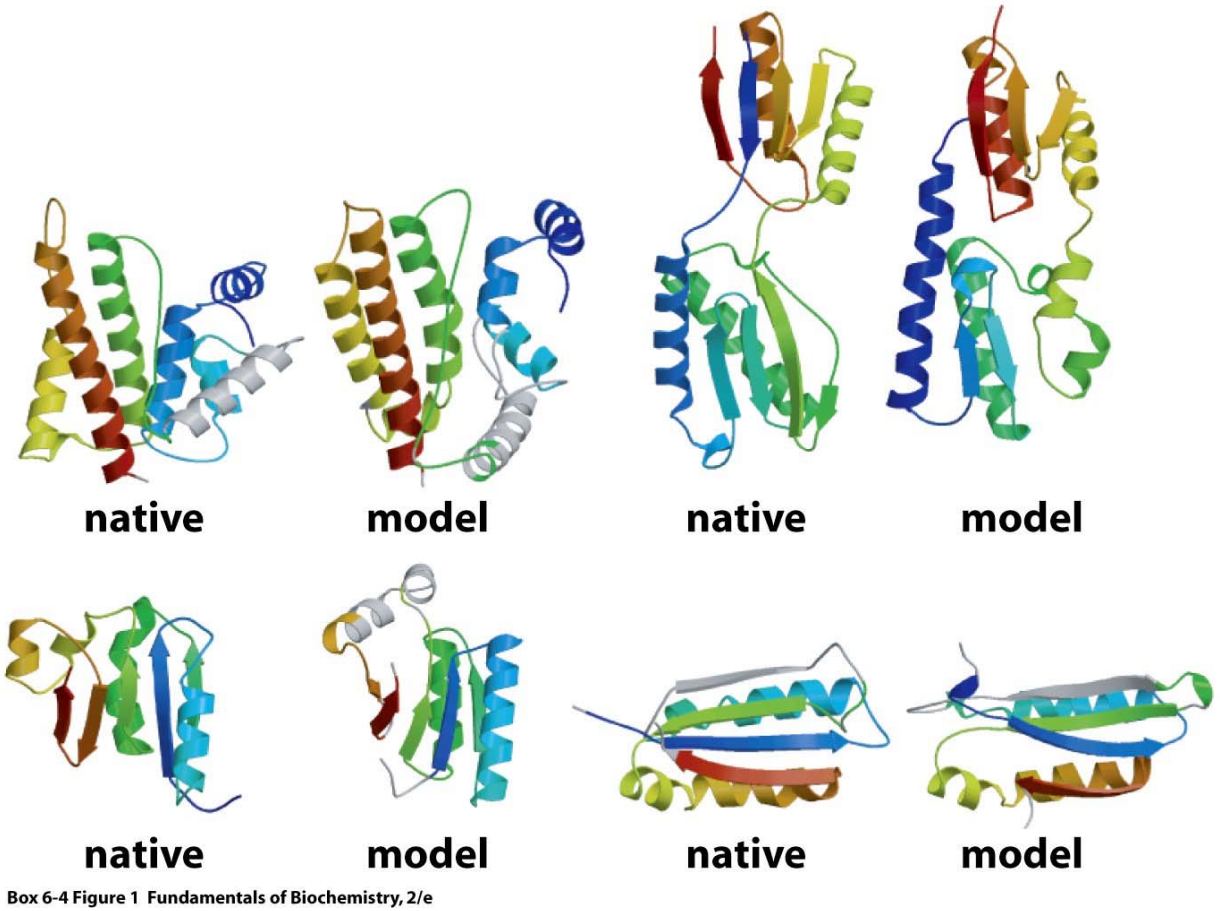
Figure 6-41 Fundamentals of Biochemistry, 2/e

Folding funnel

Energy-entropy diagram for protein folding

Protein structure prediction

Homology modeling: based on sequence homology of determined protein structure
Threading: computational technique based on the known protein structure
Ab initio (from the beginning): moderately successful

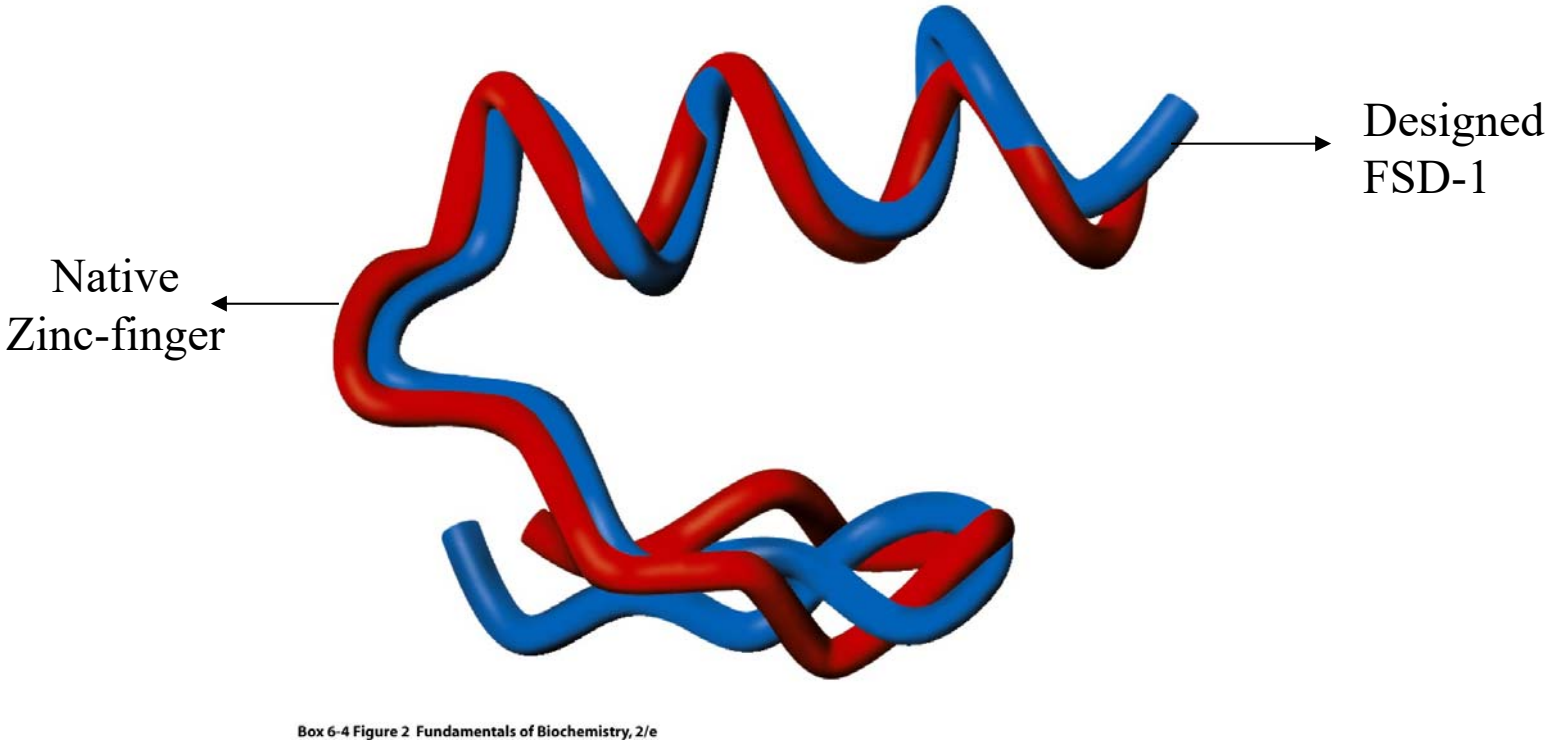


Box 6-4 Figure 1 Fundamentals of Biochemistry, 2/e

Comparison of experimentally determined (native) and predicted (model) folds of polypeptides

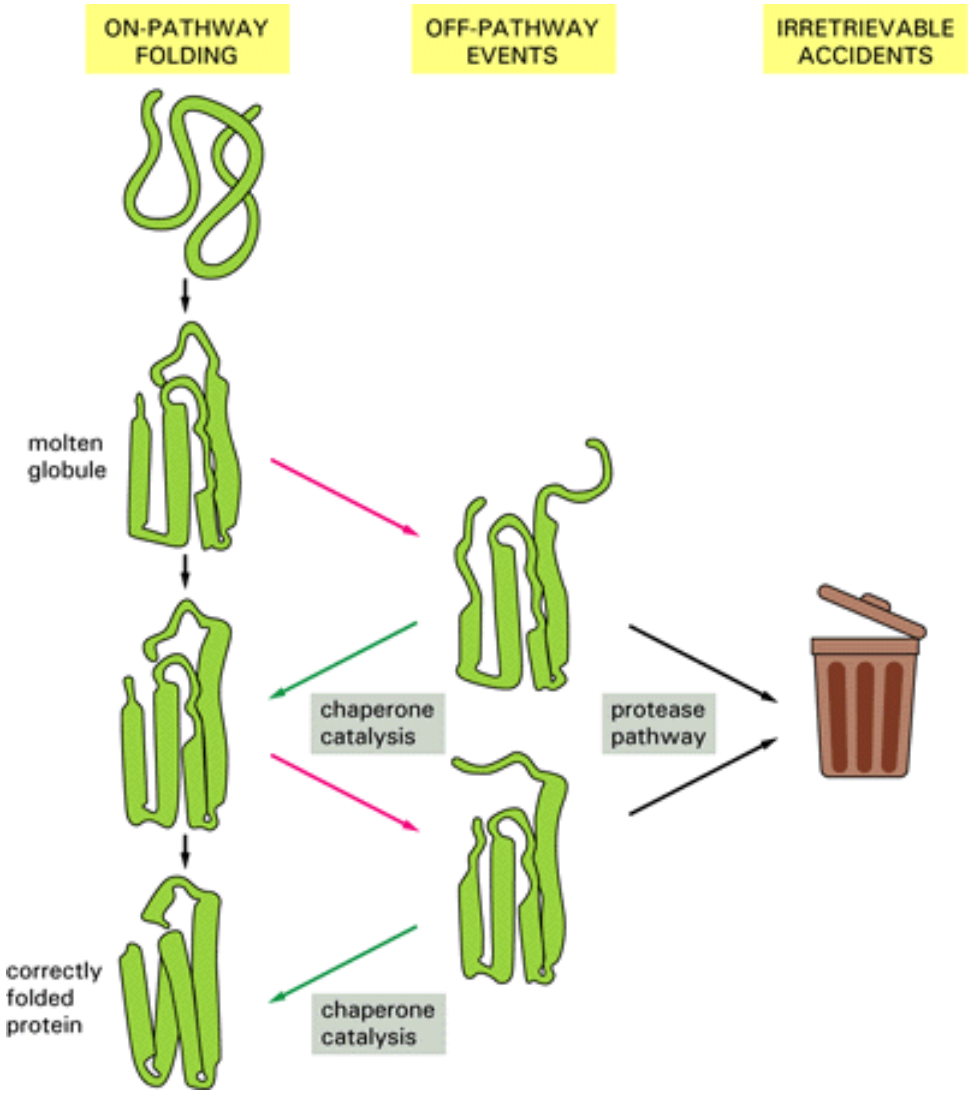
protein design

The great challenge of getting the polypeptide to fold to the desired conformation



Structure of a designed protein through computational calculation

Molecular chaperones



Mechanism of protein disulfide isomerase

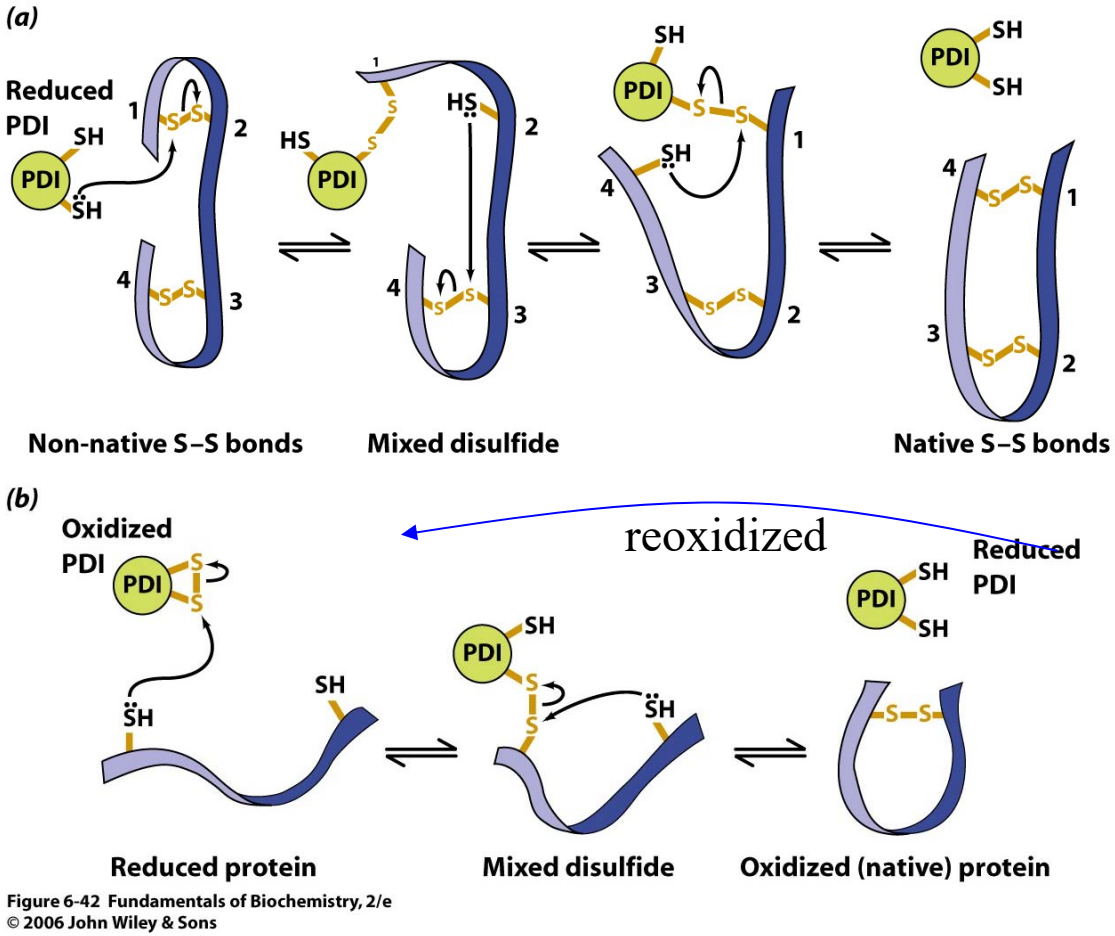


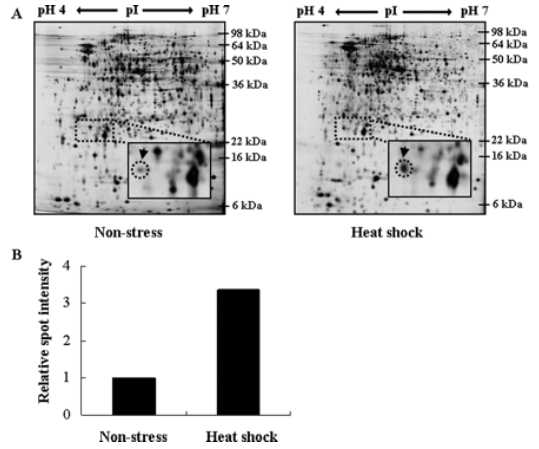
Figure 6-42 Fundamentals of Biochemistry, 2/e

Rearrangement of disulfide bond

Initial formation of disulfide bond

Molecular chaperones

Essential proteins that bind to unfolded and partially folded polypeptide chains
to prevent the improper association of exposed hydrophobic segments
that might lead to non-native folding as well as polypeptide aggregation
and precipitation



Especially important for multidomain and multisubunit proteins

Heat shock proteins (Hsp)

Several classes

- Hsp70 family: in both prokaryotes and eukaryotes
  - function in association with Hsp40 (cochaperones)
  - prevent premature folding: initial role
  - unfold & refold for membrane transport
- chaperonins: large multisubunit proteins
- Hsp90 proteins: mainly involved with signal transduction proteins
- many are ATPases

Hsps (ATP-dependent), small HSPs, cochaperones

Chaperonins

consists of Hsp60 and Hsp10
GroEL & GroES: in *E. coli*, the best characterized

X-ray structure of GroEL

homopolymer of 14 subunits (549-residues forming three domains)
D7 symmetry of hollow cylinder (inner diameter ~45 A)
constriction in the center (no passage between the rings)

(side-view)

(top-view)

3 domains
Apical
Intermediate
Equatorial

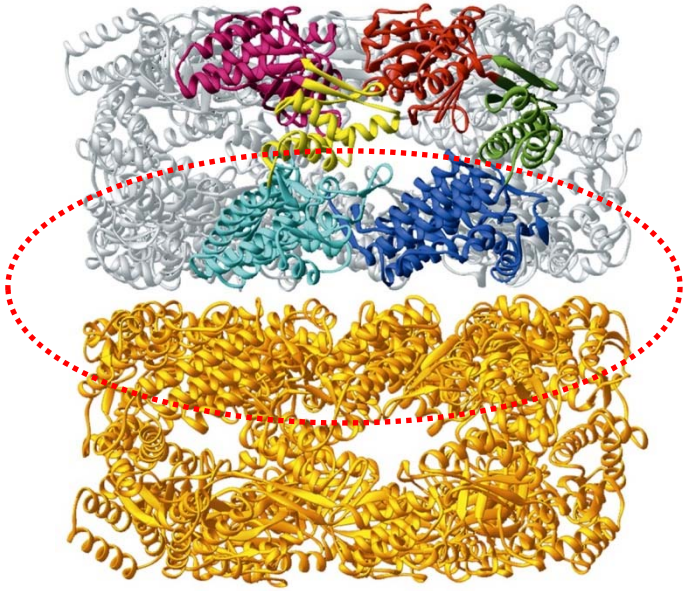


Figure 6-43a Fundamentals of Biochemistry, 2/e

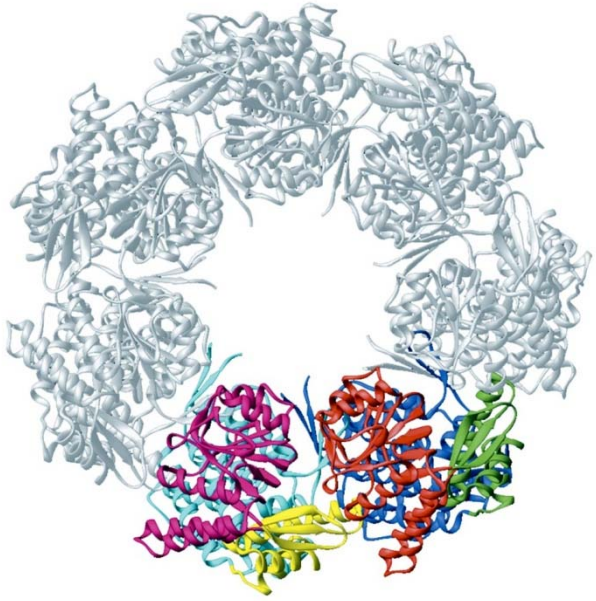


Figure 6-43b Fundamentals of Biochemistry, 2/e

X-ray structure of GroES (top-view)

heptamer with C7 symmetry

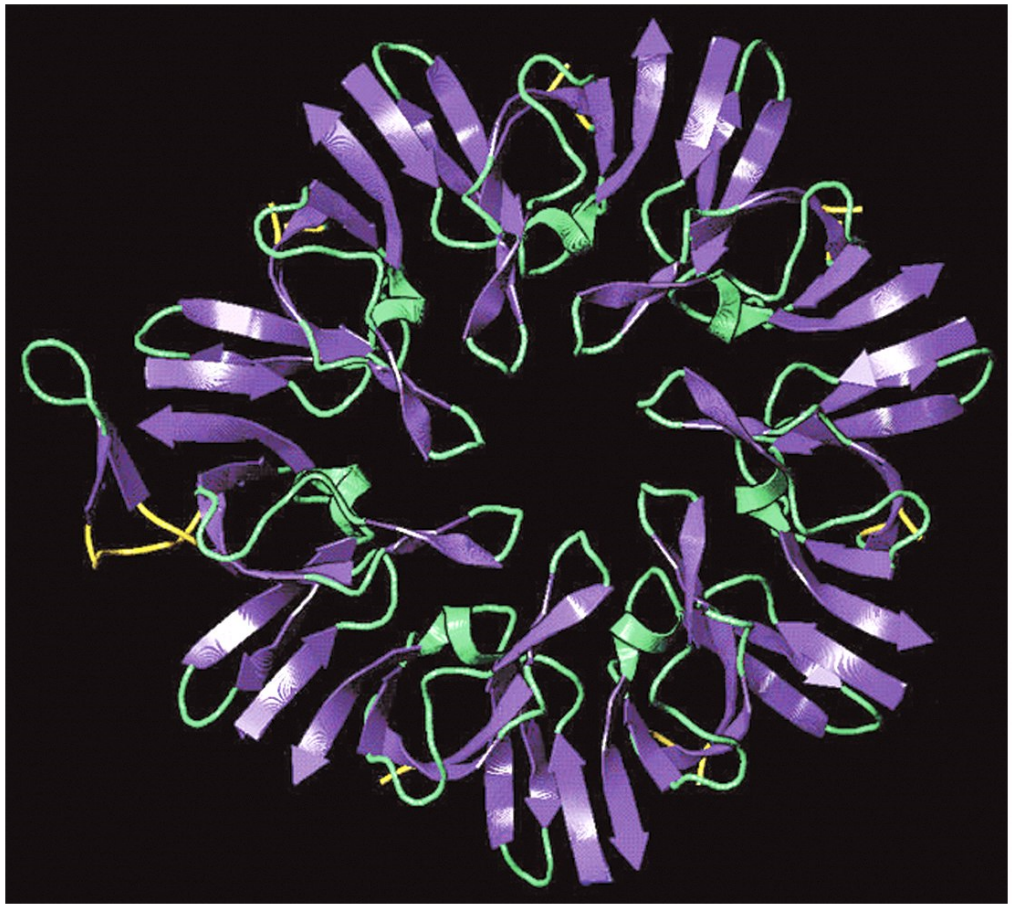


Figure 6-44 Fundamentals of Biochemistry, 2/e

X-ray structure of the GroEL-GroES-(ADP)7 complex

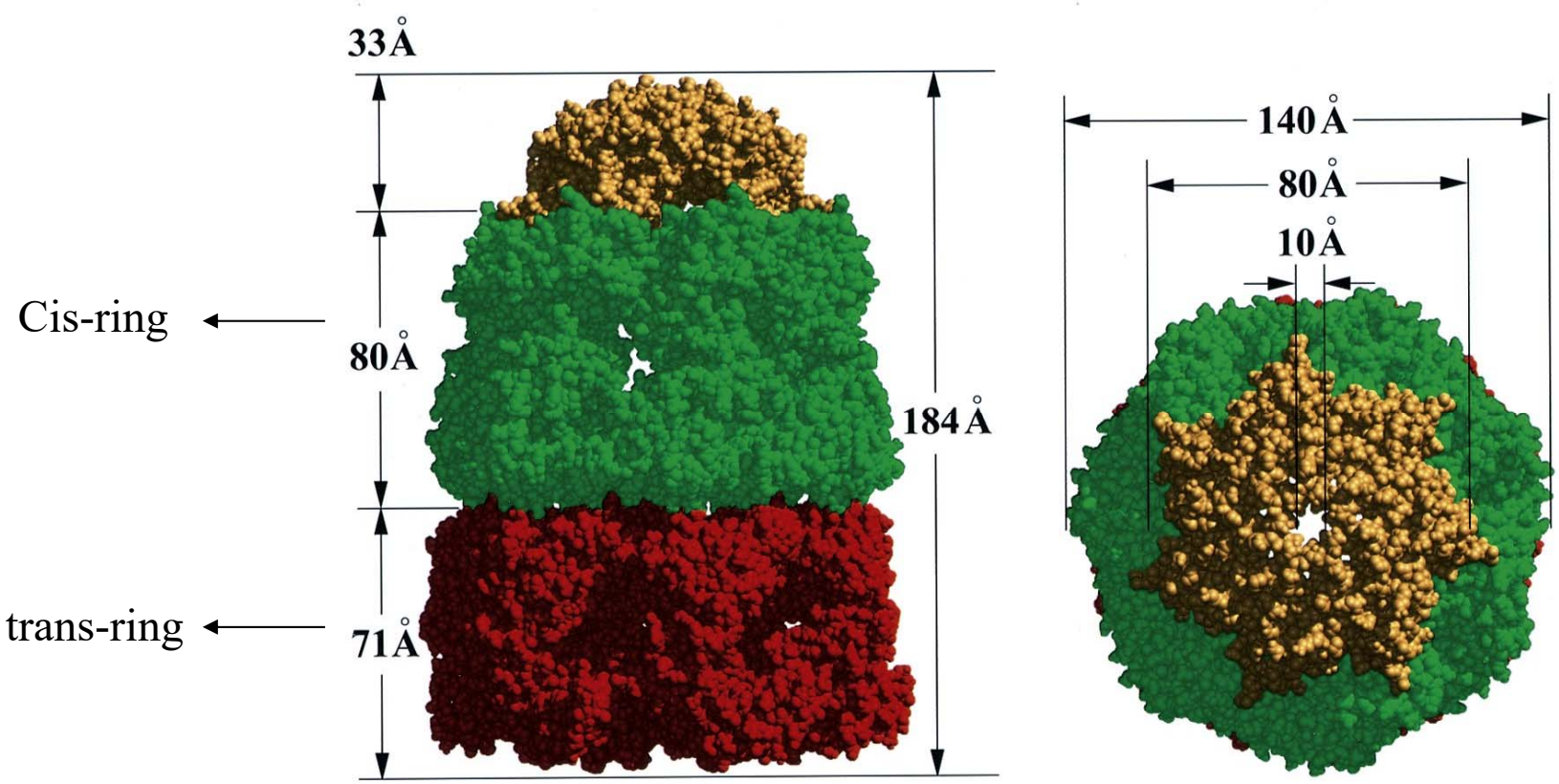


Figure 6-45ab Fundamentals of Biochemistry, 2/e

enlarged cavity

Hydrolyzed ATP

Enclose at least 70 kD

7 ADPs

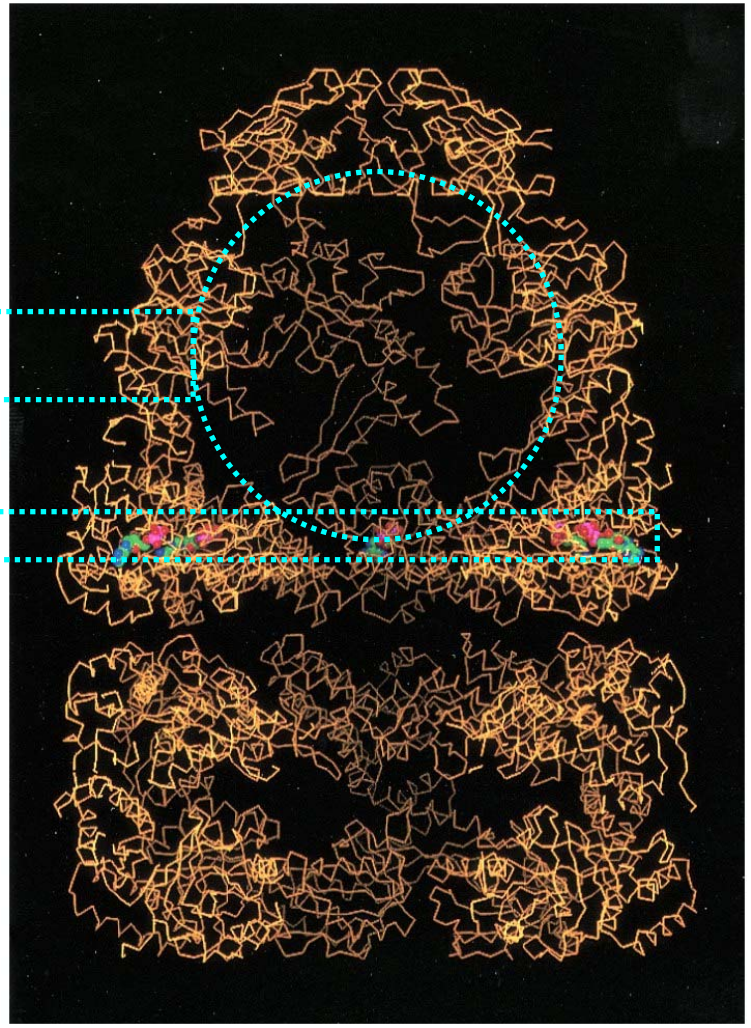


Figure 6-45c Fundamentals of Biochemistry, 2/e

Cα-backbone structure

Reaction cycle of the GroEL/ES chaperonin

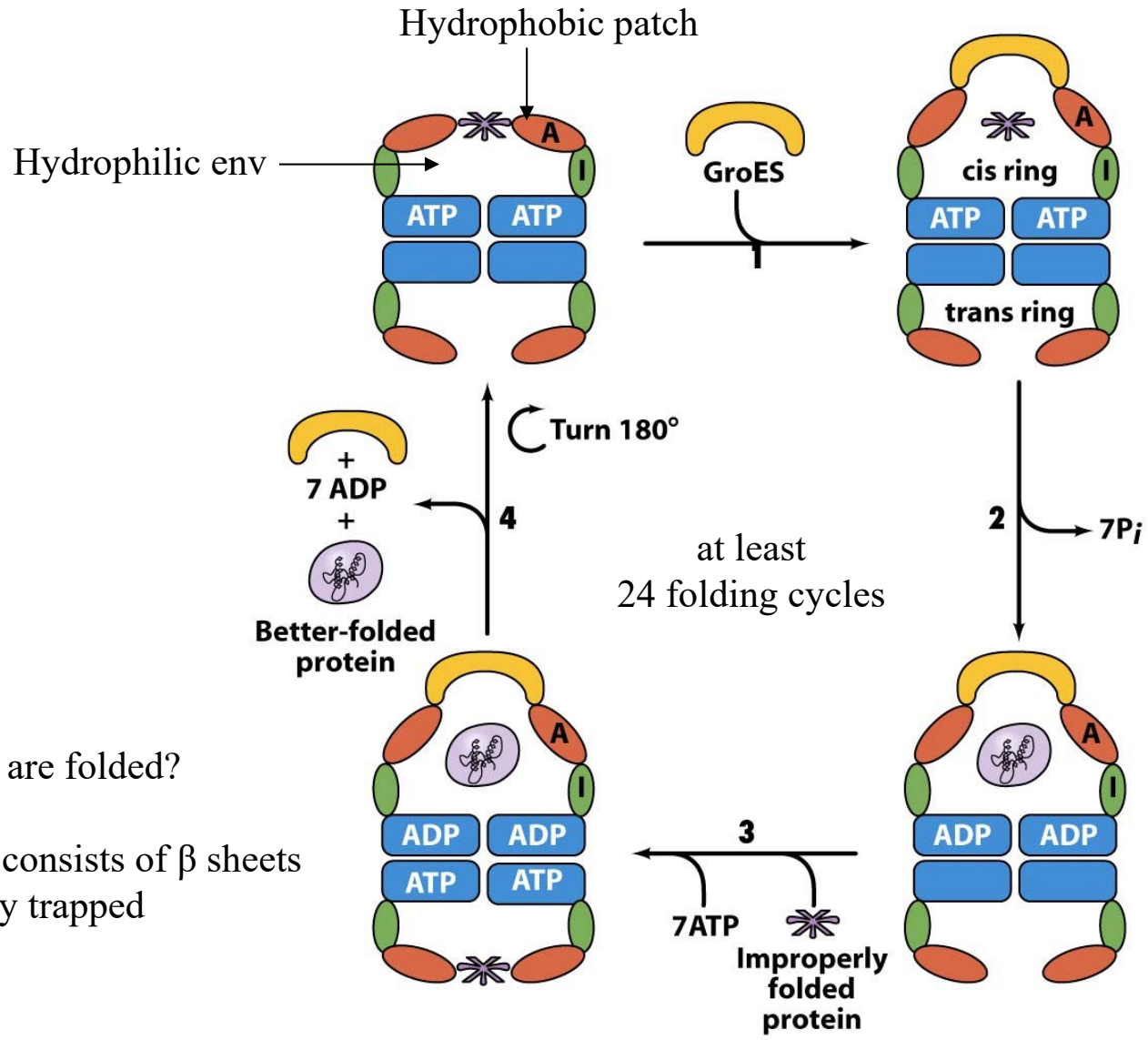


Figure 6-46 Fundamentals of Biochemistry, 2/e
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What kinds of proteins are folded?
20~60 kD proteins
>2 αβ domains mainly consists of β sheets
Misfolded or kinetically trapped

Disease caused by protein misfolding

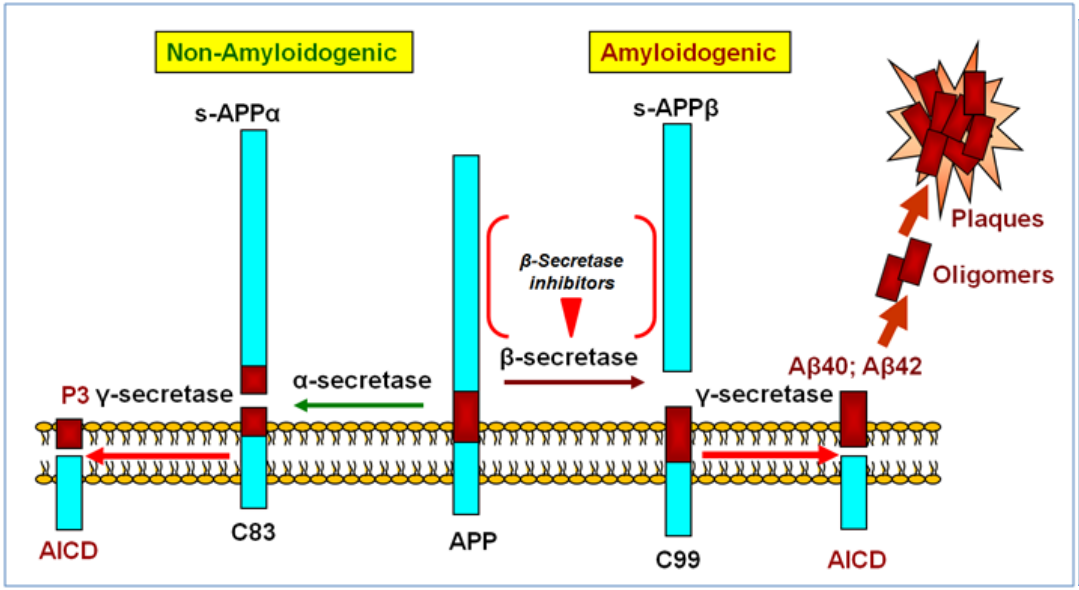
Alzheimer's disease

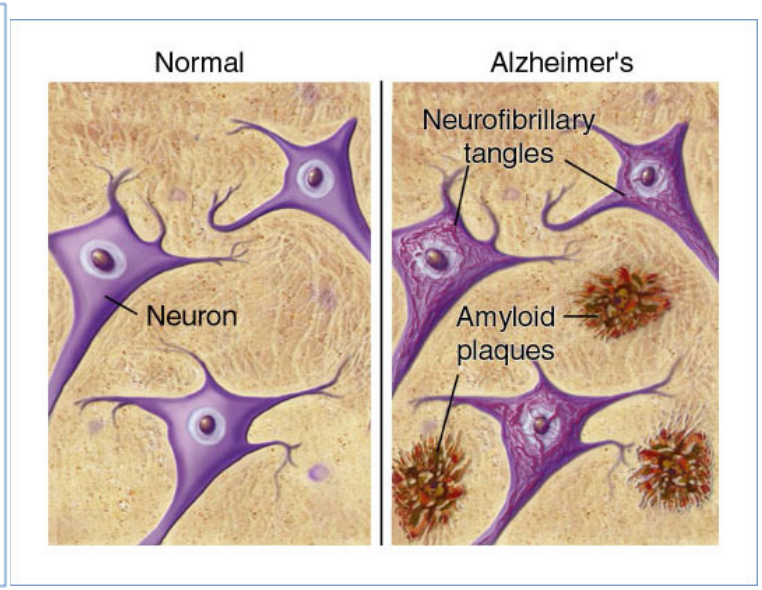
amyloid plaques= amyloid β proteins (Aβ): fibrils of a 40~42 residues

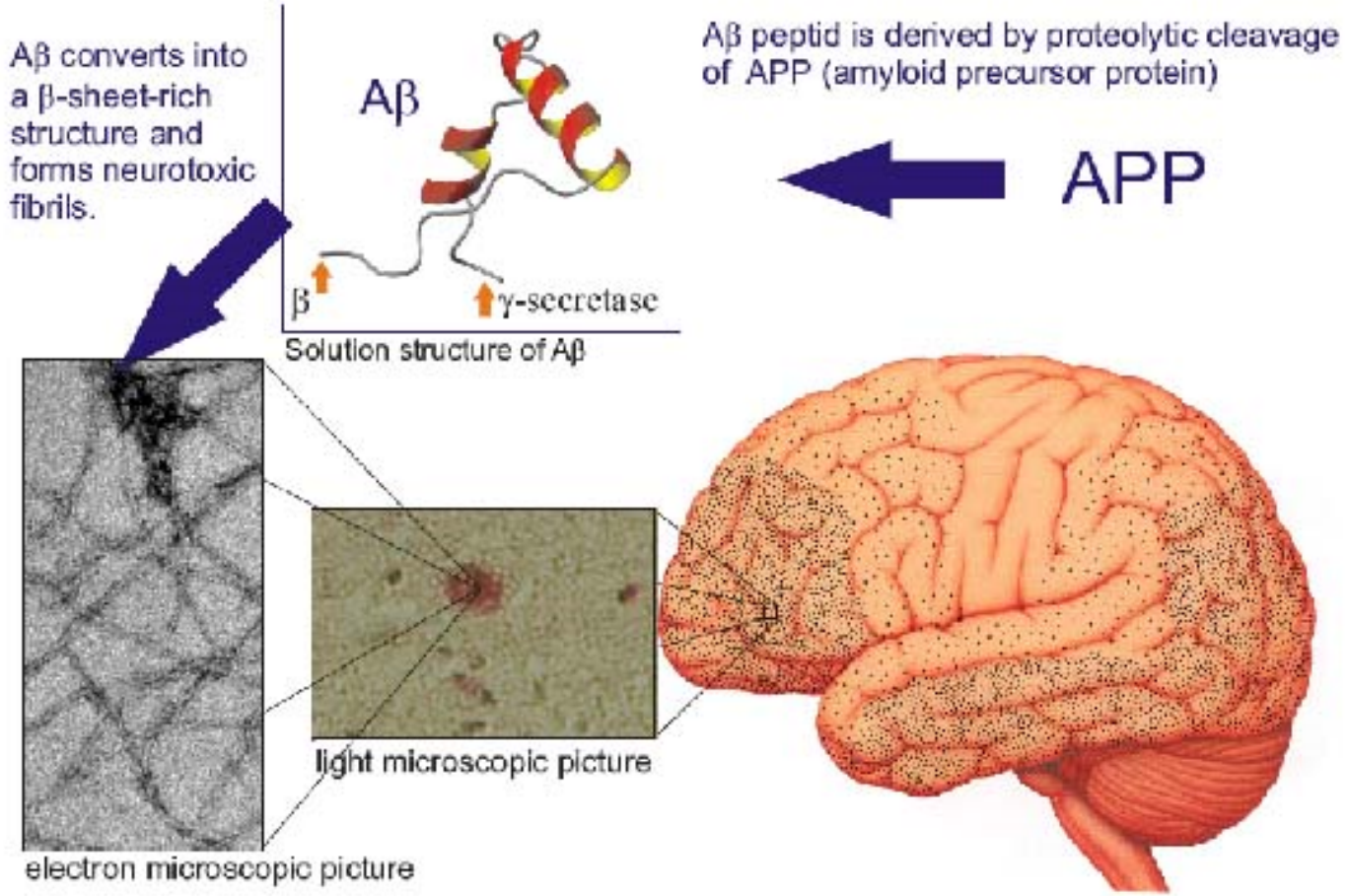
a fragment of a 770-residue membrane proteins-Aβ precursor protein (βPP)

multistep proteolytic process involving β- and γ-proteases

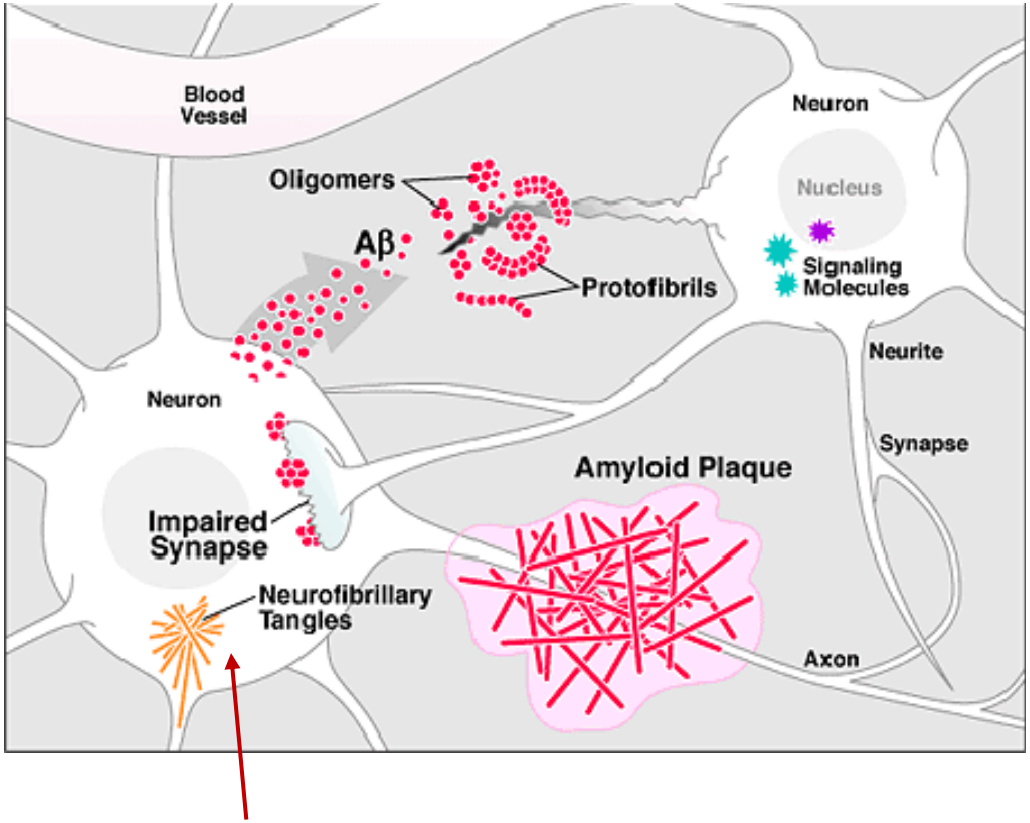
Aβ fibrils are neurotoxic even before their deposition in amyloid plaques



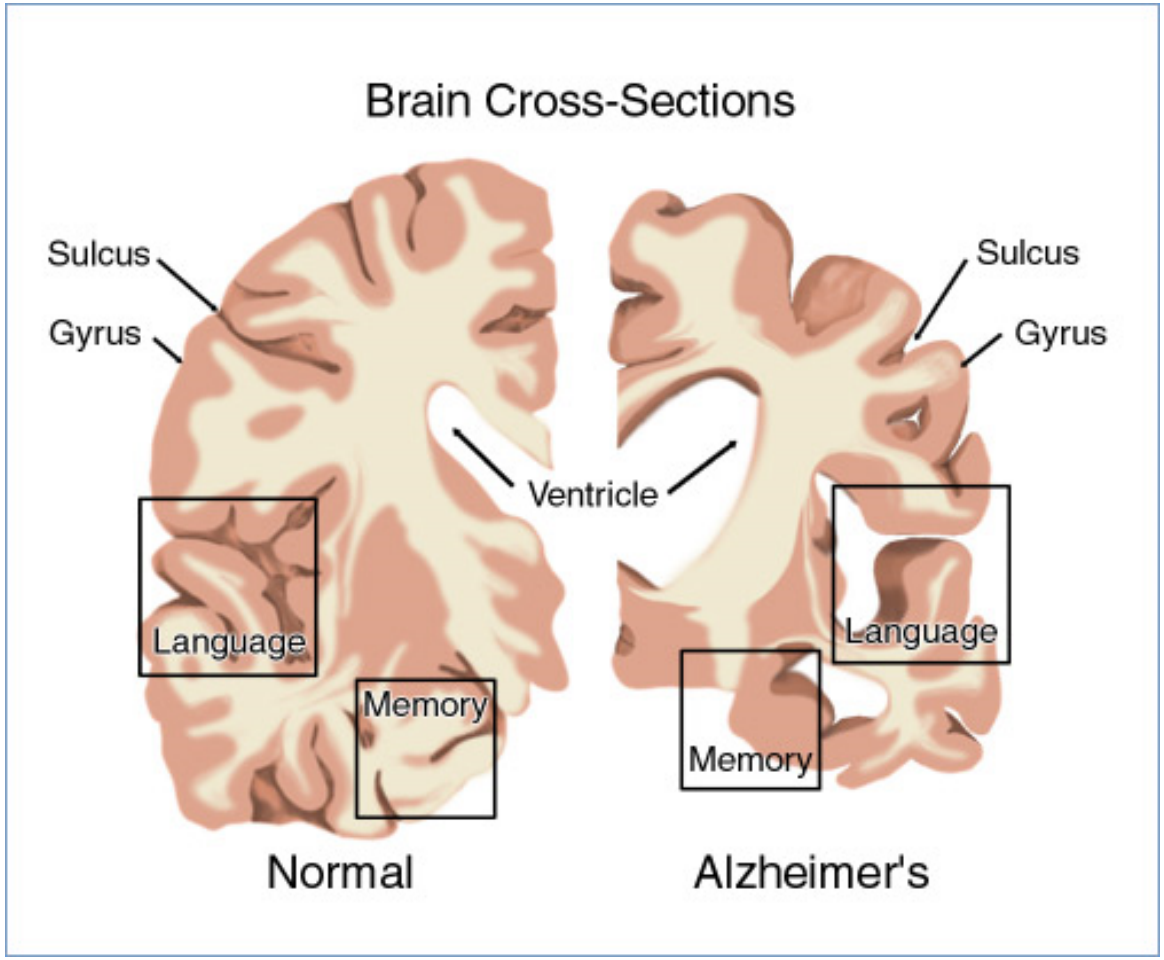




http://www.biologie.uni-duesseldorf.de/Institute/Physikalische_Biologie/Research/Topics/Alzheimer_diagnosis



A protein called tau stabilizes the microtubules when phosphorylated, and is therefore called a microtubule-associated protein. In AD, tau undergoes chemical changes, becoming hyperphosphorylated; it then begins to pair with other threads, creating neurofibrillary tangles and disintegrating the neuron's transport system.


Brain Cross-Sections
Sulcus
Gyrus
Ventricle
Language
Memory
Sulcus
Gyrus
Language
Memory
Normal
Alzheimer's

Prion diseases: transmissible spongiform encephalopathy (TSEs)

Proteinaceous infectious agent that lacks nucleic acid
Scrapie (sheep, goat)
Bovine spongiform encephalopathy (BSE or mad cow disease)
Kuru (human)
Creutzfeldt-Jakob disease (CJD) (human)

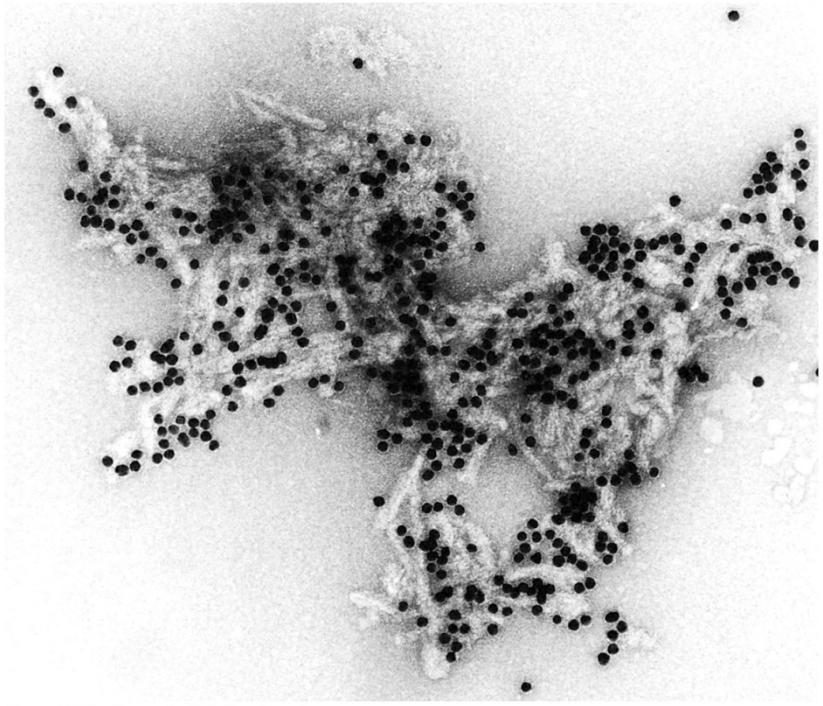
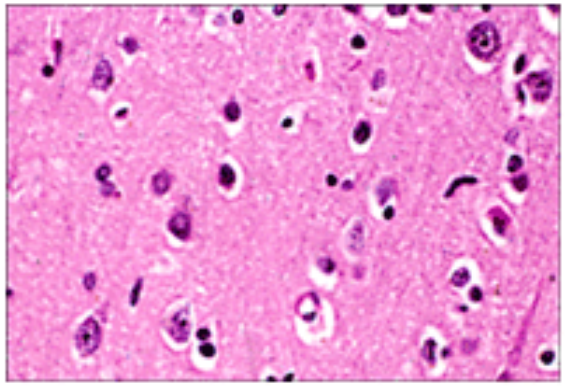


Figure 6-48 Fundamentals of Biochemistry, 2/e

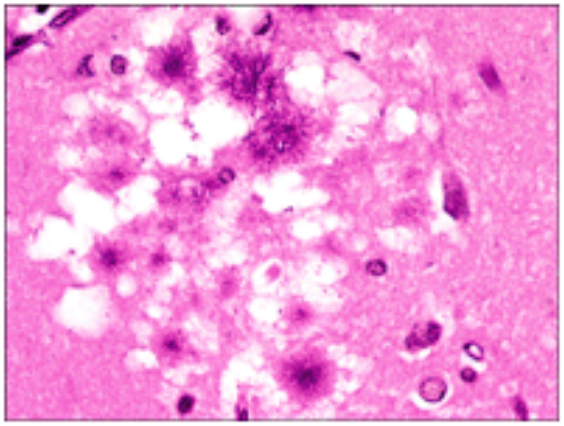
Electron micrograph of a cluster of partially proteolyzed prion rods

Figure 1.

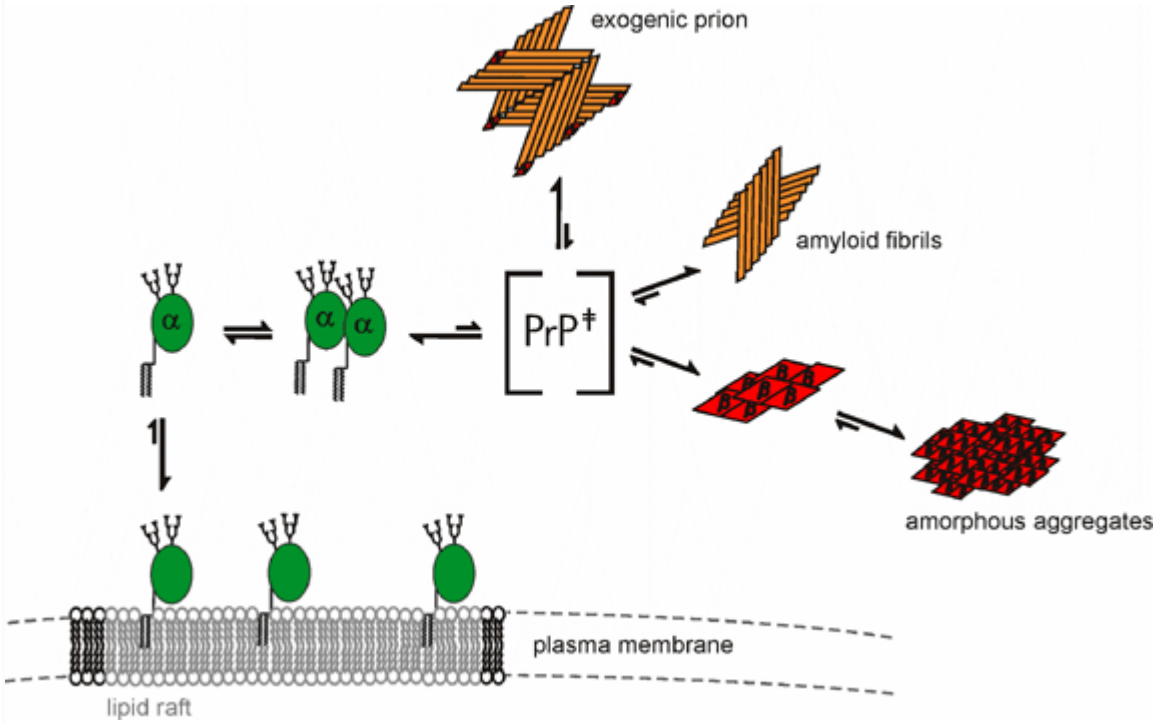
Comparison of Healthy and Prion -Infected Human Brain [3]



Normal human cerebral cortex showing no significant pathological changes



nvCJD cerebral cortex showing the "florid" plaques that consist of vacuoles (spongiform degeneration) containing amyloid plaques



209 amino acids: glycosylated and transmembrane domain
PrPc (cellular) PrpSc (scrapie)

Prion protein (PrP) conformations

flexible N-terminal residues (23-121)

PrP^{c}

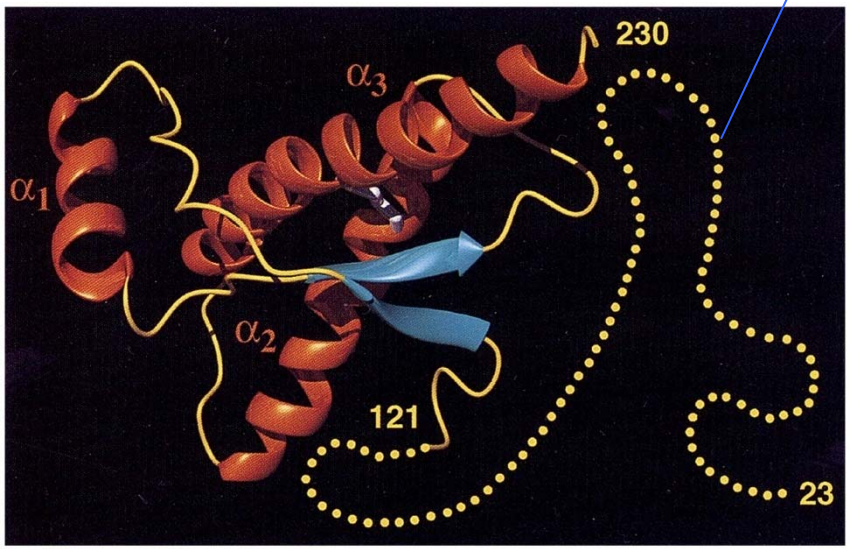


Figure 6-49a Fundamentals of Biochemistry, 2/e

PrP^{Sc}

autocatalytic

PrP^{Sc}

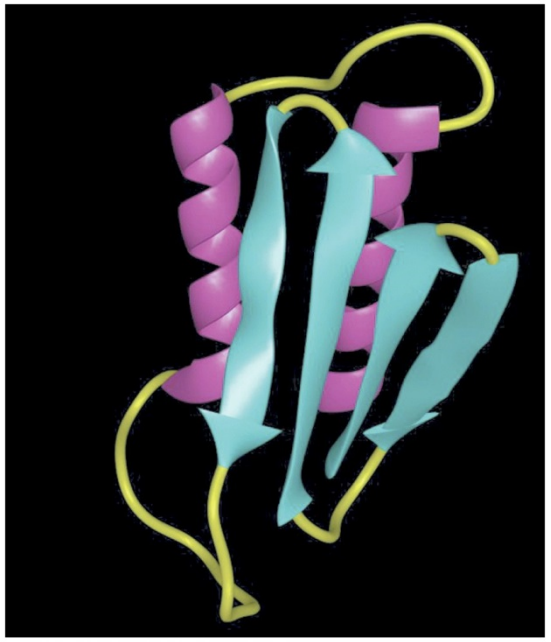


Figure 6-49b Fundamentals of Biochemistry, 2/e

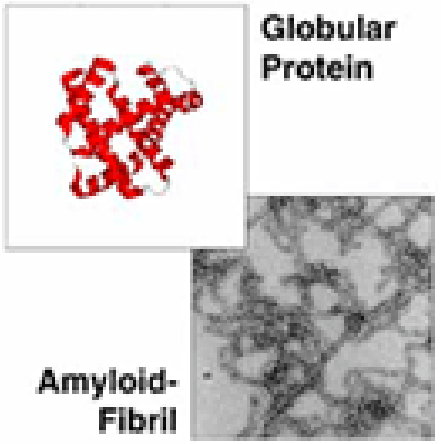
Amyloidoses

mutant forms of normally occurring proteins
amyloid fibrils are beta sheet structures
extension of partial beta domains

Under the appropriate conditions

almost any protein can be induced to aggregate

It seems likely that protein folding pathways have evolved not only to allow polypeptides to assume stable native structures but also to avoid forming interchain H bonds that would lead to fibril formation



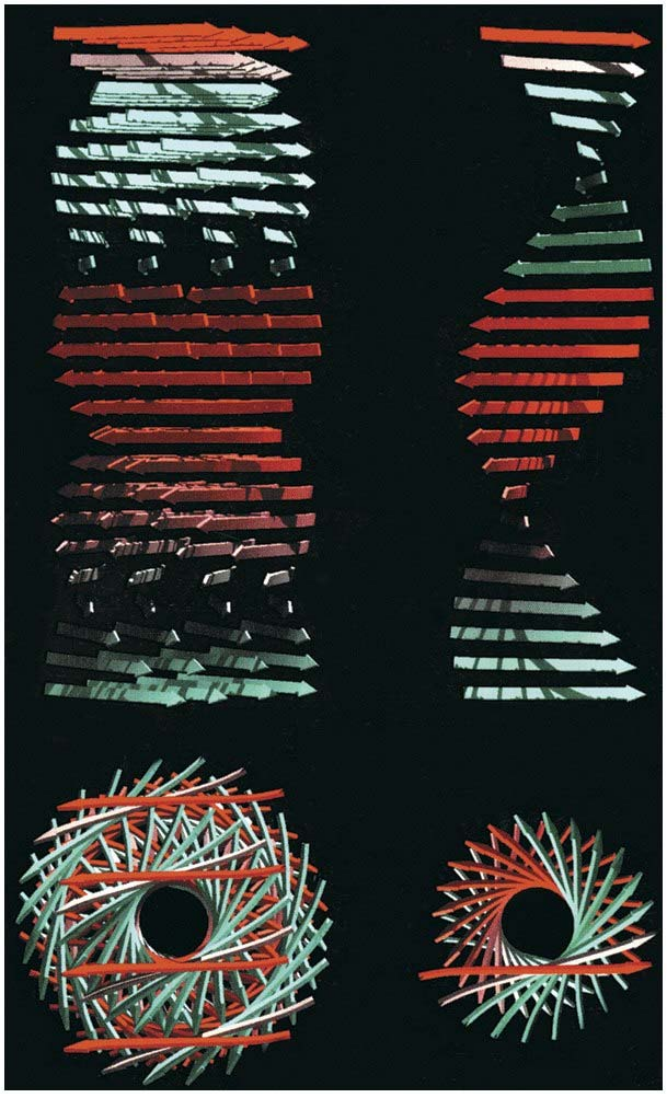
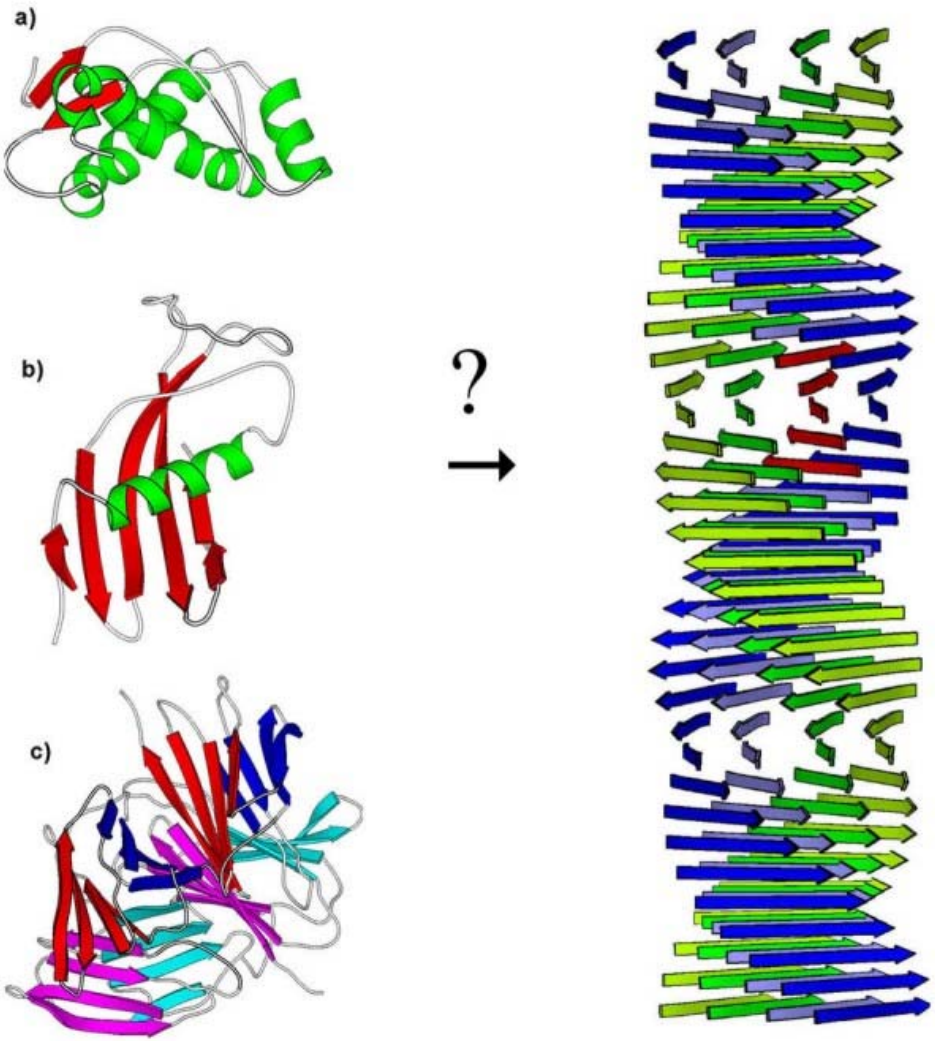
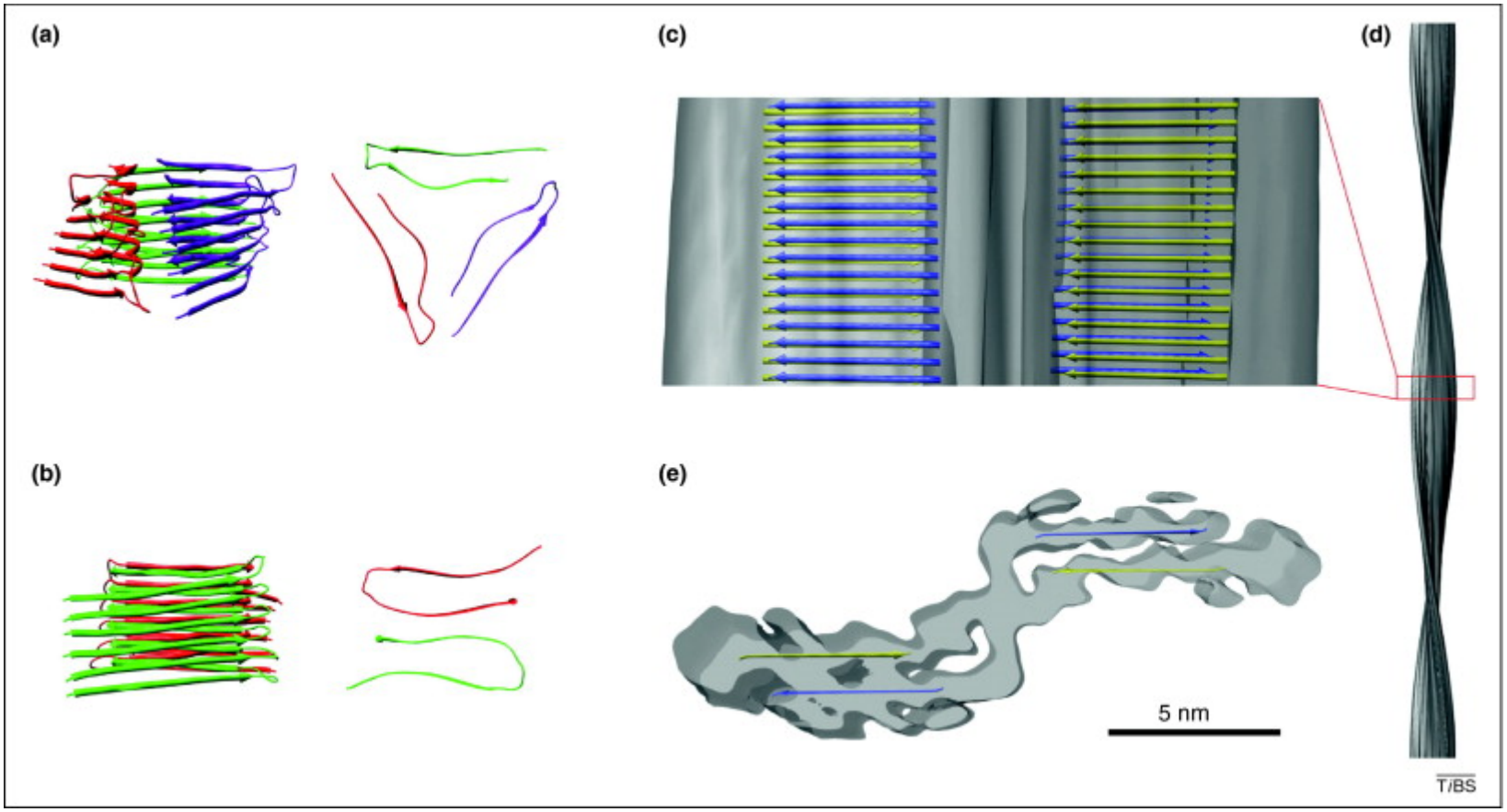


Figure 6-50 Fundamentals of Biochemistry, 2/e

A model, based on X-ray fiber diffraction measurements, of an amyloid fibril



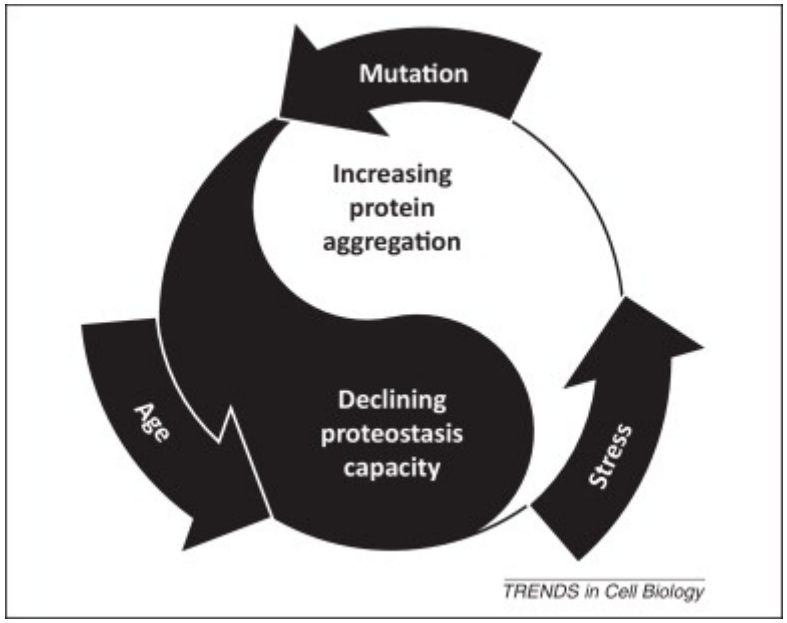
Three different proteins, **(a)** prion protein, **(b)** cystatin C and **c)** transthyretin, with three different three-dimensional structures which are known to form amyloid fibres in vivo. On the right is a proposed model for the structure of an amyloid fibril.



Recent progress in understanding Alzheimer's β-amyloid structures
TIBS Volume 36, Issue 6, June 2011, Pages 338–345

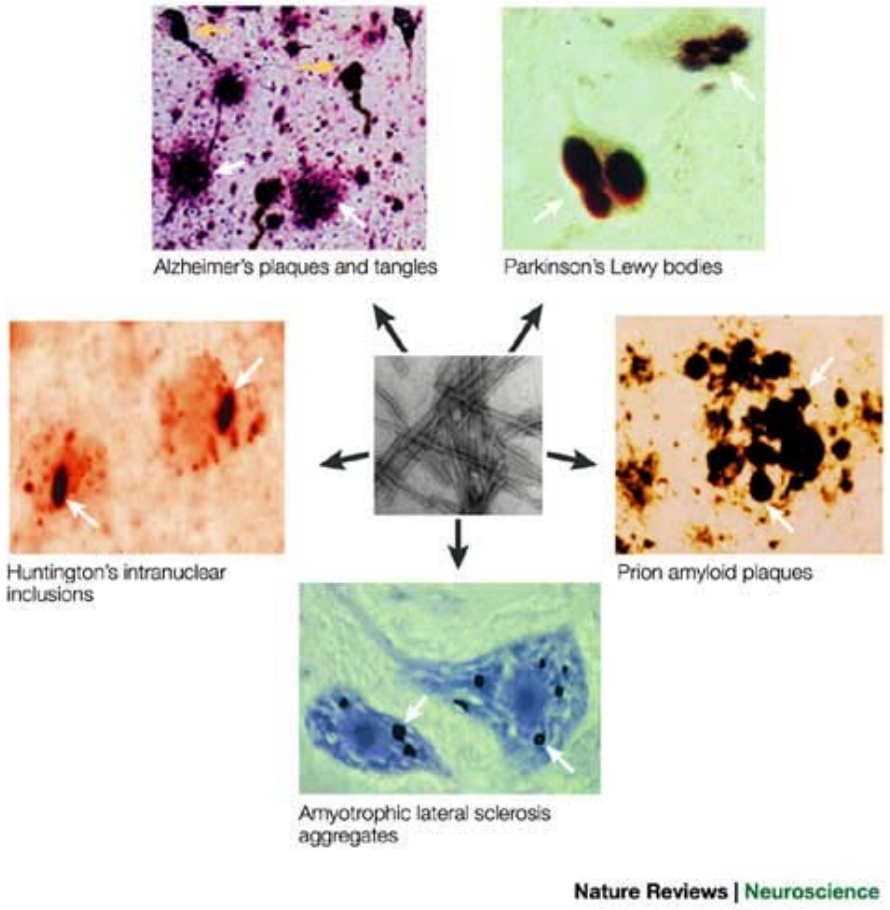
Figure 4. Structural models of Aβ fibrils. Previous structural models deviate significantly for the most highly resolved Aβ fibril structures, which were obtained by cryo-EM. Hence, the latter encompass a different peptide assembly. (a,b) Structural models assuming a U-shaped peptide fold; side views and top views shown; only residues 9–40 modeled. (a) Three Aβ(1–40) molecules per cross-sectional layer [29]. (b) Two Aβ(1–40) molecules per cross-sectional layer [24]. (c,d) Cryo-EM structure of an Aβ(1–40) fibril (0.8 nm). (c,d) side views, (e) cross-section. Images in (c,e) are superimposed with a β-sheet model, which is derived from these cryo-EM data, and highlights the peptides forming the cross-β regions in yellow or blue (the lines are not meant to imply continuous β-strands over their entire length; these regions might instead contain several shorter strands). Images in (a–c) and (e) are displayed with the same scale.

Self-propagating cycle of proteostasis decline in disease and aging



Trends in Cell Biology. Volume 24, Issue 9, p506–514 (2014)

Cerebral aggregates in neurodegenerative diseases



Extracellular amyloid plaques (white arrows) and intracytoplasmic neurofibrillary tangles (yellow arrows) are the pathological signature of Alzheimer's disease. Intracytoplasmic aggregates are typically present in the neurons of people affected by Parkinson's disease and amyotrophic lateral sclerosis. Intranuclear inclusions of huntingtin are observed in Huntington's disease patients and extracellular prion amyloid plaques that are located in different brain regions are present in some cases of transmissible spongiform encephalopathy. In spite of the different protein compositions, the ultrastructure of these deposits seems to be similar and composed mainly of a network of fibrillar polymers (centre). Nature Reviews Neuroscience 4, 49-60 (January 2003)

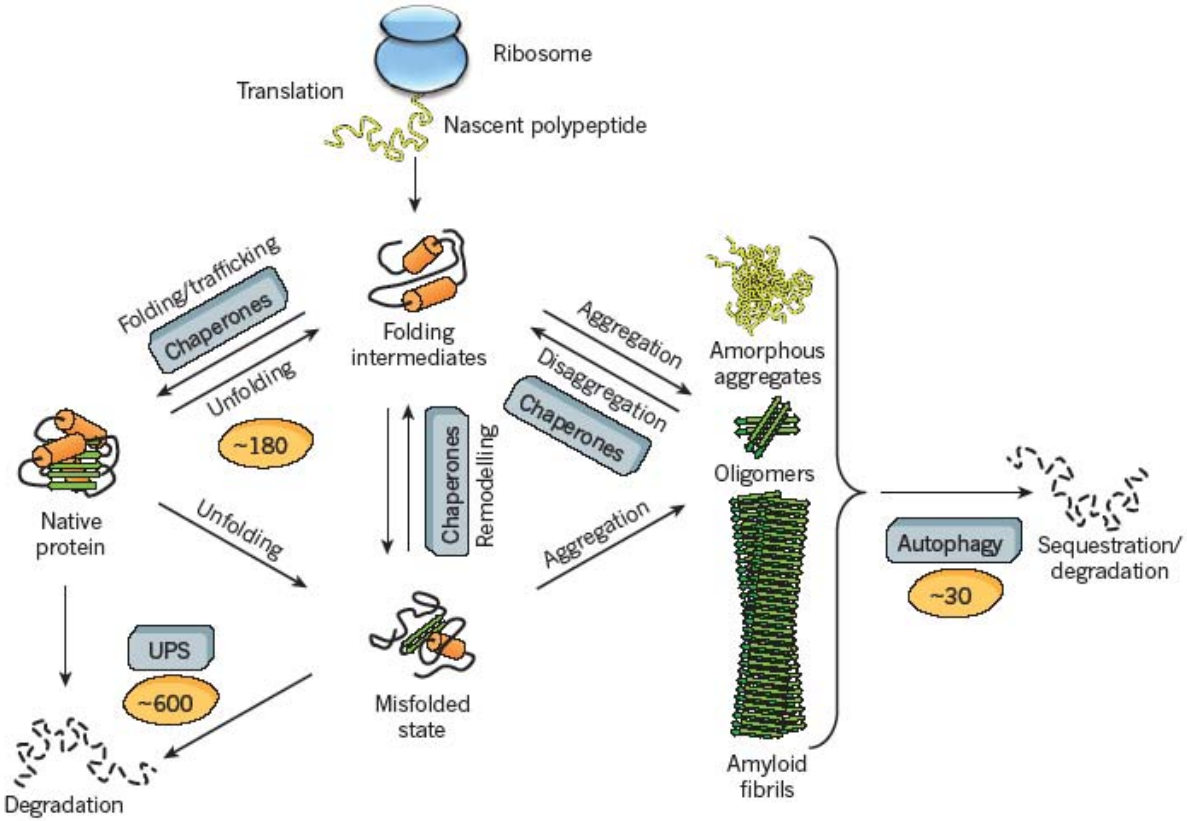


Figure 6 | Protein fates in the proteostasis network. The proteostasis network integrates chaperone pathways for the folding of newly synthesized proteins, for the remodelling of misfolded states and for disaggregation with the protein degradation mediated by the UPS and the autophagy system. Approximately 180 different chaperone components and their regulators orchestrate these processes in mammalian cells, whereas the UPS comprises ~600 and the autophagy system ~30 different components. The primary effort of the chaperone system is in preventing aggregation, but machinery for the disaggregation of aggregated proteins has been described in bacteria and fungi, involving oligomeric AAA^{+}-proteins such as HSP104 and the *E. coli* molecular chaperone protein ClpB, which cooperate with HSP70 chaperones[25]. A similar activity has been detected in metazoans, but the components involved have not yet been defined[83].

Nature (2011) 475:324-332

Table 6-3 Structural Bioinformatics Internet Addresses

Structural Databases

Protein Data Bank (PDB): http://www.rcsb.org/pdb/
Nucleic Acid Databank: http://ndbserver.rutgers.edu/
Molecular Modeling Database (MMDB): http://www.ncbi.nlm.nih.gov/Structure/index.shtml
Most Representative NMR Structure in an Ensemble: http://pqs.ebi.ac.uk/pqs-nmr.html
PQS Protein Quaternary Structure Query Form at the EBI: http://pqs.ebi.ac.uk/

Molecular Graphics Programs/Plug-Ins

Chime: http://mdli.com/products/framework/chemscape/
Cn3D: http://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml
Mage: http://kinemage.biochem.duke.edu/
Protein Explorer: http://www.umass.edu/microbio/chime/explorer/index.htm
RasMol: htttp://www.bernstein-plus-sons.com/software/rasmol/ *and* http://www.umass.edu/microbio/rasmol/
Swiss-PDB Viewer (Deep View): http://us.expasy.org/spdbv/

Structural Classification Algorithms

CATH (*C*lass, *A*rchitecture, *T*opology, and *H*omologous superfamily): http://www.biochem.ucl.ac.uk/bsm/cath/
CE (*C*ombinatorial *E*xtension of optimal pathway): http://cl.sdsc.edu/
FSSP (*F*old classification based on *S*tructure-*S*tructure alignment of *P*roteins): http://www2.ebi.ac.uk/dali/fssp/
SCOP (*S*tructural *C*lassification *O*f *P*roteins): http://scop.mrc-lmb.cam.ac.uk/scop/
VAST (*V*ector *A*lignment *S*earch *T*ool): http://www.ncbi.nlm.nih.gov/Structure/VAST/vast.shtml

Table 6-3 Fundamentals of Biochemistry, 2/e