Protein Misfolding Done Right: The Biogenesis of Bacterial Amyloid Fibers

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Curli

- Extracellular fibers produced by enteric bacteria
- Mediate binding to matrix proteins like fibronectin
- Potent inducers of the host inflammatory response
- Required for biofilm formation
- Required for persistence in a murine infection model
- Activate the contact and fibrinolytic system
- Are a ‘functional’ amyloid fiber

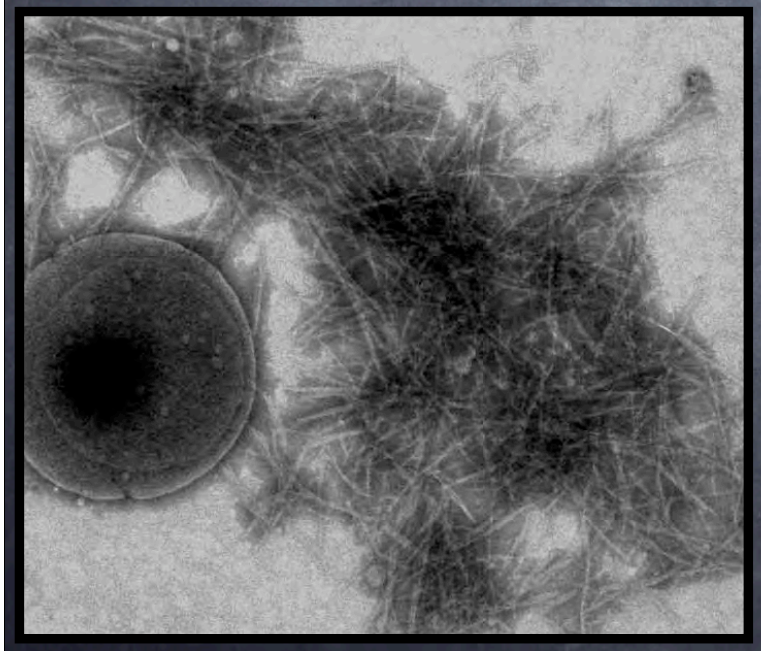
Table 2. *Fibril protein components and precursors in amyloid diseases. (Data from (21)).*

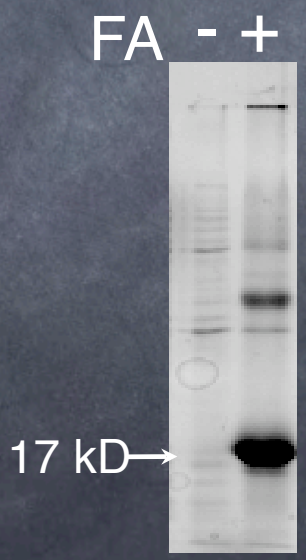
clinical syndrome	fibril component
Alzheimer's disease	Aβ peptide, 1-42, 1-43
spongiform encephalopathies	full length prion or fragments
primary systemic amyloidosis	intact light chain or fragments
secondary systemic amyloidosis	76-residue fragment of amyloid A protein
familial amyloidotic polyneuropathy I	transthyretin variants and fragments
senile systemic amyloidosis	wild-type transthyretin and fragments
hereditary cerebral amyloid angiopathy	fragment of cystatin-C
haemodialysis-related amyloidosis	β2-microglobulin
familial amyloidotic polyneuropahty II	fragments of apolipoprotein AI
Finnish hereditary amyloidosis	71-residue fragment of gelsolin
type II diabetes	fragment of islet-associafed polypeptide
medullary carcinoma of the thyroid	fragments of calcitonin
atrial amyloidosis	atrial natriuretic factor
lysozyme amyloidosis	full length lysozyme variants
insulin-related amyloid	full length insulin
fibrinogen α-chain amyloidosis	fibrinogen α-chain variants

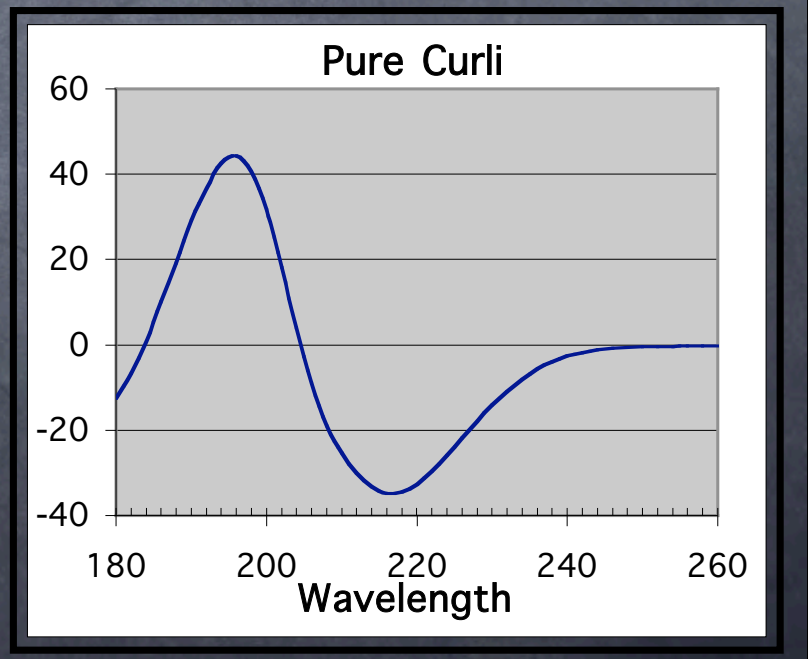
Published in "Molecular Informatics: Confronting Complexity", Martin G. Hicks & Carsten Kettner (Eds.), Proceedings of the Beilstein-Institut Workshop, May 13th - 16th 2002, Bozen, Italy

Amyloid Characteristics of Curli

- Binds to Congo Red
- Similar fibrous structure
- Insoluble and resistant to protease
- Fibers are beta-sheet rich
- Cause red shift and bind to thioflavin T

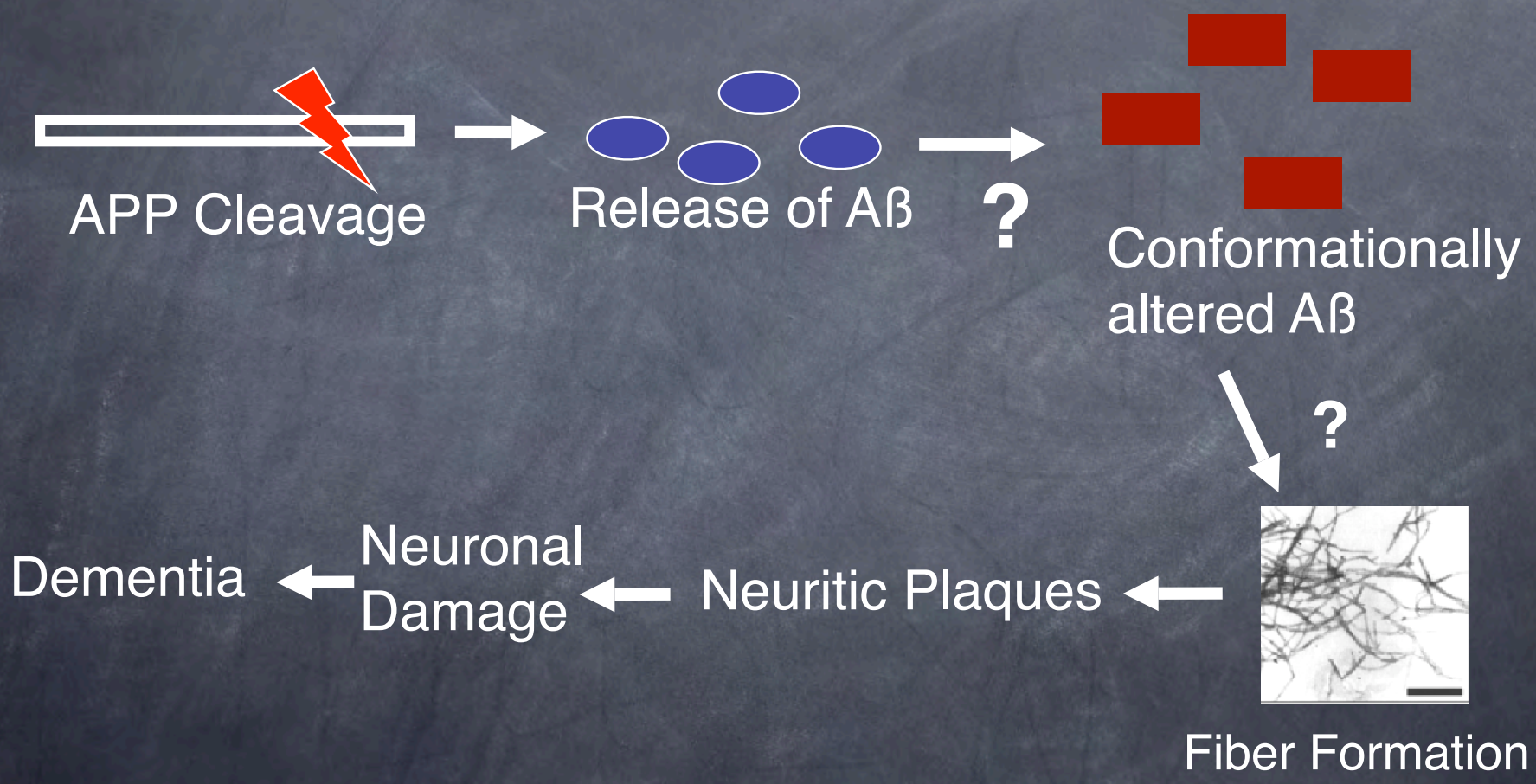


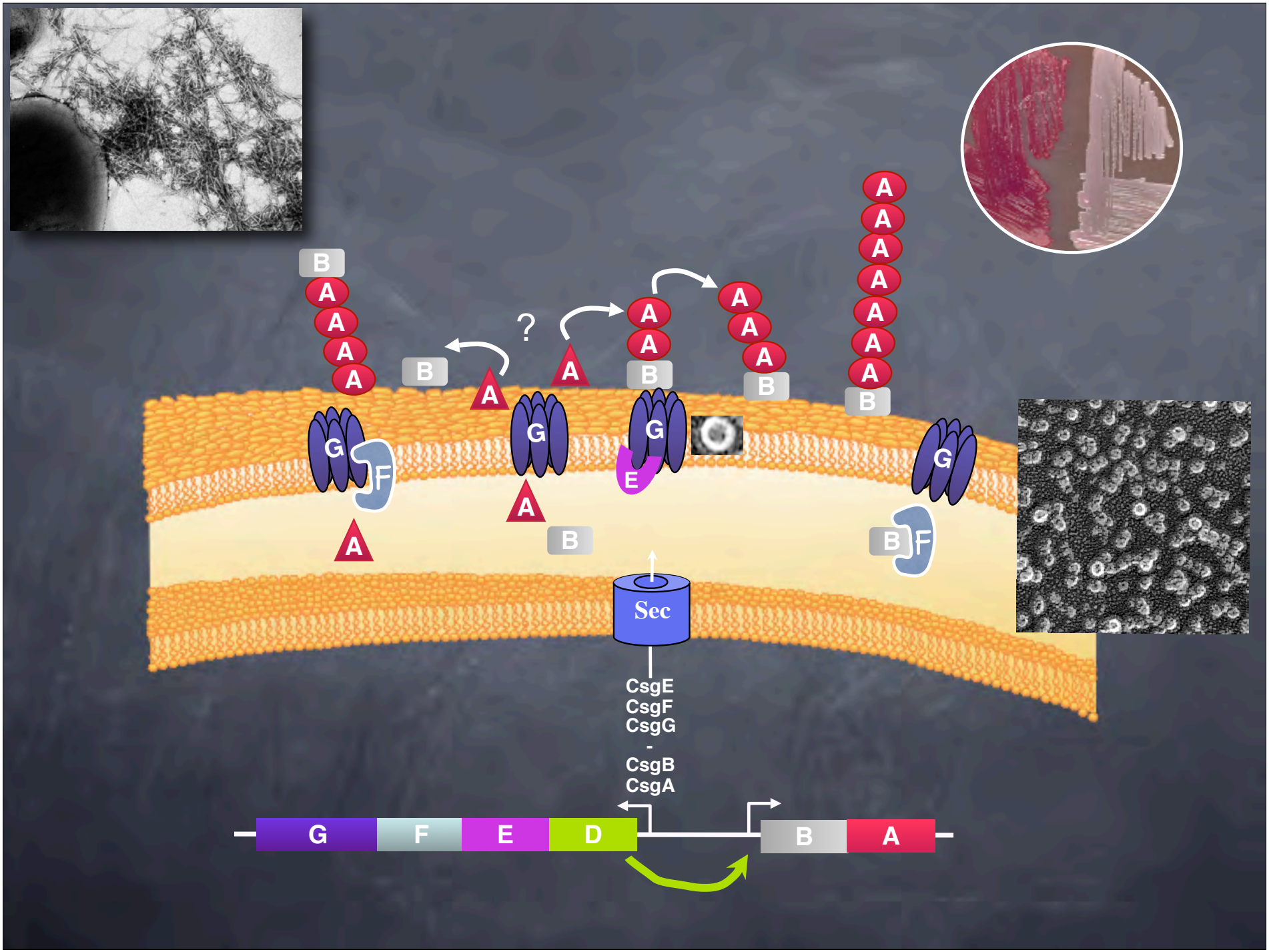




Chapman et al. 2003 *Science* 295:851-5

Progression of Alzheimer's

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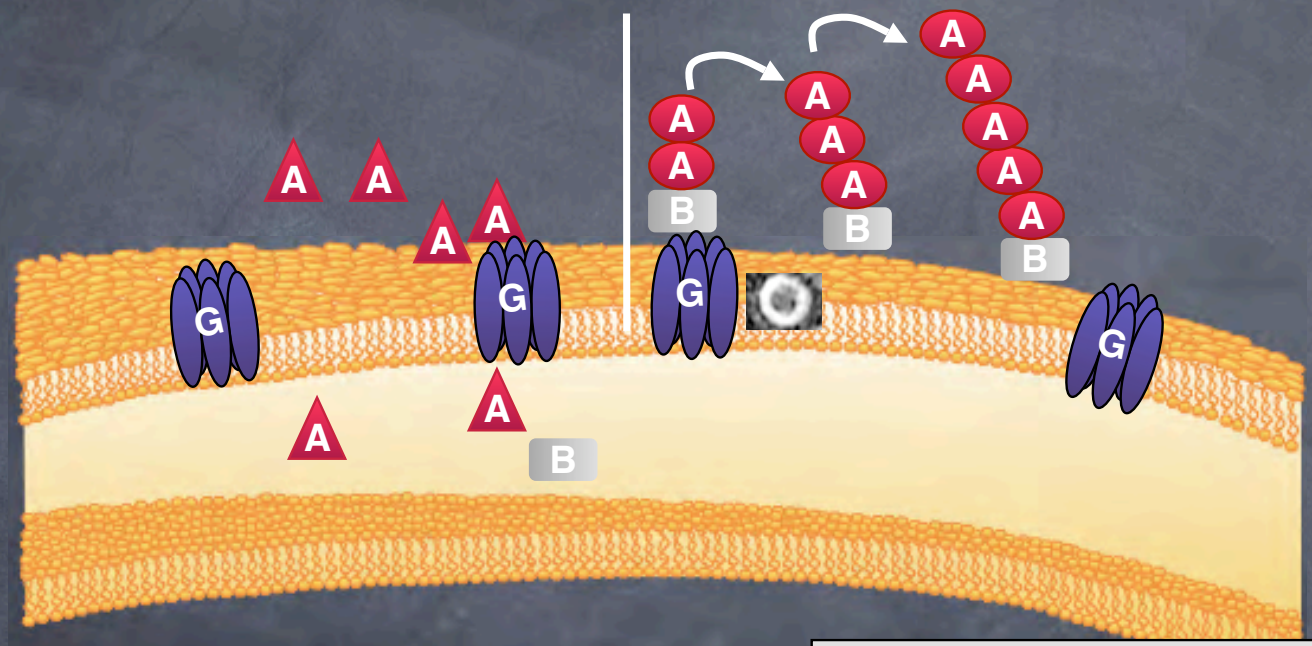
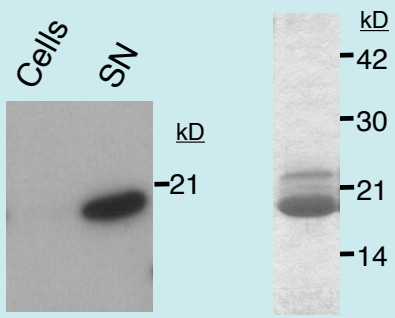
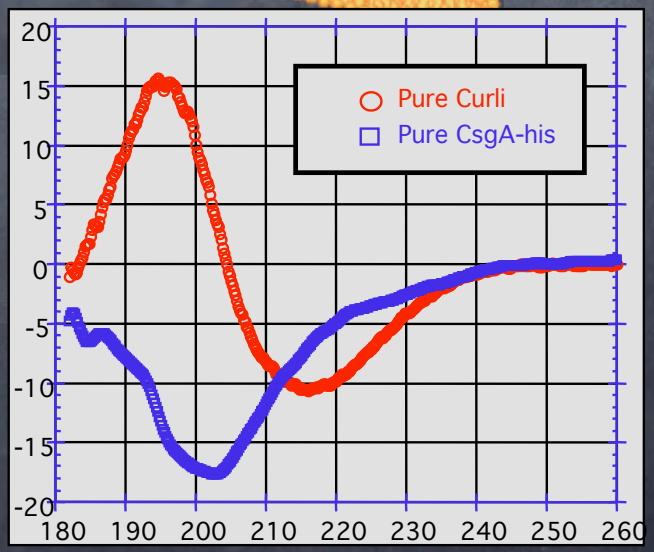
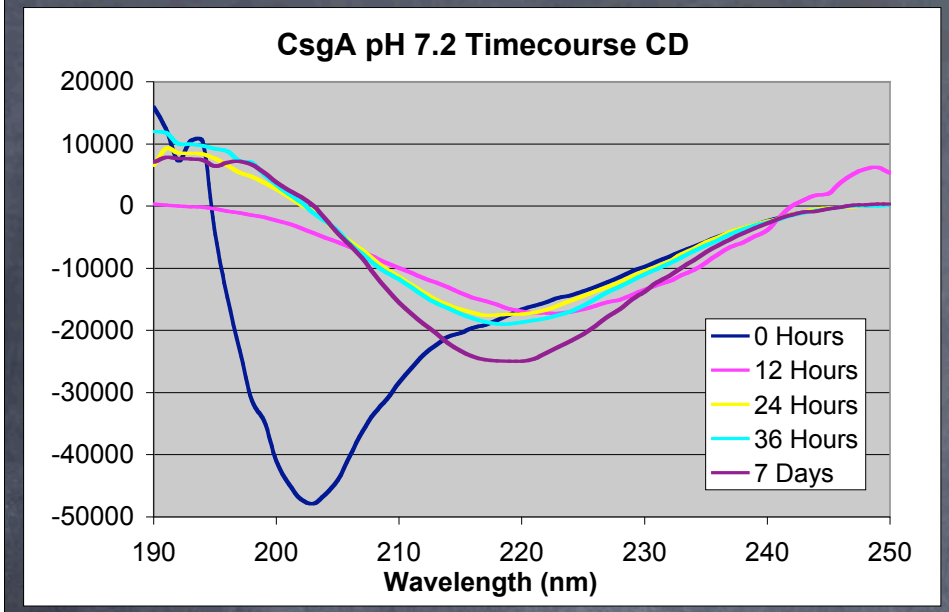
Setting Up an in vitro Polymerization System

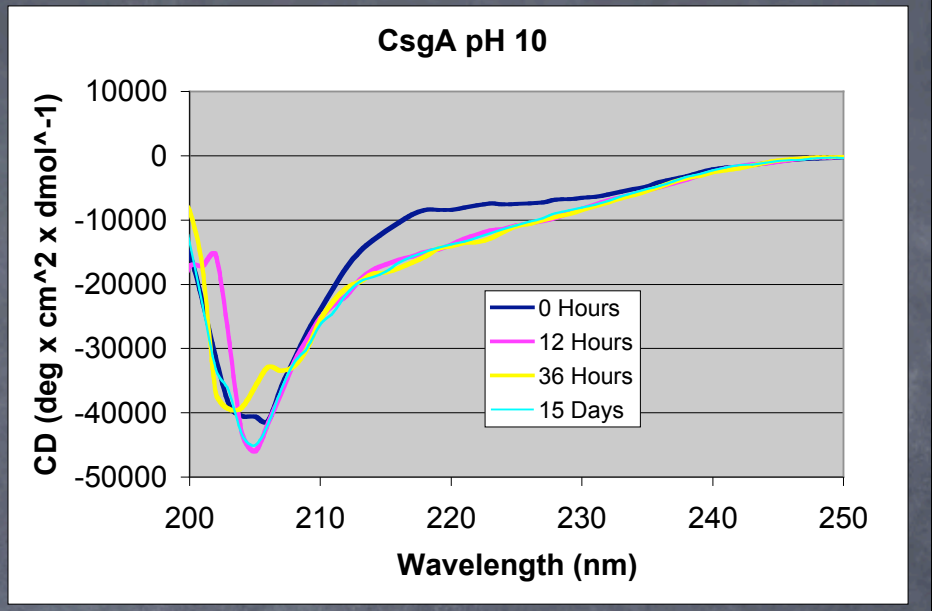


Purification of CsgA

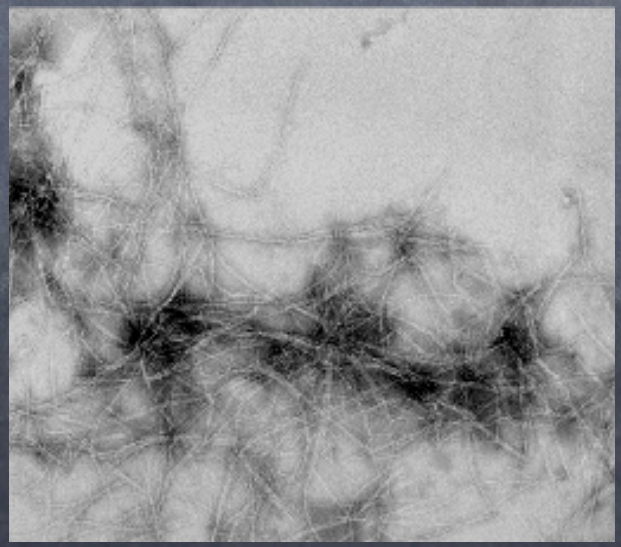




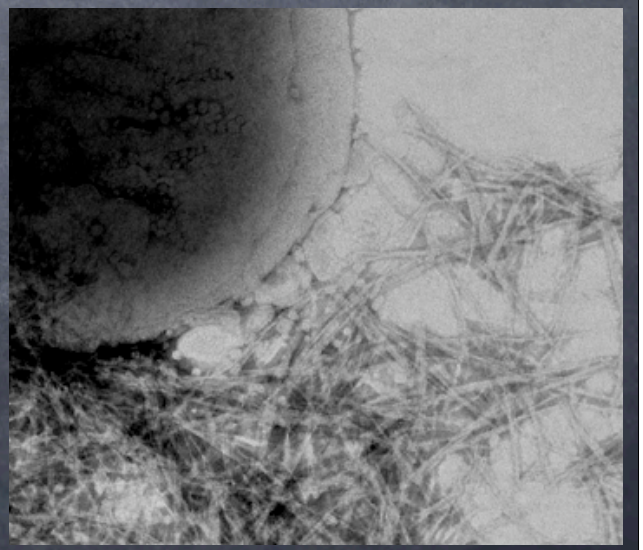




0 days

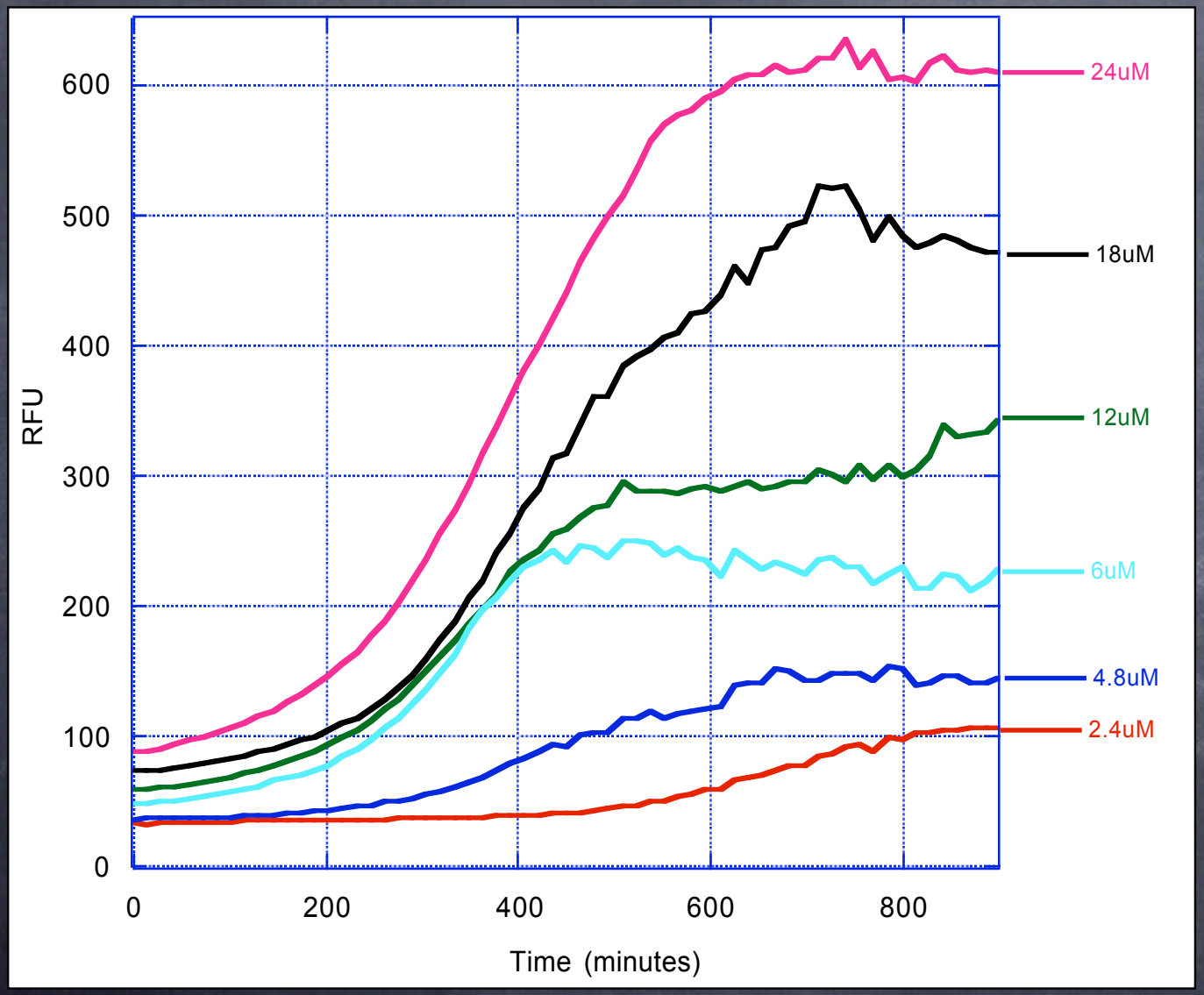


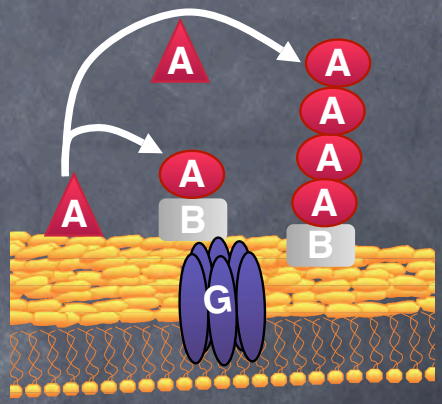
2 days

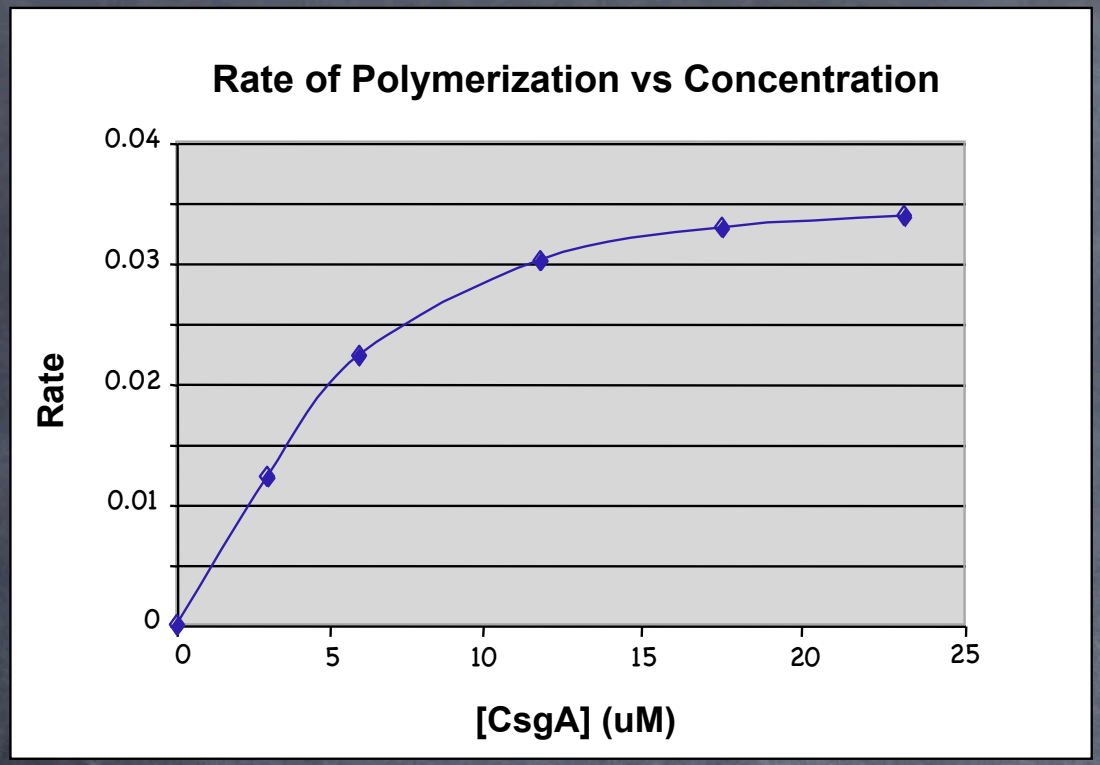


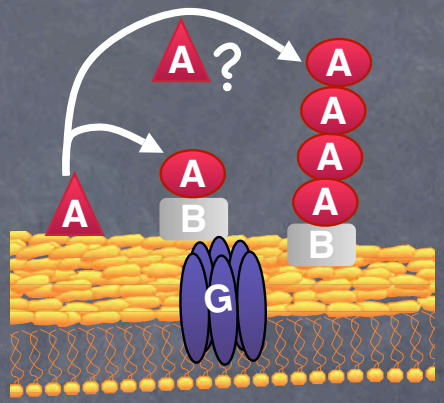
Wt *E. coli*

Using Thioflavin T to Monitor Polymerization



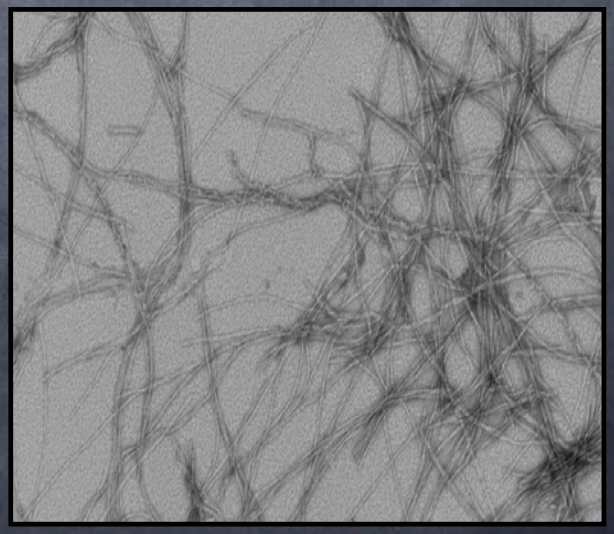




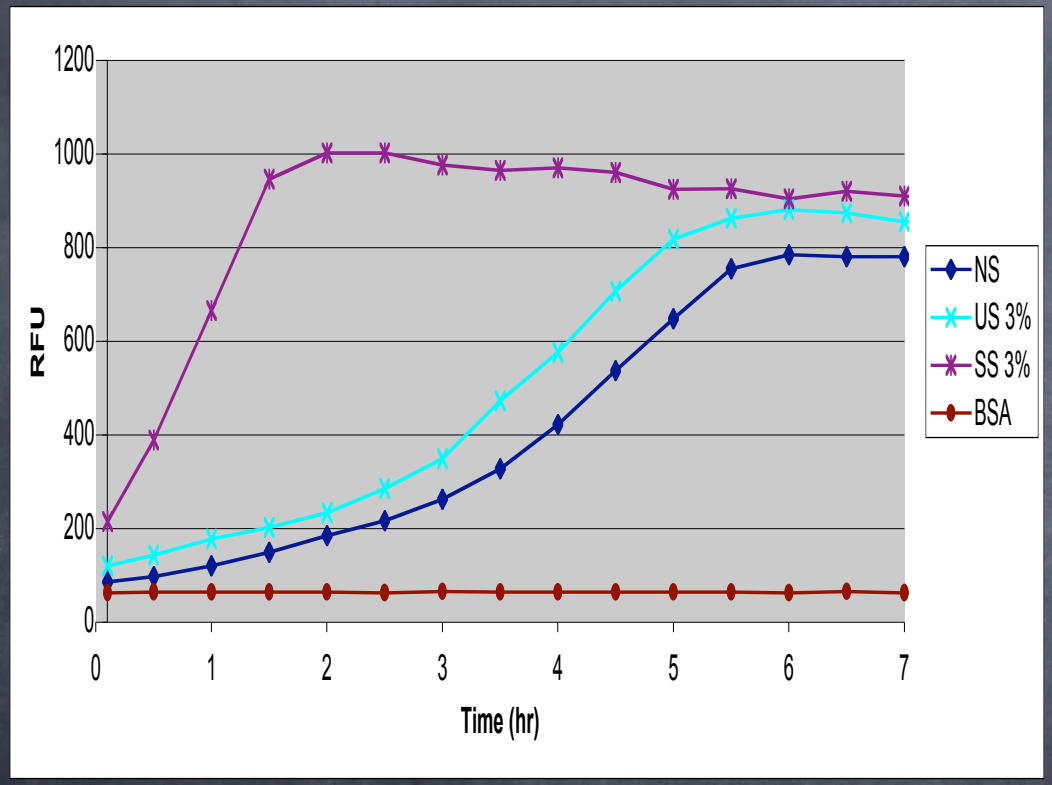


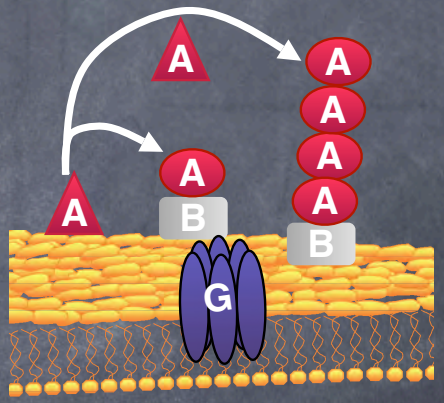
Nucleator?

$$CsgA_S \rightleftharpoons CsgA_N \longrightarrow CsgA_F \longrightarrow$$

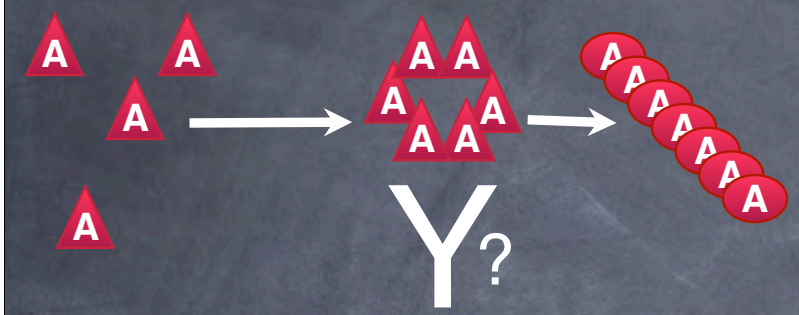


Seeding CsgA polymerization

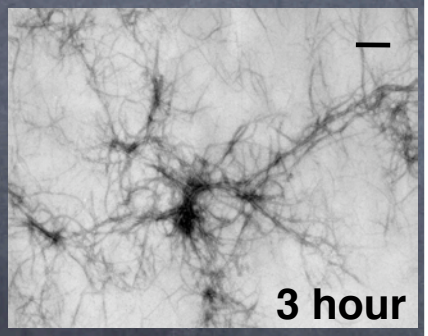


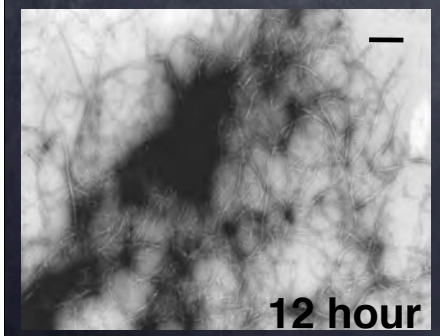


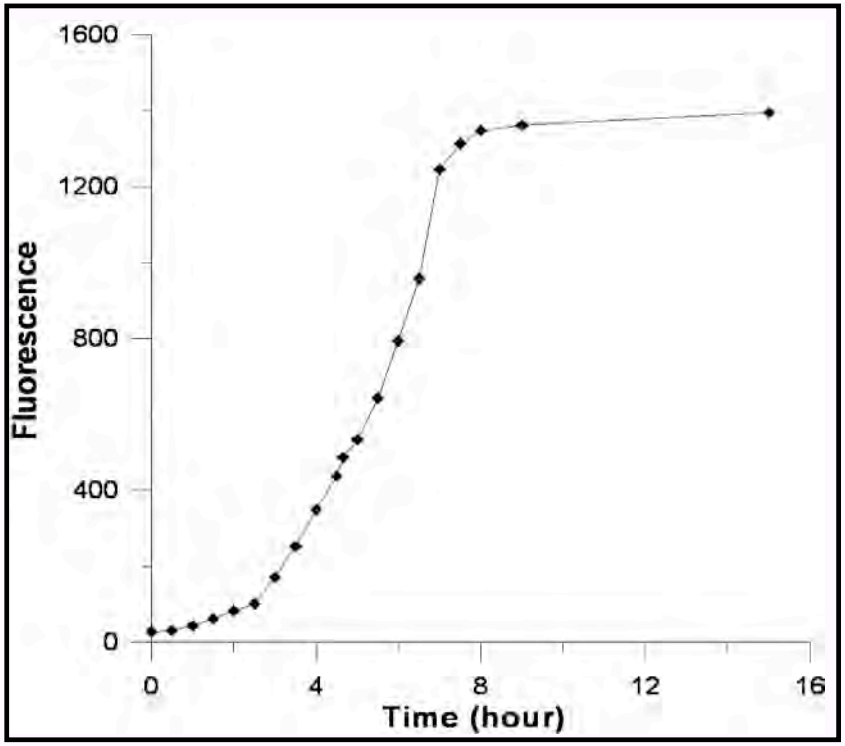
Does CsgA polymerization involve a conserved intermediate?

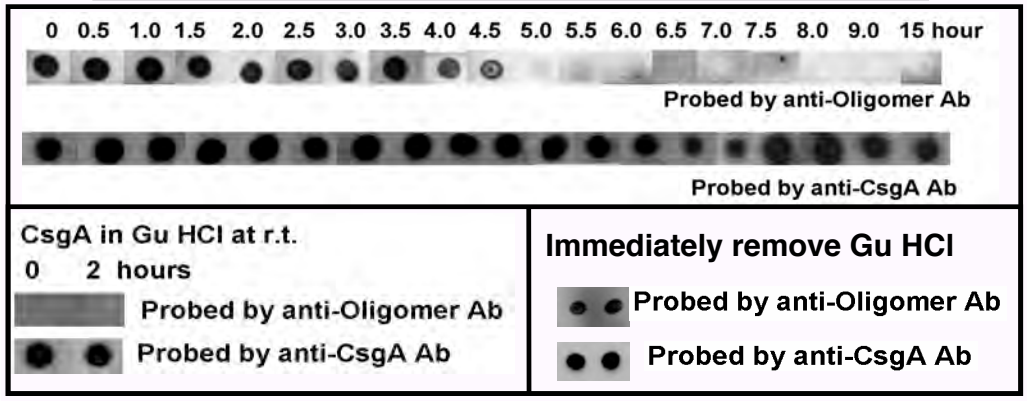


Kayed R., et al. Science. 2003 Apr 18;300(5618):486-9.

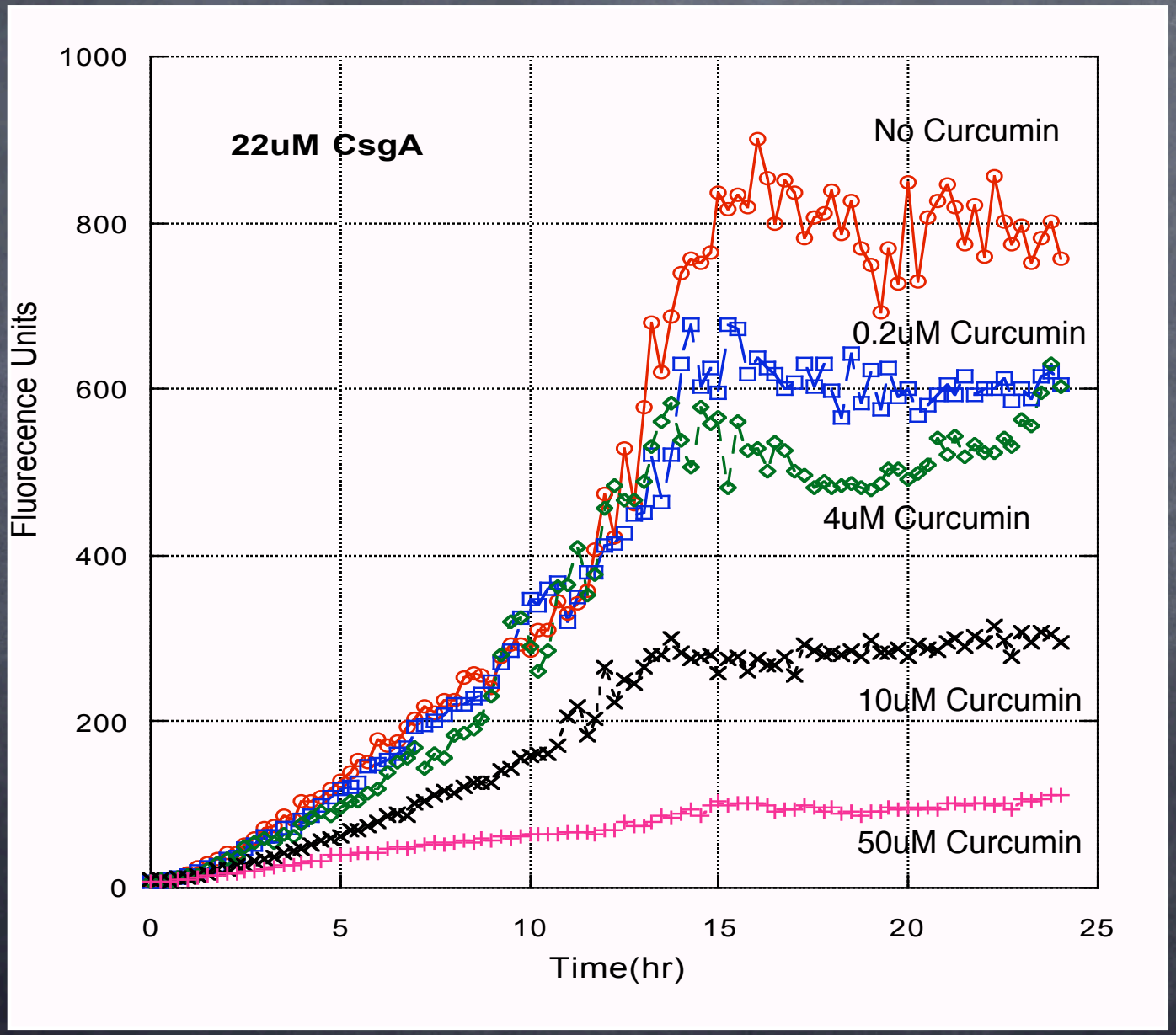








Using this in vitro assay as a part of a high-throughput screen for molecules that block amyloid formation



Strand-loop-Strand-loop motif

```
R1  S E L N I Y Q Y G G G N S A L A L Q T D A R N
R2  S D L T I T Q H G G G N G A D V G Q - G S D D
R3  S S I D L T Q R G E G N S A T L D Q W N G K N
R4  S E M T V K Q F G G G N G A A V D Q - T A S N
R5  S S V N V T Q V G F G N N A T A H Q Y
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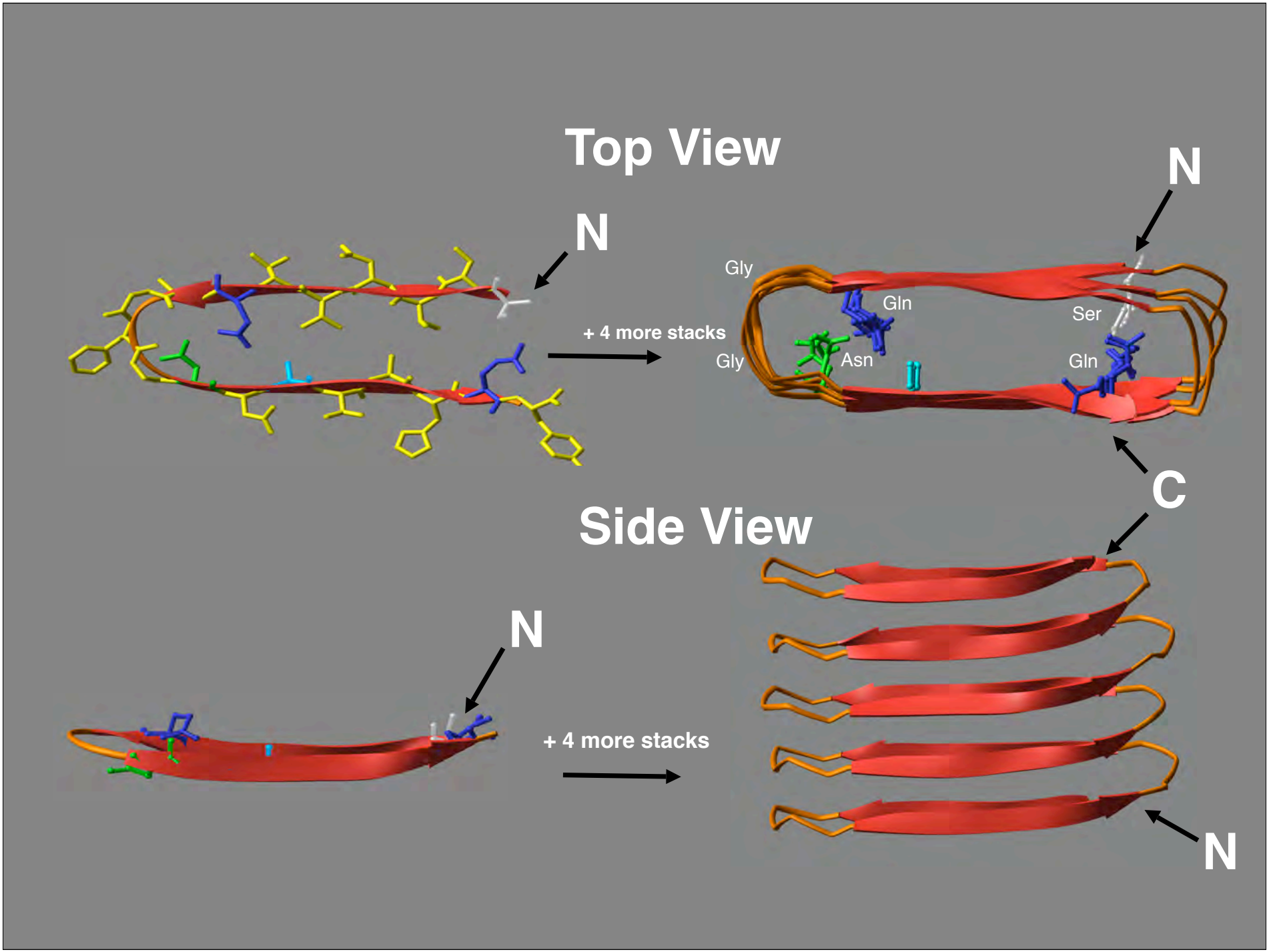
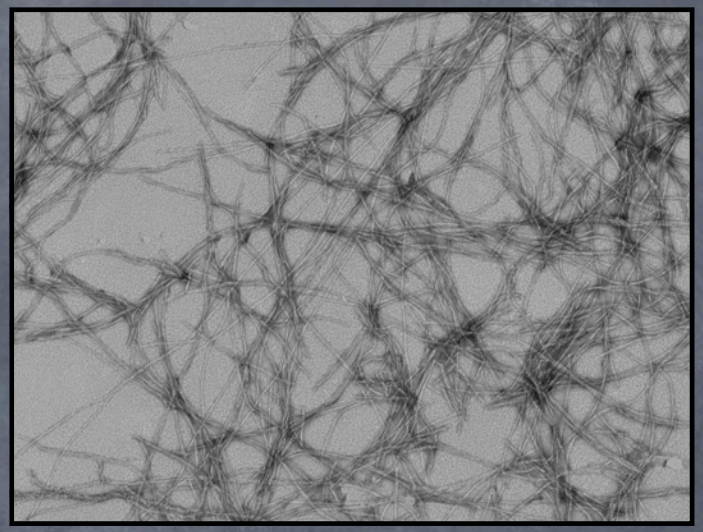
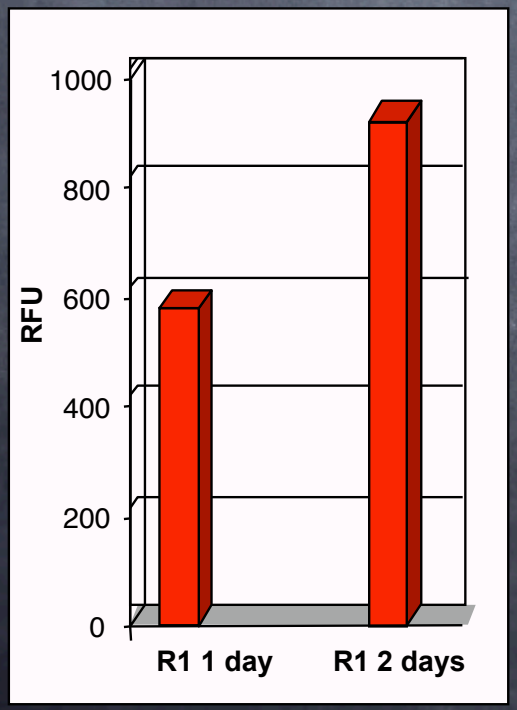
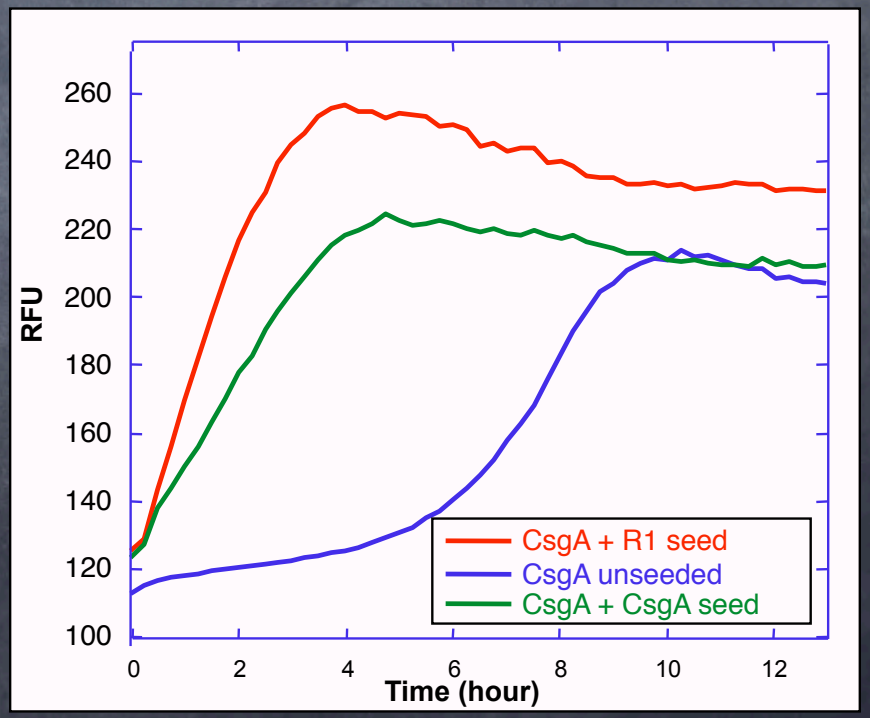
J. Mol. Biol. 290, 741-756


Top View
N
+ 4 more stacks
Gly
Gln
Ser
Gly
Asn
Gln
N
C
Side View
N
+ 4 more stacks
N

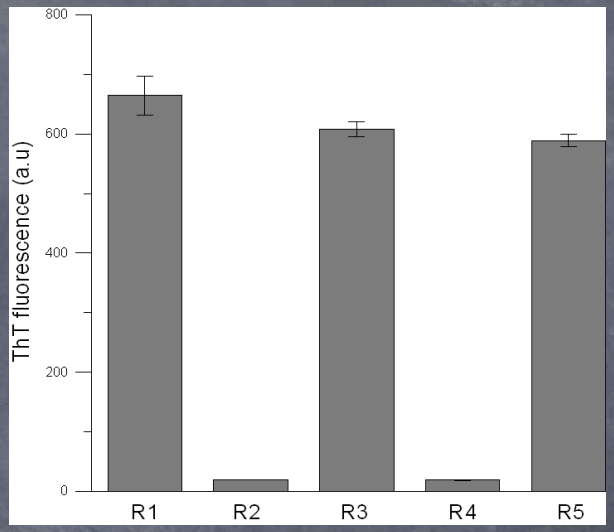
Amyloid Forming Units

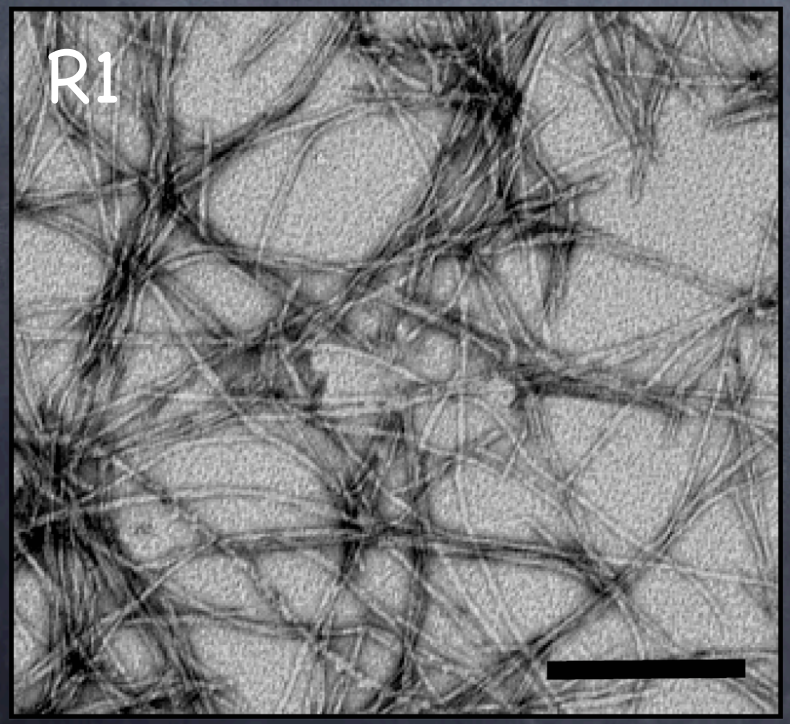


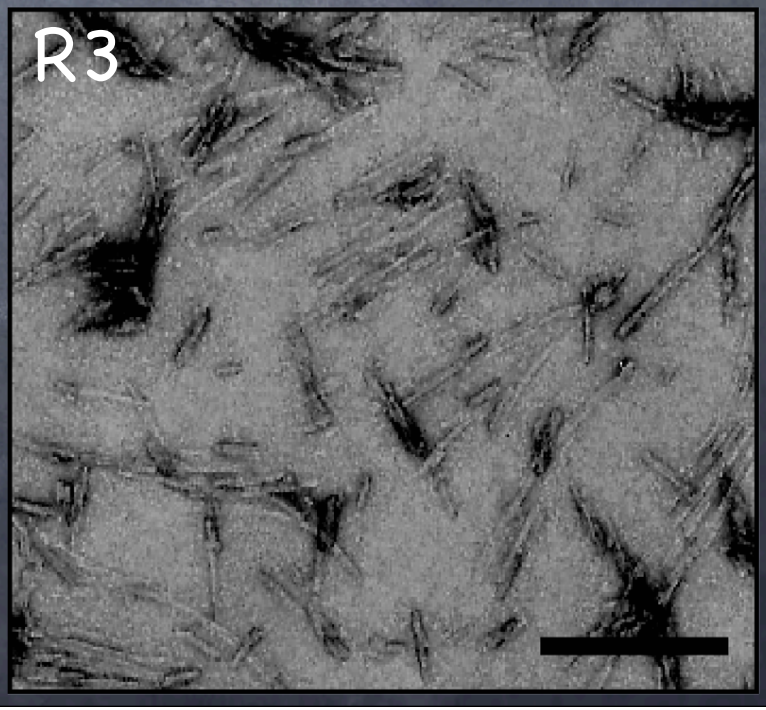




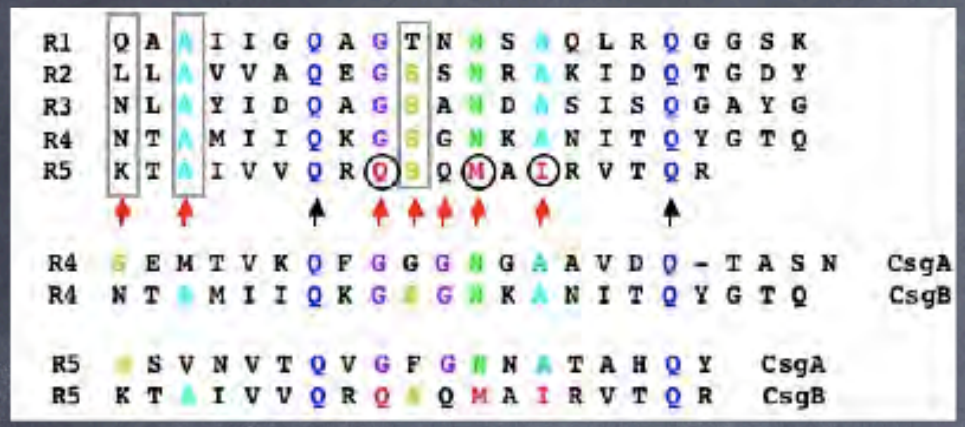
Amyloid Formation of CsgA Repeating Units

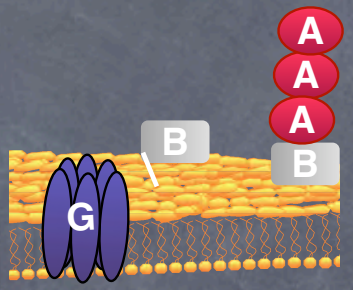


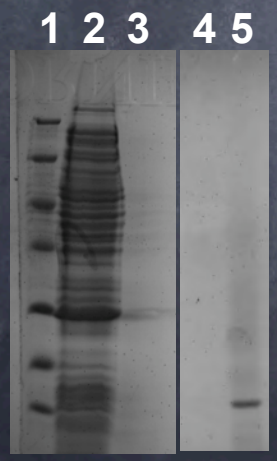


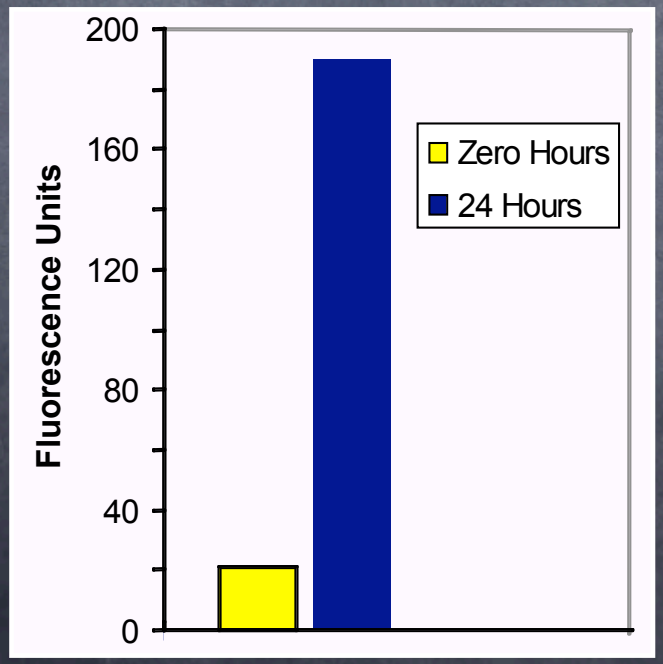


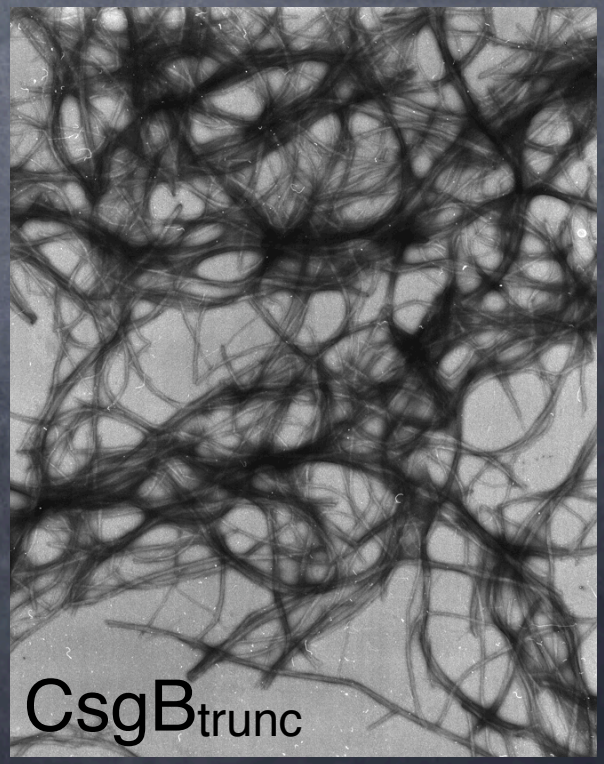
Does CsgB Contain an Amyloid-Forming Domain?











Looking Forward

1. Are functional amyloids the rule or the exception?

Functional Amyloids

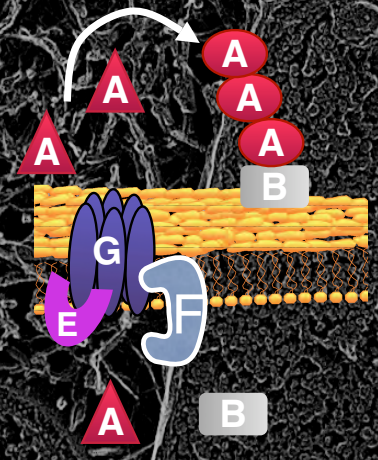
Bacteria	Yeast	Fungi	Animals
Curli Mcc	Supt35	Chaplins	Pmel17 CPEB

2. What can nature tell us about disease-associated amyloid formation?

Future Directions

•Understand the nucleation reaction. How specific is nucleation? Does CsgB specifically nucleate CsgA?

•Are “seeding” and “nucleation” mechanistically similar?



•How does CsgG mediated secretion work? Does CsgG work alone?

•What are the chaperone proteins CsgE and CsgF doing?

•Why do bacteria make curli? How prevalent is template-directed nucleation in other systems?

•Over 50 genes are required for curli formation in *E. coli*, suggesting a complex regulatory and biosynthesis pathway.

•Excellent model system for understanding amyloid formation, protein secretion, protein folding and organelle assembly.

Acknowledgments

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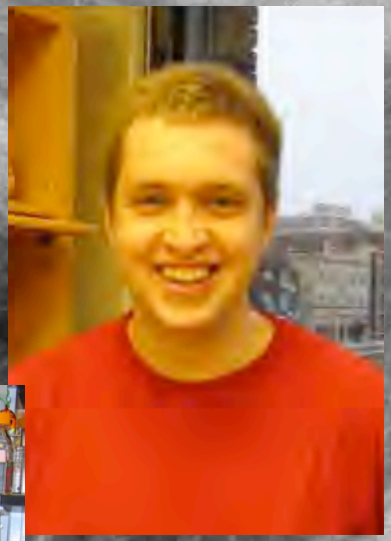
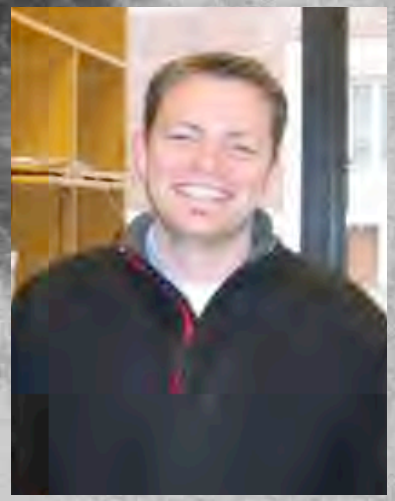
Ute Romling

Reagents
Charles Glabe





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