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**Analyses of Soft Tissue from Tyrannosaurus rex  
Suggest the Presence of Protein**

Mary Higby Schweitzer, *et al.*

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6. E. Asphaug, W. Benz, *Icarus* **121**, 225 (1996).
7. D. C. Richardson, W. F. Bottke, S. G. Love, *Icarus* **134**, 47 (1998).
8. J. L. Margot *et al.*, *Science* **296**, 1445 (2002).
9. S. J. Weidenschilling, P. Paolicchi, V. Zappalà, in *Asteroids II*, R. P. Binzel, T. Gehrels, M. S. Matthews, Eds. (Univ. of Arizona Press, Tucson, AZ, 1989), pp. 643–658.
10. W. F. Bottke, D. Vokrouhlický, D. P. Rubincam, M. Broz, in *Asteroids III*, W. F. Bottke, P. Paolicchi, R. P. Binzel, A. Cellino, Eds. (Univ. of Arizona Press, Tucson, AZ, 2002), pp. 395–408.
11. G. H. Stokes, J. B. Evans, H. E. M. Vighh, F. C. Shelly, E. C. Pearce, *Icarus* **148**, 21 (2000).
12. P. Wiegert *et al.*, American Geophysical Union, Fall Meeting 2002, abstr. #P11A-0352 (2002).
13. J. L. Margot, P. D. Nicholson, *AAS/Division of Dynamical Astronomy Meeting* **34** (2003).
14. R. Brasser *et al.*, *Icarus* **171**, 102 (2004).
15. S. Hudson, *Remote Sensing Rev.* **8**, 195 (1993).
16. J. L. Margot *et al.*, *Bull. Am. Astron. Soc.* **35**, 960 (2003).
17. The Doppler broadening of the radar echo due to rotation of the target is  $B = (4\pi D/\lambda P) \sin \alpha$ , where  $B$  is the limb-to-limb bandwidth of the echo,  $D$  is the target diameter producing the Doppler shift at the current viewing geometry and rotation phase,  $\lambda$  is the radar wavelength,  $P$  is the spin period of the target, and  $\alpha$  is the inclination of the spin axis to the line of sight.
18. S. J. Ostro, *Rev. Mod. Phys.* **65**, 1235 (1993).
19. Resolution in time delay, and equivalently range, is achieved by transmitting a time-dependent signal and analyzing the received signal according to arrival time.
- The time increment  $\tau$  used in the transmitted signal yields a range resolution  $c\tau/2$ , where  $c$  is the speed of light.
20. We typically define the limb-to-limb bandwidth as the full width of the radar echo at the level of twice the root mean square (RMS) of the off-DC, off-target noise. The exception is the strong 2004 Arecibo data, for which we use 10 times the RMS as the threshold to avoid contributions from frequency sidelobes.
21. This assumes PH5 is a principal axis (PA) rotator where the spin axis remains fixed in inertial space and aligned with the axis of maximum moment of inertia. The spin axis of PH5 must then be oriented such that the angles it makes with the lines of sight satisfy the observed bandwidths (17). The damping time scale (28) to PA rotation for PH5 is of order 0.1 million years.
22. Materials and methods are available as supporting material on Science Online.
23. The spin state solution is also validated by the phase agreement of infrared lightcurves from the Spitzer Space Telescope with synthetic lightcurves produced with our shape (27).
24. B. Gladman *et al.*, *Science* **277**, 197 (1997).
25. W. F. Bottke Jr., M. C. Nolan, R. Greenberg, R. A. Kolvoord, in *Hazards Due to Comets and Asteroids*, T. Gehrels, M. S. Matthews, A. M. Schumann, Eds. (Univ. of Arizona Press, Tucson, AZ, 1994), pp. 337–357.
26. D. J. Scheeres, *Icarus* **10.1016/j.icarus.2006.12.015** (2007).
27. M. Mueller, A. W. Harris, *IAU General Assembly Abstracts* (2006), p. 95.
28. I. Sharma, J. A. Burns, C.-Y. Hui, *Mon. Not. R. Astron. Soc.* **359**, 79 (2005).
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### Supporting Online Material

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Methods

Figs. S1 to S5

Tables S1 and S2

References

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## Analyses of Soft Tissue from *Tyrannosaurus rex* Suggest the Presence of Protein

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We performed multiple analyses of *Tyrannosaurus rex* (specimen MOR 1125) fibrous cortical and medullary tissues remaining after demineralization. The results indicate that collagen I, the main organic component of bone, has been preserved in low concentrations in these tissues. The findings were independently confirmed by mass spectrometry. We propose a possible chemical pathway that may contribute to this preservation. The presence of endogenous protein in dinosaur bone may validate hypotheses about evolutionary relationships, rates, and patterns of molecular change and degradation, as well as the chemical stability of molecules over time.

It has long been assumed that the process of fossilization results in the destruction of virtually all original organic components of an organism, and it has been hypothesized that original molecules will be either lost or altered to the point of nonrecognition over relatively short time spans (well under a million years) (1–7). However, the discovery of intact structures retaining original transparency, flexibility, and other characteristics in specimens dating at least to the Cretaceous (8, 9) suggested that, under certain conditions, remnant organic constituents may persist across geological time.

The skull, vertebrae, both femora and tibiae, and other elements of an exceptionally well-preserved *Tyrannosaurus rex* [MOR 1125 (8)]

were recovered from the base of the Hell Creek Formation in eastern Montana (USA), buried within at least 1000 m<sup>3</sup> of medium-grained, loosely consolidated sandstone interfingering with fine-grained muds, interpreted as stream channel sediments. Demineralization of femur and tibia fragments revealed the preservation of fibrous, flexible, and apparently original tissues, as well as apparent cells and blood vessels (8), but the endogeneity and composition of these structures could not be ascertained without further analyses.

We present molecular and chemical (10) analyses of tissues remaining after partial demineralization (11) of the left and right femora and associated medullary bone (12) that would, in extant bone, represent the extracellular matrix (osteoid) dominated by collagen I (13). Because

of its ordered structure as a triple helix (14, 15), collagen I has unique characteristics that are highly conserved across taxa, making validation of its presence relatively straightforward. The molecular composition of collagen incorporates glycine, the smallest amino acid, at every helical turn. Therefore, an amino acid profile of collagen results in ~33% glycine content (14). This molecular structure also results in packing of microfibrils with a banded repeat of ~70 nm (15, 16). Collagen also shows posttranslational hydroxylation of about half of all proline and some lysine residues; thus, the detection of hydroxyproline and hydroxylysine in extracts of organic material is viewed as strong evidence for the presence of collagen (17, 18). Finally, collagen is identified by polyclonal or monoclonal antibody reactivity that can distinguish between collagen types (19). We focused on identifying collagen-like compounds because in addition to being abundant and easily identified by multiple

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and independent methods, this protein is durable (20, 21) and resistant to degradation.

The fibrous nature of demineralized dinosaur tissues was demonstrated by optical (8) and electron (fig. S1) microscopy. Furthermore, regions of dinosaur cortical and medullary (12) bone demonstrated a repeat pattern with periodicity of ~70 nm when examined by atomic force microscopy (AFM) (Fig. 1, A to D), consistent with collagen in extant bone (Fig. 1, E and F) and

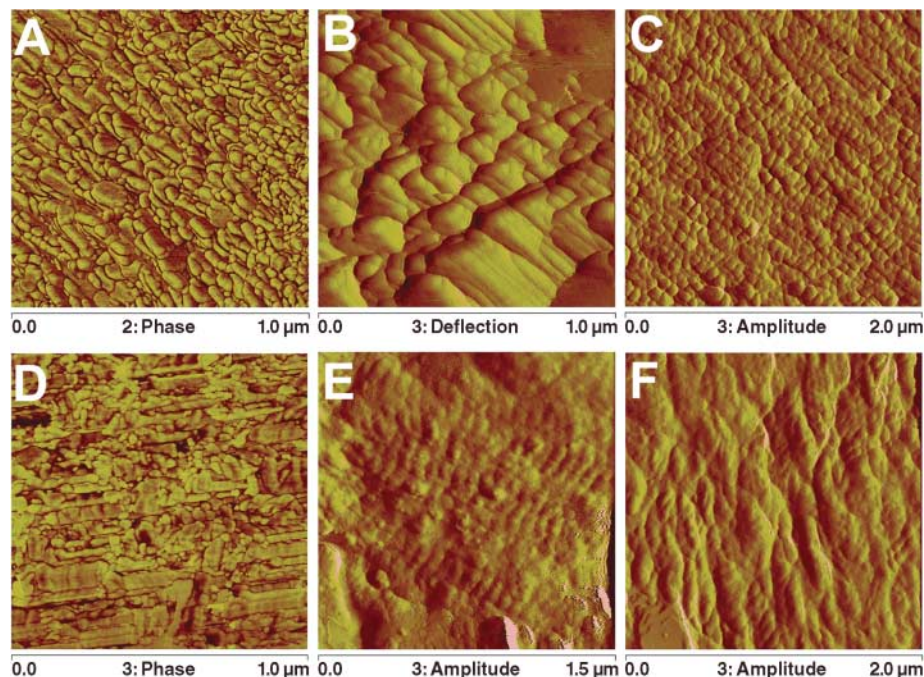
similar to that previously observed in fragments of demineralized Cretaceous avian bone (22). However, this periodic pattern was rarely observed in ultrathin sections of MOR 1125 demineralized bone by transmission electron microscopy (TEM) (fig. S1). This may be a methodological problem, or the periodic features we observe (Fig. 1, A to D) may be due to surface features generated when demineralization removed most of the apatite crystals emplaced during biomineralization, when

collagen acted as a template. Thus, the banded features may represent a type of natural molecular imprinting (23), because banded fibers have been observed by TEM for other dinosaur tissues (24).

TEM studies confirm that, unlike extant bone, dinosaur bone did not completely demineralize after prolonged incubation in EDTA (11). Selected-area electron diffraction (SAED) of the tissues (fig. S1D, inset) showed that this retained mineral is biogenic hydroxylapatite (25). It is not possible to determine this conclusively because of the similarity in structure between hydroxylapatite and fluorapatite; however, the observed diffraction circle intensities are most consistent with hydroxylapatite. This finding suggests that the bone mineral is virtually unchanged from the living state and has undergone little if any alteration.

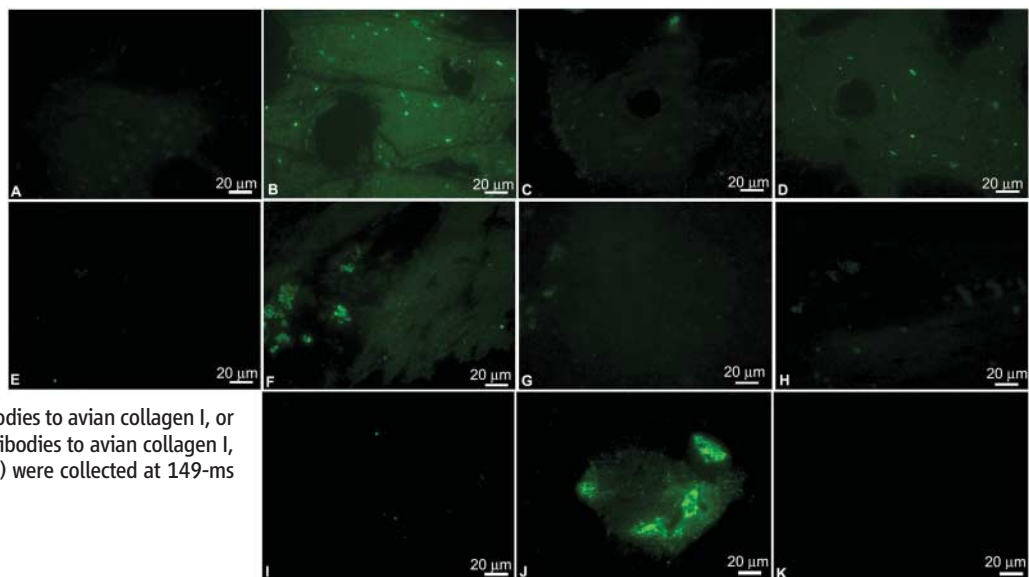
Force curve measurements of demineralized dinosaur medullary and cortical bone indicate that the elasticity of dinosaur tissues was similar to that of demineralized extant bone. We measured both embedded sections (fig. S2A) and unembedded whole mounts (fig. S2B) of demineralized bone in both air and liquid (11). The demineralized bone surface softened after exposure to buffer, allowing the AFM tip to penetrate deeper into the tissues with less resistance. Thus, the modulus of elasticity (fig. S2C) was reduced in liquid by more than three orders of magnitude (fig. S2B). Although ~2000 nN of force was required to penetrate ~40 nm into MOR 1125 bone matrix in air, only ~15 nN of force was required to depress the tip ~75 nm into the same matrix when hydrated (fig. S2B, inset).

MOR 1125 cortical and medullary whole-bone extracts showed reactivity to antibodies raised against chicken collagen I (11) when measured by enzyme-linked immunosorbent assay (ELISA), although the degree of binding varied widely. Reactivity was greatly reduced in dinosaur extracts relative to extant samples (fig. S3), but still at least twice that observed in negative con-



**Fig. 1.** AFM images of partially demineralized bones of MOR 1125 (A to D) and emu (E and F). (A) Phase image of MOR 1125 cortical bone imaged in air; (B) deflection image of MOR cortical bone imaged in phosphate-buffered saline; (C) amplitude image of embedded and sectioned MOR medullary bone imaged in air; (D) phase image of MOR 1125 medullary bone imaged in air (note longitudinal and cross-sectional orientation of fiber-like structures at right angles to each other); (E) amplitude image of emu cortical bone imaged in air; (F) amplitude image of emu medullary bone imaged in air.

**Fig. 2.** In situ immunochemistry on 300-nm sections of demineralized MOR 1125 cortical bone (A to D) and medullary bone (E to H). (A) and (E), no primary antibodies added (negative control); (B) and (F), antibodies to avian collagen I; (C) and (G), antibodies to actin protein (nonrelevant, negative control); (D) and (H), antibodies to avian collagen I, inhibited by incubating with purified chicken collagen before exposing to dinosaur tissues. All data were collected using the same parameters at 122-ms integration. (I to K) MOR 1125 cortical tissue exposed to (I) no primary, (J) antibodies to avian collagen I, or (K) collagenase digestion followed by antibodies to avian collagen I, as described (11). Data in (I), (J), and (K) were collected at 149-ms integration.



trols of coextracted sediments and buffer without sample, similarly treated.

We confirmed the antibody reactivity data by in situ immunohistochemistry in a series of experiments. We exposed thin (0.3 to 0.5  $\mu\text{m}$ ) sections of demineralized cortical (Fig. 2, A to D and I to K) and medullary (Fig. 2, E to H) dinosaur bone to antibodies raised against avian collagen I, both before (Fig. 2, B and F) and after inhibition of antibodies with chicken collagen (Fig. 2, D and H) (11). Additionally, antibody reactivity (Fig. 2J) was significantly decreased after we digested dinosaur tissues with collagenase (Fig. 2K), although this enzyme effect was not consistently observed. Reactivity to antibodies, measured by fluorescence, was significantly greater than in negative controls (Fig. 2, A, C, E, G, and I) and was localized to tissues. We also observed some binding of osteocalcin antibodies to dinosaur tissues (fig. S4). These patterns were similar to those observed with extant emu cortical and medullary bone (fig. S5). Immunoreactivity in dinosaur tissues was greatly reduced from that observed in extant bone, as illustrated by longer integration times and fainter signal, but was greater than in negative controls. Immunohistochemistry performed on sediments was negative for binding. These results imply that the concentration of reactive epitopes in the dinosaur

tissues is very low, consistent with the ELISA results. That antibody reactivity was more consistently observed in situ than in ELISA could be due to greater alteration and/or loss of organic compounds during extraction procedures, or to reduced binding of degraded antigen to ELISA plate polymers.

The presence of collagen-derived epitopes in demineralized tissues is supported by mass spectrometry data. Time-of-flight secondary ion mass spectrometry (TOF-SIMS) detects surface ions associated with molecular fragmentation with high mass resolution, and can localize signal to whole samples without subjecting them to chemical extraction. In situ TOF-SIMS analyses were performed to unambiguously detect amino acid residues consistent with the presence of protein in demineralized MOR 1125 tissues (Fig. 2 and fig. S6). We obtained ratios of glycine (Gly), the most abundant amino acid in collagen [ $\sim 33\%$  (14)], and alanine (Ala), which constitutes about 10% of collagenous amino acids, to support the presence of the specific collagen  $\alpha 1$  type 1 protein in these tissues. Small peaks representing proline (Pro) at mass/charge ratio ( $m/z$ ) 70 (Fig. 3C), lysine (Lys) at  $m/z$  84 (fig. S6A), and leucine or isoleucine at  $m/z$  86 (fig. S6B) were also detected. TOF-SIMS is highly matrix dependent, and desorption and ionization of some amino acid res-

idues, especially modified residues such as hydroxylated Pro, are less efficient than for other residues (26). These modified residues were not detected by this method but were readily identified by other mass spectrometry methods (10).

The Gly:Ala ratio for published chicken collagen  $\alpha 1$  type 1 sequence (27) is 2.5:1. The TOF-SIMS results show that the Gly:Ala ratio in medullary bone of MOR 1125 is 2.6:1 (Fig. 3, A and B). Sandstones entombing the dinosaur, subjected to TOF-SIMS as a control, showed little or no evidence for these amino acids (Fig. 3, D and E).

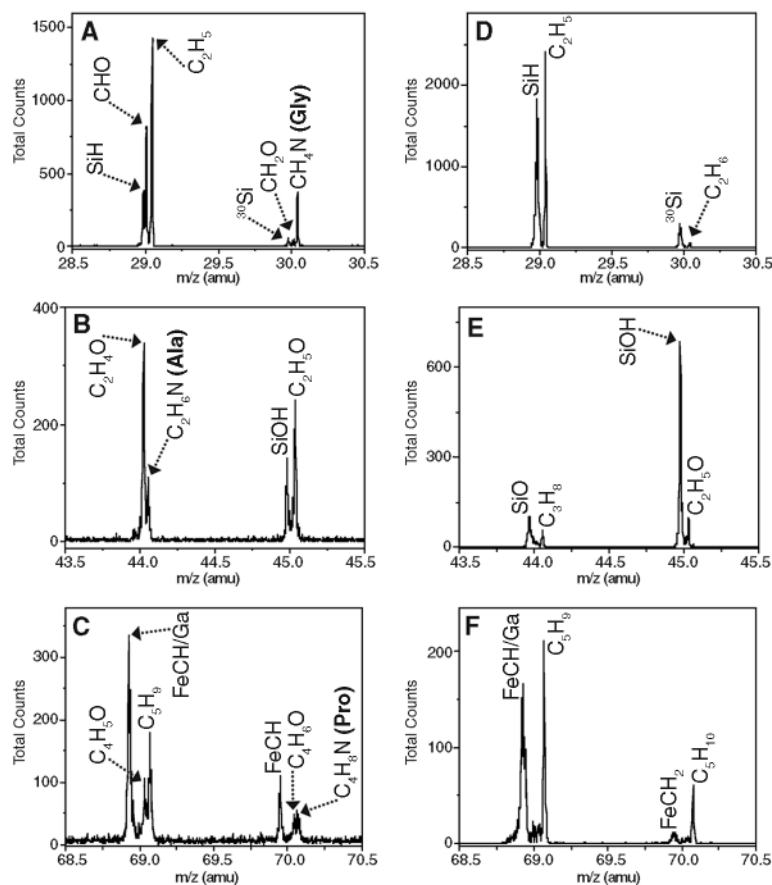
We identified a variety of nitrogen-containing species—including an alkyl amine group,  $\text{C}_7\text{H}_{18}\text{N}_2^+$ , located at 130 amu (fig. S6C)—in all dinosaur samples tested, but not in any surrounding sediments. We also observed a number of Fe-C-H species such as  $\text{FeCH}$ ,  $\text{FeCH}_2$ , and  $\text{FeCH}_3$ , associated with the dinosaur matrix (fig. S7) but not seen in extant material. Similar compounds were observed in the sediments surrounding the dinosaur. These may be microbial products, as sequences from iron-containing microbial enzymes were identified by mass spectrometry (10). We interpret these fragments as evidence that iron may help preserve soft tissue through initiation of intra- and intermolecular cross-links (9).

Dinosaur protein sequence, including collagen, should be most similar to that of birds among extant taxa, according to other phylogenetic information (28). The hypothesis that molecular fragments of original proteins are preserved in the mineralized matrix of bony elements of MOR 1125 is supported by peptide sequences recovered from dinosaur extracts, some of which align uniquely with chicken collagen  $\alpha 1$  type 1 (10).

The amount of protein or protein-like components in MOR 1125 is minimal. The percent yield after extraction and lyophilization was  $\sim 0.62\%$  for cortical bone and 1.3% for medullary bone. Protein-derived material is only a small percentage of the lyophilate relative to other material coextracted from bone, as assessed by comparison of immunoreactivity with extant samples. This is verified by mass spectrometry, which identifies only femtomole amounts of sequenceable material (10) in a heterogeneous mixture of extracted material.

Microenvironments within a single bone vary greatly, and not every fragment of bone examined yielded positive results. There was a high degree of variability between extractions, and we have also noted progressive reduction of signal in more recent extractions, indicating bone degradation in modern environments (29). Therefore, each of the analyses we report has been repeated numerous times, and we have set a minimum of three repetitions with similar results before reporting an assay as positive. Additionally, experiments have been conducted independently in at least three different labs and by numerous investigators, and the results strongly support the endogeneity of collagen-like protein molecules.

We hypothesize that these molecular fragments are preserved because reactive sites on the



**Fig. 3.** TOF-SIMS spectra of demineralized MOR 1125 medullary bone (A to C) and entombing sedimentary matrix (D to F). The imonium ions for Gly ( $m/z = 30$ ), Ala ( $m/z = 44$ ), and Pro ( $m/z = 70$ ) can be unambiguously identified for MOR 1125; no signal was observed in sediment controls that corresponded to these amino acids. See text for discussion.

original protein molecules became irreversibly cross-linked, both to similar molecules and to mineral or exogenous organic components. These cross-linking reactions may have been initiated by unstable metal ions that formed free radicals (30, 31), which in turn reacted with organic molecules to form polymers (6, 7, 9, 32). We propose that the unstable metal ions were derived from the post mortem degradation of iron-containing dinosaur biomolecules such as hemoglobin, myoglobin, and possibly cytochromes (9, 31). Once stabilized by these cross-linking reactions, the molecules were no longer available as substrates for further degradative reactions.

The intimate relationship between apatite and the organic phase of bone also contributes to the preservation of organic matter (16, 33–38), but we propose that the mineral phase may be stabilized by this relationship as well. The presence of biogenic apatite in these 68-million-year-old bones can only be rationalized by protection from an intact organic phase, which in turn is only satisfied by a synergistic relationship between collagen and mineral phases. Whereas extant bone retains no detectable calcium after days to weeks of demineralization, dinosaur bone retains a fraction of recognizable apatite crystals after months of treatment (fig. S1). Another contributing factor in the retention of original mineral may be that apatite is stabilized in the presence of calcite (33). Sandstones surrounding MOR 1125 contain abundant calcite cements.

The depositional environments may affect organic preservation in other ways. Comparison of fossils from a variety of environments indicates that those derived from sandstones are more likely to retain soft tissues and/or cells (9). We hypothesize that the porosity of sandstones may facilitate draining of enzymes of decay and suppurating fluids as the organism degrades, whereas organisms buried in nonporous mudstones or clays may be exposed to these longer and therefore may be more completely degraded.

Our findings indicate the need for optimizing methods of extraction and handling of fossil material. In particular, the decrease in signal we observed over time supports the need to establish field collection and storage of fossils according to protocols that allow future analytical studies (29).

The data presented here illustrate the value of a multidisciplinary approach to the characterization of very old fossil material and validate sequence data reported elsewhere (10). The inclusion of fossil-derived molecular sequences into existing phylogenies may provide greater resolution and may allow reconstruction of character evolution beyond what is currently possible. Elucidating modifications to ancient molecules may shed light on patterns of degradation and diagenesis. The presence of original molecular components is not predicted for fossils older than a million years (1–7), and the discovery of collagen in this well-preserved dinosaur supports the use of actualistic conditions to formulate molecular degradation rates and models, rather than relying on theoretical

or experimental extrapolations derived from conditions that do not occur in nature.

#### References and Notes

1. T. Lindahl, *Nature* **365**, 700 (1993).
2. G. Eglinton, G. A. Logan, *Philos. Trans. R. Soc. London Ser. B* **333**, 315 (1991).
3. J. L. Bada, X. Y. S. Wang, H. Hamilton, *Philos. Trans. R. Soc. London Ser. B* **354**, 77 (1999).
4. M. Hoss, *Nature* **404**, 453 (2000).
5. D. E. G. Briggs, R. P. Evershed, M. J. Lockheart, *Paleobiology* **26**, 169 (2000).
6. D. E. G. Briggs, *Annu. Rev. Earth Planet. Sci.* **31**, 275 (2003).
7. B. A. Stankiewicz *et al.*, *Geology* **28**, 559 (2000).
8. M. H. Schweitzer, J. L. Wittmeyer, J. R. Horner, J. B. Toporski, *Science* **307**, 1952 (2005).
9. M. H. Schweitzer, J. L. Wittmeyer, J. R. Horner, *Proc. R. Soc. London Ser. B* **274**, 183 (2007).
10. J. M. Asara, M. H. Schweitzer, L. M. Freimark, M. Phillips, L. C. Cantley, *Science* **316**, 280 (2007).
11. See supporting material on Science Online.
12. M. H. Schweitzer, J. L. Wittmeyer, J. R. Horner, *Science* **308**, 1456 (2005).
13. L. Zylberberg, *C. R. Palevol.* **3**, 591 (2004) and references therein.
14. M. van der Rest, *Bone* **3**, 187 (1991).
15. V. Ottani, M. Raspanti, A. Ruggeri, *Micron* **32**, 251 (2001).
16. S. Weiner, H. D. Wagner, *Annu. Rev. Mater. Sci.* **28**, 271 (1998).
17. G. Nemethy, H. A. Scheraga, *Biochemistry* **25**, 3184 (1986).
18. J. G. Bann, H. P. Bachinger, *J. Biol. Chem.* **275**, 24466 (2000).
19. D. R. Keene, L. Y. Sakai, R. E. Burgeson, *J. Histochem. Cytochem.* **39**, 59 (1991).
20. N. Tuross, L. Stathoplos, *Methods Enzymol.* **224**, 121 (1993).
21. P. Semal, R. Orban, *J. Archaeol. Sci.* **22**, 463 (1995).
22. R. Avci *et al.*, *Langmuir* **21**, 3584 (2005).
23. K. Mosbach, *Trends Biochem. Sci.* **19**, 9 (1994).
24. F. A. Rimblot-Baly, A. de Ricqles, L. Zylberberg, *Ann. Paleontol.* **81**, 49 (1995).
25. J. M. Hughes, M. Cameron, K. D. Crowley, *Am. Mineral.* **74**, 870 (1989).
26. B.-A. Gotliv *et al.*, *J. Struct. Biol.* **156**, 320 (2006).
27. Accession number P02457, NCBI nonredundant protein database.
28. T. Holtz, *Gaia* **15**, 5 (1998) and references therein.
29. M. Pruvost *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 739 (2007).
30. J. M. C. Gutteridge, D. A. Rowley, B. Halliwell, *Biochem. J.* **199**, 263 (1981).
31. P. Mladěnka, T. Simunek, M. Hubl, R. Hrdina, *Free Radic. Res.* **40**, 263 (2006).
32. F. Q. Schafer, S. Yue Qian, G. R. Buettner, *Cell. Mol. Biol.* **46**, 657 (2000).
33. F. Berna, A. Matthews, S. Weiner, *J. Archaeol. Sci.* **31**, 867 (2004).
34. M. J. DeNiro, S. Weiner, *Geochim. Cosmochim. Acta* **52**, 2415 (1988).
35. M. J. Glimcher, L. Cohen-Solal, D. Kossiva, A. de Ricqles, *Paleobiology* **16**, 219 (1990).
36. T. Schmidt-Schultz, M. Schultz, *Am. J. Phys. Anthropol.* **123**, 30 (2004).
37. G. A. Sykes, M. J. Collins, D. I. Walton, *Org. Geochem.* **23**, 1059 (1995).
38. M. Salamon, N. Tuross, B. Arensburg, S. Weiner, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 13783 (2005).
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## Protein Sequences from Mastodon and *Tyrannosaurus Rex* Revealed by Mass Spectrometry

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Fossilized bones from extinct taxa harbor the potential for obtaining protein or DNA sequences that could reveal evolutionary links to extant species. We used mass spectrometry to obtain protein sequences from bones of a 160,000- to 600,000-year-old extinct mastodon (*Mammuth americanum*) and a 68-million-year-old dinosaur (*Tyrannosaurus rex*). The presence of *T. rex* sequences indicates that their peptide bonds were remarkably stable. Mass spectrometry can thus be used to determine unique sequences from ancient organisms from peptide fragmentation patterns, a valuable tool to study the evolution and adaptation of ancient taxa from which genomic sequences are unlikely to be obtained.

Obtaining genome sequences from a number of taxa has dramatically enhanced our abilities to study the evolution and adaptation of organisms. However,

difficulties in the acquisition of DNA or RNA from ancient extinct taxa limit the ability to examine molecular evolution. Recent advances in mass spectrometry (MS) technologies have