EMBO YIP PhD COURSE
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# Protein Structure and disease

**Fabrizio Chiti**
**Università di Firenze**

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## (also known as *Protein Conformational Diseases*)

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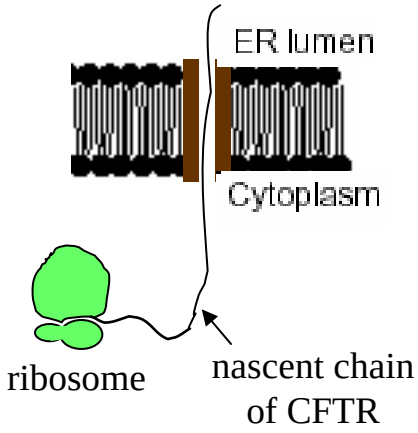

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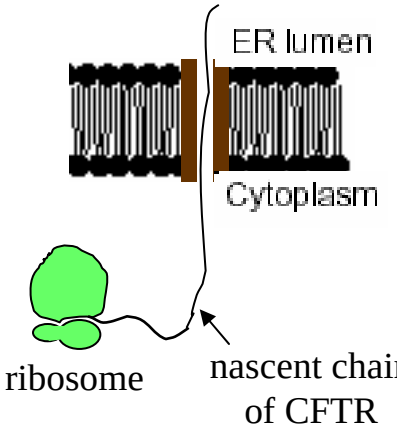


end of translation,
folding on the ER membrane,
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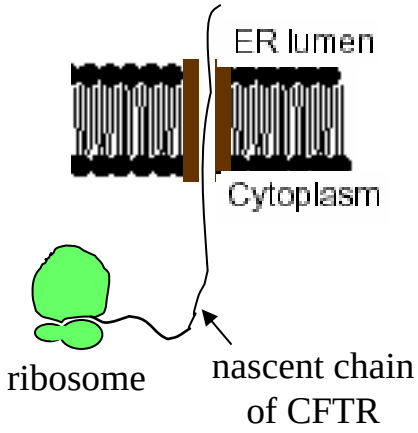

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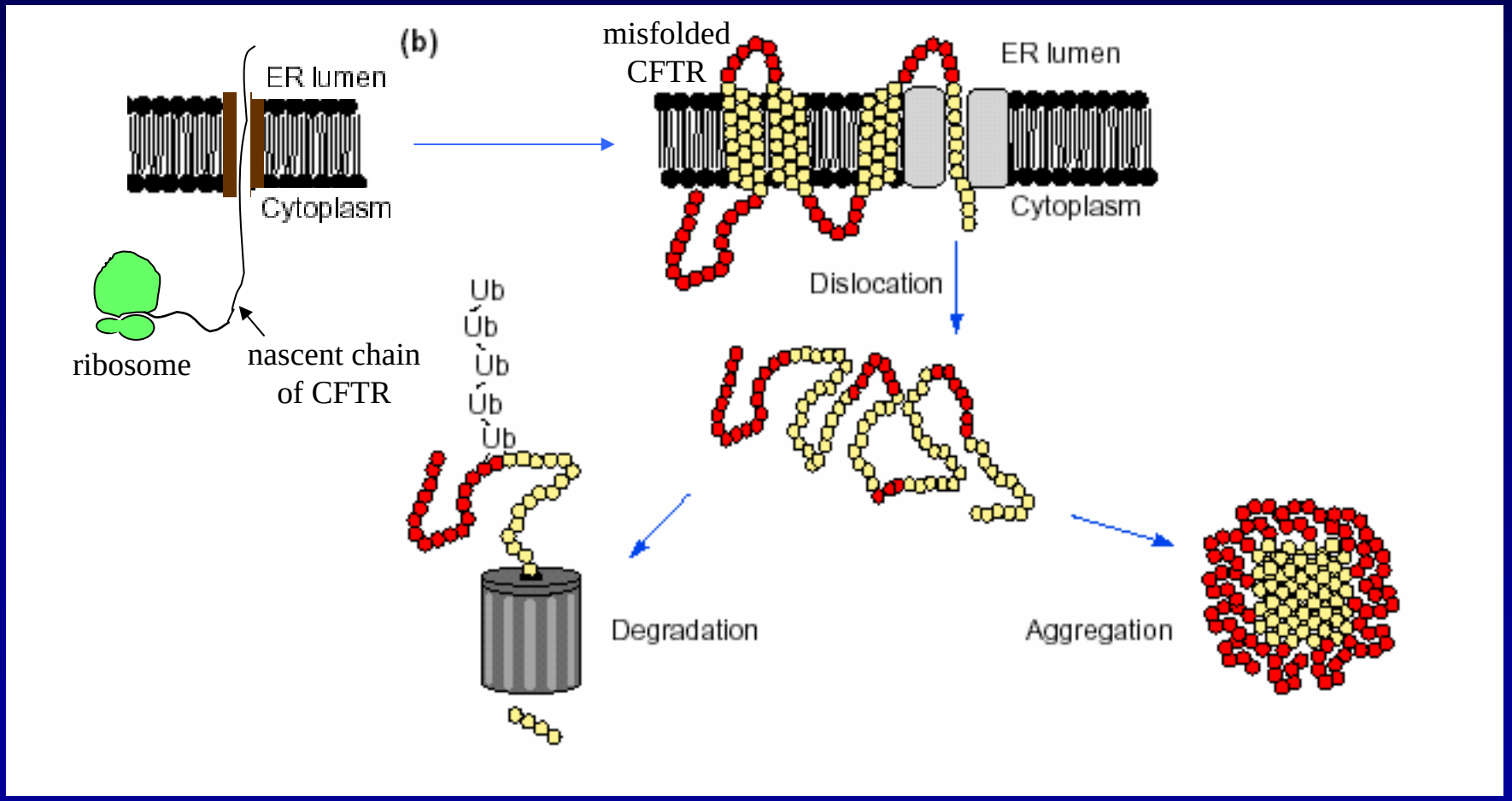

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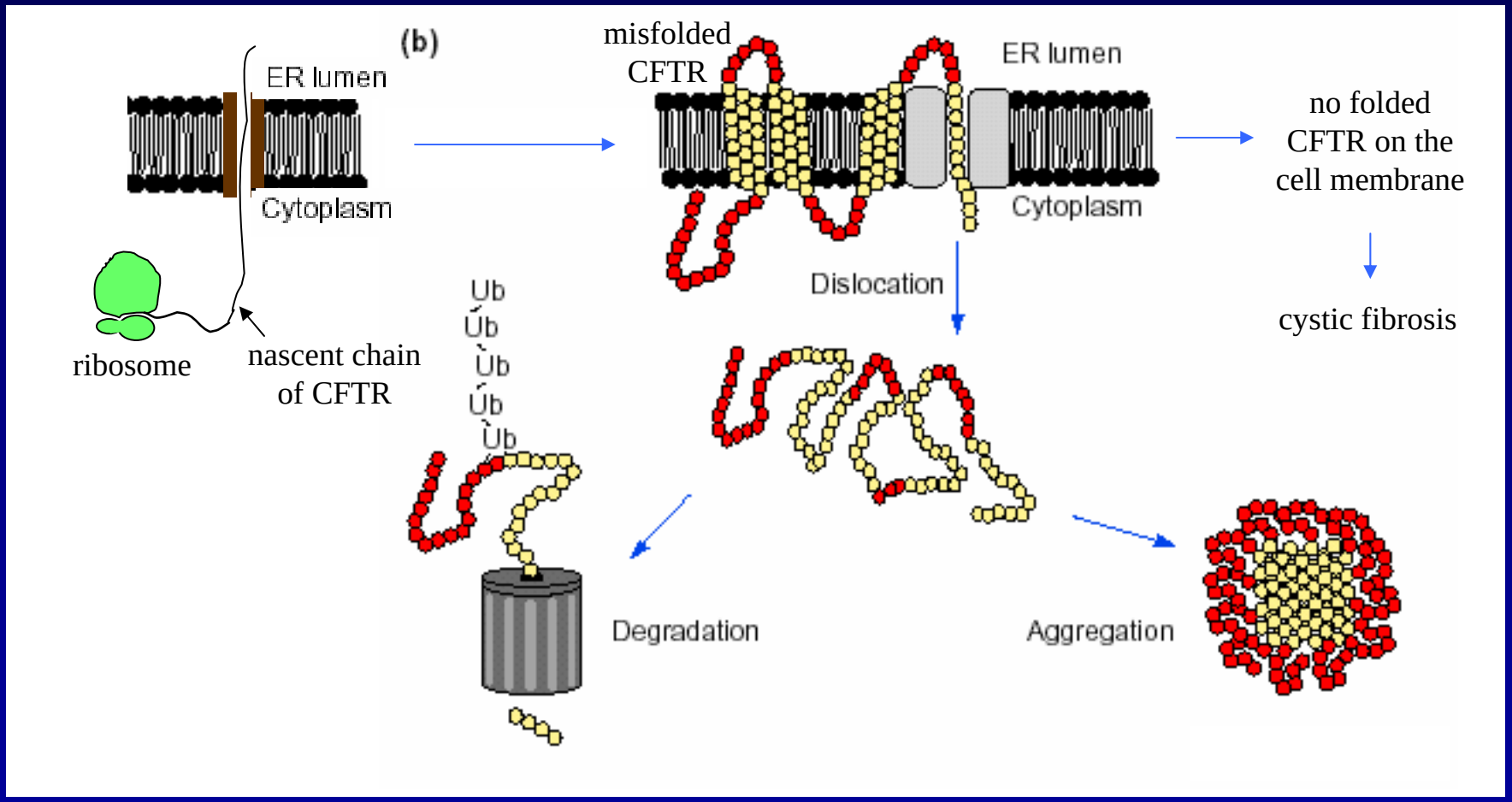

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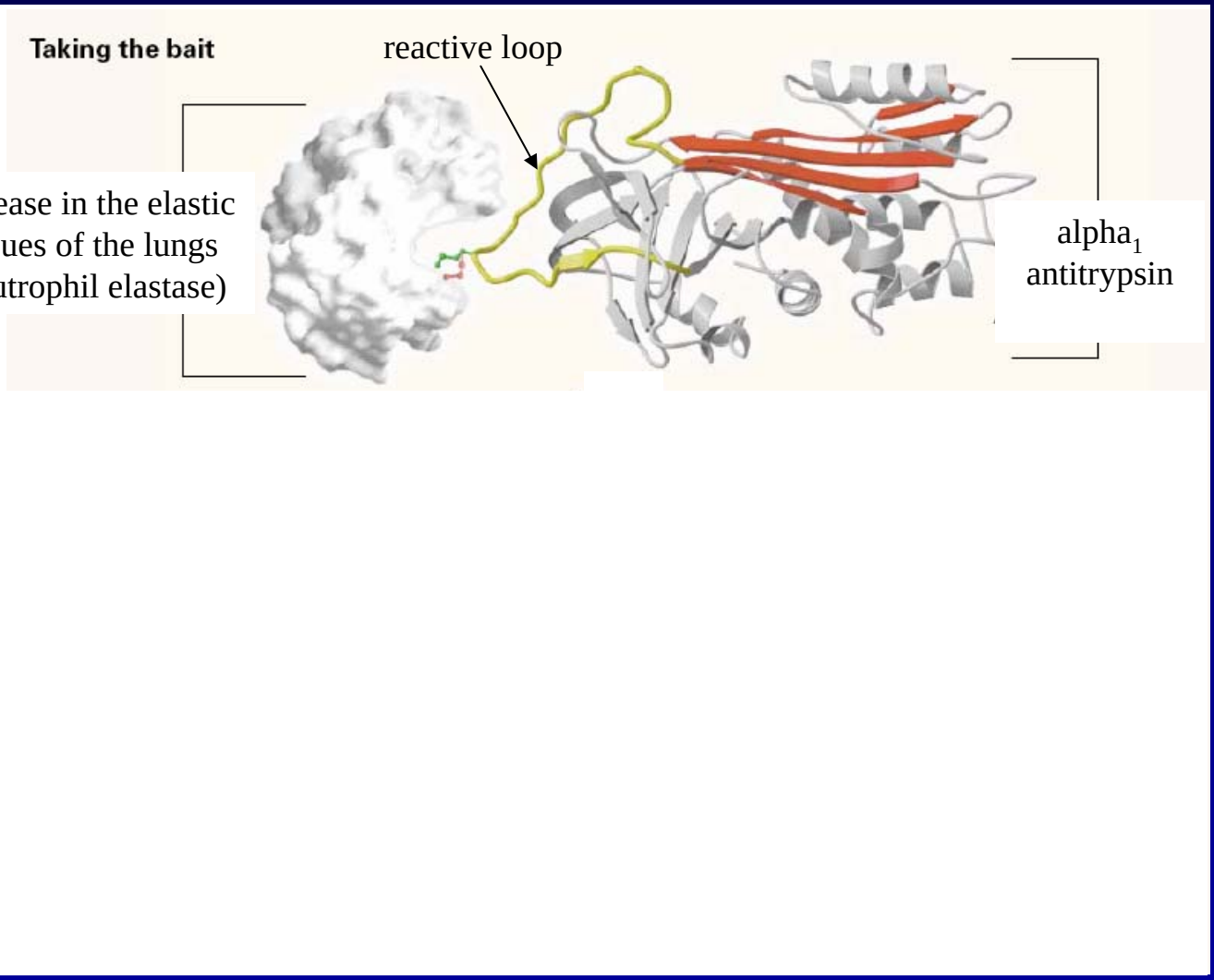

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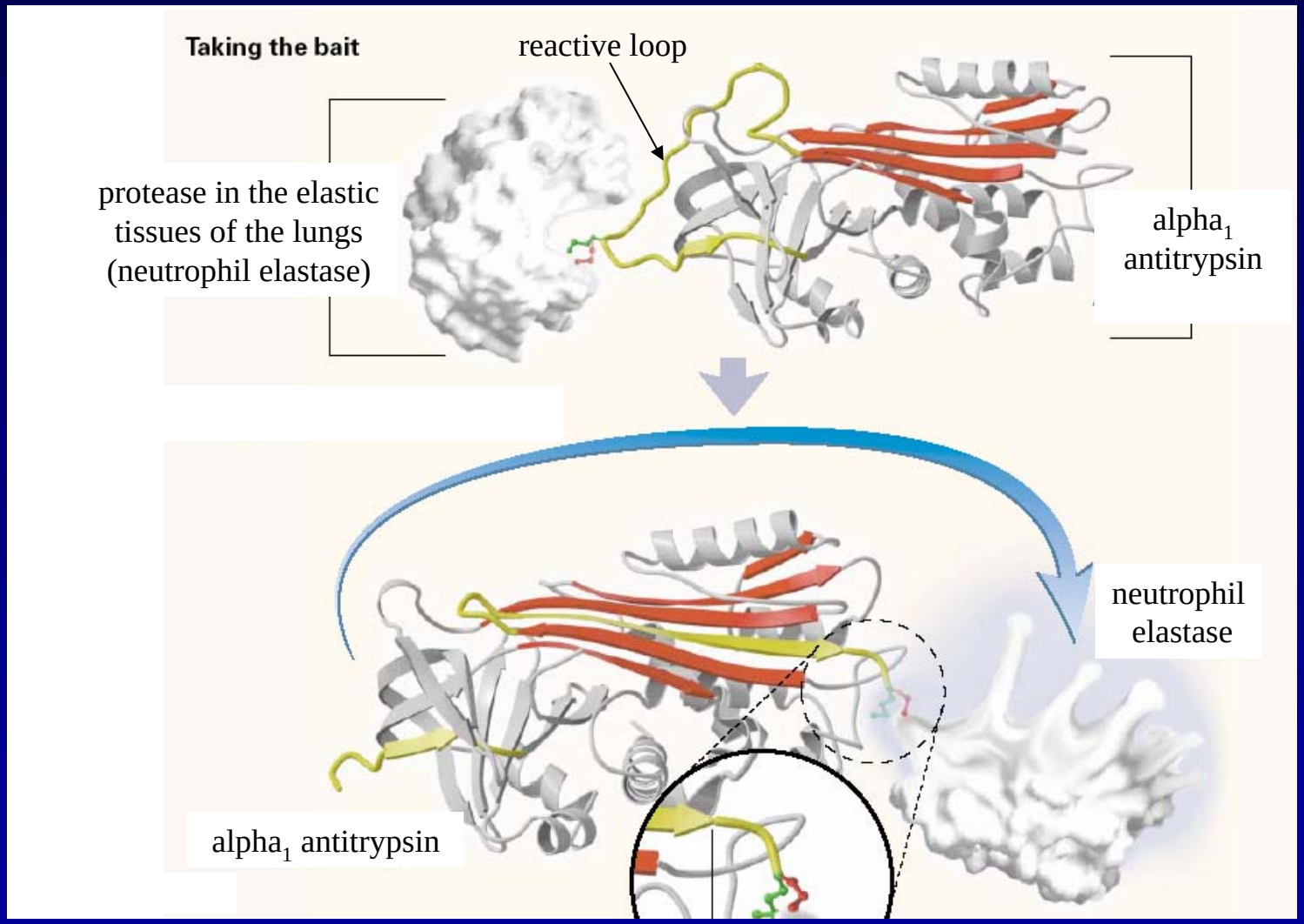

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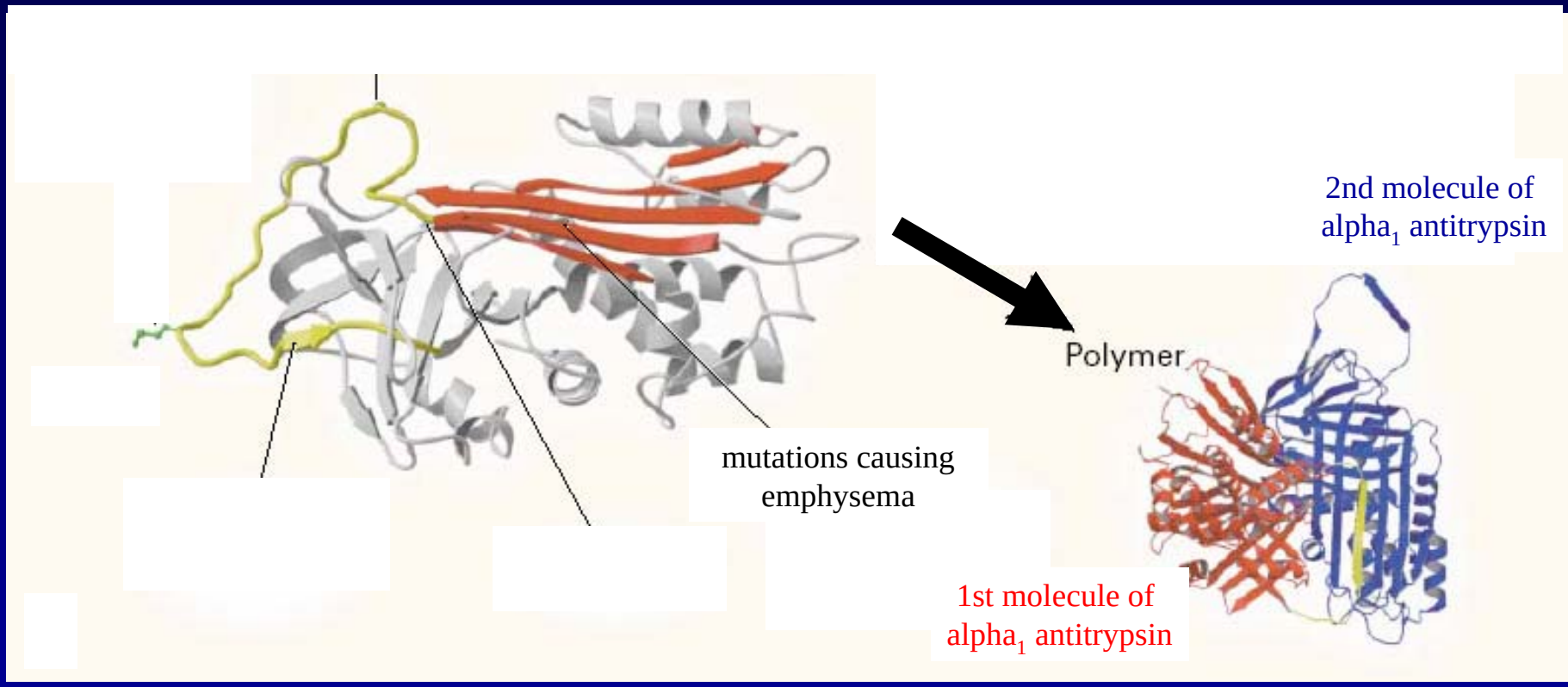

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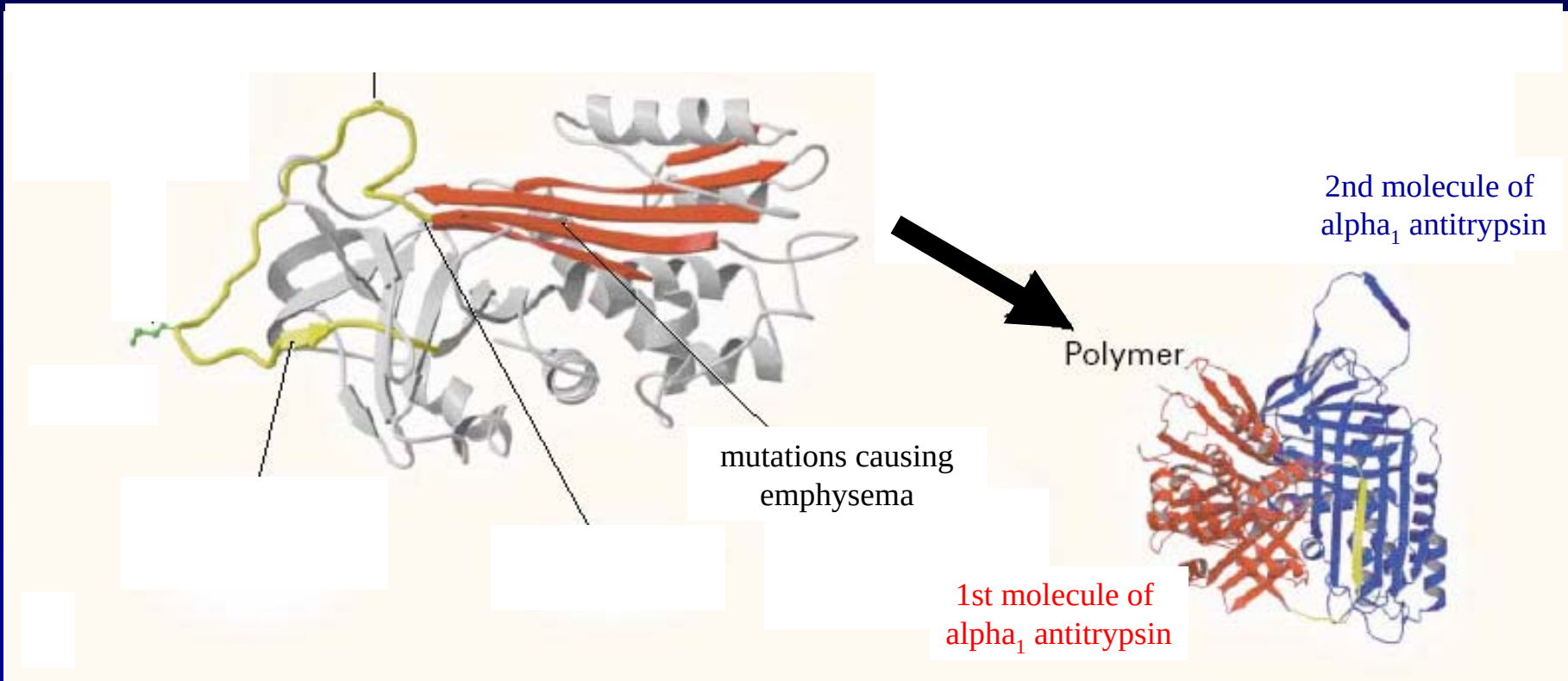
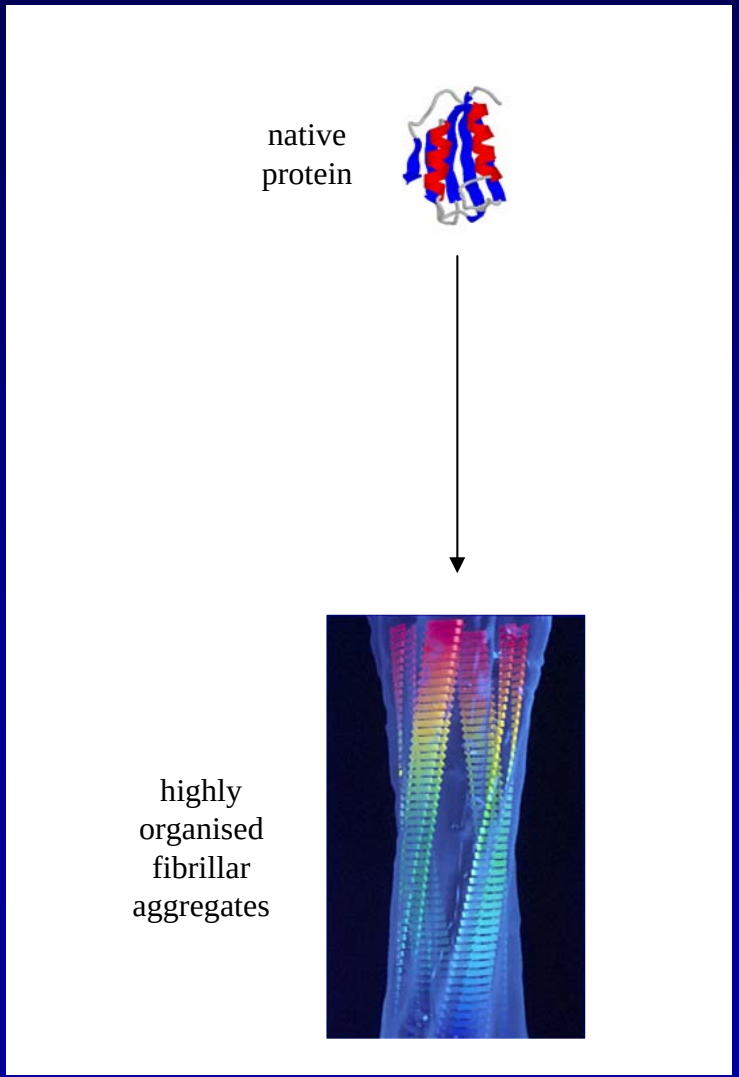



**polymers of alpha$_1$ antitrypsin molecules will form. These will be retained in the liver (where alpha$_1$ antitrypsin is produced) and will not reach the lungs**

**3. Diseases associated with the conversion of a given peptide or protein from its soluble native state into highly organised fibrillar aggregates**

**Example: Alzheimer's disease**



# Over 40 human diseases are associated with formation of extracellular amyloid fibrils or intracellular inclusions with amyloid-like characteristics

| - Neurodegenerative conditions | aggregating protein |
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| Alzheimer's disease | Aβ peptide (1-40, 1-42) |
| Spongiform encephalopathies | prion (whole or fragments) |
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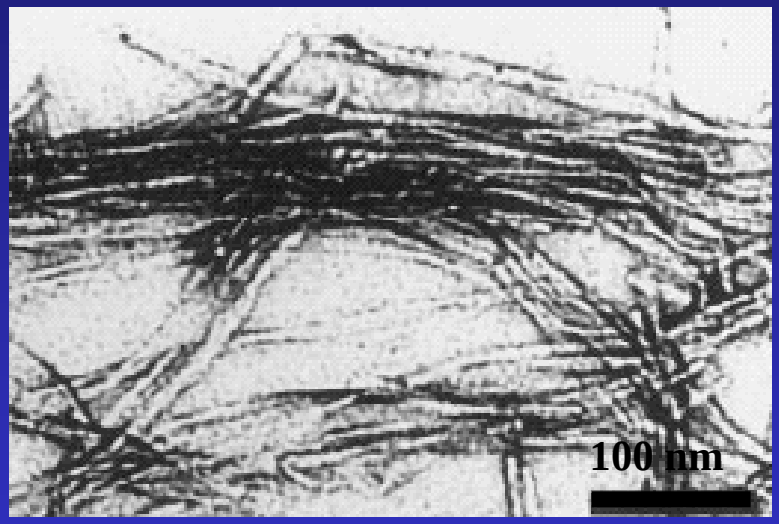
| **- Non neuropathic localised amyloidoses** | **aggregating protein** |
|---|---|
| Type II diabetes | amylin |
| Medullary carcinoma of the thyroid | calcitonin |
| Aortic medial amyloidosis | medin |

## The three hallmarks of amyloid fibrils:

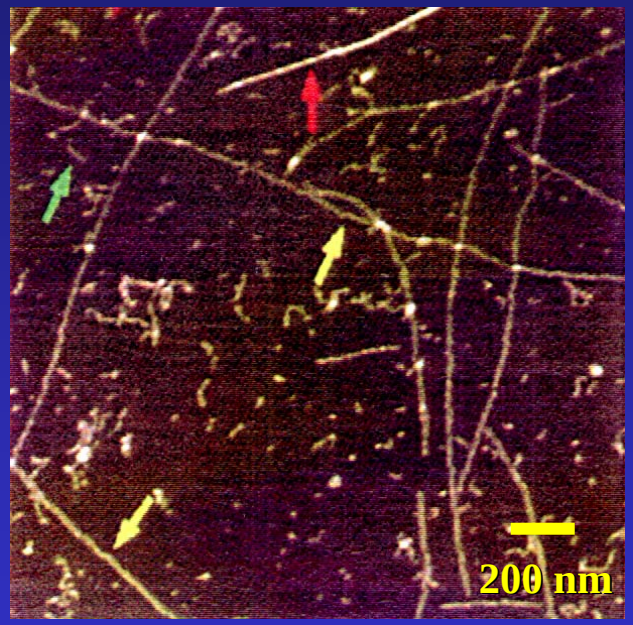
1) Fibrillar morphology observable using transmission electron microscopy
2) Cross-β structure using X-ray fibre diffraction
3) Green Congo red birefringence under cross-polarised light

**First hallmark:**

Fibrillar morphology observable using trasmission electron microscopy



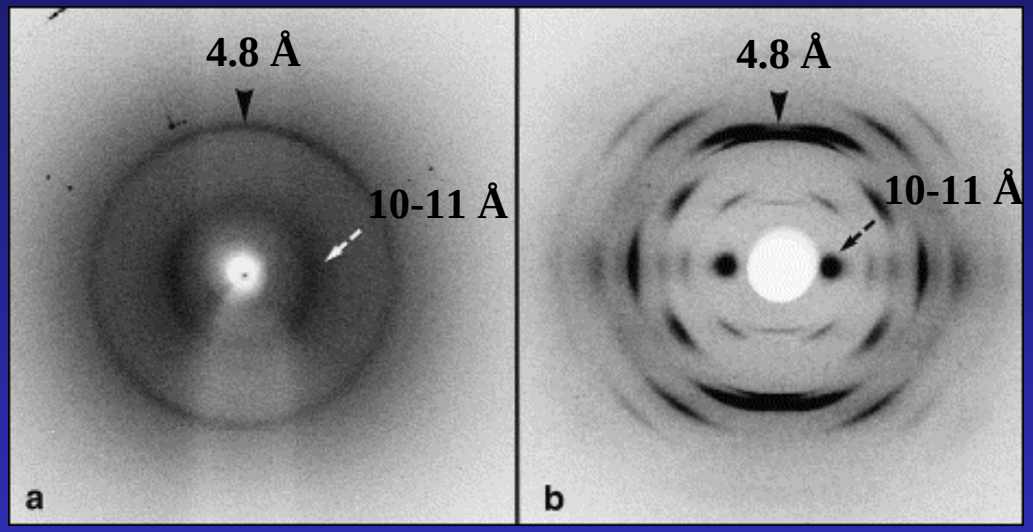

amyloid fibrils of the $A\beta_{1-40}$ peptide
by transmission electron microscopy
(Walsh et al. 1999. *J. Biol. Chem.* 274, 25945)




amyloid fibrils of $A\beta_{1-42}$ peptide
by atomic force microscopy
(Harper et al. 1997, *Chem. Biol.* 4, 119)

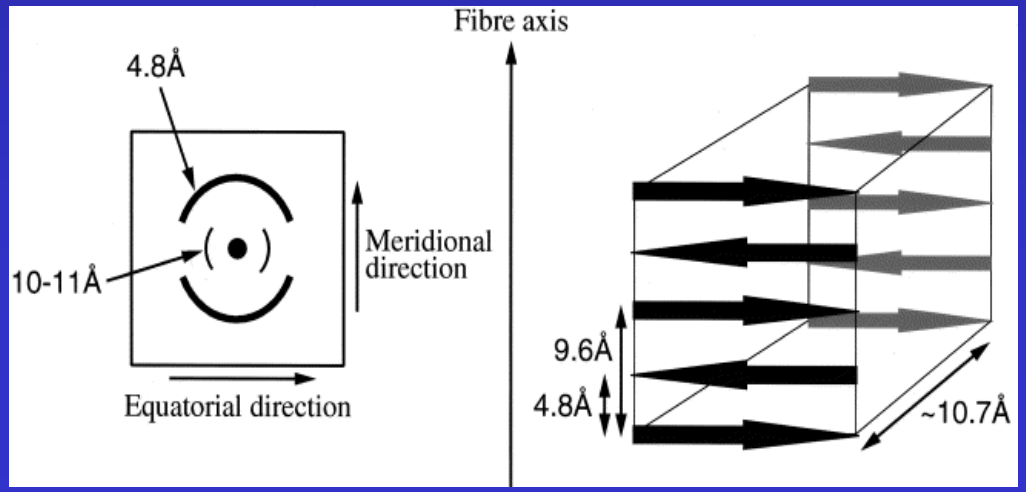
**Second hallmark:**

Cross-β structure using X-ray fibre diffraction



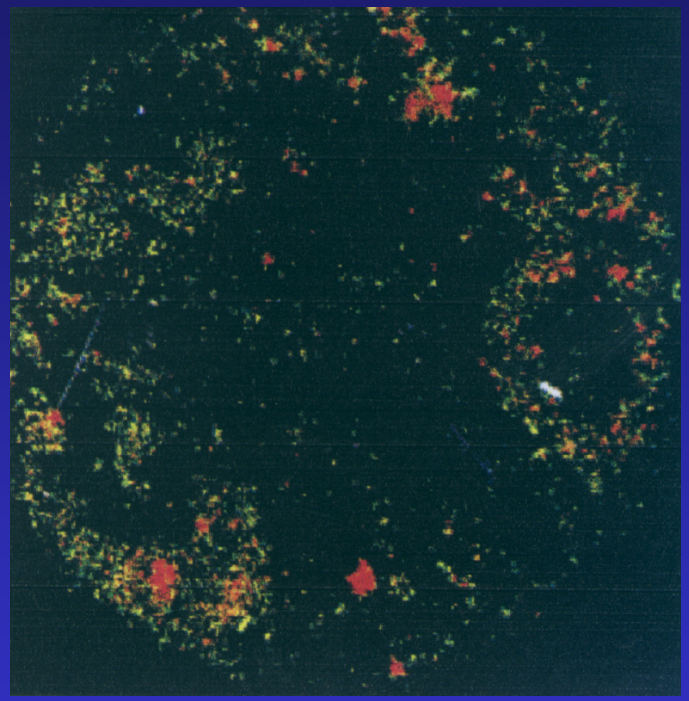

**X-ray diffraction of Aβ fibrils**

Serpell, 2000, *Bioch. Biophys. Acta* 1502, 16-30



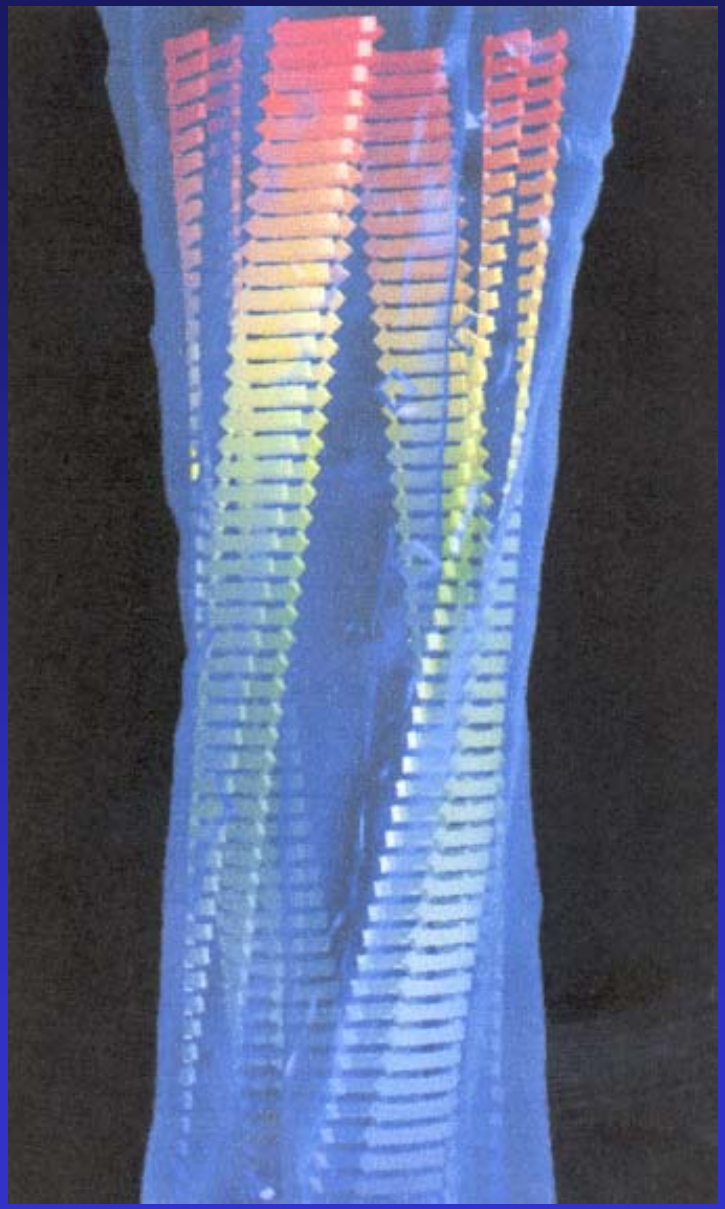
**Third hallmark:**

Green Congo red birefringence under cross-polarised light

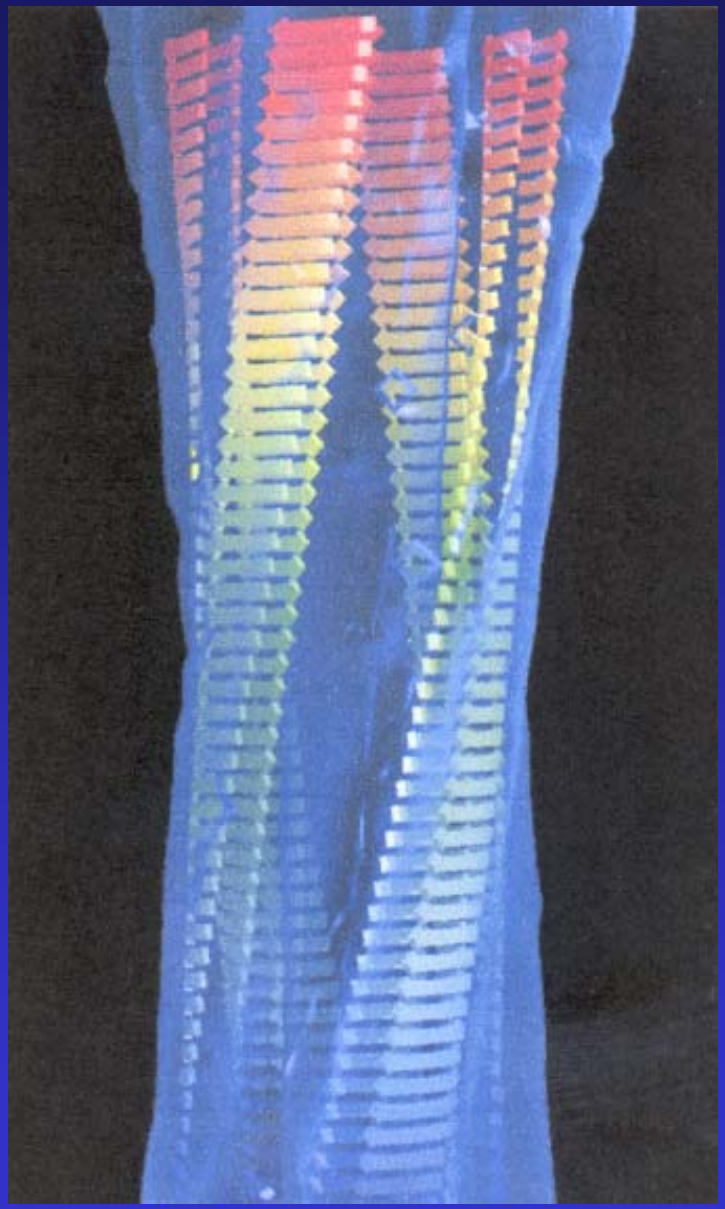


**Fibrillar deposits of β2-microglobulin**

Ivanova et al., 2003, *Biochemistry* 42, 13536-13540



- Amyloid fibrils appear to be formed from protofilaments (2-5 nm wide) that twist toghether or associate laterally to form fibrils of higher width (generally 7-13 nm but even more)



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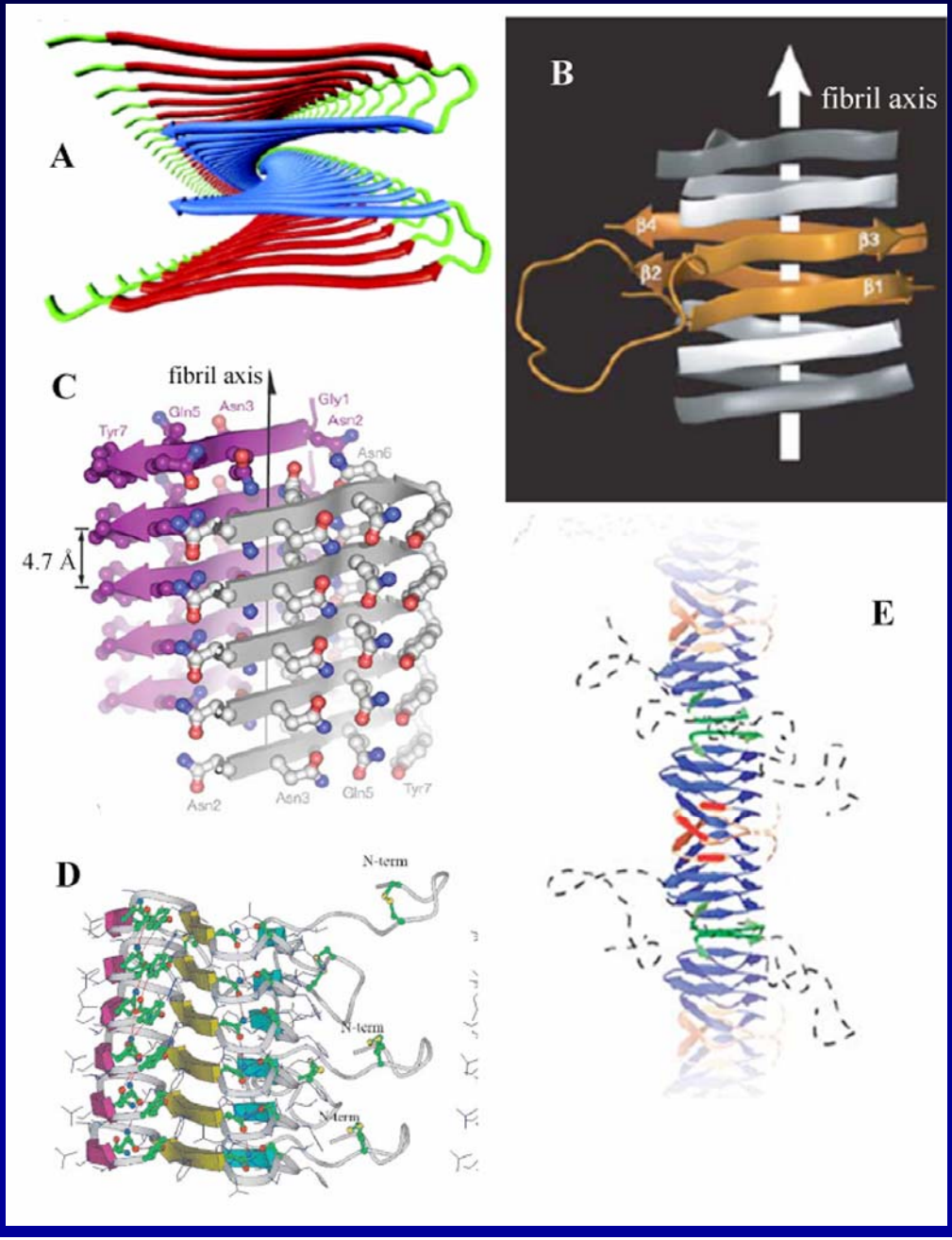
- Within individual protofilaments a β-sheet structure is present. β -strands are parallel to each other and perpendicular to the axis of the fibrils.

# Structures of amyloid-like protofilaments

Protofilament from Aβ

Protofilament from GNNQQNY peptide

Protofilament from amylin




Protofilament from HET-s

Protofilament from Sup35p

# IMPORTANT NOTE!

**In some cases the misfolded protein molecules form fibrillar aggregates which cannot be classified as amyloid fibrils (they do not have extensive cross-β structure and do not bind diagnostic dyes such as Congo red**

**(as in cell sickle anemia and serpinopathies)**

# Thorough classification

**Protein misfolding diseases**

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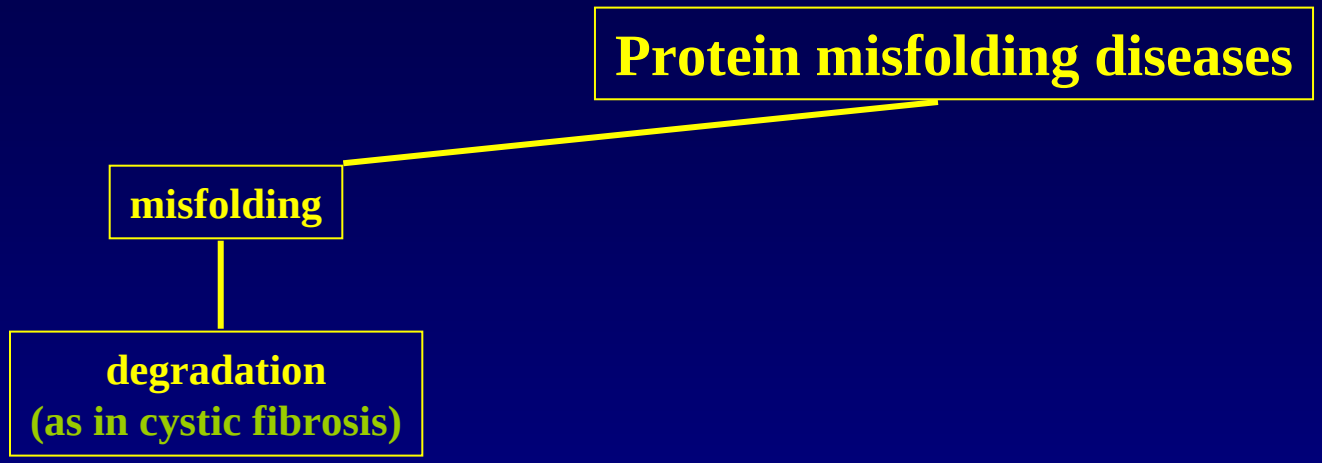

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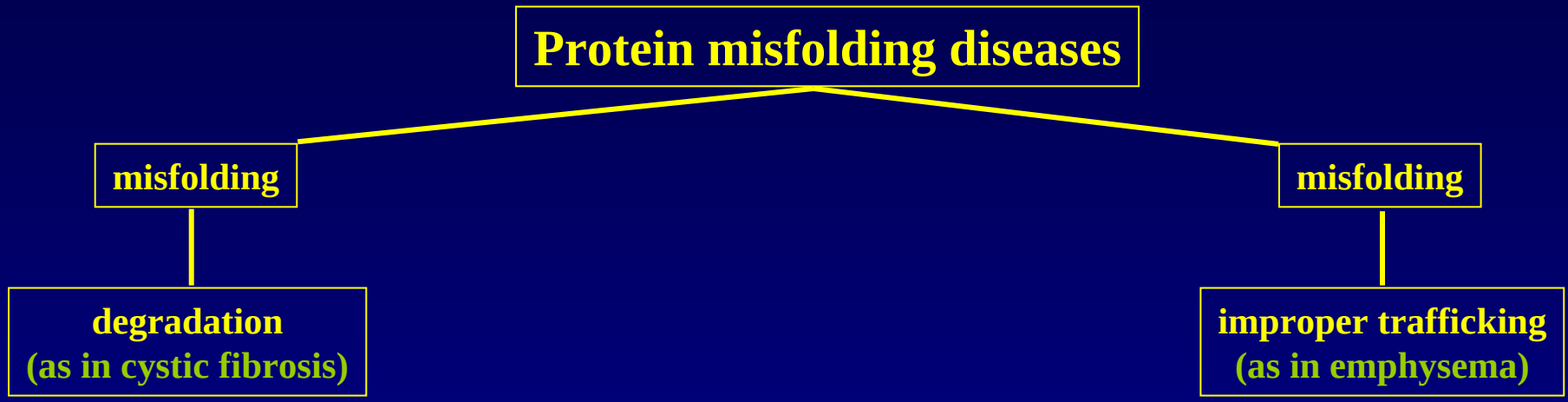

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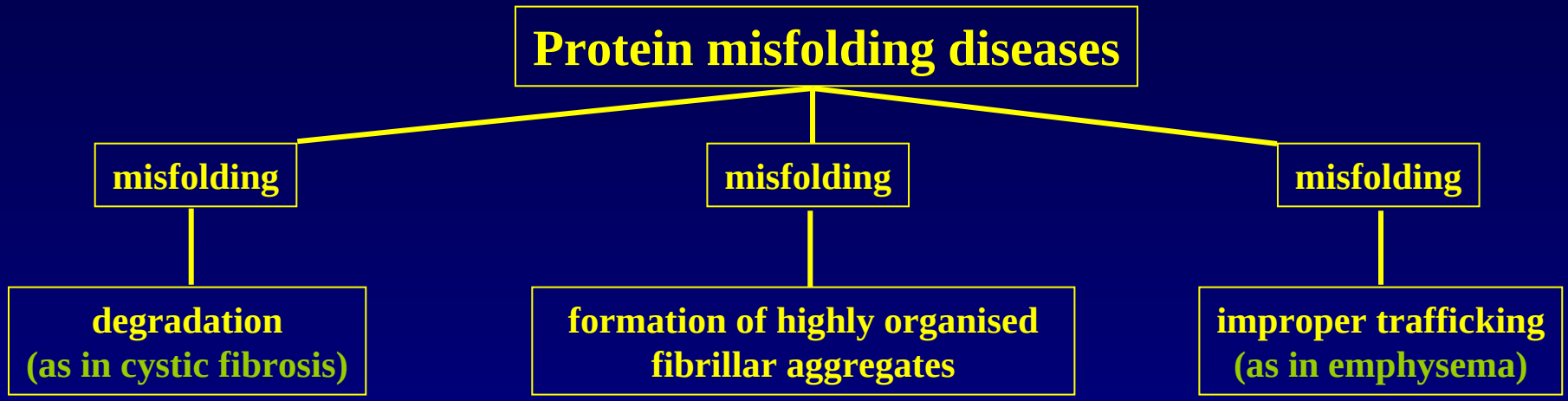

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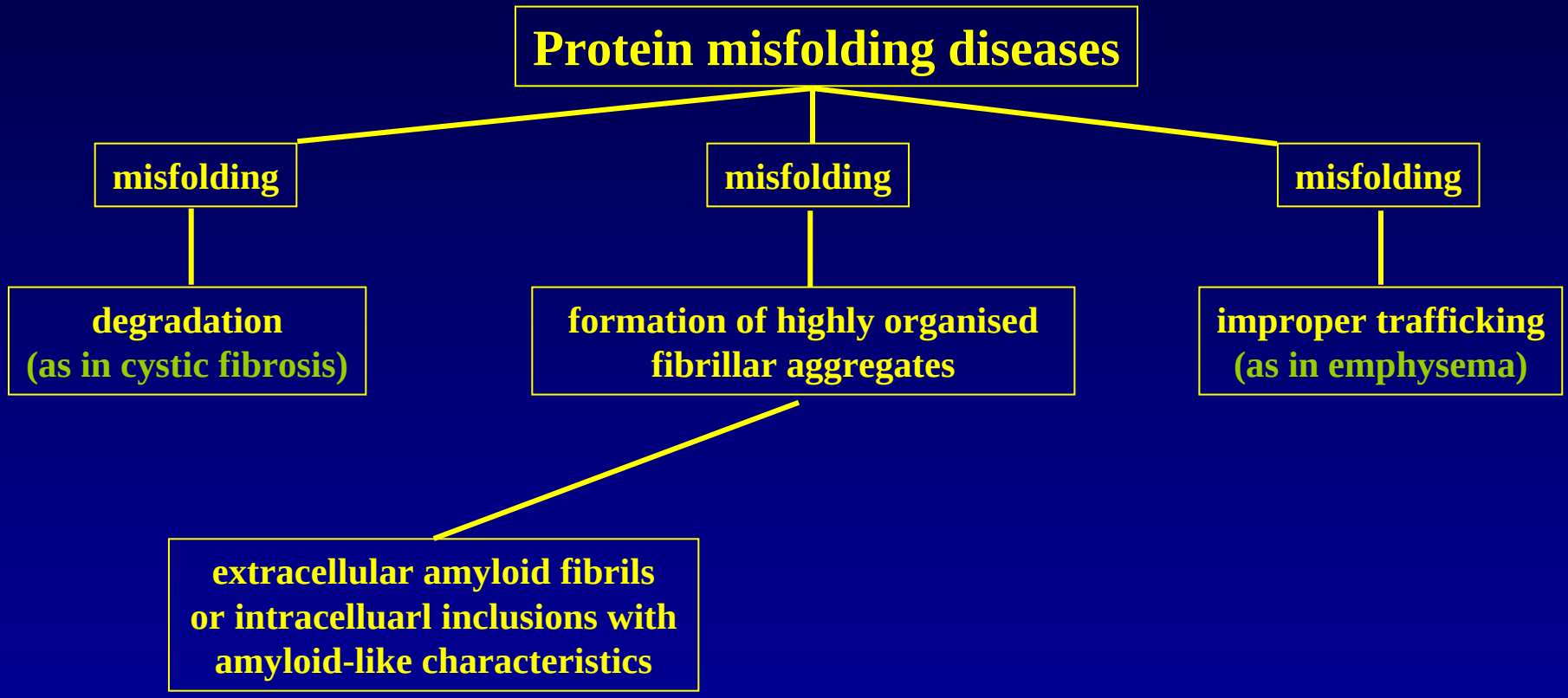

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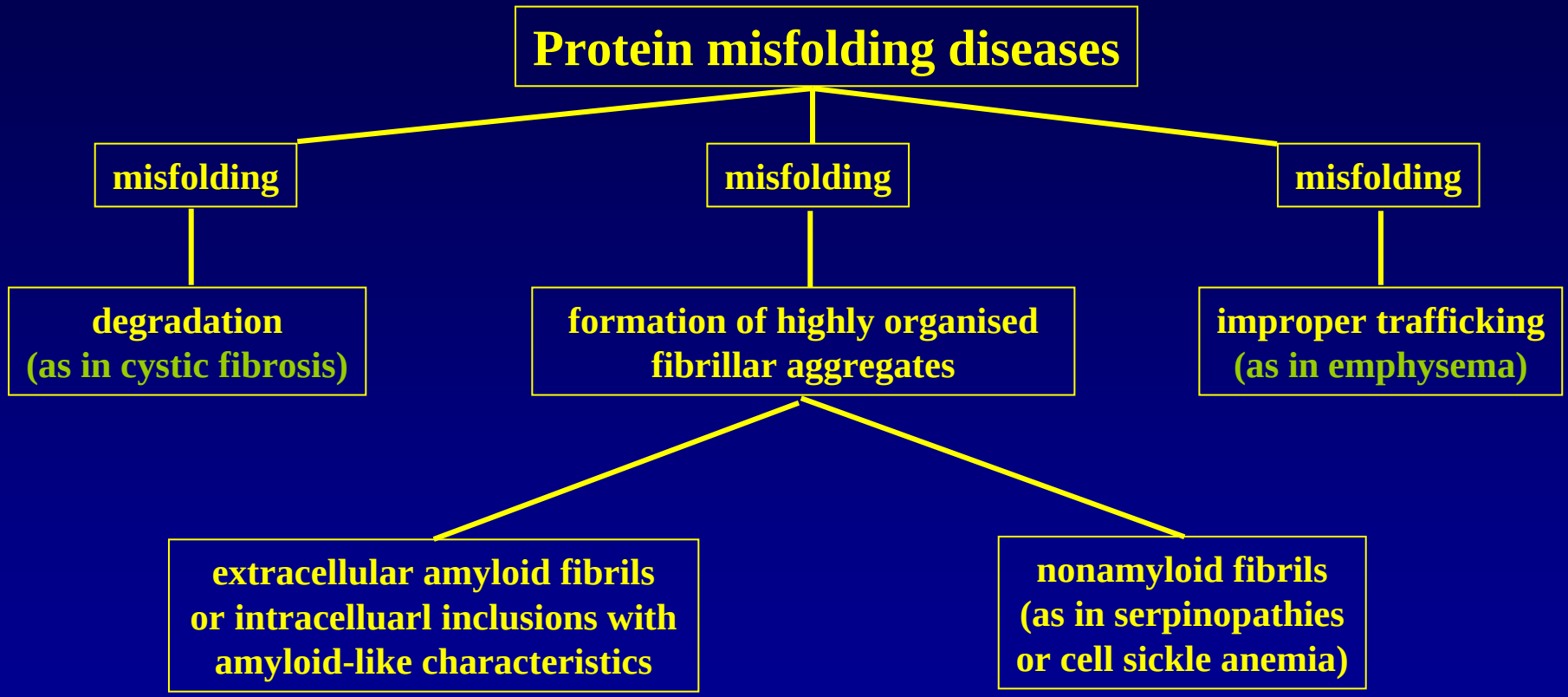

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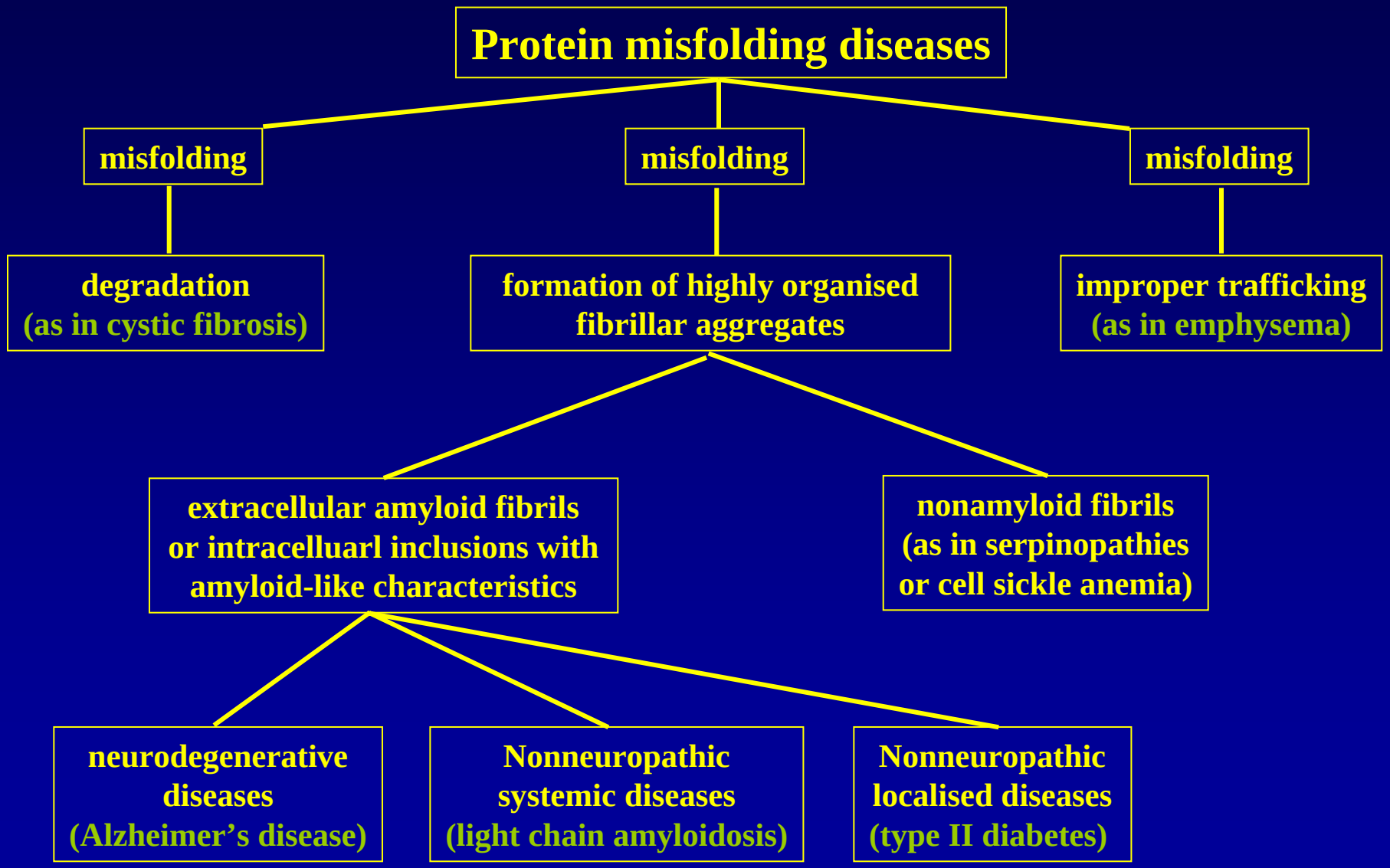
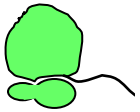


# The possible conformational changes for a polypeptide chain

ribosome



Chiti & Dobson (2006)
*Ann. Rev. Biochem.* 75, 333-366

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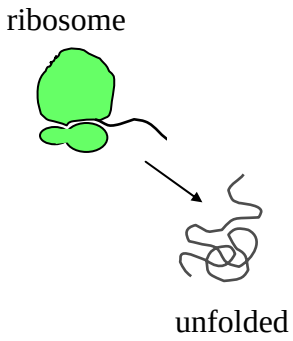


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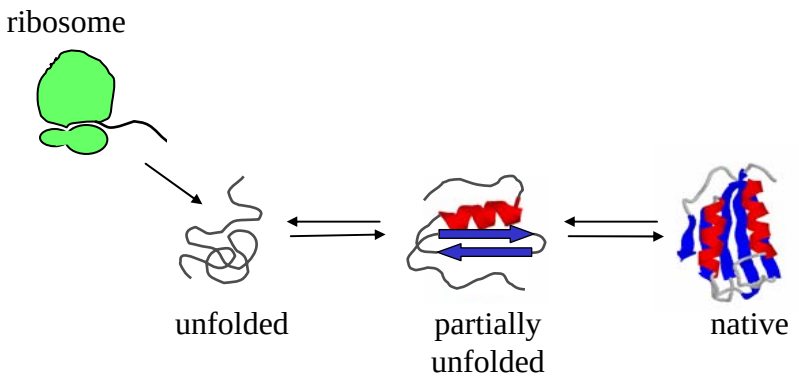


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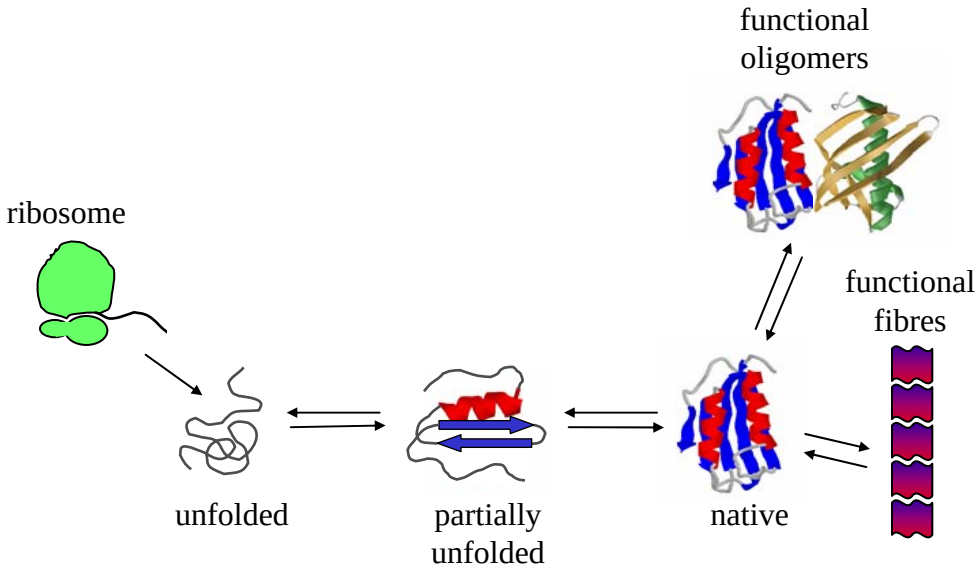


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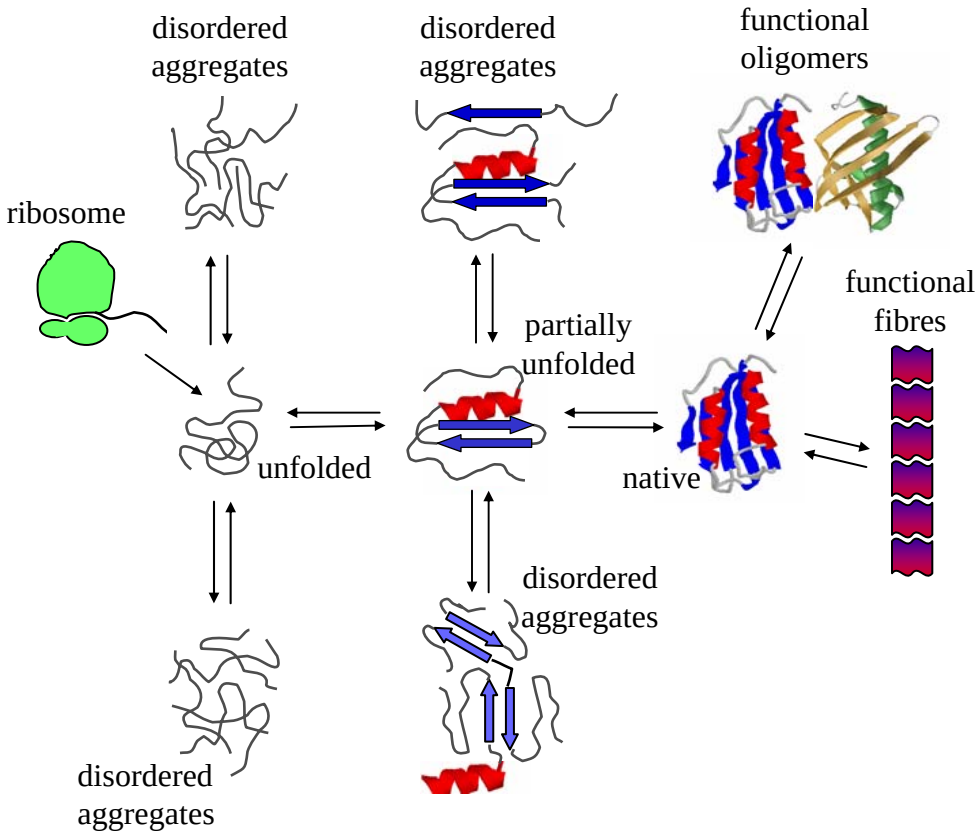


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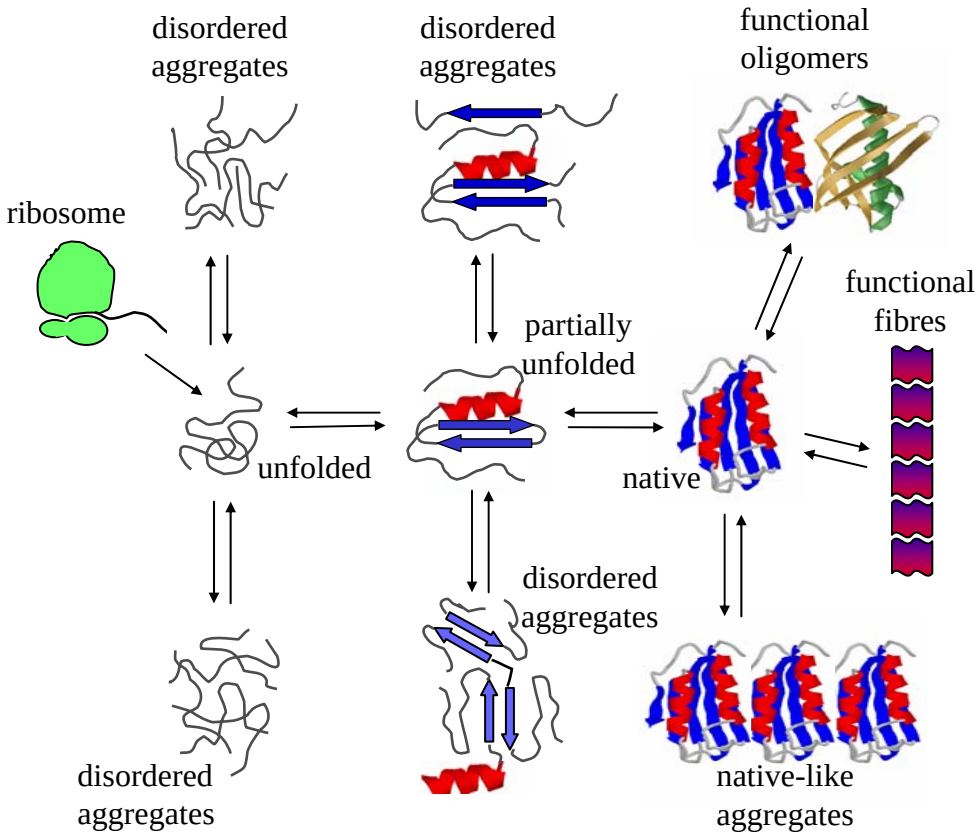


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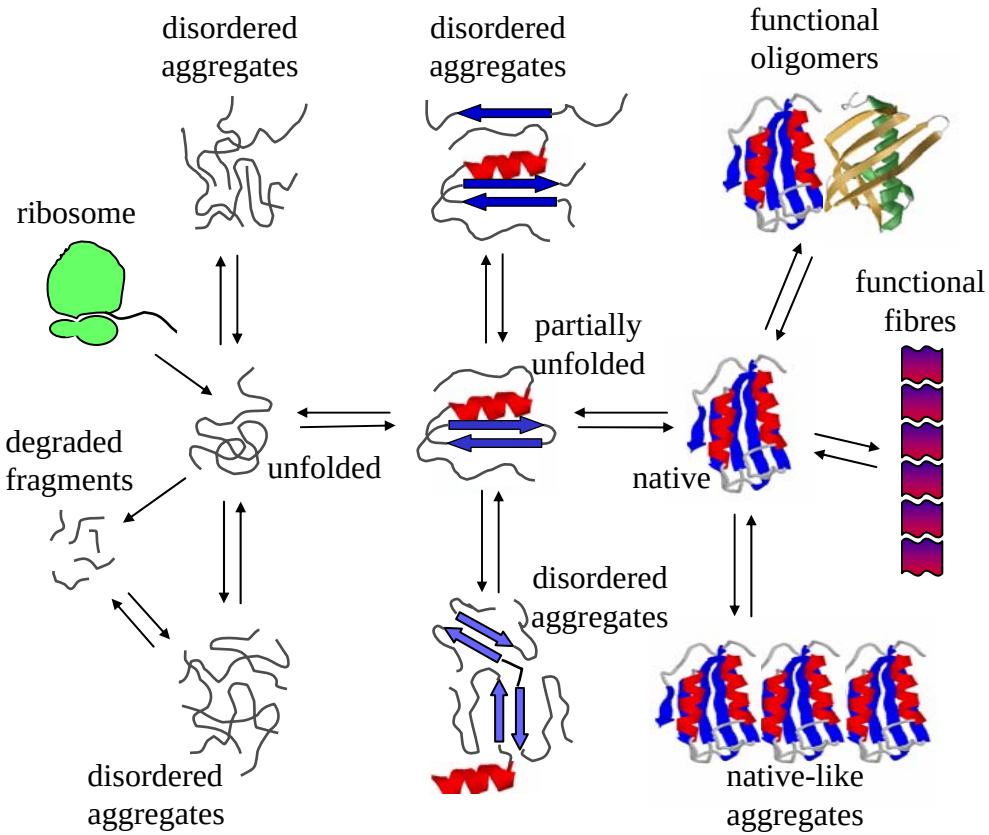


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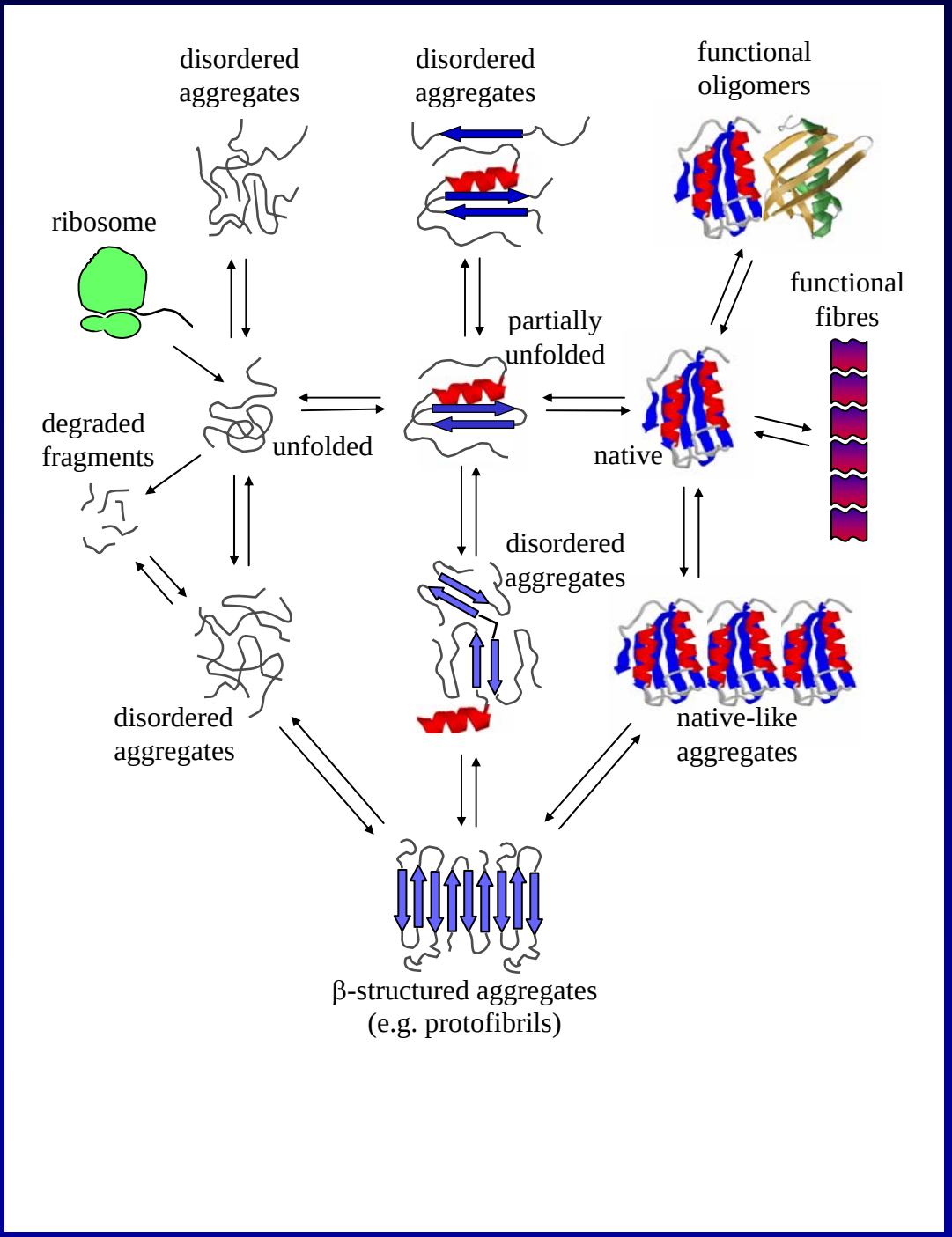


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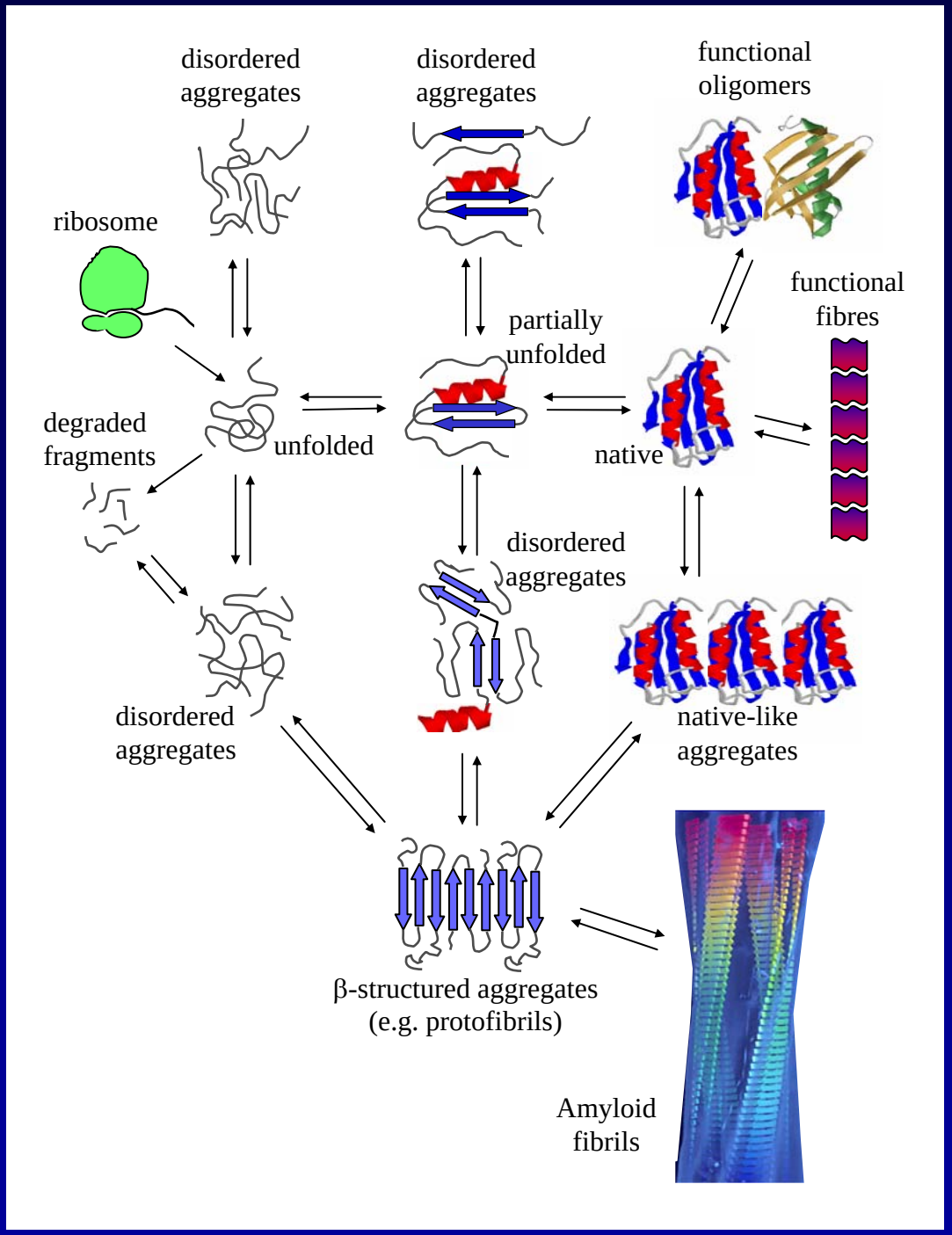


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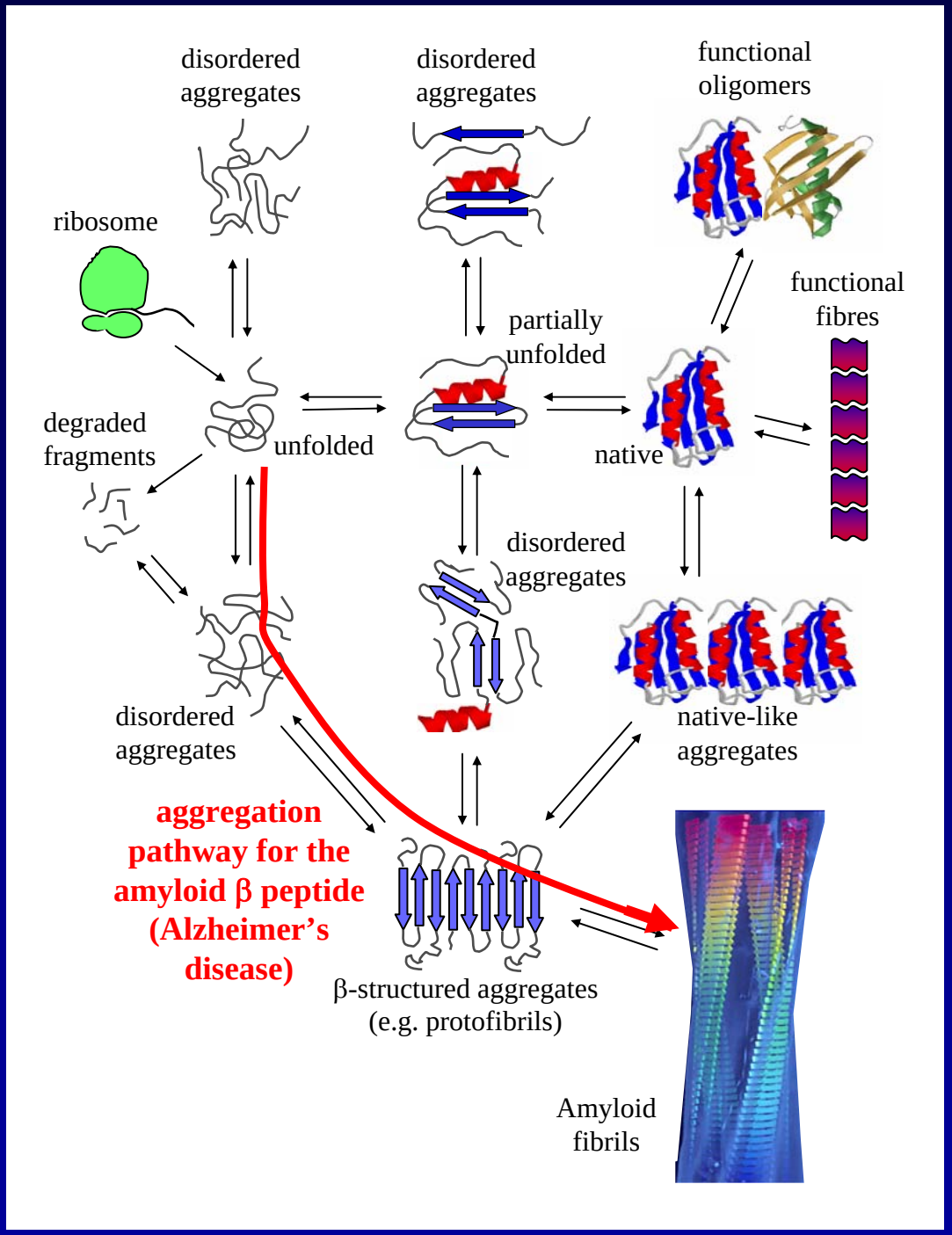


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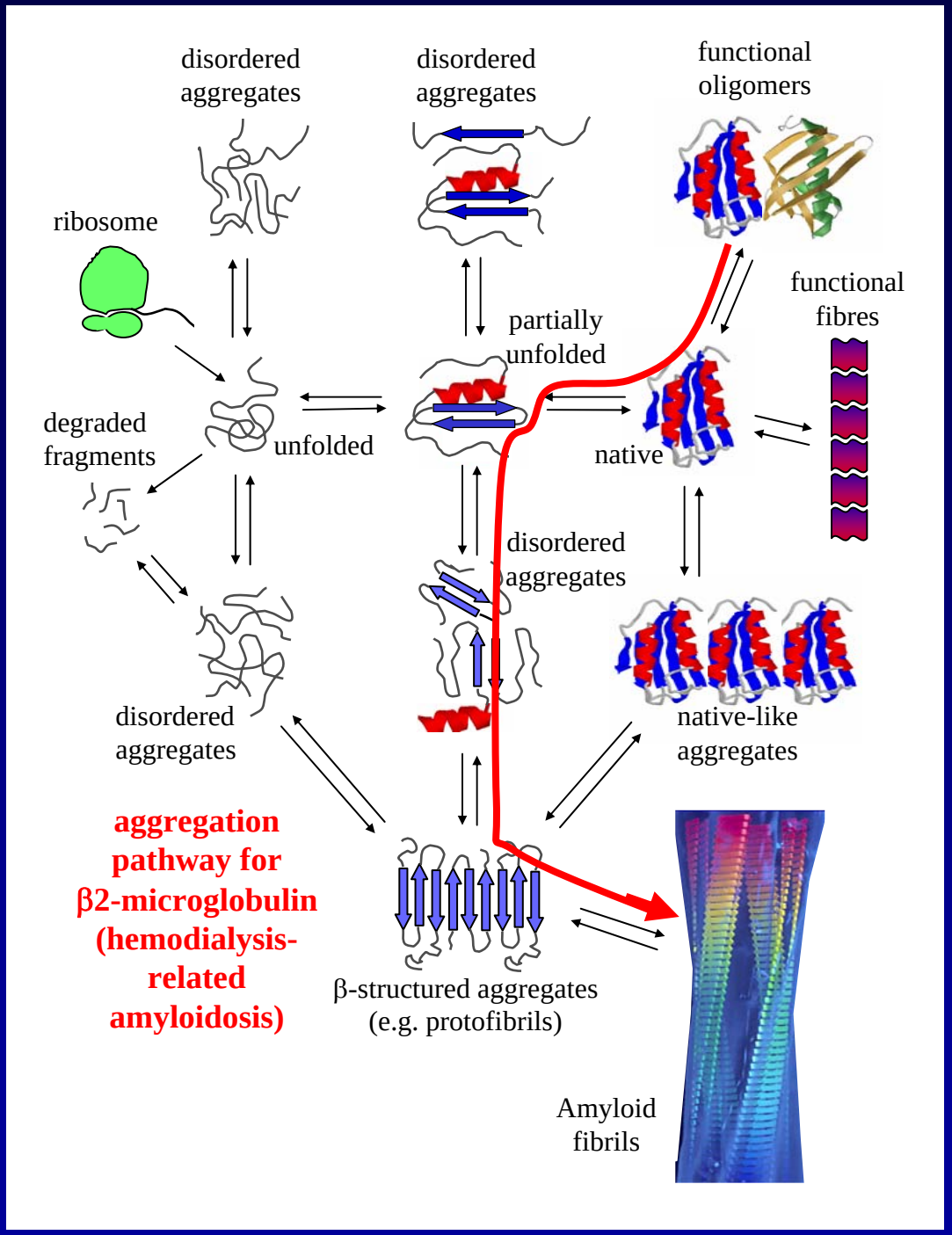


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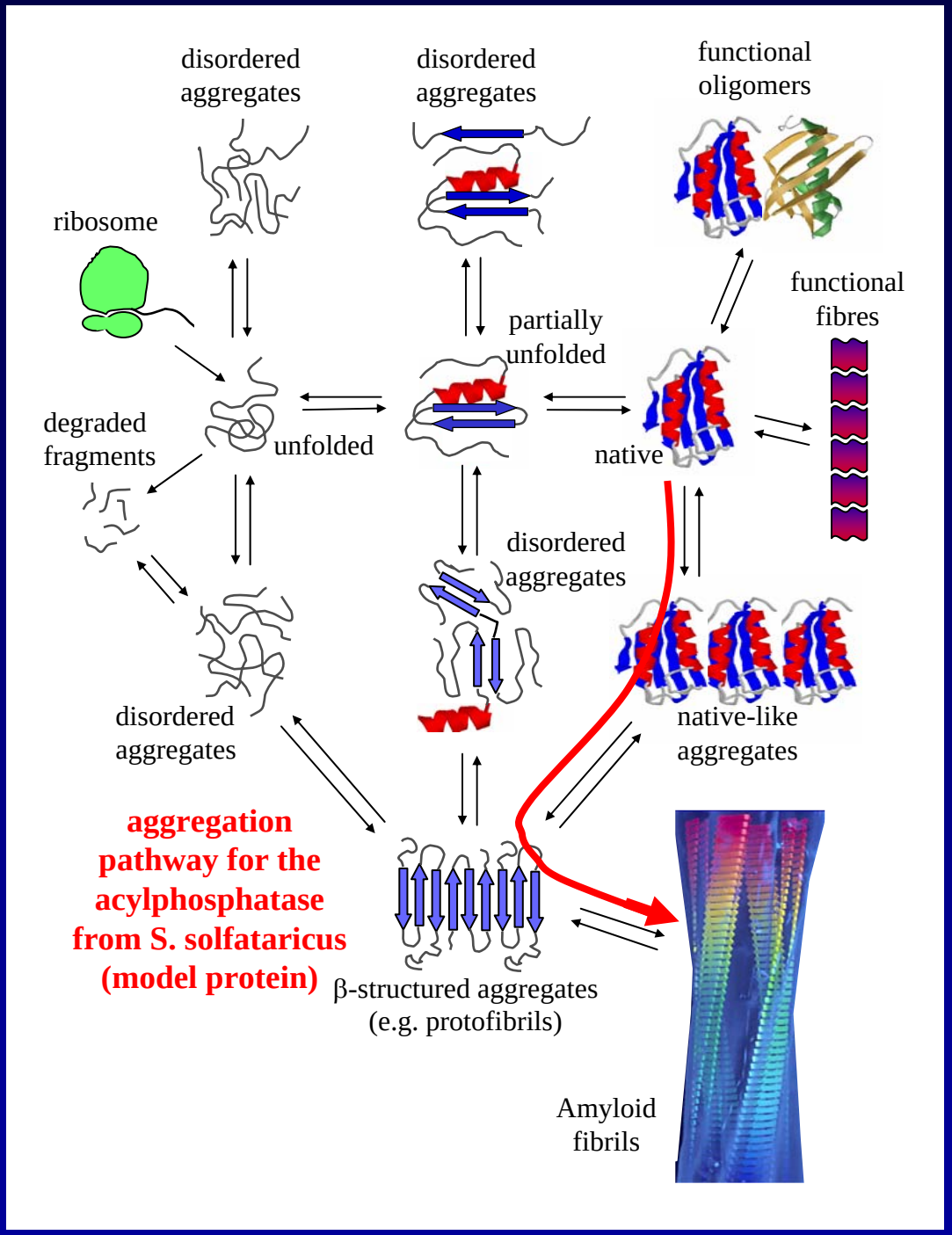


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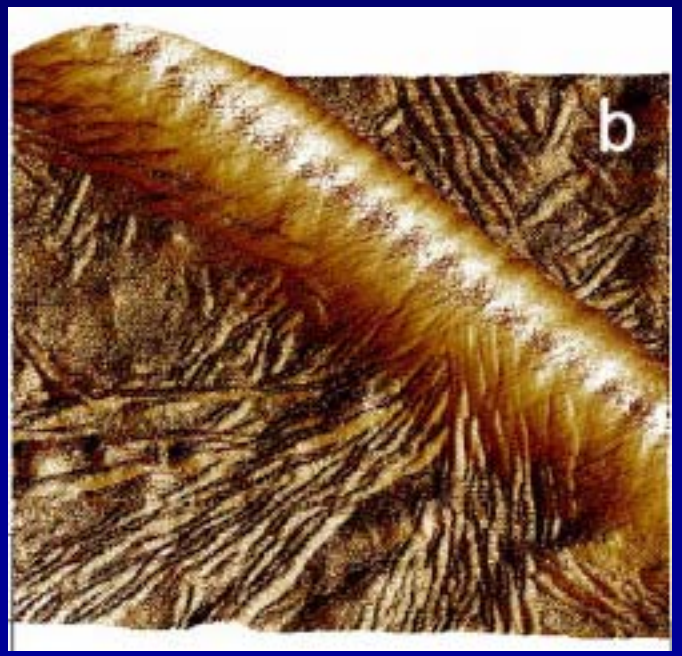
fibrils of $\beta_2$ microglobulin associated with a collagen fibre



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**- the serum amyloid P component**
**- apolipoprotein E**
**- and many others**

**All these components clearly play a role in the rate of formation and stabilisation of fibrillar aggregates and precursor aggregates**

fibrils of $\beta_2$ microglobulin associated with a collagen fibre

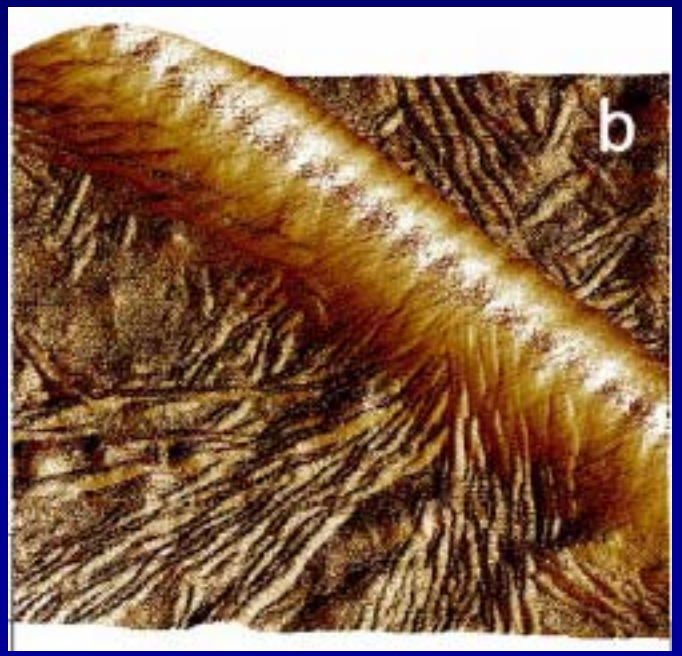

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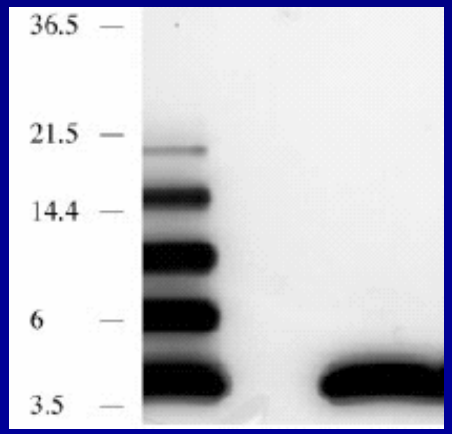
Photo-induced cross-linking of unmodified proteins (PICUP)



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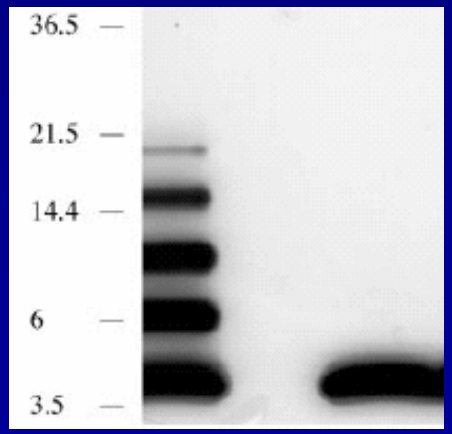
Molecular weight (kDa)




no cross-linked Aβ

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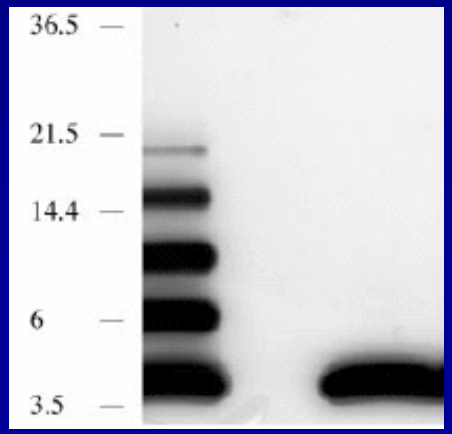
unstructured oligomer (2-4 units)

Photo-induced cross-linking of unmodified proteins (PICUP)



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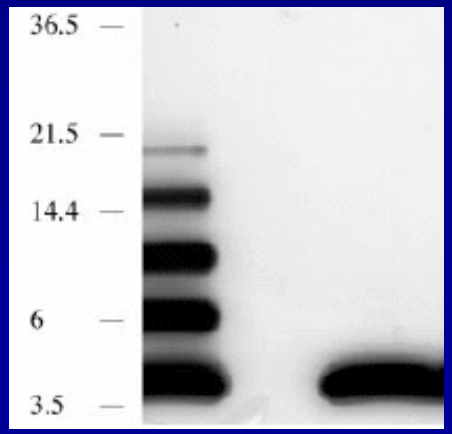

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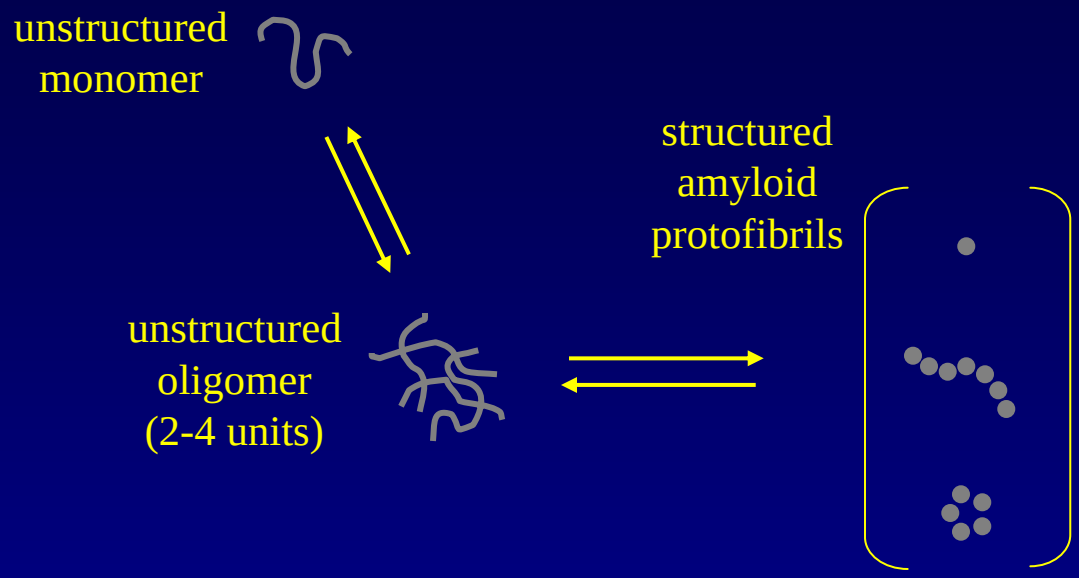


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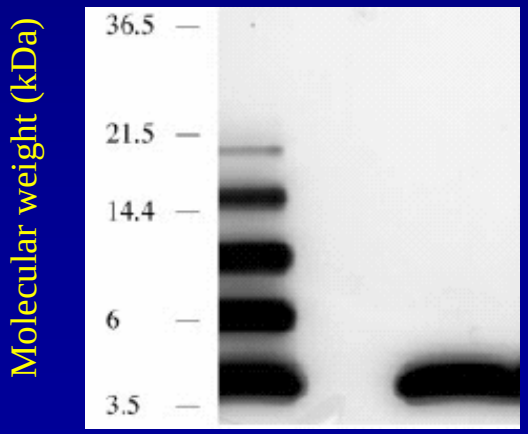

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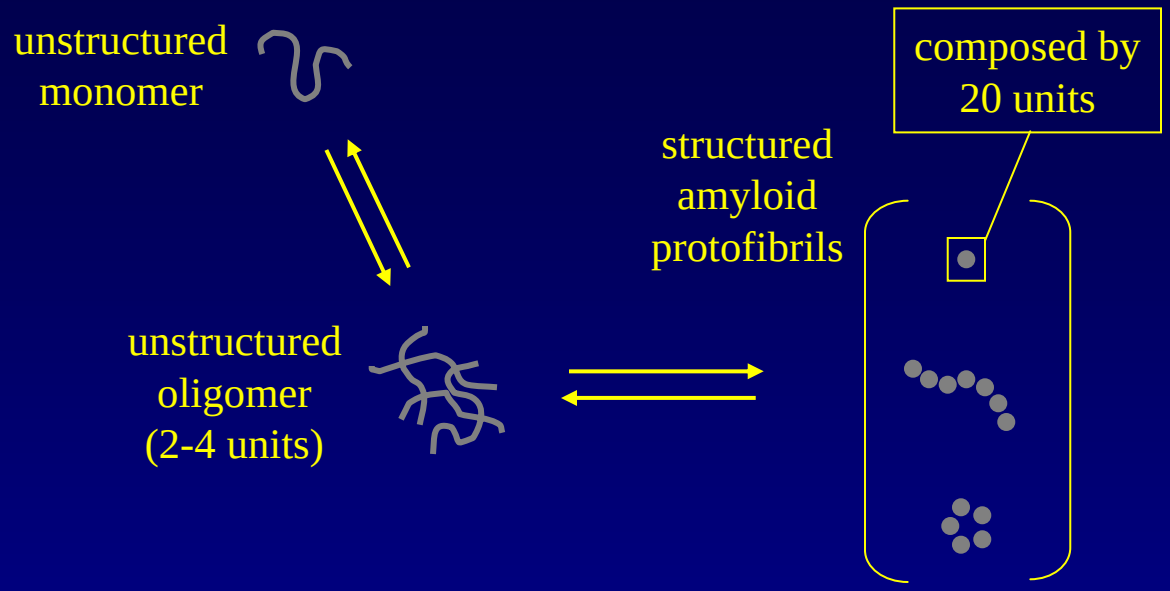


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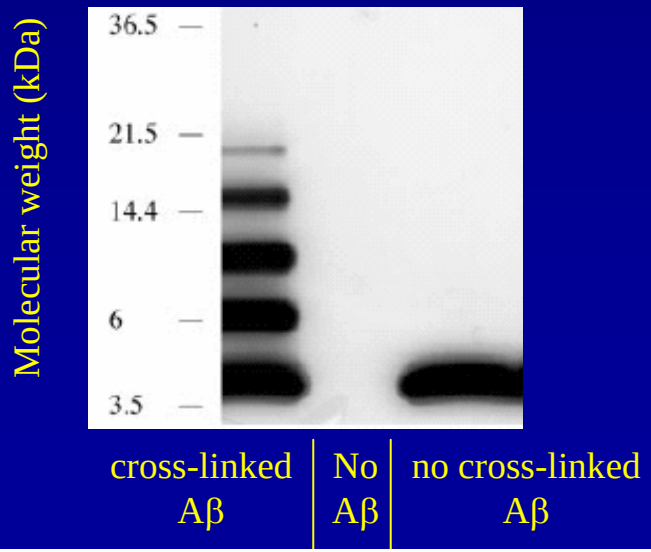

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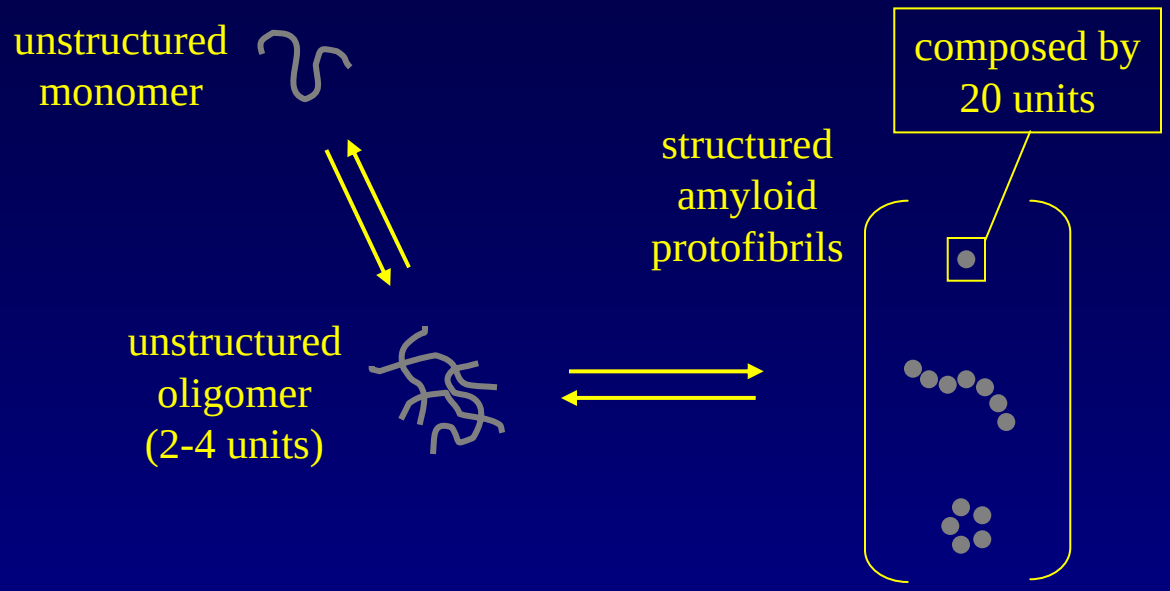


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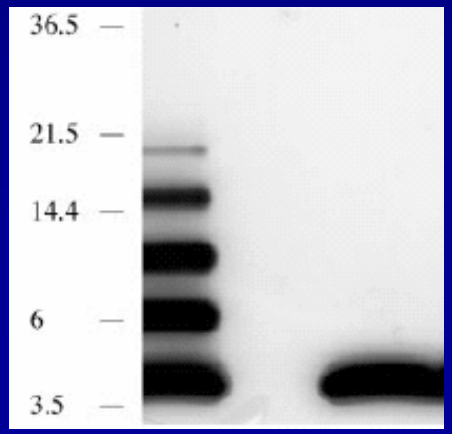


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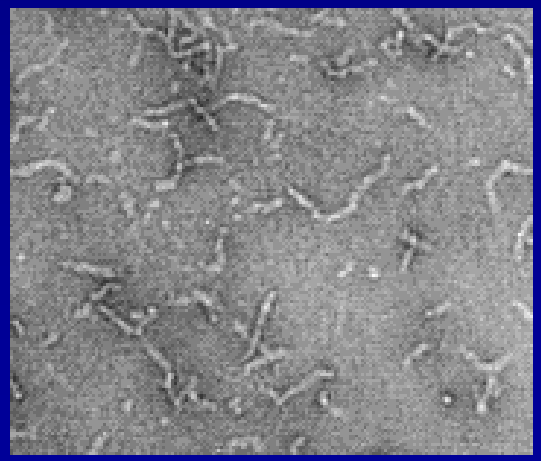

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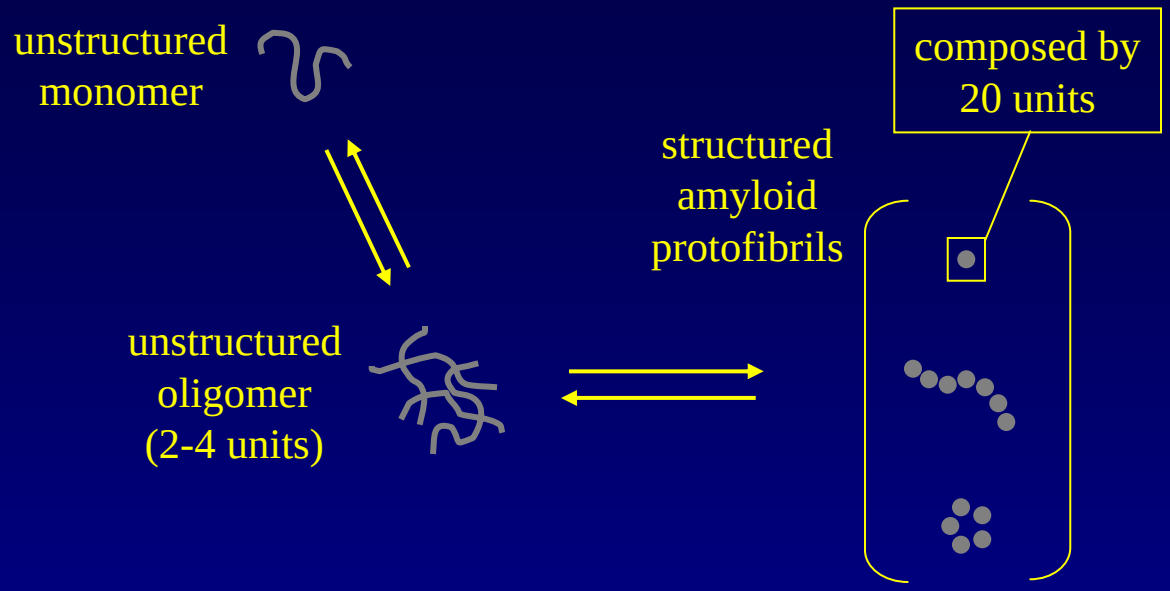


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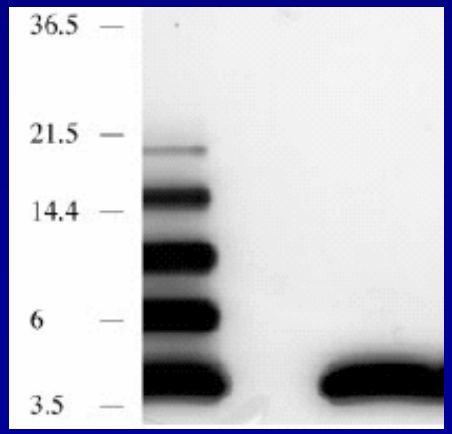


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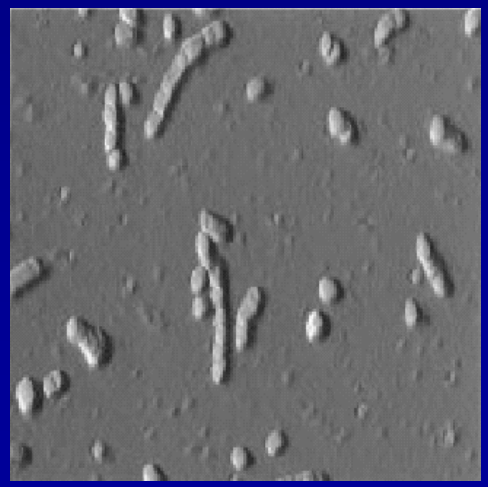

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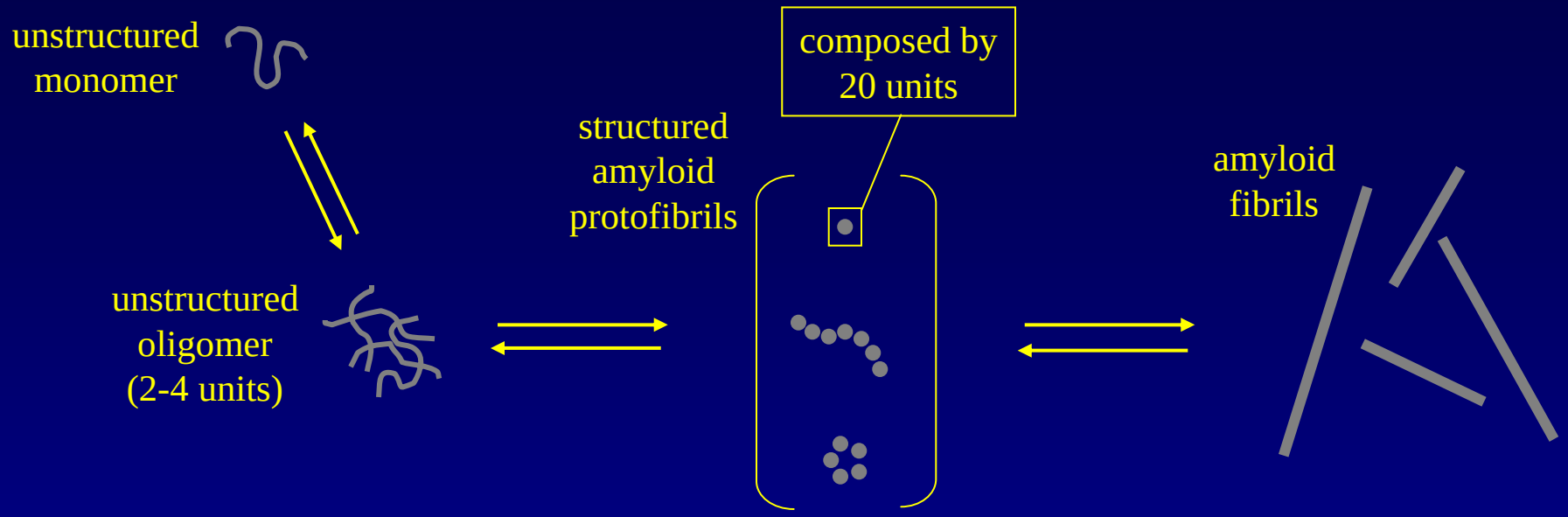


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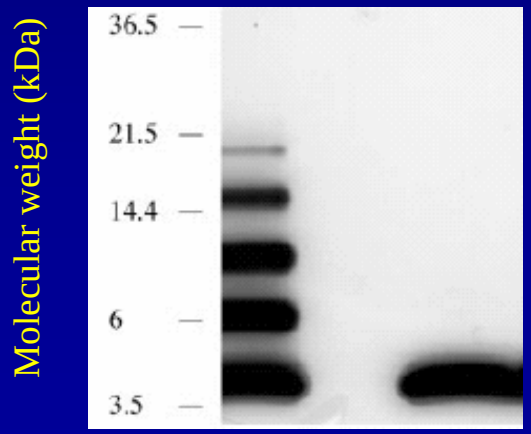


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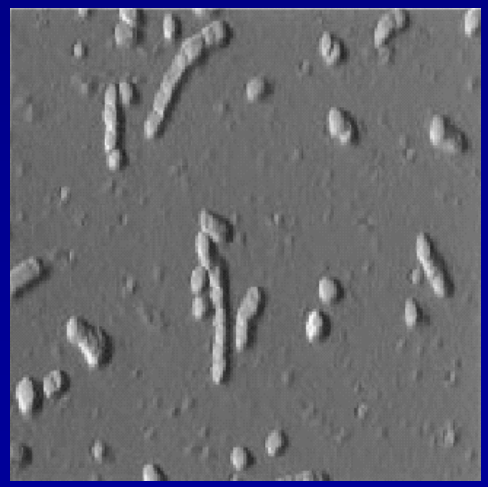

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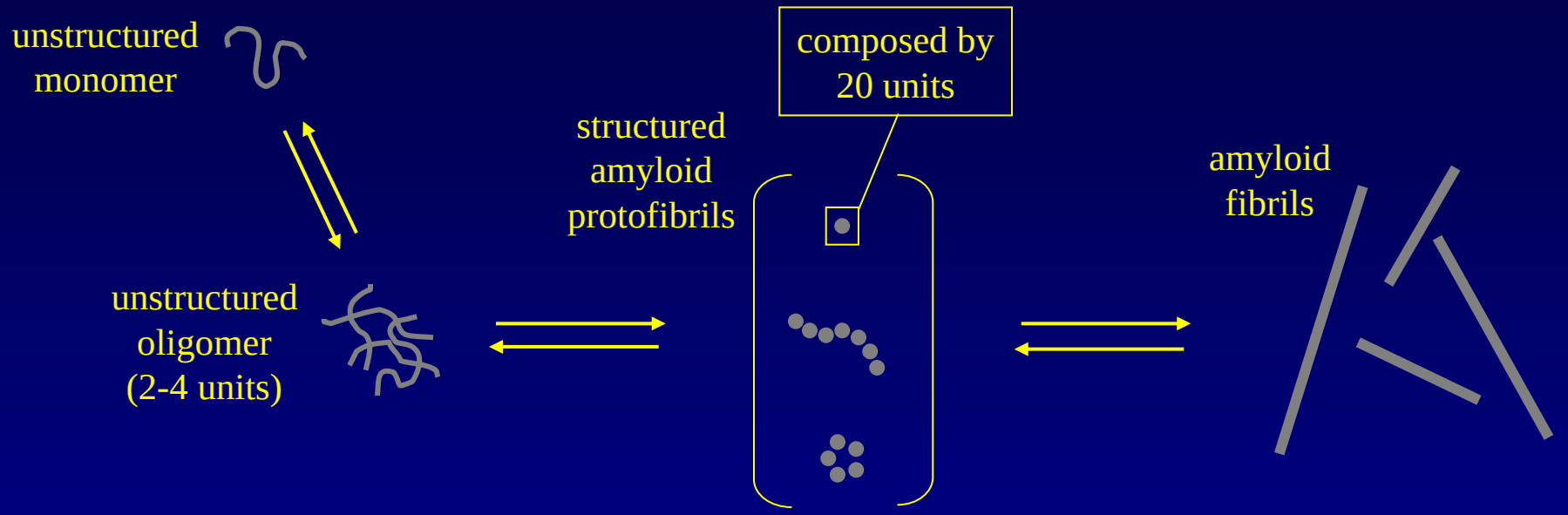


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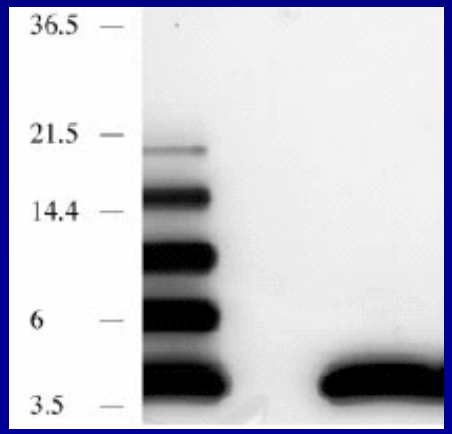


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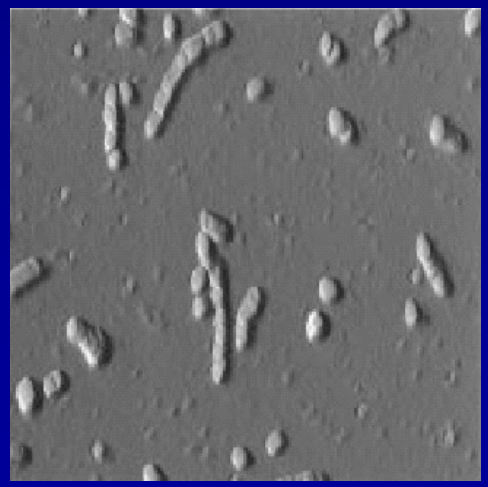

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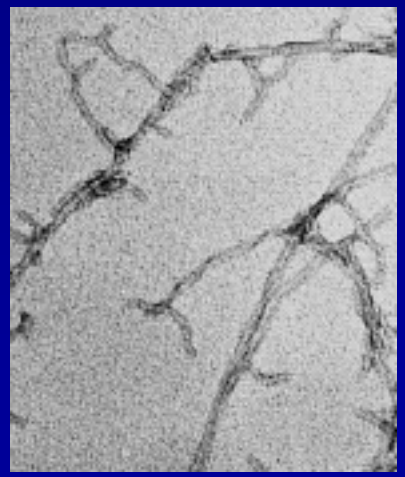

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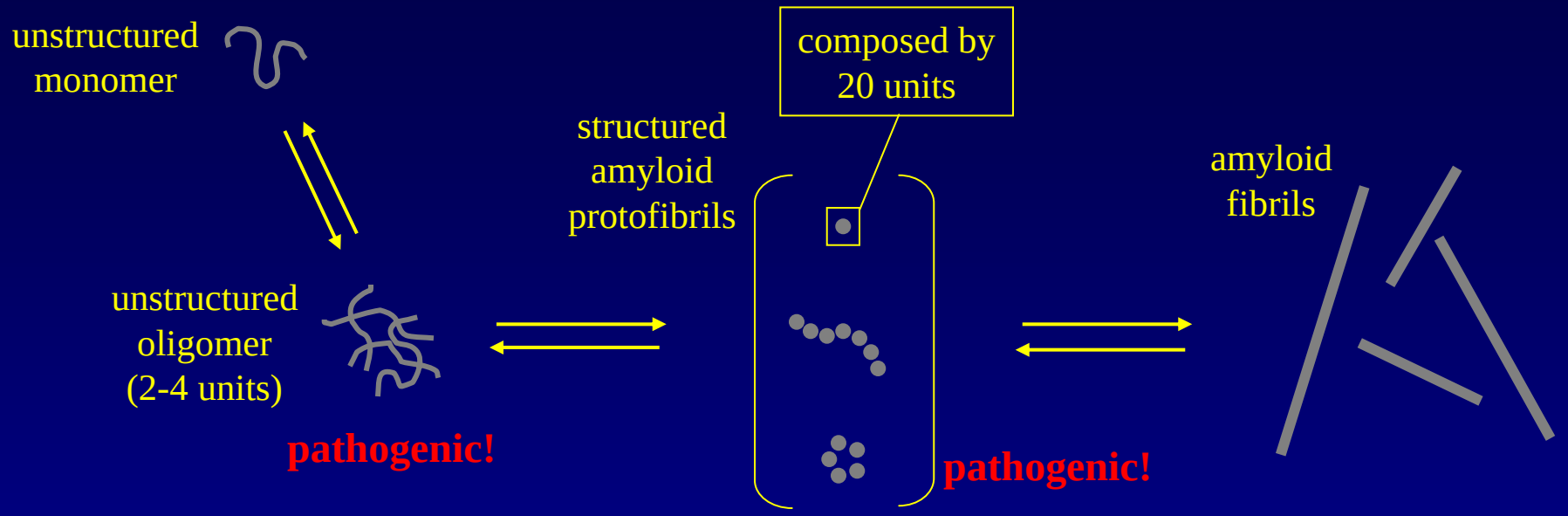


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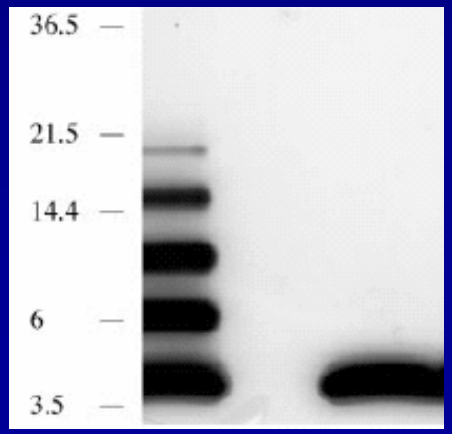


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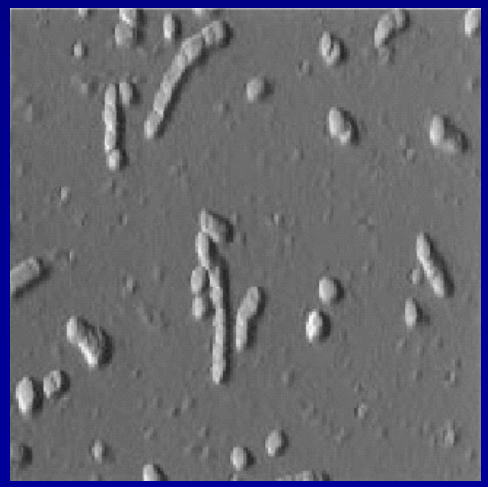

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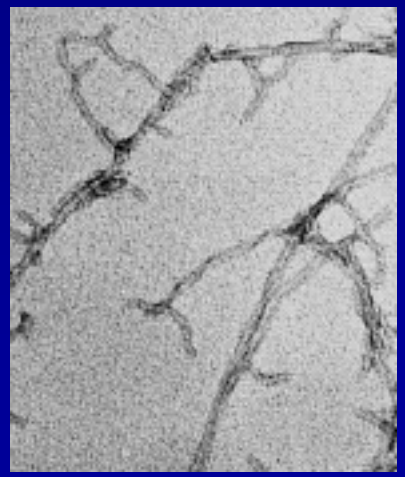
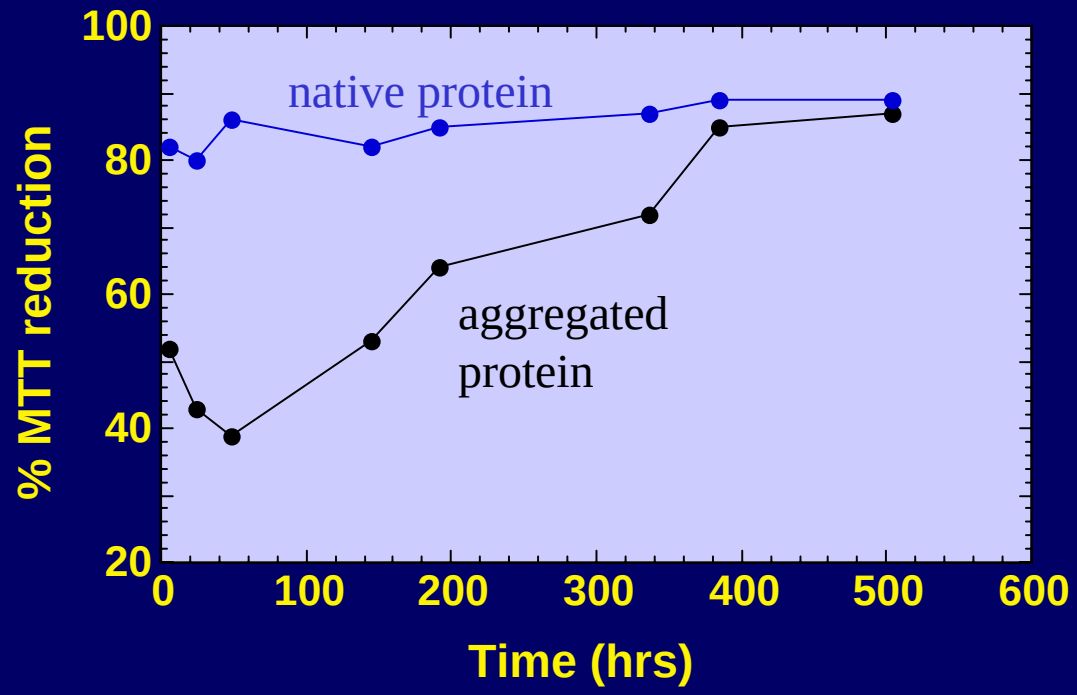


# Early-forming aggregates are more cytotoxic than fully formed fibrils




**Aggregation of HypF-N was induced in vitro. At different time points aliquots were added to the extracellular medium of cultured cells**

**Bucciantini *et al*. (2002) *Nature*, 416, 507**

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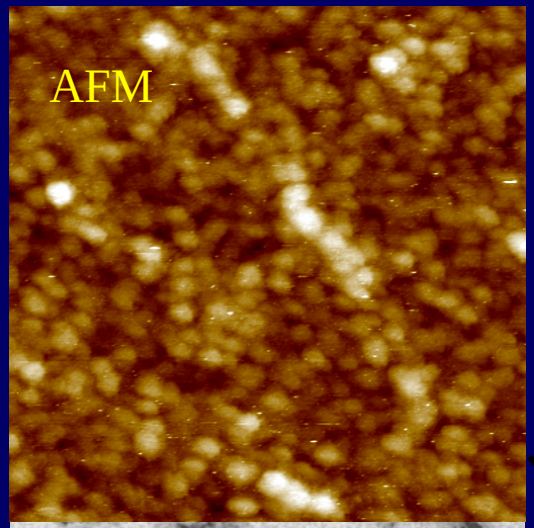


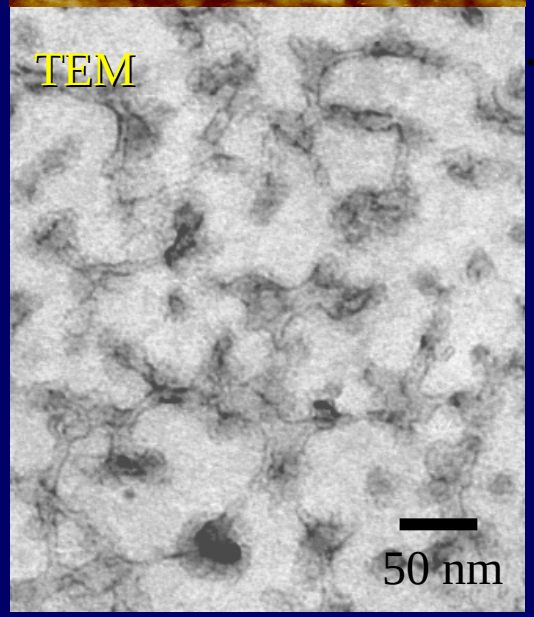


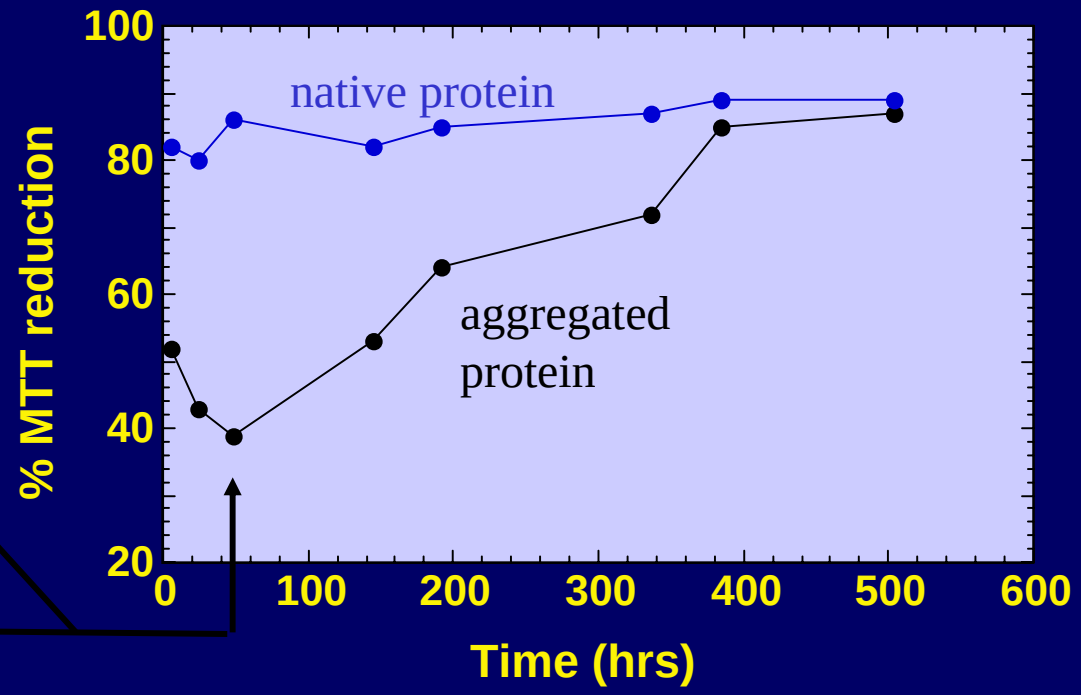


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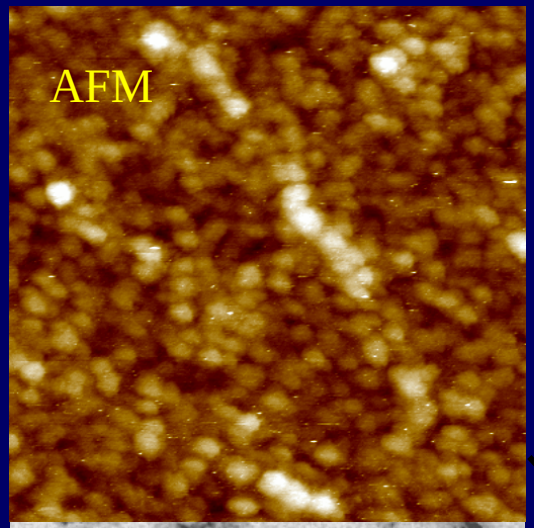


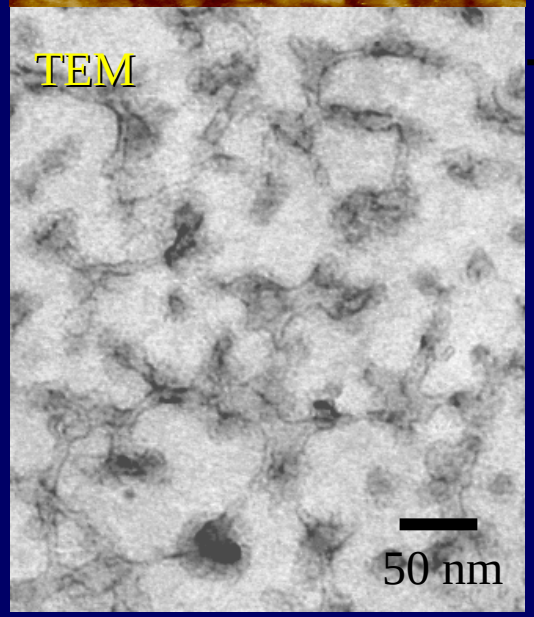


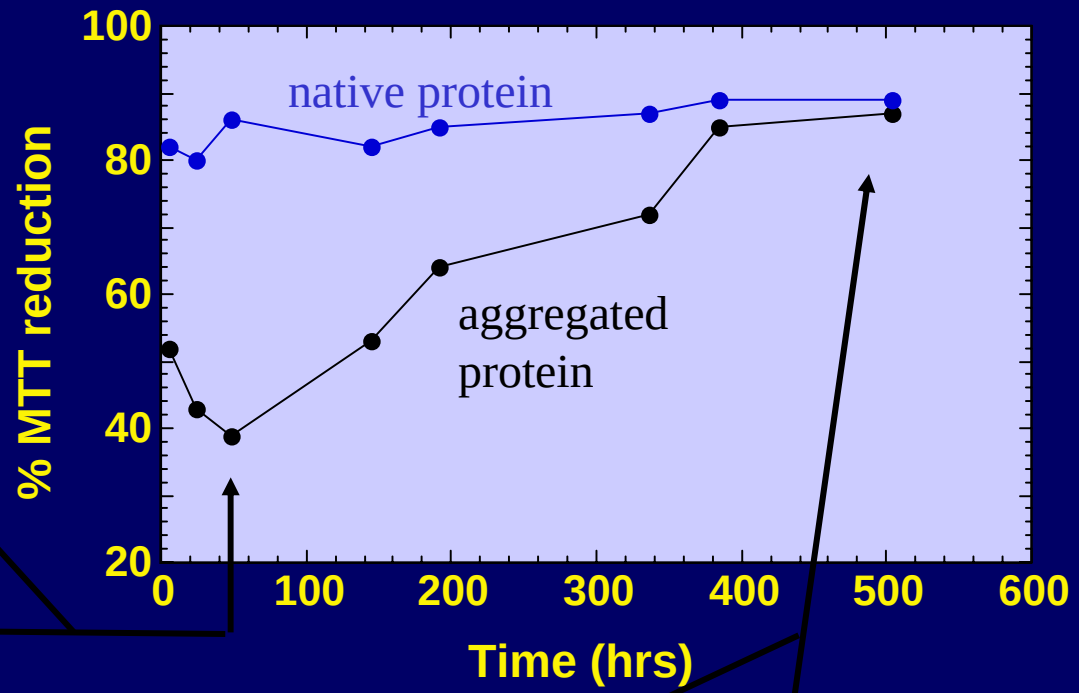


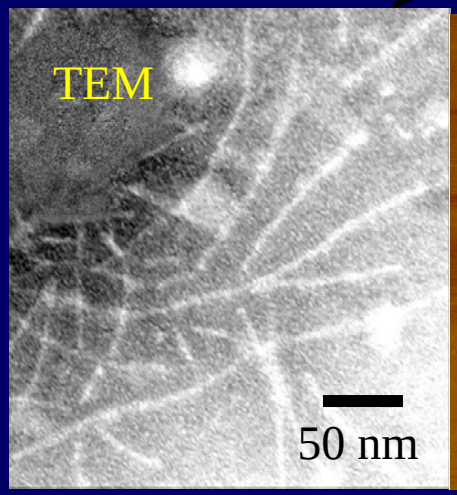


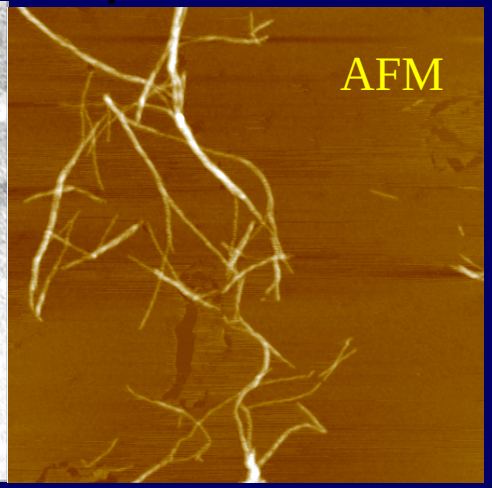


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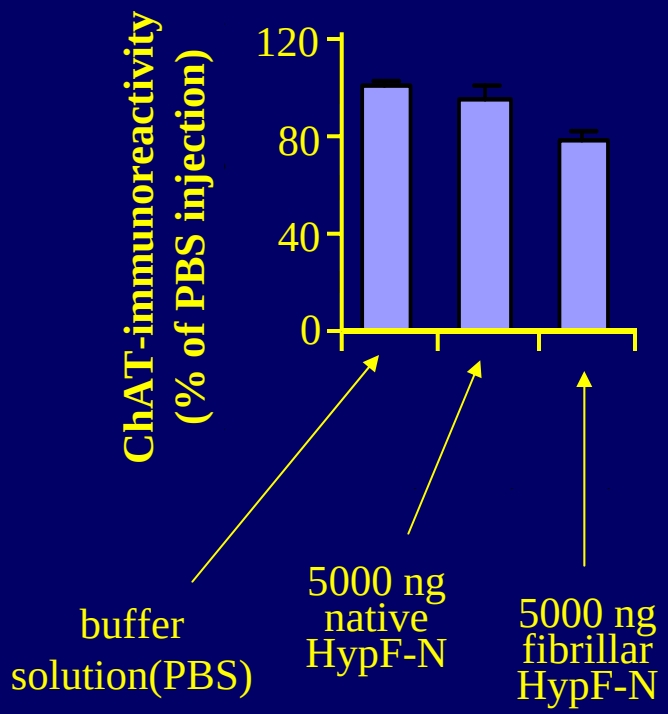


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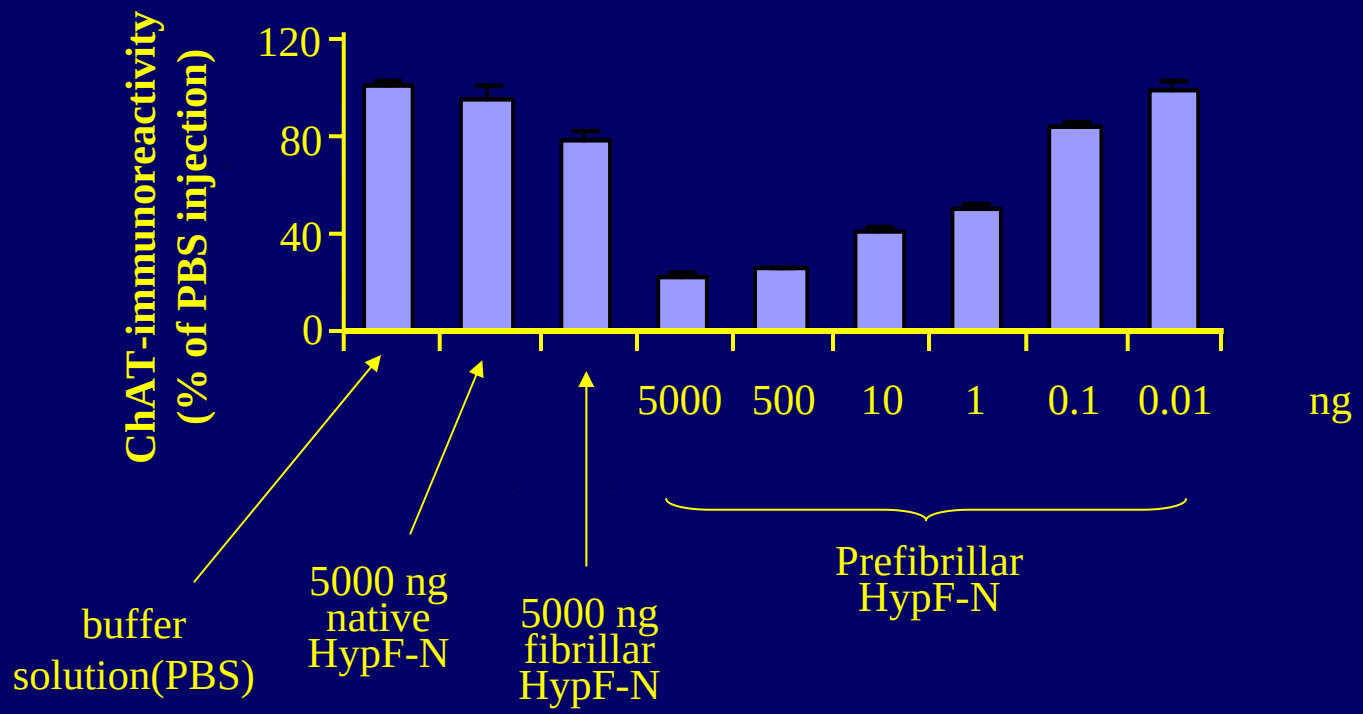


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# Proteins forming naturally nonpathological amyloid-like fibrils with specific functional roles

| Protein | Organism | Function of the resulting amyloid-like fibrils |
|---|---|---|
| Curlin | *Escherichia coli* (bacterium) | To colonize inert surfaces and mediate binding to host proteins |
| Chaplins | *Streptomyces coelicolor* (bacterium) | To lower the water surface tension and allow the development of aerial hyphae |
| Hydrophobin[a] EAS | *Neurospora crassa* (fungus) | To lower the water surface tension and allow the development of aerial hyphae |
| Proteins of the chorion of the eggshell[b] | *Bombyx mori* (silkworm) | To protect the oocyte and the developing embryo from a wide range of environmental hazards |
| Spidroin | *Nephila edulis* (spider) | To form the silk fibers of the web |
| Intralumenal domain of Pmel17 | *Homo sapiens* | To form, inside melanosomes, fibrous striations upon which melanin granules form |
| Ure2p (prion) | *Saccharomyces cerevisiae* (yeast) | To promote the uptake of poor nitrogen sources ([URE3]) |
| Sup35p (prion) | *Saccharomyces cerevisiae* (yeast) | To confer new phenotypes ([PSI+]) by facilitating the readthrough of stop codons on mRNA |
| Rnq1p (prion) | *Saccharomyces cerevisiae* (yeast) | Not well understood ([RNQ+], also known as [PIN+], phenotype) |
| HET-s (prion) | *Podospora anserina* (fungus) | To trigger a complex programmed cell death phenomenon (heterokaryon incompatibility) |
| Neuron-specific isoform of CPEB (prion) | *Aplisia californica* (marine snail) | To promote long-term maintenance of synaptic changes associated with memory storage |

**I very much hope that you have enjoyed this lecture and thank you for your kind attention!**