Silk Fibers: the interplay of their sequence / structure with mechanical properties

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Outlines

- Introduction
  - Background and superiority of spider silks and silkworm silk
- Structures of silk fibers
  - Morphological structure and chemical composition of silk fibers
  - Hierarchical structure of silk proteins
- The interplay of structure and mechanical properties
  - Experimental investigations
  - Computational evidences
- Bio-inspired silk fibers and silk-based biomaterials
- Summary and perspective

Animal fibers

Sheep

Alpaca to yak

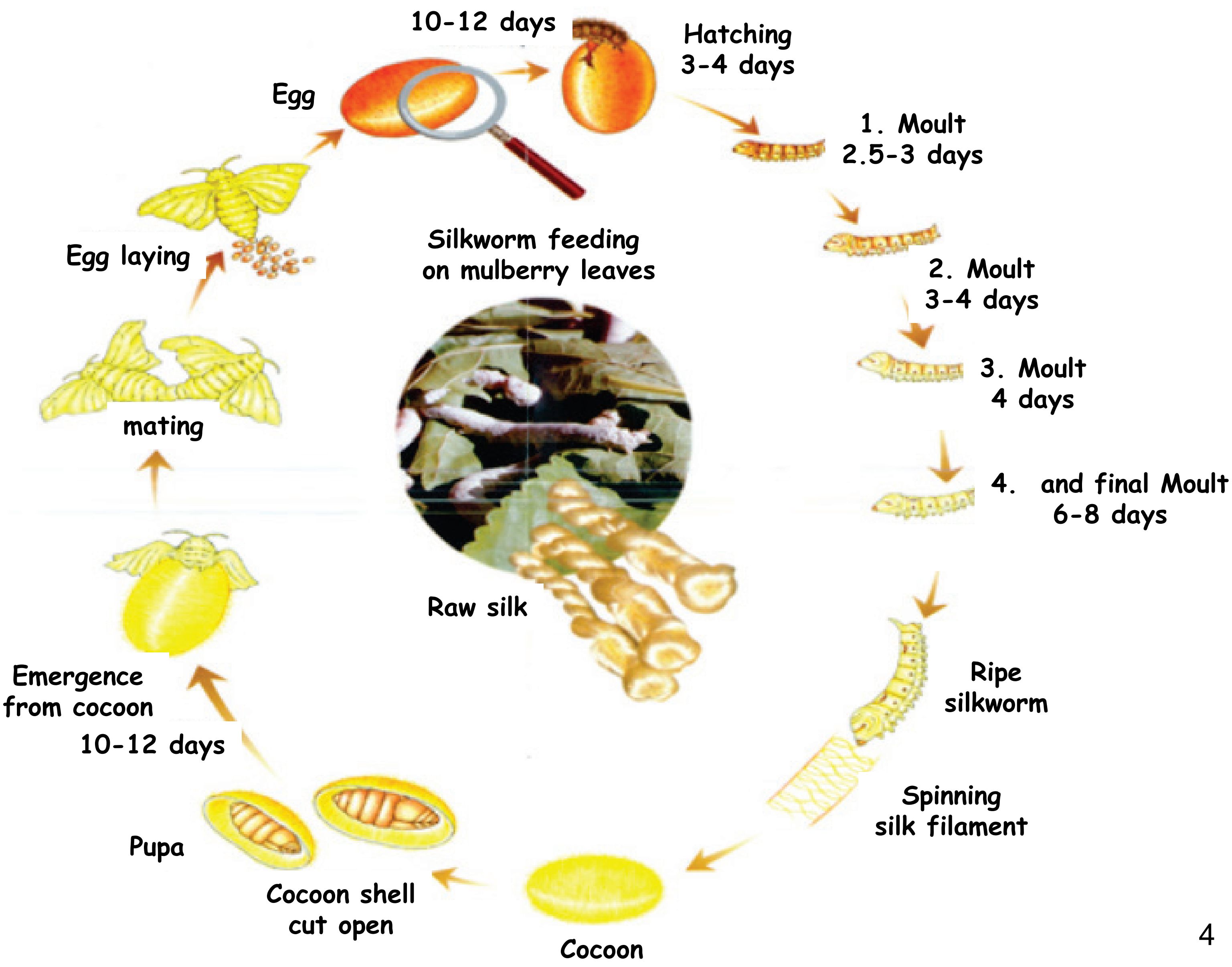
Silkworm & silkmoth

Wool

Camel fiber

Silk

Silkworm silk



10-12 days

Hatching
3-4 days

1. Moult
2.5-3 days

2. Moult
3-4 days

3. Moult
4 days

4. and final Moult
6-8 days

Ripe
silkworm

Spinning
silk filament

Cocoon

Cocoon shell
cut open

Pupa

10-12 days

Emergence
from cocoon

mating

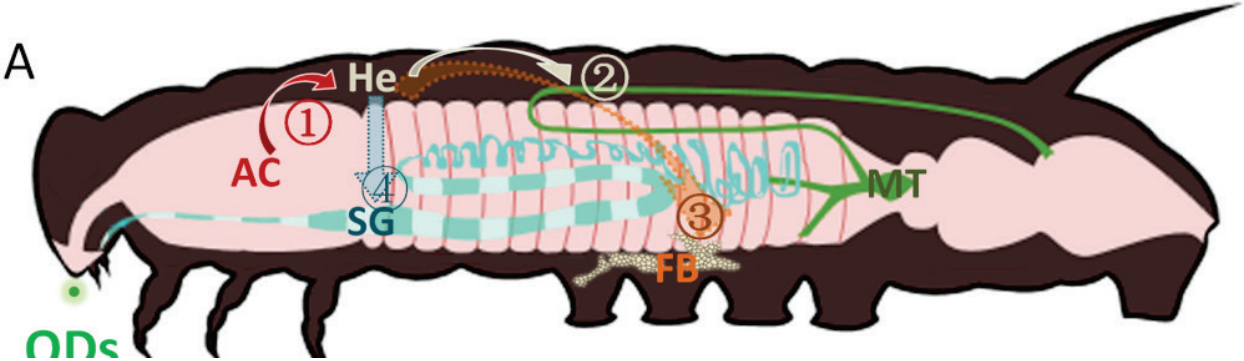
Egg laying

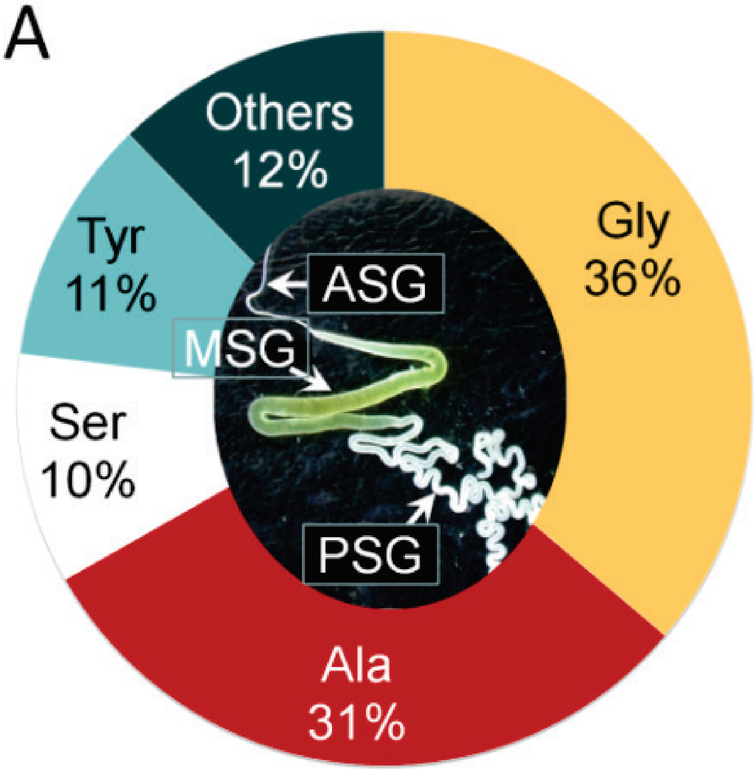
Egg

Silkworm feeding
on mulberry leaves

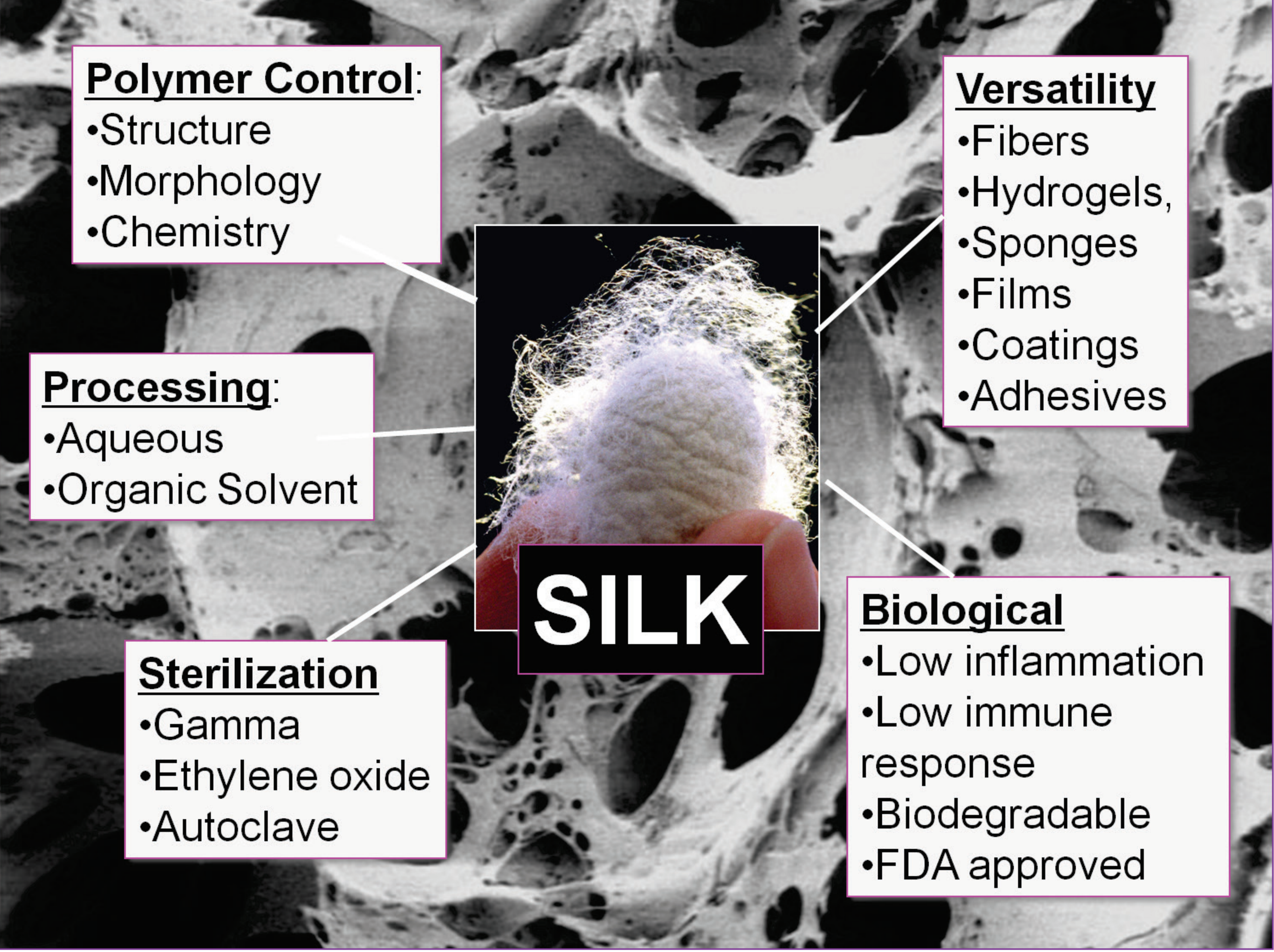
Raw silk

Silkworm silk





Silkworm silk—used as a textile



SILK

Polymer Control:
- Structure
- Morphology
- Chemistry

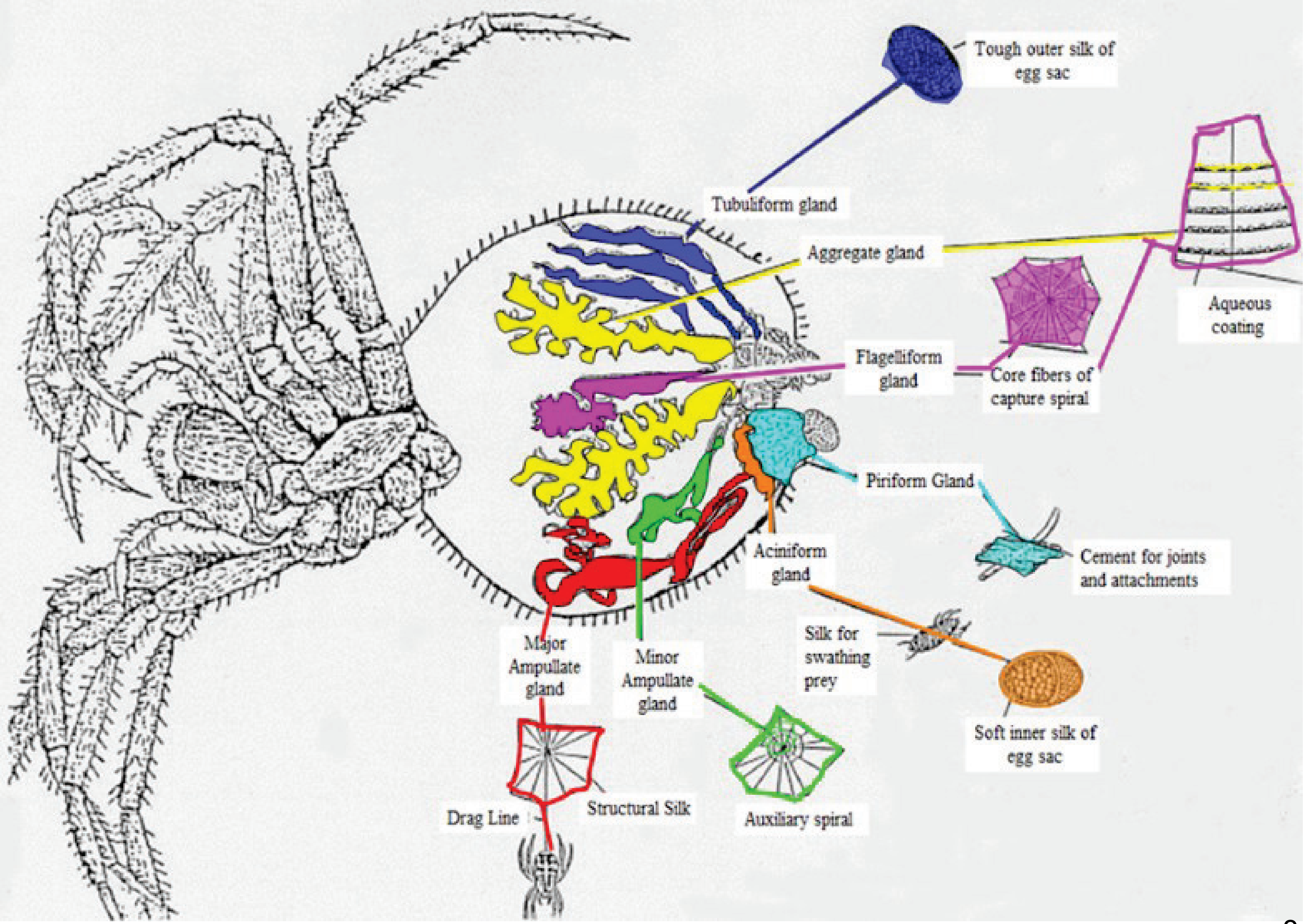
Processing:
- Aqueous
- Organic Solvent

Sterilization
- Gamma
- Ethylene oxide
- Autoclave

Versatility
- Fibers
- Hydrogels,
- Sponges
- Films
- Coatings
- Adhesives

Biological
- Low inflammation
- Low immune response
- Biodegradable
- FDA approved

Spider silks



The strongest natural fiber—spider dragline silk

Fibers	Stiffness (GPa)	Strength (GPa)	Extensibility (%)	Toughness (MJ·m^{-3})
B. mori cocoon silk	7	0.6	18	70
B. mori reeled silk	15	0.7	28	150
A. Diadematus silk (dragline)	10	1.1	27	180
A. Diadematus silk (flagelliform)	0.003	0.5	270	150
Wool (at 100% RH)	0.5	0.2	5	60
Nylon fiber	5	0.95	18	80
Kevlar 49 fiber	130	3.6	2.7	50
Carbon fiber	300	4	1.3	25
High-tensile steel	200	1.5	0.8	6

Scheibel T. et al. *Angew. Chem., Int. End.* **2009**, *48*, 3584-3596.

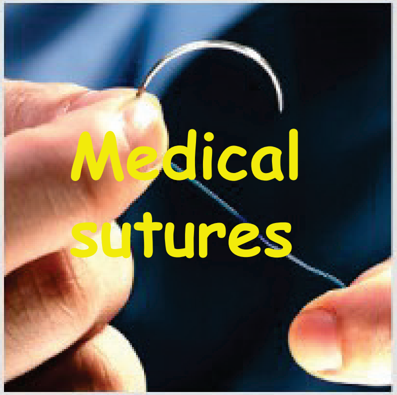
Spider silks—used as a textile

Yellow woven spider silk cape

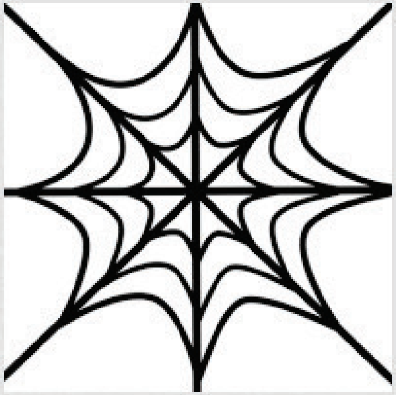
Spider silks—potential applications


Bridge cables


Airbags

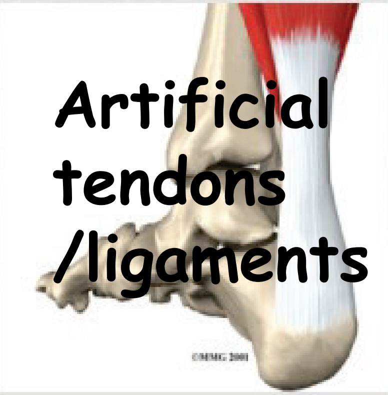

Medical sutures


Parachute cords




Body armors


Performance wear


Artificial tendons /ligaments


Seatbelts

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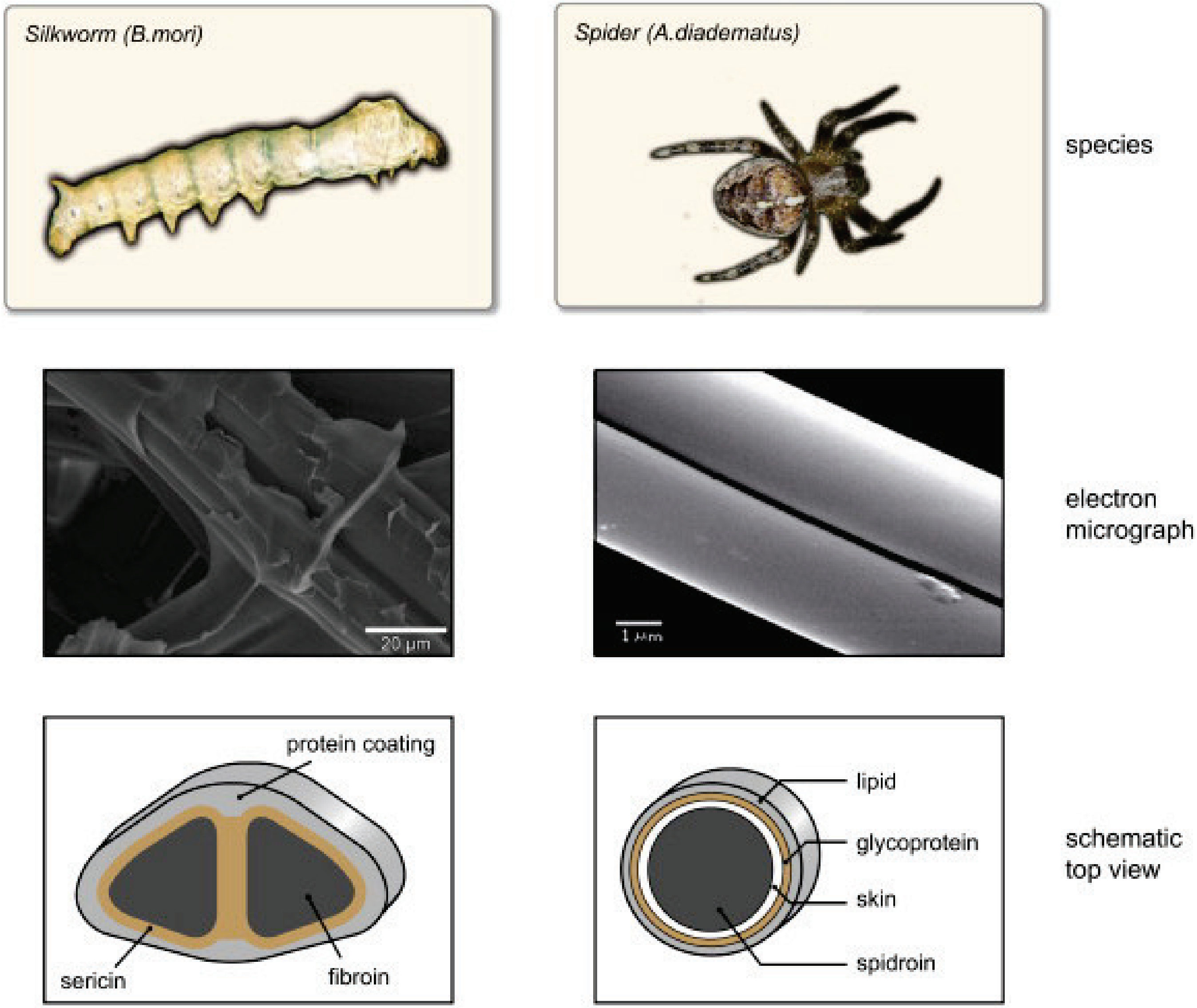
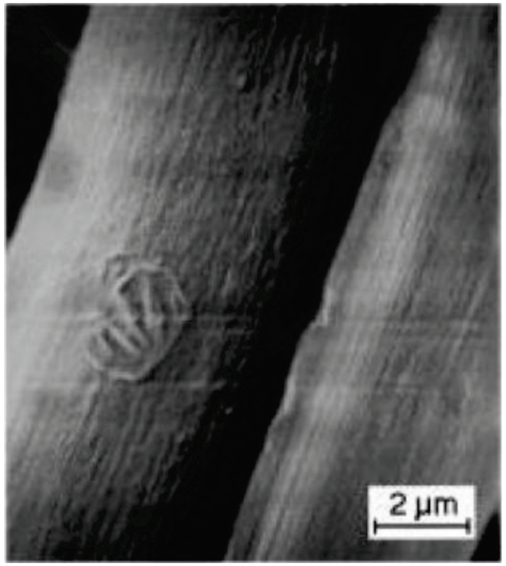
Macrostructure of silk fibers

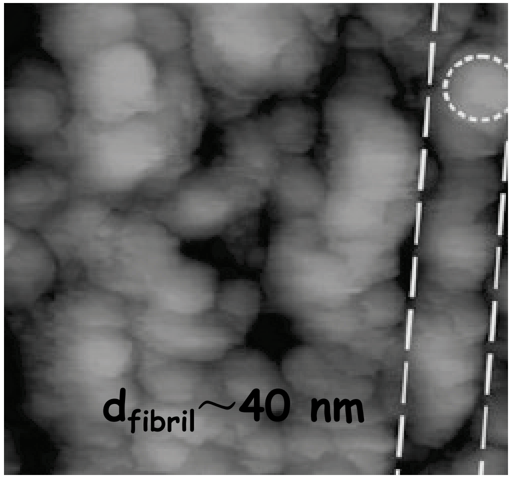


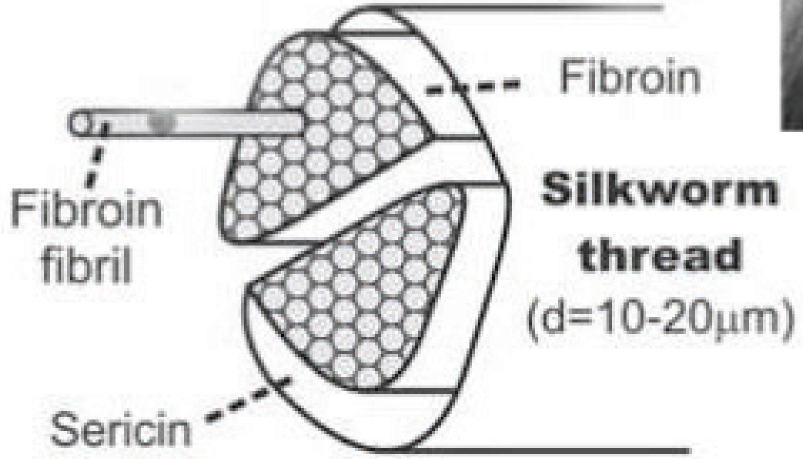
sketches are not to scale

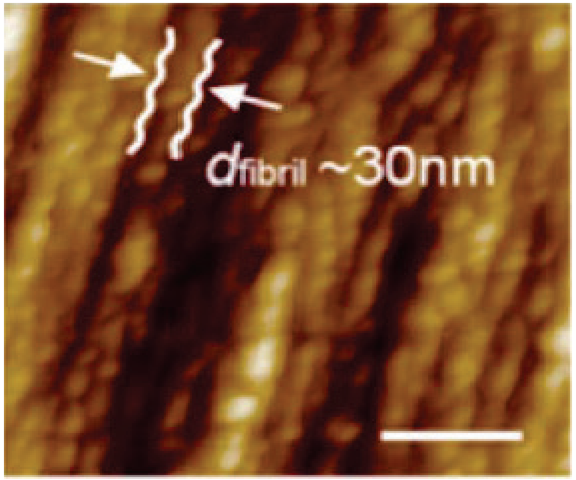
Scheibel T. R. et al. *Polymer* **2008**, *49*, 4309-4327.

Structure of silk fibers







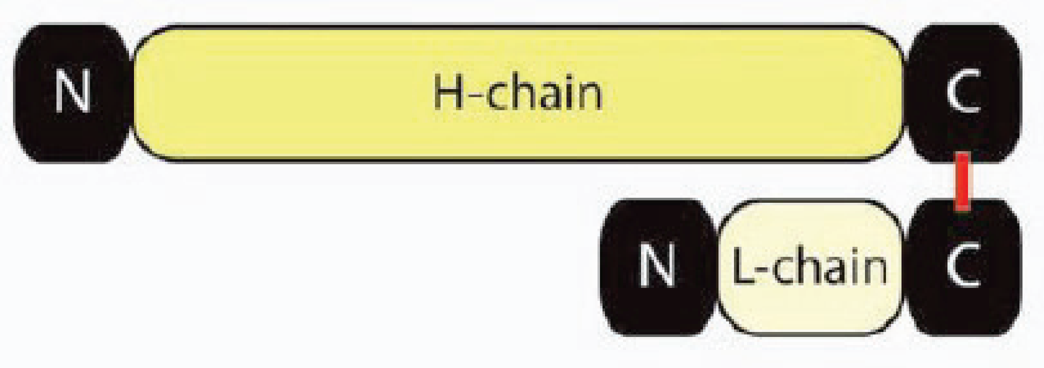


Martin D. C. *Macromolecules* **1998**, *31*, 8857-8864. Liu X. Y. et al. *Biophys. J.* **2006**, *91*, 4528-4535.
Liu X. Y. et al. *Adv. Funct. Mater.* **2011**, *21*, 772-778. Liu X. Y. et al. *Soft Matter.* **2014**, *10*, 2116-2123.

Chemical composition of silk fibers

B. mori **fibroin** consists of heavy chain (H-fibroin) and light chain (L-fibroin)

- Covalently linked at the carboxy-terminus
- MW of H-fibroin ~ 350 kDa; L-fibroin ~ 26 kDa



covalent linking

Spider fibroin (major ampullate dragline silk protein) composes of Spidroin 1 (MaSp 1) and Spidroin 2 (MaSp 2)

- MW of MaSp1 ~ 275-320 kDa; Sp1+Sp2 ~ 700-750 kDa

Scheibel T. R. et al. *Bipolymers* **2012**, *97*, 355-361.

The primary structure of silk protein

Primary structure of H-fibroin

- Sequences highly conserved
- Repetitive stretches of $(\text{Gly-Ala})_n$ sequences and tyrosine-rich domains

Bombyx mori fibroin:

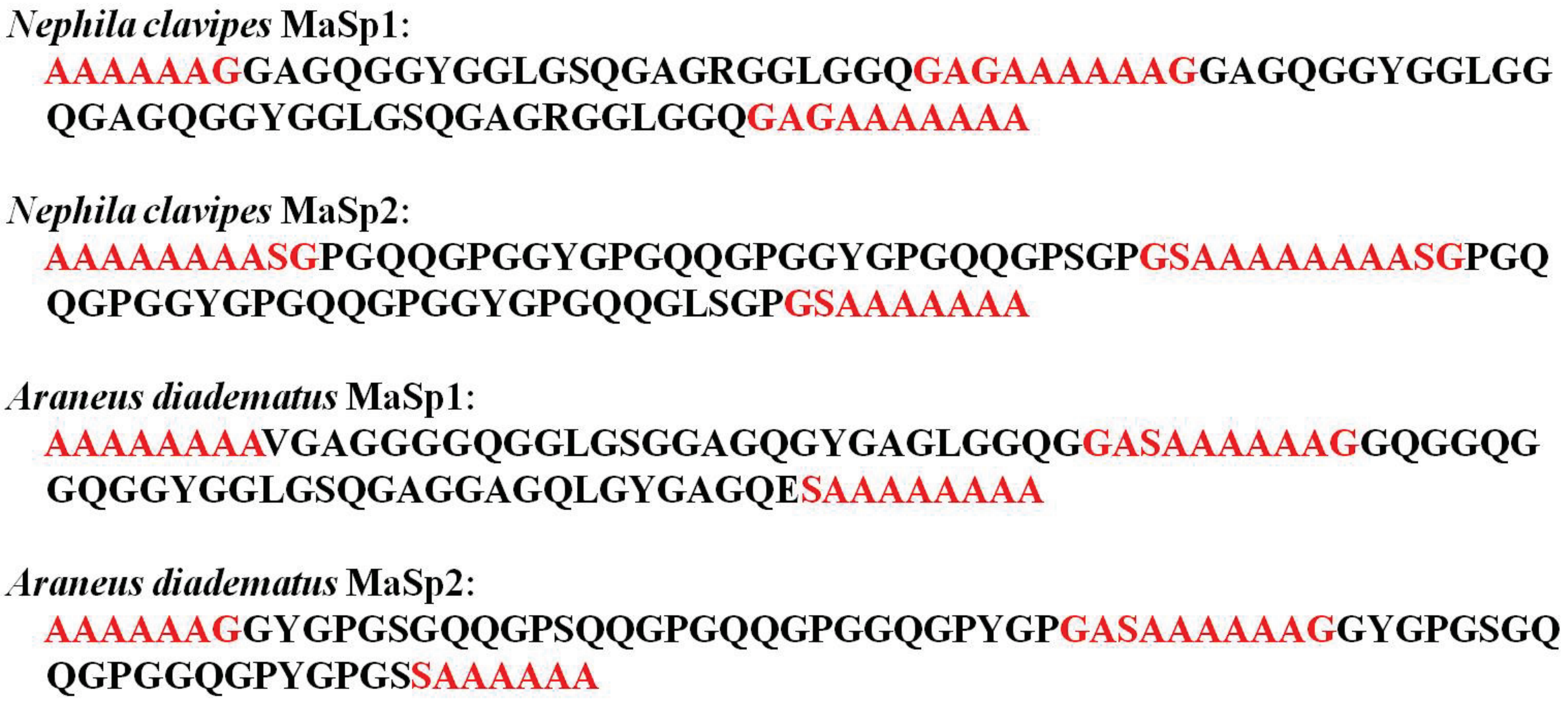
GAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGYGAGVGVGYGAGYGAGAG
AGYGAGAGSGAASGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGA
GSGAGAGSGAGAGSGAGAGSGAGVGSGAGAGSGAGAGVGYGAGAGVGYGAGAG
SGAASGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSGAGAGSG

Zhou C.-Z. et al. *Nucleic Acids Res.* **2000**, *28*, 2413-2419.

The primary structure of silk protein

Primary structure of Spidroin 1 (MaSp 1) and Spidroin 2 (MaSp 2)

- Repetitive sequences motifs of (Ala)$_n$
- Followed by several GGX motifs in MaSp 1 and GPGXX motifs (proline residues) in MaSp 2



Nephila clavipes MaSp1:
AAAAAAGGAGQGGYGGLGSQGAGRGGLGGQGAGAAAAAAGGAGQGGYGGLGG
QGAGQGGYGGLGSQGAGRGGLGGQGAGAAAAAAA

Nephila clavipes MaSp2:
AAAAAAAASGPGQQGPGGYGPGQQGPGGYGPGQQGPSGPGSAAAAAAAASGPGQ
QGPGGYGPGQQGPGGYGPGQQGLSGPGSAAAAAAA

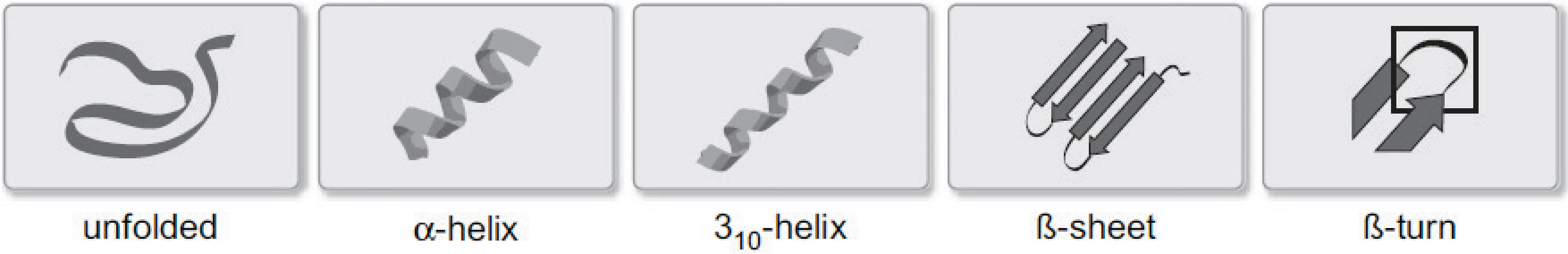
Araneus diadematus MaSp1:
AAAAAAAAVGAGGGGQGGLGSGGAGQGYGAGLGGQGGASAAAAAAGGQGGQG
GQGGYGGLGSQGAGGAGQLGYGAGQESAAAAAAAA

Araneus diadematus MaSp2:
AAAAAAGGYGPGSGQQGPSQQGPGQQGPGGQGPYGPGASAAAAAAGGYGPGSGQ
QGPGGQGPYGPGSSAAAAAA

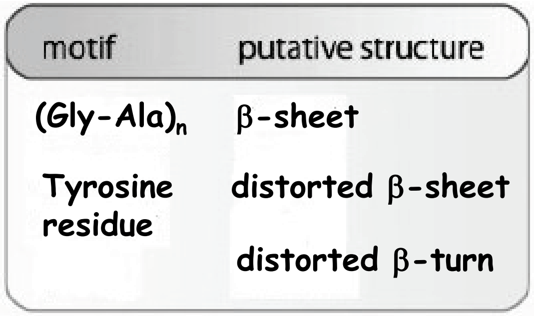
Lewis, R. V. et al. *TIBTECH* **2000**, *18*, 374-379. Knight, D. P. et al. *Nature* **2001**, *410*, 541-548.
Simmons, A. H. et al. *Science* **1996**, *271*, 84-87.

Secondary structure

Common secondary structural motifs in proteins

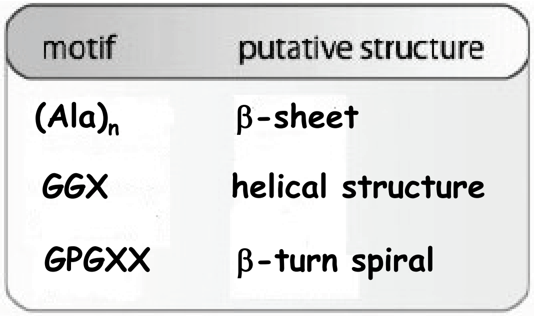


B. mori fibroin



motif	putative structure
$(Gly-Ala)_n$	β-sheet
Tyrosine residue	distorted β-sheet
	distorted β-turn

dragline silk spidroin

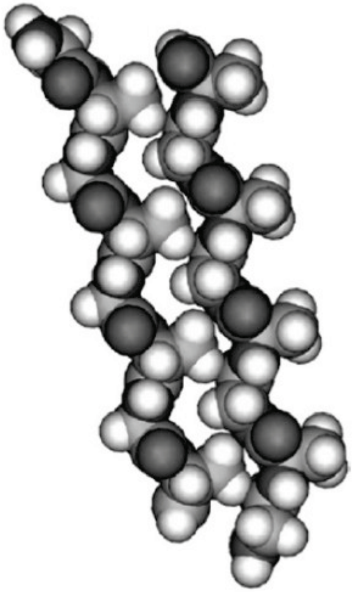


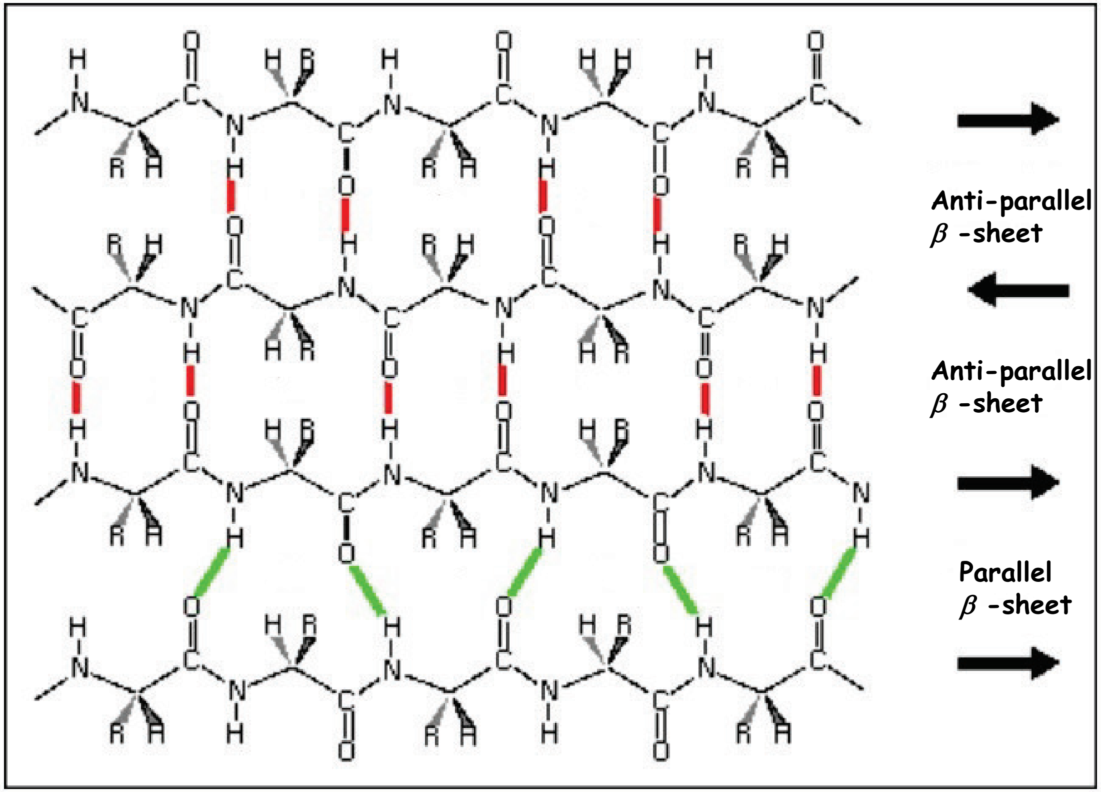
motif	putative structure
$(Ala)_n$	β-sheet
GGX	helical structure
GPGXX	β-turn spiral

Ulrich A. S. et al. *J. Am. Chem. Soc.* **2002**, *124*, 8794-8795. Scheibel T. et al. *Macromol. Biosci.* **2010**, *10*, 998-1007.

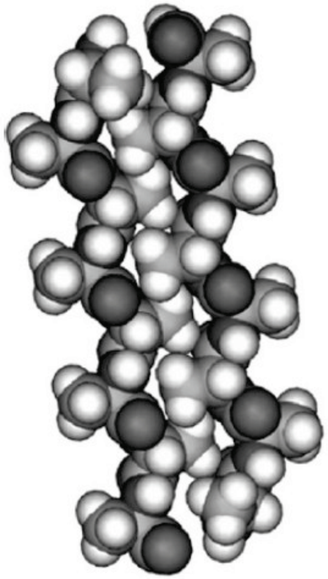
Antiparallel and parallel β-sheet structure

(glycine-alanine)$_n$





(alanine)$_n$



Ulrich A. S. et al. *J. Am. Chem. Soc.* **2002**, *124*, 8794-8795. Scheibel T. et al. *Macromol. Biosci.* **2010**, *10*, 998-1007.

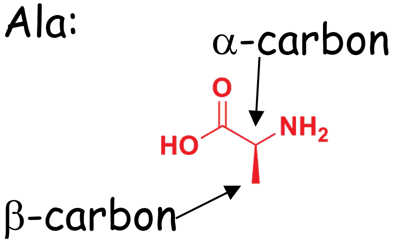
Raman spectroscopy

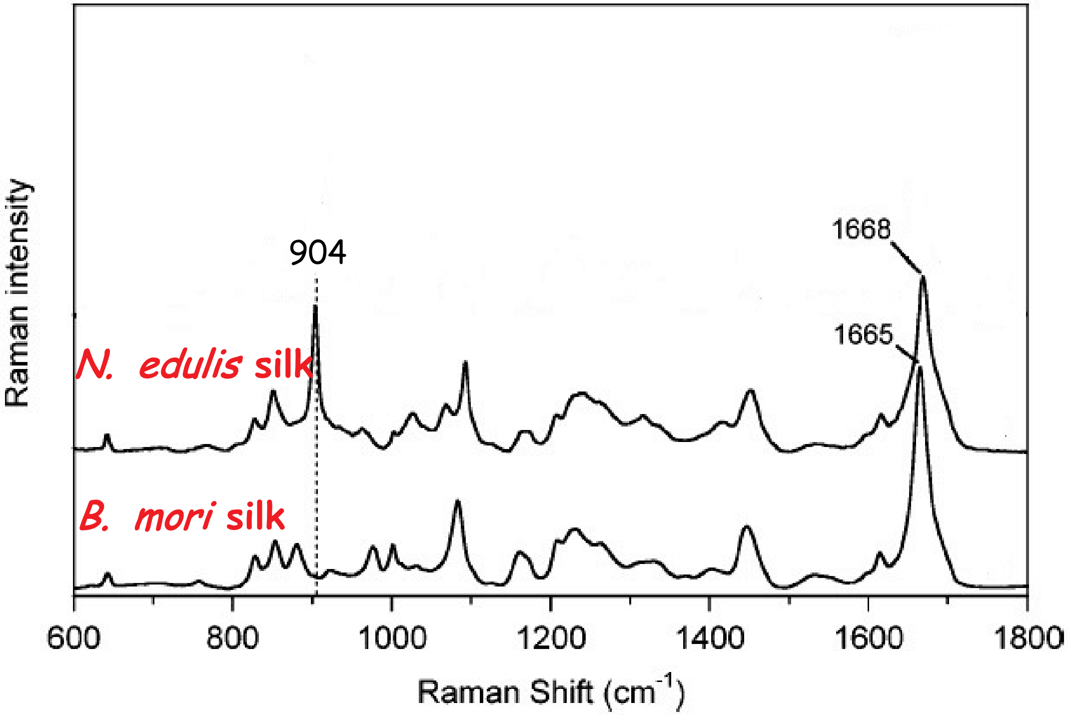
Band Assignments for the Silks Studied

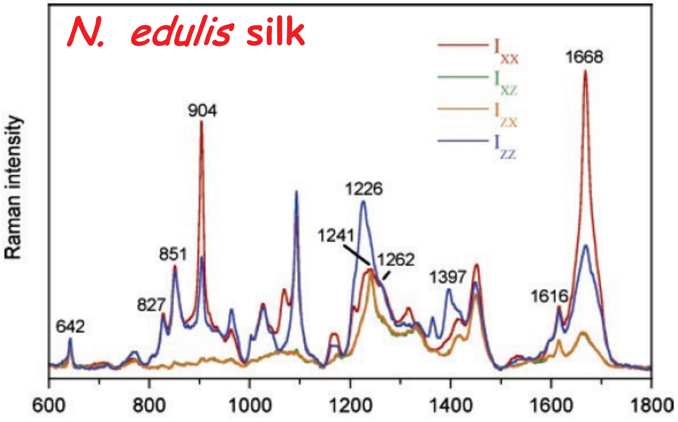
Raman shifts (cm^{-1})

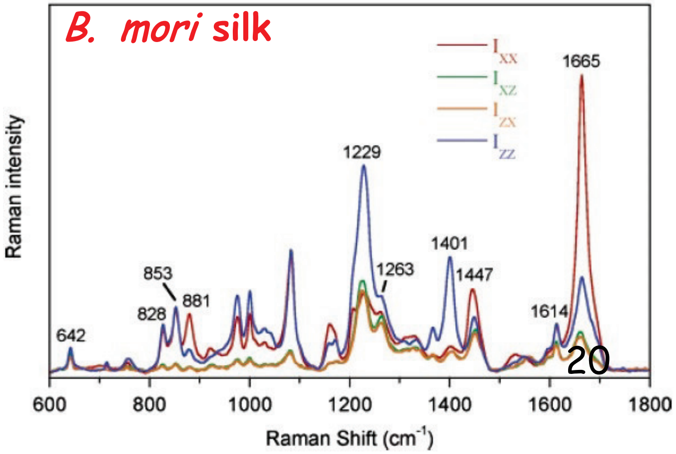
N. Edulis silk	*B. mori* silk	assignment
904		Ala C_α-C_β, Ala C_α-N-C_β , CH_3
1668	1665	amide I, C=O s in β-sheets
1699sh	1693sh	amide I, mainly C=O s in antiparallel β-sheets

Ala:





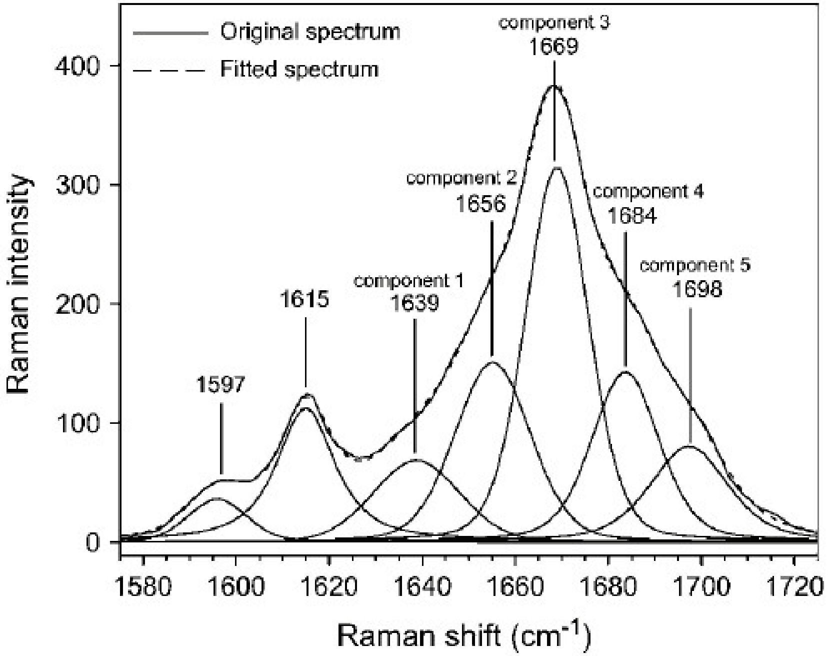




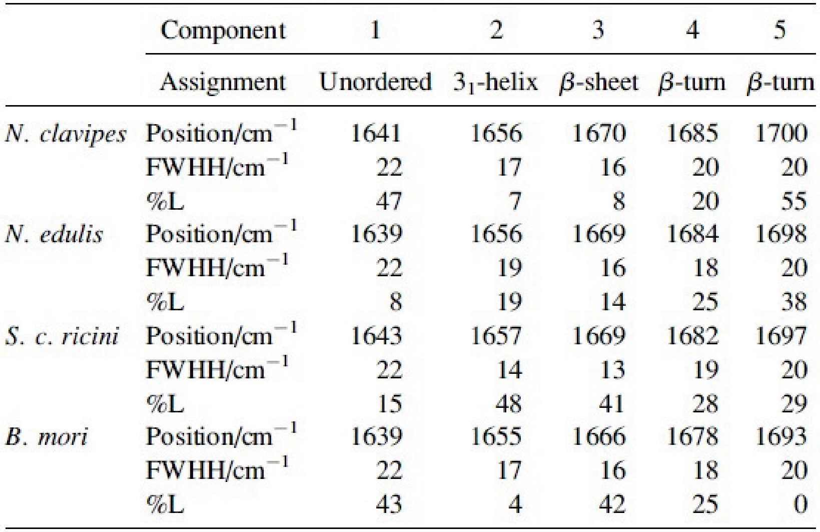
Pézolet M. et al. *Biomacromolecules* **2004**, *5*, 2247-2257.

Raman spectroscopy

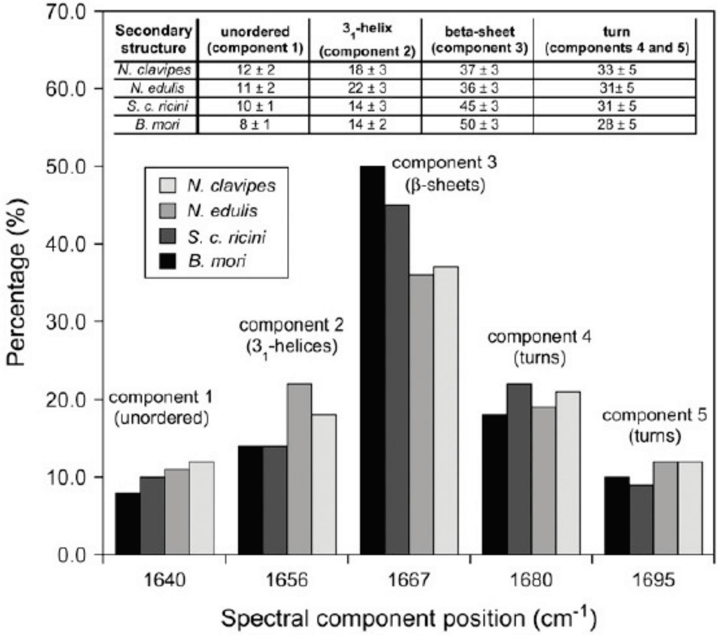
Spectral decomposition of the dragline silk monofilament of *N. edulis* in the amide I region.



Band parameters of the amide I components



	Component	1	2	3	4	5
	Assignment	Unordered	3_1-helix	β-sheet	β-turn	β-turn
N. clavipes	Position/cm^{-1}	1641	1656	1670	1685	1700
	FWHH/cm^{-1}	22	17	16	20	20
	%L	47	7	8	20	55
N. edulis	Position/cm^{-1}	1639	1656	1669	1684	1698
	FWHH/cm^{-1}	22	19	16	18	20
	%L	8	19	14	25	38
S. c. ricini	Position/cm^{-1}	1643	1657	1669	1682	1697
	FWHH/cm^{-1}	22	14	13	19	20
	%L	15	48	41	28	29
B. mori	Position/cm^{-1}	1639	1655	1666	1678	1693
	FWHH/cm^{-1}	22	17	16	18	20
	%L	43	4	42	25	0

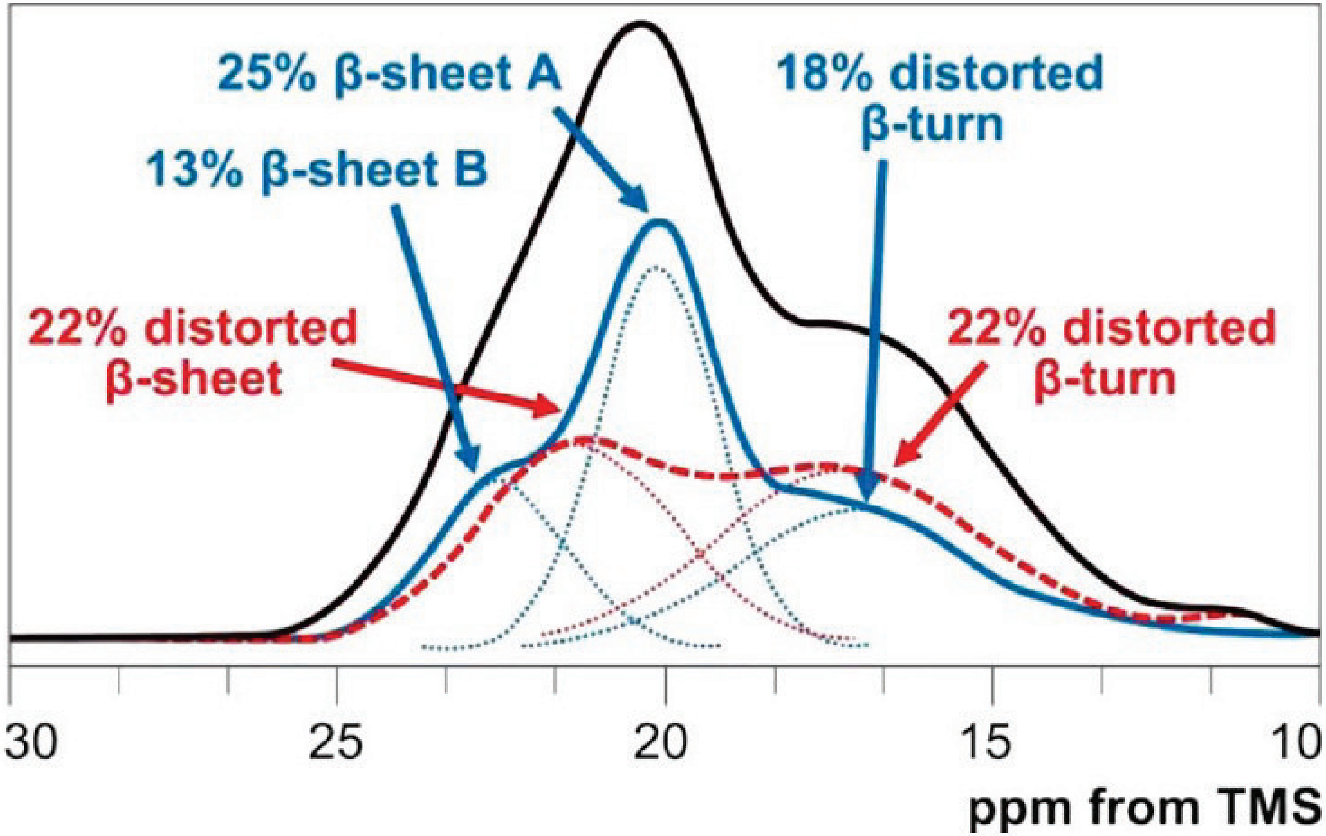


Secondary structure	unordered (component 1)	3_1-helix (component 2)	beta-sheet (component 3)	turn (components 4 and 5)
N. clavipes	12 ± 2	18 ± 3	37 ± 3	33 ± 5
N. edulis	11 ± 2	22 ± 3	36 ± 3	31± 5
S. c. ricini	10 ± 1	14 ± 3	45 ± 3	31 ± 5
B. mori	8 ± 1	14 ± 2	50 ± 3	28 ± 5

Pézolet M. et al. *Biophys. J.* **2007**, *92*, 2885-2895.

Solid state NMR

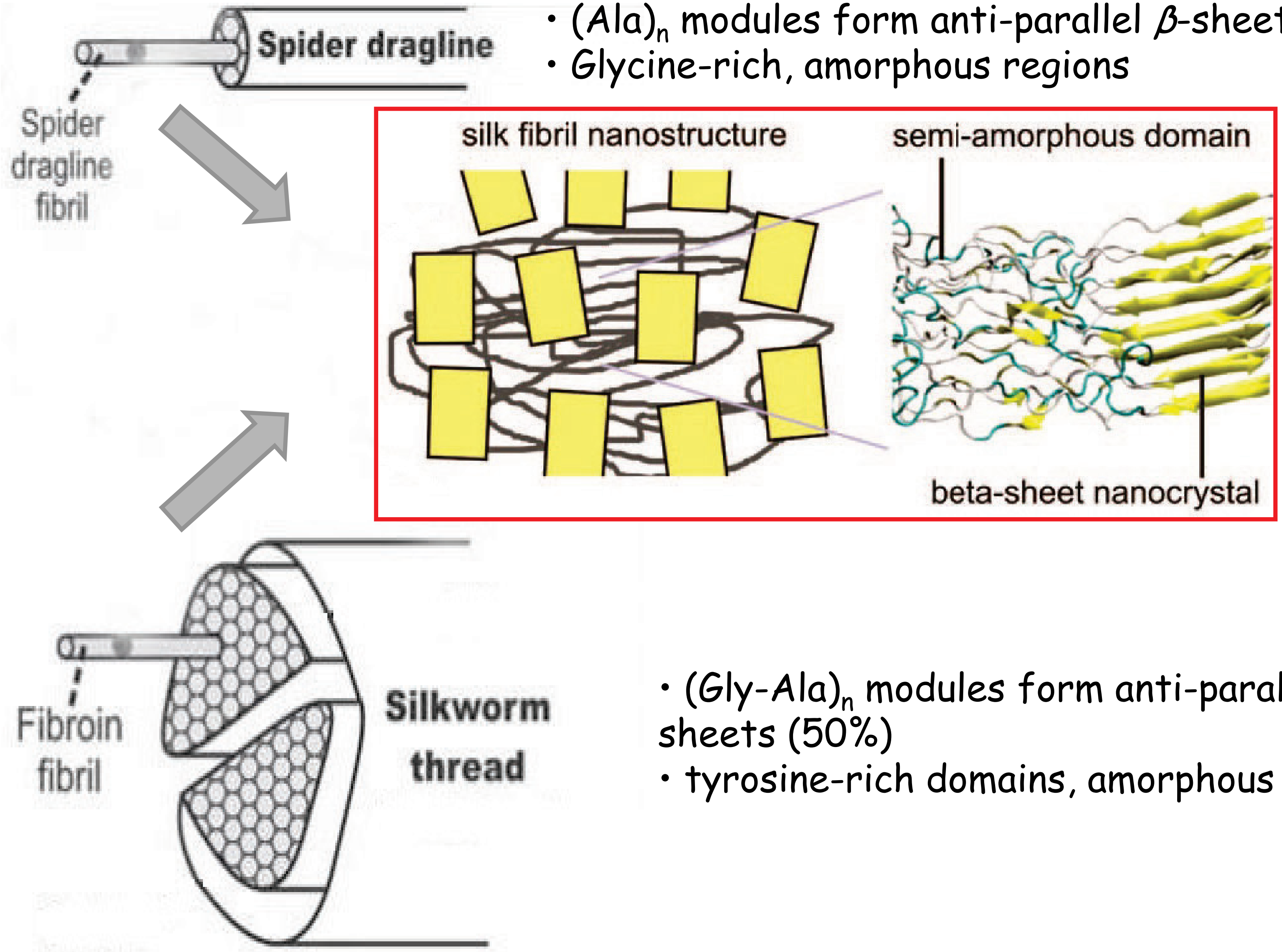
^{13}C Ala methyl peak of the ^{13}C CP-MAS NMR spectrum of [3-^{13}C]Ala *B. mori* silk fibroin fiber.



Asakura T et al. *Soft matter* **2013**, *9*, 11440-11450.

Proposed hierarchical structure model—two phases

- $(Ala)_n$ modules form anti-parallel β-sheets (~30-40%)
- Glycine-rich, amorphous regions



- $(Gly\text{-}Ala)_n$ modules form anti-parallel β-sheets (50%)
- tyrosine-rich domains, amorphous regions

The key questions

(1) How can mechanically weak structural elements such as proteins stabilized by H-bonds provide the basis to strong materials?

(2) What role do hierarchical structures play in providing overall strength and functions of a material?

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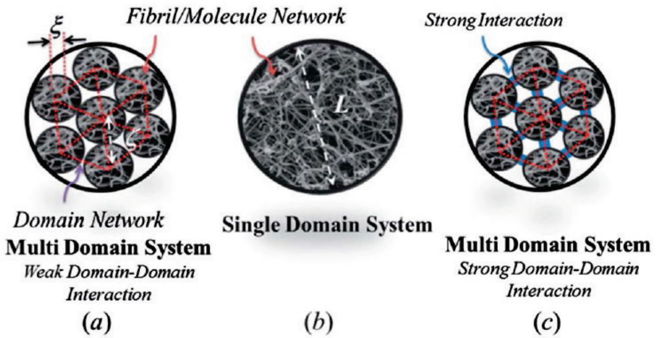
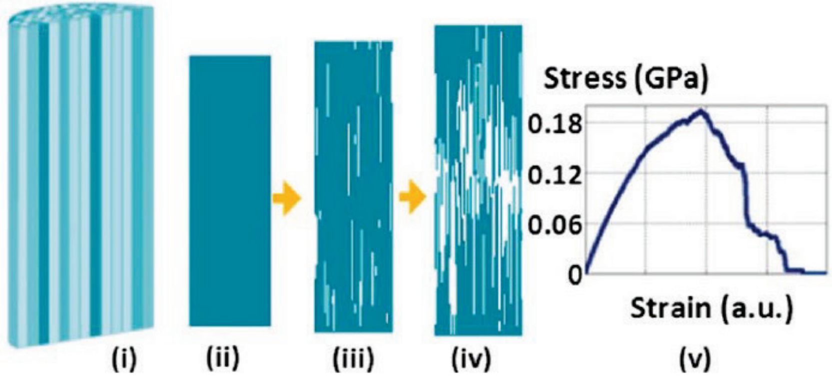
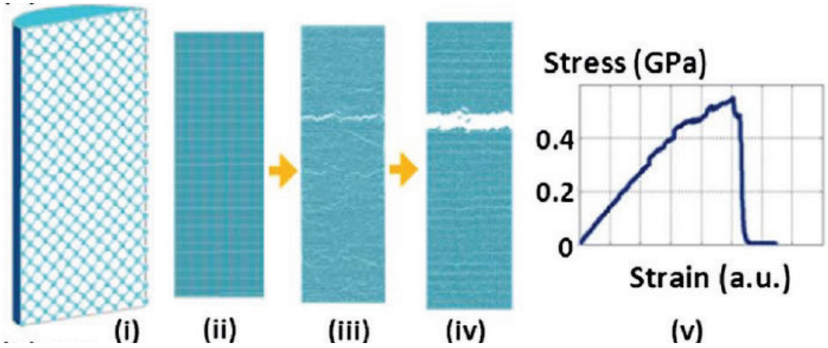
The importance of hierarchical structure – network?



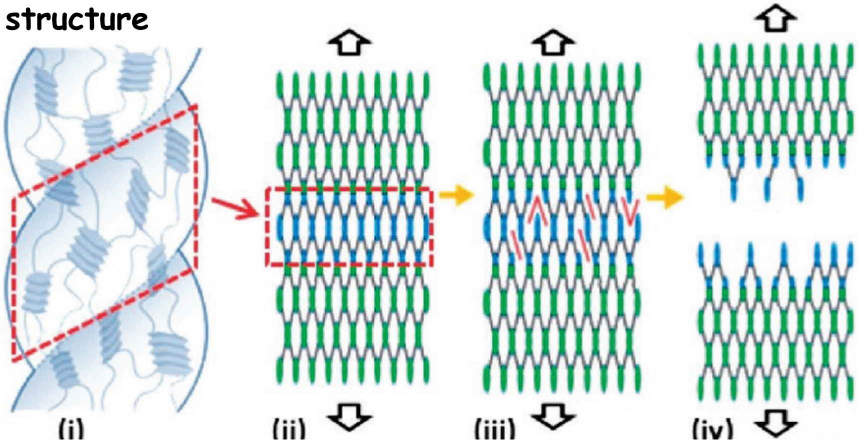
Slippery Fibril Bundle (SFB) structure

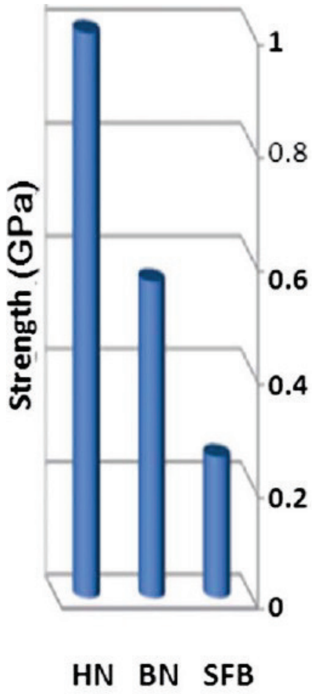


Bulk Network (BN) structure



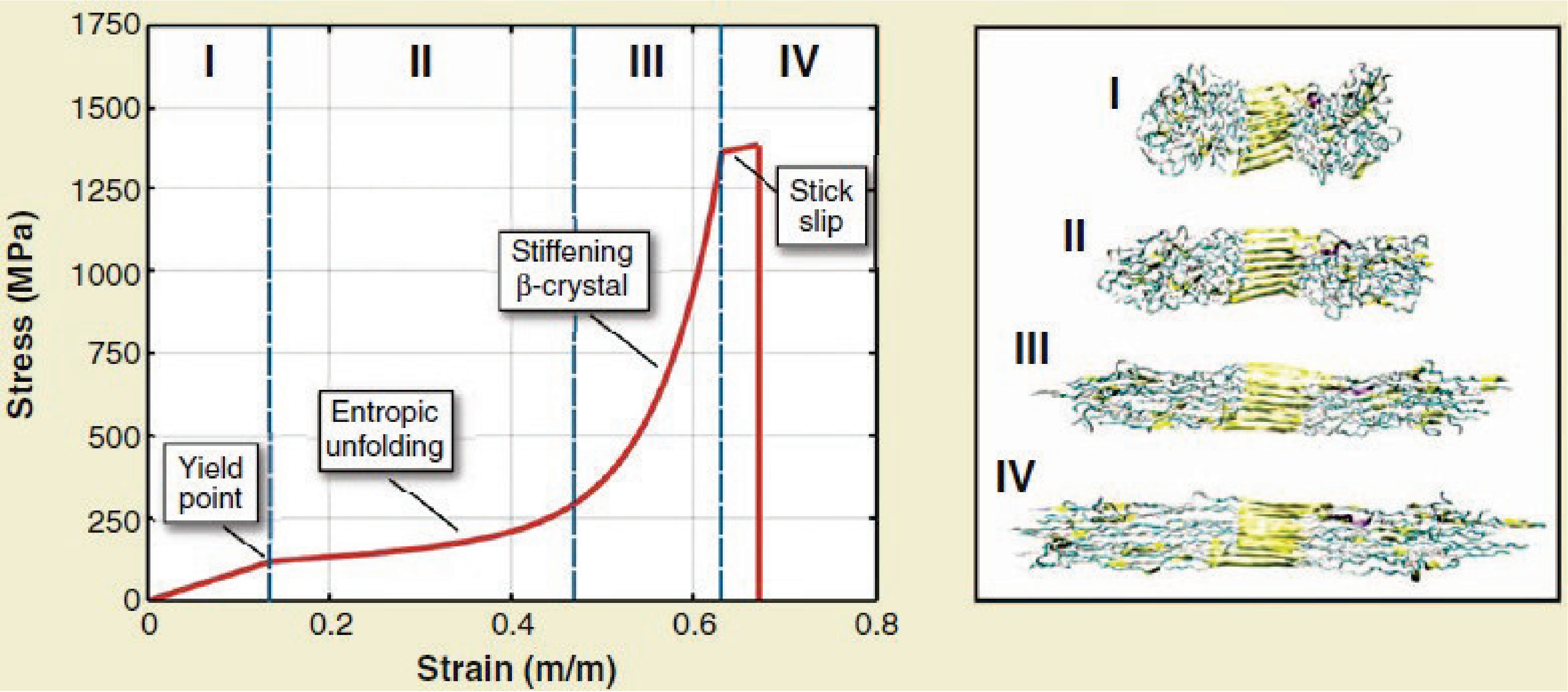
Hierarchical network (HN) structure





Liu X. Y. et al. *Soft matter* **2014**, *10*, 2116-2123.

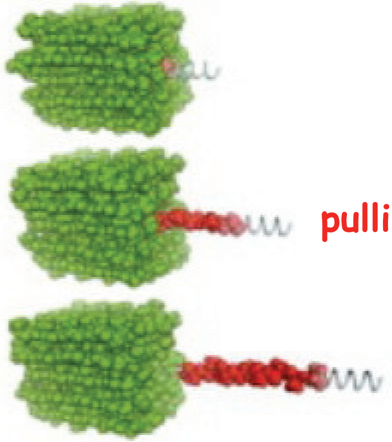
The importance of hierarchical structure in the mechanical behavior



- i. The hydrogen bonds in the semi-amorphous regions to rupture
- ii. Entropic unfolding of the amorphous strand
- iii. Load transfer to the crystalline sheet
- iv. Failure of the silk

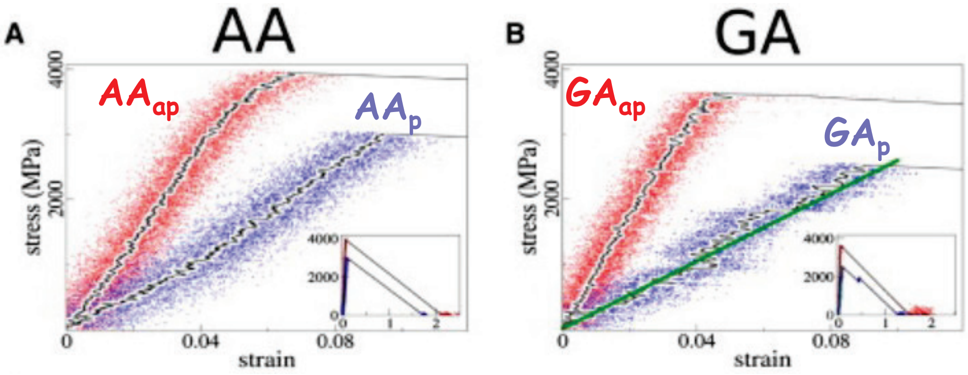
Buehler M. J. et al. *Nano. Lett.* **2010**, *10*, 2626-2634.

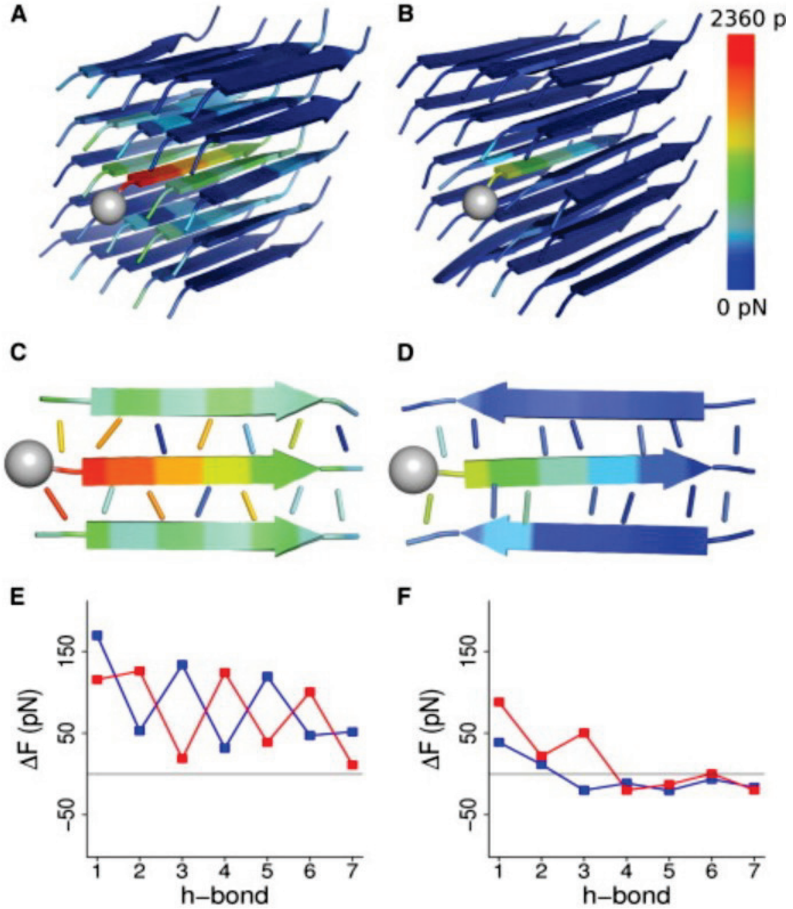
The importance of crystalline β-sheet in the mechanical behavior



Crystalline unit

pulling force

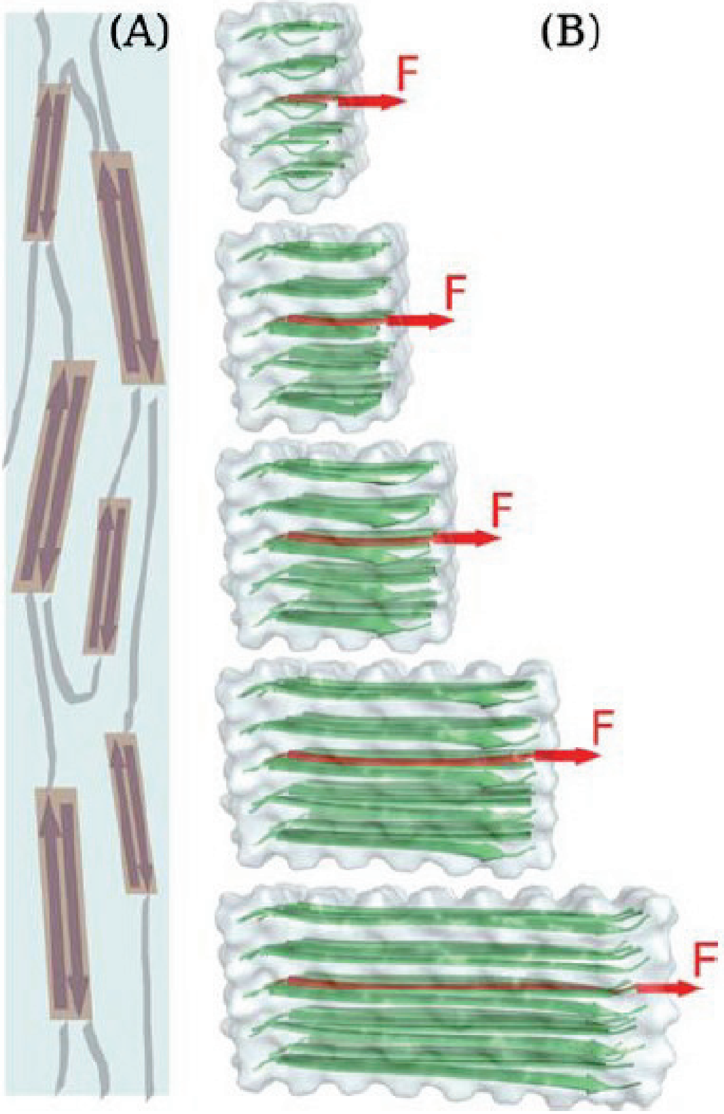


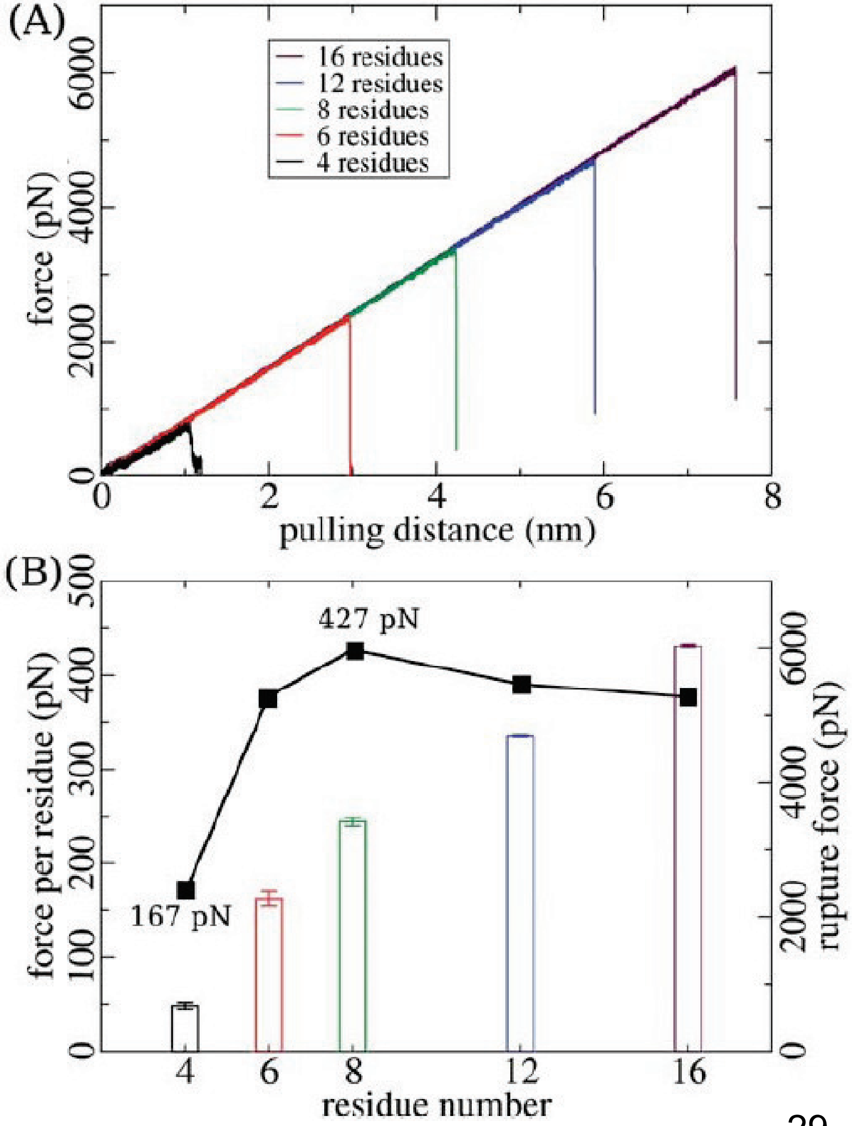


ΔF for interstrand hydrogen bonds along the strands. The upper and lower hydrogen bonds in panels *C* and *D* are shown in red and blue, respectively, starting from the point of force application in AA_p (*E*) and AA_{ap} (*F*).

Xiao S. et al. *Biophy. J.* **2009**, *96*, 3997-4005.

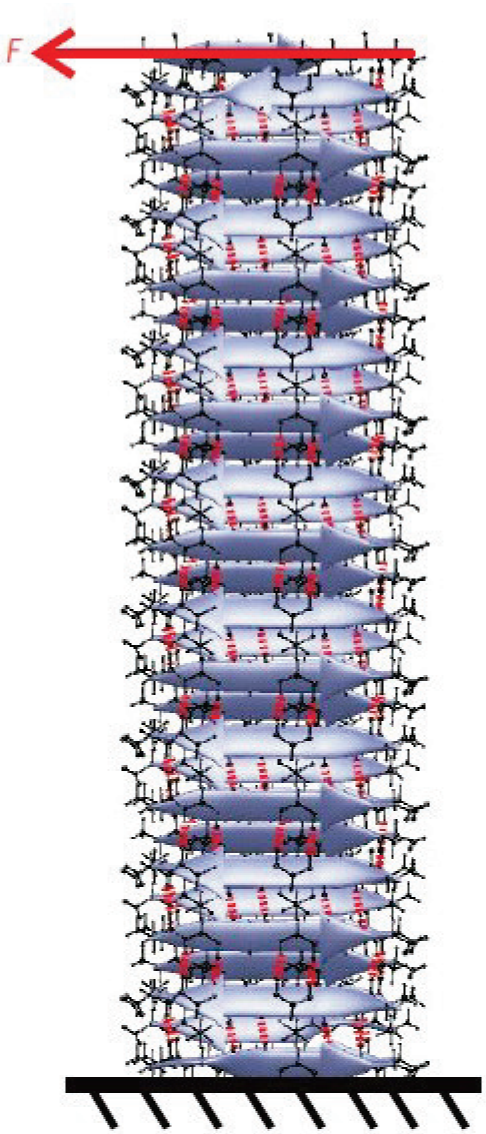
The importance of crystalline β-sheet in the mechanical behavior



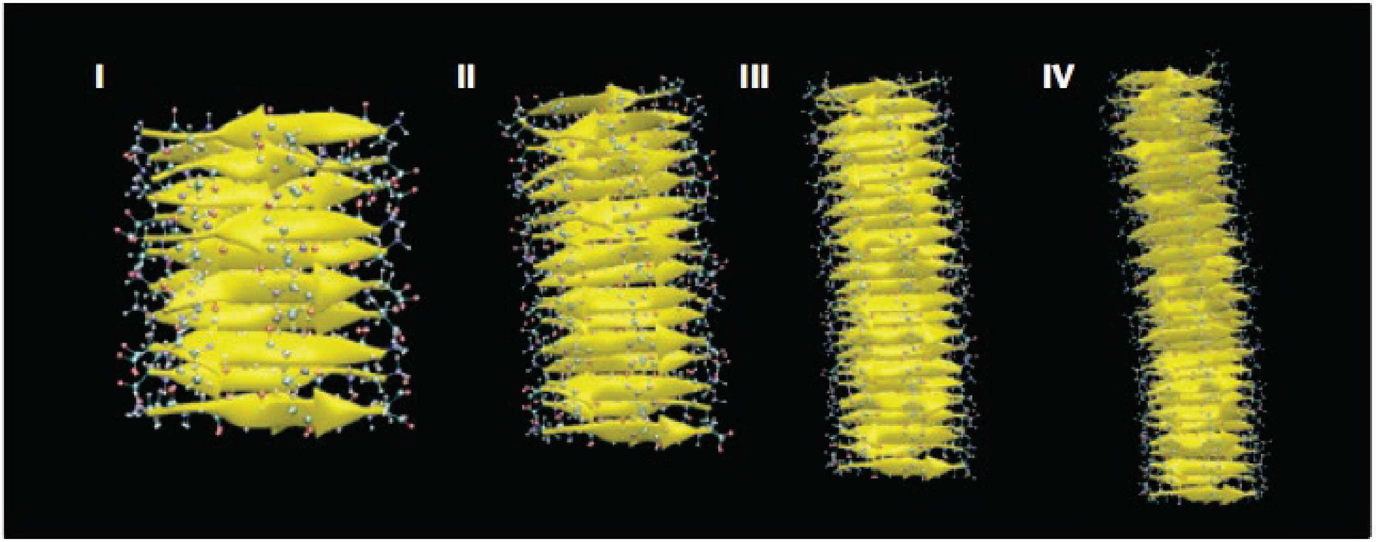


Xiao S. et al. *Soft Matter.* **2011**, *7*, 1308-1311.

The importance of crystalline β-sheet in the mechanical behavior

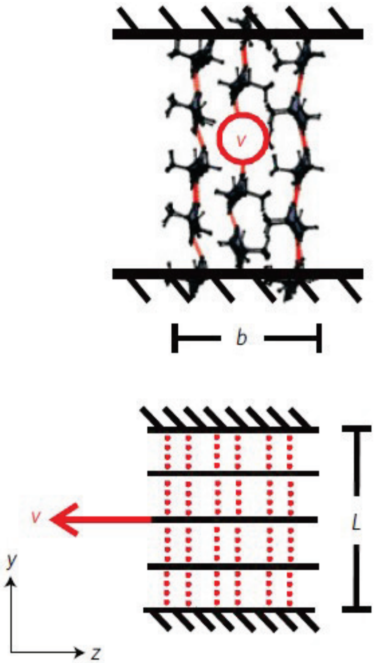


Size-dependent elastic deformation of β-sheet nanocrystals

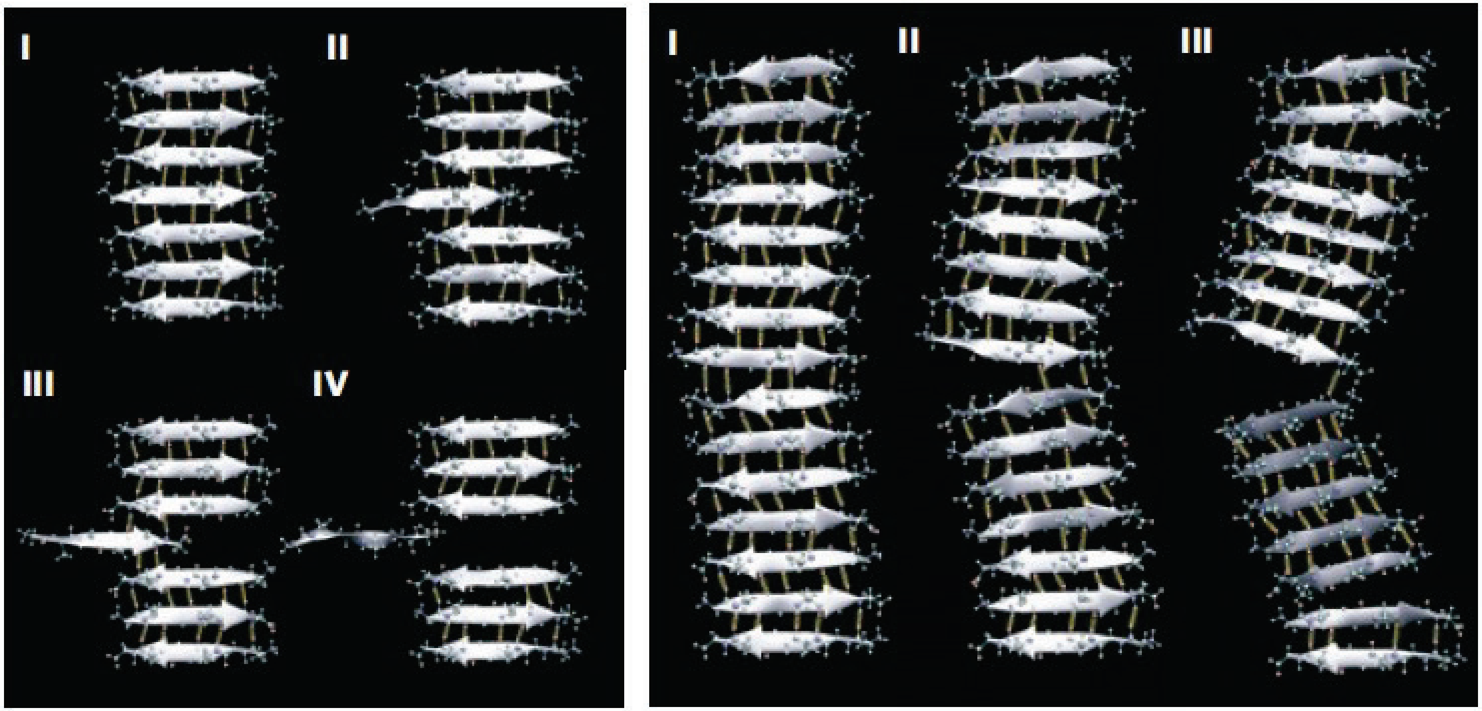


Buehler M. J. et al. *Nat. Mater.* **2010**, *9*, 359-367.

The importance of crystalline β-sheet in the mechanical behavior



Size-dependent fraction mechanism of β-sheet nanocrystals

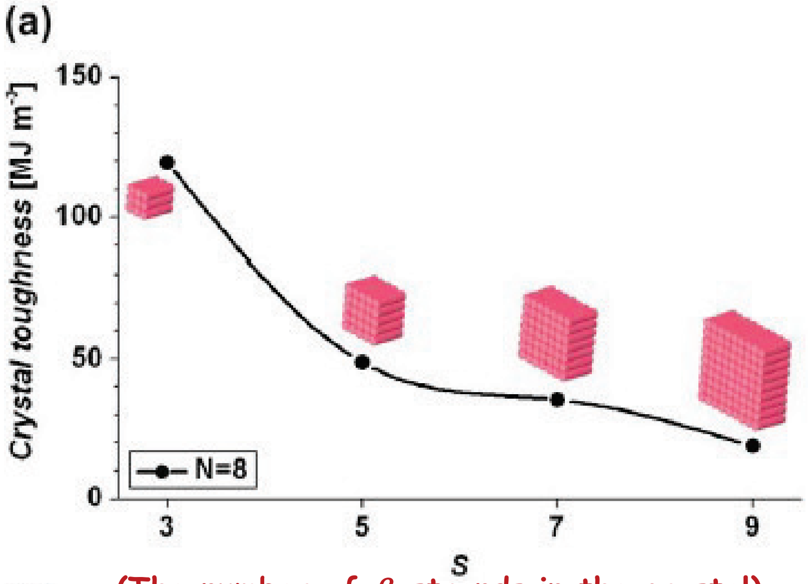


Stick-slip deformation (robust)

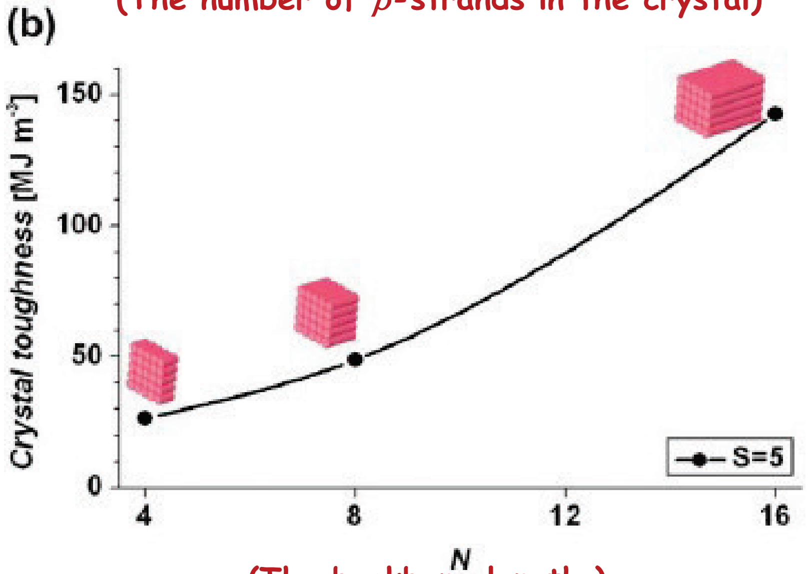
'Brittle' fracture (fragile)

Buehler M. J. et al. *Nat. Mater.* **2010**, *9*, 359-367.

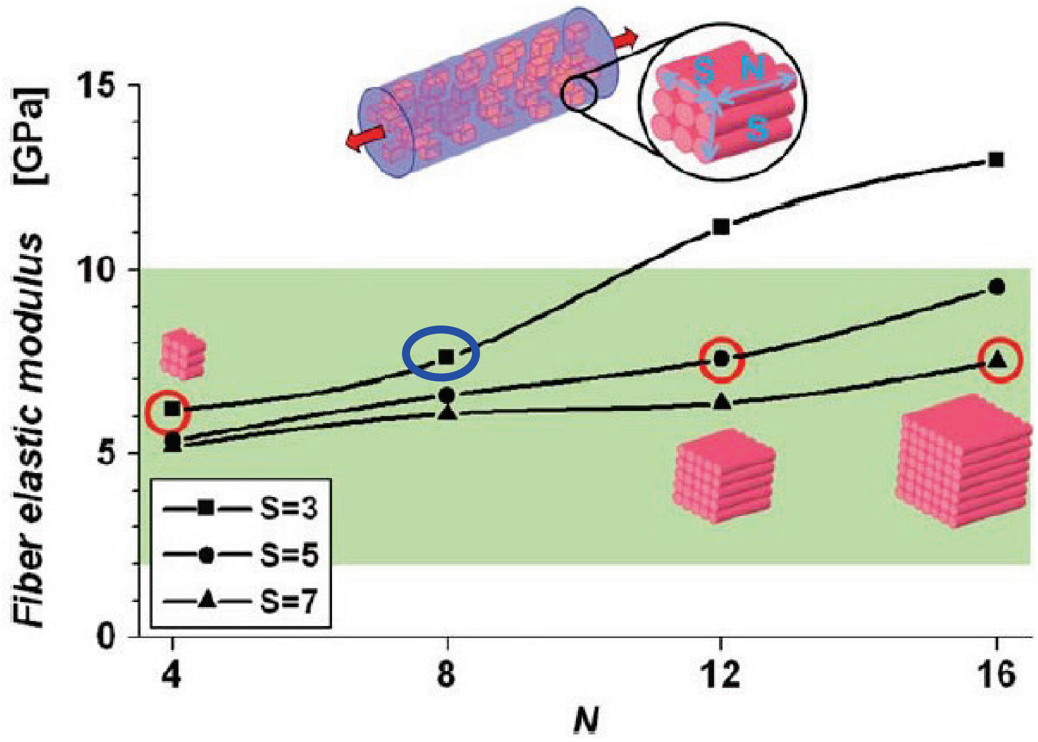
The importance of crystalline β-sheet in the mechanical behavior



(The number of β-strands in the crystal)



(The backbone length)

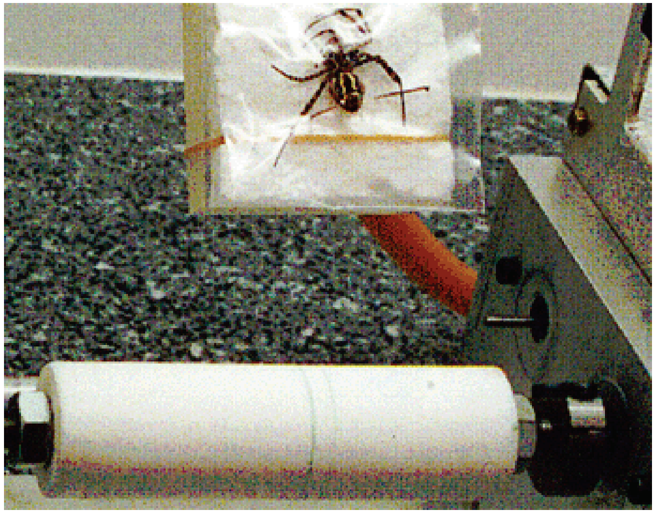


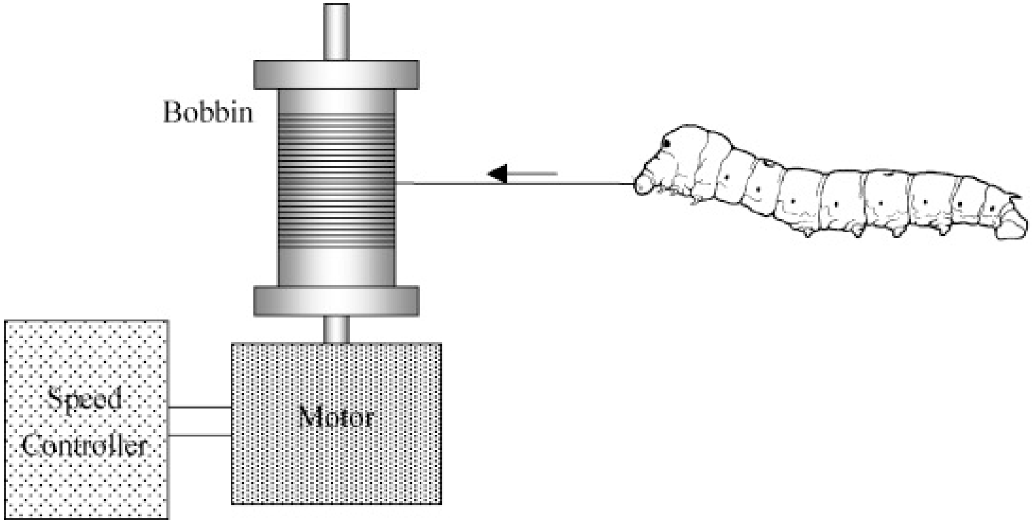
Based on the elastic modulus calculations, the N = 8, S = 3 structure shows the most efficient usage of the protein crystalline material to maximize the crystal stiffness.

Cetinkaya M. et al. *Soft Matter.* **2011**, *7*, 8142-8148.

The importance of crystalline β-sheet in the mechanical behavior

Forced silking to obtain silk fibers: Silk is pulled from the spinneret,
attached to a reel,
and drawn at a specified speed.

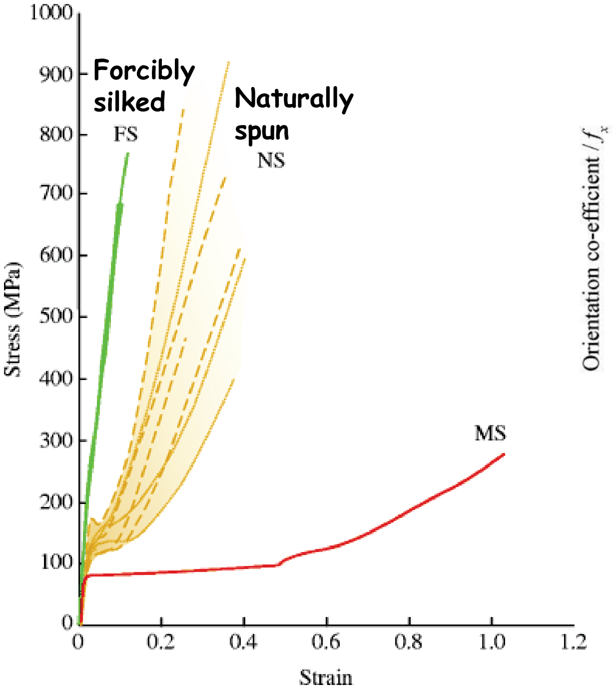


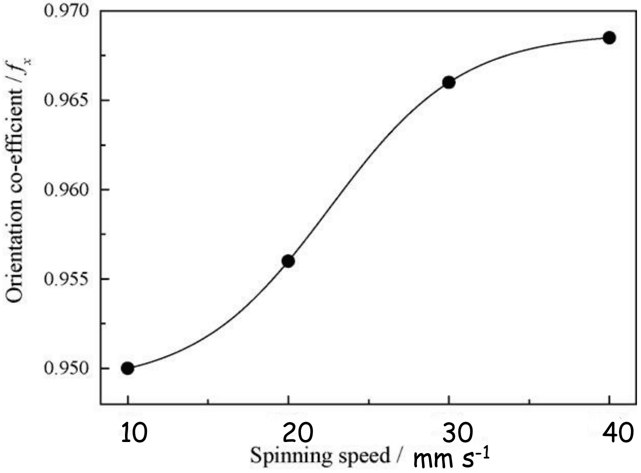


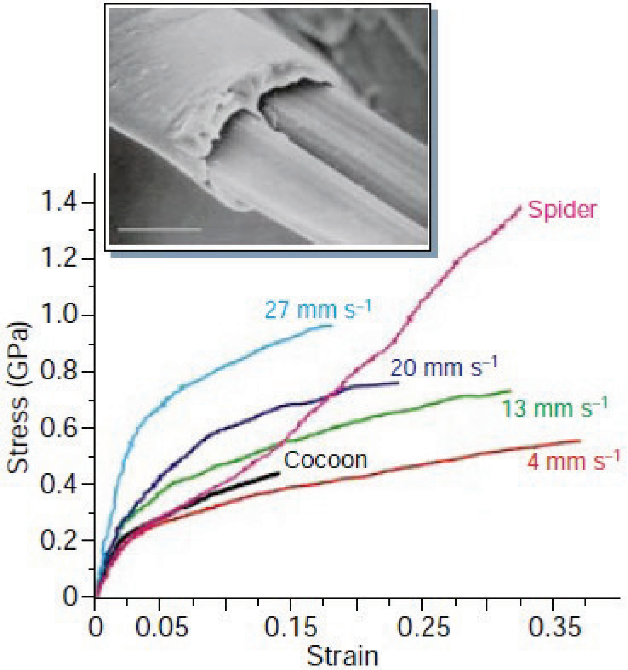
Elices, M. et al. *JOM.* **2005**, *57*, 60-66. Pérez-Rigueiro, J. et al. *J. Exp. Biol.* **2005**, *208*, 2633-2639.

The importance of crystalline β-sheet in the mechanical behavior

	Reeling speed: (mm s^{-1})	c%	Size of β-crystallites (nm)			f	n_β	A (nm^2)
			L_a	L_b	L_c			
Silkworm	1	41	2.63	3.20	11.65	0.922	13.5	0.403
	4	41	2.57	3.18	11.48	0.930	15.3	0.403
	13	41	2.55	3.17	11.49	0.944	17.3	0.403
	20	41	2.55	3.16	11.49	0.945	17.3	0.403
	27	41	2.54	3.15	11.49	0.956	17.9	0.403
Spider	1	27	2.46	2.68	6.48	0.963	22.0	0.582
	2.5	26	2.46	2.67	6.25	0.967	23.3	0.597
	10	25	2.45	2.66	6.09	0.973	23.3	0.613
	25	24	2.45	2.64	6.05	0.982	22.8	0.629







Elices, M. et al. *JOM.* **2005**, *57*, 60-66. Pérez-Rigueiro, J. et al. *J. Exp. Biol.* **2005**, *208*, 2633-2639. Morikawa, H. et al. *Int. J. Biol. Macromol.* **2008**, *42*, 264-270. Shao, Z. Z. *Nature* **2002**, *418*, 741-741.

Outlines

- Introduction
  - Background and superiority of spider silks and silkworm silk

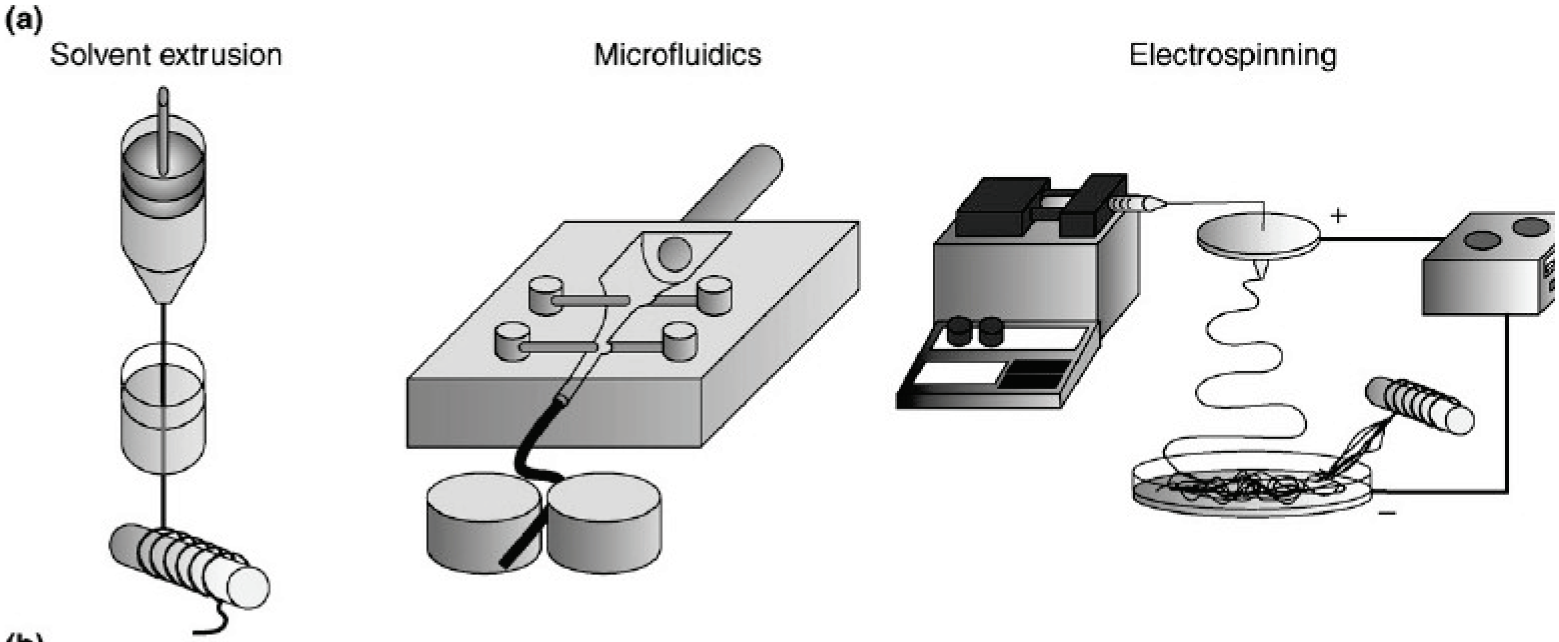
- Structures of silk fibers
  - Morphological structure and chemical composition of silk fibers
  - Hierarchical structure of silk proteins

- The interplay of structure and mechanical properties
  - Experimental investigations
  - Computational evidences

- Bio-inspired silk fibers and silk-based biomaterials

- Summary and perspective

Bio-inspired silk fibers



(b)

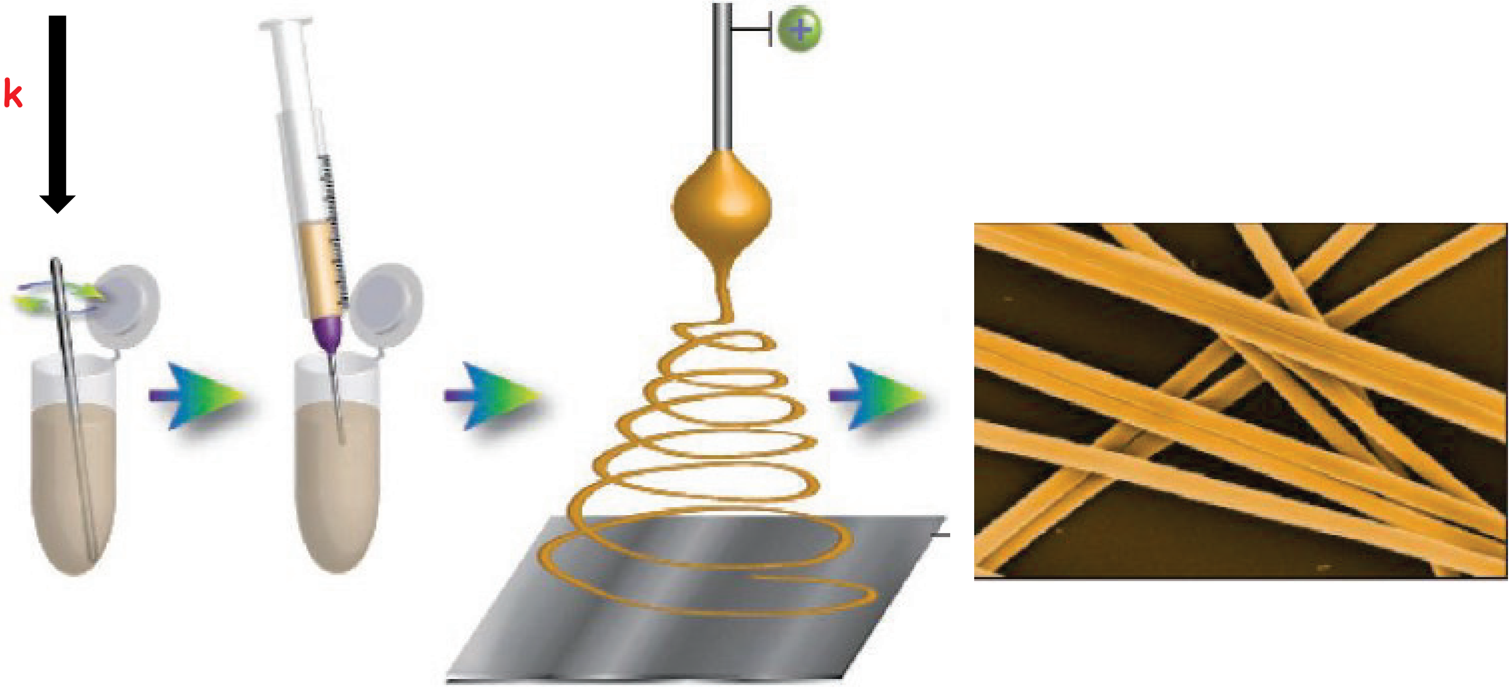
	Solvent extrusion	Microfluidics	Electrospinning
Final fiber size	Micron-scale	Micron-nano scale	Nano-scale
Advantages	Well-established, inexpensive and simple	Highly biomimetic, multiple inputs, fine process control	Extremely fine diameters, simple operation
Disadvantages	Large-scale only	Not well-established, manufacturing challenges	Poor process control, poor functional properties, small-scale only

Kaplan D. L. et al. *Trends in Biotech.* **2008**, *26*, 244-251.

Bio-inspired silk fibers

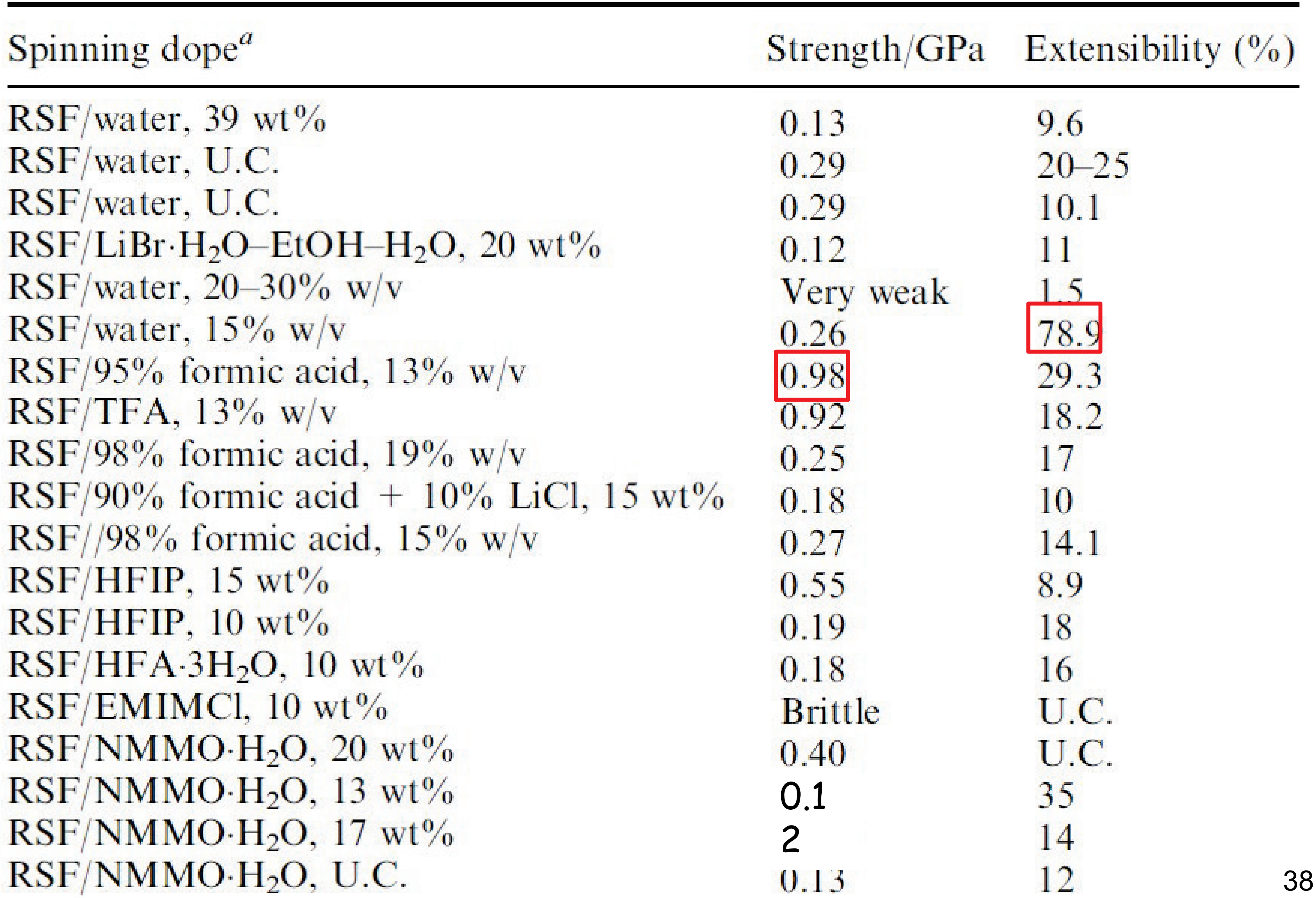
Silkworm silk

Redissolving of silk



Shao Z. et al. *Chem. Commun.* **2009**, 6515-6519.

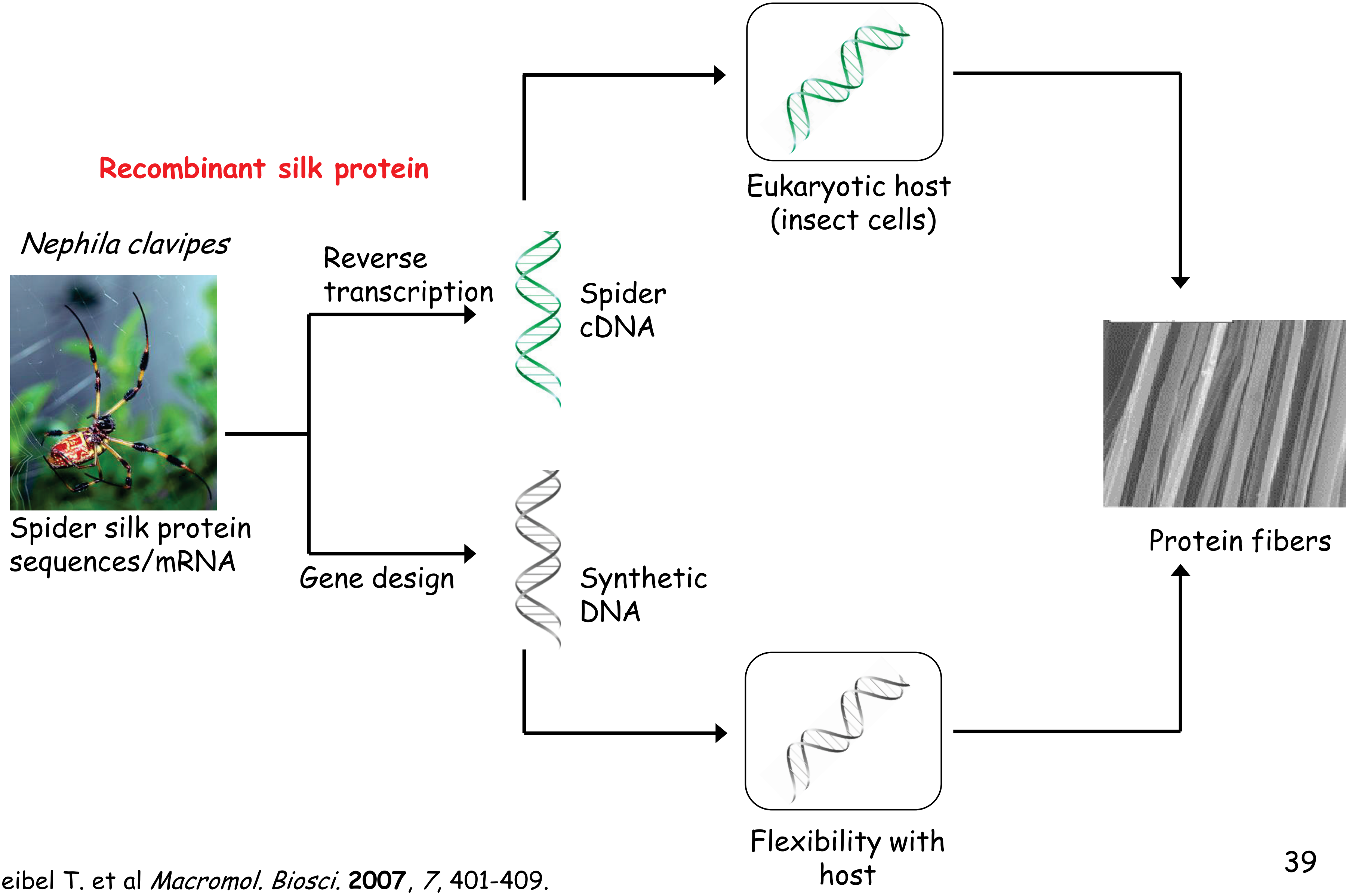
Bio-inspired silk fibers



Spinning dope[a]	Strength/GPa	Extensibility (%)
RSF/water, 39 wt%	0.13	9.6
RSF/water, U.C.	0.29	20–25
RSF/water, U.C.	0.29	10.1
RSF/LiBr·H_2O–EtOH–H_2O, 20 wt%	0.12	11
RSF/water, 20–30% w/v	Very weak	1.5
RSF/water, 15% w/v	0.26	78.9
RSF/95% formic acid, 13% w/v	0.98	29.3
RSF/TFA, 13% w/v	0.92	18.2
RSF/98% formic acid, 19% w/v	0.25	17
RSF/90% formic acid + 10% LiCl, 15 wt%	0.18	10
RSF//98% formic acid, 15% w/v	0.27	14.1
RSF/HFIP, 15 wt%	0.55	8.9
RSF/HFIP, 10 wt%	0.19	18
RSF/HFA·3H_2O, 10 wt%	0.18	16
RSF/EMIMCl, 10 wt%	Brittle	U.C.
RSF/NMMO·H_2O, 20 wt%	0.40	U.C.
RSF/NMMO·H_2O, 13 wt%	0.1	35
RSF/NMMO·H_2O, 17 wt%	2	14
RSF/NMMO·H_2O, U.C.	0.13	12

Shao Z. et al. *Chem. Commun.* **2009**, 6515-6519.

Bio-inspired silk fibers



Recombinant silk protein

Nephila clavipes

Spider silk protein sequences/mRNA

Reverse transcription

Spider cDNA

Gene design

Synthetic DNA

Eukaryotic host (insect cells)

Flexibility with host

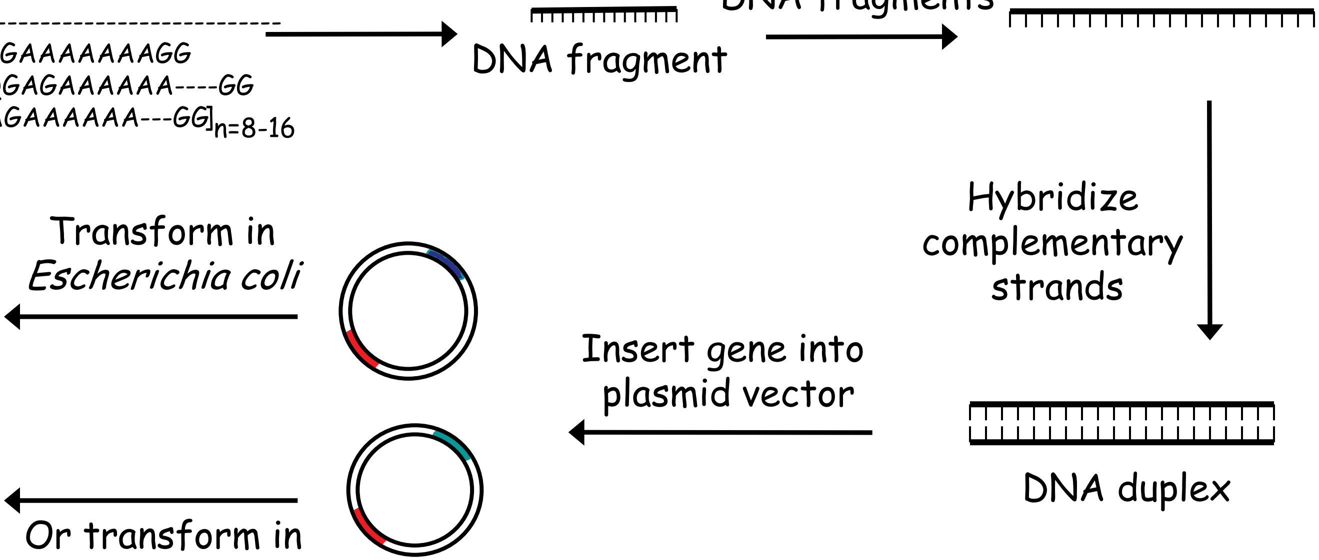
Protein fibers

Scheibel T. et al *Macromol. Biosci.* **2007**, *7*, 401-409.
Altman G.H. et al. *Biomaterials* **2003**, *24*, 401-416.

Bio-inspired silk fibers

Spidroin 1 analog: DP-1B

[AGQGGYGGLGSQG---
AGQGGYGGLGSQGAGRGGLGGQGAGAAAAAAAGG
AGQG-------GLGSQGA---------- GQGAGAAAAAA----GG
AGQGGYGGLGSQGAGRG-----GQGAGAAAAAA---GG]$_{n=8-16}$



DNA fragment

Ligate 8 or 16
DNA fragments

Hybridize
complementary
strands

DNA duplex

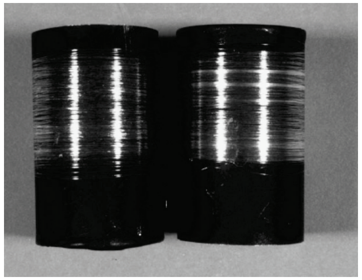
Insert gene into
plasmid vector

Transform in
Escherichia coli

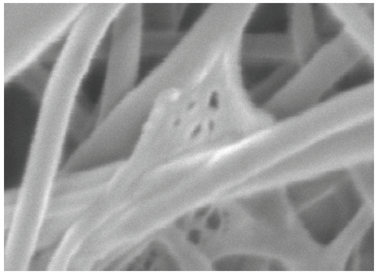
Or transform in
yeast

Protein fibers
300 mg/L

Protein fibers
1 g/L



170 nm diameter fibers

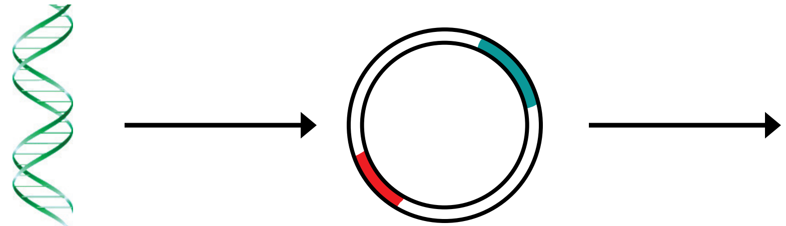


Premature termination with
expression in *E. coli*

High MW polymers from yeast

Fahnestock S. R. et al. *Appl. Microbiol. Biotechnol.* **1997**, *47*, 23-32.
Fahnestock, S. R. et al. *Appl. Microbiol.Biotechnol.* **1997**, *47*, 33-39.
O'Brien J. P. et al. *Adv. Mater.* **1998**, *10*, 1185-1195.

Bio-inspired silk fibers



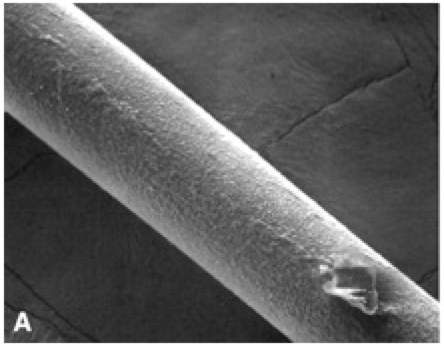
Dragline silk
gene sequence
from *A. diadematus*

Gene sequence
inserted into
expression vector

Transformation
of vector in
mammalian cells

protein
synthesis

Protein purification,
and characterization

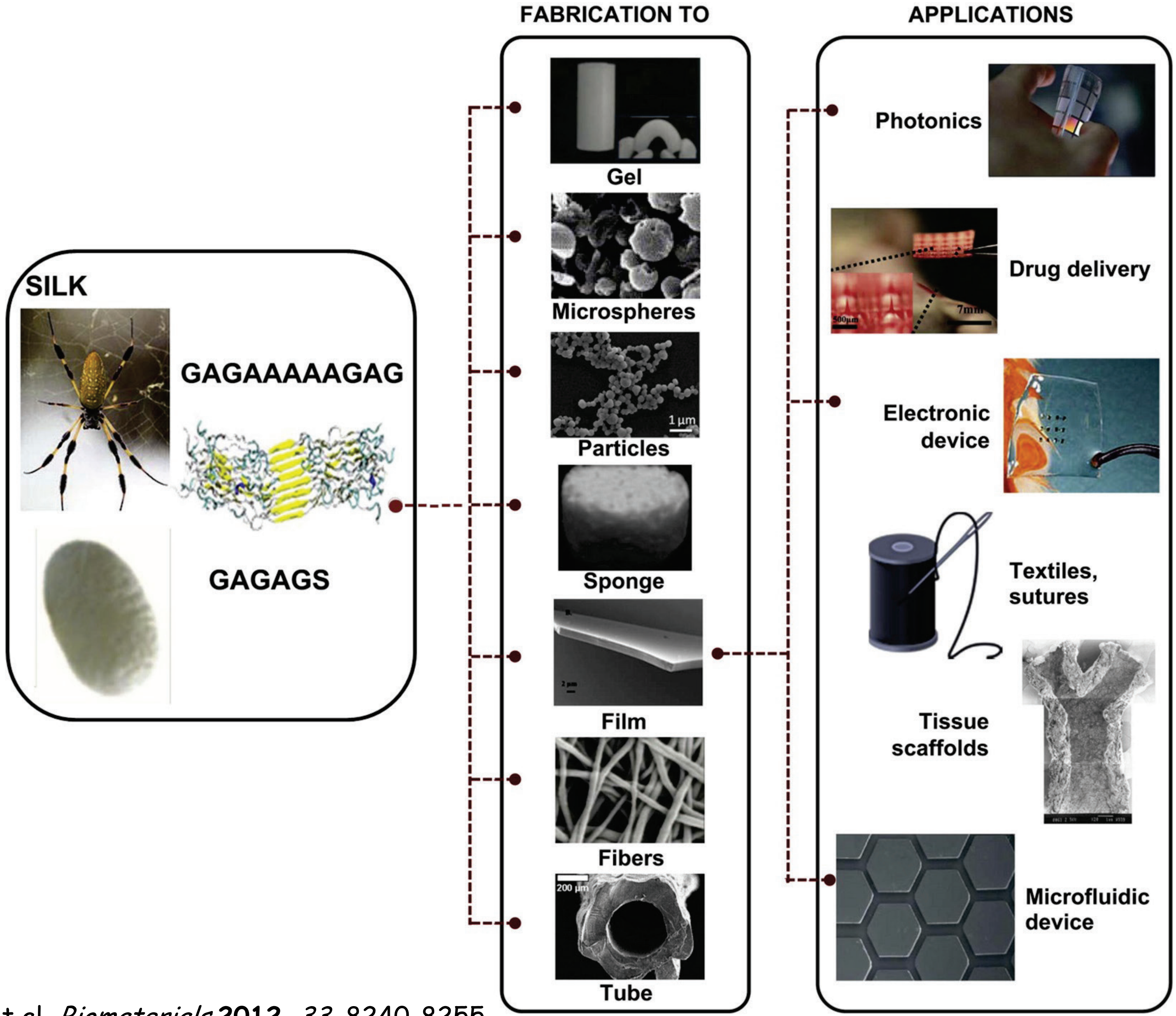


Mechanical Properties:

Protein sample	Toughness (MJ/m^3)	Modulus (GPa)	Elasticity (%)	Strength (GPa)
ADF-3	85	13	43.4	0.26
A. diadematus dragline	130	10	30	1.1

Lazaris, A. et al. *Science* **2002**, *295*, 472-476.

Silk-based biomaterials



Buehler M. J. et al. *Biomaterials* **2012**, *33*, 8240-8255.

Summary

- It turns out that the sequence of silk protein may affect the secondary conformation. The ordered structure, β-sheets, will determine the stiffness of the silk fibers.

- The hierarchical network structures strongly correlate with the strength, elasticity, and toughness of silk fibers.

Perspective

- The robustness and plasticity of network -> mechanical properties?
- The genetic modification of silk protein -> enhanced mechanical properties and novel functions?