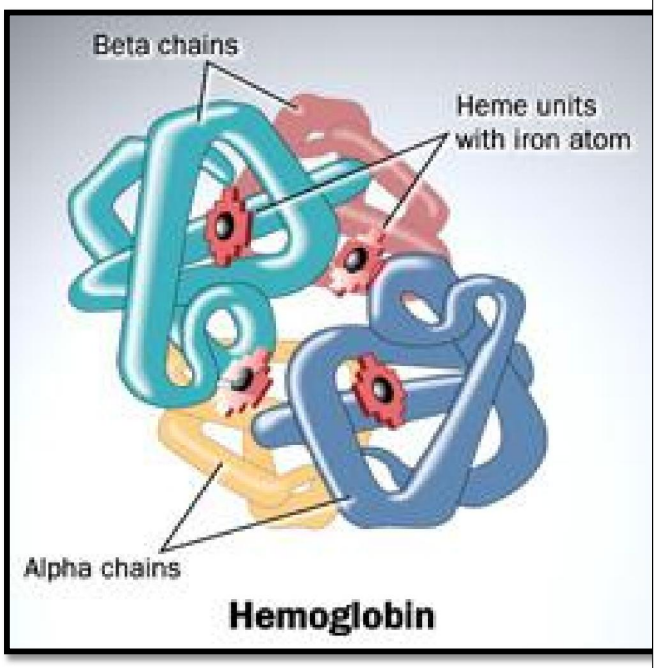
STRUCTURE OF HEMOGLOBIN

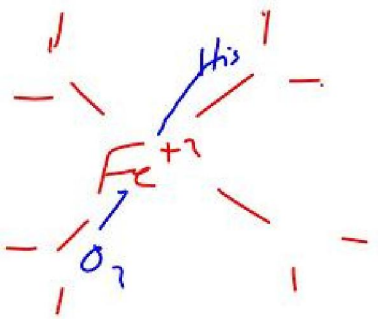
- Hb is a conjugated protein molecule consist of two pairs of polypeptide chain. (alpha & beta)
- Each globin chain bears a heme (iron- porphyrin) group whose central iron atom is the site to which O_2 attaches to hemoglobin.
- Molecular weight of Hb is 64000



Hemoglobin (Hgb A)

* transport protein (O_2)
* Globular
* Conjugated (hemoprotein)
* tetrameric ($\alpha_2\beta_2$) (4°)

141 a.a / each α
& 146 a.a / β
574 a.a → m.wt = 64,000 Da



Hgb found in RBC's (normally circular biconcave disk)

≈ 3×10^8 Hgb molecules / each RBC
(300 million)

Sickle Cell *Disease* and Sickle Cell *Trait*

1. Sickle Cell Traits (HbAS) Adequate amount of normal haemoglobin is present, they are carriers, do not have symptoms of sickle cell disorder.
2. Sickle Cell Anemia (HbSS) Most severe form of disease

HISTORY

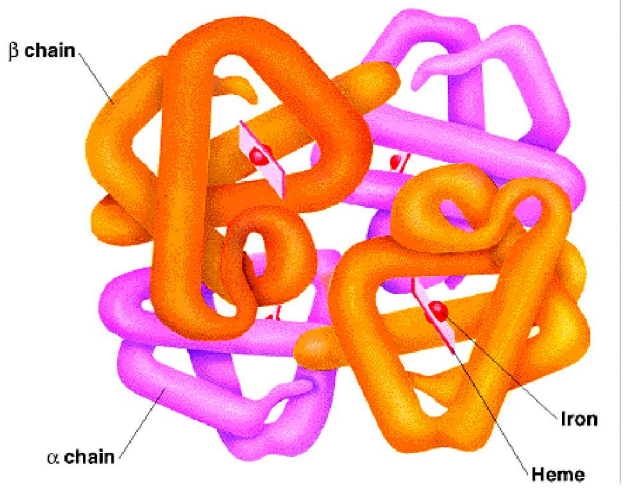
- In the year 1904 cardiologist and professor of medicine ***James B Herrick*** found peculiar elongated cells in blood of a 20 years old dental student who was suffering from anemia.
- ***Veron Mason*** in 1922 named SICKLE CELL ANAEMIA.
- ***Linus Pauling*** in 1940 demonstrated that sickling occurs as a result of abnormality in haemoglobin molecule.
- Sickle cell disease is the first genetic disorder whose molecular basis was known.
- **June 19th** is celebrated as World Sickle Cell Day

- Sickle cell anemia is a autosomal recessive genetic disease that results from the substitution of Valine from Glutamic acid in position 6 of beta globin gene leading to production of defective form of haemoglobin (Hb S).
- Hb S is a structurally defective haemoglobin.

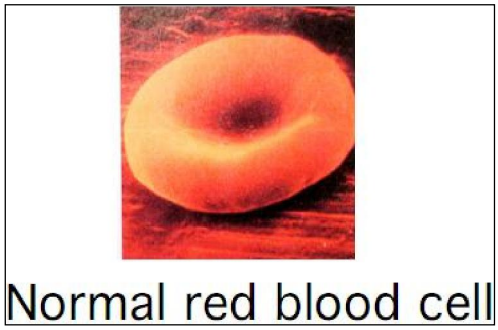
Hemoglobin (Hgb)

- Multi-subunit protein (tetramer)
  - 2 α and 2 β subunits
- Heme
  - One per subunit
  - Has an iron atom
  - Carries O_2
- In red blood cells

(about 300million Hgb molecules /RBC)



Sickle cell anemia is an autosomal recessive genetic disorder. A single amino acid substitution in hemoglobin is responsible for the development of sickle cell anemia. This single amino acid substitution (Val instead of Glu) in position 6 of each of the β subunits creates an abnormal hydrophobic sticky spot on the surface of hemoglobin molecules, resulting in the adherence of hemoglobin proteins to each other, forming long cables that can distort normal red blood cells into sickle shaped cells. Unlike the normal circular biconcave red blood cells which are flexible and can fit through small capillaries, sickle red blood cells are inflexible and have the tendency to clog blood vessels. This can result in organ damage and severe pain in individuals with sickle cell anemia.



Normal red blood cell

Sickle red blood

Sickle Cell Hemoglobin

Normal mRNA

```
GUG CAC CUG ACU CCU GAG GAG AAG
val his leu thr pro glu glu lys
 1   2   3   4   5   6   7   8
```

Normal protein

Mutation
(in DNA)

Mutant mRNA

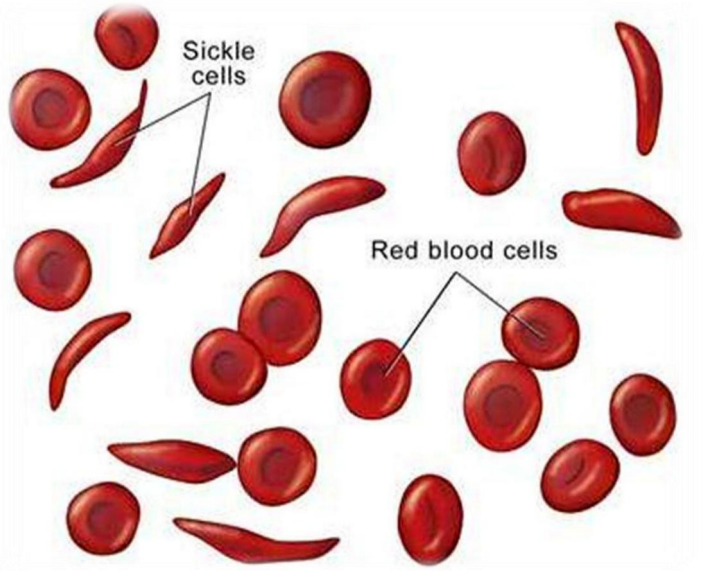
```
GUG CAC CUG ACU CCU GUG GAG AAG
val his leu thr pro val glu lys
 1   2   3   4   5   6   7   8
```

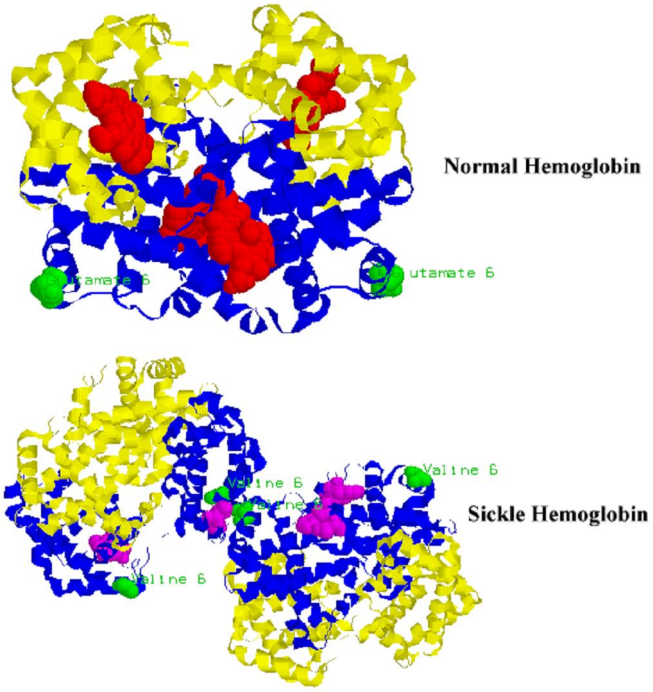
Mutant protein

Glutamate (glu), a negatively charged amino acid, is replaced by valine (val), which has no charge.

Sickle Cell Hemoglobin

Significant change in structure caused by the single mutation

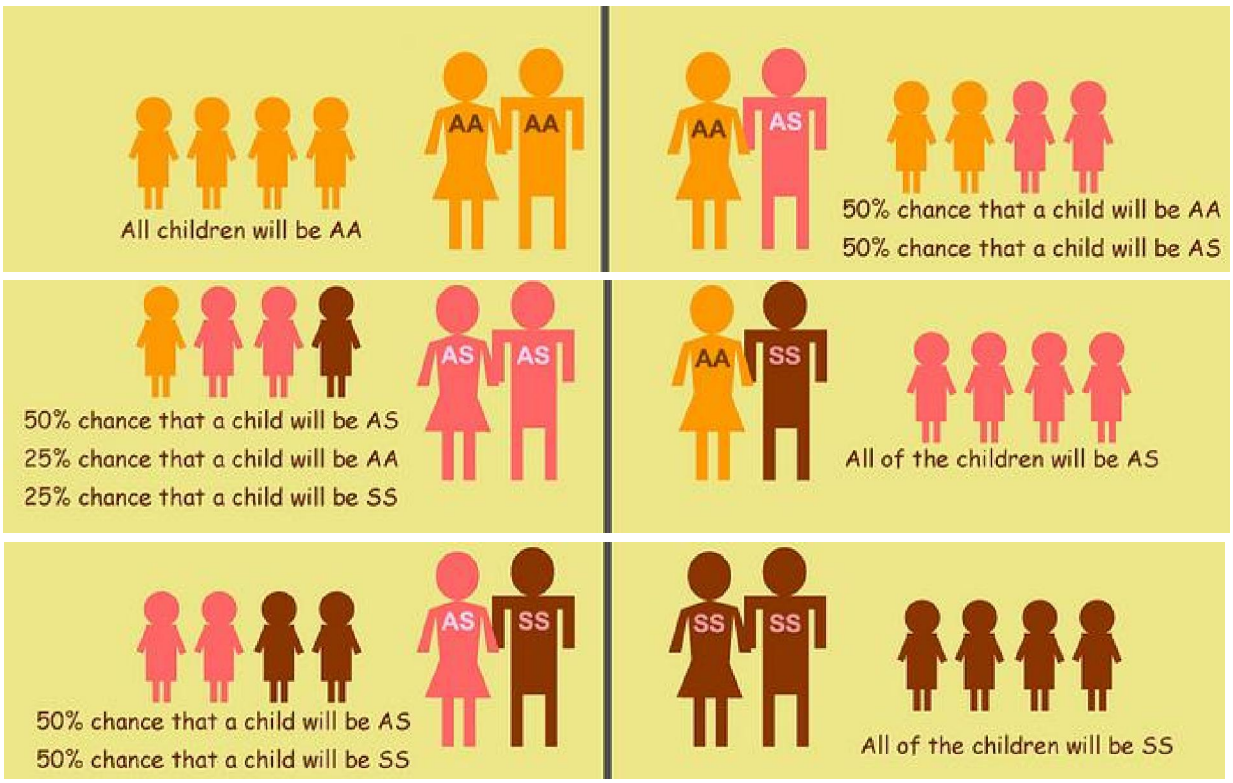




Note: The Sickle hemoglobin image is drawn at 50% of the size of the Normal hemoglobin

http://www.siskiyous.edu/class/bio1/genetics/sickle_cell.html

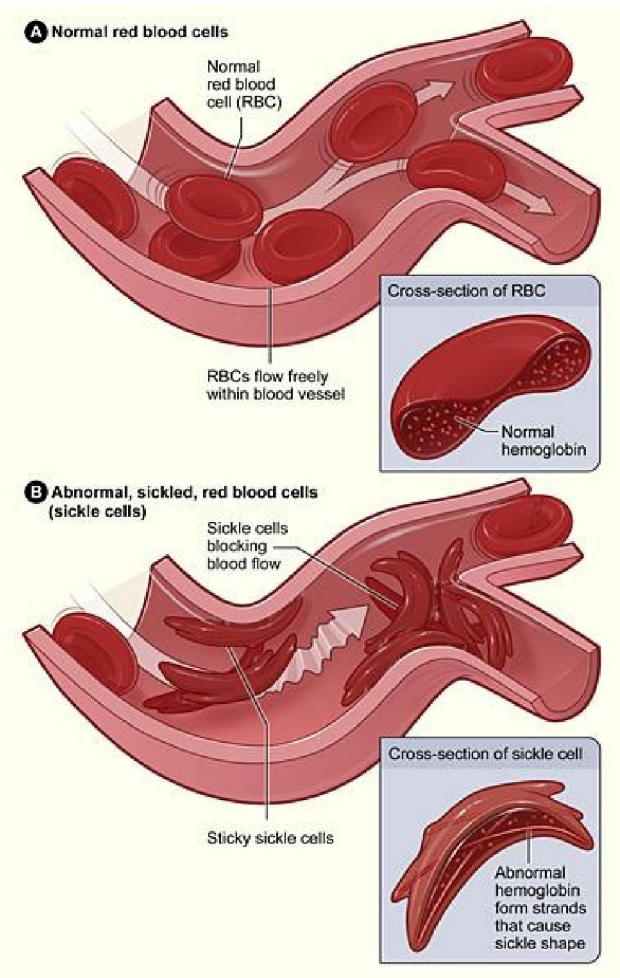
Sickle Cell Anemia Inheritance

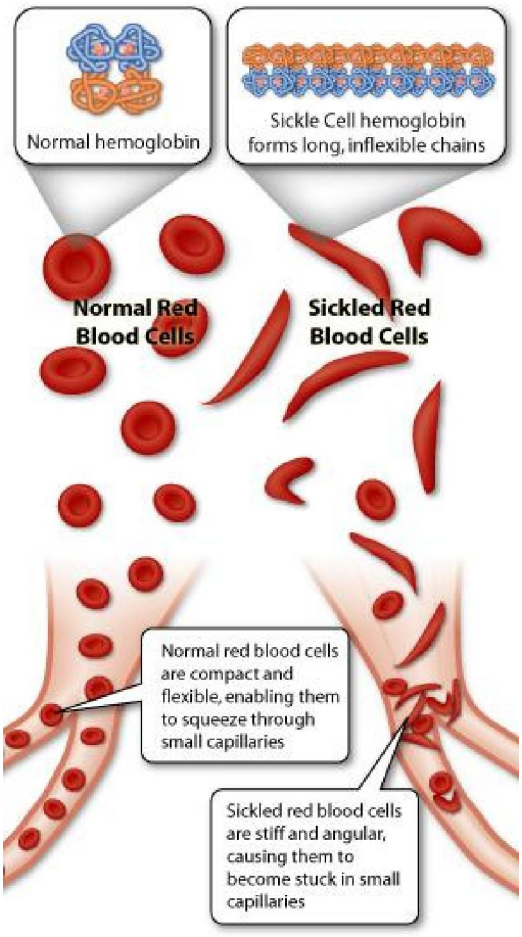


KEY:
AA- Person with no sickle-cell traits
AS- Person with sickle-cell-trait
SS- Person with sickle-cell-anaemia

Change in properties of Sickle Cell RBC

- Deoxygenation leads to hydrophobic interaction between adjacent Hb S molecules.
- Distortion of RBC into sickle form cells
- Rapid haemolysis
- Decreased elasticity of cell wall of RBC
- Decreased life span 10 – 20 days.
- Clogging of RBC in microcirculation.

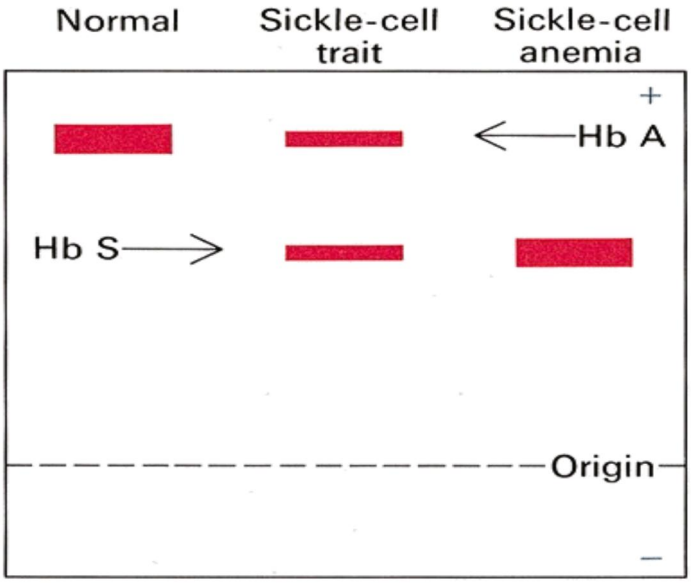




Biochemistry 24311
Dr. Awni A. Abu-Hijleh

Electrophoresis

Due to the replacement of 2Glu (Hgb A) by 2Val (HgbS), HgbA has two more –ve charges, so it will migrate further towards anode compared to HgbS.



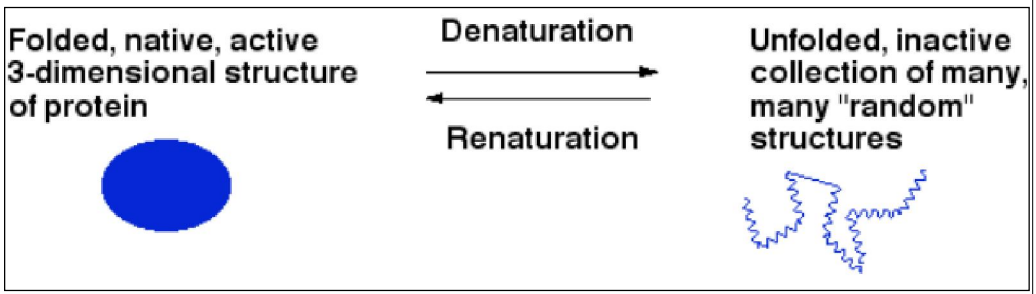
PRIMARY STRUCTURE DETERMINES TERTIARY (AND QUATERNARY) STRUCTURES.

– demonstrated by the fact that many proteins can refold from a more or less "random coil" set of conformations without "instructions" from any other cellular components

– All the information for 3-dimensional structure is provided by the amino acid sequence.

• Proteins can be *unfolded (denatured)* in vitro by chemical agents like urea, or extremes of heat or pH, and then *refolded (renatured)* by diluting out the chemical denaturant, changing the pH, etc.

- Protein denaturation is not accompanied by hydrolysis of peptide bonds (Primary structure is not affected)



Proteins that *don't* (re)fold on their own, without assistance, *don't* need other *"instructions"* -- they just need "molecular chaperones" (which are also proteins) to keep them from slipping off the folding pathway or to help them to get back on it.
– Some chaperones require "expenditure" of energy currency (hydrolysis of ATP) to carry out their function.

Many diseases are the result of defects in protein folding, e.g., neurodegenerative diseases such as the prion diseases (spongiform encephalopathies like human CJD, bovine “mad cow” disease), Alzheimer disease, Parkinson disease), Alzheimer disease, Parkinson disease, ……
— One hypothesis is that cellular degradation apparatus can’t keep up with disposal of the abnormally folded protein.

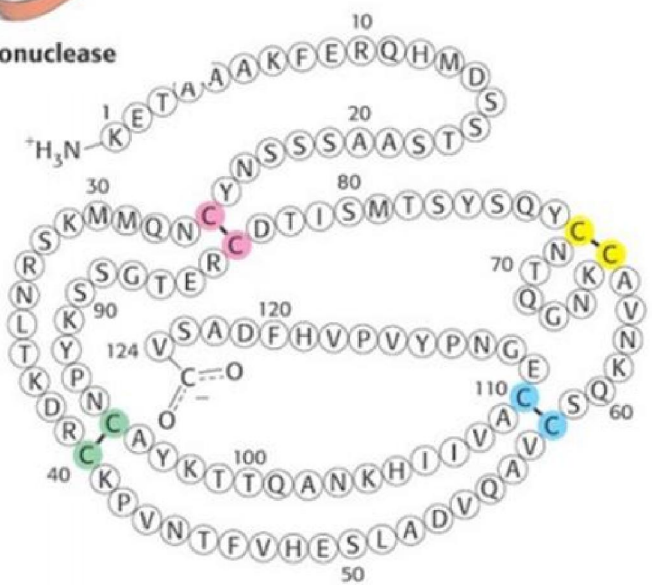
Proof that AA sequence determines 3-D structure: Anfinsen’s experiments with Ribonuclease A

Bovine ribonuclease

Human ribonuclease

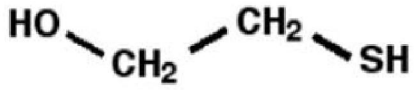
Tertiary structure of ribonuclease

Amino acid sequence (primary structure) of bovine ribonuclease
• Note the 4 disulfide bonds



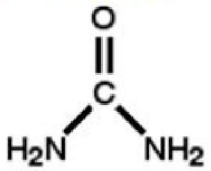
urea (denaturing agent) and β-mercaptoethanol (reducing agent to reduce disulfide bonds)

Reducing agents (donate electrons):
e.g., *thiols*, such as β-mercaptoethanol



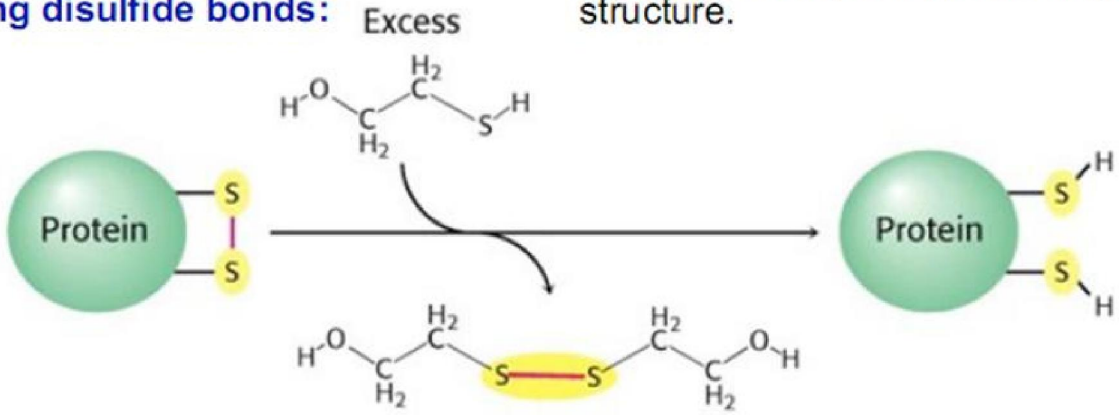
β-mercaptoethanol reduces disulfide bonds in proteins.

Denaturing agents:
e.g., urea (shown), guanidinium HCl



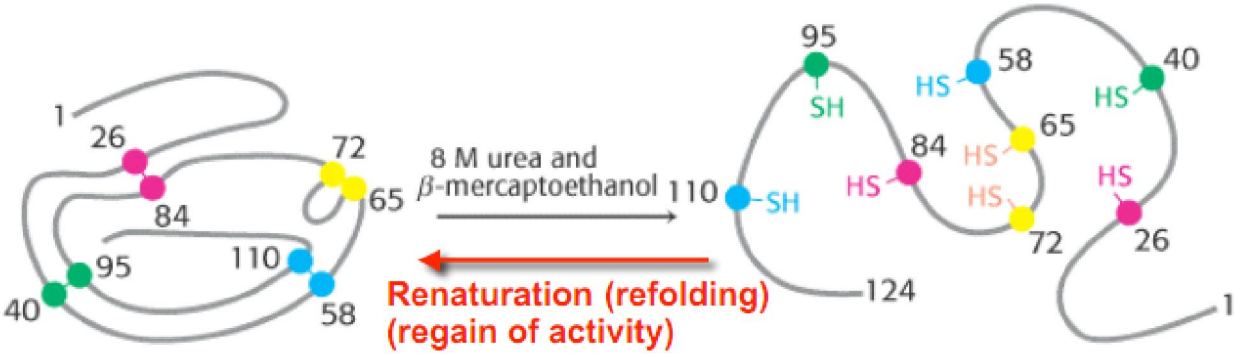
Denaturing agents like urea disrupt the noncovalent bonds within the protein that stabilize its native tertiary and quaternary structure.

Role of β-mercaptoethanol in reducing disulfide bonds:



Anfinsen's experiments: unfolding and refolding RNase

- **He unfolded RNase with denaturing agent (8 M urea).**
- **problem:** 4 S–S bonds in RNase (covalent crosslinks) stabilize some of the 3-D structure even when backbone is unfolded.
- **Solution: reducing agent (β-mercaptoethanol)** -- reduces disulfide bonds (S–S --> 2 SH groups), so "unfolded" protein is ***entirely*** unfolded.



Native ribonuclease **Denatured reduced ribonuclease**

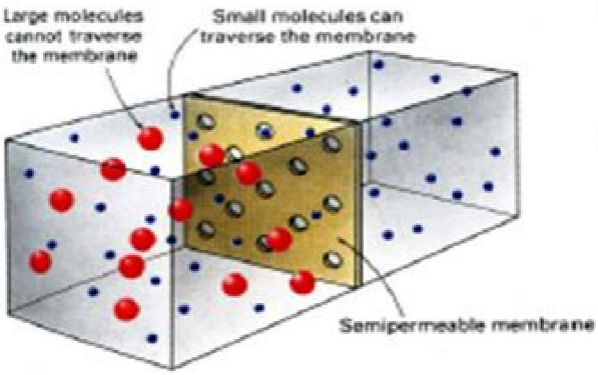
- Loss of native structure -- ***denaturation*** -- inactivates RNase.
- slow removal of urea (by dialysis) --> refolded protein
- refolded in absence of reducing agent (so O_2 in air could reoxidize SH groups to disulfides)
- **Enzyme refolded and *regained activity*** -- proof the **right combinations of S-S bonds** had formed (i.e., structure was correct)

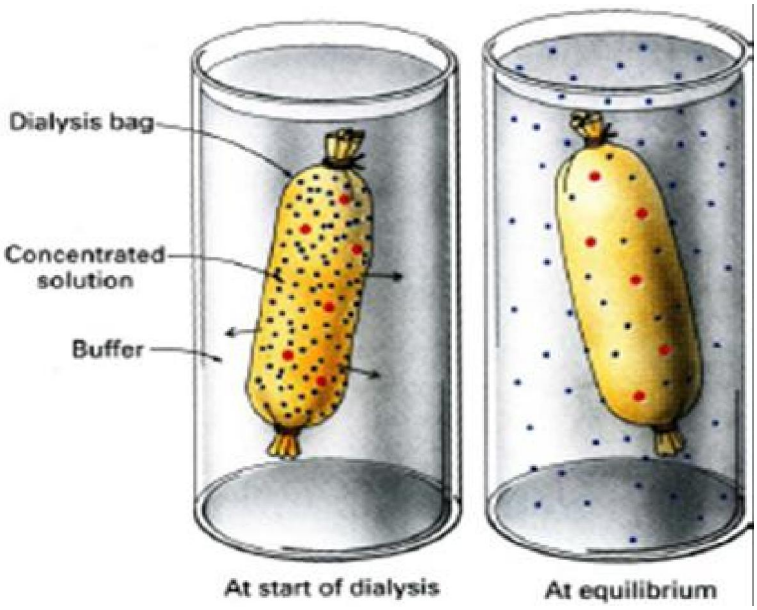
Dialysis

Passage of solutes through a semi-permeable membrane.

Pores in the dialysis membrane are of a certain size.

Protein stays in, while salts and other molecules *smaller than the pore* size pass through.





INCORRECT PROTEIN FOLDING (MISFOLDING) AND NEURODEGENERATIVE DISEASES

- The correct three-dimensional structure is essential to function, although some parts of functional proteins may remain unfolded. Failure to fold into native structure (misfolding) produces inactive proteins that are usually toxic (infectious). Several neurodegenerative and other diseases are believed to result from the accumulation of amyloid fibrills formed by *misfolded* proteins.
- Aggregated proteins are associated with prion-related illnesses such as Creutzfeldt-Jakob disease (CJD), bovine spongiform encephalopathy (mad cow disease) and amyloid-related illnesses such as Alzheimer's disease, as well as diseases such as Huntington's and Parkinson's disease. These age onset degenerative diseases are associated with the multimerization of misfolded proteins into insoluble, extracellular aggregates and/or intracellular inclusions including cross-beta sheet amyloid fibrils.

Misfolding of some proteins may cause several rare diseases

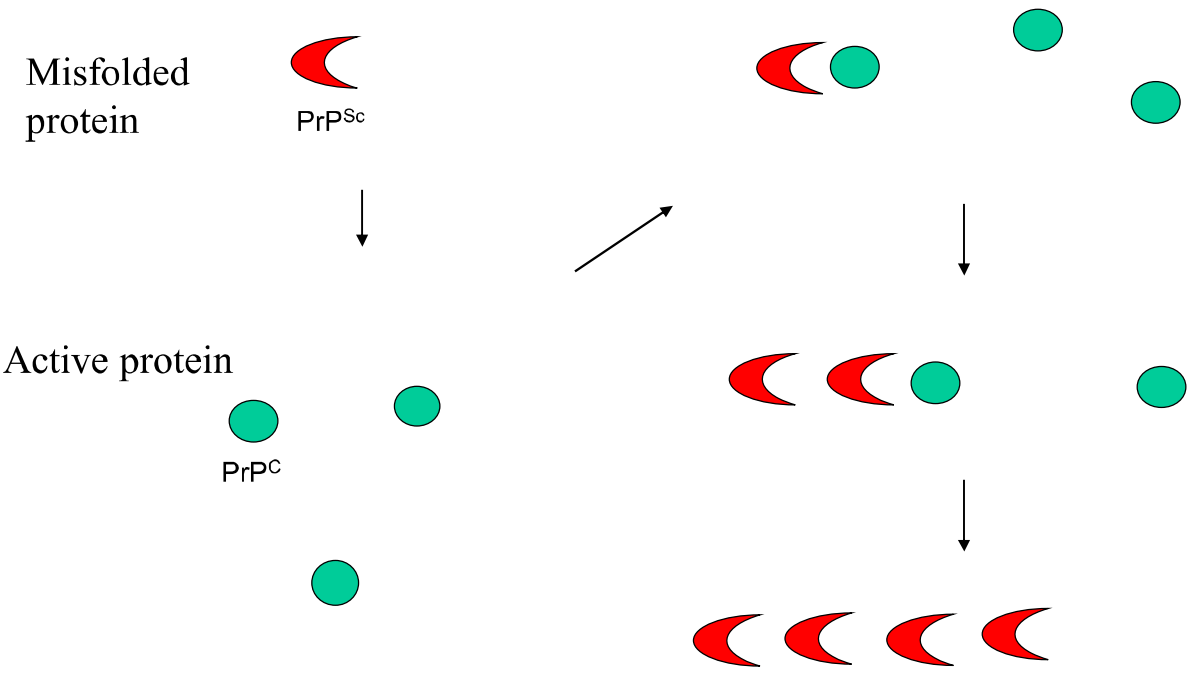
e.g. Prion diseases

An example of Prion diseases is mad cow disease, or bovine spongiform encephalopathy (BSE), which is a fatal brain disorder that occurs in cattle. Abnormal protein folding (misfolding) is considered crucial to the onset of the disease.

Evidence indicates that the infectious agent in transmissible spongiform encephalopathy is a protein (Prion protein). Stanley Prusiner pioneered the study of these proteins and received the Nobel Prize in 1997. He has named them prion proteins (referred to as PrP) or simply prions.

The normal protein is called PrP^{c} (for cellular) is a normal constituent of brain tissue in most mammals (function not well known) of a m.wt. of 28,000 Da. Its secondary structure is dominated by alpha helices. The abnormal, disease producing protein called PrP^{Sc} (for Scrapie), has the same primary structure as the normal protein, but its secondary structure is dominated by beta conformations. Interaction of misfolded form (PrP^{Sc}) with the normal PrP^{c}converts them very rapidly (domino effect) to PrP^{Sc}

Misfolded proteins can be infectious (Mad Cow's Disease, Prion proteins)

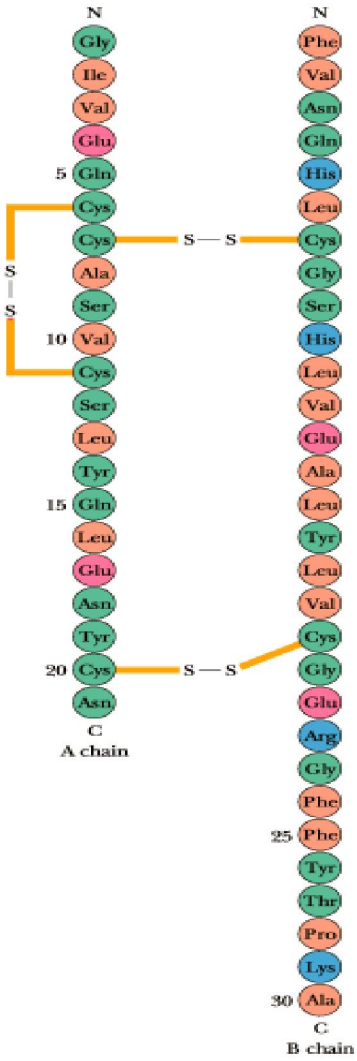


Stanely Prusiner: 1997 Nobel Prize in Medicine

PROTEIN SEQUENCING

First Sequence

- The first protein sequencing was achieved by Frederic Sanger in 1953.
- He determined the amino acid sequence of bovine insulin
- Sanger was awarded the Nobel Prize in 1958



Protein Sequencing

→ Cleavage of -S-S- bonds
① β-mercaptoethanol } followed by alkylation using iodoacetate
② DTT
③ Performic acid

→ Complete hydrolysis → mixt. of individual a. acids
⊛ 6N HCl for ≈ 24h at 110°C, in vacuo
(a. acid analyzer) separated & analyzed

→ N-terminal determination
① Sanger reagent (2,4-dinitrofluorobenzene) DNFB
② Dansyl chloride
⁂ ③ Edman reagent (phenylisothiocyanate)

→ C-terminal determination
① Carboxypeptidases (enzymatic)
② Hydrazine (H_2N-NH_2) = Hydrazinolysis

→ Internal cleavage

cleaving at specific peptide bonds using enzymatic or chemical reagents → Unique fragments

① Cyanogen bromide (CNBr)

→ C-side of R_n = Met

② Trypsin

→ C-side of R_n = Lys or Arg ($R_{n+1} \neq Pro$)

* Trypsin + M.A → C-side of R_n = Arg
* Trypsin + E.I → C-side of R_n = Lys, Arg & Cys

③ Chymotrypsin

→ C-side of R_n = Phe, Tyr, Trp + Leu

($R_{n+1} \neq Pro$)