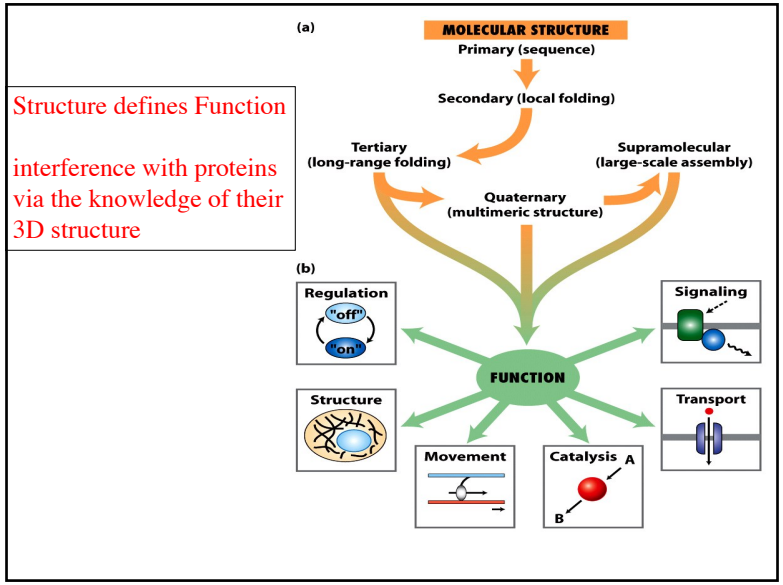
Molecular and Cellular Biology

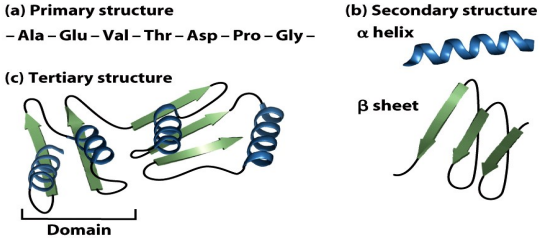
4. Proteins: Structure and Function

Prof. Dr. Klaus Heese

Structure defines Function

interference with proteins via the knowledge of their 3D structure

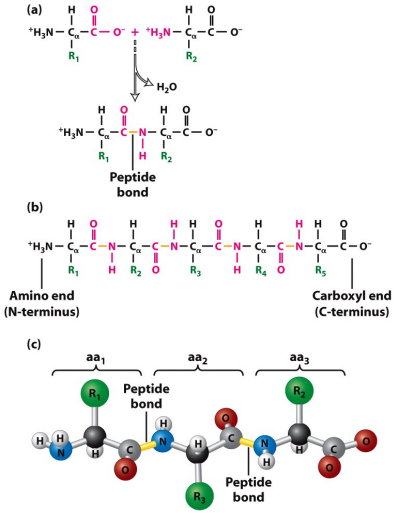


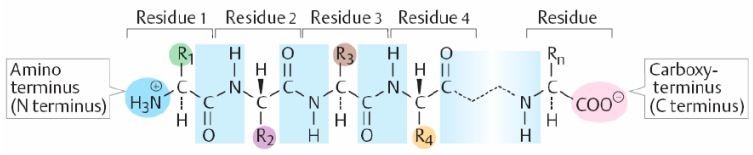


Amino acids

Peptides

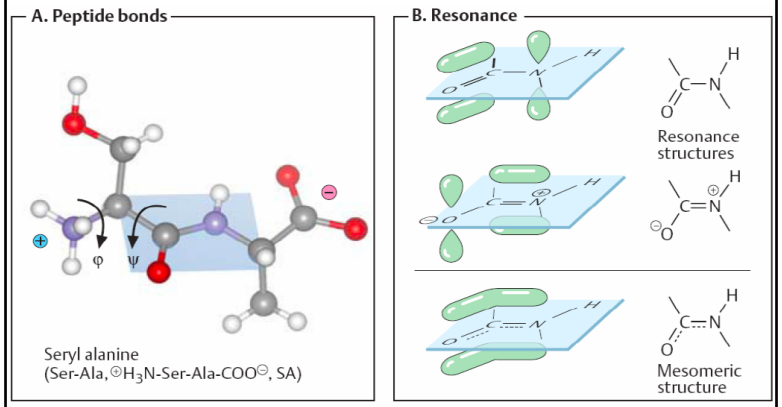
Proteins

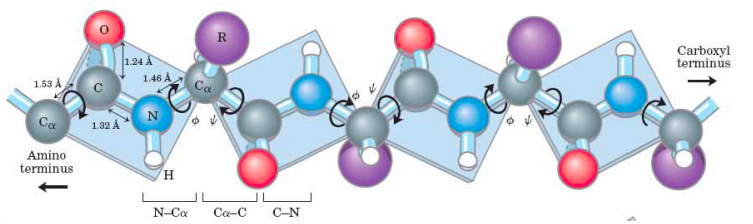




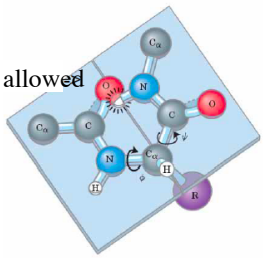
synthesis direction of the protein in ribosoms

plain, plane, layer, platform, planar

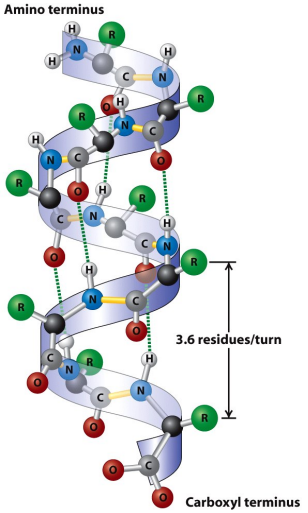




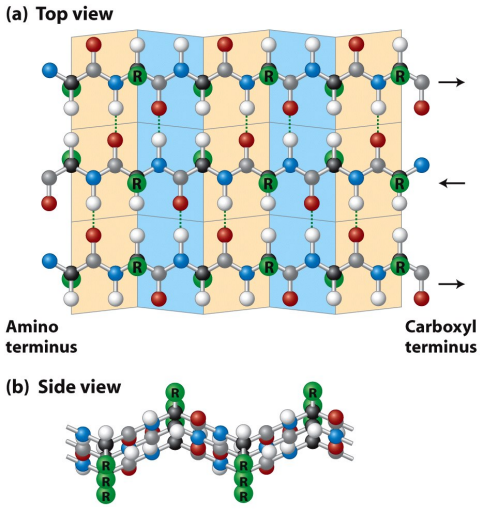
Not allowed

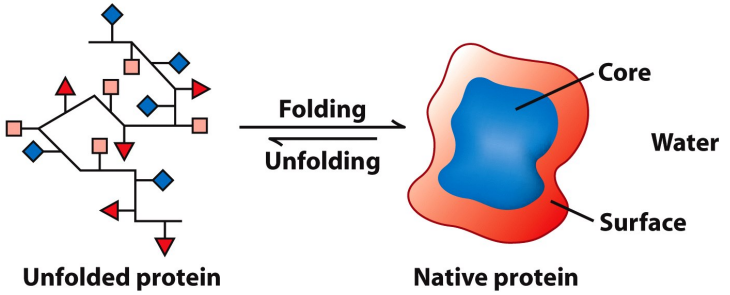


Helix



Sheet

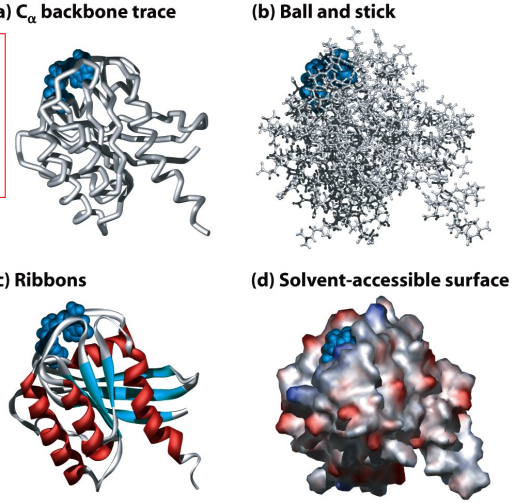




3D Protein Structures

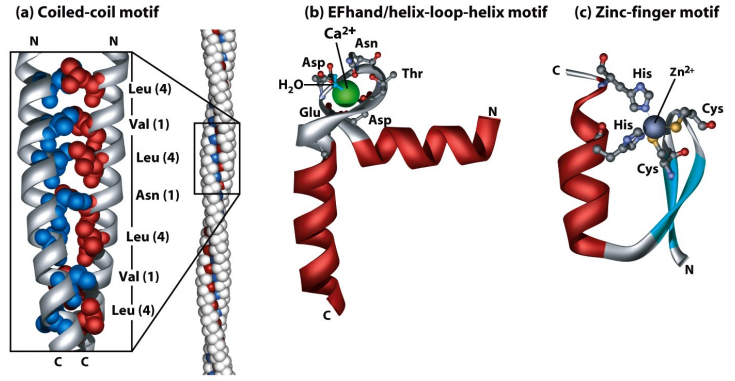
with various

Sub-Domains

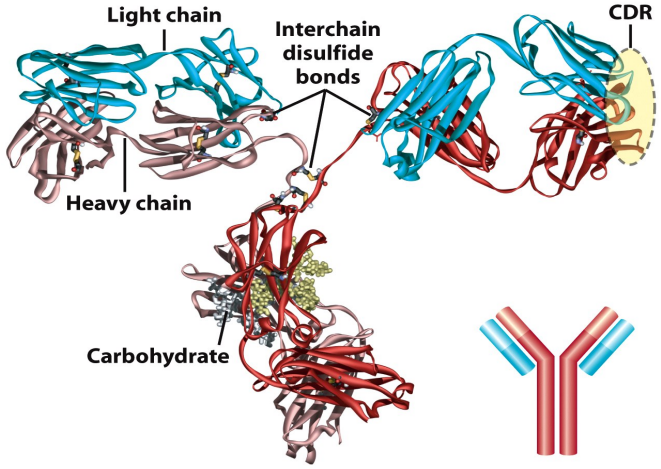


bio-computational protein structure representation using e.g. 'chimera'.

3D Protein Structures with various Sub-Domains

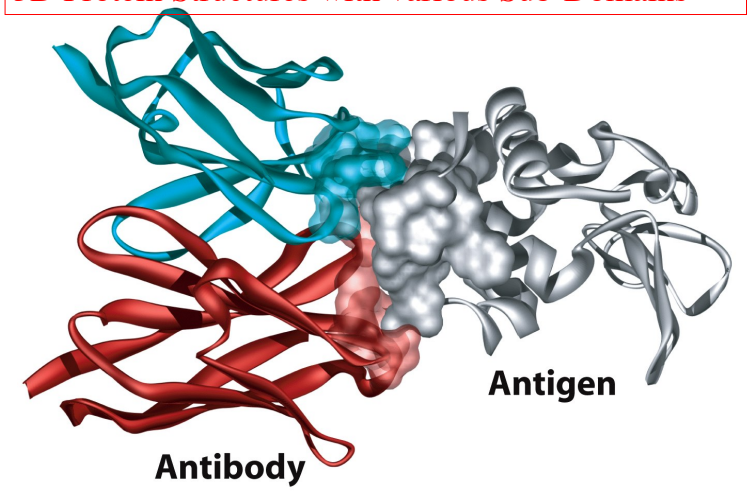


3D Protein Structures with various Sub-Domains



general structure of an antibody

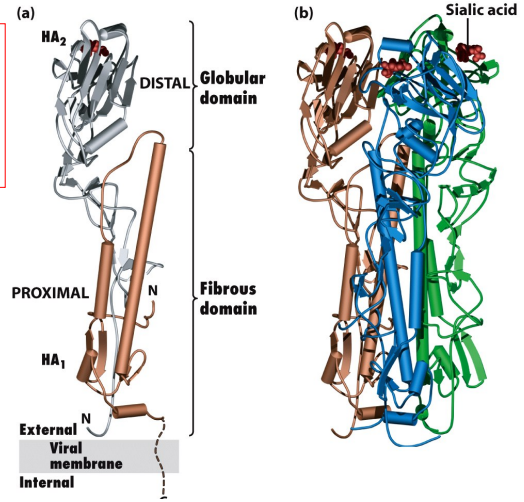
3D Protein Structures with various Sub-Domains



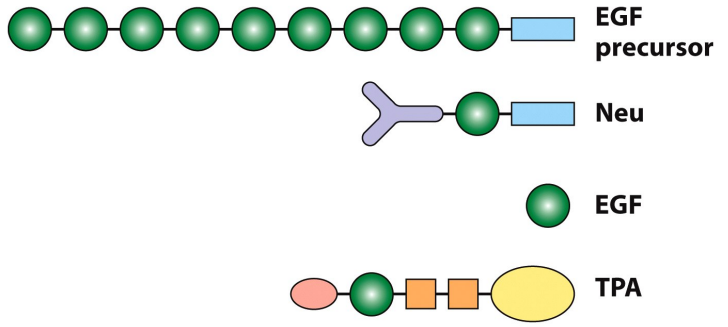
3D Protein Structures

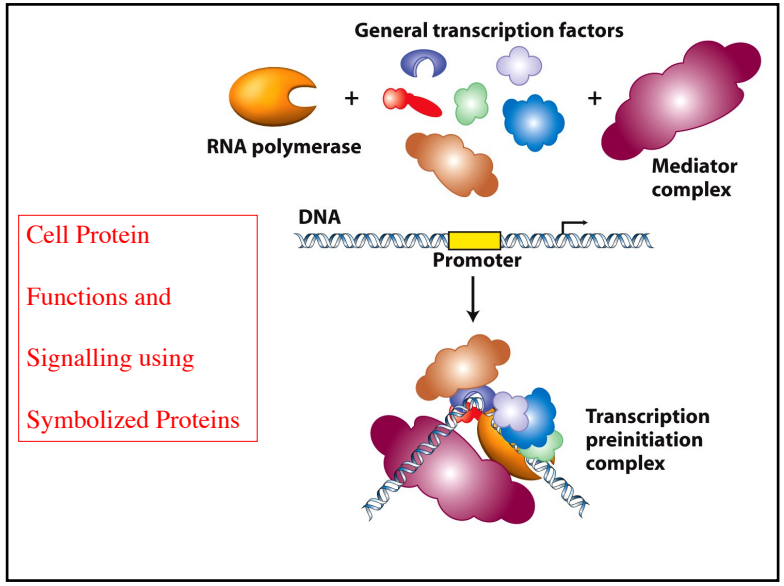
with various

Sub-Domains



Protein Structure Subunits are Symbolized



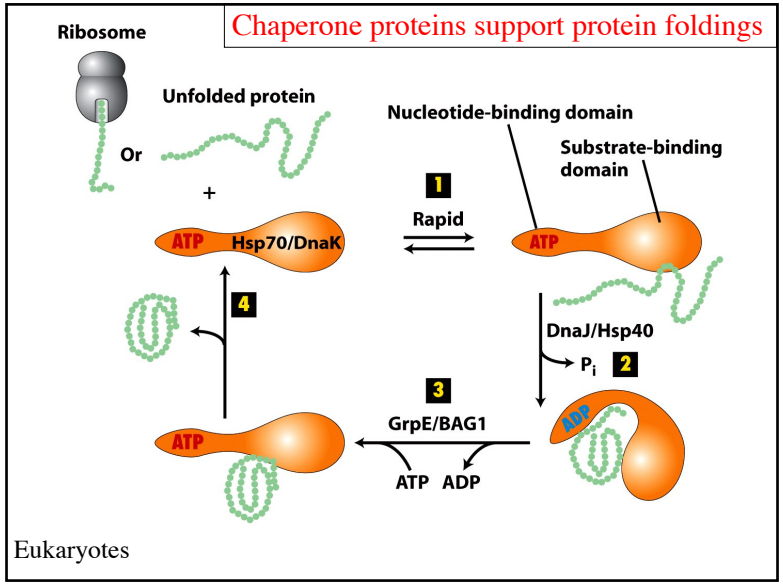


Cell Protein

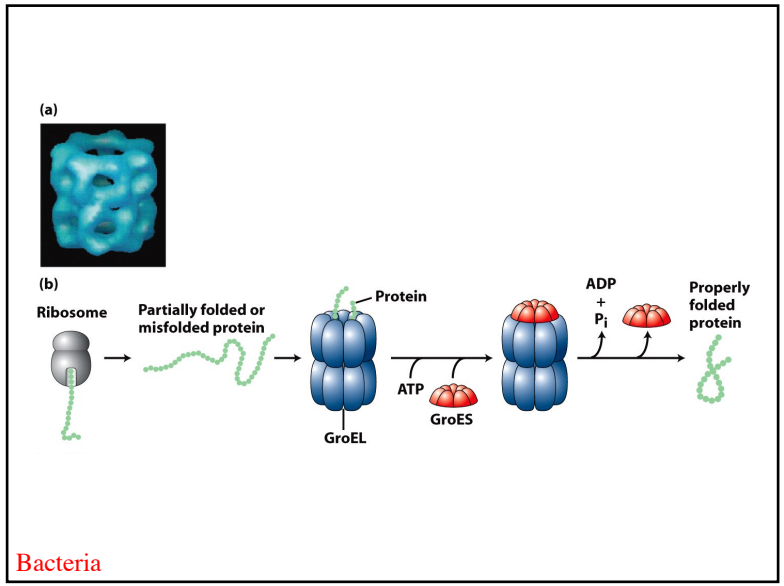
Functions and

Signalling using

Symbolized Proteins



Eukaryotes



Bacteria

Protein conformation (3D structure) change induces protein function change

http://www.youtube.com/watch?v=YAva4g3Pk6k

http://www.youtube.com/watch?v=4TGDPotbJV4

http://www.youtube.com/watch?v=CNWaMEW9QZ8&feature=watch_response

Moving vesicle

http://www.youtube.com/watch?v=y-uuk4Pr2i8

http://www.youtube.com/watch?v=B_zD3NxSsD8&feature=fvwp

Kinesin Walking (by Atomic Force Microscopy)

http://www.se.kanazawa-u.ac.jp/bioafm_center/movies/Walking_myosinV-2.gif

protein action

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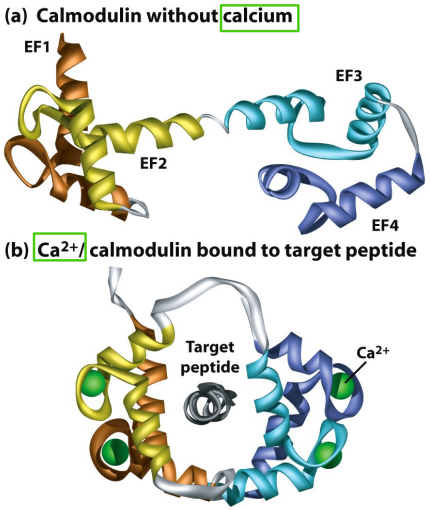
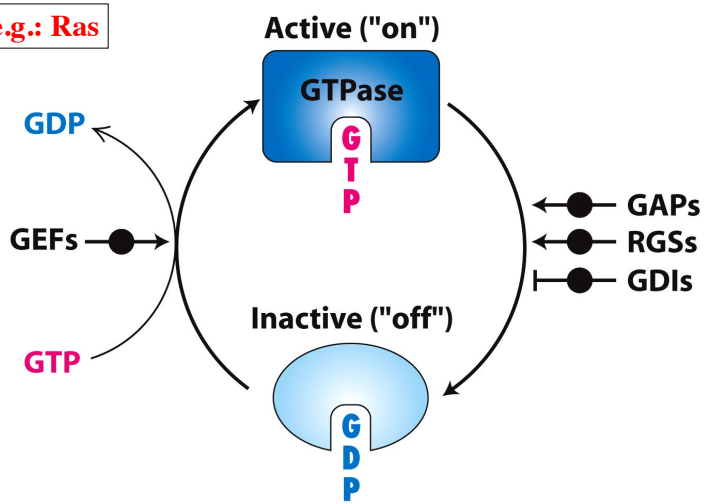
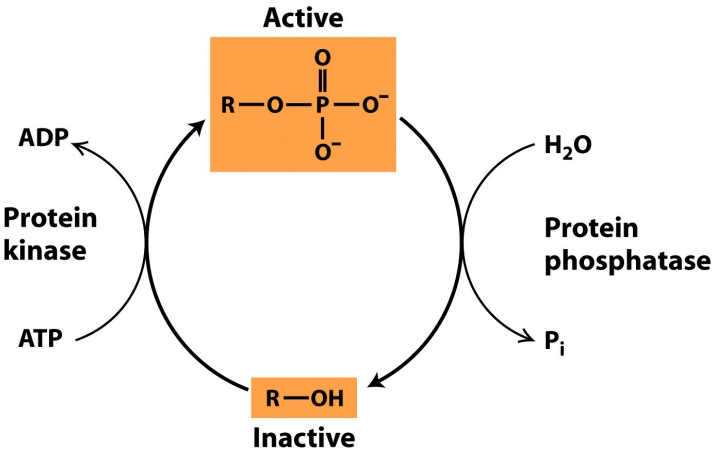
on/off



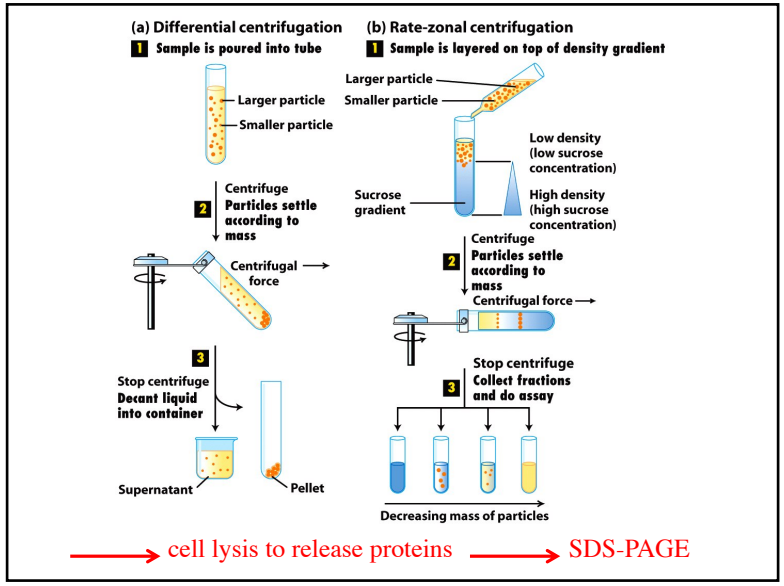
e.g.: Ras



e.g.: CaCamkII
(calcium/calmodulin-dependent protein kinase)



Practical methods in the laboratory for Protein isolation, identification and characterization



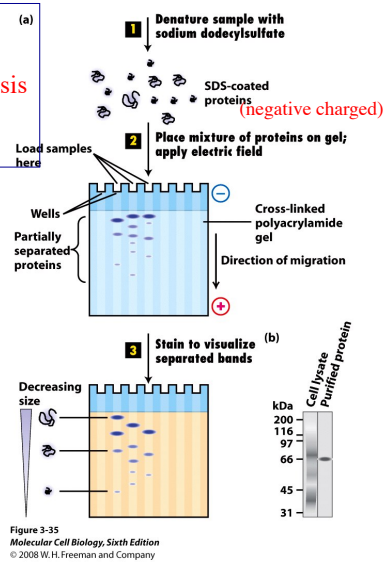
→ cell lysis to release proteins → SDS-PAGE

Sodium **D**odecyl **S**ulfate –

Poly-**A**crylamide-**G**el **E**lectrophoresis

SDS-PAGE

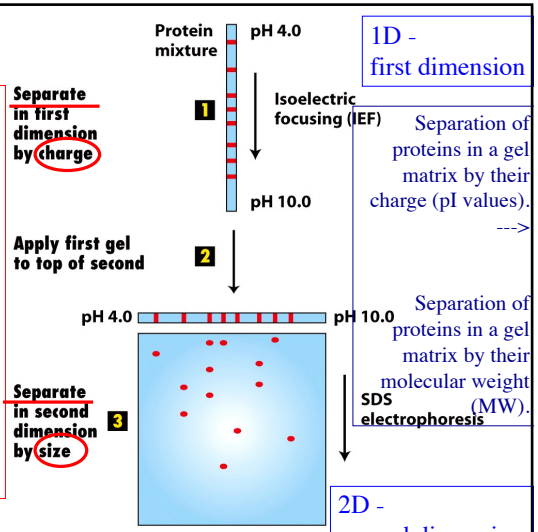
Separation of proteins in a gel matrix by their size (Molecular Weight (MW in kilo Dalton (kDa)))



2D-Gel

Separation of proteins by their charge due to different Isoelectric points.

Remember, amino acids can exist as hybrid ions, can act as ampholytes, thus, proteins move here through the pH gradient based on their specific charges.



1D - first dimension

Separation of proteins in a gel matrix by their charge (pI values). --->

Separation of proteins in a gel matrix by their molecular weight (MW).

2D - second dimension

2D Gel analysis after

a) Coloring of proteins

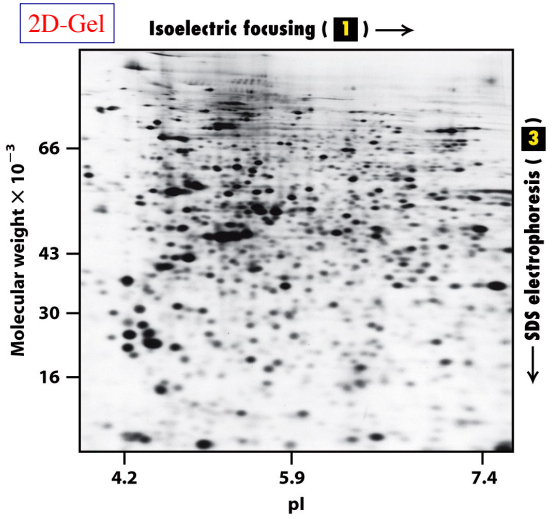
Or

b) Radioisotope labelling

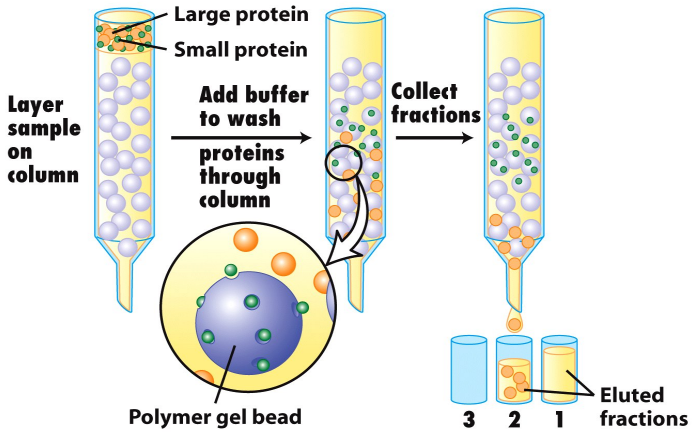
Or

c) DIG labelling

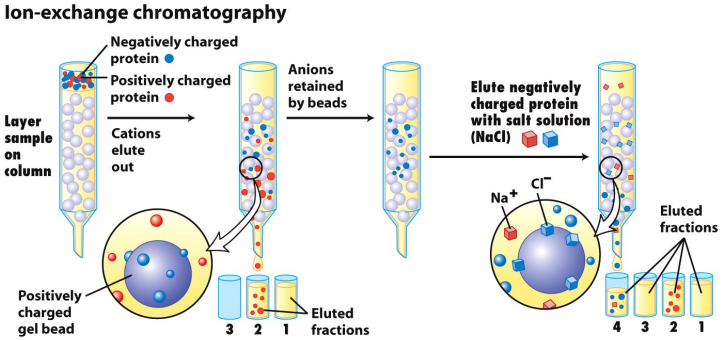
2D-Gel



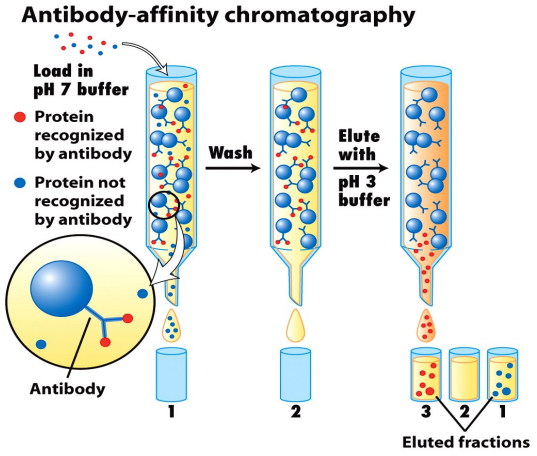
Separation of Proteins by their Size



Separation of Proteins by their Charge



Separation of Proteins by their Specific Interaction – Immune-Affinity: Antibody-Antigen-Interaction



Western-blot

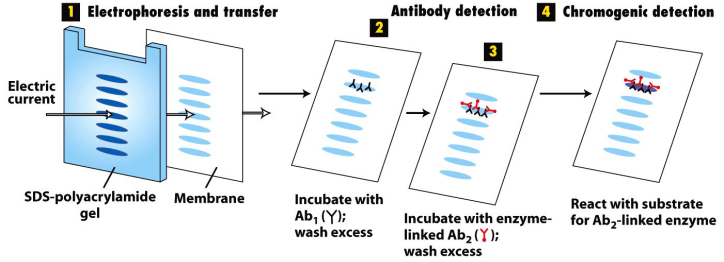


TABLE 3-1 Radioisotopes Commonly Used in Biological Research

ISOTOPE	HALF-LIFE
Phosphorus-32	14.3 days
Iodine-125	60.4 days
Sulfur-35	87.5 days
Tritium (hydrogen-3)	12.4 years
Carbon-14	5730.4 years

Protein Folding & Diseases

Box 1 | **Prion nomenclature**

α-PrP
The α-helix-rich form of PrP, represented by PrP^{C}.

β-PrP
The β-sheet-rich forms of PrP can be generated from the oxidized and from the reduced form of PrP by exposure to various chemical treatments. They can form fibrillary structures, particularly when amino-terminally truncated.

PrP^{C}
The physiologically occurring, mainly GPI-linked form of PrP, or prion protein, that can be glycosylated on one or both of two asparagine residues with a variety of glycans. As shown by NMR and X-ray crystallography, it is rich in α-helical structure and contains only a little β-sheet structure.

PrP^{bse}
A designation I propose, for any stable form of PrP that differs from PrP^{C} only by virtue of its conformation but not primary structure. Such differences may currently be detected by a variety of methods, such as reactivity to certain monoclonal antibodies, conformation-dependent immunoassay, susceptibility to proteinases, including the location of cleavage site(s), and optical measurements such as infra-red or circular dichroism. PrP^{bse} comprises, among others, PrP-res, PrP^{Sc} or $sPrP^{Sc}$, as defined below.

PrP^{Sc}
An isoform of PrP^{C} that is almost invariably detected in TSE-infected tissues and cells. It comprises a carboxy-proximal segment of about 140 residues that is resistant to defined conditions of PK treatment. The term PrP^{Sc} is used by some interchangeably with prion, a usage that should be avoided. PrP^{Sc} designates a structure, prion is a functional concept. The implication that a particular form of PrP is the only essential constituent of the prion remains to be proven.

PrP27-30
The PrP fragment remaining after controlled PK digestion of PrP^{Sc}.

PrP-res
Alternative designation for PrP^{Sc}, that has been proposed to generalize the term for all types of TSEs and not only scrapie.

PrP-sen
The designation for PrP^{C} and forms of PrP that are equally susceptible to PK digestion.

PrP^{*}
A hypothetical isoform of PrP that is the essential component of the TSE agent or prion.

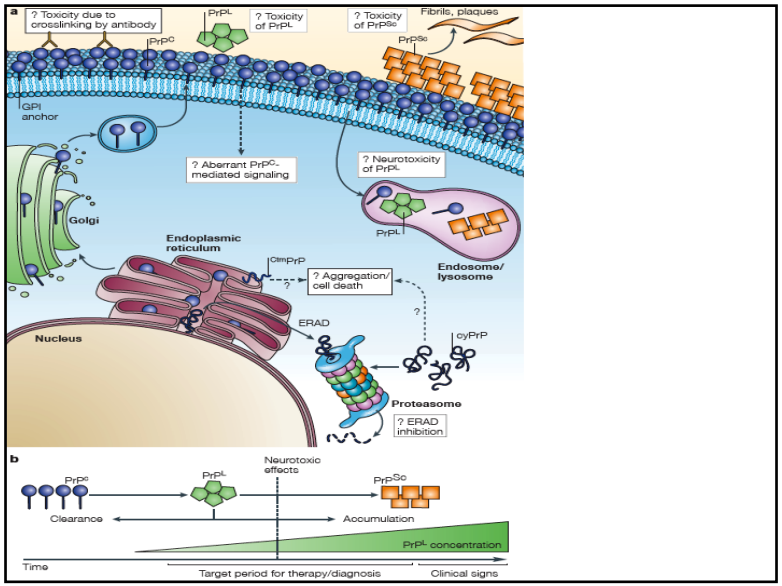
Prnp
The gene encoding PrP.

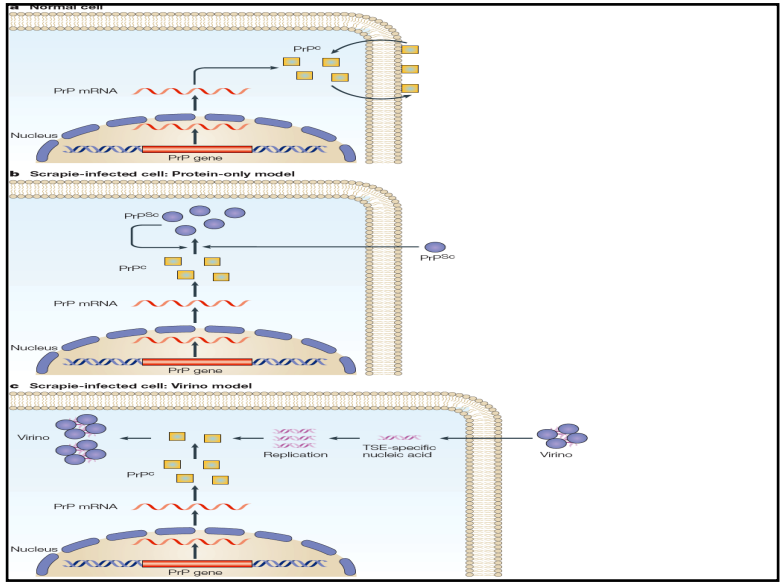
$Prnp^{a/a}$
Genotype in which both copies of the PrP gene are inactivated or ablated.

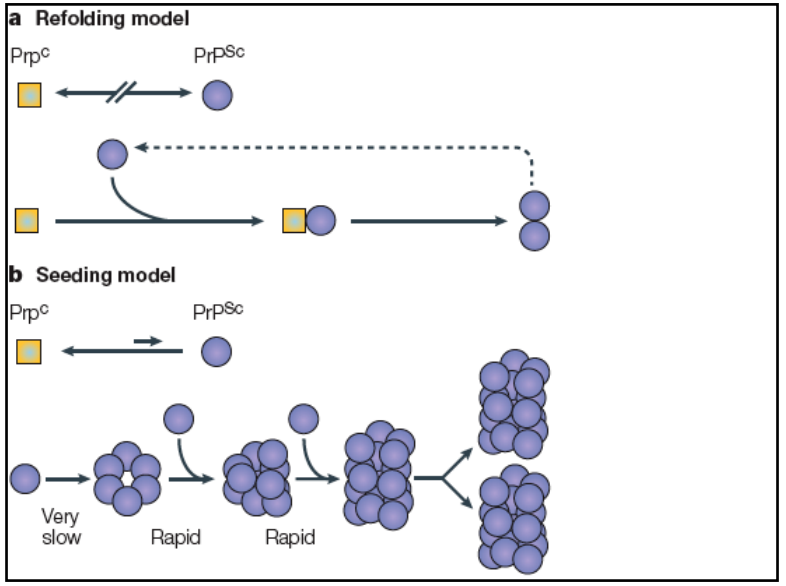
rPrP
Denotes recombinant PrP. When produced in *Escherichia coli* it lacks the GPI anchor and the glycan residues.

$sPrP^{Sc}$
A term used by Prusiner to designate a protease-sensitive isoform of PrP that is detected in prion-infected tissue. This terminology is contradictory because PrP^{Sc} was originally defined as a protease-resistant entity.

Prion:
The transmissible agent that is responsible for prion diseases, which, according to the 'protein-only' hypothesis, lacks an agent-specific nucleic acid genome and is composed principally or entirely of a conformational isomer of cellular prion protein. A term that was originally coined by Prusiner from 'proteinaceous infectious particle'. Highly expressed in brain, in neurons and glia cells, probably anti-oxidative stress function, cell adhesion, stem cell character.







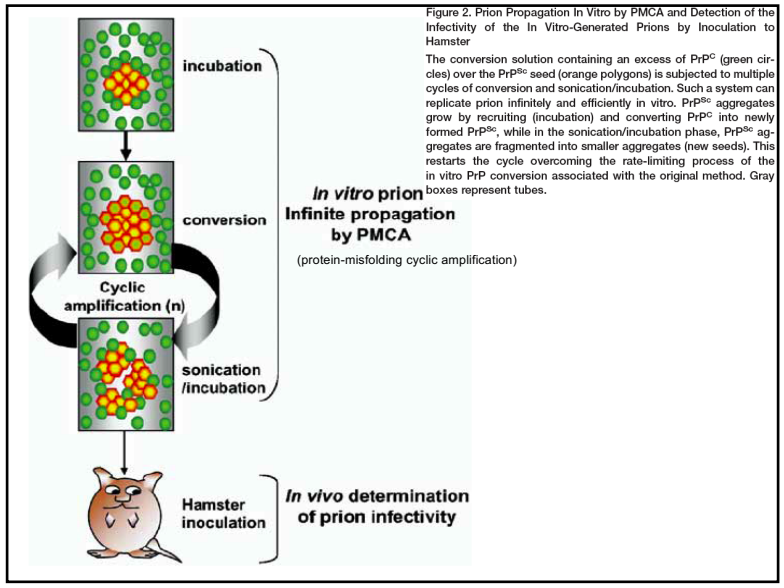
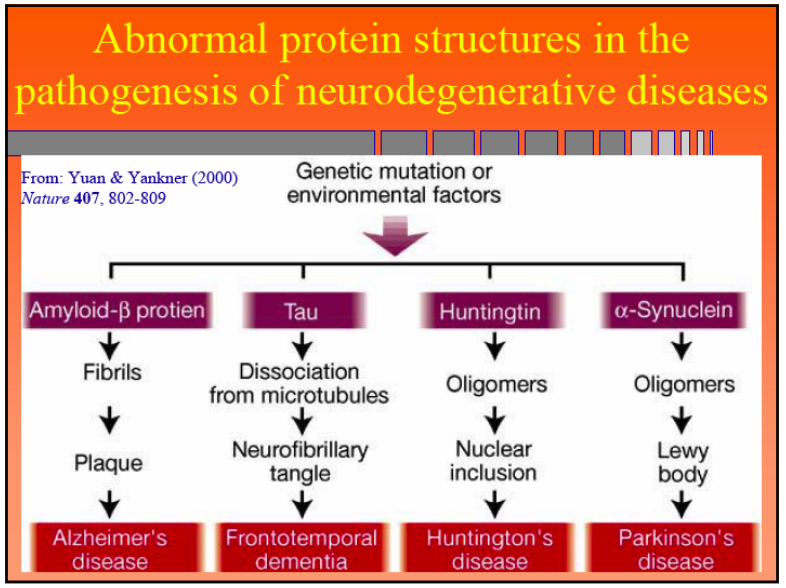
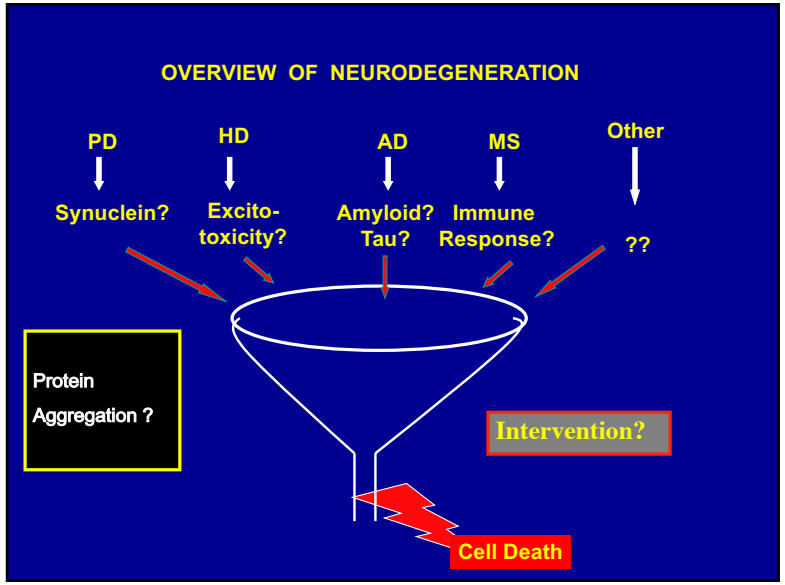
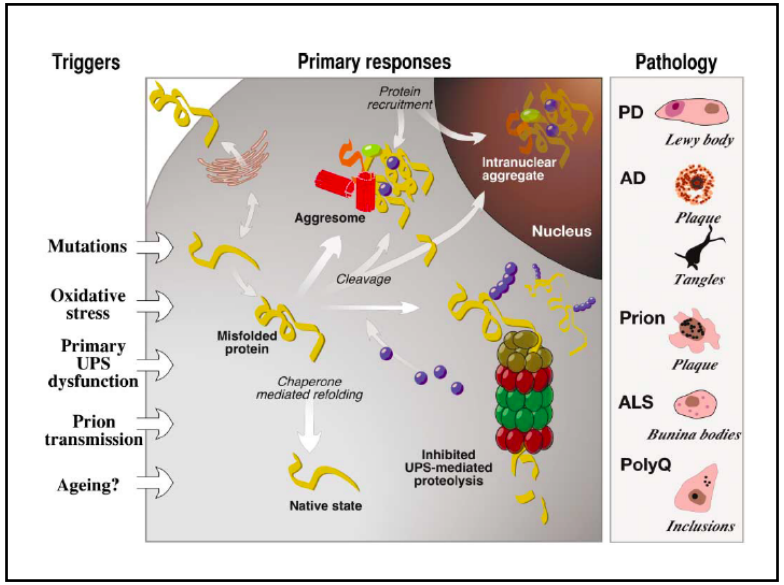


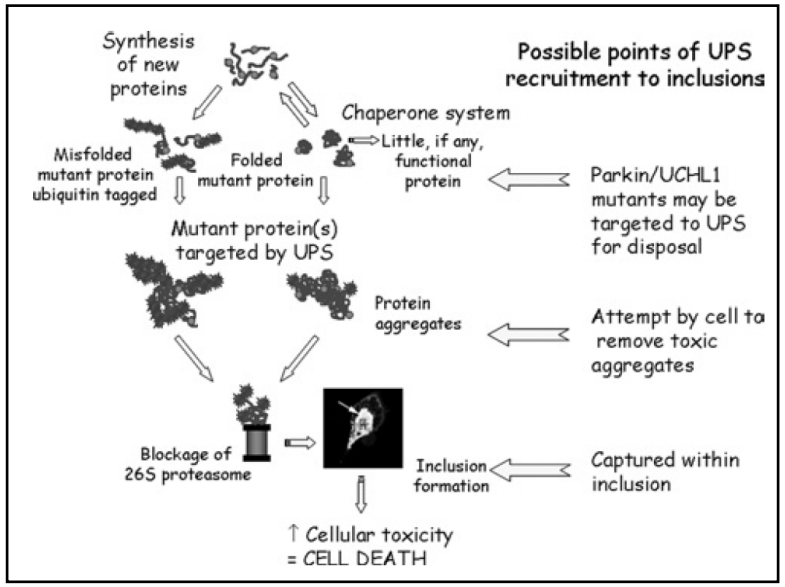
Figure 2. Prion Propagation In Vitro by PMCA and Detection of the Infectivity of the In Vitro-Generated Prions by Inoculation to Hamster

The conversion solution containing an excess of PrP^C (green circles) over the PrP^{Sc} seed (orange polygons) is subjected to multiple cycles of conversion and sonication/incubation. Such a system can replicate prion infinitely and efficiently in vitro. PrP^{Sc} aggregates grow by recruiting (incubation) and converting PrP^C into newly formed PrP^{Sc}, while in the sonication/incubation phase, PrP^{Sc} aggregates are fragmented into smaller aggregates (new seeds). This restarts the cycle overcoming the rate-limiting process of the in vitro PrP conversion associated with the original method. Gray boxes represent tubes.

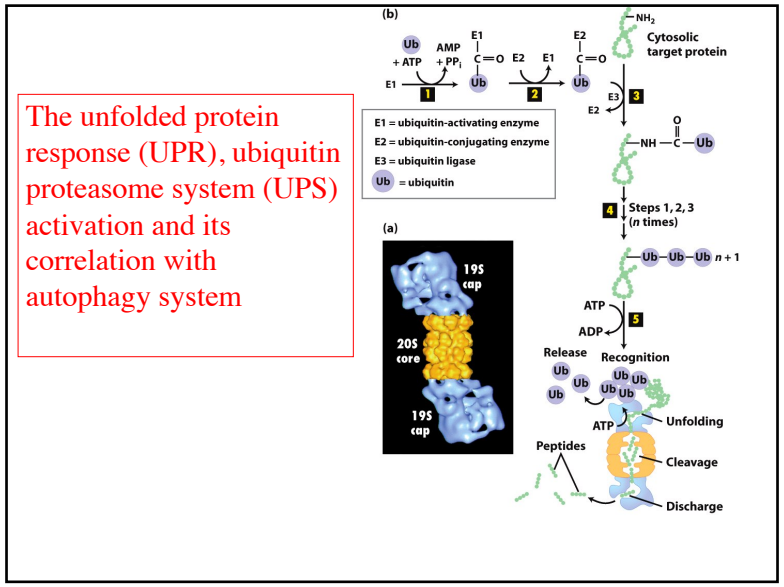








The unfolded protein response (UPR), ubiquitin proteasome system (UPS) activation and its correlation with autophagy system



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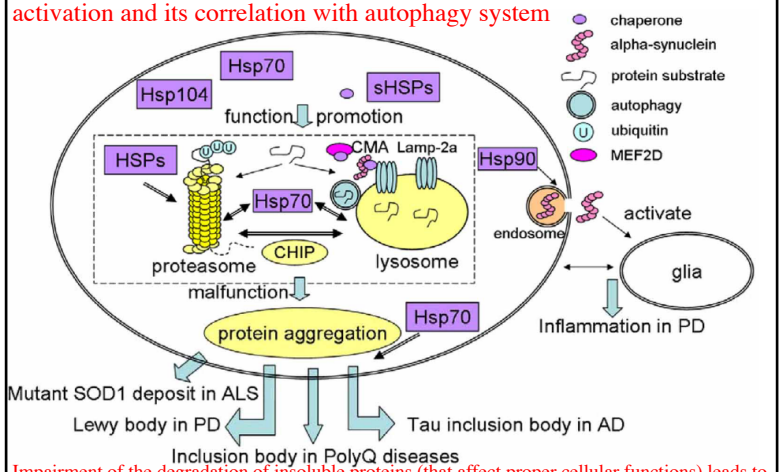

Impairment of the degradation of insoluble proteins (that affect proper cellular functions) leads to NDs such as PD

CHIP: C terminal of molecular chaperone interaction protein; MEF2D: myocyte enhancer factor 2D; CMA: chaperone-mediated autophage