Unveiling Salt-Bridge Interactions Between GAGs and Collagen Protein in Cartilage by DNP-Enhanced ssNMR Spectroscopy

Navneet Dwivedi[1], Bijaylaxmi Patra[1,2], Frederic Mentink-Vigier[3,4], Sungsool Wi[3,4*] and Neeraj Sinha[1,2,*]

1. Centre of Biomedical Research, Lucknow INDIA; 2. Academy of Scientific and Innovative Research, INDIA; 3. Florida State University, USA; 4. National High Magnetic Field Laboratory, USA

Funding Grants: K.M. Amm (NHMFL, NSF/DMR-2128556 and DMR-1644779), R.W. Schurko (NIH P41 GM122698 and NIH RM1-GM148766)

The extracellular cartilage matrix (ECM) is a complex biological structure, consisting of highly mobile glycosaminoglycans (GAGs) alongside rigid collagen proteins. The interaction between GAGs and collagen is crucial for cartilage stability, yet direct evidence of these interactions in their native state has not been observed.

To address this, we applied novel magic-angle spinning/dynamic nuclear polarization (MAS-DNP) solid-state NMR (ssNMR) methodologies to study the ECM in its natural ^{13}C-abundance state. For the first time, these approaches enabled the direct observation of dipolar-coupled ^{15}N-^{13}C and SQ-SQ ^{13}C-^{13}C interactions in 2D correlation spectra—previously unattainable in natural-abundance ^{13}C/^{15}N samples.

^{15}N-^{13}C 2D ssNMR experiments (**Fig. 1**, **left**) provide the first atomic-level confirmation of direct bonding interactions between hydroxyproline (Hyp) in collagen and GAGs, which contributes to the structural stability of the cartilage extracellular matrix. ^{13}C-^{13}C 2D correlation experiments (**Fig. 1**, **right**) identified ^{13}C-^{13}C correlations between GAGs and collagen at distances of up to 4–5 Å, revealing direct molecular interactions, including charge-pair salt-bridge interactions between the sulfate groups of GAGs and the guanidinium groups of arginine in collagen.

This approach has the provides a powerful new method for characterization of biological systems without isotopic enrichment.

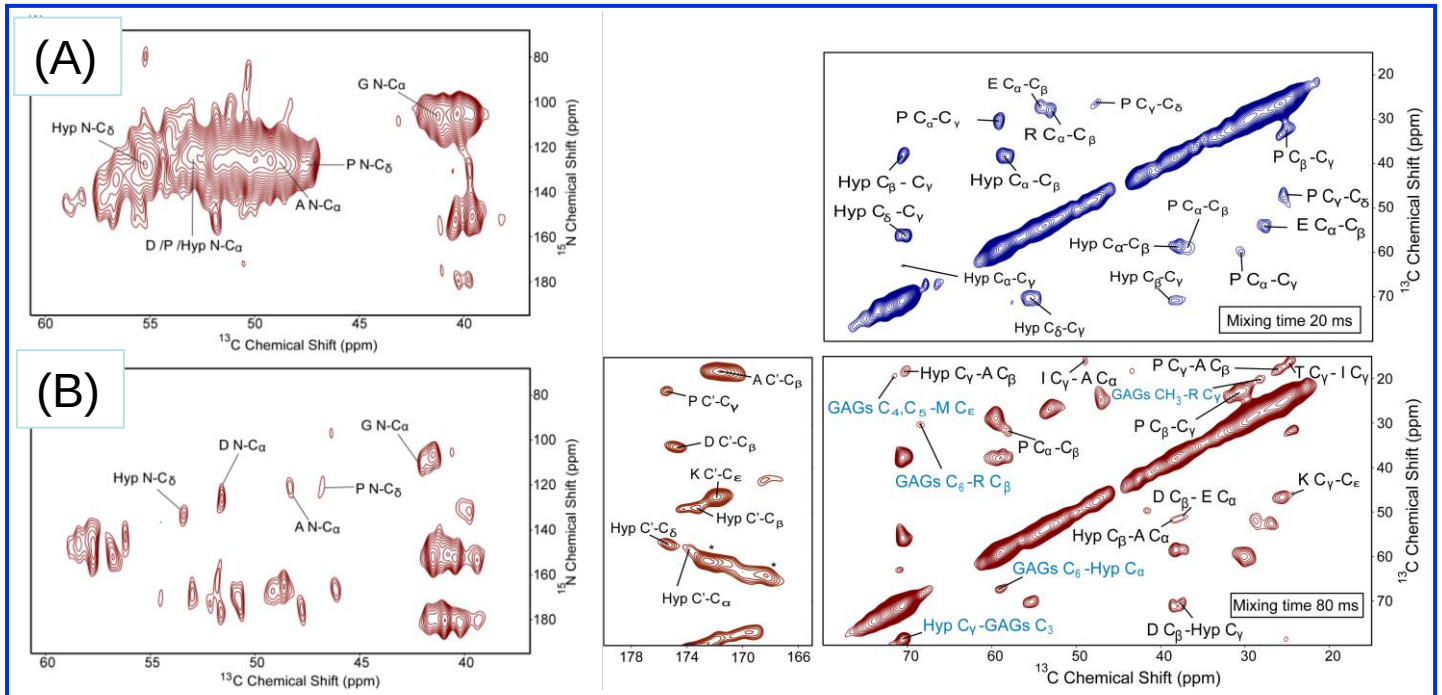

Figure 1. Left. DNP-enhanced 2D ^{15}N–^{13}C dipolar correlation NMR spectra of natural ^{15}N and ^{13}C abundance in cartilage (A) and bone (B). **Right**. Double-Quantum Filtered 2D SQ-SQ ^{13}C–^{13}C dipolar correlation spectra of cartilage by using the DARR (Dipolar Assisted Rotational Resonance) mixing scheme.

Facilities and instrumentation used: Solid-State NMR Facility - Wide-bore 600 MHz Bruker Avance III spectrometer configured with a 395 GHz gyrotron microwave source, microwave quasi-optical table, and a Bruker 3.2 mm MAS DNP ^{1}H-X-Y triple-resonance probe.

Citation: Dwivedi, N.; Patra, B.; Mentink-Vigier, F.; Wi, S.; Sinha, N., *Unveiling Charge-Pair Salt-Bridge Interaction Between GAGs and Collagen Protein in Cartilage: Atomic Evidence from DNP-Enhanced ssNMR at Natural Isotopic Abundance,* **Journal of the American Chemical Society** (2024) **doi.org/10.1021/jacs.4c05539**