Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Electronic Supplementary Information (ESI)

Selective Detection and Complete Identification of Triglycerides in Cortical Bone by High-Resolution ^{1}H MAS NMR Spectroscopy

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Experimental Section

Sample Preparation. All animal procedures were performed with the relevant protocols and standards of the University Committee on Use and Care of Animals (UCUCA) at the University of Michigan. Powdered bovine cortical bone samples were harvested from bovine femora collected at a local slaughterhouse from freshly slaughtered animals (2–4 years old). Type-I collagen from bovine Achilles tendon was purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. Prior to performing the NMR experiments, the collagen and bone samples were lyophilized in vacuum chamber for 24 hours to reduce the strong surface water signal. The lipid extractions from bone and collagen were released as follows: The bone powder (or collagen) was homogenized with chloroform: methanol (2:1, v/v) solvent to 10 times the volume of the tissue sample. After dispersion, the whole mixture was sonicated for about 30 minutes, followed by overnight (12 h) incubation (4^{o}C). Then, the homogenate was filtrated to recover the lipid phase. Firstly, the lipid phase was evaporated under a nitrogen stream to remove all the solvent. To remove all the residual water and solvent, the lipid was further evaporated under a vacuum chamber overnight. For liquid-state NMR experiments, the lipids were redissolved in $CDCl_3$.

NMR Spectroscopy. Liquid-state NMR experiments were carried on a Varian 400M NMR spectrometer, while solid-state NMR experiments were performed on a Bruker DSX300 NMR spectrometer using a 4-mm double-channel probe and zirconia rotors All solid experiments were performed at room temperature under 10 kHz magic angle spinning (MAS) condition. The ^{1}H MAS NMR spectra were recorded using either a single-pulse excitation or a spin-echo sequence ($\pi/2$---$\tau/2$---π---$\tau/2$), with a $\pi/2$ ^{1}H pulse of 2.5 μs. The ^{13}C NMR spectra were recorded using single-pulse excitation, ramped Cross-Polarization (CP) and Refocused INEPT pulse sequences respectively. A 50 kHz SPINAL-16 decoupling sequence was used during acquisition for all the ^{13}C NMR experiments. The assignment of proton peaks was aided by COSY-type spectrum recorded using constant time uniform-sign cross-peak (CTUC) COSY sequence, which is a variation of the corresponding ^{13}C NMR sequence, achieved by removing the CP part. This experiment was performed with a recycle delay of 2 s, an inter-pulse delay of 20 ms, and a z-filter delay of 10 ms; 64 increments were collected in the indirect dimension with 4 scans each.

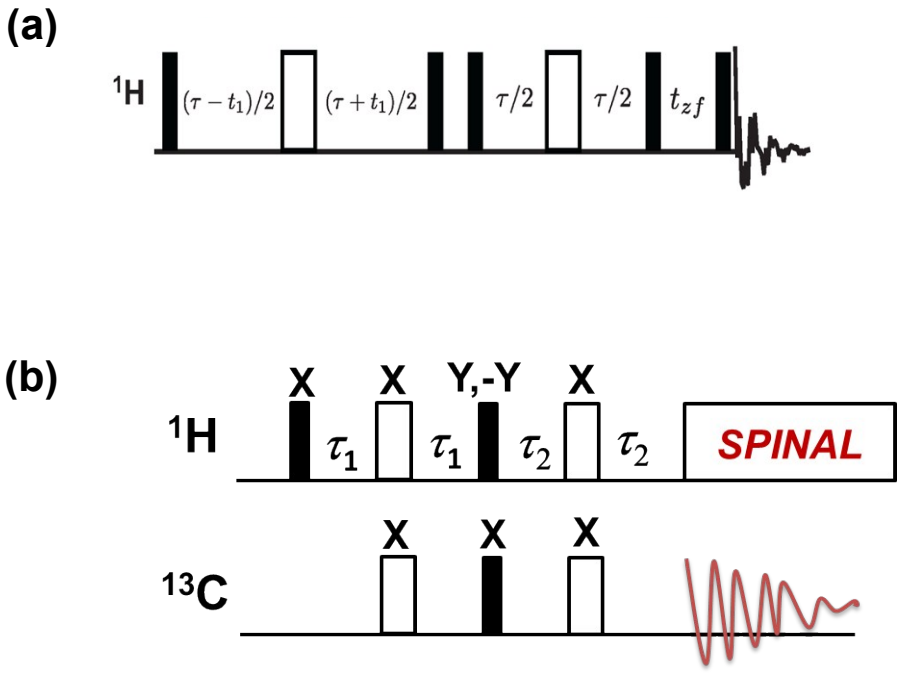

Figure S1. Radio-frequency pulse sequences for the constant-time uniform-sign cross-peak (CTUC) 2D COSY-type **(a)** and RINEPT **(b)** experiments used in this study. π/2 and π pulses are represented by solid and blank rectangles, respectively.

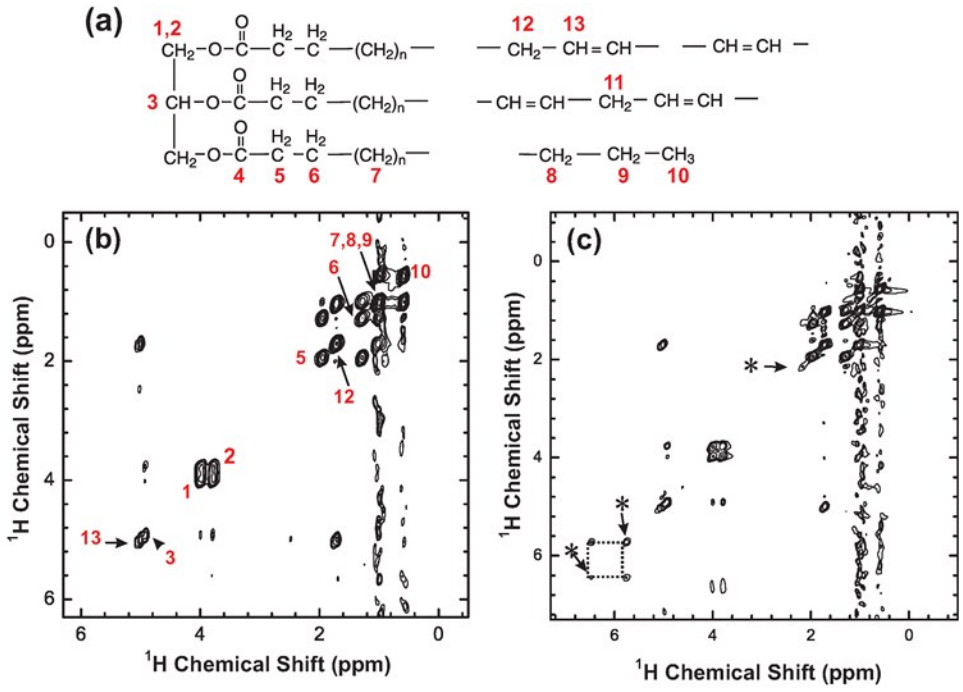

Figure S2. (a) Molecular fragments of triglyceride and fatty acids, along with the labeling on carbon atoms. Two-dimensional ^{1}H/^{1}H NMR chemical shift correlation spectra recorded at 10 kHz MAS using the CTUC 2D COSY-type NMR pulse sequence on bovine cortical bone (b) and type-I collagen (c). The CTUC 2D COSY spectrum of type-I collagen shows similar features to that of cortical bone, except for few additional peaks (indicated by *) that may be due to other mobile lipids that are in fast molecular motion.

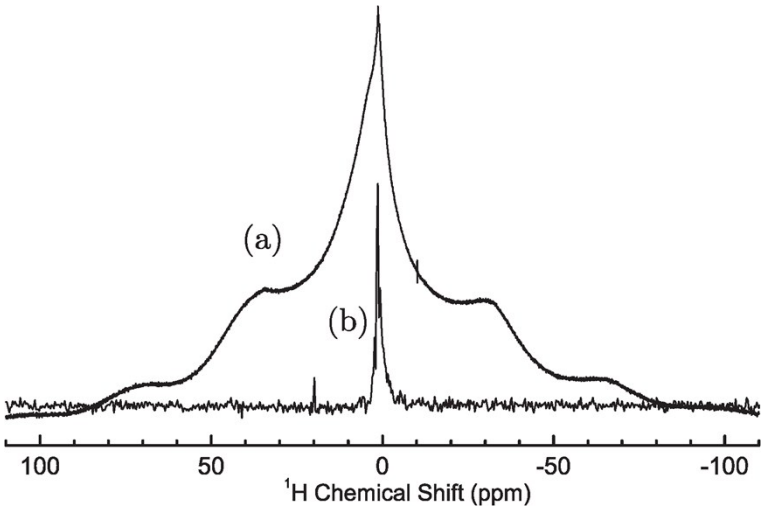

Figure S3. ^{1}H NMR spectra of type-I collagen collected before **(a)** and after **(b)** incubation in 2:1 chloroform:methanol solution. Both spectra were collected under 10 kHz MAS. Spectrum (a) was collected with a ^{1}H single-pulse excitation pulse while spectrum (b) was collected using a spin-echo sequence ($\pi/2$---$\tau/2$--- π --- $\tau/2$) with τ = 2 ms. The ^{1}H NMR spectrum in (a) is a typical spectrum of proteins in solid-state, in which the overall and internal motions of protein molecules are slow or anisotropic. The central peak arises from the relative mobile side chains and water, while the two broad shoulders come from dipolar interactions. The ^{1}H NMR spectrum recorded using a 2 ms spin-echo shows sharp peaks only from water and methyl protons in protein.

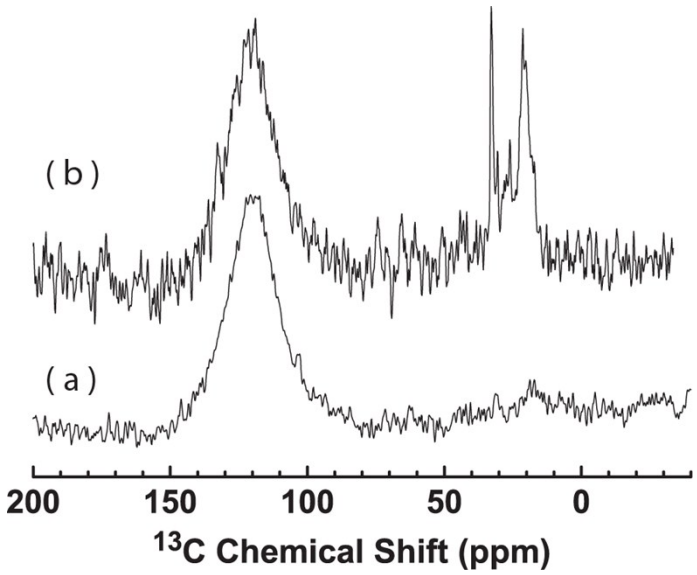

Figure S4. ^{13}C NMR spectra of cortical bone powder before **(a)** and after **(b)** incubation in 2:1 chloroform:methanol solution. Both spectra were collected using a ^{13}C single-pulse excitation under 10 kHz MAS.