Amyloid proteins, human diseases and neurodegeneration

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Amyloid research : landmarks

1838: Mathias Schleiden (“amyloid” in plants)
1854: Rudolf Virchow (“amyloid” in human liver)
1927: Paul Divry (Congo red staining brain amyloid)
1959: A. Cohen and E. Calkins (EM of amyloid fibrils)
1967: E. Eanes and G. Glenner (X-ray diffraction)
1971: G. Glenner (First aa sequence of amyloid)

1984: G. Glenner (identification of amyloid in AD)

PubMed (05/02/11)

“Amyloid”	46306 articles
“Amyloid Alzheimer’s disease	21877 articles

TOPICS

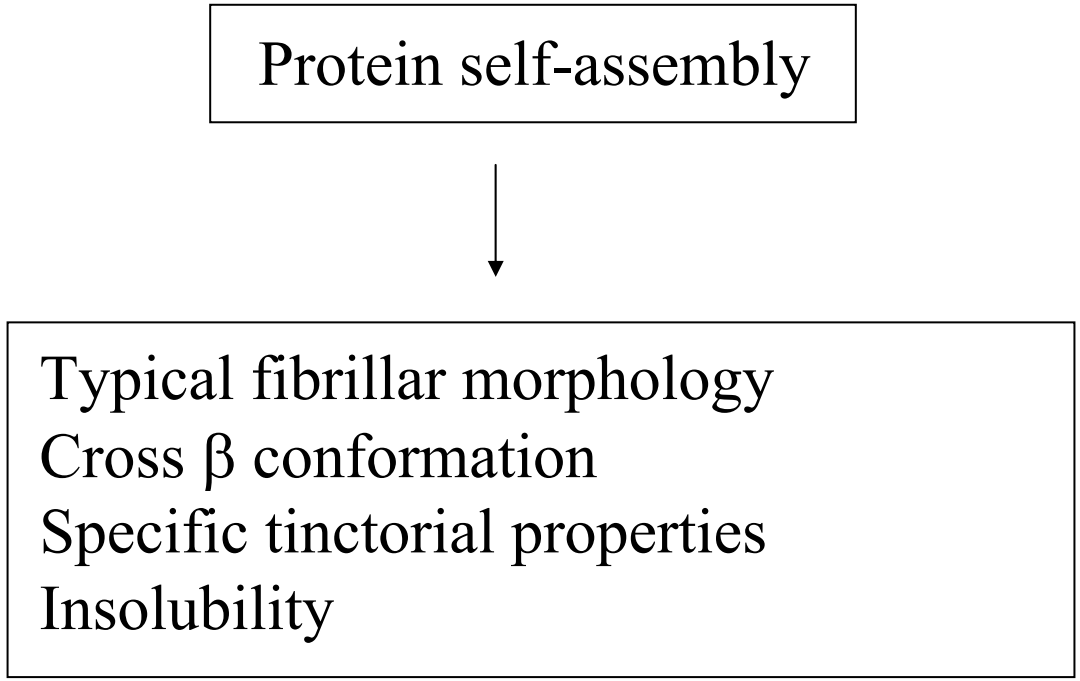
1) What is “amyloid”?

2) What are the factors involved in amyloid formation?

3) How are amyloid proteins related to human disease?

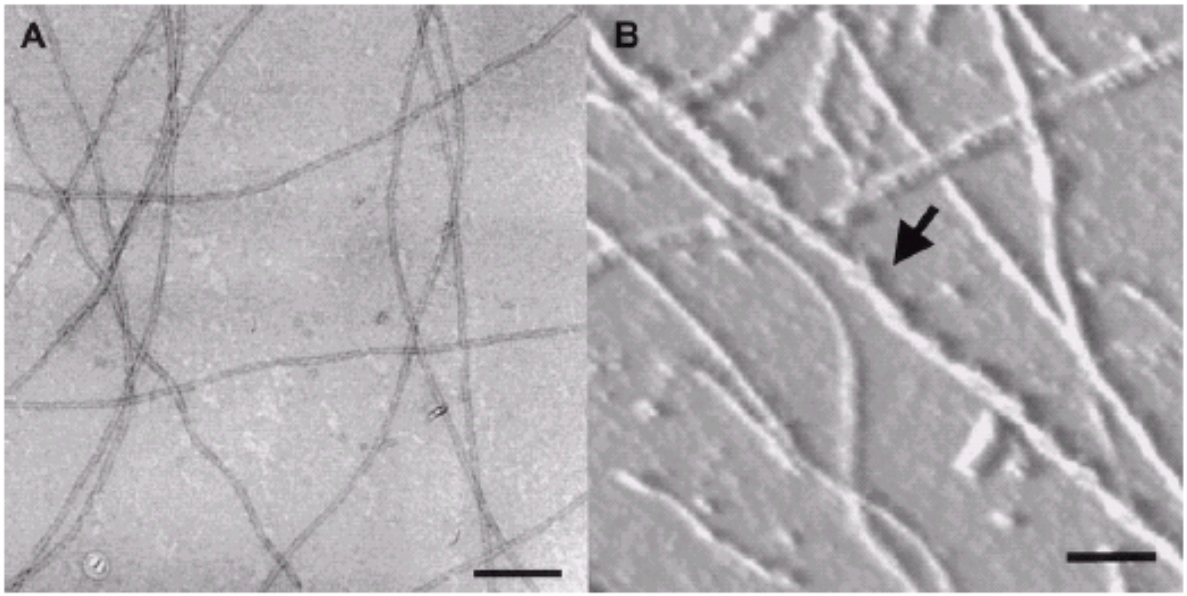
4) Are amyloid proteins neurotoxic?

Amyloid definition



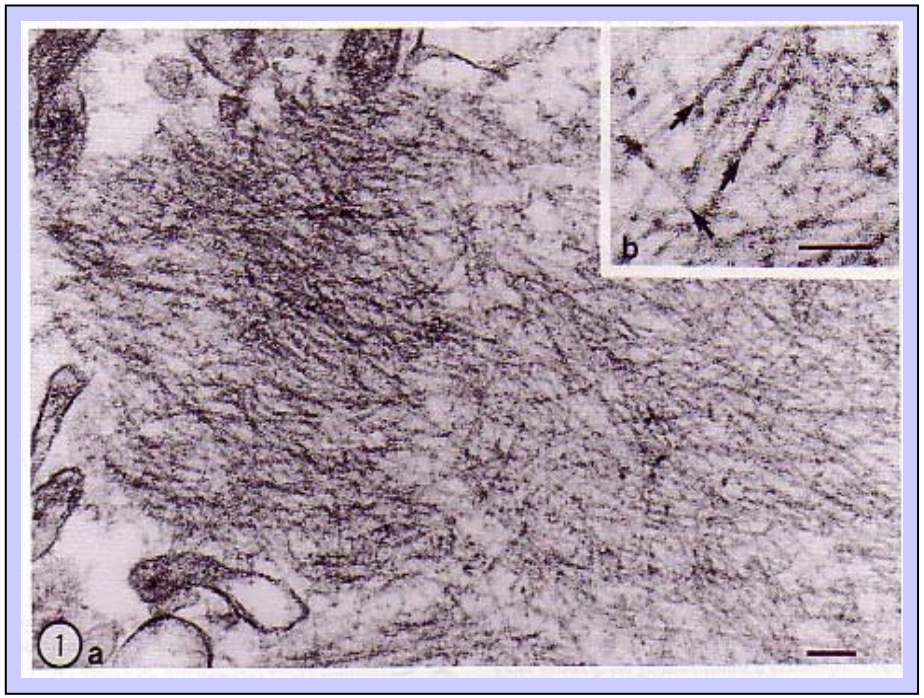
Typical morphology of amyloid fibrils TEM and AFM

TEM AFM



8-12 nm wide
Non-branching
Very long
(several microns)

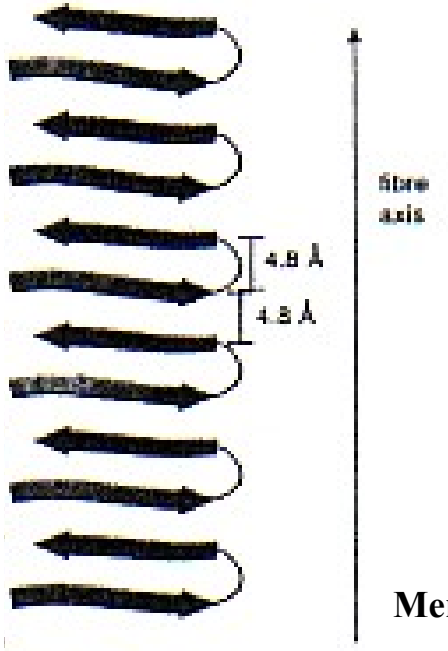
Electron microscopy of amyloid deposits *in vivo*

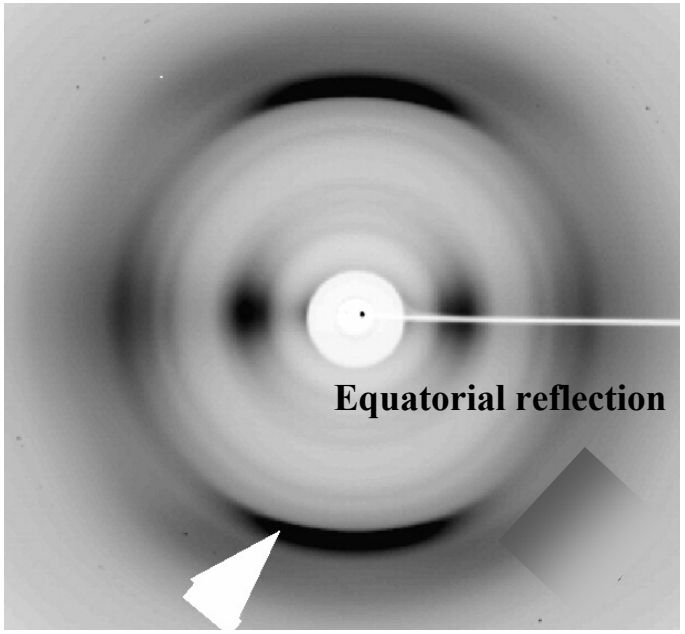


Spleen, AA

Brain, PrP (GSS)

X-ray diffraction: periodicity of β strands and sheets: "cross β conformation"

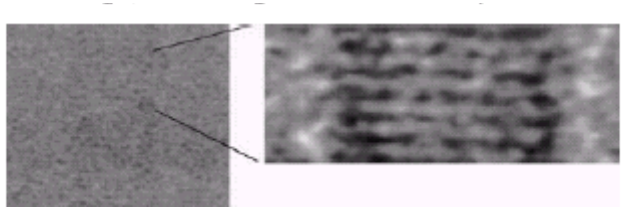




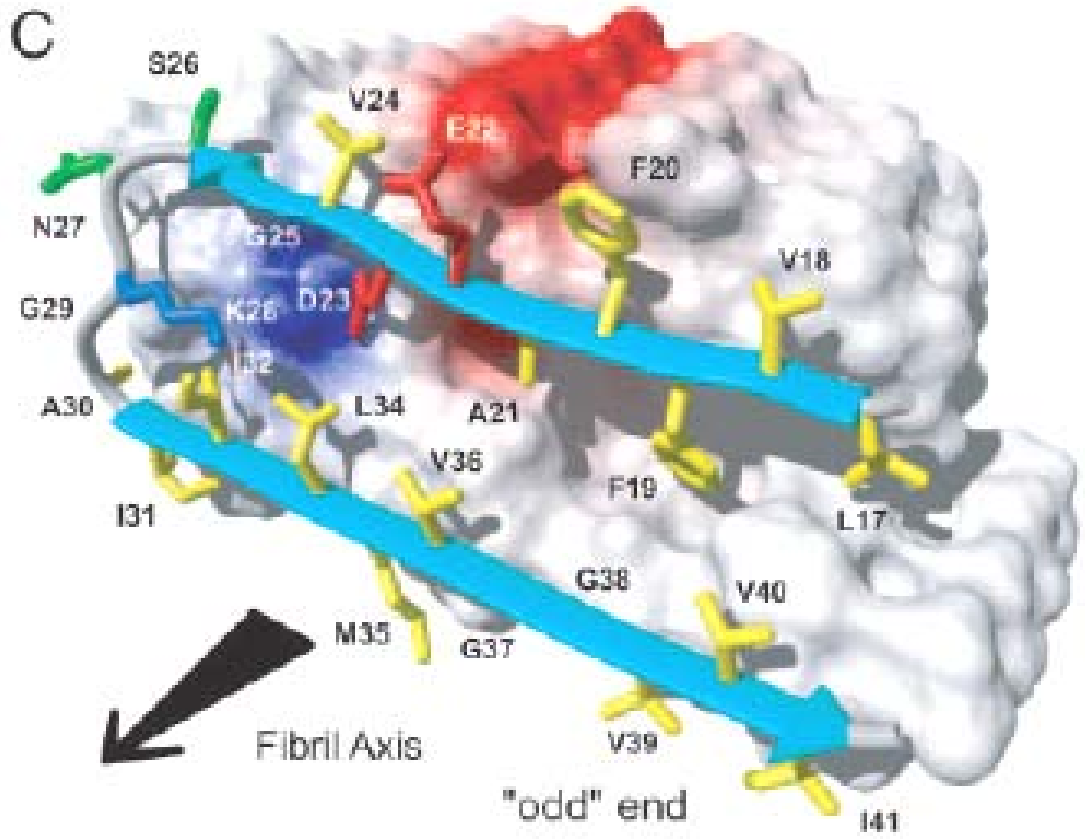
Meridional reflection

Top view

Cryo-EM



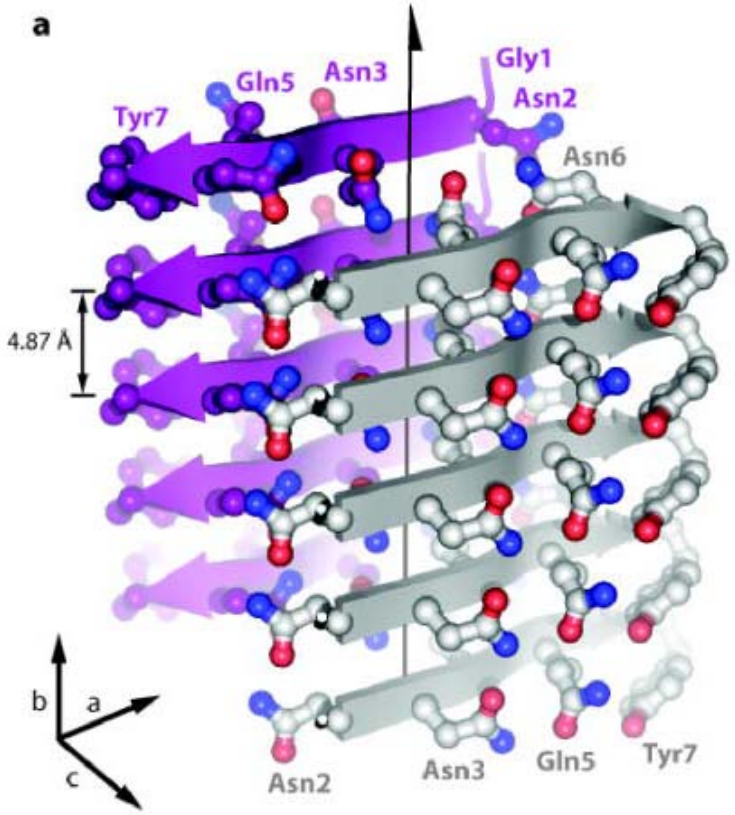
Solid state NMR of amyloid β 1-42 fibrils



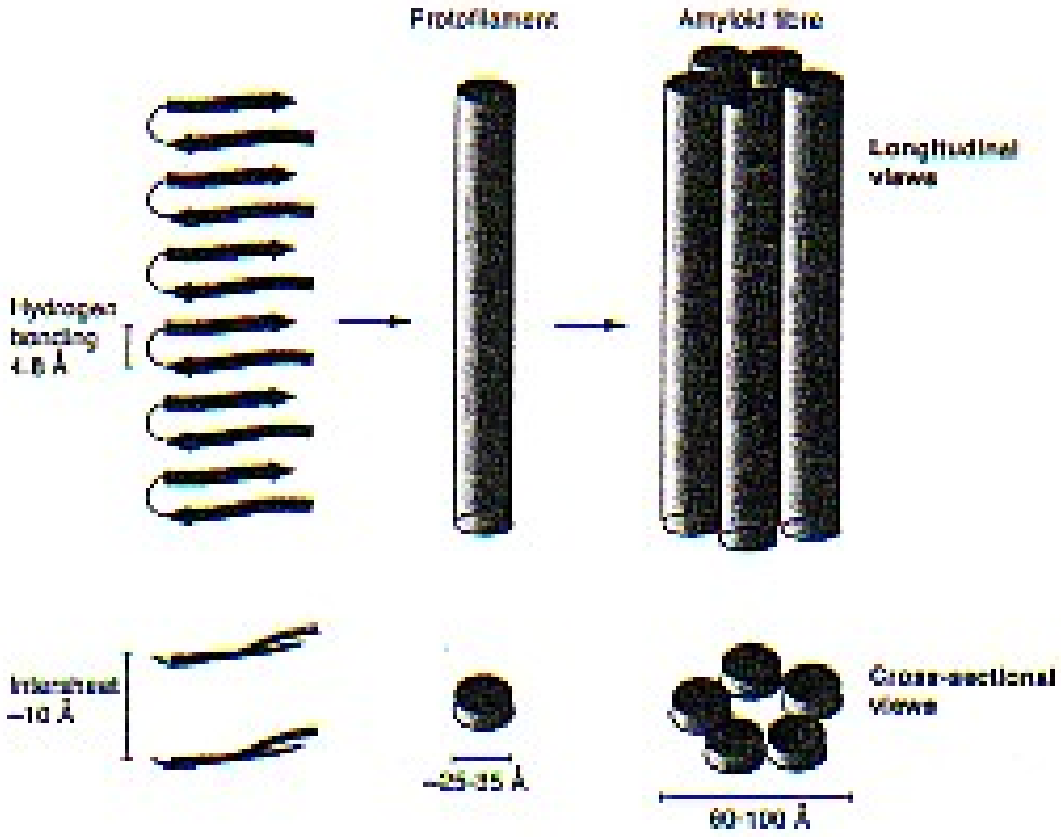
PDB 2beg

Luhrs T et al, PNAS 2005; 102:17342

Crystal structure of amyloid peptide GNNQQNY from yeast sup35 protein



Simple model of organization of amyloid fibrils



Number and twisting of protofilaments yield different fibrils (insulin model)

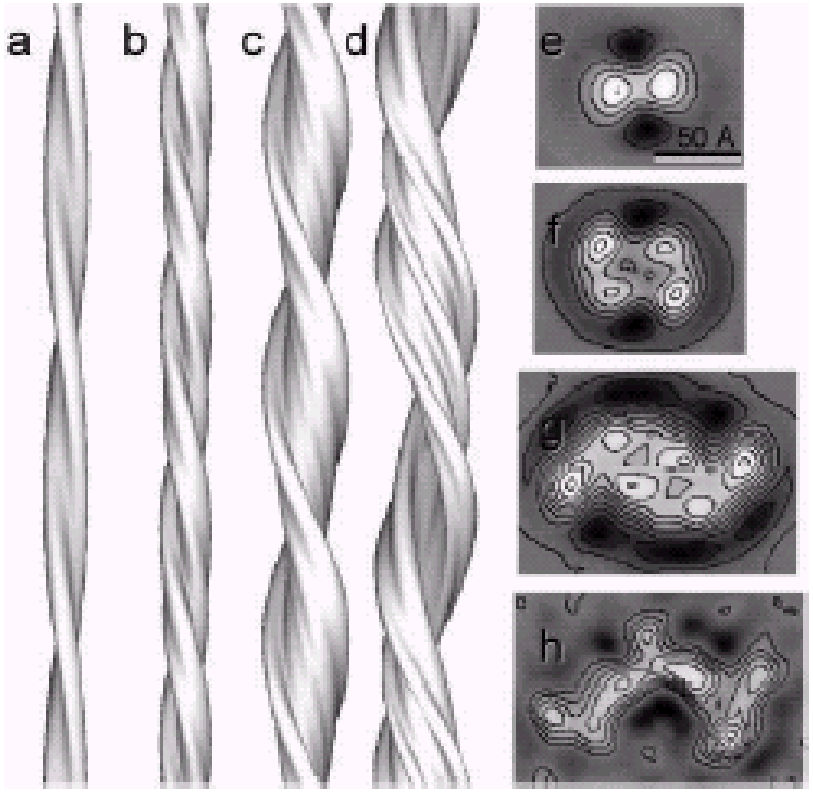
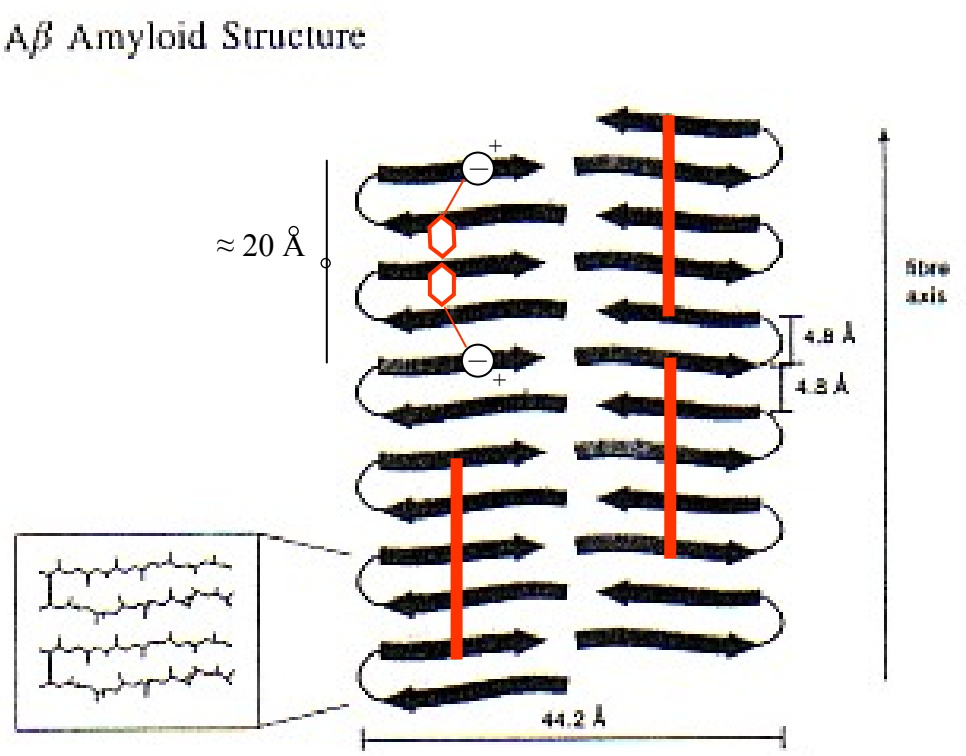
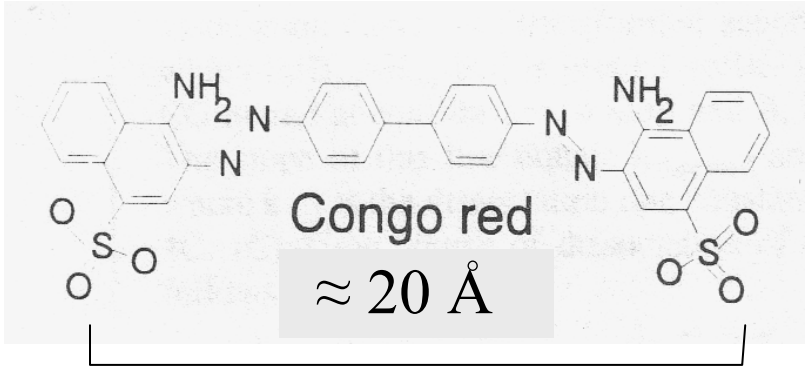
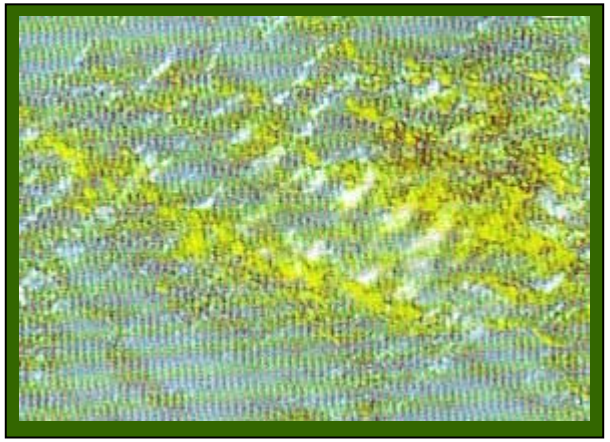


Fig. 3. Surface representation of 3D maps and contoured density cross sections of the four insulin fibril structures shown in Fig. 2. (*a* and *e*) Structure of the fibril with a pair of protofilaments twisting around each other. (*b* and *f*) The four-protofilament compact fibril. (*c* and *g*) The six-protofilament fibril. (*d* and *h*) The twisted ribbon. The protofilaments are well resolved in the first three structures, but are less clear in the twisted ribbon. (*e–h*, bar = 50 Å.)

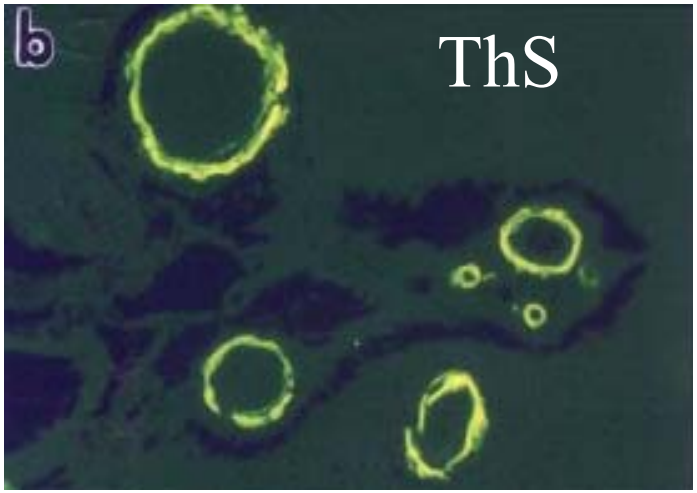
Congo red staining reflects the order of amyloid proteins

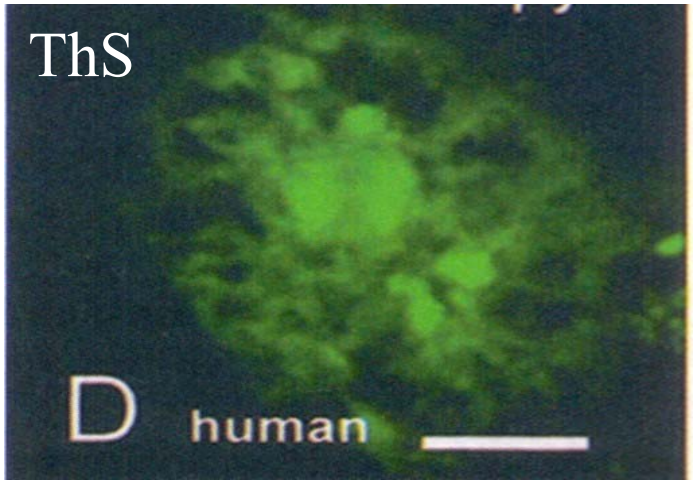


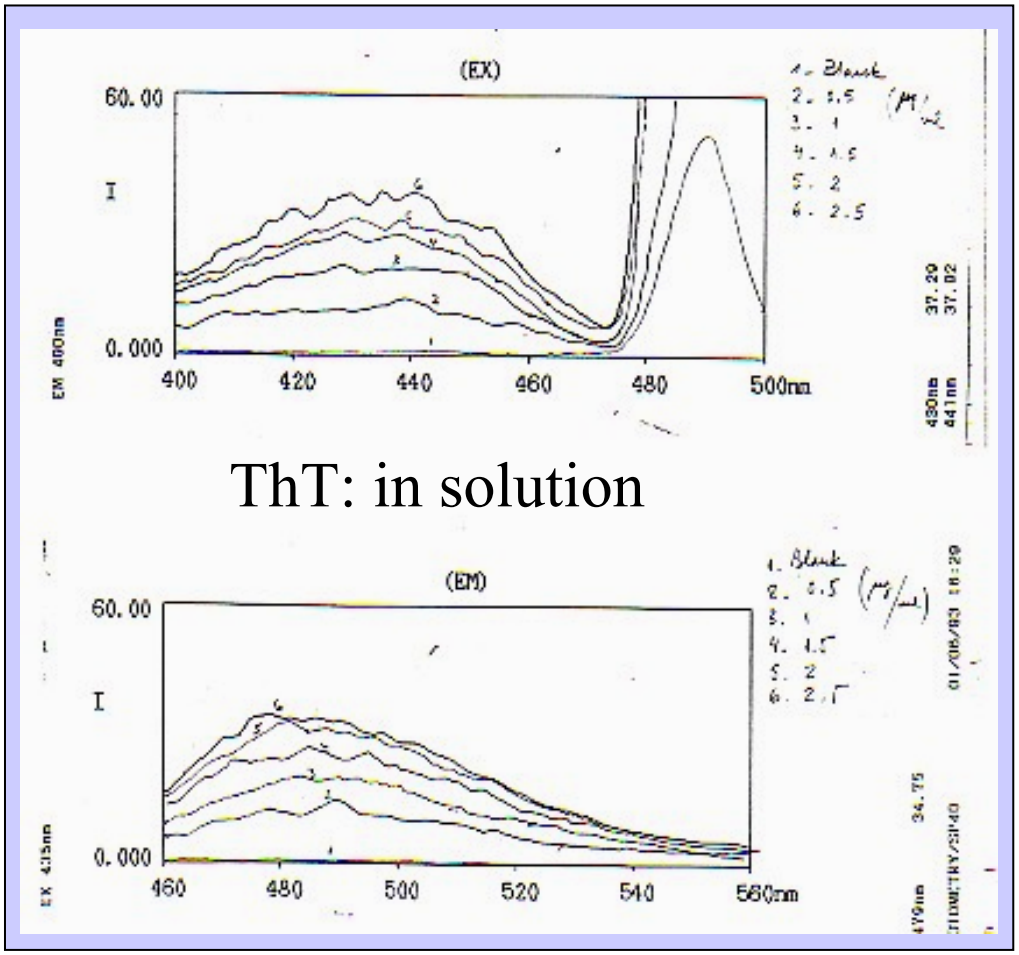




Congo red and Thioflavine S/T staining defines amyloid deposits

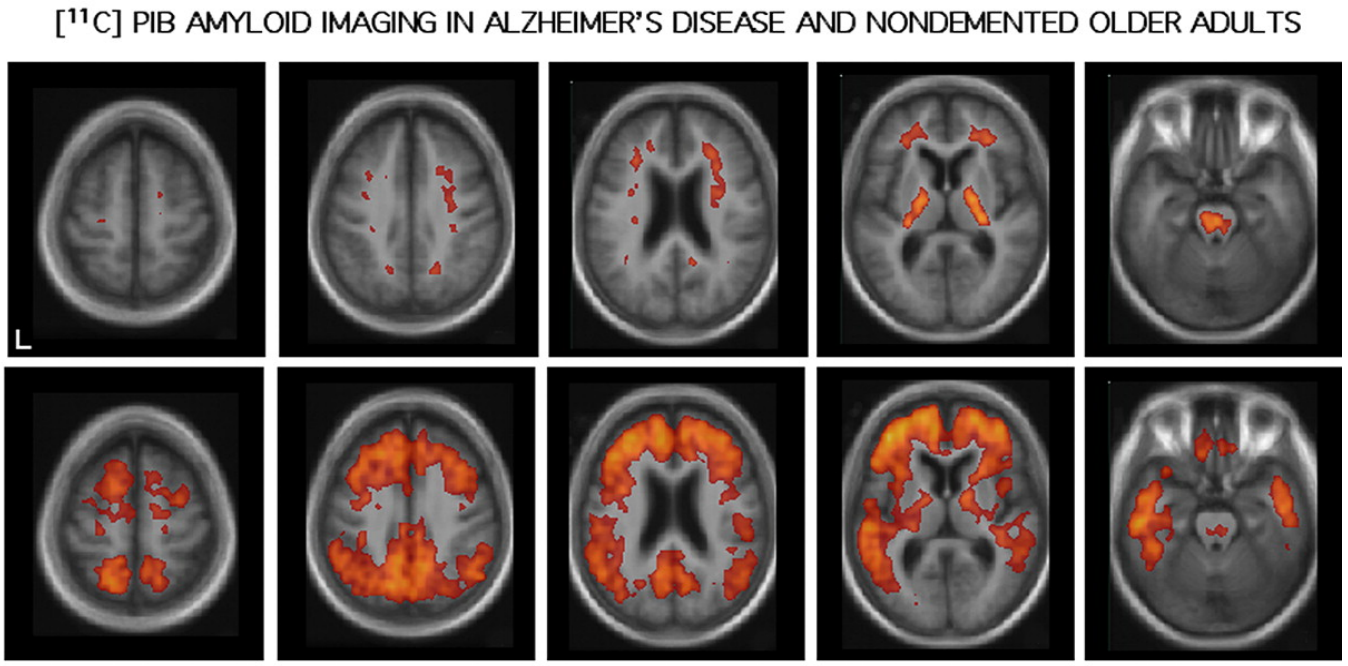




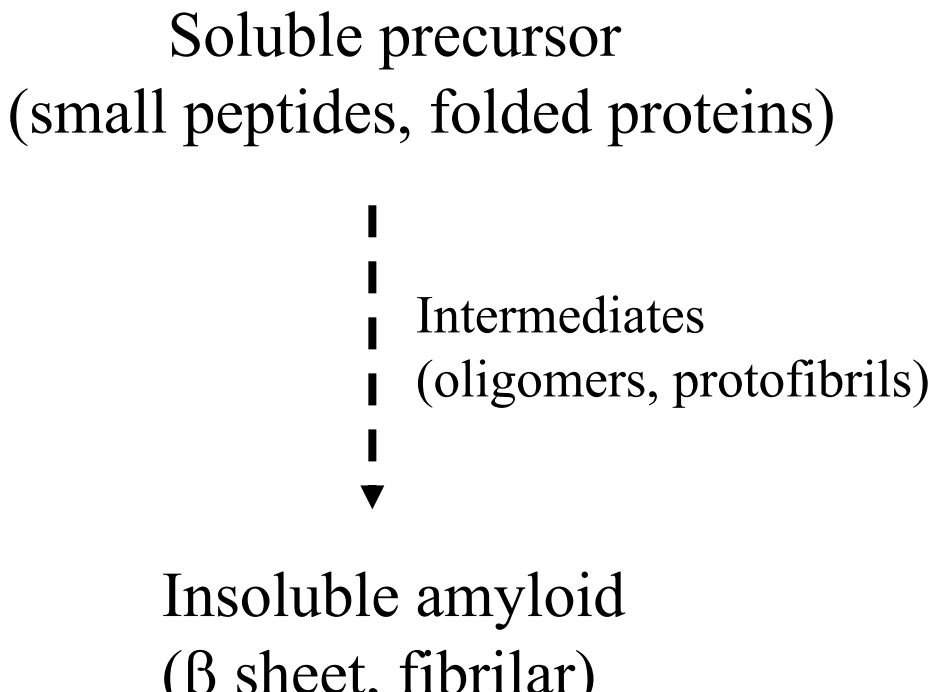


ThT: in solution

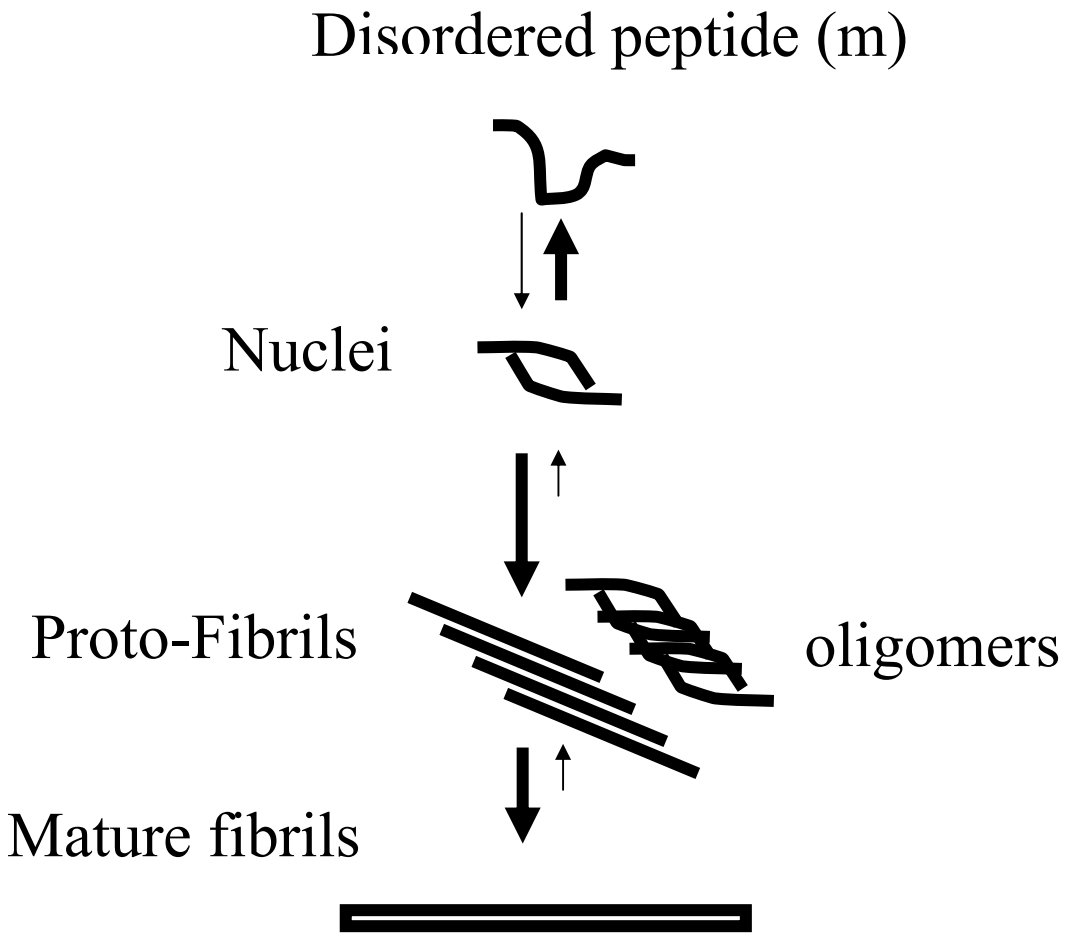
Synthetic compounds to label amyloid in vivo



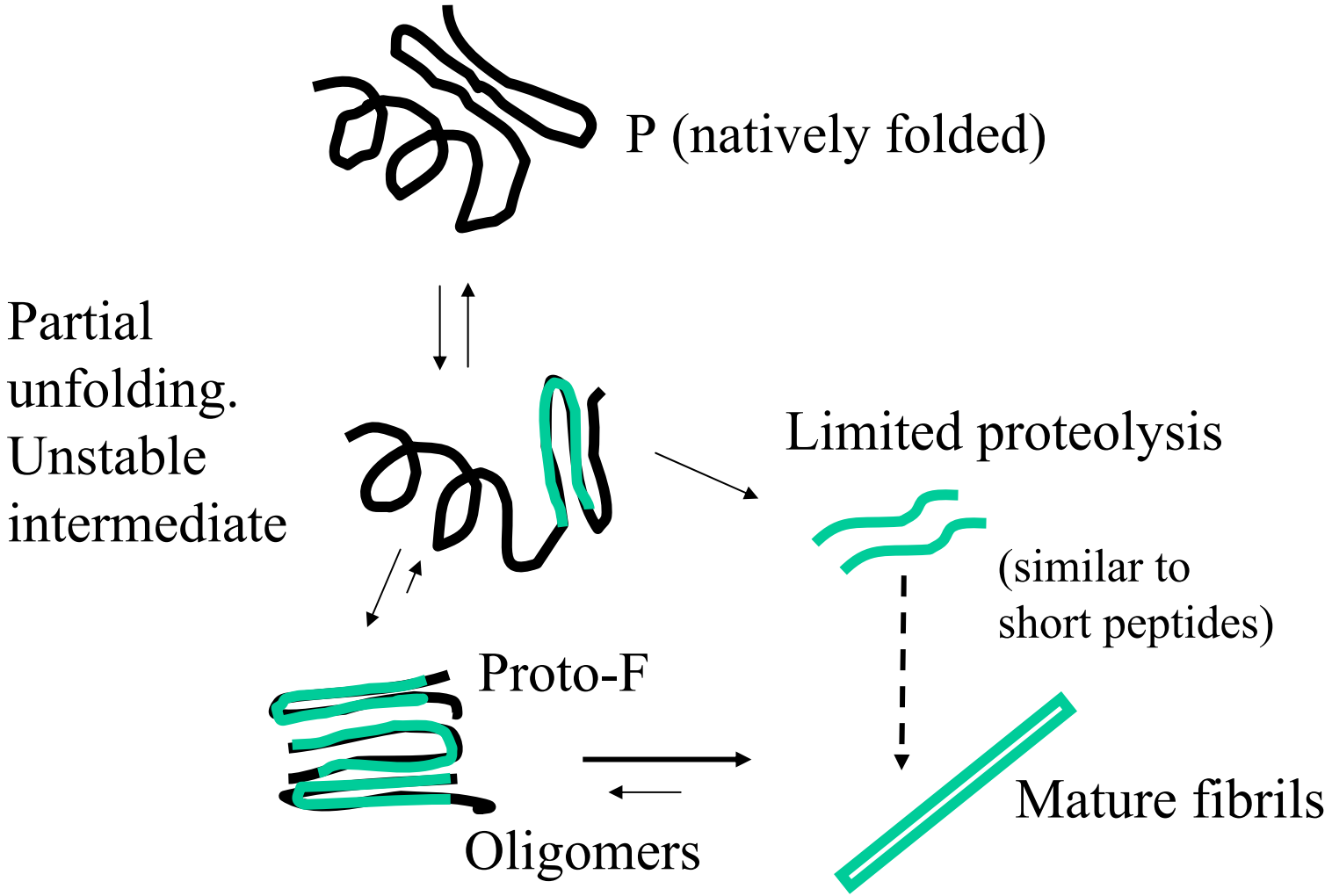
Amyloid formation involves a conformational change followed by self-assembly



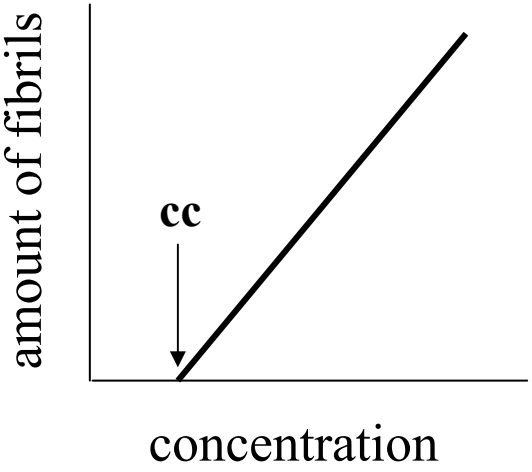
Mechanisms involved in amyloid formation *in vitro:*
a) Short peptides

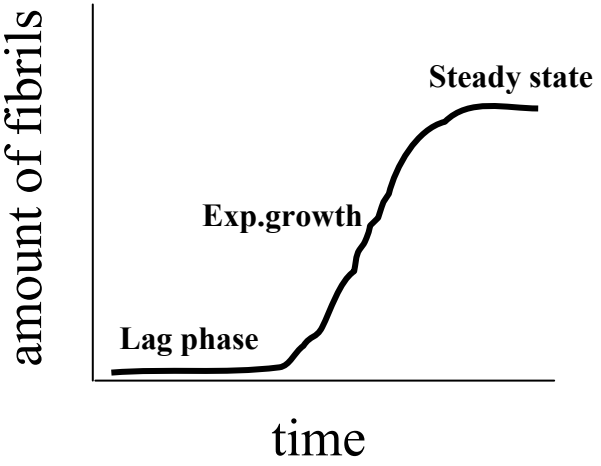


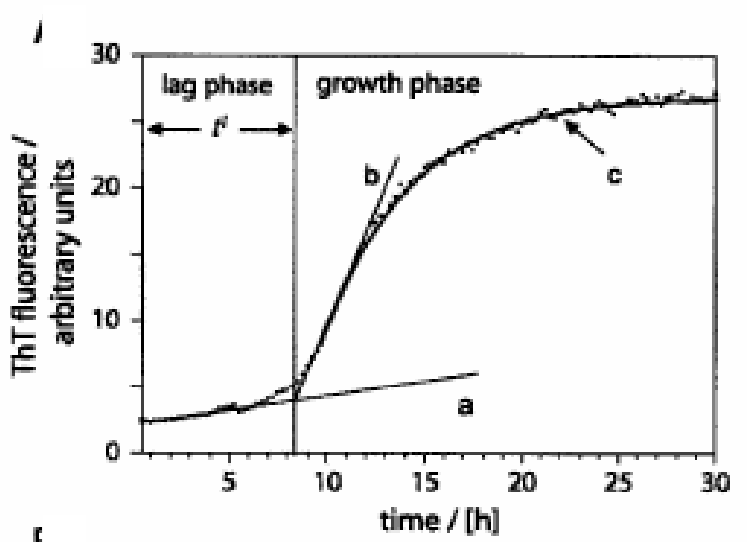
Mechanisms involved in amyloid formation *in vitro:*
b) Folded proteins

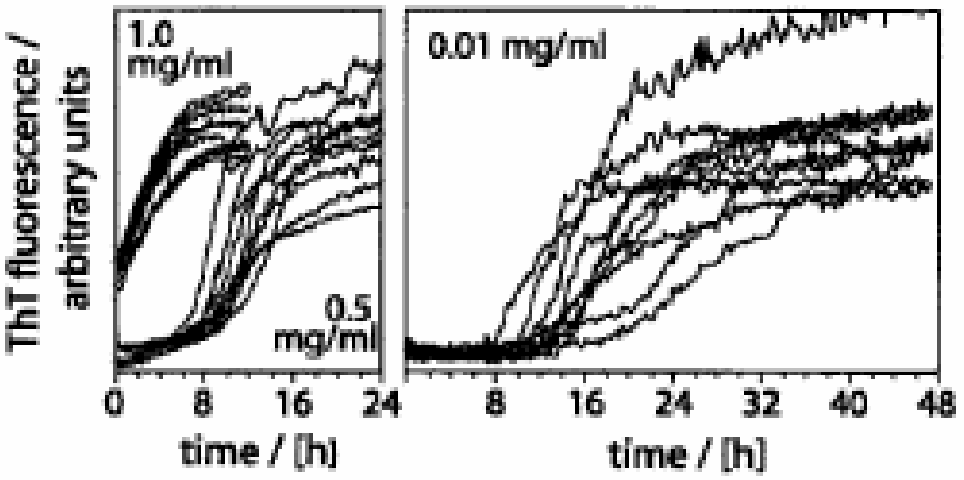


Nucleation-dependent kinetics of amyloid formation

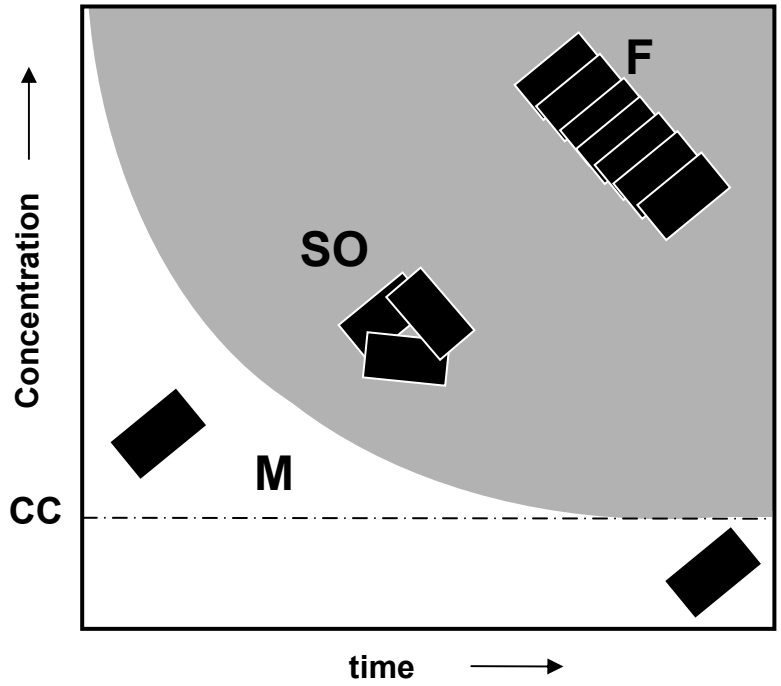








Nucleation kinetic of amyloid formation: concentration, cc and time



Factors that determine amyloid formation *in vivo*

1) Intrinsic property of the amyloid precursor (primary structure)

2) Concentration

3) Interactions (ions, proteins, polysacharides)

Primary structure of amyloid precursor

1) Species-specific (i.e. amylin)

2) Normal polymorphisms (i.e. Ig)

3) Mutations: autosomal dominant familial disease

Primary structure of amyloid precursor: Species-specific

Key residues determine amyloidogenicity of pancreatic diabetes-associated amylin in different species

```
          1                  20                37
Human     KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY
Monkey    -----------------R------T---------D--
Cat       ----------------IR----L-----P--------
Rat       -----------------R----L-PV-PP--------
Mouse     -----------------R----L-PV-PP--------
Degu      ------------T----R--H-L--A-PP-K------
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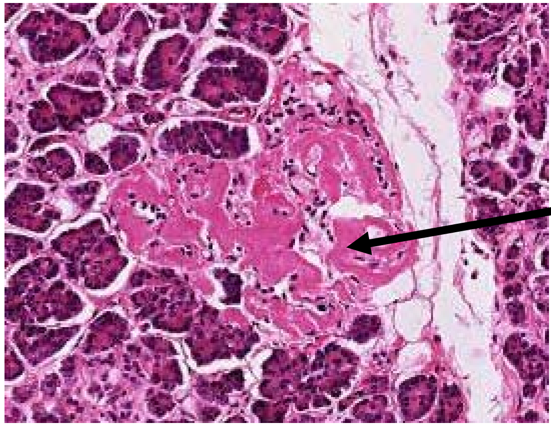
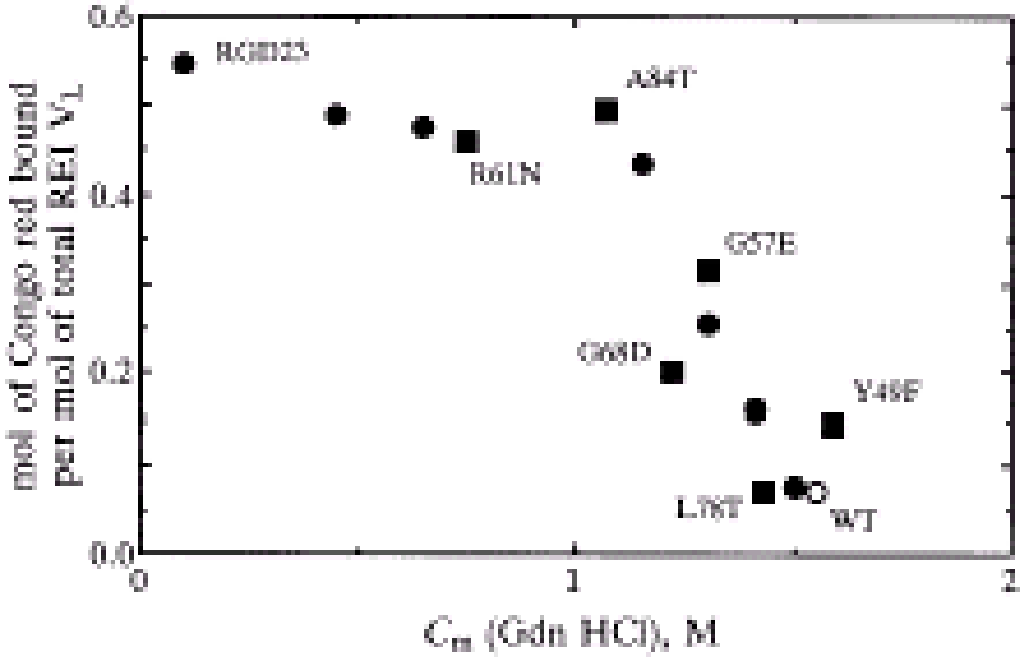
Unable to form amyloid



Pancreatic amylin deposits in Type II Diabetes Mellitus

Primary structure of amyloid precursor:normal polymorphisms

destabilizing replacements in variable regions of human Ig



Primary structure of amyloid precursor: mutations

MUTATIONS

Outside amyloid sequence

*Processing (APP>Aβ)

*Intronic (ARN splicing)

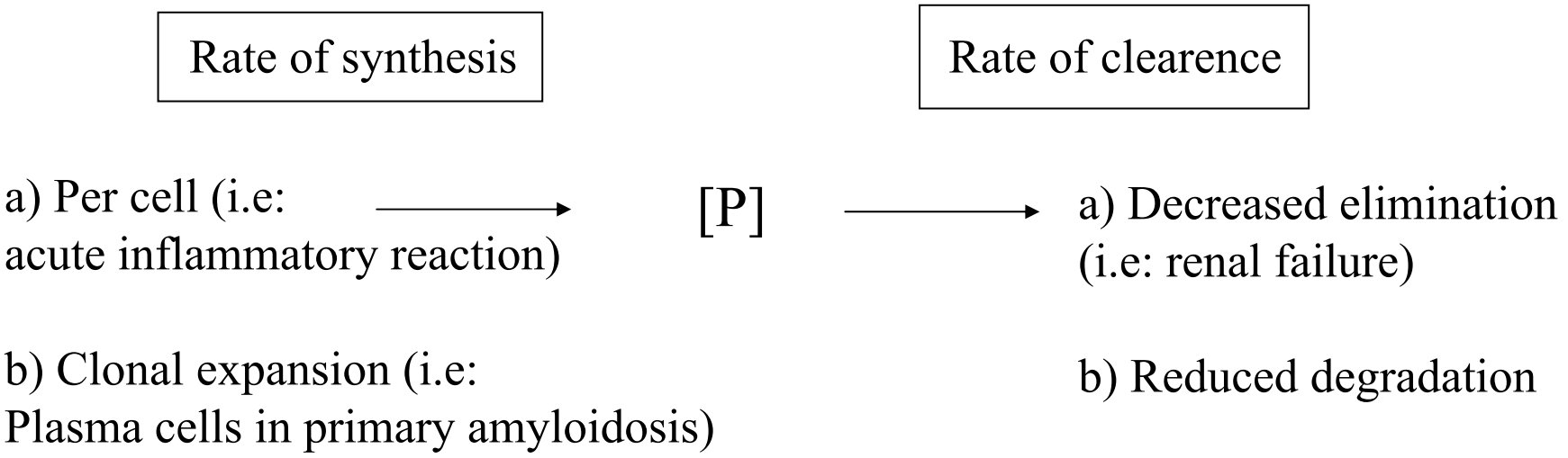
Within amyloid sequence

* Destabilization of native folding

*Charge effect

*Hydrophobicity

Steady-state concentration of amyloid precursors



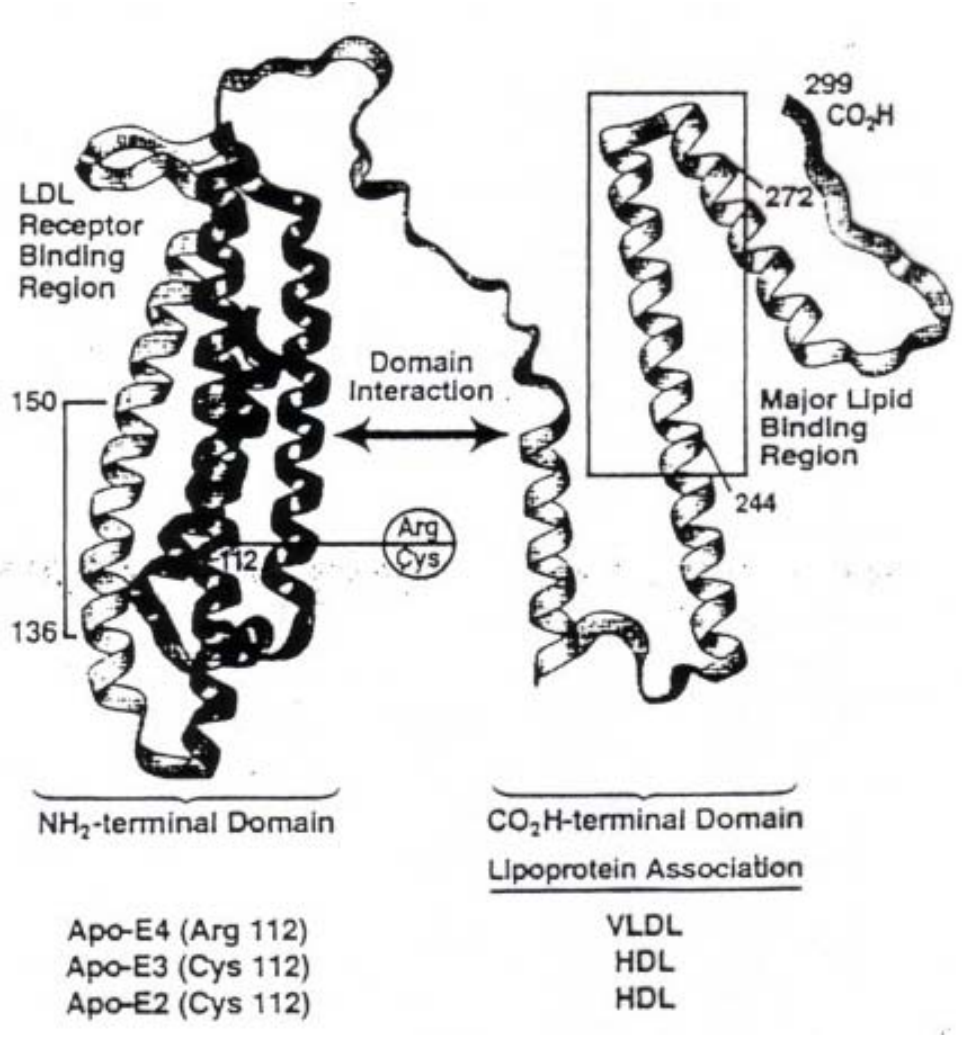
Interactions of amyloid precursor

a) With other proteins (i.e: apolipoprotein E, amyloid P component)

b) With glycosaminoglycans (i.e: heparan-sulfate)

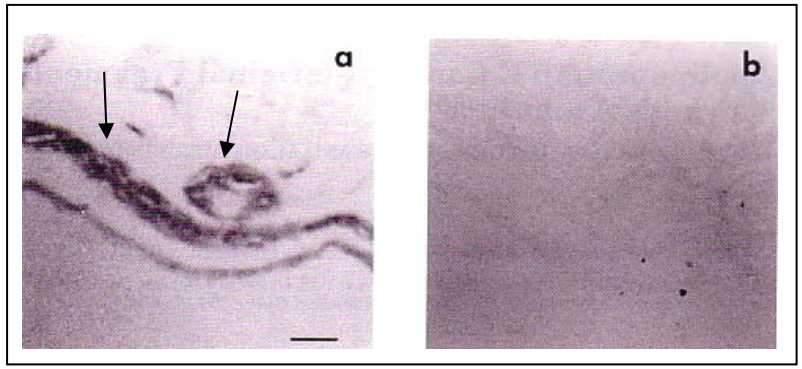
c) With metal ions (i.e; zinc)

Amyloid-associated proteins:Apolipoprotein E



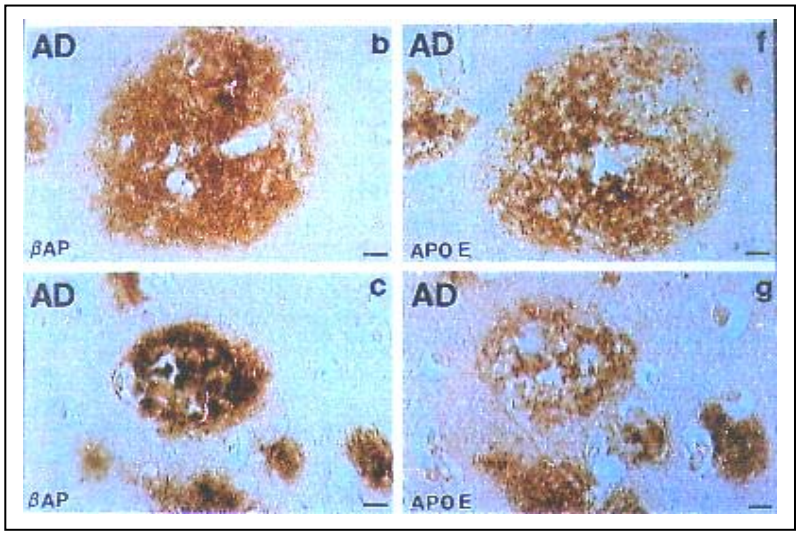
ApoE is co-deposited with all types of amyloid proteins

Anti-apoE



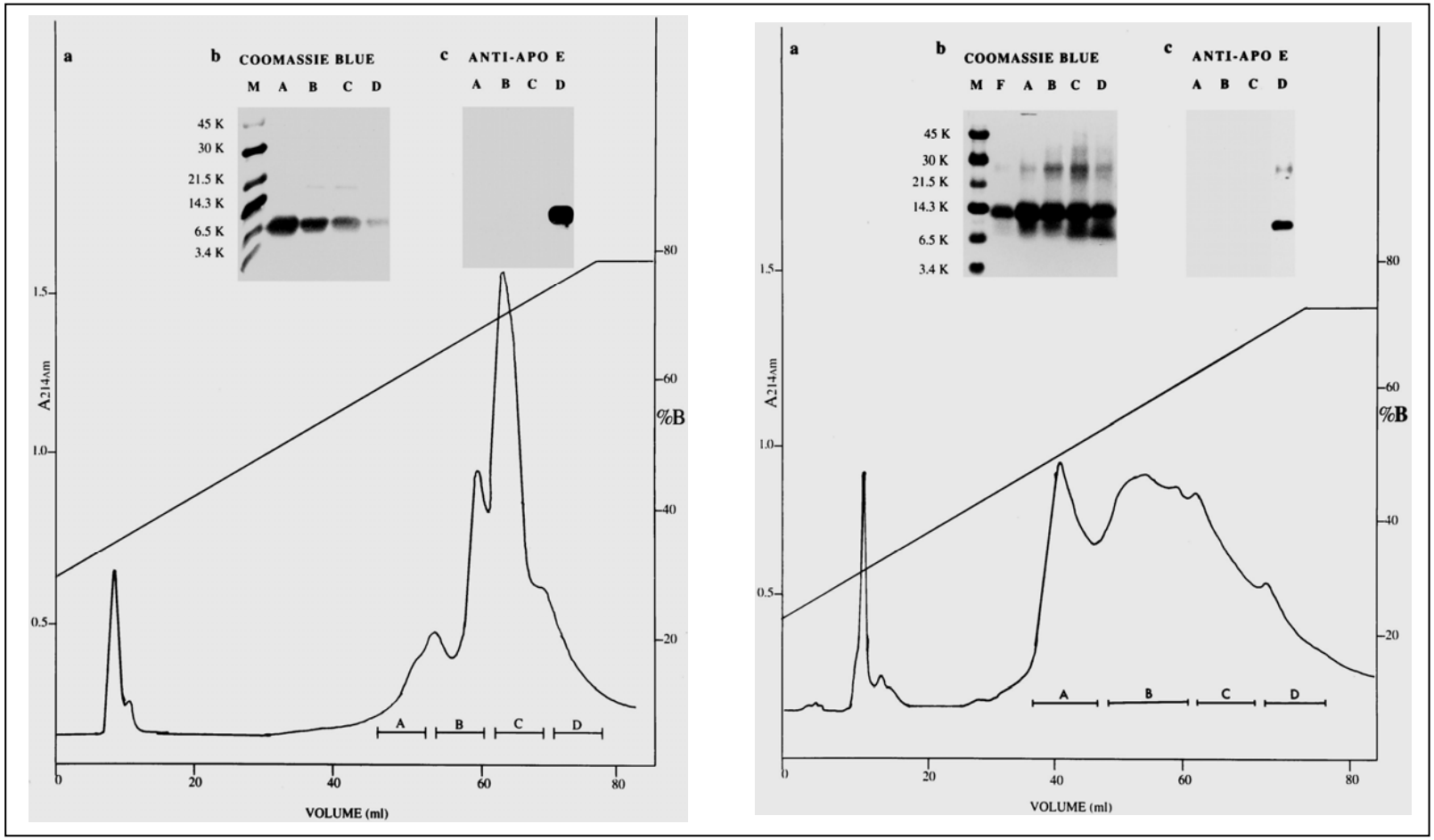
AL spleen Normal spleen

Anti-Aβ Anti-apoE



Alzheimer's disease brain

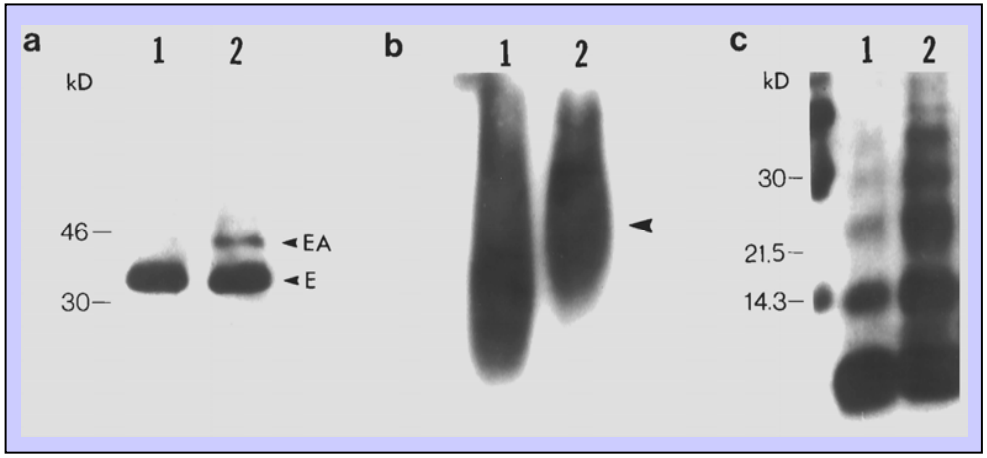
ApoE lipid-binding domain is associated with amyloids

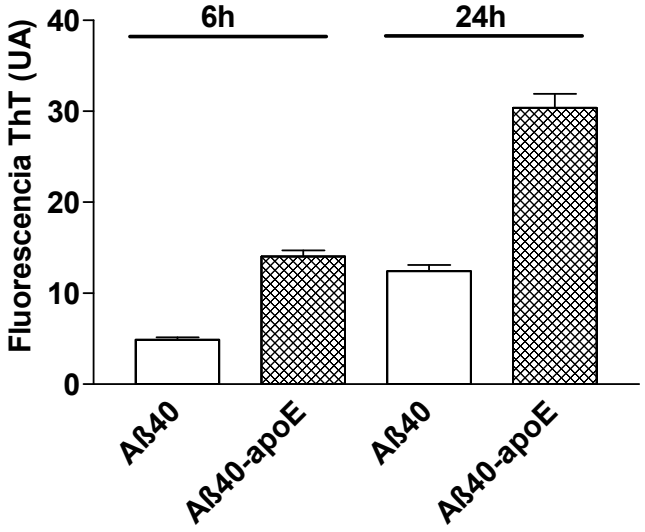


Amyloid A

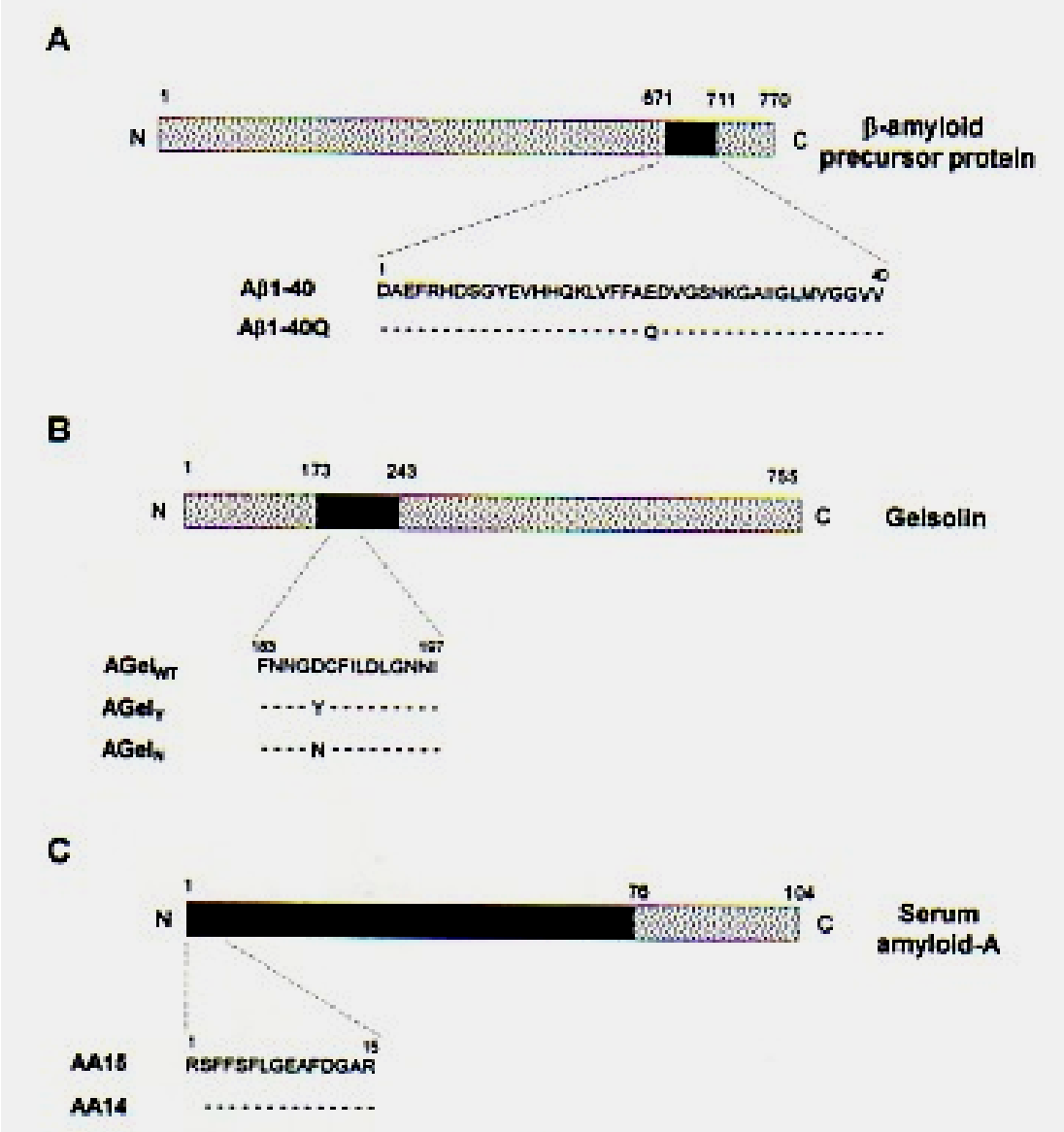
Amyloid L

ApoE promotes the aggregation of amyloid proteins





ApoE incubation with different amyloid peptides



\+ ApoE

↓

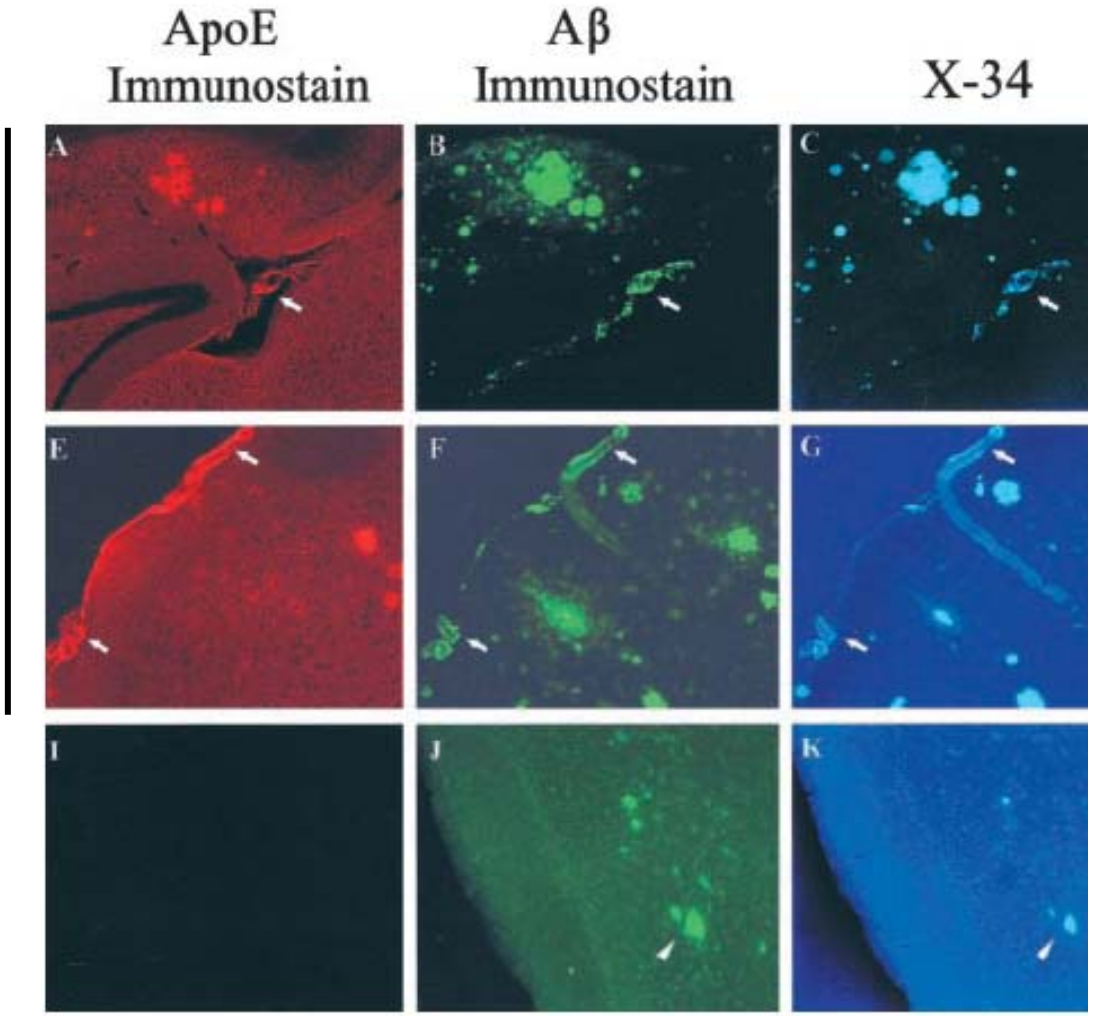
ThT and EM

ApoE increases the fibrillogenesis of different amyloids

Table 1
Effect of human plasma apoE on amyloid formation by Agel, AA and Aβ analogs

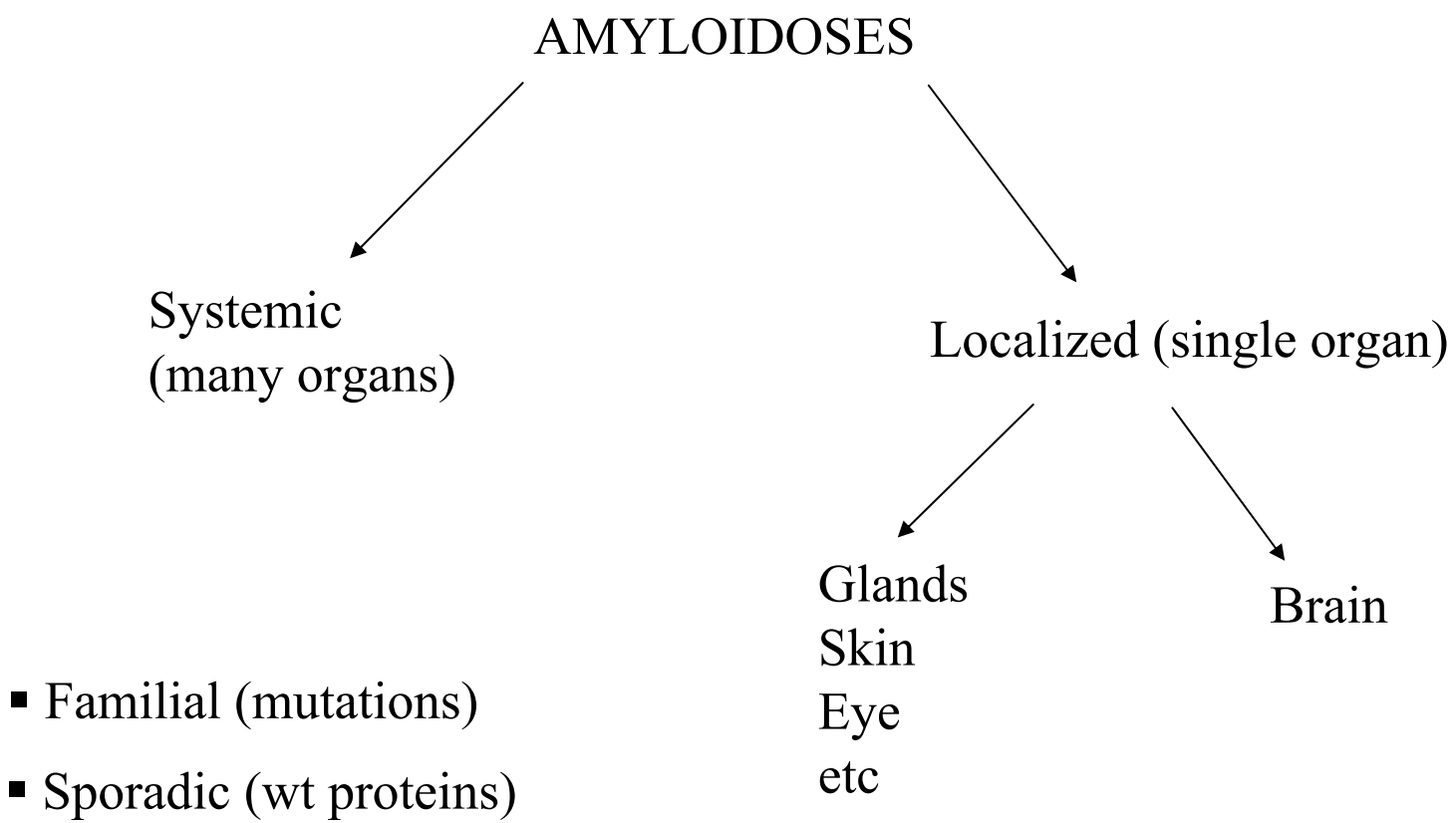
Peptide	ThT-fluorescence		apoE[b] enhancement
	Peptide alone	Peptide with apoE	
AGel183–197^{WT}	3.6 ± 0.2	7.7 ± 0.4	2.14
AGel183–197^{Y}	1.6 ± 0.3	13.9 ± 2.3	8.69
AGel183–197^{N}	17.6 ± 1.9	22.2 ± 2.2	1.26
Gel165–182	0.53 ± 0.1	0.58 ± 0.05	1.09
AA14	2.9 ± 0.6	16.9 ± 3.7	5.83
AA15	14.1 ± 0.7	14.3 ± 1.5	1.01
Aβ1–40[a]	15.2 ± 1.1	33.8 ± 2.9	2.22
Aβ1–40Q[a]	33.8 ± 0.4	38.1 ± 2.5	1.13
Aβ1–16	0.39 ± 0.04	0.34 ± 0.07	0.87

Cerebrovascular amyloidosis in APPswe transgenic mice: effect of apoE



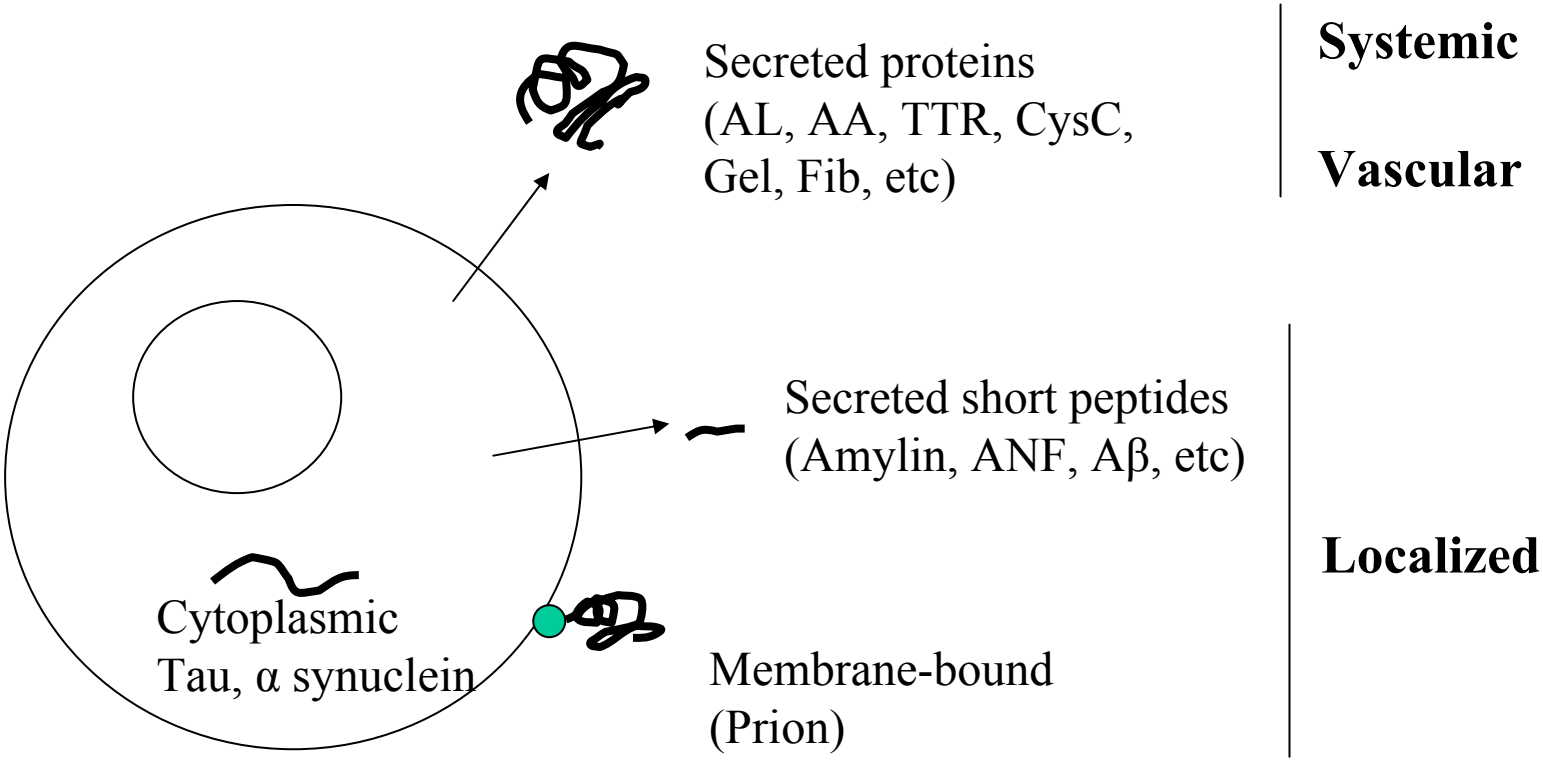
Fryer JD.et al J Neurosci 2003; 23: 7889

Amyloid proteins and human disease: classification



- Familial (mutations)
- Sporadic (wt proteins)

A variety of intra and extracellular proteins/peptides may form amyloid deposits



Human Amyloidoses: classification (systemic)

Amyloid	Precursor	Type/variant	Clinical/ affected organs
AL	Ig light chains	λ / κ	Primary, myeloma, *localized*
AH	Ig heavy chains	IgG1	Primary, myeloma
AA	SAA apo	SAA_{1-2}	Secondary, FMF
Aβ2M	B2 microglobulin	wt	Chronic hemodialysis-bones
AFib	Fibrinogen (α chain)	R554L, E526V, Deletions	Renal familial amyloidosis
ATTR	Transthyretin	>70 mutants TTRwt	FAP type I-II Senile cardiac amyloidosis
AApoAI	Apolipoprotein AI	9 mutants	FAP type III
AApoAII	Apolipoprotein AII	P5Q, Stop78G,S	Renal familial amyloidosis
ALys	Lysozyme	I56T, D67H	Renal familial amyloidosis
AGel	Gelsolin	D187N/ T	FAP type IV
Medin	Lactadherin	wt	Aortic amyloidosis

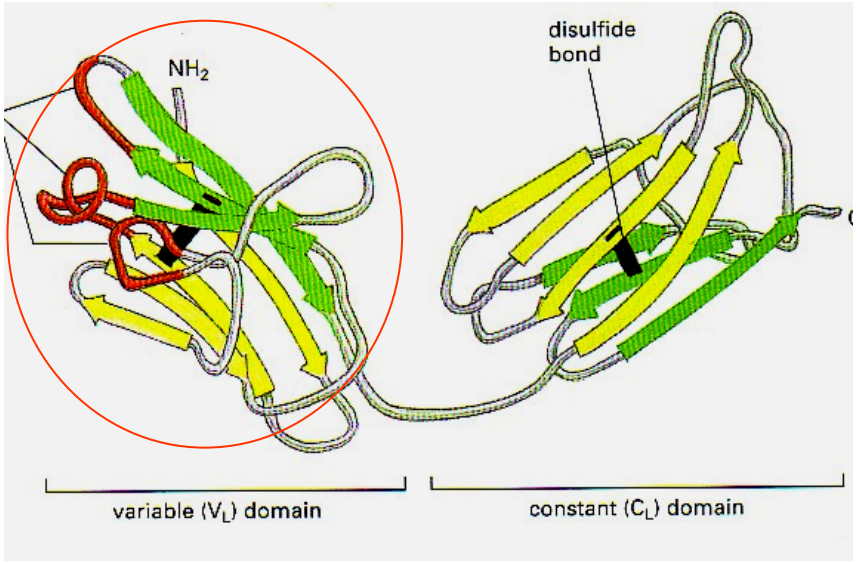
Human Amyloidosis: classification (localized)

Amyloid	Precursor	Type/variant	Clinical/affected organ
ACal	Procalcitonin	wt	Medullar thyroid carcinoma
AIAPP	Amylin	wt	B-islet cells/ Diabetes type II
APro	Prolactin	wt	Prolactinoma/ senile pituitary
AIns	Insulin	wt	Sites of injection
AANF	Atrial natriuretic peptide	wt	Atrial amyloidosis
ALac	Lactoferrin	wt	Corneal familial amyloidosis

Human Amyloidosis: CNS

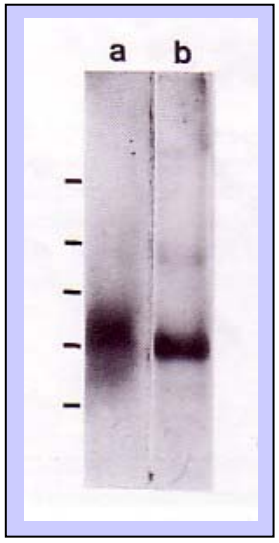
ACys	Cystatin C	L68Q	Familial amyloid angiopathy (Icelandic)
APrP	Prion protein	wt-mutants	Spongiform encephalopaties
ASyn	Alpha-synuclein	wt-mutants	Parkinson Disease
Aβ	APP	wt- >30 mutants	Alzheimer, Down, SCA, Familial amyloid angiopathies
Tau	P-tau	mutants	Frontotemporal dementia
ABri	BRI	R267stop	Familial British Dementia
ADan	BRI	795 ins	Familial Danish Dementia

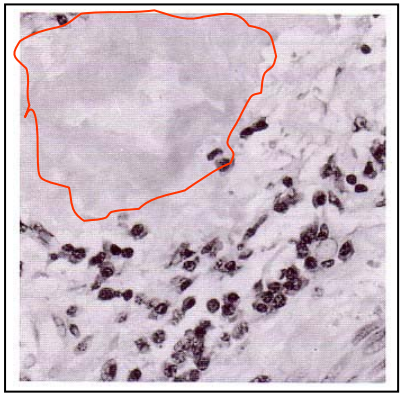
Immunoglobulin light-chain amyloidosis (AL)



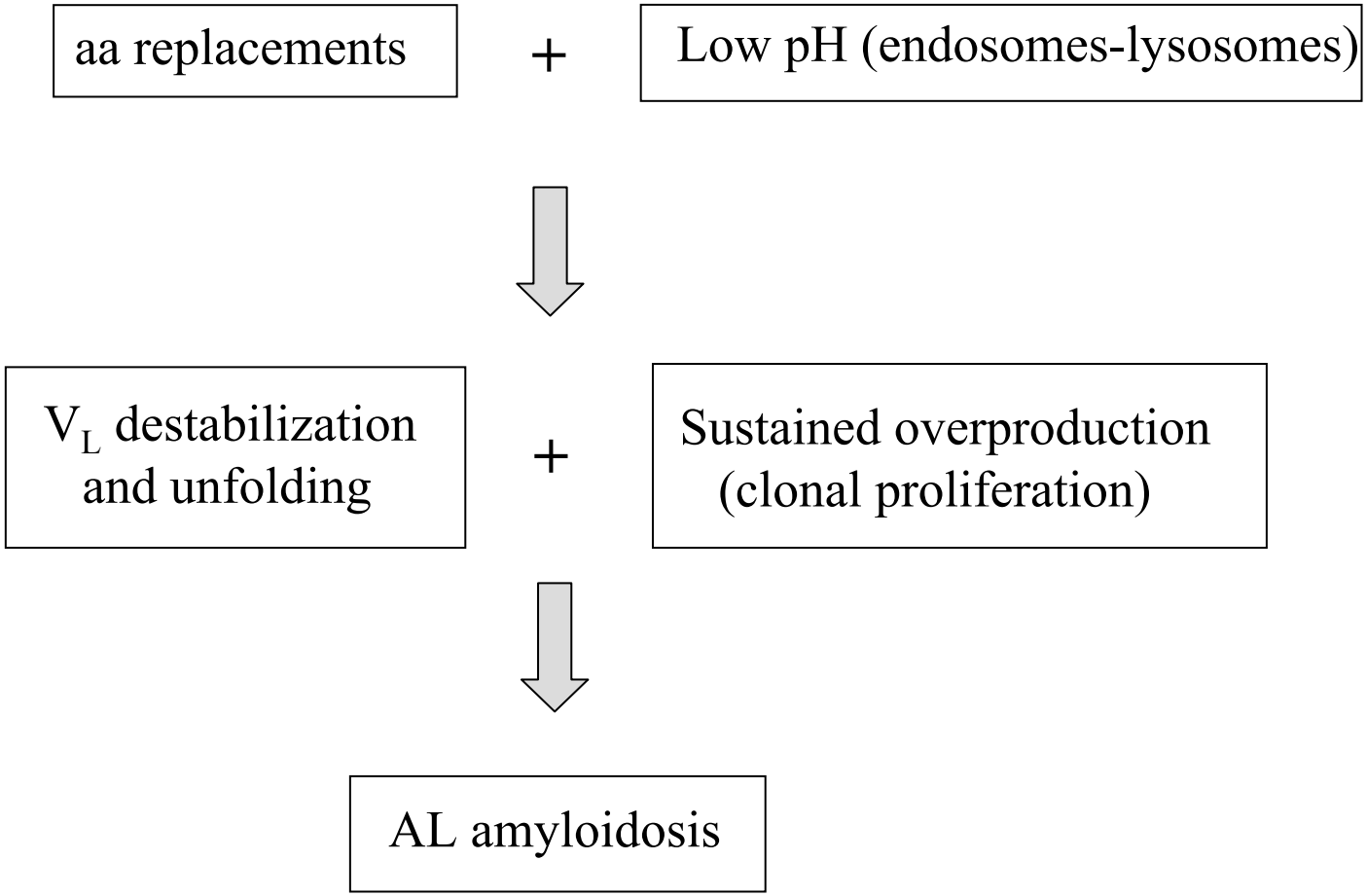
1) Systemic ("primary")
Kidney, spleen, liver, tongue, skin, peripheral nerves. First amyloid sequenced (Glenner,G. 1971)
In 90% monoclonal component can be detected

2) Localized (amyloidomas)
Ureter, lungs, skin, CNS
Local monoclonal production

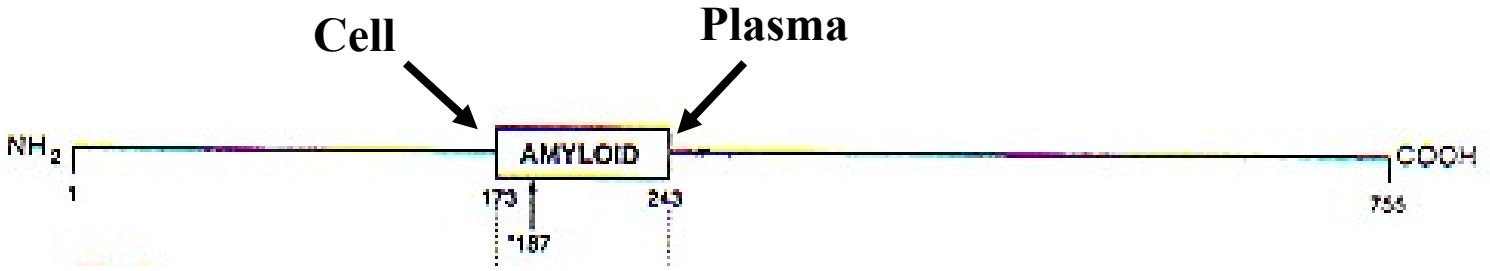




AL: a typical example of multiple factors in amyloid pathogenesis



Gelsolin amyloidosis: destabilizing mutations and proteolytic release of amyloidogenic peptide



93 kDa gelsolin

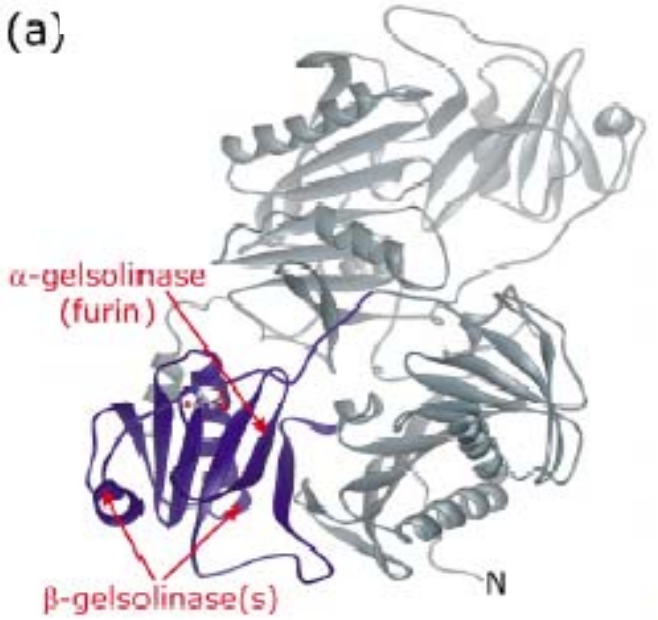
D187N
D187Y

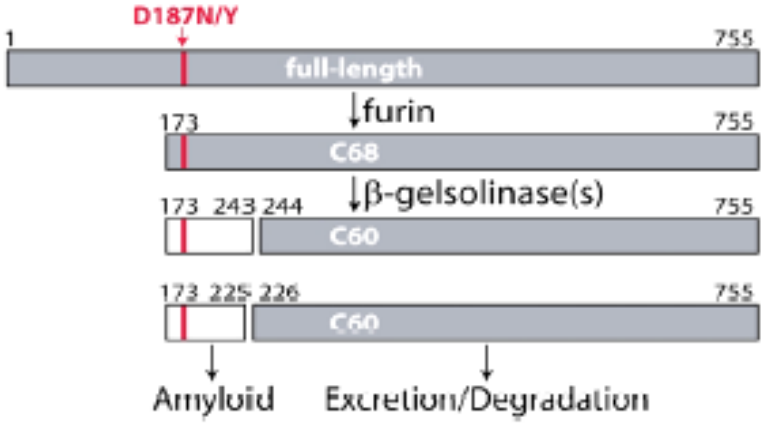
internal proteolysis

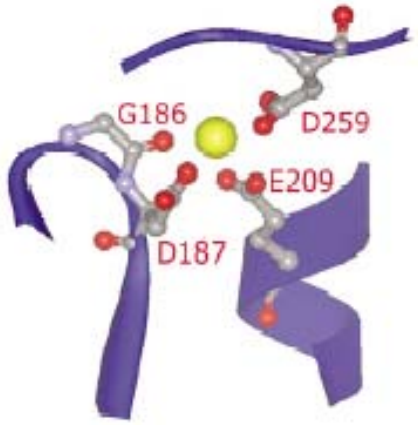
"Finnish Amyloidosis"

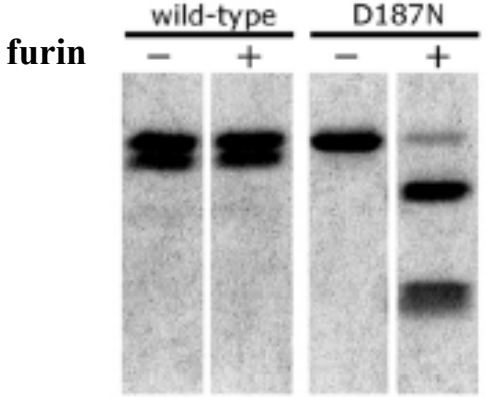
8 kDa AGel
Deposition
(cornea, skin,
nerves, kidney)

Loss of Ca^{2+} binding in Gel D187N increases susceptibility to proteolysis



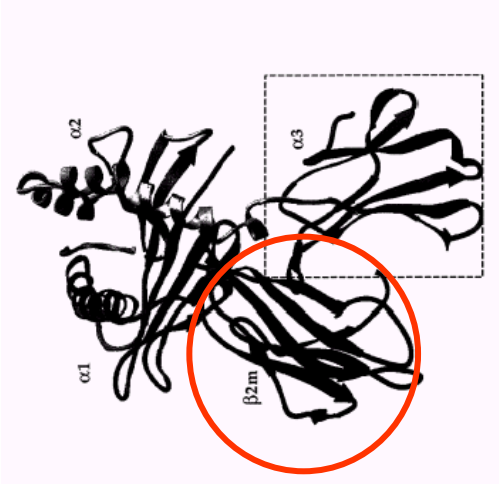






β2 microglobulin: chronic renal failure amyloidosis

MHC class I



CRI
Long-term dialysis

High levels of β2M in plasma

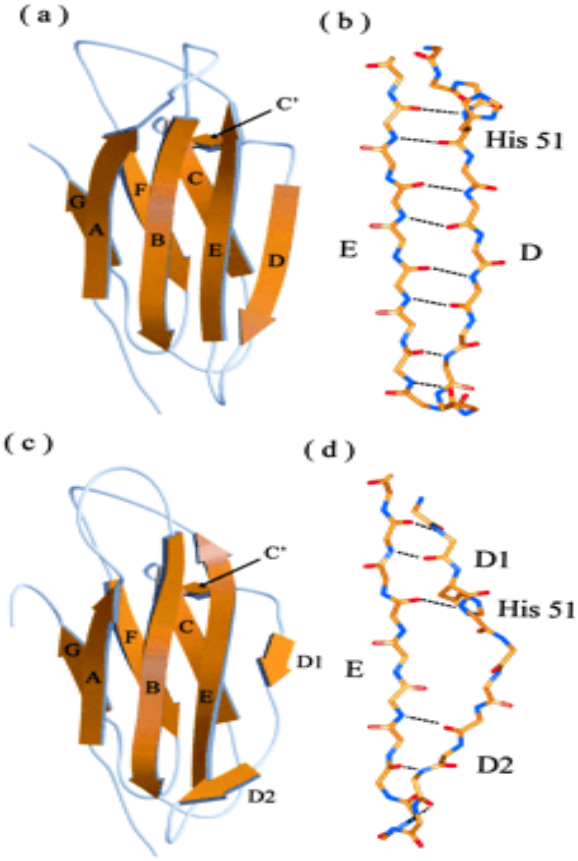
(60-fold)

Deposition of intact 10 kDa β2M in bones and synovial membranes

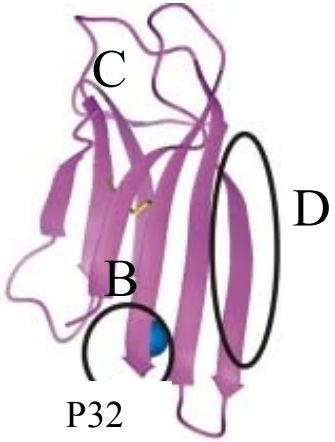
β2M removal by affinity matrix (plasmapheresis)

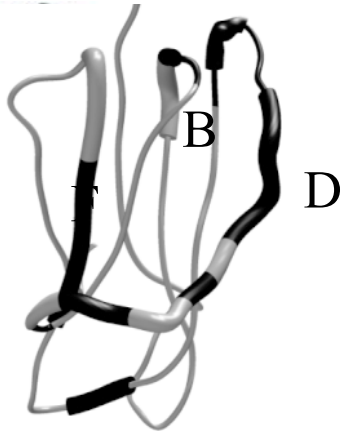
β2M: defective clearance + conformational changes as key pathogenic mechanisms

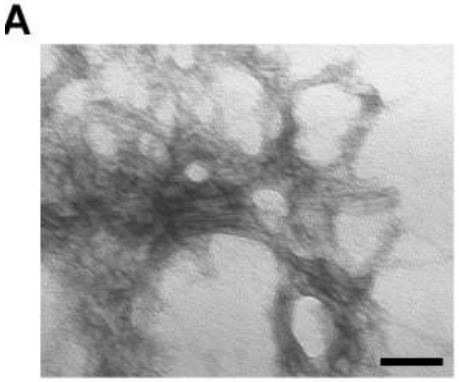
Free β2Mm

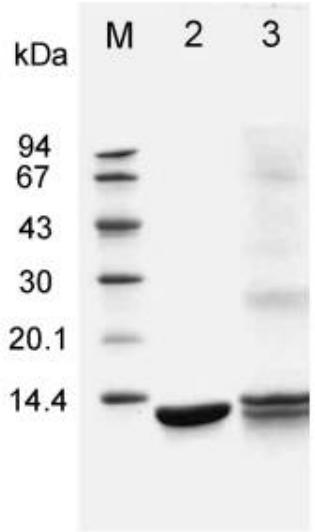


Bound to α1MHC









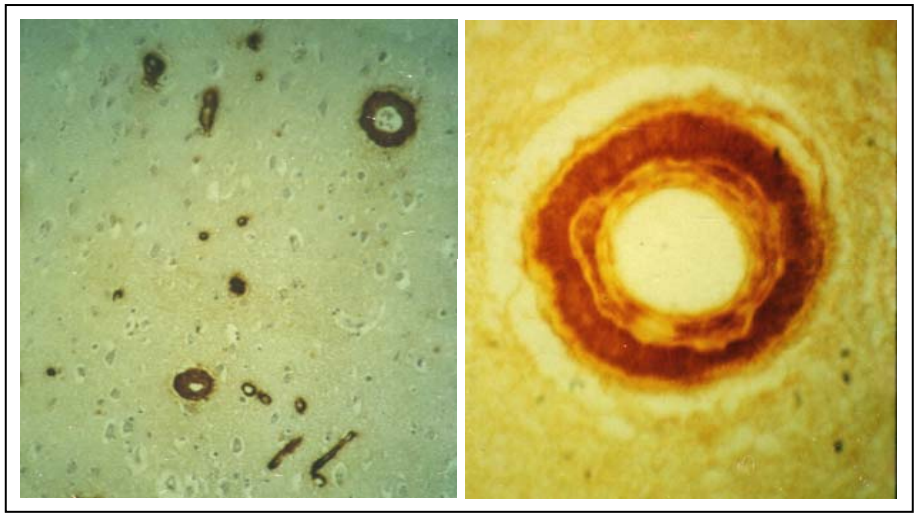
Hereditary cerebral hemorrhage with amyloidosis, Dutch type (HCHWA-D)

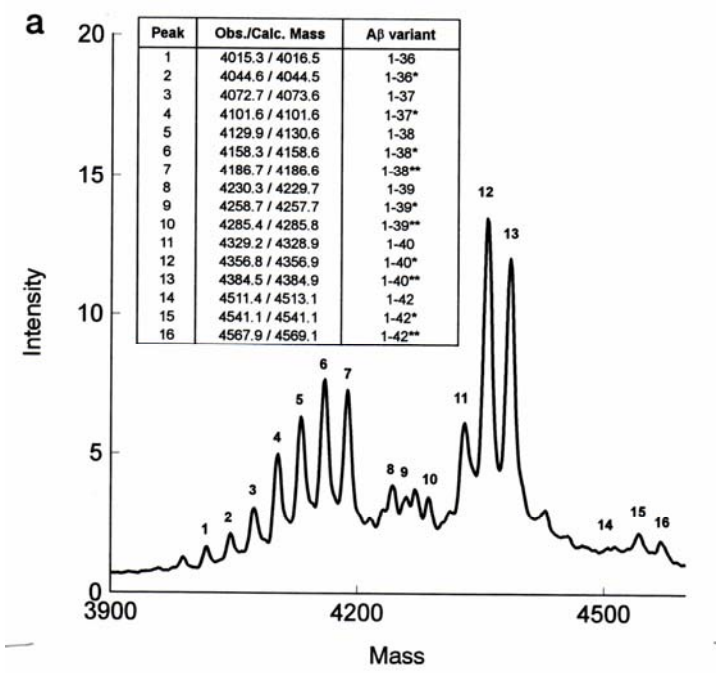
1) Autosomal dominant, high penetrance
2) Stroke at 50-55 years
3) First missense mutation in AβPP gene: substitution of E22 for Q

Aβ

$_{1}$DAEFRHDSGYEVHHQK LVFFAEDVGSNKGAIIGLMVGGVV$_{40}$

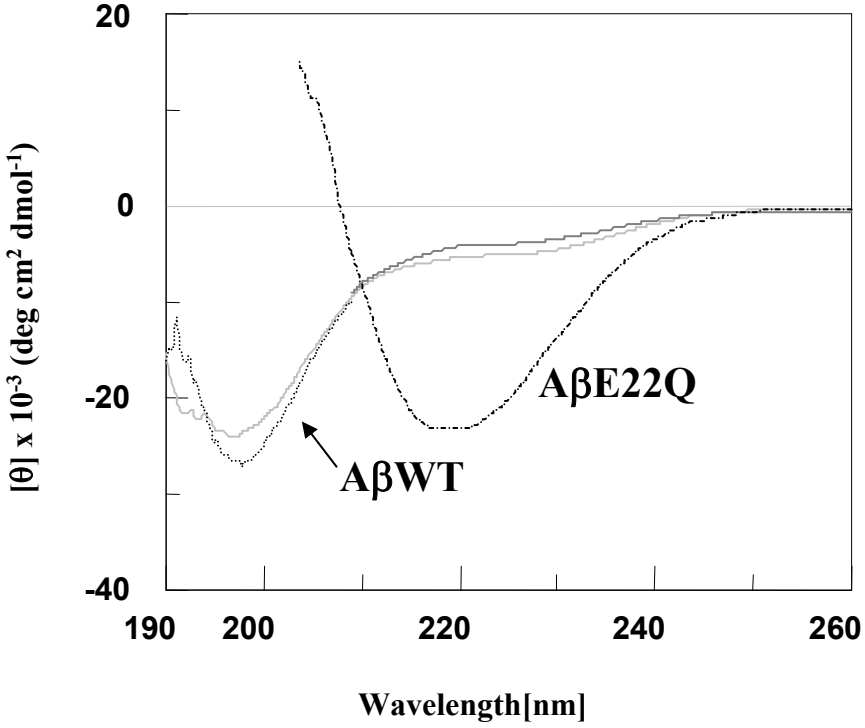
Q

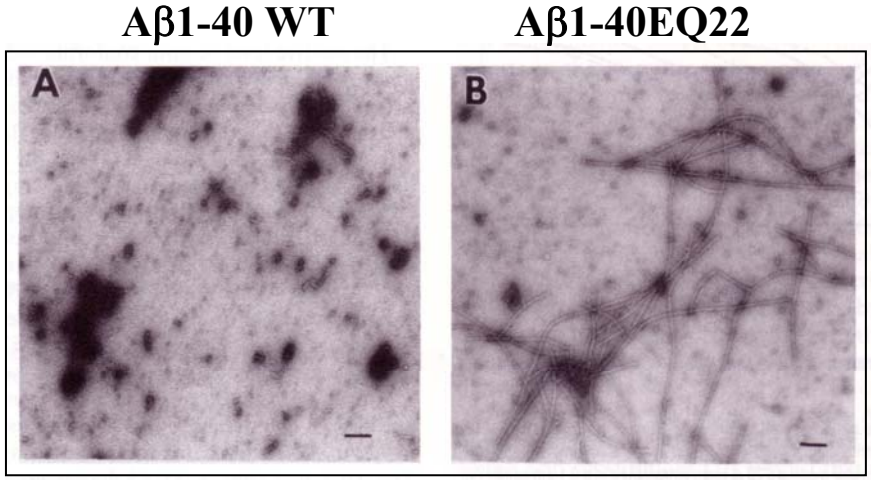


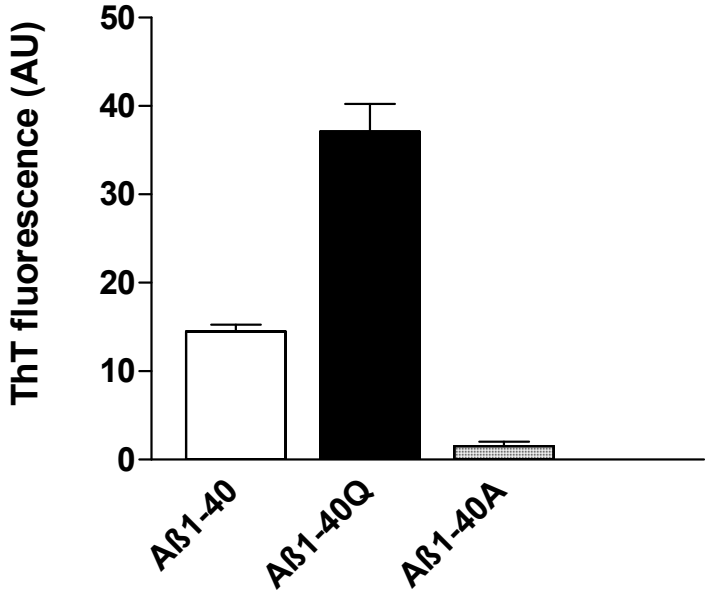


Peak	Obs./Calc. Mass	Aβ variant
1	4015.3 / 4016.5	1-36
2	4044.6 / 4044.5	1-36*
3	4072.7 / 4073.6	1-37
4	4101.6 / 4101.6	1-37*
5	4129.9 / 4130.6	1-38
6	4158.3 / 4158.6	1-38*
7	4186.7 / 4186.6	1-38**
8	4230.3 / 4229.7	1-39
9	4258.7 / 4257.7	1-39*
10	4285.4 / 4285.8	1-39**
11	4329.2 / 4328.9	1-40
12	4356.8 / 4356.9	1-40*
13	4384.5 / 4384.9	1-40**
14	4511.4 / 4513.1	1-42
15	4541.1 / 4541.1	1-42*
16	4567.9 / 4569.1	1-42**

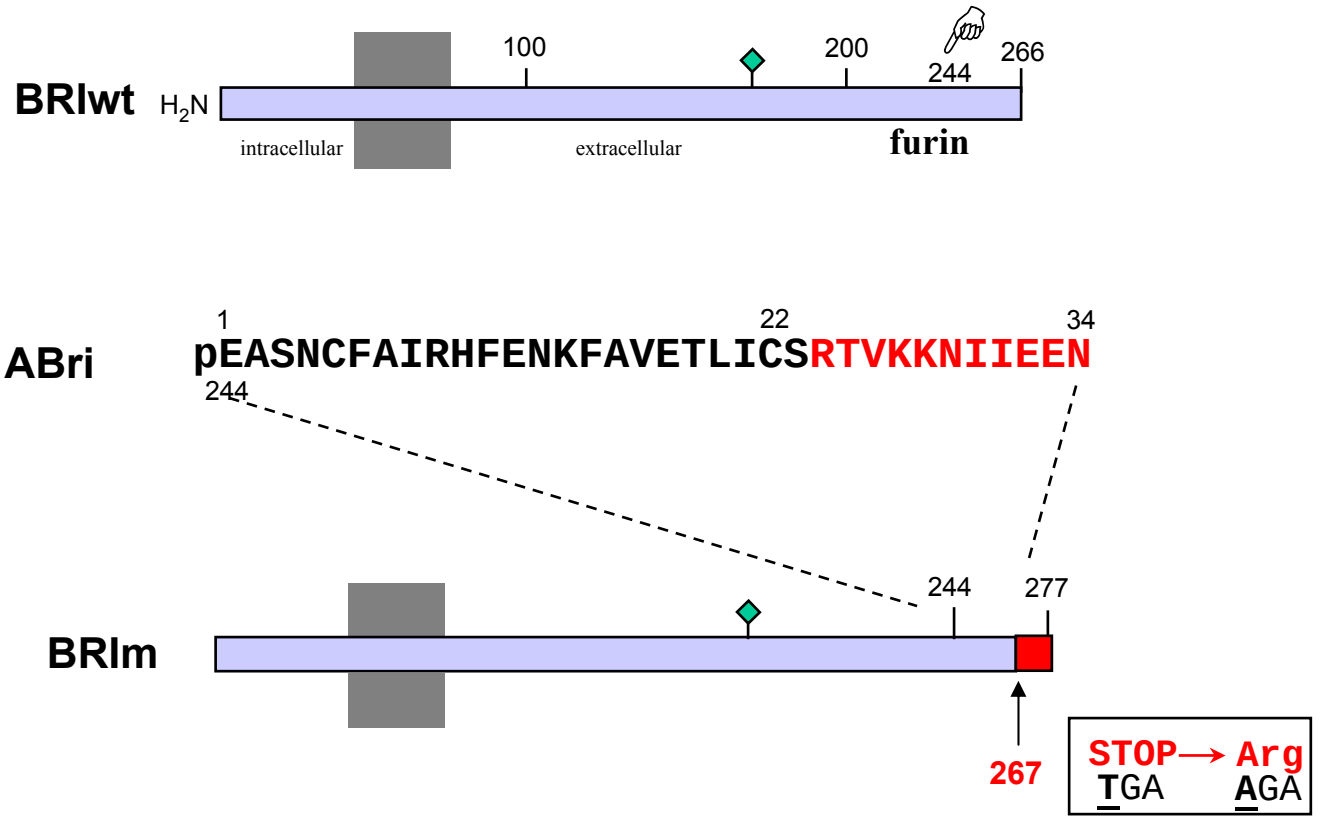
Effect of E22Q substitution on Aβ ordered aggregation







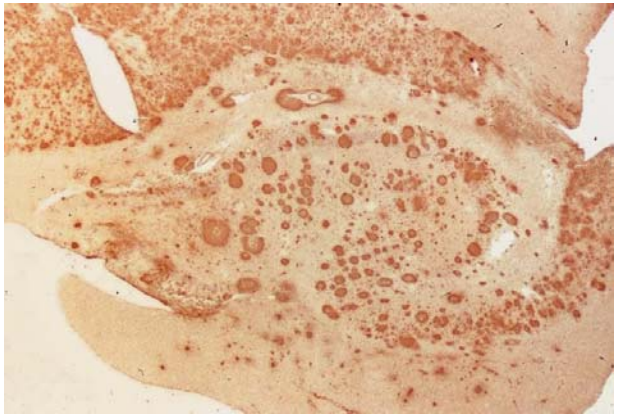
Familial British Dementia



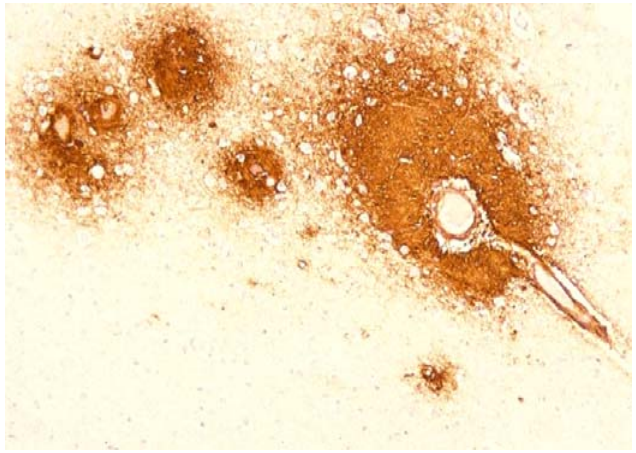
Familial British Dementia: plaques, vascular amyloid deposits and neuronal degeneration

Anti-ABri

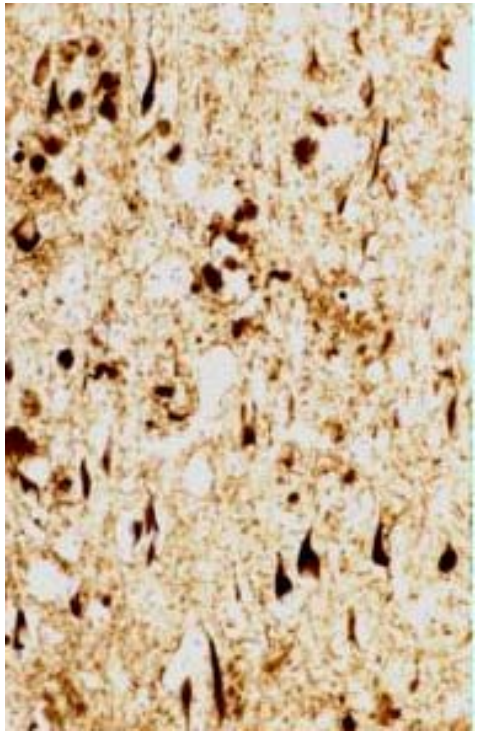
Hippocampal plaques



Vascular deposits



Neurofibrilar degeneration



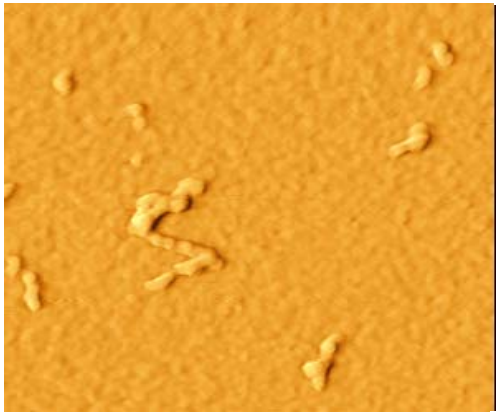
Pathogenic effect of amyloid proteins

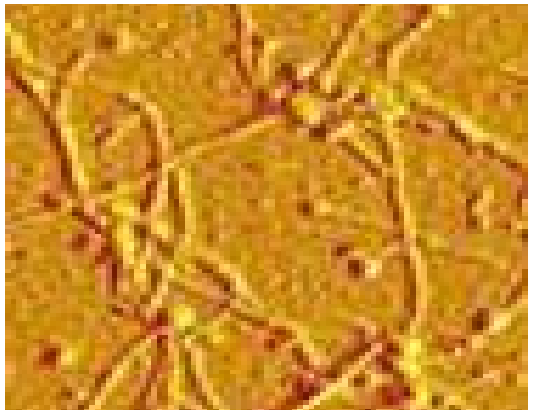
① Progressive accumulation of fibrils ("bulk" effect): AL, AA, AGel, etc

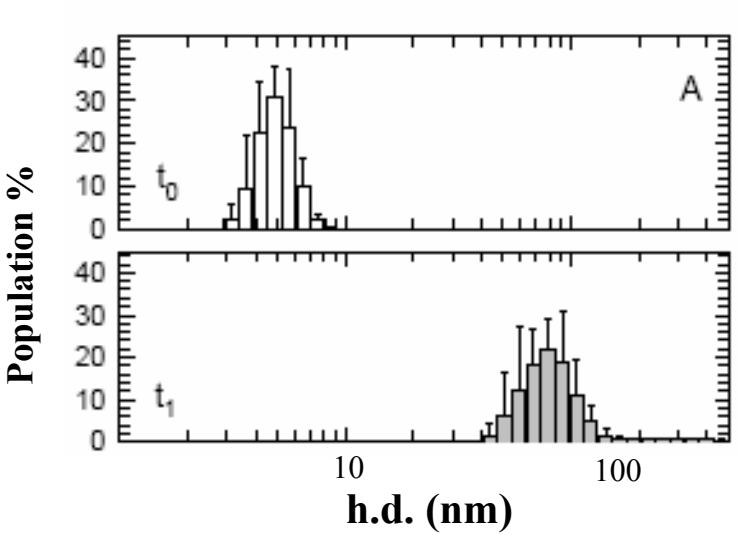
② Cellular toxicity: membrane damage, apoptosis, oxidative stress: the role of "soluble" oligomers

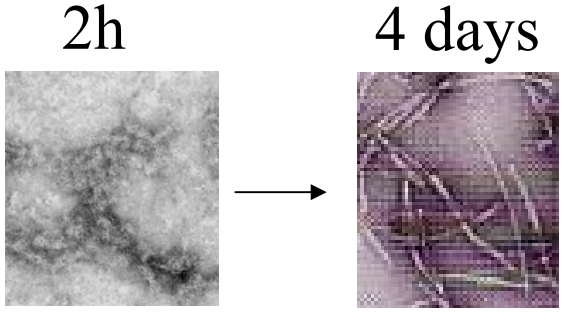
Amyloid proteins and neurotoxicity

Soluble Oligomers vs Amyloid fibrils

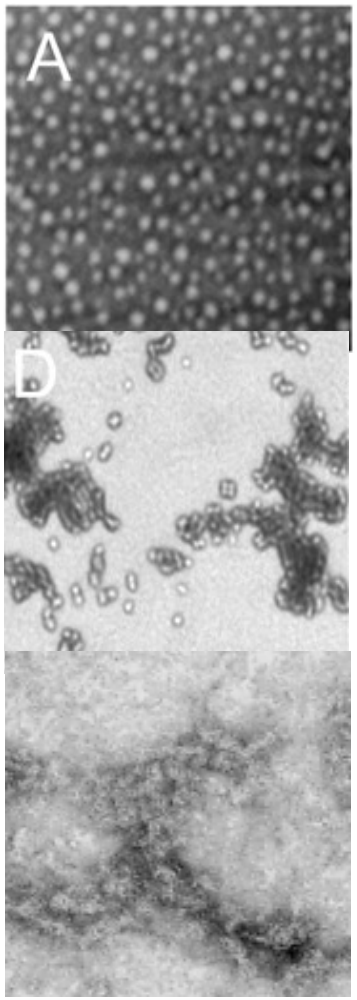


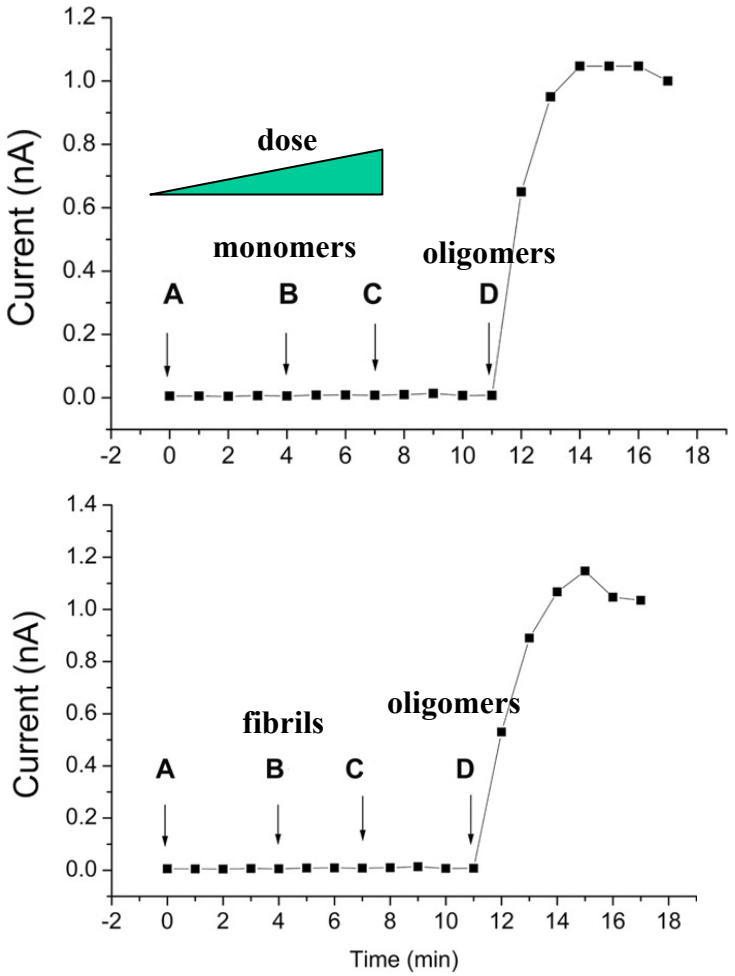




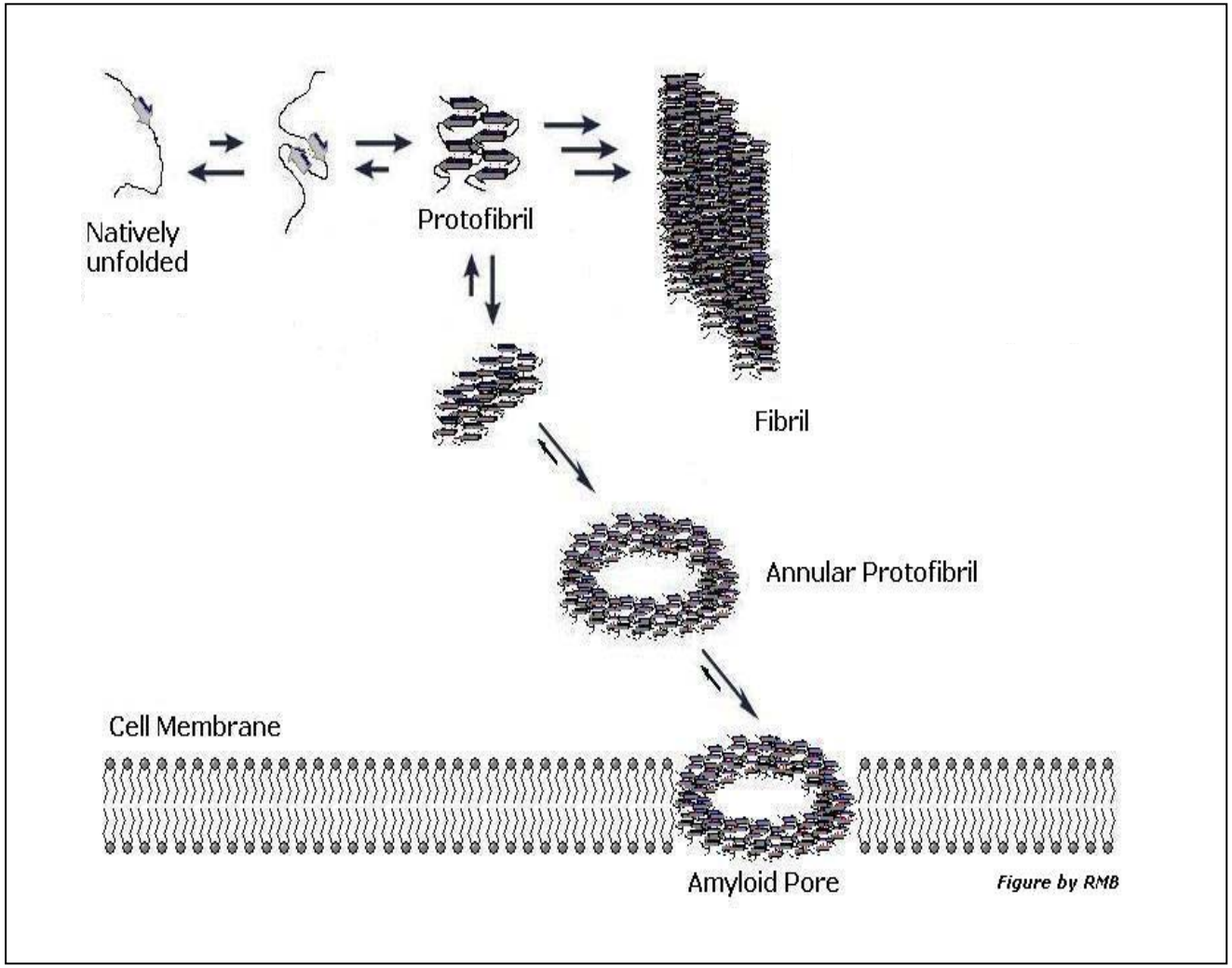


Soluble oligomers of amyloid precursors increase membrane conductivity





Membrane pore formation hypothesis

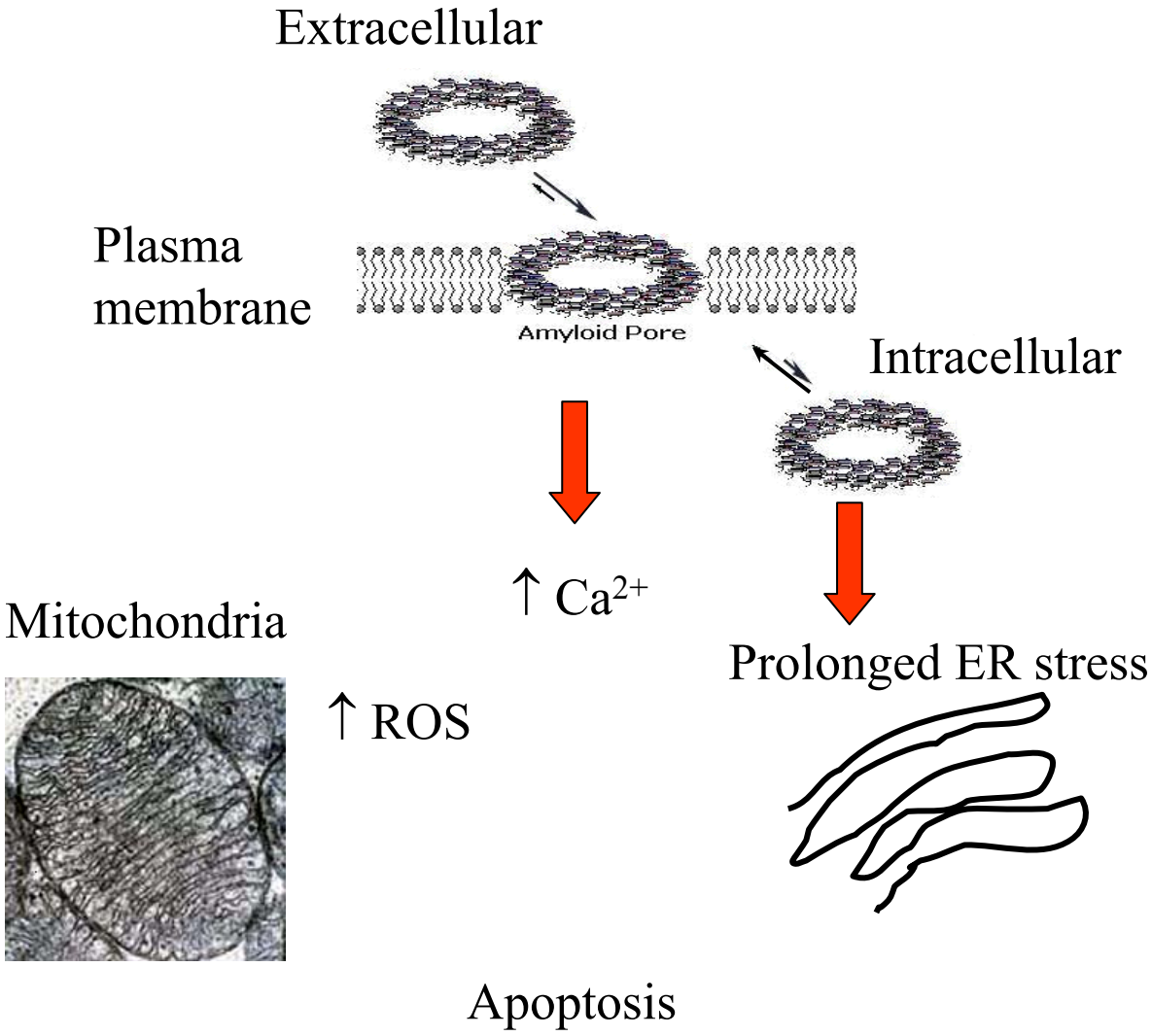


Amyloid proteins can form ion channels *in vitro*

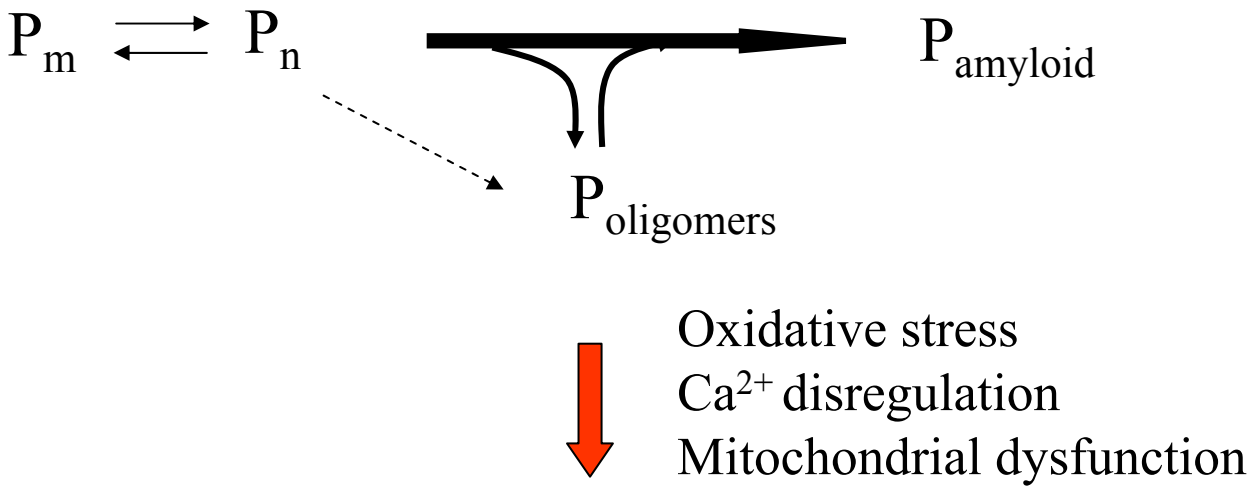
Table 1
Properties of channels formed by amyloid peptides

	Voltage-dependence	Single channel conductance (pS)	Ion selectivity (permeability ratio)	Blockade by zinc	Inhibition by Congo red	Reference
Aβ 25–35	Dependent[a]	10–400	Cation ($P_K/P_{Cl}=1.6$)	+	+	[34]
Aβ 1–40	Independent	10–2000	Cation ($P_K/P_{Cl}=1.8$)	+	N.D.	[20]
Aβ 1–40	Independent	50–4000	Cation ($P_K/P_{Cl}=11.1$)	+	N.D.	[1–5]
Aβ 1–42	Independent	10–2000	Cation ($P_K/P_{Cl}=1.8$)	+	+	[20]
Islet amyloid polypeptide (amylin)	Dependent[b]	7.5	Cation ($P_K/P_{Cl}=1.9$)	+	+	[37]
PrP 106–126	Independent	10–400	Cation ($P_K/P_{Cl}=2.5$)	+	+	[37]
PrP 106–126	Independent	Various	Cation (variable)	N.D.	N.D.	[29]
Serum amyloid	Independent	10–1000	Cation ($P_K/P_{Cl}=2.9$)	+	+	[20]
CT 105	Independent	120	Cation	+	+	Kim et al. (1999)
C-type natiuretic peptide	Independent		Cation ($P_K/P_{Cl}=4.6$)	+	+	[30,31]
β2-Microglobulin	Independent		Non-selective ($P_K/P_{Cl}=1.0$)	+	+	Hirakura and Kagan (2000)
Transthyretin	Independent		Cation (variable)	+	+	Azimov et al. (2001)

Common downstream toxicity of soluble oligomers



The protein misfolding-amyloid hypothesis in neurodegenerative diseases



Neuronal damage