PROTEIN MISFOLDING

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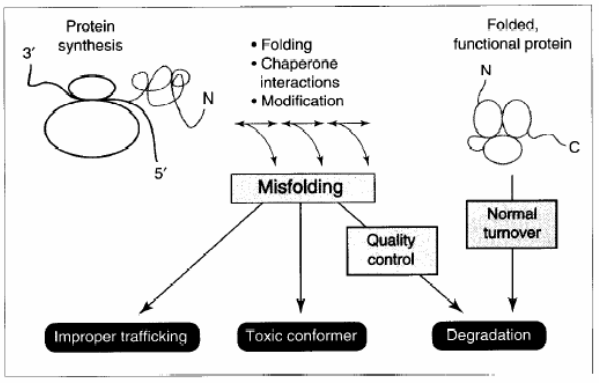


Figure 1

Diversity of protein misfolding in disease. The ability of a protein to perform its function in the cell depends upon its ability to assume a functional conformation. Thus, mutations and environmental changes that destabilize the native state, or divert proteins from their normal folding pathway, underlie several human diseases. For example, alteration of the thermodynamic stabilities of the native state and crucial folding intermediates may prevent folding into the functional conformation on a biological time scale. The cell's quality control apparatus must then recognize the misfolded and partially folded products and mark them for recycling. Off-pathway traps can be caused by aggregation, mistargeting into an inappropriate cellular location, or proteolysis of the polypeptide. Proteins and peptides that are aggregated into amyloid plaques are often resistant to degradation. Furthermore, the formation of these deposits, rather than the lack of native protein, may be responsible for the cellular pathology.

Table I. Some putative protein folding diseases

Disease	Mutant protein/protein involved	Molecular phenotype
Inability to fold		
Cystic fibrosis	CFTR	Misfolding/altered Hsp70 and calnexin interactions
Marfan syndrome	Fibrillin	Misfolding
Amyotrophic lateral sclerosis	Superoxide dismutase	Misfolding
Scurvy	Collagen	Misfolding
Maple syrup urine disease	α-Ketoacid dehydrogenase complex	Misassembly/misfolding
Cancer	p53	Misfolding/altered Hsp70 interaction
Osteogenesis imperfecta	Type I procollagen pro α	Misassembly/altered BiP expression
Toxic folds		
Scrapie/Creutzfeldt-Jakob/ familial insomnia	Prion protein	Aggregation
Alzheimer's disease	β-Amyloid	Aggregation
Familial amyloidosis	Transthyretin/lysozyme	Aggregation
Cataracts	Crystallins	Aggregation
Mislocalization owing to misfolding		
Familial hypercholesterolemia	LDL receptor	Improper trafficking
α_1-Antitrypsin Deficiency	α_1-Antitrypsin	Improper trafficking
Tay–Sachs disease	β-Hexosaminidase	Improper trafficking
Retinitis pigmentosa	Rhodopsin	Improper trafficking
Leprechaunism	Insulin receptor	Improper trafficking

Prion diseases include:

Sheep: Scrapie

Cattle: Bovine Spongiform Encephalopathy (BSE) also called "mad cow disease"

Human: Creutzfeldt-Jacob disease (CJD), **kuru**, Gerstmann-Straussler-Scheinker (GSS), Fatal Familial Insomnia (FFI)

Scrapie 18th century

(*la tremblante*)

This ewe is affected with scrapie. Notice the severe weight loss and wool loss from scratching. Photo from: Gates' Practical Guide to Sheep Disease Management.

Creutzfeldt-Jakob disease (CJD)

- Found by H.G. Creutzfeldt & A. Jakob in 1920
- A rare ($1/10^6$) and fatal neurodegenerative disease of unknown cause. Patients are usually aged between 50 and 75
- 記憶喪失及混淆, 慢性痴呆, 運動神經失調, 不自主動作, 失明, 喪失語言能力

1940s-1960
In 1957 >200 new cases per year

Kuru (shaking or tremor)
Also called *negi nagi (silly or foolish person)*

Kuru

1966
Intracerebral inoculation of kuru brain into chimpanzee

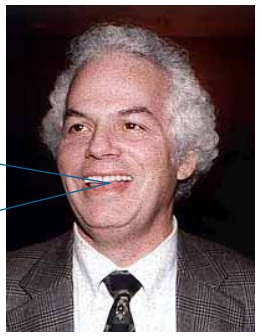
The Nobel Prize in Physiology or Medicine 1976

Dr. Carleton Gajdusek
NIH, USA

Prion Disease is a class of diseases caused by the misfolding of prion protein

PRION: PROteinaceous INfectious particle

The disease is caused by protein only. This protein is called prion protein



The 1997 Nobel Prizes Sciences

-Stanley B. Prusiner
University of California, San Francisco

Mad Cow Disease (Bovine Spongiform Encephalopathy): one of the neuron degenerative disorders

3 ~ 7 years old

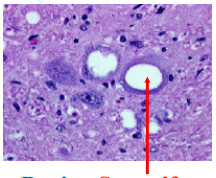
Meat and Bone Meal (MBM) from the offal of the infected sheep

Lose weight
Difficulty standing
Drool
Wave head
Threaten other animal

After a few months

> 180,000 BSE cases in UK

Infected brain tissue



Bovine Spongiform Encephalopathy

Near 5 million of cows were slaughtered !

Raw Material Reception and Processing

The rendering process of raw material at the factory, involves six basic stages:

- Crushing/grinding of Raw Material
- Cooking/dehydration
- Separation of Fat
- Sterilisation of Meal
- Sterilisation of Tallow
- Tallow Filtration

MBM food

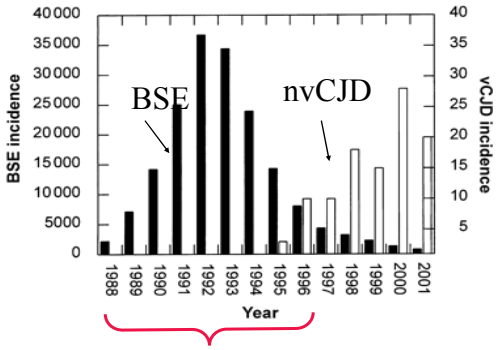
New variant CJD (nvCJD) in human

Depressed and paranoid

Lose the ability to walk and swallow

Grow blind and comatose

……..die



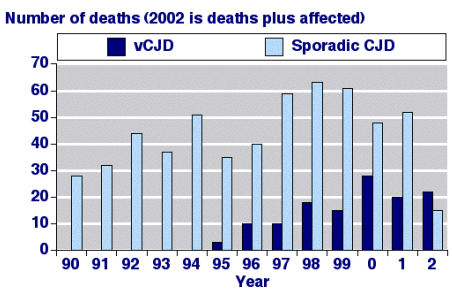
MBM food export

Incurable!!!!

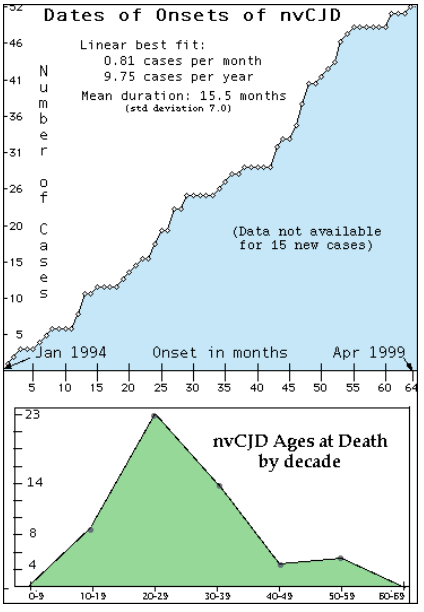
CJD &vCJD

vCJD之臨床徵狀包括下列數項：

- **發病或死亡的年齡早。(範圍自18-41歲，平均年齡27.6歲，)**
- **疾病病程較CJD長。(由7.5-24個月，平均為13.1個月)**
- **主要表現為焦慮、憂鬱、退縮及行為逐漸改變等精神症狀。**

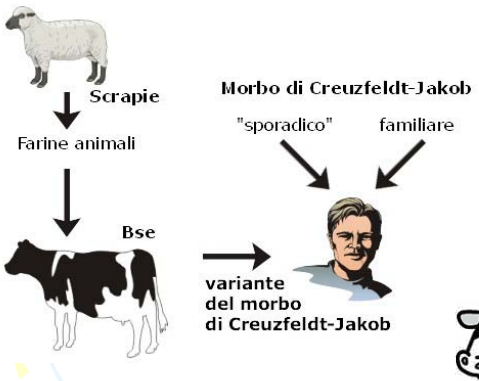


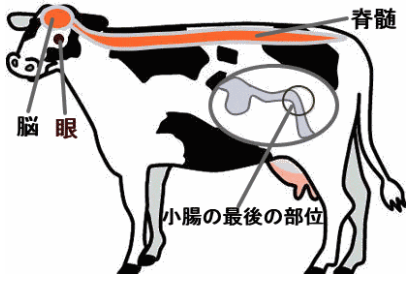
Sporadic and variant CJD in the UK



狂牛症的影響

- 鉅額經濟損失: 在過去廿年間，牛肉相關產業佔英國農業產出的15%-18%。1995年時，英國牛肉產業的生產總值達二兆六千億英鎊。世界各國的貿易禁令對英國畜牧業者自然造成嚴重打擊
- 對全世界的影響: 1988年至1996年英MBM製造商大量輸出MBM至歐盟、中東、亞洲、非洲等七十國
- 感染風險: 飲食, 血液傳染。
- 禁止傳染力高的腦、脊髓、扁桃腺、胸腺、脾、腸等組織）進入人或動物的食物鏈
- 法國已規定，凡是在1980年到1996年期間，在英國或愛爾蘭居住超過一年者都不能在法國捐血。瑞士紅十字會則不接受曾在英國居住超過兩個月的人捐血。美國聯邦食品暨藥物管理局 (FDA)原規定，禁止1980至1996年間在英國停留超過六個月的人捐血，1980年以後在英國居住六個月以上的人不能在美國捐血。並考慮限制1980年以後，在法國或葡萄牙地區居住或旅行累積時間超過十年的人捐血。但美國紅十字會則傾向採取更嚴格措施，禁止所有1980年後曾在西歐任何地方居住過的人捐血。
- 變種庫賈氏症 vCJD: 無法早期偵測,無法治療





Prion diseases can be

Inherited: FFI, GSS (human)

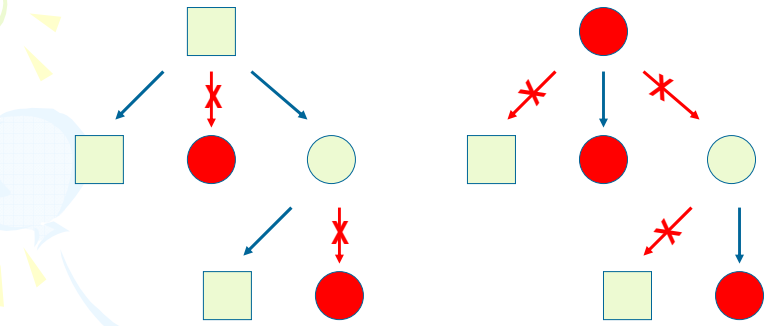
Sporadic: CJD (human)

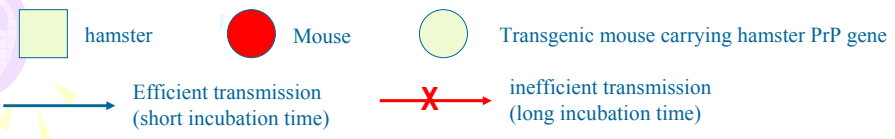
Transmissible: kuru (human – to- human)

vCJD (cow – to- human)

(with species barrier)

Susceptibility of a host to prion infection is co-determined by the prion inoculum and the prion gene



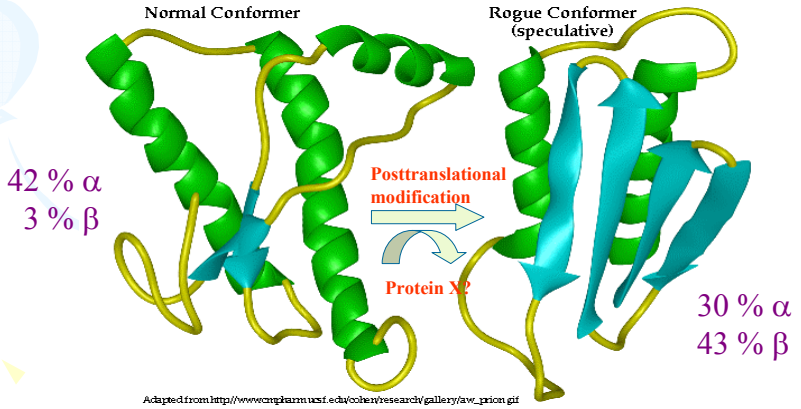


Prion disease formation:structural transition

Cellular form PrP^{C} → PrP^{Sc} Scrapie form

Protease sensitive — Protease resistant

About half of α-helical and coil structure turns into β-sheet



Critical concentration

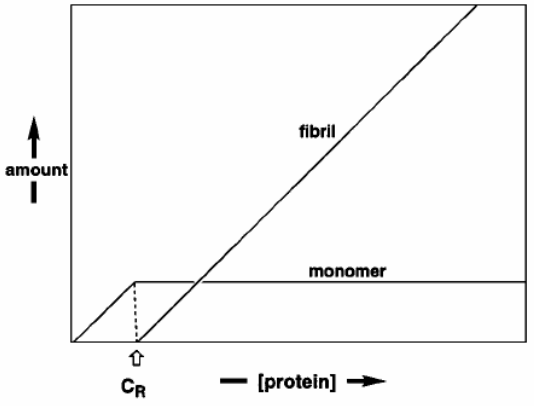
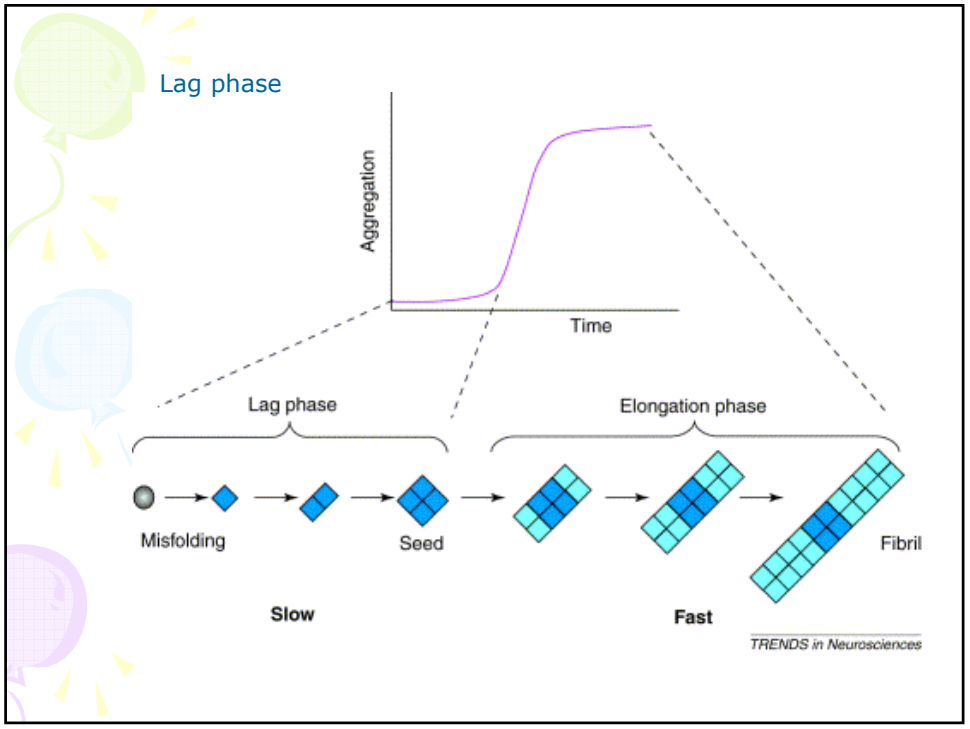
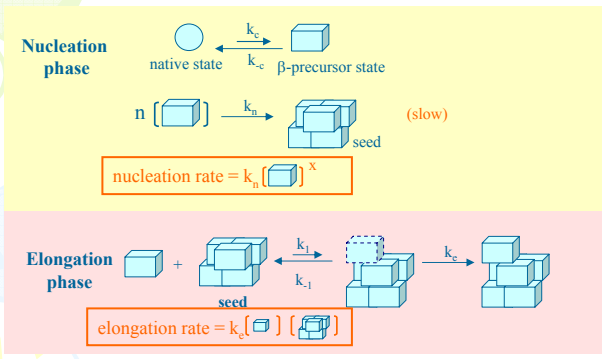
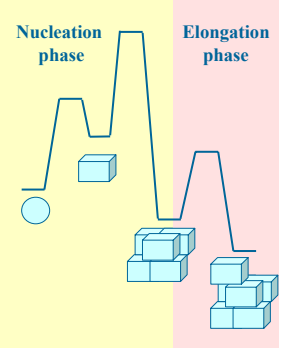


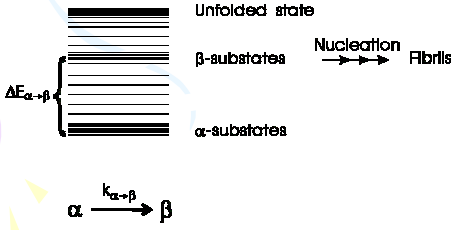
Figure 3 Fibril formation is concentration dependent. Below the critical concentration (C_R), no fibril formation will occur. Above that concentration, all added protein will be incorporated into the fibrils such that the monomer concentration never exceeds C_R. This behavior is seen at equilibrium.

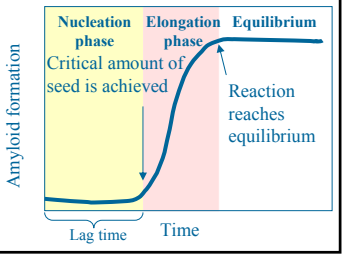


Nucleation-dependent polymerization model









Seeding

When seed is provided from the environment,

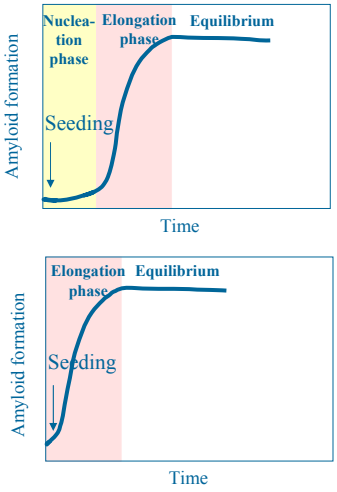
if [ ] < [critical amount of seed]

⇒ There is still a lag time, but it is reduced

During the lag time, nucleation is going on until
[produced seed] + [provided seed] = [critical amount of seed]

if [ ] ≧ [critical amount of seed]

⇒ Elongation starts immediately



Amyloid fibrils are found as deposits of insoluble aggregation in patients with a range of diseases

Table 1
Amyloidogenic proteins and amyloid-based clinical disorders

Disease-associated amyloidogenic proteins

Amyloidogenic protein	Type of structure	Disease	Tissue distribution of protein deposits
Prion protein and its fragments	N-terminal fragment (23–121) is natively unfolded; C-terminal domain (121–230) is α-helical (predominantly)	Creutzfeld–Jacob disease (CJD) Gerstmann–Straussler–Schneiker syndrome (GSS) Fatal familial insomnia (FFI) Kuru Bovine spongiform encephalopathy (BSE) and scrapie	Brain
Amyloid-β and its fragments	Natively unfolded	Alzheimer's disease (AD)	Brain
		Dutch hereditary cerebral hemorrhage with amyloidosis (HCHWA, also known as cerebrovascular amyloidosis)	
		Congophilic angiopathy	Brain, spinal cord
ABri	Natively unfolded	Familial British dementia	Brain
Cystatin C	α/β	Hereditary cystatin c amyloid angiopathy (HCCAA)	Brain
Huntingtin	α-Helical (but exon 1 is unfolded and forms fibrils)	Huntington Disease	Brain
Androgen receptor protein	Ligand-binding (LBD) and DNA-binding domains (DBD) are α-helical; amino-terminal domain (NTD) is natively unfolded	Spinal and bulbar muscular atrophy (SBMA)	Brain, scrotal skin, dermis, kidney, heart, and testis, spinal cord
Ataxin-1	Unknown (likely natively unfolded)	Spinocerebellar ataxia (SCA) Neuronal intranuclear inclusion disease (NIID)	Brain, spinal cord Central and peripheral nervous system
DRPLA protein (atrophin-1)	Unknown (likely natively unfolded)	Hereditary dentatorubral-pallidoluysian atrophy (DRPLA)	Brain
Serum amyloid A and its fragments	α/β	AA amyloidosis (inflammation-associated reactive systemic amyloidosis)	Bladder, stomach, thyroid, kidney, liver, spleen, gastrointestinal tract
Medin (245–294	β-Sheet	Aortic medial amyloidosis	Aortic smooth muscle

Continued

α-Synuclein	Natively unfolded	Parkinson's disease (PD) Diffuse Lewy bodies disease (DLBD) Lewy bodies variant of Alzheimer's disease (LBVAD) Dementia with Lewy bodies (DLB) Multiple system atrophy (MSA) Hallervorden–Spatz disease	Brain
NAC (central fragment of α-synuclein)	Natively unfolded	Alzheimer's disease (AD)	Brain
Fibrinogen and its fragments	β-Sheet	Hereditary renal amyloidosis	Kidney
Atrial natriuretic factor	"Small protein"	Atrial amyloidosis	Heart
Insulin	Predominantly α-helical	Injection-localized amyloidosis	Skin, muscles

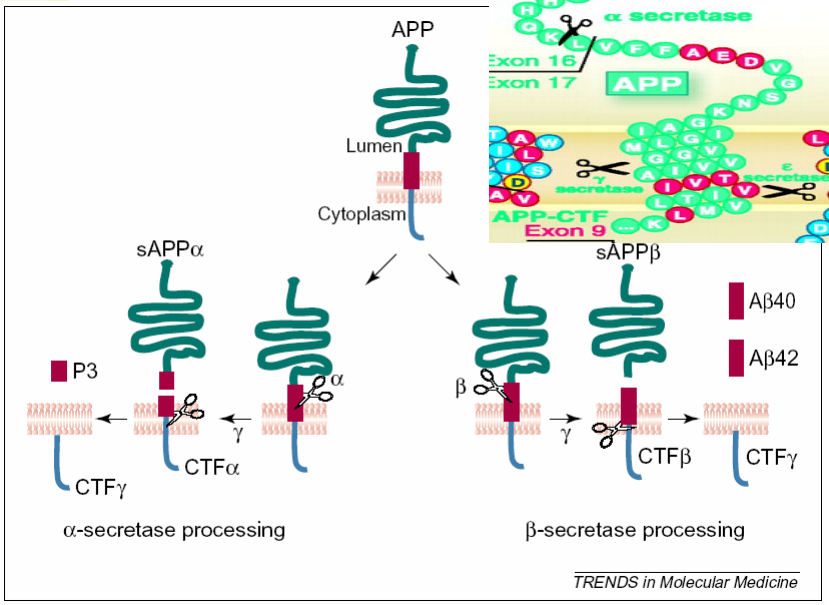
Continued

Islet amyloid polypeptide (IAPP, Amylin)	Natively unfolded	Pancreatic islet amyloidosis in late-onset diabetes (type II diabetes mellitus)	Pancreas
Calcitonin	Natively unfolded	Medullary carcinoma of the Thyroid (MCT)	Thyroid
Lysozyme	α + β	Hereditary systemic amyloidosis	Several visceral organs and tissues
Gelsolin	α/β	Hereditary systemic amyloidosis Finnish-type familial amyloidosis	Several visceral organs and tissues
Transthyretin	β-Sheet (predominantly)	Senile systemic amyloidosis (SSA) (or senile cardiac amyloidosis)	Almost all organs and tissues, including heart, gland, arteries, bones, liver, digestive tract, etc.
		Familial amyloid polyneuropathy (FAP)	Various organs and tissues.
Apolipoprotein A1	α-Helical	Hereditary systemic amyloidosis	Eyes
β_2-Microglobulin	β-Sheet	Amyloid associated with hemodialysis (AH or Aβ2M) (athropathy in hemodialysis)	Musculoskeletal tissues (large and medium-sized joints, bones, muscles), peripheral nervous system, gastrointestinal tract, tongue, heart, urogeniteal tract
Tau protein	Probably natively unfolded	Alzheimer's disease (AD), Pick's disease, Progressive supranuclear palsy (PSP)	Brain
Immunoglobulin light chain variable domains	β-Sheet	Light chain associated amyloidosis or AL amyloidosis	Almost all organs and tissues, including heart, kidneys, liver, spleen, gastrointestinal tract, skin, tongue, endocrine glands, peripheral nervous system, etc.
		Light chain deposition disease or LCDD	Liver, spleen, bone marrow, vessel walls, parenchymal tissue, kidneys, heart, liver, skin, lungs, tongue, ovary, pancreas, etc.
		Light chain cast nephropathy	Kidneys
		Light chain cardiomyopathy	Heart

Non-disease-related amyloidogenic proteins and peptides

Protein (peptide), reference	Type of structure	Protein (peptide), reference	Type of structure
Betabellins 15D and 16D [294]	β-Sandwich	Prothymosin α [41]	Natively unfolded
Cytochrome c_{552} [133]	α-Helical	Myoglobin [38]	α-Helical
Methionine aminopeptidase [35]	α-Helical	Muscle acylphosphatase [32]	α/β
Phosphoglycerate kinase [34]	α/β	Hen egg white lysozyme [33]	α + β
Hen egg white lysozyme, β-domain [33]	β-Sheet	Acidic fibriblast growth factor [42]	β-Barrel
PI3-SH3 domain [27]	β-Barrel	OspA protein, BH^{9-10} peptide [36]	β-Turn
β-Lactoglobulin [44]	β-Sheet (predominantly)	De novo αtα peptide [37]	α-Helix-turn-α-helix
Monellin [29]	α/β	Lung surfactant protein C [40]	α-Helix
Immunoglobulin light chain LEN [87,88]	β-Sheet	α-Lactalbumin [45]	α + β
HypF, N-terminal domain, [295]	α/β	V_L domain of mouse antibody F11 [296]	β-Sheet
Human complement receptor 1, 18–34 fragment [297]	Unfolded	Apolipoprotein C-II [213–215]	Natively unfolded
Human stefin B [298]	α/β	Cold shock protein A [299]	β-Barrel
GAGA factor [300]	Natively unfolded	Protein G, B1 Ig-binding domain [79,301]	Four-stranded β-sheet with a flanking α-helix
Yeast prion Ure2p [302]	α-Helical/unfolded	Cold shock protein B, 1–22 fragment [303]	Unfolded
Herpes simplex virus glycoprotein B fragment [304]	β-Structural	De novo proteins from combinatorial library [305]	β-Structural
The fiber protein of adenovirus, 355–396 peptide from shaft [306]	Fibrillar	Soluble homopolypeptides, [262]: poly-L-lysine poly-L-glutamic acid poly-L-threonine	Unordered

APP Processing Pathways

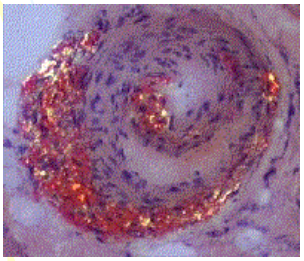


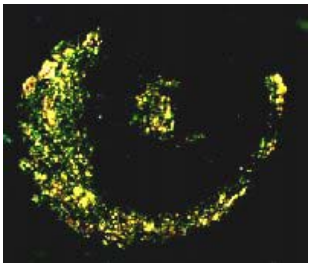
Methodologies of studying protein misfolding

Absorption spectroscopy
Fluorescence spectroscopy
Circular Dichroism spectroscopy (CD)
Fourier Transform InfraRed spectroscopy (FTIR)
Nuclear Magnetic Resonance spectroscopy (NMR)
Electron Microscopy (EM)
Atomic force microscopy (AFM)

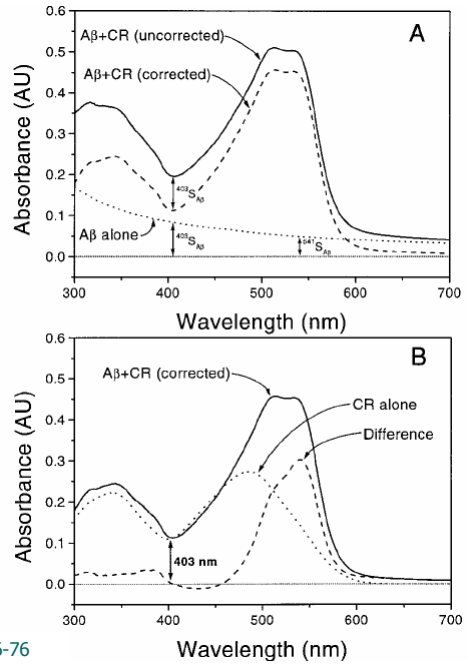
Properties of amyloid fibril:

- 1920s, Divry and Florkin demonstrated that Congo-red stained deposits exhibit apple-green birefringence under polarized light





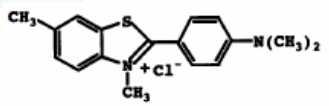
Congo red binding



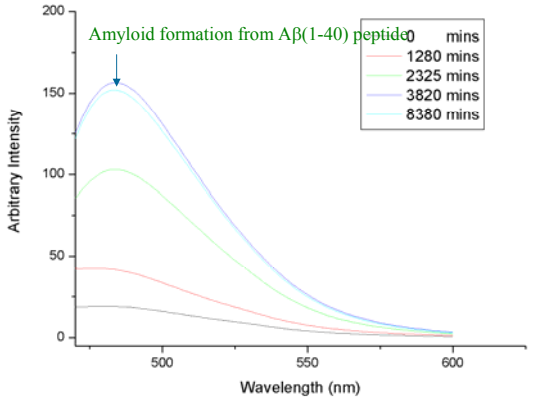
Anal Biochem (1999) 266, 66-76

Fluorescence of amyloid fibril

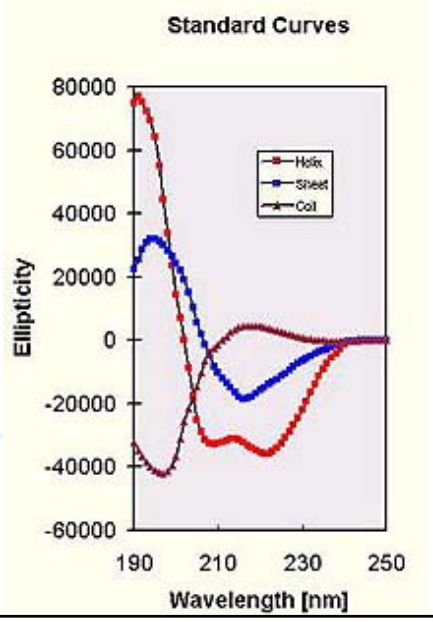
- When bind with Thioflavin T, amyloid fibril emit fluorescence at 482 nm (Ex 450nm).



Thioflavin(ThT)

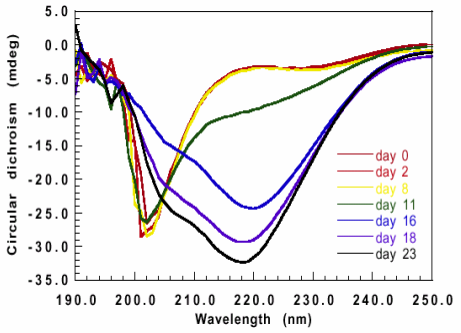


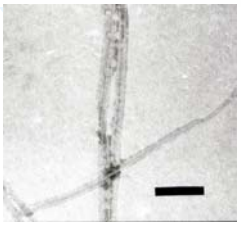
CD



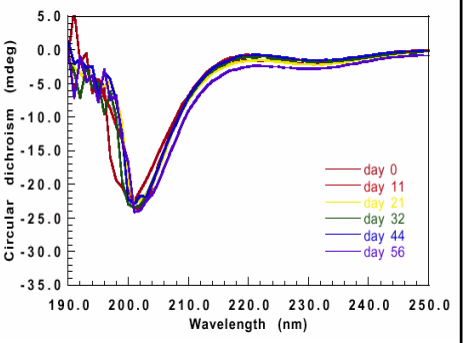
Peptides were incubated in the buffer of 20 mM NaOAc, 140 mM NaCl, pH 3.7. The Circular dichroism spectra were recorded.

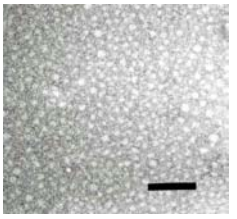
PrP(108-144)





S135-α-GalNAc

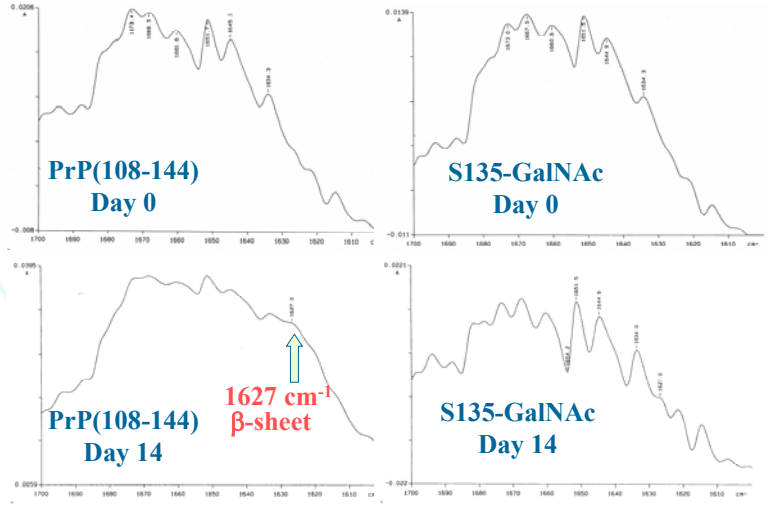




Characteristic FTIR bands

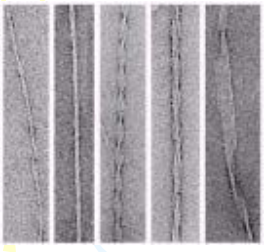
Antiparallel β-sheet and aggregates	1675-95 cm^{-1}
3_{10}-helix	1660-70
α-helix	1648-60
unordered	1640-48
β-sheet	1625-40
Aggregates	1610-28

FT-IR of PrP(108-144) and S135-α-GalNAc



Electron Microscopy

- Twisted nonbranched fiber with 7-12 nm diamet



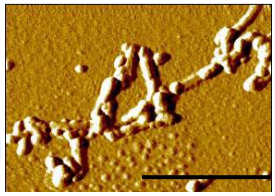
Negative stain EM images of insulin fibrils, showing some of the diverse fibril morphologies found in the samples

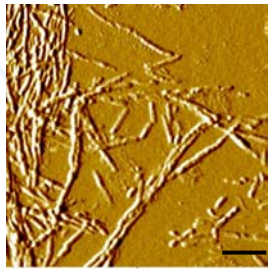
3D maps of 4 different insulin fibril morphologies. The distance between helical crossovers is around 100 nm. The fibrils are made up of different numbers of component strands (protofilaments; from left to right: 2,4,6,and probably 6). The protofilaments are twisted around eachother in either compact or ribbon-like arrangements

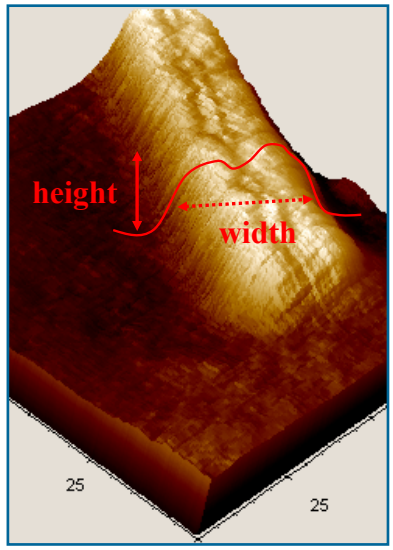
A molecular model of the compact, 4-protofilament insulin fibrils

Jimenez, JL, Nettleton, E, Bouchard, M, Dobson, CM, Robinson, CV & Saibil, HR (2002) Proc. Natl. Acad. Sci. USA 99, 196-201)

Atomic Force Microscopy







Funnel-like protein folding energy landscape