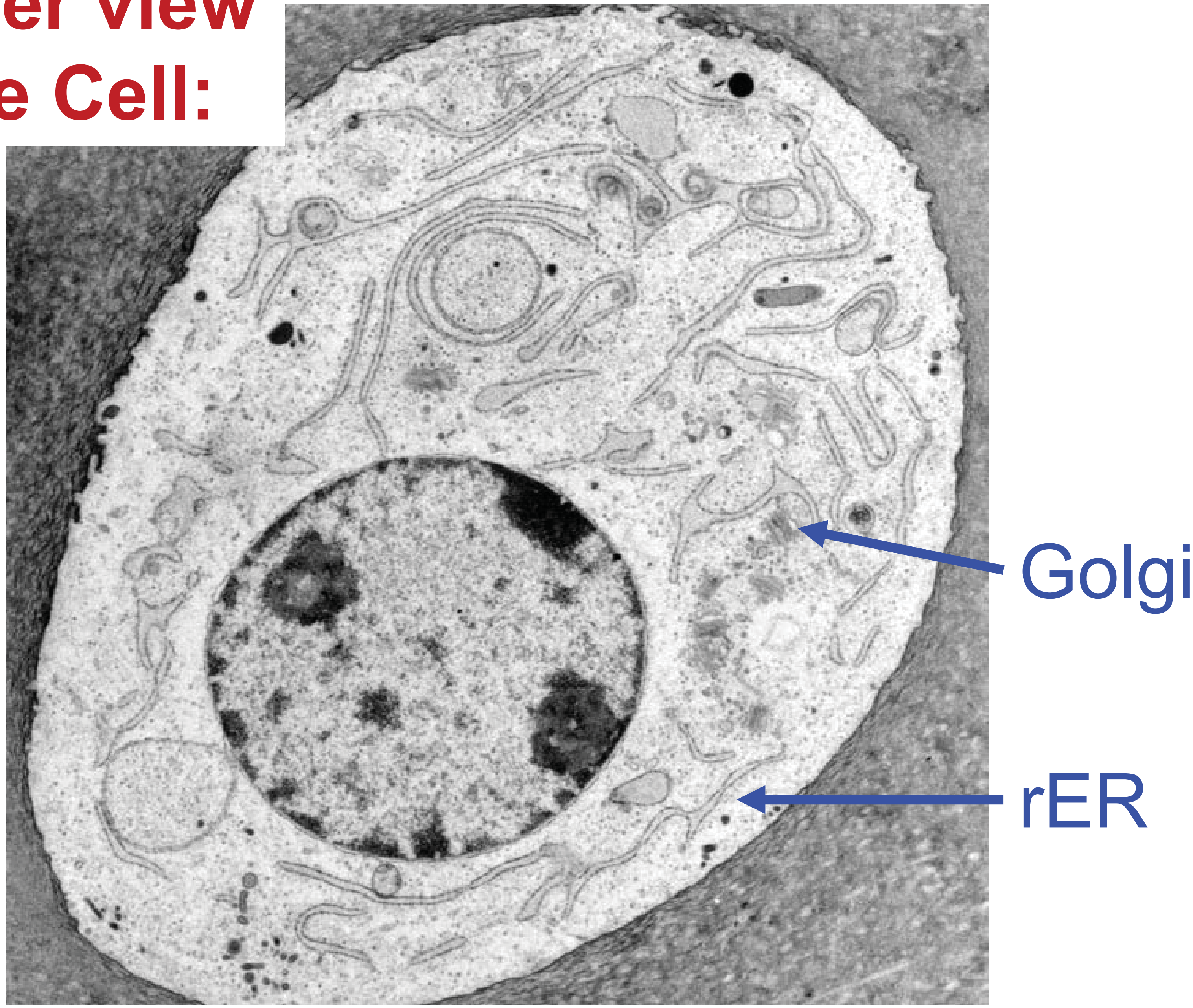
Intracellular molecules: Cytoskeleton, DNA…

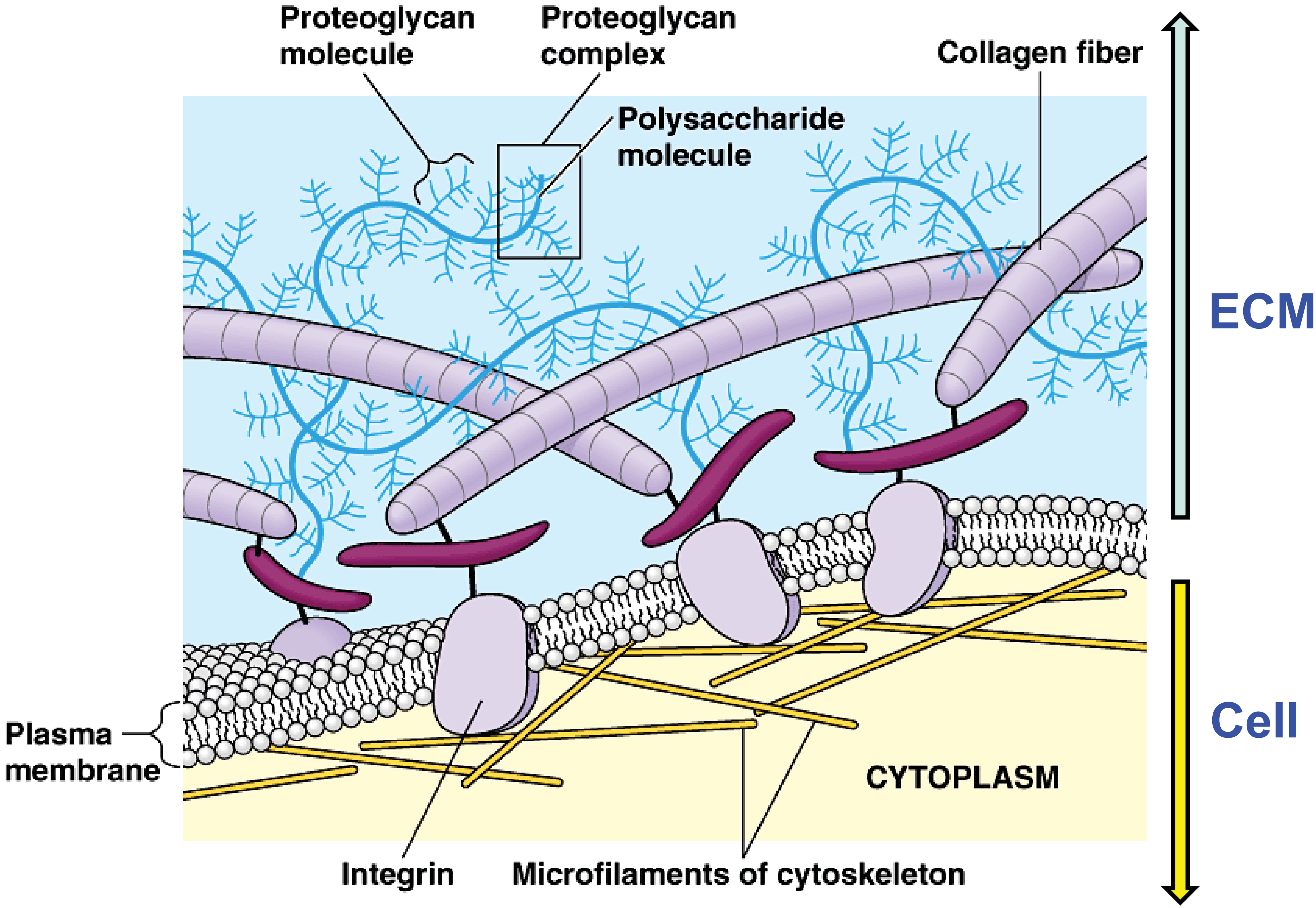
Microfilaments **Microtubules** **Intermediate filaments**

Images of microfilaments, microtubules, intermediate filaments, and DNA removed due to copyright restrictions.

DNA

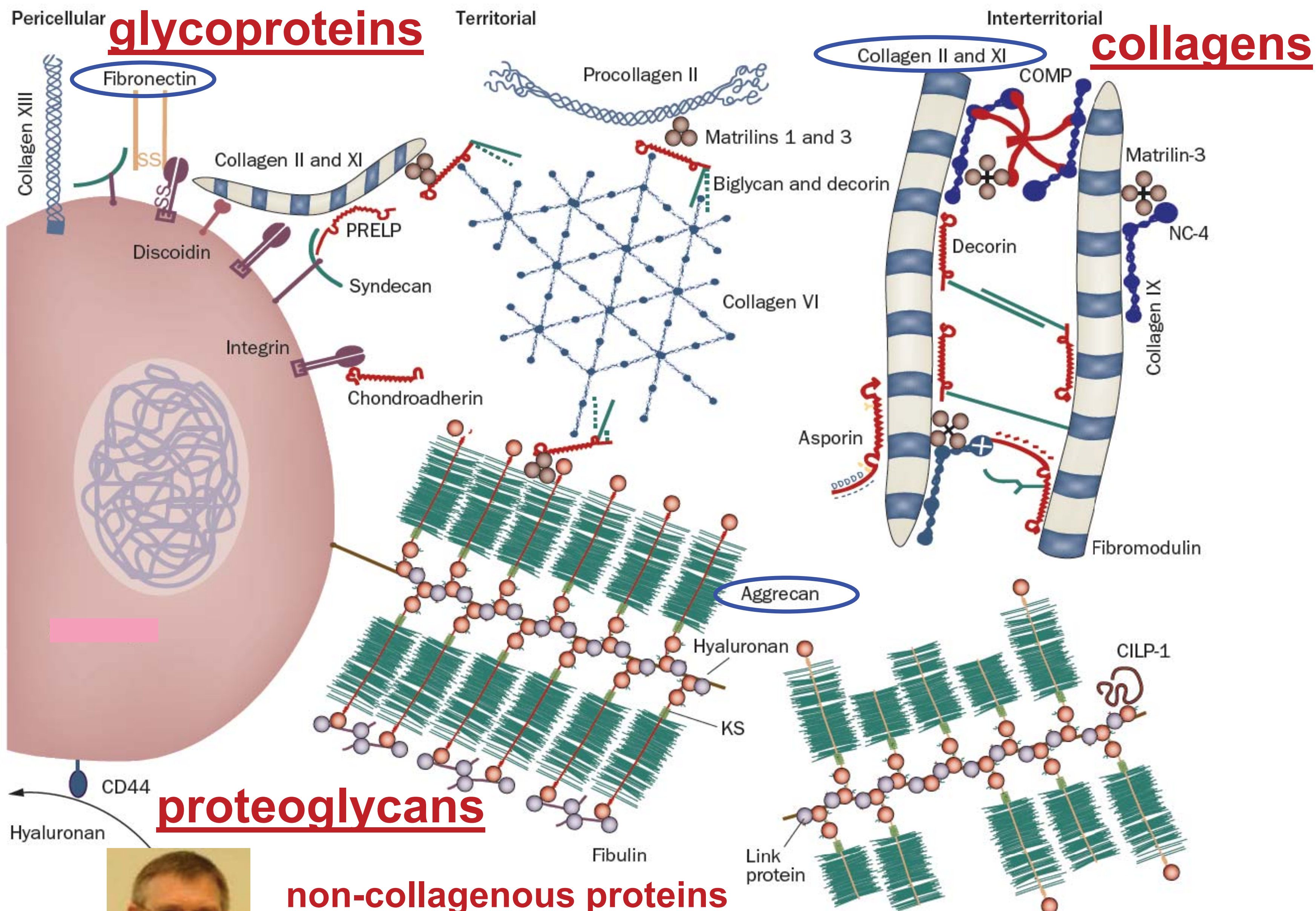
Another view of the Cell:





Proteoglycan molecule
Proteoglycan complex
Collagen fiber
Polysaccharide molecule
ECM
Cell
Plasma membrane
CYTOPLASM
Integrin
Microfilaments of cytoskeleton

Cells Synthesize 100s of Extracellular Matrix Macromolecules



(Dick Heinegård, Nature Revs. Rheumatology 2010)

Source: Heinegård, Dick, and Tore Saxne. "The Role of the Cartilage Matrix in Osteoarthritis." *Nature Reviews Rheumatology* 7, no. 1 (2011): 50-6.

Mechanics↔Biology: Organ, Tissue, Cell, & Molecular Levels

Images removed due to copyright restrictions.

Collagen fibrils (from sciatic nerve)

(AJ Hodge, electron microscopy; Textbook, page 17)

How much **Force** is required to stretch a typical strand of DNA by 10% of its contour length?

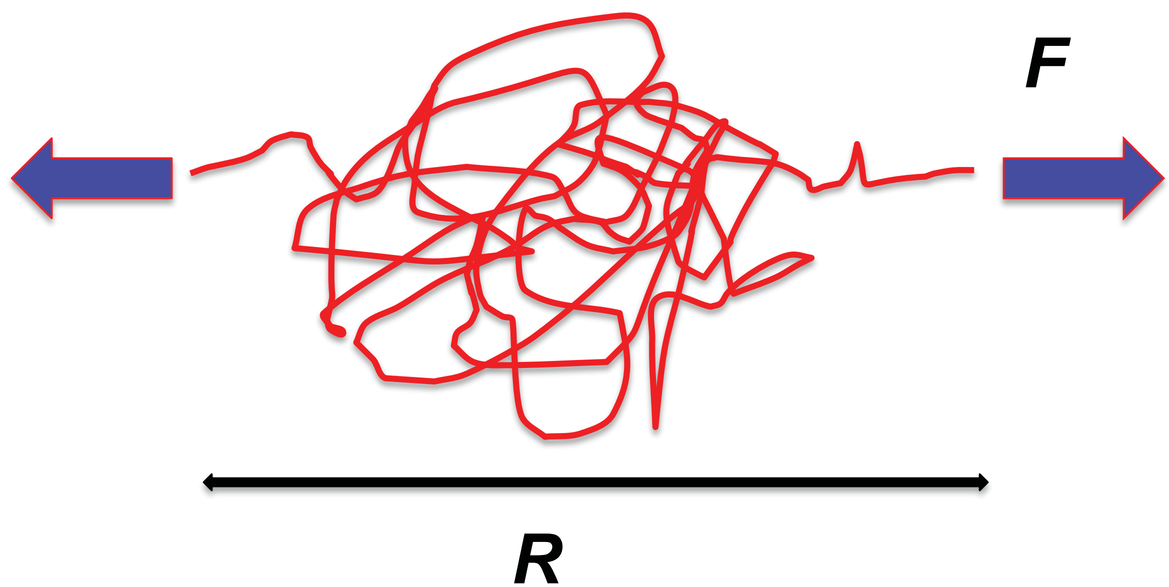
k_BT = 4 pN • nm

L_p = 50 nm

R/L_c = 0.01

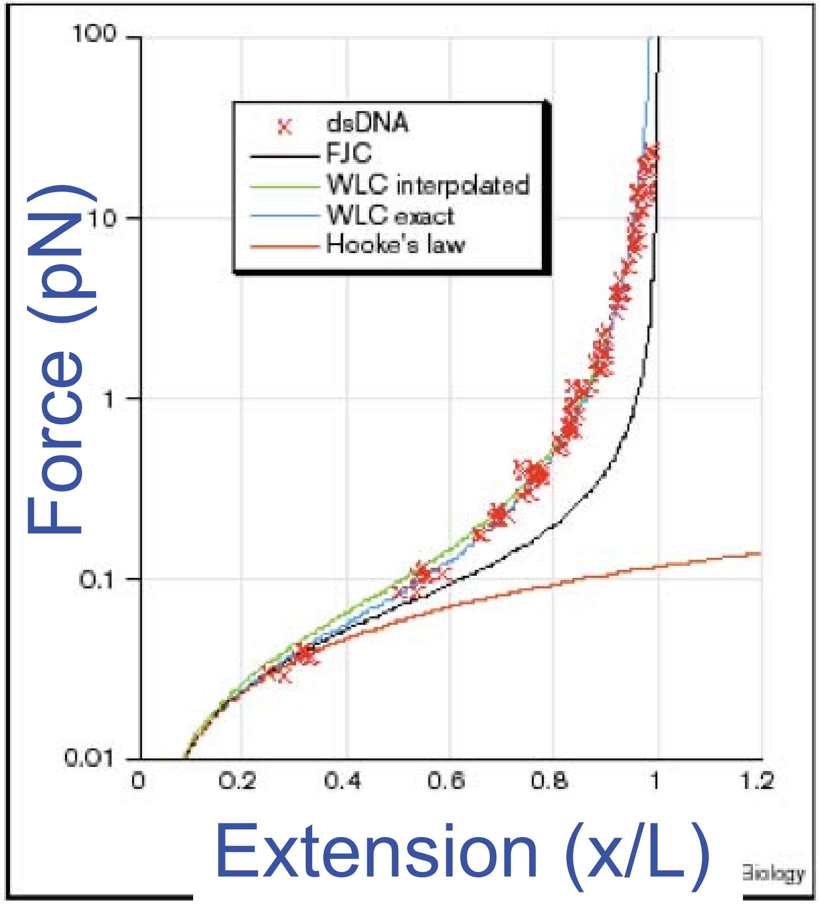
L_c = 15 μm

F = ??



DNA extension --

Best fit is with
"worm-like chain"



Force versus extension data (red crosses) for λ phage dsDNA (48,502 bp) pulled by magnetic beads in 10 mM Na+ buffer [4]. The data are fit to a WLC model solved numerically (WLC exact) or using Equation 3 (WLC interpolated), both assuming P = 53 nm. The FJC curve assumes b = 2P = 106 nm. The Hooke's law force curve is from Equation 2.

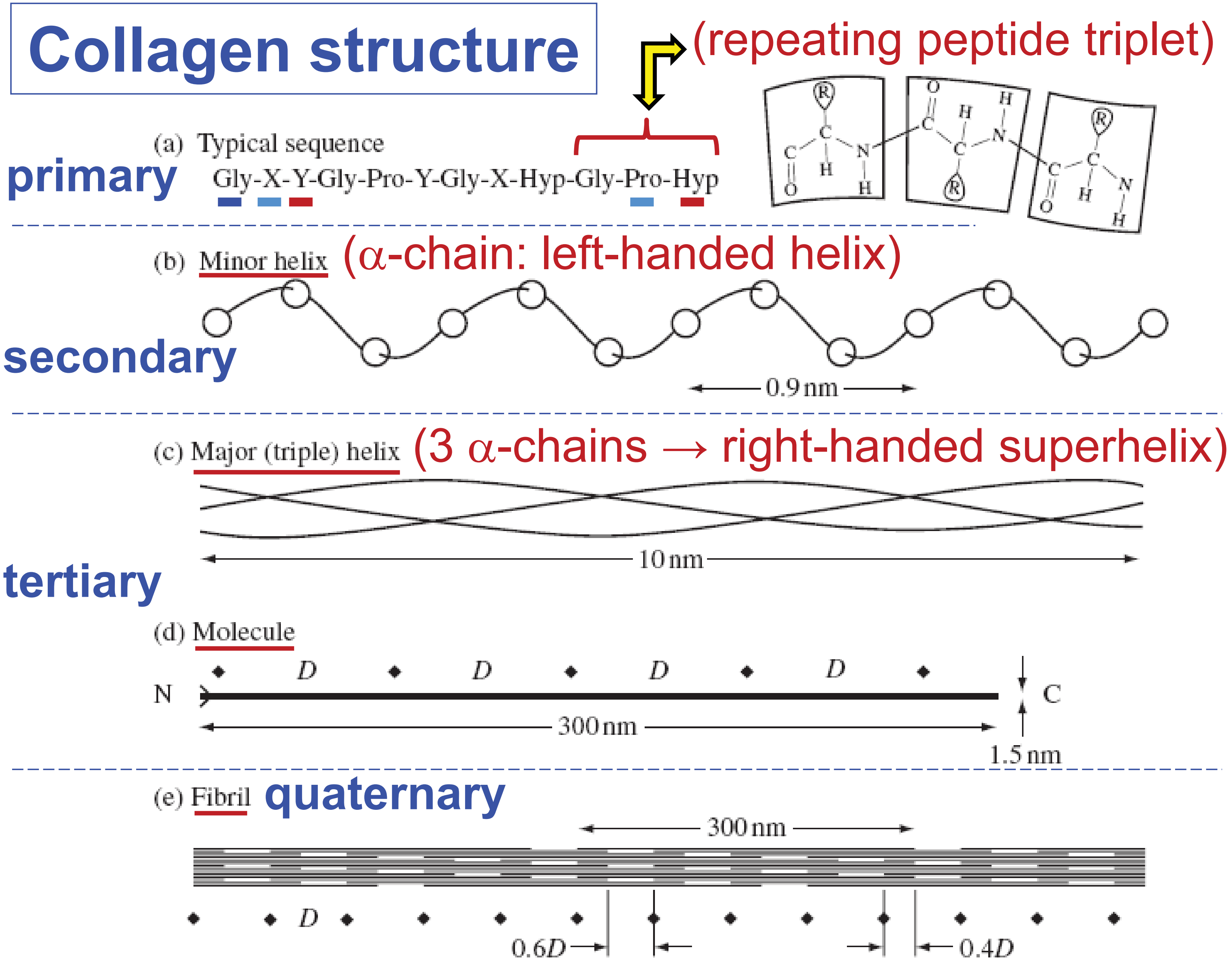
Bustamante et al. 2001

Collagen fibrils (from sciatic nerve)

Images removed due to copyright restrictions.

(AJ Hodge, electron microscopy; Textbook, page 17)

Collagen structure



(Textbook, page 16) (molecules quarter staggered; X-links)

Work leading up to "announcement" of Structure of Type I Collagen:

November 12, 1955 NATURE 915

THE STRUCTURE OF COLLAGEN

By Dr. ALEXANDER RICH* and Dr. F. H. C. CRICK
Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems, Cavendish Laboratory, Cambridge

VERY recently, Ramachandran and Kartha[1] have made an important contribution by proposing a coiled-coil structure for collagen. We believe this idea to be basically correct but the actual structure suggested by them to be wrong.

⋮

We believe this structure to be wrong for two reasons. (1) It is stereochemically unsatisfactory. In particular, there is a very short C_α—C_α contact of 3·3 A. (normally 3·6–4·0 A.) and an extremely short C_α—O contact of 2·6 A. (normally 3·2–3·5 A.). In addition, the hydrogen bond angles are on the outside limit of the values usually found. (2) It is not compatible with recent work[3] on the amino-acid sequence, which shows that

—gly—pro—hypro—

Source: Rich, Alexander, and Francis Crick. "The Structure of Collagen." *Nature*, no. 176 (1955): 915-916.

The Amino-acid Composition and Titration Curve of Collagen

By JOANE H. BOWES and R. H. KENTEN
The British Leather Manufacturers' Research Association, London, S.E. 1

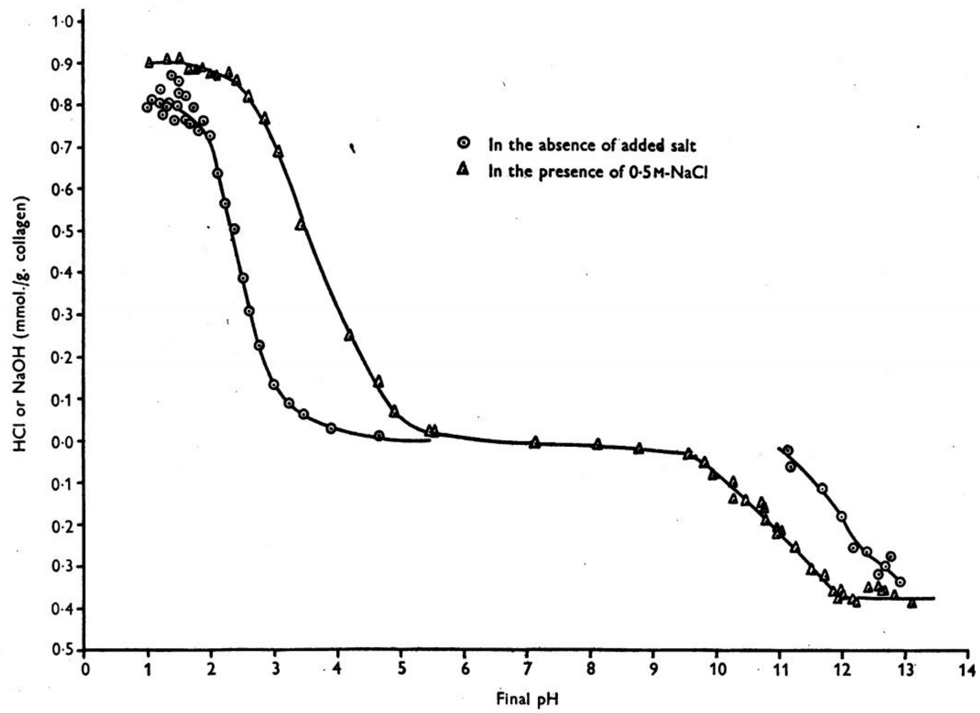
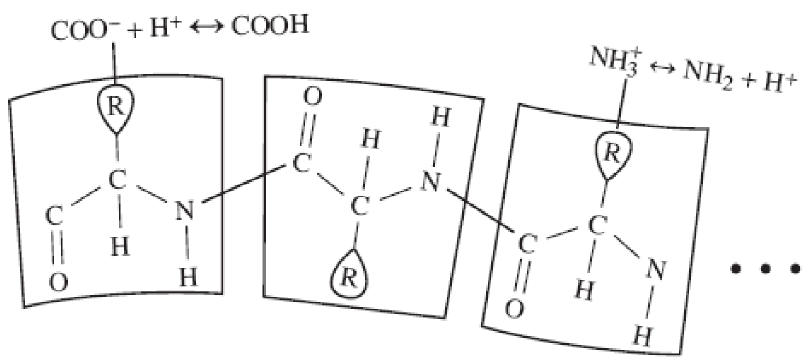


Fig. 2. Titration curves of collagen with and without sodium chloride.

Source: Bowes, Joane H., and R. H. Kenten. "The Amino-acid Composition and Titration Curve of Collagen." *Biochemical Journal* 43, no. 3 (1948): 358.

(Textbook, page 18) . . .



The Collagen Superfamily

Ricard-Blum et al., *Top Curr Chem*, 2005

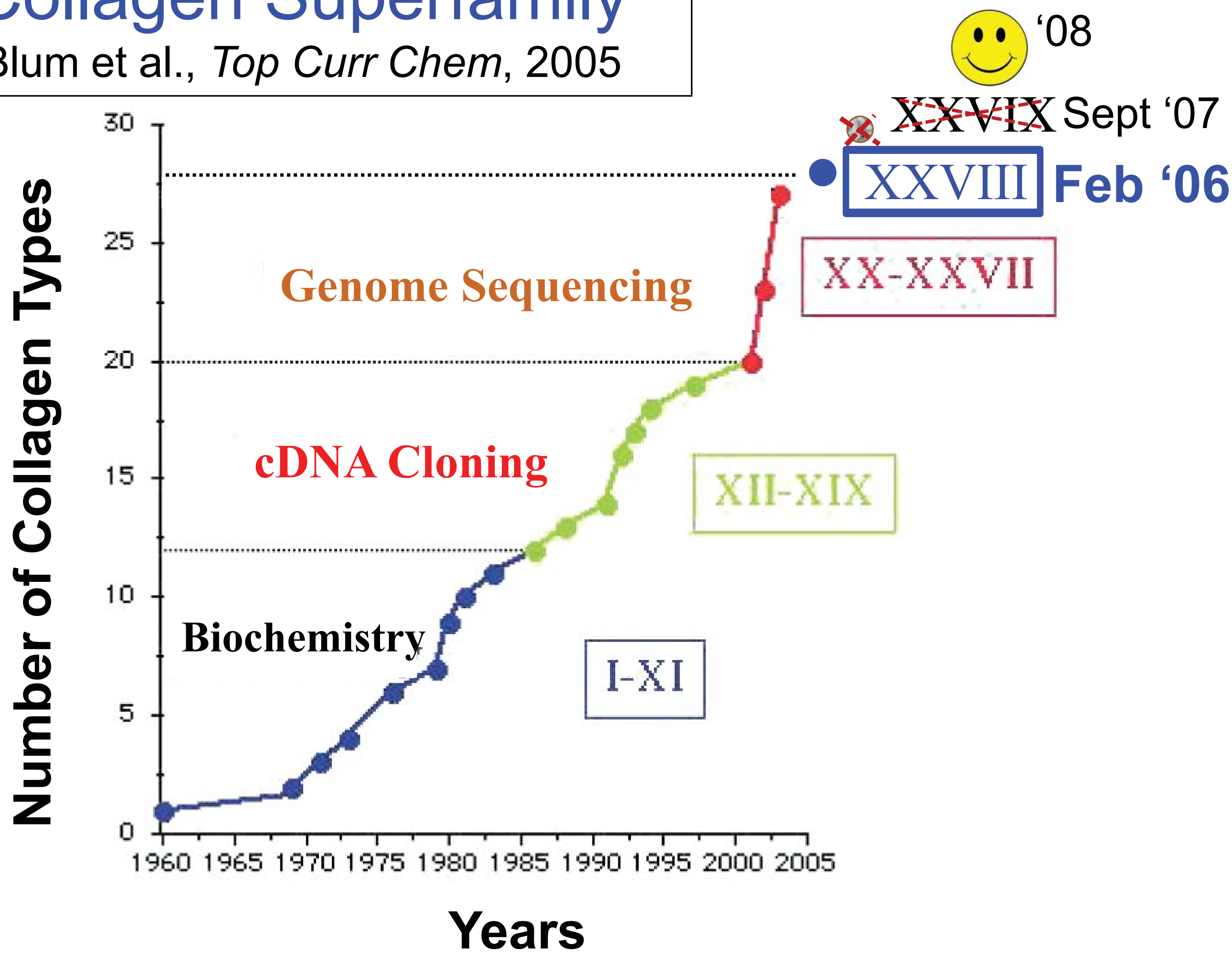
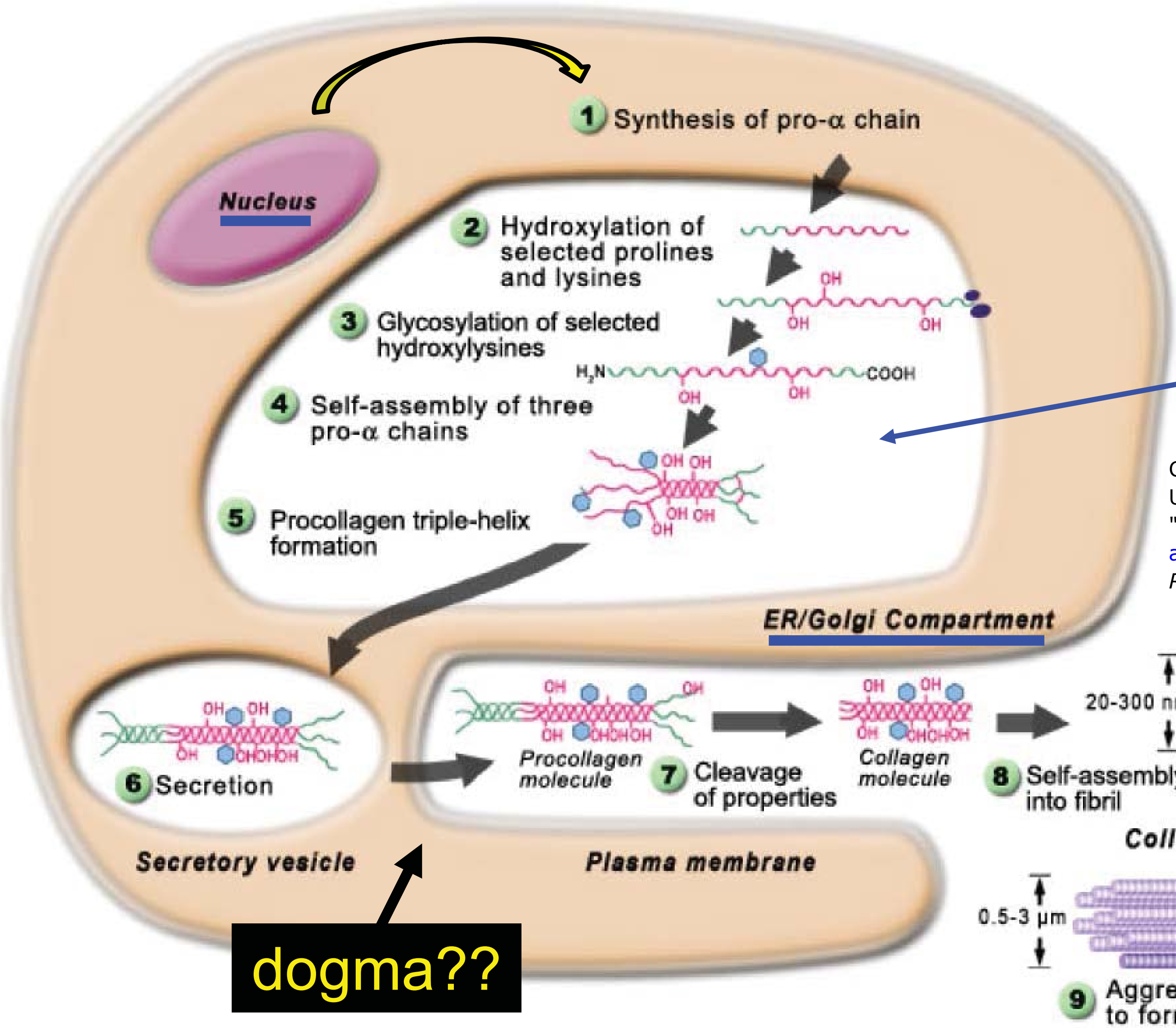
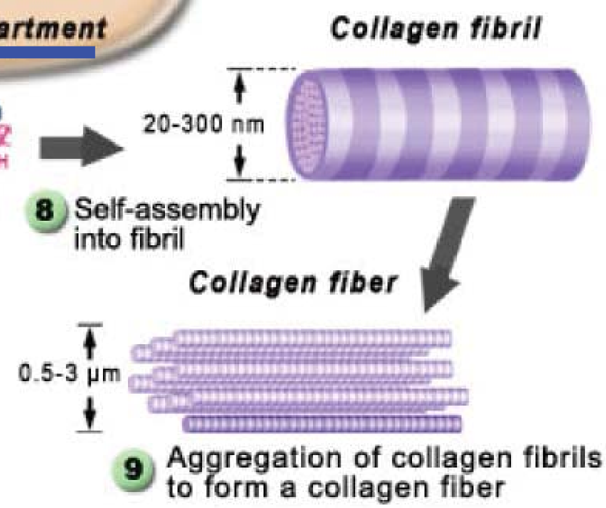


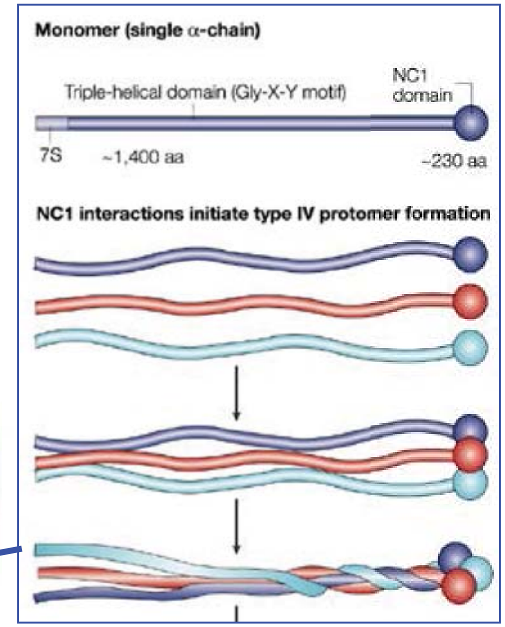
Fig. 1 Discovery of the collagen super family members: a 50-year story

Source: Ricard-Blum, Sylvie, Florence Ruggiero, and Michel van der Rest. "The Collagen Superfamily." In *Collagen.* Springer Berlin Heidelberg, 2005, pp. 35-84.

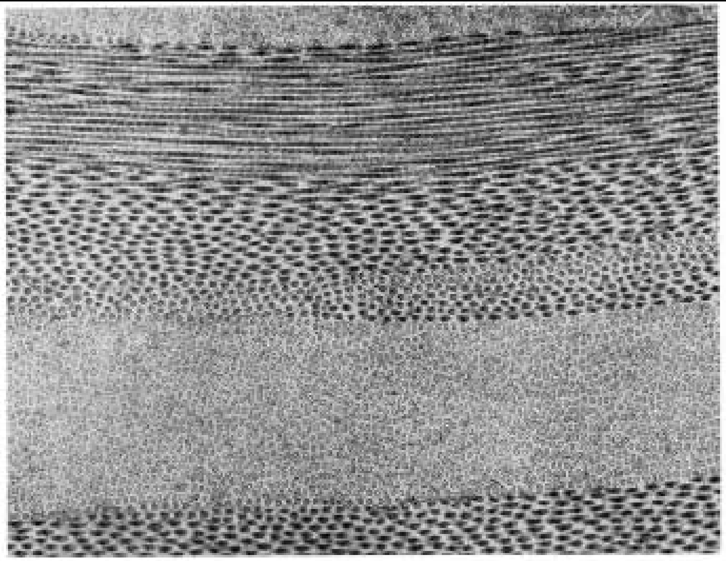
How do cells make collagen molecules and regulate "fibrillogenesis" ??

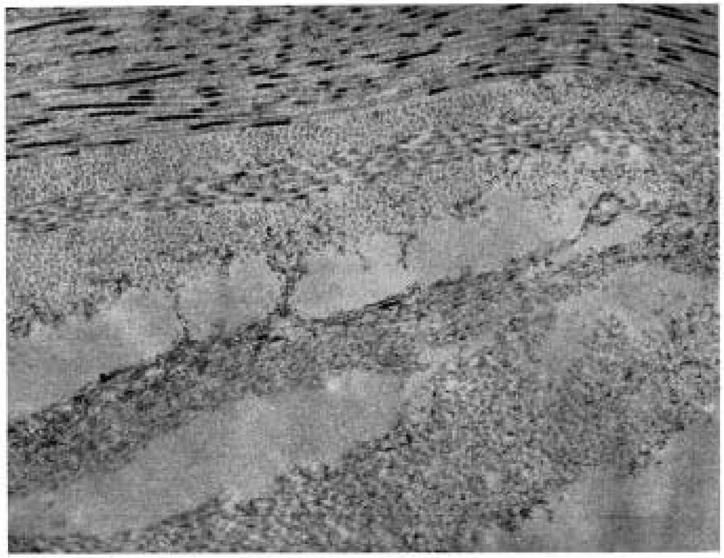






Courtesy of Macmillan Publishers Limited. Used with permission. Source: Kalluri, Raghu. "Basement Membranes: Structure, Assembly and Role in Tumour Angiogenesis." *Nature Reviews Cancer* 3, no. 6 (2003): 422-33.



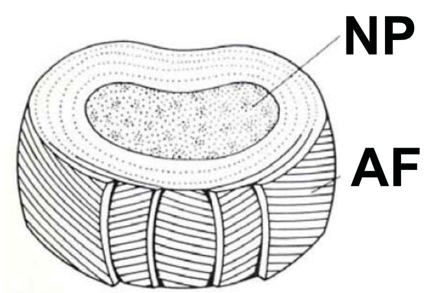


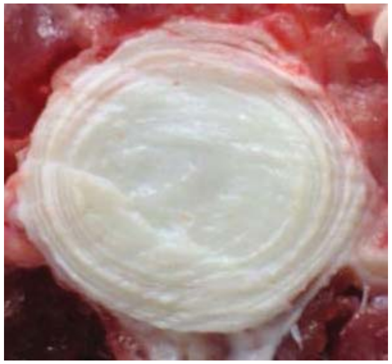
Corneal Stroma

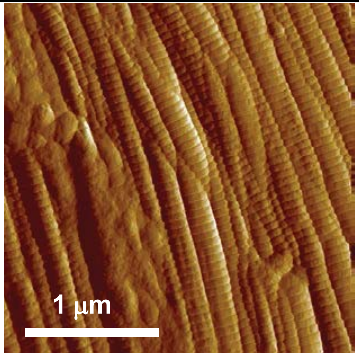
(normal: top; cataract: below)

(Text book page 241)

Human **Intervertebral Disc**



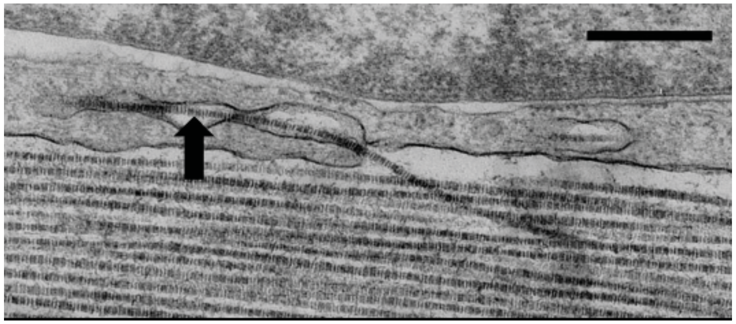




Tendon Fibers

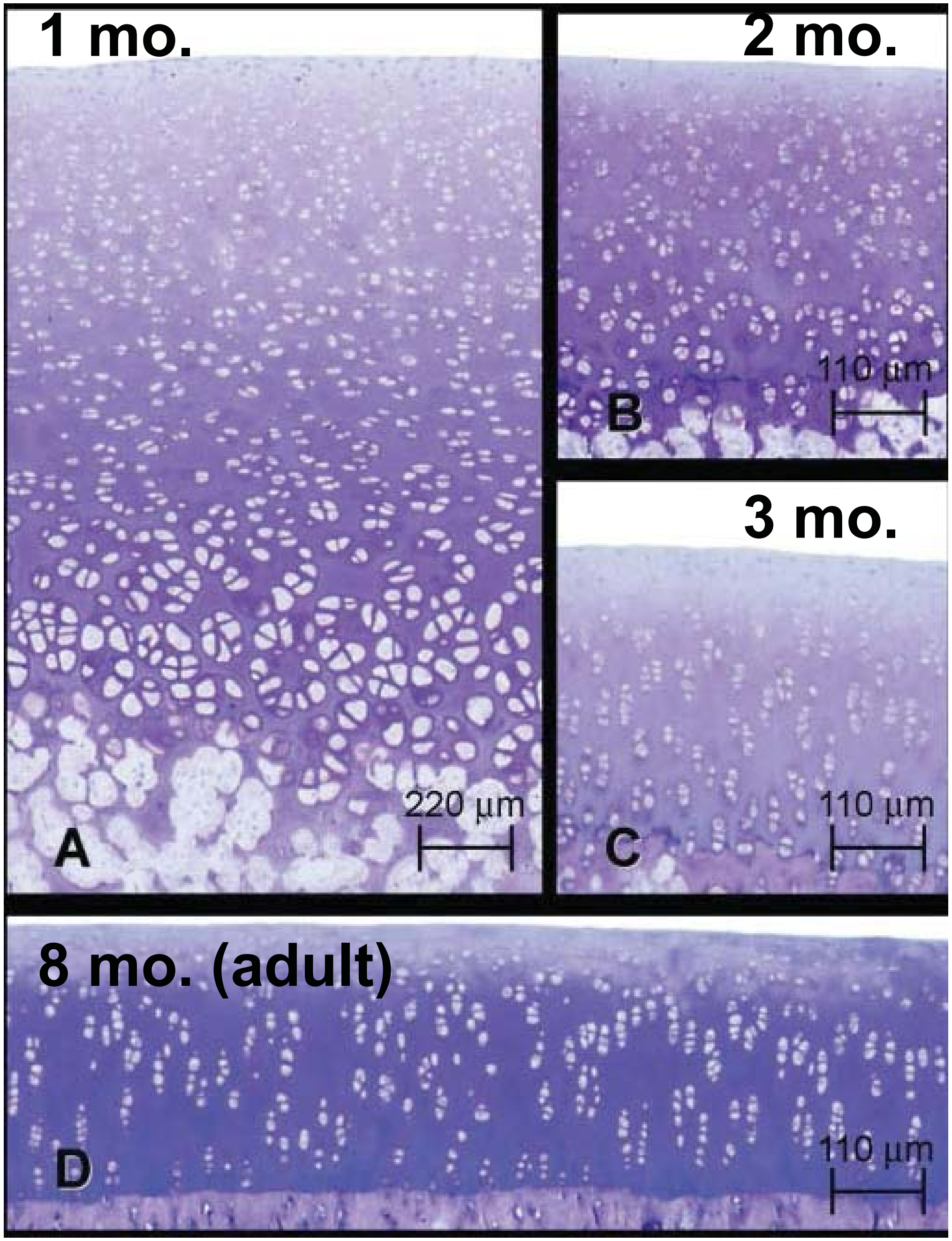
~200 nm diam

(Lin Han, AFM)



28 nm diam (TEM, Karl Kadler; embryonic chick tendon, JCB '04)

Collagen Architecture in Articular Cartilage
(Rabbit)



(Text book page 241)

(Hunziker+, 2007)

Source: Hunziker, E. B., et al. "The Structural Architecture of Adult Mammalian Articular Cartilage Evolves by a Synchronized Process of Tissue Resorption and Neoformation during Postnatal Development." *Osteoarthritis and Cartilage* 15, no. 4 (2007): 403-13.

Electron micrographs of the interterritorial matrix of articular cartilage from the medial femoral condyle of an eight-month-old rabbit removed due to copyright restrictions. Source: Figure 3 in Buckwalter, J. A. and J. J. Mankin. "Articular Carilage: Part I." *The Journal of Bone and Joint Surgery* 79-A, no. 4 (1997): 600.

Collagen Superfamily: Types I – XXVIII

Table 1 Collagen α chains, number of amino acids (*aa*), signal peptide, *vWC* von Willebrand factor C domain, *FNII*

Collagen α chain	Number of amino acids
α1(I)	1464 (includes 22 aa SP)
α2(I)	1366 aa (includes SP)
α1(II)A	1487 aa (includes 25 aa SP)
α1(II)B	Same as a1(II)A but lacks vWC
α1(III)	1466 aa (includes 23 aa SP)
α1(IV)	1669 aa (includes 27 aa SP)
α2(IV)	1712 aa (includes 25 aa SP)
α3(IV)	1670 aa (includes 28 aa SP)
α4(IV)	1690 aa (includes 38 aa SP)
α5(IV)	1685 aa (includes 26 aa SP)
α6(IV)	1691 aa (includes 21 aa SP)
α1(V)	1838 aa (includes SP)
α2(V)	1499 aa (includes SP)

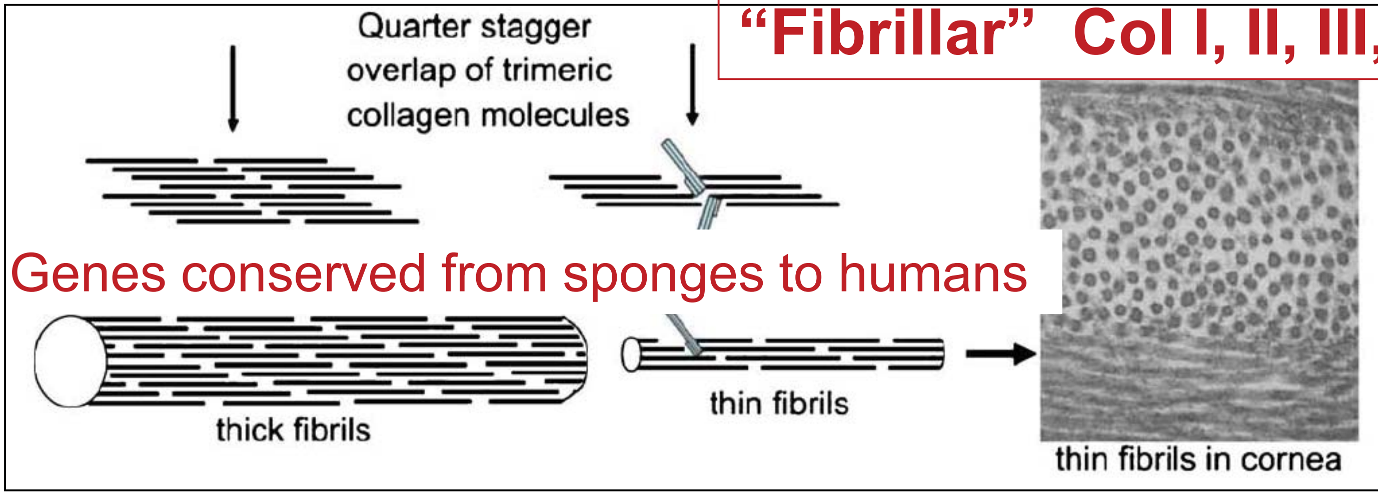
α1(XXI)	957 aa (includes 22 aa SP)
α1(XXII)	1626 aa (includes SP)
α1(XXIII)	540 aa (transmembranous)
α1(XXIV)	1714 aa (includes SP)
α1(XXV)	654 aa (transmembranous) Isoform 2 is 642 aa
α1(XXVI)	439 aa (includes SP)
α1(XXVII)	1860 aa (includes 41 aa SP)
α1(XXVIII)	1125 aa (includes SP)

Nomenclature: α-chains of the different collagen types

(Gordon & Hahn, Cell Tiss Res, 2010)

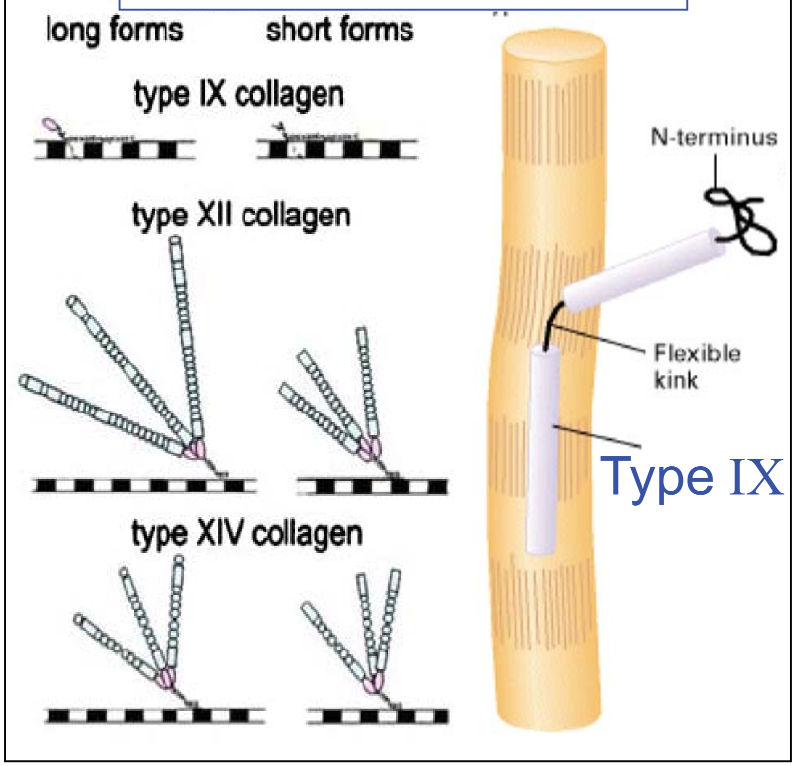
"Fibrillar" Col I, II, III, V, XI

Genes conserved from sponges to humans

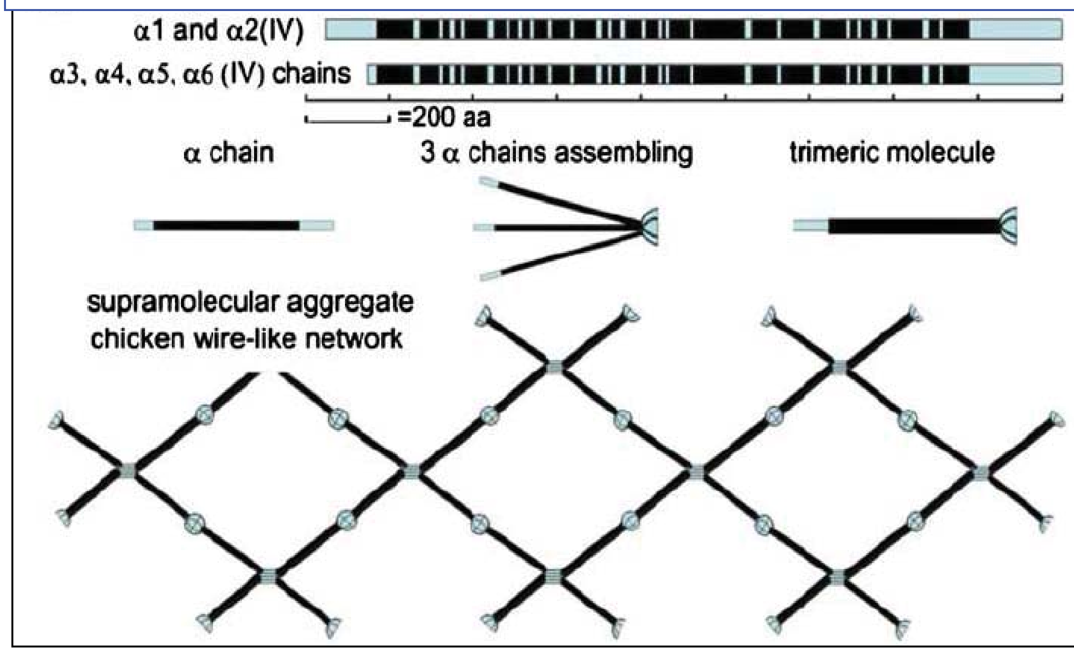


Collagen forms fibrils, networks, other aggregates…

"FACIT" Col IX, XII, …



Type IV (network) Collagen: Basement mem



(Gordon & Hahn, Cell Tiss Res, 2010)

Source: Gordon, Marion K., and Rita A. Hahn. "Collagens." *Cell and Tissue Research* 339, no. 1 (2010): 247-57.

Biophys J, Feb 2012

Structural and Mechanical Differences between Collagen Homo- and Heterotrimers: Relevance for the Molecular Origin of Brittle Bone Disease

Shu-Wei Chang,[†] Sandra J. Shefelbine,[‡] and Markus J. Buehler[†§¶*]

[†]Laboratory for Atomistic and Molecular Mechanics, Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts; [‡]Department of Bioengineering, Imperial College London, London, United Kingdom; and [§]Center for Materials Science and Engineering and [¶]Center for Computational Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts

- ABSTRACT Collagen constitutes one-third of the human proteome, providing mechanical stability, elasticity, and strength to organisms. **Normal type I collagen is a heterotrimer**… A homotrimer is found in fetal tissues, fibrosis, and human cancers, and in a mouse model brittle bone disease, osteogenesis imperfect…. Experimental studies of *oim* tendon and bone show reduced mechanical strength…
- Here, fully atomistic simulations of … mouse type I heterotrimer and homotrimer collagen … are developed …
- Homotrimer L_p is half that of the heterotrimer (9.6 nm vs. 21.5 nm), indicating it is more flexible and confirmed by direct mechanical testing. …Implications for reduced intermolecular cross-linking reduced mechanical strength.

Stretching type II collagen with optical tweezers

Yu-Long Sun[a], Zong-Ping Luo[b], Andrzej Fertala[c], Kai-Nan An[a]

(J Biomechanics, 2004)

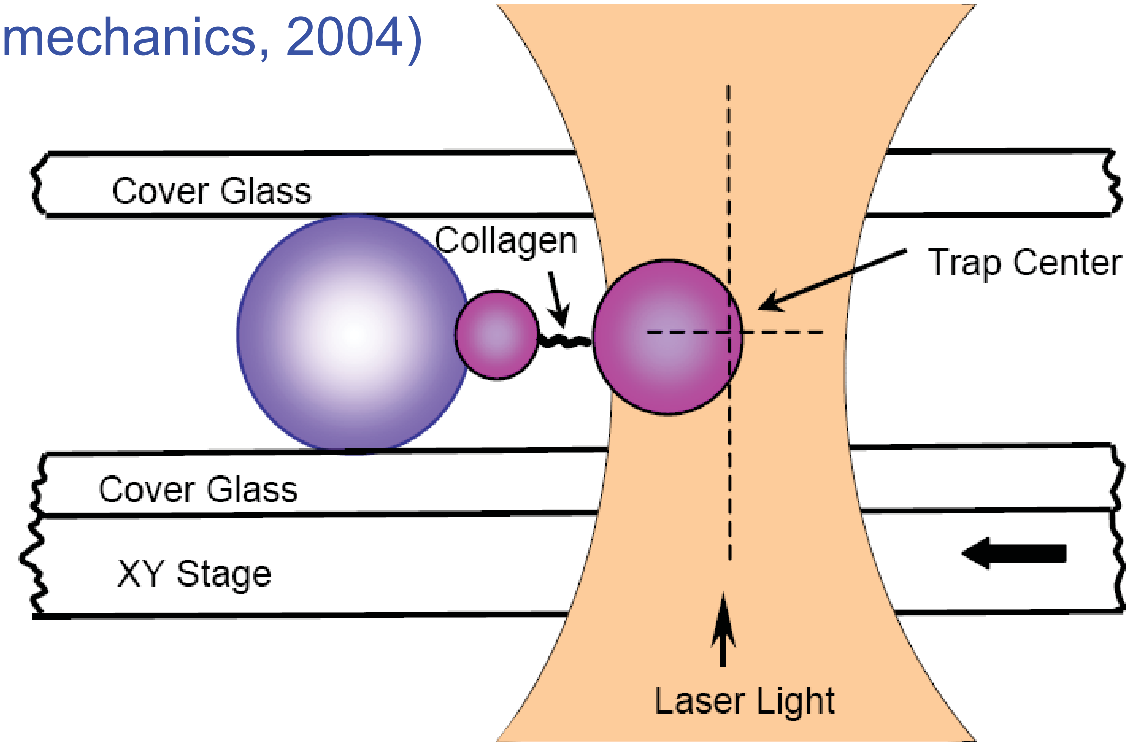
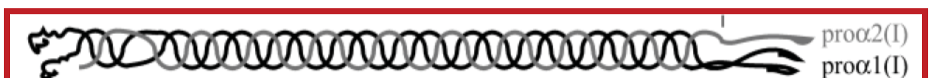


Fig. 1. Stretching a single collagen molecule using optical tweezers. One end of collagen is attached to the bead trapped by optical tweezers. The other end is attached to the small bead fixed with a big bead, which is clamped with two cover glass. Before collagen is stretched, the small bead and the trapped bead are touched. The

Source: Sun, Yu-Long et al. "Stretching Type II Collagen with Optical Tweezers." *Journal of Biomechanics* 37, no. 11 (2004): 1665-9.



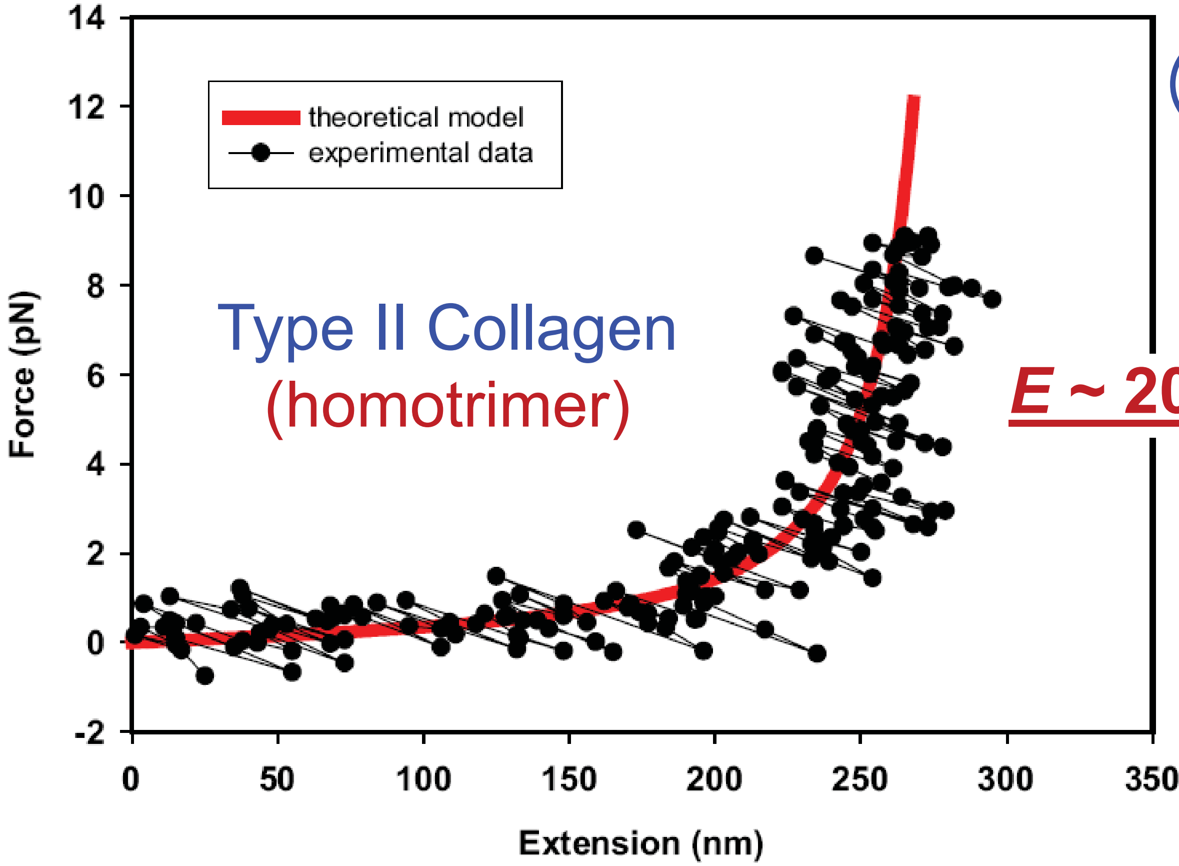
~300 nm

biotin -- streptavidin

Magnetic Tweezer

Image removed due to copyright restrictions.

Worm-like chain model was fit to the data: L_p = 11.2 ± 8.4 nm



$EI = k_B T L_p$

***E* ~ 200-300 MPa**

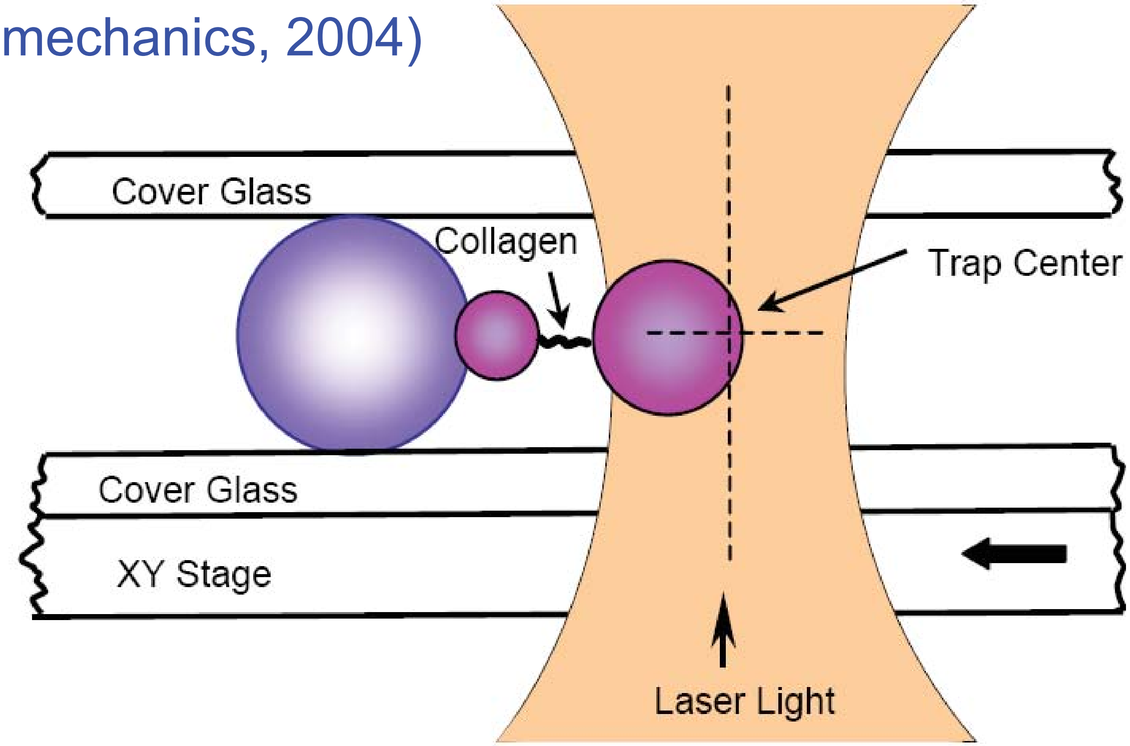
Fig. 2. The force-extension curve for stretching a single type II collagen molecule. The data were fitted to Marko–Siggia entropic elasticity model. The molecule length and persistence length of this sample is 300 and 7.6 nm, respectively.

(Sun+, J Biomechanics, 2004)

Stretching type II collagen with optical tweezers

Yu-Long Sun[a], Zong-Ping Luo[b], Andrzej Fertala[c], Kai-Nan An[a]

(J Biomechanics, 2004)



Source: Sun, Yu-Long et al. "Stretching Type II Collagen with Optical Tweezers." *Journal of Biomechanics* 37, no. 11 (2004): 1665-9.

$$F = \left(\frac{k_B T}{L_p}\right)\left[\frac{1}{4(1 - \boldsymbol{R}/L_c)^2} - \frac{1}{4} + \frac{\boldsymbol{R}}{L_c}\right]$$

Worm-like chain model

Worm-like chain model

- Polymer is treated as a flexible rope rather than a collection of freely-jointed rigid rods
- **Bending stiffness accounted for directly**
- Enthalpic contributions important

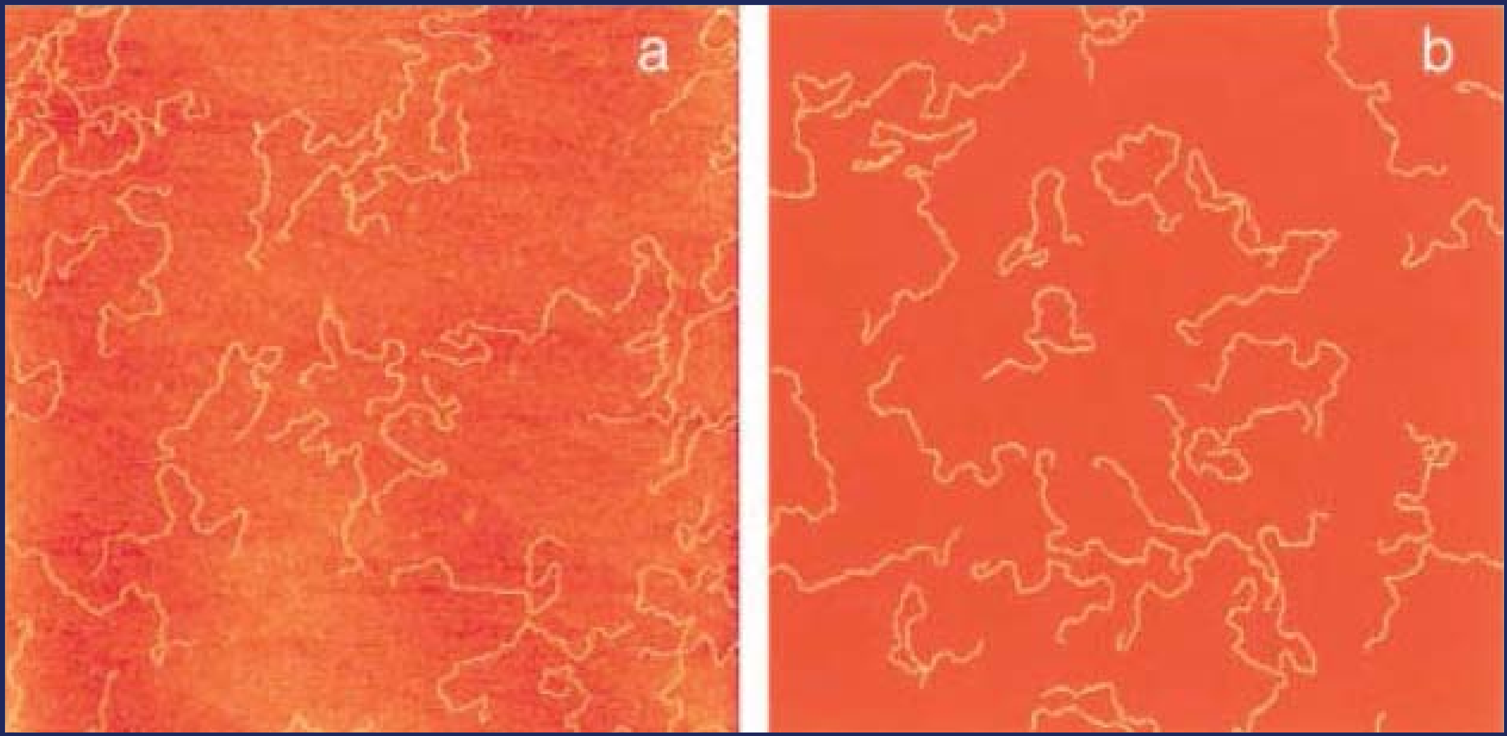
$$F_{WLC} = \frac{kT}{L_p}\left[\frac{1}{4}\left(1 - \frac{R}{L_c}\right)^{-2} - \frac{1}{4} + \frac{R}{L_c}\right]$$

…where L_p = persistence length

L_c = contour length

R = end-to-end distance

Persistence Length L_p : the length scale over which a polymer remains roughly straight (~ 50 nm for DNA)

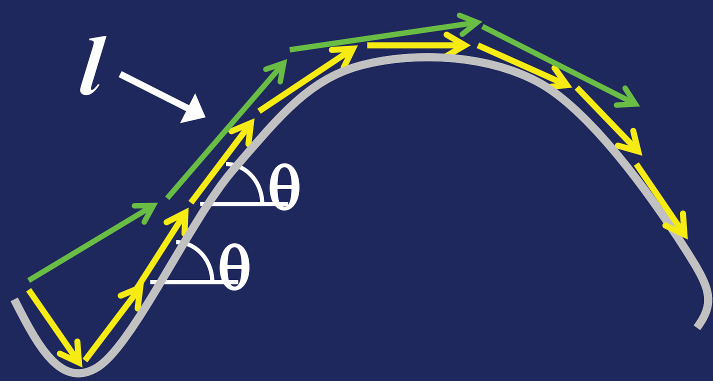


4 µm

AFM image of DNA on 2D mica surface

Simulated worm-like Chain in 2D

Estimation of Persistence Length, L_p



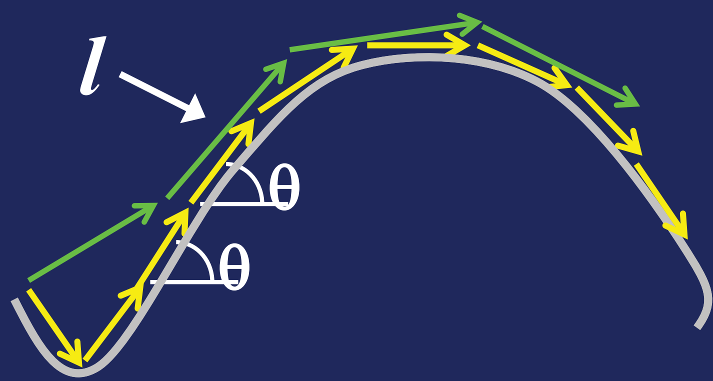
L_p is calculated from WLC model:

$$< \theta^2(l) >_{2D} = \frac{l}{L_p}$$

Equilibration of a molecule on a 2D surface is manifested by a Gaussian distribution of the angles formed by the projected vectors, ***l***

(Rivetti, Bustamante, J Molec Biol, 1996: estimated L_p of DNA from AFM images on 2D mica surfaces)

Estimation of Persistence Length, L_p



$L_p \sim L_c$ stiff needle-like molecule

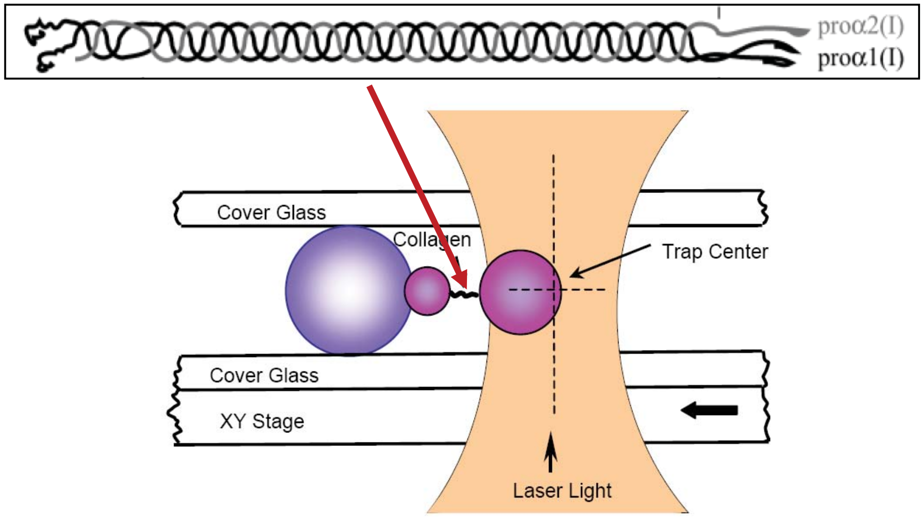
$L_p << L_c$ "floppy" molecule

$$L_p = \frac{E\,I}{k_b T}$$

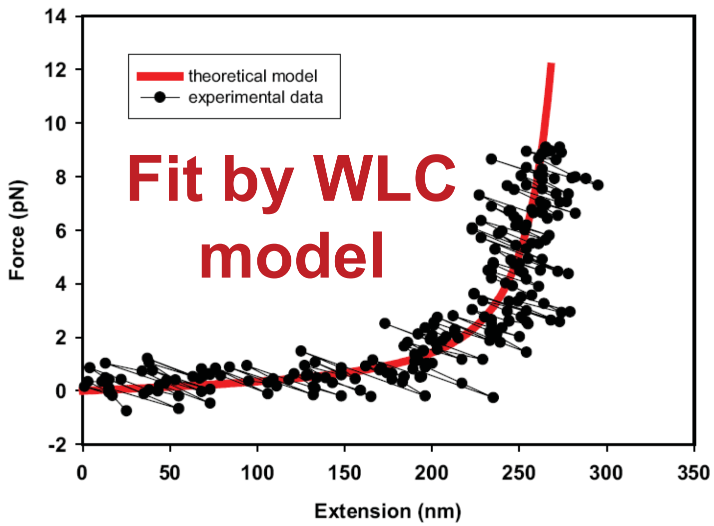
E = *"Young's modulus"*
I = *area moment of inertia*

Rivetti, Bustamante, *J Molec Biol*, 1996: estimated L_p of DNA from AFM images on 2D mica surfaces)

Pro-collagen molecule →



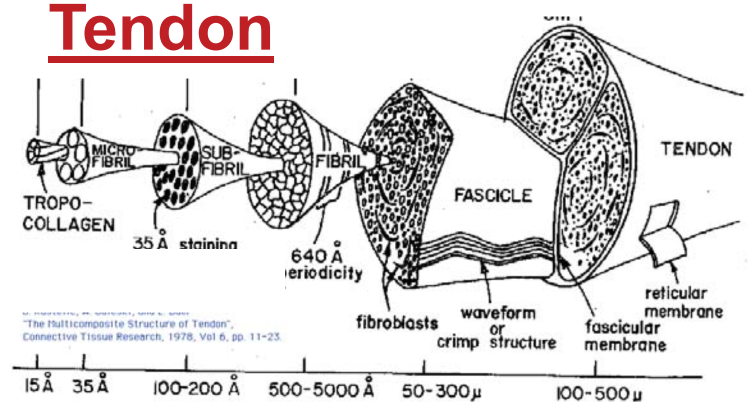
Force - extension



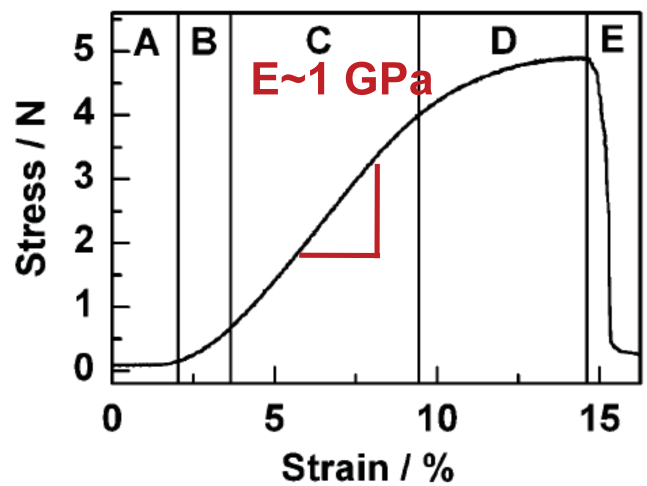
(Sun+, J Biomechanics, 2004)

Source: Sun, Yu-Long, et al. "Stretching Type II Collagen with Optical Tweezers." *Journal of Biomechanics* 37, no. 11 (2004): 1665-9.

Tendon



Source: Kastelic, J., A. Galeski, et al. "The Multicomposite Structure of Tendon." *Connective Tissue Research* 6, no. 1 (1978): 11-23.

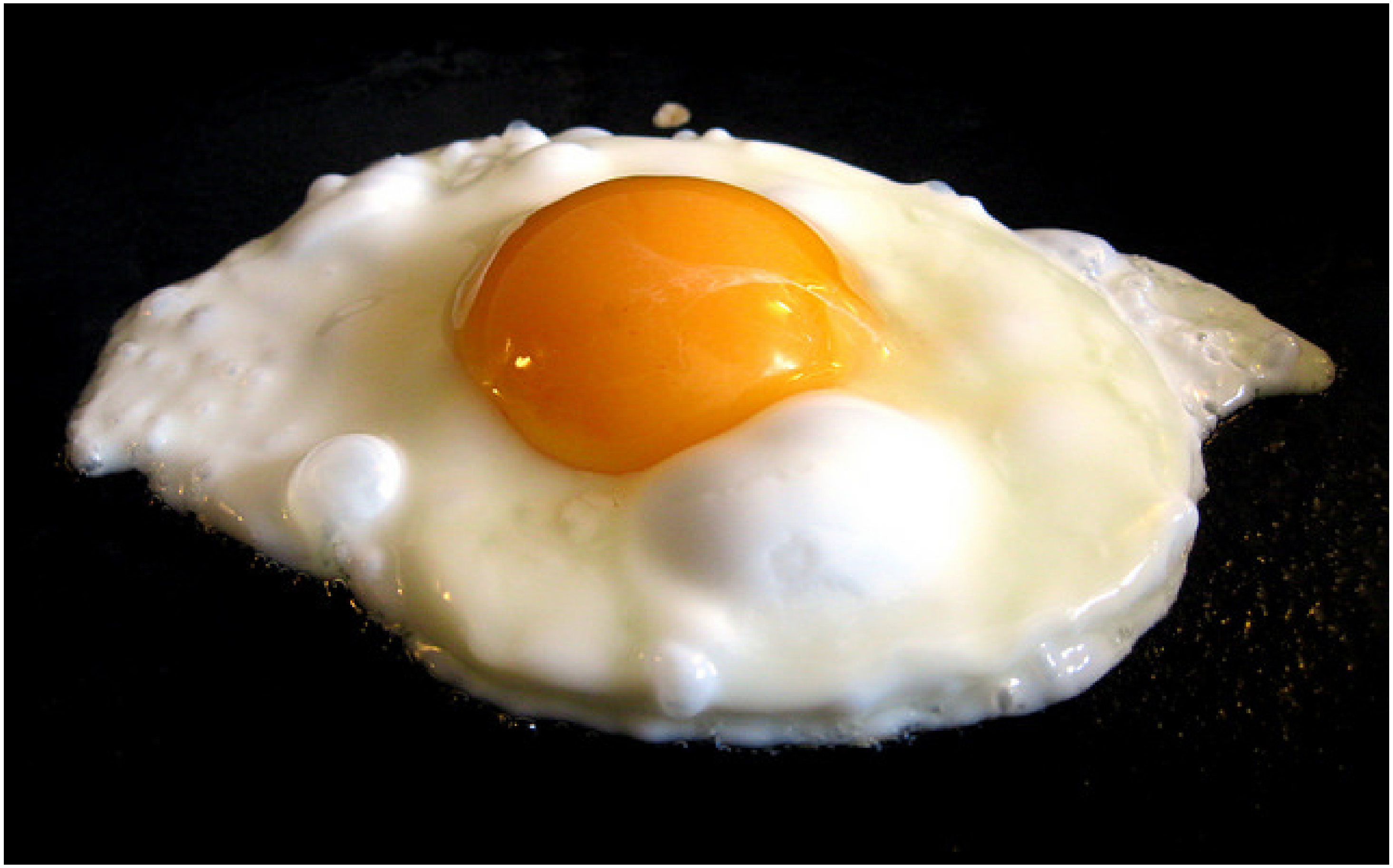


Source: Gutsmann, Thomas, et al. "Force Spectroscopy of Collagen Fibers to Investigate their Mechanical Properties and Structural Organization." *Biophysical Journal* 86, no. 5 (2004): 3186-93.

Stress vs strain curve of a rat tail tendon: (A-B) Toe - heel region, **(C) linear region**, (D) plateau, (E) rupture of the tendon.

(Gutsmann+, Biophys J, 2004)

Collagen is a Protein: What about Thermal Denaturation!?



Courtesy of Matthew Murdoch.

Collagen Molecules in Solution

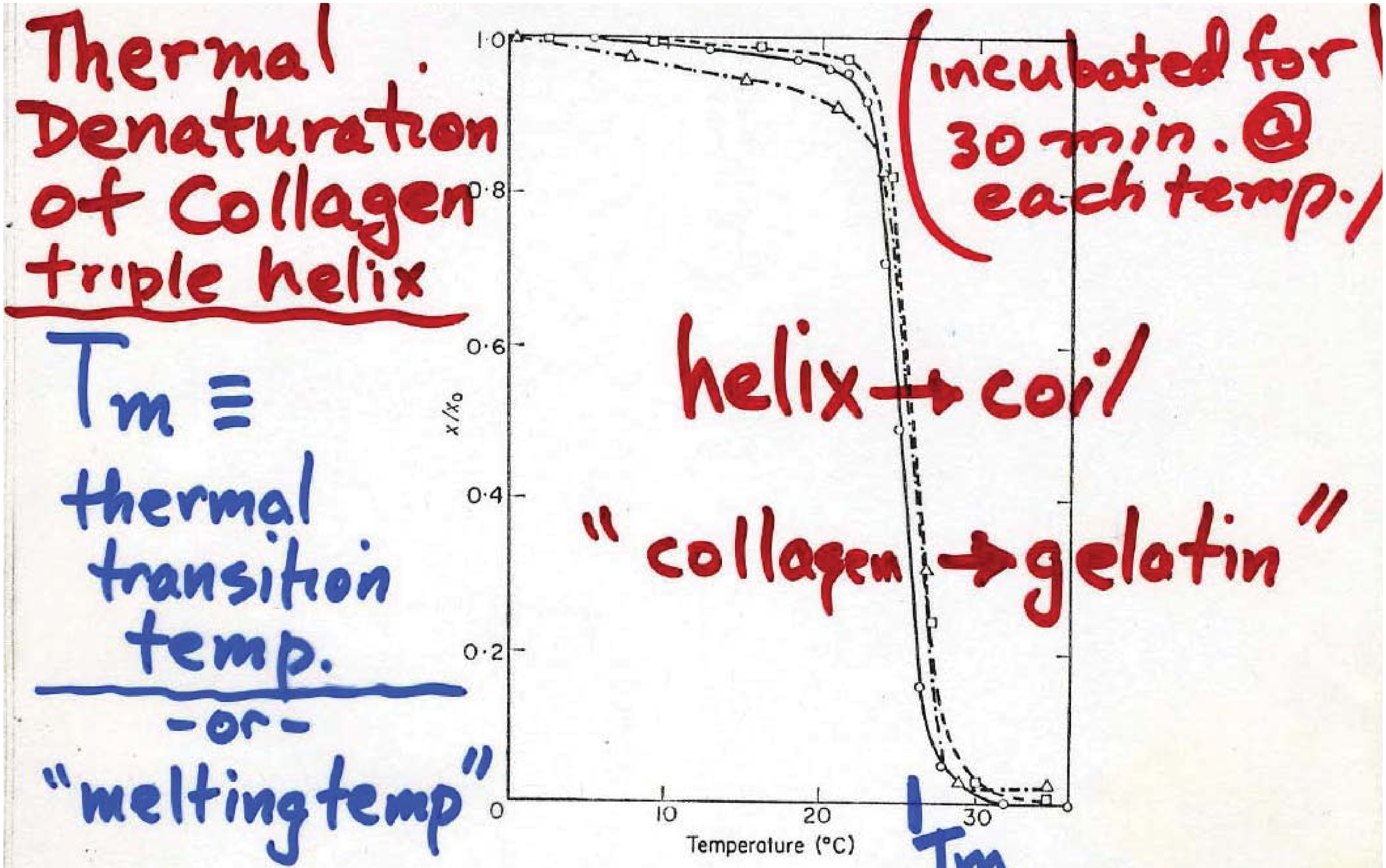


FIG. 6. Melting curves for ichthyocol collagen in 0·5 M $CaCl_2$, pH 7, protein concentration = 0·7 mg/ml; ○, optical rotation, equilibrium curve; □, optical rotation, "30 min" curve (incubated for 30 min at each temperature prior to reading); Δ, viscosity, "30 min" curve. $x/x_0 = ([\alpha]_T - [\alpha]_{40°})/([\alpha]_{5°} - [\alpha]_{40°})$ or $\eta_{sp,T}/\eta_{sp,5°}$. Reproduced with permission from von Hippel and Wong (1963a).

Source: von Hippel, Peter H., and Kwok-Ying Wong. "The Collagen [UNK] Gelatin Phase Transition. I. Further Studies of the Effects of Solvent Environment and Polypeptide Chain Composition*." *Biochemistry* 2, no. 6 (1963): 1387-98.

You Are JELLO ?

Thermal Denaturation of Collagen triple helix

Helix → Coil ☹

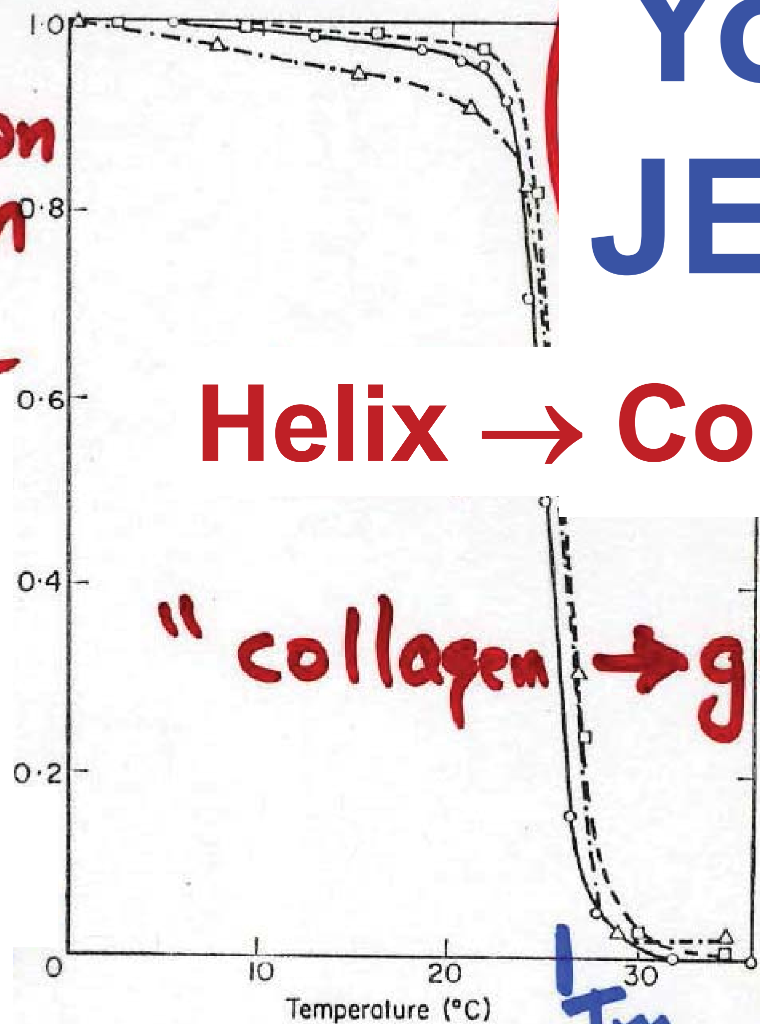


FIG. 6. Melting curves for ichthyocol collagen in 0·5 M $CaCl_2$, pH 7, protein concentration = 0·7 mg/ml; ○, optical rotation, equilibrium curve; □, optical rotation, "30 min" curve (incubated for 30 min at each temperature prior to reading); Δ, viscosity, "30 min" curve. $x/x_0 = ([\alpha]_T - [\alpha]_{40°})/([\alpha]_{5°} - [\alpha]_{40°})$ or $\eta_{sp,T}/\eta_{sp,5°}$. Reproduced with permission from von Hippel and Wong (1963a).

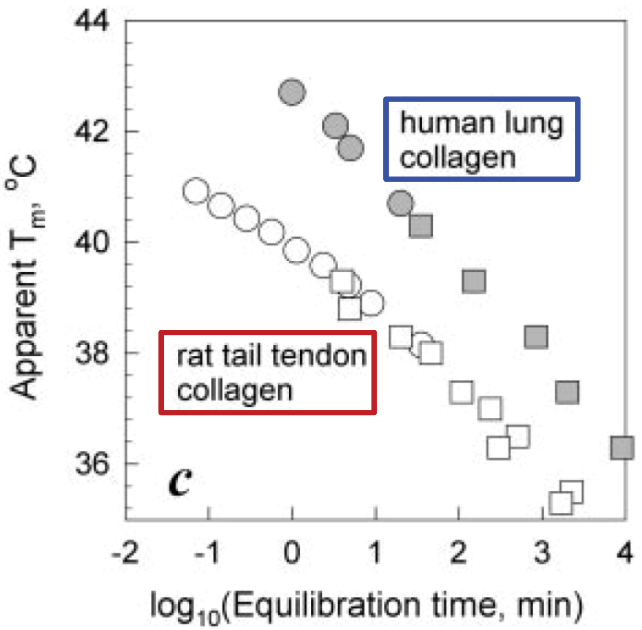
Source: von Hippel, Peter H., and Kwok-Ying Wong. "The Collagen [UNK] Gelatin Phase Transition. I. Further Studies of the Effects of Solvent Environment and Polypeptide Chain Composition*." *Biochemistry* 2, no. 6 (1963): 1387-98.

Type I collagen is thermally unstable at body temperature

E. Leikina, M. V. Mertts, N. Kuznetsova, and S. Leikin*

National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892

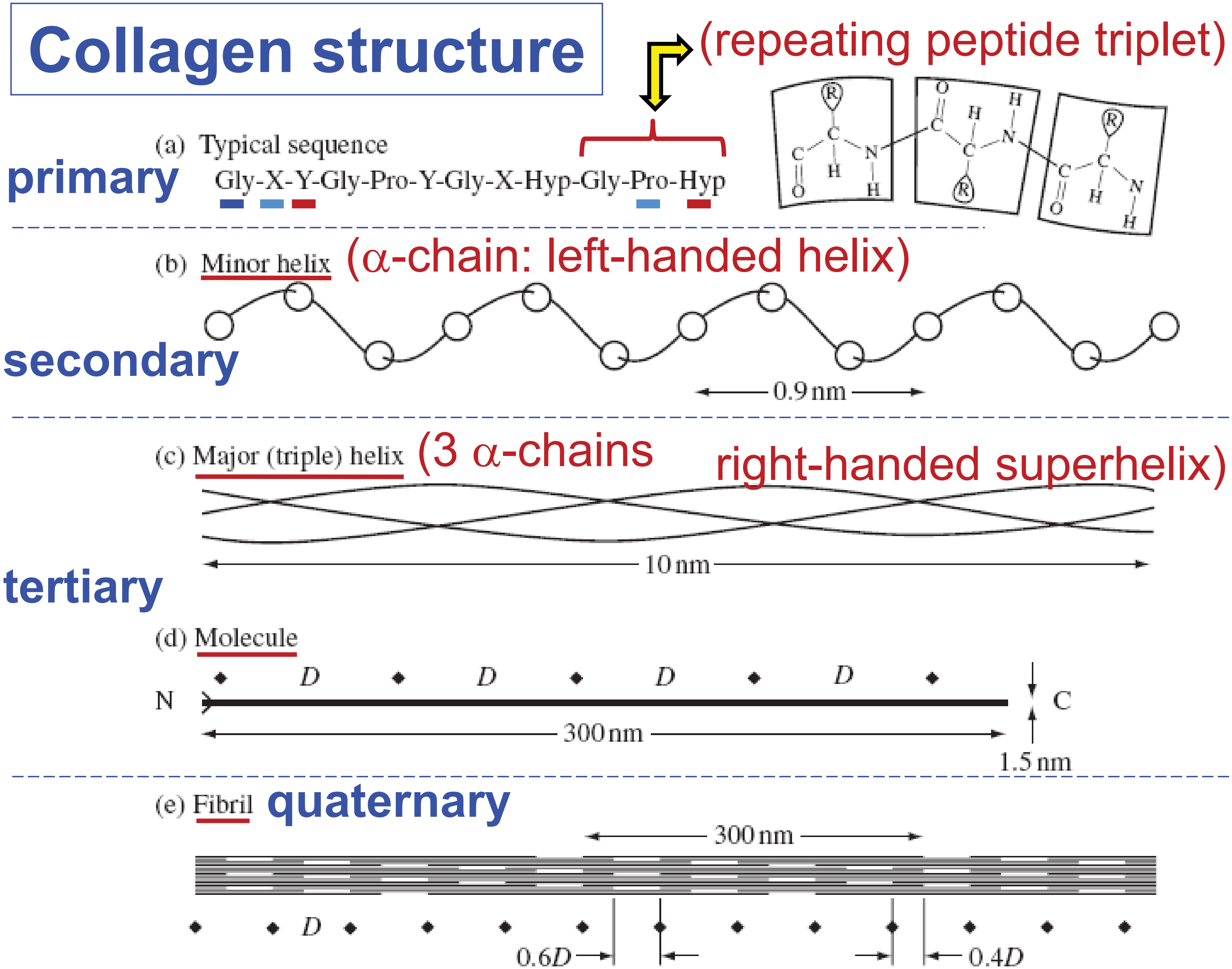
(PNAS, 2002)



The Equilibrium State of Monomeric Type I Collagen at Body Temperature Is a Random Coil Rather than Helix. It follows from Fig. 2*c* that at 37°C type I collagen from human lungs converts to random coils within 2 to 3 days. Type I collagen from rat-tail tendon is even less stable. In contrast to the existing consensus, both proteins undoubtedly melt even several degrees below body temperature and their thermodynamically favored conformation at body temperature is a random coil. (CD spectra similar to

Source: Leikina, E. et al. "Type I Collagen is Thermally Unstable at Body Temperature." *Proceedings of the National Academy of Sciences* 99, no. 3 (2002): 1314-8.

Collagen structure



(Textbook, page 16) (molecules quarter staggered; X-links)

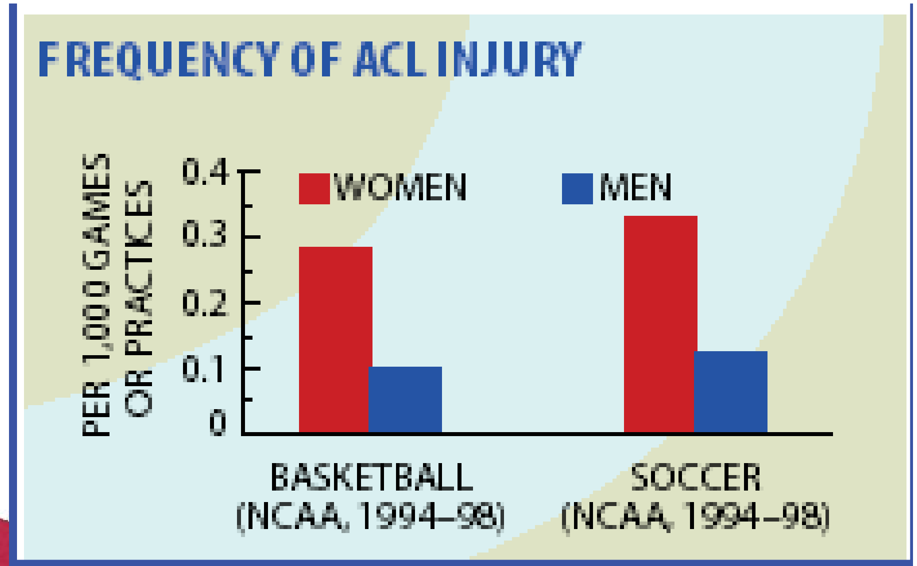
Demo:

<u>Mechanochemistry</u> and <u>Biomechanical Properties</u> at Molecular and Tissue Levels

ACL Tear: Molecular / Tissue-Level Injuries

(ACL = anterior cruciate ligament)

- 82% of Women with a torn ACL show the beginning stages of OA 12 years after injury (by age 31 (ave)) (Lohmander, 2004)

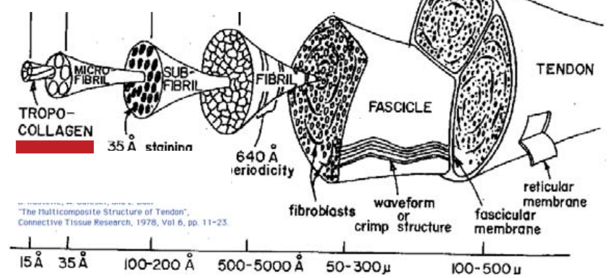


Mechanical and Biological Consequences….

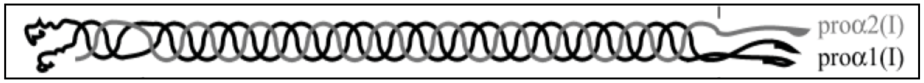
Scientific American, 2000

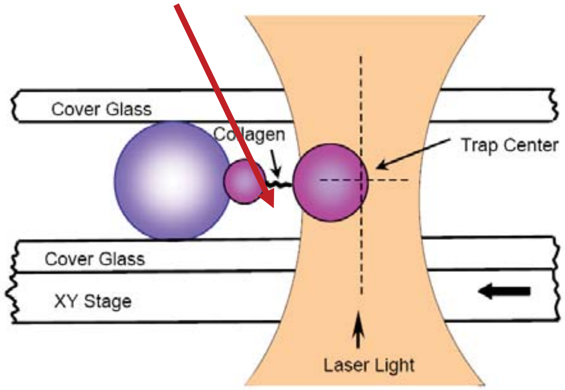
Tropo-collagen molecule →

Tendon



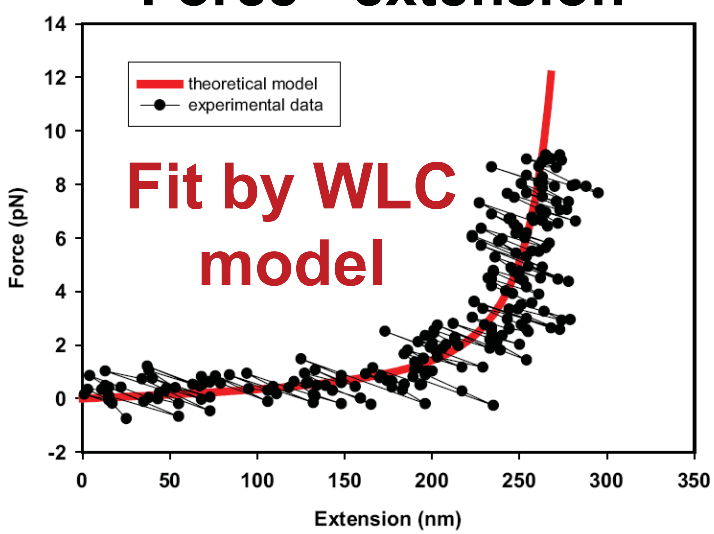
Source: Kastelic, J., A. Galeski, et al. "The Multicomposite Structure of Tendon." *Connective Tissue Research* 6, no. 1 (1978): 11-23.





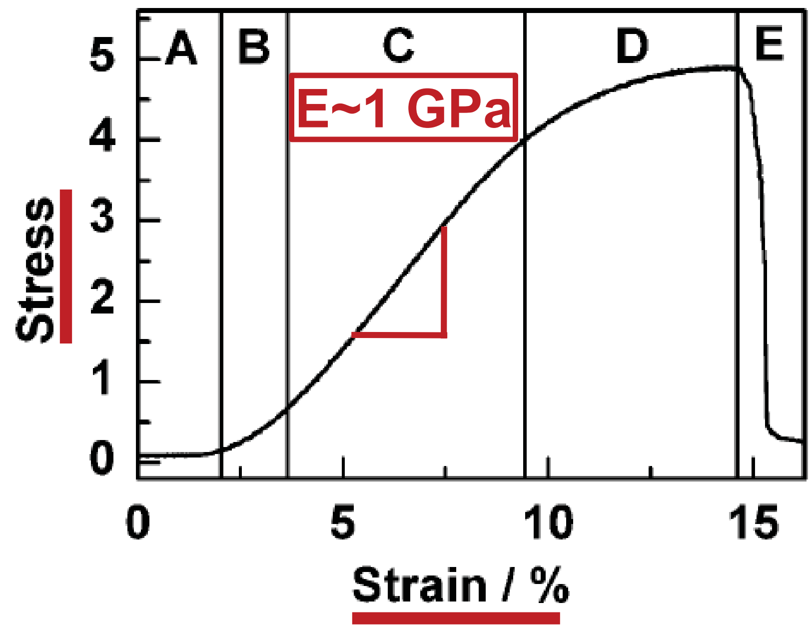
E~ 200-300 MPa

Force - extension



(Sun+, J Biomechanics, 2004)

Source: Sun, Yu-Long et al. "Stretching Type II Collagen with Optical Tweezers." *Journal of Biomechanics* 37, no. 11 (2004): 1665-9.

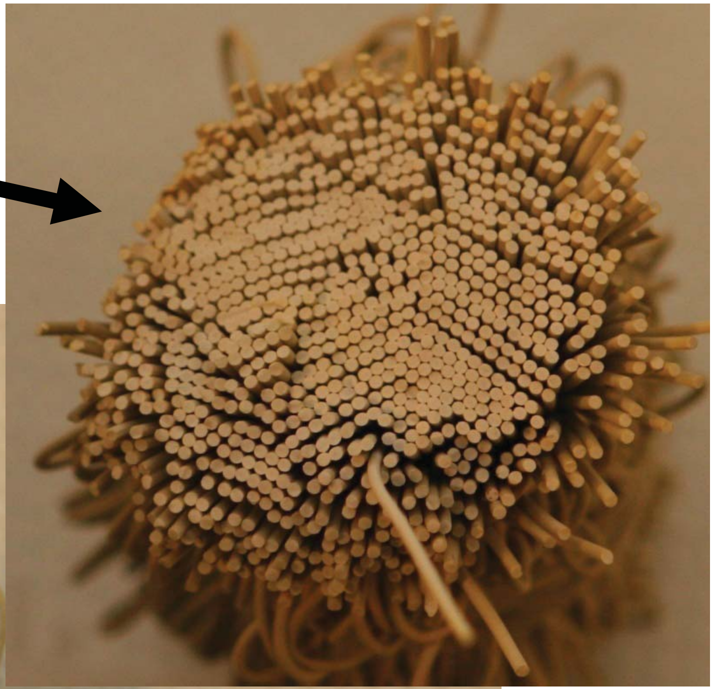


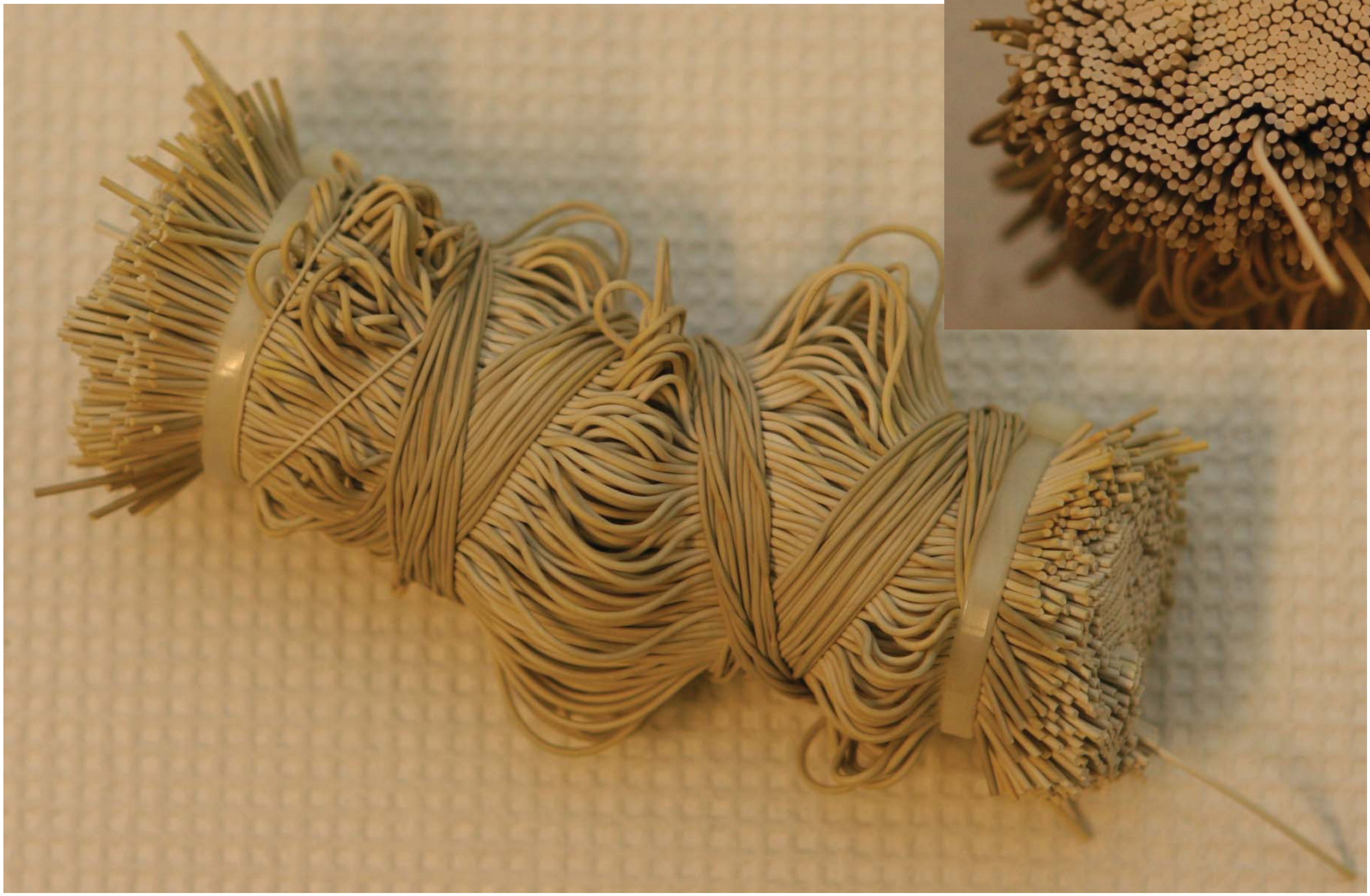
Source: Gutsmann, Thomas, et al. "Force Spectroscopy of Collagen Fibers to Investigate their Mechanical Properties and Structural Organization." *Biophysical Journal* 86, no. 5 (2004): 3186-93.

Stress vs strain curve of a rat tail tendon:
(A-B) "Toe" region,
(C) linear region, (D) plateau,
(E) rupture of the tendon.

(Gutsmann+, Biophys J, 2004)

A structure with No intra- or inter-molecular X-links…..





The Original Bungy Jump: Kuwarau River Gorge, Queenstown, New Zealand

(from AJ Hackett website)

Risks - Extreme Bungy Jumping:

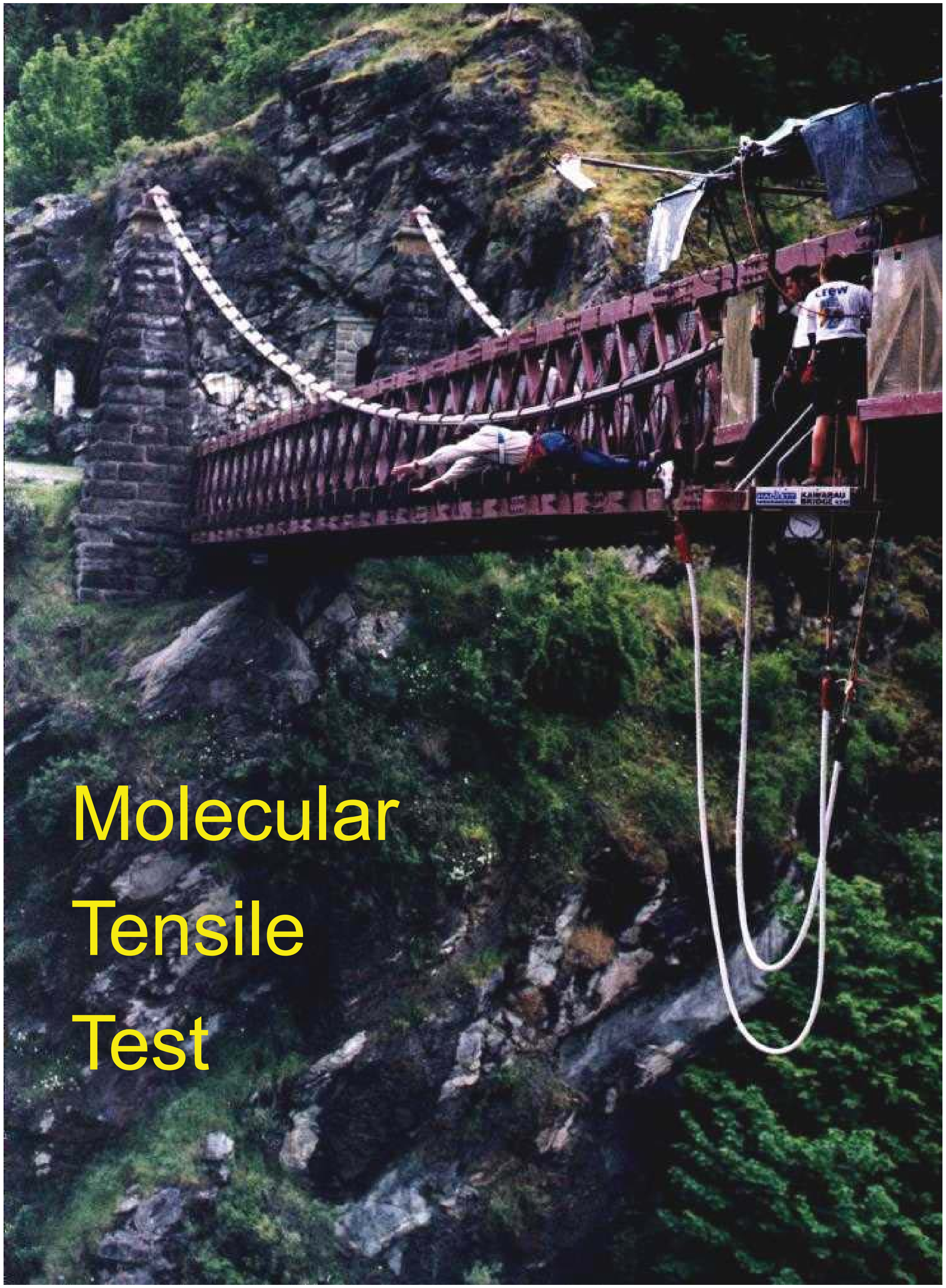
- **If the bungy cord collapses, one can be injured.**
- Other injuries include eye trauma, back injury, rope burn, whiplash, bruises, dislocations…..

All-Rubber Bungy Cord:

- Developed in New Zealand, all-rubber cords are collection of more than 1000 individual strands of rubber tied together …….

Kuwarau River Gorge, Queenstown, New Zealand

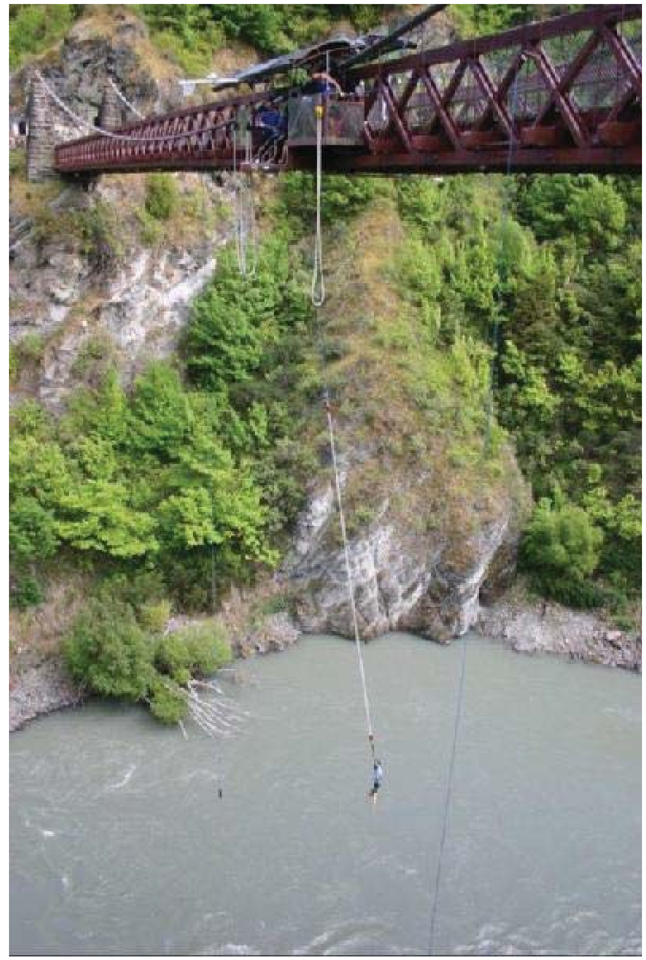
(the original bungy jump)



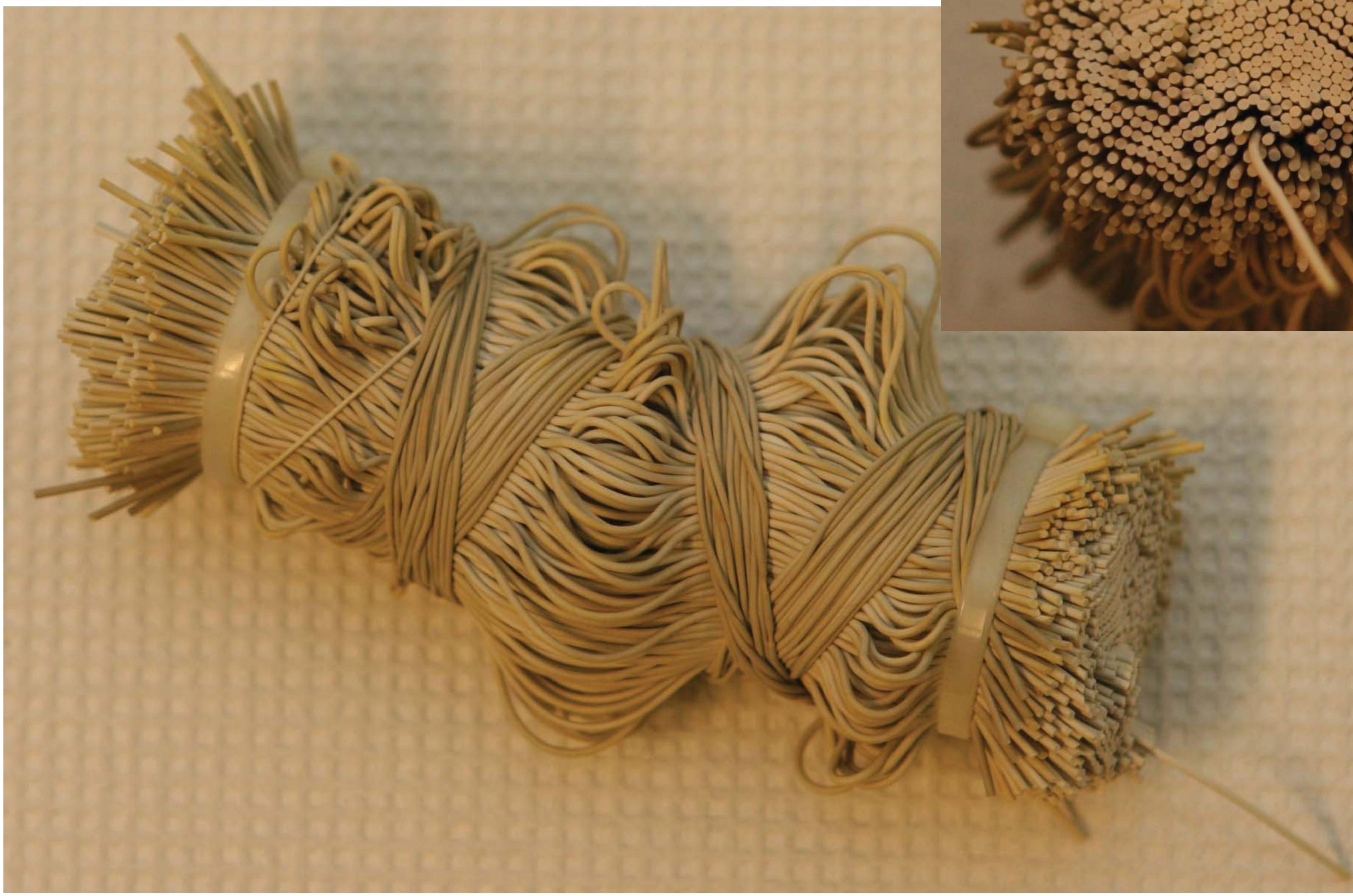
The Original Bungy Jump:

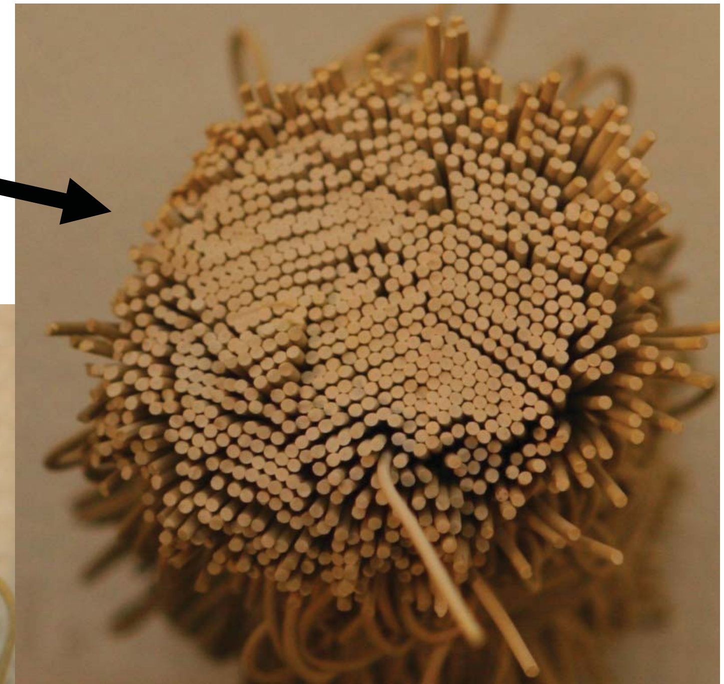
over the

Kuwarau River Gorge, Queenstown, New Zealand

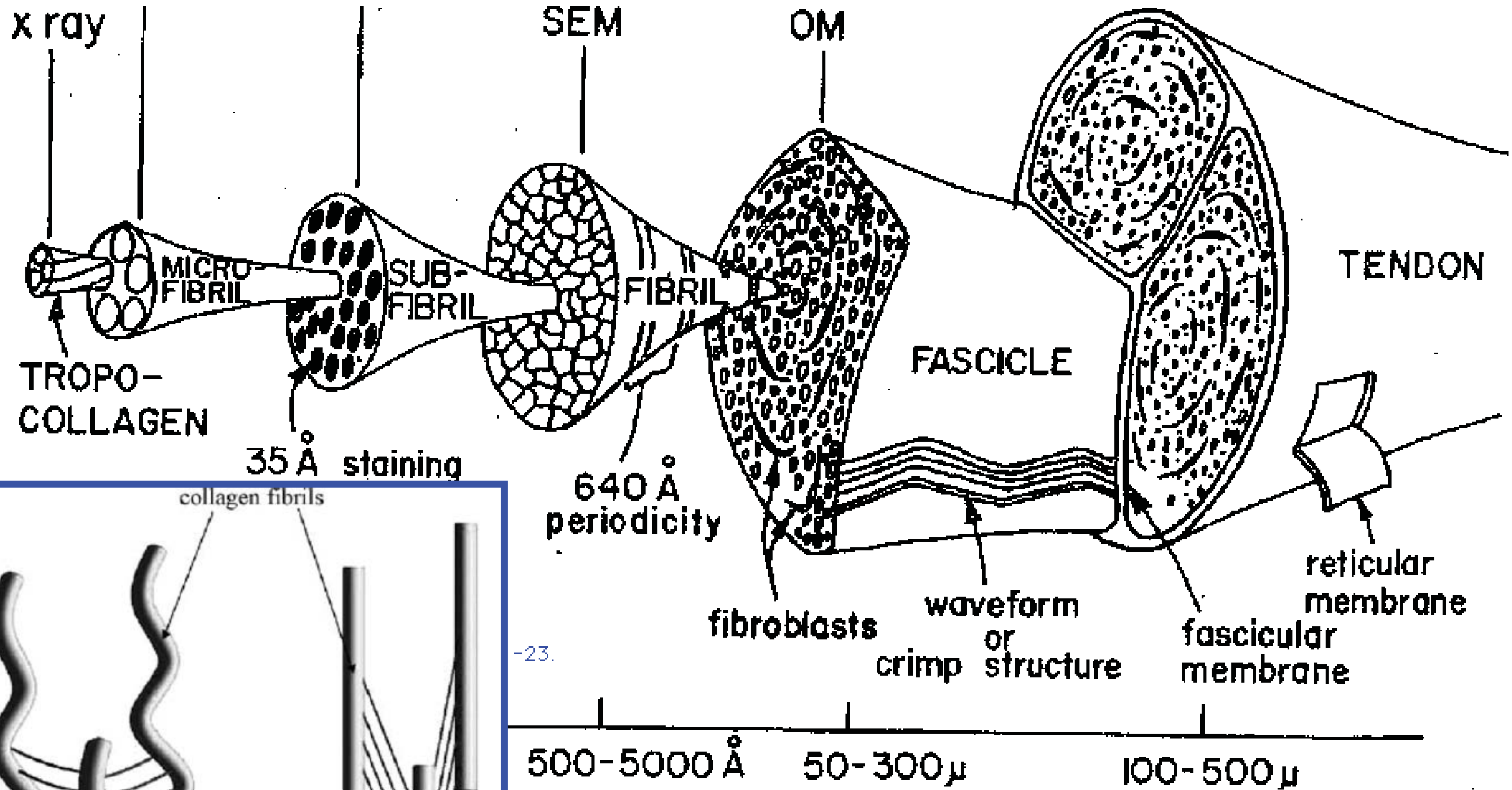


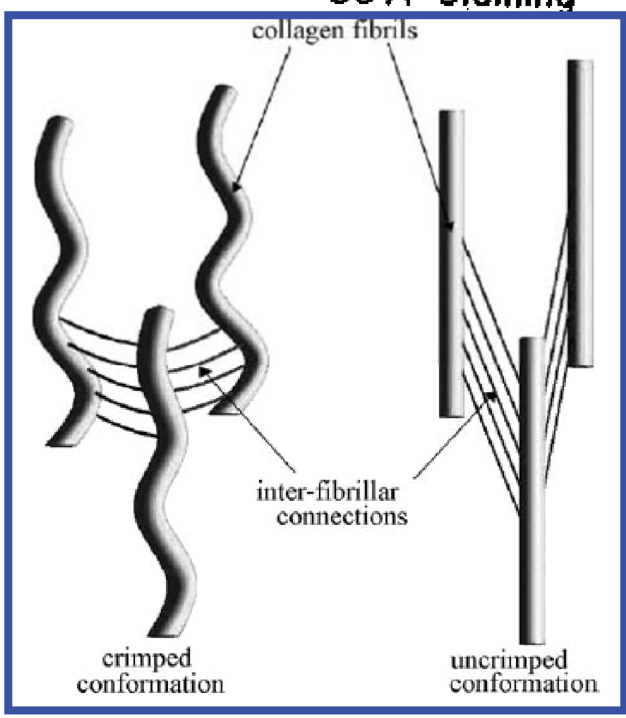
No intra- or inter-molecular crosslinks…..
what were they thinking?





Tendon Structure





Source: Kastelic, J., A. Galeski, et al. "The Multicomposite Structure of Tendon." *Connective Tissue Research* 6, no. 1 (1978): 11-23.

Lots of Intra-molecular and Inter-molecular Crosslinks!!