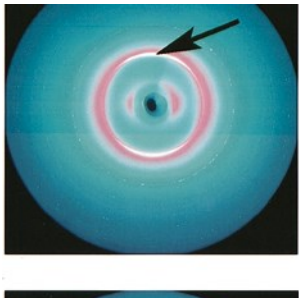
Common Core Structure of Amyloid Fibrils by Synchrotron X-Ray Diffraction



Michael Foody

May 5th, 2015

M. Sunde, L.C. Serpell, M. Bartlam, P. Fraser, M. Pepys, C. Blake, *J. Mol. Biol.* **273**, 729-739, (1997)

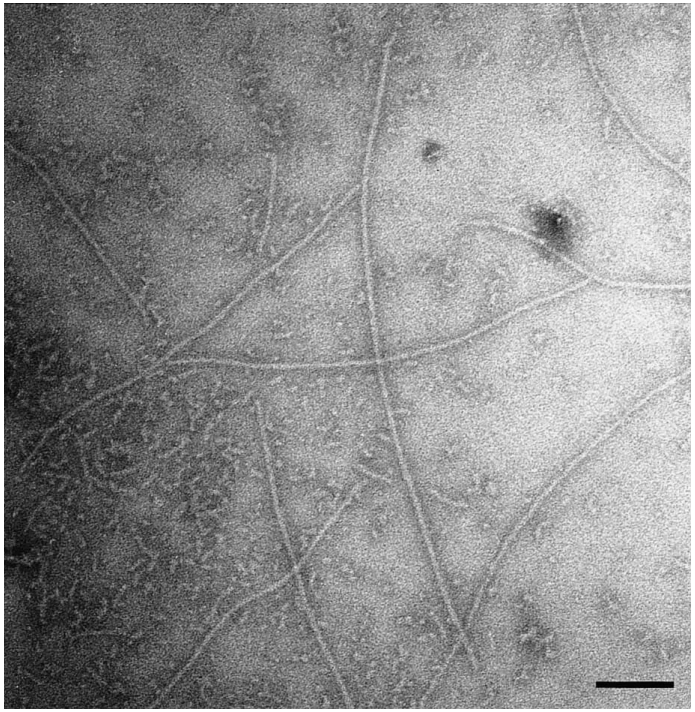
Outline

1. Background
   - Amyloid fibrils
   - X-Ray Fiber Diffraction
2. Experimental design & method
3. Findings
   - Meridional Reflections
   - Equatorial Reflections
4. Conclusions

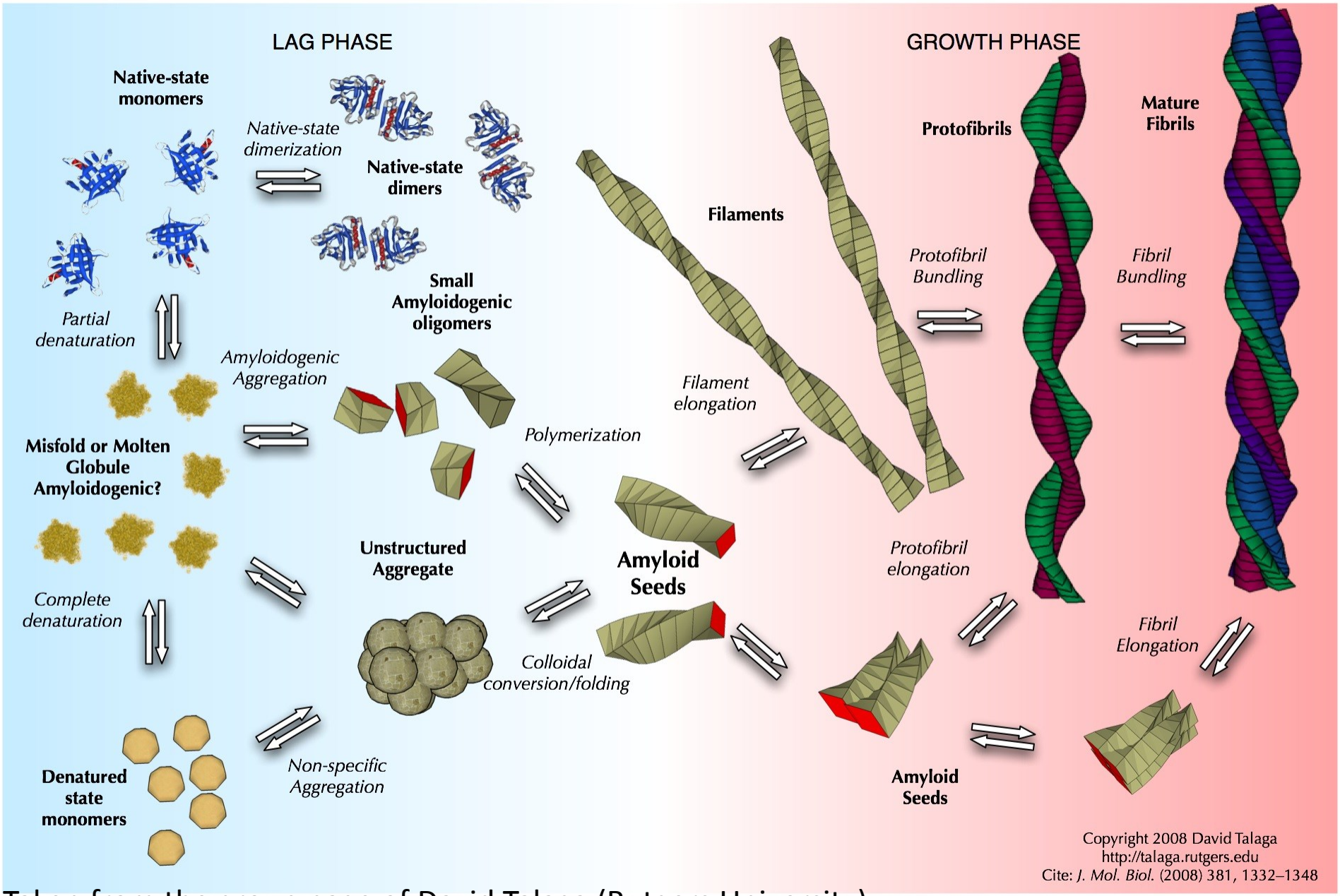
Amyloid Fibrils

- Extracellular deposition of insoluble protein fibrils[1]
- Wide variety of protein subunits
- Disrupt tissue functions
- No treatment

2



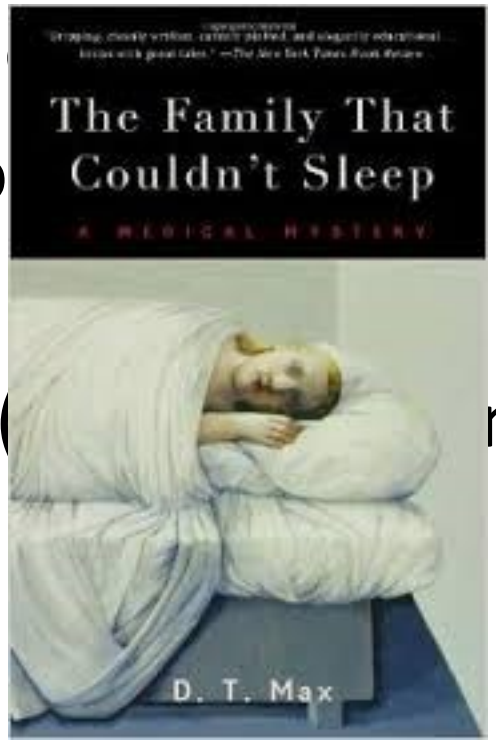
[1]M. Sunde, L.C. Serpell, M. Bartlam, P. Fraser, M. Pepys, C. Blake, *J. Mol. Biol.* **273**, 729-739, (1997)
[2]L. C. Serpell, *Biochimica. et Biophysica. Acta*, **1502**, 16-30 (2000)



Taken from the group page of David Talaga (Rutgers University)
http://talaga.rutgers.edu/research/index.php

Disease

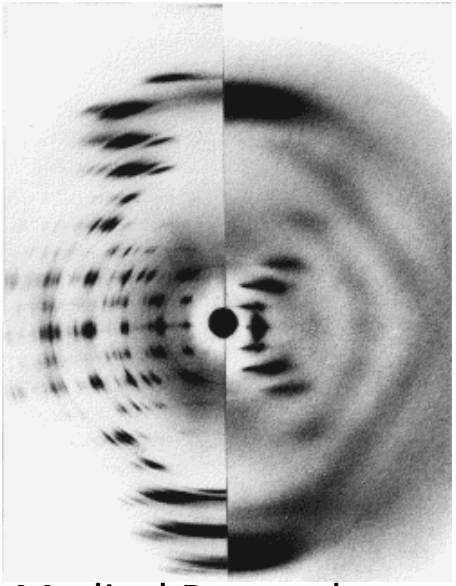
- Alzheimer's Dis
- Systemic amylo
- Maturity onset
- Prion Diseases (ncephalopathy)
- No treatment



The Family That Couldn't Sleep (Random House)
D.T. Max

X-Ray Fiber Diffraction

- Many biological molecules do not crystallize
- Fibers can be oriented in the same direction
- Fibers have one of their crystallographic axes along fire axis (*c*) – Periodic order
  - Azimuthimal orientation is random

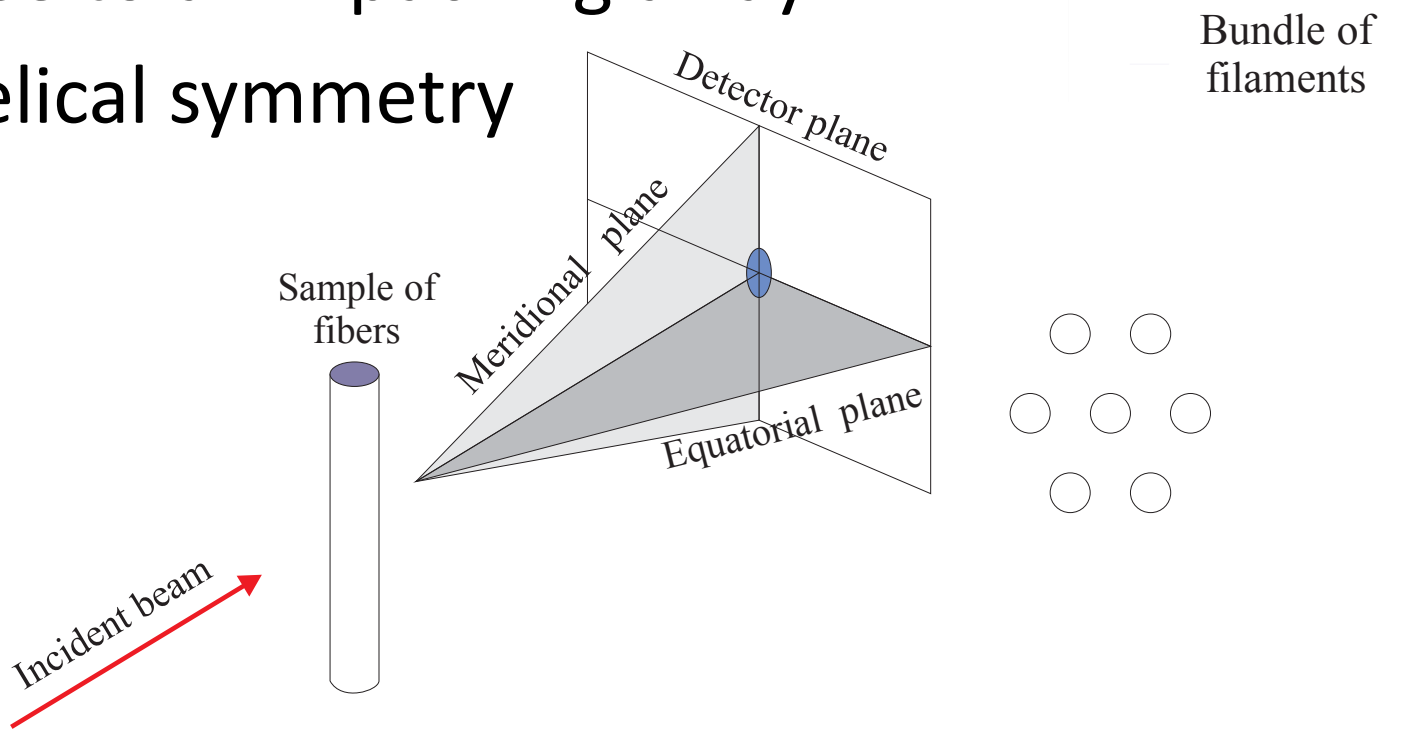


[1]

[1]"Max Planck Institute for Medical Research." Max Planck Institute for Medical Research. Web. 5 May 2015. <http://www.mpimf-heidelberg.mpg.de/en>.

Fiber XRD – Background

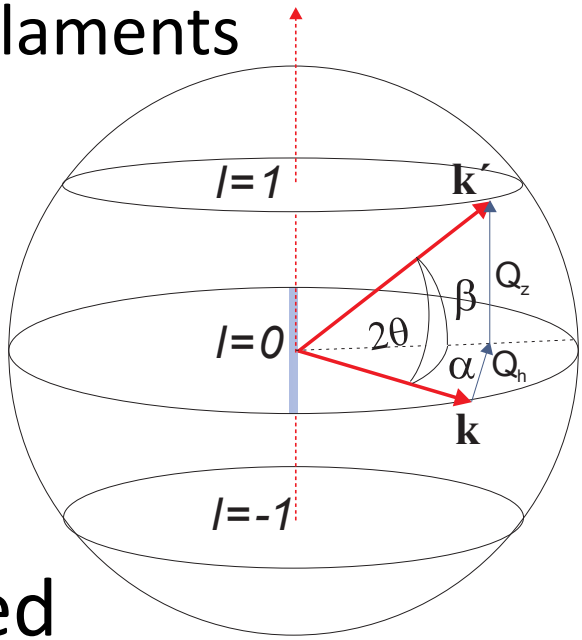
- Monochromatic beam (**k**), perpendicular to fiber axis
- Condense to a 2D packing array
- Show helical symmetry



Elements of Modern X-Ray Physics (2nd Edition), Wiley, (2011)

Fibre XRD – Background

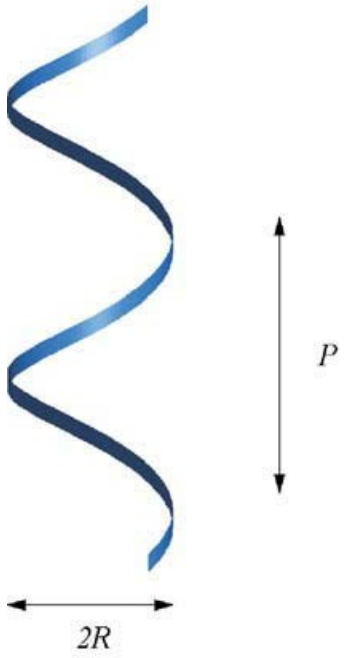
- Fiber is rotated (β)
- The azimuthimal angle (α) changes
- Bragg reflections:
  - Equatorial (horiz.) from bundles of filaments
  - Meridional (vert.) from periodicity
- Scattering vector (**Q**) is separated
  - $Q_z = lc^*$ (only l=±1, ±2, …) & Q_h
- $\cos(2\theta)=\cos(\beta)\cos(\alpha)$
- For cetain α, $\lambda = 2d_{hkl}\sin(\theta)$ is fulfilled

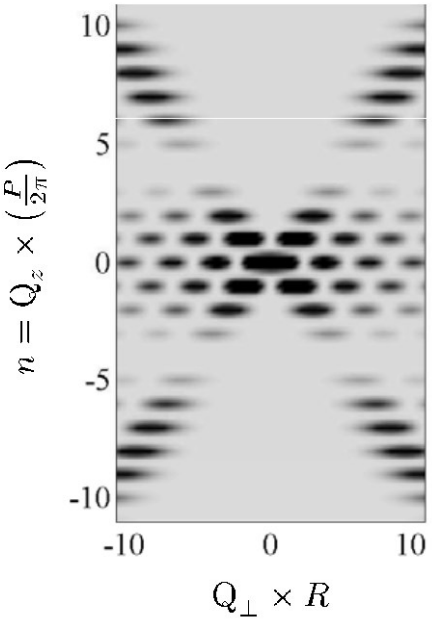


Elements of Modern X-Ray Physics (2nd Edition), Wiley, (2011)

Fiber XRD – Helix

- Layer lines result from *c*-axis structure
- Characteristic "helix cross"
  - Comes from order of Bessel Function
- Position of first strong peak is R^{-1}
- Spacing of layer lines is the P^{-1}
  - Large separation means small pitch





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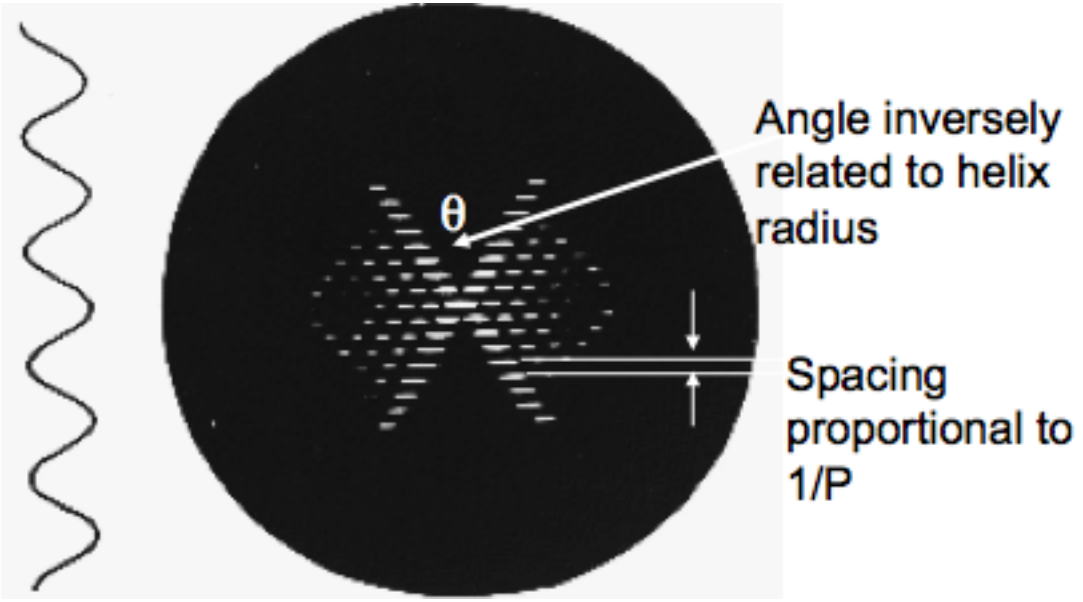


"Max Planck Institute for Medical Research." Max Planck Institute for Medical Research. Web. 5 May 2015. <http://www.mpimf-heidelberg.mpg.de/en>.

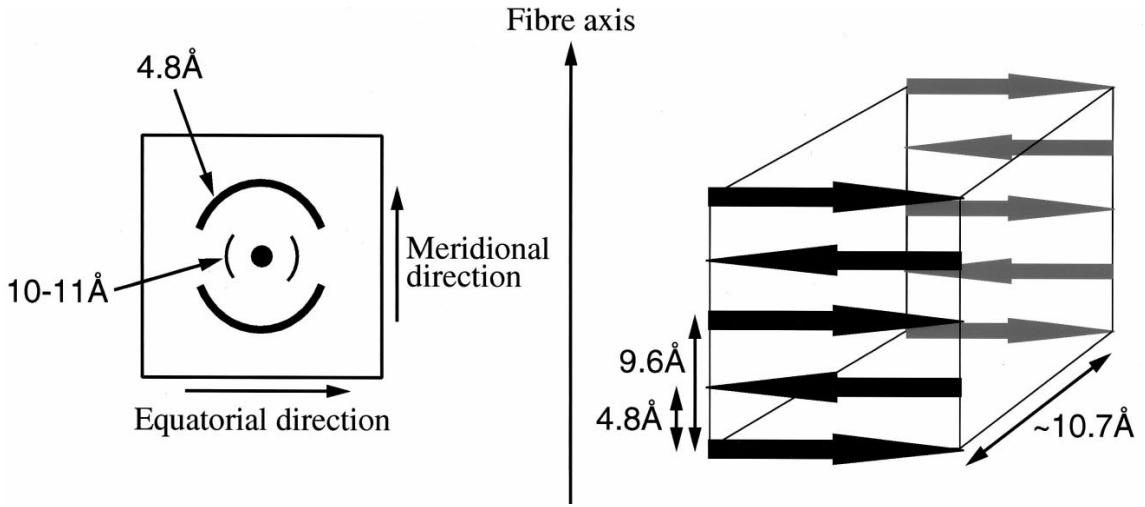
Early Characterization

- Amyloid fibrils
  - Difficult to study using conventional techniques
  - Intermediate length
  - 70-120Å in diameter
  - Unbranched
- 4.7-4.8Å Meridonial Reflections[1]
- 10Å Equatorial Reflections
- Cross-β structure

E.D. Eanes, G.G. Gleener, *Journ. of Histochem. and Cytochem.*, **16**, 673-677 (1968)

Early Characterization

- Characteristic of a cross-β structure
- High degree of structural similarity from different precursors
- But how much?



L. C. Serpell, *Biochimica. et Biophysica. Acta*, **1502**, 16-30 (2000)

Experimental Purpose

"to determine the extent and nature of this similarity we have used intense synchrotron X-ray beams to obtain the first high-resolution diffraction patterns from a range of different *ex vivo* and synthetic amyloid fibrils."

M. Sunde, L.C. Serpell, M. Bartlam, P. Fraser, M. Pepys, C. Blake, *J. Mol. Biol.* **273**, 729-739, (1997)

Experimental Details

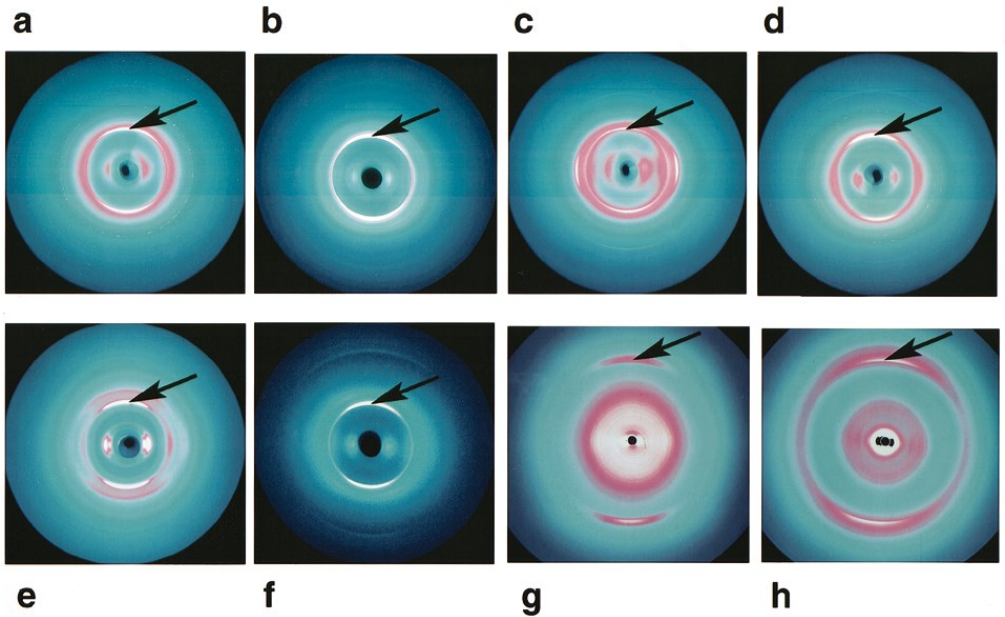
- Fibril/H_2O suspensions on stretch frame oriented with **B** (2T) while H_2O dries
- Experiment performed at ESRF
- X-Ray detectors are 18-30cm diameter
  - MaRResearch Image Plate

Samples

a. ATTR2, Gly47Val transthyretin
b. ATTR1, Val30Met transthyretin
c. AAPOAI, Leu60Arg apolipoprotein A-I
d. AL, monoclonal λ immunoglobulin light chain
e. FTTR, peptide with the sequence of the A-strand of human transthyretin
f. FIAPP, peptide with the sequence of residues 20 to 29 of the islet-associated polypeptide
g. AA, amyloid A protein
h. ALys, Asp67His lysozyme

Findings

- Patterns dominated by cross-β reflections (meridional)
- Also have some additional previously unreported reflections
- Folding patterns of ***different*** precursors are ***very*** similar



Meridional Findings

- Reveals periodicity along *c*-axis
- Reflections go out to 2Å – Highly structured
- Intense reflections at 4.7-4.8Å – H-bonded β-strands
  - b, d, e, and f show doublets (4.62Å, 4.84Å)
- Reflections at 2.39-2.41 are 2nd harmonic of 4.82Å
- Very vague reflections around 2-3.2Å
  - Well defined and closely similar structures

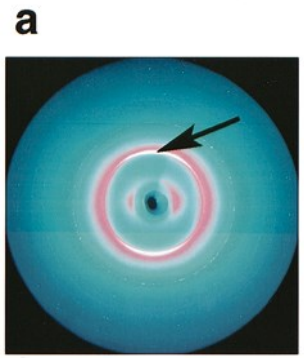
Meridional Reflections

- Unit cell: about 115Å (repeat)

σ	0.59	0.37	0.099	0.46	0.28	0.61	0.47	0.35
	ATTR1	ATTR2	AL	AApoAI	ALys	AA	FTTR	FIAPP
Order								
24	4.84	4.84	4.82	4.79	4.80	4.76	4.80	4.83
25	4.64	4.60	4.62	4.64	4.63	4.60	4.58	4.60
26				4.42	4.46			
27								
(n-i)	4.11		4.13	4.12	4.13	4.14		
28								
29								
30								
(o-m)	3.83	3.85	3.85					3.83
31				3.73	3.73	3.72	3.71	
32			3.61					
33								
34				3.40				
35								
36							3.21	
37				3.13				
38								
39								
40				2.90				
41		2.82					2.82	
42								
43								
44							2.61	
45								
46								
47								
48	2.39	2.41	2.41	2.39	2.40		2.39	2.41
49								
50								
51			2.27				2.25	
52								2.22
53				2.17				
54								
55								
56								
57								
58				2.00			2.00	
cell	115.37	115.39	115.61	115.46	115.56	115.12	115.13	115.48

Equatorial Reflections

- Relates to fibril structure perpendicular to fibril direction
  - Weaker and broader in intensity
- Display typical 5Å & 10Å sheets for β-sheet
- More sensitive to protein makeup
- Other studies show difference in number of proto-filaments in fibril



Equatorial Reflections

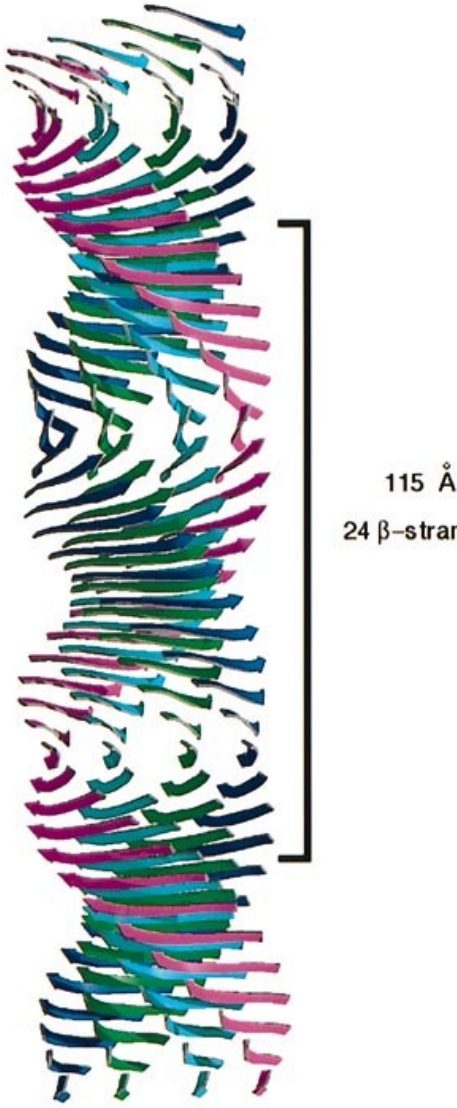
ATTR1	ATTR2	AL	AApoAI	ALys	AA	FTTR	FIAPP
						26.3	
16.0			15.17	14.60			
12.60		12.55	11.78	12.32		11.51	11.42
	10.62				10.96		
10.10		9.70	9.79	10.35		8.92	8.60
7.56	7.94	7.56					
6.05		6.11	5.98	5.74		5.72	5.53
5.32	5.46	5.26	5.37			4.53	
3.94			3.88	3.89		3.43	3.28

Spacing (in Å) of all equatorial reflections measured from diffraction patterns. Samples as in Table 2.

- Suggests presence of proto-filament packing
- Larger variation of results and difficult to interpret

Conclusions – Generic Fibril Structure

- Structure on proto-filament level
  - Suggests 4 proto-filaments per fibril
- Meridional:
  - Lowest reflection is at 4.8Å (24^{th} order)
  - Suggests 24 β-strands per rotation
  - Twist of 15^{o} per beta sheet



Transthyretin

Conclusions – Similarity of Fibrils

Clinical syndrome	Fibril subunit	Cross-β pattern	Structure of precursor
Monoclonal protein systemic (AL) amyloidosis	Full-length or fragments of V_L domain of Ig light chain	*	All β
Reactive systemic (AA) amyloidosis	76-residue N-terminal fragment of amyloid A protein	*	α/β
Familial amyloidotic polyneuropathy	Full-length or fragments of transthyretin variants	*	All β
Hereditary apoAI amyloidosis	N-terminal fragments (~90 residues) of apoAI variants	*	(α/β)
Hereditary lysozyme amyloidosis	Full-length lysozyme variants	*	α + β
Type II diabetes mellitus	37-residue fragment of islet-amyloid polypeptide	*	Unknown
Alzheimer's disease	1-39 to 43 residue Aβ protein	†	α, β or coil
Insulin-related amyloid	Full-length wild-type insulin	†	α + β
Transmissible spongiform encephalopathies	Full-length or fragments of prion protein	†	α + β
Medullary carcinoma of the thyroid	Fragments of calcitonin	†	Unknown
Senile systemic amyloidosis	Full-length or fragments of wild-type transthyretin	‡	All β
Hemodialysis-related amyloidosis	Full-length, wild-type β_2-microglobulin	‡	All β
Isolated atrial amyloidosis	Atrial natriuretic factor	‡	Unknown
Hereditary cerebral amyloid angiopathy	110-residue fragment of variant cystatin-C	‡	α + β
Finnish hereditary amyloidosis	71-residue fragment of gelsolin variants	‡	α/β
Hereditary fibrinogen α-chain amyloidosis	Fragments of fibrinogen α-chain variants	‡	Unknown

Key to symbols: *, this work; †, see the text for reference; ‡, no diffraction evidence; (α/β), secondary structure prediction.

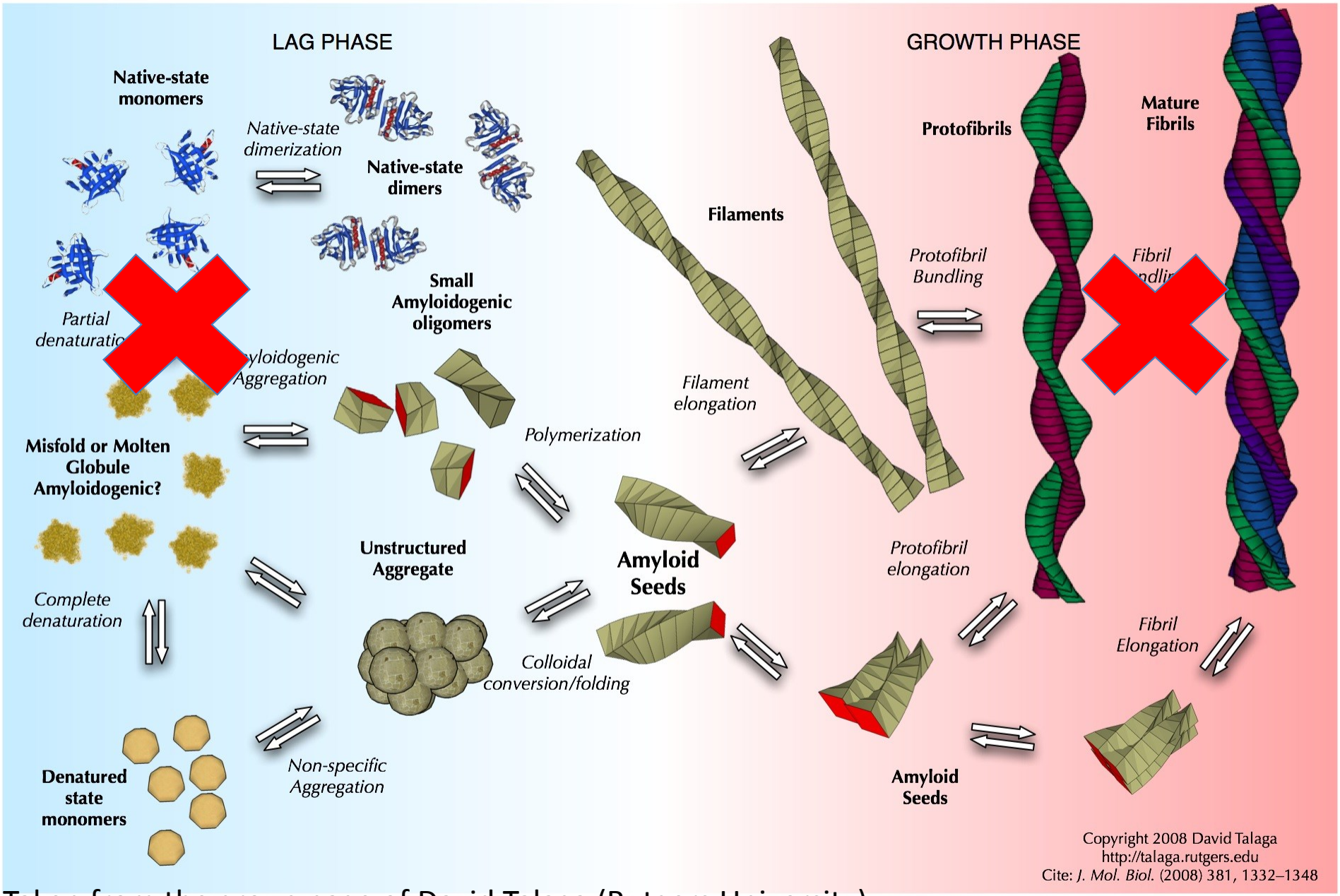
- Precursor proteins show wide range of structures
- All amyloid fibrils display same cross-β skeleton
- Implies a significant structural change happening

Summary of Work

- Amyloid plaques are extracellular deposits of insoluble protein fibrils
- Best characterized using XRD methods
- Synchrotron Fiber XRD gives highest resolution yet

Summary of Work

"The present work demonstrates that, although the amyloidogenic proteins have very different precursor structures, they can all undergo a structural conversion, perhaps along a similar pathway, to a mis-folded form that is the building block of the β-sheet helix proto-filament"



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