The role of amyloid beta peptide variability toward progress of Alzheimer disease

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Brain Disorders and Therapeutics
London, August 26

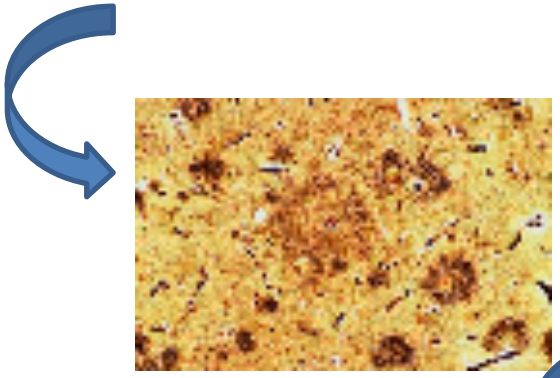
UNIVERSITY OF TWENTE.

ULB

VIB

Alzheimer's disease

Amyloid β peptide



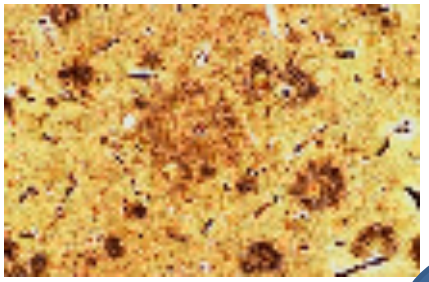
Alzheimer's disease

Amyloid β peptide

Genetic preposition

Neuroinflammation

Tau



Apolipoprotein E isoform

Mitochondrial dysfunction

Aging

Environmental factors

Hyperphosphorylated tau

The aggregation process of Aβ is characterized by intermediates that may have toxic activity

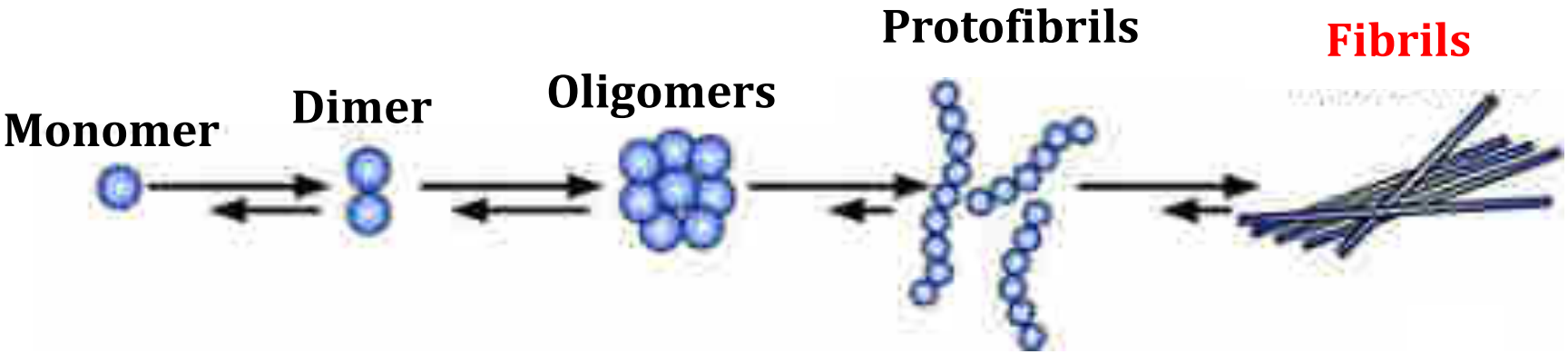


Figure adapted from Kumar *et al* *EMBO J (2011)*

The aggregation process of Aβ is characterized by intermediates that may have toxic activity

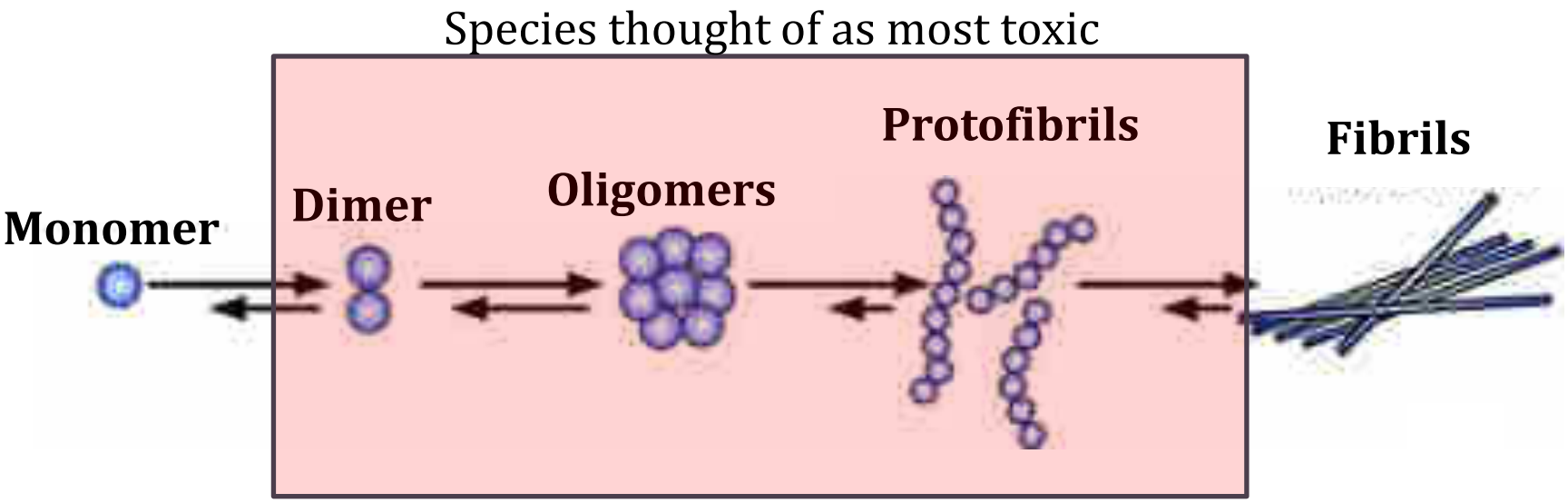
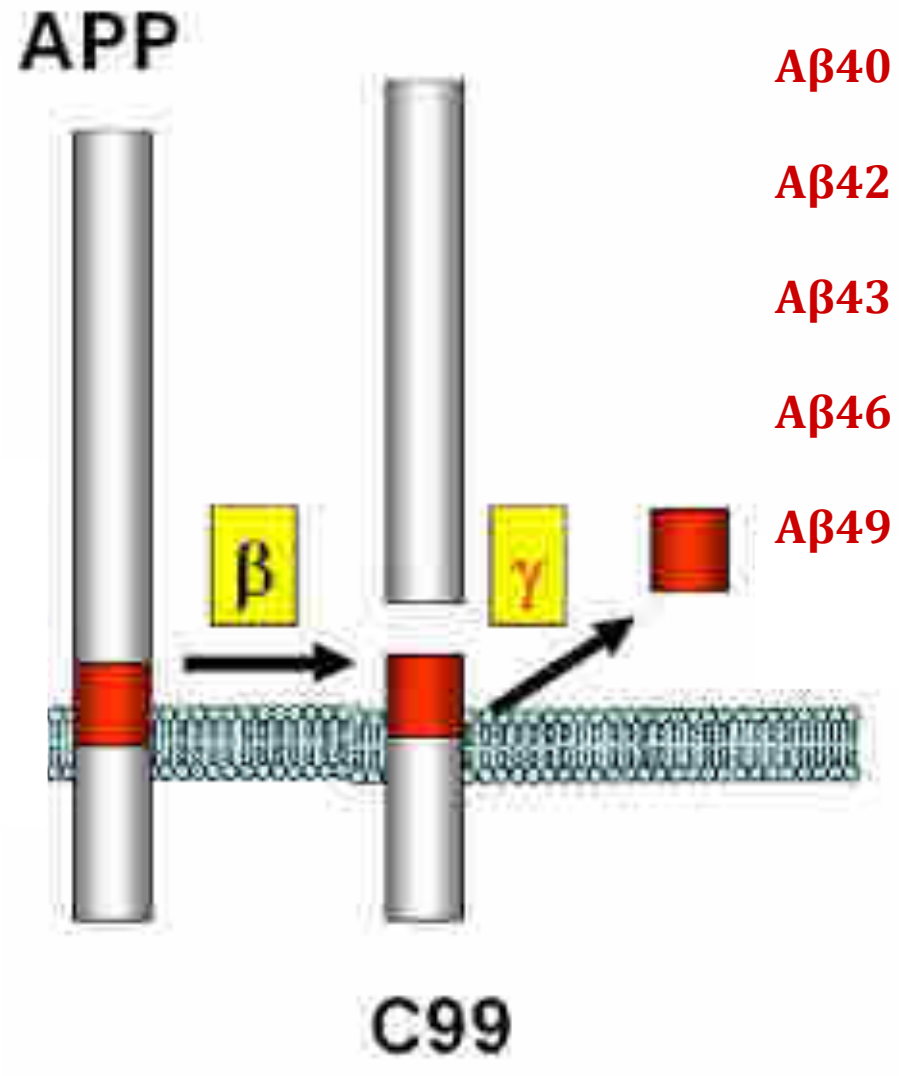


Figure adapted from Kumar *et al* *EMBO J (2011)*

Aβ variation – result of heterogeneous γ-secretase activity



Aβ37

Aβ38

Aβ40

Aβ42

Aβ43

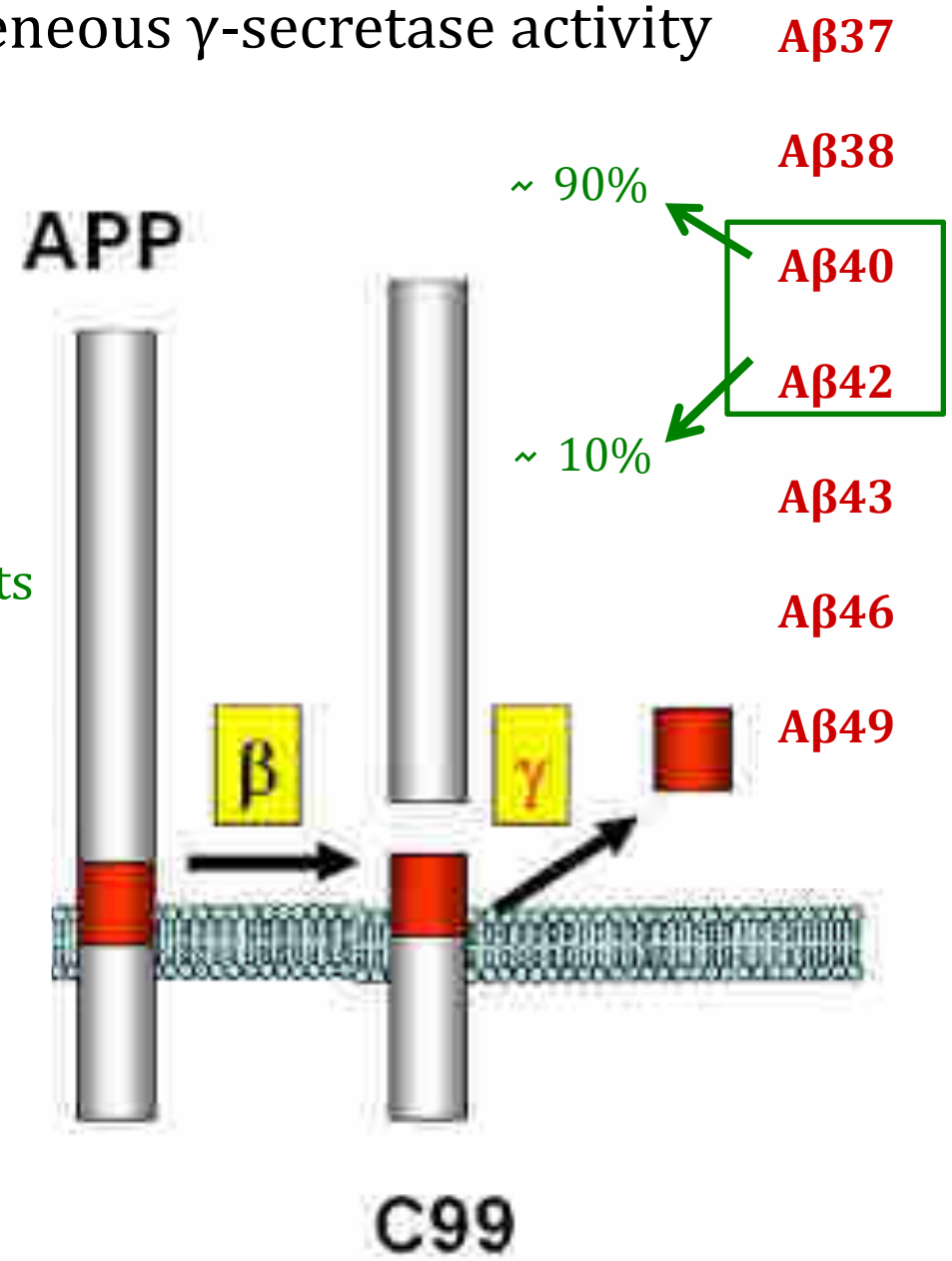
Aβ46

Aβ49

Aβ variation – result of heterogeneous γ-secretase activity

Aβ production in healthy aged subjects

Aβ42: Aβ40 = 1:9

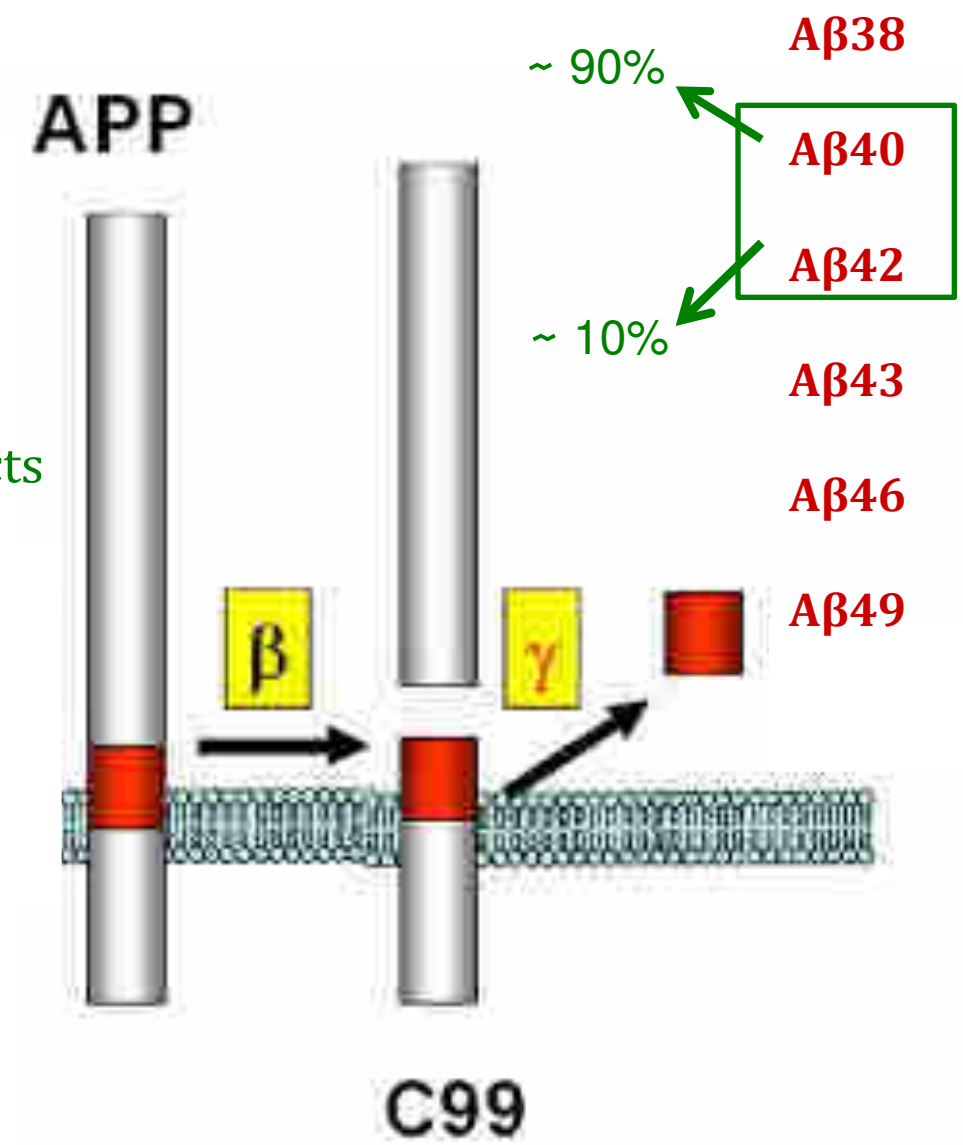


Aβ variation – result of heterogeneous γ-secretase activity

Aβ production in healthy aged subjects

Aβ42: Aβ40 = 1:9

Highly amyloidogenic



Aβ variation – result of heterogeneous γ-secretase activity

Aβ production in healthy aged subjects

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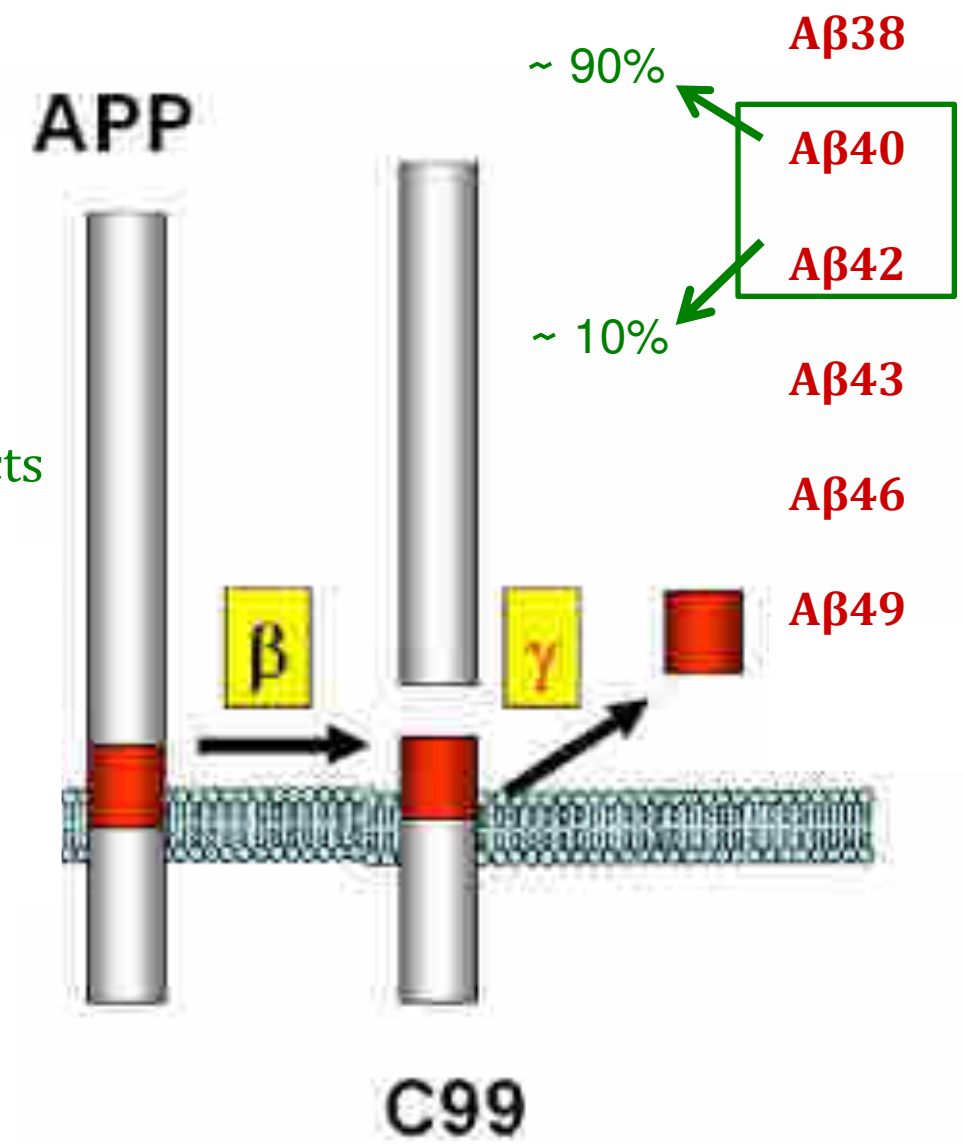
↓

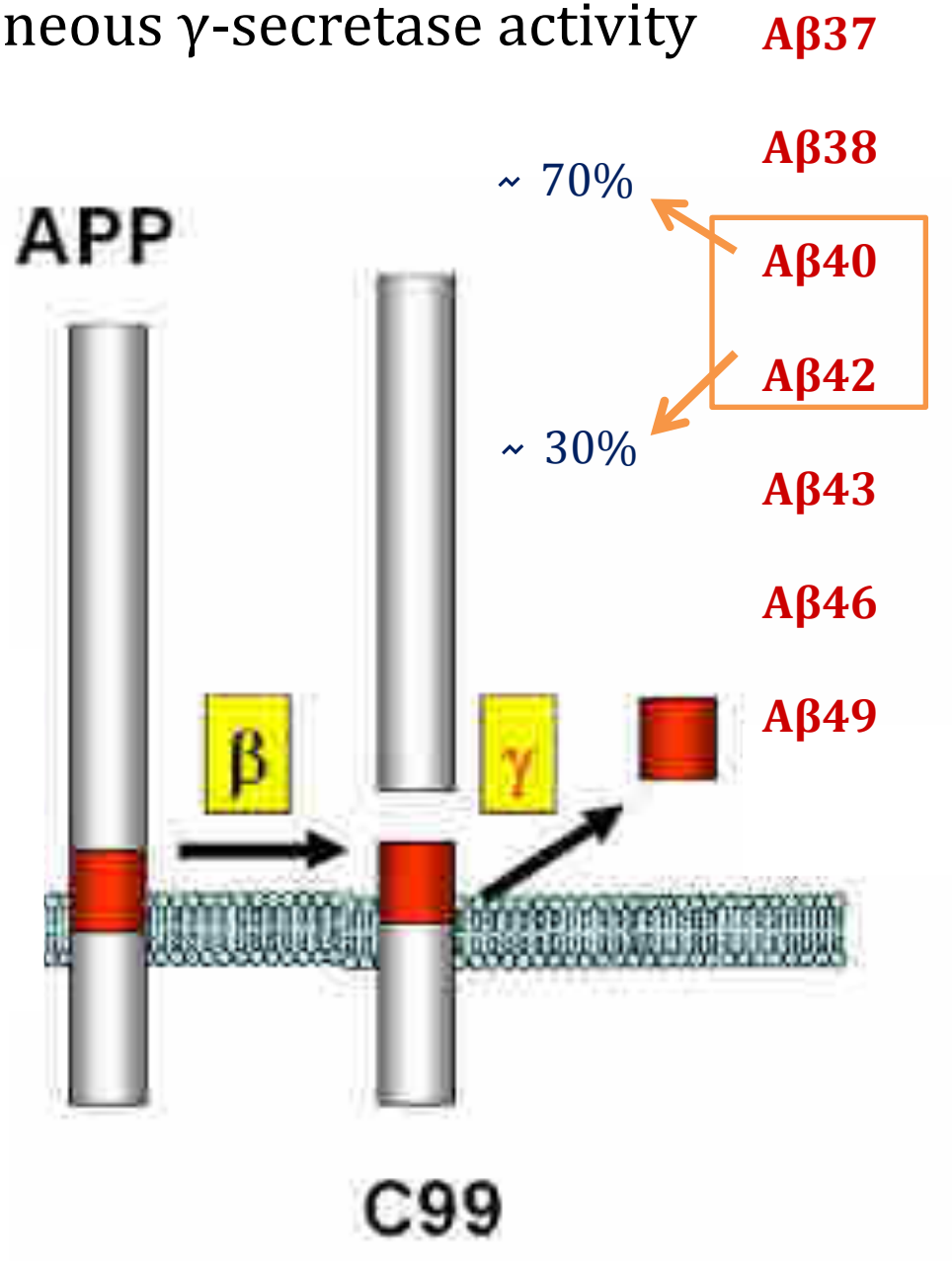
Little amyloidogenic



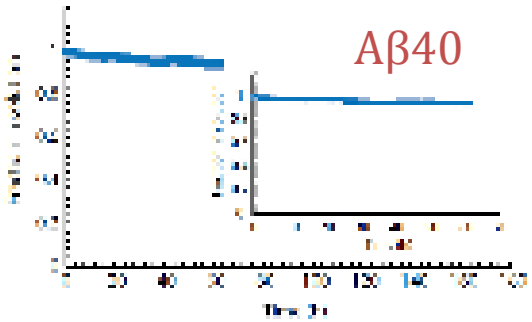
Aβ variation – result of heterogeneous γ-secretase activity

Aβ production in aged subjects with Alzheimer pathology

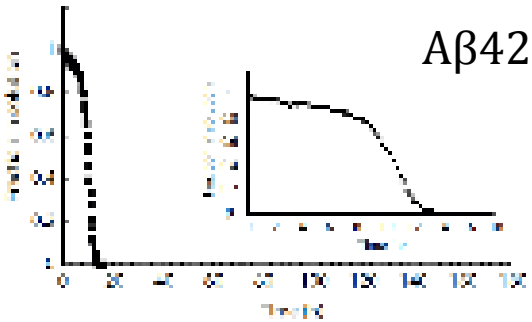
Aβ42:Aβ40 = 3:7



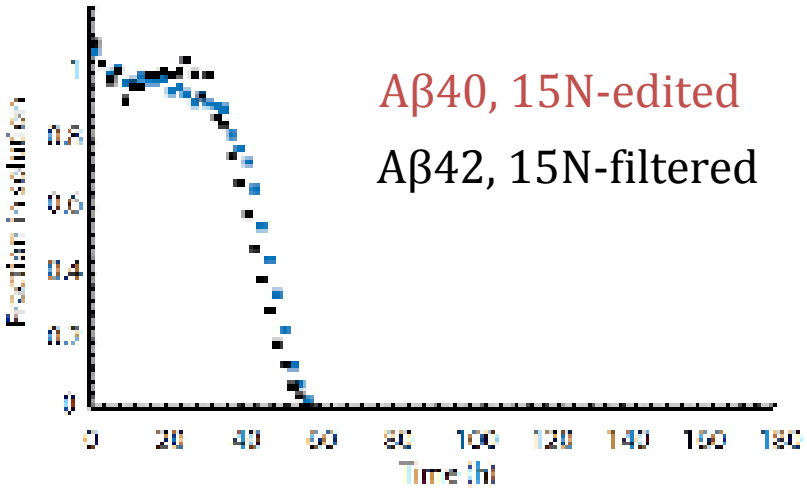
Aβ heterogeneity affects aggregation



Aβ40

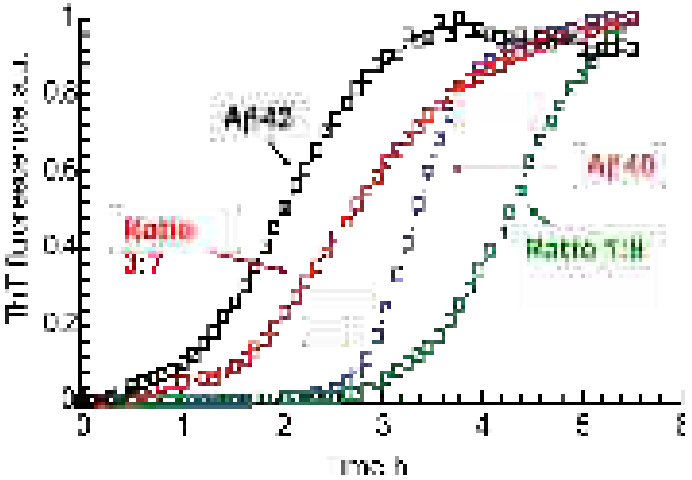


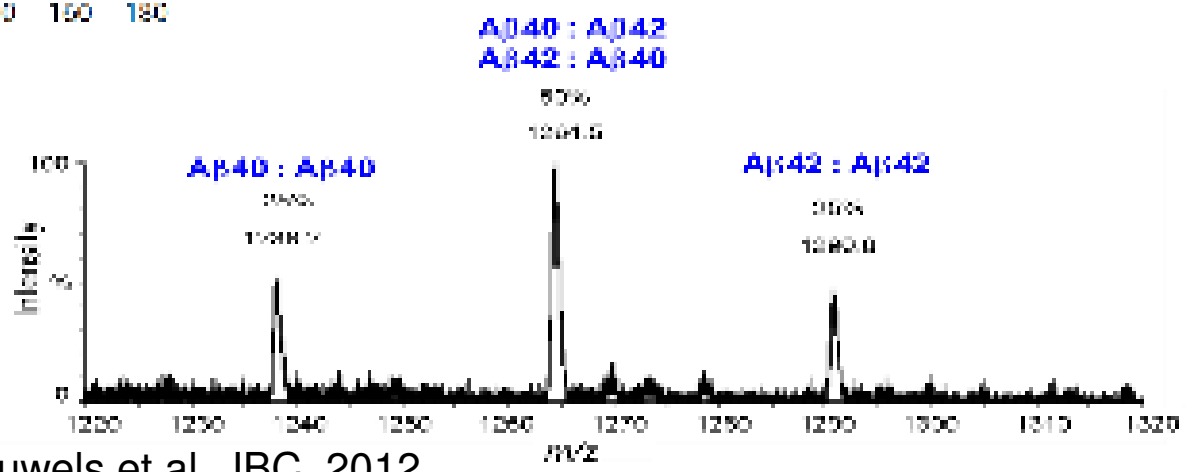
Aβ42



Aβ40, 15N-edited

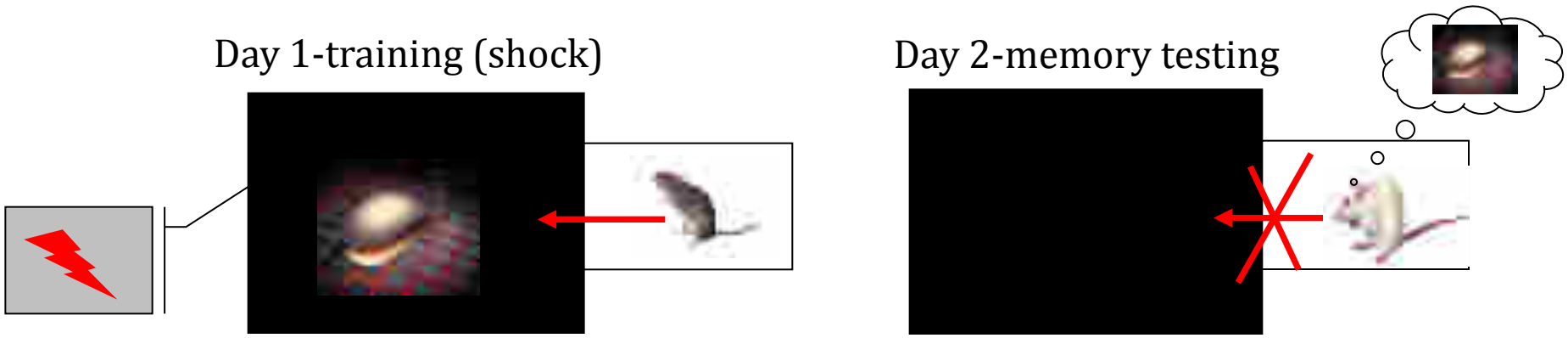
Aβ42, 15N-filtered

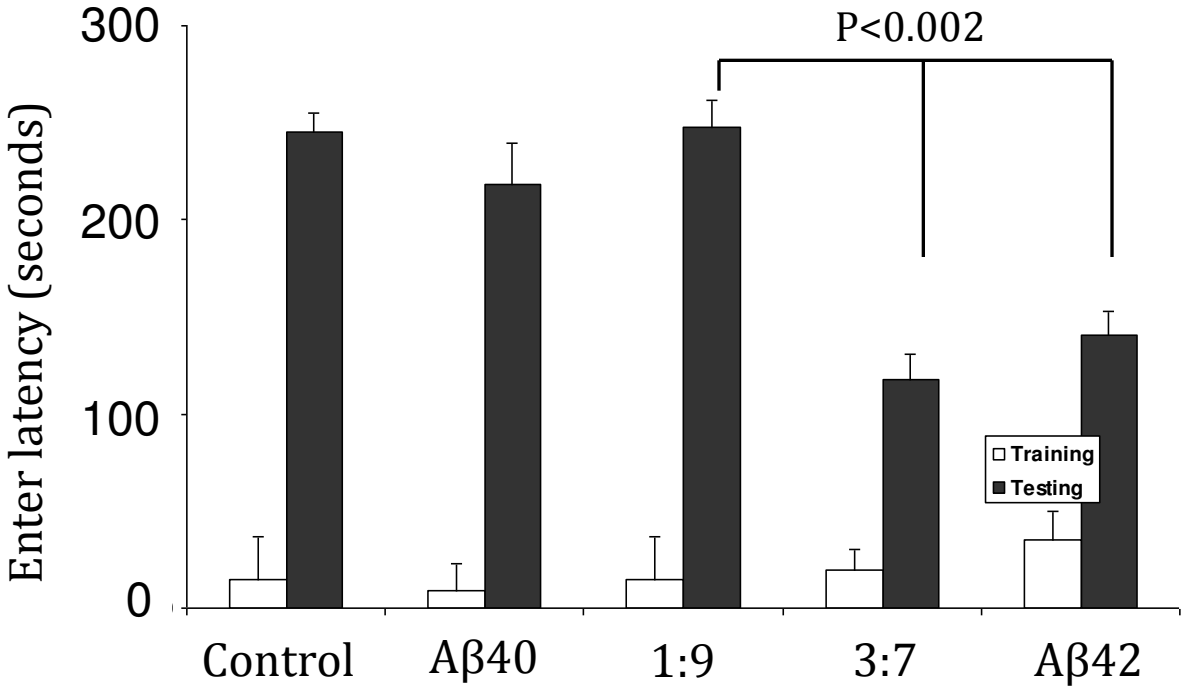




Kuperstein et al. EMBO J. 2010, Pauwels et al. JBC. 2012.

Aβ heterogeneity affects toxicity





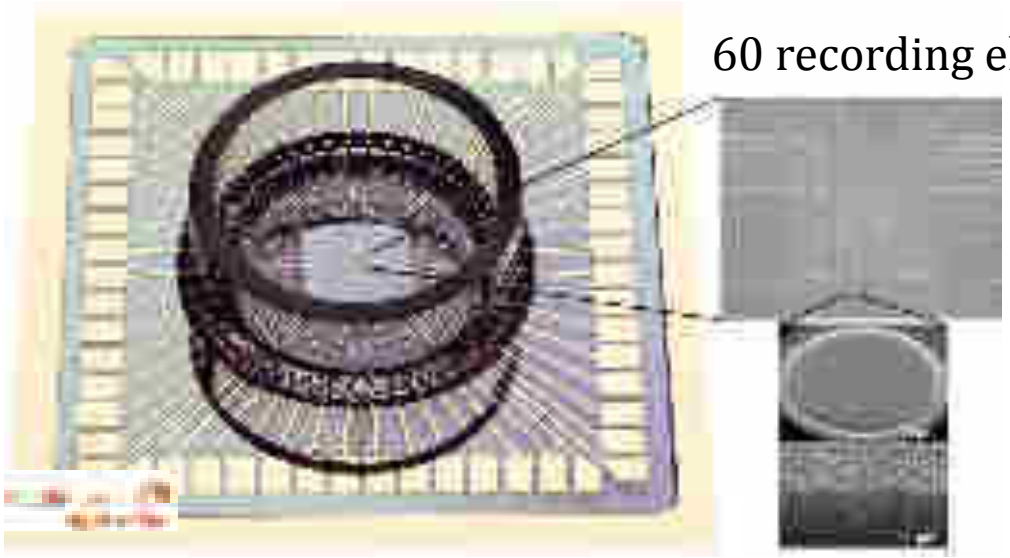
3:7 Aβ42:Aβ40 ratio causes memory and learning impairment in mice

Passive avoidance response

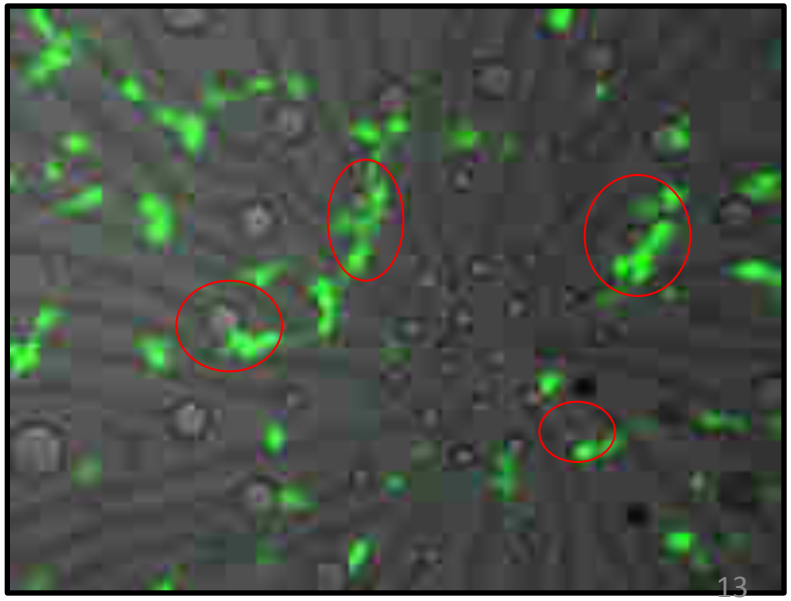
Kuperstein et al., EMBO J. 2010

Aβ heterogeneity affects toxicity

Microelectrode array: synaptic activity recordings in neuronal network grown on chip

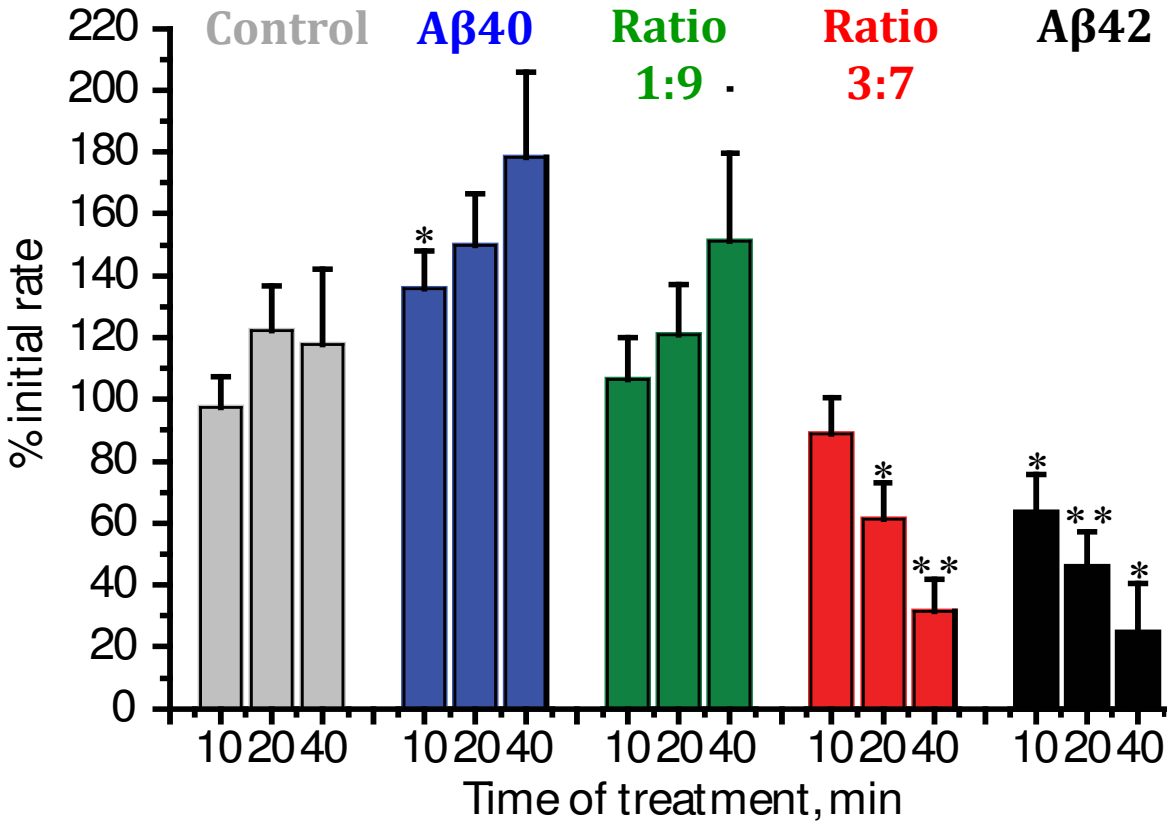


60 recording electrodes



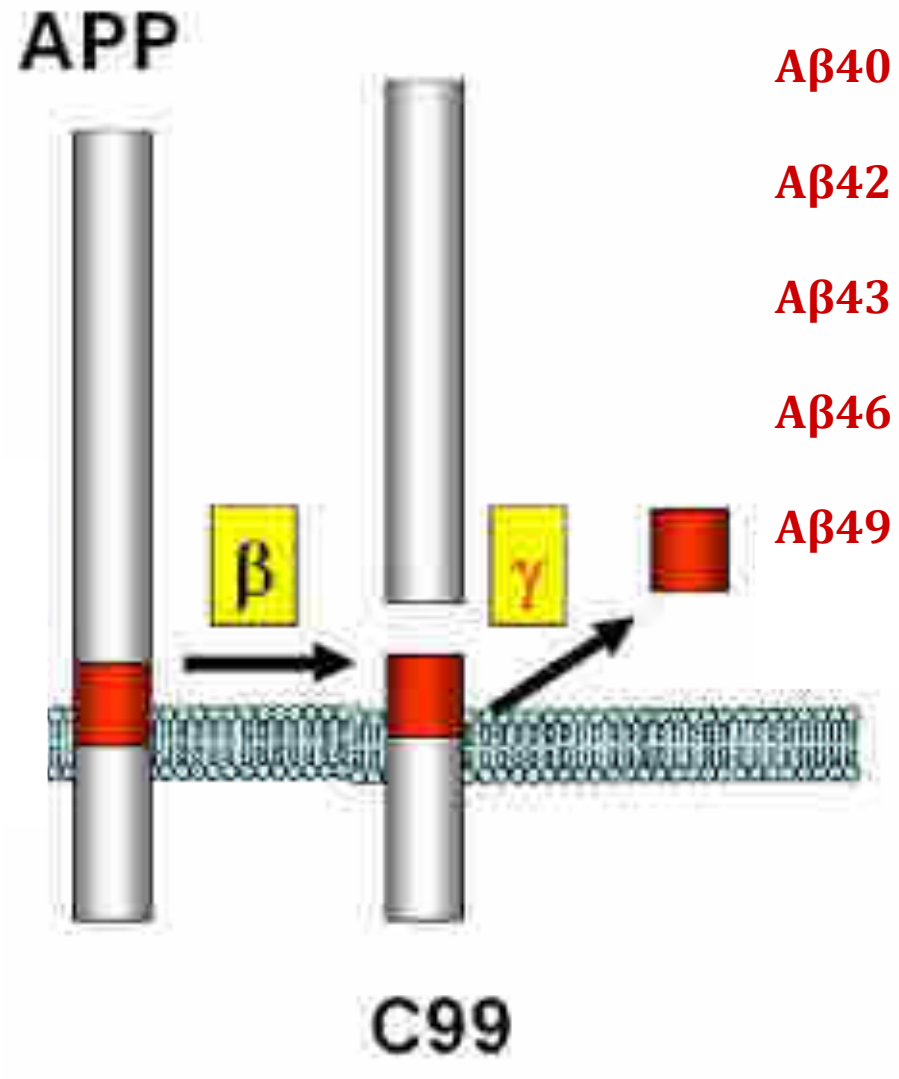
Kuperstein et al. EMBO J. 2010

Pathological mixtures of Aβ40 and Aβ42 induce acute synaptotoxicity

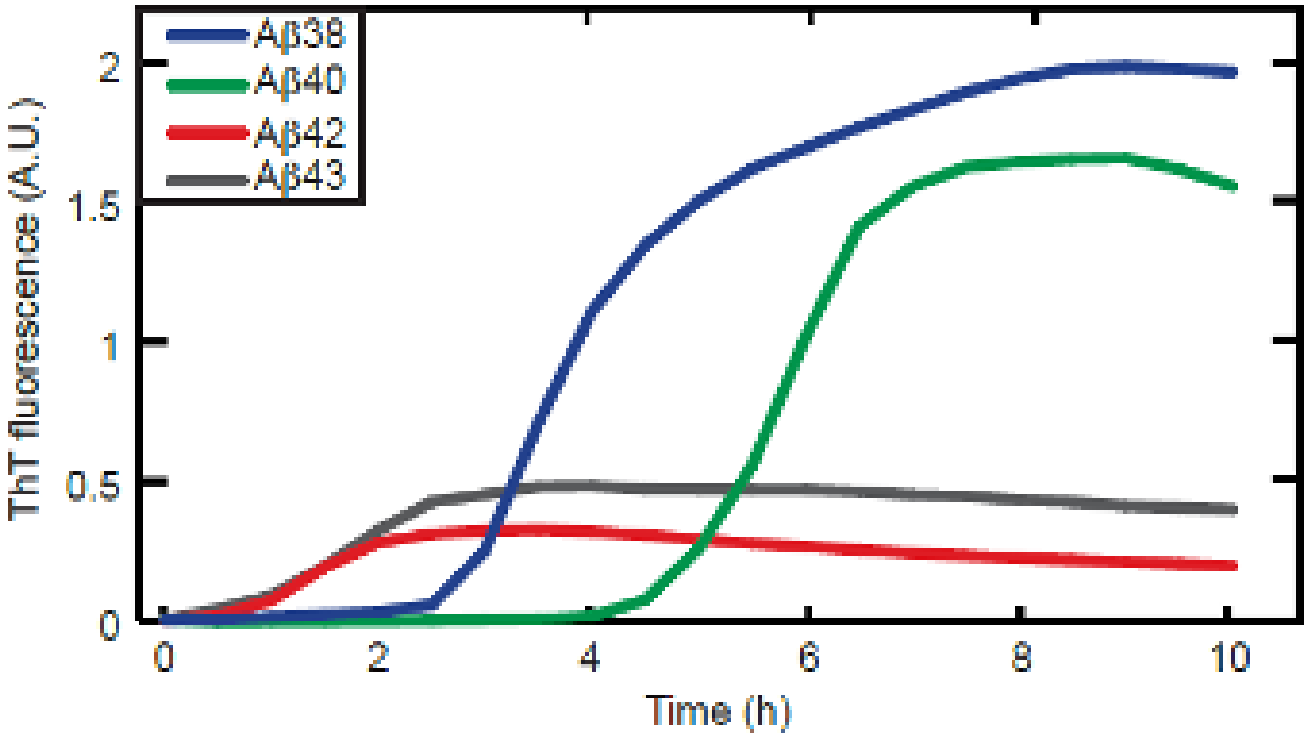


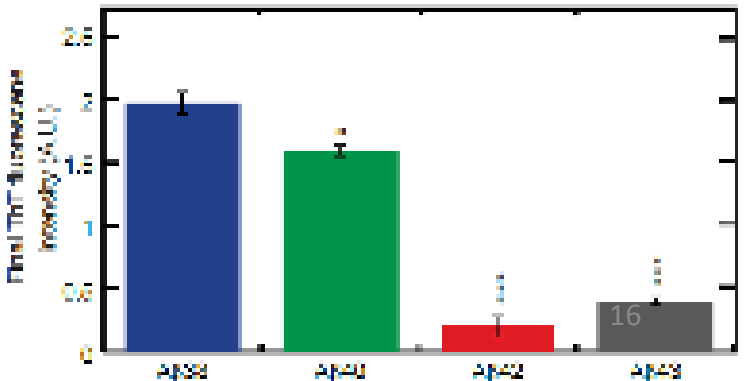
Kuperstein et al. EMBO J. 2010

Aβ variation – result of heterogeneous γ-secretase activity



Aβ peptide length affects aggregation rate

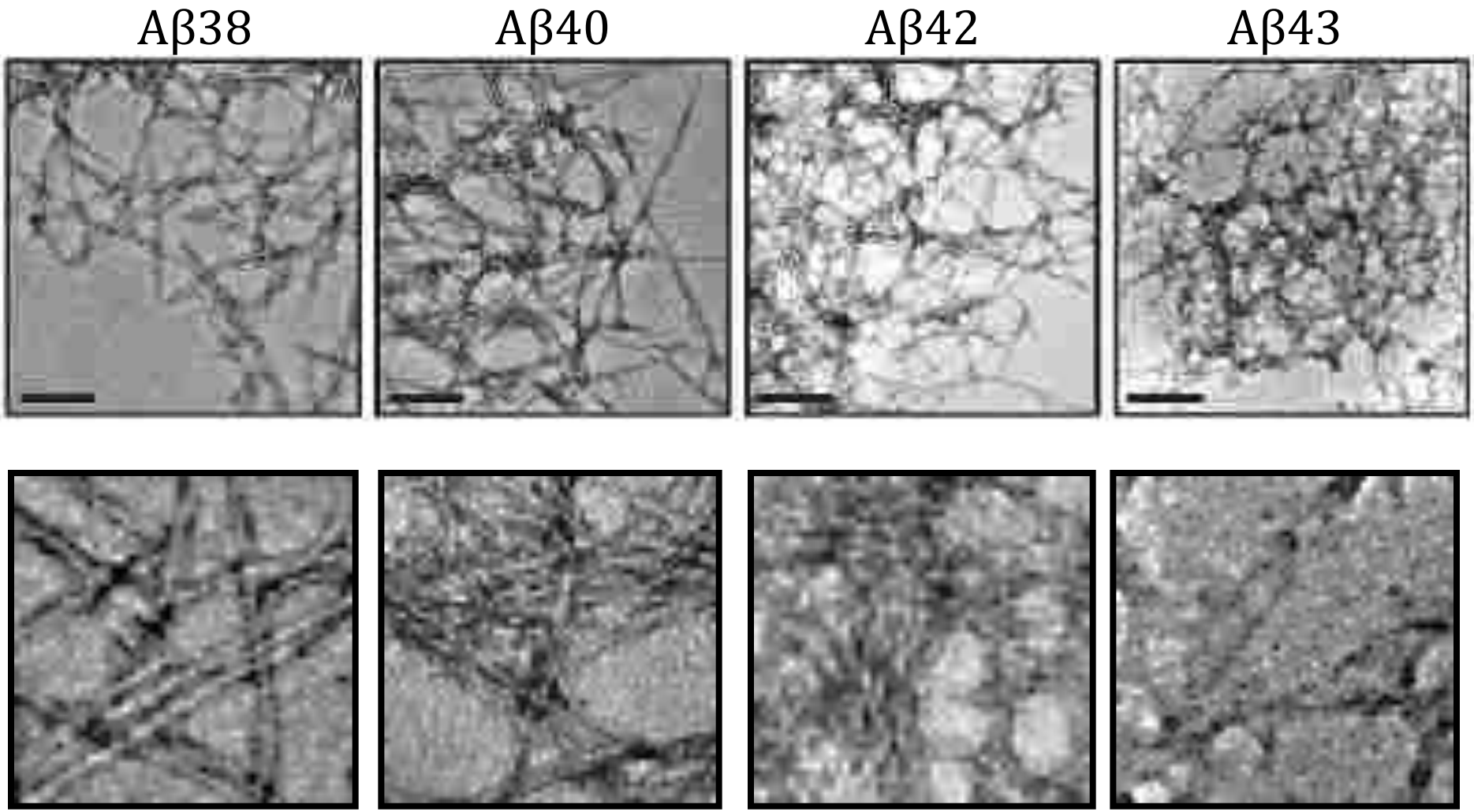




Vandersteen et al (2012) J Biol Chem 287: 36732-36743

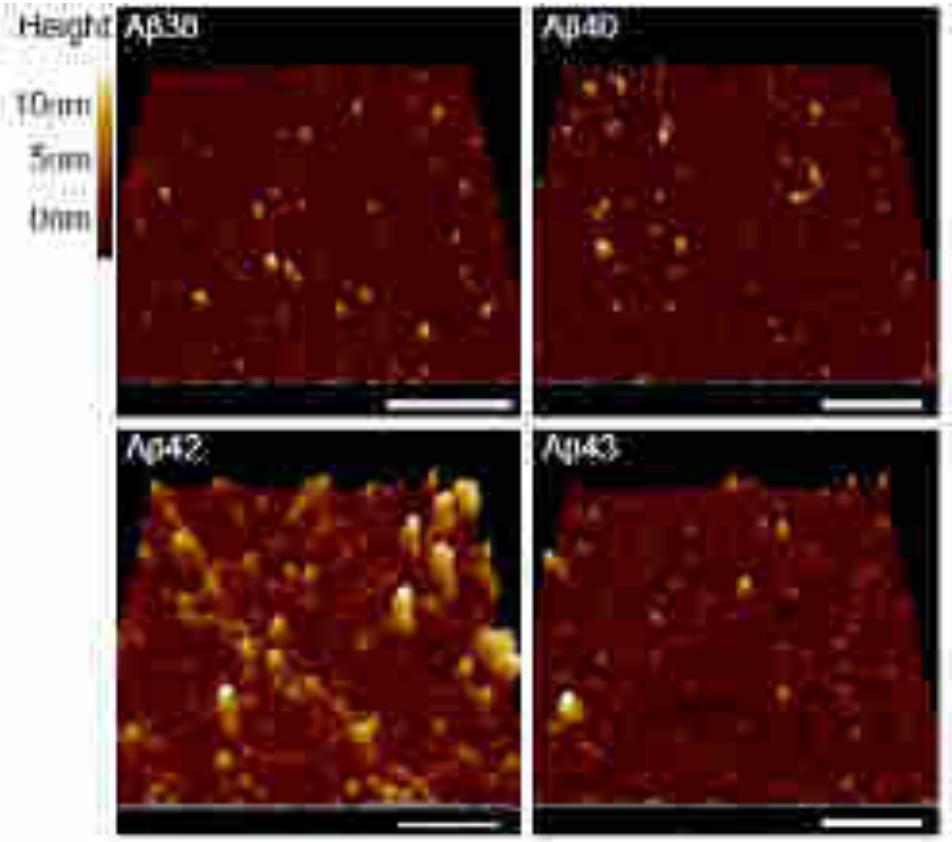
Tendency to form amyloid fibrils is independent of Aβ peptide length

… but morphological differences

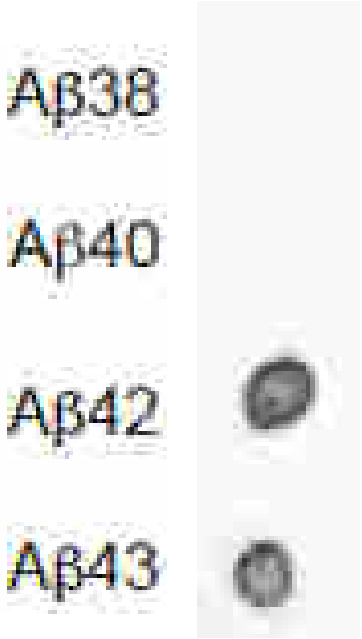


Vandersteen et al (2012) J Biol Chem 287: 36732-36743

All tested Aβ peptide lengths form oligomers but with different structural properties.



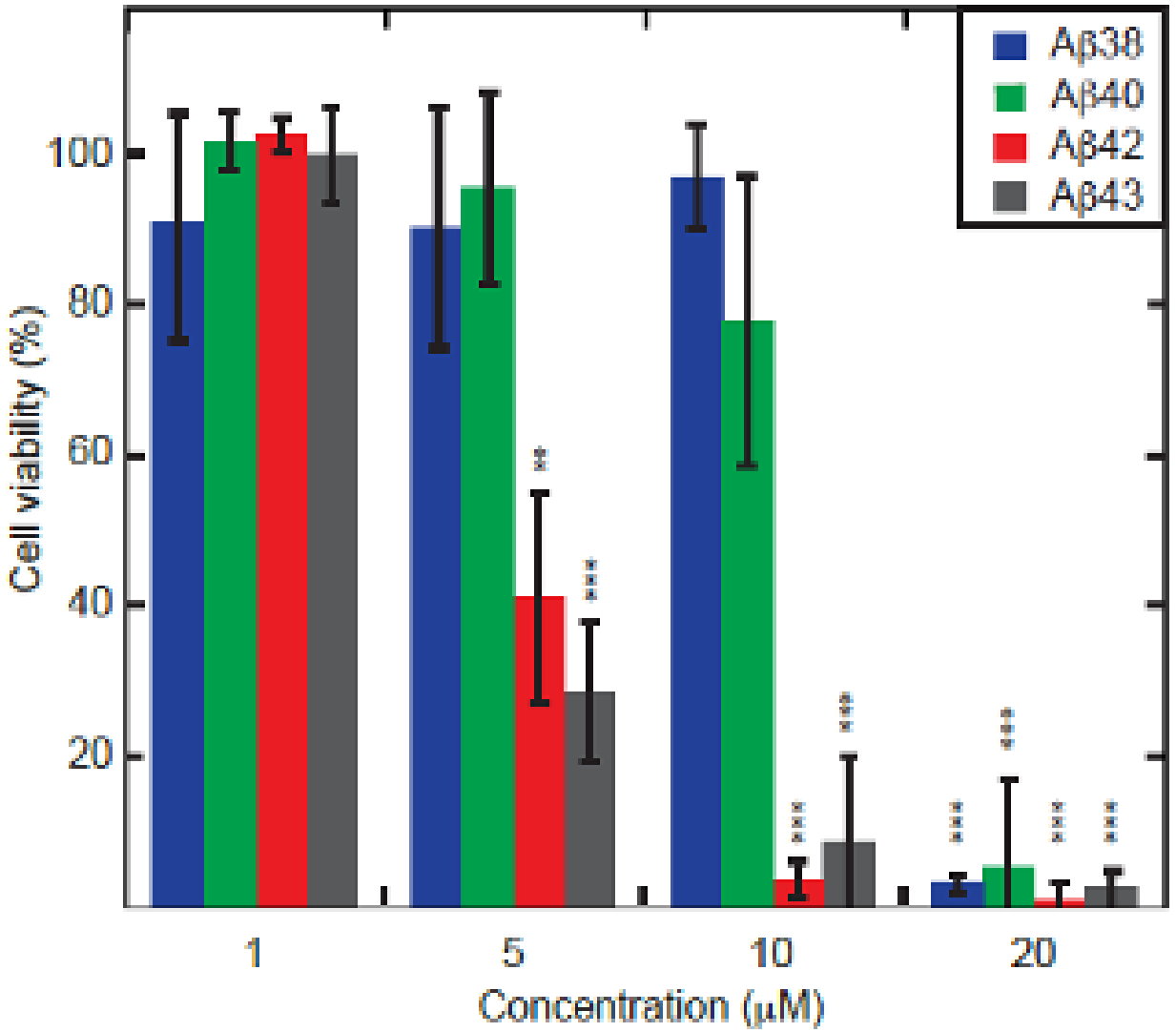
Recognition of oligomeric Aβ by **A11 antibody.**



Vandersteen et al (2012) J Biol Chem 287: 36732-36743

Aβ peptide length affects cytotoxicity: unexpected toxicity of Aβ38

Cell titer blue **cell viability assay** of neuroblastoma SHSY-5Y cells by incubation with oligomeric Aβ.



Chavez-Gutierrez et al (2012) EMBO J 31: 2261-2274

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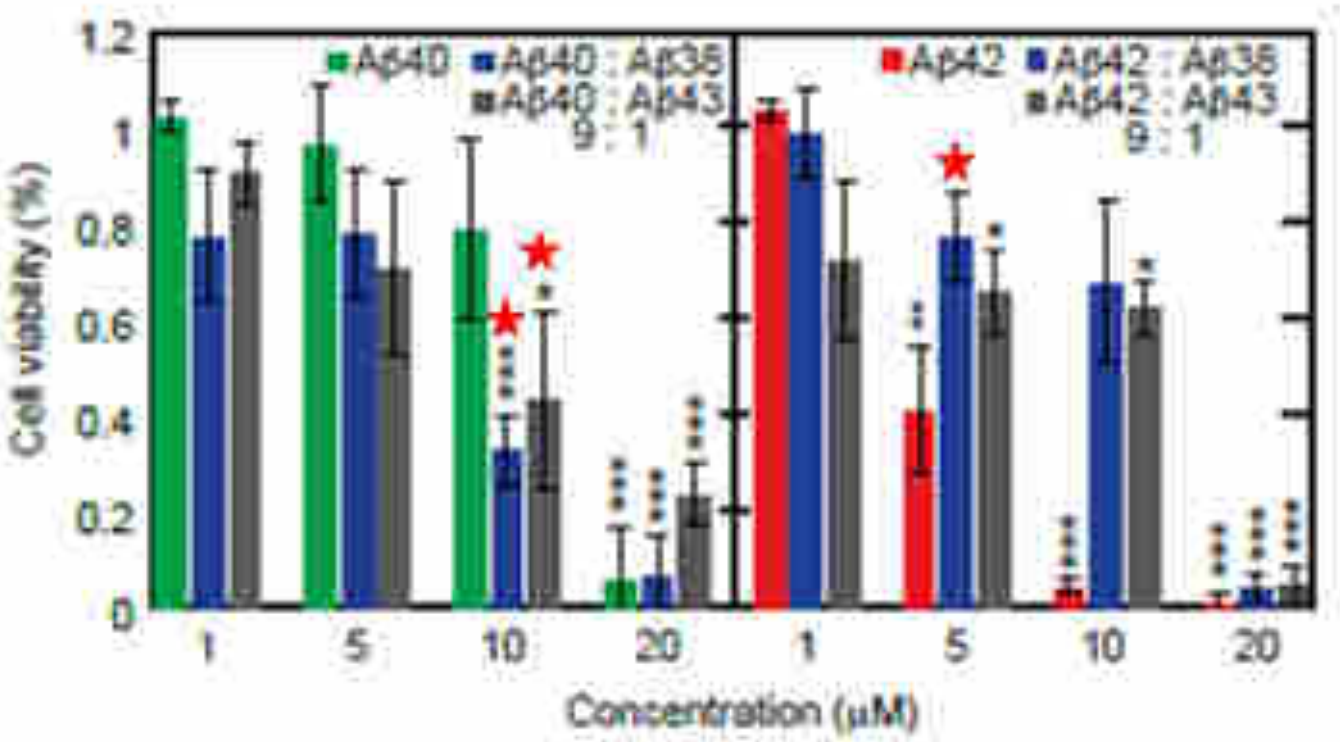

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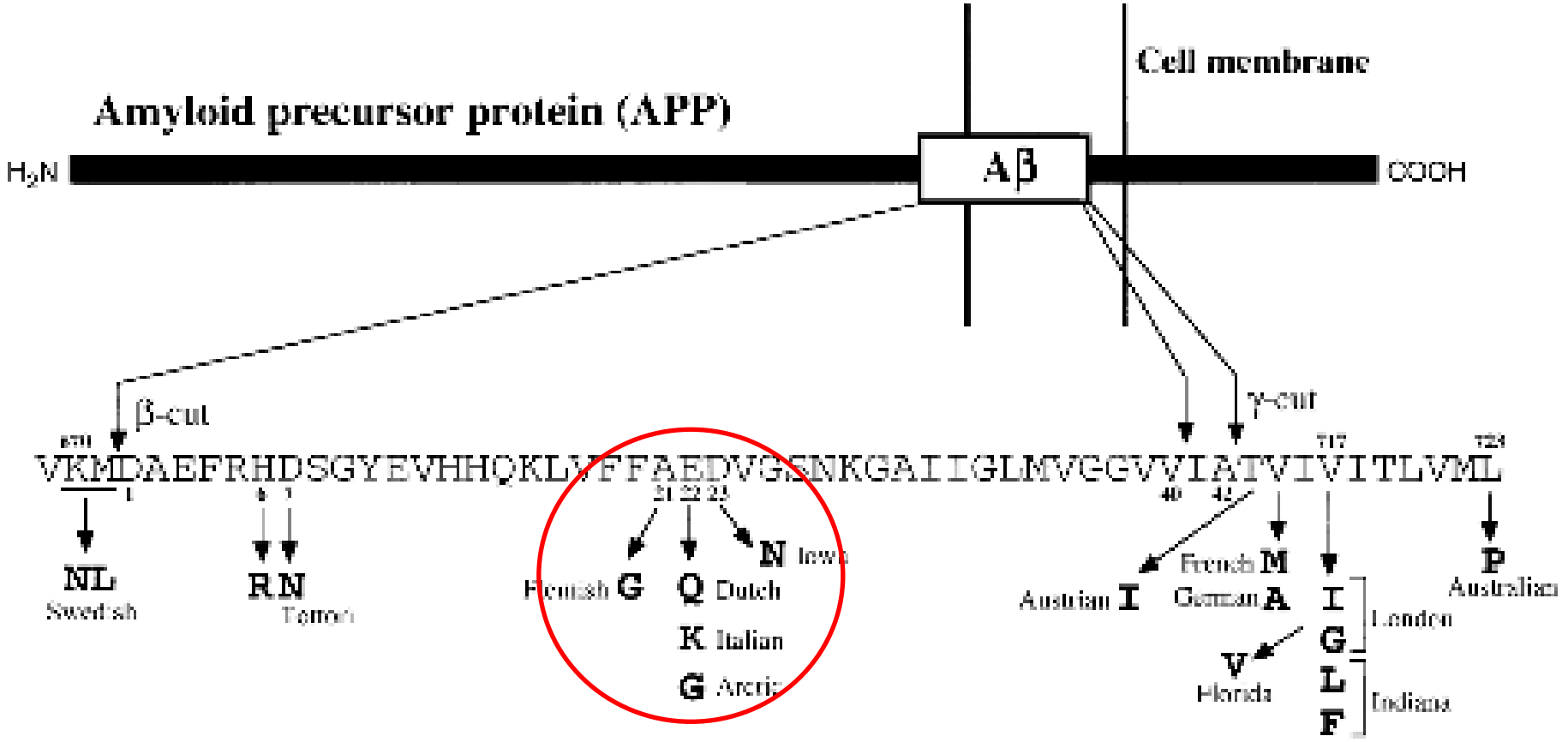
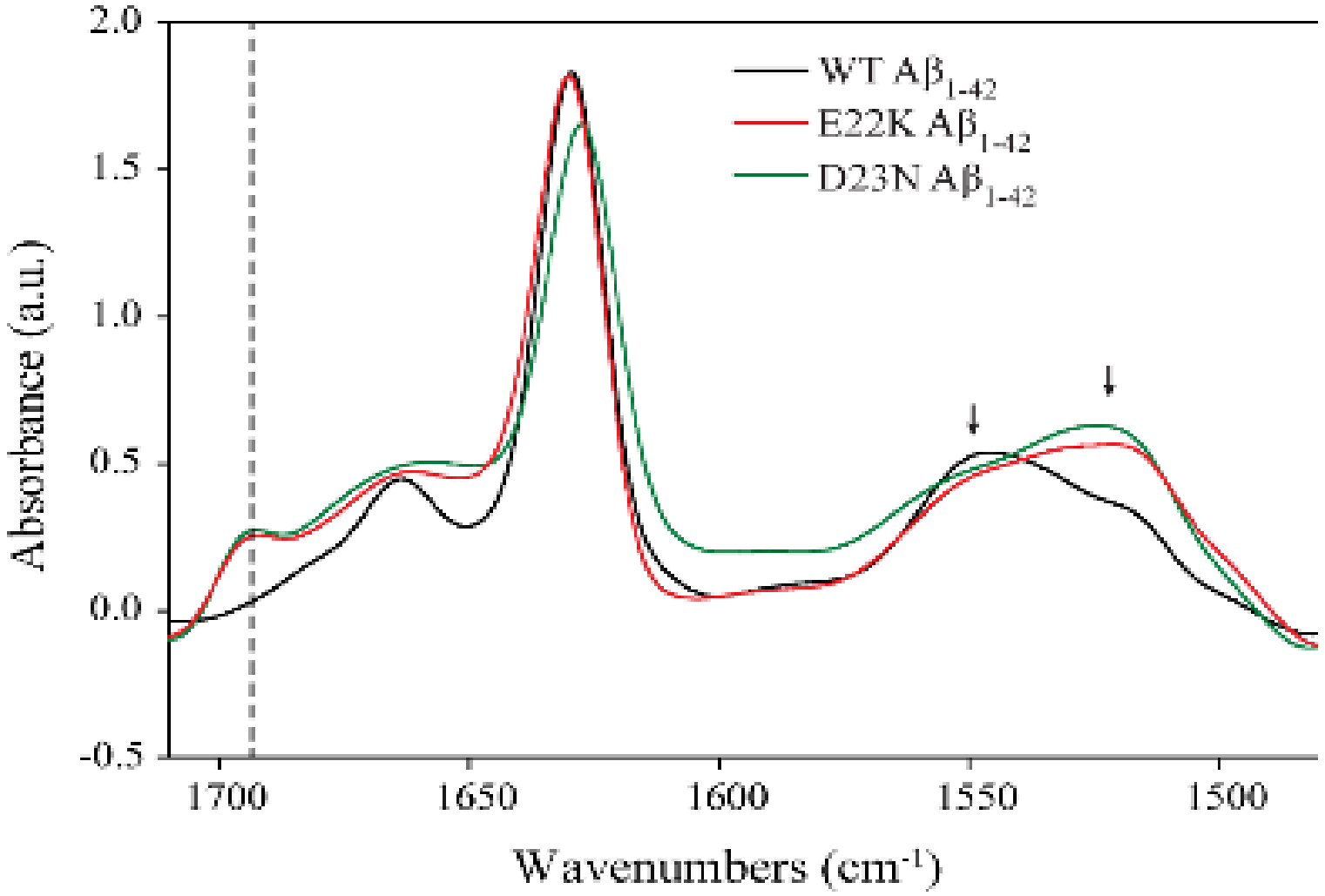
Vandersteen et al (2012) J Biol Chem 287: 36732-36743

Aβ mutations



D23N (Iowa mutant) and E22K (Italian mutant) Aβ42 form antiparallel β-sheet fibrils



Qiang et al (2012) PNAS → Aβ40.

Vandersteen et al (2012) FEBS Letters

Alzheimer's disease – Cerebral amyloid angiopathy (CAA)
CAA is a common clinical symptom of early-onset familial AD

BRAIN
Amyloid-beta (Aβ) plaques

Bugiani et al 2010.

BLOOD
Aβ deposits in cerebral blood vessel walls

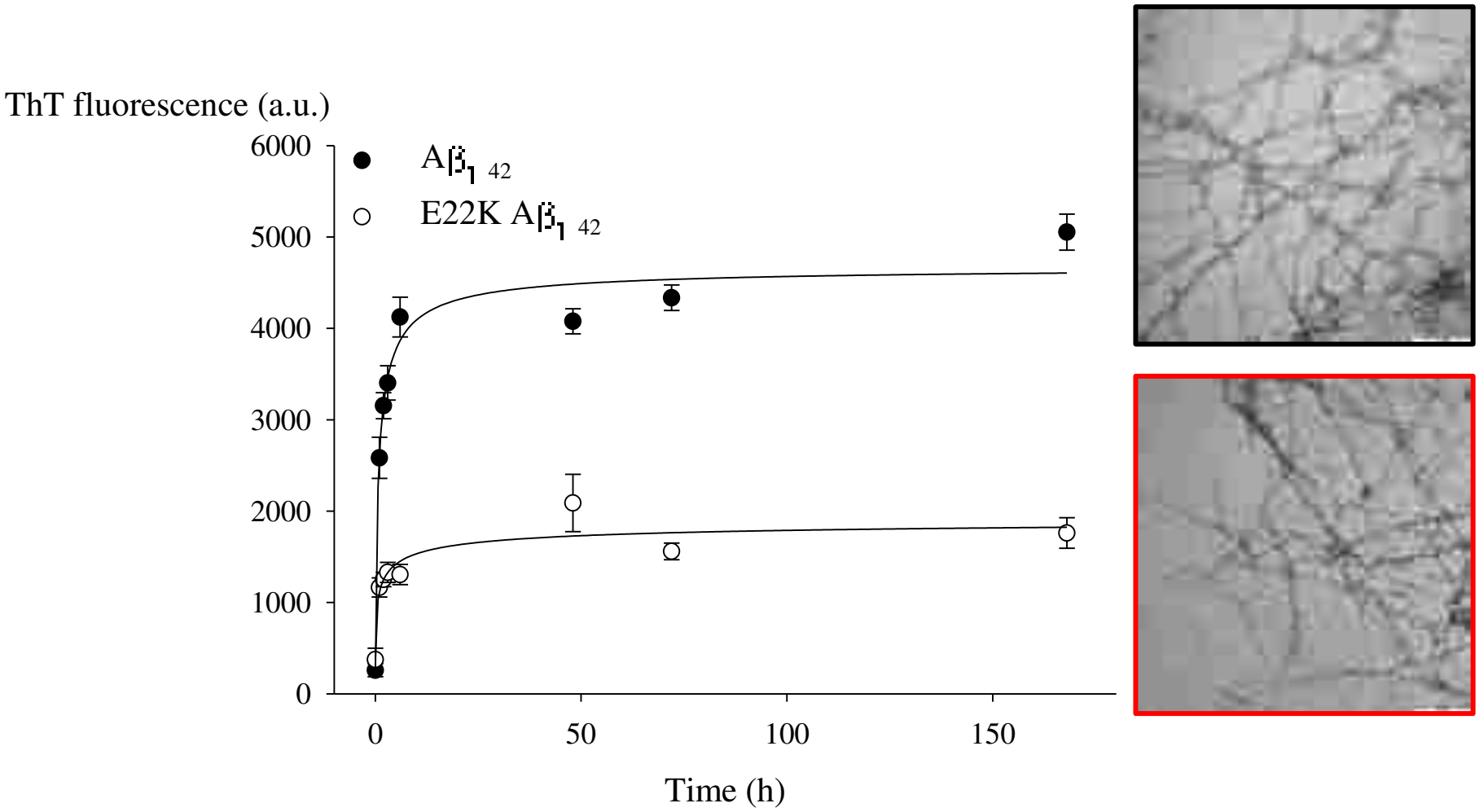
⇩

Vessel walls prone to rupture
Narrows lumina

⇩

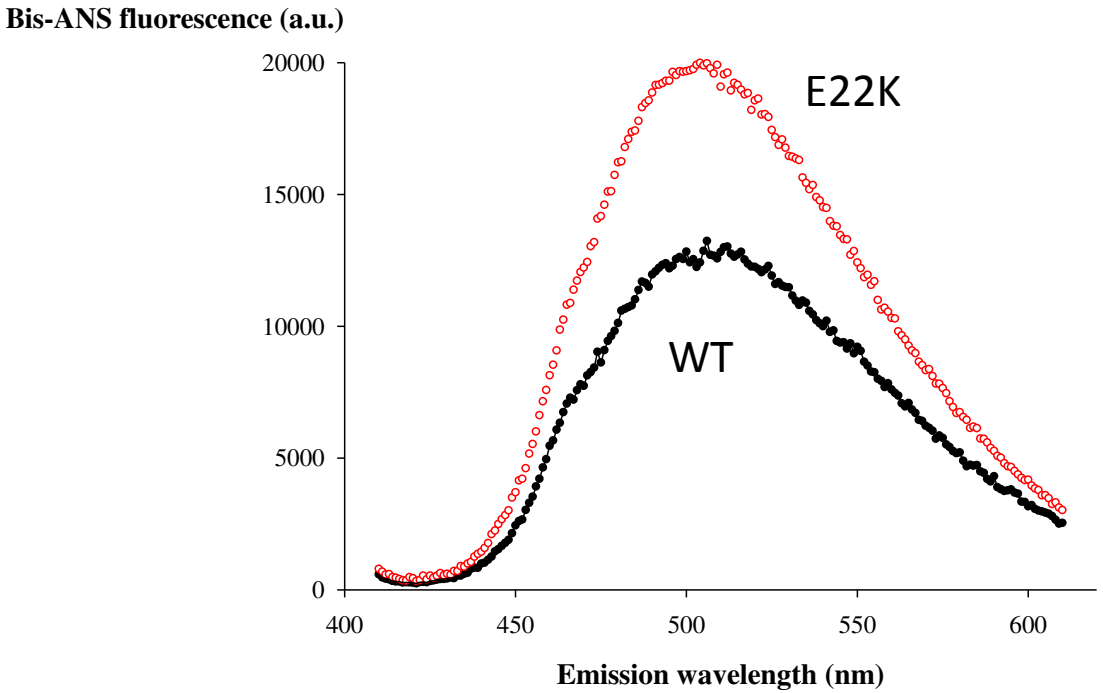
Intracerebral and subarachnoid bleeding
Infarcts
Periventricular oedema

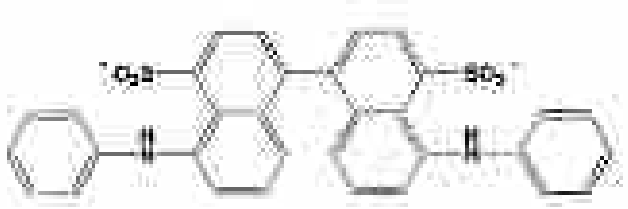
ThT fluorescence intensity of fibrils varies with mutation



Hubin et al. Cell Mol Life Sci 2015

Wild type and E22K $A\beta_{1-42}$ fibrils interact differently with conformation-dependent fluorescent probes (ThT and bis-ANS)



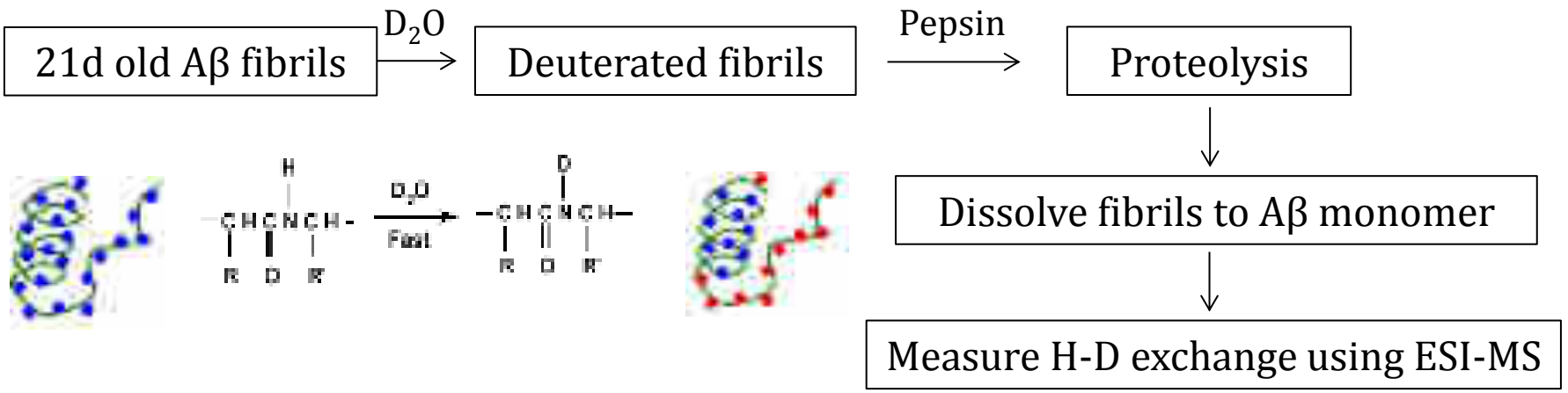


Bis-ANS: binds to hydrophobic patches

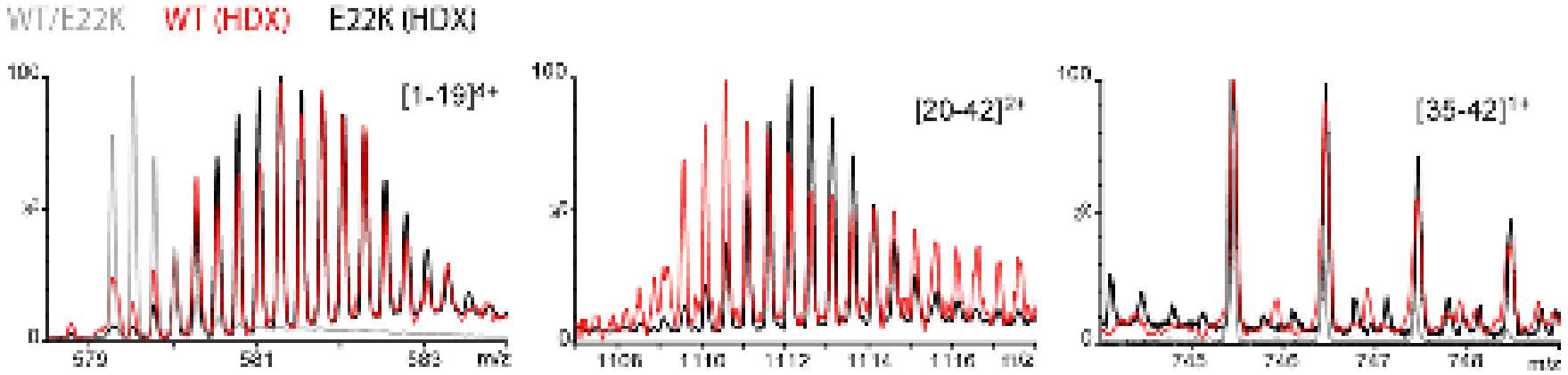
LeVine H. (2002)

Hubin et al. Cell Mol Life Sci 2015

Wild type and E22K $A\beta_{1-42}$ differ in solvent accessibility in the Aβ region comprising residues [20-34]

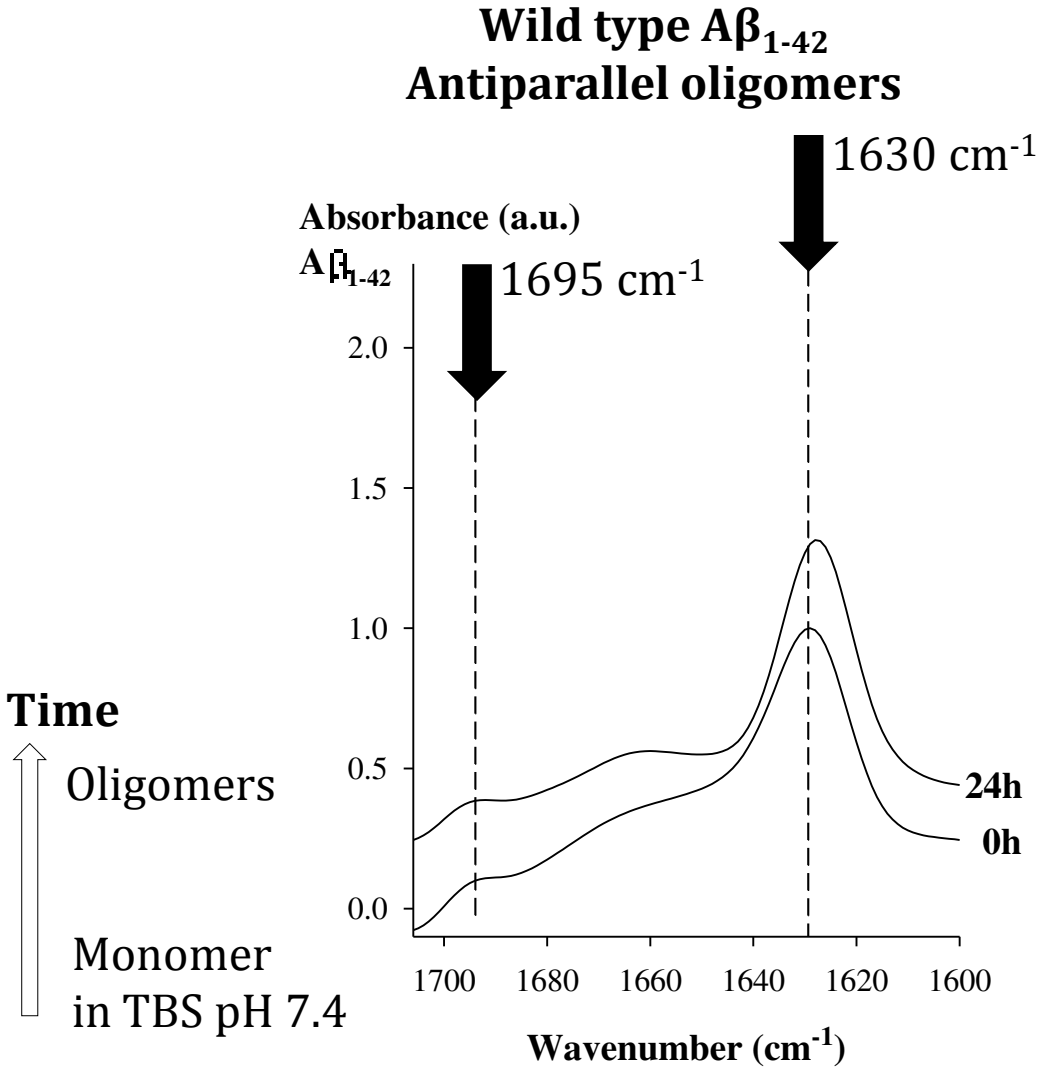


H/D exchange - Mass Spectrometry with proteolytic fragmentation



Hubin et al. Cell Mol Life Sci 2015

Changes in secondary structure during Aβ aggregation can be monitored by ATR-FTIR



Cerf *et al.* Biochem J (2009); Sarroukh *et al.* BBA Biomembrane (2013)

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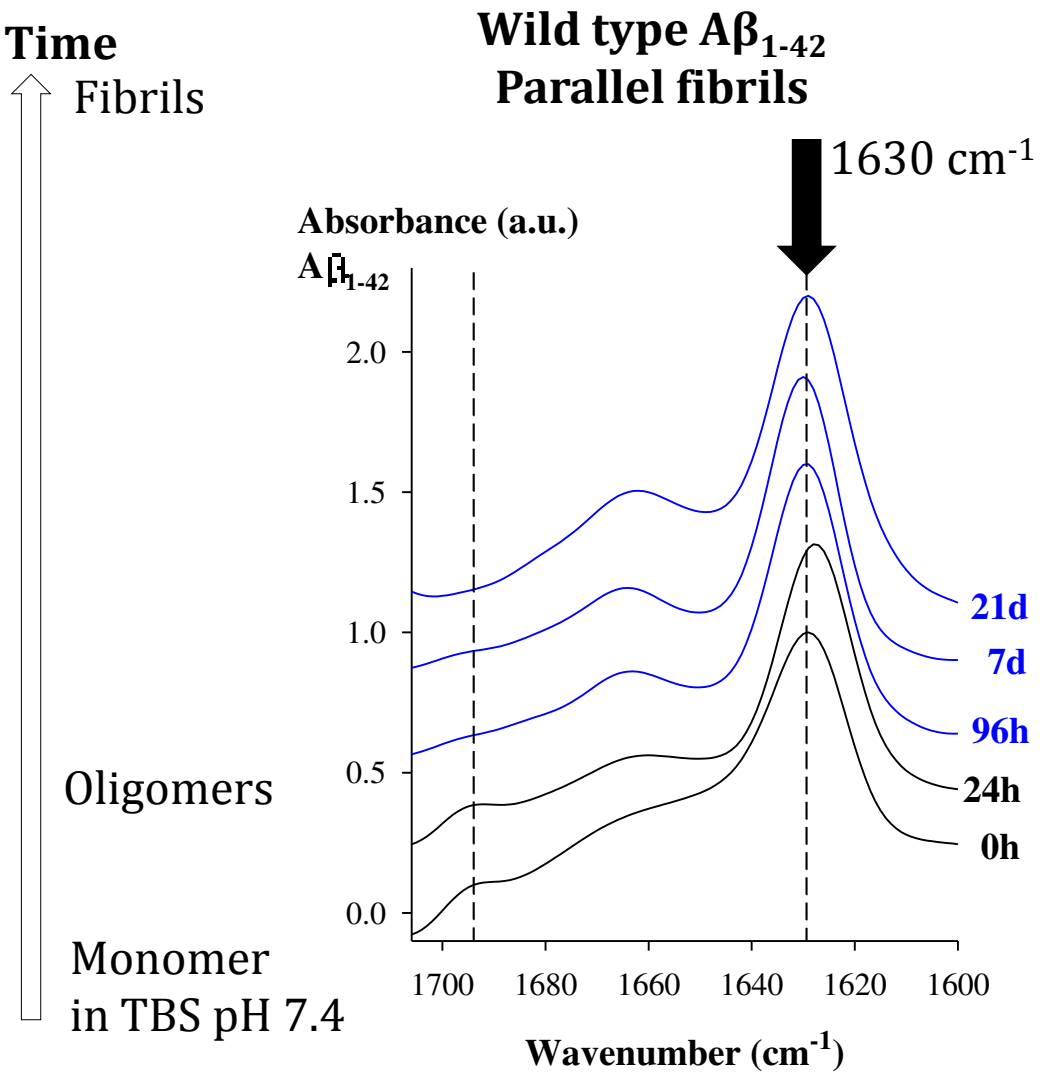

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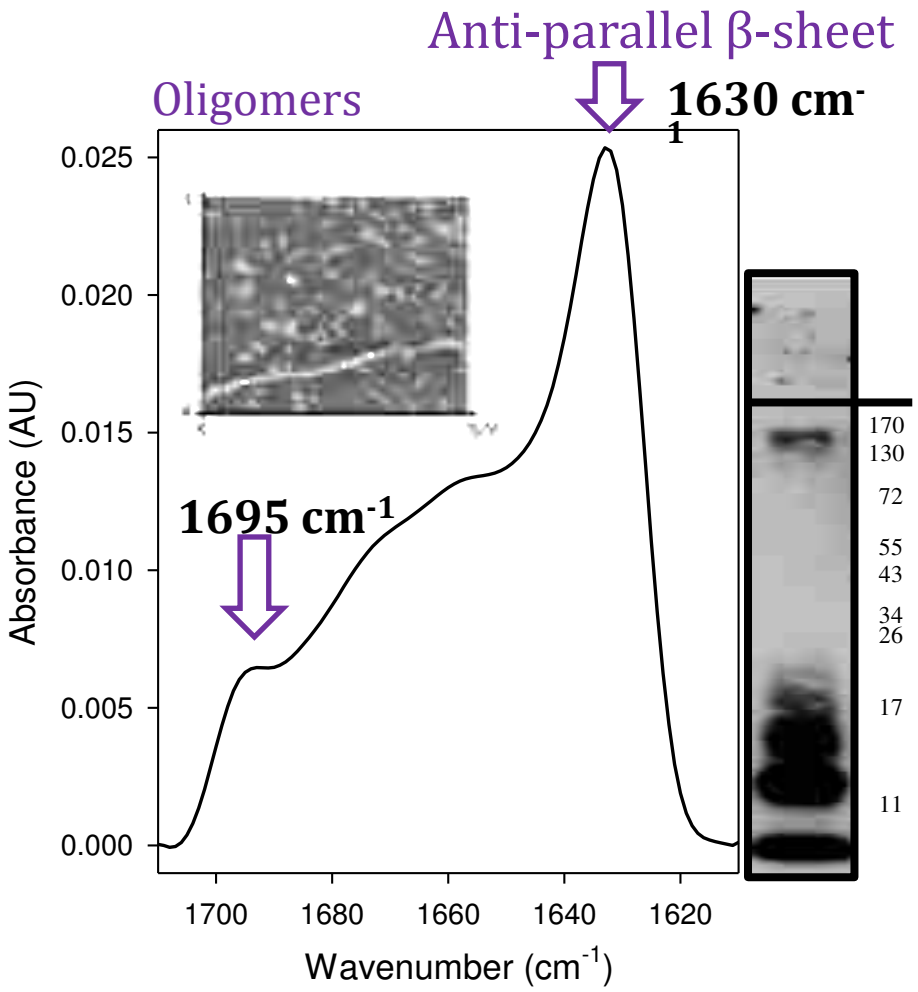
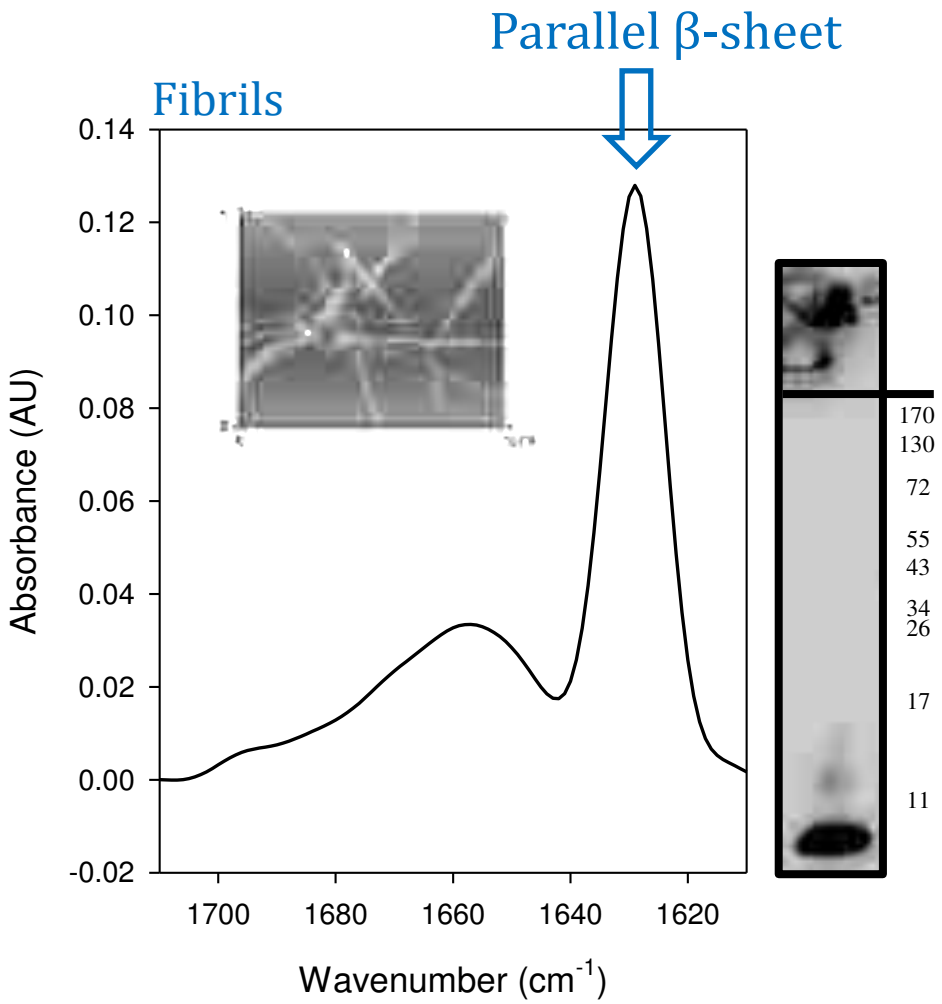
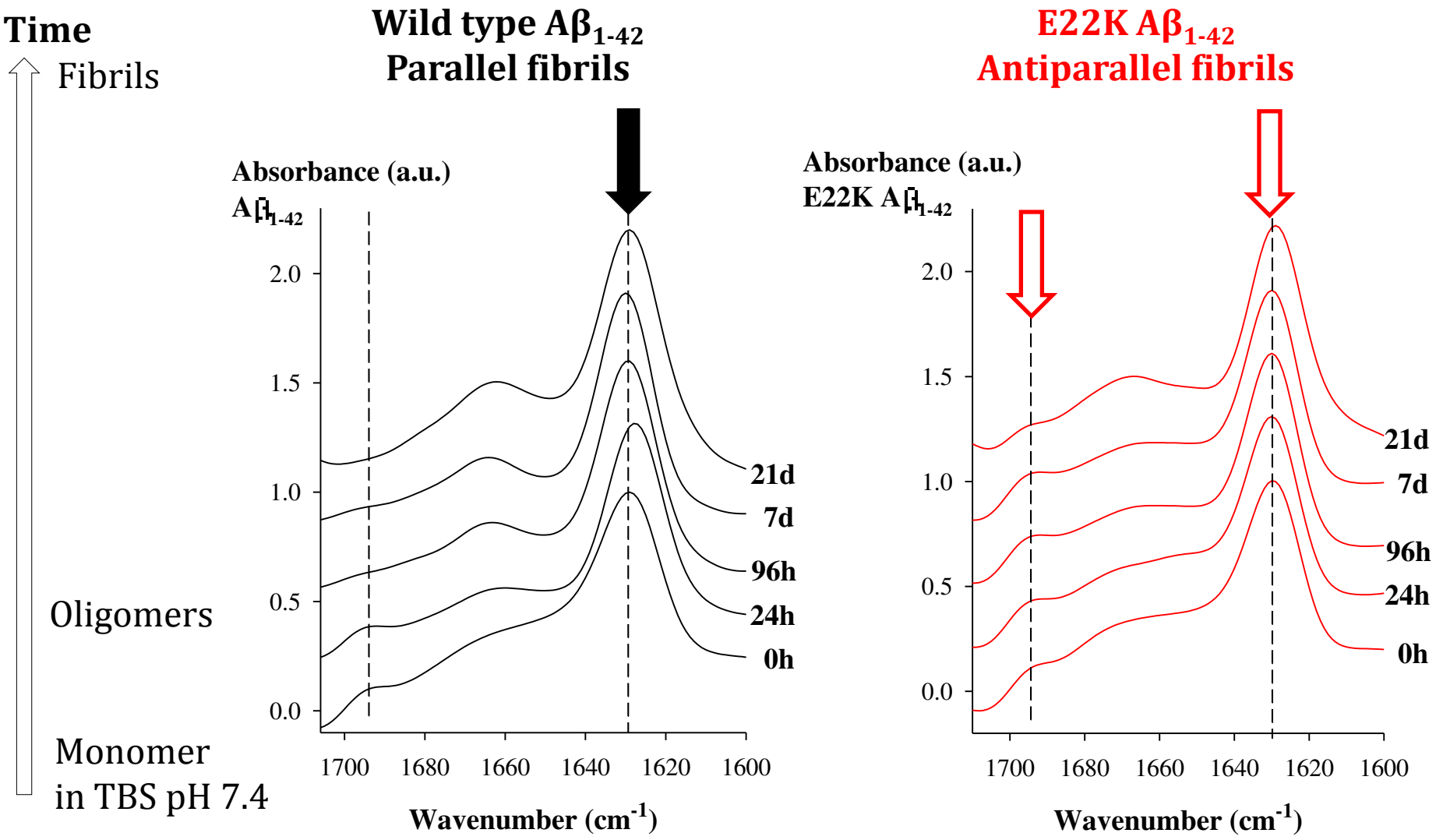
ATR-FTIR reveals major change in secondary structure during transformation of wild type Aβ oligomers into fibrils





Cerf *et al.* Biochem J (2009); Sarroukh *et al.* BBA Biomembrane (2013)

A minor peak around 1695 cm^{-1}, representative of antiparallel β sheets, persists during E22K $A\beta_{1-42}$ aggregation



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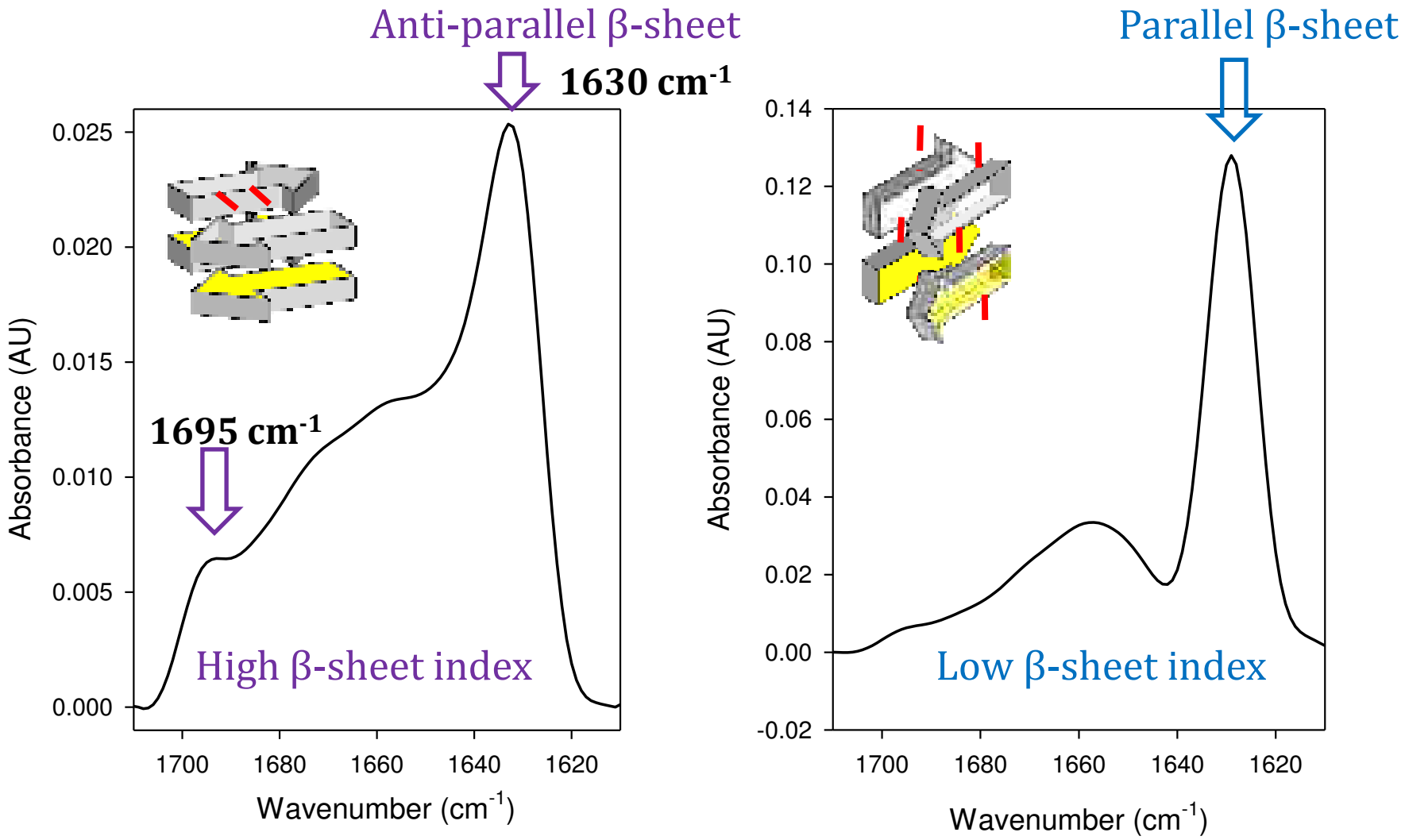
The β-sheet index (1695/1630 intensities ratio) is proportional to the percentage of antiparallel arrangement of β-strands in a β-sheet



Sarroukh R. *et al* (2010)

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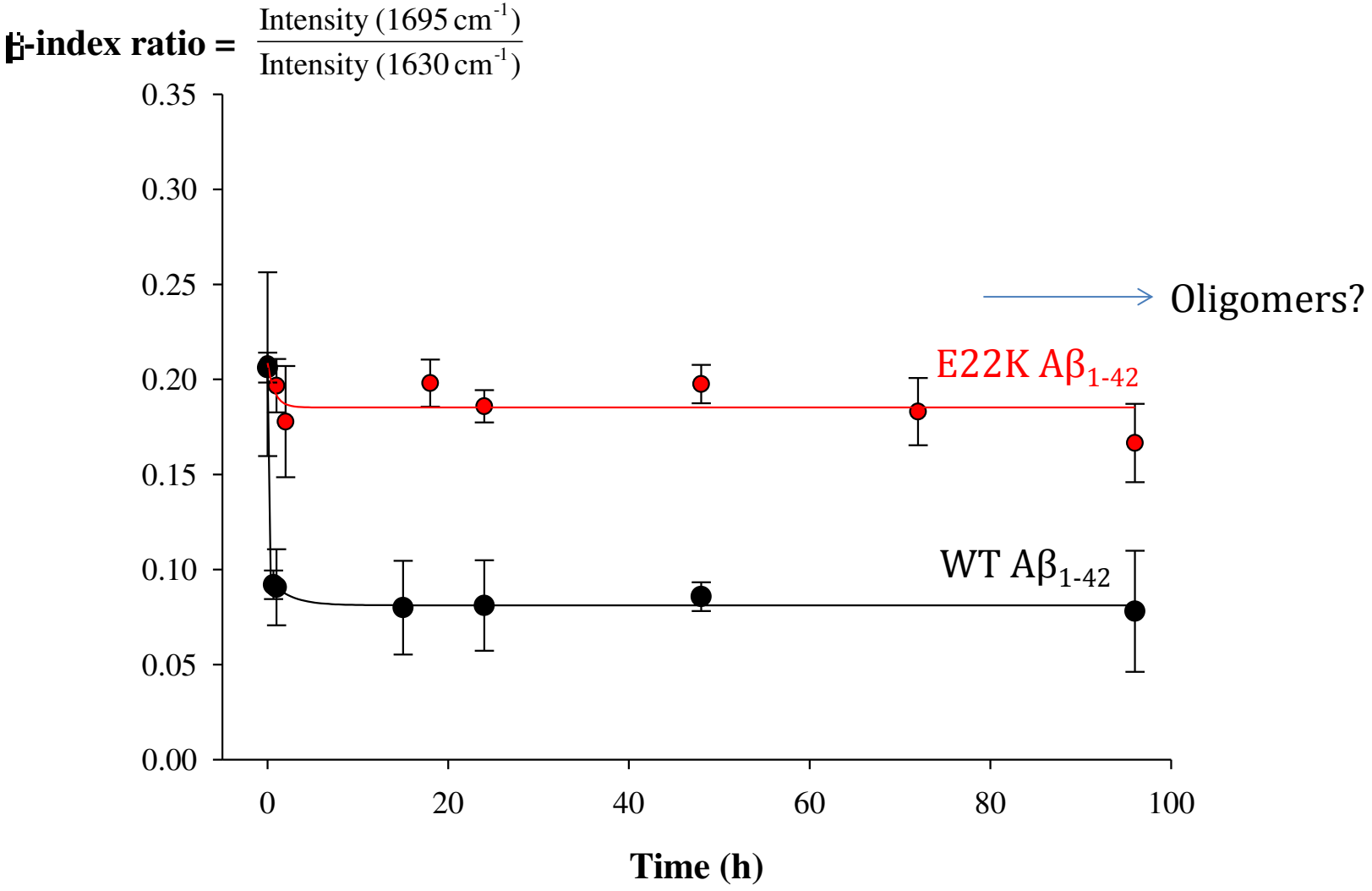
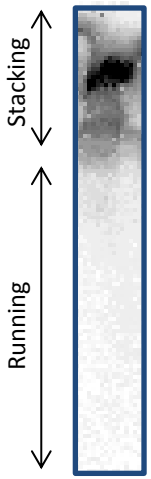
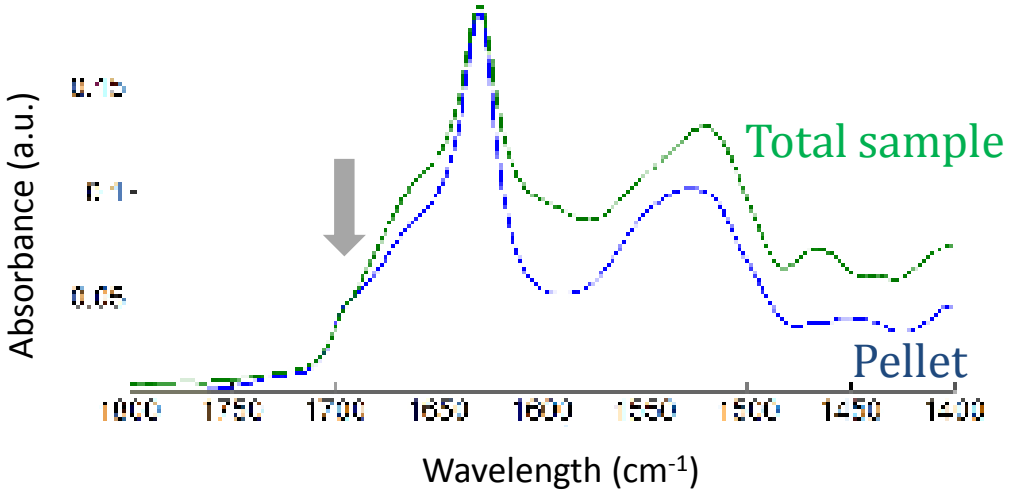


Hubin et al. Cell Mol Life Sci 2015

But: Fibrils and not remaining oligomers are responsible for observed antiparallel β-sheet conformation



Centrifugation
+
Resuspension pellet



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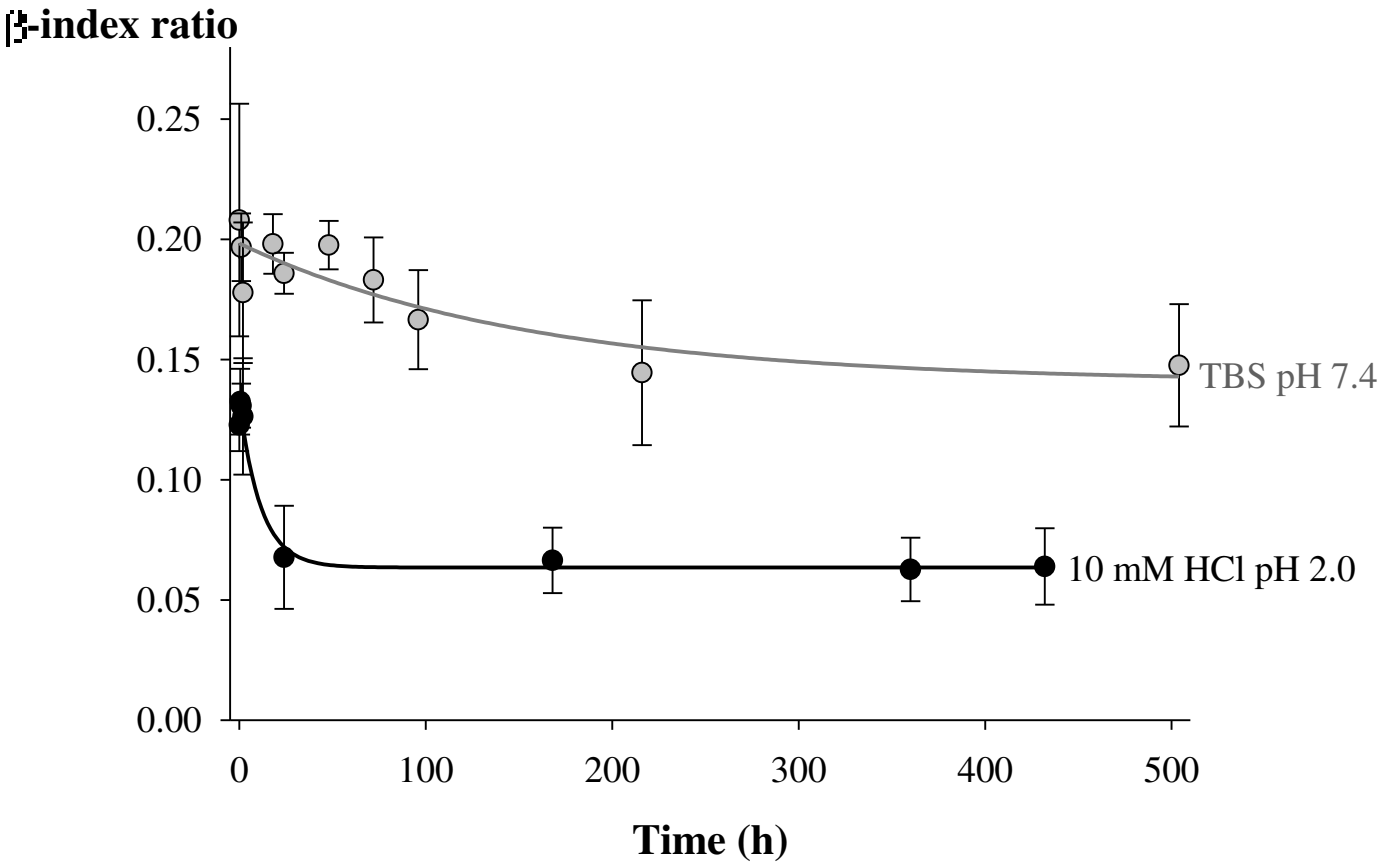
E22K $A\beta_{1-42}$ fibrils can convert from antiparallel to parallel β-sheet structure in an acidic environment (switch from pH 7.4 to pH 2.0)



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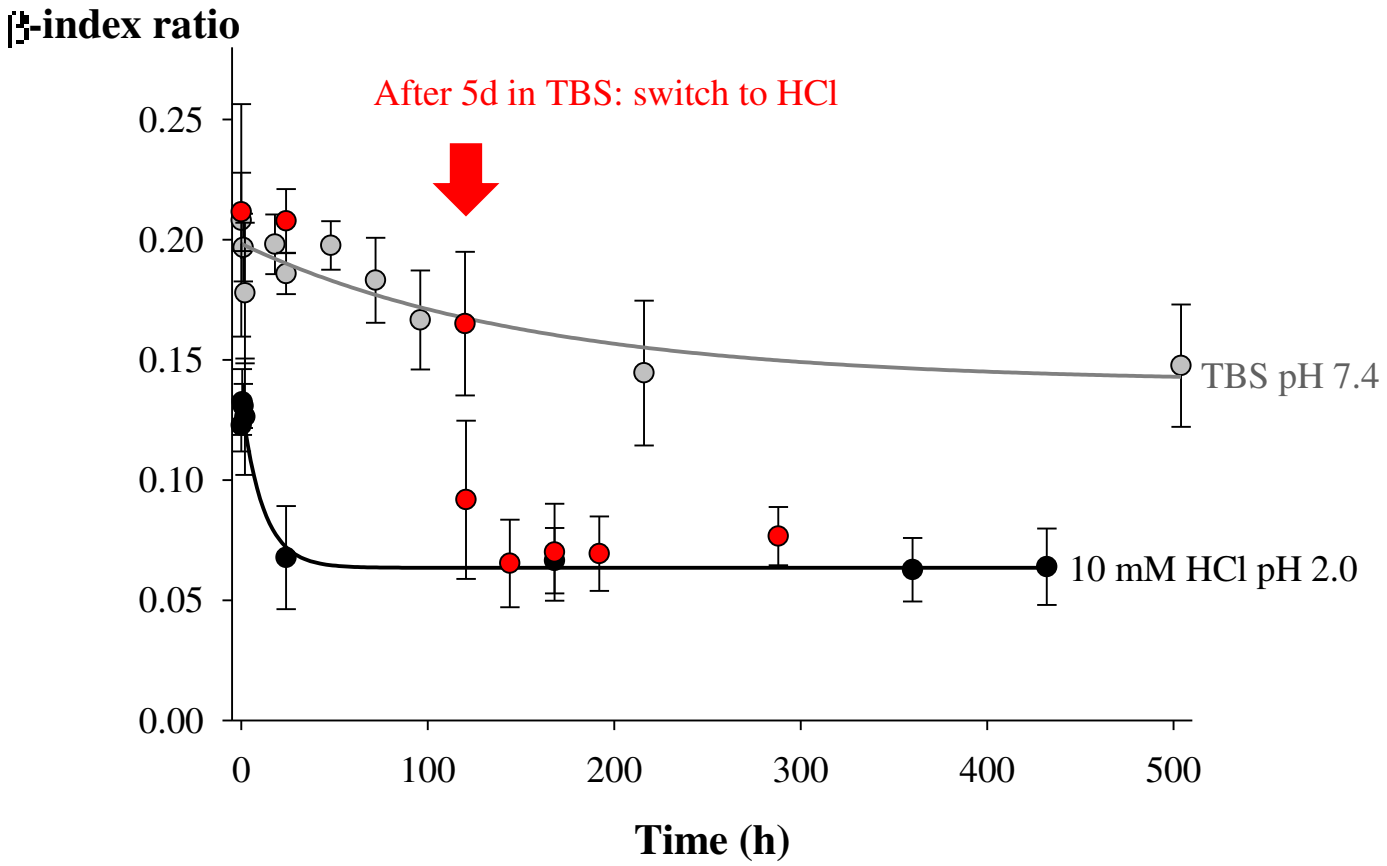

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Protease resistant?
Impact on BBB permeability?

Conclusions

‘Physiologically’ relevant mixtures of Aβ peptides may behave differently from what you would expect based on their isolated characteristics.

What is the perfect in vitro read-out for AD-related toxicity?

Antiparallel β-sheet fibrils exist, are remarkably stable but can also be converted upon changing environmental conditions.

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Kris Pauwels

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Joost Schymkowitz
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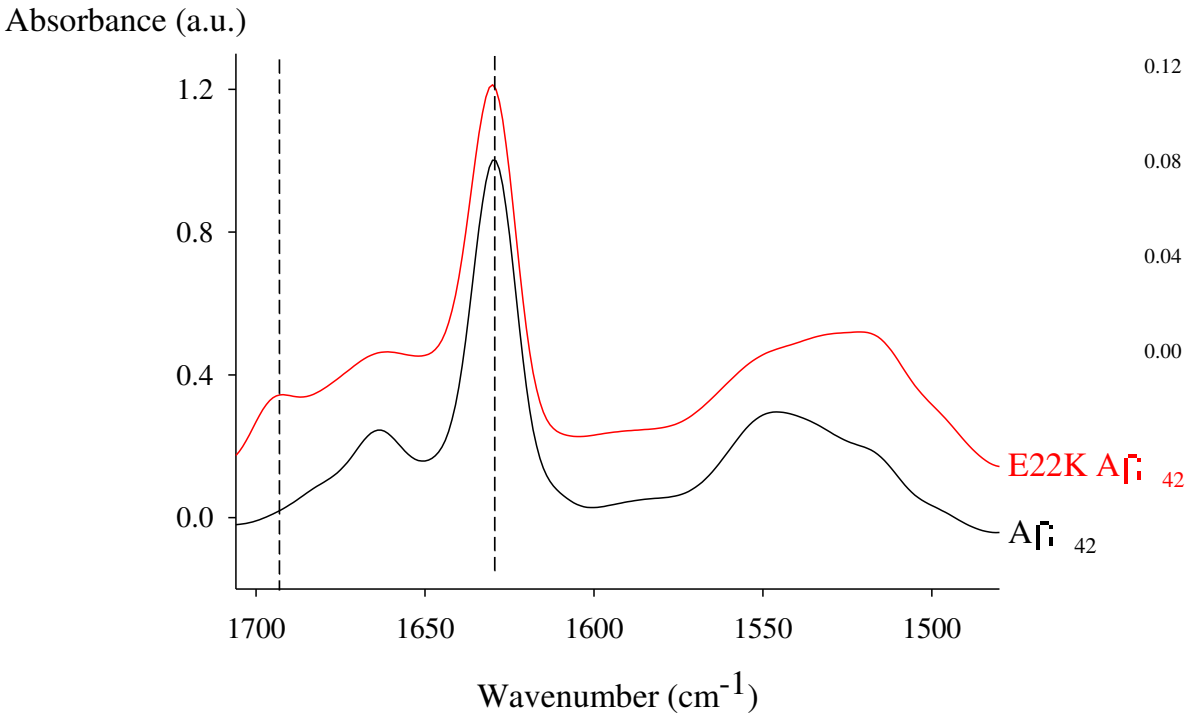
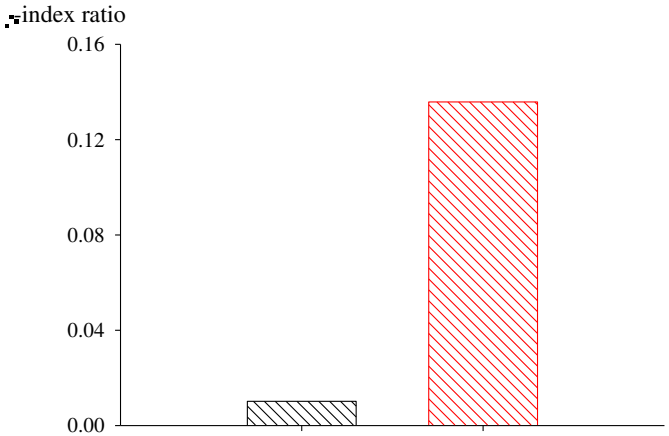
Geoff Kelly

University of Sussex
Louise Serpell
Kyle Morris
Thomas Williams

Funding

E22K Aβ: more random coil and turn at the expense of β-structure.

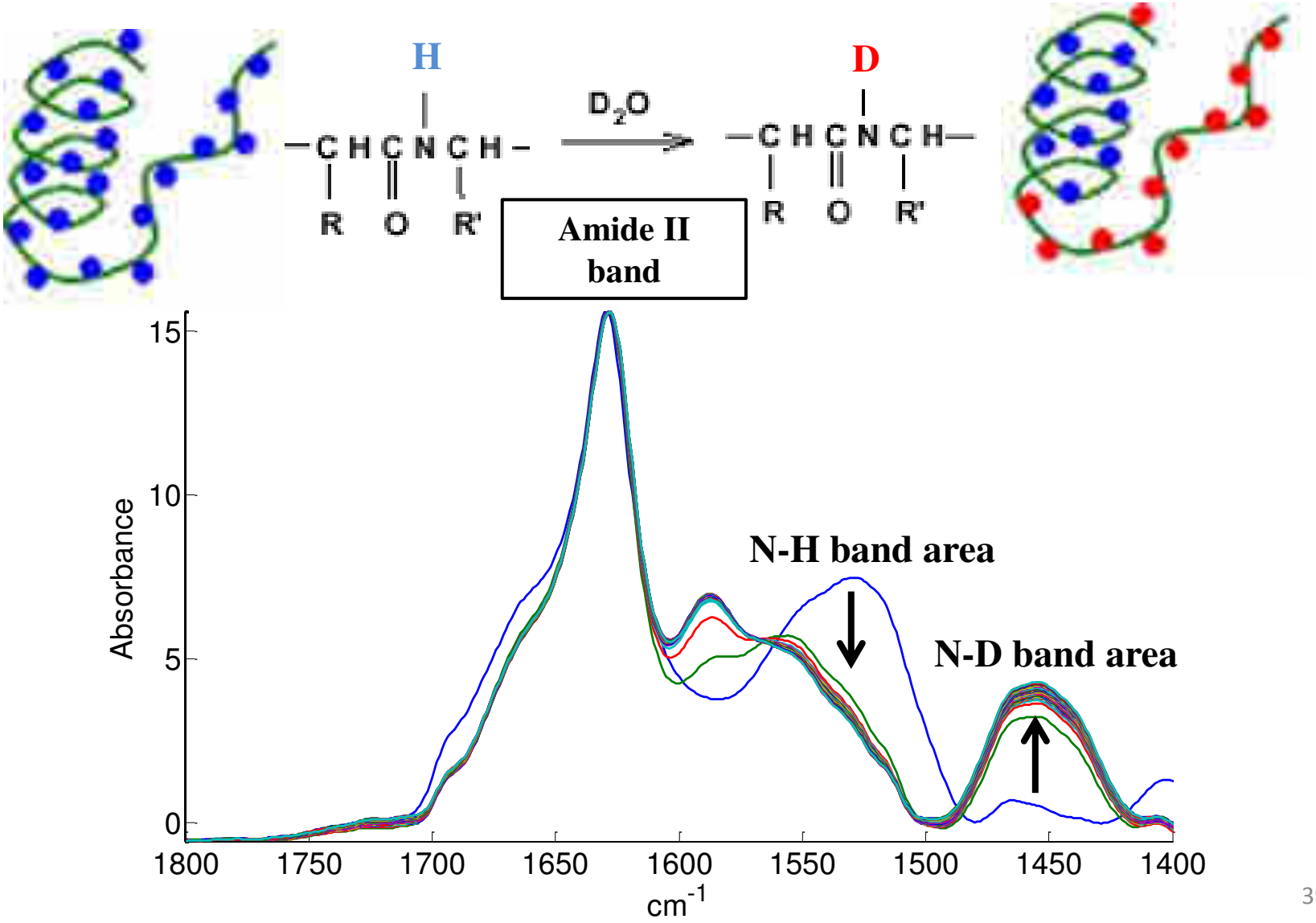




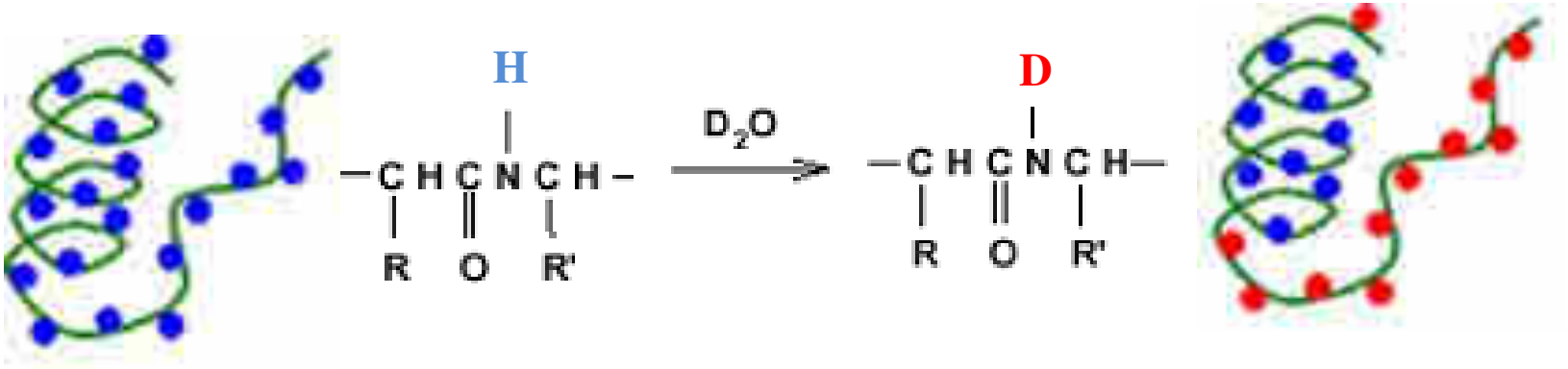
Approx. 8 residues

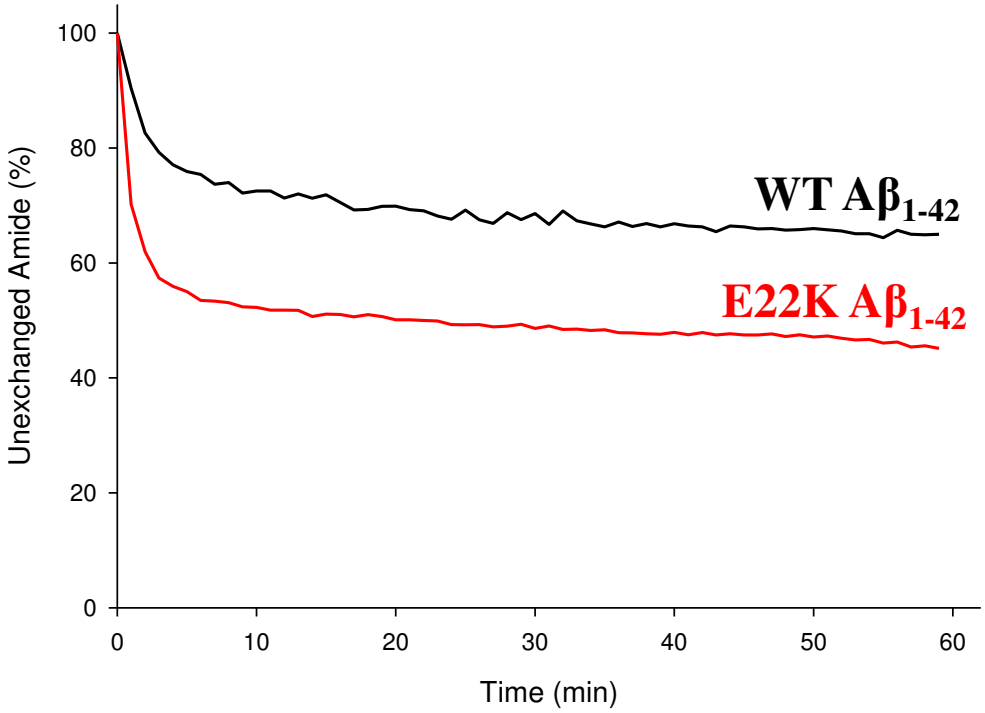
	$A\beta_{1-42}$	E22K $A\beta_{1-42}$
β-structure	**73**	**59**
α-helix	**8**	**10**
random	**11**	**18**
turn	**8**	**13**

H/D exchange shows 20 % difference in accessibility of backbone amide hydrogens between wild type and E22K $A\beta_{1-42}$ fibrils



H/D exchange shows 20 % difference in accessibility of backbone amide hydrogens between wild type and E22K $A\beta_{1-42}$ fibrils





H/D exchange monitored by ESI-MS and pepsin proteolysis reveals a higher solvent accessibility for the Italian mutant in the central region

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

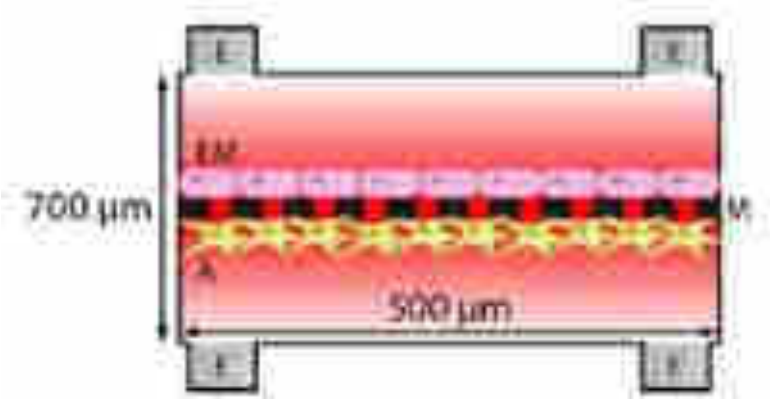
K

Peptide		Backbone amide protons exchanged	Protected NH/total
[1-19]	WT	14.0 ± 0.5	4/18
	E22K	14.3 ± 0.4	3.7/18
[20-42]	WT	7.3 ± 0.7	14.7/22
	E22K	9.6 ± 0.8	12.4/22
[35-42]	WT	3.1 ± 0.5	3.9/7
	E22K	3.4 ± 0.3	3.6/7

Conclusions & perspectives: is there a link between the underlying β-sheet orientation of Aβ fibrils and disease-associated pathology?

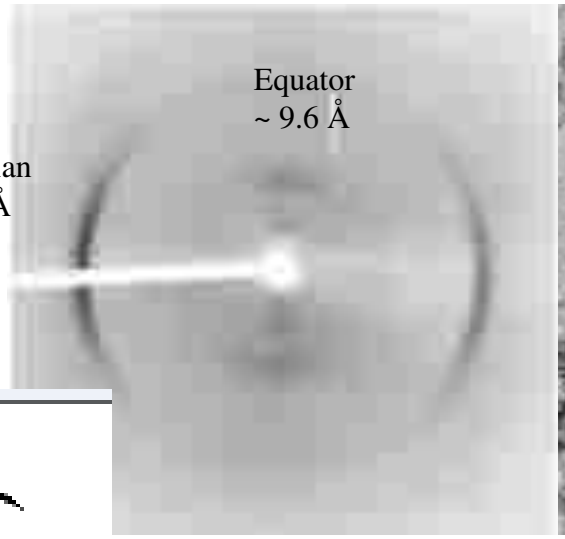
- E22K Aβ42 (and D23N Aβ40 and Aβ42) arrange into anti-parallel β-sheet fibrils.
- E22K fibrils are structurally different from WT Aβ42.
- Antiparallel β-sheet fibrils from E22K Aβ42 can rapidly (< 30 min) converse into parallel by changing the pH.

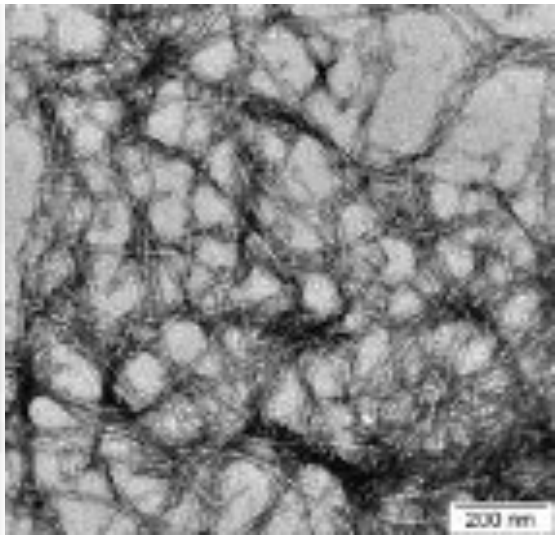
Work in progress – relation with CAA:
Do antiparallel β-sheet fibrils disintegrate the blood-brain barrier → blood-brain-barrier-on-a-chip technology.

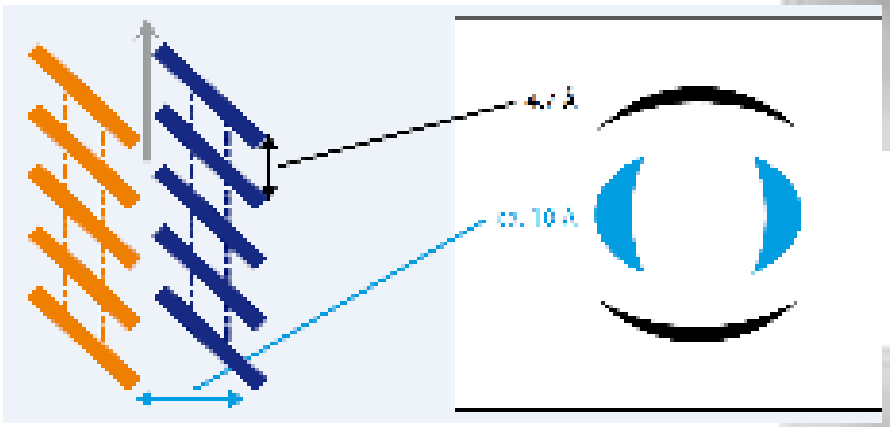


In our search for additional confirmation …

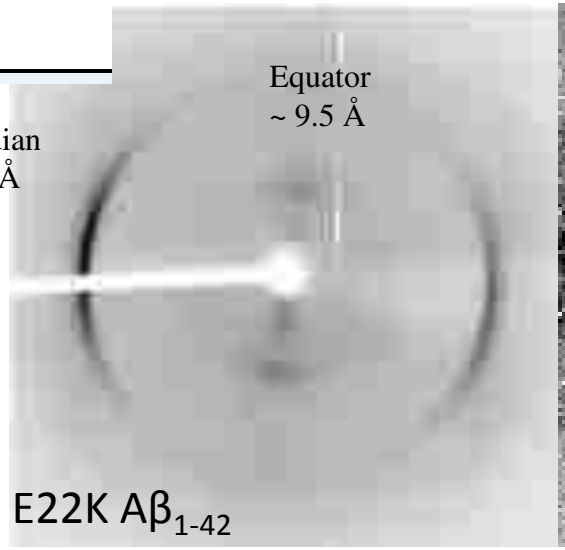
$A\beta_{1-42}$

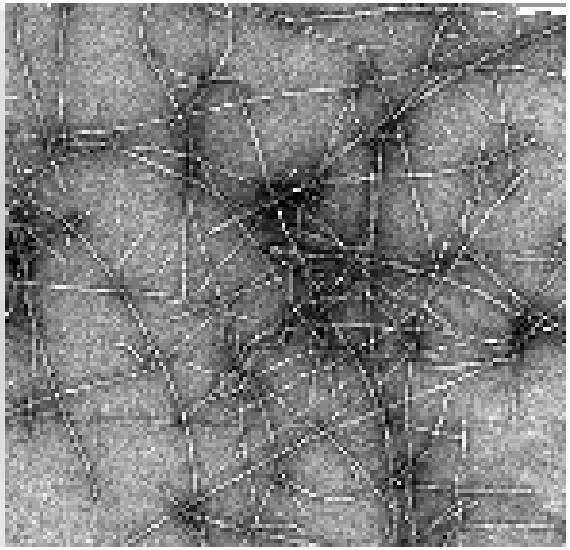






X-ray fiber diffraction → two reflections but no differences in structure.

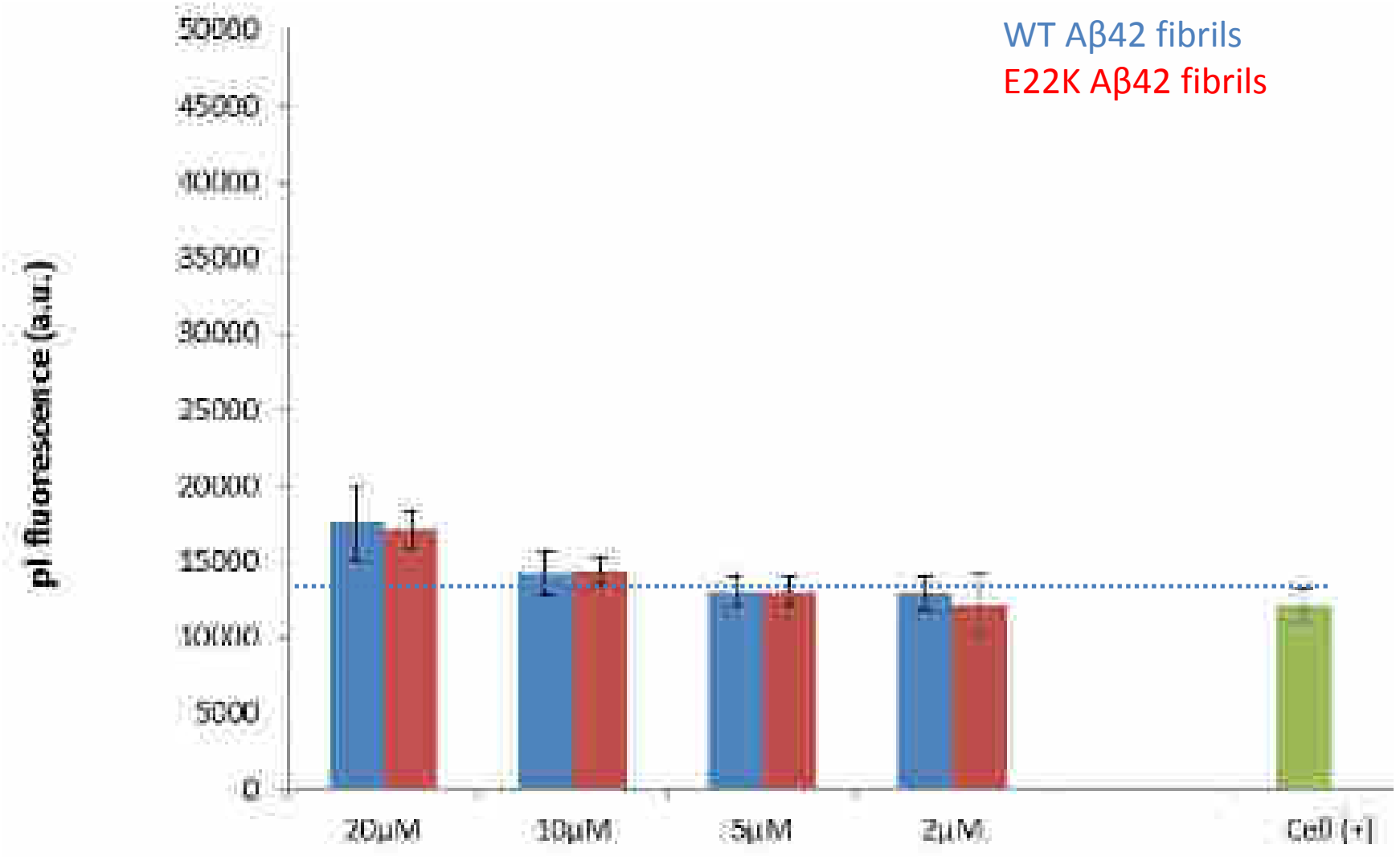




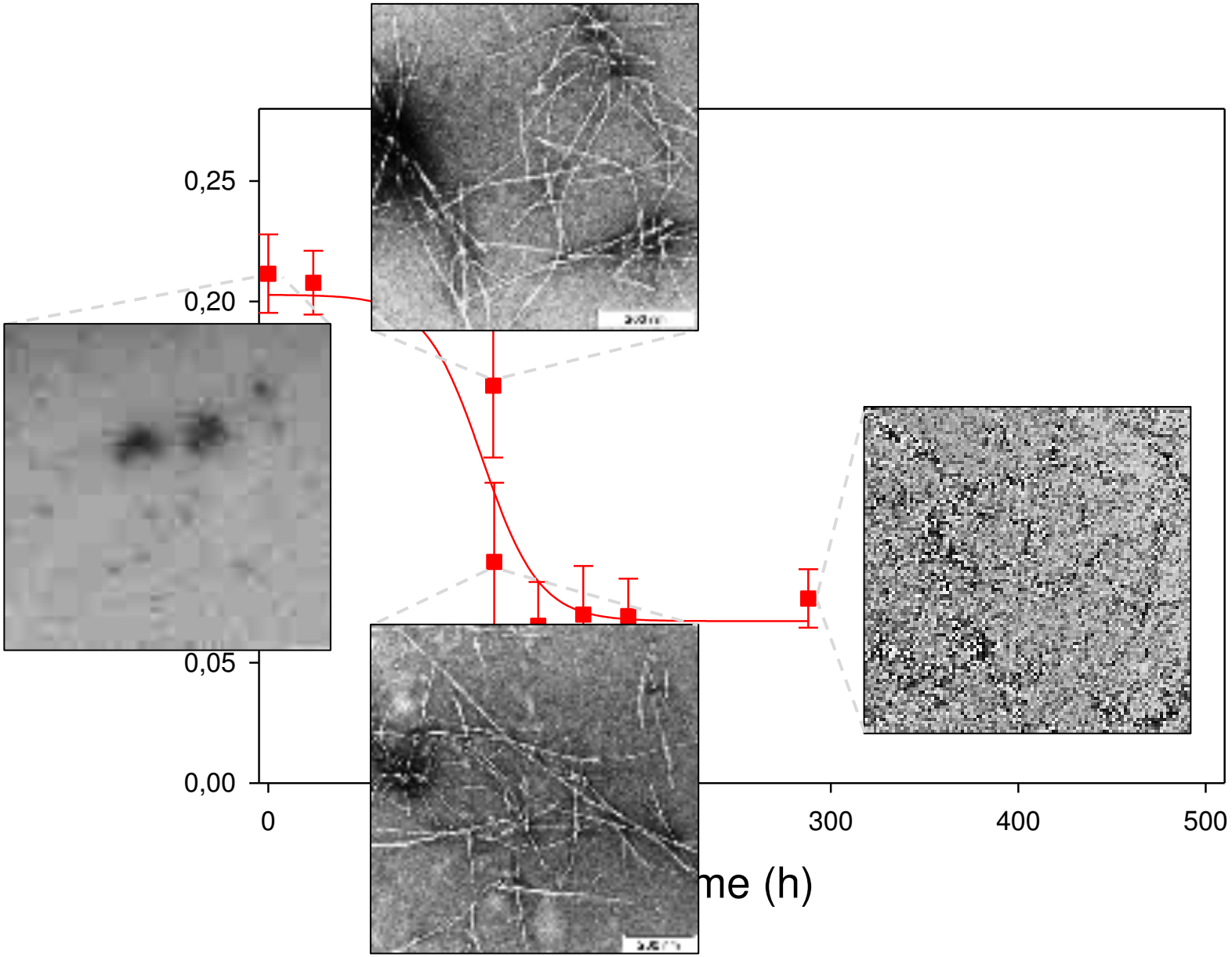
$E22K\ A\beta_{1-42}$

X-ray fibre diffraction in collaboration with L. Serpell (University of Sussex, UK), Morris KL & Serpell L (2012).

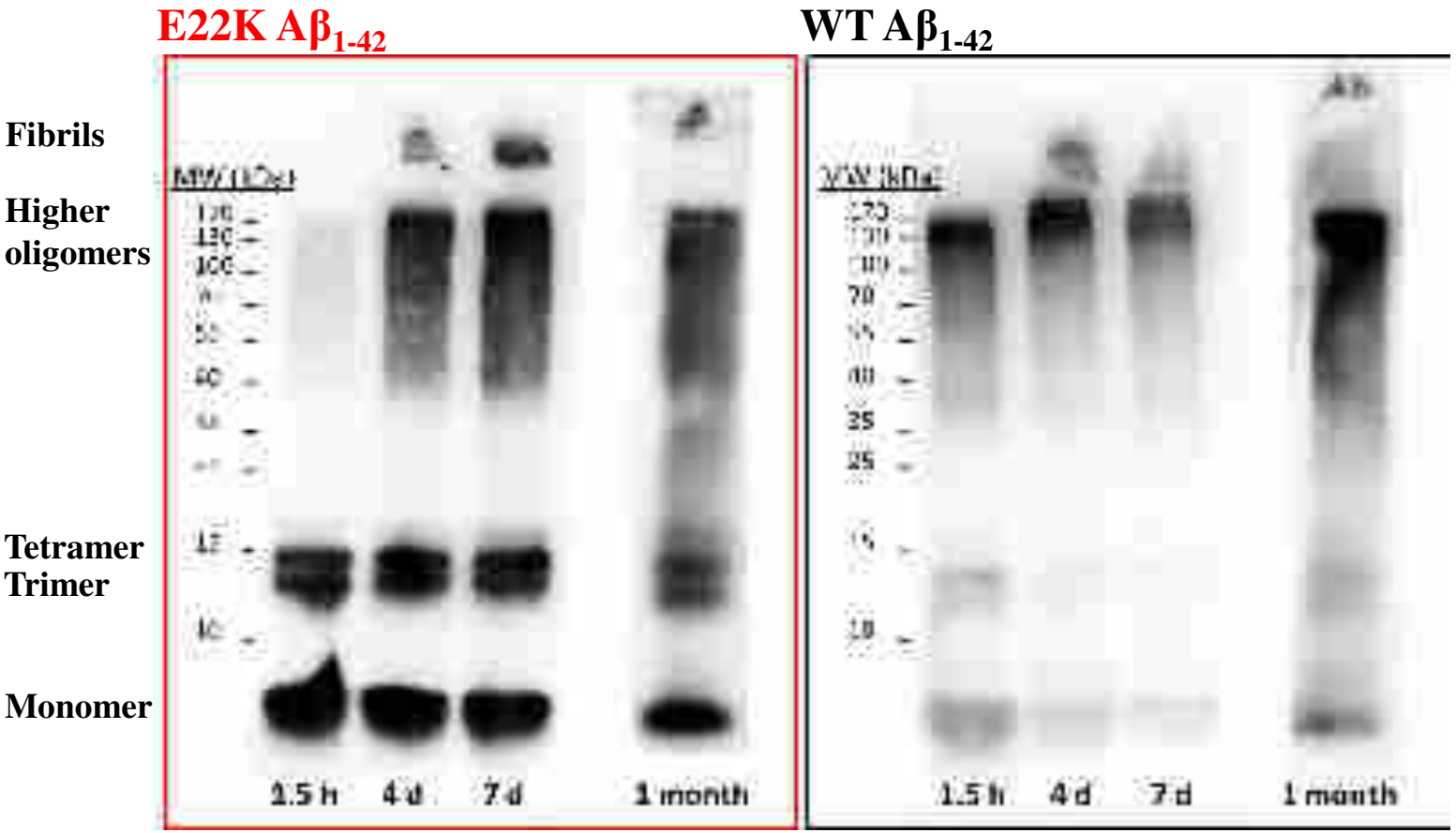
Fibrils from E22K and WT Aβ42 are similarly ‘toxic’ to astrocytes (and neuroblastoma cells).



Mature E22K $A\beta_{1\text{-}42}$ fibrils can convert from antiparallel to parallel structure

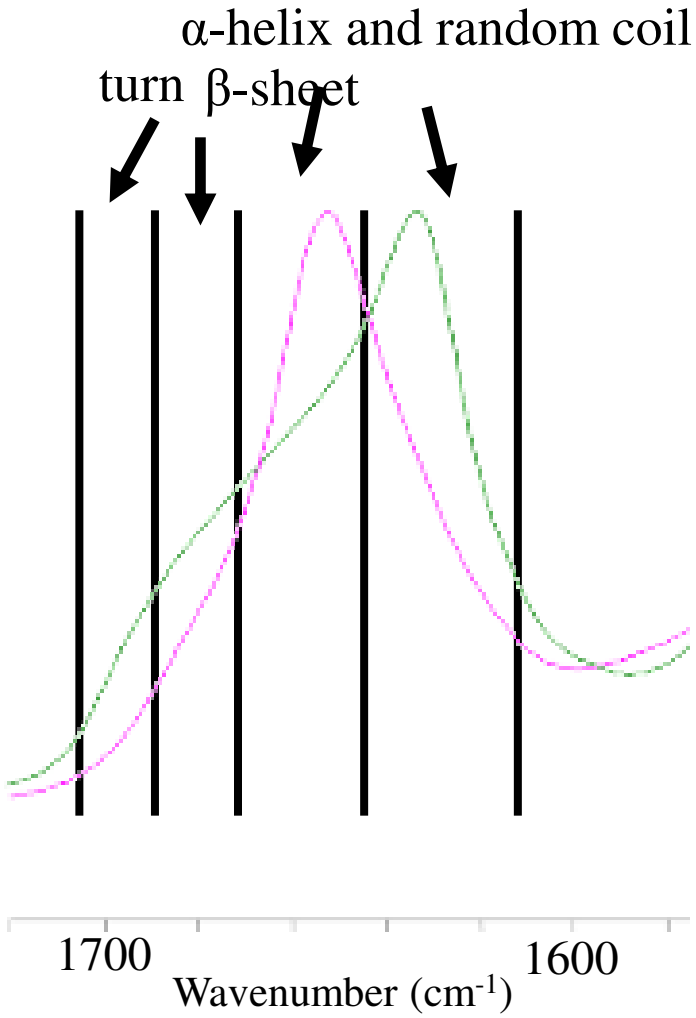


E22K $A\beta_{1-42}$ shows sustained (SDS-stable) oligomer formation.



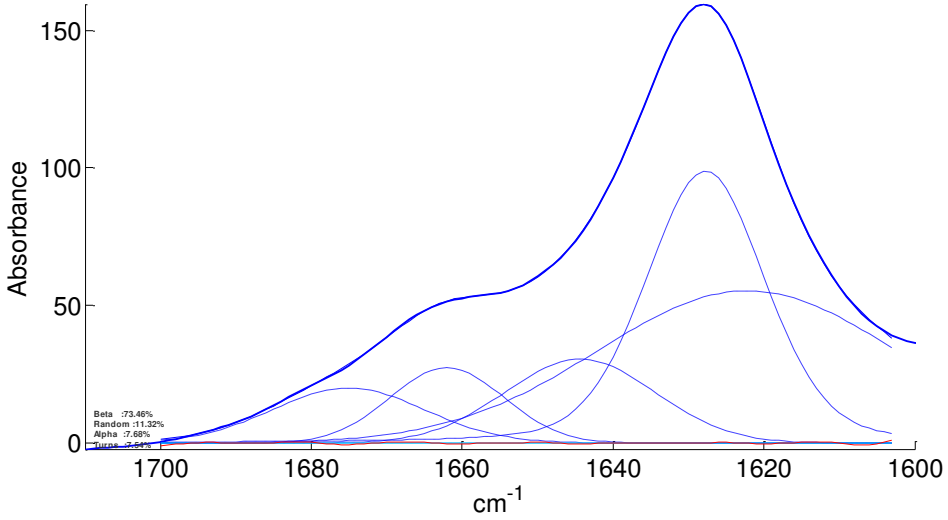
SDS-PAGE/WB (antibody 6E10)

Quantitative analysis consists of deconvolution of the amide I band into its primary components, followed by a curve fitting



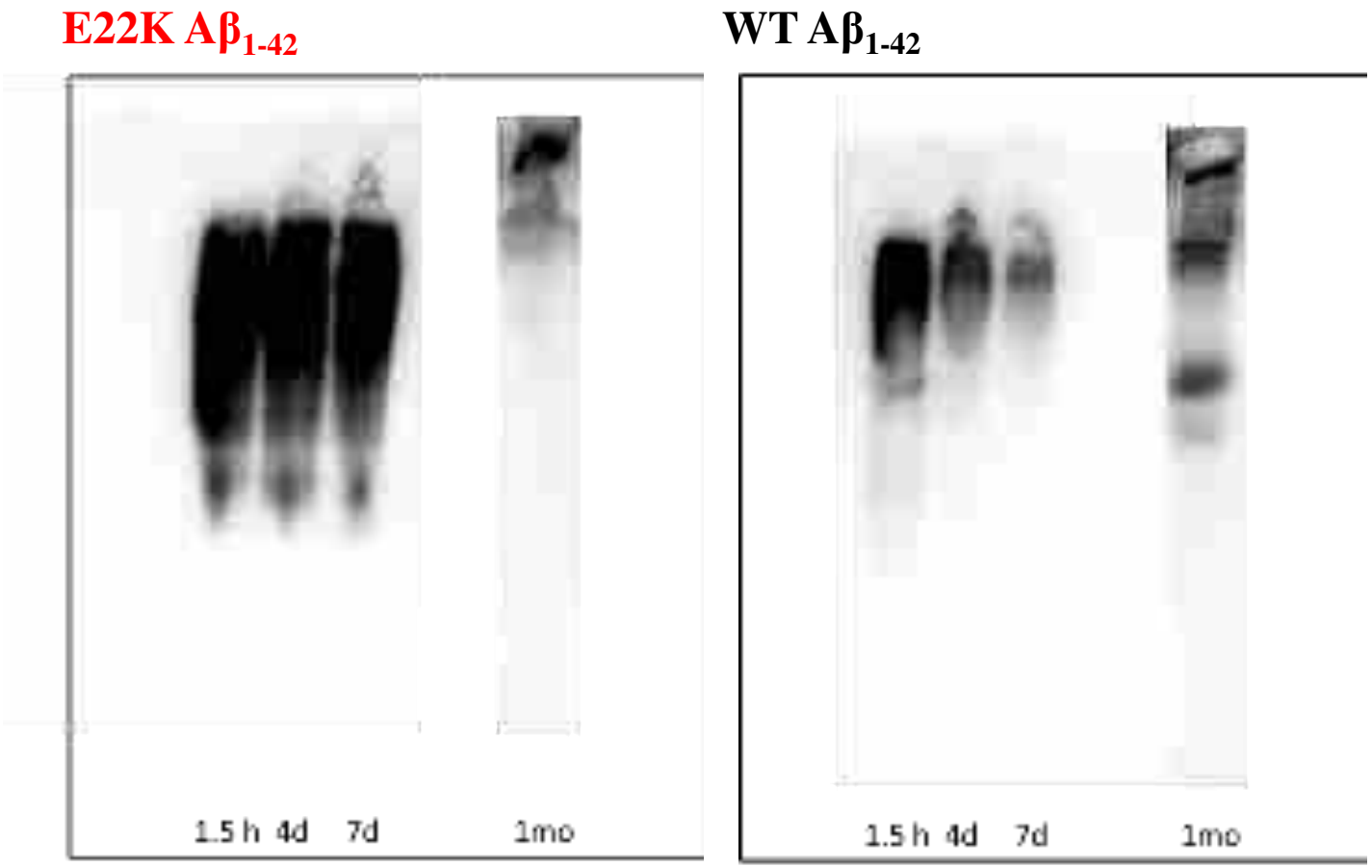
Parallel β-sheets:
1 peak at 1630 cm^{-1}

Antiparallel β-sheets:
peaks at 1630 cm^{-1} and 1695 cm^{-1}



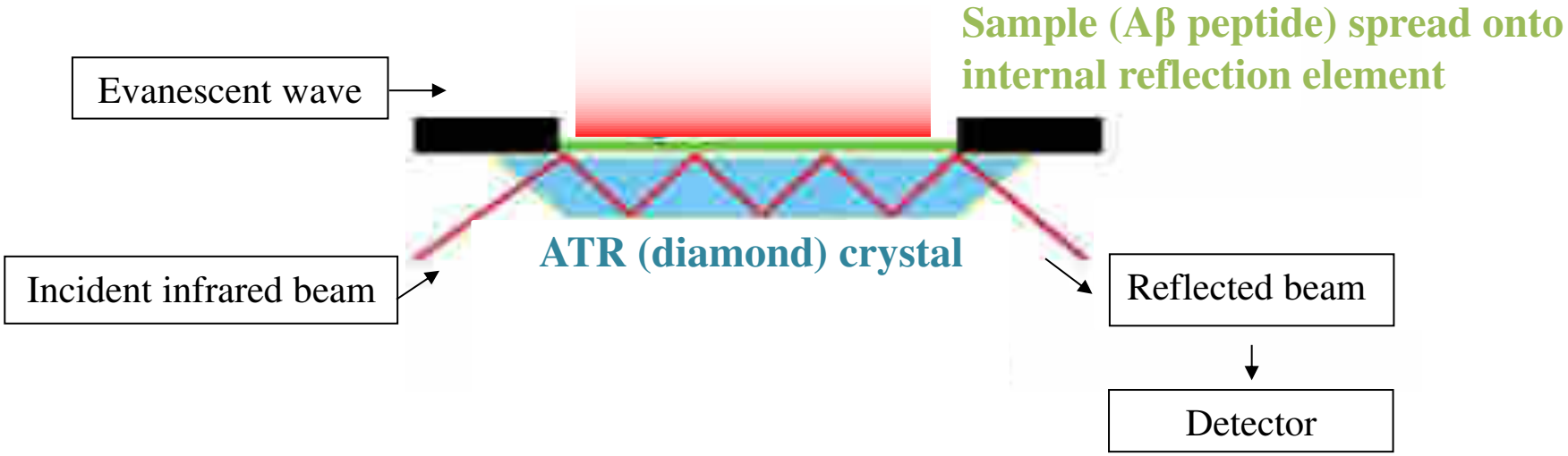
Goormaghtigh E. *et al* (1999)

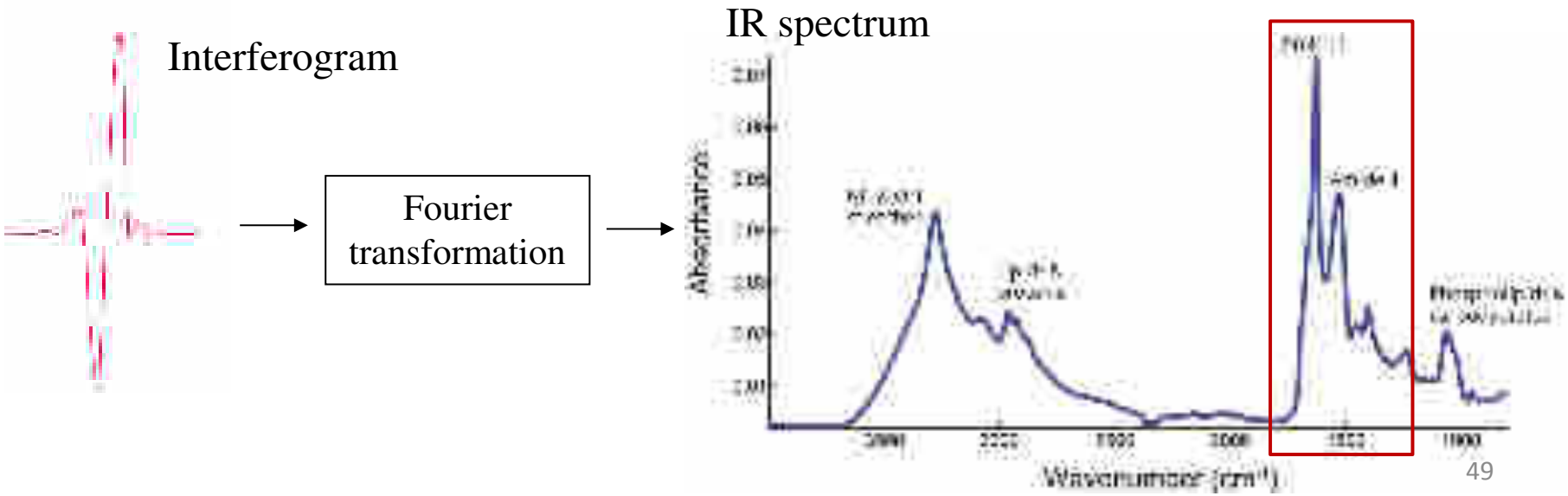
E22K $A\beta_{1-42}$ shows sustained (SDS-stable) oligomer formation.



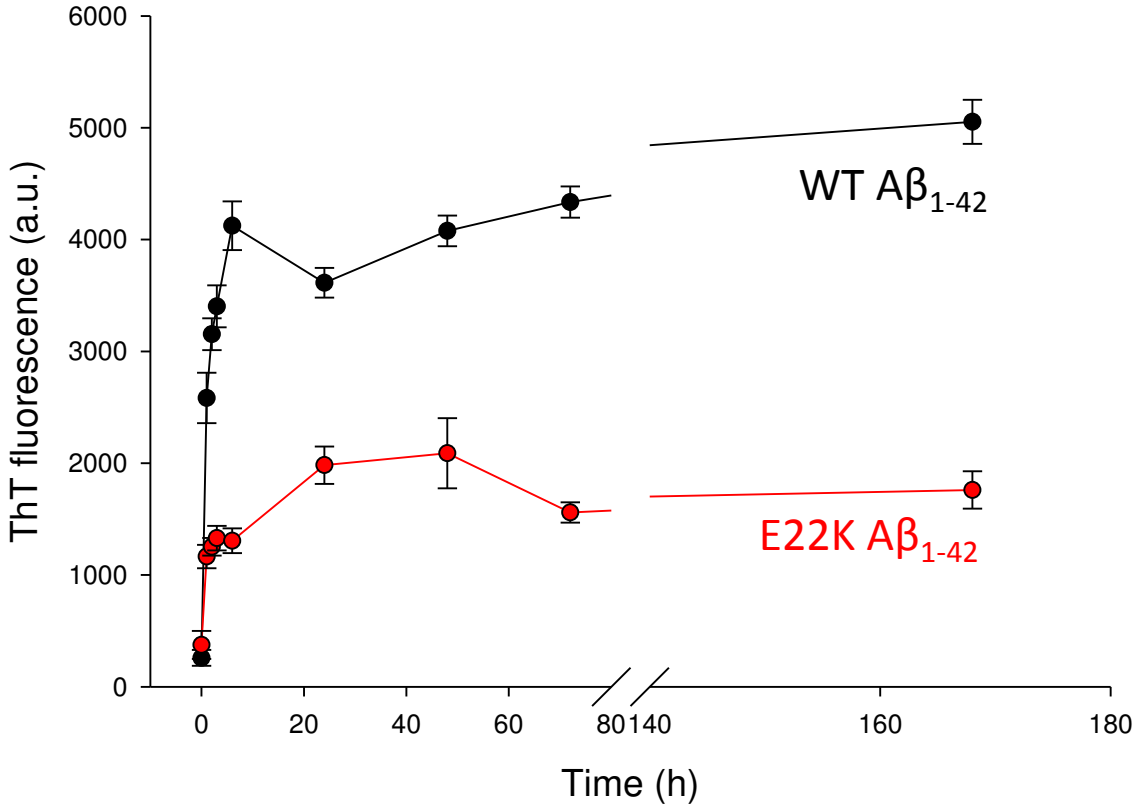
Native PAGE/WB (antibody 6E10)

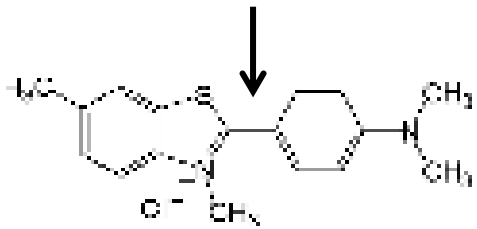
Attenuated Total Reflectance (ATR) – FTIR spectroscopy has the capability to identify functional groups (C=O, C-H, N-H, ...)



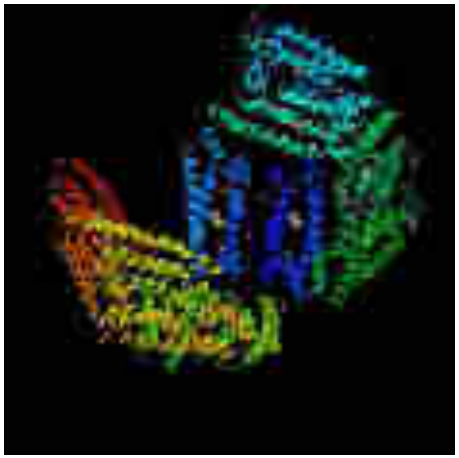


Thioflavin T (ThT) is less easily locked in its excited comformation in antiparallel fibrils, leading to a lower final ThT fluorescence intensity

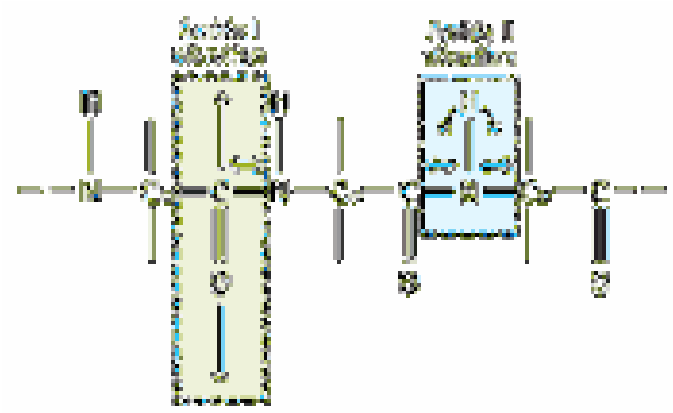


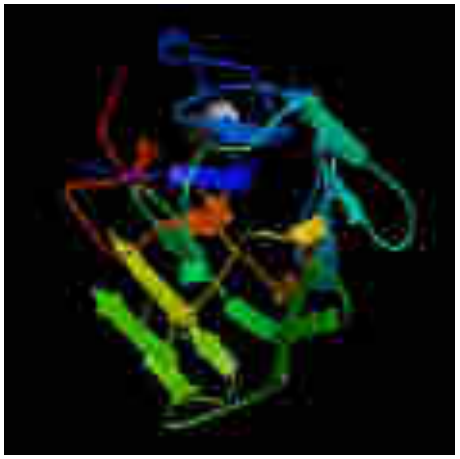


Amide I and Amide II bands contain secondary structure information

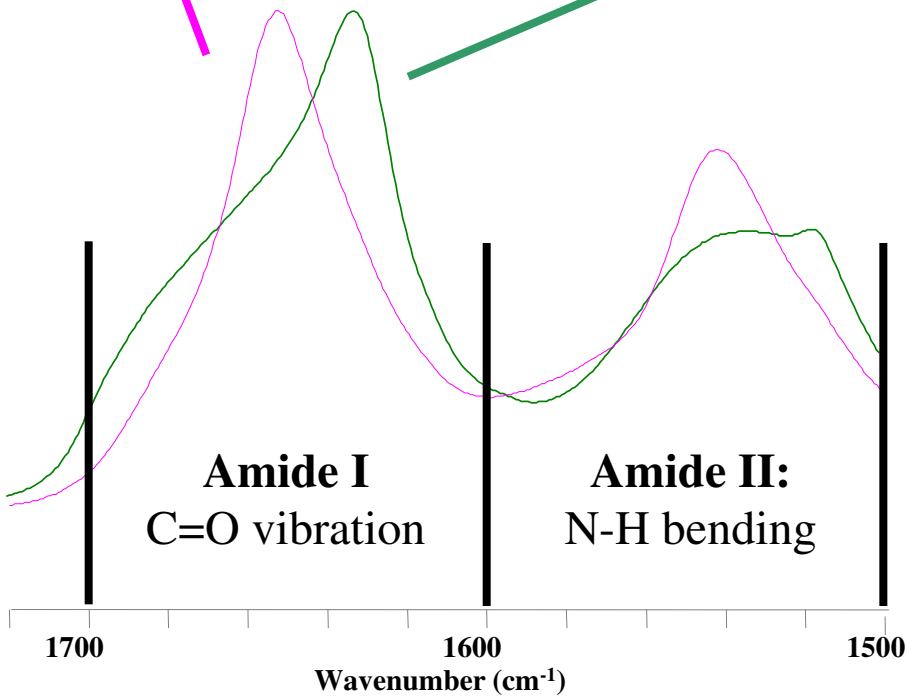


Ferritin

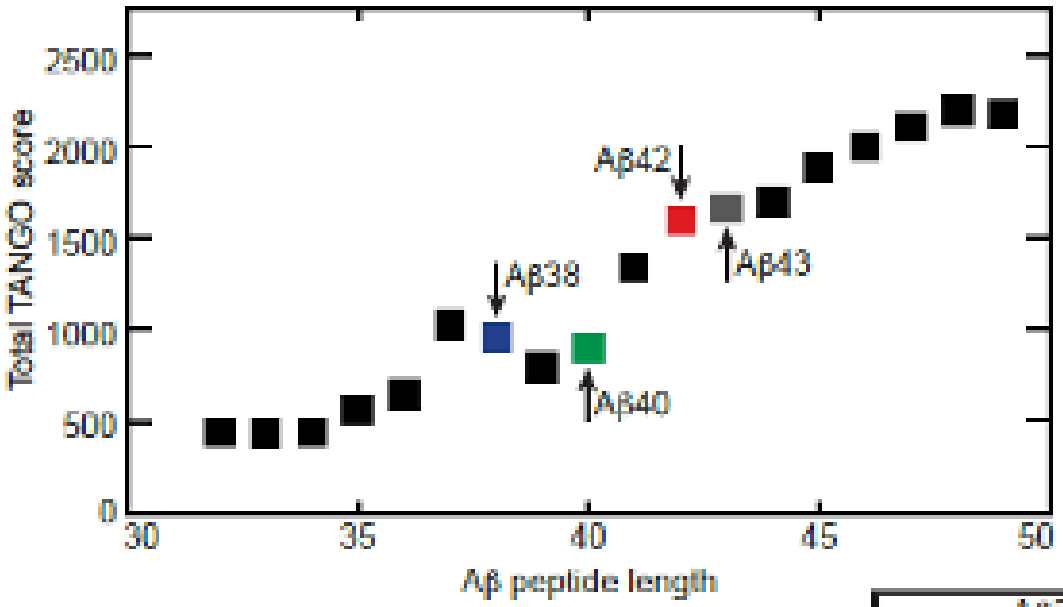




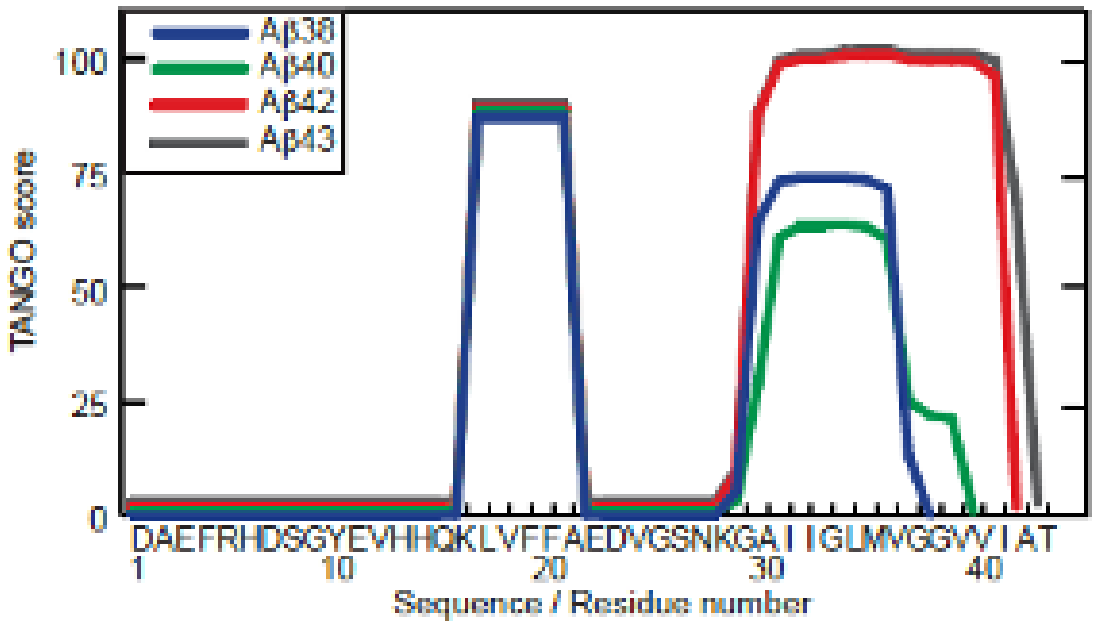
Concanavalin A



Aggregation of Aβ peptide is predicted to vary with peptide length



TANGO is an algorithm designed predict the aggregation propensity of peptide sequences.
www.tango.switchlab.org



Vandersteen et al (2012) J Biol Chem 287: 36732-36743

Antiparallel β-sheet architecture of Italian mutant E22K $A\beta_{1-42}$ fibrils

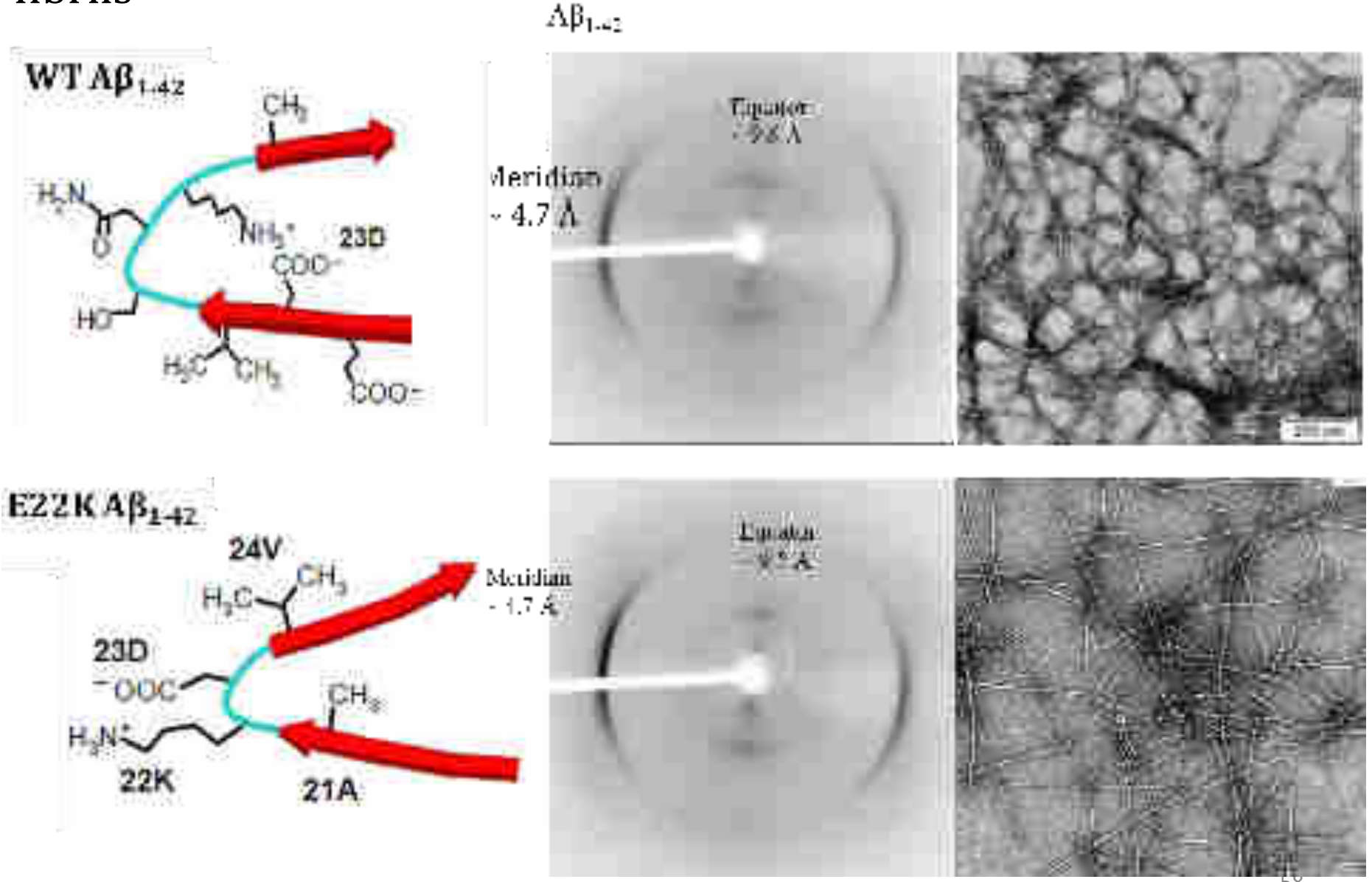


Figure adapted from Masuda *et al.* Bioorg & Med Chem (2005), FDX: Louise Serpell, Sussex University, UK