Collagen Crosslinking Mechanisms

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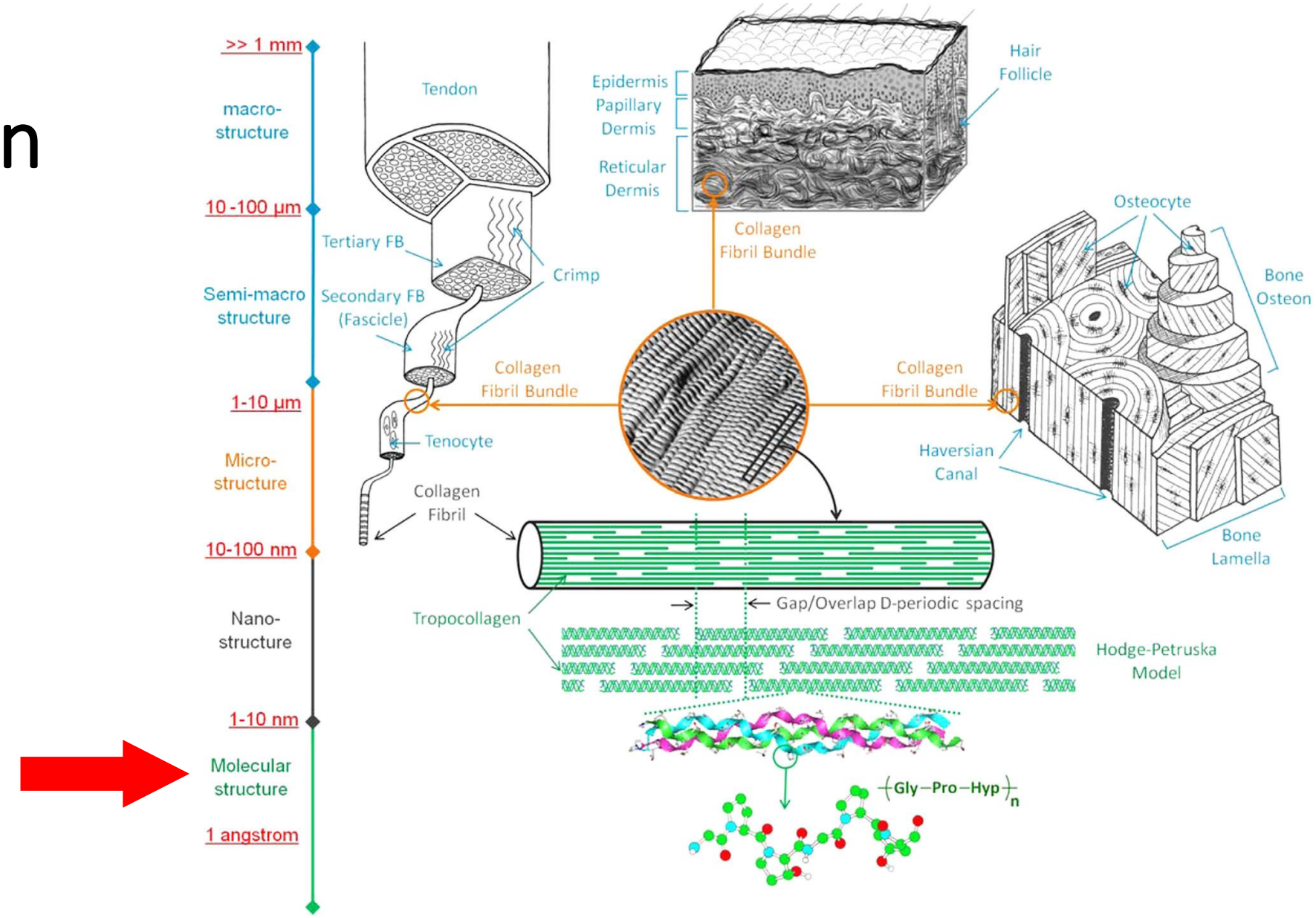
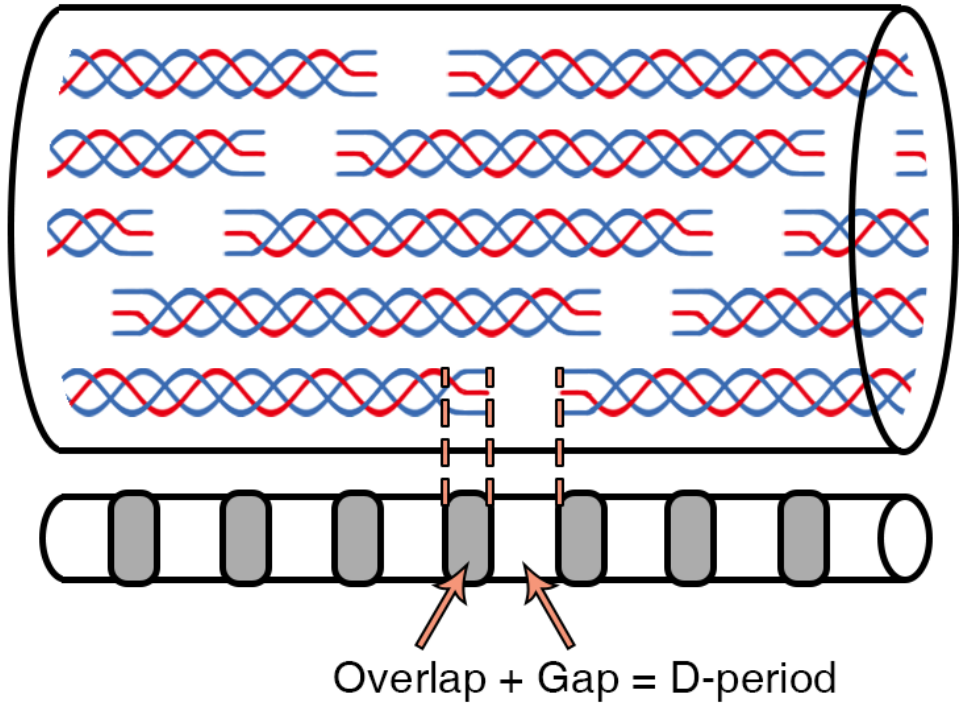
Collagen



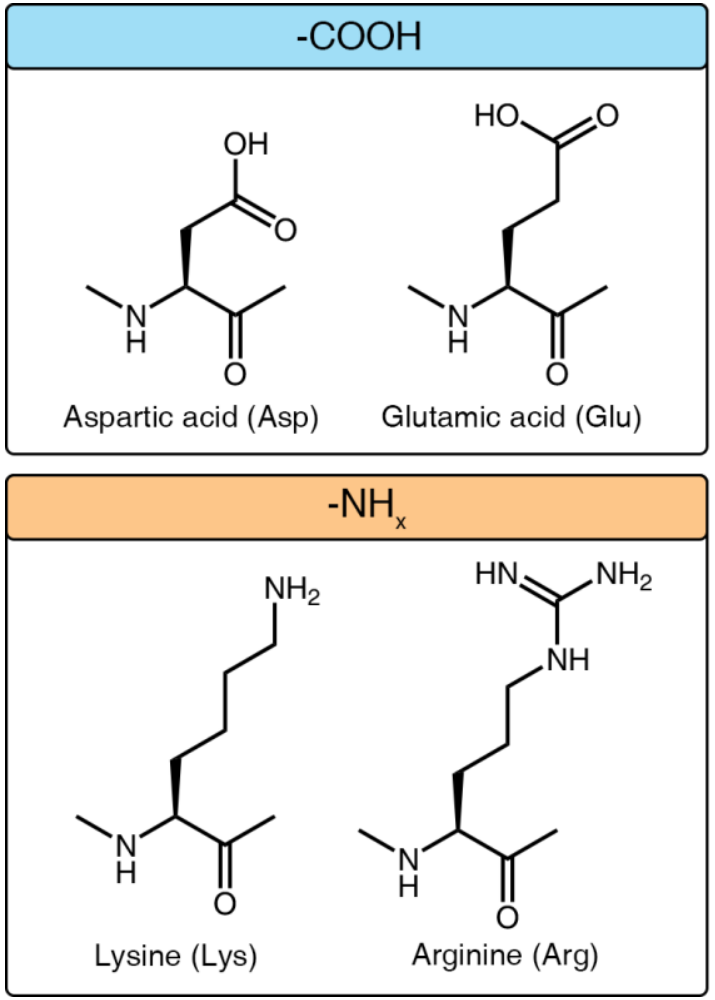
Fang, M., Goldstein, E.L., Turner, A.S., Les, C.M., Orr, B.G., Fisher, G.J., Welch, K.B., Rothman, E.D. and Banaszak Holl, M.M., 2012. Type I collagen D-spacing in fibril bundles of dermis, tendon, and bone: bridging between nano-and micro-level tissue hierarchy. *ACS nano*, *6*(11), pp.9503-9514.

Intermolecular Structure



Amino Acid Side Chains

- –COOH
- –NH_2
- –OH



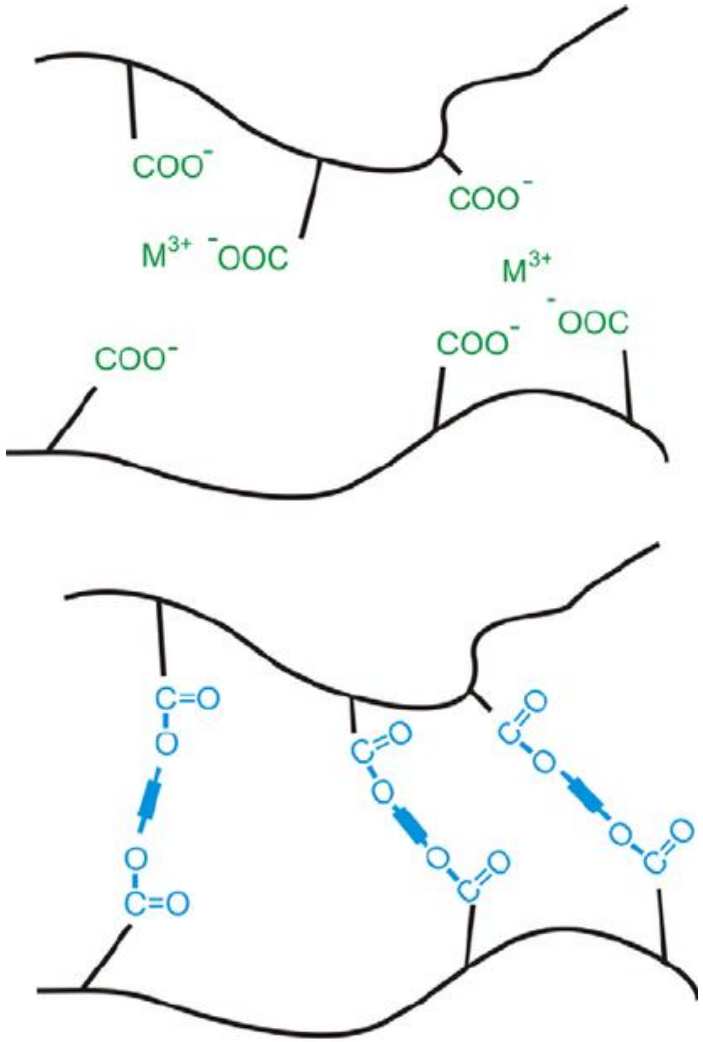
Types of Crosslinking

By bonding types:

- Covalent
- Electrostatic
- Hydrogen bond
- Van der Waals

By species involved:

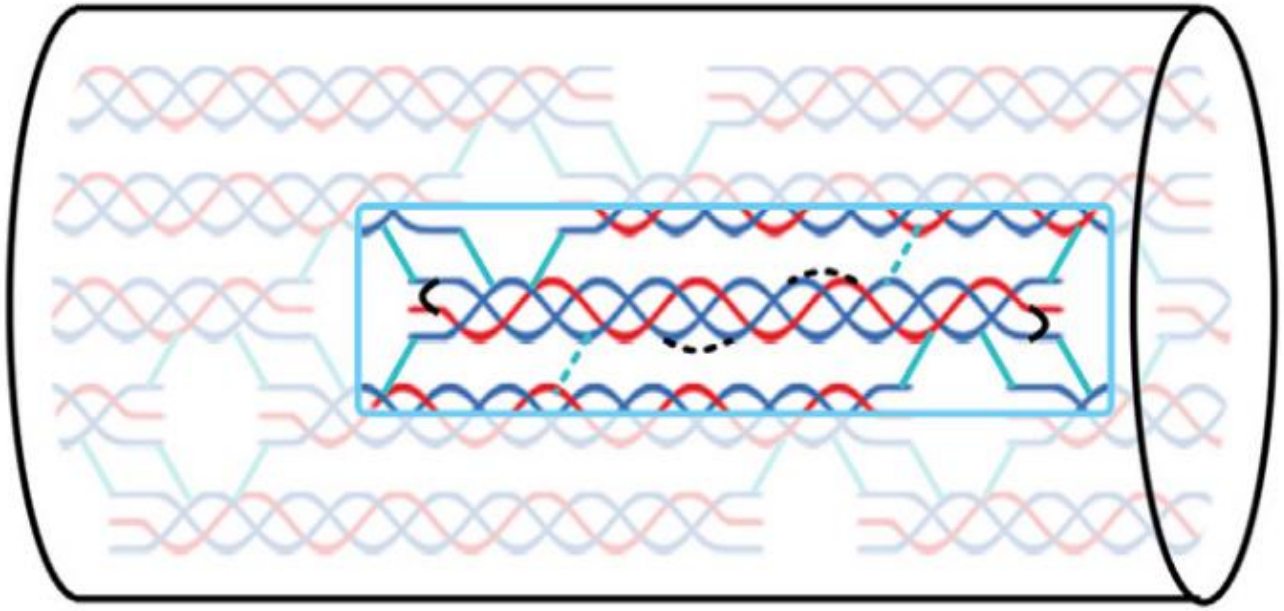
- Inorganic
- Organic
- Zero-linker



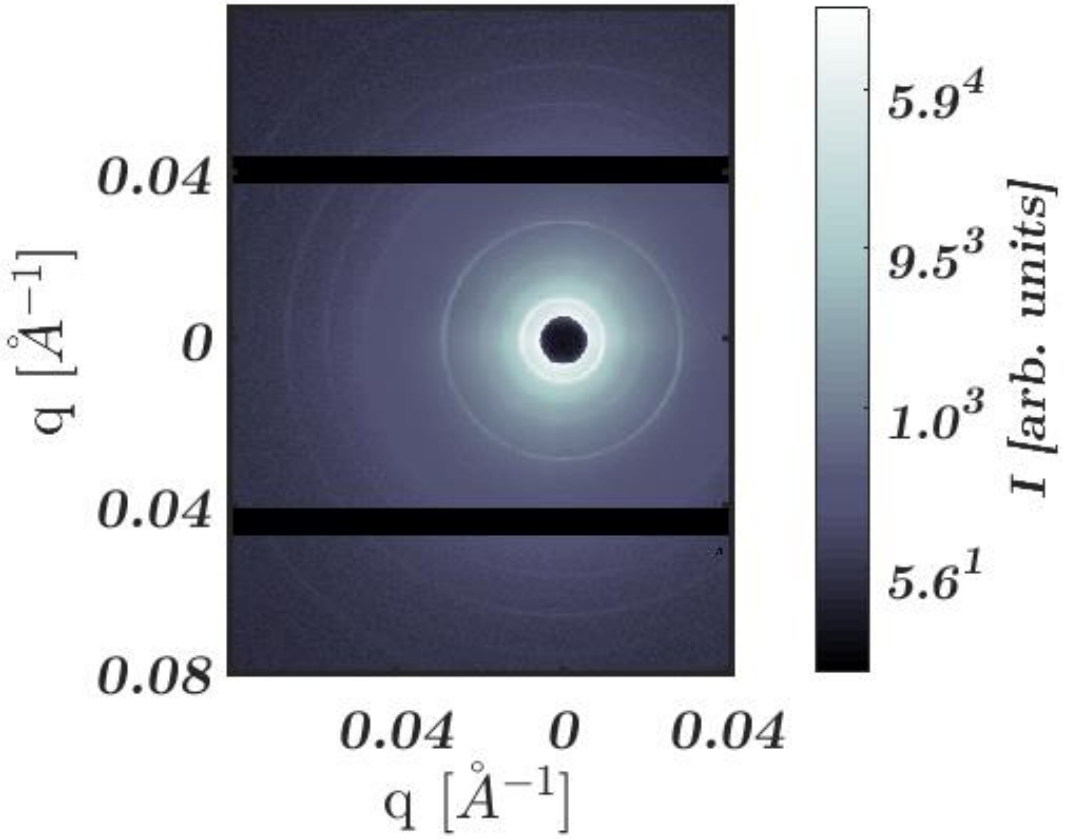
Types of Crosslinking

By binding sites:

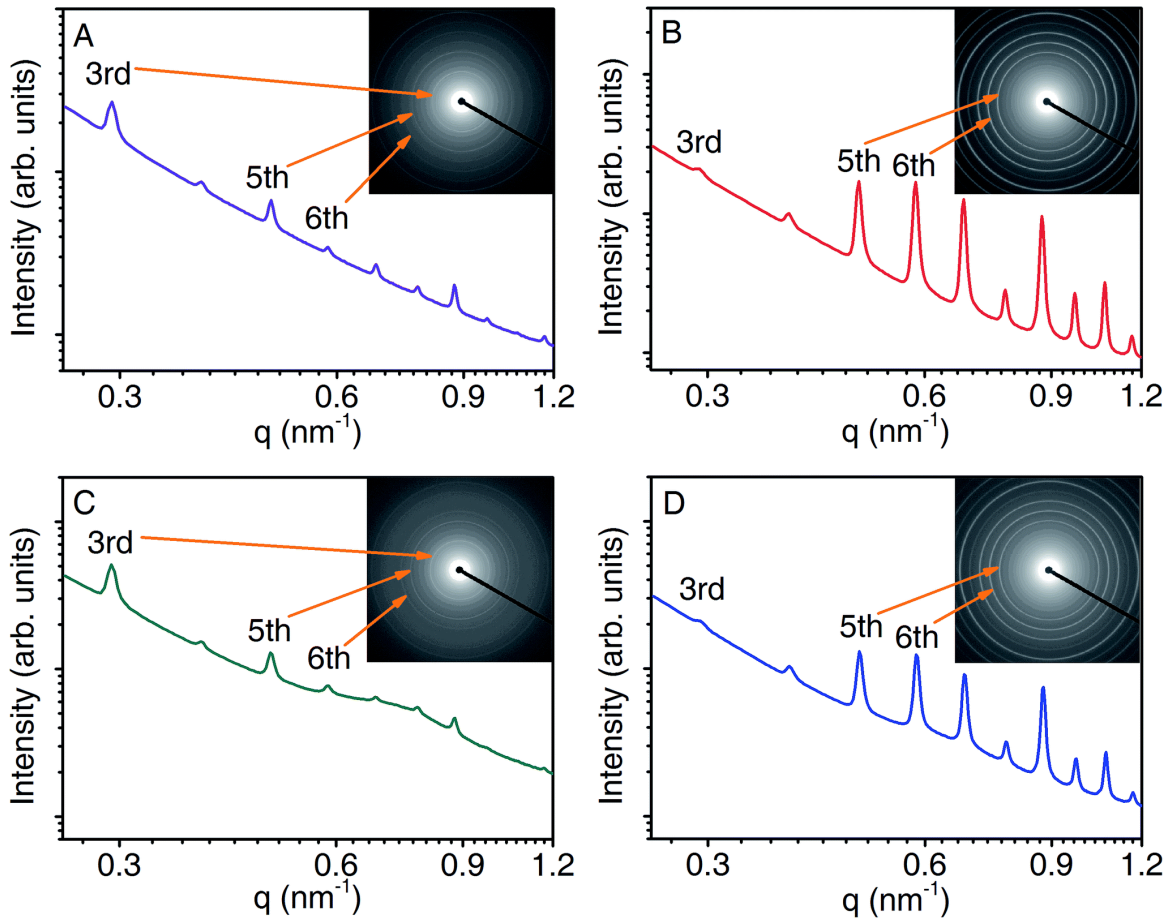
- Intra / Intermolecular
- Telopeptidyl / Helical



Synchrotron X-ray Scattering



Diffraction Peaks

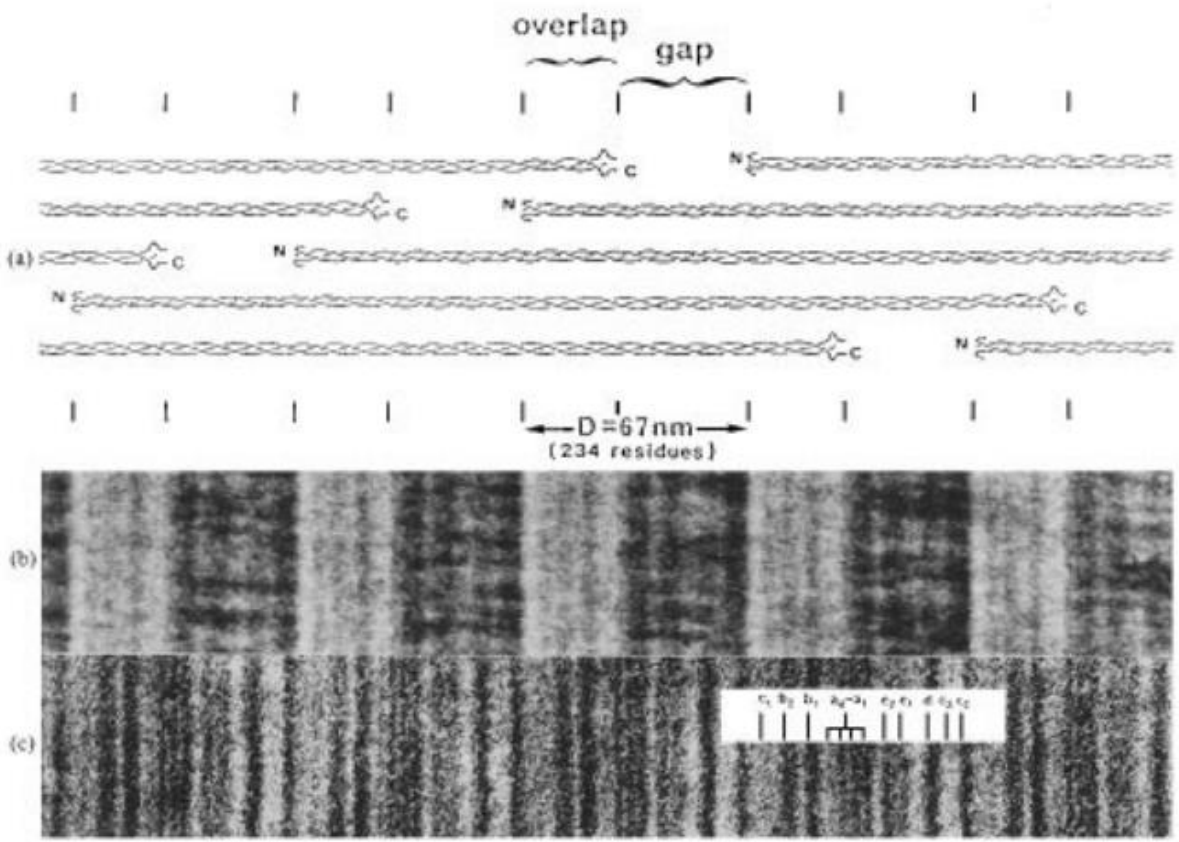


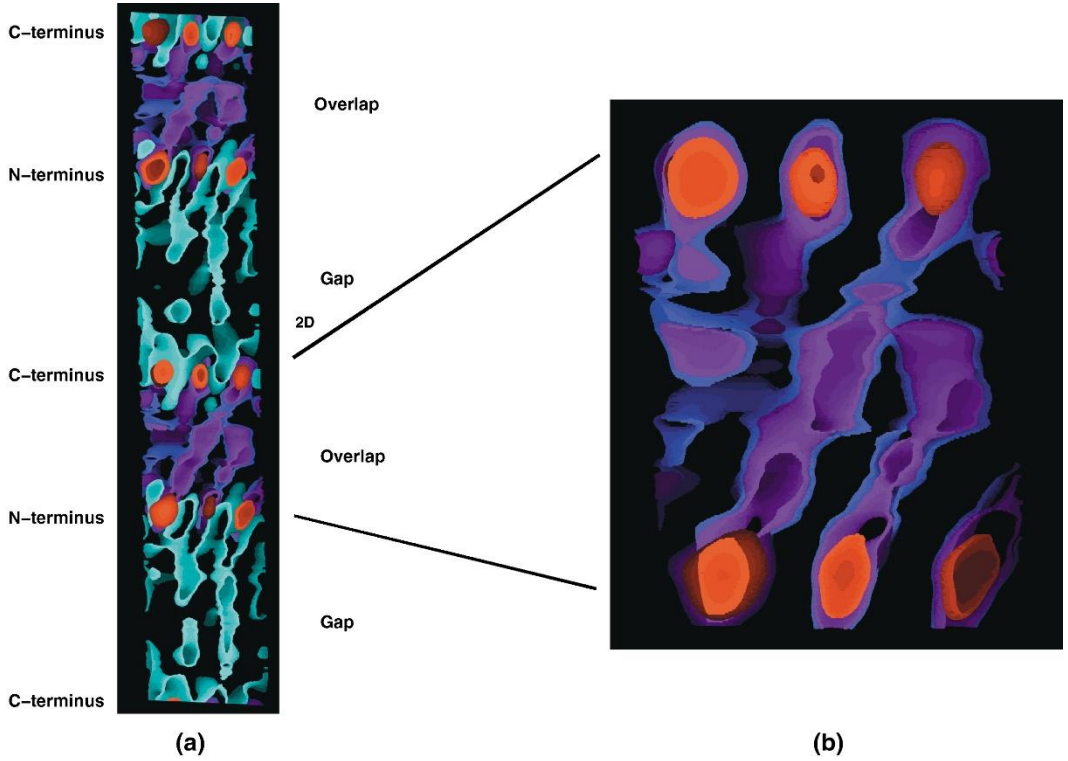
Peak information:

- Position (D-period)
- Intensity (electron density)

Zhang, Y., Ingham, B., Leveneur, J., Cheong, S., Yao, Y., Clarke, D.J., Holmes, G., Kennedy, J. and Prabakar, S., 2017. Can sodium silicates affect collagen structure during tanning? Insights from small angle X-ray scattering (SAXS) studies. *RSC advances*, *7*(19), pp.11665-11671.

Electron Density Contrast





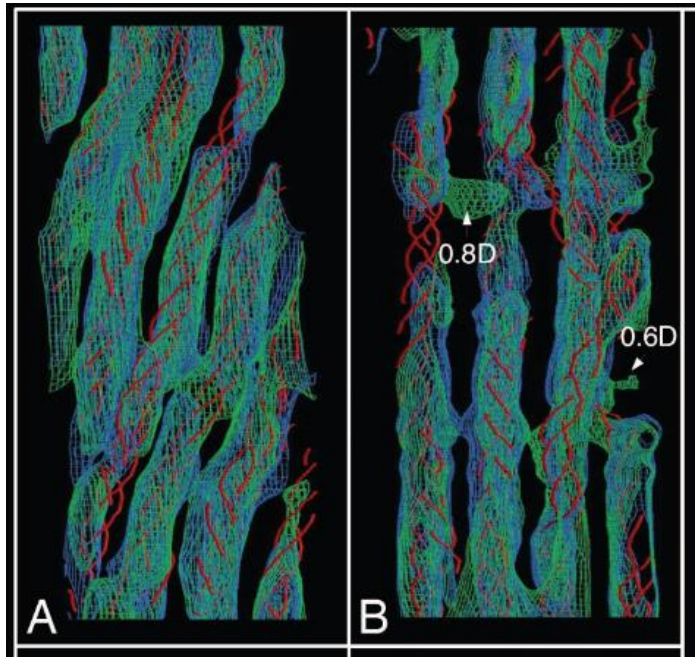
Kadler, K.E., Holmes, D.F., Trotter, J.A. and Chapman, J.A., 1996. Collagen fibril formation. *Biochemical Journal*, *316*(1), pp.1-11.
Orgel, J.P., Miller, A., Irving, T.C., Fischetti, R.F., Hammersley, A.P. and Wess, T.J., 2001. The in situ supermolecular structure of type I collagen. *Structure*, *9*(11), pp.1061-1069.

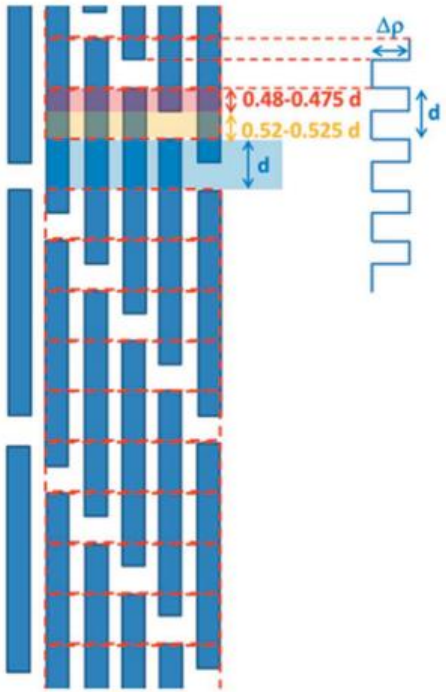
Data Analysis

Efficiency and Accuracy:

- Detailed molecular modelling
- Rough modelling
- Overall peak analysis

(Odd/even order peaks, representative peaks)





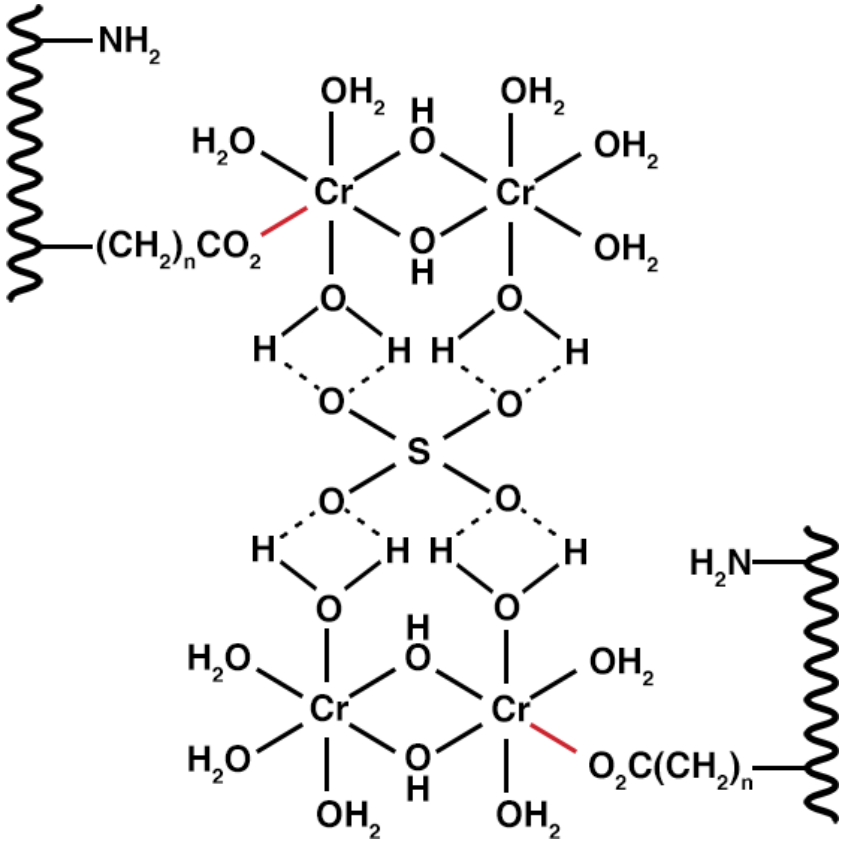
(a)

Orgel, J.P., Irving, T.C., Miller, A. and Wess, T.J., 2006. Microfibrillar structure of type I collagen in situ. *Proceedings of the National Academy of Sciences*, *103*(24), pp.9001-9005.
Giannini, C., De Caro, L., Terzi, A., Fusaro, L., Altamura, D., Diaz, A., Lassandro, R., Boccafoschi, F. and Bunk, O., 2021. Decellularized pericardium tissues at increasing glucose, galactose and ribose concentrations and at different time points studied using scanning X-ray microscopy. *IUCrJ*, *8*(4), pp.621-632.

Crosslinking (Binding) Mechanisms

- **Cr(III), Zr(IV)**
- GA (dialdehydes), EDC, Oxazolidine, THPS
- Vegetable tannins

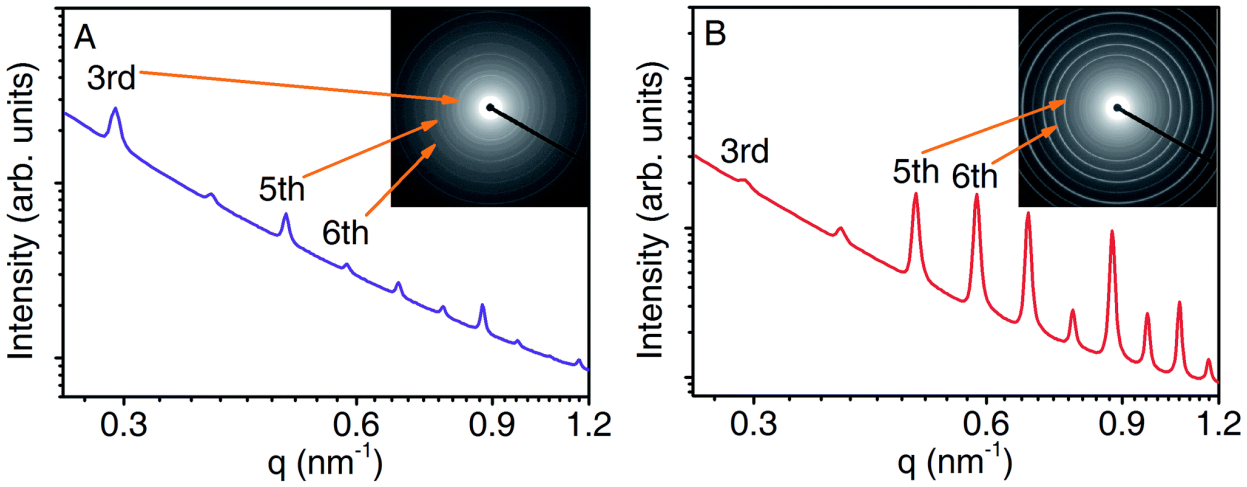
Chromium (III)



Proposed mechanism:

- -COO–Cr (covalent)
- SO_4^{2-} bridge

Chromium (III)



Peak intensity changes:

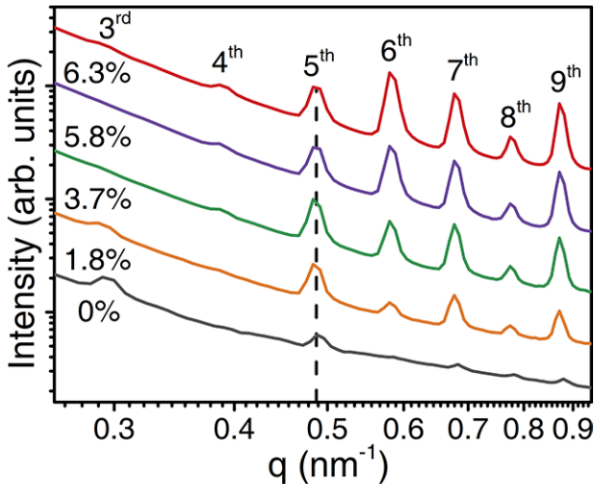
- 3rd decreases, 5th onwards increases
- R(5/3): 0.22 for pickled, ~5 for wet blue

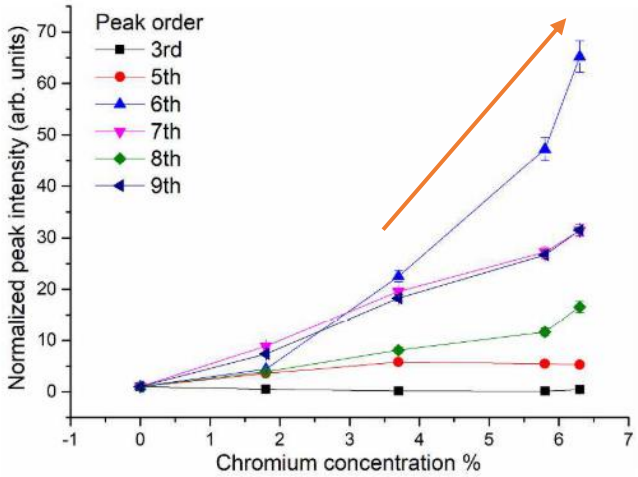
Mechanism:

- Site-specific binding

Zhang, Y., Ingham, B., Leveneur, J., Cheong, S., Yao, Y., Clarke, D.J., Holmes, G., Kennedy, J. and Prabakar, S., 2017. Can sodium silicates affect collagen structure during tanning? Insights from small angle X-ray scattering (SAXS) studies. *RSC advances*, *7*(19), pp.11665-11671.

Chromium (III): Concentration





Peak intensity changes:

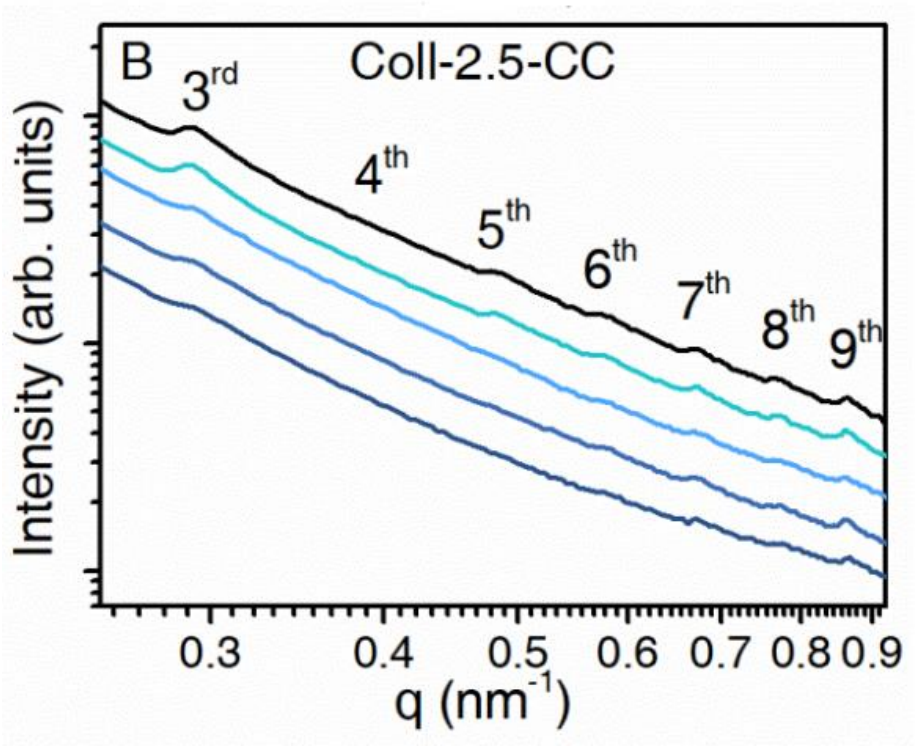
- Overall (especially 6th) increase above certain concentration

Mechanism:

- Covalent binding occurs first, electrostatic binding when adding excess

Zhang, Y., Mansel, B.W., Naffa, R., Cheong, S., Yao, Y., Holmes, G., Chen, H.L. and Prabakar, S., 2018. Revealing molecular level indicators of collagen stability: minimizing chrome usage in leather processing. *ACS Sustainable Chemistry & Engineering*, *6*(5), pp.7096-7104.

Chromium (III): Anion



Peak intensity changes:

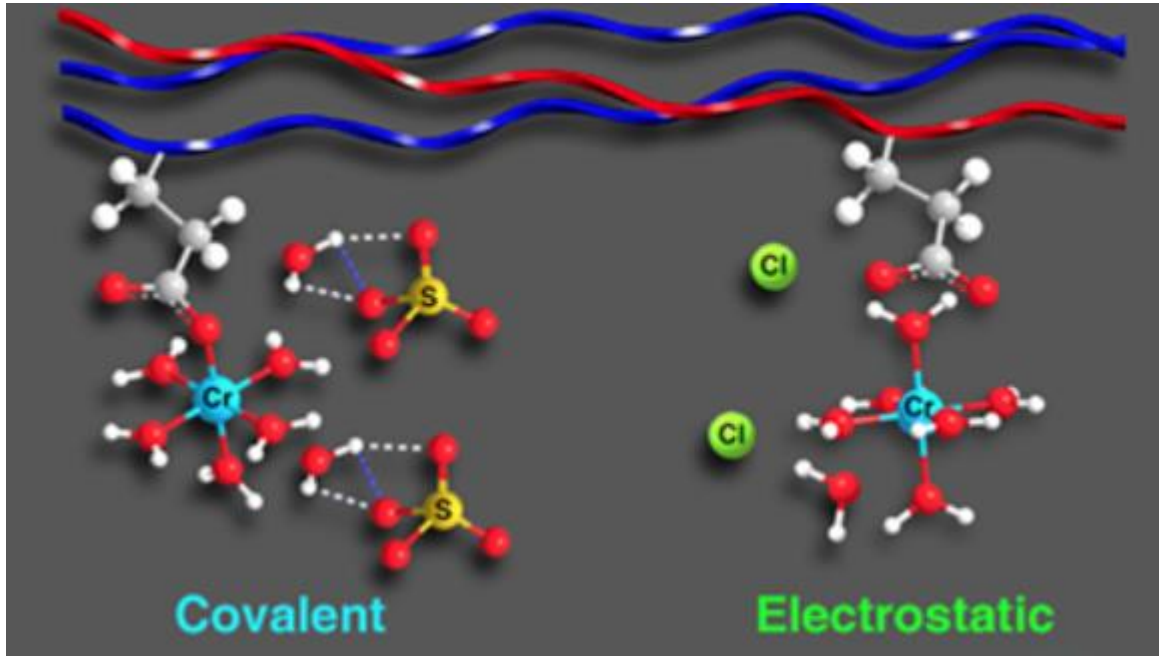
- SO_4^{2-} leads to intensity changes, Cl^- does not
- Weakened peaks

Mechanism:

- Both occurs in the matrix
- Priority regulated by anions

Zhang, Y., Mehta, M., Mansel, B.W., Ng, H.W., Liu, Y., Holmes, G., Le Ru, E.C. and Prabakar, S., 2020. Anion-regulated binding selectivity of Cr (III) in collagen. *Biopolymers*, *111*(11), p.e23406.

Chromium (III): Anion



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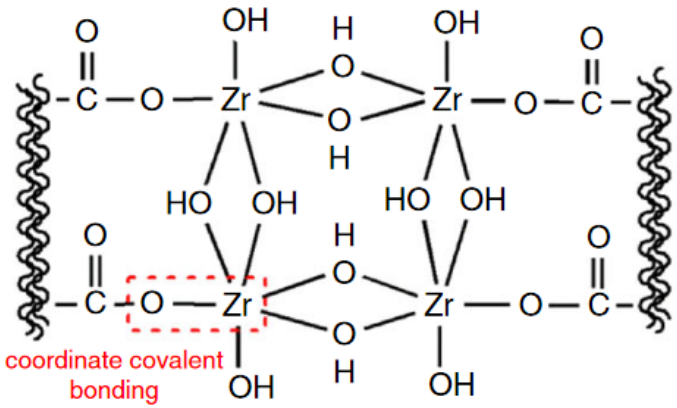
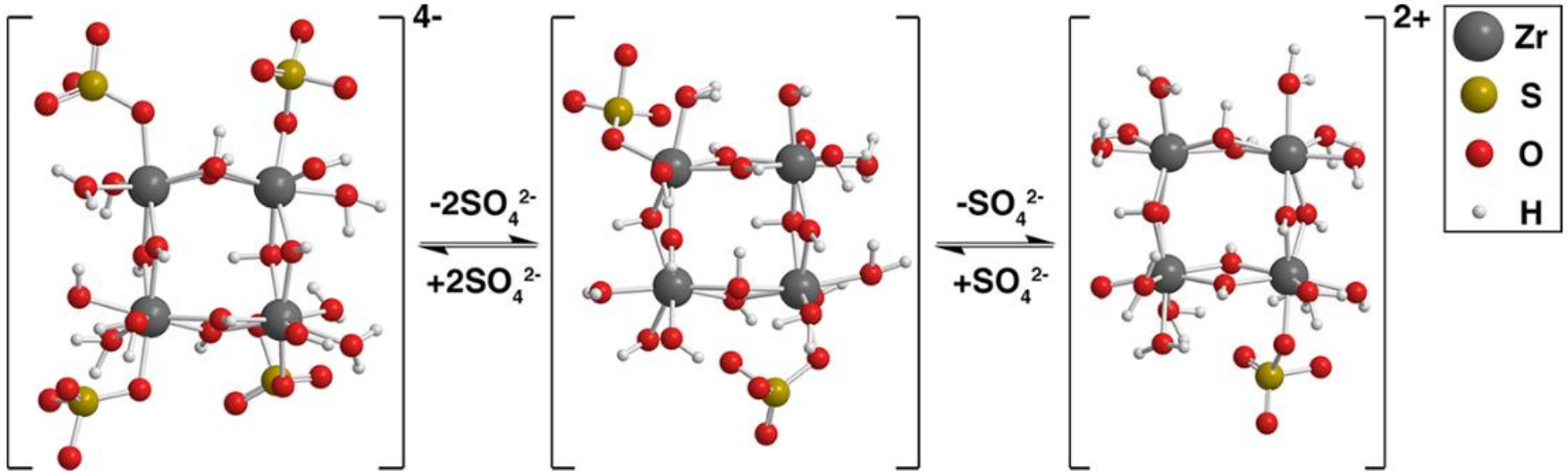
Zirconium (IV)

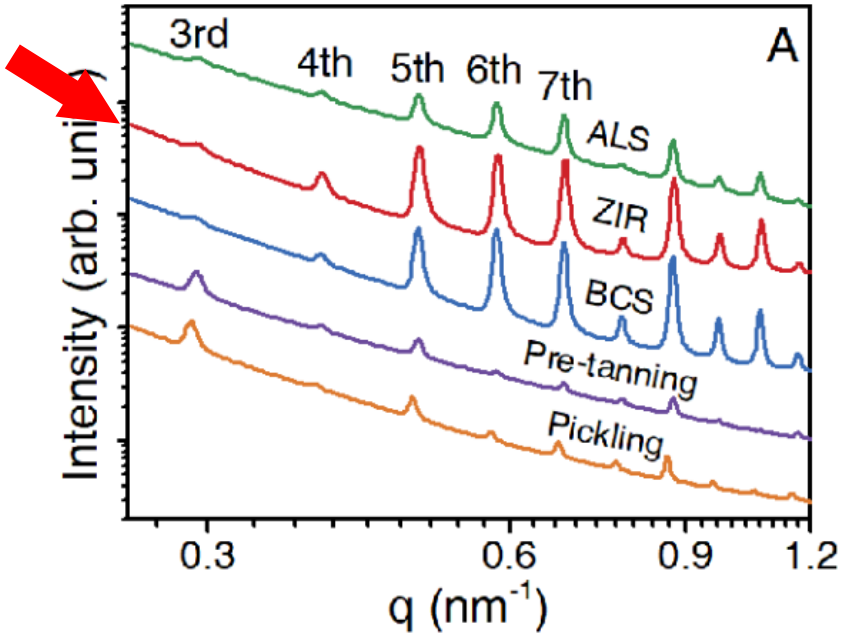
Proposed mechanism:

- Mixed binding mechanisms





Zirconium (IV)



Peak intensity changes:

- Strong peaks, 3rd decreases, 4th onward increases
- R(5/3): ~5 for Zr(IV) tanned

Mechanism:

- Site-specific binding dominates, similar to Cr(III)

Zhang, Y., Ingham, B., Cheong, S., Ariotti, N., Tilley, R.D., Naffa, R., Holmes, G., Clarke, D.J. and Prabakar, S., 2018. Real-time synchrotron small-angle X-ray scattering studies of collagen structure during leather processing. *Industrial & Engineering Chemistry Research*, *57*(1), pp.63-69.

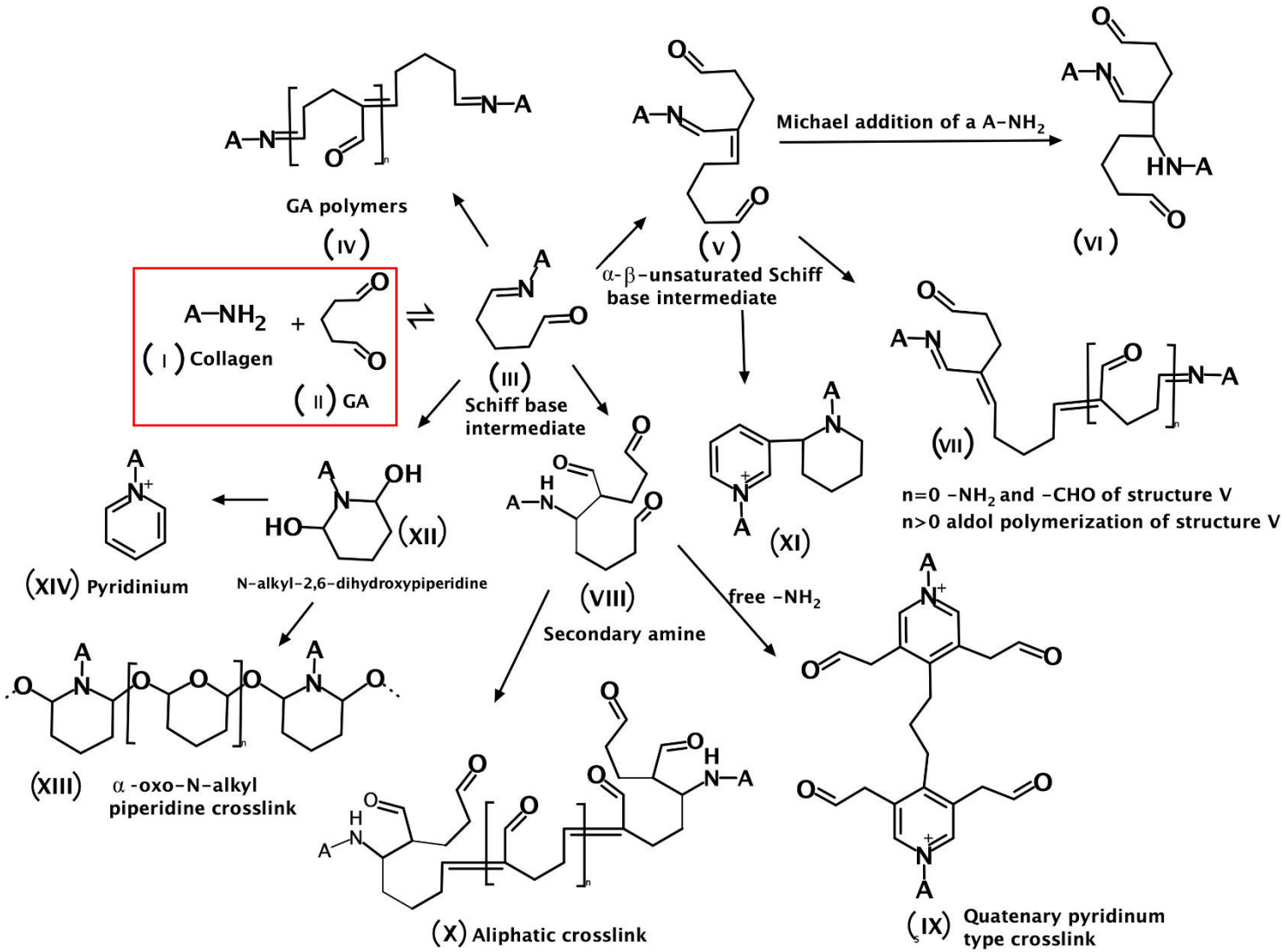
Crosslinking (Binding) Mechanisms

- Cr(III), Zr(IV)
- **GA (dialdehydes), EDC, Oxazolidine, THPS**
- Vegetable tannins

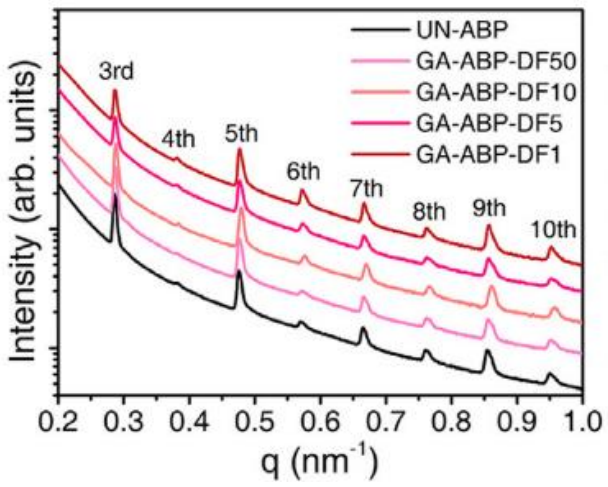
Glutaraldehyde

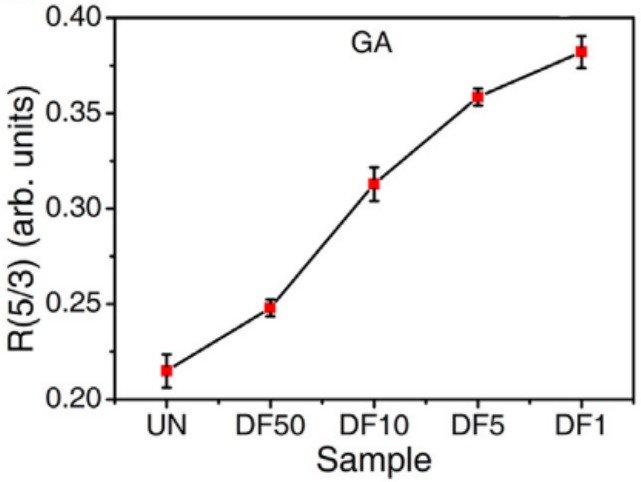
Proposed mechanism:

- Many routes
- Potentially polymerise



Glutaraldehyde





Peak intensity changes:

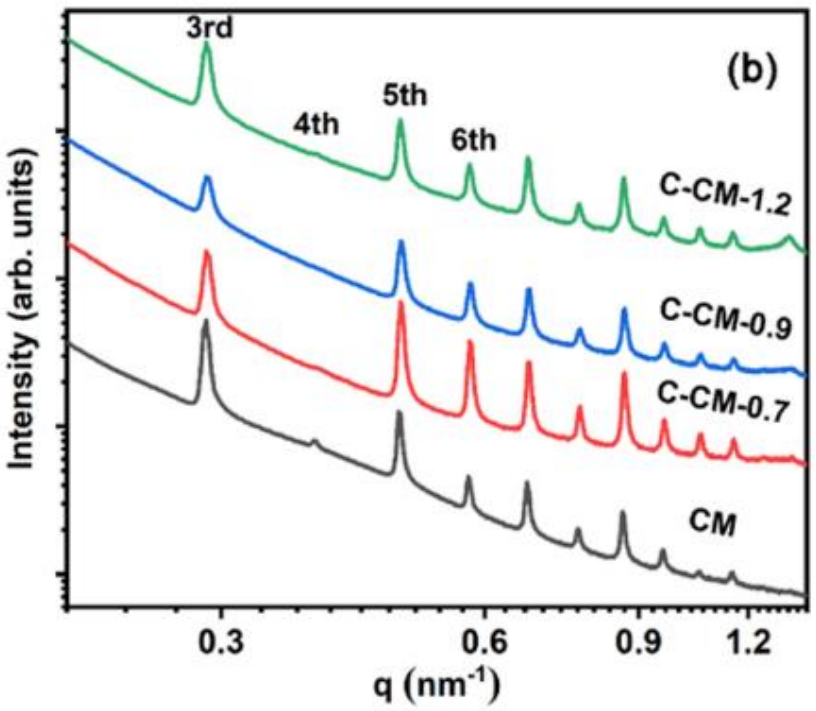
- Minor changes (all concentrations)
- R(5/3): from 0.22 to 0.38

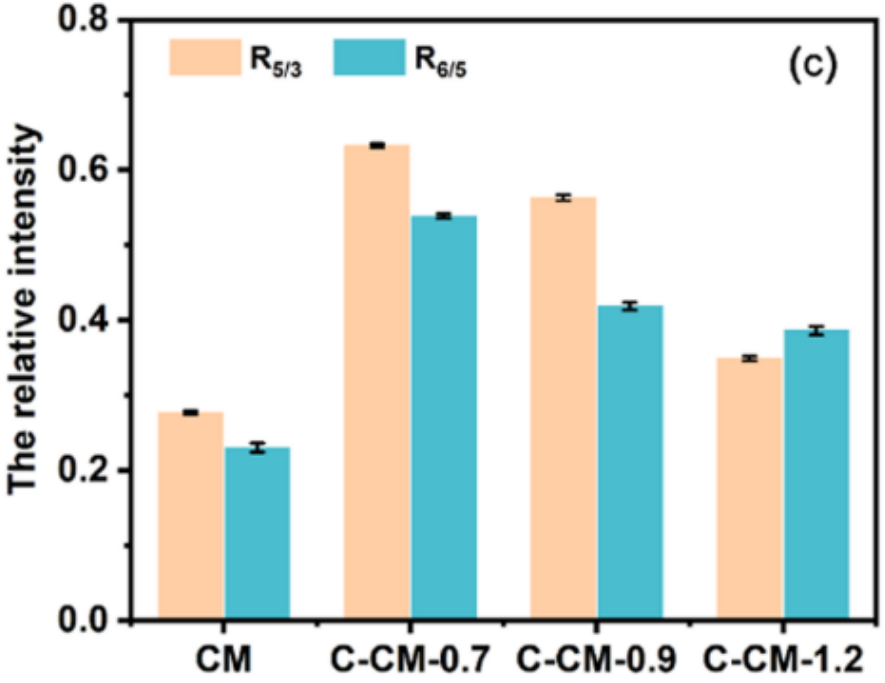
Mechanism:

- Site-specific binding

Zhang, Y., Zhang, W., Snow, T., Ju, Y., Liu, Y., Smith, A.J. and Prabakar, S., 2022. Minimising chemical crosslinking for stabilising collagen in acellular bovine pericardium: Mechanistic insights via structural characterisations. Acta Biomaterialia.

Dialdehydes



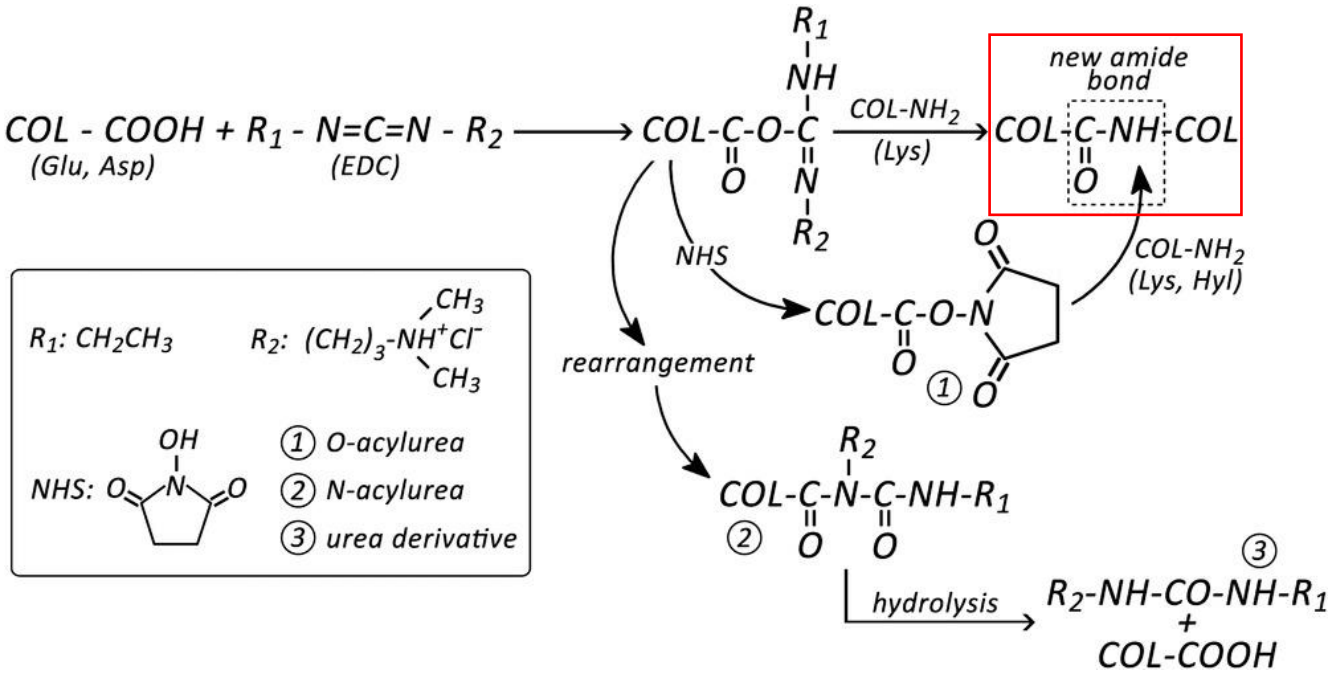


Yi, Y., Zhang, Y., Mansel, B., Wang, Y.N., Prabakar, S. and Shi, B., 2022. Effect of Dialdehyde Carboxymethyl Cellulose Cross-Linking on the Porous Structure of the Collagen Matrix. *Biomacromolecules*, *23*(4), pp.1723-1732.

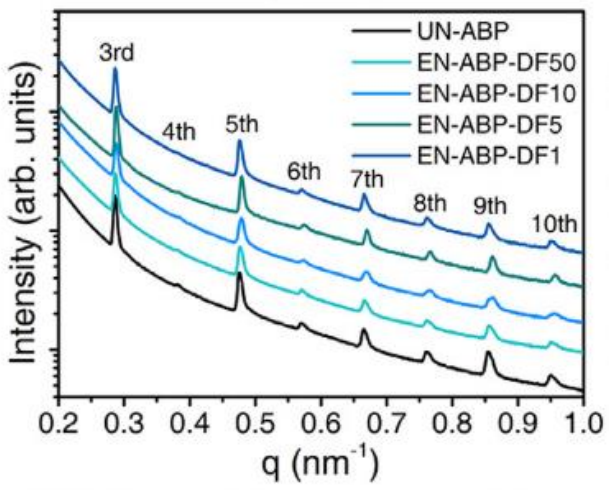
EDC

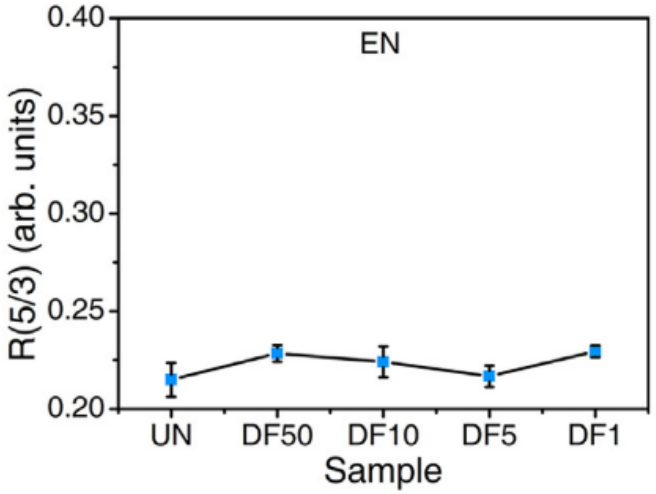
Proposed mechanism:

- Peptide bonds (Zero-linker)



EDC





Peak intensity changes:

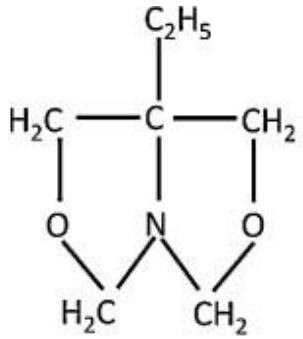
- Zero-linker
- R(5/3): unchanged

Mechanism:

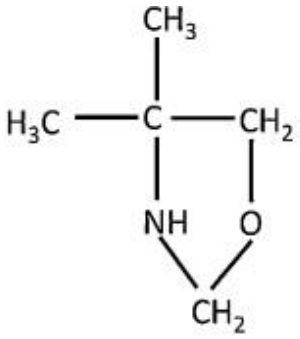
- Triggering the crosslinking

Zhang, Y., Zhang, W., Snow, T., Ju, Y., Liu, Y., Smith, A.J. and Prabakar, S., 2022. Minimising chemical crosslinking for stabilising collagen in acellular bovine pericardium: Mechanistic insights via structural characterisations. Acta Biomaterialia.

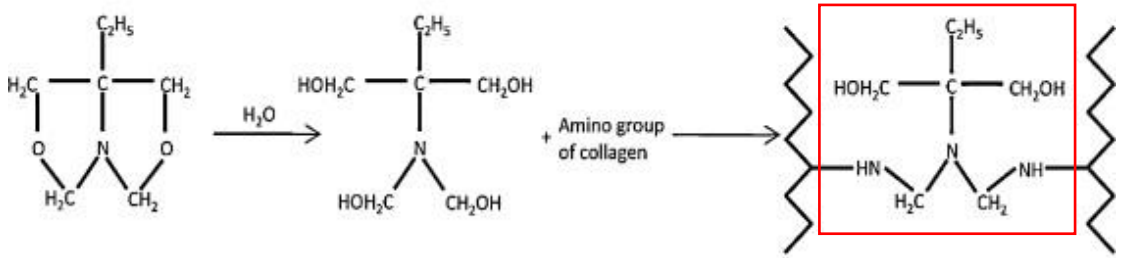
Oxazolidine



a. Bicyclic oxazolidine



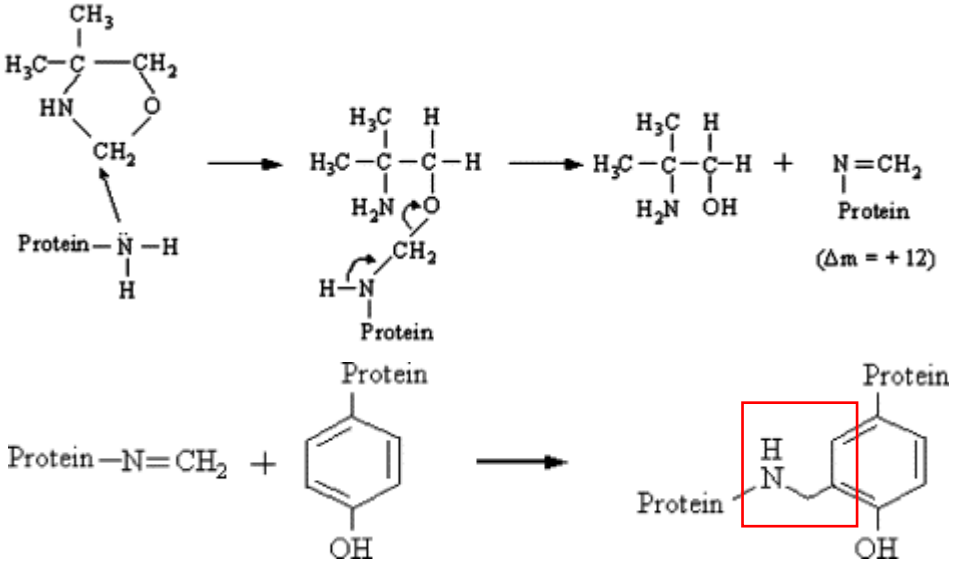
b. Monocyclic oxazolidine



c. Possible oxazolidine - collagen interaction

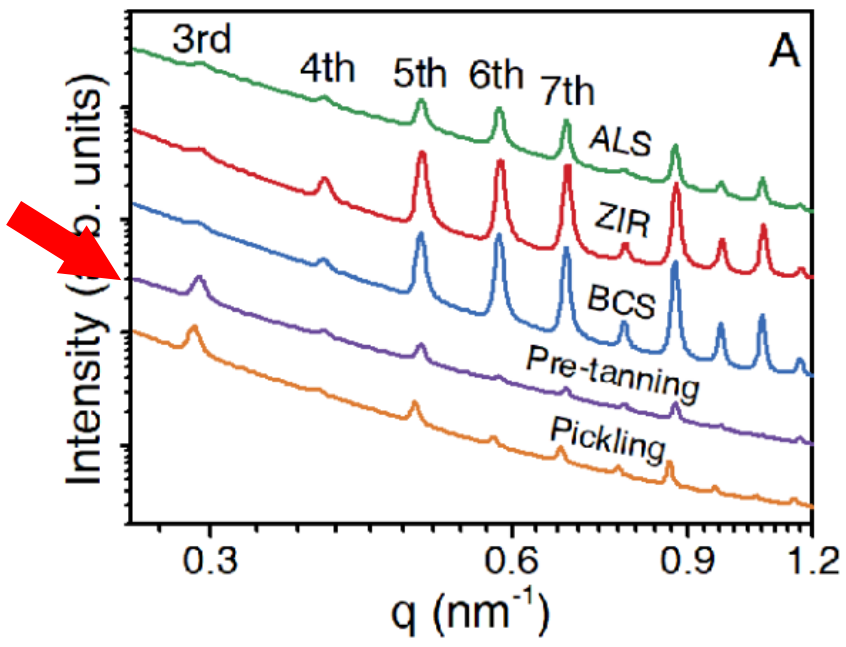
Proposed mechanism:

- Covalent linkage with $-NH_2$ groups
- Triggering or involving



Sundarapandiyan, S., Brutto, P.E., Siddhartha, G., Ramesh, R., Ramanaiah, B., Saravanan, P. and Mandal, A.B., 2011. Enhancement of chromium uptake in tanning using oxazolidine. Journal of hazardous materials, 190(1-3), pp.802-809.
Choudhury, S.D., DasGupta, S. and Norris, G.E., 2007. Unravelling the mechanism of the interactions of oxazolidine A and E with collagens in ovine skin. International journal of biological macromolecules, 40(4), pp.351-361.

Oxazolidine



Peak intensity changes:

- Minor changes (2% offer)
- R(5/3) remains at 0.22

Mechanism:

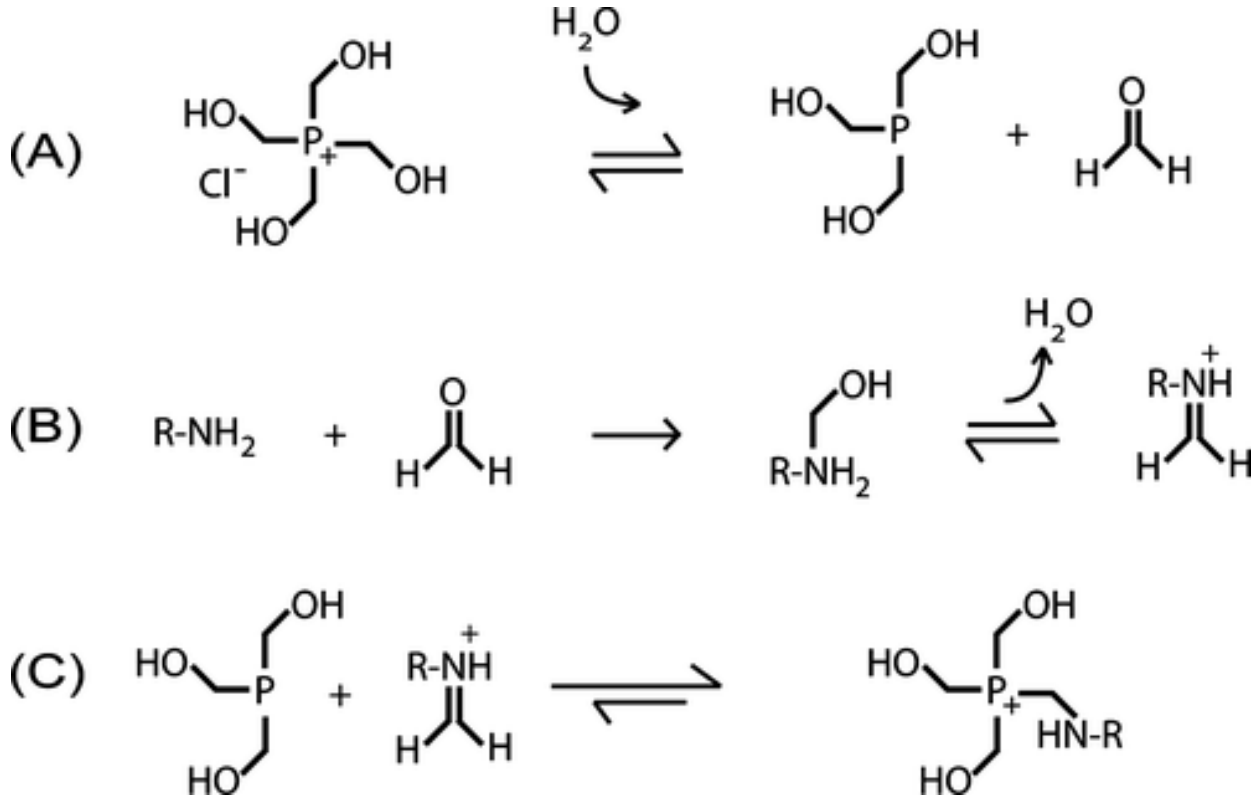
- Triggering the crosslinking, similar to EDC

Zhang, Y., Ingham, B., Cheong, S., Ariotti, N., Tilley, R.D., Naffa, R., Holmes, G., Clarke, D.J. and Prabakar, S., 2018. Real-time synchrotron small-angle X-ray scattering studies of collagen structure during leather processing. *Industrial & Engineering Chemistry Research*, *57*(1), pp.63-69.

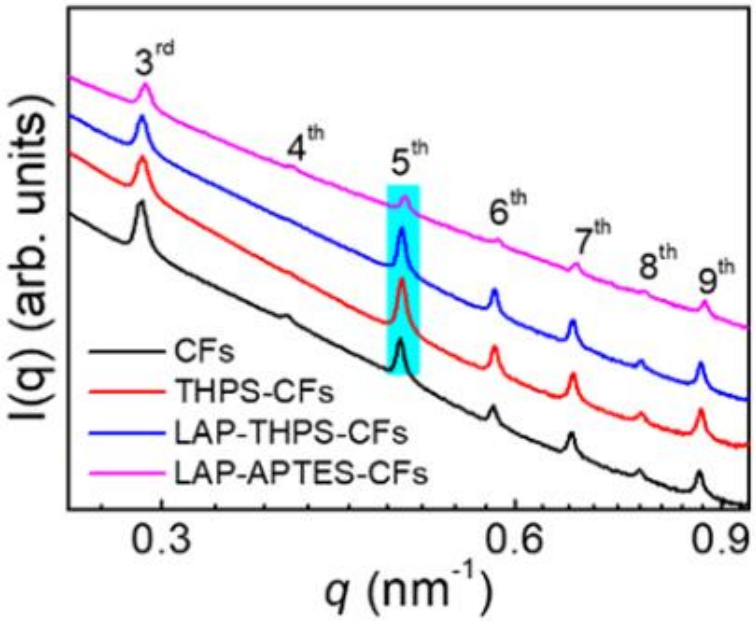
THPS

Proposed Mechanism:

- Covalent linkage with $–NH_2$ groups



THPS



Peak intensity changes:

- Consistent changes to a moderate extent
- R(5/3): from 0.22 to 0.47

Mechanism:

- Site-specific binding, similar to GA

Shi, J., Zhang, R., Yang, N., Zhang, Y., Mansel, B.W., Prabakar, S. and Ma, J., 2020. Hierarchical Incorporation of Surface-Functionalized Laponite Clay Nanoplatelets with Type I Collagen Matrix. Biomacromolecules, 22(2), pp.504-513.

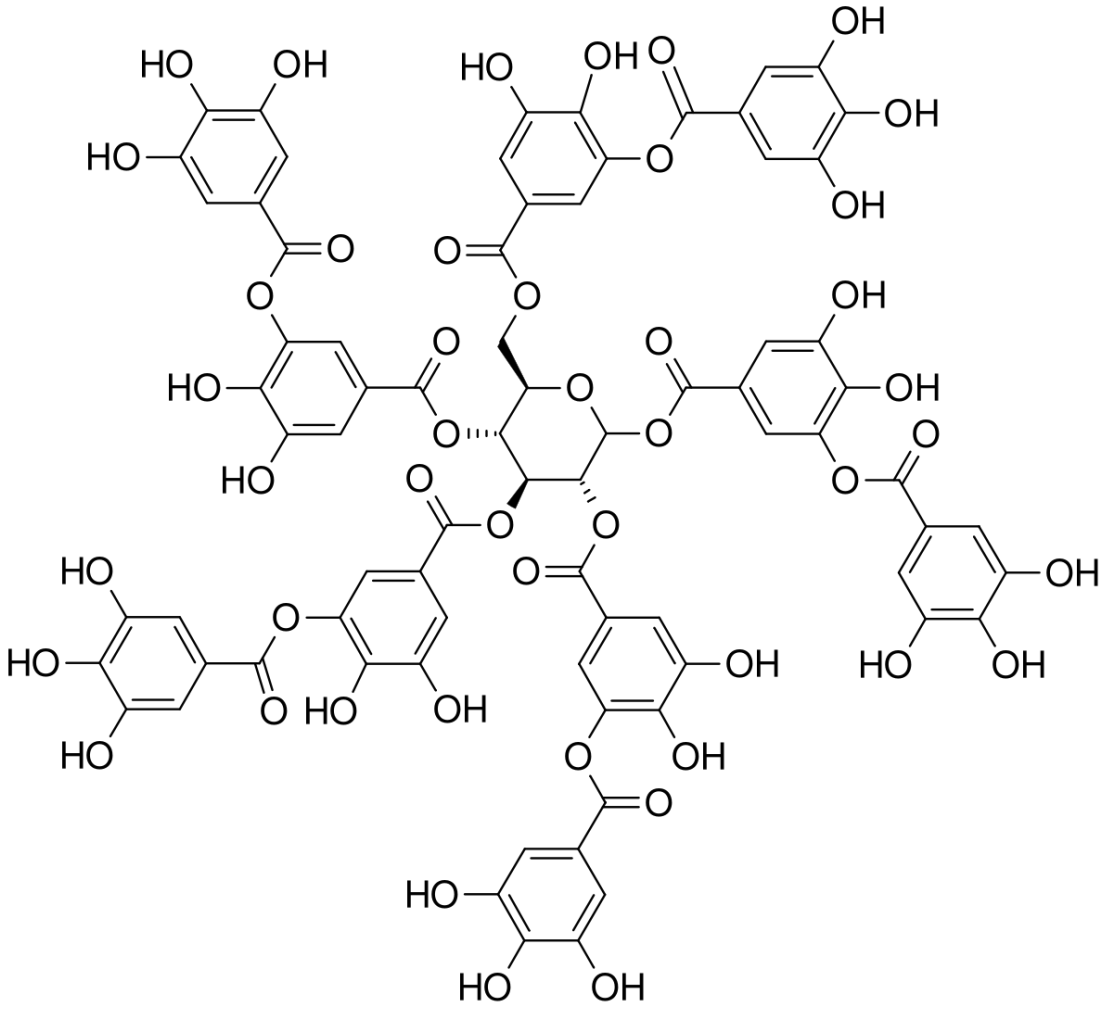
Crosslinking (Binding) Mechanisms

- Cr(III), Zr(IV)
- GA (dialdehydes), EDC, Oxazolidine, THPS
- **Vegetable tannins**

Vegetable Tannins

Proposed mechanism:

- Hydrogen bonding



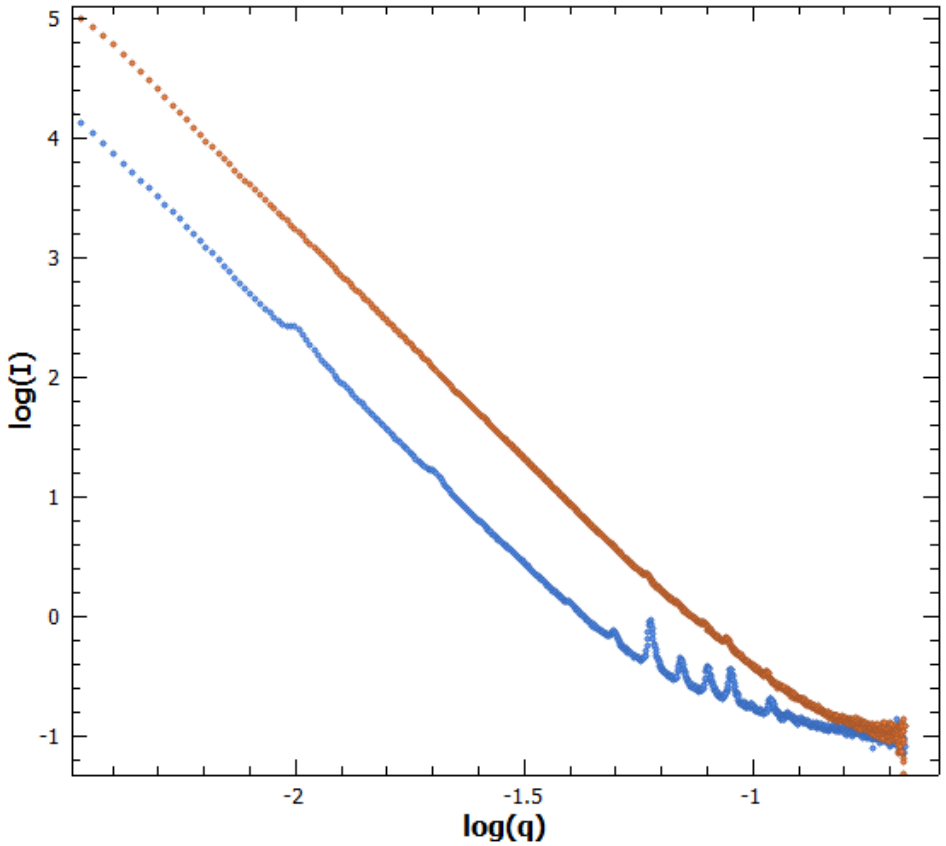
Vegetable Tannins

Peak intensity changes:

- Background enhanced, peak weakened
- R(5/3): largely indifferent from uncrosslinked

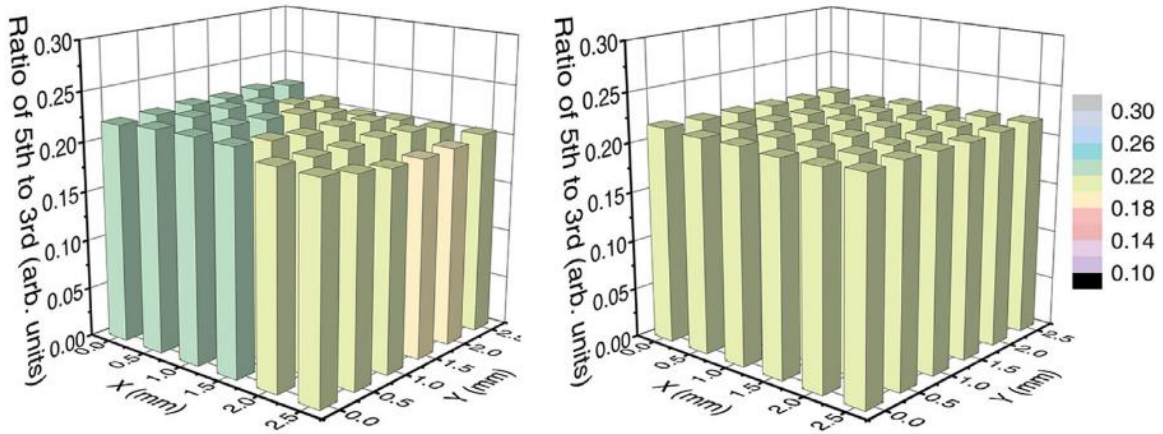
Mechanism:

- Unspecific binding, similar to Cr(III) when electrostatic binding dominates



Summary

- Molecular level insights without interference from unbound species
- Microscale mapping for uniformity



- Other ratios (such as R(6/5)) representing hydration levels, which relate closely to thermal properties

Zhang, Y., Zhang, W., Snow, T., Ju, Y., Liu, Y., Smith, A.J. and Prabakar, S., 2022. Minimising chemical crosslinking for stabilising collagen in acellular bovine pericardium: Mechanistic insights via structural characterisations. Acta Biomaterialia.

Acknowledgement

NSRRC

diamond

ANSTO
Science. Ingenuity. Sustainability.

Thanks for listening!