

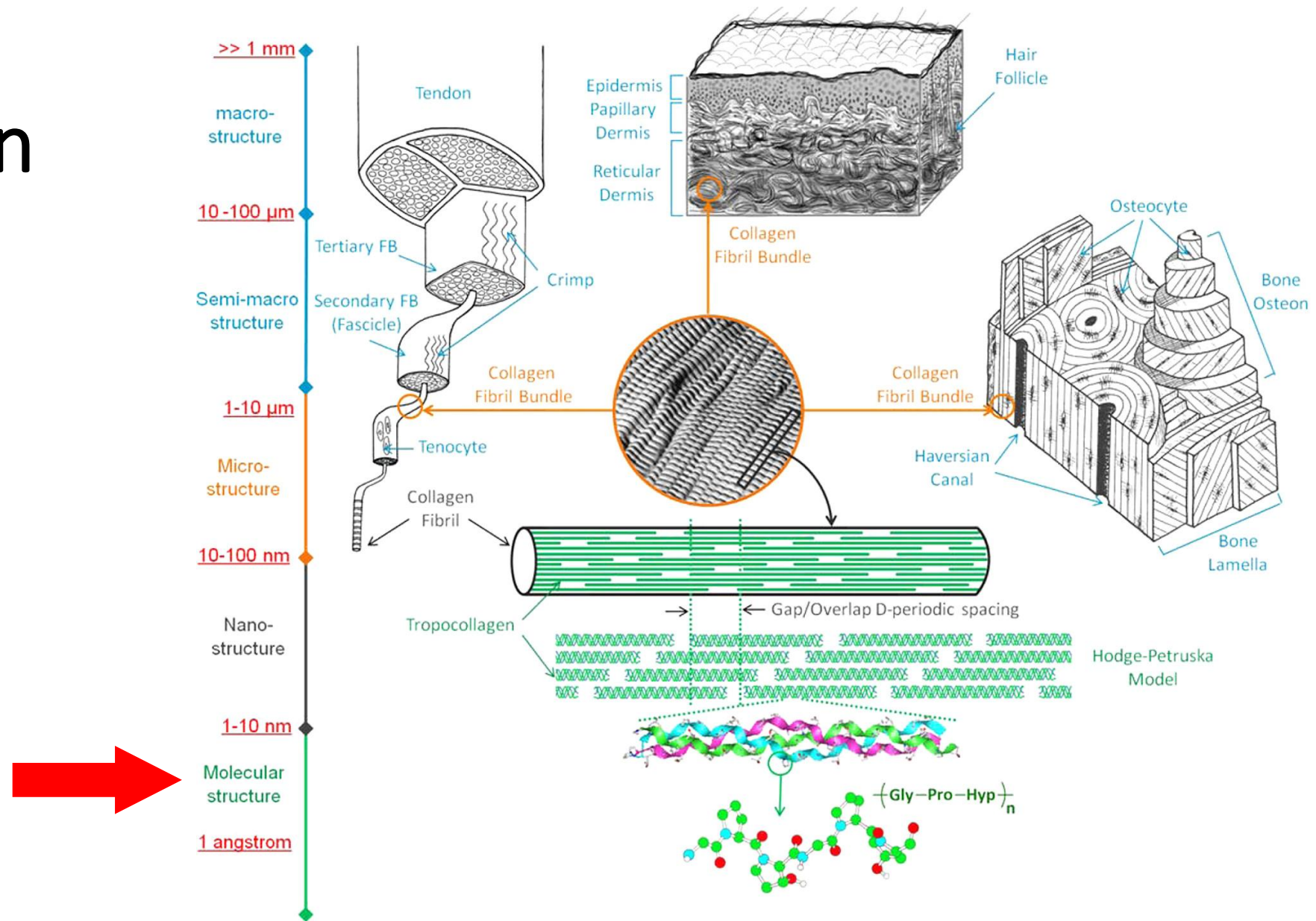
Collagen Crosslinking Mechanisms

Yi (Ethan) Zhang

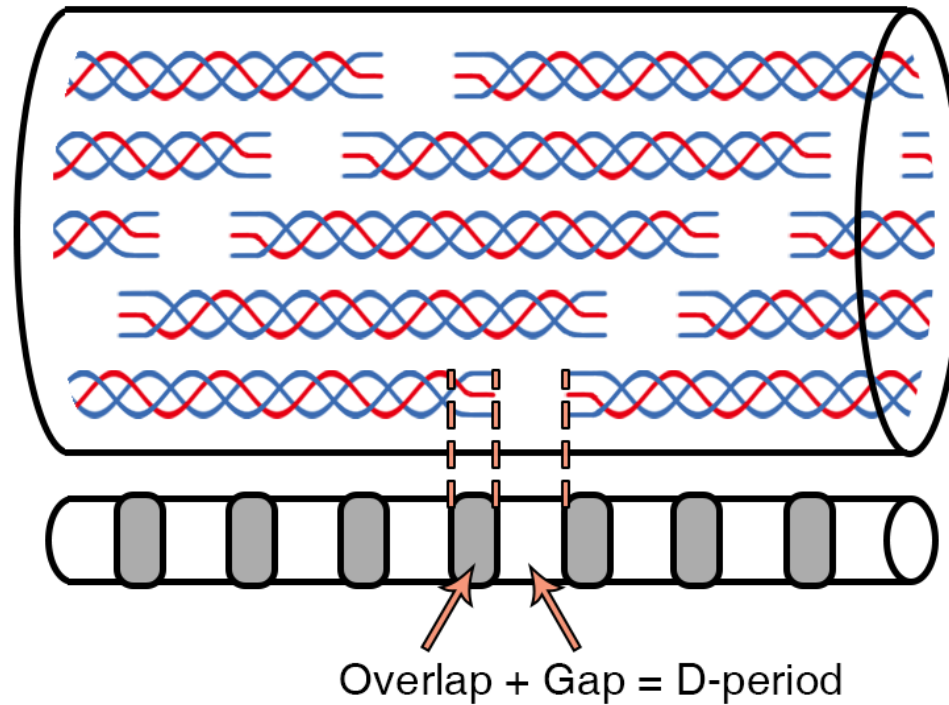
New Zealand Leather and Shoe Research Association (LASRA)



Collagen

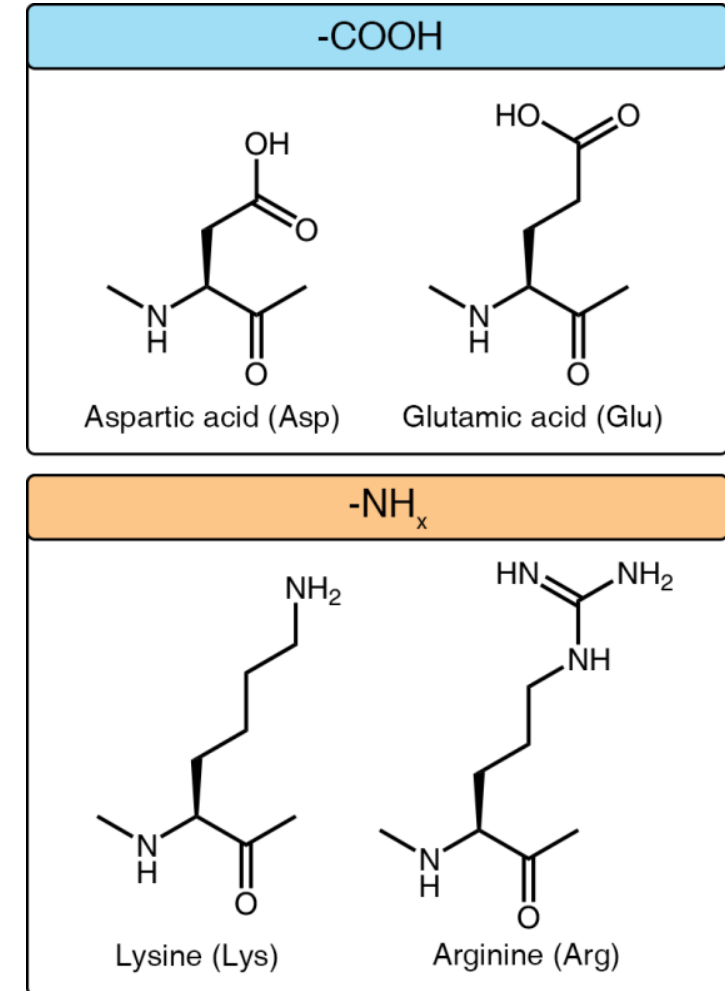


Intermolecular Structure



Amino Acid Side Chains

- $-\text{COOH}$
- $-\text{NH}_2$
- $-\text{OH}$



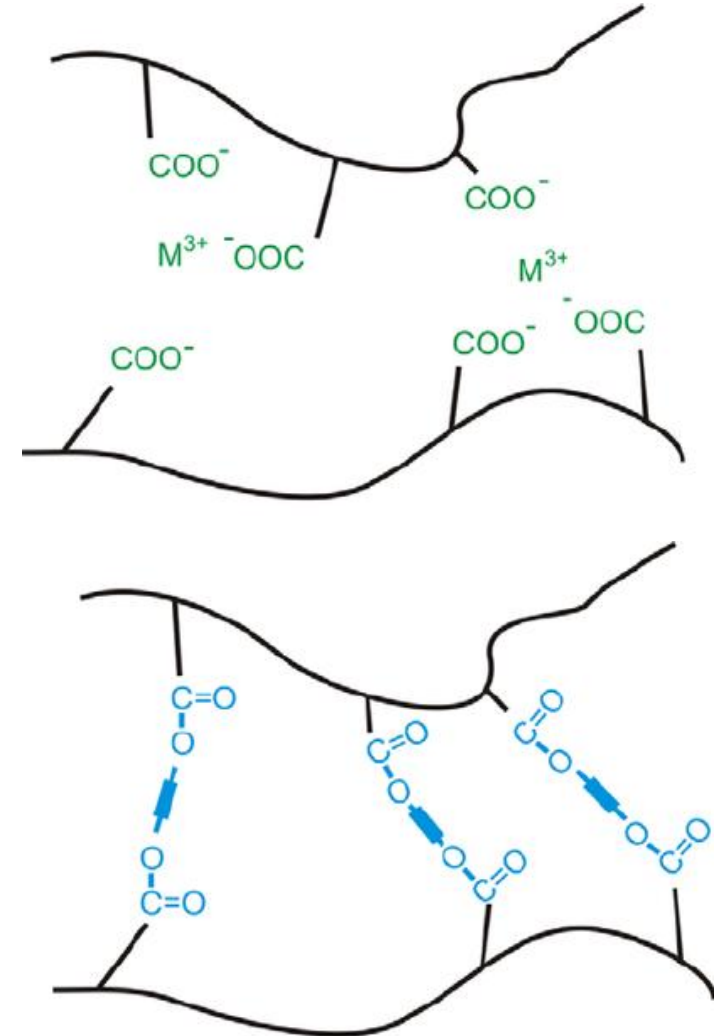
Types of Crosslinking

By bonding types:

- Covalent
- Electrostatic
- Hydrogen bond
- Van der Waals

By species involved:

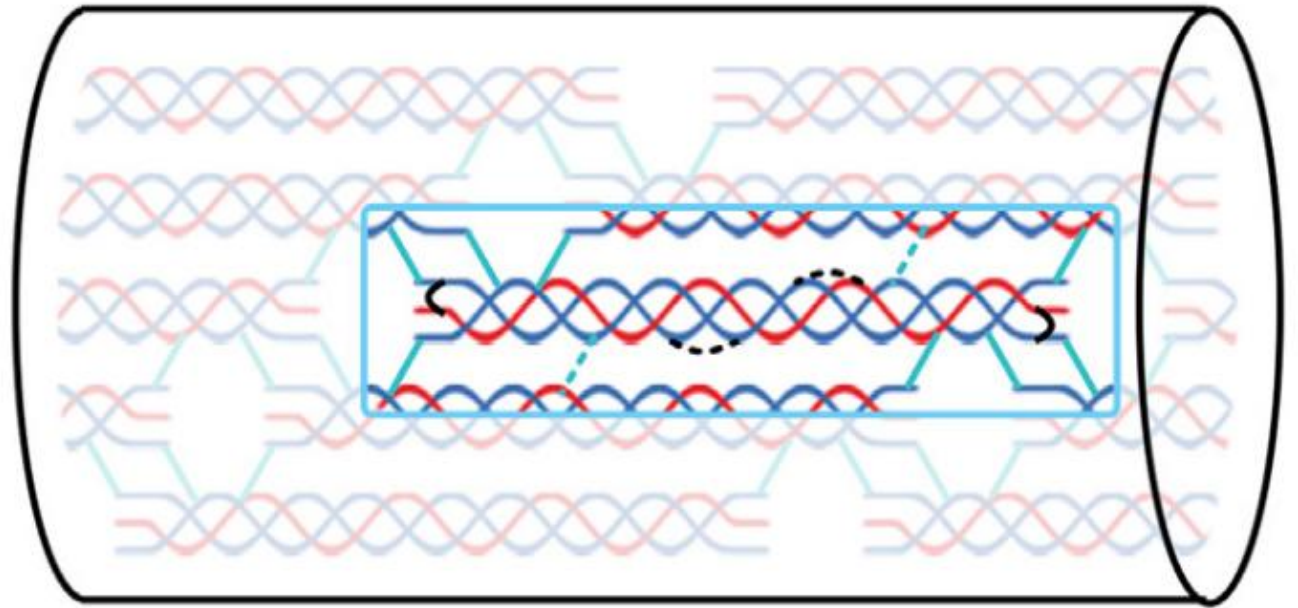
- Inorganic
- Organic
- Zero-linker



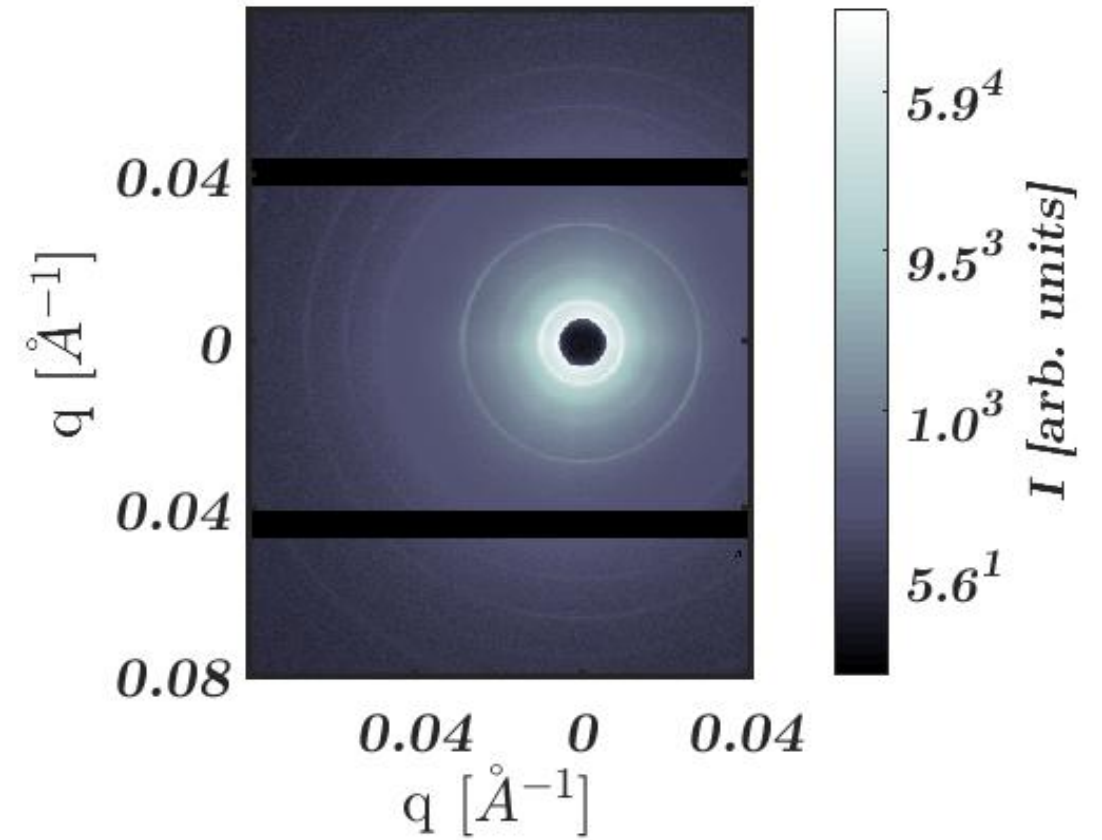
Types of Crosslinking

By binding sites:

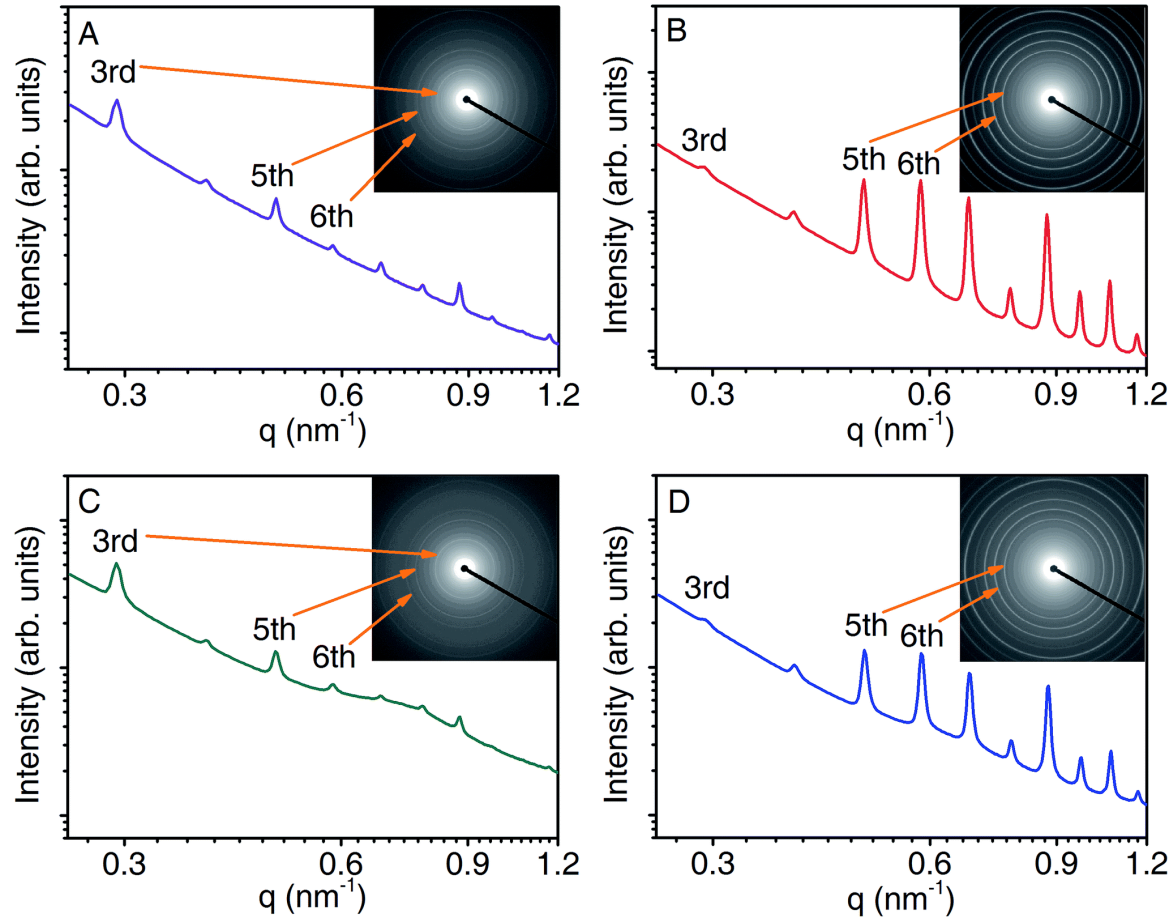
- Intra / Intermolecular
- Telopeptidyl / Helical



Synchrotron X-ray Scattering



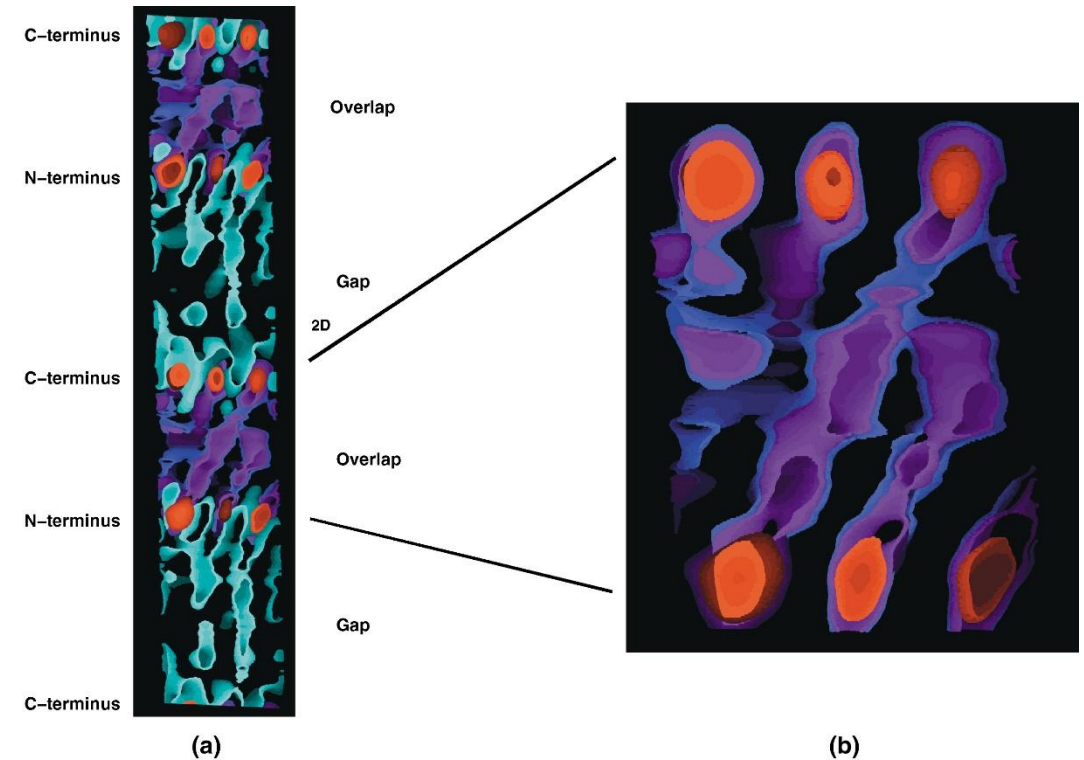
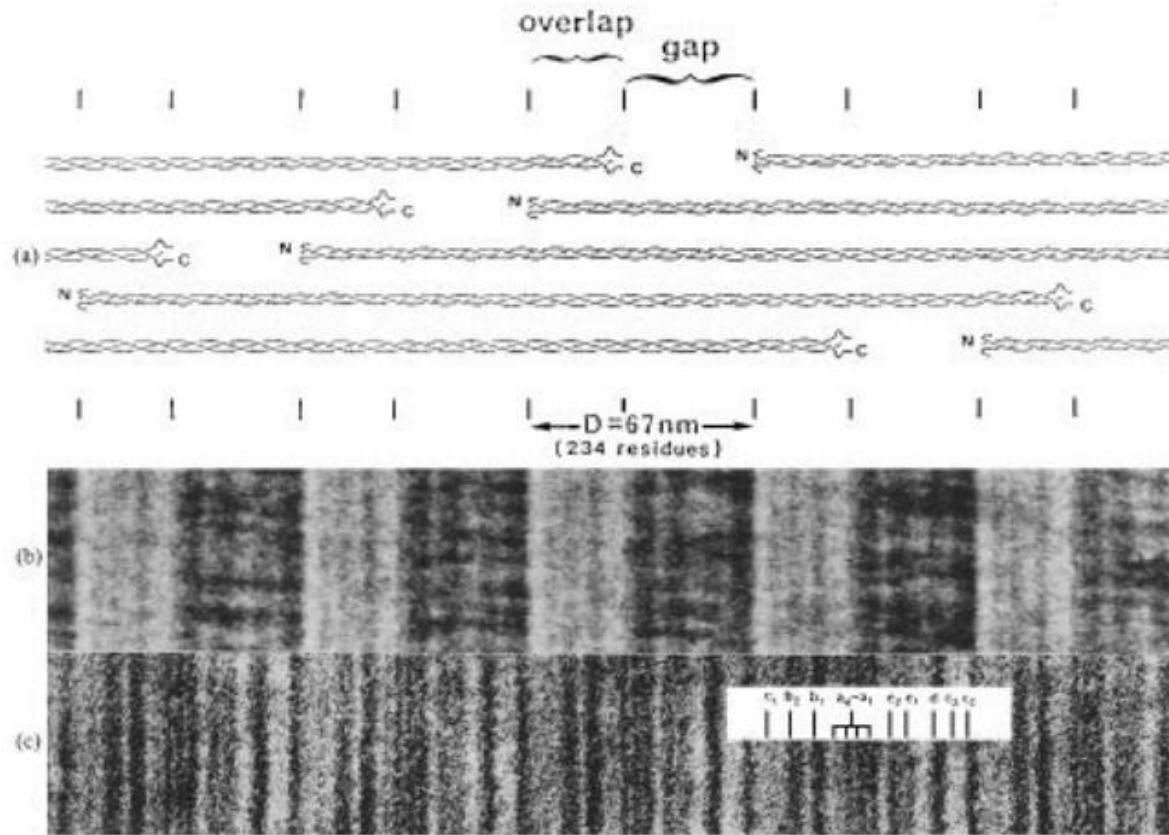
Diffraction Peaks



Peak information:

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

Electron Density Contrast

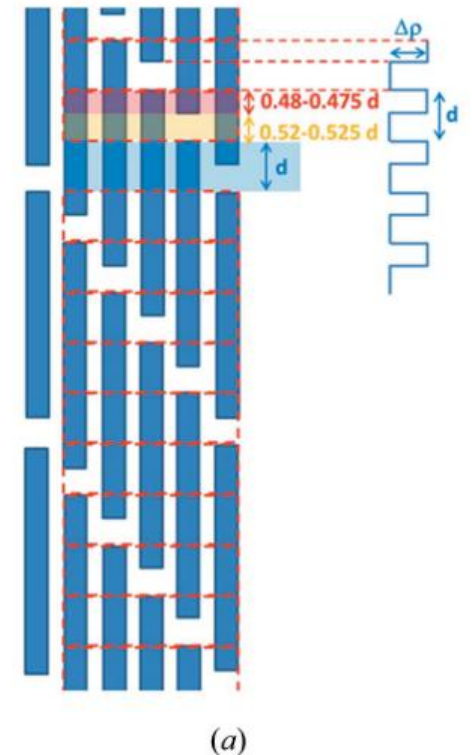
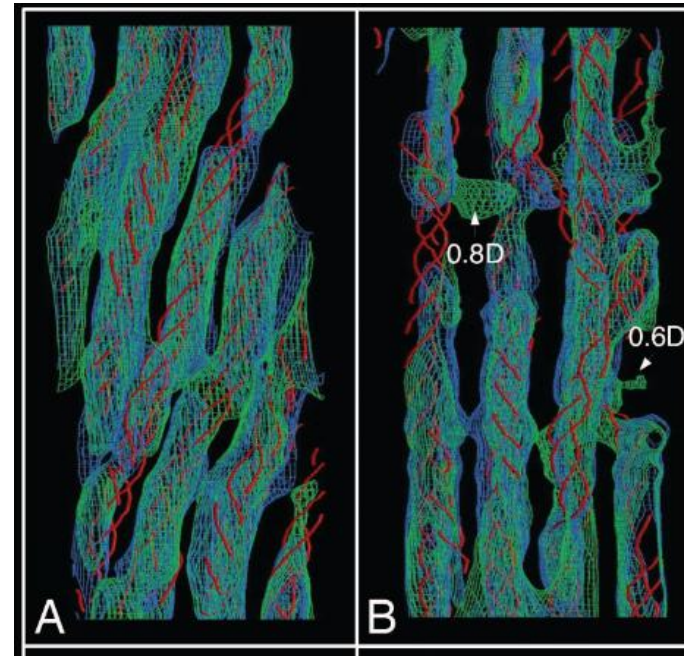


Data Analysis

Efficiency and Accuracy:

- Detailed molecular modelling
- Rough modelling
- Overall peak analysis

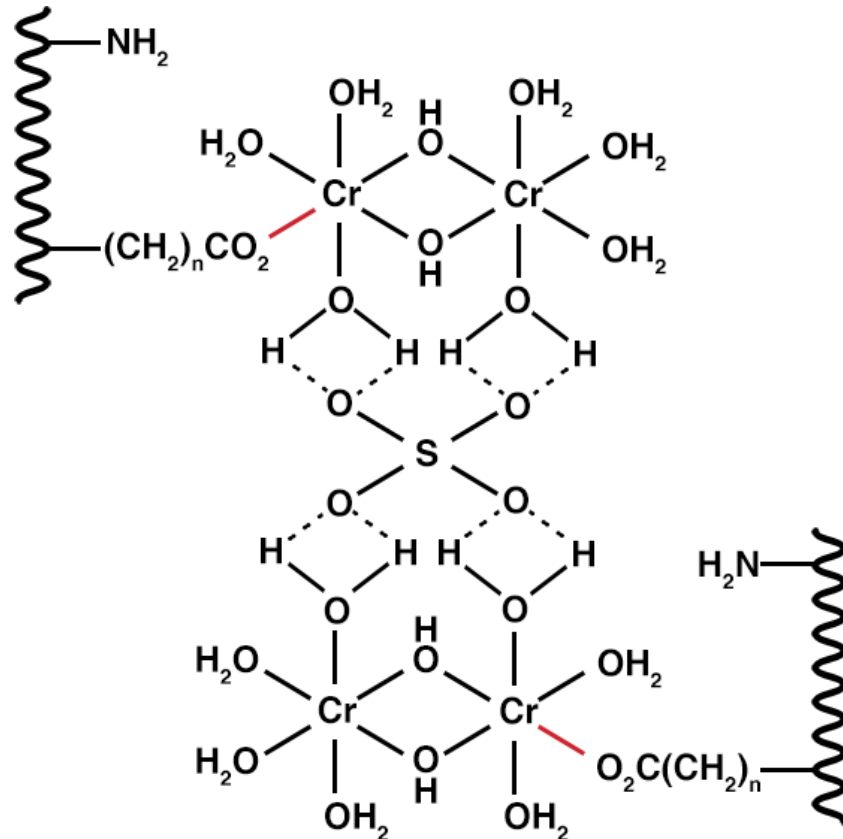
(Odd/even order peaks,
representative peaks)



Crosslinking (Binding) Mechanisms

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

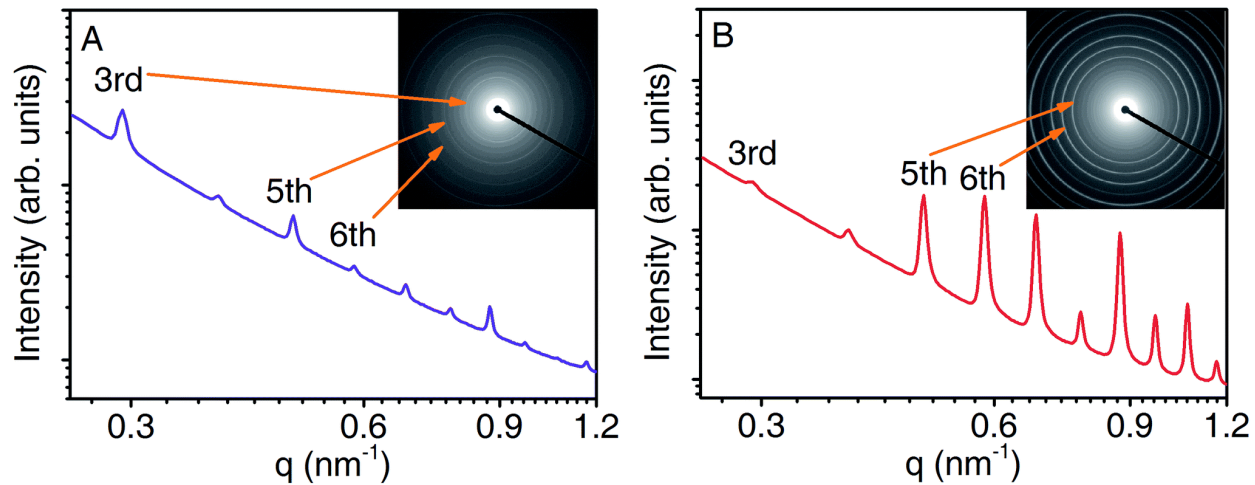
Chromium (III)



Proposed mechanism:

- $-\text{COO}-\text{Cr}$ (covalent)
- SO_4^{2-} bridge

Chromium (III)



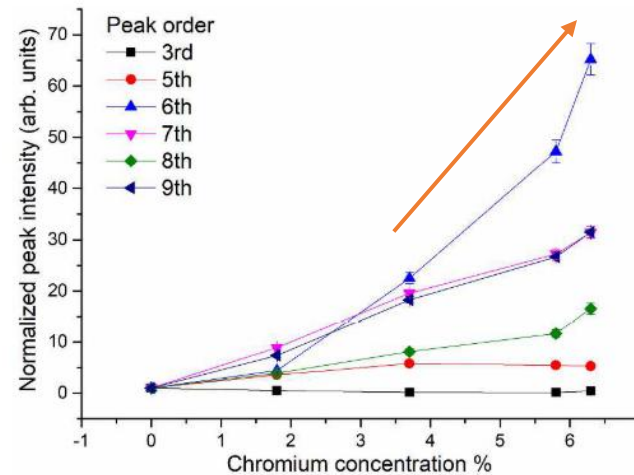
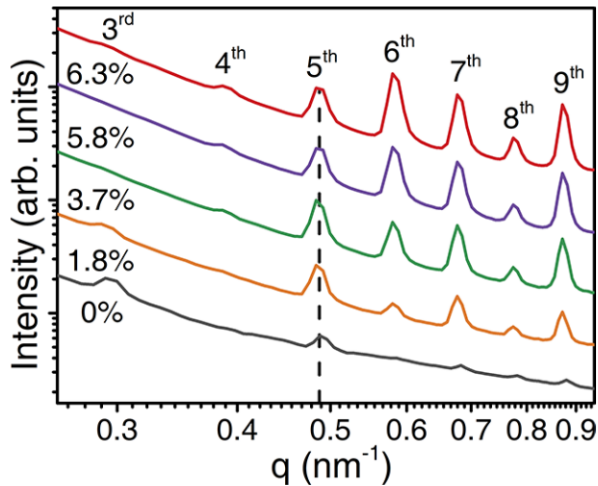
Peak intensity changes:

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

Mechanism:

- Site-specific binding

Chromium (III): Concentration



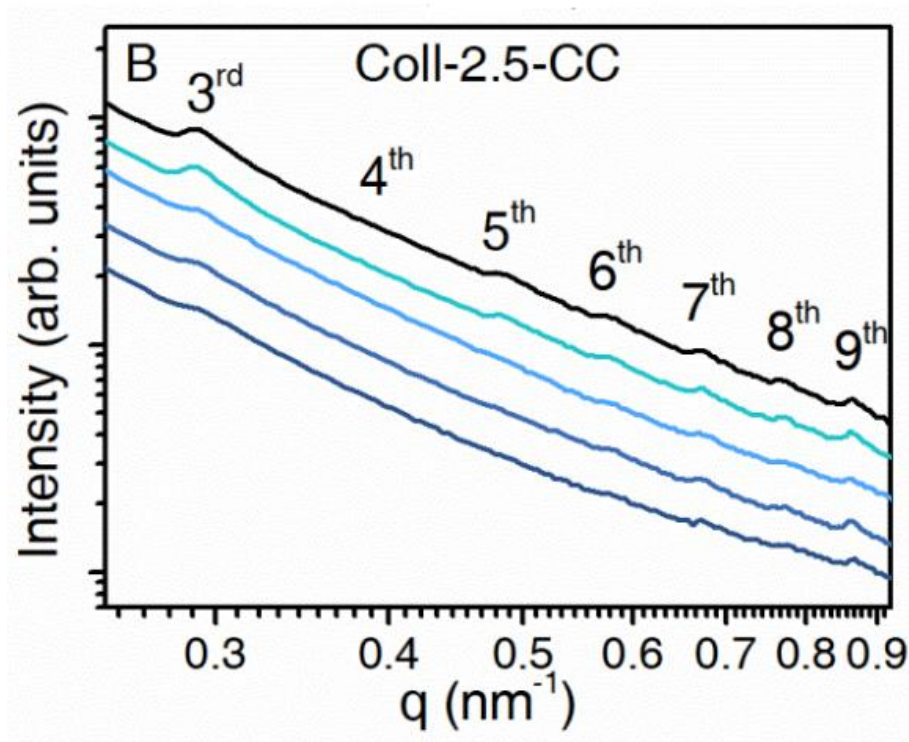
Peak intensity changes:

- Overall (especially 6th) increase above certain concentration

Mechanism:

- Covalent binding occurs first, electrostatic binding when adding excess

Chromium (III): Anion



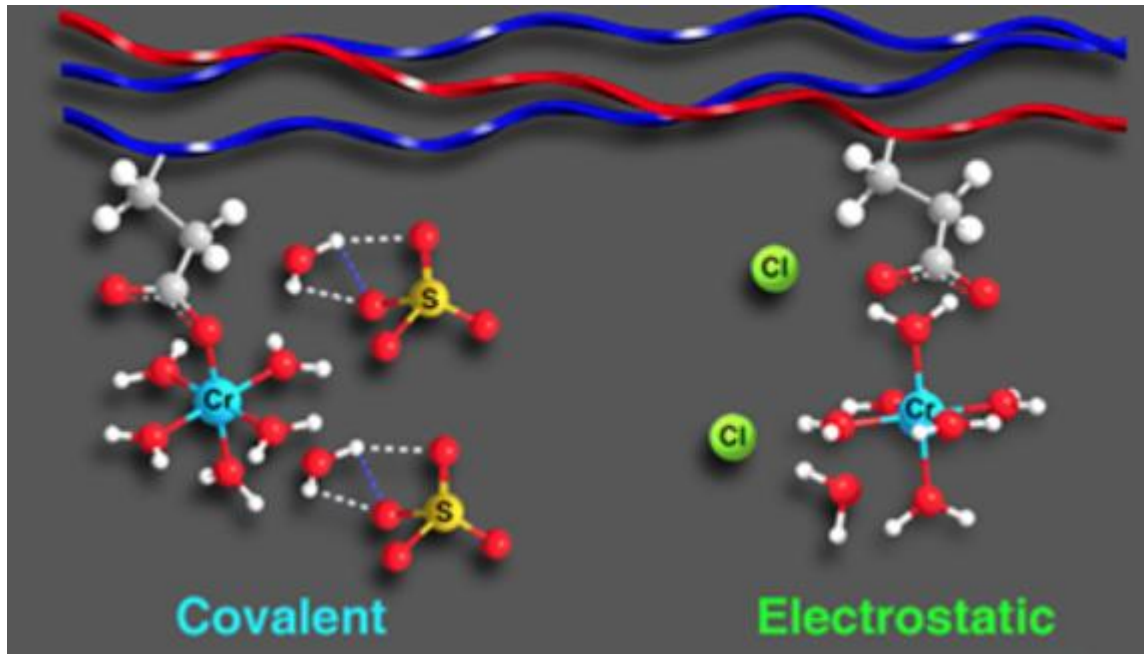
Peak intensity changes:

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

Mechanism:

- Both occurs in the matrix
- Priority regulated by anions

Chromium (III): Anion



Peak intensity changes:

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

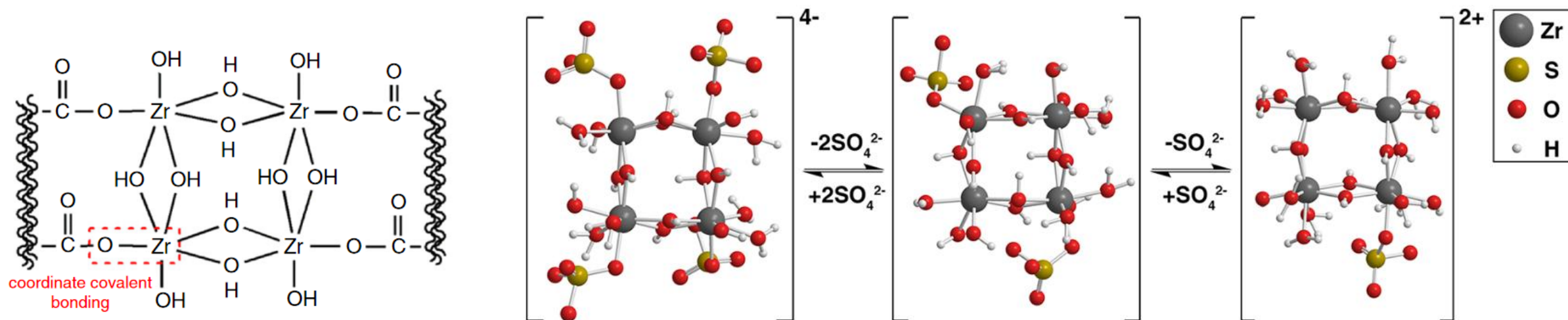
Mechanism:

- Both occurs in the matrix
- Priority regulated by anions

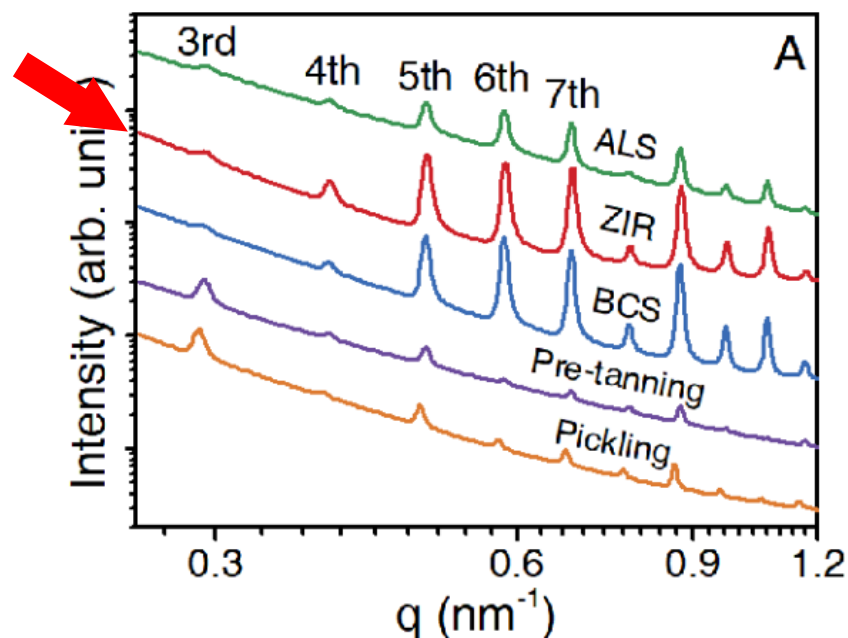
Zirconium (IV)

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)

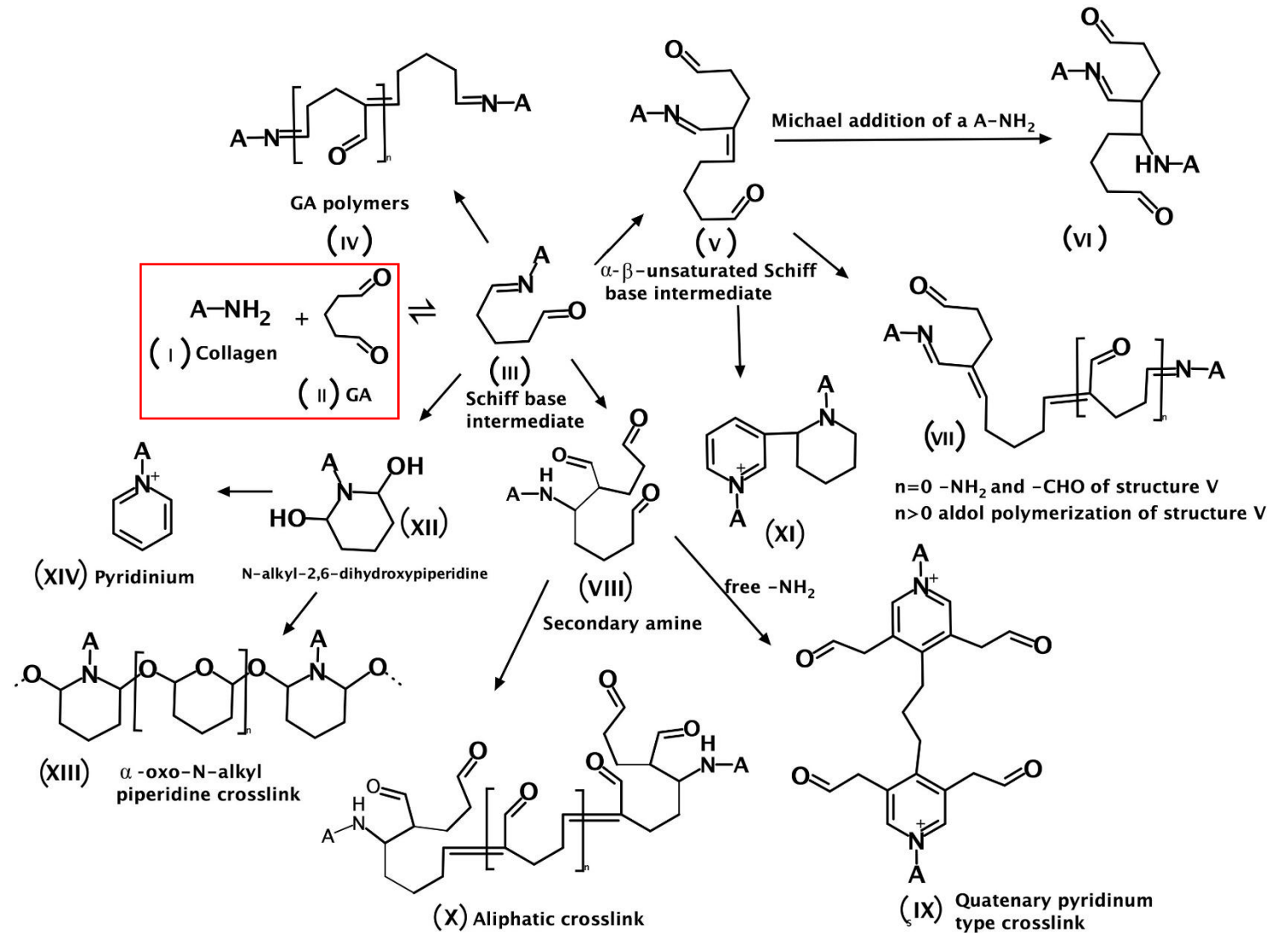
Crosslinking (Binding) Mechanisms

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

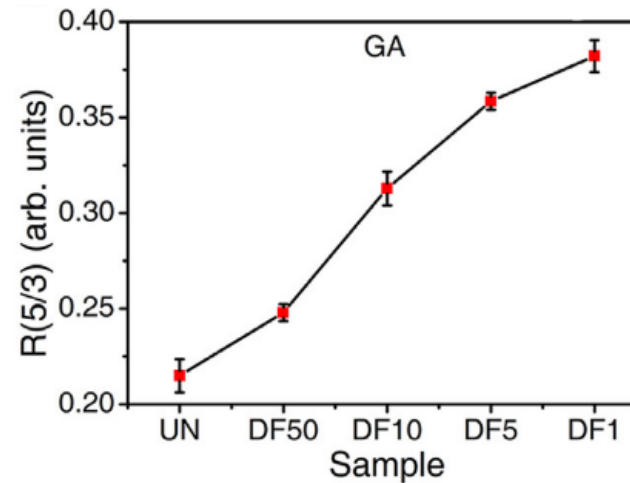
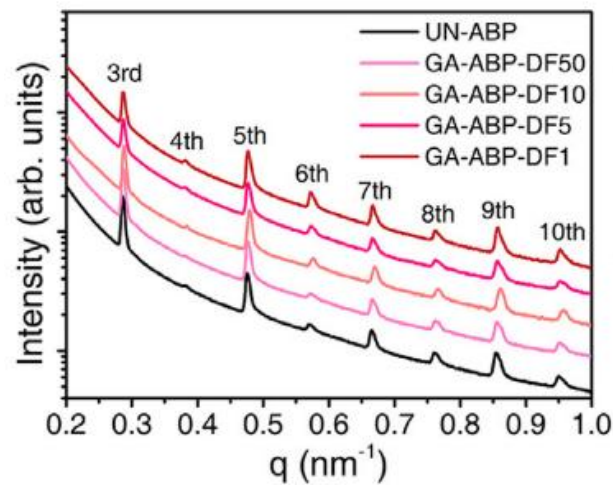
Glutaraldehyde

Proposed mechanism:

- Many routes
- Potentially polymerise



Glutaraldehyde



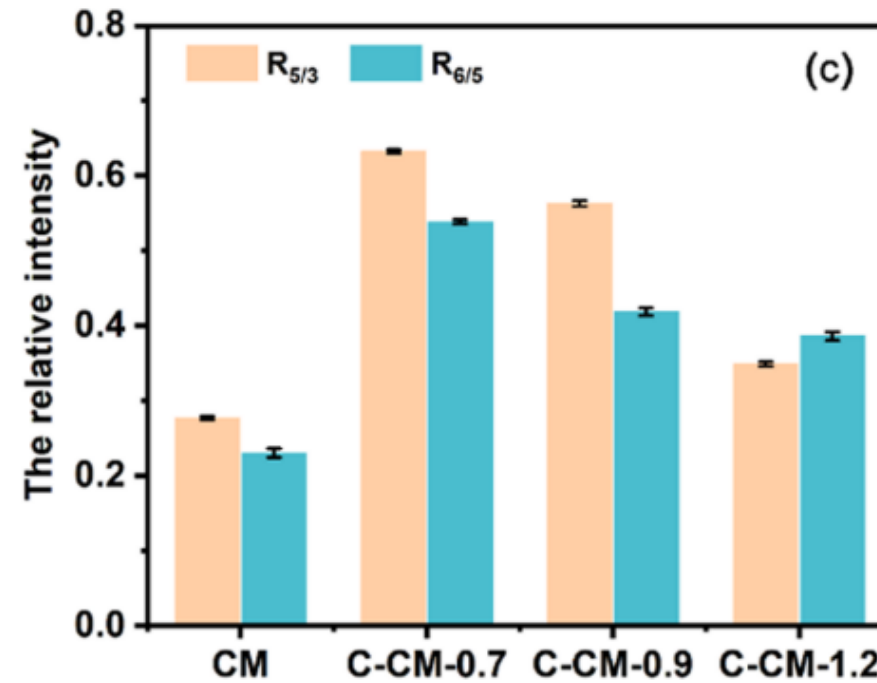
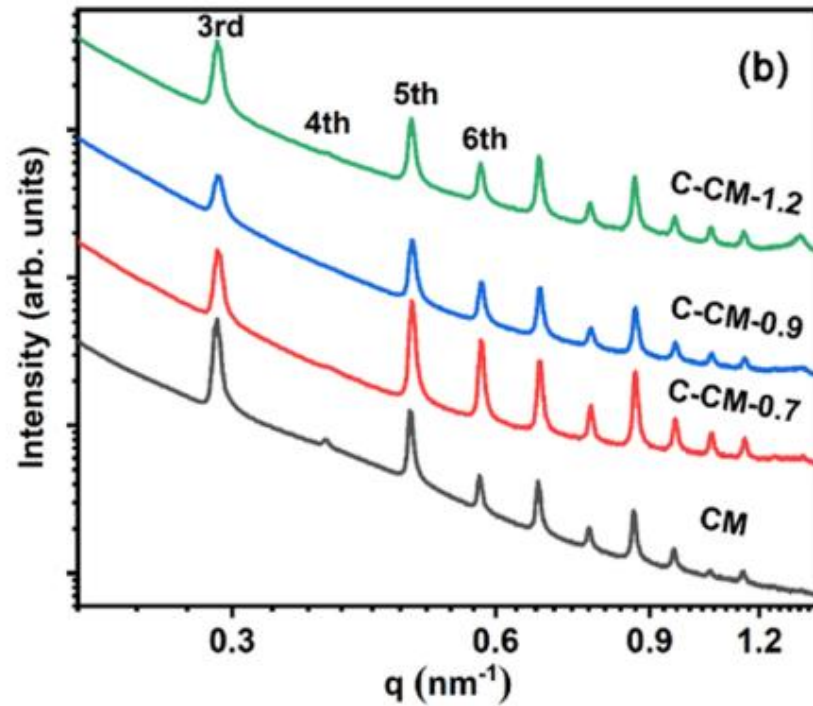
Peak intensity changes:

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

Mechanism:

- Site-specific binding

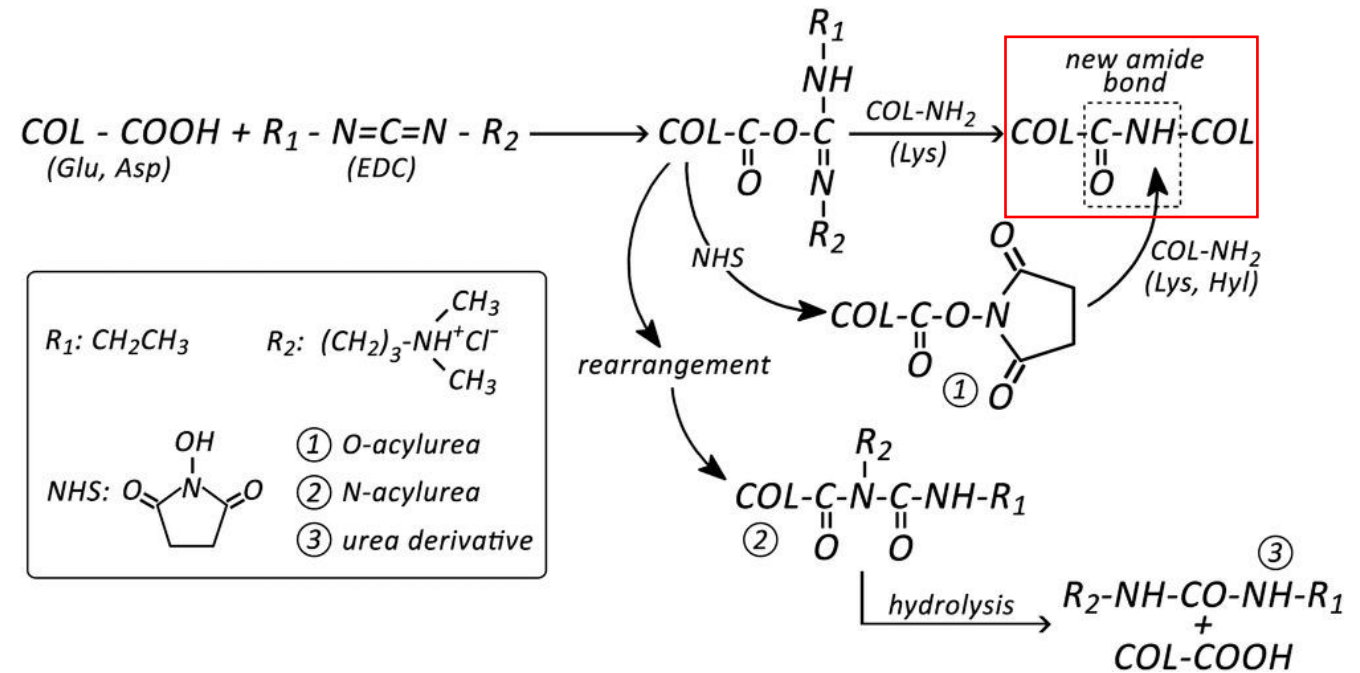
Dialdehydes



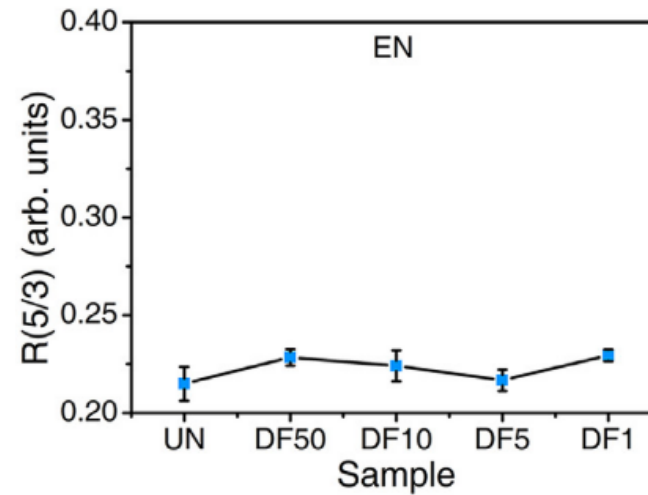
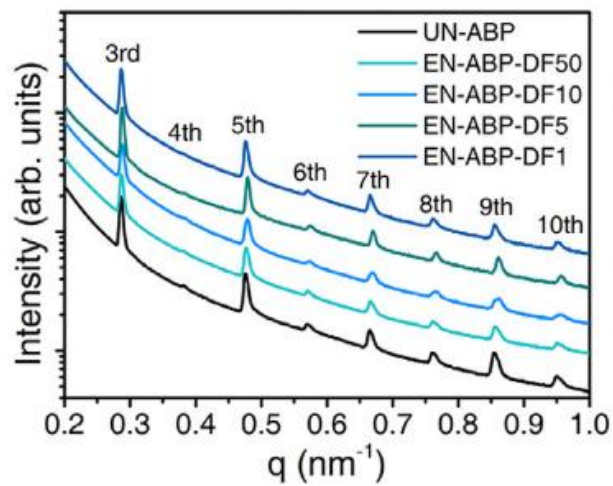
EDC

Proposed mechanism:

- Peptide bonds (Zero-linker)



EDC



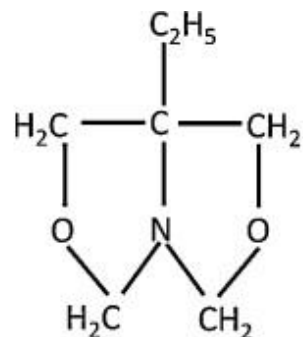
Peak intensity changes:

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

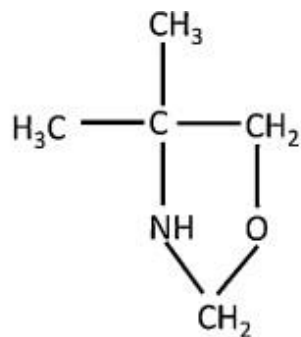
Mechanism:

- Triggering the crosslinking

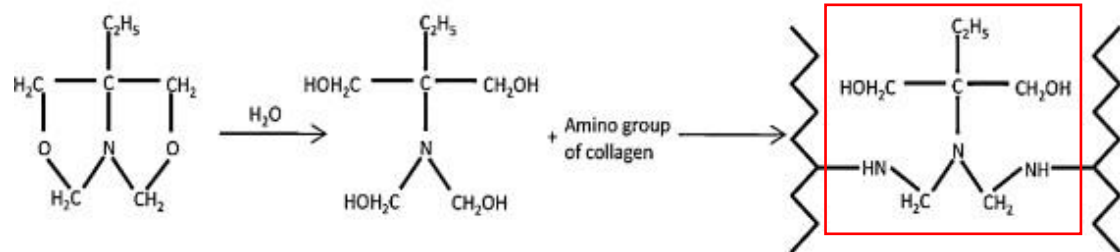
Oxazolidine



a. Bicyclic oxazolidine



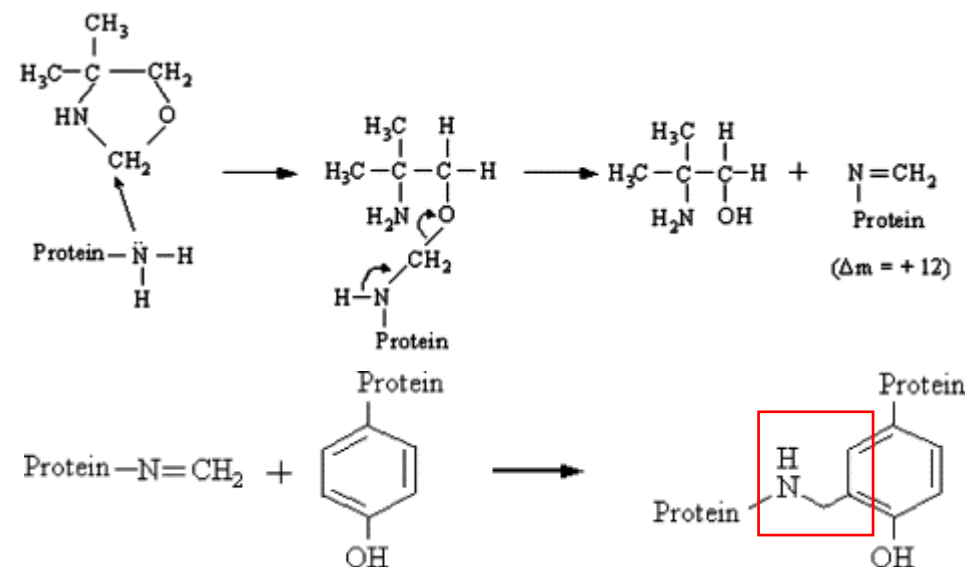
b. Monocyclic oxazolidine



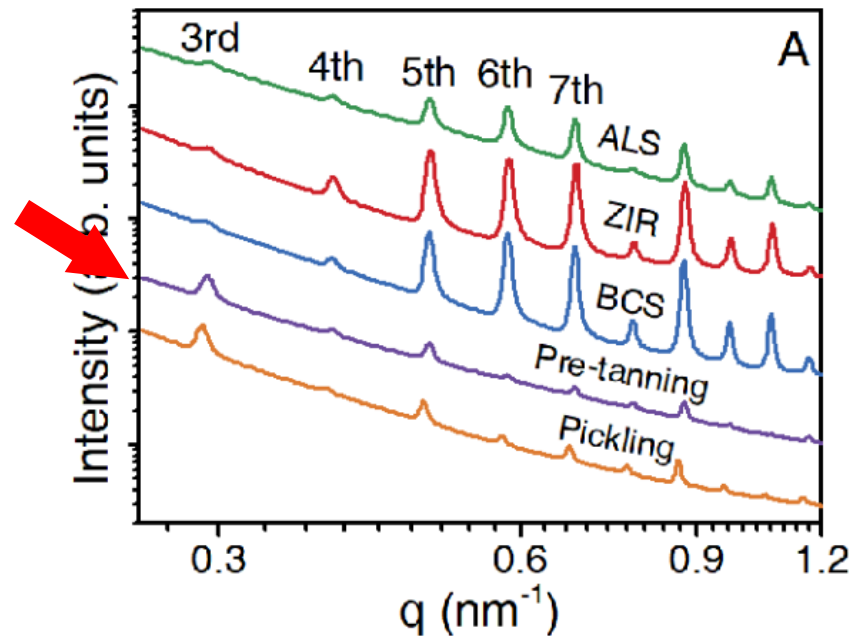
c. Possible oxazolidine - collagen interaction

Proposed mechanism:

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



Oxazolidine



Peak intensity changes:

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

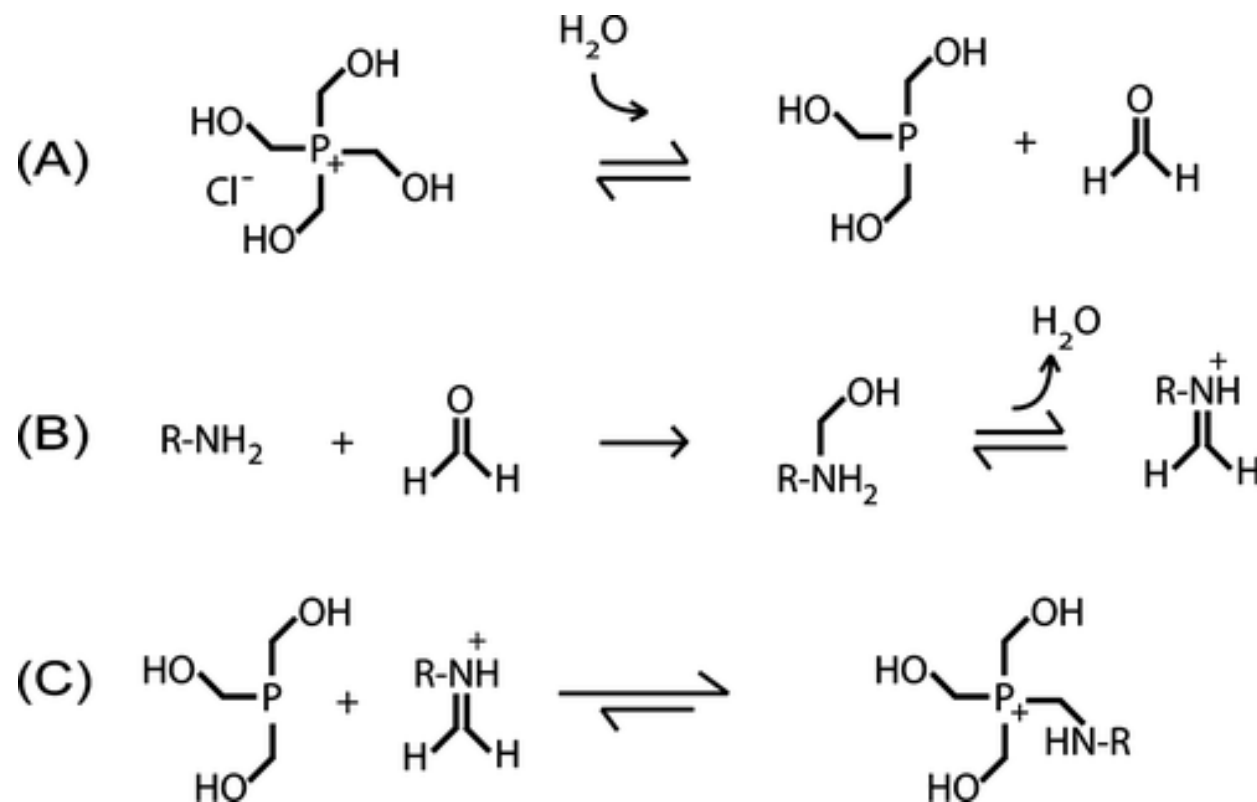
Mechanism:

- Triggering the crosslinking, similar to EDC

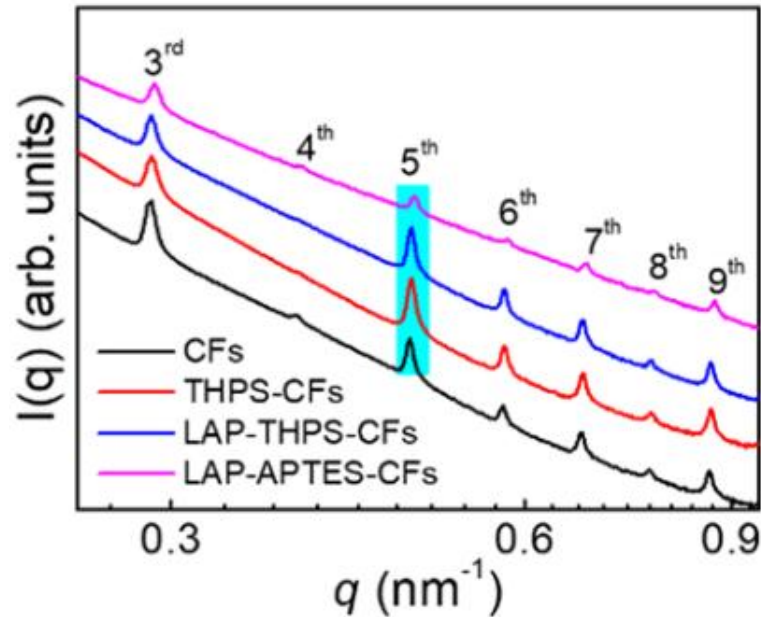
THPS

Proposed Mechanism:

- Covalent linkage with -NH_2 groups



THPS

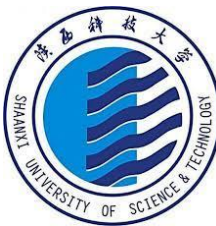


Peak intensity changes:

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

Mechanism:

- Site-specific binding, similar to GA



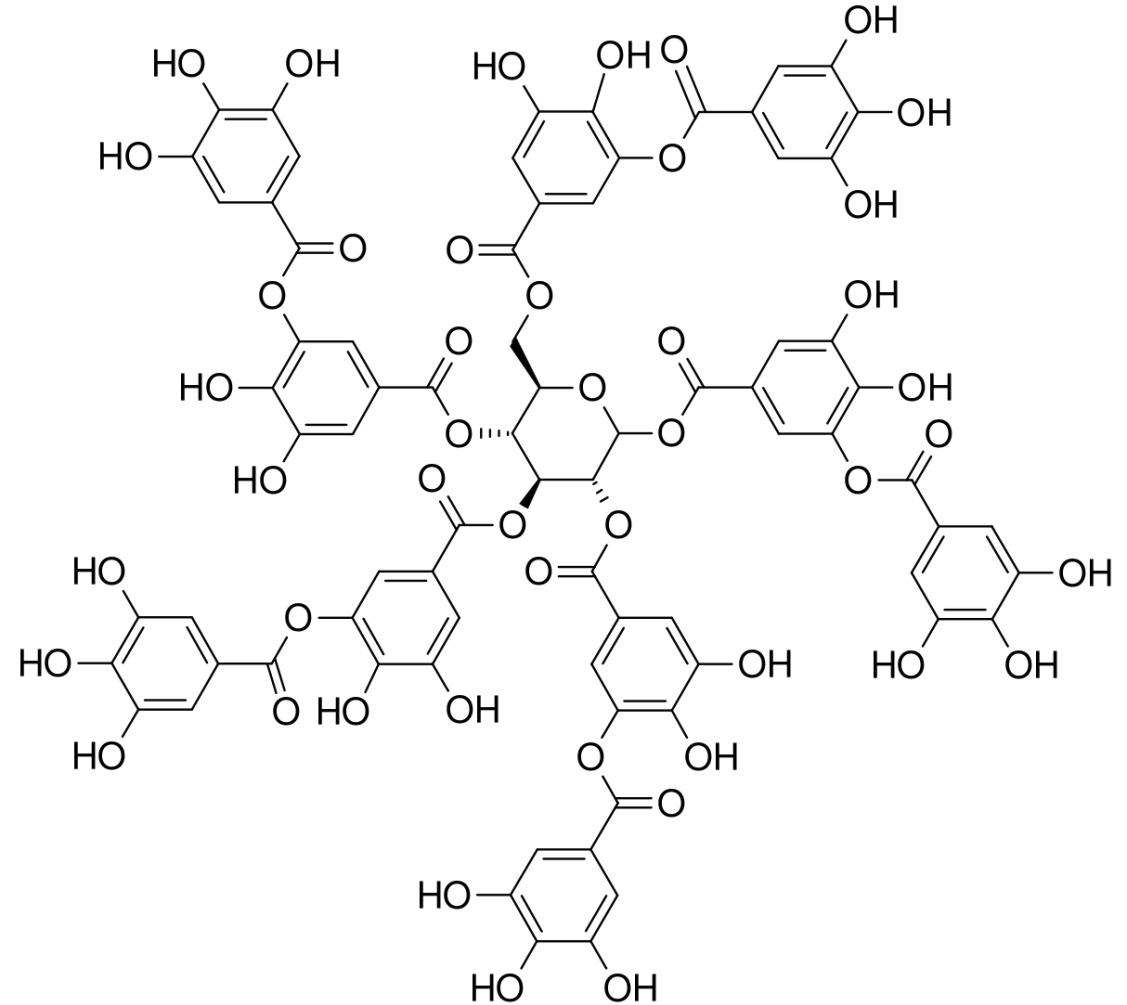
Crosslinking (Binding) Mechanisms

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

Vegetable Tannins

Proposed mechanism:

- Hydrogen bonding



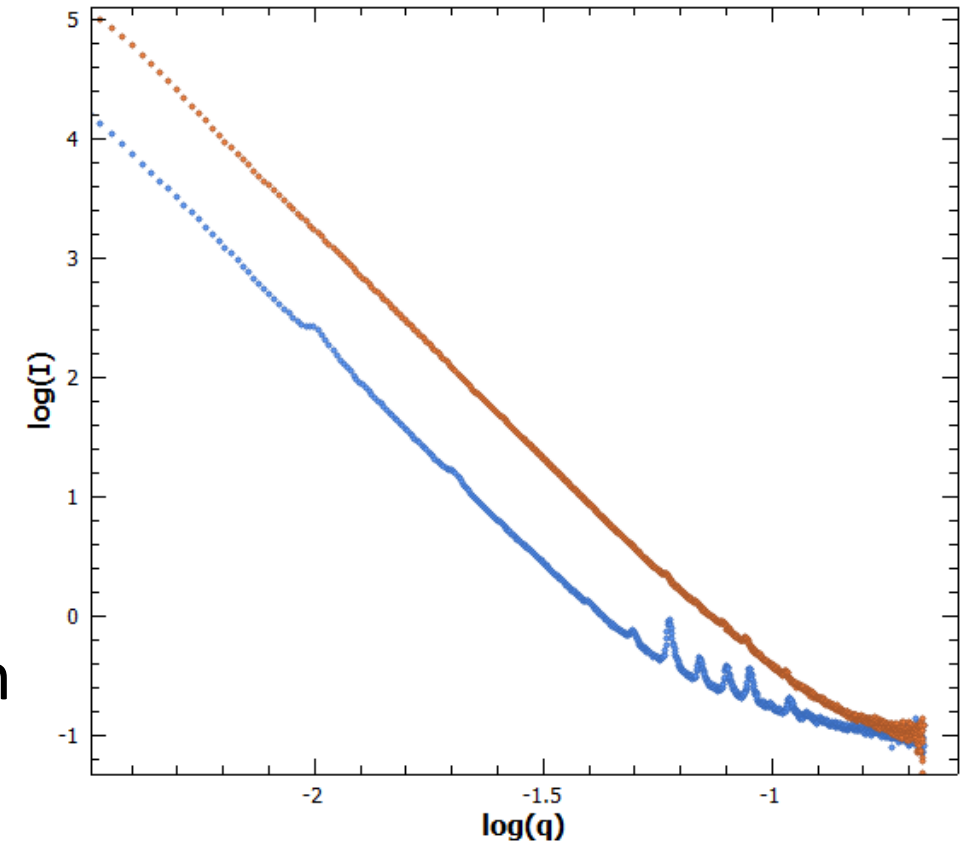
Vegetable Tannins

Peak intensity changes:

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

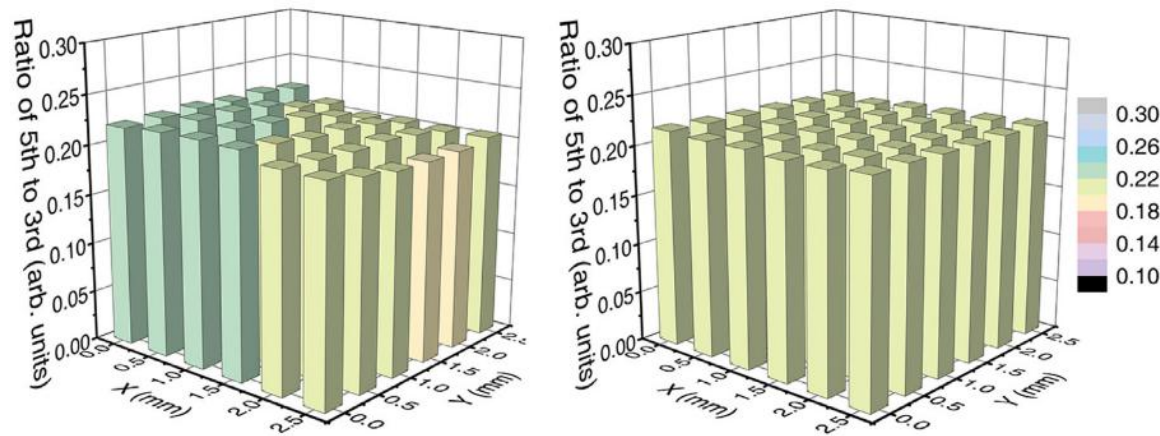
Mechanism:

- 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

Acknowledgement



Thanks for listening!