Unraveling nature's secrets with AFM (part 2)

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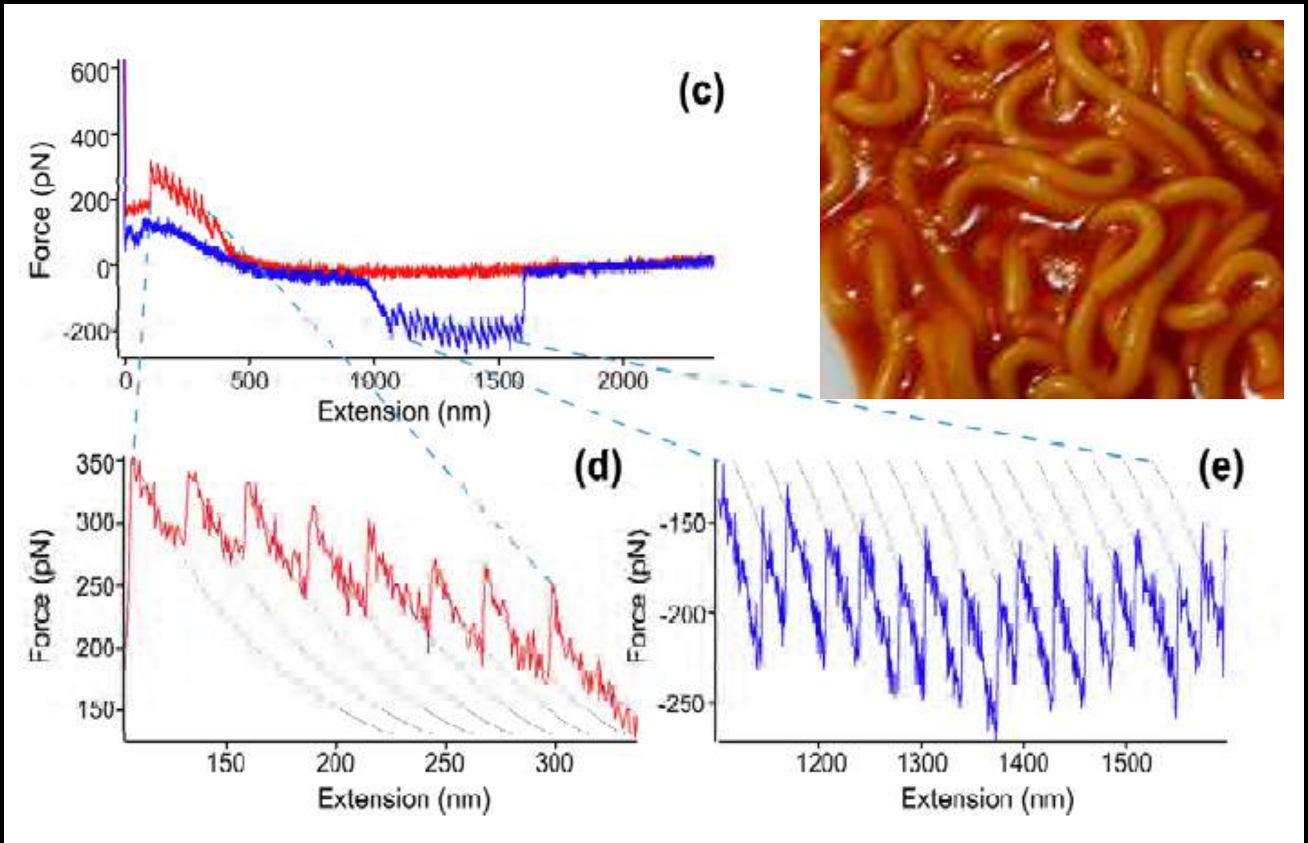
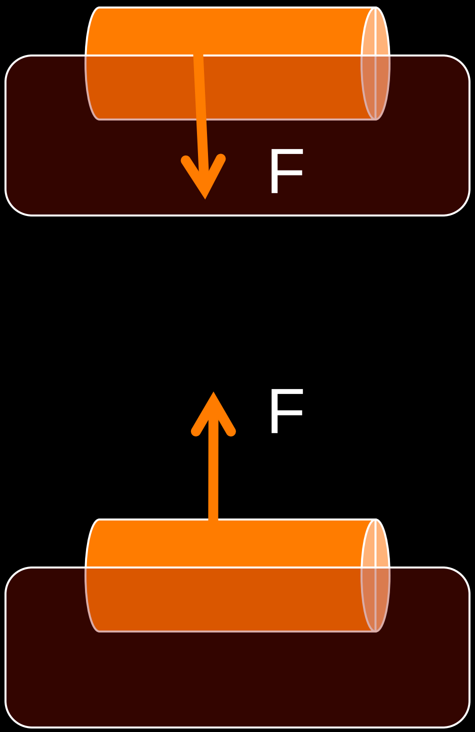
http://www.nanofunction.org

Contract!

- This is a ‘safe space’ where everyone is free to make mistakes.
- Everyone in the room participates.





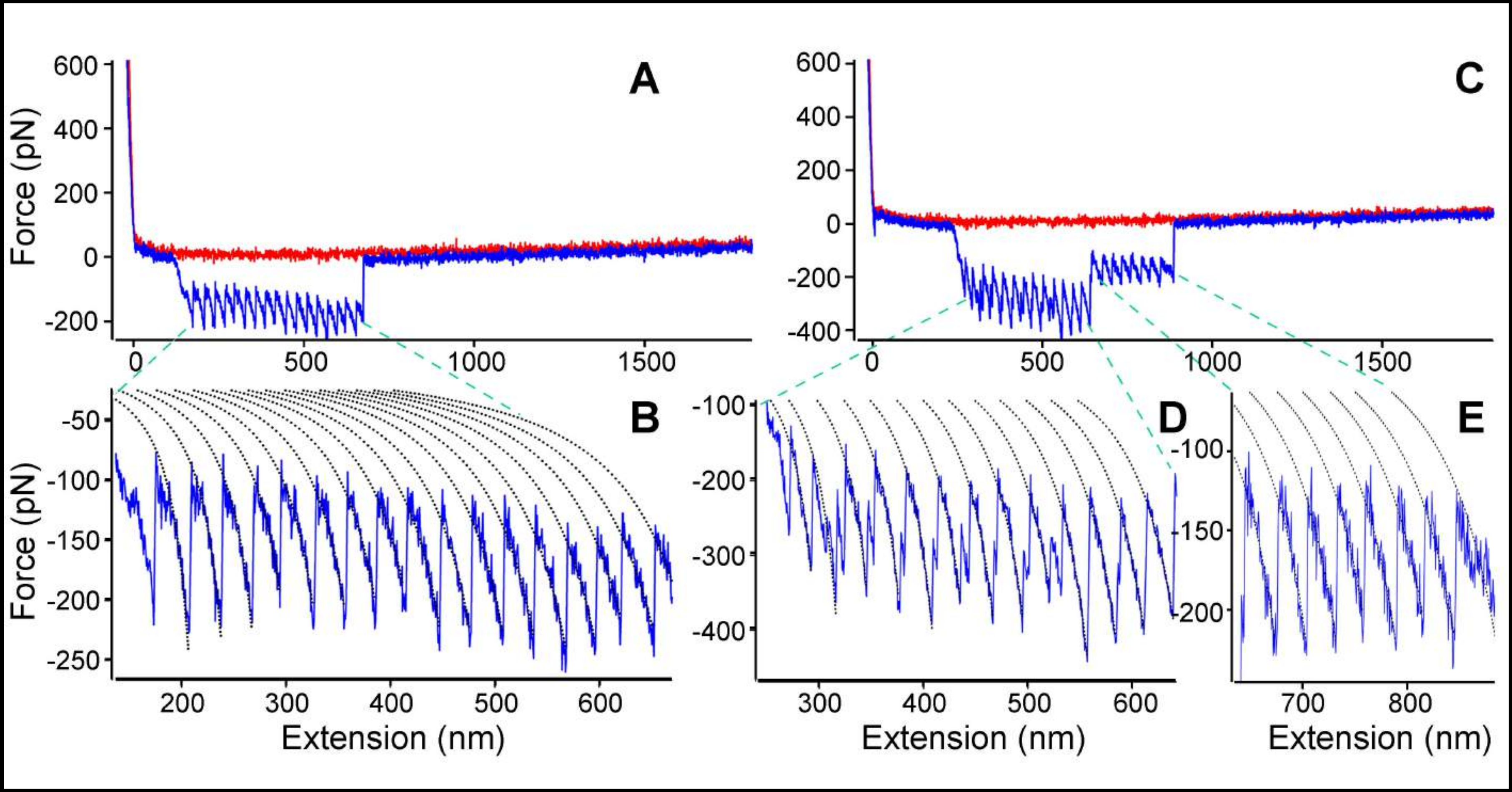
Mean persistence length = 0.08 nm **Mean persistence length = 0.45 nm**

Persistence length is an indication of 'net' stiffness

What happened here?

persistence length = 0.22 nm

persistence length = 0.21/0.41 nm



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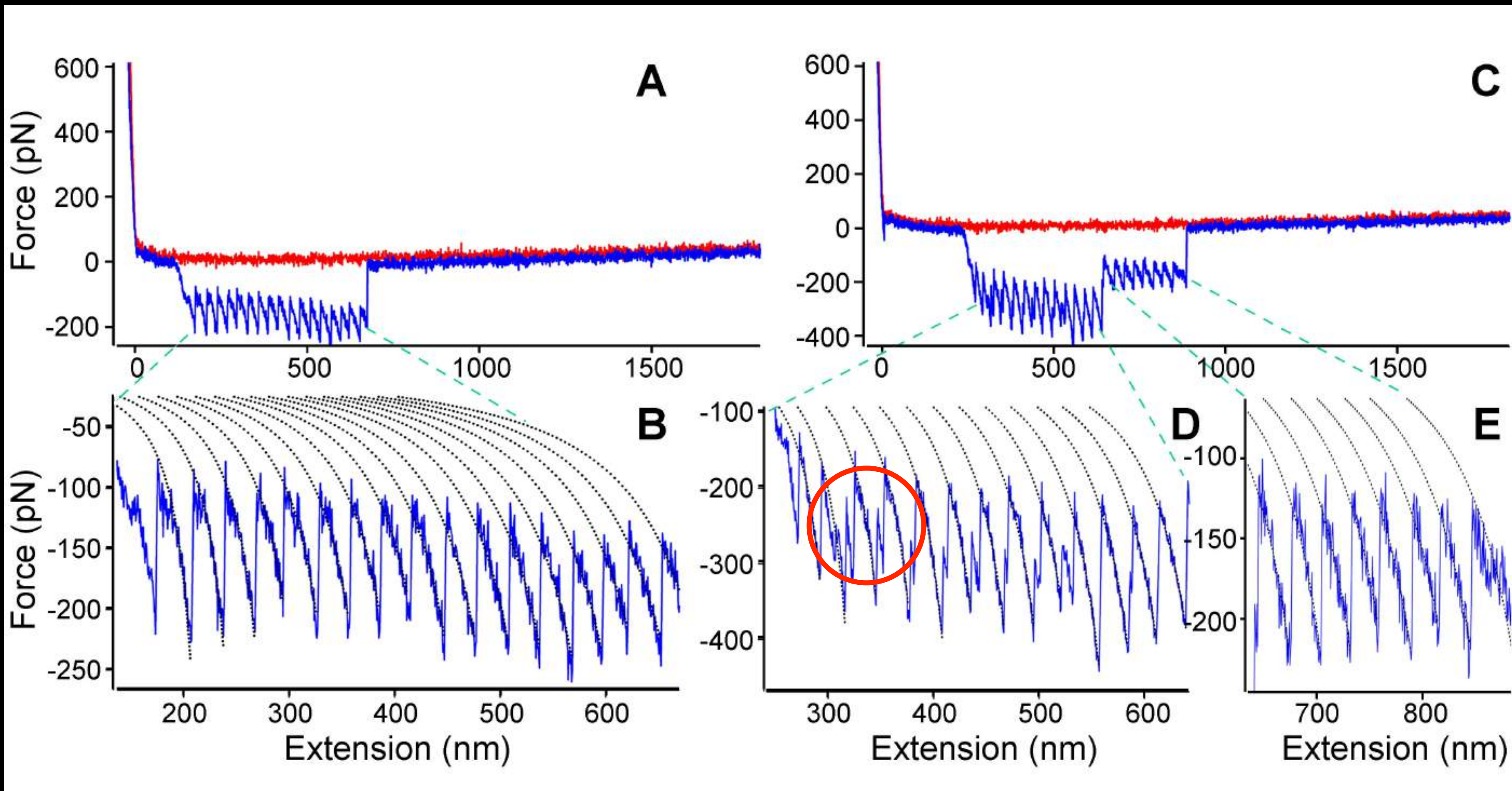

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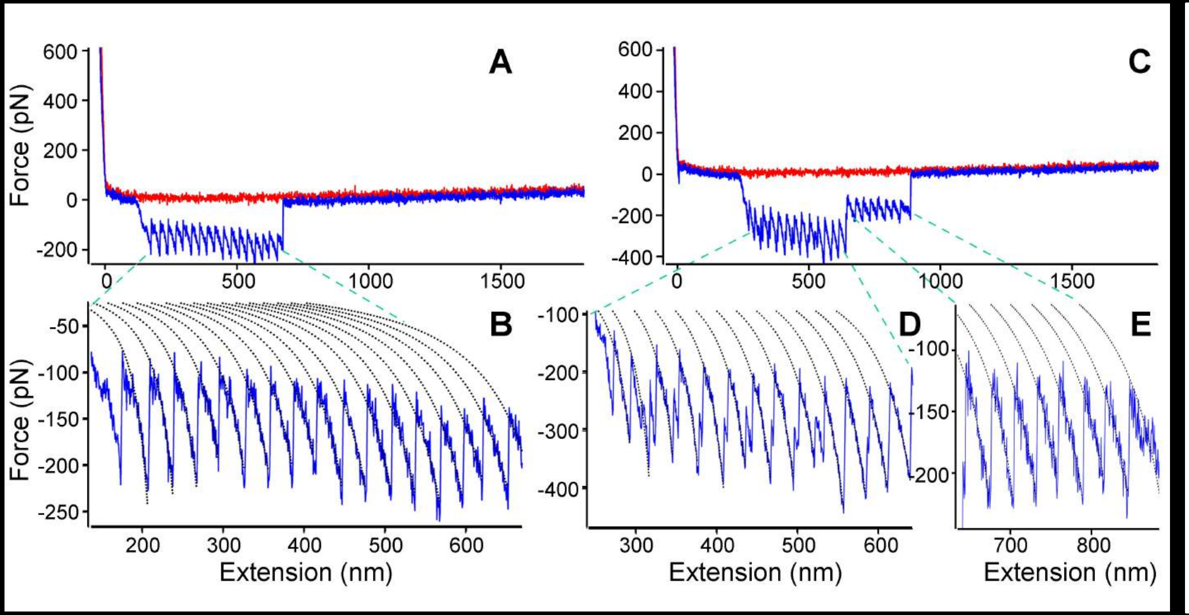
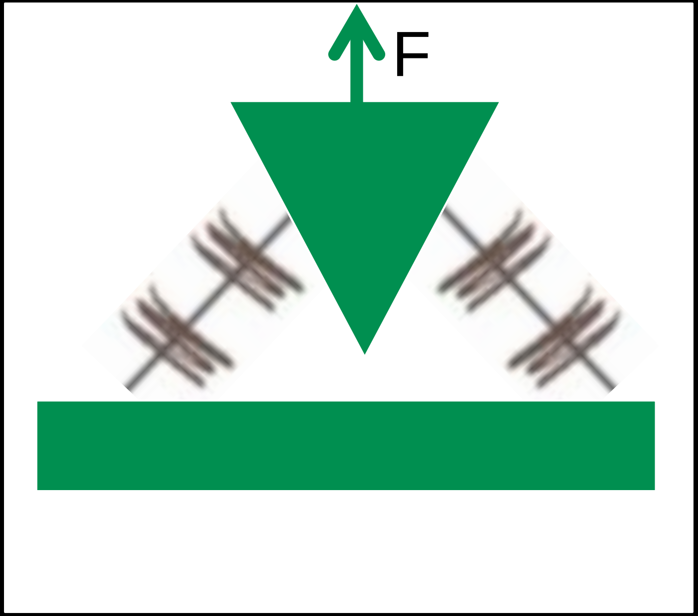
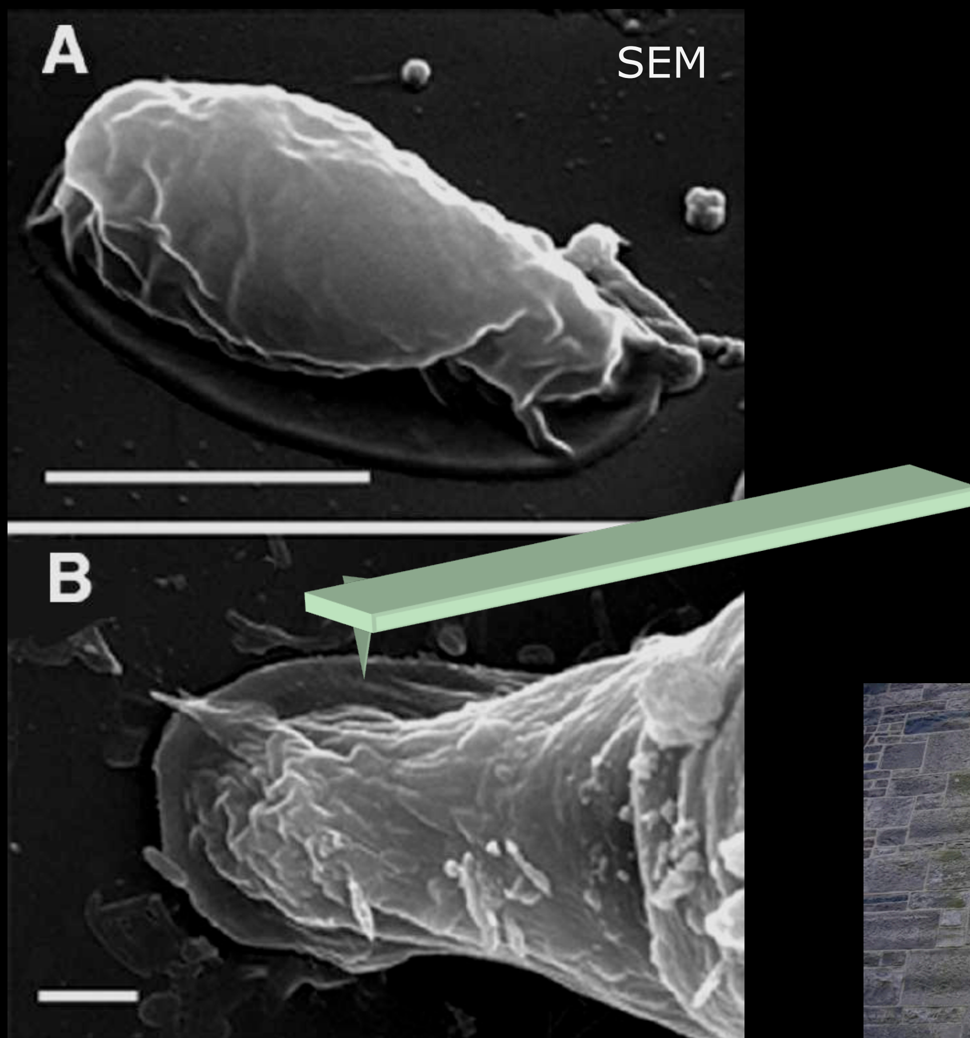




Parallel structures with same ordered arrangement
Two springs in parallel = twice the stiffness, half the persistence length

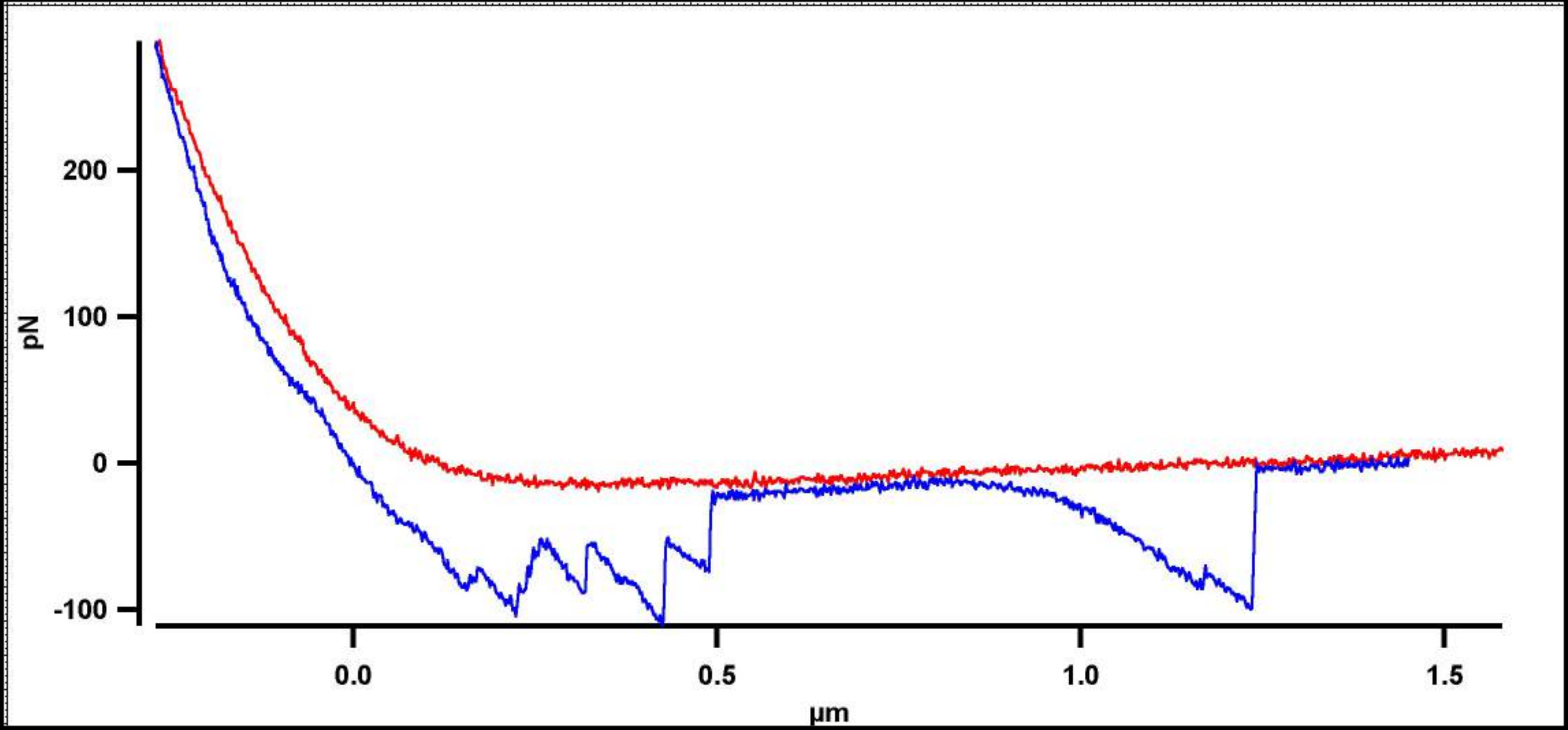
Nanoscale mechanics of natural adhesive



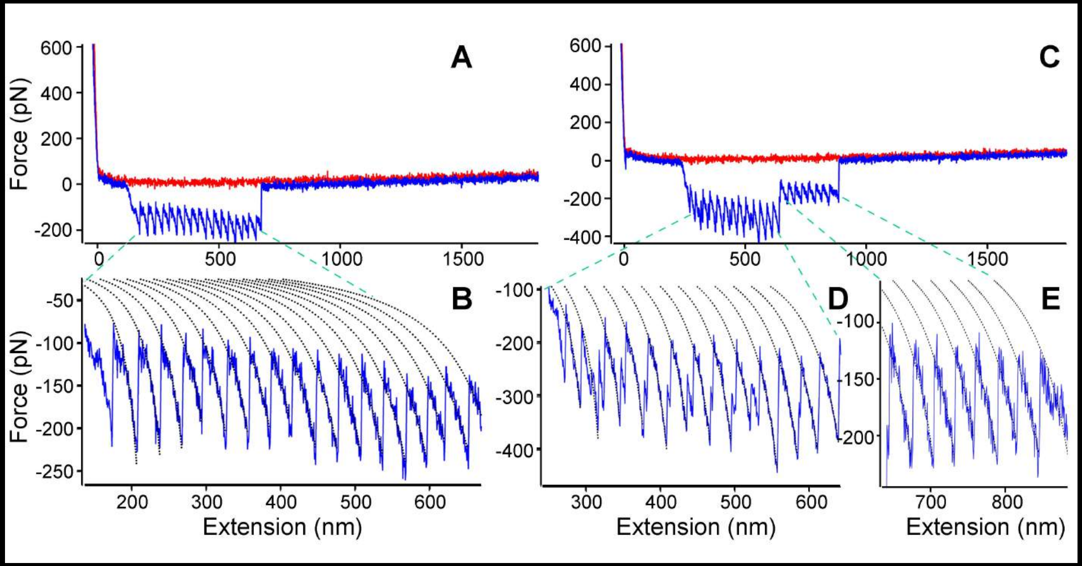
Scale = 2 µm

Probing the adhesive interface

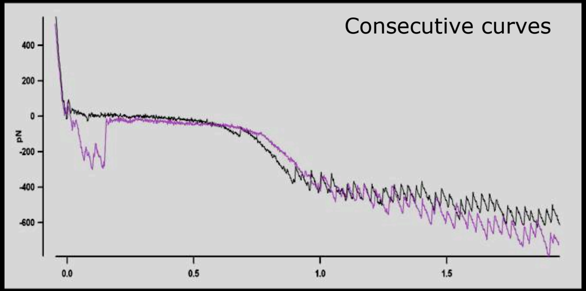
Random force-extension curve



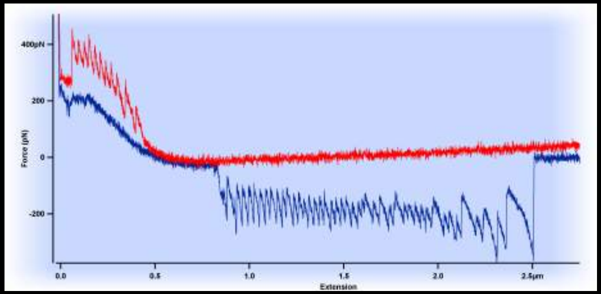
Very unusual force curves



Mechanical strength (sawtooths), parallel springs



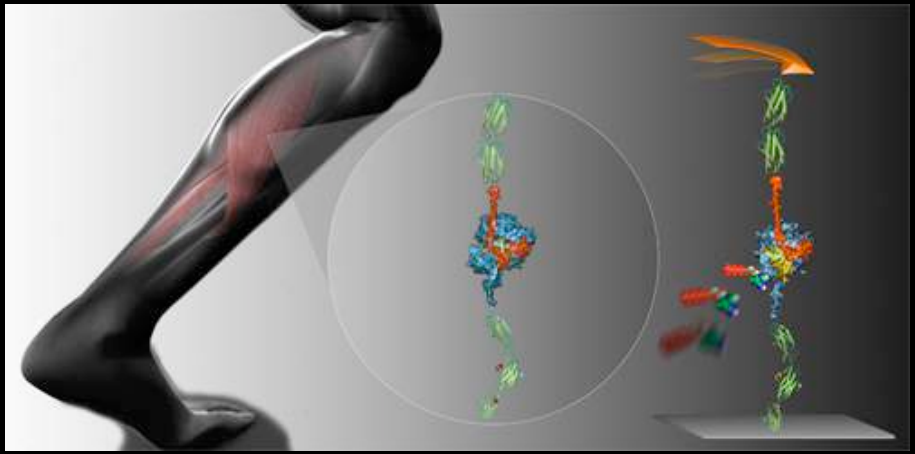
Refolding/Self-healing



High degree of symmetry

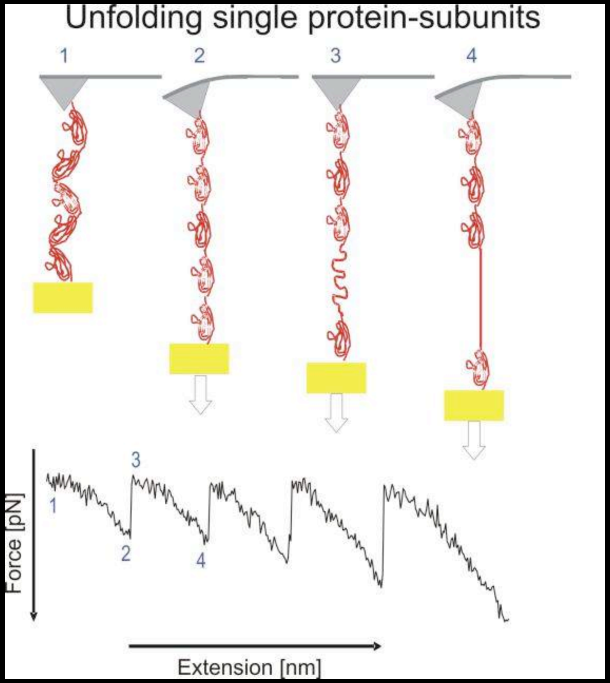
Mostaert & Jarvis 2007 Nanotechnology 18, 044010
Jarvis & Mostaert 2007 Imaging & Microscopy 9, 25-28

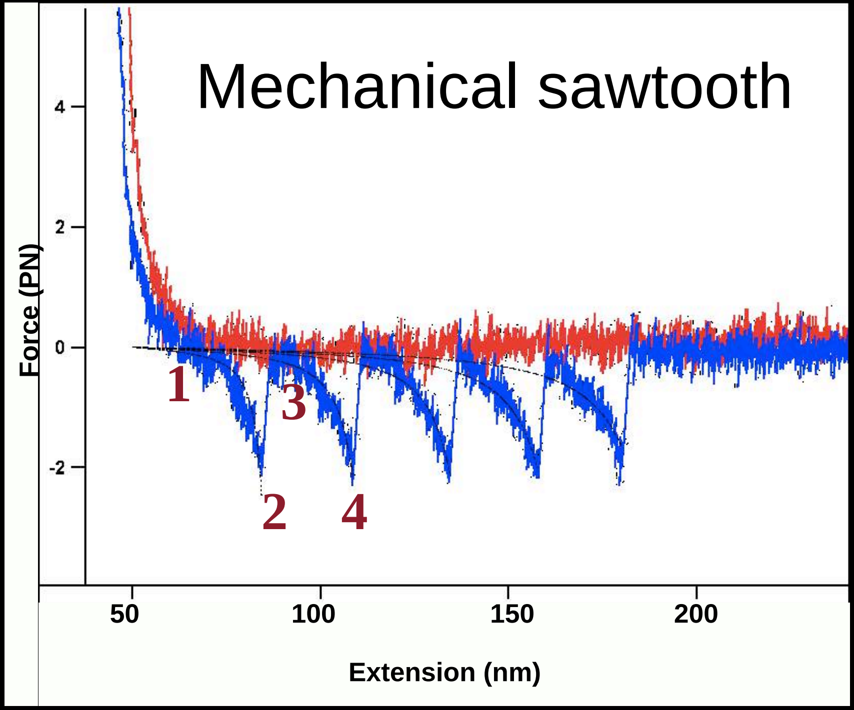
Modular proteins measured with AFM



Worm Like Chain Fitting

Mean persistence length = 0.4 nm (amino acid)
Mean domain length = 28 nm (hidden length)
Mean unfolding forces = 200 pN (sacrificial bonds)





Peer pressure!

3676 Biophysical Journal Volume 89 December 2005 3676–3677

New and Notable

Fingerprinting Single Molecules In Vivo

the experiments being carried out, underpinning an explosive understanding

the identification of a protein with a level of rigor comparable to that of the

4252 Biophysical Journal Volume 89 December 2005 4252–4260

Single Adhesive Nanofibers from a Live Diatom Have the Signature Fingerprint of Modular Proteins

Biophysical Journal Volume 90 April 2006 2987–2993 2987

Diatom Adhesive Mucilage Contains Distinct Supramolecular Assemblies of a Single Modular Protein

Biophysical Journal: Biophysical Letters

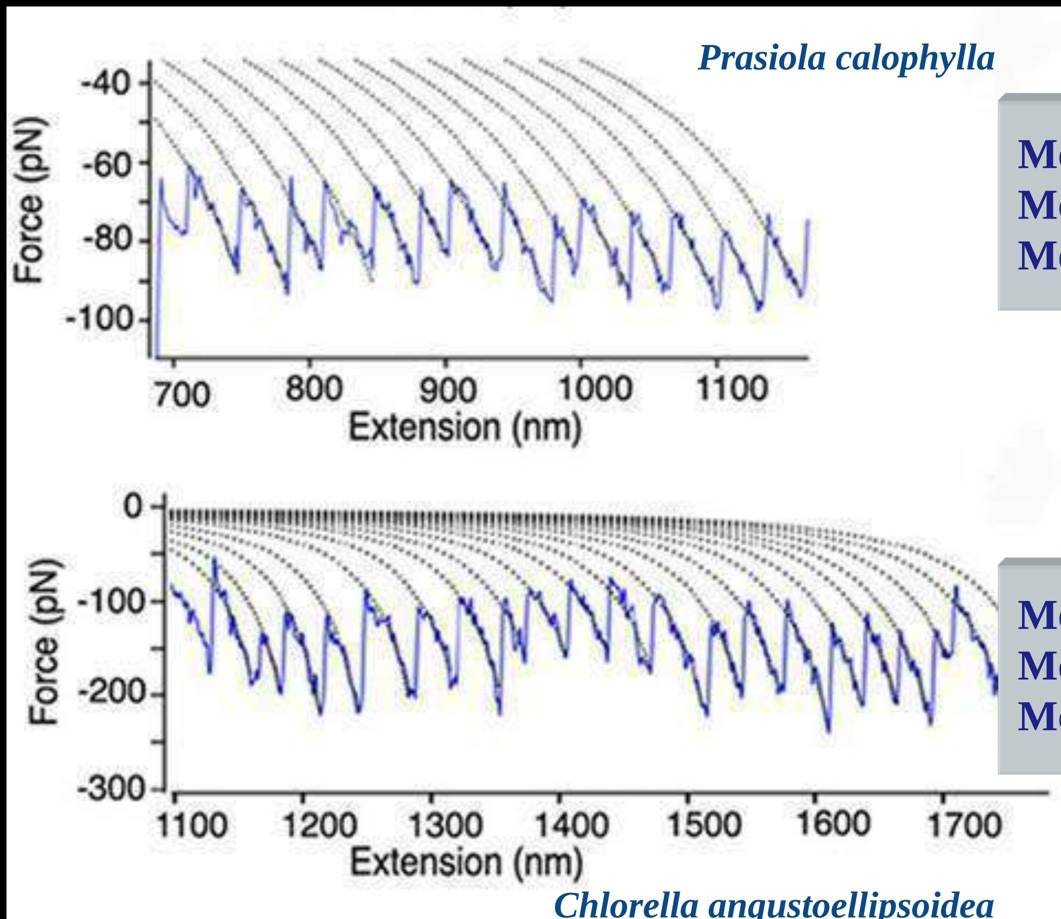
Adhesive Modular Proteins Occur in the Extracellular Mucilage of the Motile, Pennate Diatom *Phaeodactylum tricornutum*

Biofouling, 2006; 22(4): 245–250 Taylor & Francis Taylor & Francis Group

An *in situ* study of the nanomechanical properties of barnacle (*Balanus amphitrite*) cyprid cement using atomic force microscopy (AFM)

some force curves also contained a ‘fingerprint’ of modular protein unfolding.

Sawtooth fit to the WLC model



Prasiola

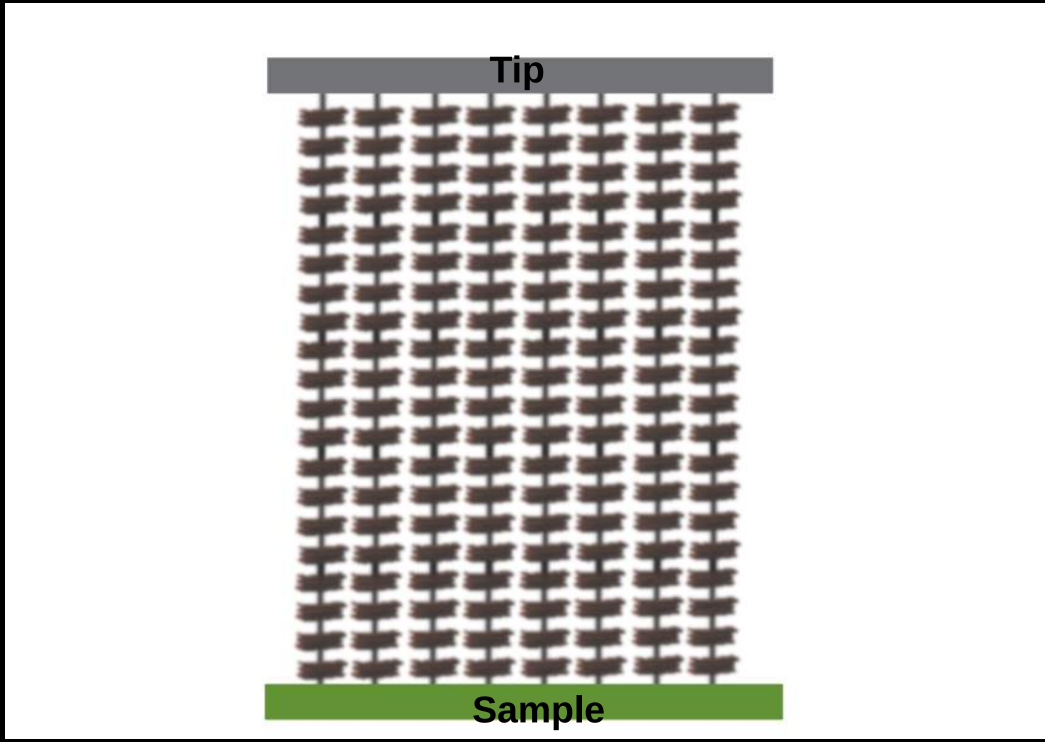
Mean persistence length = 0.5 nm
Mean domain length = 37 nm
Mean unfolding forces = 97 pN

Chlorella

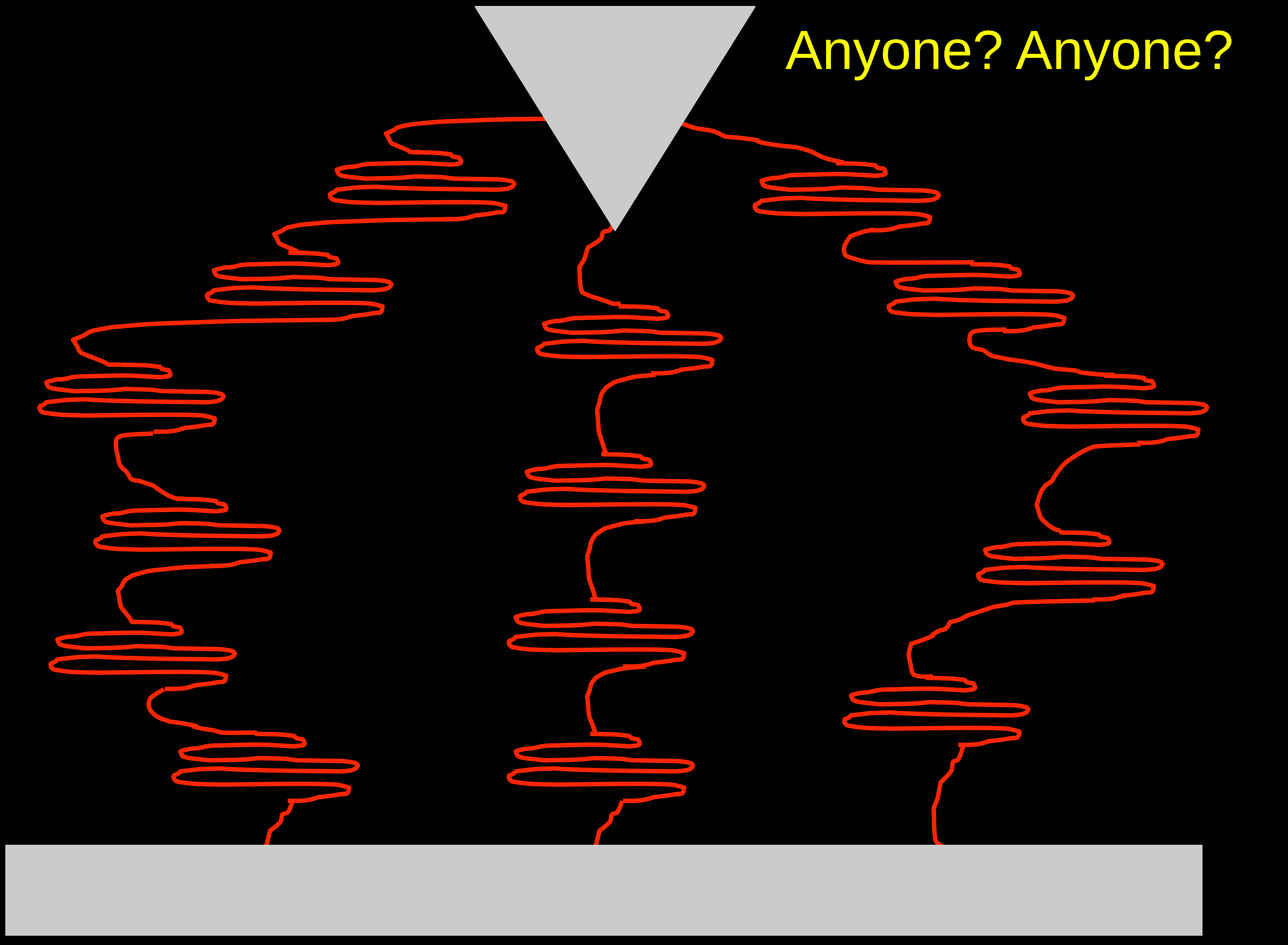
Mean persistence length = 0.4 nm
Mean domain length = 38 nm
Mean unfolding forces = 225 pN

Are these huge modular proteins? Evolution?

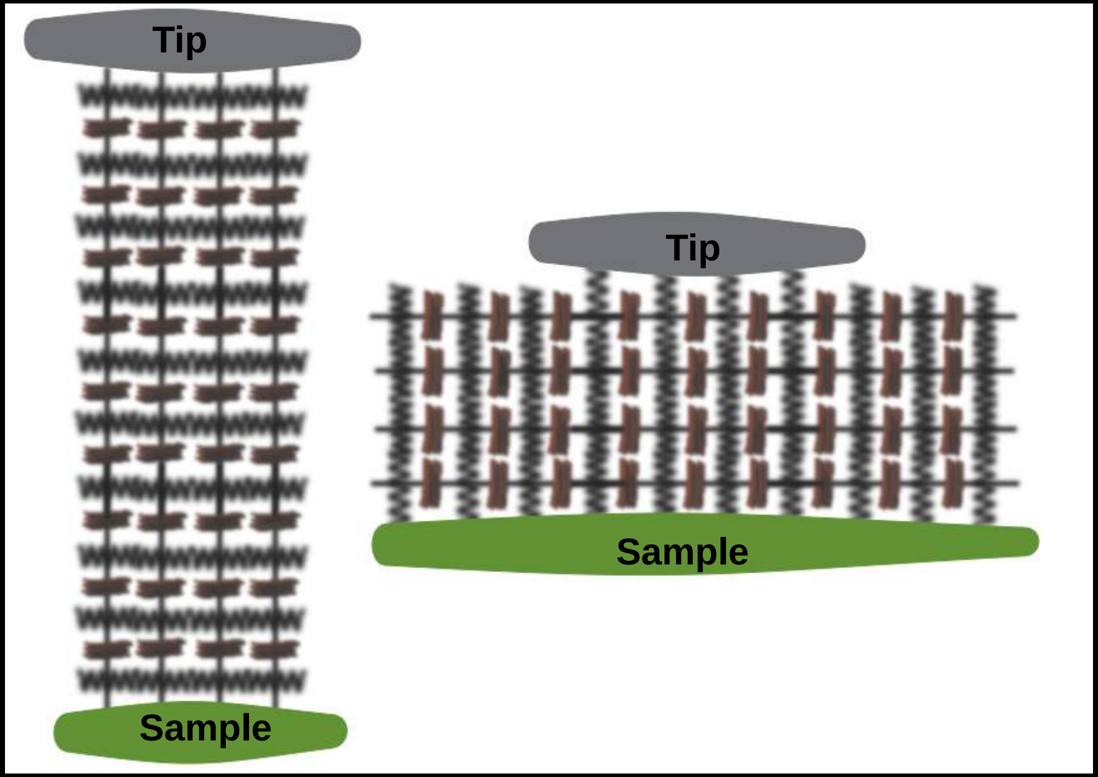
“ It is most likely that the adhesive nanofiber is a cohesive unit composed of multiple modular proteins aligned in parallel and functioning in synchrony.”*



*Dugdale, T.M., Dagastine, R., Chiovitti, A., Mulvaney, P. & Wetherbee, R. *Biophysics Journal* 89, 4252 - 4260 (December 1, 2005)


Anyone? Anyone?

Glycoprotein with modular backbone?

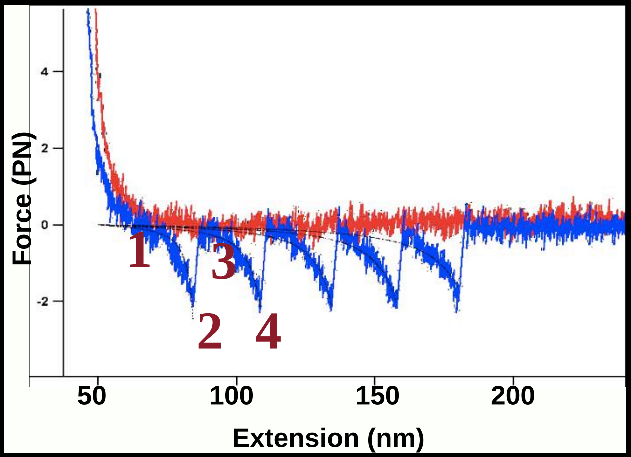


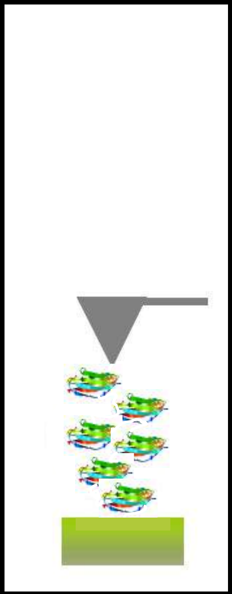
Even single modular proteins rarely attach by their ends: why would parallel molecules do so?

Mechanical involvement of side chains removes any uniform mechanical signature.

Is this really the most likely scenario?

Fingerprint, signature or dogma?





$2 + 2 = 4$

$2 + 2 = 4$

$2 + 2 = 4$

$2 + 2 = 4$

$2 + 2 = 4$

$? = 4$

$1 + 0 \neq 4$

$2 + 4 \neq 4$

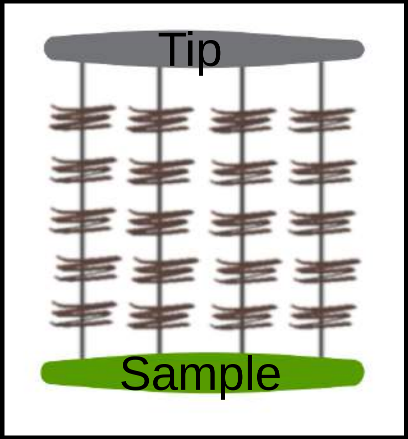
$5 + 2 \neq 4$

$3 + 9 \neq 4$

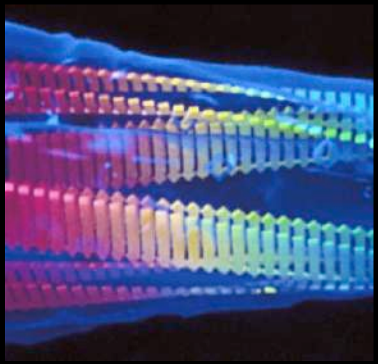
$4 + 0 = 4$

$7 + 3 \neq 4$

$3 + 1 = 4$



Amyloid?



Intrinsically highly ordered structure

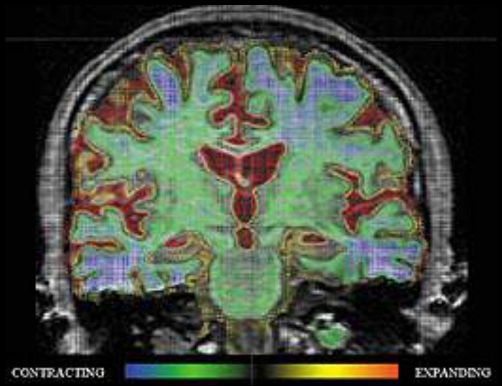
High probability of ‘parallel’ mechanical unfolding (multiple levels of structure)

Multidirectional mechanical strength

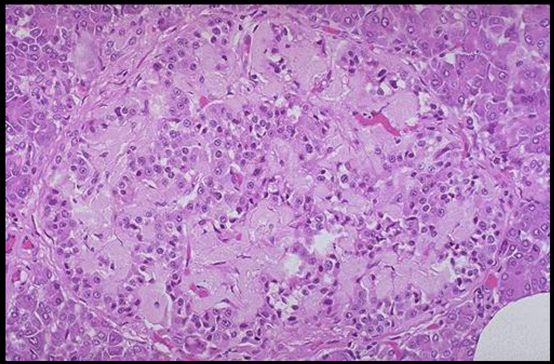
Amyloid is a generic alternative protein fold which could be produced from a wide variety of amino acid sequences

Difficult to identify experimentally or using bioinformatics

Pathogenic amyloid



Neurodegenerative diseases - Alzheimer's, Parkinson's



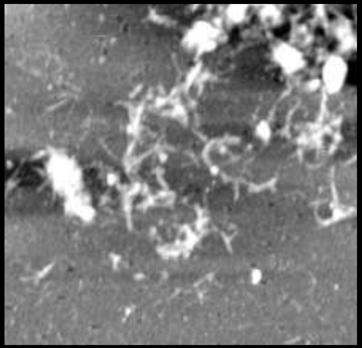
Type II diabetes -Islets of Langerhans (pancreas)

A generic alternative protein structure?

A number of emerging amyloid based disease e.g. Trasmissible spongiform encephalopathies, glaucoma…

Amyloidosis: A universal disease of aging?

Physiological amyloid



Curli - E.coli biofilm

Hydrophobin
fungal coat

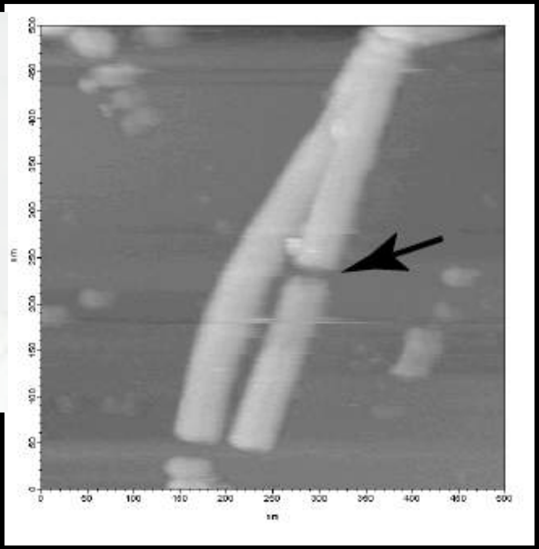
Spider silk production

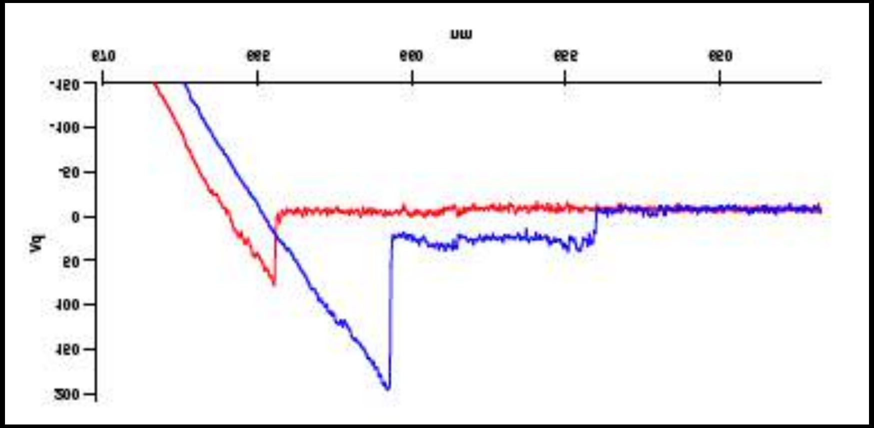
Hagfish lubricant

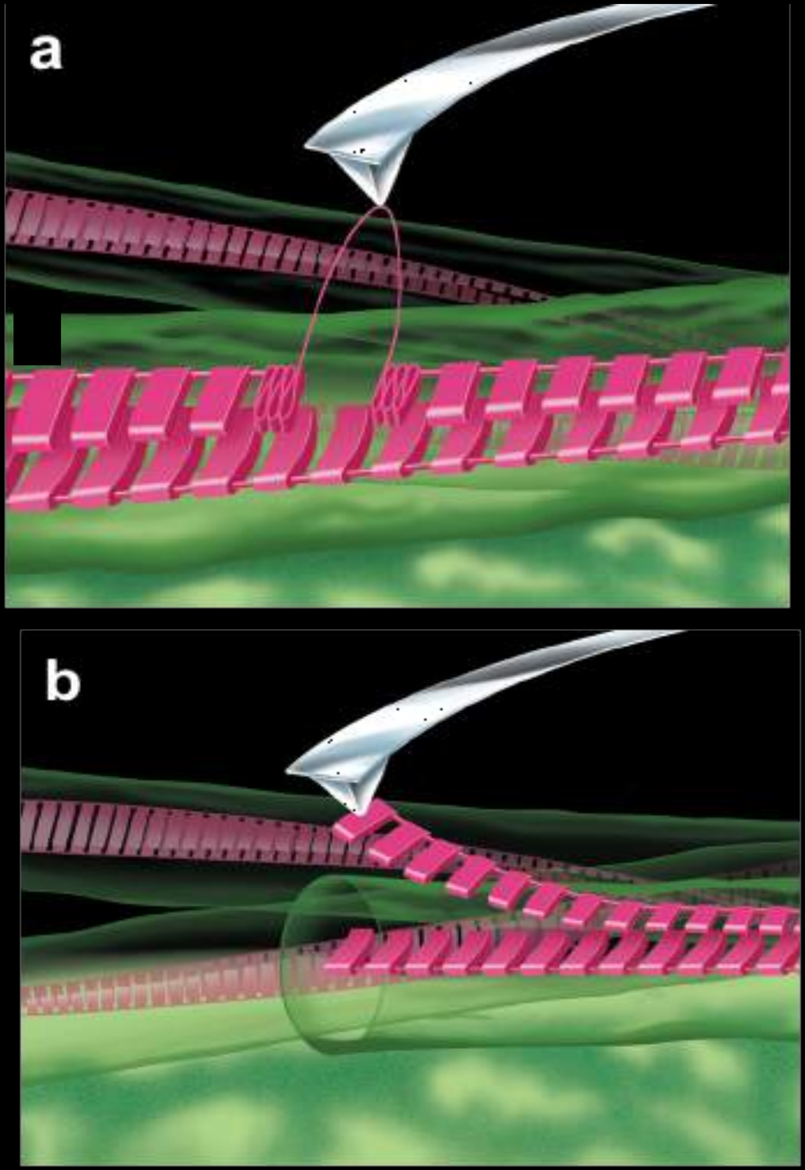
Apparent mechanical connection

Defeating dogma is difficult!

Mechanics of model Amyloid Fibrils







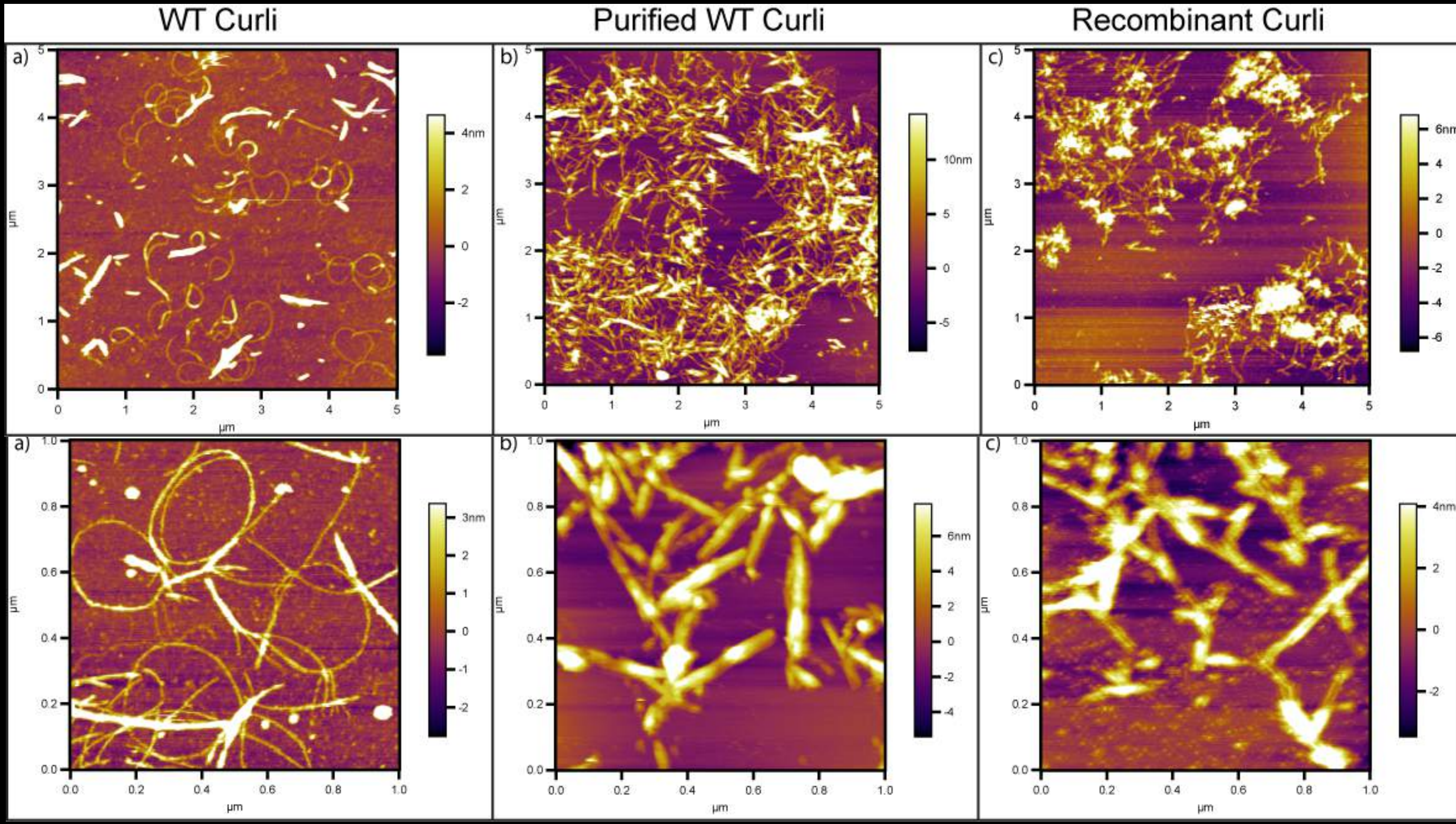
Fukuma, Mostaert and Jarvis (2006) Tribology Letters 22, 233. (Open access)

Known Physiological Amyloid

Curli E.coli adhesive biofilm formation

Bian et al. The EMBO J. 16, 5827 (1997)

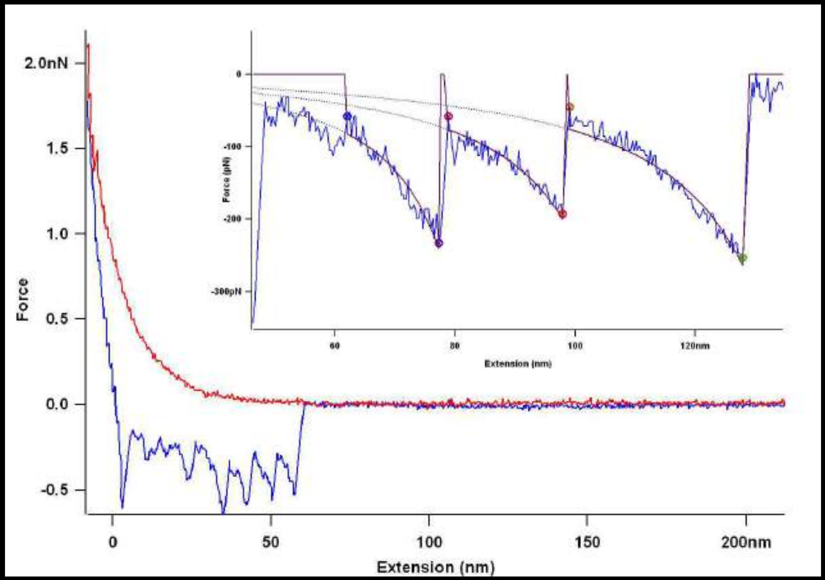
Three types of curli morphology (AFM images)



Recombinant sample supplied by Paul Barker and Adrian Nickson, University of Cambridge

Mechanics of wild type Curli

21% of curve show multiple sawtooth events

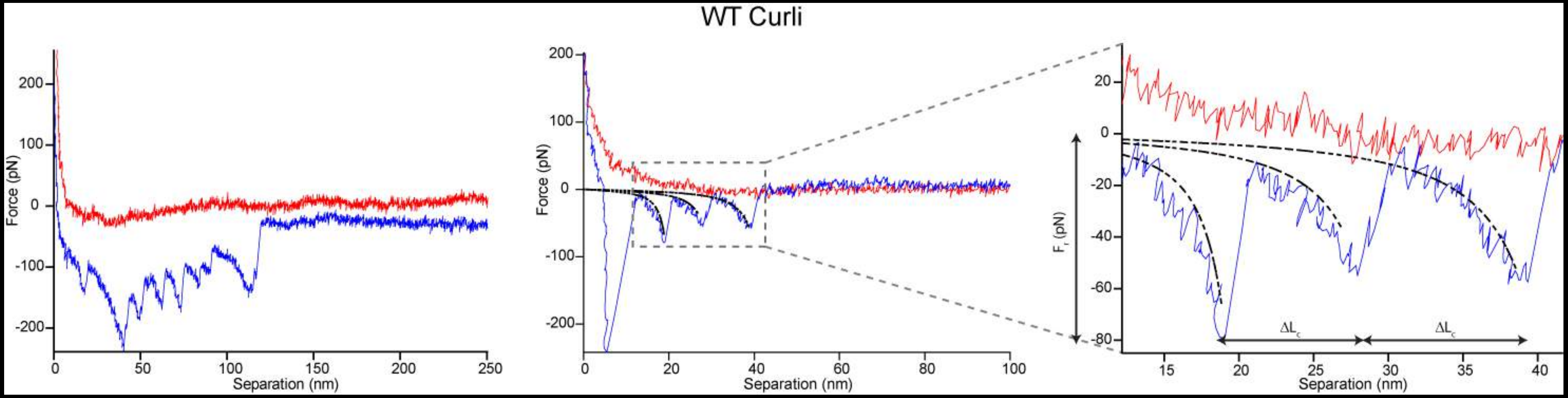


Worm Like Chain Fitting For Polymers

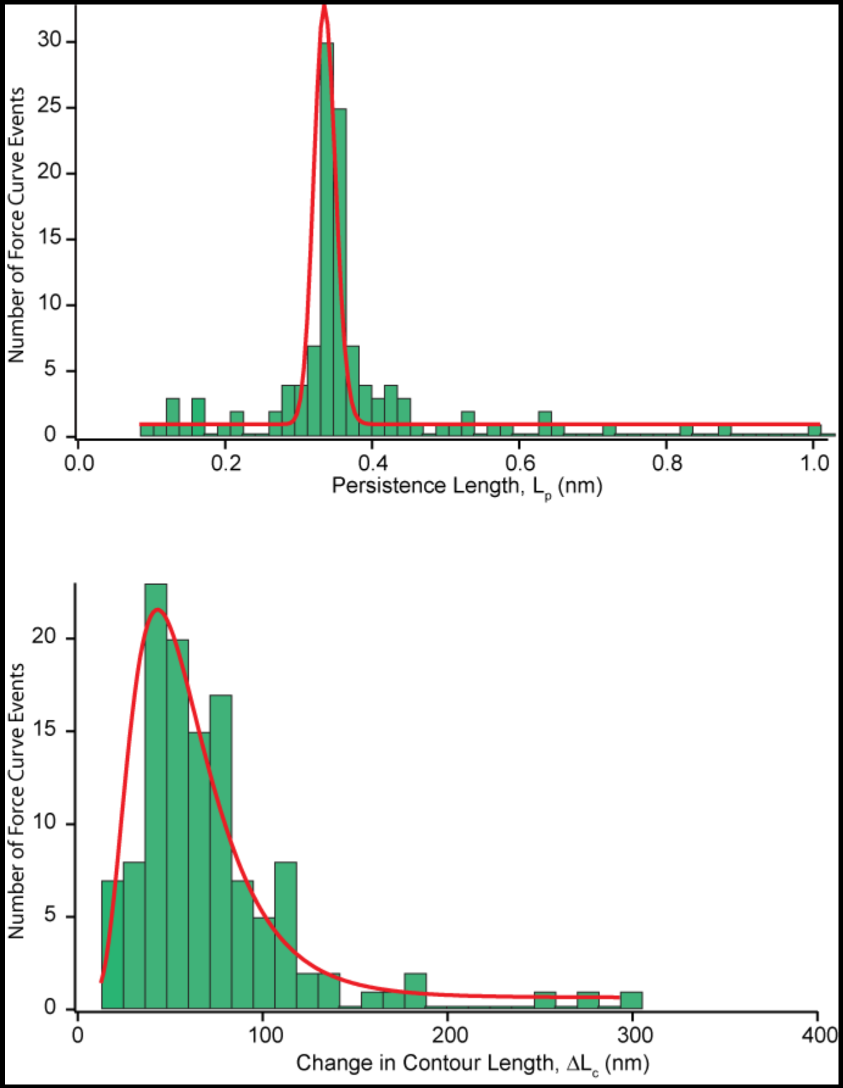
Mean persistence length ≈ size of amino acid

Change in contour length ≈
hidden length within folded protein

Mean unfolding forces ≈
total bonds within the folded protein



Mechanics of wild type Curli

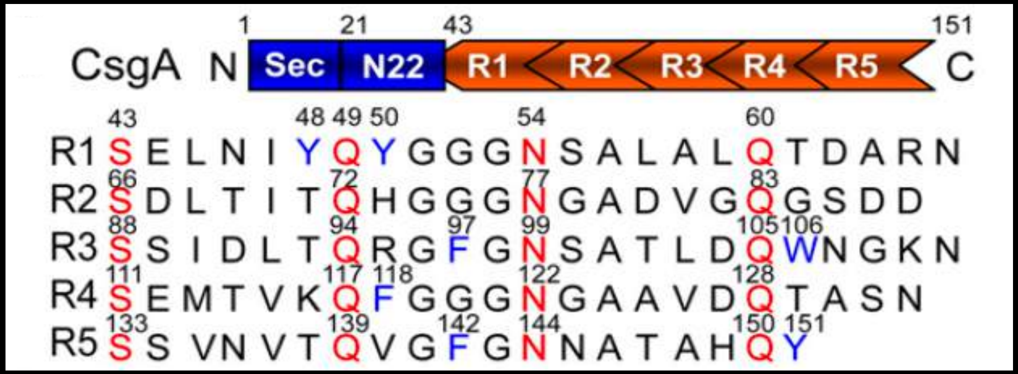


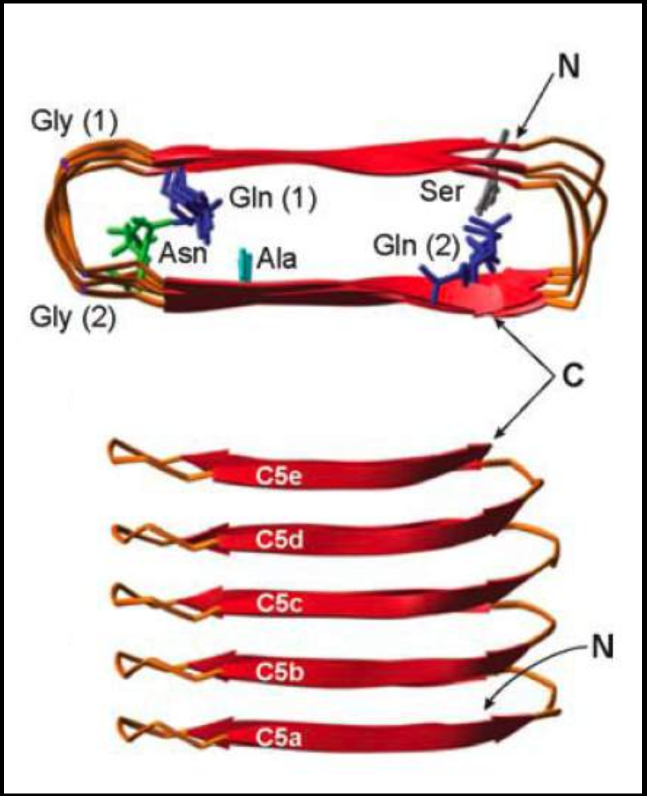
Worm Like Chain Fitting

Persistence length = 0.34 nm (SD 0.01)
Contour length = 43.2 nm (SD 1.9)
Mean unfolding forces = 54 pN (SD 1.0)

Mechanics of wild type Curli

M.R. Chapman *et al.*





Total length = 130 x 0.36 = 46.8 nm
(109 aa in amyloid core)

Folded length = 2.5 nm

Total length = folded length + hidden length (contour length)

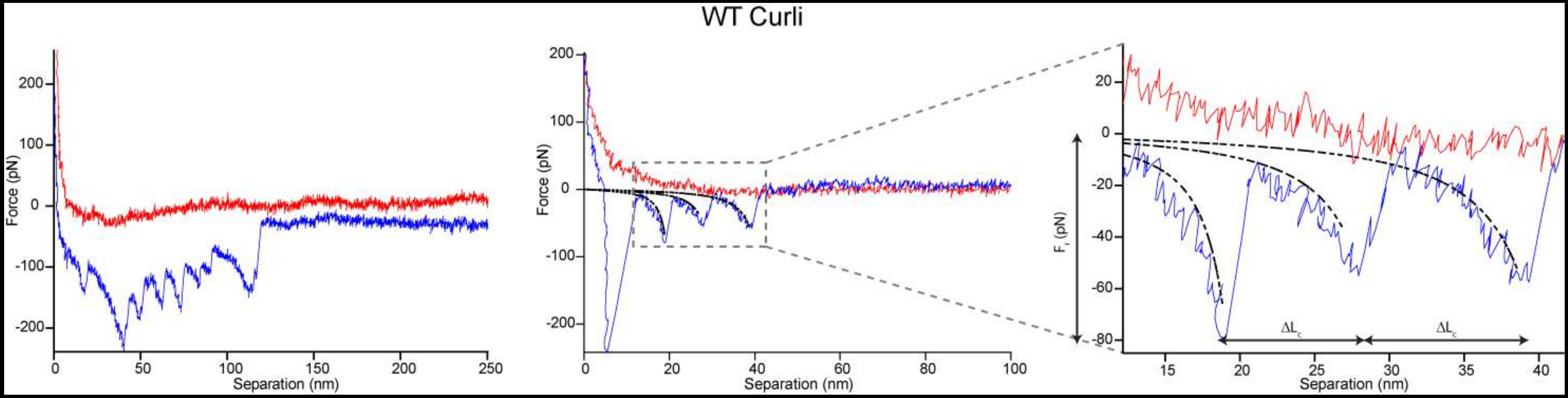
= 2.5 + 43.2 = 45.7 nm (SD 1.9)

Mechanics of wild type Curli

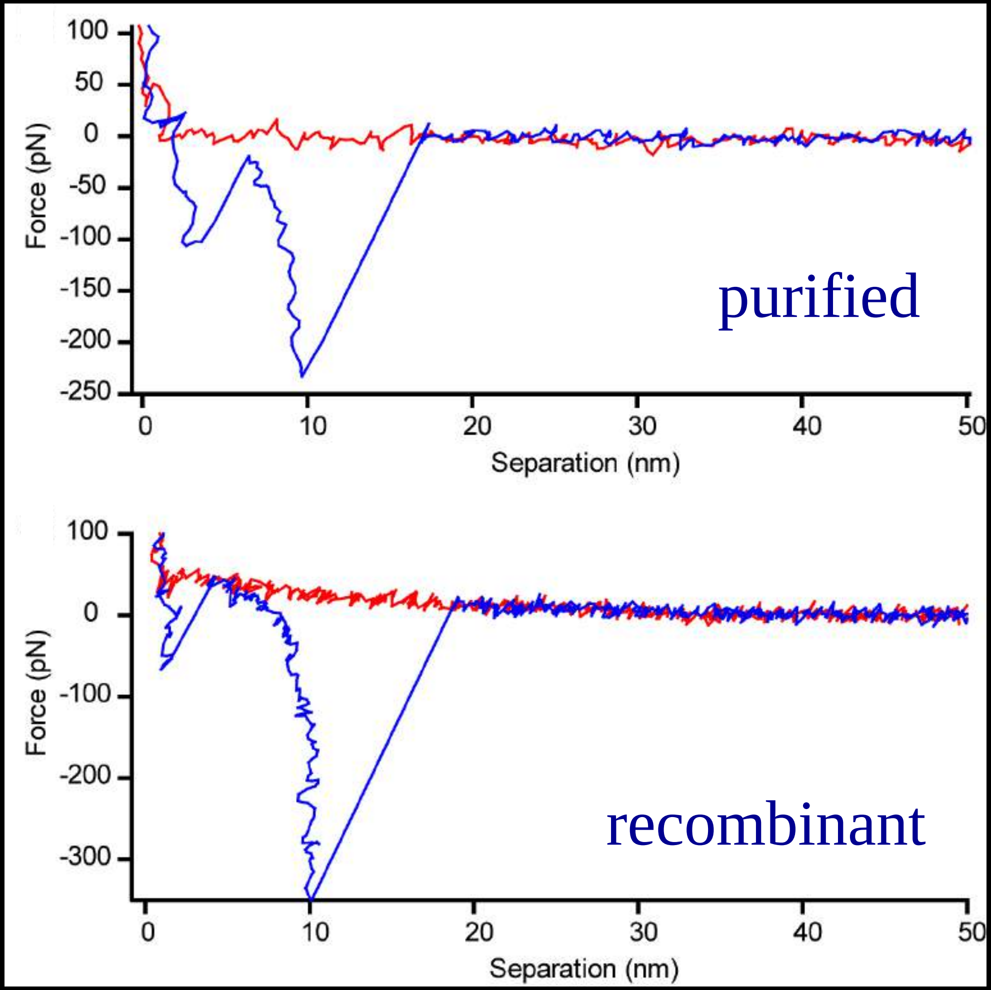
Monomer length = folded length + contour length

Therefore each sawtooth corresponds to a monomer

The interaction between each monomer must be stronger than the interaction within each monomer to produce a multiple sawtooth response.



Mechanics of purified and recombinant Curli

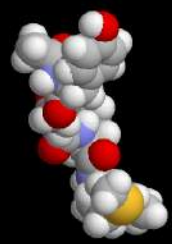
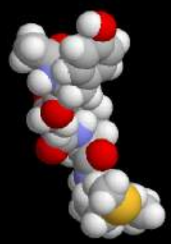
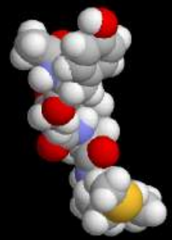


Less than 2% of curves show a single elastic response

No multiple sawtooth responses were observed (n=6000)

The interaction between each monomer must be weaker than the interaction within each monomer

Specific peptide segments aggregating *in vitro*

 +  + 

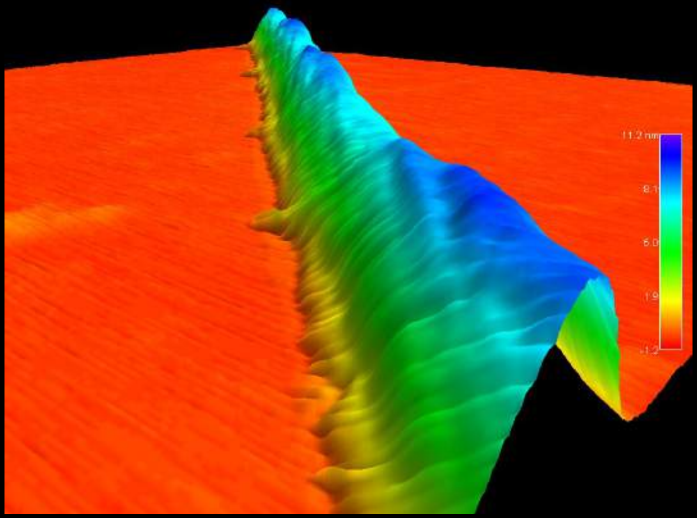
+

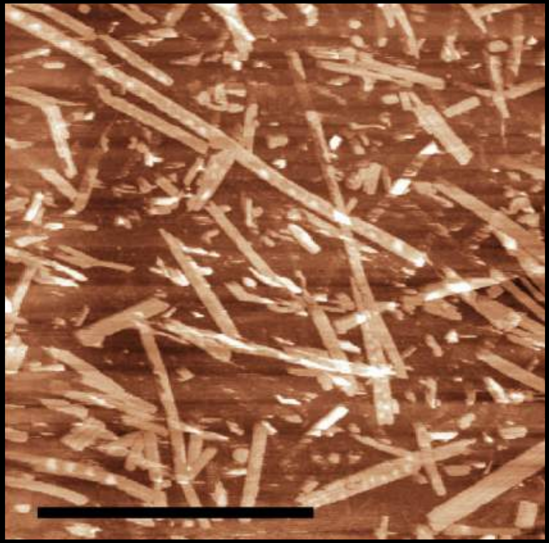
Low pH (2.5)

Purified single peptides in bulk liquid

No crowding, no competition and usually not physiological solutions

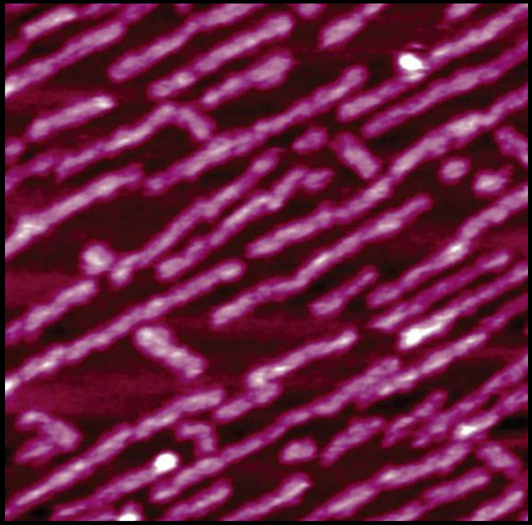
Result in very uniform ‘crystalline’ structures



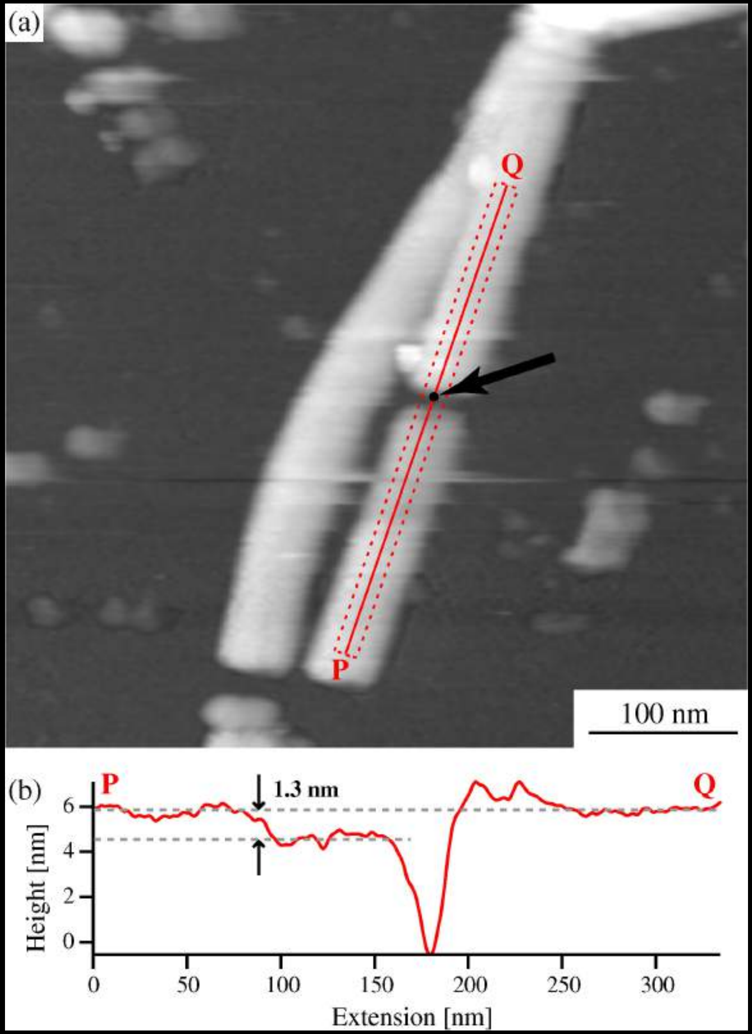


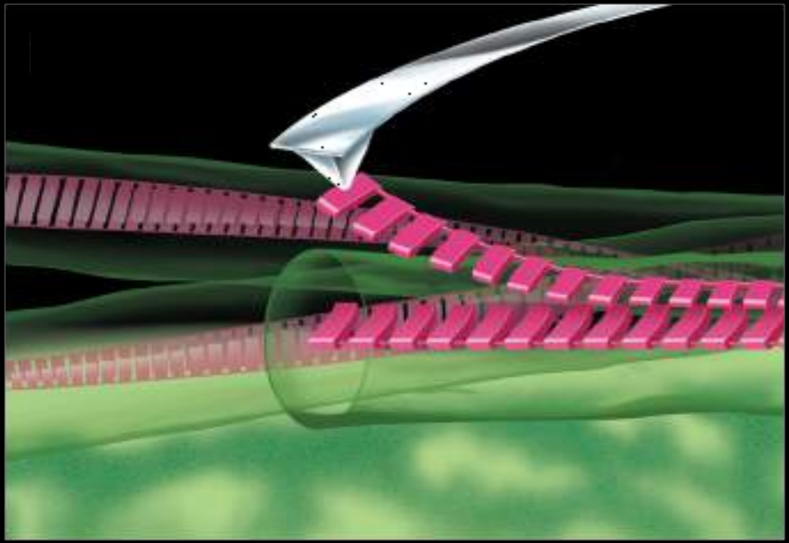
In vitro

Scan size 250 nm



Crystalline *in vitro* fibrils are very fragile



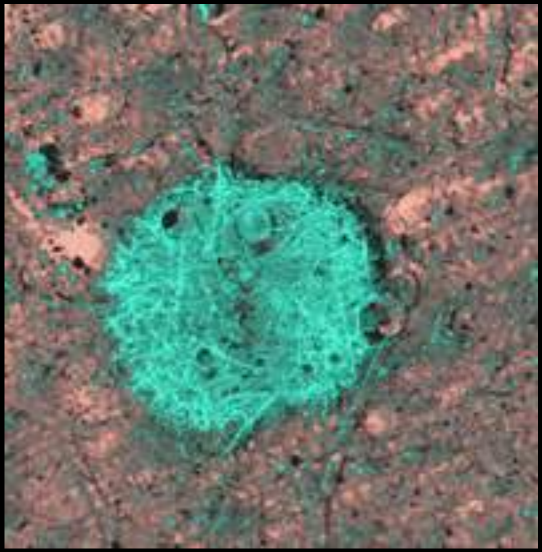


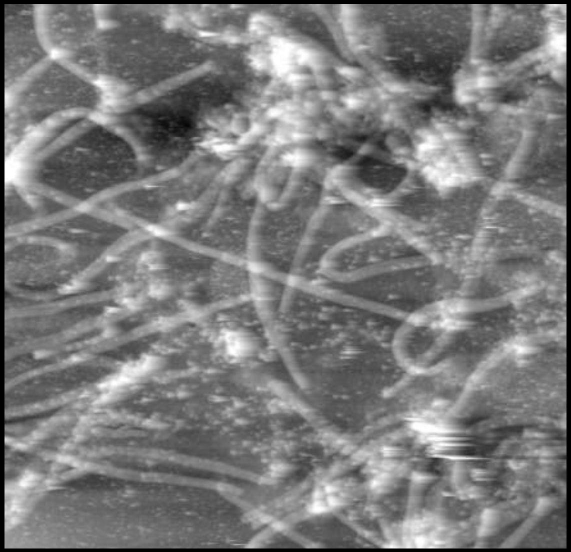
ß-sheets are broken and peeled from the bulk of the fibril

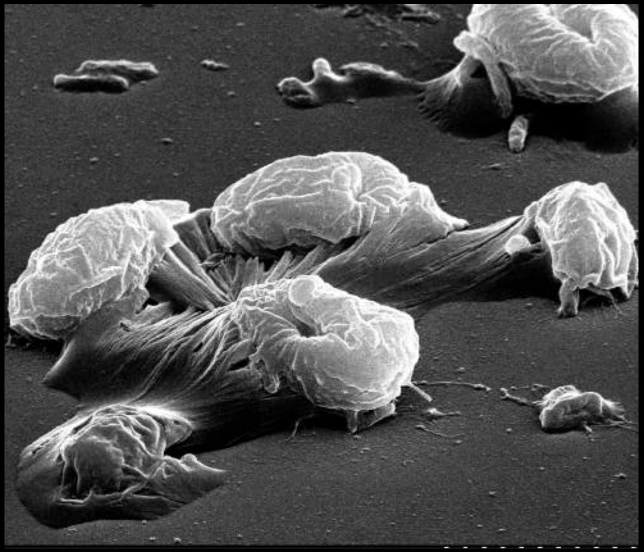
No defects to pin crack propagation

Fukuma, Mostaert and Jarvis (2006) Tribology Letters 22, 233. (Open access)

In vivo structures are very different







Med. Mic. Sciences,
Cardiff Uni.

'Gooey sticky fiberous clumps of amyloid plaque causing brain cell dysfunction and death'

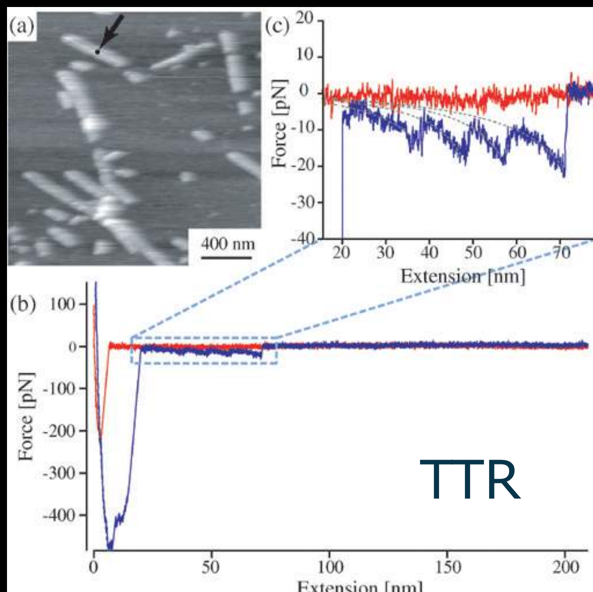
clumps of amyloid in natural adhesive

Quantitative comparison of amyloid

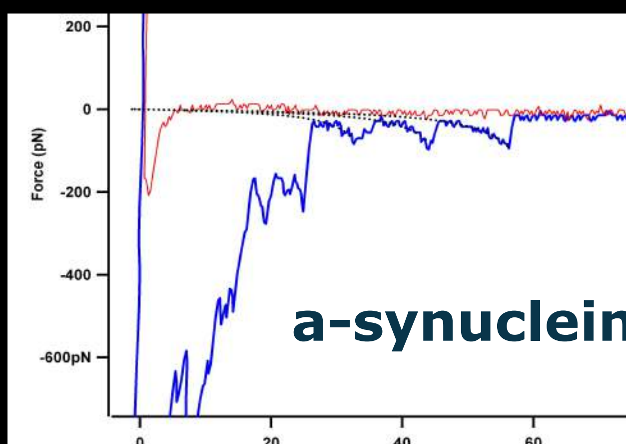
relative toughness

Pathogenic

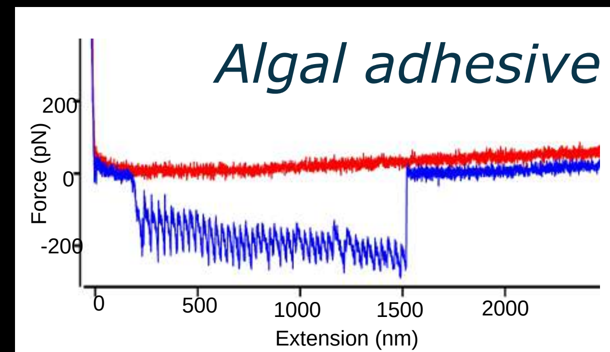
0.2 aJ



1.2 aJ

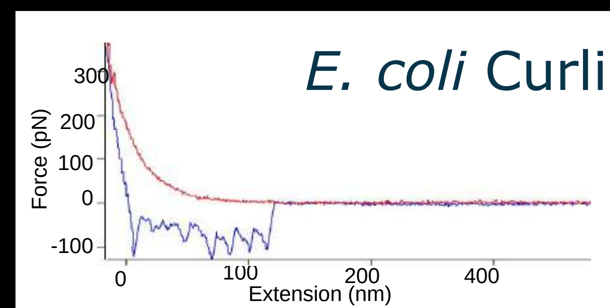


Physiological

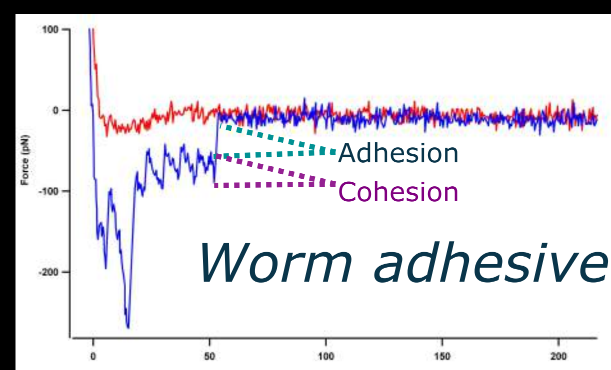


>100 aJ

Cross-linking?



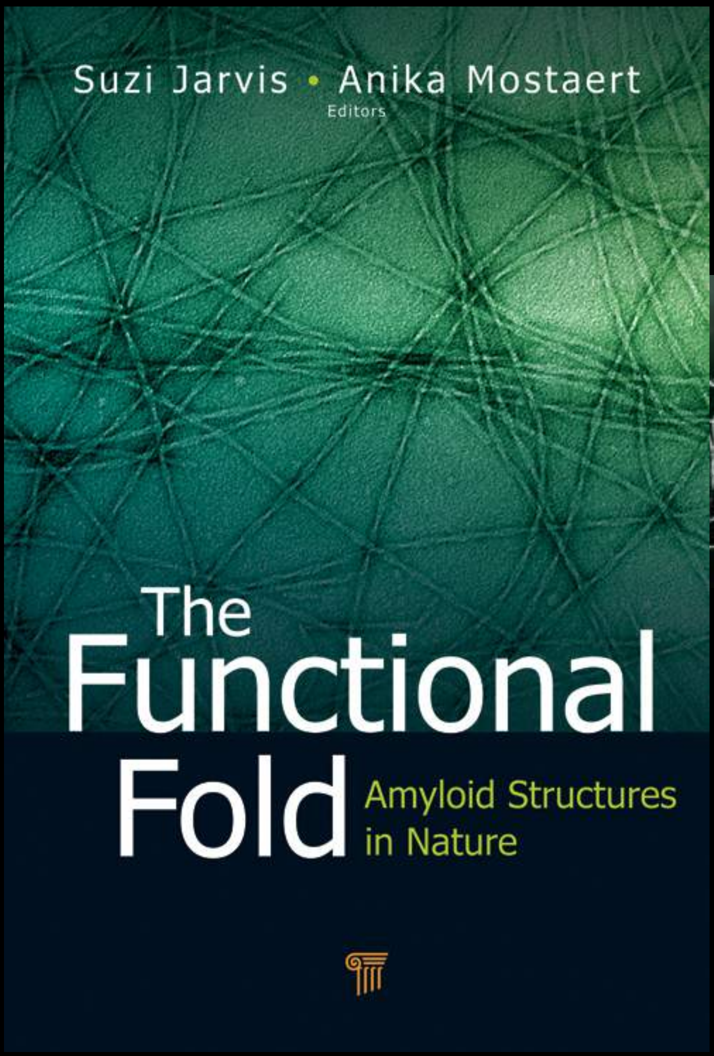
7.2 aJ



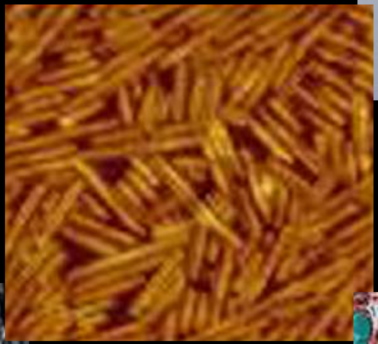
2.3 aJ

Functional amyloid

Non-pathogenic, physiological amyloid
Materials applications in nature

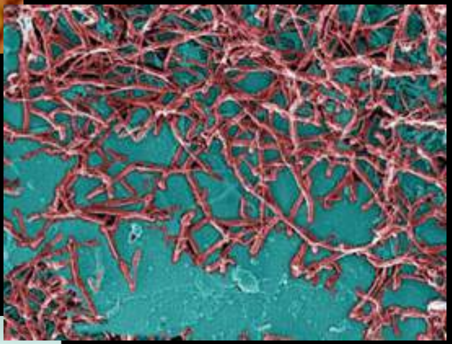


Chorion - Silkmoth & Fish



Hydrophobins - Fungal coats

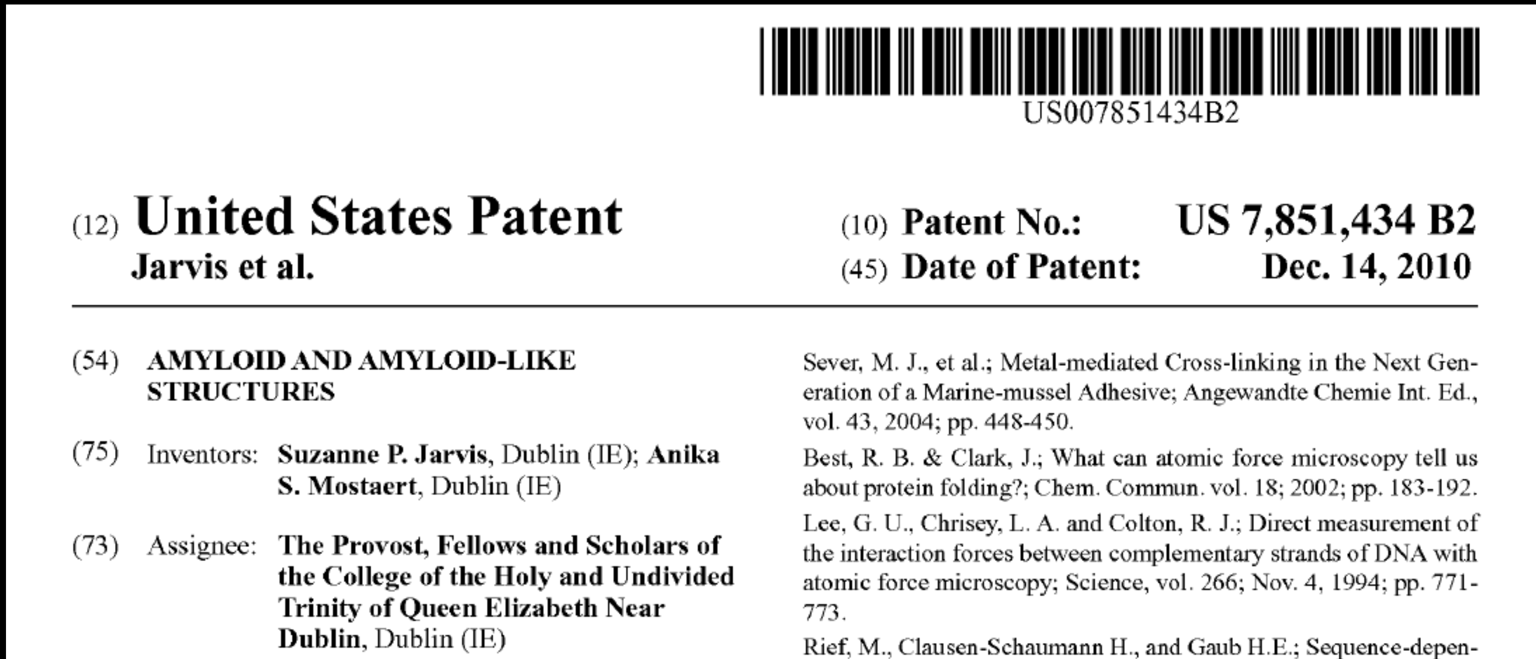
Silk production



Curli - *E. coli* biofilm

Hagfish slime

Applications



US007851434B2

(12) **United States Patent**
Jarvis et al.

(10) **Patent No.: US 7,851,434 B2**
(45) **Date of Patent: Dec. 14, 2010**

(54) **AMYLOID AND AMYLOID-LIKE STRUCTURES**

(75) Inventors: **Suzanne P. Jarvis**, Dublin (IE); **Anika S. Mostaert**, Dublin (IE)

(73) Assignee: **The Provost, Fellows and Scholars of the College of the Holy and Undivided Trinity of Queen Elizabeth Near Dublin**, Dublin (IE)

Sever, M. J., et al.; Metal-mediated Cross-linking in the Next Generation of a Marine-mussel Adhesive; Angewandte Chemie Int. Ed., vol. 43, 2004; pp. 448-450.

Best, R. B. & Clark, J.; What can atomic force microscopy tell us about protein folding?; Chem. Commun. vol. 18; 2002; pp. 183-192.

Lee, G. U., Chrisey, L. A. and Colton, R. J.; Direct measurement of the interaction forces between complementary strands of DNA with atomic force microscopy; Science, vol. 266; Nov. 4, 1994; pp. 771-773.

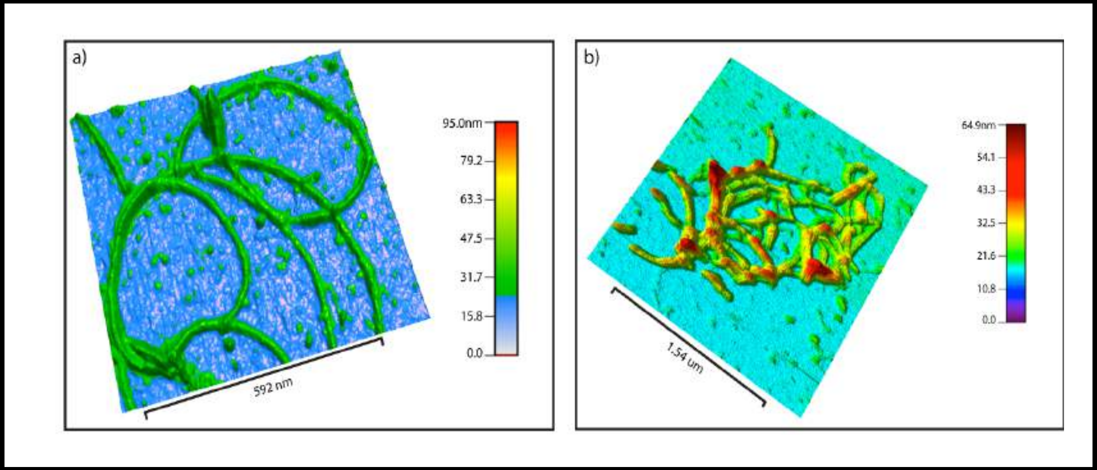
Rief, M., Clausen-Schaumann H., and Gaub H.E.; Sequence-depen-

Market gap:
water-based, biocompatible adhesive with good performance and reliability

Market for compromise solutions:
>$2billion in USA growing at 19% p.a.

Scientific Summary

- Mechanically functional amyloid found in natural adhesives.
- Amyloid could be a widespread mechanism for mechanical strength in a many natural materials.
- Amyloid fibrils vary in morphology, mechanical strength and inter-molecular interactions within the amyloid core.
- *In vivo* formed fibrils have greater toughness when compared to *in vitro* formed fibrils, with consequences for the production of biomaterials.



Values trump facts