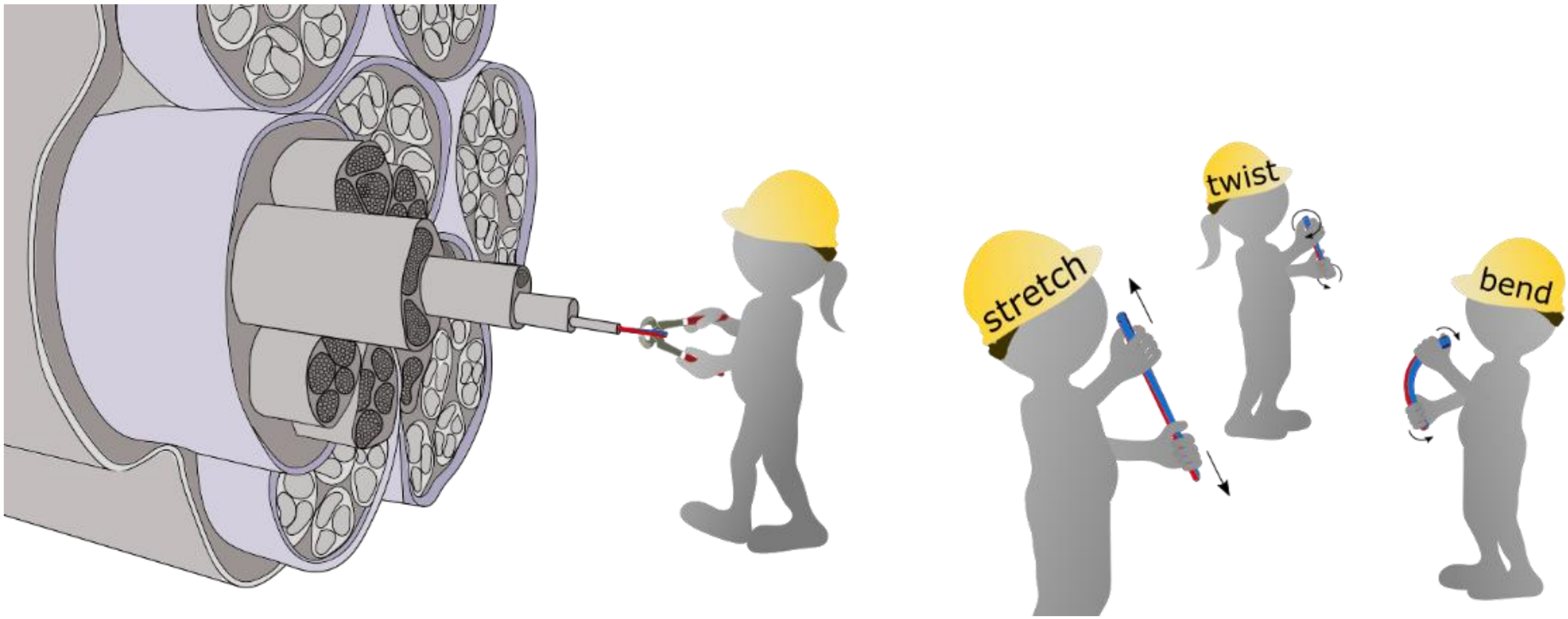
Building tools and models to characterize biological mechanics at the nanoscale



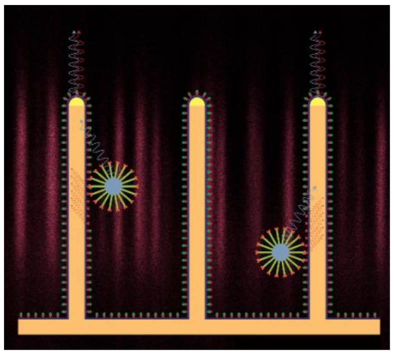
Nancy Forde

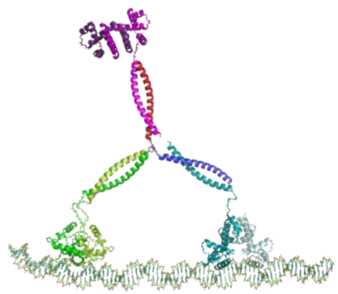
Department of Physics
also affiliated with Department of Chemistry and Department of Molecular Biology and Biochemistry
Simon Fraser University, Burnaby, BC, Canada

Photos credit: SFU Community Trust

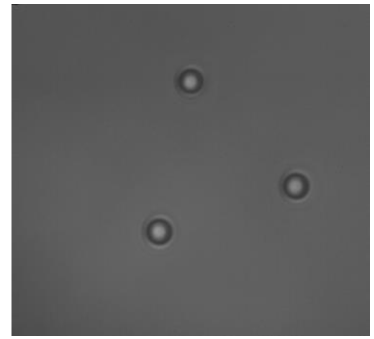
Research interests

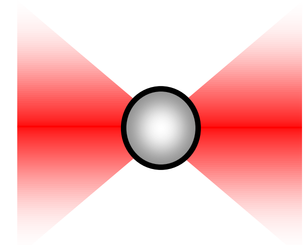
Synthetic molecular motors





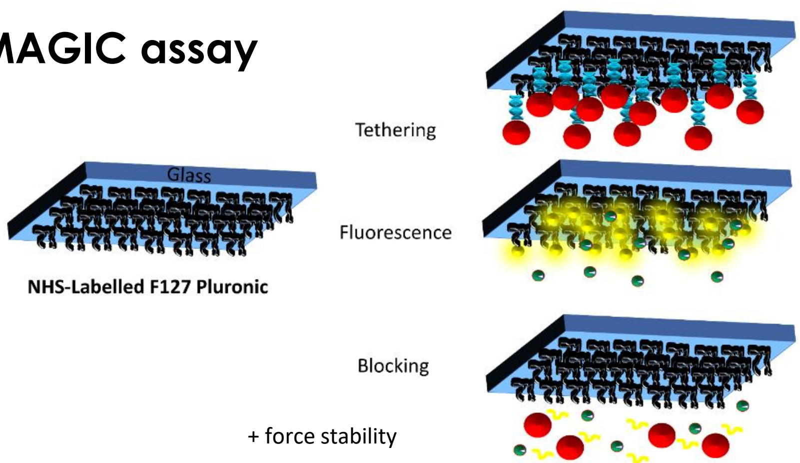
Optical manipulation



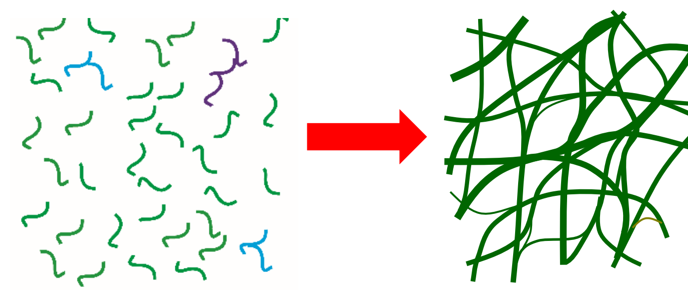


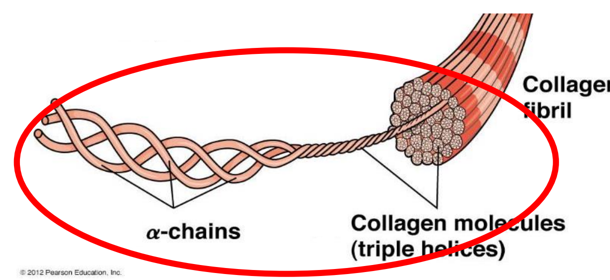
Surface chemistry

MAGIC assay

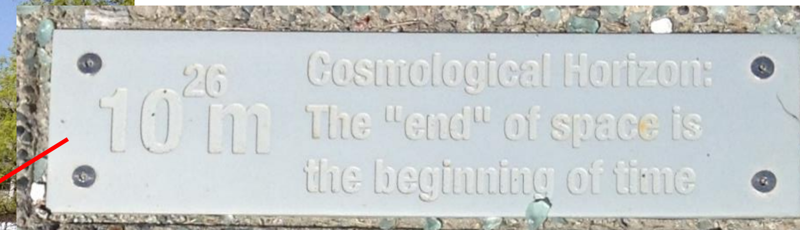


Hierarchical protein mechanics





Length scales in the universe



10^{26} m Cosmological Horizon:
The “end” of space is
the beginning of time

10^{-18} m Electroweak unification

From humans to quarks

Voyage into the world of atoms – from CERN

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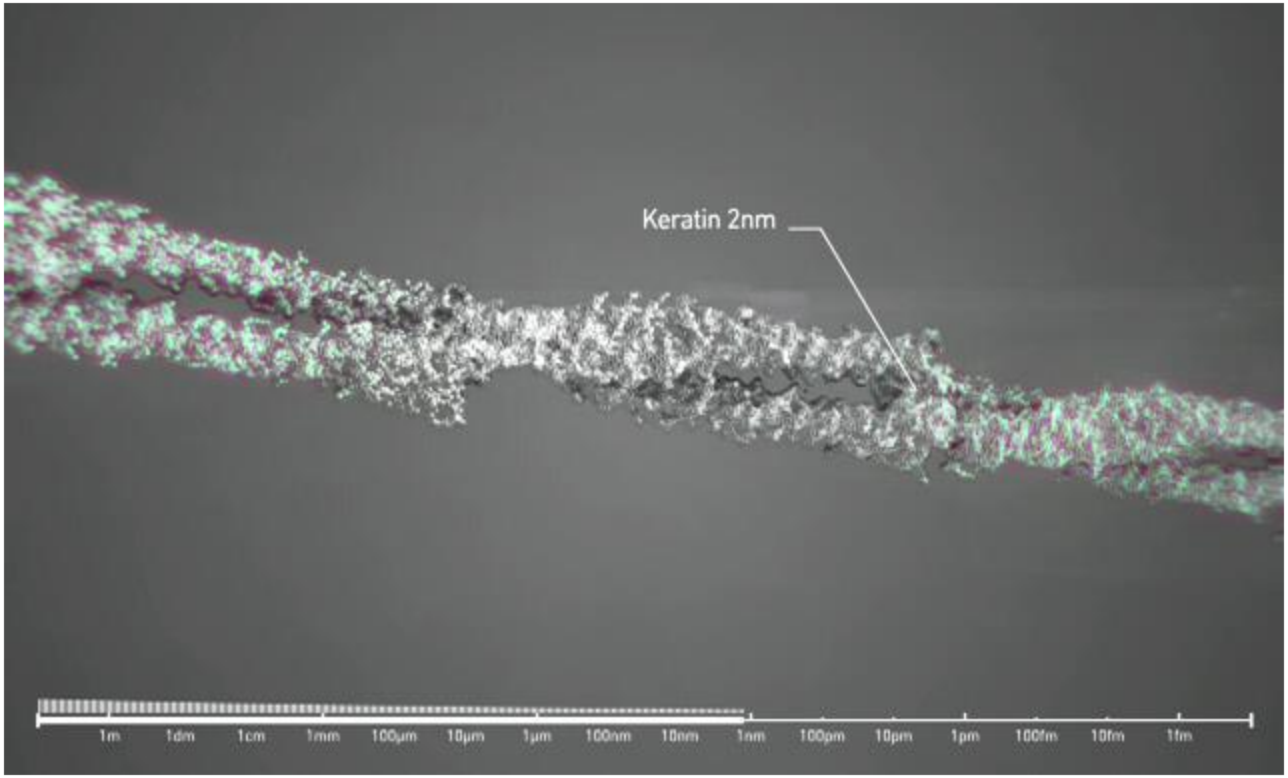
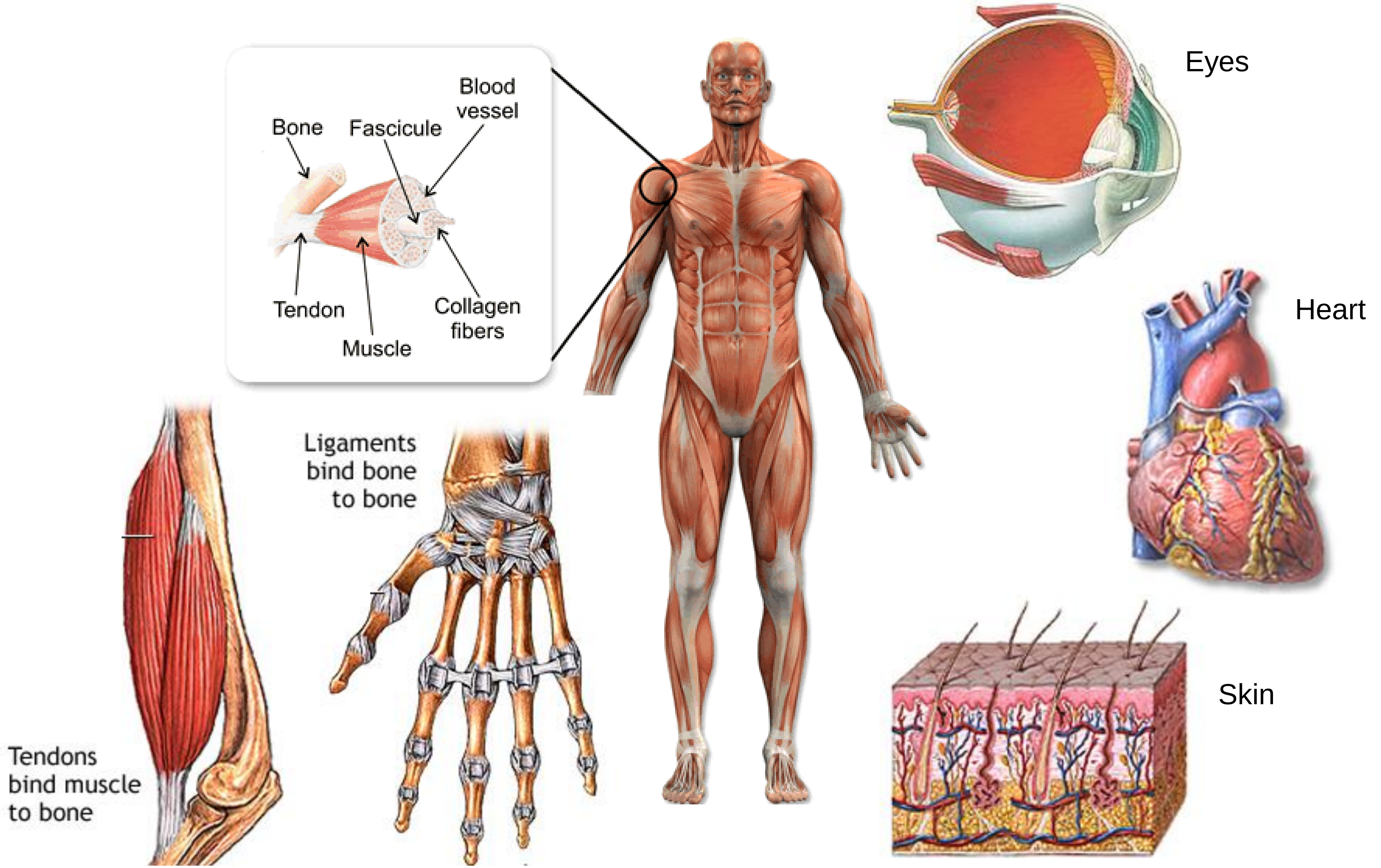
FORDE LAB

Proteins: nanometer length scale



Collagen is similar in size…

Collagen holds us together



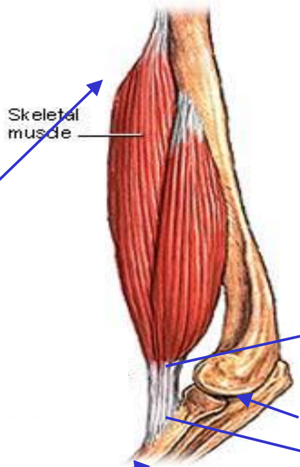
Images: http://vsearch.nlm.nih.gov/

Collagen: tensile material & template

Applications of collagen mechanics

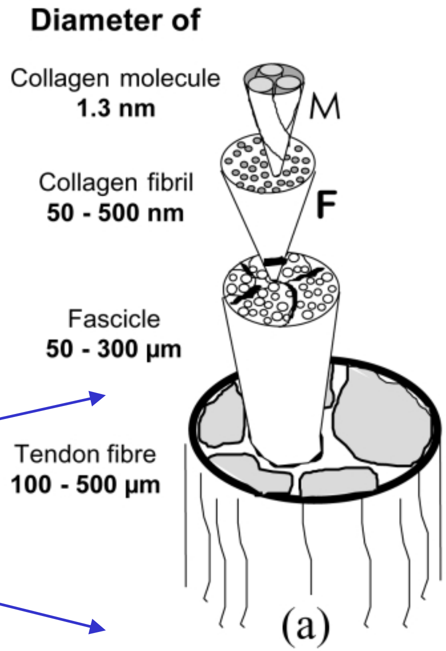
Prosthetics; (Bio)materials design; Tissue engineering

Terry Fox, former SFU student
(photo from wikipedia)



New York Times

Connective tissue function (disease & aging)

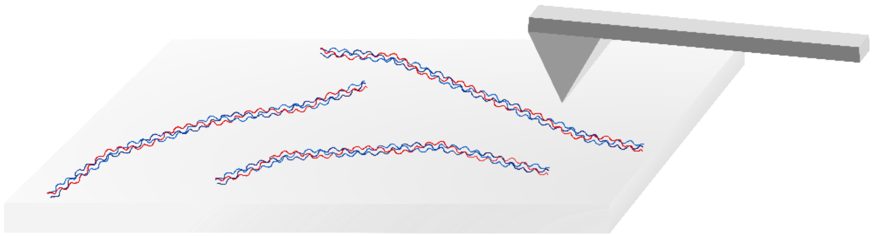


Fratzl, *Curr. Opin. Colloid Interface Sci.* **8,** 32 (2003)

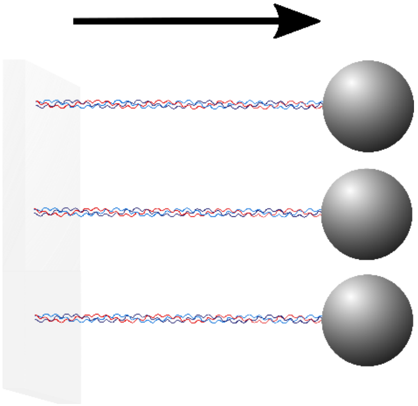
Influence of extracellular mechanics on cell fate (cancer, fetal development,…)

Mechanics of single collagen proteins

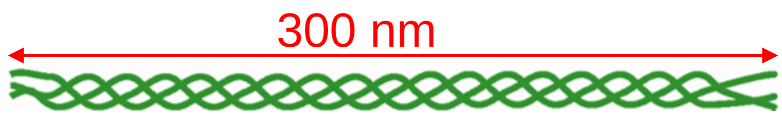
Flexibility



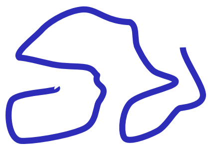
Force response

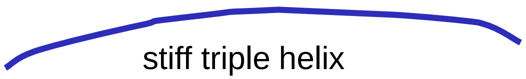


Flexibility of single collagen proteins



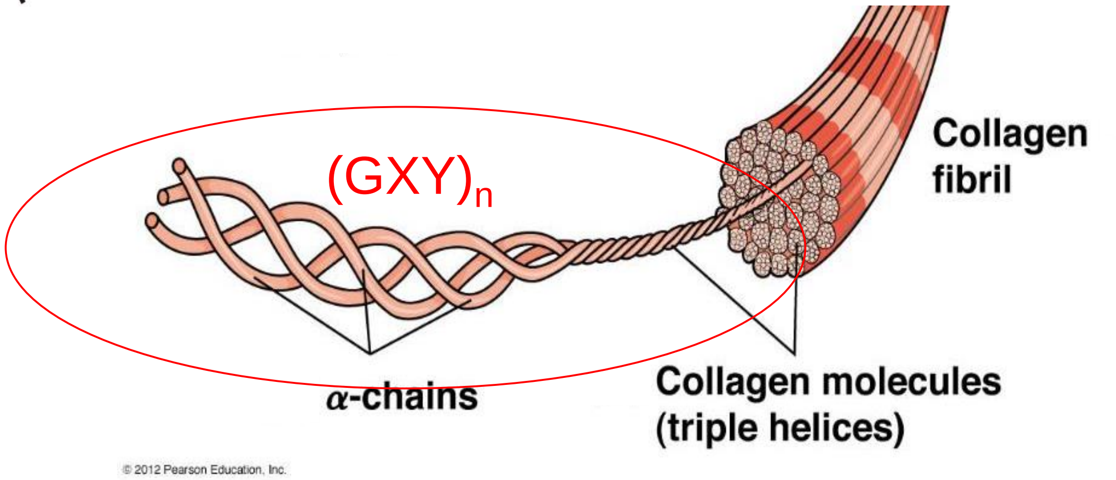
Method	Persistence length, p (nm)
Molecular dynamics	10-22
AFM imaging	12
Optical tweezers stretching	11-65
Electron microscopy	40-57
Coarse-grained molecular dynamics	51
Viscometry	130
AFM imaging	135-165
Rheology	161-167
Dynamic light scattering	160-165





Rezaei, Lyons & Forde, *Biophys. J.* 2018

Flexibility of single collagen proteins



Structural models of hierarchical materials

- Mechanics of fundamental building blocks

Flexibility relates to protein structure

- Denaturation of triple helix → flexibility
- Local denaturation / unwinding necessary for controlled degradation *in vivo*

Cell biology

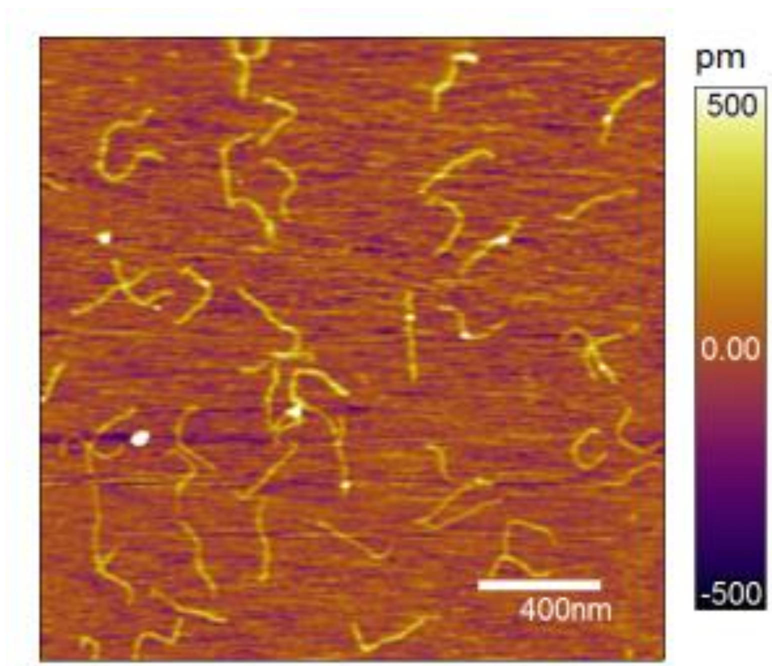
- How compact is collagen during secretion from cells?

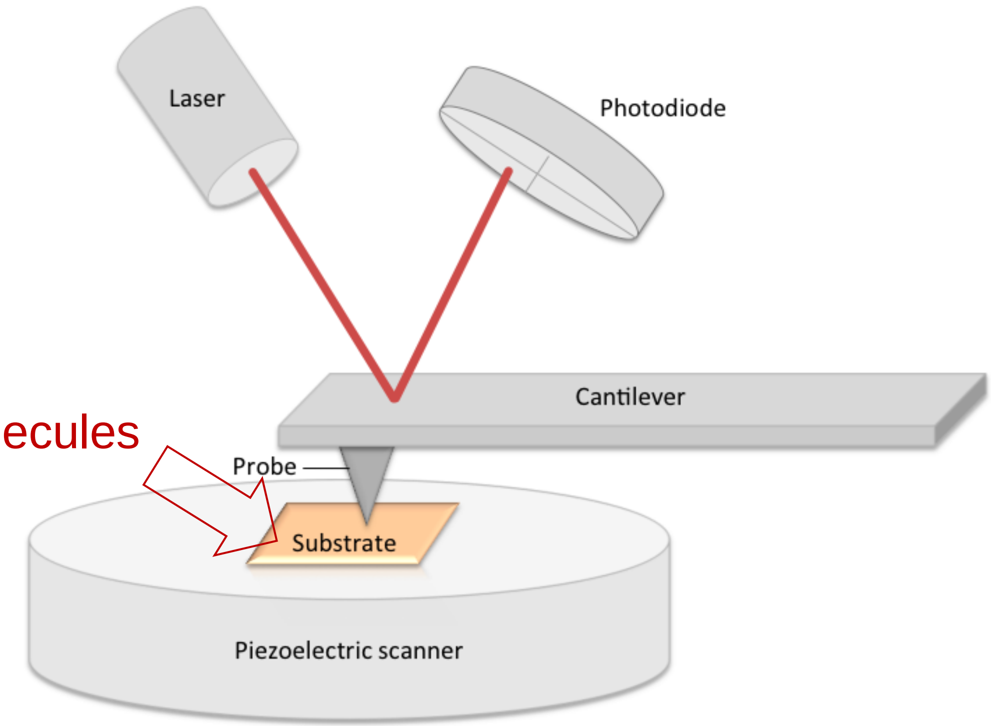
Polymer physics

- Limited examples of semiflexible chains; underdeveloped theory

Atomic Force Microscopy (AFM)

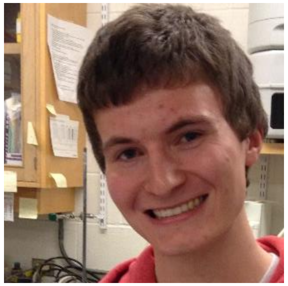
Collagen molecules





deposited, then dried prior to imaging


Naghmeh Rezaei


Aaron Lyons

Rezaei, Lyons & Forde, *Biophys. J.* 2018

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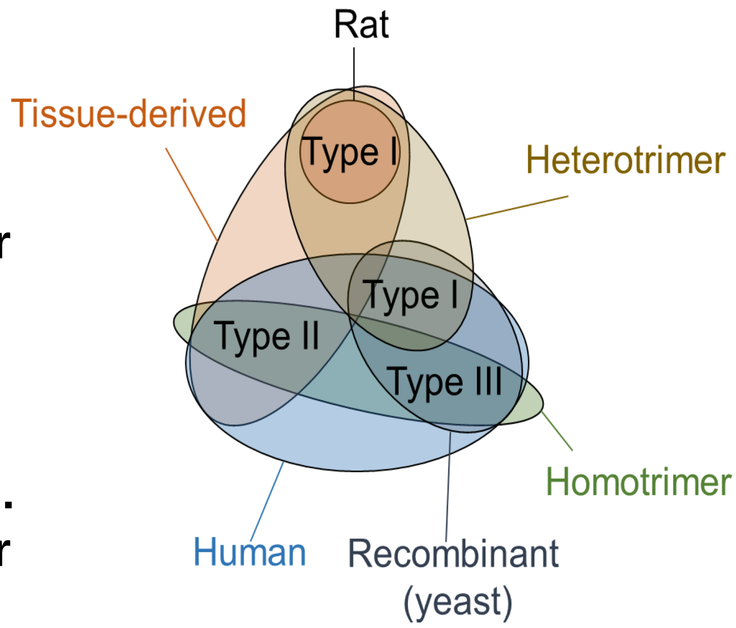
Fibrillar collagen types

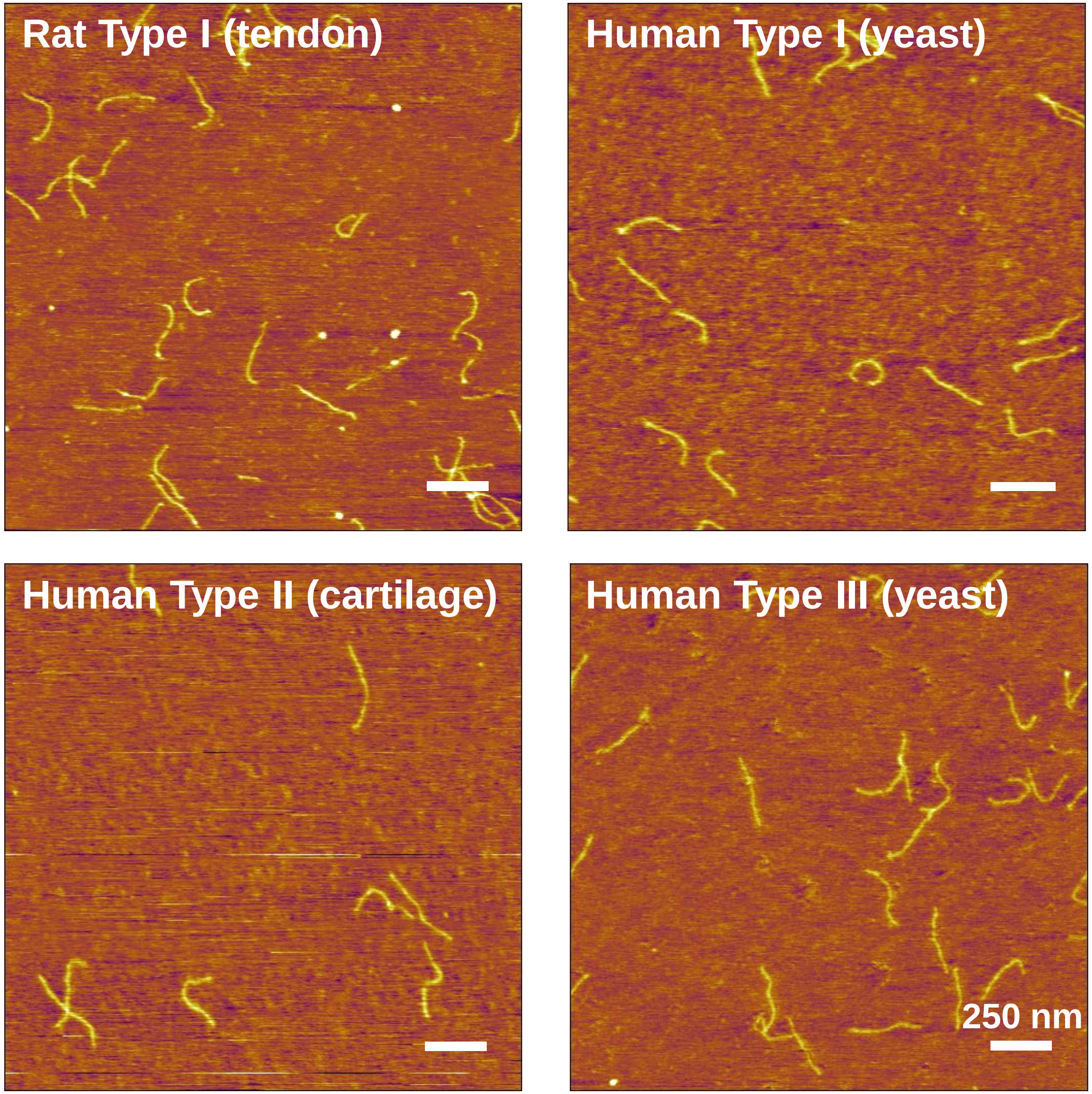
Type I
- tendon, bone, skin…
- heterotrimer

Type II
- cartilage
- homotrimer

Type III
- skin, lung…
- homotrimer



AFM imaging of different collagen types



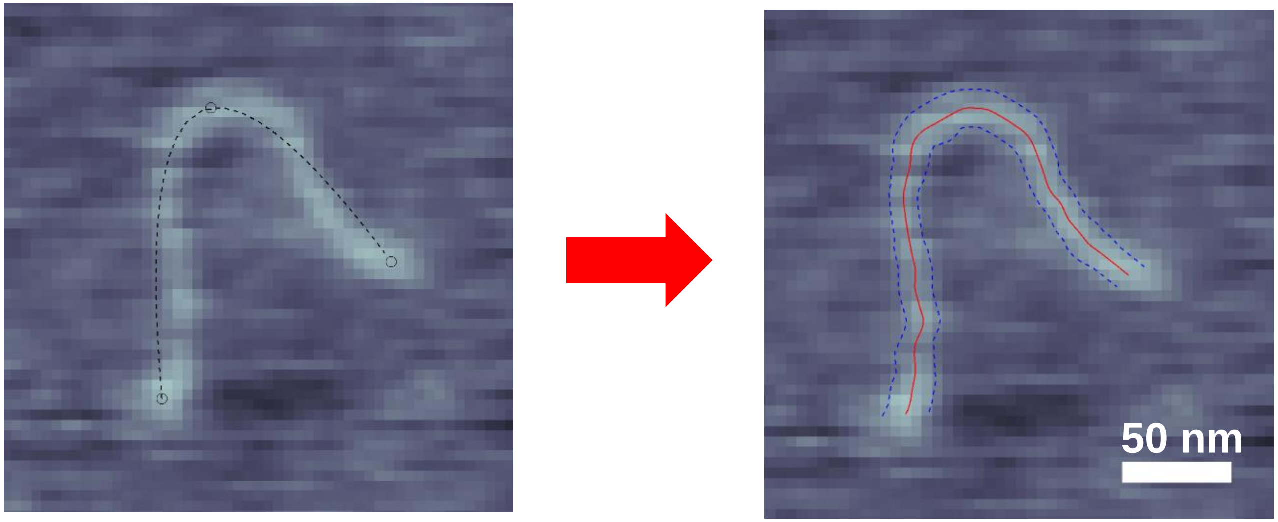
Naghmeh Rezaei

N. Rezaei, A. Lyons and N.R. Forde, *Biophys J* 2018

Aaron Lyons

Chain analysis principles

SmarTrace algorithm



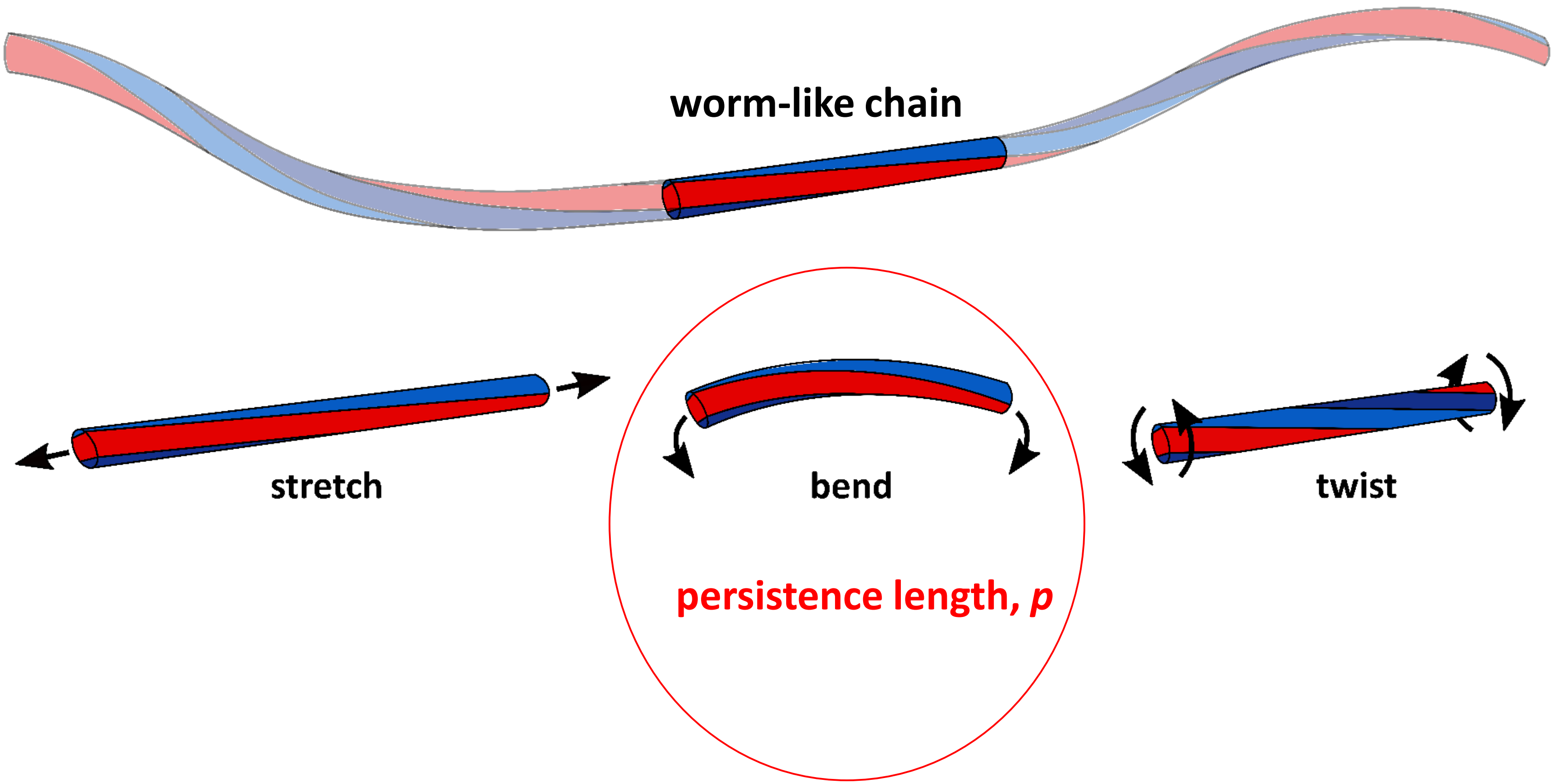
User Input Spline

Refined Spline

Naghmeh Rezaei

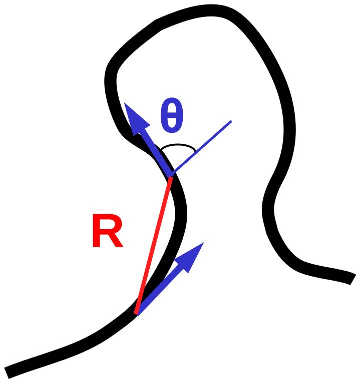
N. Rezaei, A. Lyons and N.R. Forde, *Biophys J* 2018

Chain analysis principles: semiflexible rod



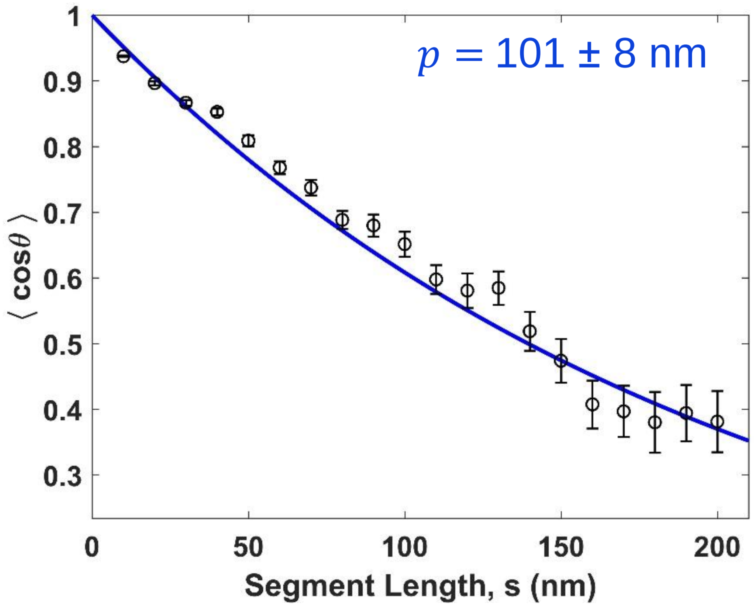
M.W.H. Kirkness, K. Lehmann and N.R. Forde, submitted

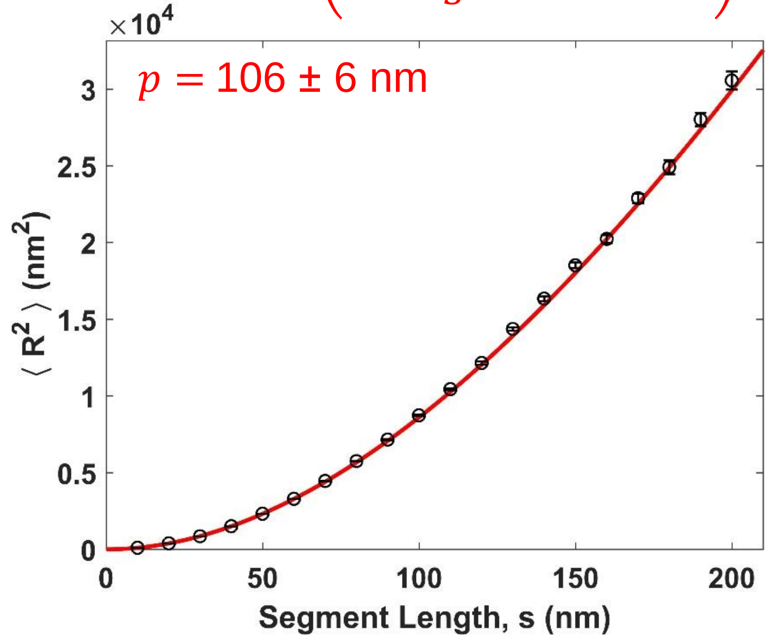
Chain analysis principles: WLC



$$\langle \hat{\boldsymbol{t}}_1 \cdot \hat{\boldsymbol{t}}_2 \rangle = \langle \cos\theta \rangle = e^{-\frac{s}{2p}}$$

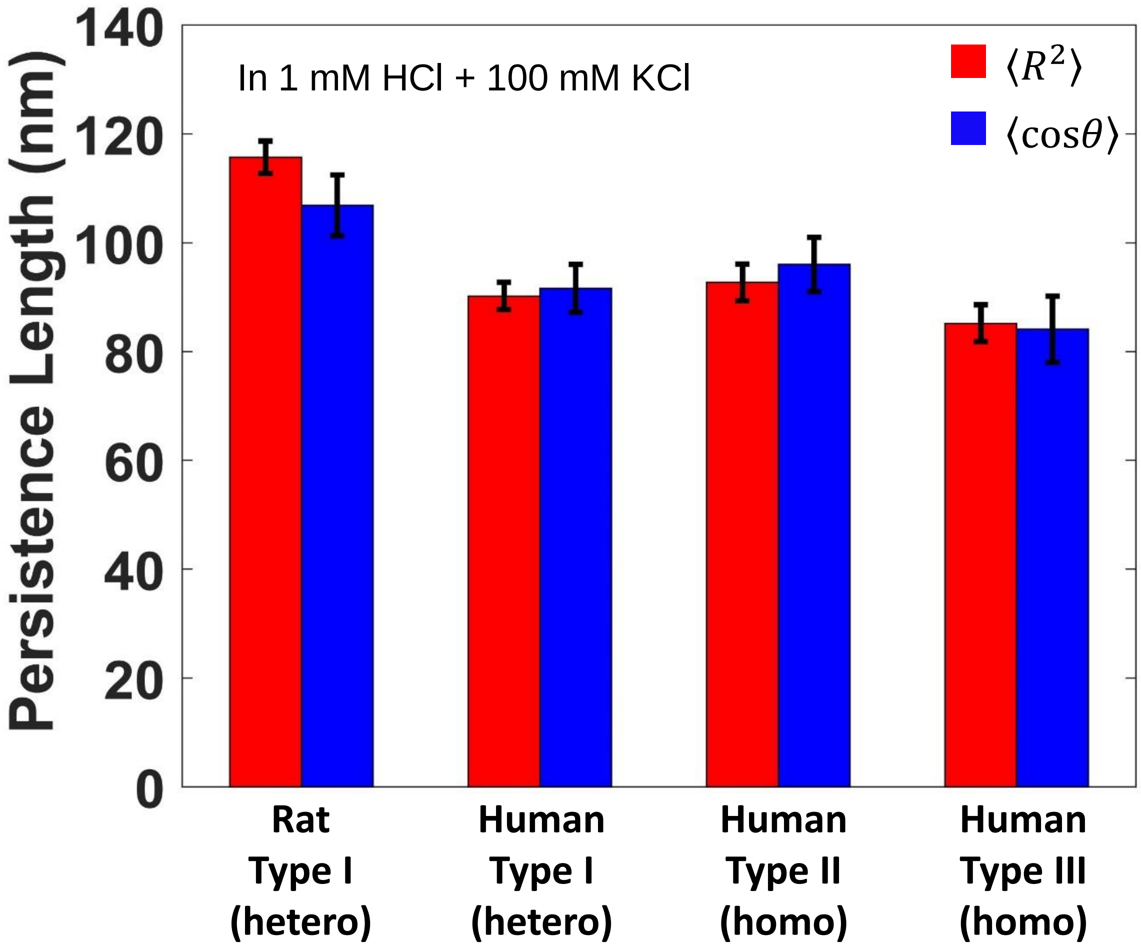
$$\langle R^2 \rangle = 4sp\left(1 - \frac{2p}{s}\left(1 - e^{-\frac{s}{2p}}\right)\right)$$





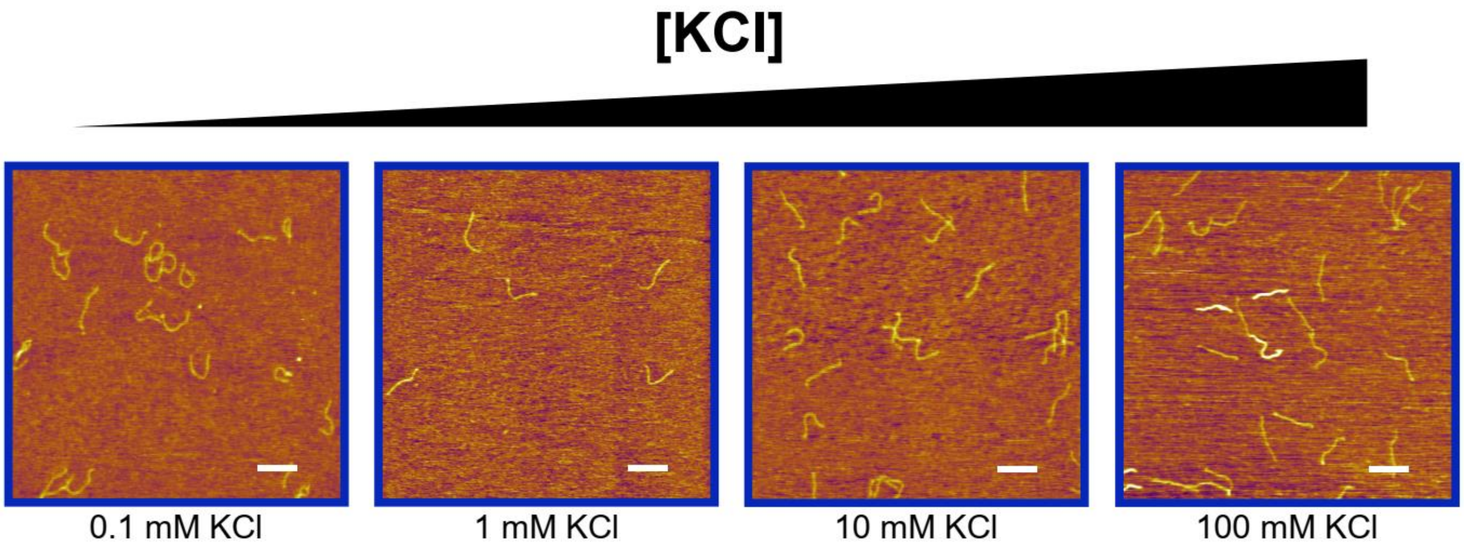
Rezaei, Lyons & Forde, *Biophys. J.* 2018

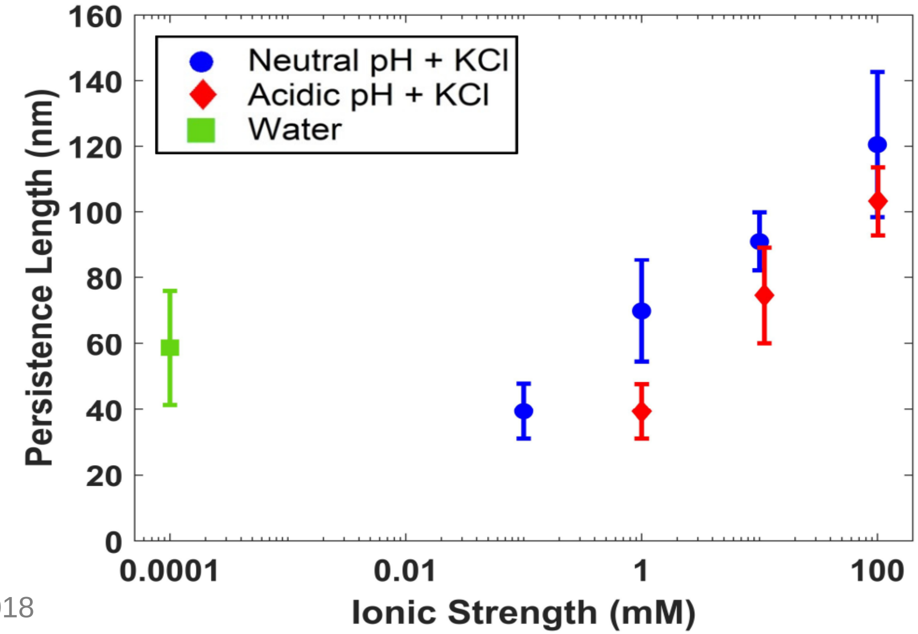
AFM imaging of different collagen types



N. Rezaei, A. Lyons and N.R. Forde, *Biophys J* 2018

Ionic strength and flexibility

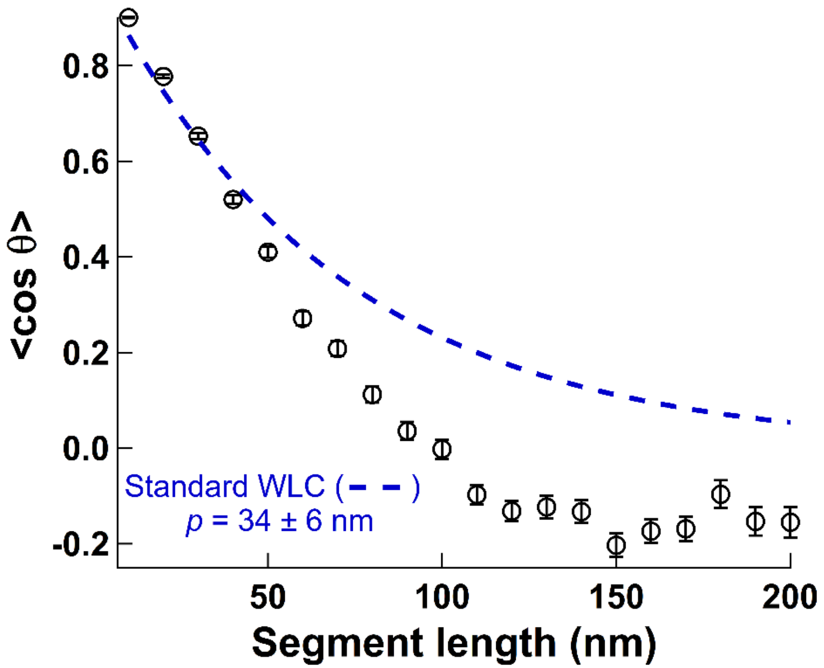


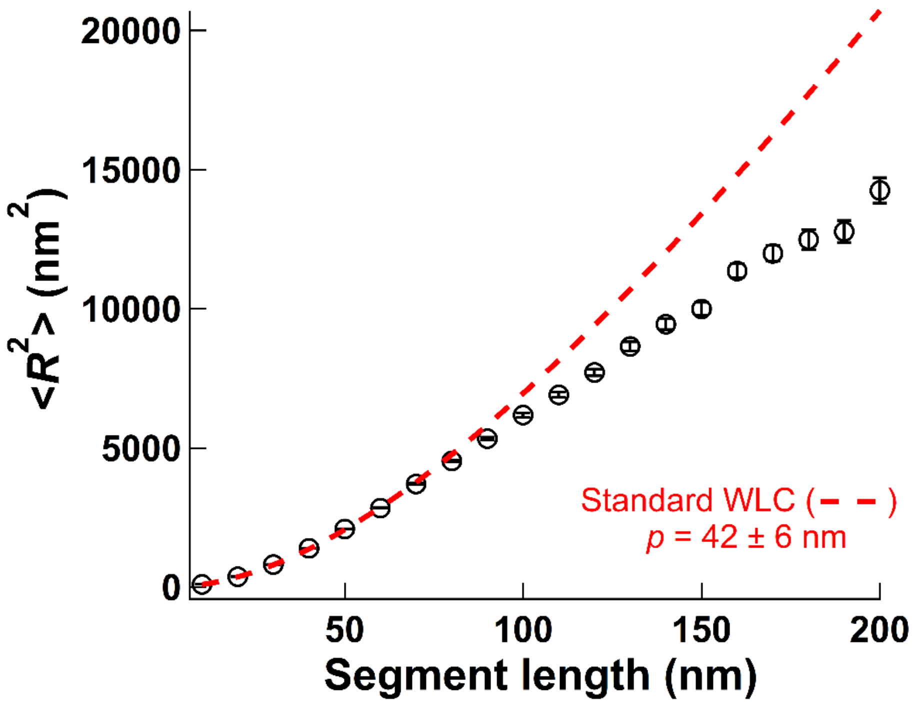


Rezaei, Lyons & Forde, *Biophys. J.* 2018

Is collagen a worm-like chain?

Low ionic strength, acidic pH





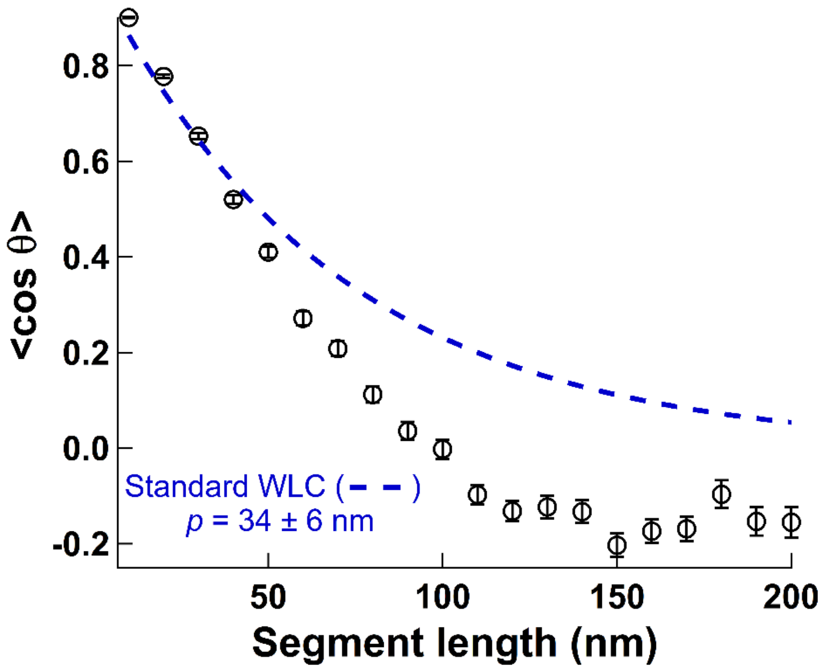
$$\langle \cos\theta \rangle = e^{-\frac{s}{2p}}$$

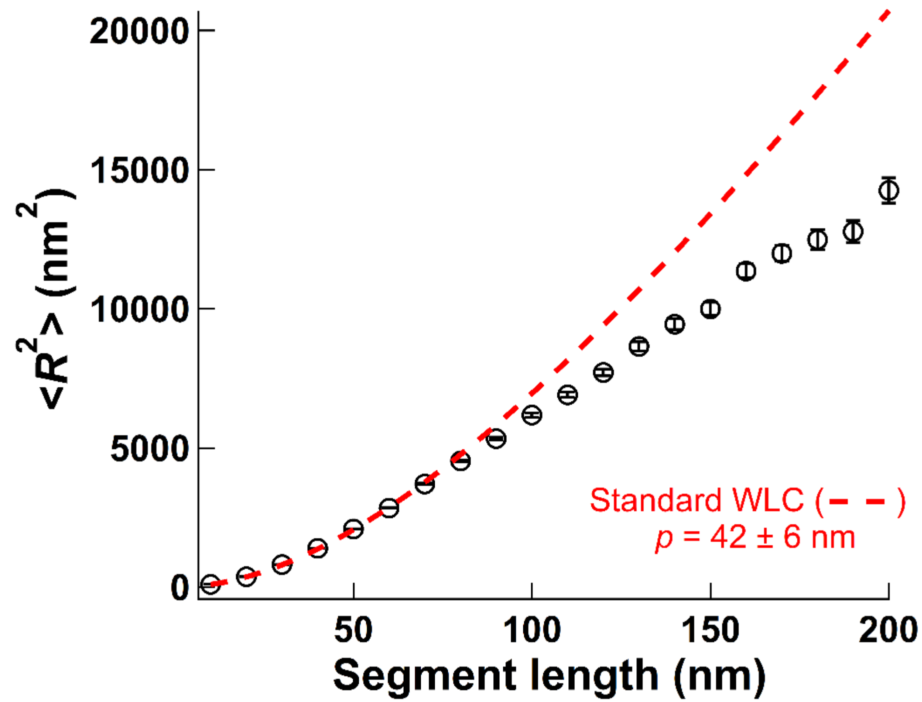
$$\langle R^2 \rangle = 4sp\left(1 - \frac{2p}{s}\left(1 - e^{-\frac{s}{2p}}\right)\right)$$

Rezaei, Lyons & Forde, *Biophys. J.* 2018

Collagen: a curved WLC?

Low ionic strength, acidic pH





$$\langle \cos\theta \rangle = e^{-\frac{s}{2p}}$$

↓

$$\langle \cos\theta\,(s) \rangle_c = \cos(\kappa_0 s)\, e^{-\frac{s}{2p}}$$

$$\langle R^2 \rangle = 4sp\left(1 - \frac{2p}{s}\left(1 - e^{-\frac{s}{2p}}\right)\right)$$

Rezaei, Lyons & Forde, *Biophys. J.* 2018

Collagen: a curved WLC?

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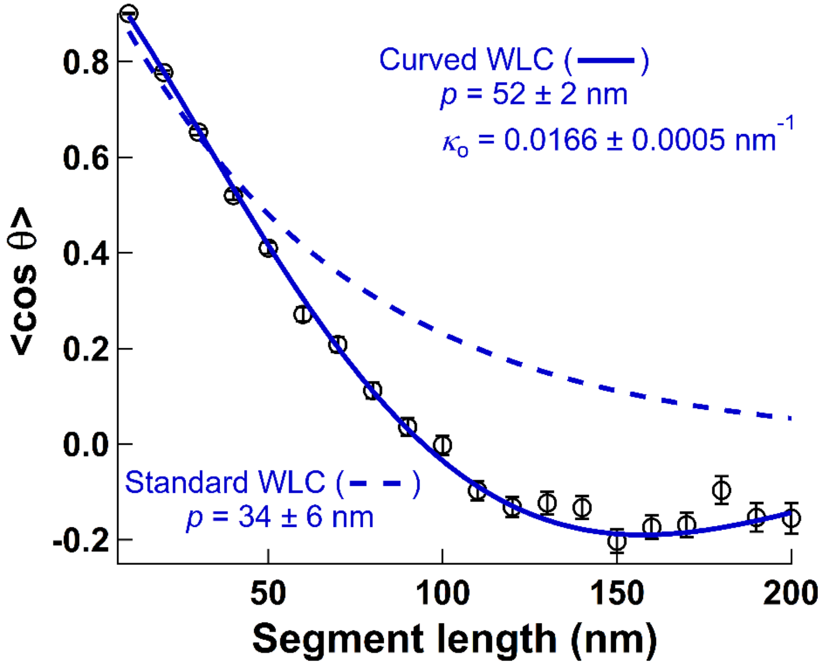
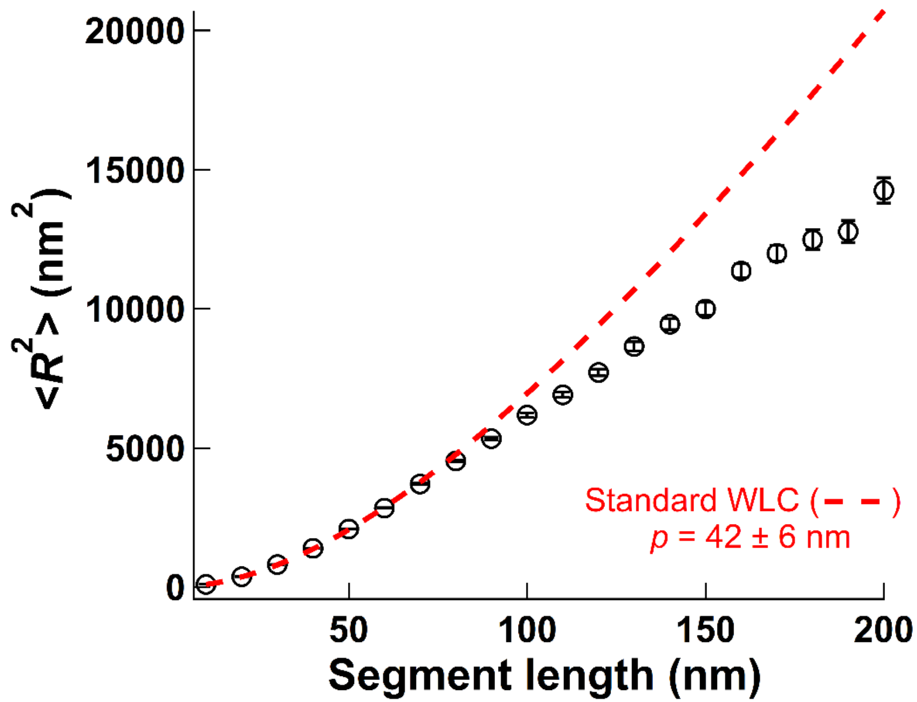




$$\langle \cos\theta \rangle = e^{-\frac{s}{2p}}$$

$$\langle \cos\theta\,(s)\rangle_c = \cos(\kappa_0 s)\, e^{-\frac{s}{2p}}$$

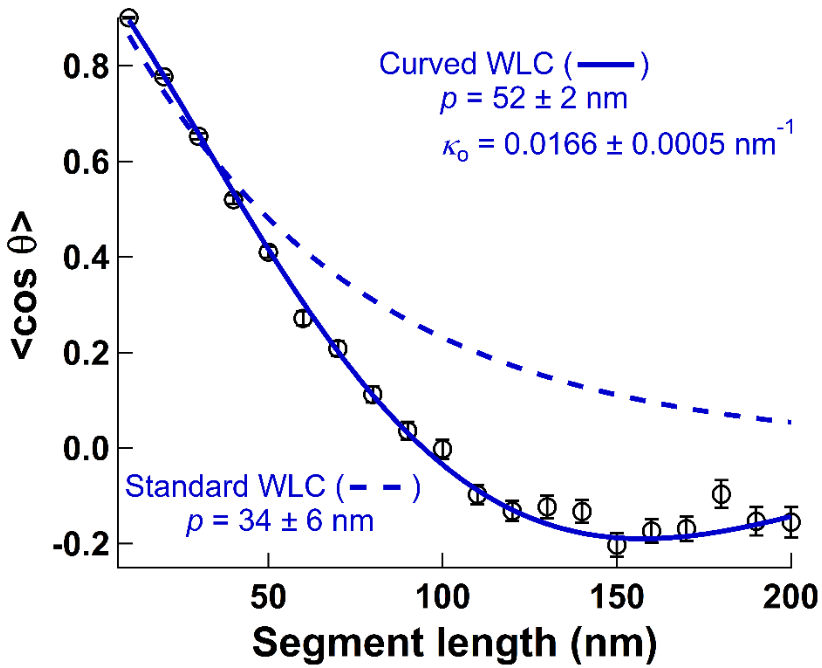
$$\langle R^2 \rangle = 4sp\left(1 - \frac{2p}{s}\left(1 - e^{-\frac{s}{2p}}\right)\right)$$

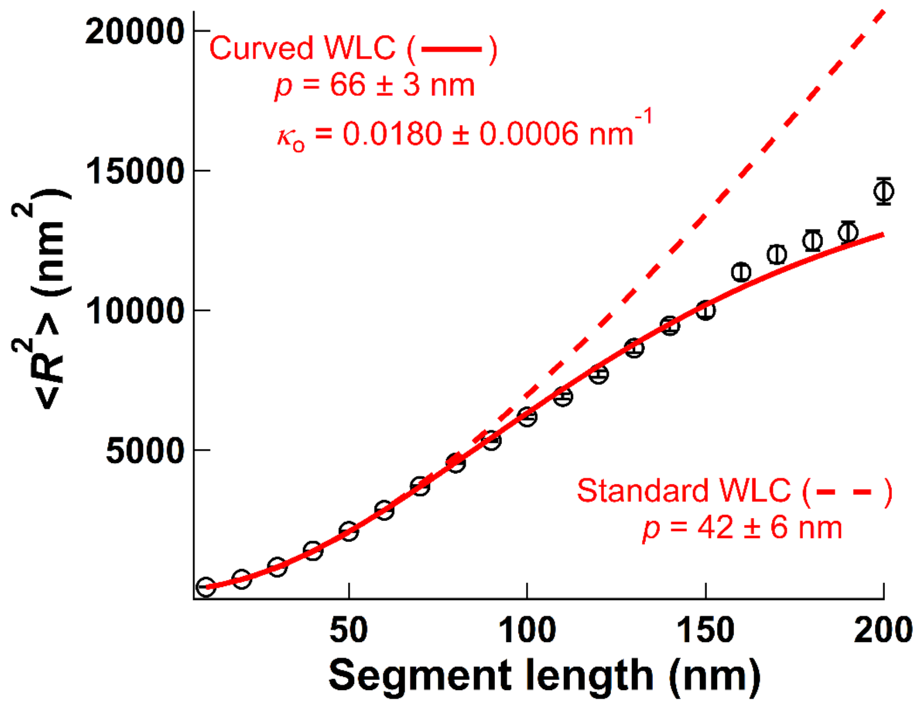
$$\langle R^2(s)\rangle_c = \frac{4sp}{(1+4\kappa_0{}^2p^2)^2}\left\{1 - \frac{2p}{s}(1-4\kappa_0{}^2p^2)\left[1-\cos(\kappa_0 s)\, e^{-\frac{s}{2p}}\right] + \frac{4\kappa_0 p^2}{s}\left[\kappa_0 s - 2\sin(\kappa_0 s)\, e^{-\frac{s}{2p}}\right]\right\}$$

Rezaei, Lyons & Forde, *Biophys. J.* 2018

Collagen: a curved WLC?

Low ionic strength, acidic pH





$$\langle \cos\theta \rangle = e^{-\frac{s}{2p}}$$

$$\downarrow$$

$$\langle \cos\theta\,(s) \rangle_c = \cos(\kappa_0 s)\, e^{-\frac{s}{2p}}$$

$$\langle R^2 \rangle = 4sp\left(1 - \frac{2p}{s}\left(1 - e^{-\frac{s}{2p}}\right)\right)$$

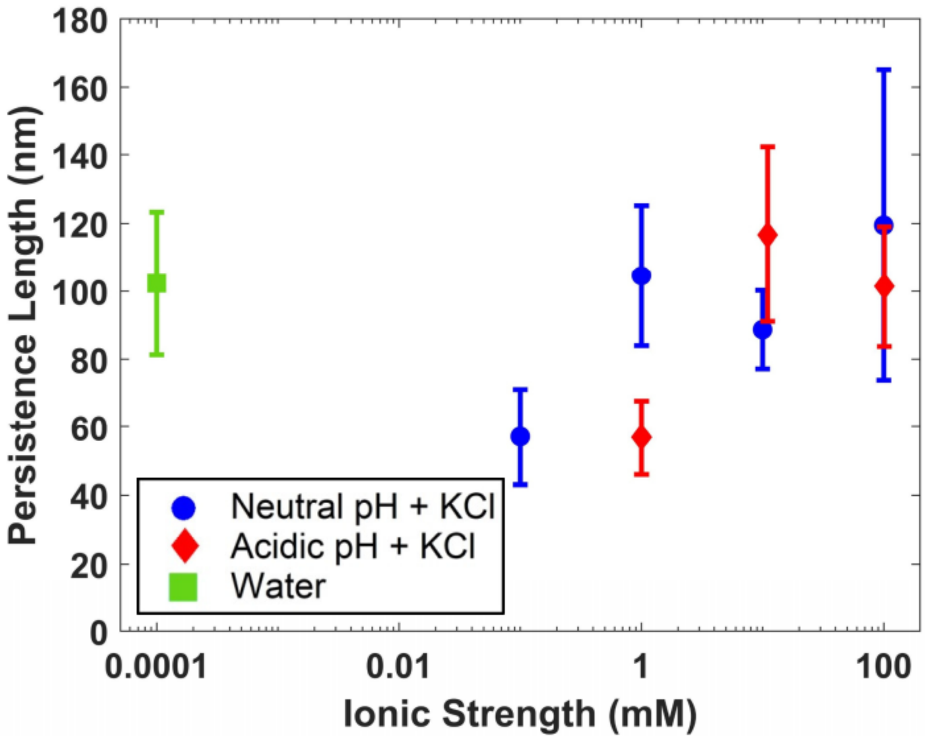
$$\downarrow$$

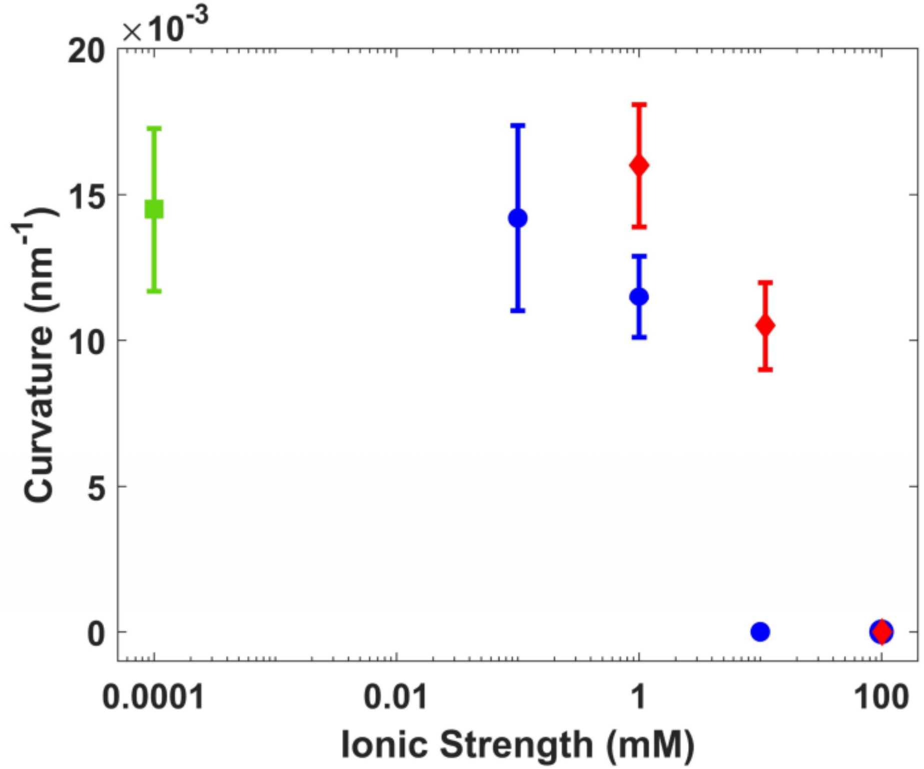
$$\langle R^2(s) \rangle_c = \frac{4sp}{(1+4\kappa_0{}^2p^2)^2}\left\{1 - \frac{2p}{s}(1-4\kappa_0{}^2p^2)\left[1-\cos(\kappa_0 s)\, e^{-\frac{s}{2p}}\right] + \frac{4\kappa_0 p^2}{s}\left[\kappa_0 s - 2\sin(\kappa_0 s)\, e^{-\frac{s}{2p}}\right]\right\}$$

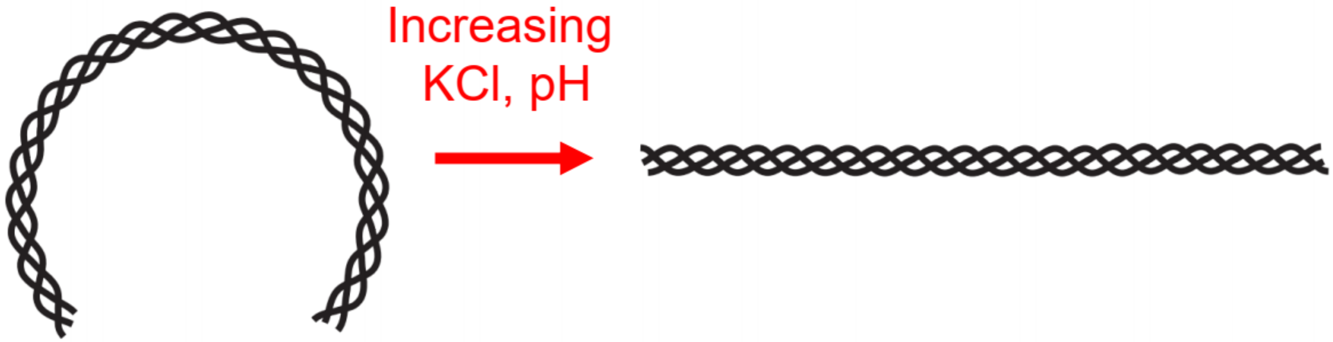
Rezaei, Lyons & Forde, *Biophys. J.* 2018

BIOPHYSICS @ SFU
FORDE LAB

Ionic strength and ~~flexibility~~ curvature







Rezaei, Lyons & Forde, *Biophys. J.* 2018

Outlook

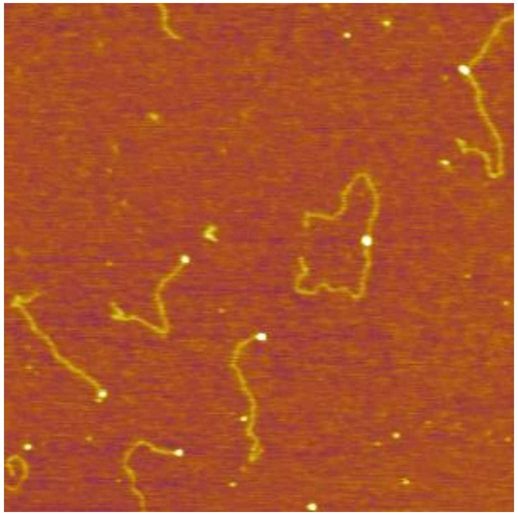
Other applications of curved worm-like chain model?

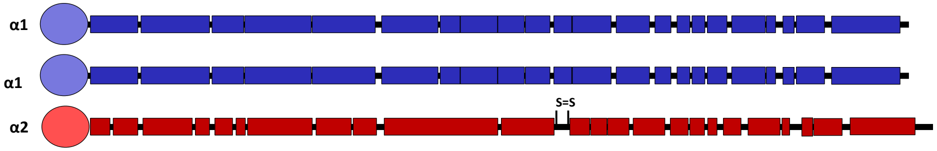
- Tropomyosin, Amyloids, DNA, FtsZ, …

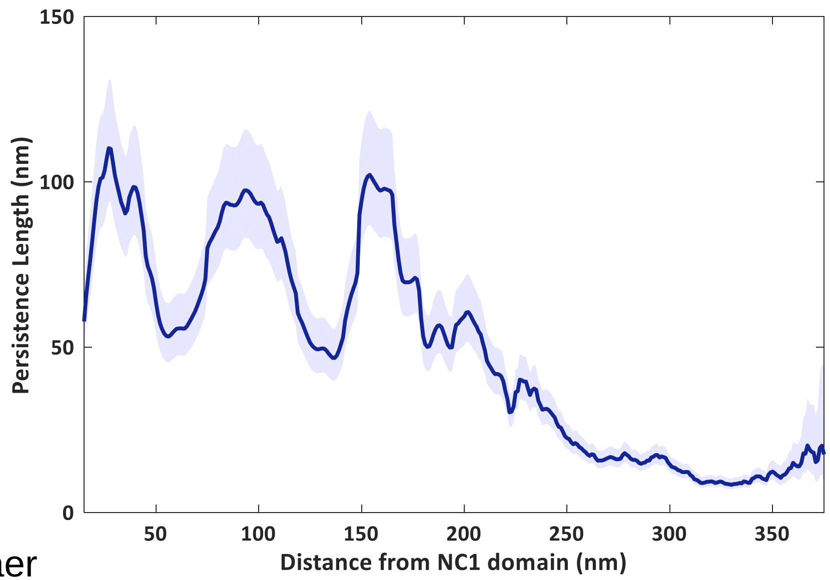
Adapt SmarTrace to study sequence-dependent flexibility

- Fibrillar & nonfibrillar collagens

Type IV collagen – triple helix with interruptions



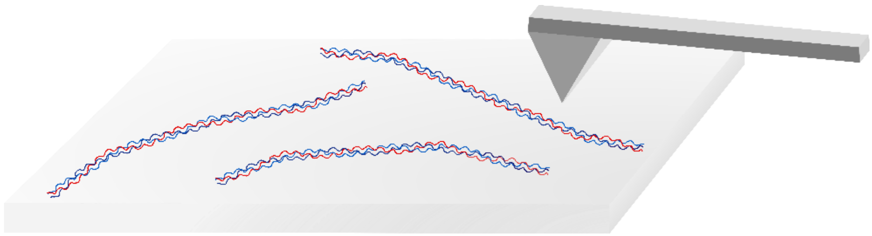




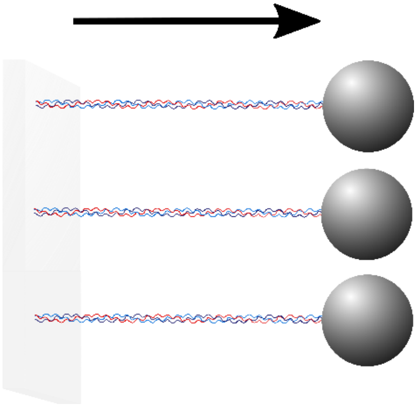
Alaa Al-Shaer

Mechanics of single collagen proteins

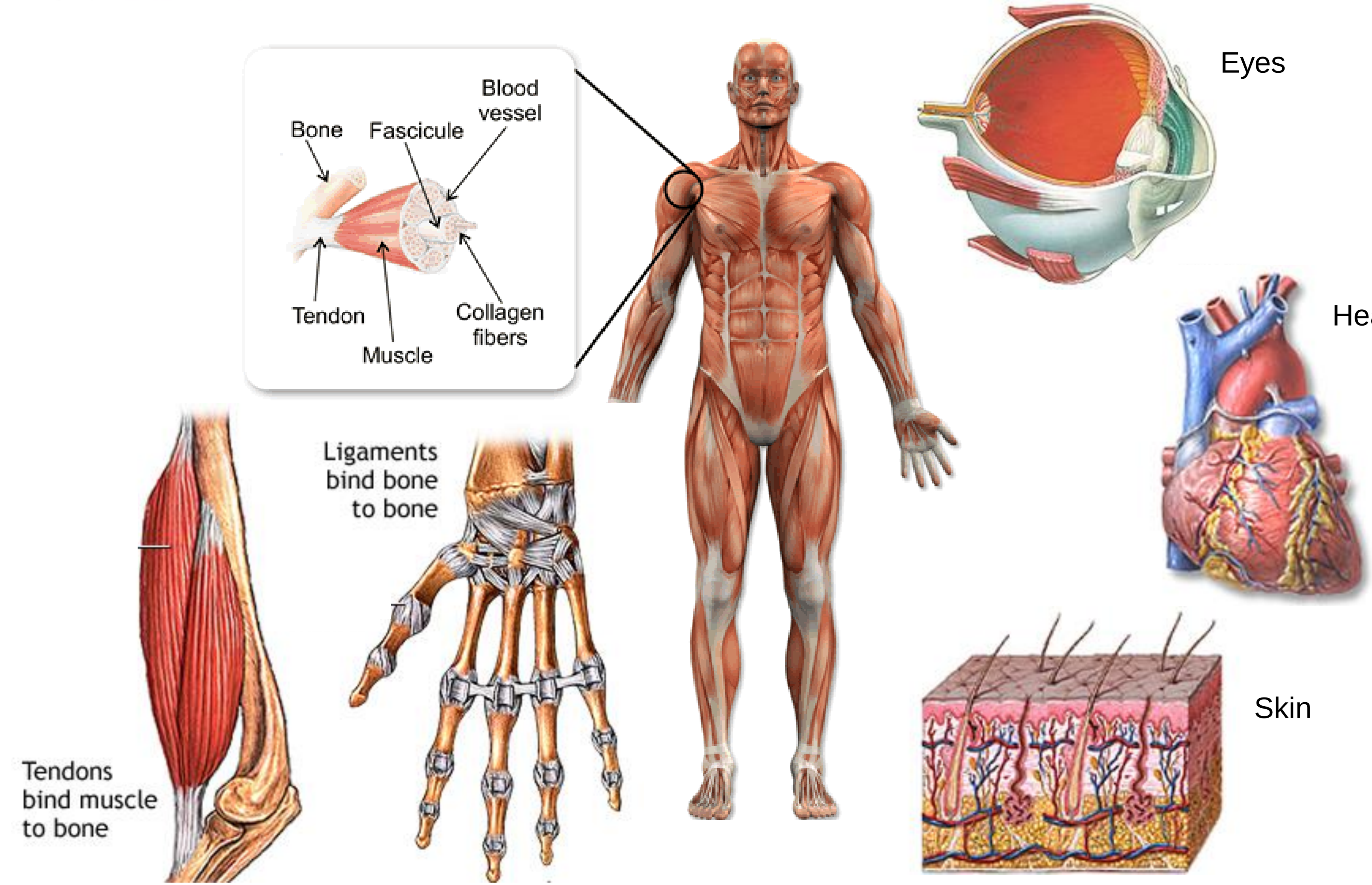
Flexibility



Force response



Collagen holds us together



Collagen: tensile material & template

Images: http://vsearch.nlm.nih.gov/

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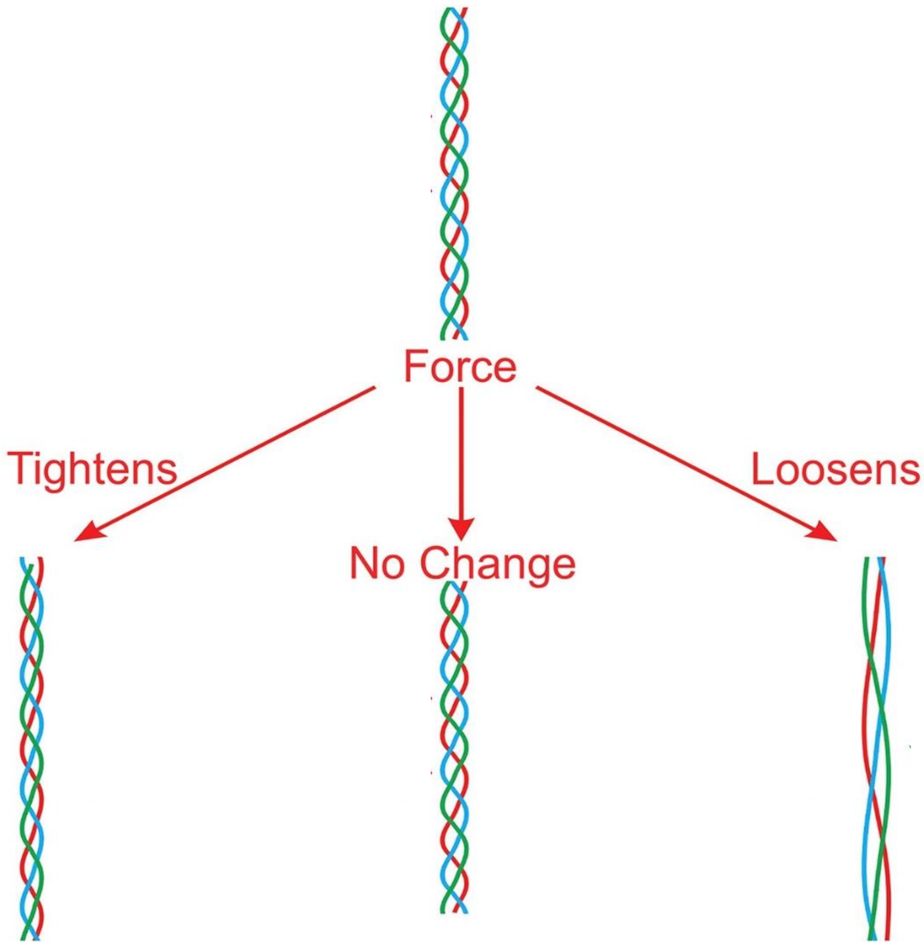
Forde Lab

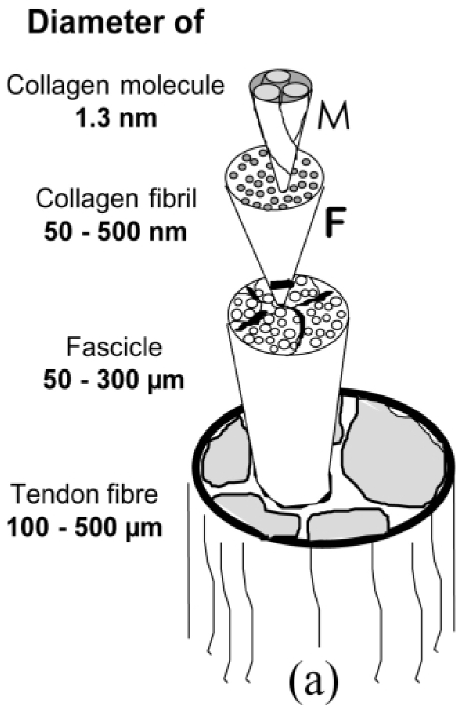
Force-dependent collagen structure



Ruberti lab

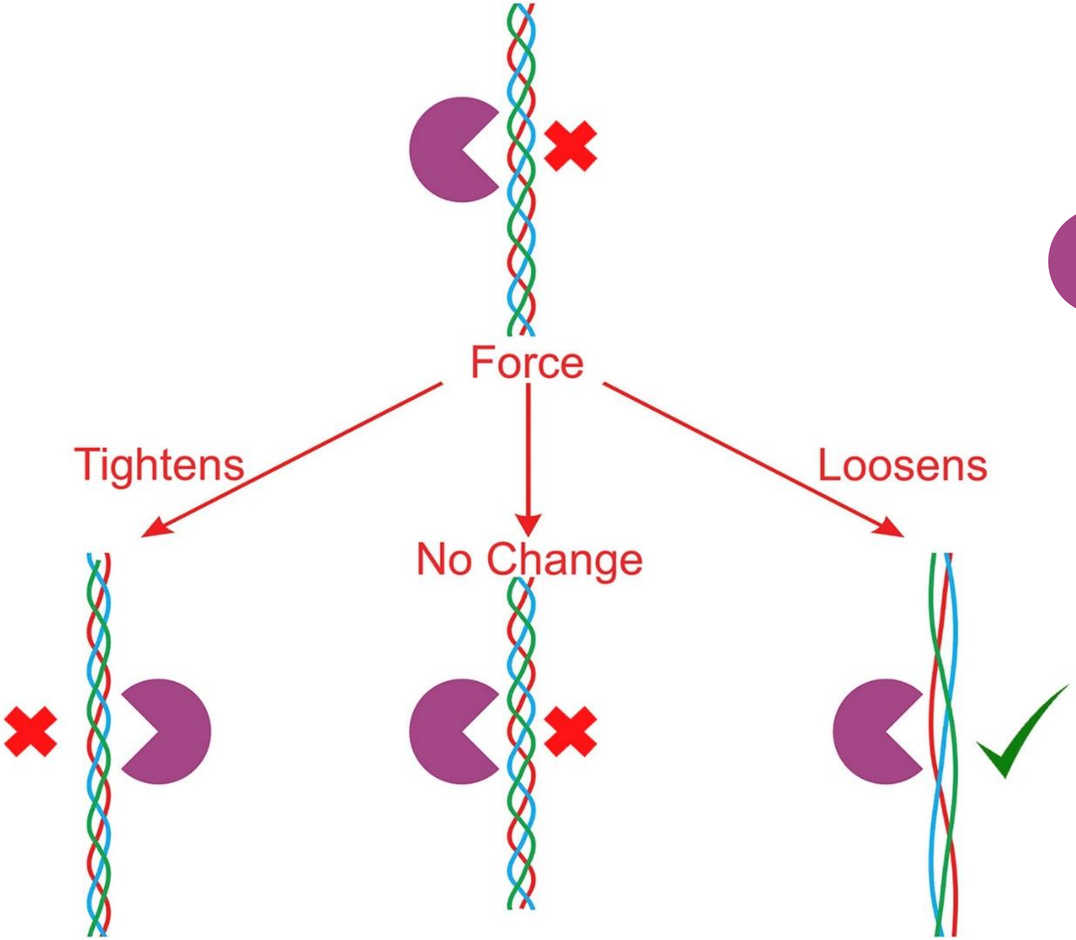
Dunn lab

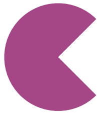
JACS 2011, 2012



Fratzl, *Curr. Opin. Colloid Interface Sci.* 2003

Force-dependent collagen structure



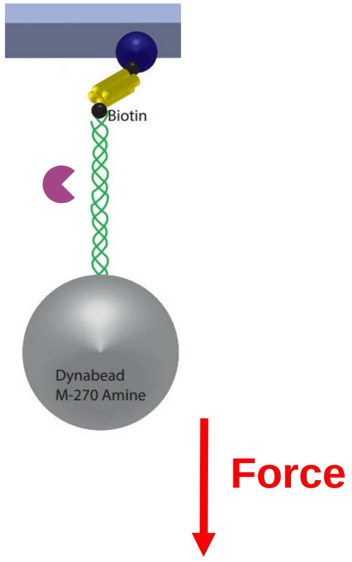


= trypsin
(can only cut single chains)

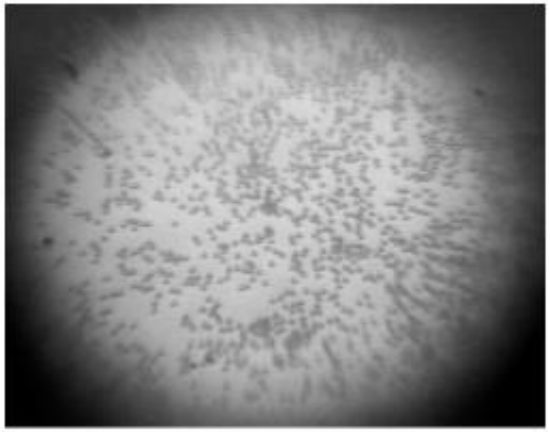
Mike Kirkness

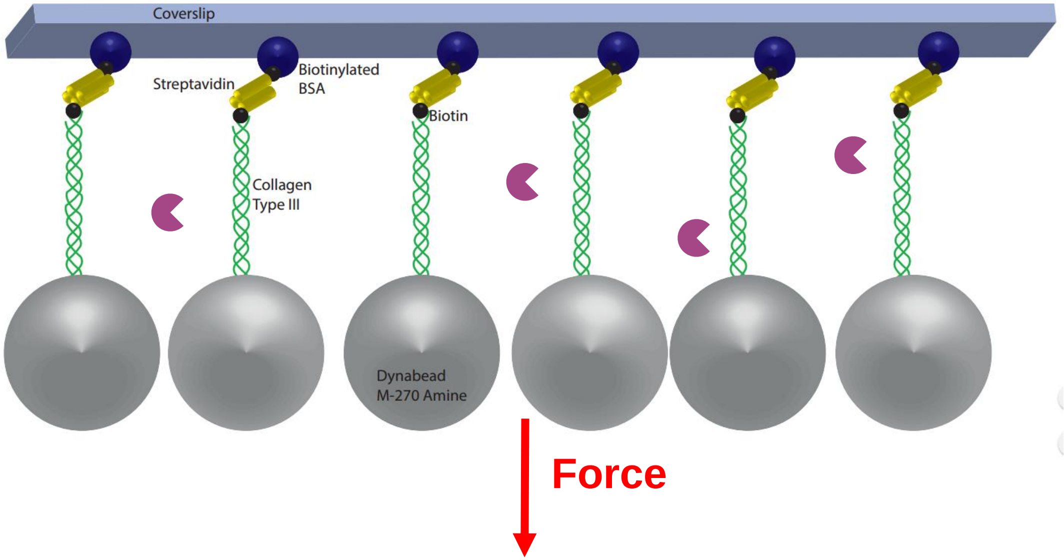
Bruckner & Prockop, *Anal. Biochem* 1981

Measuring collagen's response to force

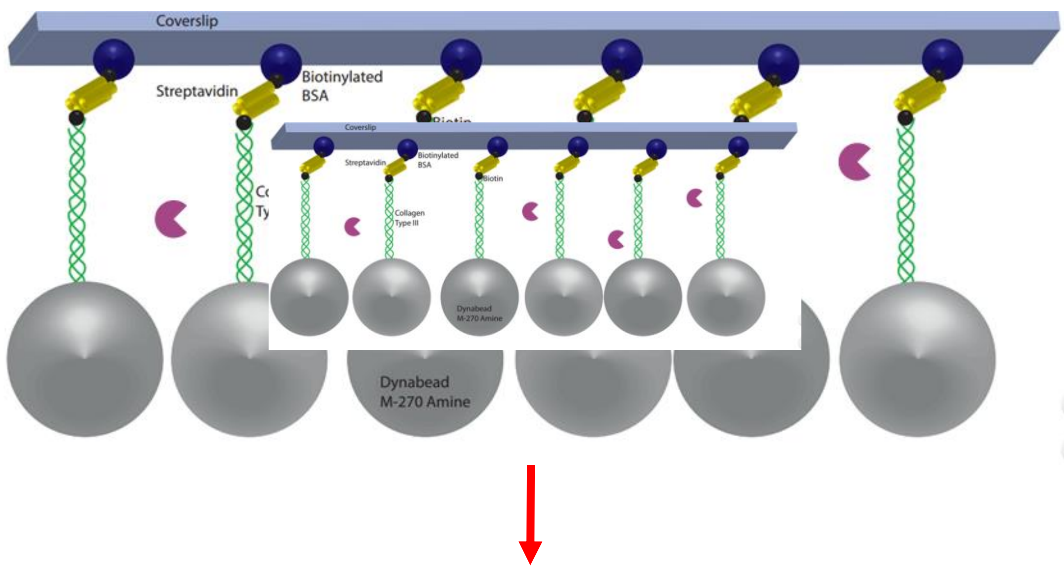


Measuring collagen's response to force

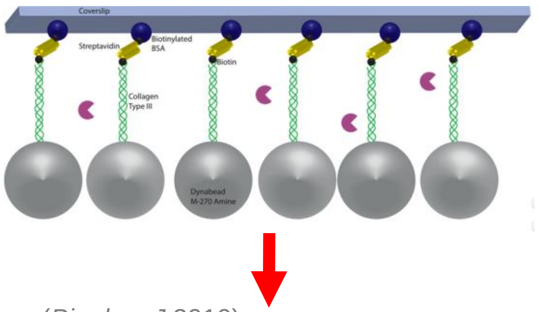




Measuring collagen's response to force



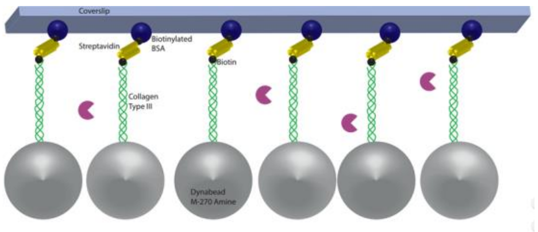
Measuring collagen’s response to force



$$F = m\omega^2 R$$

1st Centrifuge Force Microscope (CFM): Halvorsen & Wong (*Biophys J* 2010)

Measuring collagen’s response to force



$$F = m\omega^2 R$$

1st Centrifuge Force Microscope (CFM): Halvorsen & Wong (*Biophys J* 2010)

Centrifuge force microscope

Kirkness and Forde, *Biophys. J.* (2018)

Centrifuge force microscope

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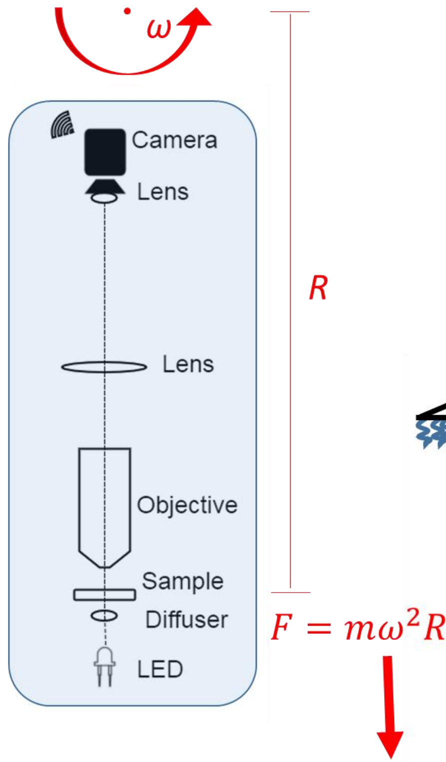

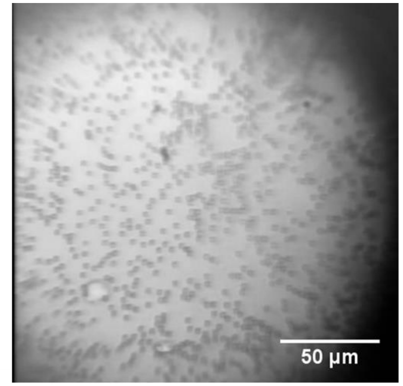

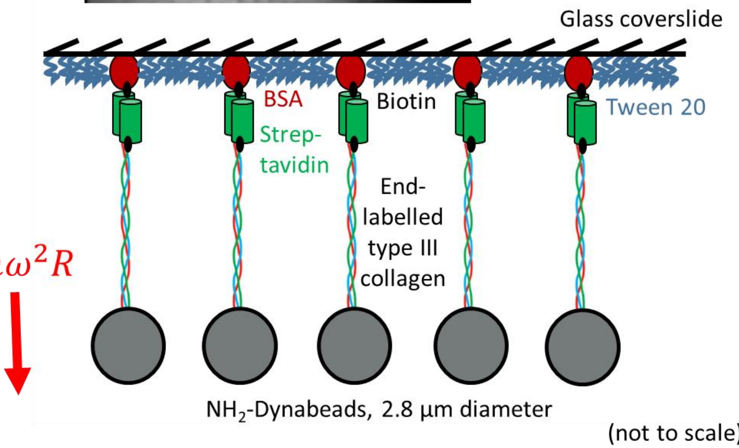

- Microscope withstands >1000 g
- Real-time, video-rate wireless communication
- Wide possible force range (tested 70 fN → 70 pN)
- *N* = 100s-1000s of simultaneous, constant-force single-molecule measurements
- Cost ~ $500

Kirkness and Forde, *Biophys. J.* (2018)

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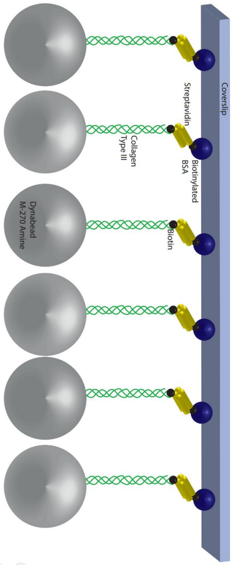
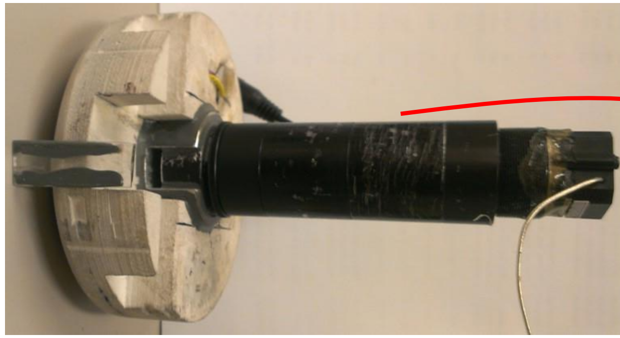
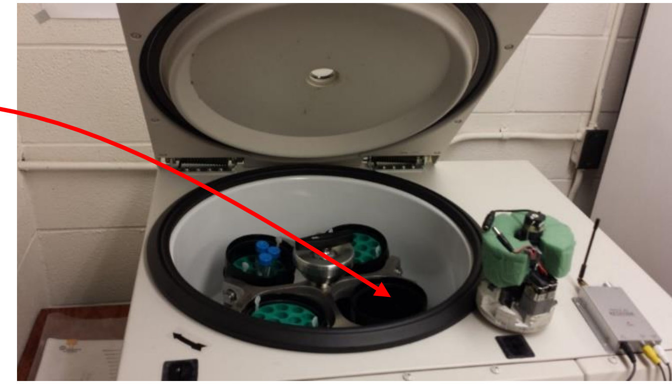
Highest acceleration?

$F = m\omega^2 R$

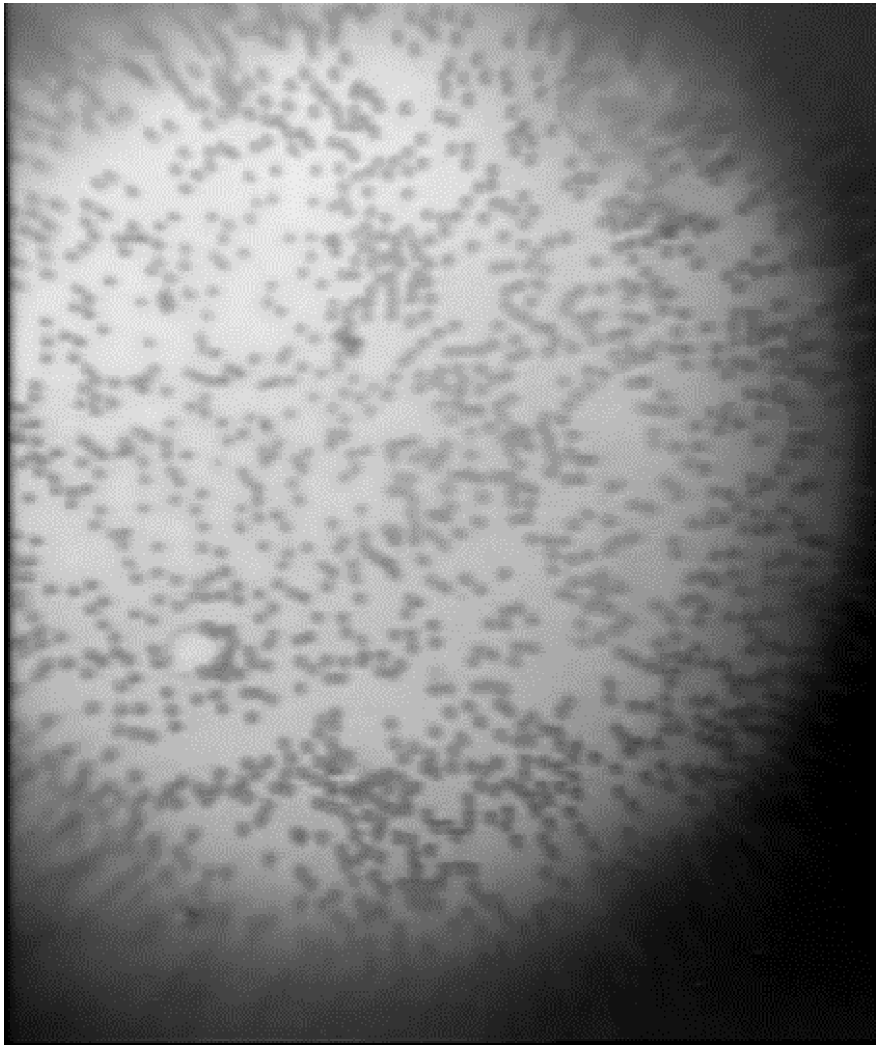


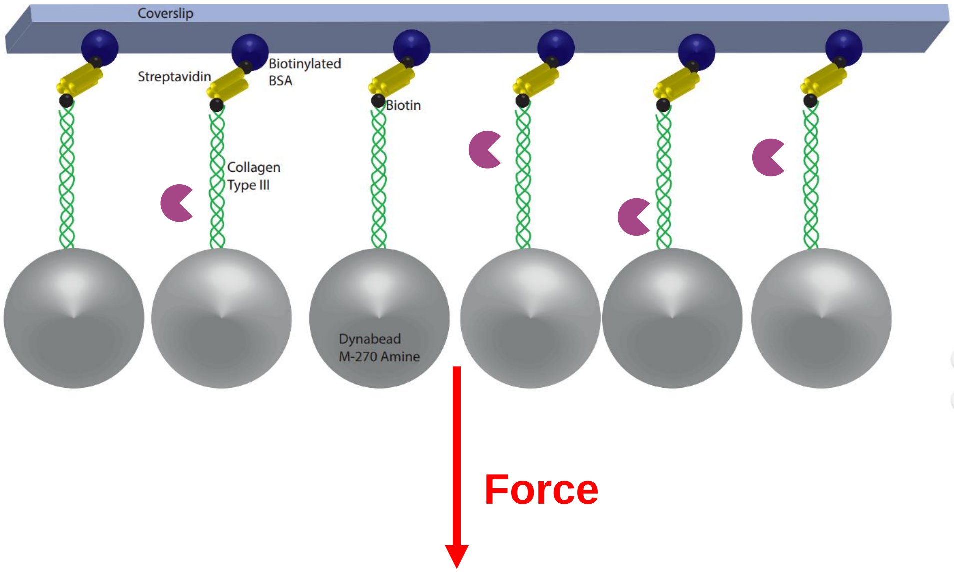




Collagen cleavage under force

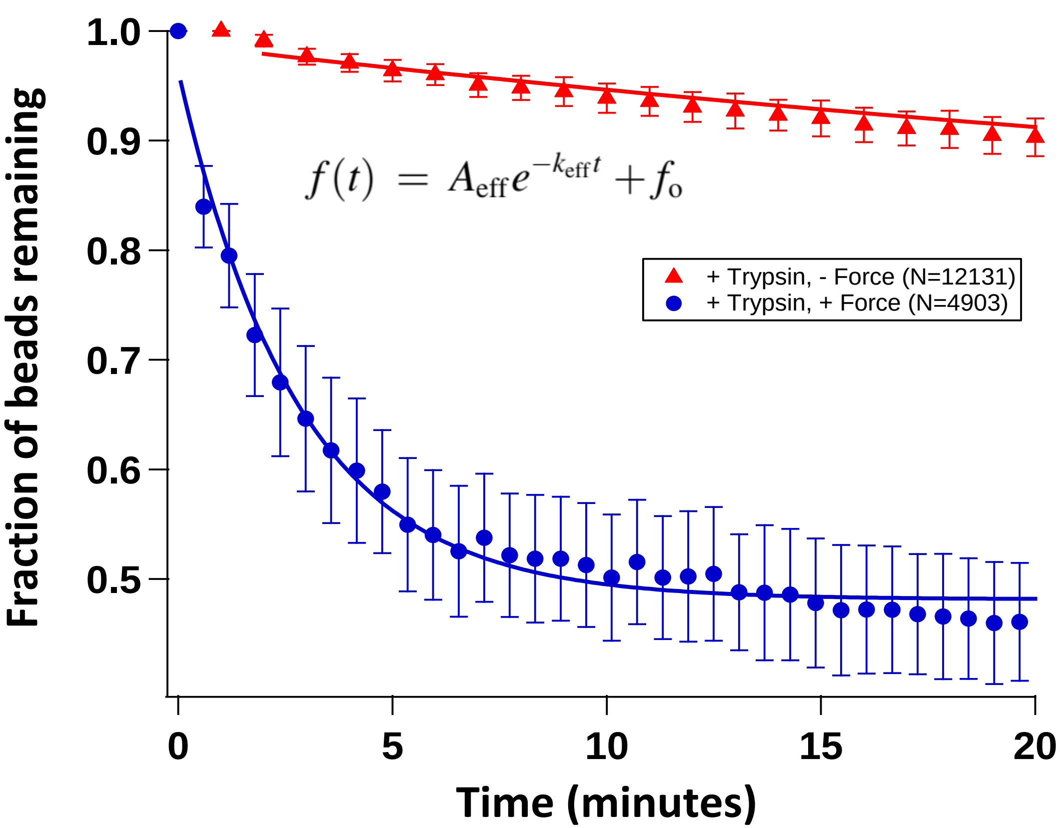
F=9 pN, room temperature (10X real time)

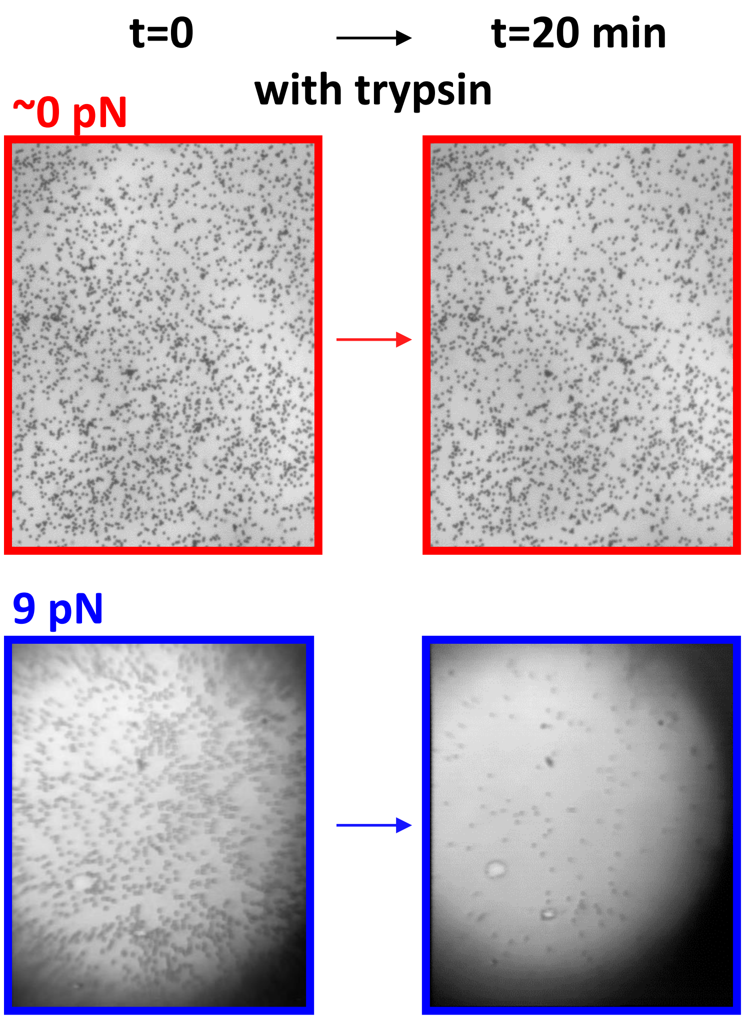




Kirkness and Forde, *Biophys. J.* (2018)

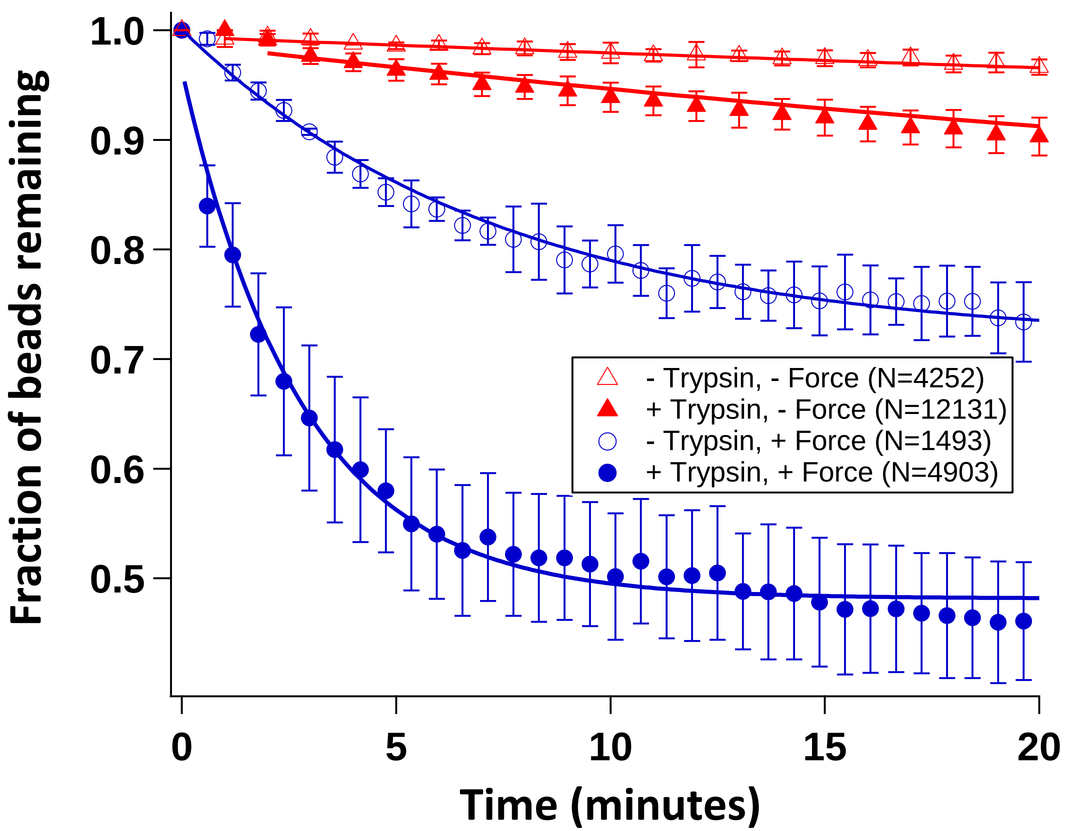
Collagen cleavage under force





Kirkness and Forde, *Biophys. J.* (2018)

Collagen cleavage under force



$$f(t) = A_{\text{eff}} e^{-k_{\text{eff}} t} + f_{\text{o}}$$

$$f_{\text{ns},\, i}(t) = A_{\text{ns},\, i} e^{-k_{\text{ns},i} t} + f_o$$

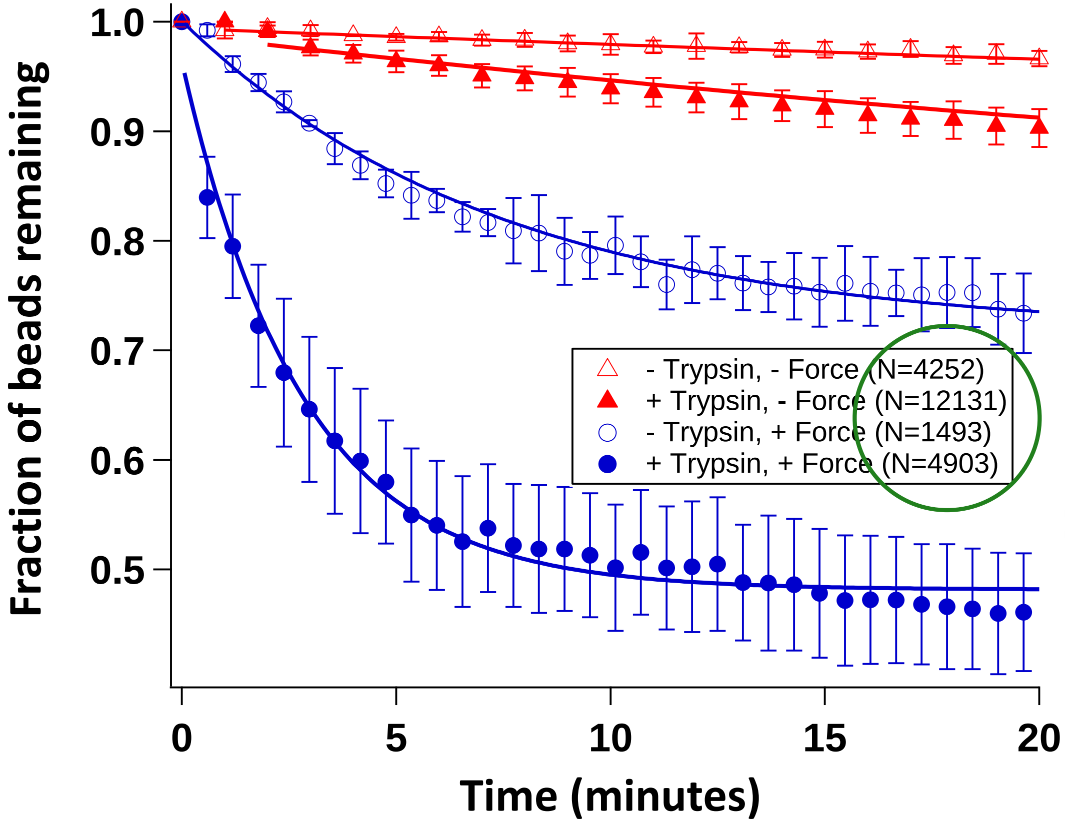
$$f_{\text{Tr},\, i}(t) = A_{\text{eff},\, i} e^{-\left(k_{\text{Tr},i} + k_{\text{ns},i}\right) t} + f_o$$

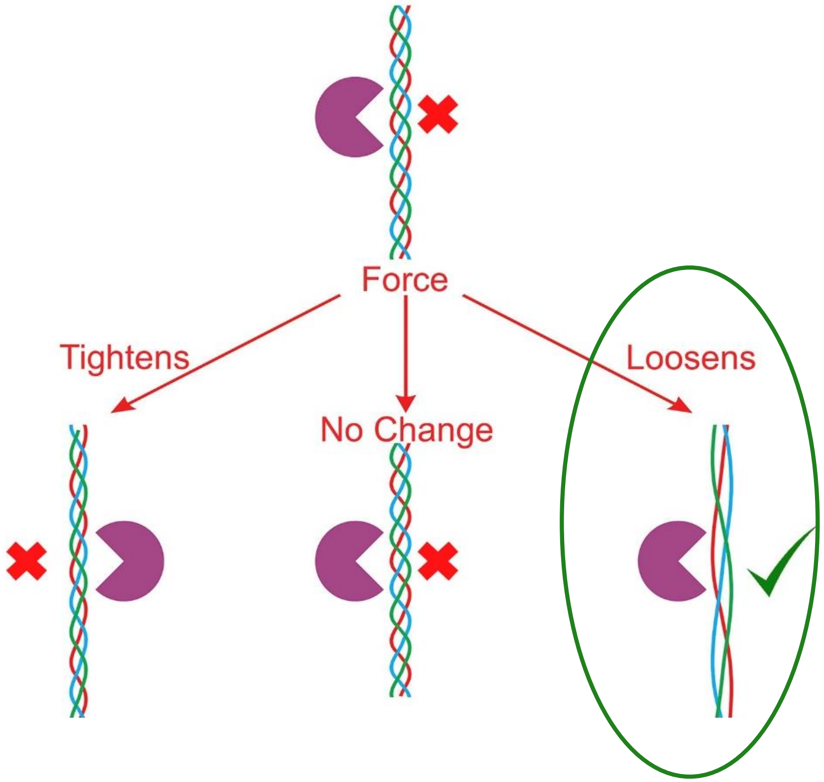
$k_{\text{Tr, 0 pN}} = 0.009 \pm 0.013\ \text{min}^{-1}$
$k_{\text{Tr, 9 pN}} = 0.222 \pm 0.018\ \text{min}^{-1}$

force enhances collagen cleavage rate ~ 20-fold

Kirkness and Forde, *Biophys. J.* (2018)

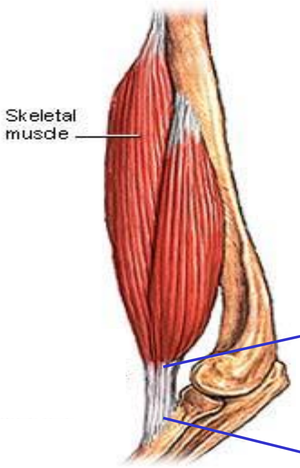
Collagen cleavage enhanced by force



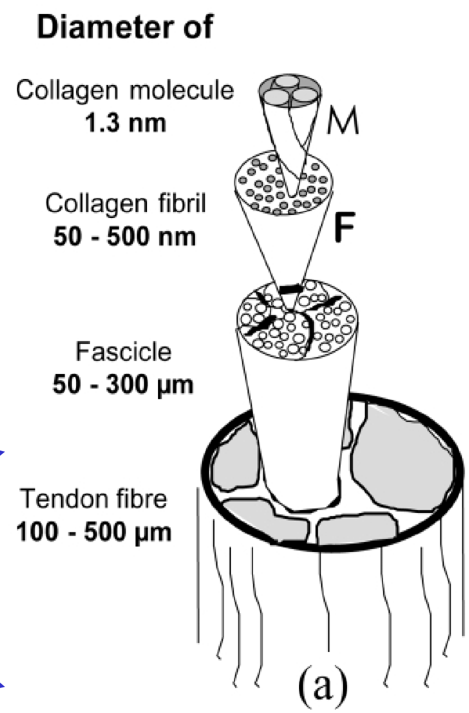


Kirkness and Forde, *Biophys. J.* (2018)

Fibrillar/tissue context



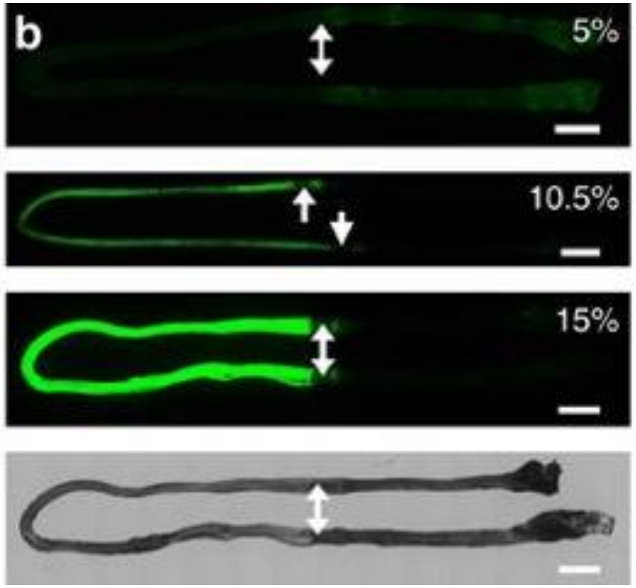
New York Times

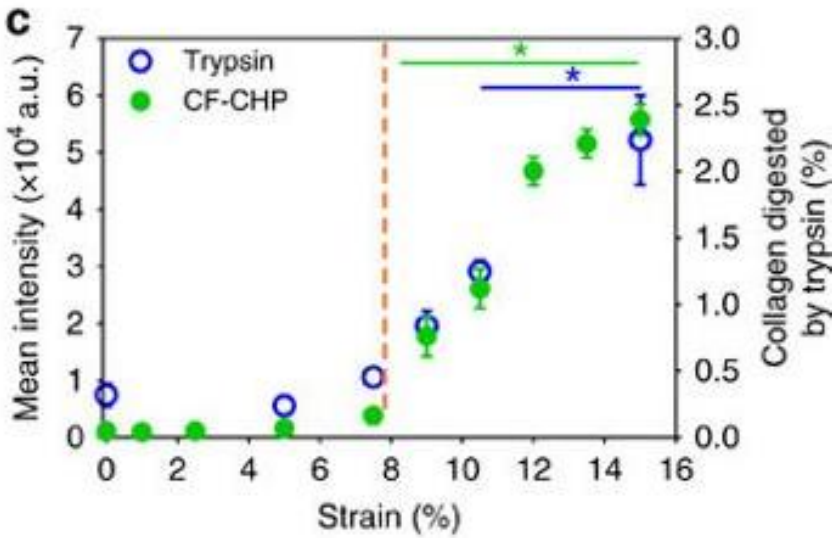


Fratzl, *Curr. Opin. Colloid Interface Sci.* 2003

Fibrillar/tissue context

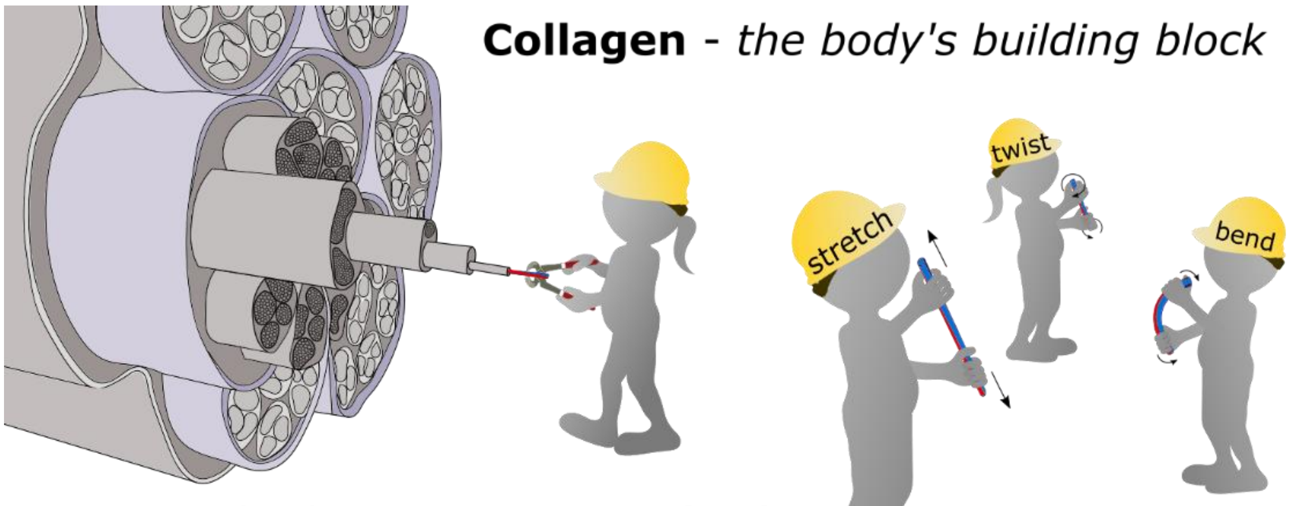
Strained tendon fascicles





Brighter = more triple helix damage

Zitnay et al., *Nature Commun.* **8**, 14913 (2017)



Uncovering the molecular basis for **collagen mechanics**:

- Collagen's curvature (on mica) depends strongly on solution conditions
- Collagen's triple helical structure appears to destabilize with force

Facilitated by **technical and theoretical developments**:

- SmarTrace algorithm, appropriate for tracing noisy images of relatively short polymers
- curved worm-like chain polymer model
- centrifuge force microscope (CFM) for highly parallel single-molecule force spectroscopy

SFU


Forde group, April 2018

NSERC CRSNG