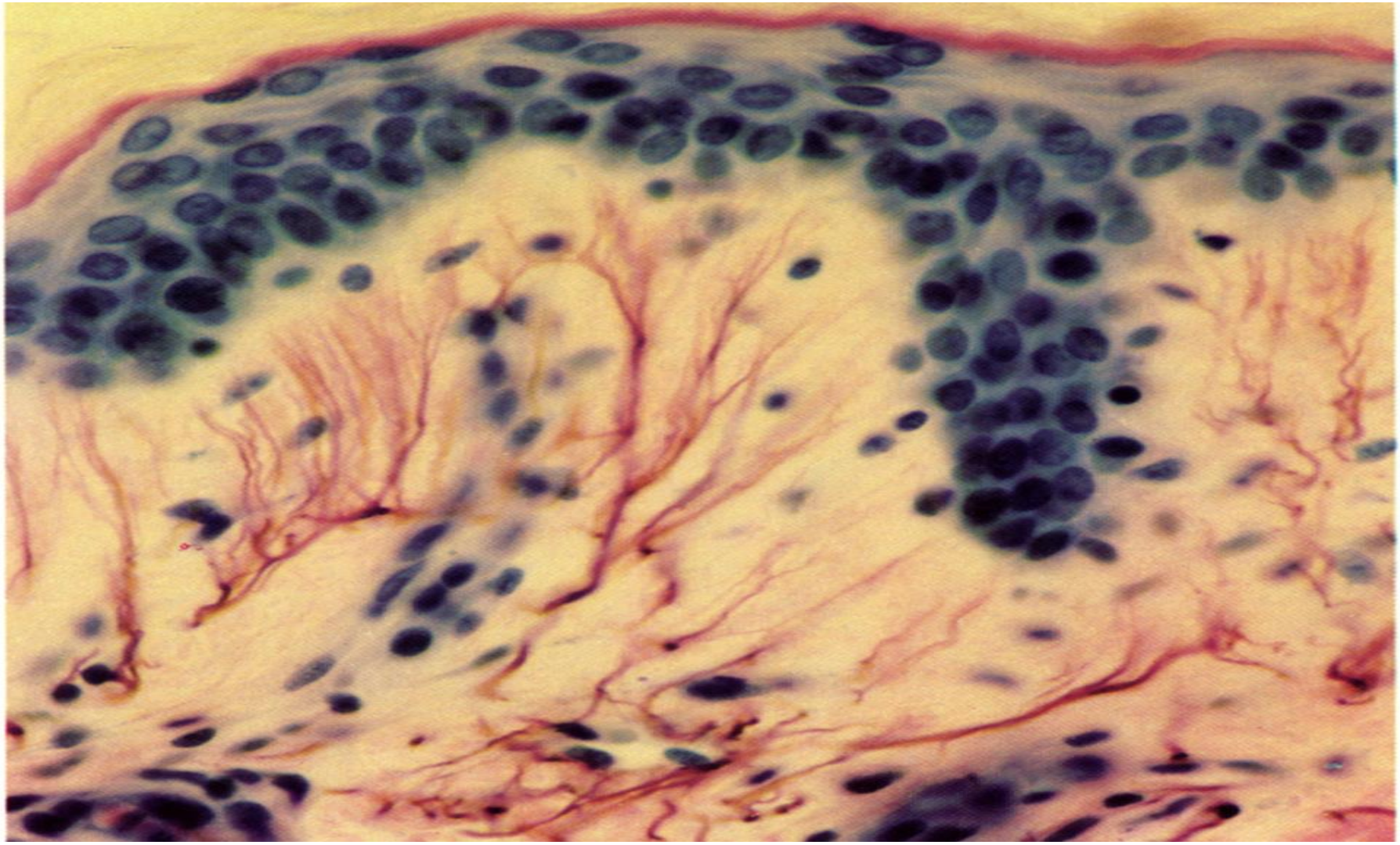
Effects of Temperature and Sodium Hyaluronate on Fluorescence of type I Calfskin Collagen Solutions at Physiological pH

Julian M Menter, PhD
Morehouse School of Medicine
Atlanta GA 30310

Histology of Normal Human Skin



What Photochemical Fluorescence Spectra and Fading Properties Can Also Tell Us

- Can give insight into collagen molecular dynamics (e.g. changes in physical state, helix – coil transition, photochemical transformations)

- Can give insight into photophysical properties (properties and energy levels of fluorescent and photo – reactive states)
-

Some properties of skin collagens

- Skin collagens belong to a genetically distinct group of structural proteins.

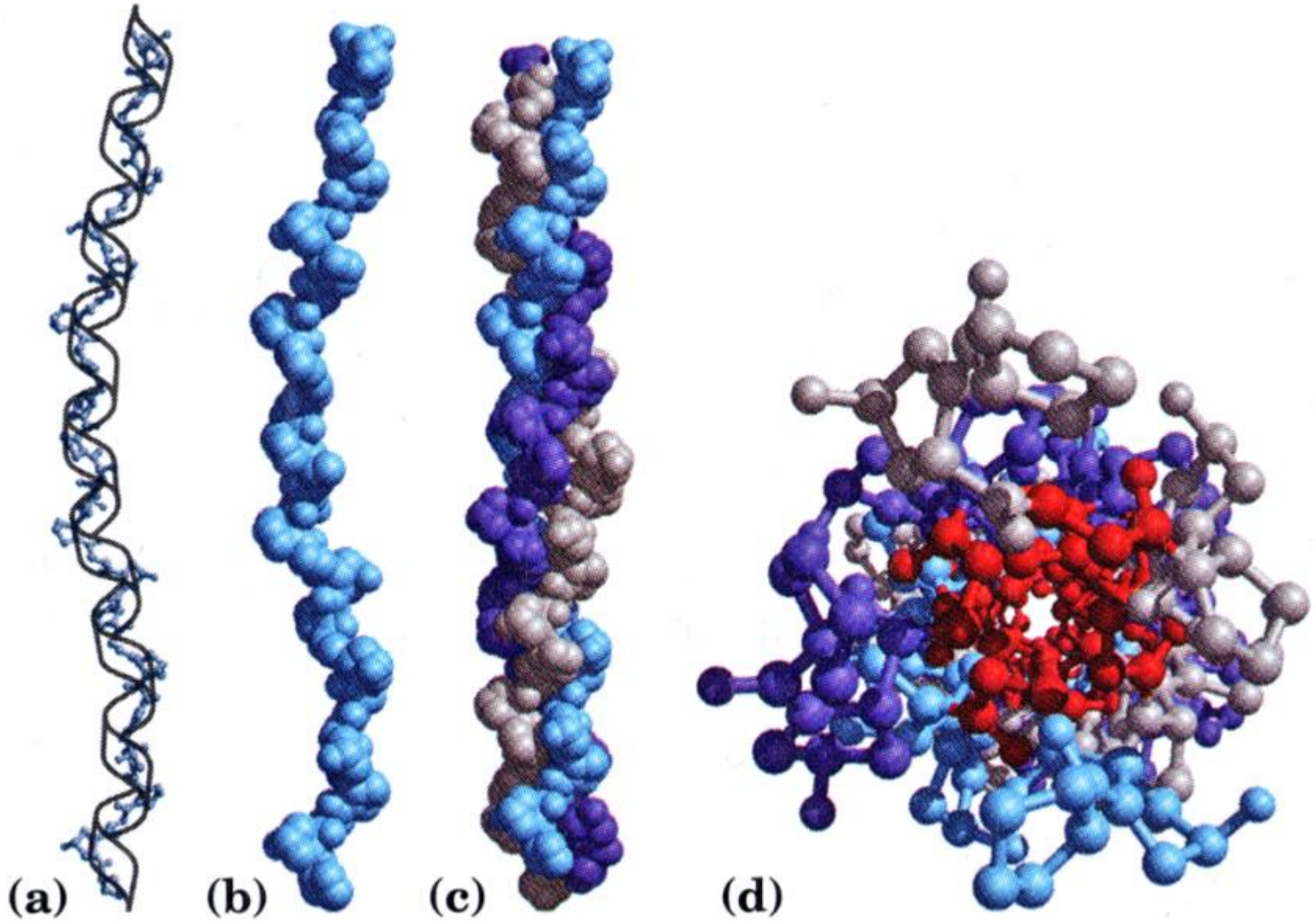
- Type I and Type III Collagens Are the Predominant Collagens in the Dermis, in the Ratio ~ 85:15.

- In this work, we focus on Type I collagen

Structural Aspects of Type I Collagen (1)

Tropocollagen is the basic structural unit of skin collagen; it consists of 2 α chains and 1 β chain. These chains have a left – handed helical structure, and they are wound in a right – handed superhelix.

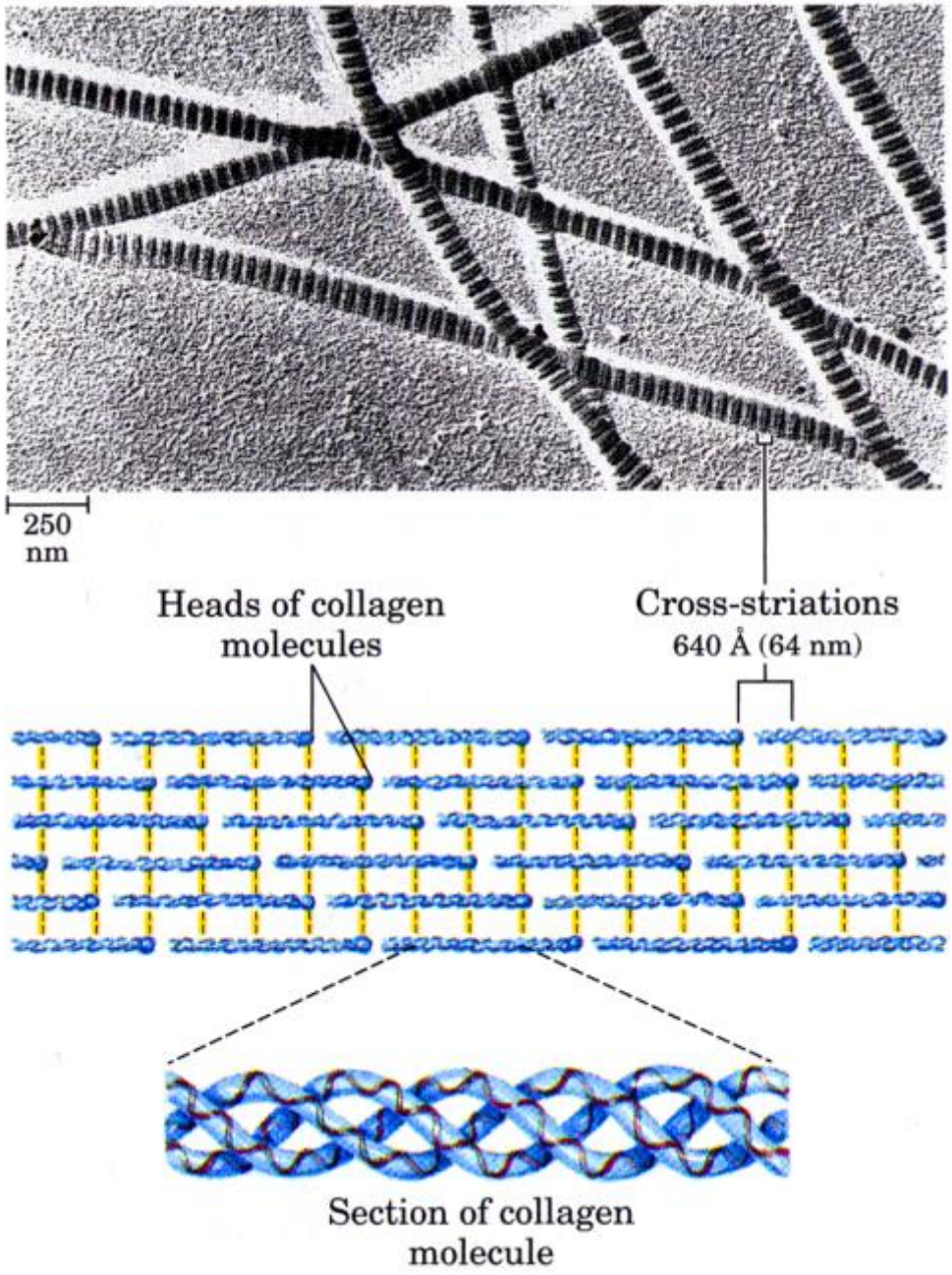
It has the general formula X – Y – Gly, and contains ~10 – 15% each of Proline and Hydroxyproline


(a)
(b)
(c)
(d)

Structural Aspects of Type I Collagen (2)

Tropocollagen forms fibrils by cross – linkage with other tropocollagen molecules.

These “normal” linkages can involve one of several molecules, but the “fundamental” linkage involves the formation of Schiff base between free amino groups of (hydroxy)lysine and nearby aldehydes (formed by lysyl oxidase on some of the (hydroxy)lysine residues


250
nm
Heads of collagen
molecules
Cross-striations
640 Å (64 nm)
Section of collagen
molecule

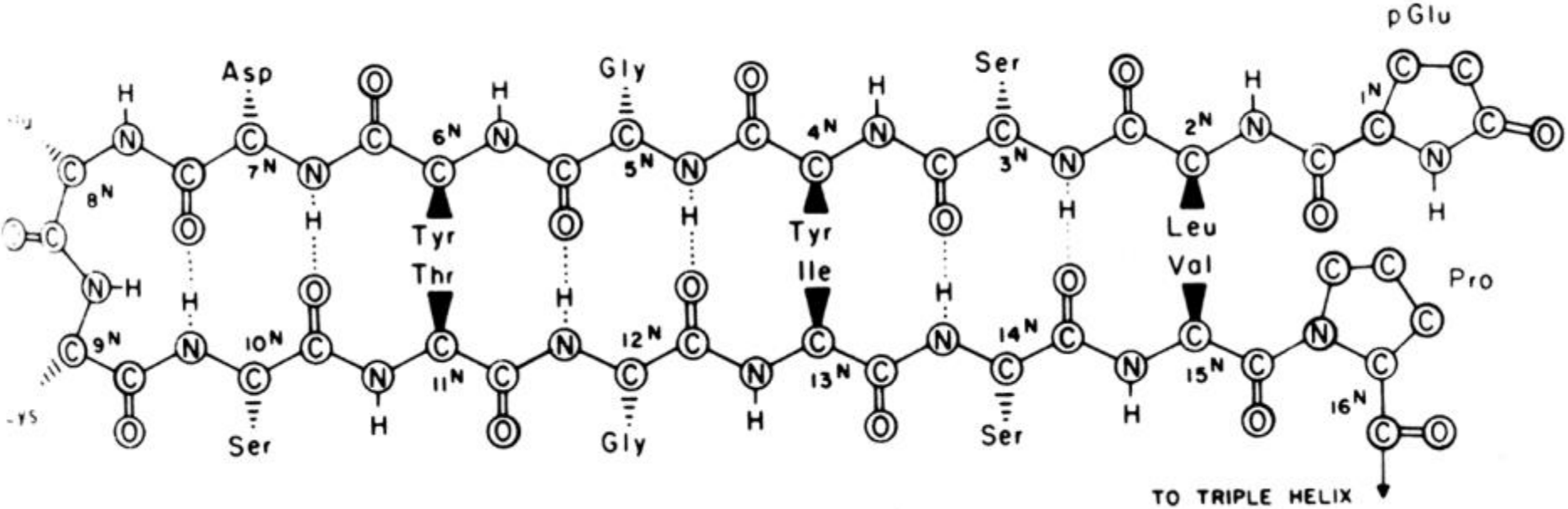
Telopeptides are Non – Helical Portions and the N- and C- Terminal Ends of the Collagen Molecule

- (a) Telopeptides have an antiparallel β – pleated sheet*
- (b) Telopeptides are high in tyrosine and phenylalanine residues, low in arginine and (hydroxy)lysine residues**
- (c) Tyrosine residues in favorable position to form dimers, "excimers" and higher oxidation products**
- (d) Telopeptides (Mainly N- telopeptides) are necessary for fibrillogenesis

- * D. Helseth et al, *Biopolymers* 18 *3005 – 3014 (1979)*
- * *A.L. Rubin et al, *Science* 139 *37 – 38 (1963)*

Proposed Structure of Collagen N-Telopeptide

Note that the hydrophobic AA face in towards the center of the molecule and the hydrophilic AA face out towards the surface

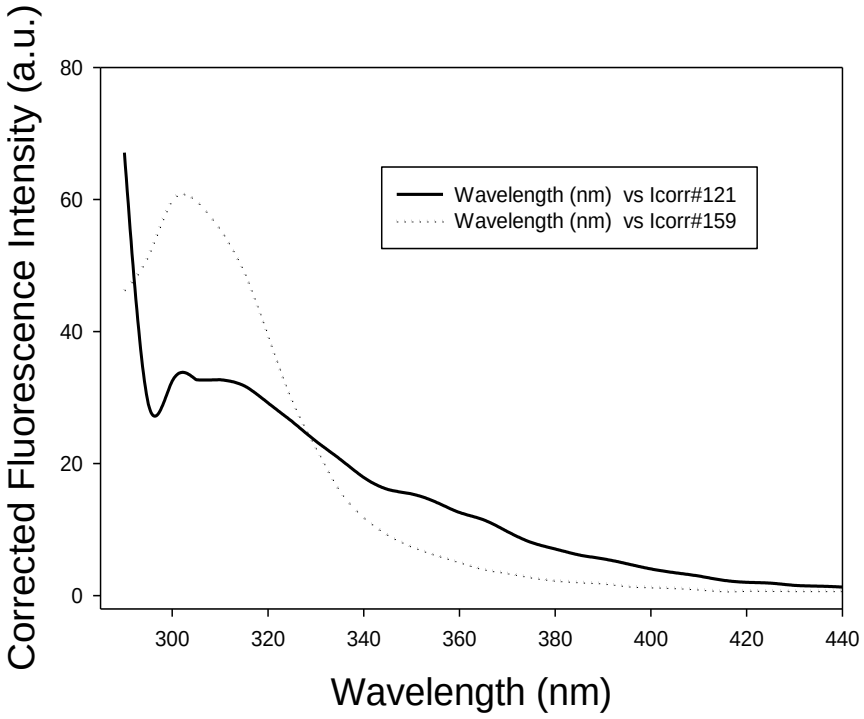


Schematic representation of proposed secondary structure of N - telopeptide

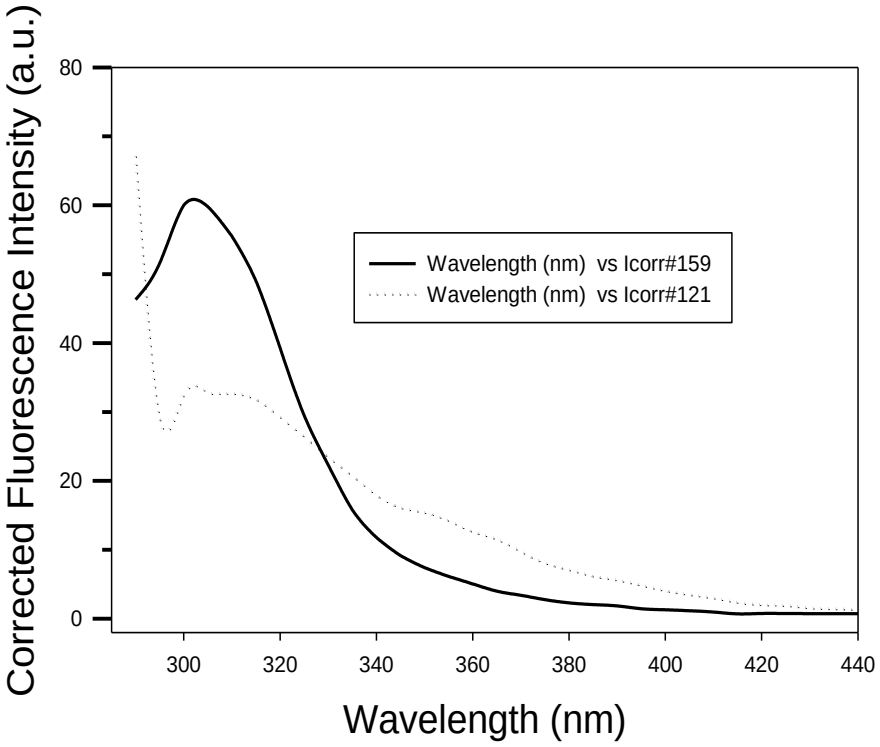
Tyrosine Can be Degraded by Thermal Oxidation or UV radiation. Degraded Collagen Has Different Fluorescence Properties than Normal

Spectral Differences in Calf Skin Collagen Depend on Previous History

Lot# 121 (5 years old; kept in refrigerator in dark at 4o C)



Lot# 159 "New" (obtained Feb 2012 and used July 2012)



Temperature Dependence of Collagen Photochemical Fluorescence Fading

- (1) Fluorescent State (*radiative*) *not* the same as the photochemical state (*radiationless*)

- (2) In general, fluorescence intensity decreases with temperature with concomitant increase of photochemical or other radiationless transistions (*e.g. vibration*) back to the ground state.

Autoxidation vs. UV Photolysis

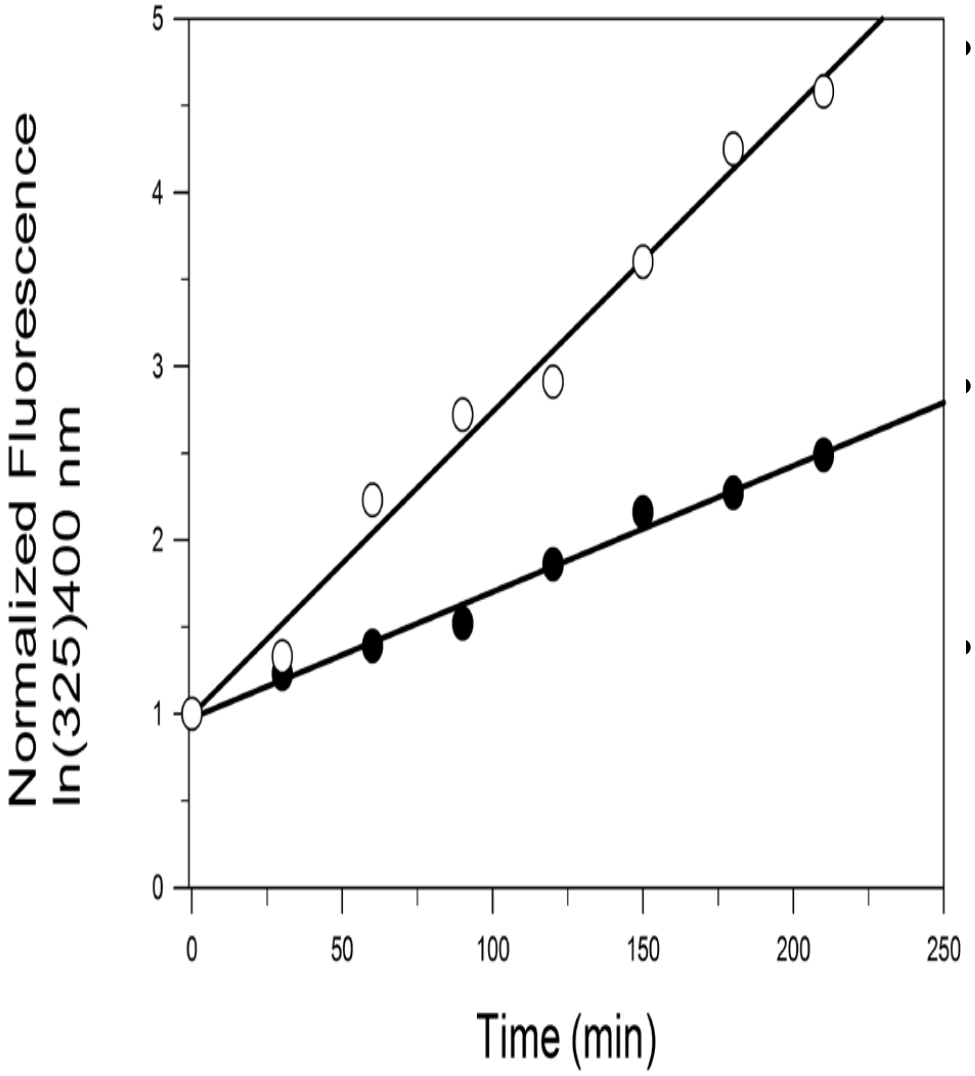
- Autoxidation is Oxygen – Dependent
- UV Photodimerization Does *Not* Depend on Oxygen

Effect of Temperature on Fading and Fluorescence Emission

- *Higher* Temperatures *Favor* Photochemical Reaction (“Fading”) and *Disfavor* Fluorescence Emission

- *Lower* Temperatures *Favor* Fluorescence Emission and *Disfavor* Photochemical Fading

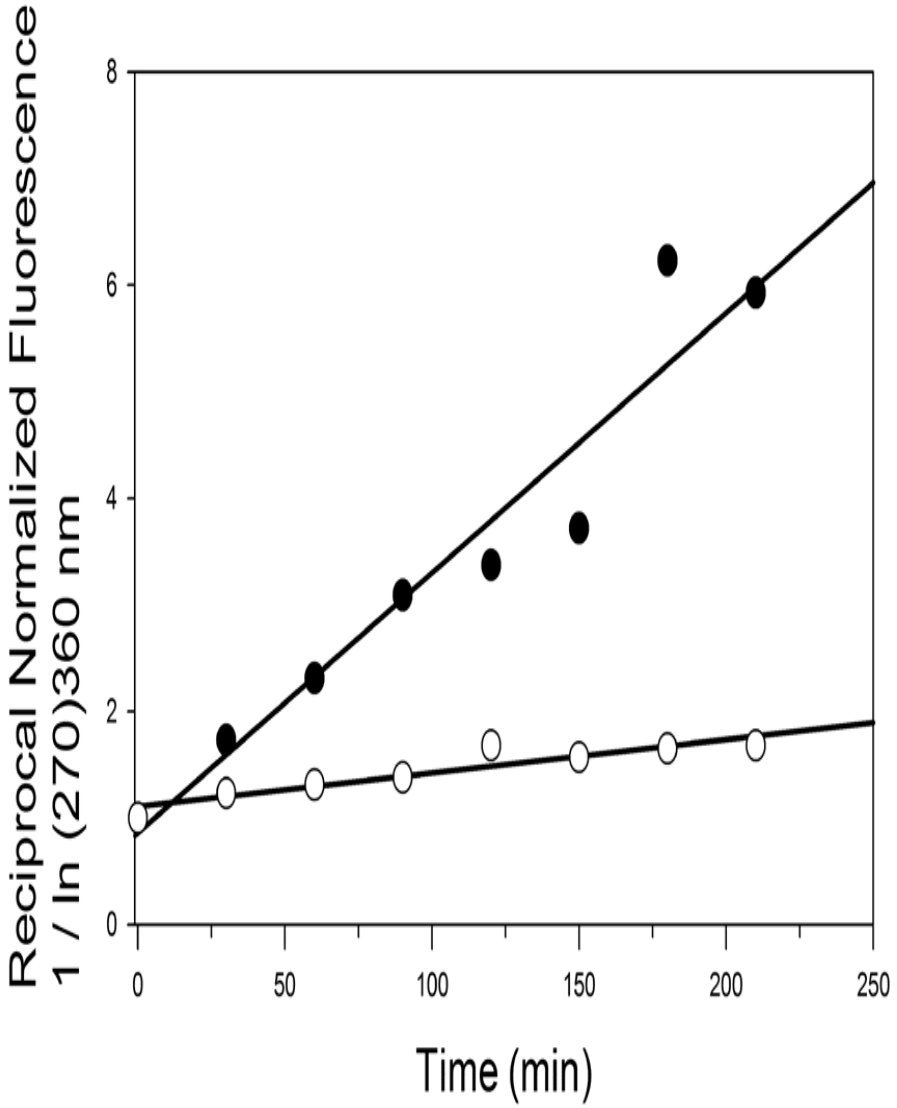
Tyrosine Dimerization to Dityrosine *Decreased* in Autoxidized Collagen



- Fresh Collagen was irradiated with UVC (254 nm) as a function of time.
- Black Dots - "aged" collagen sample
- White Dots - fresh collagen sample

See O Shimizu Photochem Photobiol 18(3) 123 – 133, 1973

Photodestruction of DOPA Oxidation Product is *Increased* in Autoxidized Collagen



- Oxidized Sample “aged” in dark at 4 C for ~ 5 years was irradiated with UVC (254 nm) as a function of time
- Black Dots – “aged” collagen sample
- White Dots - new collagen sample

Discussion:

- Common Intermediate is A*:

- Oxidation to AO_2 takes place at the expense of dityrosine formation

Discussion: (2)

- Photolability of Oxidation Product Indicates That Thermal Oxidation *Destablizes C*ollagen Molecule

- Dityrosine is *Unstable* to Longer UVA Wavelengths (not shown)

Kinetics of dityrosine formation

- Reaction
- (1) A + hγ ----> A*
  (2) A* ----> A + Δ
- (3) A* + A ----> A – A

- (a) Rx (3) is slow step in UV photodimerization.

- (b) System (3) must "build up" to a steady – state that requires $t > 0$

- (c) **As t ----> 0 , rate of A-A formation is maximum; dA*/dt ~ k[A] and is quasi - linear (A* is << A)**

- Rate
- $I_{abs}\,\Phi rx = k^*[A]$
- $-\,k_T\,[A^*]$
- $-\,k_{AA}\,[A][A^*]$

-

Effect of ECM on Collagen Fading

(1) Important because ECM surrounds collagen in dermal tissue

(2) ECM has ionic interactions with collagen

(3) We wish to inch towards the “physiological condition”

What is THE "extracellular matrix (ECM)"?

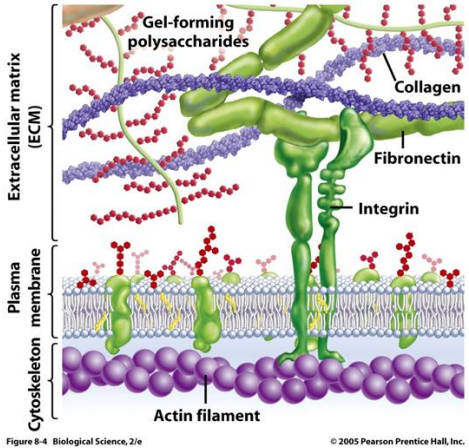
- The ECM is a network of macromolecules that fill the space between cells

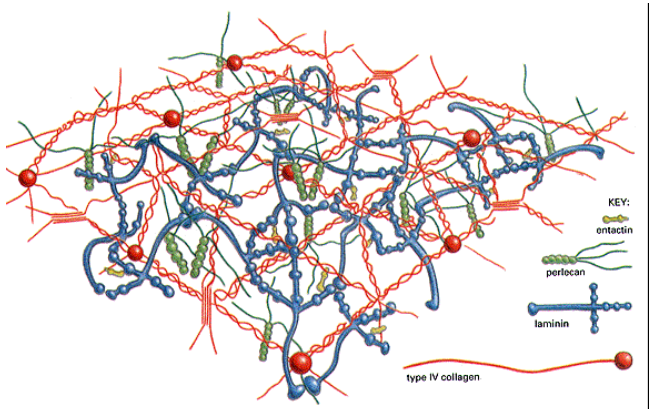
- Two main macromolecules make up the ECM

1. Polysaccharide chains of glycosaminoglycans (GAG) that is usually linked to proteins to form proteoglycans.
2. Fibrous proteins of two functional types:

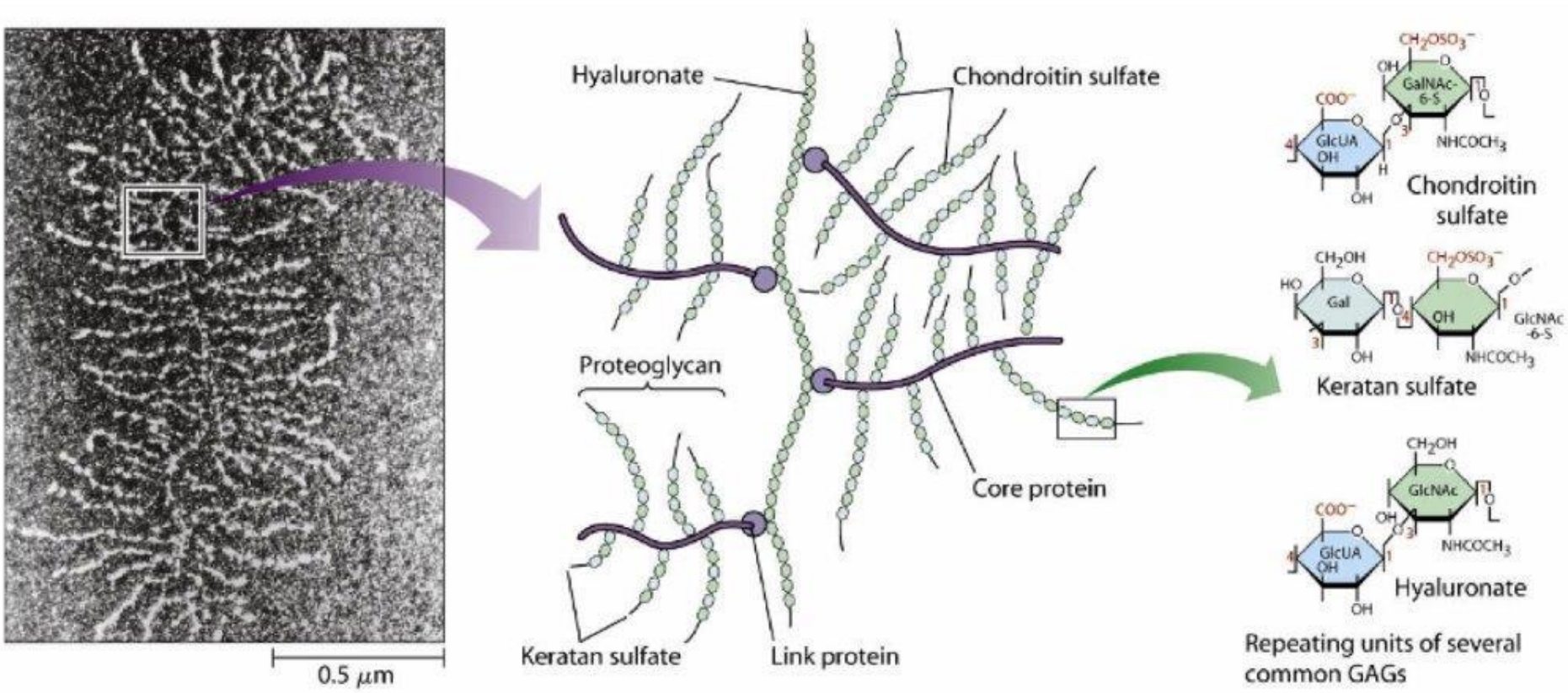
    a) mainly structural (e.g. collagen and elastin)

    b) mainly adhesive (e.g. fibronectin and laminin)





DERMAL PROTEOGLYCAN AGGREGATE



Preliminary Data (on HA)

- Excess Hyaluronate Does Not Seem to Affect Collagen Fading Rate as Evidenced By:

- (1) Fluorescence Spectral Shape

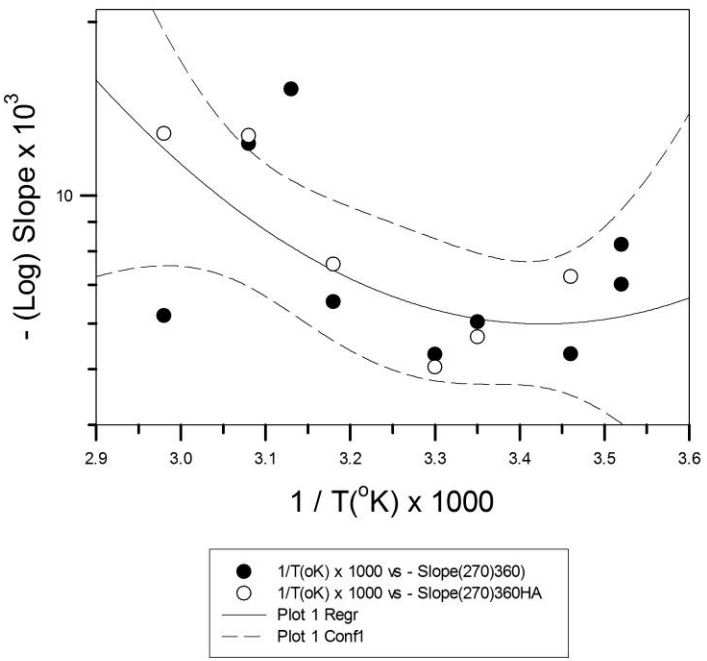
- (2) Fluorescence Fading/Buildup Rate

- (3) Arrhenius Plots

Hyaluronate Has Only Marginal Effect on Fluorescence Fading of DOPA – Oxidation Product

Arrhenius-Type Plot for Calf - Skin Collagen (Black) and Collagen - HA (white)

λ_{ex} = 270 nm λ_{em} = 360 nm



(1) Curved regression line in Arrhenius plot reflects collagen change of phase (melting) at ~ 36 C

(2) There is no statistical difference between Collagen alone and Collagen + Added HA at the 95% confidence level

Possible Reasons

Tyrosine is buried in hydrophobic region of telopeptide

Photochemical reaction involves *free radical* mechanism

Collagen/HA molecules are not packed close enough to each other as they are in skin.

(C. *Nagorski et al Res. Comm. Mol .Pathology and Pharm 89(2) 179 – 186*

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