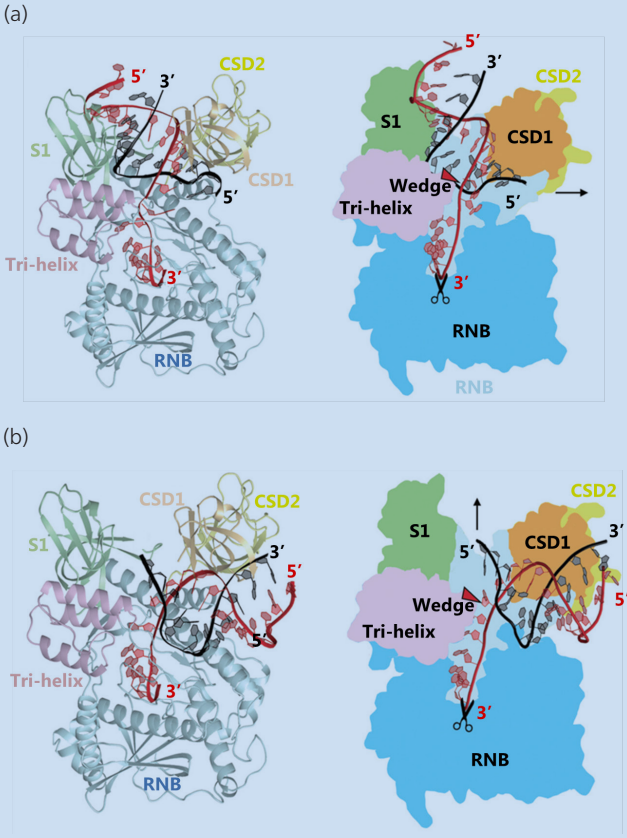

This report features the work of Hanna S. Yuan and co-workers published in Nucleic Acid Res. ***45****, 12015 (2017).*

TLS 13C1 SW60 – Protein Crystallography

- MR, SIR, MIR
- Protein Crystallography

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Fig. 2: (a) & (b) Two possible working models for RNA binding, unwinding and degradation for RNase R. [Reproduced from Ref. 3]

Preserved Collagen in an Early Jurassic Sauropodomorph Dinosaur

Protein preservation in a terrestrial vertebrate is revealed inside the Haversian canals of a rib of a 195-million-year-old Lufengosaurus. This study was selected as one of the Discover's 100 top stories of 2017.

The opportunity to reveal a genomic connection between extinct ancient animals and extant animals is strongly dependent on the DNA species in the fossil; fossilized organic remains are therefore crucial sources of possible genomic information to relate biological and evolutionary information.[1] The half-life of DNA after an animal death is predicted to be ~521 years, based on the statistics of bone fossil from moa; it is quite rare to extract the DNA molecules from a multimillion-year-old fossil. Yao-Chang Lee (NSRRC) and Robert Reisz (University of Toronto) together with their co-workers reported SR-FTIR spectral evidence of protein preservation in a terrestrial vertebrate found inside the Haversian canals of a rib of a 195-million-year-old Lufengosaurus, in which the blood vessels and nerves would normally have been present in a living organism.[2] The FTIR spectra acquired on utilizing synchrotron radiation-based Fourier-transform infrared (SR-FTIR) measurements *in situ* revealed the characteristic IR absorption bands of amides A and B, amides I, II and III of collagen. Using a confocal Raman microscope, aggregated hematite particles (α-Fe_2O_3) of diameter about 6–8 mm were also identified inside the Haversian canals, in which the collagen and protein remains were preserved. These authors proposed that iron(II) ions likely had an antioxidant role in the preservation of the proteins before the formation of the micrometre-sized hematite particle, and might be remnants partially contributed from hemoglobin and other iron-rich proteins from the original blood.

Rib fossils of an adult Lufengosaurus were collected and studied (specimens housed in the ChuXiong Prefectural Museum, catalogue CXPM Z4644). Rare or no evidence of soft tissue preservation exists for

transversely sectioned fossil samples after a few trials of SR-FTIR measurement. The authors set up a longitudinal sectioning process coupled with washing with DI water for most procedures; alcohol was utilized in the last stage of washing sectioned rib samples. Some transparent flat fragments and infilling material mixed with dark-red aggregated micrometre-sized hematite particles were found along and inside the osteonal central Haversian canals, as indicated in **Figs. 1(b)–1(h)**. Preserved organic remains inside the Haversian canals, transparent flat preserved protein fragments, were identified using SR-FTIR spectra *in situ*; dark-red aggregated hematite particles in both Haversian canals and osteocyte housing, the so-called lacunae, were also clearly observed on using SR-TXM as shown in **Figs. 1(i)–1(m)**. The SR-TXM tomographic image of the dark-red particles showed an aggregate-lamellar structure inside the Haversian canals, and an amorphous structure when found within the lacunae.

They utilized SR-FTIR spectra *in situ* to measure the preserved infilling material and transparent flat fragment on the surface of the longitudinal sectioned rib. SR-FTIR spectral lines of the preserved infilling material within the central vascular canals were observed at 3279, 3052, 1649, 1637, 1545, 1292 and 1260 cm^{-1} as shown in **Fig. 2**, which were consistent with the characteristic IR absorption lines of collagen type I and elastin of an extant animal, and assigned to amide A band, amide B band, amide I band, triple helix of collagen type I, amide II band and amide III band attributed to the C–N stretching vibration and the N–H deformation absorption of collagen and elastin, respectively.

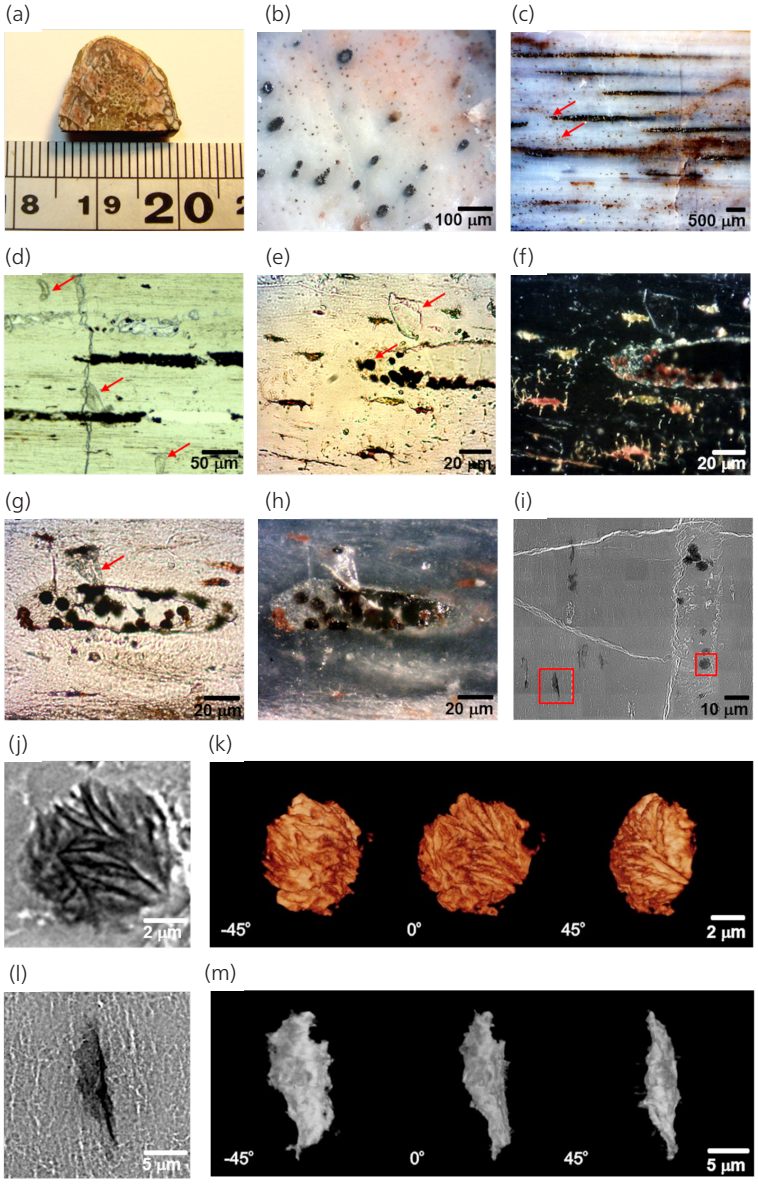

Fig. 1: Rib fragment (CXPM Z4644) of Lufengosaurus. (a) & (b) Transversely sectioned rib; dark-red circles are the central Haversian canals in the osteons. (c) Longitudinal section of the rib showing a distribution of infilled Haversian canals. (d)–(h) Close-up of preserved collagen infilling materials within the Haversian canals of the rib; flat transparent preserved protein fragments that were washed out from the cut canals are indicated with red arrows. (f) & (h) are dark-field images of (e) & (g), respectively. (i) SR-TXM image of hematite within the Haversian canal, indicated with red squares. (j) Microcrystals of hematite inside the Haversian canal. (k) SR-TXM images of hematite-aggregated particles at varied angles of view. (l) Lacuna within a bone matrix and (m) SR-TXM images of lacunae at varied angles of view. [Reproduced from Ref. 2]

Figure 2 reveals that SR-FTIR spectra of the transparent flat protein fragments on the bone surface were similar to those of the preserved collagen infilling material inside the Haversian canals, with weak amide III bands at 1292 and 1260 cm^{-1}. These infrared absorption lines of protein material were also matched as characteristic IR absorption bands with extant collagen type I extracted from the skin of a modern calf. Transparent flat preserved protein fragments were found inside the Haversian canals and near, around and along the canals, adhering to the bone surface as indicated in **Figs. 1(d)–1(h)**. The SR-FTIR spectra also exhibit that the protein remains within the rib were mixed with carbonated apatite of the bone matrix, as shown in **Fig. 2**.

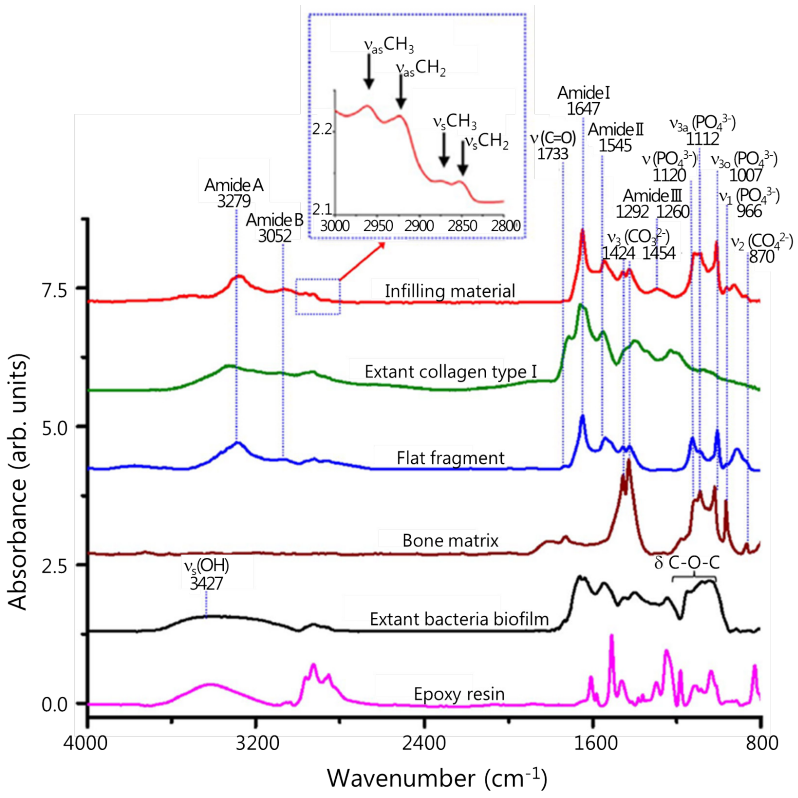

Fig. 2: Representative baseline-corrected and normalized characteristic infrared spectra; line assignment of SR-FTIR spectra of infilling material, extant collagen type I, flat fragment, bone matrix, extant bacteria biofilm and epoxy resin. [Reproduced from Ref. 2]

SR-FTIR spectra *in situ* were employed to provide undeniable and clear spectral evidence to exclude contamination attributed to the bacteria biofilm and epoxy resin used as embedding material herein. There has been no or rare observation of the IR lines characteristic of absorption of bacteria, hydroxyl group (–OH) and glycosidic bonds (–C–O–C–) of polysaccharides in the ranges 3700–3100 cm^{-1} and 1200–900 cm^{-1}, normally attributed to the absorption of the cell wall of bacteria 25 as in the extant bacterial biofilm of *Saccharomyces cerevisiae*.

Herein end stations at **TLS 14A1** for SR-FTIR microspectra *in situ* and at **TLS 01B** for confocal-Raman spectra and SR-TXM were utilized non-destructively to identify the protein or collagen remains and the aggregated hematite microcrystals as compositional constituents of fossils. The result of investigation proved the oldest known organic remains, collagen type I and protein, inside a dinosaur fossil and more than 100 million years older than that of previous investigations without chemical treatment to prevent chemical contamination. Finally, the research team made a breakthrough for the duration of preservation of collagen type I or other organic remains across geologic time scales greater than previously considered possible. (Reported by Chun-Jung Chen)

This report features the work of Yao-Chang Lee, Robert Reisz, and their co-workers published in Nat. Commun. ***8****, 14220 (2017).*

TLS 01B SWLS – X-ray Microscope
TLS 14A1 BM – IR Microscope

- Fourier-transform Infrared Spectra, Confocal-Raman Spectra, Transmission X-ray Microscope
- Dinosaurs, Collagen, Fossil, Life Science

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The photo of the research team – (left to right) Cheng-Cheng Chiang (NSRRC), Rong-Seng Chang (National Central University), Yao-Chang Lee (NSRRC), Robert R. Reise (University of Toronto), was taken in Dinosaur Mountain, Yunnan Province, China.