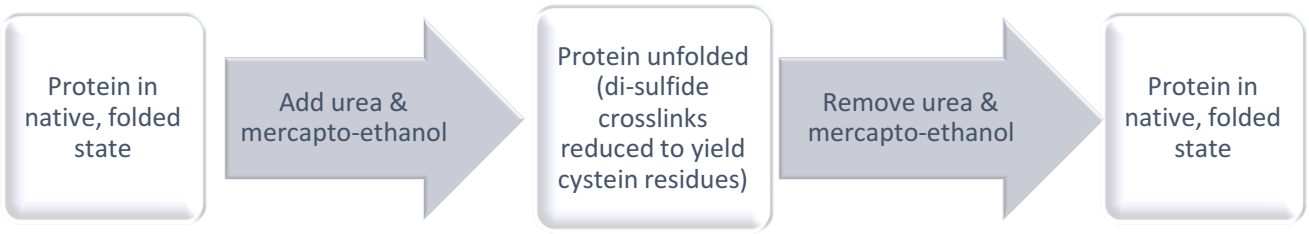
Lecture 13

Afinsens experiment

- Urea → breaks H-bonds
- Mercaptoethanol → breaks di-sulfide bonds



How do proteins fold?

- Protein folding can be described as a **free energy tunnel**
- **Unfolded** proteins have ↑ **free energy** and a **large number of species**
- Proteins explore different conformation states; equilibrium b/w many different possible structures
- As folding procees, intermediates form which restrict the number of species present
- At the bottom of the funnel, a **single native state** is present with relatively ↓ free energy

- 3 classic folding mechanisms
  - Secondary Structure Formation (Framework) → followed by diffusion/collision
  - Nucleation → followed by growth
  - Hydrophobic Collapse → chain collapses around hydrophobic side chains

Mechanism of amyloid formation

Amyloid: extracellular (or intracellular) **fibrillar deposits** associated with disease
- strong, irreversible and water resistant

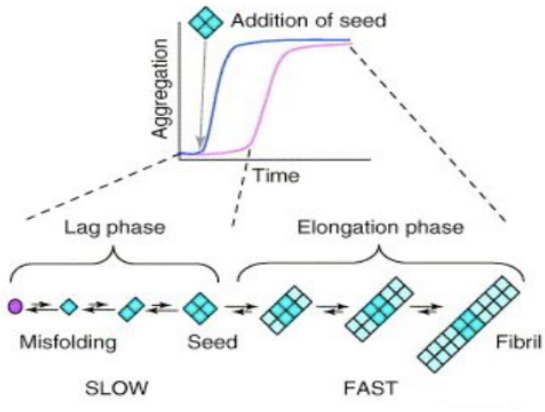
Amyloidoses (diseases associated with amyloid formation) → **slow-onset** and **degenerative**
Amyloidogenic proteins → distinct amino acid sequence and native fold

Some animals convert their normal proteins into amyloid fibrils which have important functions (as opposed to causing disease)

e.g. Spidroin → spider silk
Curlin → *E. coli* use curlins to colonise surfaces and bind to host proteins

Kinetics of Fibril Formation

Blue Line:
- 'Seed' (amyloid-prone sequence which has already begun to form fibrils and aggregate..the nuclei is already formed) is taken and put into a new solution
- More rapid extension of the fibril protein + faster aggregation to produce amyloid
- Short '**Lag**' phase



Pink Line:
- A normal sequence prone to form amyloid is placed in a tube
- Initially, very shallow curve with long '**Lag**' phase

Lag phase: formation of 'nuclei'
Lag phase is followed by a rapid exponential **elongation** phase (monomers or oligomers associate with the nucleus)

Structure of amyloid

Technique	Finding
Electron Microscopy	• fibrils are straight, 7-12nm in diametre
X-Ray Fibre Diffraction	• cross-β structure
NMR and X-Ray Crystallography	• β-sheets hydrogen bonded 4.7Å apart • Sequence dictates length of strands, length of loops & turns and parallel/anti-parallel
Circular dichroism (CD) and Fourier transform infra red spectroscopy	• ↑ β-sheet composition

Toxicity of amyloid

Loss of Function

- incorrect folding may lead to loss of function
- aggregation could also cause loss of protein function
- misfolding may cause incorrect trafficking

Gain of Function

- interaction b/w aggregate and cellular components
- non-neurological amyloidosis due to large deposits of aggregated protein in/around vital organs
    - *e.g.* Transthyretin → β-sheet rich protein predisposed to form amyloids
        - carries thyroid hormone in serum and CSF
        - mutant form (transthyretin) forms amyloids in the blood and aggregates around tissue/organs

Pre-Fibrillar Aggregates (short aggregates) are more toxic to cells compared to longer aggregates (mature fibrils) as the mature forms are benign and thus less toxic.

- Protofibrils (Pre-fibrallar aggregates) have hydrophobic side chains and other regions of the protein exposed
- Mature fibrils are inert and resistant to proteolysis and degradation