

Background

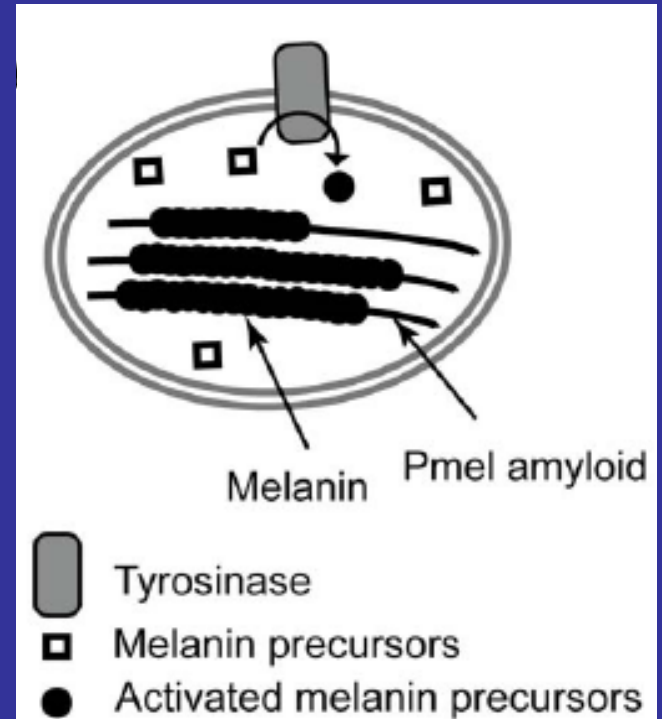
Amyloidosis

- A group of disorders caused by abnormal folding, aggregation, and accumulation of amyloid fibrils in tissues
- Deposits composed of amyloid fibrils that progressively interfere with the structure/function of affected organs throughout the body
- Two dozen proteins known to form amyloid fibrils in vivo

Biochim Biophys Acta 2005;1753:11
Neth J Med 2004;62:104

Functional Mammalian Amyloid

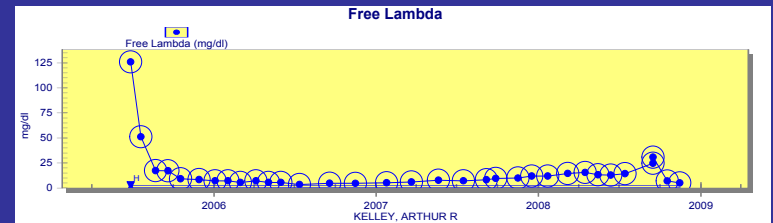
- “Amyloidin”
- Forms, degrades rapidly
- Template for melanin production
- Highly regulated
- Compartment specific
- Limits diffusion of toxic melanogenic precursors



Amyloidosis

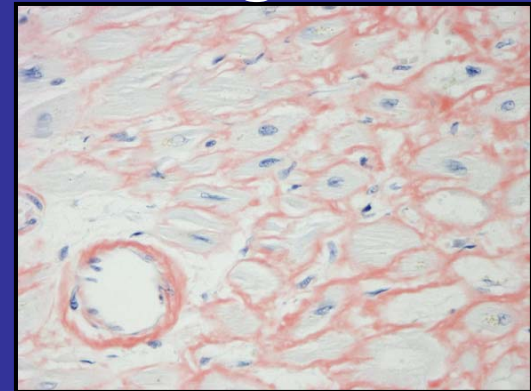
- Protein conformation disorder
 - Misfolding
 - Toxic intermediates
 - Fibril formation*

- Concentration



- Tissue deposits
- Vascular changes
- Cellular injury
- Organ dysfunction

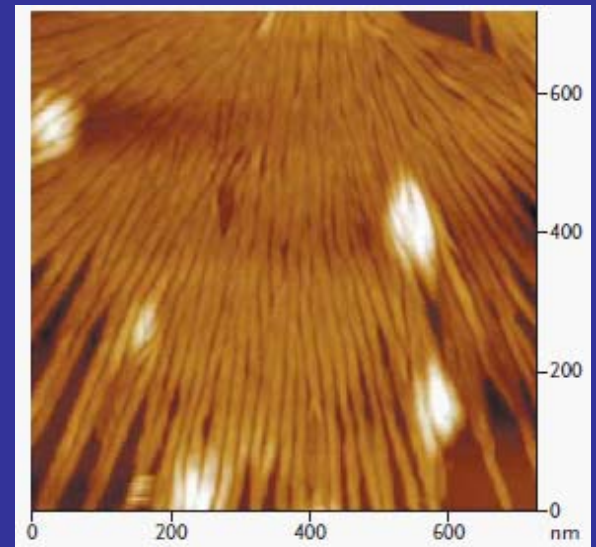
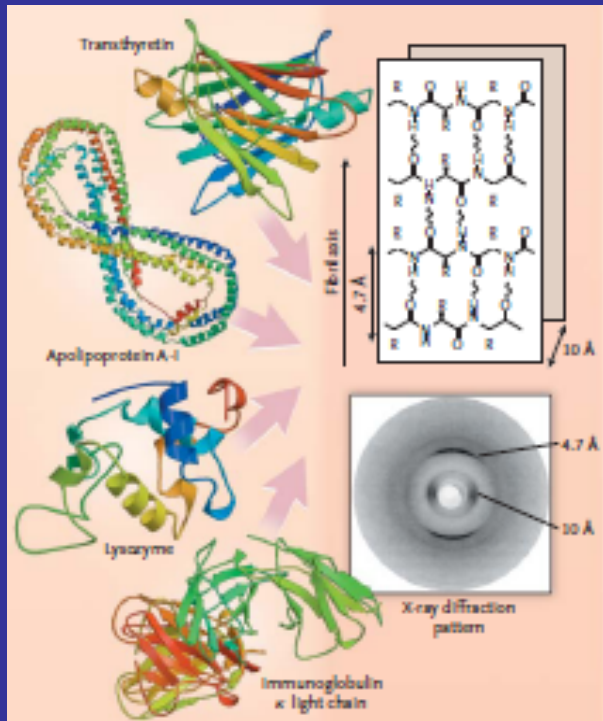
- Congo red



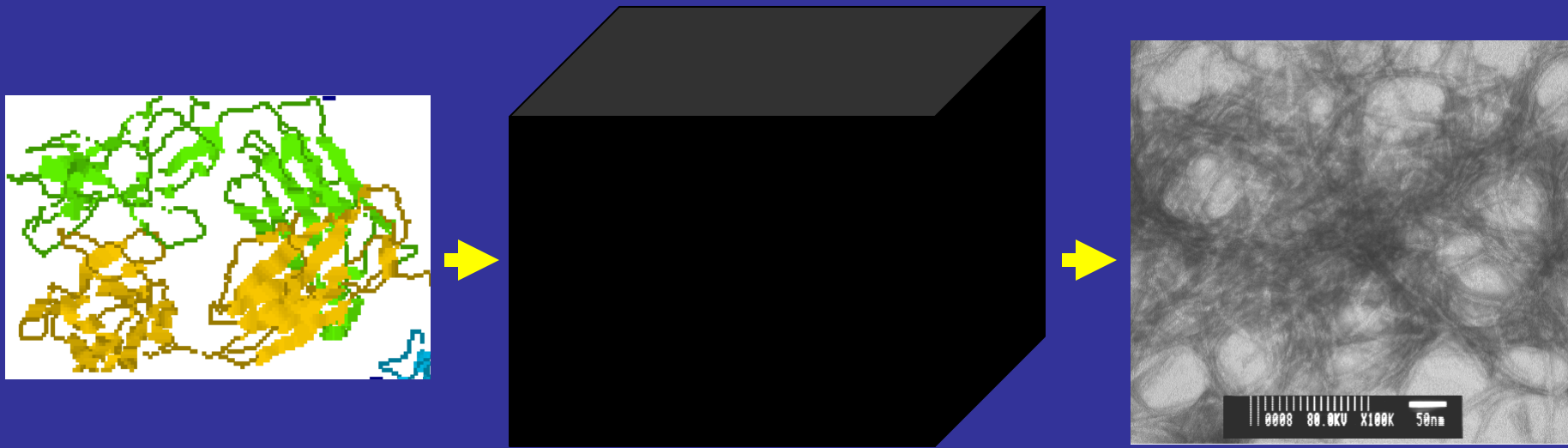
* Fibrils – a less toxic form until the tissue deposit threshold is passed?

Precursor Protein to Fibrils

- ~25 Precursors
- Common endpoint
- Intermediates
- Filaments
- Fibrils



Precursor Protein to Fibrils



Blood 2009;114:3147

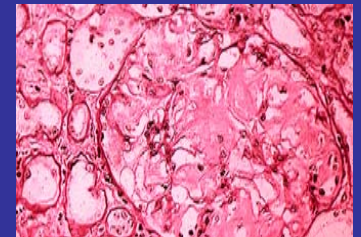
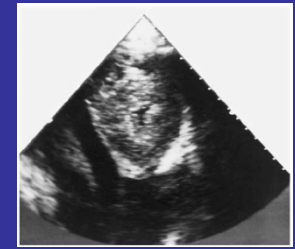
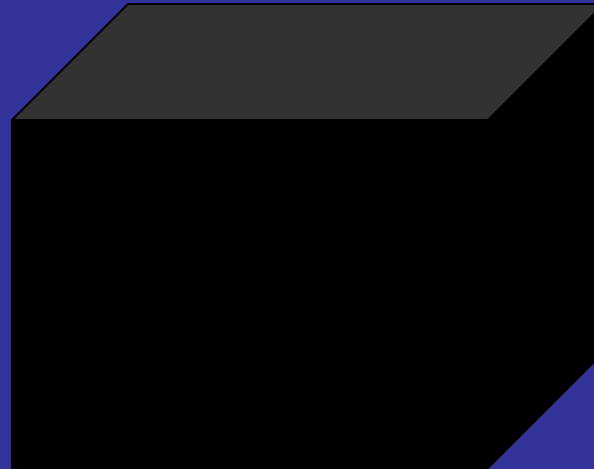
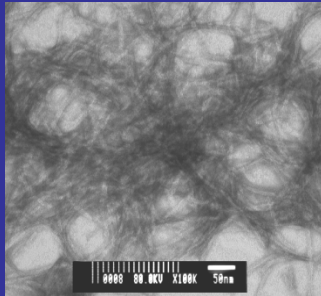
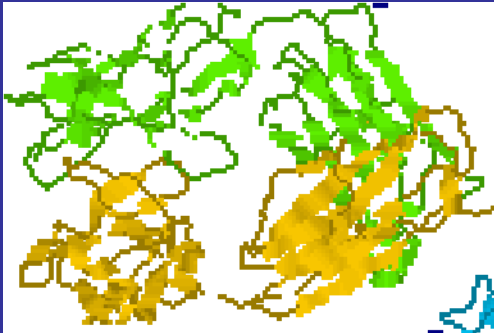
Over Two Dozen Types

- Nomenclature
- Precursor designation
- AL
- ATTR – mutant and wt
- AFib, AApoAI, ALys
- AA
- Systemic & Localized
- Significant Risk of Misdiagnosis

Amyloid 2007;14:179

NEJM 2002;346:1786

Precursor Protein & Fibrils To Organ Disease



NEJM 1997;337:898

Systemic Disease

<u>Type</u>	<u>Heart</u>	<u>Kidneys</u>	<u>Liver/ GI Tract</u>	<u>PNS</u>	<u>ST</u>
AL*	✓	✓	✓	✓	✓
ATTRm**	✓	[✓]		✓	
ATTRwt	✓		[✓]		[✓]
AFib		✓	✓		
AApoAI	✓	✓	✓		
ALys		✓	✓		[✓]

* Monoclonal gammopathies are 2x more common in African-Americans

**V122I mutant TTR occurs in 4% of African-Americans

Physical and Chemical Properties of Amyloid Fibrils

- All types of fibrils share the same characteristics
 - Amorphous eosinophilic appearance on light microscopy after hematoxylin and eosin staining [possible image]
 - Apple-green birefringence observed under polarized light after Congo red staining [possible image]
 - Characteristic fibrillar structure observed on electron microscopy [possible image]
 - Beta pleated sheet structure observed by x-ray diffraction
- Solubility in water and buffers of low ionic strength

J. Biol Chem 2008;283:17279-17286

J Pathol 2008;216:253-261

J Mol Biol 2007;366:711-719

Probable Pathologic Properties of Amyloid Deposition

- **Disrupted tissue architecture**
- **Local cytotoxicity**
- **Free radical injury**
- **Induction of apoptosis**

Curr Drug Discov Technol 2006;3:145
Protein Pept Lett 2006;13:219
Circ Res 2004;94:1008

Toxic Intermediates

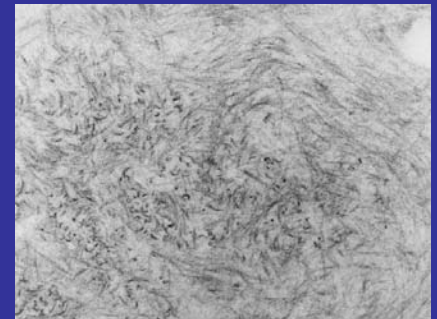
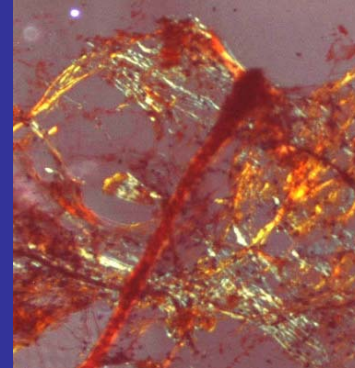
- Misfolded light chains
 - Intermediate forms
 - Auto-aggregates
- Rat Cardiomyocyte Model
 - Impaired contractile function
 - Altered intracellular calcium
- Rat Cardiac Fibroblast Model
 - Internalization of FLC
 - Alteration of proteoglycans
- Computational Biology
- Reversibility of symptoms
- Measurable Ig light chains

stirring time (hours)	peak position ^b (cm ⁻¹)	secondary structure assignment	area under the curve ^c (%)
0	1691	tums	5.5
	1676	β -sheet	20
	1662	tums/loops	23
	1652	α -helix	3
4	1637	β -sheet	48.5
	1691	tums	8.5
	1679	β -sheet	14
	1665	tums/loops	29.5
	1652	α -helix	3
12	1637	β -sheet	45
	1694	tums	4.5
	1685	β -sheet	6.5
	1664	tums/loops	33
80	1641	unordered	23
	1625	β -sheet	33
	1695	tums	3.5
	1686	tums/loops	4.5
	1674	β -sheet	21
	1654	tums/loops	27.5
	1635	β -sheet	17.5
	1626	β -sheet	26

Biochemistry 2003;42:8094
Circ Res 2004;94;1008
Am J Pathol 2005;166:197
J Mol Biol 2007;374:917

Surrogate Biopsy: Abdominal fat pad aspirate

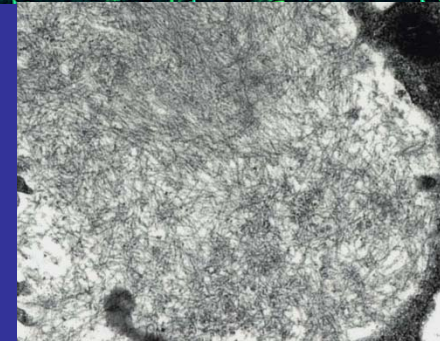
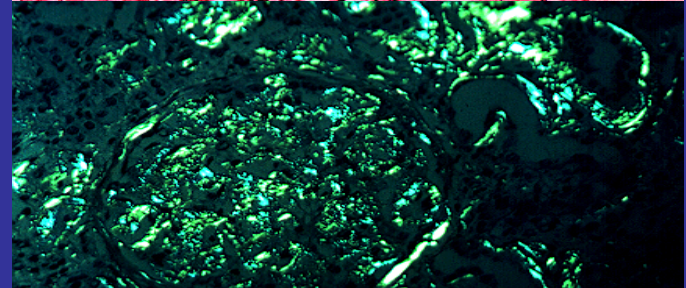
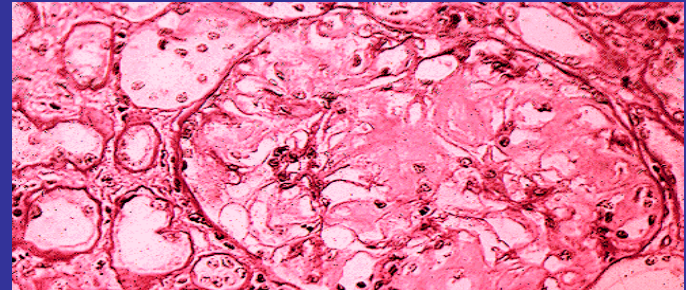
- 190 patients
- 57% with amyloid
- Positive predictive value = 89%
- Negative predictive value = 90%
- Sensitivity = 93%
- Specificity = 85%



Clark BD, Comenzo RL.
Unpublished observations

Involved Organ Biopsy: Renal

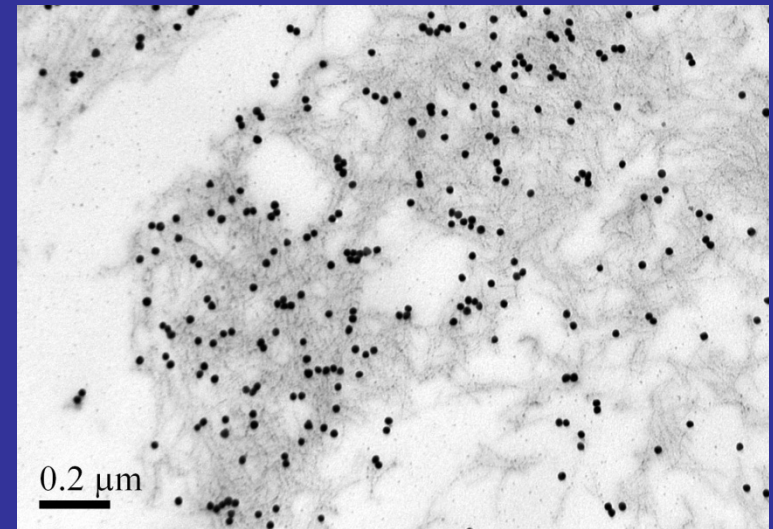
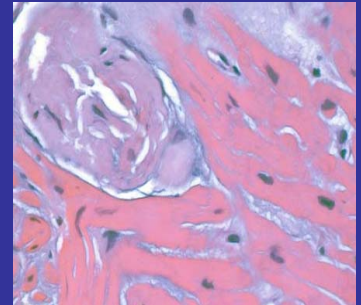
- 3% show amyloidosis
- Diagnostically reliable
- Immunostains
variably reliable
- MIDD & amyloidosis
- Ultrastructural studies



N Engl J Med 2002;346:1786
Blood 2006;107:3489

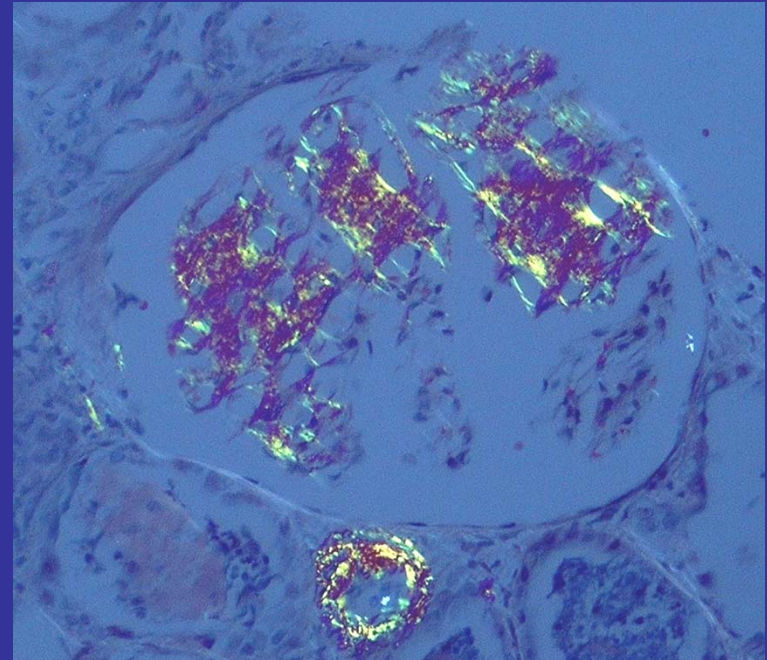
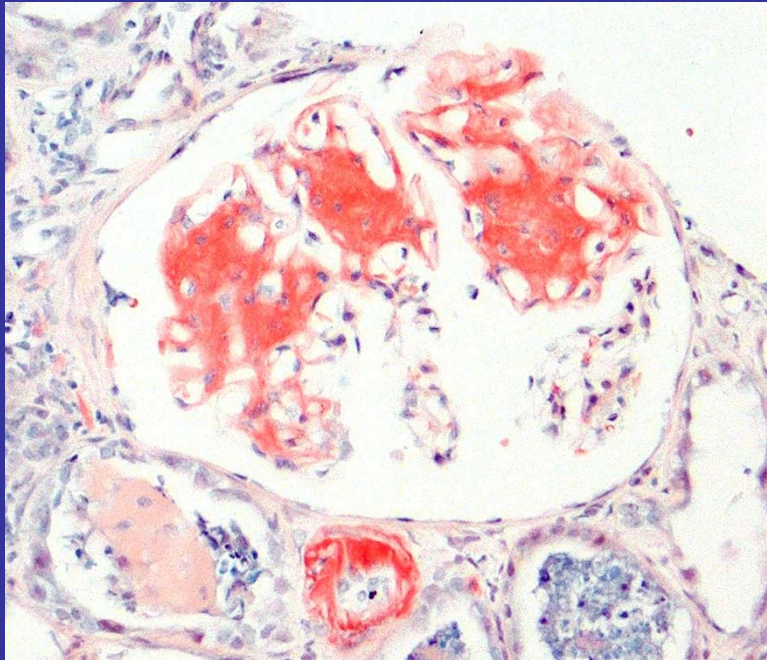
Involved Organ Biopsy: Heart

- Systematic Approach
- Specimens for
 - Light microscopy (Congo red)
 - Ultrastructural studies (IEM)
 - Proteomics
 - Archive (snap frozen)
- Diagnosis
- Typing
- Patient & Family
- Patentable Discoveries



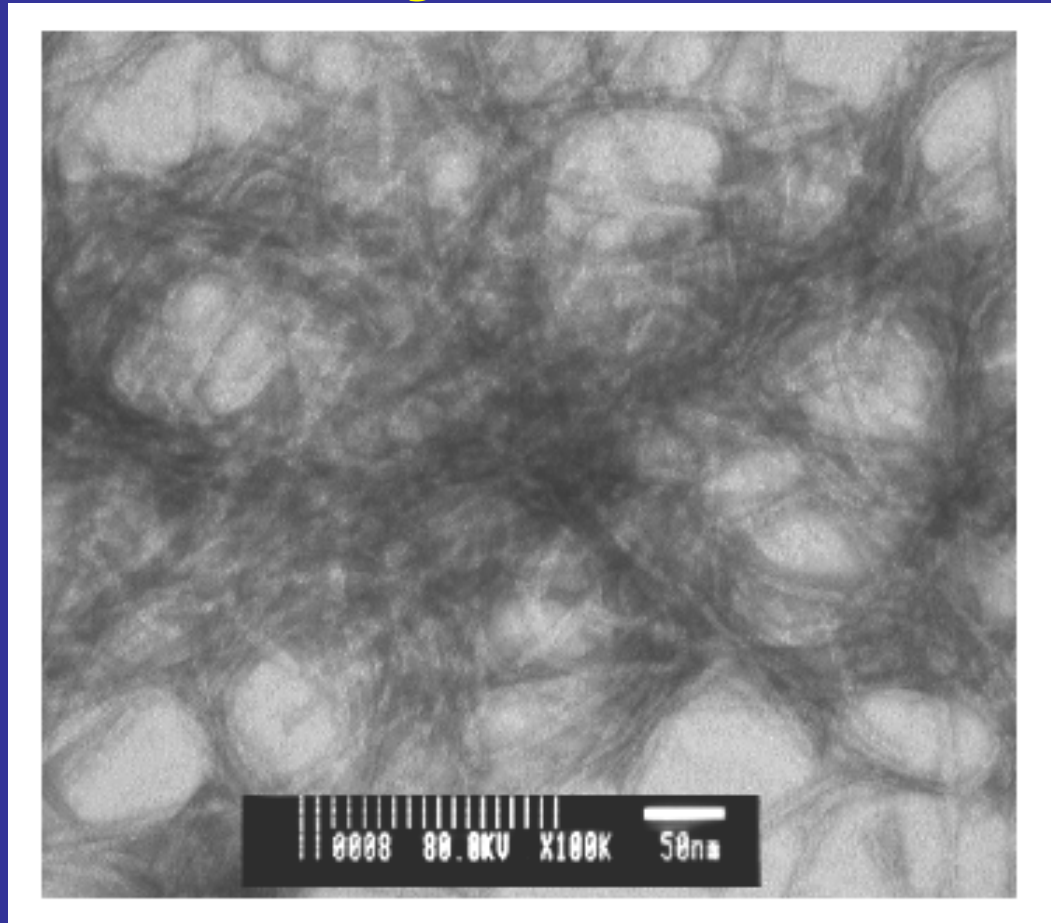
J Struct Biol 1998;124:1
Am J Med. 2001;111:243

Congo red Stain



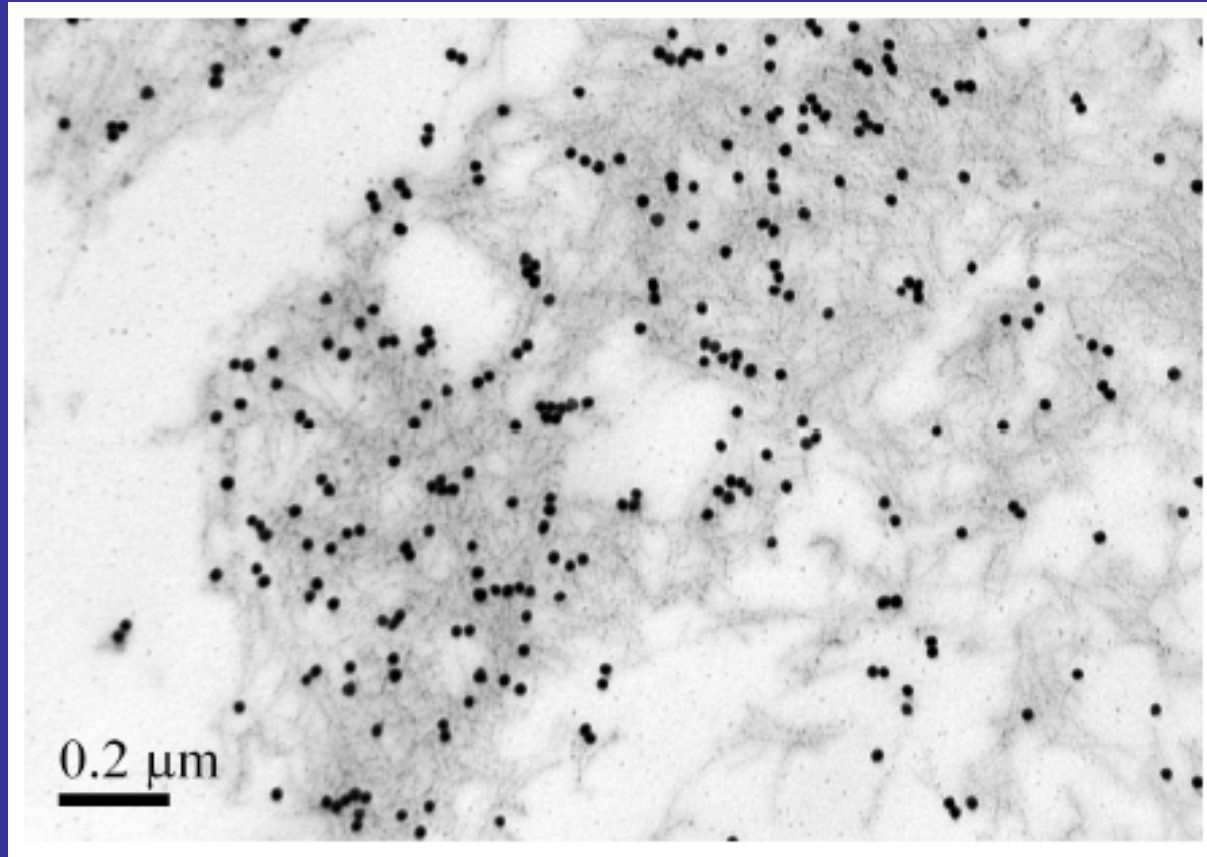
Blood 2009;114:3147

Electron Microscopic Image of Amyloid Fibrils



Blood 2009;114:3147

Immunogold Electron Microscopy



Blood 2009;114:3147

Arch Pathol Lab Med 2007;131:917

Ann Diagn Pathol 2000;4:174