The Secret Life of Atoms in Collagen in Bone

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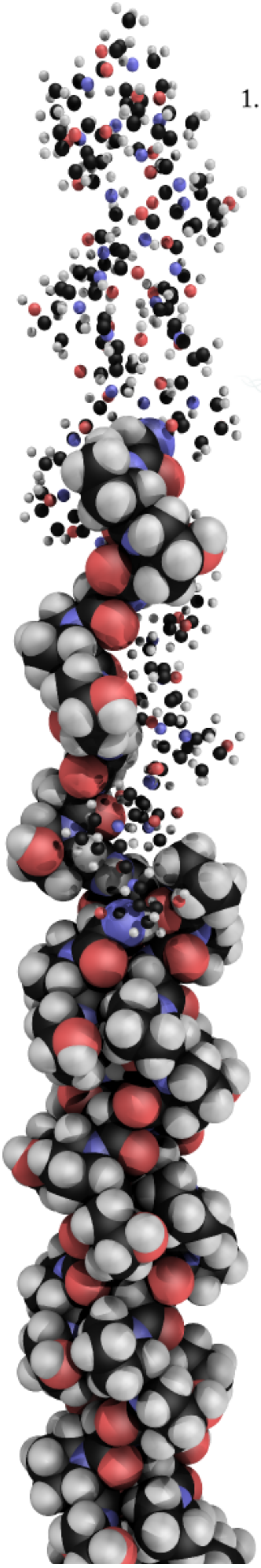


1. Collagen

Collagen is an important protein found in many solid structural tissues in the body. From skin and eyes to joints and bone, collagen is an key component of the material surrounding cells: the extracellular matrix (ECM). It has a triple helical structure, where three protein strands coil together to form a long, cable-like molecule (left). In health and in disease, collagen in the ECM interacts with cells and plays complex roles that we still do not fully understand.

There is a lack of methods to study the atomic structure of collagen in intact tissues.

2. Solid-state NMR

Solid-state nuclear magnetic resonance (ssNMR) can give atomic-level information on solid samples. By placing the sample in a strong magnet (9.4 T) and applying radiofrequency pulses, some of the nuclei within the sample will induce an electric signal. The frequency of this electric signal depends on the chemical environment.

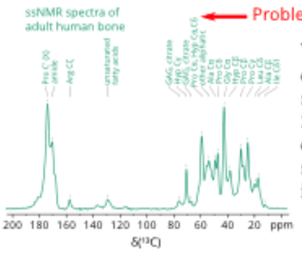
ssNMR is suitable for investigating large protein complexes that are difficult to investigate by X-ray diffraction or solution-state NMR.

In order to enhance the ssNMR signal, it is usual to enrich the samples in ^{13}C and ^{15}N. We have taken new approaches to obtain enriched mammalian collagen proteins.

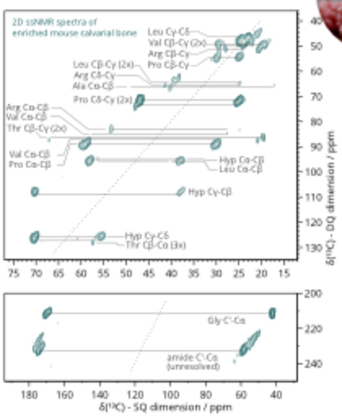
Samples are usually dried and packed directly into ssNMR rotors in small pieces. No extra purification or staining is required.

How can ssNMR report on atomic structure of collagen in tissue?

3. Identifying Specific Atoms

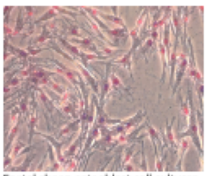


We kept mice on an NMR-active diet, so we can acquire 2D NMR spectra and resolve NMR signals for atoms in mouse bone.

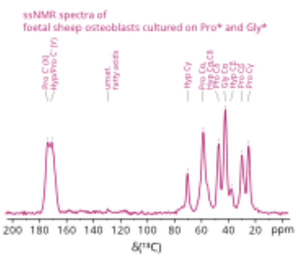


Using 2D mouse data, we developed an osteoblast cell culture protocol that mimics healthy developing bone.

Bone tissue can be broken into pieces and directly packed into an insert.

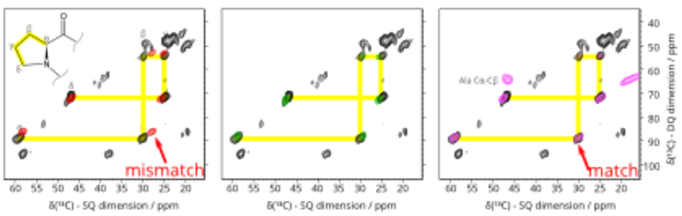


Foetal sheep osteoblast cell cultures provide a tunable biological model for developing bone. Samples are usually freeze-dried before packing into an insert.



This protocol can be easily adapted for cell-based models of diabetes, heart disease, cancer and aging.

4. Proline Ring Atoms: Models vs Real Life



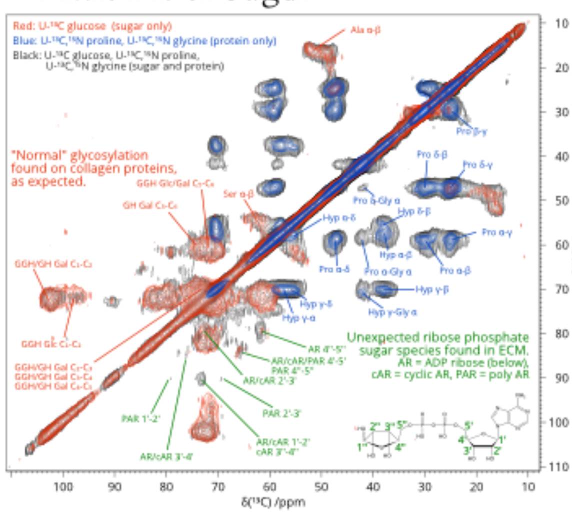
Model A: (GPO)$_5$**GPO**(GPO)$_5$ "Ideal" peptide sequence Helix-coil transition T (expt): 62°C MOST STABLE (BEST?)

Model B: (GPO)$_5$GP**PG**PO(GPO)$_4$ Hyp→Pro Helix-coil transition T (est): 60°C

Model C: (GPO)$_5$GP**PA**PO(GPO)$_4$ Hyp→Pro, Gly→Ala Helix-coil transition T (expt): 29°C LEAST STABLE (WORST?)

ssNMR show the limitations of our current models. We now have a quick "fingerprint" test for whether a biomaterial is similar to bone, which can be used to assess tissue engineering materials.

5. A Little Bit of Sugar



Our ssNMR approach enabled us to identify ribose phosphates that may play a part in bone mineralisation (good) and diabetes (bad).

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