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From bone to fossil: a review of the diagenesis of bioapatite

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Abstract

The preservation of bone or bioapatite over geologic time has presented paleobiologists with long-standing and formidable questions. Namely, to elucidate the mechanisms, processes, rates, and depositional conditions responsible for the formation of a fossil from a once living tissue. Approaches integrating geochemistry, mineralogy, physics, hydrology, sedimentology, and taphonomy have all furthered insights into fossilization, but several fundamental gaps still remain. Notably, our understanding of: (1) the timing of processes during diagenesis (e.g., early and/or late), (2) the rate of bioapatite transformation into thermodynamically more stable phases, (3) the controls imparted by depositional environment, and (4) the role of (micro)biology in determining the fate of bone bioapatite (dissolution or preservation) are limited. The versatility of fossil bioapatite to provide information on the biology of extinct vertebrates rests on our ability to identify and characterize the changes that occurred to bioapatite during diagenesis. This review will evaluate our current understanding of bioapatite diagenesis and fossilization, focusing on the biogeochemical transformations that occur during diagenesis to the mineral and

organic components of bone (excluding teeth and enamel), the analytical approaches applied to evaluate fossilization processes, and outline some suggestions for future promising directions.

Keywords: fossilization, bioapatite, diagenesis, geobiology

Biologically precipitated apatite (bioapatite)

Vertebrates, by definition, develop a mineralized internal skeleton composed of bioapatite that provides the animal with structural and mechanical support (e.g., Akkus et al. 2004), a reservoir of ions to maintain acid-base homeostasis (Green and Kleeman 1991), and permits terrestrial locomotion. Bioapatite consists of an organic and inorganic fraction forming a composite material that provides the skeleton and bones with a degree of flexibility as well as strength (e.g., Alexander et al. 2012). The composition of the inorganic, or mineralized, fraction of bioapatite is a non-stoichiometric apatite phase most similar in structure and composition to hydroxylapatite, with additional minor elements incorporated in the lattice (Na_y $(Ca,Mg)_{10-x-y}[(PO_4)_{6-x-y}(CO_3)_{x+y}](OH)_{2-x}$) (Li and Pasteris 2014b). In a living organism, bioapatite of bone is in a dynamic state of equilibrium with the body, undergoing precipitation and dissolution over the lifetime of the animal (Green and Kleeman 1991). For example, bone provides the body with a reservoir of Na, Ca, P, Mg (e.g., Green and Kleeman 1991), as well as other important sorbed species, such as citrate (e.g., Dickens 1941; Hu et al. 2010). The composition of bioapatite in bone reflects vital processes occurring over the animal's lifetime. For paleobiologists, the use of isotopes to reconstruct past diet, climate, and ecology of extinct animals is enabled by the preservation of endogenous indicators of vital processes in the form of isotopes (e.g., Nd and Sr;

Tütken et al. 2011; $\delta^{13}C$ from tooth enamel; Cerling et al. 1993; $\delta^{18}O$; Kohn 1996; Suarez et al. 2014).

At the macro- and micro-scale, the bioapatite found in vertebrate teeth (enamel) and bones is distinct. Tooth enamel has larger, well-ordered bioapatite crystallites that contain less carbonate and more fluorine compared to bones (Wopenka and Pasteris 2005). Additionally, enamel has a low organic content (<1 % by volume), which contrasts with bone (32-44 % by volume; Olszta et al. 2007). The presence of organics plays a critical role in diagenesis (discussed below).

From living tissue to fossil

Once removed from an organism, bioapatite undergoes necrolysis, biostratinomy, and diagenesis, potentially transforming the original living tissue into fossil bioapatite phase(s) (Figs. 1, 2). The process of diagenesis—the chemical, physical, and biological interactions that result in the transformation of an original compound—is divided into two broad intervals for describing fossilization: early and late. For bones, early diagenesis generally refers to the initial alteration of bone once introduced into a geochemical system, although there is some ambiguity regarding the timing of this period (Trueman et al. 2008a, b). Early diagenetic processes specific to bone include the removal of soft tissues (i.e. muscle and skin), degradation of collagen (abiotic and biotic), and initial chemical and structural changes to the mineralized component of bone, bioapatite, ultimately resulting in decomposition or potentially in preservation (e.g., Greenlee 1996; Sponheimer and Lee-Thorp 1999). The removal of organic compounds, including collagen, from within in the bone provides a critical mechanism for opening the bone and bioapatite lattice to subsequent fluid movement (Fig. 2). Migration of fluid derived from the

surrounding environment facilitates the substitution of ions in bioapatite to form a thermodynamically more stable phase (e.g., Hinz and Kohn 2010). Late diagenetic alteration includes further structural and chemical modification to the apatite lattice, resulting in the formation of a new apatite phase, and potentially whole-scale replacement of the original bioapatite.

Alteration to the mineralized fraction of bone

Apatite was once referred to as "Nature's trashcan," an apt description given the extreme flexibility of the lattice for replacements at every site within the mineral structure (e.g., Pan and Fleet 2002). Trace metals including Fe, Mn, Sr, and Mg as well as rare earth elements (REE) may be incorporated in place of calcium at both the Ca(I) and Ca(II) sites (e.g., Trueman et al. 2008a, b; Koenig et al. 2009; Herwartz et al. 2011). Protonation of the hydroxyl ion facilitates the incorporation of trace halogens like F^- and Cl^- forming fluorine- or chlorine-enriched phases. The incorporation of carbonate (CO_3^{2-}) ions in place of phosphate (PO_4^{3-}) or OH^- readily occurs under a range of pH conditions (Berna et al. 2004) as well as *in vivo* (Bergstrom and Wallace 1954; Green and Kleeman 1991; Rollin-Martinet et al. 2013). The flexibility of apatite for substitutions plays a critical role in the subsequent diagenesis of bone and preservation over geologic time (e.g., Trueman 1999; Berna et al. 2004; Keenan et al. 2015).

In fossils, the composition of the resulting apatite phase varies widely (e.g., Trueman 1999; Goodwin et al. 2007). The vast majority of fossilized bone exists as fluorine and/or carbonate-enriched apatite phases in both archaeological (e.g., Berna et al. 2004) and paleontological (e.g., Sponheimer and Lee-Thorp 1999; Trueman 1999) materials. Modeled fluid saturation states with respect to selected apatite and phosphorus (P)-bearing mineral phases

provides a first approximation of the predicted fate of each mineral phase, and helps to explain the persistence of fossil bioapatite as altered phases (Fig. 3). Bone, closest compositionally to hydroxylapatite (HAP), is predicted to be unstable under low total P concentrations and under acidic to circumneutral pH (Fig. 3). If fluids have high total P, stability shifts towards HAP supersaturation across a wider range of pH conditions. If recrystallized to a phase approaching a stoichiometric end-member such as fluorapatite (FAP) or carbonated fluorapatite (CO_3-FAP), stability shifts towards supersaturation, even under low total P and low pH conditions (Fig. 3). From purely a thermodynamic perspective, in this modern system inhabited by aquatic and semi-aquatic vertebrates, bone will only be preserved if altered to a different apatite phase, such as FAP or CO_3-FAP. For teeth, larger crystallite sizes, reduced carbonate content, low collagen content, and the presence of F^- in enamel *in vivo* results in a thermodynamically more robust material compared to bone (e.g., Wopenka and Pasteris 2005).

These predictions broadly match observed fossil bone chemistry where F enrichment is widely observed and bone is recrystallized to a new apatite phase. Additionally, the major and trace element composition of fossil bone is highly site-specific, and varies even within a single bone or between bones preserved at the same site (e.g., Trueman and Benton 1997; Suarez et al. 2010). This variability reflects the intimate connection between fossil composition and site geochemistry, with recrystallization driven by mineral stabilities, dissolved ions in solution, site mineralogy, and sediment porosity. For example, in the modeled aqueous solution discussed above (Fig. 3), varying one dissolved constituent (P) drastically altered predicted saturation states. Integrating observations of conditions present in modern depositional environments can help to guide interpretations about diagenetic conditions present in the geologic past facilitating bone recrystallization.

Alteration and degradation of collagen in bone

The association of organics, predominantly type I collagen, with the mineral fraction of bioapatite imparts bone with characteristic strength and a certain degree of flexibility in life (Olszta et al. 2007; Alexander et al. 2012). The process of bone biomineralization still eludes biologists and materials scientists, where the initial phases of formation are unclear (e.g., apatite nucleation and growth on collagen, or development from an amorphous precursor phase) (e.g., Olszta et al. 2007), in addition to the nanostructure (e.g., Alexander et al. 2012). Regardless of the factors controlling growth and mineralization of bone and the underlying nanostructure, the resulting composite material contains collagen fibers arrayed in characteristic association with the bioapatite crystallites (Fig. 2; Collins et al. 2002; Alexander et al. 2012). Collagen is intimately associated with crystallites in a parallel and staggered arrangement, resulting in a series of regularly spaced gaps or grooves (~ 67 nm) with bioapatite crystallites found occupying intrafibrillar, interfibrillar, and extrafibrillar regions relative to the collagen (e.g., Olszta et al. 2007; Alexander et al. 2012). The association of organics and mineral becomes important for understanding processes associated with the diagenesis of bone.

After host death, bone is deposited in a natural environment outside the relative homeostasis experienced within a living vertebrate. Collagen is removed through autolytic (e.g., via thermal destabilization; Leikina et al. 2002) or biologic activity (Grupe 1995; Balzer et al. 1997; Collins et al. 2002; Jans et al. 2004), opening pore spaces in bone to the movement of fluids, dissolved ions, and microorganisms (Fig. 2). The combined effects of abiotic and biotic processes drive pore space opening and subsequent alteration of bioapatite crystallites. Type I collagen is a ubiquitous protein found not only in bone but also in the skin, tendon, and muscles

of vertebrates (Collins et al. 2002). While collagen is stable *in vivo*, if exposed to varying temperatures *in vitro*, collagen can begin to denature (Leikina et al. 2002). Destabilization due to relaxation of the triple helix structure opens the system to further decomposition. Exposure to aqueous solutions can result in swelling of collagen, a process used to explain fracturing observed in bones deposited in aqueous environments (Pfretzschner 2004).

The role of microorganisms in bone-associated collagen degradation has received some direct investigation (e.g., Grupe 1995; Balzer et al. 1997). However, the mechanisms by which microorganisms break down collagen are unclear. For example, it is unclear if enzymatic processes (i.e., release of collagenase enzymes), or physical modification exposing collagen to subsequent biochemical alteration (i.e., fungal hyphae penetration of bone), occur in a specific order (e.g., Nicholson 1996; Grupe and Turban-Just 1998). Microbes (*sensu stricto* "bacteria" and "fungi") have been implicated in bone breakdown in the fields of archaeology and paleontology, where they are believed to actively scavenging the carbon and nitrogen-rich constituent amino acids forming the complex collagen molecule (Child 1995; Jans et al. 2004; Jans 2008). But, at present, limited direct testing through experimental approaches places significant ambiguity as to the precise role of microorganisms in bone collagen as well as bone mineral degradation. Additionally, the potential for site-specific processes to accelerate or retard (e.g., role of humics; Nicholson 1996) collagen decomposition presents another level of ambiguity. One possibility is the presence of microbial communities specializing in the production of collagenolytic proteases, forming the first line of attack on bone collagen (Watanabe 2004). Unraveling the timing and processes associated with collagen degradation, often invoked as the first step in diagenesis (Collins et al. 2002), is critical for estimating the preservation potential of bioapatite over geologic time.

Preservation of biomolecules

One of the goals of a paleobiologist is to reconstruct the biology of an extinct organism, including the color of its skin or feathers (Vinther et al. 2010; Lindgren et al. 2012), diet (Varricchio 2001; Chin et al. 2003), thermophysiology (e.g., Barrick and Showers 1994) as well as the use of oxygen and carbon isotopes to source drinking water and diet (e.g., Koch 2007). Unfortunately, the processes of diagenesis and fossilization have the potential to alter an endogenous, biogenic signature, both in the form of soft tissues and chemical information held in the mineralized fraction of bone. Despite the recovery and successful sequencing of DNA extracted from archaeological remains, the upper-limit for DNA preservation is generally considered to be less than a million years for bacterial DNA (Willerslev and Cooper 2005), and ~65 kyr for vertebrates (e.g., bison; Gilbert et al. 2004; Allentoft et al. 2012). Perhaps one of the most controversial and transformative studies, resulting in the development of a new field of paleobiology—molecular paleontology—was the discovery of endogenous biomolecules from fossil dinosaur bone preserved in sandstone (Schweitzer et al. 1997). Not only was the fossil material significantly older than previously believed to be able to host any original biogenic organics, but the specific molecules uncovered also indicated that the *Tyrannosaurus rex* was an actively reproducing female, providing biological insights never before deemed possible by paleobiologists. Further discovery of preserved original biomolecules in fossils from the Recent to the Cretaceous, from marine as well as terrestrial sediments, suggests that the process of fossilization may not completely destroy an original, biogenic signal (Lindgren et al. 2011; Schweitzer 2011). Despite the hotly contested results of this research (Buckley et al. 2008), they have held up to subsequent replication (Bern et al. 2009; Schweitzer et al. 2009) and inclusion of

additional bones and localities (Schweitzer et al. 2007; Lindgren et al. 2011), suggesting fossilization may not operate in a predictable manner at every site. One of the unifying themes that links exceptional preservation of biomolecules relates to inhibition of microbial degradation through physical and/or chemical controls (e.g., growth of secondary minerals in pore spaces, preventing recrystallization). Molecular paleontology will undoubtedly continue to fundamentally transform our understanding of process, rate, and duration of bioapatite diagenesis and the potential for preservation of endogenous biomolecules (see review by Schweitzer (2011) and references therein).

Analytical approaches to investigate fossils

Fossils are used widely in paleobiology and archaeology to address a variety of questions related broadly to paleoclimate, paleoecology, taphonomic processes, and vital processes, to name a few. Driving the development and evolution of these questions are simultaneous advancements in analytical capabilities. The integration of newly emerging analytical techniques, singularly and in combination with long-established tools, has revolutionized our understanding of bone as a whole, from both compositional and diagenetic perspectives (e.g., Trueman and Benton 1997; Reiche et al. 2003; Koenig et al. 2009; Dumont et al. 2009) (Table 1).

Some of the earliest attempts to characterize the mineralogy and geochemistry of fossil bones integrated petrographic assessment (e.g., Hubert et al. 1996; Wings 2004), X-ray diffraction (XRD), electron microprobe (EMP) analyses (Person et al. 1995; Hubert et al. 1996), as well as bulk chemical measurements (e.g., Samoilov and Benjamini 1996). These as well as other early attempts to quantify the chemical composition of fossil bone provided some fundamental insights into composition as well as mechanisms involved in diagenesis. The

application of these analytical techniques to evaluate fossil composition is still widely used today, and provides a way to quantify, visualize, and spatially resolve elemental compositions (Fig. 4). Examining the petrography of fossils, including both the development of secondary phases (e.g., Wings 2004) and histological modification (e.g., Jans 2008), provides a visual means of assessing diagenesis.

The stable isotopic composition of bones are routinely used to evaluate diet (e.g., Koch 2007) and thermophysiology (e.g., Barrick and Showers 1994) of extinct taxa, and more recently, growing appreciation for the potential overprinting by diagenetic conditions as led to an assessment of the integrity of bioapatite stable isotopes as an archive of an original, biogenic signature (e.g., Kohn and Law 2006). The $\delta^{13}C$ and $\delta^{18}O$ isotopic composition of bioapatite carbonate and $\delta^{18}O$ of phosphate have the potential to preserve an original biogenic signature for reconstructing paleodiet (see Koch (2007) for an in-depth review). Additionally, the isotopic composition may be exchanged with pore fluids during diagenesis, resulting in a record of conditions during diagenesis rather than a biogenic signal (e.g., Wang and Cerling 1994; Kohn and Law 2006).

Subsequent diagenesis-specific studies capitalized on initial observations of rare earth element (REE) enrichment by using spatially-resolved REE patterns collected from laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) were used to assess transport history and bone provenance (e.g., Trueman and Benton 1997; Metzger et al. 2004; Suarez et al. 2007), rates of ion uptake and exchange (e.g., Millard and Hedges 1996; Kohn 2008; Kohn and Moses 2013), and mechanisms of uptake (e.g., Kohn 2008; Herwartz et al. 2011, 2013; Kohn and Moses 2013). The integration of novel tools for REE-based studies marked a major turning point in diagenesis-related studies. The ability to not only quantify but to also spatially-resolve the

chemical composition of fossil bone fundamentally transformed our understanding of the changes that occur to bone over geologic time. Diagenesis of bone was recognized to be a dynamic process, one controlled by a variety of bone-specific and environment-specific parameters, such as redox, groundwater composition, climate, and host sediment composition (e.g., Berna et al. 2004; Koenig et al. 2009; Suarez et al. 2010). The interplay of all of these components influences the composition of fossil bone, and the processes and mechanisms are gradually being resolved.

The integration of other analytical tools, particularly synchrotron-based techniques (Table 1), has furthered our understanding of bone as a material and diagenesis. Several approaches, including micro X-ray fluorescence (μ-XRF) and synchrotron rapid scanning X-ray fluorescence (SRS-XRF), allow for high-resolution mapping of elemental concentrations and distributions (e.g., Janssens et al. 1999; Dumont et al. 2009; Bergmann et al. 2010) in various fossil specimens. A related technique, proton induced X-ray emission (PIXE) (e.g., Goodwin et al. 2007), provides an additional tool to spatially-resolve elemental composition as well as detailed tissue morphology and microstructure. The application of X-ray spectroscopic techniques, such as X-ray absorption near-edge structure (XANES) and extended X-ray fine-edge structure (EXAFS) spectroscopy, to modern and fossil bone, refined our understanding of underlying atomic-level configurations, bonding, and electron sharing within the apatite lattice for specific elements including Mn (Reiche and Chalmin 2009), and Ca and P (Keenan et al. 2015). Recent investigations into the atomic-level structure of fossils from a range of depositional settings and ages revealed that fossil apatite converged on a uniform lattice structural arrangement, suggesting both physical and chemical controls on fossil preservation at the atomic-level (Keenan et al. 2015). Decades of research on fossil bone geochemistry provided numerous

253 insights into the process of fossilization, and led to a major conclusion, namely that fossil
254 geochemistry is a reflection of site-specific conditions, and argued for an overarching chemical
255 control on preservation. However, a uniform lattice arrangement in geochemically distinct bones
256 (Keenan et al. 2015) also suggests that there are physical constraints on preservation, and we
257 cannot invoke chemistry alone as a driving mechanism of preservation.

258 Continued development of techniques, including Raman spectroscopy, led to the
259 identification of highly carbonated bioapatite (e.g., Li and Pasteris 2014a, b), transforming our
260 understanding of biomineralization. *In situ* characterization of bone undergoing repair (e.g.,
261 Penel et al. 2005), age-related changes to bone (Gao et al. 2015), and the mineralization of bone
262 (Crane et al. 2006), permit nanoscale assessment of bioapatite development and growth in
263 vertebrates. These studies help to refine our understanding of the structural, chemical, and
264 physical properties of bioapatite *in vivo*, which become critical when assessing diagenesis. For
265 example, the potential for elevated carbonate in bioapatite in certain taxa, such as whales (Li and
266 Pasteris 2014a, b), influences bioapatite crystallite size and solubility. The application of these
267 newer tools in combination with more traditional analyses (i.e., petrography, XRD, FTIR,
268 Raman, EMP) will continue to drive novel insights into fossilization processes.
269

270 **Actualistic taphonomy and contributions from forensics**

271 The process of fossilization, starting with early diagenesis and persisting through late
272 diagenesis, renders reconstruction of endogenous biogenic signatures or physical processes
273 difficult, if not impossible. One approach to evaluating the chemical and physical processes
274 occurring during early diagenesis is through actualistic taphonomy—controlled evaluation of
275 decomposition, decay, and alteration of an organism following death. There are numerous

276 questions that may be addressed using an actualistic taphonomic approach. Questions range in
277 terms of spatial or temporal scales as well as whether the objective is an understanding of
278 physical and/or chemical processes (e.g., Behrensmeyer 1978; Weigelt 1989; Grupe 1995;
279 Nicholson 1996, 1998). Additionally, the type of environment or climate under which
280 experiments are conducted will also influence the physical and chemical processes. Evaluating
281 changes to bone chemistry and structure in modern systems through actualistic taphonomy can
282 be used to make inferences regarding processes occurring in the geologic past.

283 Some of the earliest studies incorporating actualistic taphonomy focused on large-scale,
284 physical assessment of bone transport over time in various depositional systems. One of the first
285 (paleo)biologists to evaluate decomposition in modern systems was Johannes Weigelt (1989). He
286 observed a range of vertebrate taxa decomposing over time in a suite of depositional systems
287 ranging from fluvial to estuarine environments. His observations provided a predictive
288 framework for assessing the accumulation history of bones in the fossil record (Weigelt 1989).

289 Additional seminal work focusing on modern bone taphonomy is a multi-decadal study of
290 bone alteration in Kenya by Behrensmeyer and colleagues (Behrensmeyer 1978; Behrensmeyer
291 et al. 2000), which resulted the identification of discrete weathering stages. Unfortunately, long-
292 term studies like this for other environments and climates are notably lacking (although see
293 Nicholson 1996, 1998 for temperate terrestrial decomposition studies).

294 Perhaps the most in-depth and longest running research programs evaluating the
295 alteration of bone in modern systems relate to forensics applications. Starting with whole
296 carcasses of a range of animals, including humans (e.g., Carter et al. 2007), decomposition
297 progresses through several well-characterized stages until skeletonization occurs, exposing bones
298 to ambient physiochemical conditions. For forensics, bones and DNA preserved within bones are

299 frequently the only materials available to identify the remains (e.g., Mundorff and Davoren
300 2014). A rapidly evolving area in forensics research is developing a way to use microbial
301 ecology associated with the exposed and decomposing bone as a marker of postmortem interval
302 (PMI) (e.g., Metcalf et al. 2013; Damann et al. 2015). Preliminary results from these studies
303 suggest that microbial community structure associated with bone decomposition changes during
304 each stage of decomposition, culminating in microorganisms largely derived from the soil
305 (Damann et al. 2015). These results provide an important first step in understanding the
306 (micro)biological controls on bone diagenesis, although the target substrate (i.e. organic or
307 mineral) is unknown.

308 Surprisingly, a large amount of research on the role of biology in decomposing bone
309 comes from studies of whale falls (e.g., Goffredi et al. 2005; Goffredi and Orphan 2010). The
310 introduction of nutrient-rich reservoirs into an otherwise nutrient starved system stimulates rapid
311 vertebrate, invertebrate, and microbial responses, and results in a long-lived 'hot spot' for
312 benthic organisms, largely invertebrate and microbial communities (e.g., Goffredi and Orphan
313 2010). In whale falls, invertebrate polychaete worms (*Osedax*) have evolved a tight
314 (endo)symbiotic relationship with intracellular microbes that aid with the physical and chemical
315 breakdown of bone, targeting collagen-derived proteins and cholesterol (Goffredi et al. 2005).
316 The mm-cm sized holes cause by the activity of boring polychaetes opens the bone to further
317 utilization occurs by bacteria and fungi derived from the environment.

318 With improved analytical techniques and capabilities, questions relating more specifically
319 to the chemical changes that occur to bone during diagenesis are now possible, and stand as the
320 next step for actualistic taphonomy. For example, even a basic question like, how long can bone
321 survive in a natural environment, is far from understood. This lack of knowledge is not trivial,

particularly for trying to understand the preservation potential of bioapatite over geologic time and biases in the fossil record. Recent experimental work assessing the timing of early diagenesis in bones buried in a wetland and simulated wetland conditions revealed chemical and structural changes to bone within weeks to months of exposure to aggressive environmental fluids and exogenous soil microorganisms (Keenan and Engel *in preparation*). Further experimental work focused on characterizing the role of biology in early transformations of bone in a range of depositional settings are warranted. In particular, combining geochemical tools with microbial ecology will provide novel insights into early diagenesis (Table 2).

Implications: outlook and future directions

Despite the incredible progress made towards evaluating the composition, structure, and mechanisms of preservation of bone (Table 1), there are still significant gaps in our understanding of the process of fossilization. Based on rates of uptake and exchange, fossilization likely occurs on timescales ranging from thousands to 10's of thousands of years (e.g., Millard and Hedges 1996; Kohn and Law 2006), although recently transformations of bone during the early diagenetic period immediately following host death suggest changes may occur even earlier (Keenan et al. in preparation). The role of site-specific conditions in controlling apatite diagenesis is somewhat understood, including important roles played by pH (e.g., Berna et al. 2004; Fig. 3), redox (e.g., Suarez et al. 2010), and sediment porosity, but the boundary conditions are not well-defined. Perhaps most importantly, the role of biology in fossilization processes is not well understood. We know that (micro)biology can physically and chemically alter bone (e.g., Child 1995; Jans 2008), but exact mechanisms, timing, rates, and ways in which biology alters local (micro-scale) geochemistry are unknown. Additional unknowns that

currently stand at the fore-front of diagenesis-related research include: the degree of the primary signal preserved in recrystallized bone, particularly with respect to isotopes, and a better understanding of the nano-scale (and atomic-scale) information preserved within bioapatite, both modern and fossil.

The introduction of a carcass in both terrestrial and aquatic systems provides a significant input of nutrients, particularly carbon and nitrogen, stimulating microbial growth (e.g., Carter et al. 2007; Cobaugh et al. 2015). Progress in evaluating the changes to soil microbial communities associated with animal decomposition promises to provide insights into biogeochemical cycling of nutrients. The role of microbes in the physical and chemical breakdown of bioapatite in marine and terrestrial systems is vastly understudied. For example, it is unclear if destabilization of the apatite lattice and bone is driven by organic (collagen) degradation or if certain microbial communities actively target apatite, leading to mineral breakdown. With the advent of affordable and accessible molecular techniques (e.g., 16S rRNA-based sequencing of the community), evaluating the role of microbes in bone decomposition (or preservation) is an achievable goal.

As technological innovations continue to drive research, the future of unraveling fossilization and diagenesis processes sit at the convergence of novel analytical approaches through unusual collaborations. Paleobiology is poised for transdisciplinary collaborative research, bridging disciplines as seemingly disparate as physics and geomicrobiology. Only by approaching a long-held question in paleontology from different and unconventional perspectives will we continue to shed light on the ‘black-box’ of the fossilization of bone. The versatility of apatite in living tissue, and ultimately in fossils, provides an invaluable tool for understanding extinct life and biotic and climatic changes over geologic time.

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Figure Legends

Figure 1: Schematic view of diagenesis from the life to death of an animal. (**a-b**) Following the death of an animal, such as the alligator illustrated here, the bones may be deposited within the same system occupied in life. (**c**) The activity of scavengers, microorganisms, and physical processes may remove some of the bones resulting in a fragmented and incomplete fossil record. Burial and diagenesis, which transform the original bone into fossil apatite, enhance preservation potential. (**d**) Subsequent erosion of overlaying sediments may bring the fossilized bone back to the surface. Each of these stages of diagenesis results in physical and chemical modification of the original bioapatite (from Keenan 2014).

Figure 2: Schematic view of diagenesis of bioapatite. (**a**) *In vivo*, bioapatite consists of interlayered mineral and organic phases. (**b**) Following the deposition of a bone in an environmental system, the degradation of collagen (autolytic or biologic) opens pore spaces to the movement of fluids carrying dissolved ions. (**c**) Substitution of elements in the bioapatite lattice results in the formation of secondary mineral phases, with reduced porosity, and increased crystallite size (from Keenan 2014; modified after Trueman and Tuross 2002).

Figure 3: Representative mineral stabilities (K_{sp}) for apatites and vivianite in a natural environment across a range of pH conditions at 25°C. Using water chemistry from a fluvial system in Louisiana with high total dissolved solids, models of predicted saturation states with respect to selected mineral phases were developed under two end-member conditions: low (0.6 µmol/L) and high (48.4 µmol/L) total phosphorus concentrations. Models were developed using PHREEQC-I (Appelo and Postma, 2005). Values that plot within the region marked

400 'supersaturation' indicate that solution chemistry is predicted to be supersaturated with respect to
401 the mineral phase (i.e., mineral phase is stable or actively precipitating), and values within
402 'undersaturation' indicates mineral phases are predicted to dissolve. HAP refers to
403 hydroxylapatite; FAP is fluorapatite; CO_3-FAP is carbonated fluorapatite.
404
405 **Figure 4**: Electron microprobe (EMP) false-color and mixed element maps for a dinosaur fossil
406 (Hell Creek Formation, Montana; HCDO03). (**a**) Backscatter electron image of the bone held
407 within a sandstone matrix. The bone is more apparent in the false-color maps. (**b**) False-color
408 element map of phosphorus. Greater color intensity corresponds to elevated elemental
409 concentrations. (**c**) False-color element map of iron (as Fe^{2+}) distribution in the bone and
410 sediment. (**d**) False-color map of strontium distribution within the bone and adjacent sediment.
411 (**e**) Mixed element map of P, Sr, Fe, and F in bone and sediment. Some compositional grading is
412 evident in the bone fragments with a zone of P depletion following structural features.

413 # Tables

414 **Table 1**: Summary of some of the analytical methods and tools used to investigate physical
415 and/or chemical properties of bone, key results for each method, and selected publications. This
416 table is not exhaustive, but rather touches on a wide range of techniques currently in use.

Analytical Method or Tool	Results of analyses	Resolution	Selected Publications
X-ray absorption near-edge structure spectroscopy (XANES)	Atomic-level composition; arrangement of bonds; electron sharing	Angstrom	Reiche and Chalmin (2008); Keenan et al. (2015)
Synchrotron rapid scanning X-ray florescence (SRS-XRF)	Spatially-resolved chemical composition	Angstrom	Janssens et al., 1999; Dumont et al. (2009); Bergmann et al. (2010)
Proton induced X-ray emission (PIXE)	Elemental composition; spatially-resolved chemical composition through mapping	Angstrom	Reiche et al. (2003); Goodwin et al. (2007); Bradley et al. (2010)
Extended X-ray fine structure spectroscopy (EXAFS)	Atomic-level composition; arrangement of bonds; electron sharing	Angstrom	Peters et al. (2000)
Fourier-transform infrared spectroscopy (FTIR)	Vibrational modes of constituent compounds; organics; surface mapping; proportion of carbonate species; crystallinity	Micron	Sponheimer and Lee-Thorp (1999); Pucéat et al. (2004); Keenan et al. (2015)
Raman spectroscopy	Vibrational modes of constituent compounds; surface visualization; mapping; organics; quantify carbonate content and species	Micron	Penel et al. (2005); Crane et al. (2006); Li and Pasteris (2014a,b)
Electron microprobe (EMP) analyses	Elemental composition (quantitative and qualitative); visualization of surface; elemental mapping	Micron	Greenlee (1996); Hubert et al. (1996); Keenan et al. (2015)
Transmission electron microscopy (TEM)	Size and structure of crystallites	Micron	Reiche et al. (2003)
Scanning electron microscopy (SEM)	Surface morphology; elemental composition when combined with energy dispersive X-ray	Micron	Reiche et al. (2003)
Atomic-force microscopy (AFM)	Surface morphology; material properties, including strength	Nanometer	Gao et al. (2015)
Histology	Macroscale morphology	Nanometer	Tütken et al. (2004); Straight et al. (2009)
Computed tomography (CT)	Physical structure	Nanometer to micrometer	Straight et al. (2009)
Thermogravimetric analyses (TGA)	Weight loss informs water, organic, carbonate content		Mkukuma et al. (2004); Keenan et al. (2015)

X-ray diffraction (XRD)	Mineralogy	Person et al. (1995); Peters et al. (2000); Piga et al. (2009); Keenan et al. (2015)
X-ray florescence (XRF)	Elemental composition (quantitative)	Piga et al. (2009)
Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS)	Elemental composition (bulk or spatially resolved); trace element composition (e.g., REE)	Trueman et al. (2008a); Koenig et al. (2009); Suarez et al. (2010); Herwartz et al. (2011, 2013)
Nuclear magnetic resonance (NMR)	Bonding and structure of Ca-bearing phases	Laurencin and Smith (2013)
Stable isotope geochemistry (variety of techniques)	Insight into paleodiet, paleoclimate, paleoecology	Cerling et al. (1993); Barrick and Showers (1994); Wang and Cerling (1994); Kohn (1996); Tütken et al. (2004); Kohn and Law (2006); Koch (2007); Tütken et al. (2011); Suarez et al. (2014)
Molecular Methods		
Microbial ecology, community composition (DNA-based)	Genetic ID of microbes (bacteria, fungi, archaea) associated with bone diagenesis	Reeb et al. (2011); Damann et al. (2015)*associated with bone, role in diagenesis not demonstrated
Microbial ecology, community functioning (RNA-based)	Functional insights into metabolic processes occurring (e.g., P-mineralization, collagen degradation)	*none*

Table 2: Timeline of key recent advances and suggested future directions in bone diagenesis related research.

Timeline of Recent Major Advances and Suggested Future Advances
1990's
Fossil bone is chemically altered
Enrichment of F, trace elements compared to modern
Largely bulk analysis-based approaches using XRD, XRF, etc.
Crystallite-scale characterization using FTIR, Raman
Biology, specifically microbiology, plays a role in diagenesis of bone
2000's
Fossil bone is chemically zoned, reflecting diagenetic processes
Application of synchrotron-based techniques takes off
REE chemistry a major field in paleontology
Field of 'molecular paleontology' begins to emerge
2010-15
Continued application of novel tools (e.g., synchrotron-based)
Some application of molecular techniques (e.g., Reeb et al., 2011)
Continued 'molecular paleontology' (e.g., Schweitzer et al., 2014)
2015-onwards
Microbial role in bone alteration (from molecular perspectives: community function and composition)
Integrate conceptual models of bone stability with experimental data
Combined analytical techniques to address element- or process-specific questions (e.g., what is the dominant phase of Fe in bone?)
Transdisciplinary collaborations

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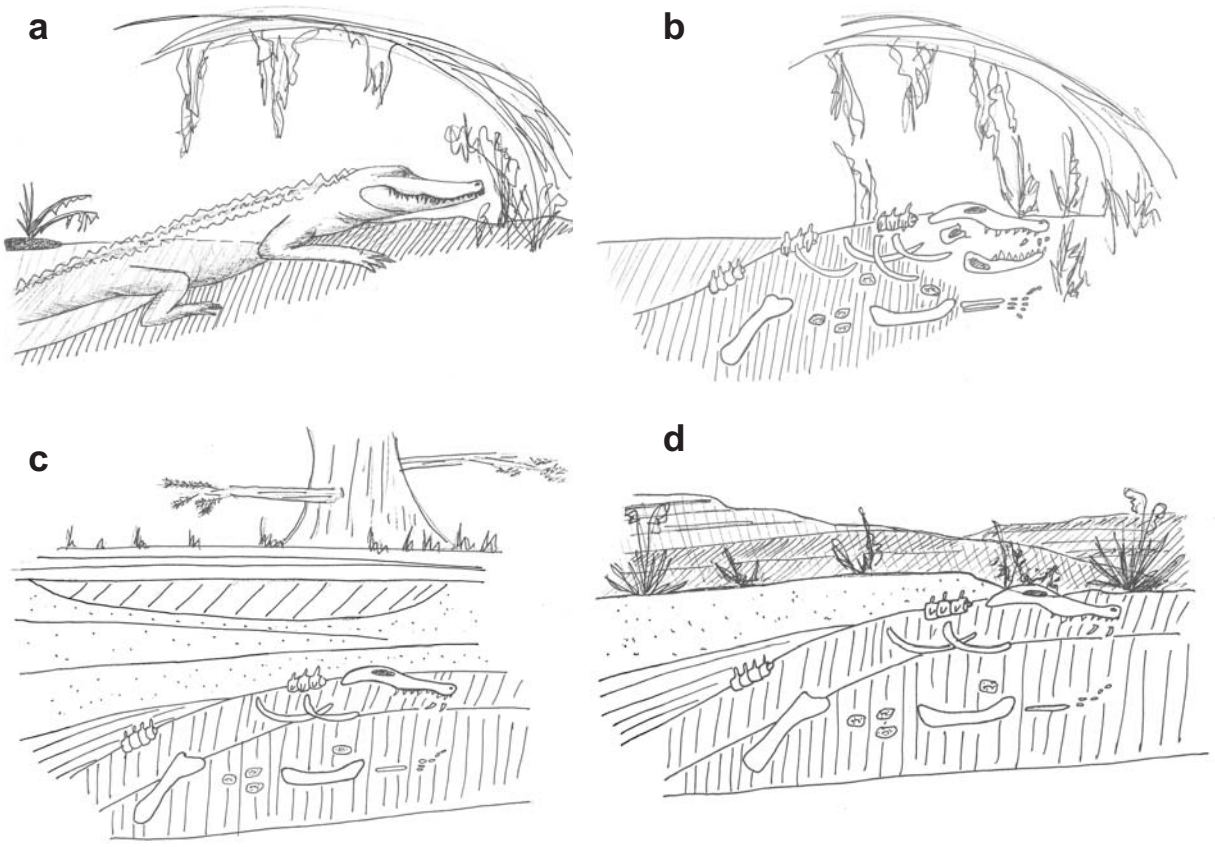
Figure 1



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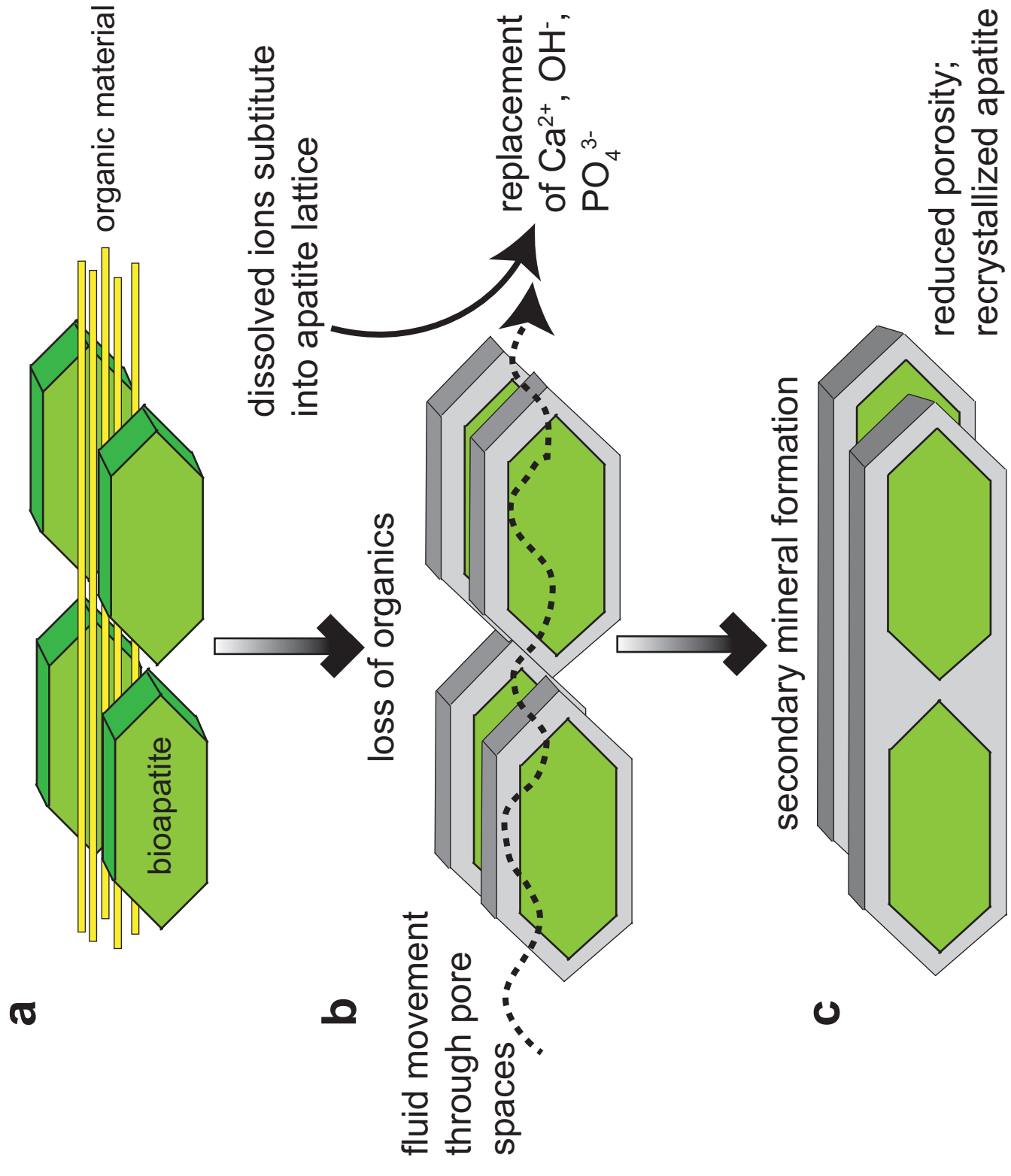

Figure 2

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Figure 3

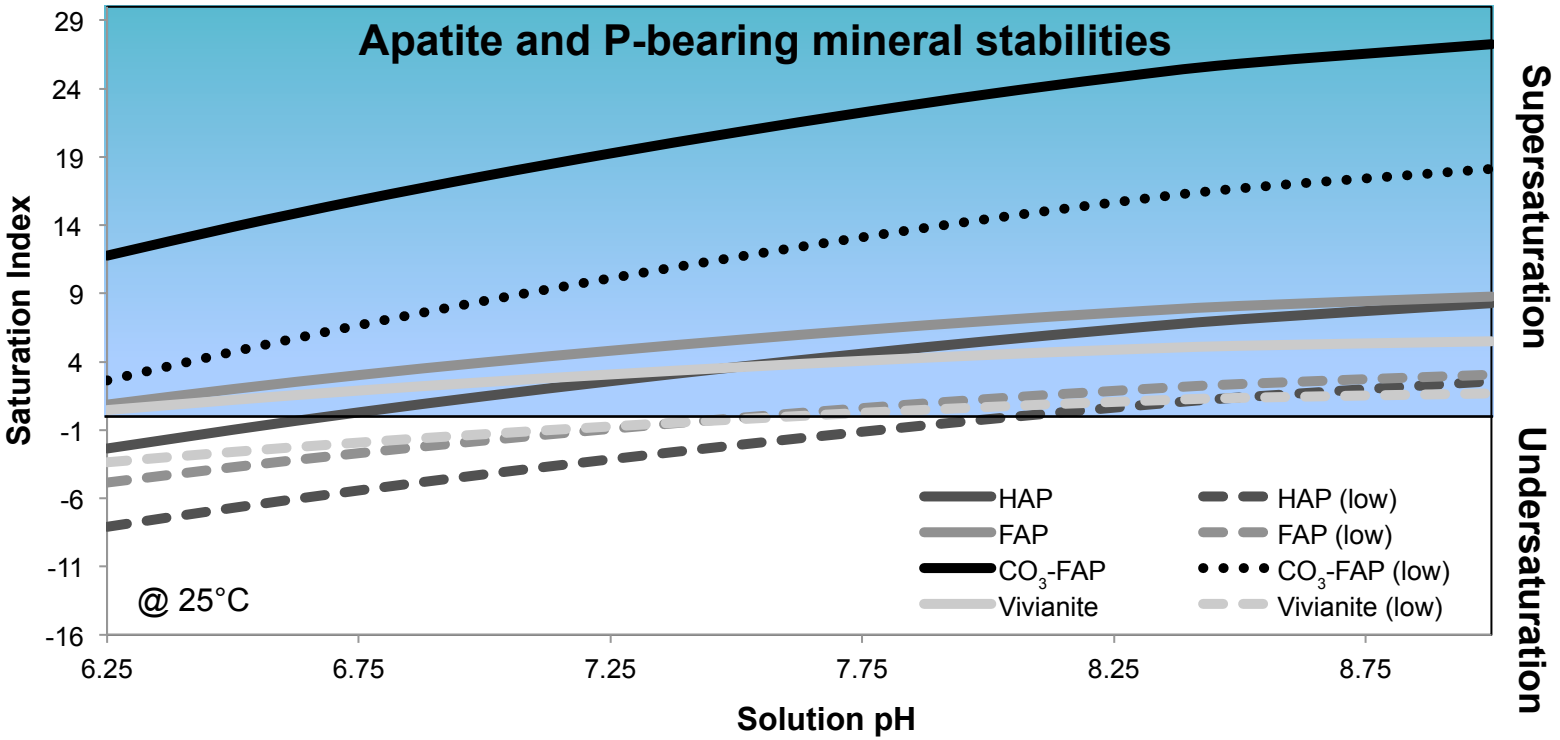

Figure 4

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