Structure, plasticity and robustness of collagens

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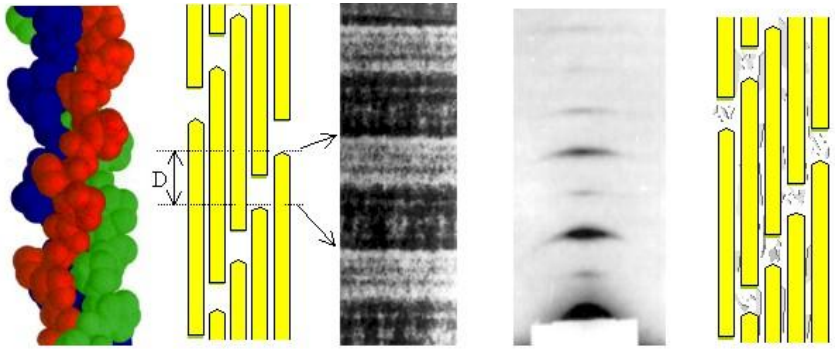
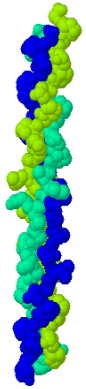
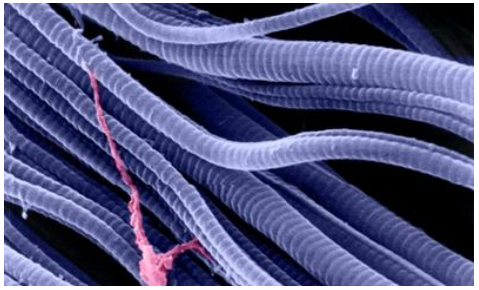


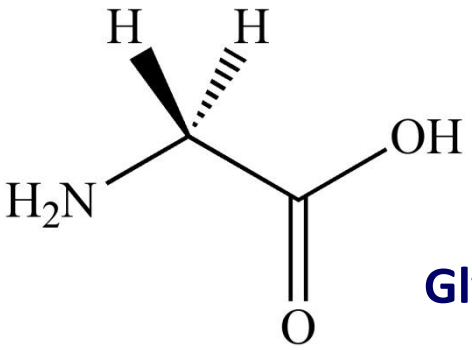
Plasticity and robustness of collagens at molecular and supramolecular levels



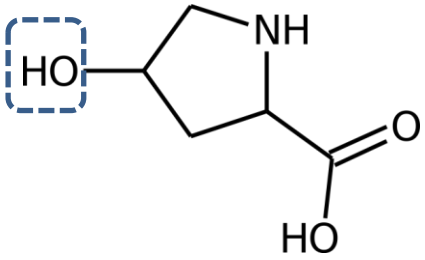
- Individual α **chains** (sequence, local structure)
- The collagen **triple helix**
- Supramolecular assemblies: collagen **fibrils**
- **Cross-linking** and plasticity



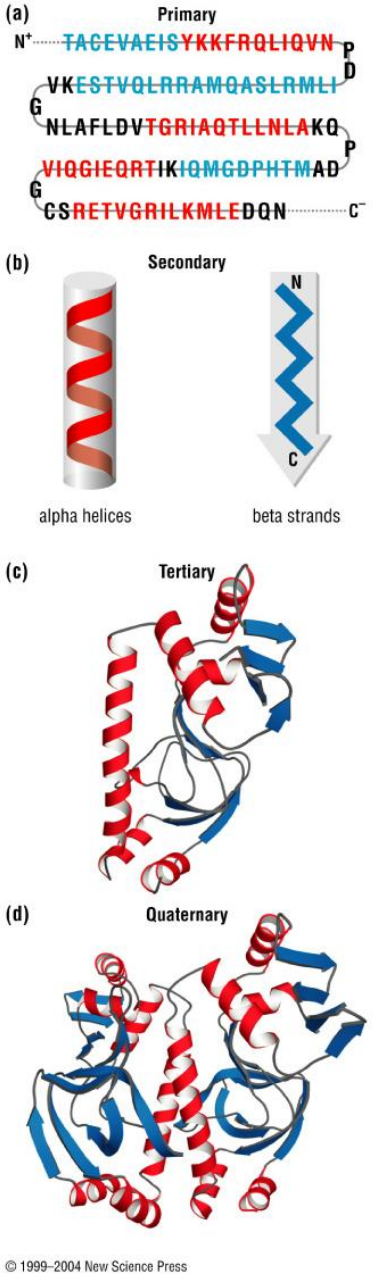
Protein structure



Glycine



Proline
Hydroxyproline



Plasticity and protein structure

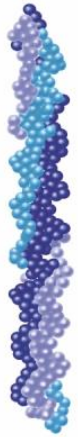
Conformational plasticity

Protein flexibility

Plasticity and physical properties of proteins

(Faísca and Gomes. On the relation between native geometry and conformational plasticity. Biophys Chem 2008 138:99-106)

The collagen superfamily

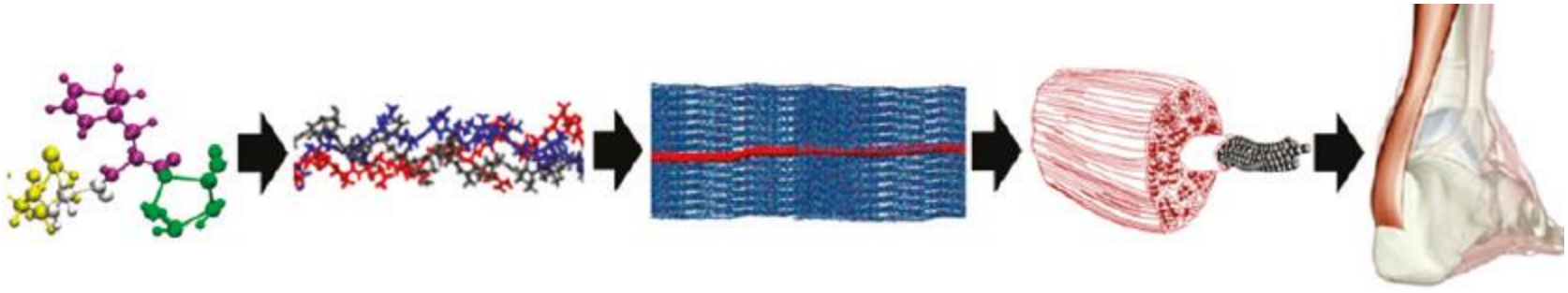


- Collagens are the **most abundant** proteins of the body (30%)

- **Different collagen types** (sequences, domains, assemblies)

- Contribution to the **shape, stiffness** and **mechanical properties** of tissues

Collagens: from amino acids to tissues



α chain	Triple helix	Supramolecular assembly Cross-linking	Molecular context

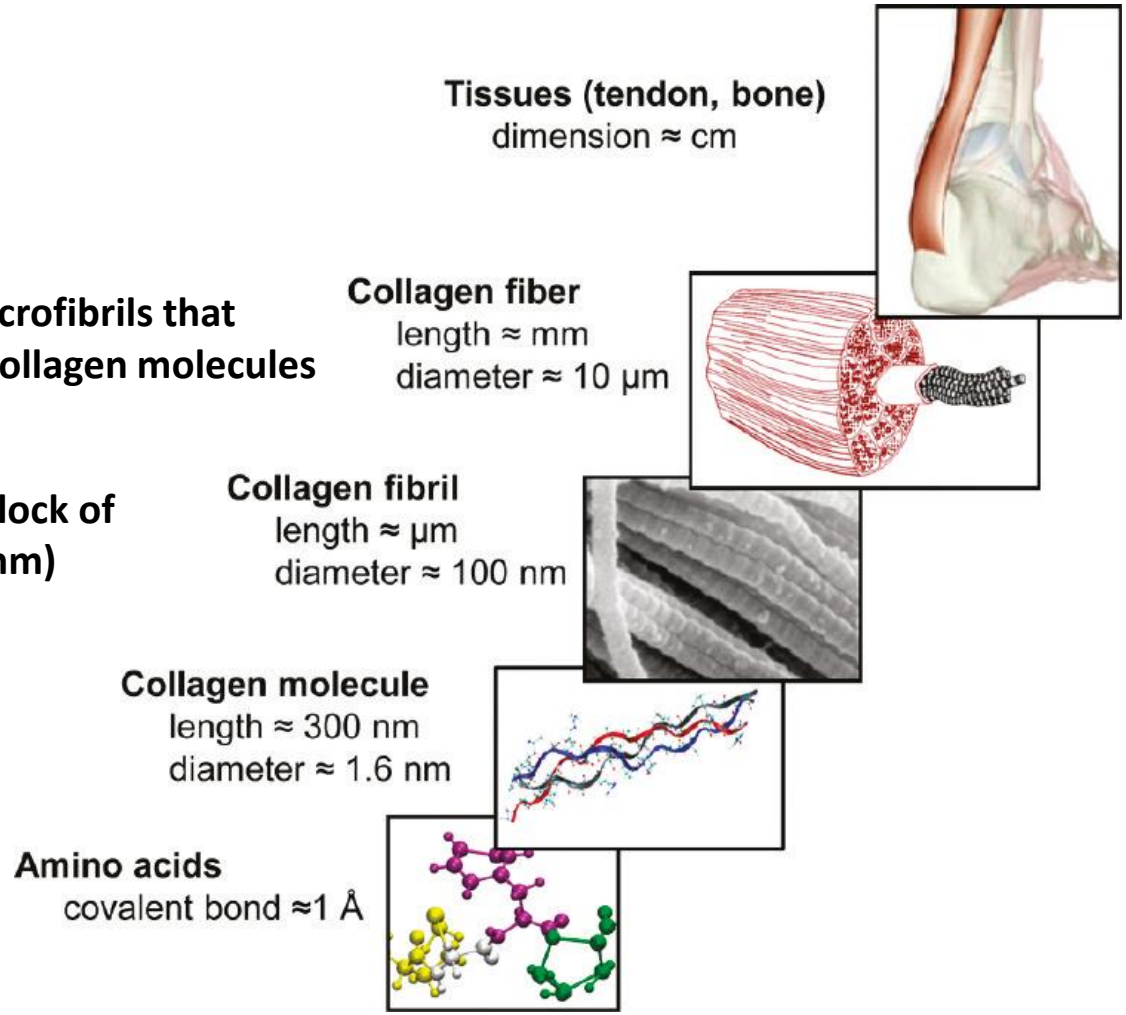
Proteoglycans
Hydroxyapatite

(Gautieri et al. 2011 Nano Lett 11:757-766)

Hierarchical structure of collagens

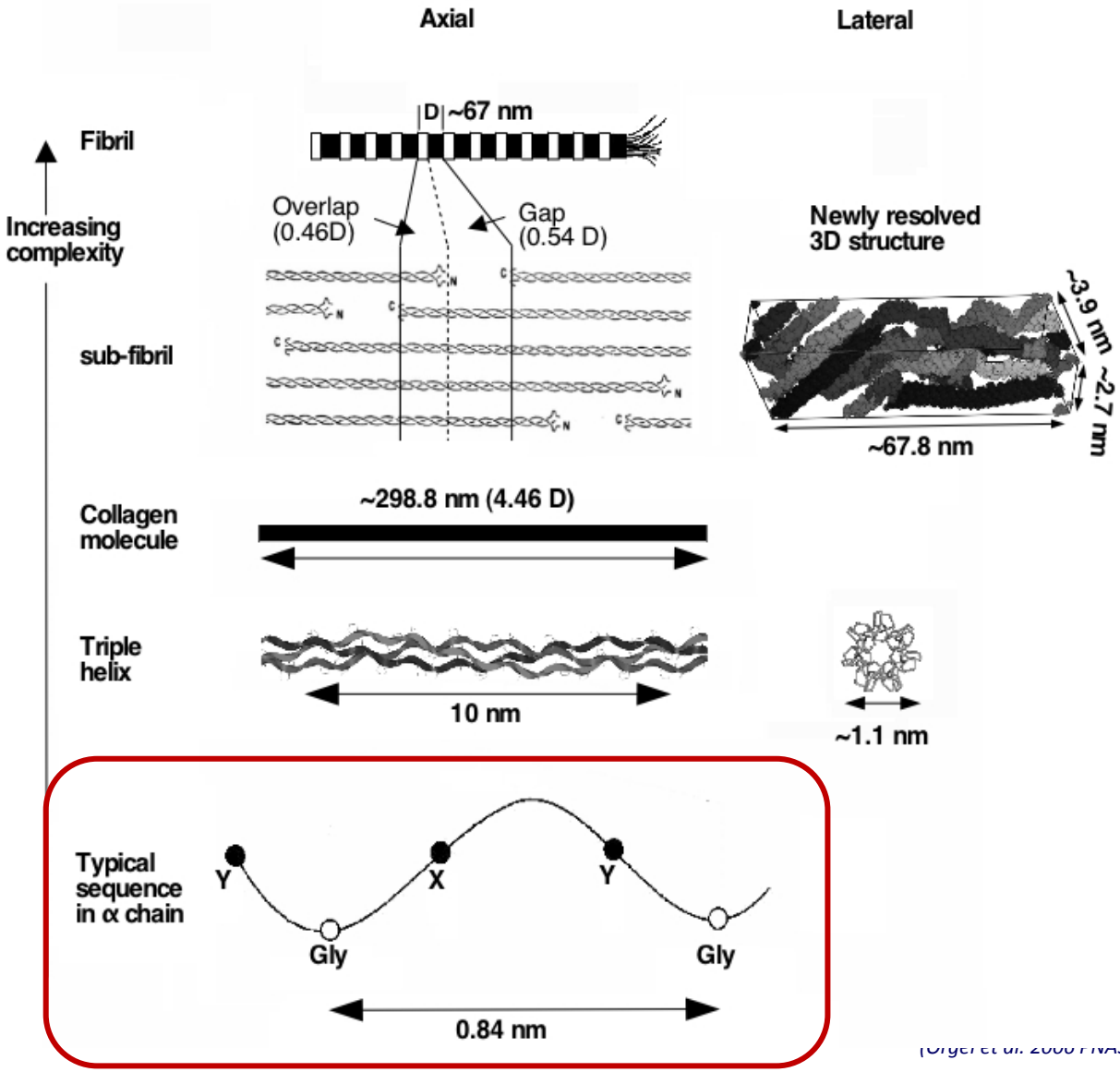
Bundling of several microfibrils that each contain clusters of 5 collagen molecules

Microfibril: the basic building block of the collagen fibril (30 - 500 nm)



(Gautieri et al. 2011 Nano Lett 11:757-766)

Collagens: from the α chains to fibrils



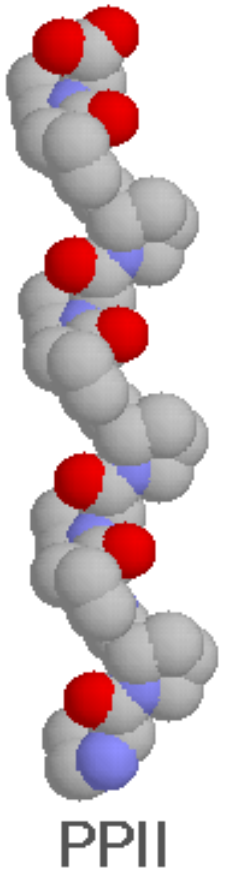
(Orgel et al. 2006 PNAS 103 :9001-9005)

Collagen α chains: polyproline II helices

- The α chains fold into a **polyproline II helix**

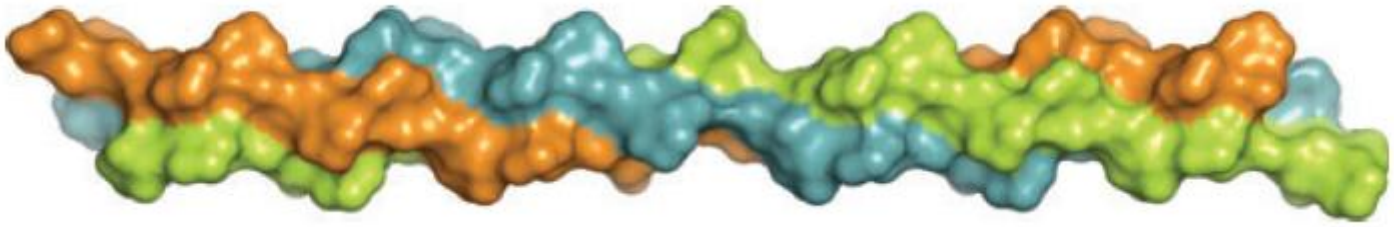
- Polyproline II helices are considered as unstable conformations

- **Structural plasticity** of **collagen α chain**



(Kramer et al. J Mol Biol 2001 311:131-47, Shoulders & Raines Ann Rev Biochem 2009 78: 929-58) *(Peysselon et al., Mol Biosyst 2011 7:3353-65)*

A common structural motif of collagens: the triple helix



300 nm x 1.5 nm

Supercoiling

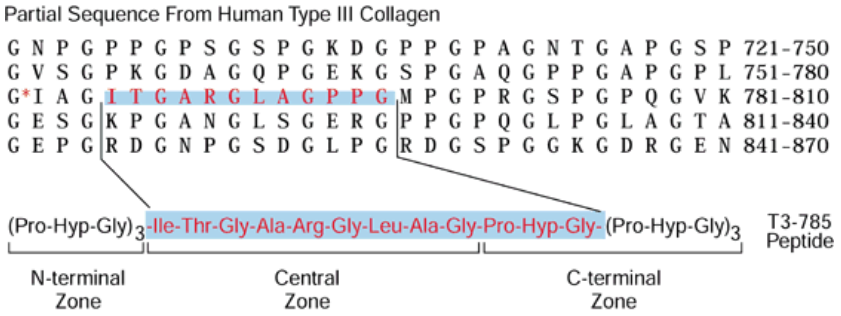
- The 3 α chains **wind around one another with a one-residue stagger**

- The formation of the triple helix requires **a specific amino acid residue** (glycine) **as every 3rd residue**

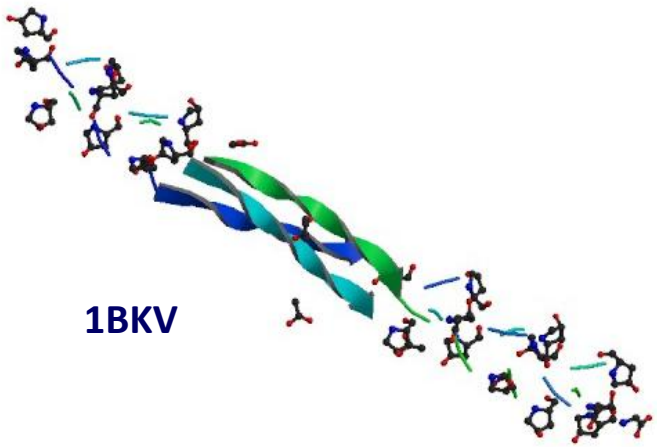
- **Sequence of collagen α chains: (Gly**-X-Y**)n**

(Leitinger Annu Rev Cell Dev Biol 2011 27:265–90)

The crystal structure of the collagen-like model peptide



3 identical chains of **12 residues** from collagen III capped with **$(Pro\text{-}Hyp\text{-}Gly)_3$** to aid helical stability

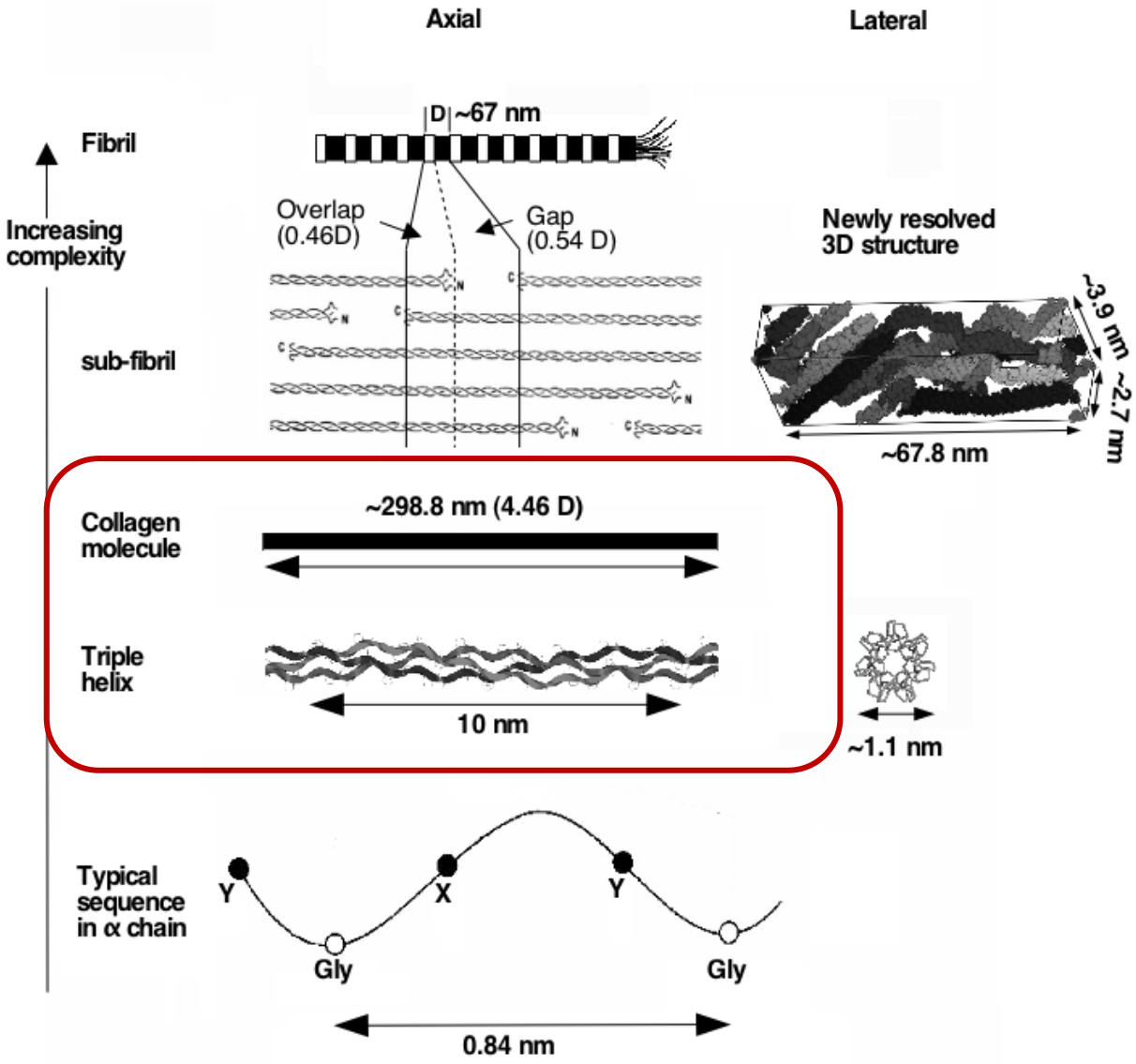


1BKV

Model to study how the amino acid sequence of collagen defines distinctive **local conformational variations** in **triple-helical structure**

(Kramer et al. Sequence dependent conformational variations of collagen triple-helical structure Nat Struct Biol 1999 6: 454-457, Kramer et al. J Mol Biol 2001 311:131-47)

Collagens: from the α chains to fibrils



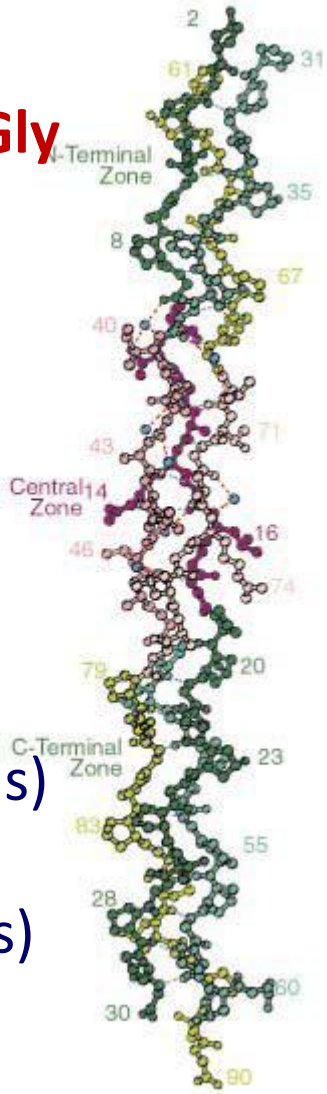
(Orgel et al. 2006 PNAS 103 :9001-9005)

Dependence of the triple-helical twist on sequence

$(Pro\text{-}Hyp\text{-}Gly)_3$ - **Ile - Thr - Gly - Ala - Arg - Gly - Leu - Ala - Gly - Pro - Hyp - Gly** -$(Pro\text{-}Hyp\text{-}Gly)_3$

Helical supercoiling changes into the 3 zones

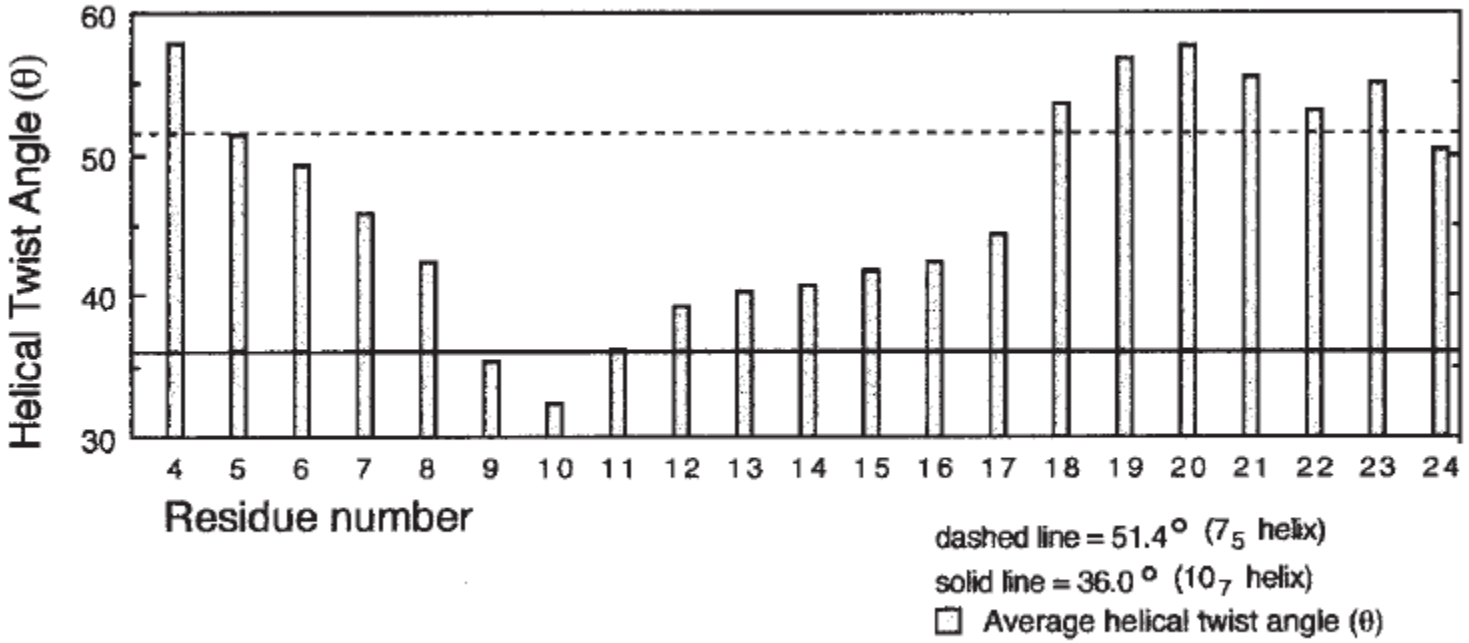
→ Sequence dependence of twist



- **Terminal** zones ~7-fold symmetry (high Pro and Hyp levels)
- **Central** zone 10-fold symmetry (low Pro and Hyp levels)

(Kramer et al. J Mol Biol 2001 311:131-47, Bella J Struct Biol 2010 170: 377-3

Dependence of the triple-helical twist on sequence



Graph of average helical twist angle for the 3 chains

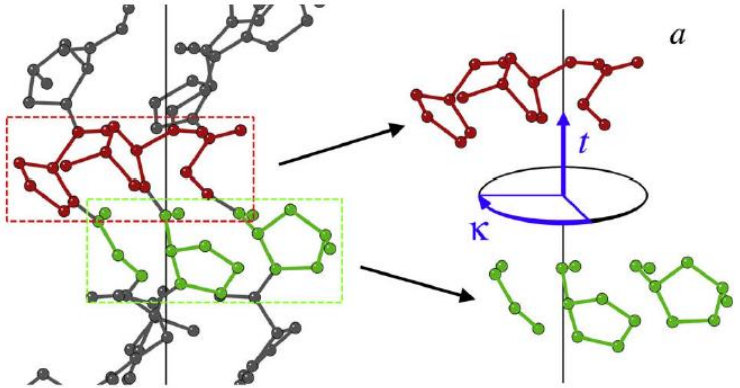
(Kramer et al. J Mol Biol 2001 311:131-47)

Dependence of the triple-helical twist on sequence

- The triple helix displays a **continuously changing triple helical conformation**

- Gradual **variation of its internal triple helical twist with its amino acid sequence** (local distribution of imino acid residues)

The rotation angle is the internal triple-helical twist (κ)



(Bella J Struct Biol 2010 170: 377-391)

The collagen sequence can influence local conformational changes in triple-helical structure

• Collagen triple helices tolerate significant local changes in **helical twist** to respond to **Sequence variability**

Imino acid content

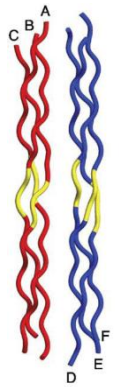
Gly-X-Y interruptions

• Changes in hydrogen bonding pattern (water-mediated interchain hydrogen bonds)

• Changes in hydration patterns

(Kramer et al. J Mol Biol 2001 311:131-47 - Bella et al., J. Mol. Biol. 2006 362:298-311

Plasticity of the collagen triple helix: role of Gly-X-Y interruptions



Asymmetric unit of the Hyp– crystal structure

- The **main conformational effect** occurs in the interruption **zone**

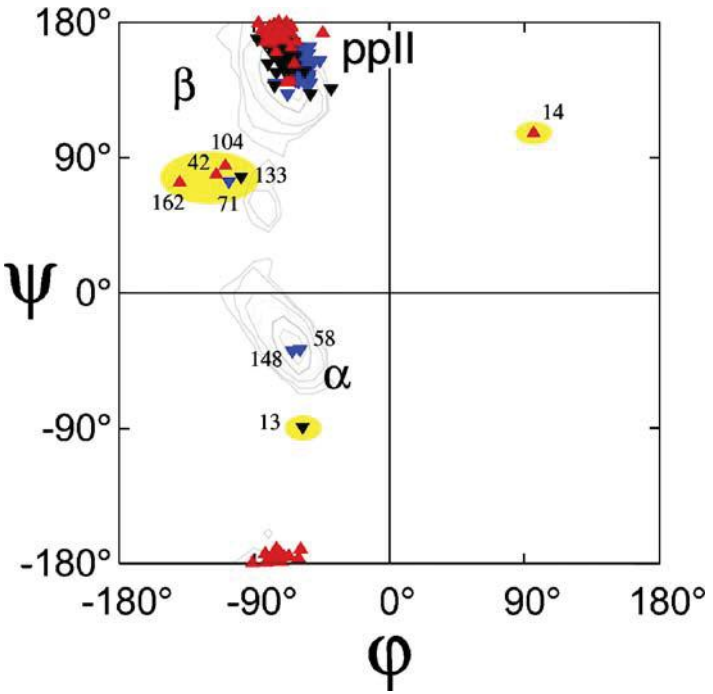
- **Highly localized** structural deviations

- The standard features* are **regained within 1 to 2 residues on either side**

***Hydrogen bonding topology**, **torsion angles**, **helical**, and **superhelical parameters**

(Bella et al., J. Mol. Biol. 2006 362:298-311)

Ramachandran map of the triple helical peptide with interruption



Pro residues: black triangles

Hyp residues: blue triangles

Gly residues: red triangles

Typical secondary structures α, α-helix;

β, β-sheet; ppII, polyproline II and collagen

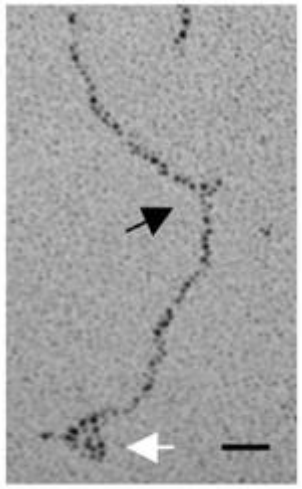
- Residues highlighted in yellow are from the interruption zone and depart from the typical conformational angles of the collagen triple helix
- Several residues in the central zone adopt conformational **angles outside the polyproline II region**

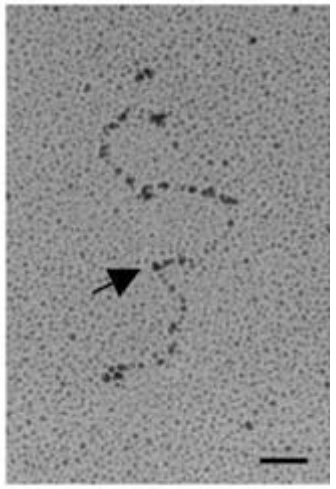
(Bella et al., J. Mol. Biol. 2006 362:298-311)

Plasticity of the collagen triple helix: role of Gly-X-Y interruptions

- These **interruptions define regions of flexibility** and **molecular plasticity**
- They **may** or **may not (plasticity)** result in **kinks** or **bends**

Rotary shadowing EM of collagen XVIII purified from chicken vitreous. Flexible or kinky regions ➔, likely due to the presence of the non-triple-helical domains that interrupt the triple-helix. Scale bar: 17 nm



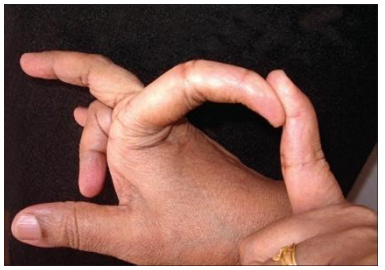


(Bella et al., J. Mol. Biol. 2006 362:298-311)

(Marneros et al., 2004 EMBO J 23:89-99)

Plasticity of the collagen triple helix: role of Gly-X-Y interruptions

- Interruptions of the -Gly-X-Y- repeating collagen sequence can
  - have severe **pathological effects in some collagens**
  - **be perfectly tolerable in others**

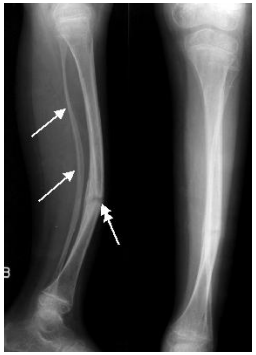


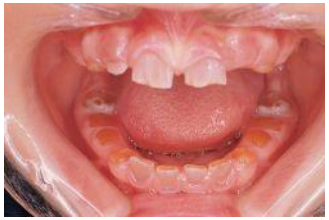
- The structural signatures **depend** on **the nature, location** and **extent of the breaks**

- The **tolerance of collagens** to **interruptions** in their **-Gly-X-Y-pattern** is **site specific**

(Bella et al., J. Mol. Biol. 2006 362:298-311)

Plasticity of the collagen triple helix and mutations

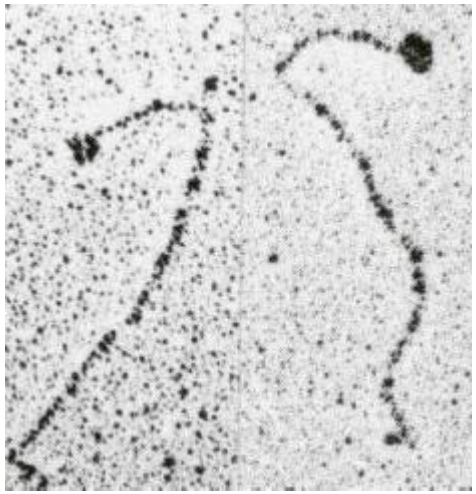




- A single-base mutation **Gly748Cys** in the α1 chain of procollagen I

- Lethal variant of **osteogenesis imperfecta**

- A significant fraction of the procollagen molecules had a **kink**



(Vogel et al. J Biol Chem 1988 263: 19249-19255)

Mutant procollagen by EM after rotary shadowing

Plasticity of the collagen triple helix

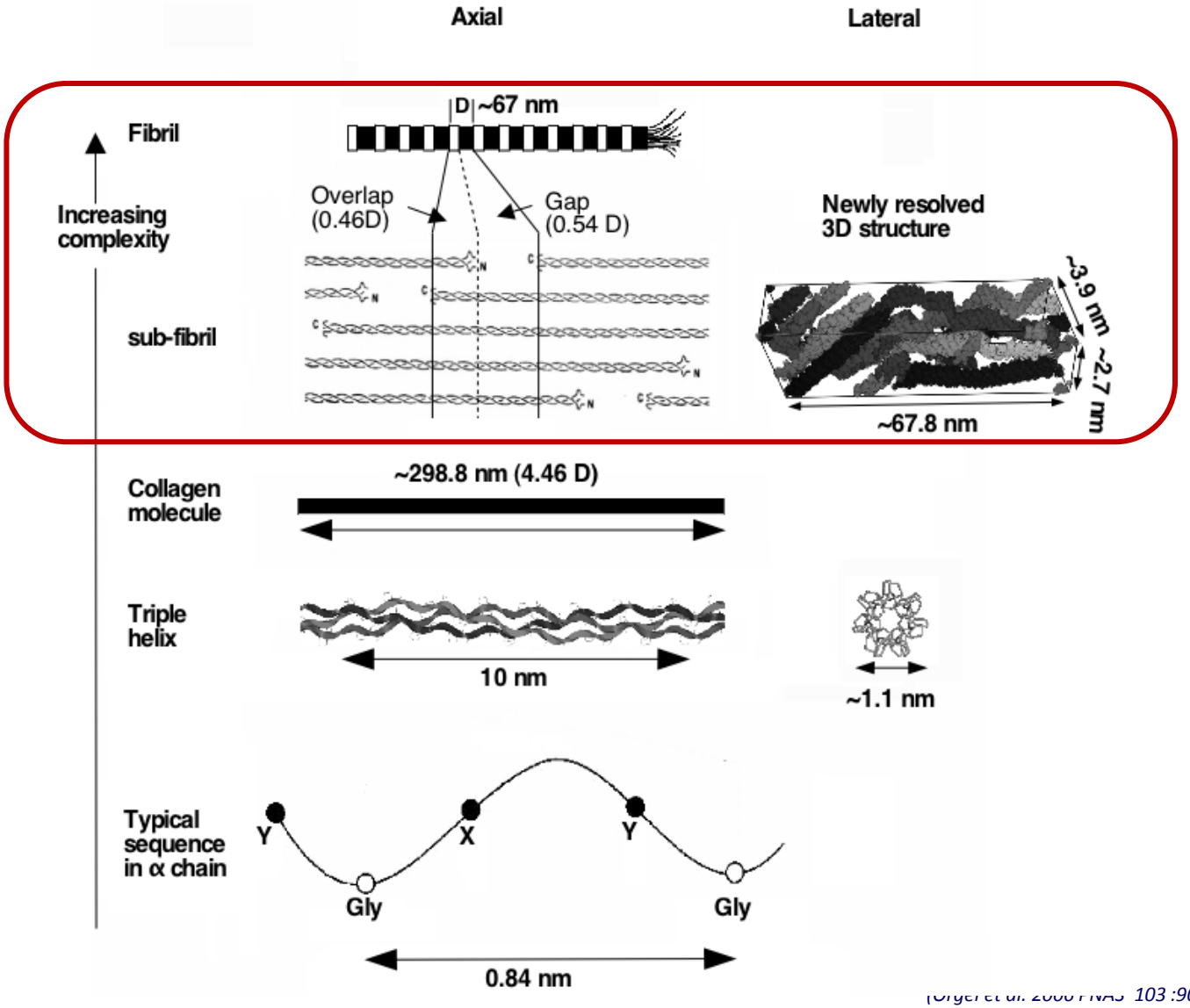
- **Flexible sites** (molecular plasticity) or **permanent kinks**

- A **macroscopic model for this plasticity** is a **3-stranded rope** that can be twisted or relaxed locally

- This **rope may react differently to torque forces along its length**, with perhaps local "rigid" spots at which further twisting (or relaxing) may not be feasible

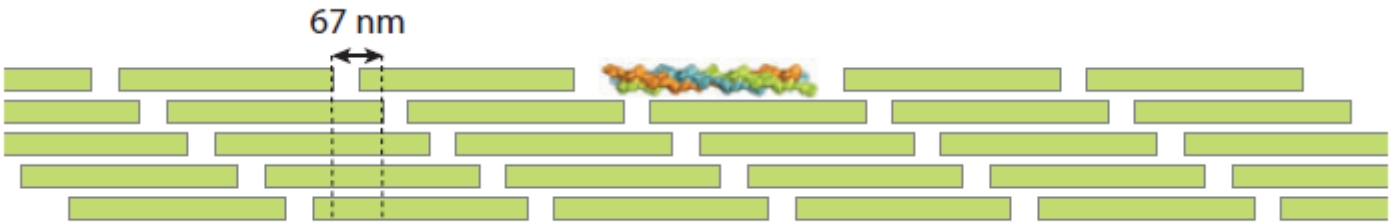
(Bella et al., J. Mol. Biol. 2006 362:298-311)

Collagens: from the α chains to fibrils



(Orgel et al. 2006 PNAS 103 :9001-9005)

From the triple-helix to the fibril



- Collagens form **fibrils** with a **banding pattern** (periodicity : 64-67 nm)

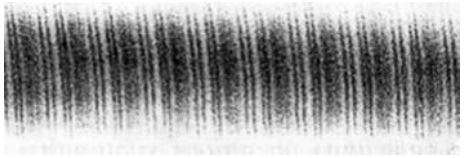
- Individual triple helices assemble into a **quarter-staggered array to form a collagen fibril**

- The triple helices are staggered by 67 nm (D-period)

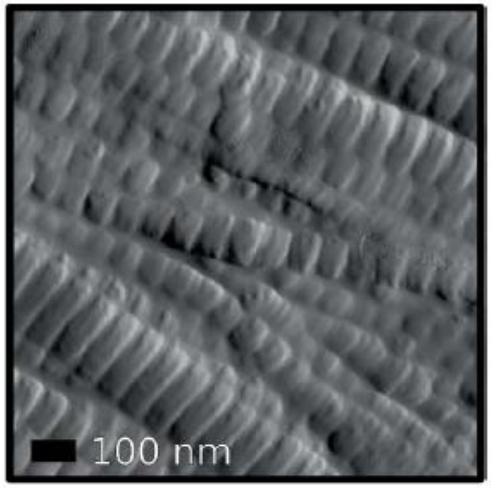
(Ricard-Blum, Cold Spring Harb Perspect Biol 2011 3:a004978)

(Leitinger Annu Rev Cell Dev Biol 2011 27:265–90)

Collagen fibrils

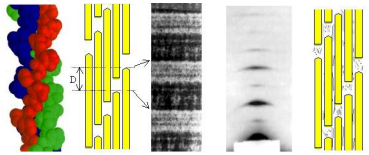


Fibrils

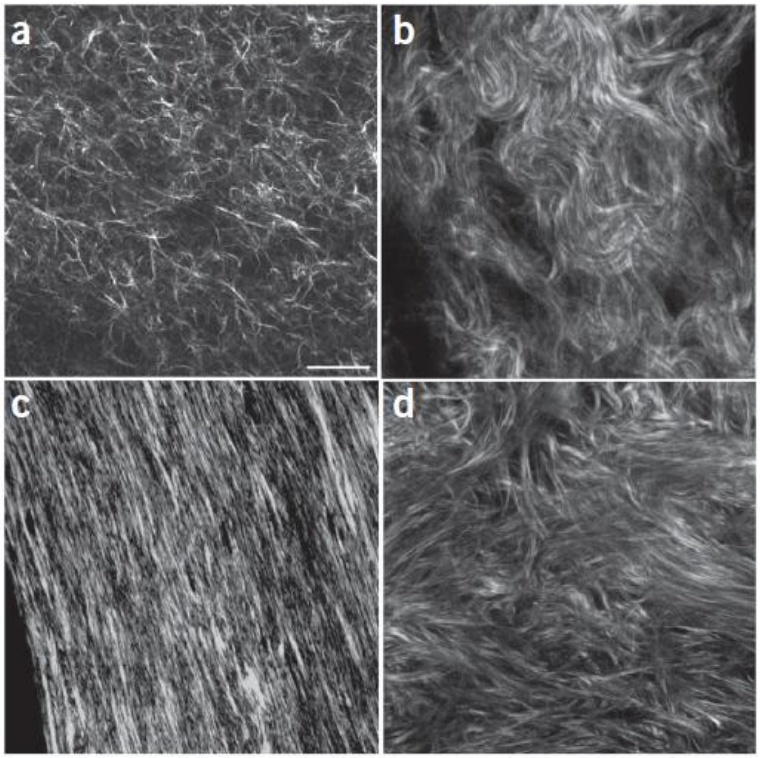


AFM of lamprey notochord, collagen II

Orgel et al. 2011 Connect Tissue Res 52: 18–24



Plasticity of collagen assemblies in tissues: a matter of context



a) Self-assembled collagen gel
b) Mouse dermis
c) Mouse bone
d) Human ovary
Scale bar: 30 µm

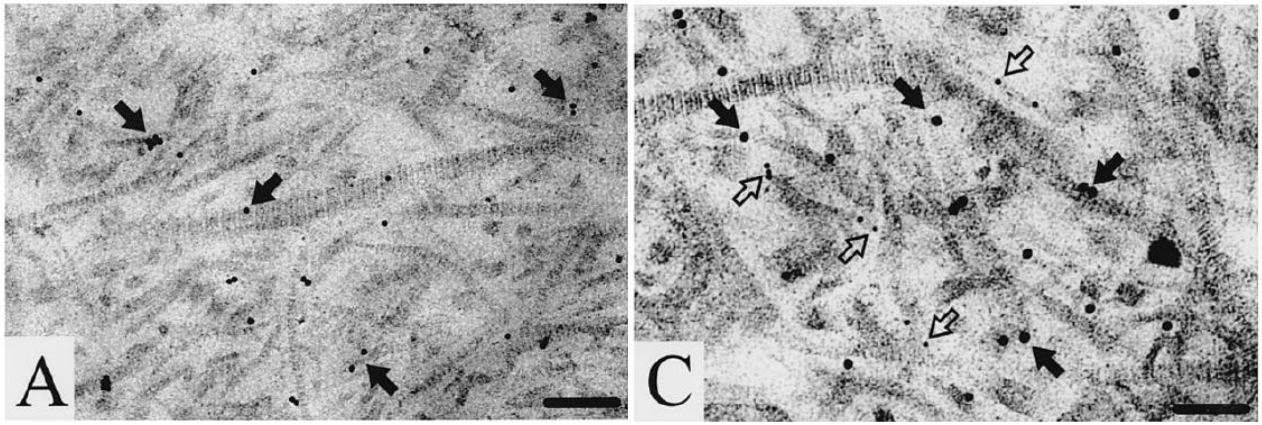
***Second Harmonic Generation**

- SHG* microcopy to **visualize** the **supramolecular assembly of collagens in tissues**
- Highly **sensitive to changes that occur in diseases** (e.g. cancer, fibrosis)

(Chen et al. Nat Protoc 2012 7: 654-669)

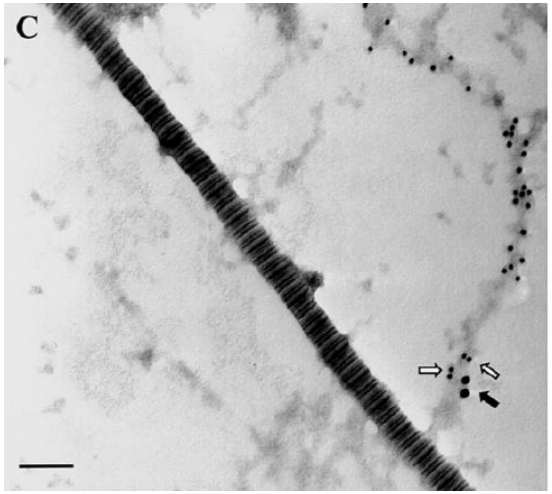
One collagen type, two tissue-specific assemblies

Collagen fibrils in cartilage



Collagen XVI ➡
Collagen IX ⇨
Bar: 0.18 µm

Beaded microfibrils in skin

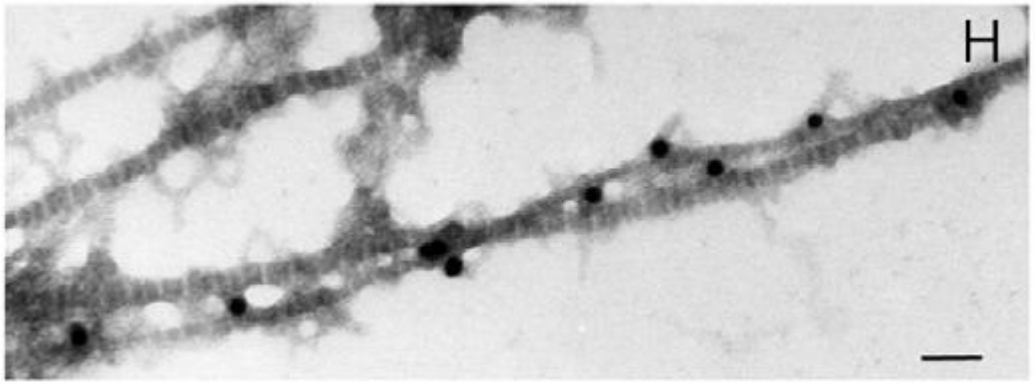


Collagen XVI ➡
Fibrillin-1 ⇨
Bar: 0.12 µm

(Kassner et al. Matrix Biol 2003 22:131–143)

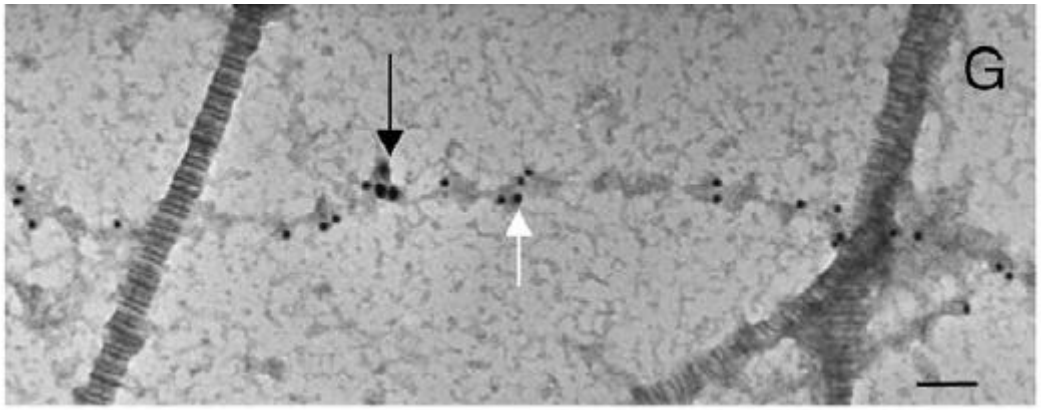
Plasticity of supramolecular assemblies: a matter of context

Collagen fibrils in cartilage



Bar: 0.18 µm

Beaded microfibrils in skin



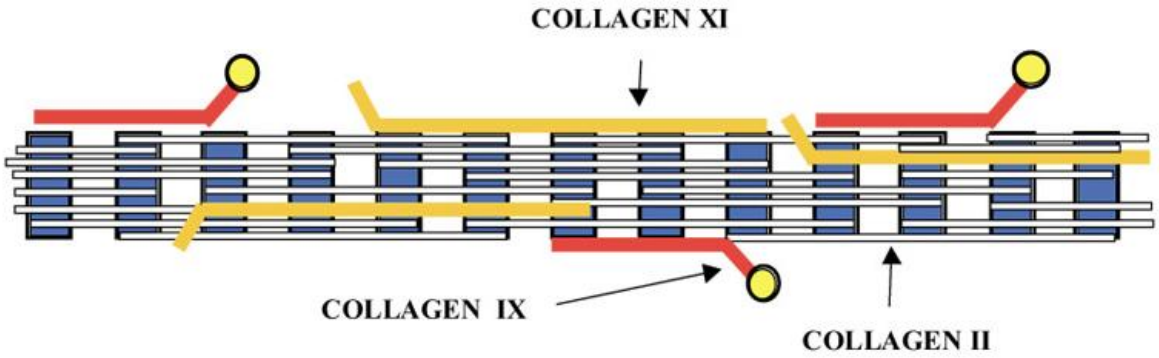
Bar: 0.12 µm

Collagen XVI →
Fibrillin-1

Grassel & Bauer Matrix Biol 2013 32: 64–73)

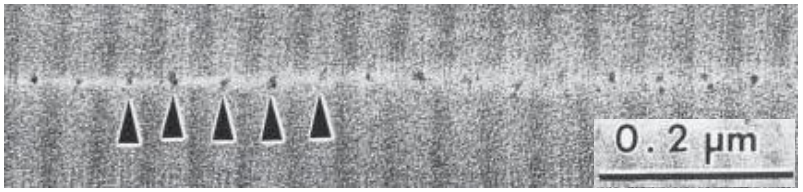
Collagen fibrils are macromolecular alloys

Several collagen types



(Reginato, Olsen Arthritis Res 2002 4:337-345)

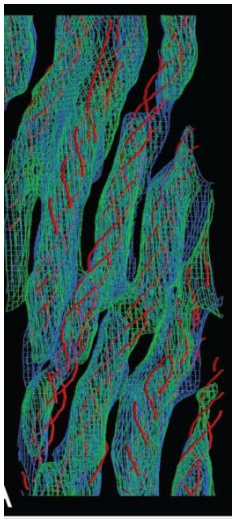
Proteoglycans bind via their protein core to collagen I fibrils within the d and e bands of the gap zone



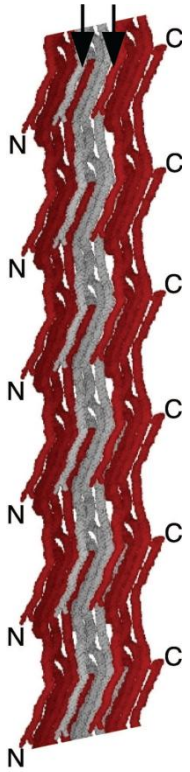
(Danielson et al. 1997 J Cell Biol 136:10729–743)

Microfibrillar structure *in situ*

3D arrangement of collagen molecules in naturally occurring fibrils



The common tilt of the 5 collagen segments within the overlap region



3 microfibrils

Molecular packing topology of the collagen molecule
Packing neighbors are arranged to form a supertwisted (discontinuous) right-handed microfibril that **interdigitates** (🡻) with neighboring microfibrils

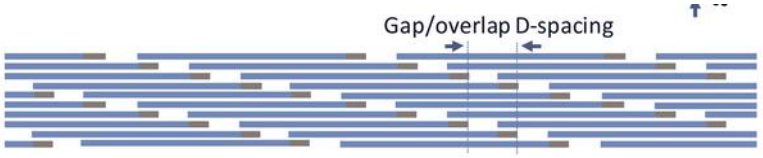
(Orgel et al. 2006 PNAS 103 :9001-9005)

Structural plasticity: a mechanism of the time-dependent stabilization of collagen fibrils?

- **Axial order is established immediately** (high specificity of the non-covalent interactions that drive assembly)

- X-ray diffraction measurements of native fibrils show **varying degrees of lateral disorder**

- **The fibril contains disordered regions**, especially in the gaps between the molecules



- They acquire **more order over time** by **internal rearrangements**

(de Wild et al., Biophys J. 2013 105:200-10)

Plasticity of protein structure

Conformational plasticity

Protein flexibility

Plasticity and physical properties of proteins

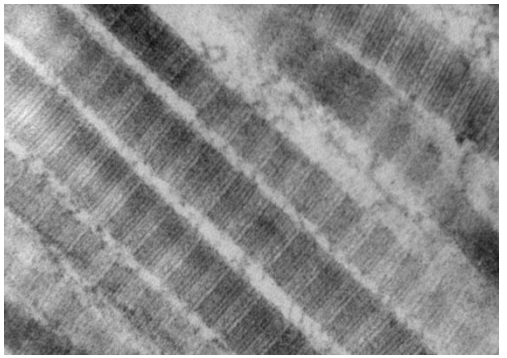
(Faísca and Gomes. On the relation between native geometry and conformational plasticity. Biophys Chem 2008 138:99-106)

Plasticity and physical properties of proteins

• **Plasticity: ability to remodel under applied loads** into anisotropic and inhomogeneous structures

• When tendons or ligaments are subjected to prolonged, or excessive, forces the elastic limits of the tissue may be exceeded

• The tissue enters the **plastic range:**
- **It** is **permanently deformed**
- **It** is **no longer able to return to its original state** following removal of the deforming force



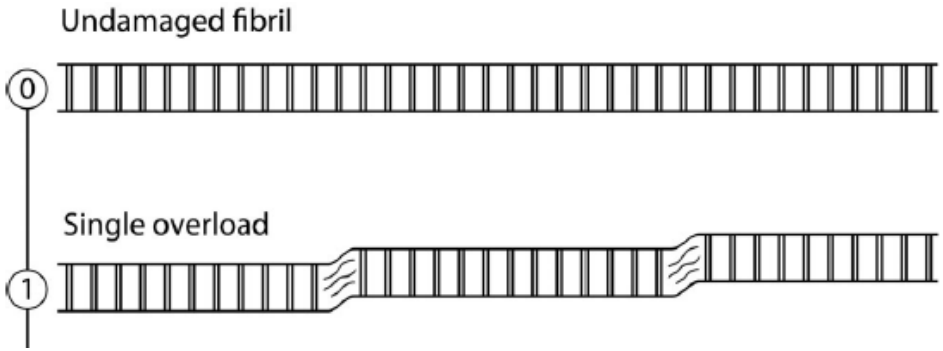
(Vader et al. 2009 PLoS One 4:e5902)

(William Weintraub Tendon and Ligament Healing: A New Approach to Sports and Overuse Injury 2003 Second revised and expanded edition Paradigm publications)

Nanostructural motif characteristic of overloaded collagen fibrils: discrete plasticity

• **Overloading tendons** causes **collagen fibrils** to develop **repeating kinks along their length**

Damage: **discrete plasticity**



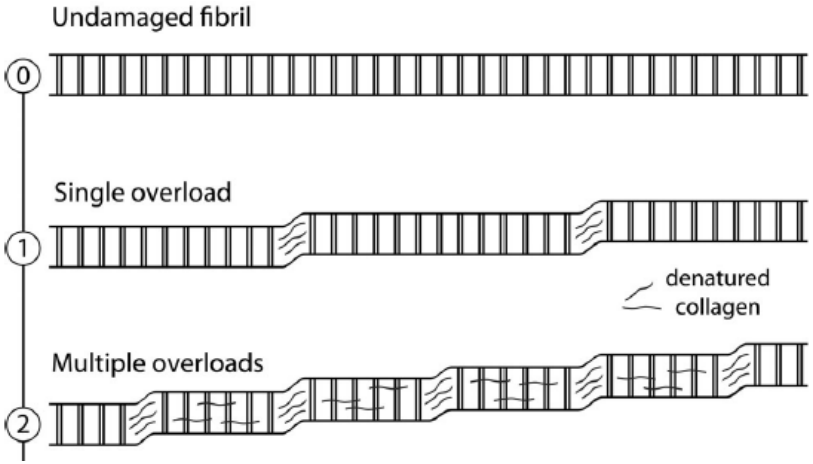
• The zones of plastic deformation created as a result of overload **do not progress to failure**

• **Discrete plasticity** does **not diminish the load bearing capacity of fibrils**

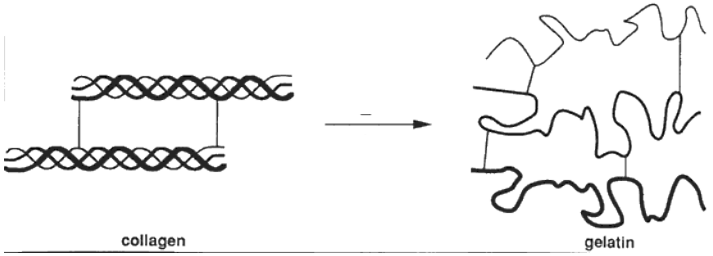
(Veres et al., J Orthop Res 2013 31:731-7)

Tensile overload causes discrete plasticity in fibrils

- With **successive overload** cycles, **fibrils** develop an **increasing number** of **kinks**



- The **kinks, discrete zones** of **plastic deformation,** contain **denatured collagen molecules**

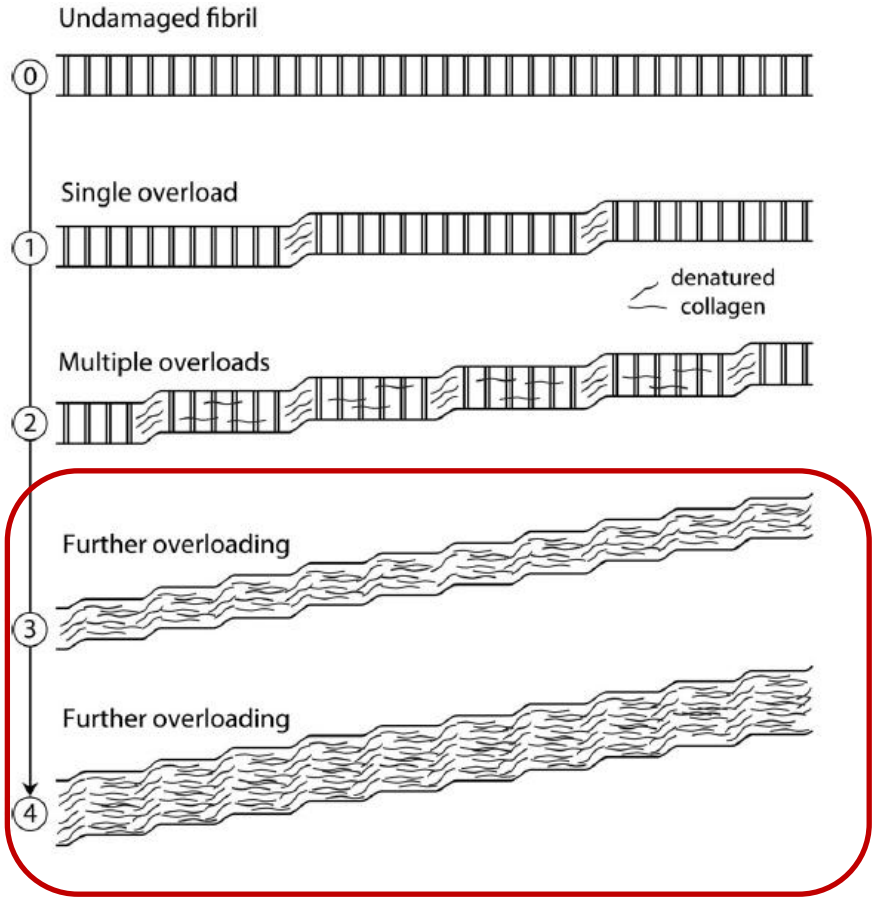


(Veres et al., J Orthop Res 2013 31:731-7)

Tensile overload causes discrete plasticity in fibrils

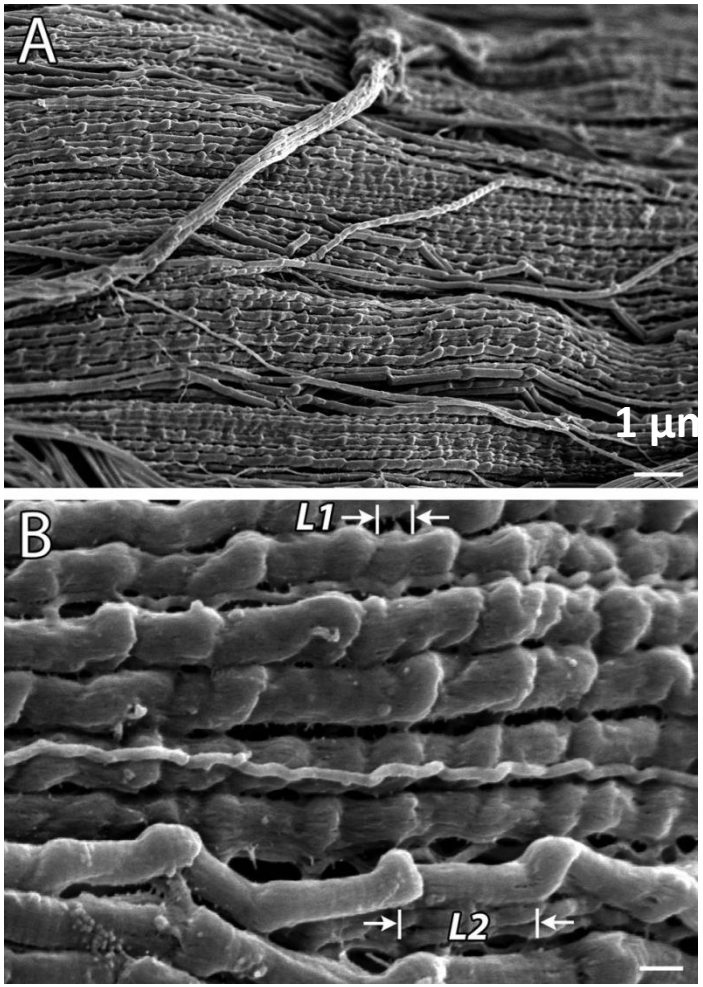
- **Progressive** and eventual **total loss of D-banding** along **the surface of fibrils**

- Loss of native molecular packing and further molecular denaturation



(Veres et al., J Orthop Res 2013 31:731-7)

Nanoscaled discrete plasticity damage in tendon fibrils



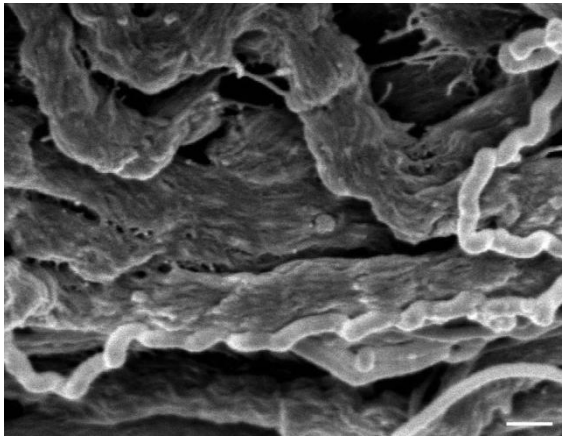
5 overload cycles

- **Kinked fibrils** are usually found in **large arrays**: a damaged fibril is bordered by many damaged fibrils

- **Severely damaged fibrils** with **short distances** between **successive kinks** and a **complete loss of D-banding** (L1=165 nm)
- A less damaged fibril with a greater separation distance between successive kinks and slightly obscured D-banding (L2=635 nm)

(Veres et al., J Orthop Res 2013 31:731-7)

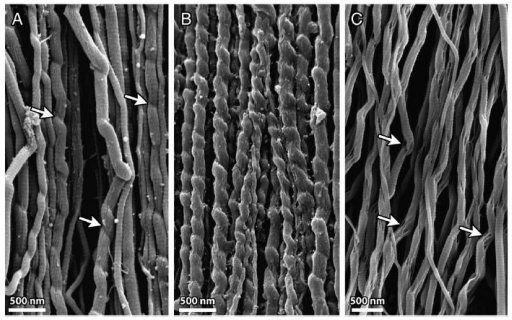
The progression of discrete plasticity damage in fibrils with increasing subrupture overloading



Eventually, fibrils expand and acquire a loose fibrous appearance as the **lateral cohesion between subfibrillar components is lost**

(Veres et al., J Orthop Res 2013 31:731-7)

Kinks collagen fibrils are digested by proteases



- Fibrils develop discrete, repeating zones of pronounced distortion → **nanoscale kinked appearance**

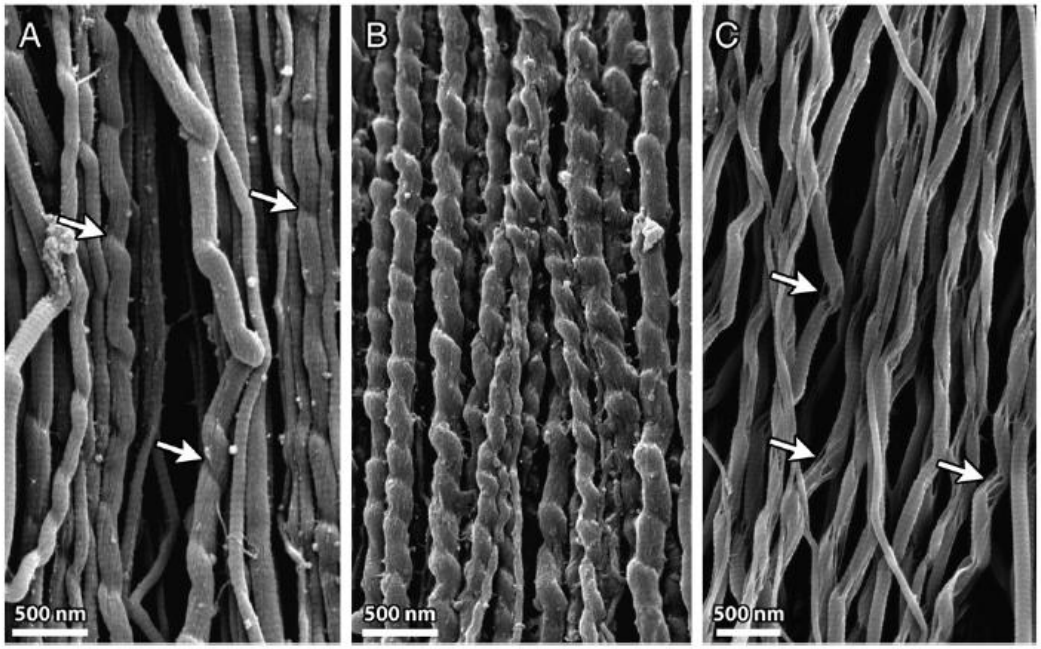
- With **repetitive** overloading, **new** discrete **zones of plasticity** develop between the preexisting zones

- The **dissolution of the overloaded fibrils by enzymes** is mostly at the location of the **kinks**

- **Tensile overload** does not simply destabilize collagen molecules promoting micro-unfolding: it places them in a **stable, denatured state**

(Veres et al. Matrix Biol 2014 33: 54-59)

Kinks of collagen fibrils are digested by proteases



A Pulling tendons to rupture causes fibrils to develop kinks (arrows) that repeat every ~300-800 nm along their length

B Repeated, **subrupture overloading** causes the number of kinks that fibrils have to increase

C Exposing fibrils with discrete plasticity damage to **proteases that can only digest non-helical collagen** causes partial dissolution of the kinks. Note that the kinks marked by arrows in image C (tendon pulled to rupture and then digested at 30 °C with trypsin) are missing material compared to the kinks marked by arrows in A (tendon pulled to rupture but not digested)

Images A & B taken at 30,000×. Image C taken at 25,000×

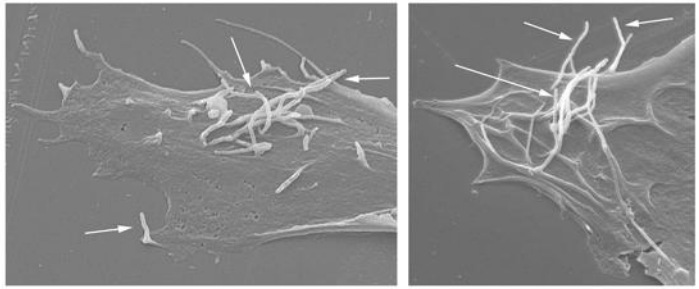
(Veres et al. Matrix Biol 2014 33: 54-59)

Collagen fibril discrete plasticity is detected by cells

• **Macrophage-like cells** respond specifically to overload-damaged collagen fibrils.

• When adherent to damaged **collagen fibrils, the cells clustered less, showed ruffled** membranes, and frequently spread, increasing their contact area with the damaged substrate

Membrane ruffling is the formation of a motile cell surface

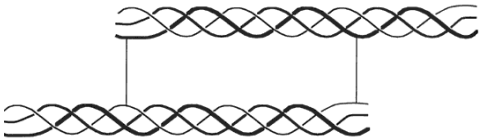


Membrane ruffles as viewed by scanning electron microscopy

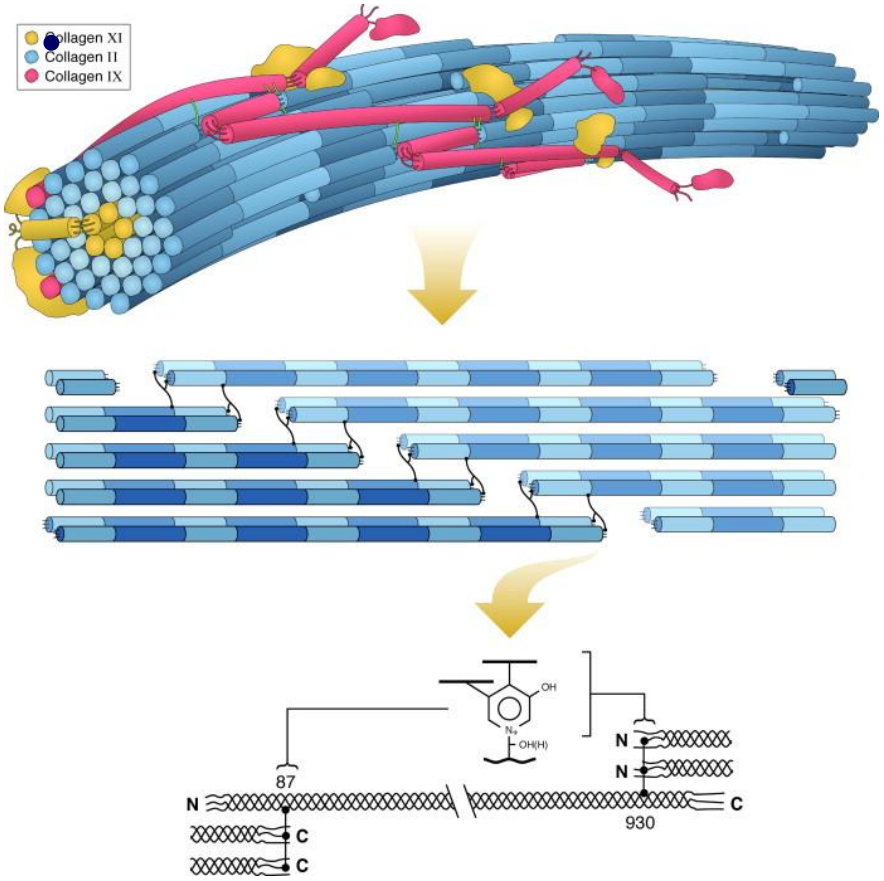
• **Pericellular collagenolysis of damaged collagen** but not of control collagen

(Veres et al. J Biomed Mater Res A. 2014 Mar 10) *http://www.reading.ac.uk/cellmigration/ruffling.htm*

Collagen cross-linking and mechanical properties



- **Shape**
- **Mechanical properties**
- **Stiffness of tissues** which modulates cell proliferation and differentiation



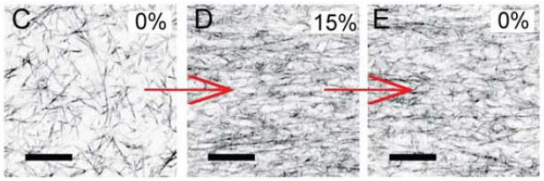
(Robins 2008 Biochem Soc Trans. 2007 35:849-52, Eyre et al. Methods 2008 45:65-74, Eyre et al. J Biol Chem 2010 285:16675-82)

Strain and plasticity: role of cross-linking
Spatial variations of collagen fiber alignment and densification

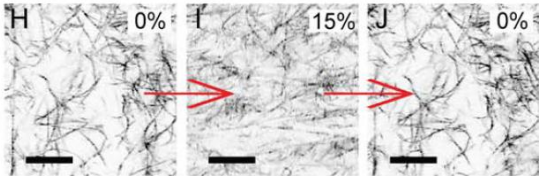
• The experimental setup mimics a deformation profile imposed by **cells** *in vivo*

• **Fiber alignment** and **densification** occur as a function of applied **strain** for uncross-linked and cross-linked collagenous networks

• This alignment is **irreversibly imprinted in uncross-linked collagen** networks

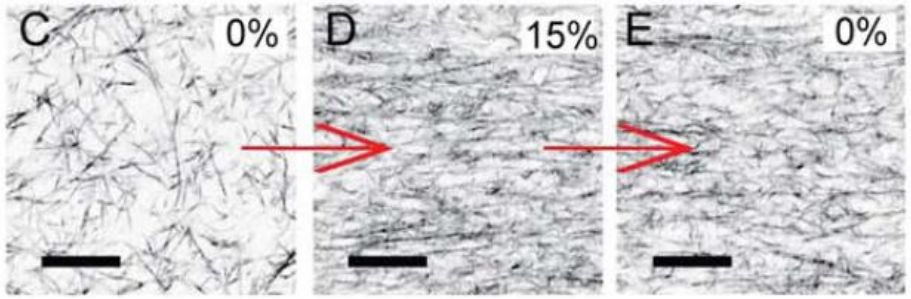


• **Cross-linked** networks display similar fiber alignment and the same geometrical properties as uncrosslinked gels, but with **full reversibility**



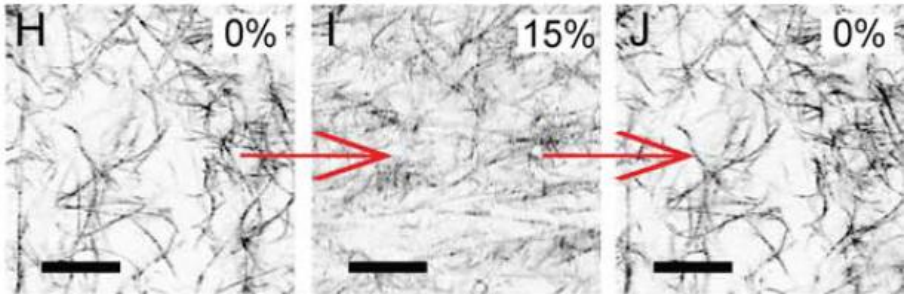
(Vader et al., 2009 PLoS One 4:e5902)

Strain and plasticity: role of cross-linking



Bars = 50 µm

Confocal reflectance images



Bars = 25 µm

Uncross-linked collagen gels

Cycled 4 times up to 15% stretch and back to 0%

C Beginning of 1st stretch cycle

D Middle of 1st stretch cycle

E End of 1st stretch cycle

Gels cross-linked with glutaraldehyde

Cycled 4 times up to 15% stretch and back to 0%

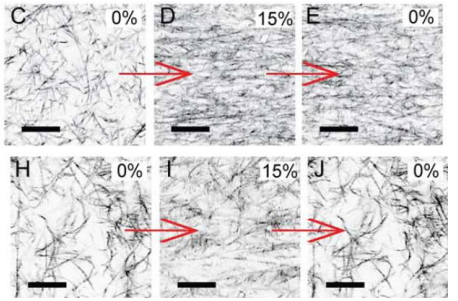
H Beginning of 3rd stretch cycle

I Middle of 3rd stretch cycle

J End of 3rd stretch cycle

(Vader et al. 2009 PLoS One 4:e5902)

Cross-linking and plasticity



- **Plasticity is not required to align fibers**

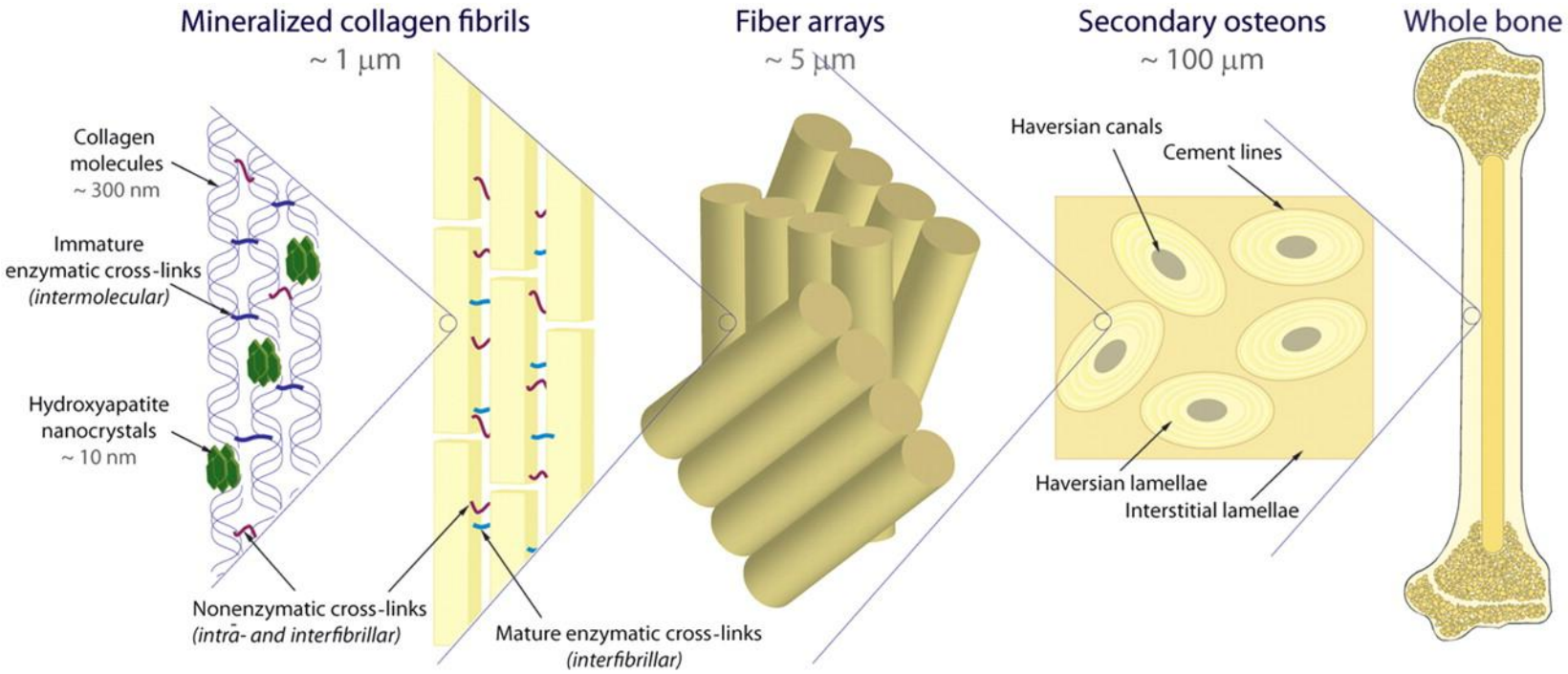
- **Microscopic origin** of the permanent texturization occurring in uncrosslinked samples?

- **Fibril-fibril junctions** are likely to be where **plastic deformation occurs** allowing **fibrils to slide** with respect to one another inducing **irreversible changes** in the network topology

- **Cross-linking** strengthens these junctions and **reduces the amount of plastic reorganization** allowed at the network level

(Vader et al. 2009 PLoS One 4:e5902)

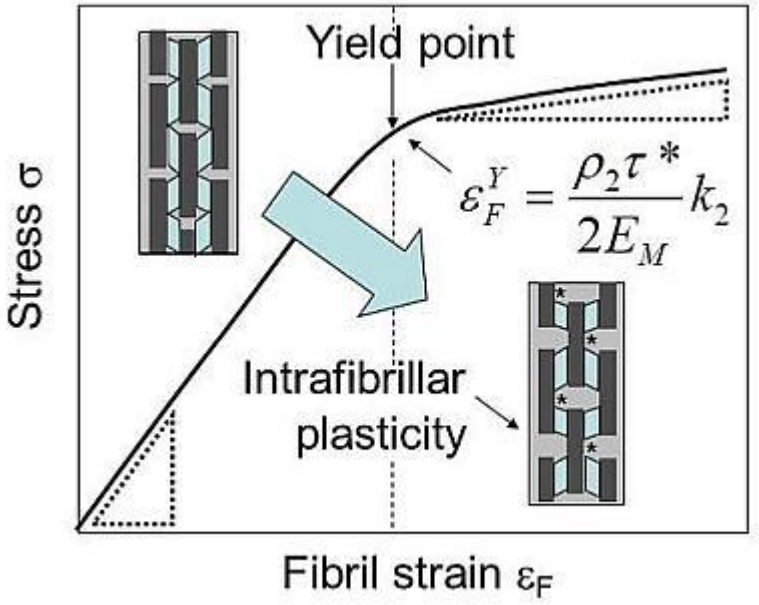
Plasticity in bone collagen



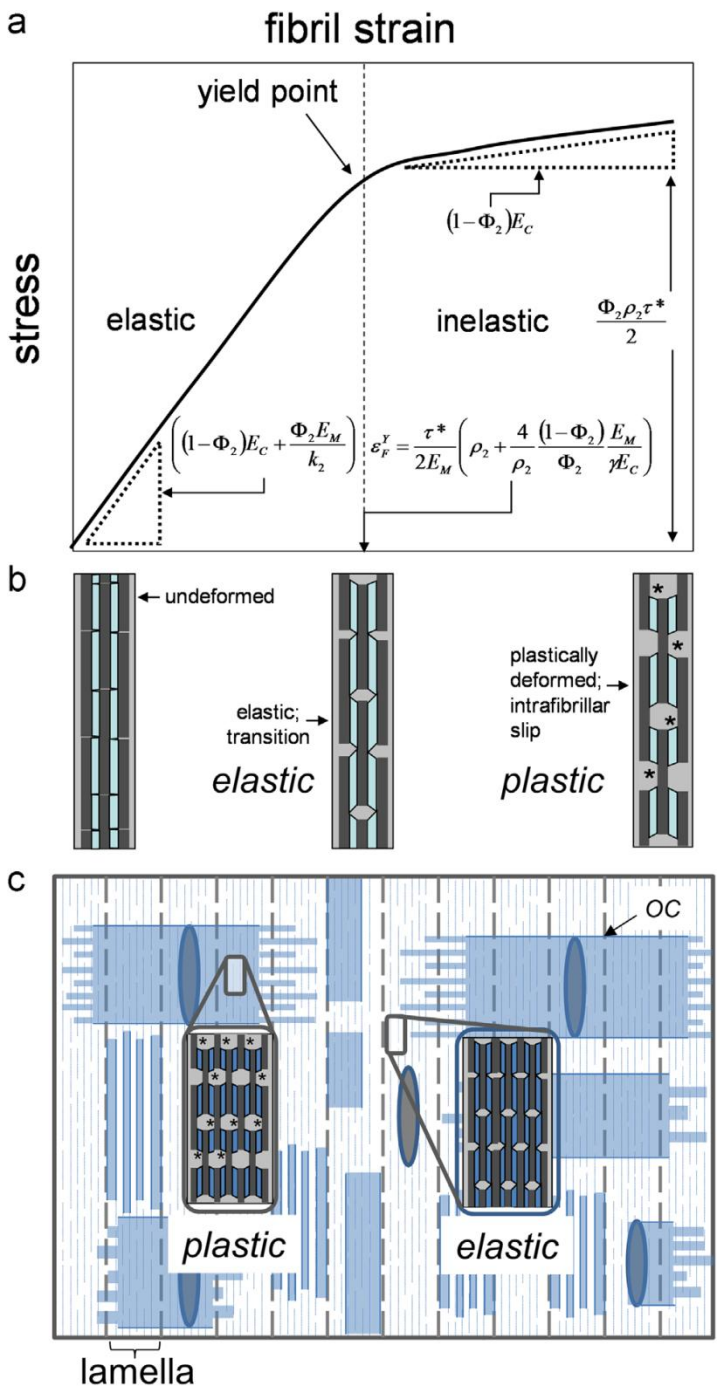
(Zimmermann et al. Proc Natl Acad Sci USA 2011 108:14416-21)

Collagen intrafibrillar plasticity

Intrafibrillar plasticity through mineral/collagen sliding is the dominant mechanism for the extreme toughness of antler bone



(Gupta et al., J Mech Behav Biomed Mater. 2013 28:366-82)

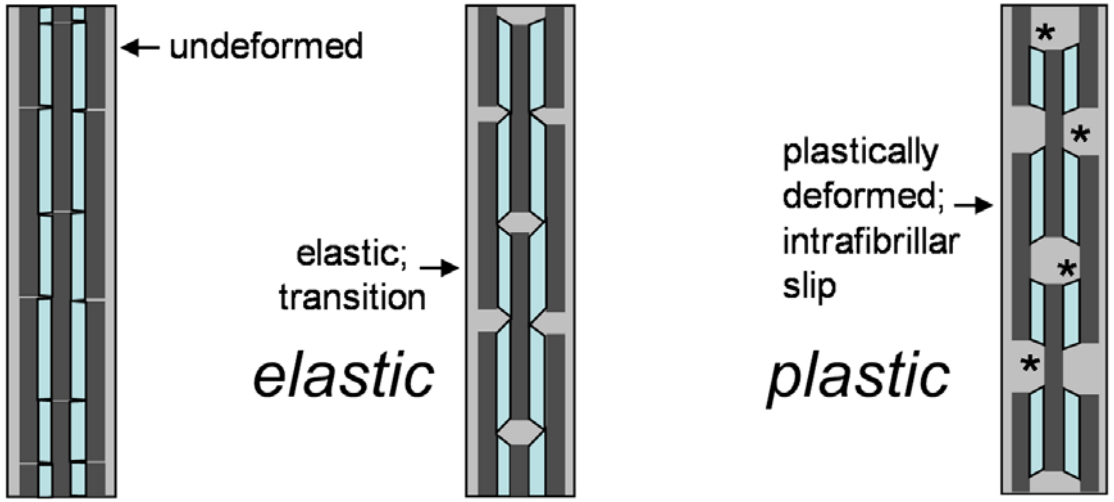


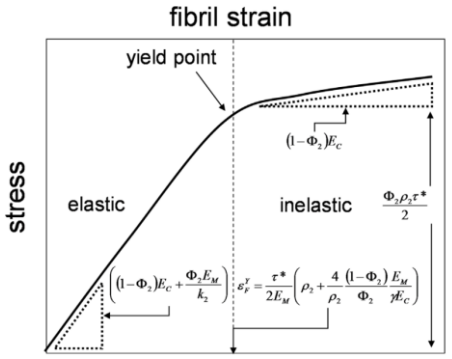
Collagen intrafibrillar plasticity

Model of intrafibrillar and fibril-array level inelastic deformation

(Gupta et al., J Mech Behav Biomed Mater. 2013 28:366-82)

Collagen intrafibrillar plasticity



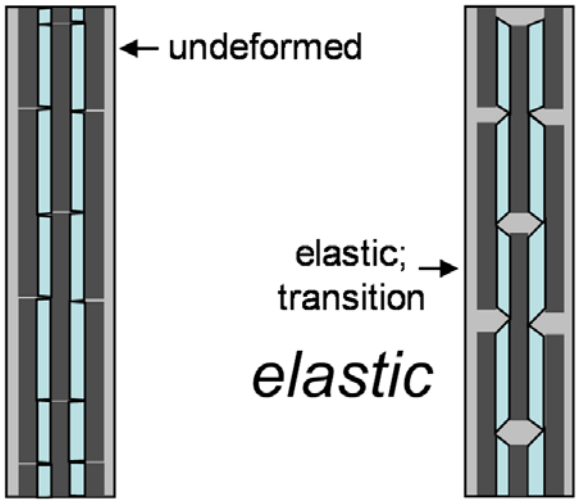


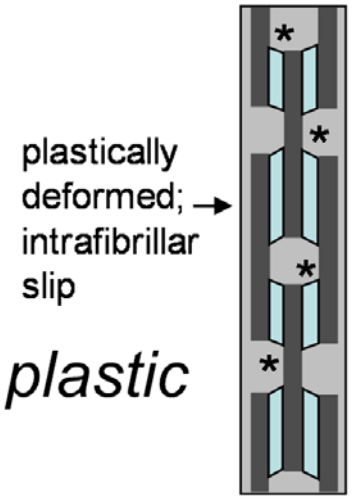
Undeformed **Deformed elastically** **Plastically deforming**

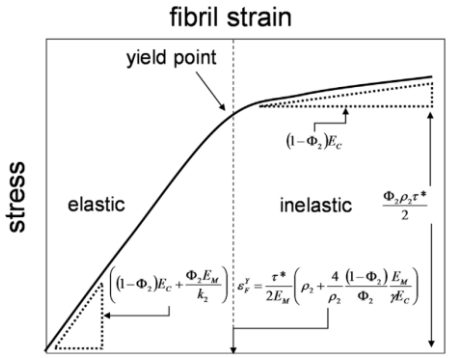
Intrafibrillar sliding between **mineral** and **collagen** leads to **permanent plastic strain** at both the **fibril** and the **tissue** level

(Gupta et al., J Mech Behav Biomed Mater. 2013 28:366-82)

Collagen intrafibrillar plasticity







Synchrotron small-angle X-ray diffraction

Undeformed fibril

Deformed elastically (at or just before the yield point)

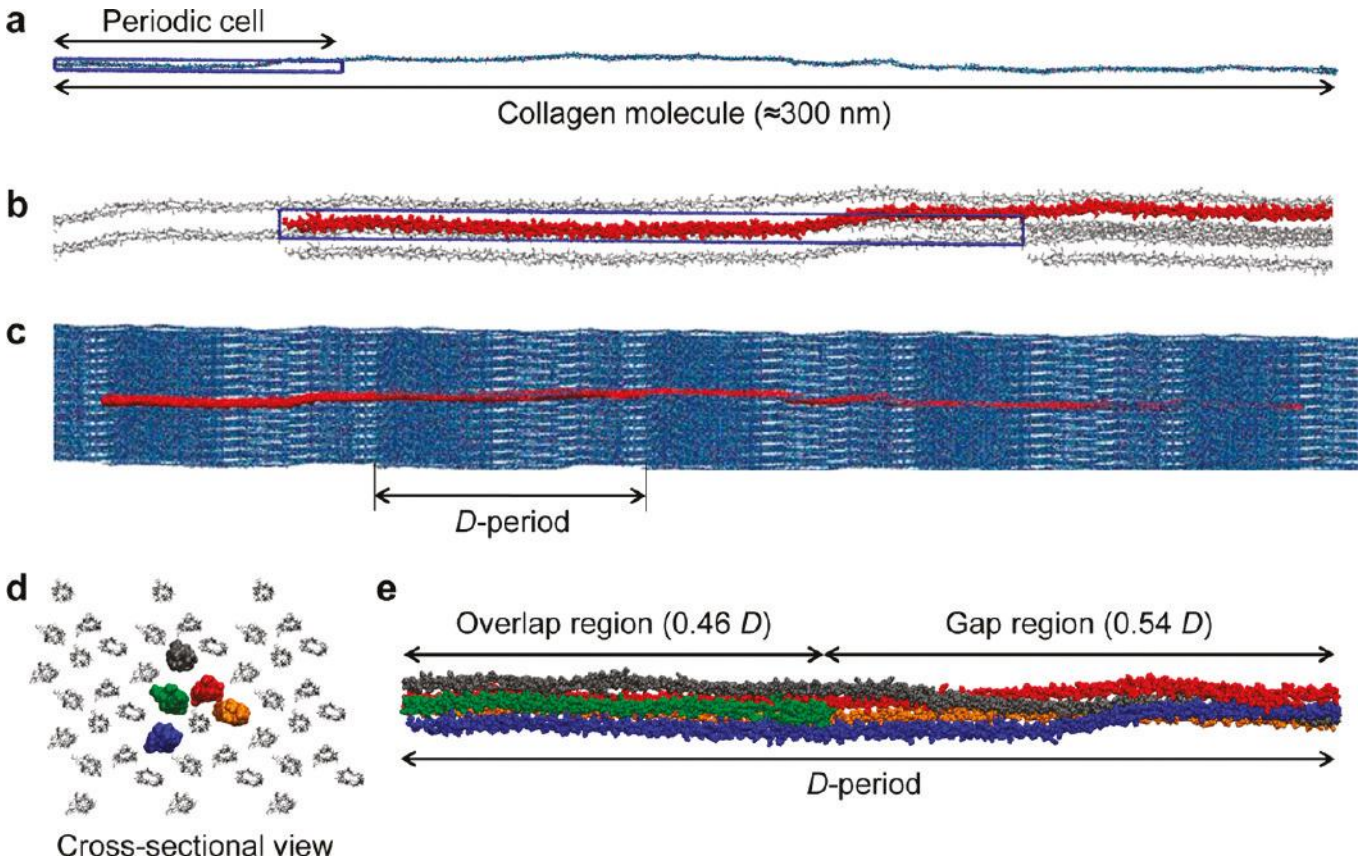
Plastically deforming with intrafibrillar slip as shown by the displaced shear parallelepipeds *

Schematic of intrafibrillar deformation in elastic and inelastic zones

(Gupta et al., J Mech Behav Biomed Mater. 2013 28:366-82)

Atomistic-based hierarchical multiscale modeling

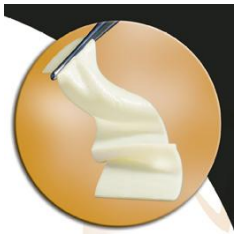
Atomistic model of the collagen microfibril



Quasi-hexagonal packing of collagen molecules, which interdigitates with neighboring molecules to form a supertwisted right-handed microfibril

(Gautieri et al. 2011 Nano Lett 11:757-766 V, esentini et al., Muscles, Ligaments and Tendons Journal 2013 3: 23-34)

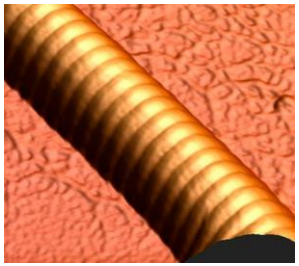
Engineering new collagen materials



• The availability of a model may lead to the design of new biomaterials for regenerative medicine

• A **fibrous architecture** in combination with **constituents that exhibit internal plasticity** creates **a material whose mechanical response adapts to external loading conditions**

• The imino acid composition and distribution of a collagen sequence can be used to predict its average triple helical conformation → **Design of artificial collagen sequences with different triple-helical twists**



(Münster et al.. Proc Natl Acad Sci U S A. 2013 110:12197-202)