



A chemical framework for the preservation of fossil vertebrate cells and soft tissues

Landon A. Anderson

Department of Biology, North Carolina State University, Raleigh, NC, USA

ARTICLE INFO

Keywords:

Soft tissue
Crosslinking
In-situ polymerization
Carbonization
Fossil
Diagenesis
Vertebrate

ABSTRACT

Reports of preserved cells and other soft tissues in ancient vertebrates, including dinosaurs, have been met with controversy within the field of vertebrate palaeontology. To explain such reports, Schweitzer et al. (2014) hypothesized that iron-mediated radical crosslinking preserves ancient soft tissues in a manner somewhat analogous to histological tissue fixation. In 2018, Wiemann et al. proposed a second hypothesis that these soft tissues were preserved as advanced glycation/lipoxidation end products (AGEs/ALEs). The chemistry underlying these hypotheses, however, remains poorly described for fossil vertebrates.

This review posits a chemical framework describing the persistence of biological “soft” tissues into deep time. The prior iron-mediated radical crosslinking and AGE/ALE mechanisms are re-described in context of established chemistry from a diversity of scientific fields. Significantly, this framework demonstrates the hypotheses presented by Schweitzer et al. (2014) and Wiemann et al. (2018) are, in many cases, subsequent steps of a single, unified reaction mechanism, and not separate hypotheses. Knowledge of the chemical mechanisms underlying vertebrate soft tissue preservation has direct implications for molecular archaeology and palaeontology, including efforts at molecular sequence recovery within the ancient DNA and palaeoproteomic communities. Such implications that are immediately apparent from examining the chemical framework are discussed.

1. Introduction

A growing number of studies have reported the recovery of “cells” and other “soft tissues” within ancient vertebrate remains, including those of dinosaurs (see Fig. 1) (Schweitzer et al., 2005; Schweitzer et al., 2007; Manning et al., 2009; Schweitzer et al., 2009; Lindgren et al., 2011; Schweitzer, 2011; Armitage and Anderson, 2013; Schweitzer et al., 2013; Cadena, 2016; Surmik et al., 2016; Lee et al., 2017; Lindgren et al., 2018; Boatman et al., 2019; Cadena, 2020). These reported soft tissues initially generated controversy within the field of vertebrate palaeontology and were challenged as being exogenous biofilms (Kaye et al., 2008; Saitta et al., 2019). Later studies disputed such claims, and reported experimental evidence in support of these “soft tissues” being endogenous (Armitage and Anderson, 2013; Schweitzer et al., 2016; Wiemann et al., 2018). However, the notion that cells and soft tissues are unlikely to preserve within mineralized vertebrate remains is questionable on its own. The fossil fuels used daily by society consist of original biomolecules of ancient plants and microorganisms that have been chemically transformed into carbonaceous macromolecules referred to as kerogens (Tissot and Welte, 1984; Tegelaar et al., 1989;

Vandenbroucke and Largeau, 2007). In cases such as with coalified fossil wood, for example, this conversion of biomolecules towards kerogen macromolecules can preserve original tissue morphology (Gupta et al., 2007a; Gupta, 2015; Mustoe, 2018). The fields of soil and petroleum science even accept that recalcitrant biomarkers can preserve through time as portions of these highly crosslinked kerogen macromolecules (Westbroek et al., 1979; Philp and Gilbert, 1987; Gupta, 2014; Ferrer et al., 2018). Further, the preservation of biological tissues is a phenomenon known to occur within invertebrate fossils (Stankiewicz et al., 1997; Gupta et al., 2007c; Cody et al., 2011; Ehrlich et al., 2013; Wysokowski et al., 2014). For example, pre-Pleistocene arthropod cuticles have been experimentally shown to consist of hydrocarbons, and in some cases nitrogenous residues (Stankiewicz et al., 1997; Gupta et al., 2007c; Cody et al., 2011). Such findings support that the original cuticle is still present in some form and has not been fully replaced by exogenous minerals. Multiple publications have hypothesized that such preservation is the result of crosslinking and chemical transformation of the original cuticular chitin and proteins (Goth et al., 1988; Stankiewicz et al., 1997; Gupta et al., 2007c; Cody et al., 2011; Gupta, 2014). The possibility that similar mechanisms are responsible for the persistence of

E-mail address: laander2@ncsu.edu.

<https://doi.org/10.1016/j.earscirev.2023.104367>

Received 8 August 2022; Received in revised form 1 January 2023; Accepted 22 February 2023

Available online 2 March 2023

0012-8252/© 2023 The Author. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

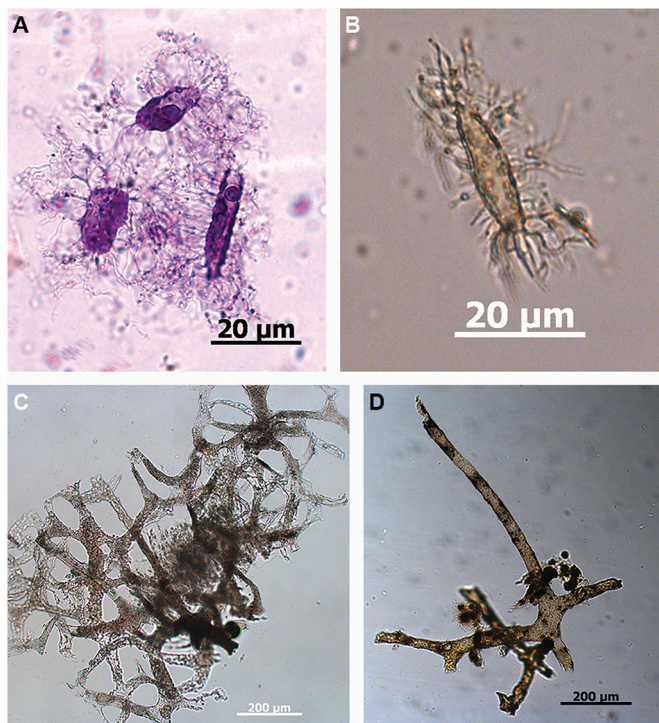


Fig. 1. Microscopy images of “soft tissues” isolated from bones of the Cretaceous dinosaur *Brachylophosaurus canadensis* (MOR 2598 and GPDM 328), along with extant *Struthio camelus* analogs. (A) Light microscope image of extant *S. camelus* osteocytes (bone cells). Cells were stained with 1% toluidine blue to aid visualization. Unstained, the extant osteocytes were observed to be transparent. (B) Light microscope image of a structure morphologically consistent with an osteocyte isolated from MOR 2598. Numerous filipodia are observed branching from the structure. (C) Blood vessels isolated from an extant *S. camelus* long-bone, with some collagenous matrix still attached. Anastomosing structure is observable. (D) Light microscope image of a structure consistent with “blood vessels” from GPDM 328. A hollow, bifurcating, tubular structure is observable. Methods: Specimens MOR 2598 and GPDM 328 were prepared in a lab dedicated to the processing of ancient materials, separate from the extant *S. camelus* specimen. All specimens were demineralized in 0.5 M EDTA (pH 8.0) for ~ 1–3 days. The extant *S. camelus* bone required a subsequent collagenase digestion (1 mg/mL in Dulbecco’s phosphate-buffered saline (PBS)) overnight at 37 °C to isolate blood vessels and osteocytes; multiple thorough washes in sterilized water were performed prior to collagenase digestion to remove residual EDTA. Isolated vessels/vessel-like structures and cells/cell-like structures from both ancient and extant specimens were placed in PBS prior to imaging. *S. camelus* osteocytes were stained with 1% toluidine blue to enhance visibility due to the cells’ transparency. Vessels/vessel-like structures and cells/cell-like structures were imaged using a Zeiss Axioskop 2 plus microscope and saved/stored as “Tiff” files. Prior to use, tools and surfaces used for preparation of ancient specimens were sterilized with a 10% bleach solution followed by a 75% ethanol solution, and consumables were autoclaved. Solutions used for sample preparation were vacuum filtered (0.220 µm) prior to use. During the preparation of ancient specimens, a lab coat, gloves, bouffant cap, and surgical mask were worn at all times.

endogenous soft tissues, and potentially even biomarkers or other biomolecules, protected within the mineralized remains of dinosaurs and other ancient vertebrates could thus be a viable hypothesis.

Indeed, two mechanisms (Collins et al., 1998; Schweitzer et al., 2014; Wiemann et al., 2018; Boatman et al., 2019) have been proposed to explain reports of soft tissues and biomolecules within ancient vertebrates. Both result in the intra- and intermolecular crosslinking and chemical transformation of reactant biomolecules, a process referred to as *in-situ* polymerization (Stankiewicz et al., 2000; Gupta et al., 2007a) within geological contexts (a comprehensive list of definitions is given in Table 1). The first proposed mechanism, iron-mediated radical

formation, hypothesizes that redox-active iron catalyzes the breakdown of hydroperoxides to free hydroxyl radicals (Schweitzer et al., 2014; Boatman et al., 2019). The free hydroxyl radical is unstable and highly reactive, and quickly abstracts a hydrogen atom (H·) from a biomolecule such as a protein, lipid, DNA, etc., and forms a radical on the biomolecule itself. The newly formed radical biomolecule can then react through one of several mechanisms with neighboring biomolecules to form intermolecular crosslinks (Roberfroid and Calderon, 1995; Catalá, 2010). The hypothesis of redox-active iron as a catalyst for this radical mechanism was supported by numerous reports of the presence of iron oxides, specifically goethite, in association with fossil vertebrate soft tissues (Kaye et al., 2008; Schweitzer et al., 2014; Cadena, 2016; Surmik et al., 2016; Boatman et al., 2019; Cadena, 2020).

The second proposed mechanism (Collins et al., 1998; Poinar et al., 1998; Poinar and Stankiewicz, 1999; Schweitzer et al., 2014; Wiemann et al., 2018; Boatman et al., 2019) is the formation of advanced glycation and lipoxidation end products (AGEs/ALEs) which involves the condensation of proteins, nucleotides, etc., with oxidatively damaged carbohydrates and/or lipids. Specifically, the conversion of carbohydrates to reactive carbonyl species, and the formation of aldehydes and ketones via lipid peroxidation generates substrates containing electrophilic carbonyl carbons with which nucleophilic groups of proteins and nucleic acids can condense. This results in the formation of large macromolecules generally termed melanoidins (Vistoli et al., 2013).

Both the iron-mediated radical and AGE/ALE pathways convert original tissue biomolecules into large, highly crosslinked macromolecules that are structurally analogous to melanoidins, humic substances (i.e. fulvic acids, humic acids, humins), immature kerogens, kerogen precursors, protokerogens, etc. (Fig. 2) (Harvey et al., 1983; Harvey and Boran, 1985; Vandenbroucke and Largeau, 2007; Vistoli et al., 2013; Schweitzer et al., 2014; Wiemann et al., 2018). These highly crosslinked macromolecules are herein referred to as humic-like macromolecules (HLMs) or alternatively, more specific to soft tissues, immature tissue macromolecules (ITMs). Extensive heat/pressure and/or timespans can further transform ITMs into carbonaceous macromolecules with molecular structures analogous to kerogens. Herein, such carbonaceous macromolecules are referred to as kerogen-like macromolecules (KLMs) (Briggs and Summons, 2014) or alternatively, more specific to soft tissues, mature tissue macromolecules (MTMs). Examples of potential molecular structures for kerogens are given in Fig. 2.

A chemical framework (Fig. 3) is herein hypothesized that explains reported cell and tissue preservation within vertebrate fossils. Currently, a detailed chemical framework does not exist within the primary literature for the fossilization of vertebrate cells and tissues. The iron-mediated radical (Schweitzer et al., 2014; Boatman et al., 2019) and AGE/ALE (Wiemann et al., 2018; Wiemann et al., 2020) mechanisms are currently understood to be competing hypotheses (Wiemann et al., 2018; Boatman et al., 2019) for vertebrate soft tissue preservation, and neither have been well-described in terms of vertebrate fossil biogeochemistry. Both hypotheses are herein re-described in terms of established chemistry across a diversity of scientific fields. Importantly, this framework will demonstrate that in many cases, the previously hypothesized iron-mediated radical (Schweitzer et al., 2014; Boatman et al., 2019) and AGE/ALE (Wiemann et al., 2018; Wiemann et al., 2020) mechanisms are not separate hypotheses. Rather, the hypotheses presented by Schweitzer et al. (2014) and Wiemann et al. (2018) will, in several instances, be shown to be subsequent steps of a single, unified reaction mechanism.

The reaction mechanisms discussed in this paper apply not only to vertebrate cell and tissue preservation but rather to the biomolecules of organismal tissues in general, whether archaeological or palaeontological and fossil or subfossil. The chemical framework presented here has implications for any field investigating the biogeochemistry of ancient tissues, cells, and biomolecules, including molecular palaeontology and archaeology, ancient DNA analyses, palaeoproteomics, and soil and petroleum science. Particularly, direct implications of this

Table 1
Definitions list.

Biomarker; ancient biomolecule; taphonomically altered biomolecule	A biomolecule, whether bound or unbound, that has undergone taphonomic alteration but is still recognizable as to its biological precursor
Biomolecule	An organic compound of biological origin; refers to carbohydrates, lipids, proteins, and nucleic acids
Biostratinomy	The chemical and physical properties and/or processes that affect an organism's preservational state from death up until burial
Bound biomolecule	A biomolecule, either unaltered or taphonomically altered, that has undergone <i>in-situ</i> polymerization
Carbonization	The maturation of <i>in-situ</i> polymerization products towards aliphatic, and primarily aromatic, macromolecules lacking heteroatoms
Carbonized film; carbonized trace	Preserved soft tissue morphology with molecular compositions consistent with carbonization products
Diagenesis	The chemical and physical properties and/or processes that determine an organism's preservational state after burial and up until the potential onset of catagenesis
Heteroatoms	Atoms other than carbon or hydrogen, such as oxygen (O), nitrogen (N), or sulfur (S); generally in reference to organic macromolecules
Humics	Sedimentary organic macromolecules that have generally undergone extensive crosslinking but limited carbonization. Can be classified operationally as fulvic acid, humic acid, or humin based on solubility
Humic-like macromolecule; immature tissue macromolecule (ITM)	Large, highly crosslinked macromolecules resulting from <i>in-situ</i> polymerization; have only undergone limited carbonization. "ITM" refers specifically to such substances as relates to soft tissue diagenesis
<i>In-situ</i> polymerization; crosslinking	Taphonomic polymerization/crosslinking of unaltered or taphonomically altered biomolecules towards HLMs/ITMs
Kerogens	Sedimentary organic macromolecules that have undergone extensive crosslinking and carbonization. Defined operationally as sedimentary organic matter that is insoluble in common organic solvents
Kerogen-like macromolecule (KLM); mature tissue macromolecule (MTM)	Large, highly crosslinked macromolecules resulting from <i>in-situ</i> polymerization and extensive carbonization. "MTM" refers specifically to such substances as relates to soft tissue diagenesis
Kerogenization	The overall process of converting biomolecules into kerogens, KLMs/MTMs, etc. Includes both <i>in-situ</i> polymerization and extensive carbonization
Non-polymerizing (NonP) taphonomic alteration; non-polymerizing (NonP) taphonomically altered	Taphonomic modification to functional group/s of an unaltered or previously taphonomically altered biomolecule that does not involve <i>in-situ</i> polymerization
Non-polymerized taphonomically altered biomolecule	A biomolecule that has undergone NonP taphonomic alteration but not <i>in-situ</i> polymerization: e.g. glutamine deamidation, fatty acid hydroxylation
Osseous soft tissues (OSTs)	The structures that make up the nonmineral portion of bone tissue: e.g. collagen matrix, osteocytes, intra-osseous vascular tissue, intra-osseous nerve tissue, collagen protein fibrils
Soft tissues	The endogenous, organic portion of an organism/specimen's biological tissues
Soft tissue morphology	The structural form of soft tissue; does not always correlate with the presence of endogenous biomolecules
Taphonomy	The study of processes affecting the decay and preservation of an organism, including all processes of biostratinomy and diagenesis
Unaltered biomolecule	A biomolecule that has not undergone NonP taphonomic alteration or <i>in-situ</i> polymerization
Unbound biomolecule; free biomolecule; labile biomolecule	A biomolecule, either unaltered or taphonomically altered, that has not undergone <i>in-situ</i> polymerization

framework for predicting degree of biomolecule preservation within fossil and subfossil specimens, including for DNA and protein sequences, are discussed.

2. Chemical framework

2.1. Post-mortem tissue decay

Cellular tissues consist primarily of lipids, carbohydrates, proteins, and nucleic acids (Alberts et al., 2002). The exterior as well as interior compartments of a eukaryotic cell, including all vertebrate cells, are bounded by lipid membranes consisting largely of phospholipids and some sterols with various associated proteins that allow interaction with the environment. Bounded by these membranes, the interior of the cell is filled with an aqueous solution termed cytosol which contains dissolved ions, metabolites, biomolecules, etc. Additionally, an internal protein scaffolding, termed the cytoskeleton, is intimately associated with the lipid membranes and provides structural support for the cell. Finally, in general, the cellular genome, which consists of strands of millions to billions of polymerized nucleotide bases, resides within a double membrane bound organelle termed the nucleus (Alberts et al., 2002).

Upon the death of an organism, this cellular structure typically begins to rapidly decompose. Within minutes to hours (Prescott, 1912; Donaldson and Lamont, 2013), cellular autolysis occurs and fermentation increases. Endogenous bacteria migrate from the intestinal tract into surrounding tissues, expediting carbohydrate fermentation and initiating putrefaction (Prescott, 1912; Butzbach, 2010; Donaldson and Lamont, 2013; Nolan et al., 2020). Phospholipids and glycerides are rapidly hydrolyzed, releasing their fatty acid moieties which are either aerobically metabolized via the β -oxidation pathway or, in the absence of free oxygen, are converted to free and crosslinked hydroxy fatty acids

(adipocere) (Schoenen and Schoenen, 2013). A buildup of gasses from these processes in the body cavity leads to bloat which stretches, deforms, and disarticulates tissues and skeletal elements, further exposing the body to external decomposers (Butzbach, 2010; Donaldson and Lamont, 2013; Schoenen and Schoenen, 2013; Nolan et al., 2020). These processes eventually result in complete breakdown and recycling of components, leaving no trace remains to be incorporated into the fossil record. For original soft tissues and biomolecules to preserve in fossils, these degradative processes must have been slowed or arrested shortly post-mortem so that the original cells and tissues were not completely decomposed (Briggs et al., 2000). This can potentially occur via rapid burial and mineralization of organismal remains (Vandenbroucke and Largeau, 2007) or via rapid freezing, as in the case of permafrost specimens (Jensen and Sheehan, 2014), or via desiccation (Lennartz et al., 2020).

Regarding mineralization specifically, biologically mineralized elements such as bone and teeth offer a layer of protection to the soft tissues they harbor. Further, rapid mineralization of tissue remains has been hypothesized to inhibit the initial stages of decomposition as described above (Lees, 1989; Tuross, 1989; Child, 1995; Sykes et al., 1995; Collins et al., 2000; Salmon et al., 2000; Salamon et al., 2005; San Antonio et al., 2008; Schweitzer et al., 2014; Wadsworth and Buckley, 2014; Demarchi et al., 2016). Mineralization of tissues renders the constituent biomolecules sterically less accessible to microbial enzyme active sites, thus inhibiting microbial decomposition (Lees, 1989; Child, 1995; Trueman and Martill, 2002; Schweitzer et al., 2014). This allows the pathways proposed within this framework a chance to take place and not be outpaced by microbial decay shortly post-mortem. Furthermore, close association/binding of biomolecules with mineral matrix has been demonstrated to stabilize molecular bonds against direct chemical degradation (Keil et al., 1994; Sykes et al., 1995; Collins et al., 2000;

Salmon et al., 2000; Salamon et al., 2005; Demarchi et al., 2016). Studies have shown that mineral association can, for example, protect ancient biomolecules from degradation when incubated with the strong oxidant sodium hypochlorite (bleach) (Sykes et al., 1995; Salamon et al., 2005). Additionally, kerogen isolated from Cenomanian shale has been observed to undergo rapid oxidation upon release from its protective mineral matrix, degrading its *in-situ* molecular structure (Salmon et al., 2000). Association of biomolecules with mineral matrix may even limit the reactions of this framework, thus facilitating the enhanced preservation of some informative biomolecules. The reactions of this proposed framework, while potentially preservative of soft tissue morphology, are generally degradative of underlying biomolecular structure. The section of this review “Biomolecule and biomarker preservation” discusses this in more detail, including the distinguishment between preservation of soft tissue morphology and of biomolecules themselves.

Permafrost freezing of post-mortem remains is a unique mode of preservation. Rapid freezing of both non-biomineralized and osseous soft tissues (OSTs) arrests or greatly slows molecular diagenesis (Jensen and Sheehan, 2014). Unaltered lipids, proteins, and nucleic acids have been recovered from permafrost frozen specimens (Kreps et al., 1981; Orlando et al., 2013; Kostyukevich et al., 2018; Yamagata et al., 2019). Even original RNA transcripts have been reported (Smith et al., 2019). This indicates that both microbial and chemical degradation of biomolecules in general has been inhibited by the freezing process.

However, the freezing process does form large ice crystals which are destructive to organic tissues (Ferrer et al., 2007; Orlando et al., 2013; Yamagata et al., 2019).

Finally, tissue desiccation removes the presence of available water, without which microbial degradation, and much chemical degradation (e.g. hydrolysis), generally does not occur. This process can result in the preservation of “mummified” tissue remains (Mustoe, 2018; Lennartz et al., 2020). Tissue desiccation is further discussed a bit more in-depth later in this review under the section “Thermally/time-mediated *in-situ* polymerization”. Ultimately, regardless of mechanism, the reaction pathways described within this framework assume that degradative processes were sufficiently arrested to allow for soft tissue preservation.

2.2. Non-polymerizing taphonomic alteration

Post-mortem, biomolecules of soft tissues that are not completely decomposed undergo taphonomic alterations. Taphonomic alterations are herein defined as either being *in-situ* polymerizing (and thus termed *in-situ* polymerization) or as being non-polymerizing (NonP). NonP taphonomic alteration refers to biostratinomic and/or diagenetic chemical modifications to labile biomolecules that do not result in intra- or intermolecular crosslinking. One such example would be cytosine deamination occurring within a strand of DNA. Additionally, compounds within vertebrate tissues may undergo chemical reactions

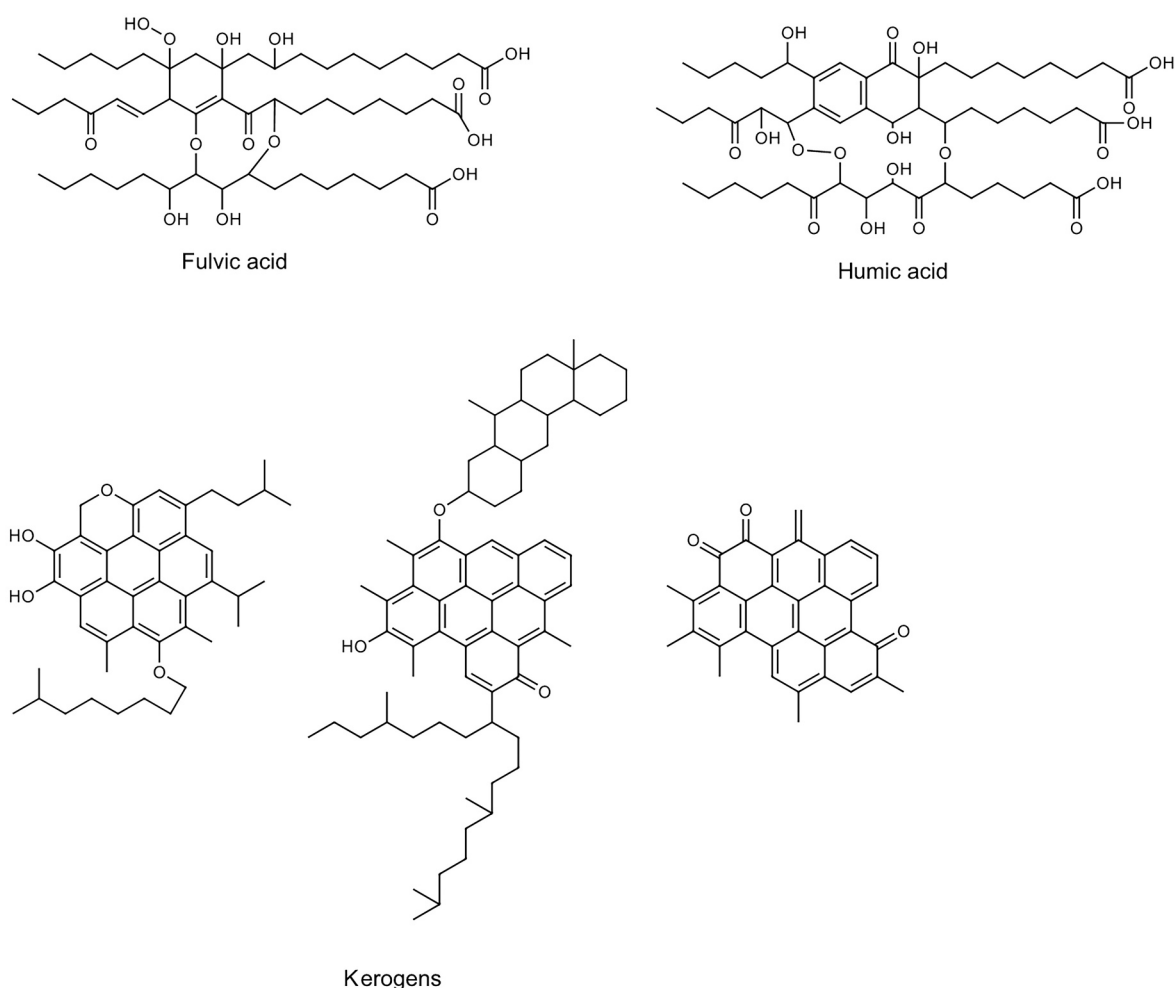


Fig. 2. Potential molecular structures for humics and for various relatively mature kerogens. Humics and kerogens typically consist of high-molecular weight aromatic ring systems functionalized to varying degrees with aliphatics and heteroatoms. The chemical composition of humics typically consists of a higher heteroatom:carbon ratio and possesses less aromaticity than kerogens. There is a broad range of potential molecular structures for humics and kerogens as the reactions that form them are complex and can incorporate a variety of biomolecules (Harvey et al., 1983; Rouzaud et al., 2015). Thus, the structures depicted here are only representative.

towards more stable NonP taphonomically altered forms (e.g. steranes) that are sometimes recoverable and identifiable from fossil specimens of ancient strata (You-Xing et al., 2007; Shinmura and Sawada, 2010); these are generally referred to as biomarkers (Philp and Gilbert, 1987; Farrimond et al., 1998; Toporski and Steele, 2002; Grice and Eiserbeck, 2014; Ferrer et al., 2018; Nguyen et al., 2019).

NonP taphonomic alteration of proteins and nucleic acids in fossil vertebrates is not particularly well studied, especially in specimens of pre-Pleistocene strata, due in part to the assumption that these molecules will not preserve (Evershed et al., 1997; Poinar et al., 1998; Briggs et al., 2000; Simoneit, 2002; Schweitzer et al., 2019). However, amino acid side chains of proteins are known to undergo NonP taphonomic alteration. Deamidation of glutamine and asparagine to glutamic and aspartic acid, respectively, is one such example in proteins (Schiffner, 2011; Cleland et al., 2015a; Cleland et al., 2021). Deamination of cytosine to uracil is an example in nucleic acids (Hofreiter et al., 2001; Lewis et al., 2016). Both proteins and DNA can also readily undergo degradative cleavages (Lindahl, 1993; Jones, 1999; Bada et al., 1999; Poinar and Stankiewicz, 1999; Dabney et al., 2013). Specifically, the peptide bonds of protein chains undergo hydrolytic cleavage (Poinar and Stankiewicz, 1999). For DNA, depurination via hydrolysis of the purine-deoxyribose *N*-glycosyl bond leads to subsequent cleavage of the phosphate-sugar backbone via a β -elimination reaction (Lindahl, 1993; Bada et al., 1999; Dabney et al., 2013).

Lipids also undergo NonP taphonomic alteration processes. For example, fatty acids are rapidly hydrolyzed from the glycerol unit in phospholipid and glyceride diagenesis (Briggs et al., 2000; Kostyukovich et al., 2018; Nolan et al., 2020); the resultant fatty acids can then be

hydroxylated or methylated, among other NonP taphonomic alterations (Stefanova and Disnar, 2000). While NonP taphonomically altered lipids and sterols often serve as biomarkers within sedimentary organic matter (Mackenzie et al., 1981; Mackenzie et al., 1982; Philp and Gilbert, 1987; Briggs and Summons, 2014; O'Reilly et al., 2017), such biomarkers have not often been reported within vertebrates prior to the Neogene (Briggs et al., 2000; O'Reilly et al., 2017). However, in one instance, lipid biomarkers were reported from the uropygial gland of an Eocene bird (O'Reilly et al., 2017).

2.3. *In-situ* polymerization

In-situ polymerization occurs when the labile biomolecules of a soft tissue form intra- and intermolecular crosslink bonds that ultimately result in the formation of HLMs/ITMs (Harvey et al., 1983; Hatcher et al., 1983) that may or may not contain heteroatoms (Stankiewicz et al., 2000; Vandenbroucke and Largeau, 2007; Gupta et al., 2009; Schweitzer et al., 2014; Osés et al., 2017). Within laboratory settings, this process can be initiated by the addition of elevated heat and pressure (Stankiewicz et al., 2000; Gupta et al., 2007d; Vandenbroucke and Largeau, 2007). In geological settings, *in-situ* polymerization has been proposed to proceed at lower temperatures and pressures over longer periods of time (Kirschenbauer, 1960; Harvey et al., 1983; Tissot and Welte, 1984; Stankiewicz et al., 2000; Vandenbroucke and Largeau, 2007) or via other hypothesized pathways that are faster, such as catalysis by redox-active transition metals (Harvey et al., 1983; Schweitzer et al., 2014; Smith et al., 2018; Boatman et al., 2019) and AGE/ALE formation (Collins et al., 1998; Schweitzer et al., 2014;

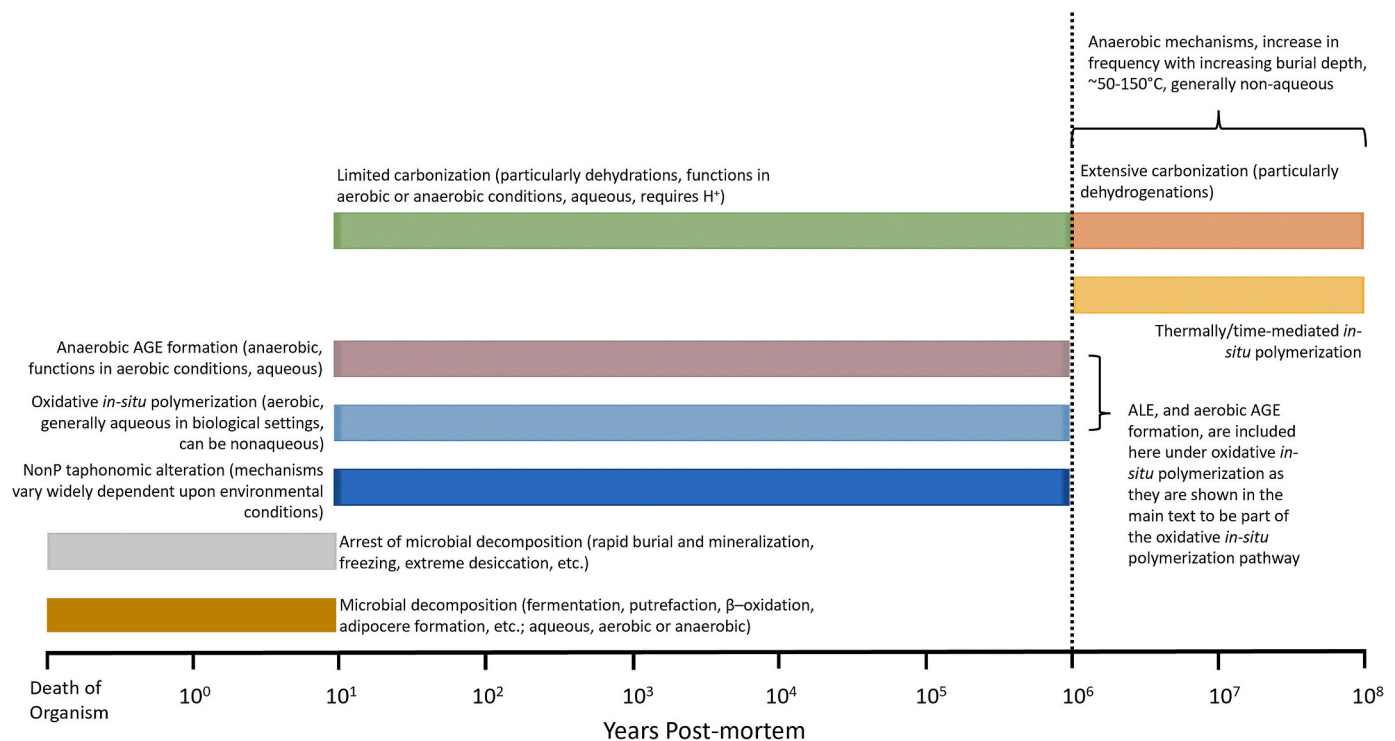


Fig. 3. Proposed chemical framework for vertebrate cell and tissue preservation. Depicted above are approximate/relative timeframes over which the processes for vertebrate soft tissue preservation described within this review are hypothesized to have occurred according to generally accepted geologic settings. The sulfurization process is not shown as evidence supporting its applicability to vertebrate soft tissue preservation is currently limited (McNamara et al., 2016). A caveat, the time frames illustrated for each set of processes are approximations, relative, and can vary substantially depending upon environmental conditions. For example, total carbonization of biological tissues can occur in a matter of minutes or hours if enough heat/pressure is applied (Vandenbroucke and Largeau, 2007; Buseck and Beyssac, 2014). However, such conditions are generally not experienced during fossilization and longer time periods (10^6 – 10^8 years) at lower temperatures (~ 50 – 150 °C) are generally hypothesized to have been typical, as shown in the diagram (Oberlin, 1984; Stankiewicz et al., 2000; Vandenbroucke and Largeau, 2007; Buseck and Beyssac, 2014). Adjustment of any such factors, such as local moisture and oxygen content, as well as heat and pressure, and even the species of dissolved ions present, can significantly accelerate or slow/inhibit the above processes. The dashed line sets an approximate boundary separating diagenetic processes from those that are catagenetic.

Wiemann et al., 2018; Boatman et al., 2019).

The covalent crosslinks formed *via in-situ* polymerization are proposed to preserve overall tissue morphology in a manner analogous to histological tissue fixation. Laboratory tissue fixation induces crosslinks through the reactions of compounds such as glutaraldehyde (Srinivasan et al., 2002; Singh et al., 2019) and osmium tetroxide (Singh et al., 2019) with labile functional groups of endogenous biomolecules. Bonds formed by these compounds reduce the affinity of microbial enzymes for binding the fixed tissue, thus inhibiting its degradation (Singh et al., 2019). The genesis of new covalent crosslinks also increases a tissue's physical rigidity, making it resistant to structural deformation (Talman and Boughner, 1995; Thavarajah et al., 2012). Likewise, *in-situ* polymerization is proposed to crosslink the original biomolecules of ancient remains such that enzymatic degradation is inhibited and overall structural integrity is enhanced.

The following hypothesized pathways of *in-situ* polymerization are by no means mutually exclusive, nor comprehensive. Indeed, these may work in concert to facilitate preservation of fossil soft tissues. Furthermore, the proposed reaction mechanisms result from processes that are not fully replicable in the laboratory. However, they do provide a structured framework, in addition to NonP taphonomic alteration and carbonization, within which the conversion of unaltered biomolecules towards stable polymers within vertebrate soft tissues can be understood and tested.

2.3.1. Oxidative *in-situ* polymerization

Lipids containing fatty acid moieties, including phospholipids and glycerides, are primary components of cellular membranes and the cytoplasm and thus must be accounted for in the preservation of ancient cells and tissues, including those of vertebrates (Alberts et al., 2002; Hulbert et al., 2014; Casares et al., 2019). An autoxidative mechanism was originally proposed and tested by Harvey et al. (1983) to explain the production of marine fulvic and humic acids from microbial phospholipids and glycerides and their eventual conversion to the kerogens of marine shales (Harvey et al., 1983; Harvey and Boran, 1985). This same reaction mechanism should be extendable to vertebrates as phospholipids and glycerides are ubiquitous among eukaryotic cells. Indeed, outside of soil/petroleum science, this autoxidative mechanism, referred

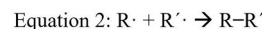
to as lipid peroxidation within biological fields (Vercellotti et al., 1992; Catalá, 2010), is recognized to initiate the oxidative crosslinking of cellular membranes. Lipid peroxidation is a focus of biomedical research as it has been linked to ageing within the human body (Dmitriev and Titov, 2010; Hulbert et al., 2014); it could function *via* similar mechanisms to preserve cellular tissues. Thus, lipid peroxidation is proposed to be a type of oxidative *in-situ* polymerization that may contribute to preservation of soft tissues throughout time; this was first suggested by Schweitzer et al. in 2014 as a part of iron-mediated radical formation (Schweitzer et al., 2014).

Lipid peroxidation begins with the formation of radical intermediates along the hydrocarbon tails of fatty acid moieties (Fig. 4) (Simic et al., 1992; Roberfroid and Calderon, 1995; Min and Ahn, 2005; Catalá, 2010; Domínguez et al., 2019). Under oxidative settings, transition metals, such as Fe²⁺ complexed with molecular oxygen (ferryl and perferryl ions), can directly catalyze the formation of radical intermediates:



where R is the fatty acid moiety and R· is a fatty acid radical intermediate

on fatty acid carbons (Schafer et al., 2000; Min and Ahn, 2005; Repetto et al., 2010). These intermediates can then combine directly with radical intermediates of neighboring fatty acid moieties, or even other biomolecules:



where R· is a fatty acid radical intermediate and R'· is a radical biomolecule

thus inducing crosslinking (Kirschenbauer, 1960; Harvey et al., 1983; Tegelaar et al., 1989; Strlic and Kolar, 2005; Gryn'ova et al., 2011; Smith et al., 2018). These radical intermediates form several orders of magnitude faster on allylic carbons, that is, carbons directly adjacent to an alkene π-bond (for example, C=C-C· is preferred to C-C-C·) (Shahidi and Zhong, 2010; Smith et al., 2018). This is because the electron density of the unstable radical intermediate is delocalized *via*

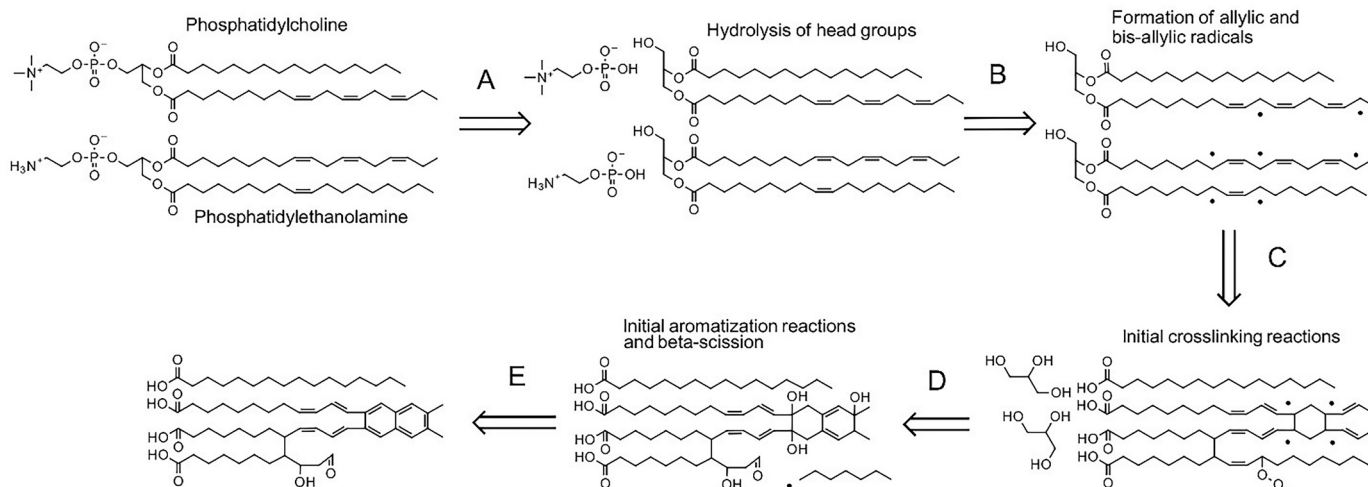
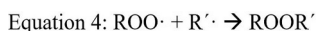
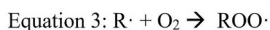
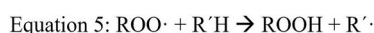


Fig. 4. Representative mechanism proposed for the oxidative *in-situ* polymerization and initial aromatization of phospholipids. The reactions depicted here are representative pathways. There is considerable variation as to how and when each reaction occurs within this sequence. For example, the radicals of the intermediate between (B) and (C) would not likely form all at once, but rather one or two at a time; however, here they are depicted simultaneously for simplicity and clarity. Additionally, an oxidizing environment is assumed. (A) Hydrolysis of the phospholipid head groups occurs early during diagenesis. (B) Radicals form preferentially on allylic and bis-allylic carbons, either through hydrogen atom abstraction or C-H bond scission. Initiation of radical formation can occur via heat/pressure or catalysis via transition metals. (C) Radicals form covalent bonds (crosslinks) with neighboring radicals or react with O₂ to form peroxyl radicals. Glycerol is eventually cleaved from the individual fatty acids. (D-E) Some aromatization begins to occur, possibly through dehydration reactions. Additionally, peroxide functional groups can decompose to alkoxy radicals which undergo β-scission to form aldehydes/ketones and new hydrocarbon radicals.

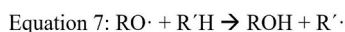
participation in resonance stabilization with the adjacent π -bond (Min and Ahn, 2005; Catalá, 2010; Domínguez et al., 2019). Furthermore, the radical intermediates, instead of directly forming covalent bonds with intermediates of neighboring molecules, can react with O_2 to form peroxy radicals which then proceed to combine with adjacent radical intermediates (Harvey et al., 1983; Gryn'ova et al., 2011; Smith et al., 2018):



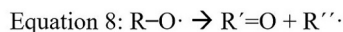
Alternatively, the peroxy radical can abstract a hydrogen atom from a neighboring molecule to form a lipid peroxide (Gardner, 1986; Roberfroid and Calderon, 1995; Gryn'ova et al., 2011; Smith et al., 2018). In the presence of heat and/or transition metals, this lipid peroxide can then decompose to form an alkoxy radical and a new hydroxyl radical (Min and Ahn, 2005; Smith et al., 2018):



this results in rapid propagation of the overall autoxidation mechanism (Shahidi and Zhong, 2010; Smith et al., 2018). The alkoxy radical in particular is significant as it can either abstract a neighboring hydrogen atom to form a hydroxyl group:



or undergo β -scission (Gardner, 1986; Gryn'ova et al., 2011; Smith et al., 2018). The β -scission pathway results in cleavage of the hydrocarbon chain, but importantly it can form aldehyde and ketone products (Nawar, 1984; Gardner, 1986; Vistoli et al., 2013):



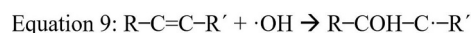
where $R'=O$ and $R''\cdot$ are the cleaved halves of the original R hydrocarbon chain

Such aldehyde and ketone products serve as precursors for ALE genesis via crosslinking with nucleophilic (electron dense) functional groups of proteins, nucleic acids, and phospholipid head groups (Vistoli et al., 2013). Thus, the pathway of oxidative radical formation proposed by Schweitzer et al. (2014) generates the chemical precursors necessary for ALE formation proposed by Wiemann et al. (2018). *ALE formation, and in this case oxidative in-situ polymerization, are subsequent steps of a single, unified reaction mechanism, and should not be viewed as competing hypotheses for soft tissue preservation.* Likewise, in some cases, the carbonyl precursors necessary for AGE formation arise via autoxidation of reducing sugars, such as glucose and fructose (Fig. 5(A)) (Wolff and Dean, 1987). This is another case in which oxidative radical formation is directly linked with AGE formation and in which the papers by Schweitzer et al. (2014) and Wiemann et al. (2018) form a single, unified hypothesis for soft tissue and biomolecule preservation through deep time.

In addition to lipids, proteins and nucleic acids are found throughout biological tissues of all taxonomic groups (Alberts, 1998; Alberts et al., 2002), and it is herein assumed this holds for prehistoric organisms (Cappellini et al., 2012; Orlando et al., 2013; Rybczynski et al., 2013). Attempts to recover protein-related data, and even that of DNA (Schweitzer et al., 2013; Bailleul et al., 2020), from pre-Pleistocene specimens have received interest within the field of molecular palaeontology (Asara et al., 2007; Schweitzer et al., 2009; Rybczynski et al.,

2013; Cleland et al., 2015b; Surmik et al., 2016; Lee et al., 2017; Schroeter et al., 2017; Lindgren et al., 2018). The potential preservation of such proteins has been hypothesized to occur via transition metal-mediated oxidative *in-situ* polymerization (radical formation). *In-situ* polymerization of proteins and nucleic acids via the formation of radical intermediates is well known to occur within biological systems exposed to transition metal catalysts (Roberfroid and Calderon, 1995). Additionally, substantial amounts of goethite have been reported associated with the OSTs of ancient vertebrates (Kaye et al., 2008; Schweitzer et al., 2014; Cadena, 2016; Surmik et al., 2016; Boatman et al., 2019; Cadena, 2020). This supports the hypothesis that at some point post-mortem the endogenous tissues encountered redox-active iron which could have functioned to catalyze *in-situ* polymerization.

Regarding the oxidative crosslinking of proteins and nucleic acids, transition metal catalyzed radical formation proposes that redox-active transition metals catalyze the breakdown of peroxide groups to free hydroxyl radicals ($\cdot OH$) (Schweitzer et al., 2014; Boatman et al., 2019); these peroxide groups can arise from free hydrogen peroxide (H_2O_2) (Gardner, 1986; Simic et al., 1992; Roberfroid and Calderon, 1995; Winterbourn, 1995; Catalá, 2010; Repetto et al., 2010), such as may be present in an organism immediately post-mortem, or a previous autoxidation pathway, such as with lipid peroxidation (Eq. 6) (Simic et al., 1992; Min and Ahn, 2005; Smith et al., 2018; Domínguez et al., 2019). The free hydroxyl radical, being a highly reactive species, can quickly abstract a hydrogen atom from a peptide/protein or nucleic acid and form a radical on the biomolecule itself (see Eq. 1 regarding lipid peroxidation) (Roberfroid and Calderon, 1995; Kehrer, 2000). Alternatively, the hydroxyl radical can bond covalently to an alkene bond via an addition reaction (Anbar et al., 1966; Roberfroid and Calderon, 1995):

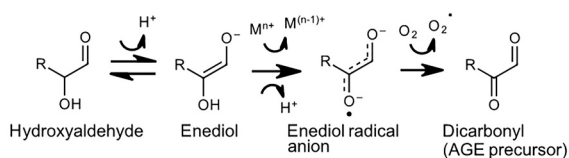


Hydroxyl radicals typically react via addition reactions with tyrosine, cysteine, phenylalanine, histidine, tryptophan, and methionine. In contrast, hydrogen atom abstraction is favorable at carbons directly adjacent to hydroxyl or amine functional groups such as those of serine, threonine, asparagine, glutamine, aspartic acid, and glutamic acid, as well as the guanidine group of arginine. For both addition and hydrogen atom abstraction, these differences are primarily due to the potential for resonance stabilization of the radical intermediate (Anbar et al., 1966; Roberfroid and Calderon, 1995; Hawkins and Davies, 2001). Newly formed radical amino acid moieties can covalently bond through one of several mechanisms with neighboring biomolecules to form intermolecular crosslinks (Eq. 2), or preferentially react with O_2 to form peroxide functional groups (Eq. 3, Eq. 5) (Hawkins and Davies, 2001). Examples of both hydrogen abstraction and an addition reaction facilitating protein crosslinking are depicted in Fig. 5(B). Further, nucleic acids, specifically DNA, also readily undergo reactions with free hydroxyl ions to incur oxidative damage. Many such reactions result in inter-strand crosslinking (Fig. 5(C)) as well the formation of crosslinks between DNA and proteins (Dizdaroglu et al., 1989; Nackerdien et al., 1991; Roberfroid and Calderon, 1995; Hong et al., 2006).

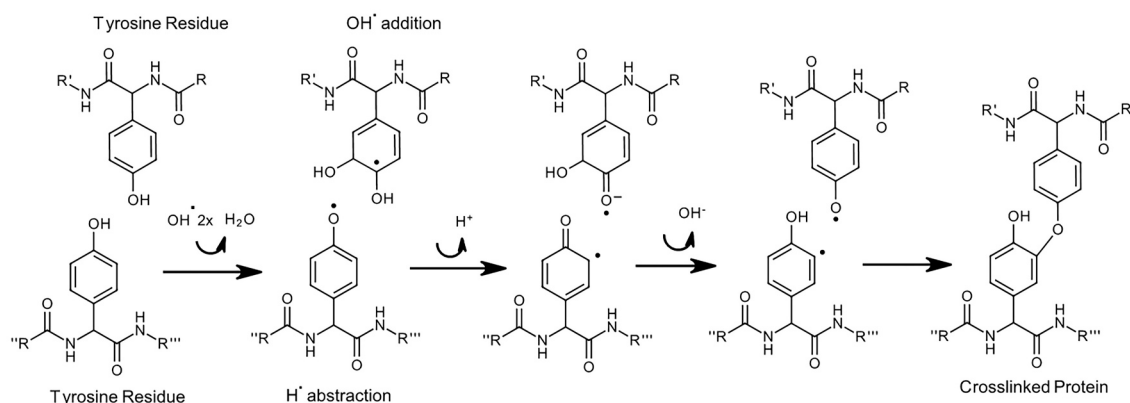
2.3.2. Anaerobic AGE formation

In addition to the mechanisms of radical crosslinking (oxidative *in-situ* polymerization) outlined above, a second proposed hypothesis explaining the preservation of fossil vertebrate soft tissues is the formation of AGEs and ALEs from individual biomolecules (Collins et al., 1998; Poinar et al., 1998; Poinar and Stankiewicz, 1999; Schweitzer et al., 2014; Wiemann et al., 2018). Specifically, lipids and reducing sugars are oxidized to reactive carbonyl substrates with which nucleophilic groups of proteins and nucleic acids can irreversibly condense. The end products of such reactions are large macromolecules termed melanoidins (Vistoli et al., 2013) which would be analogous to HLMs/ITMs.

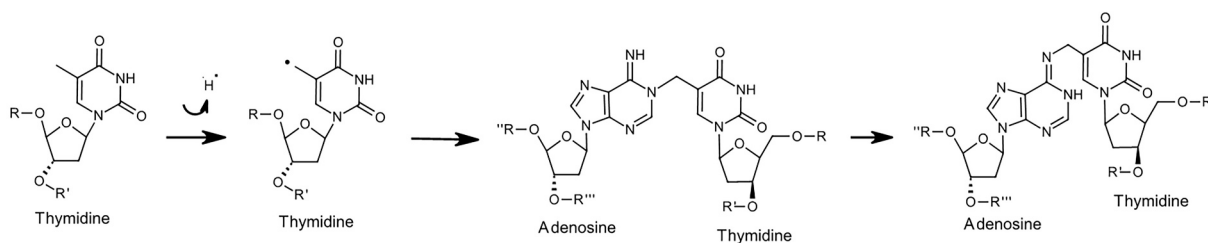
A.



B.



C.



D.

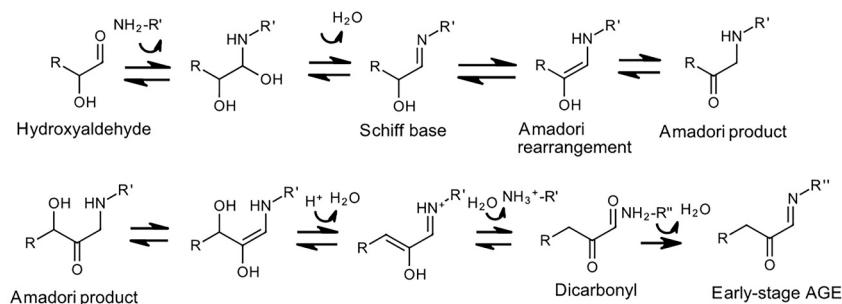


Fig. 5. Representative *in-situ* polymerization mechanisms for proteins and nucleic acids. The crosslinking mechanisms depicted here are only representative, many other mechanisms are possible depending on the reactants and environmental conditions. Additionally, the mechanisms only show the initial crosslinking and not further chemical transformation of the polymerized biomolecules. (A) The α -hydroxyaldehyde functional group of a linear reducing sugar can undergo a reversible transition to an enediol form. Then, transition metal (M^{n+}) catalyzed oxidation converts the enediol to a radical intermediate. Finally, transfer of the radical electron from the enediol to free oxygen forms an α -ketoaldehyde (dicarbonyl) functional group that can serve as a precursor for AGE formation (Wolf and Dean, 1987). (B) Two tyrosine residues are depicted undergoing crosslinking via formation of radical intermediates. One residue undergoes hydrogen atom abstraction at the side chain hydroxyl group while the other undergoes an addition reaction to the side chain aromatic ring (Hawkins and Davies, 2001). (C) Crosslinking reaction between inter-strand thymidine and adenosine nucleotides (Hong et al., 2006). (D) Formation of an early-stage AGE at the hydroxyaldehyde group of D -glucose. Only one potential pathway is shown after formation of the Amadori product, but multiple others have been well characterized and are dependent on environmental conditions, particularly pH. Additionally, the early-stage AGE, once formed, can then undergo a broad range of reactions dependent on local reactants and conditions (Vistoli et al., 2013).

As discussed under the oxidative *in-situ* polymerization section, precursors for ALEs (Gardner, 1986; Gryn'ova et al., 2011; Smith et al., 2018), and some AGEs (Wolff and Dean, 1987), form directly from oxidative *in-situ* polymerization. Thus, this review considers ALE and aerobic AGE formation to be equivalent pathways to oxidative *in-situ* polymerization. AGEs, however, can also form *via* a separate anaerobic mechanism independent of oxidative *in-situ* polymerization through the conversion of reducing sugars into dicarbonyls (Fig. 4(D)). In this pathway, a primary amine reacts with the carbonyl group of the aldehyde or ketone of a reducing sugar to form a Schiff base (imine). The imine product then undergoes an Amadori rearrangement, and subsequent hydrolysis converts the imine back to a carbonyl group and the original primary amine. The original reducing sugar is now a reactive dicarbonyl species capable of irreversibly condensing with nucleophilic groups of proteins, nucleic acids, etc. and thus crosslinking them (Vistoli et al., 2013). Other anaerobic mechanisms can lead to the formation of carbonyl precursors for AGEs as well (Hayashi and Namiki, 1981; Namiki and Hayashi, 1983; Thornalley et al., 1999; Manini et al., 2006; Vistoli et al., 2013), including one involving retroaldol fragmentation of reducing sugars, such as glucose (Thornalley et al., 1999; Manini et al., 2006). The review by Vistoli et al. (2013) provides a more in-depth overview of such mechanisms (Vistoli et al., 2013).

Within biomedical research, AGE formation, as well as lipid peroxidation (including ALE formation), has been linked to the ageing of biological tissues. As tissues age, their proteins undergo crosslinking to form AGEs and ALEs. Such modifications inhibit protein functionality and are involved in various disease pathologies (Tessier, 2010; Nagaraj et al., 2012; Gill et al., 2019; Moldogazieva et al., 2019). Additionally, Maillard (Maillard, 1912a; Maillard, 1912b; Maillard, 1916) hypothesized that AGEs were a source for soil humus (immature kerogens) and that these served as precursors for the formation of mature kerogens (Tissot and Welte, 1984; Vandenbroucke and Largeau, 2007).

Evidence of AGEs and ALEs have been noted within prehistoric biological tissues (Evershed et al., 1997; Poinar et al., 1998; Cleland et al., 2015a; Wiemann et al., 2018; Wiemann et al., 2020). Studies of Egyptian plant specimens dated to 1500 B.C.E. have demonstrated AGE/ALE compounds and suggested these play a role in the preservation of biological tissues (Evershed et al., 1997). Data obtained *via* Raman spectroscopy has been used to support that OSTs of Mesozoic organisms are preserved in this manner (Wiemann et al., 2018; Wiemann et al., 2020). However, in a recent publication, the signal detected within some of these Raman spectra was suggested to be the result of instrumental artefacts; thus further experimentation with more diverse and appropriate methodologies is necessitated (Alleon et al., 2021). Additionally, this Maillard reaction pathway has, in at least one case, been observed as the initial mechanism for *in-situ* polymerization of DNA. Specifically, a coprolite recovered from a late Pleistocene cave deposit was tested for the preservation of endogenous DNA. Initial analyses yielded no DNA amplification; however, after incubation of extracts with N-phenacylthiazolium bromide, a chemical known to cleave glucose-derived Maillard product bonds (Dabney et al., 2013), DNA amplification was achieved (Poinar et al., 1998). Therefore, as biological tissues age post-mortem, assuming they have been stabilized against microbial degradation, their constituent biomolecules are hypothesized to undergo similar crosslinking towards AGEs and ALEs.

As a final point of clarification, Schweitzer et al. (2014) and Wiemann et al. (2018) can be considered equivalent reaction mechanisms *only* when the reactive carbonyl substrates for AGE/ALE formation are generated *via* oxidatively formed radicals. Reactive carbonyl precursors to ALEs generally form *via* lipid peroxidation (Vistoli et al., 2013), thus ALE formation is generally an example in which the hypotheses of Schweitzer et al. (2014) and Wiemann et al. (2018) form a unified reaction mechanism. However, AGE precursors (and the resultant crosslinks) can form *via* non-oxidative, radical-independent mechanisms, such as with the Maillard reaction pathway (Fig. 5(D)), or even through the independent fragmentation of reducing sugars such as glucose

(Thornalley et al., 1999; Manini et al., 2006). In such cases, the hypothesis proposed by Wiemann et al. (2018) is *not* equivalent to that of Schweitzer et al. (2014) and is a standalone reaction mechanism. Likewise, Schweitzer et al. (2014) is a standalone hypothesis when radicals formed during oxidative *in-situ* polymerization do not yield products that serve as precursors to AGEs/ALEs. The direct crosslinking of two amino acid residues, such as in Fig. 5(B), is an example of this.

2.3.3. Thermally/time mediated *in-situ* polymerization

Continued deposition of overlying sedimentary strata exposes an organism's remains to increasing levels of heat and pressure, and the burial environment becomes increasingly depleted of oxygen (Tissot and Welte, 1984; Vandenbroucke and Largeau, 2007). While this depletion of oxygen inhibits oxidative *in-situ* polymerization, the elevated heat and pressure eventually initiates hydrogen atom abstraction, or potentially even bond scission, and thus radical formation on a given biomolecule:



where R is the biomolecule

These intermediates can then combine directly with radical intermediates of neighboring biomolecules (Eq. 2), thus inducing crosslinking (Fig. 6) (Kirschenbauer, 1960; Harvey et al., 1983; Tegelaar et al., 1989; Strlic and Kolar, 2005; Gupta et al., 2007b; Gryn'ova et al., 2011; Smith et al., 2018). This pathway is slower relative to oxidative *in-situ* polymerization, partly because it lacks propagation *via* hydroxyl radical formation (Eq. 6; the cleavage of the peroxide group generates multiple new radicals), and radicals must generally combine directly with one another (Eq. 2) (Hawkins and Davies, 2001; Gupta et al., 2007b; Smith et al., 2018).

Experimental evidence shows that thermally-mediated radical formation results in the crosslinking of lipids (Nawar, 1978; Harvey et al., 1983; Nawar, 1984; Harvey and Boran, 1985; Gupta et al., 2007b; Tian, 2013); this likely can occur for other biomolecules as well (Tian, 2013) although it is poorly studied. In laboratory settings, thermally mediated radical crosslinking occurs with increasing rate up to a temperature of ~470–500 °C (Harvey et al., 1983; Tissot and Welte, 1984; Harvey and Boran, 1985; Gupta et al., 2007b; Vandenbroucke and Largeau, 2007). However, in geological conditions, such temperatures generally are not encountered; rather, these radical forming reactions have been hypothesized to have occurred over longer timescales of a minimum of ~10⁶ years and at temperatures up to ~150 °C (Harvey et al., 1983; Harvey and Boran, 1985; Vandenbroucke and Largeau, 2007; Gupta, 2015). Under such conditions, these reactions would have been slow, time-dependent processes requiring an initial stabilization of soft tissues against degradation to have taken place.

The oxidative *in-situ* polymerization and anaerobic AGE crosslinking pathways have been hypothesized to help stabilize vertebrate soft tissues against degradation within a relatively short time interval (~10¹–10⁴ years) post-mortem under oxidative conditions (Schweitzer et al., 2014; Wiemann et al., 2018; Boatman et al., 2019). Such stabilization would have given a potentially slower heat and/or time-dependent chemical mechanism a chance to proceed. Therefore, oxidizing conditions would be hypothesized to promote *in-situ* polymerization and thus soft tissue preservation, as has been suggested in prior studies (Schweitzer et al., 2014; Wiemann et al., 2018; Muscente et al., 2019; Wiemann et al., 2020). For organisms initially buried under conditions of anoxia, some mechanism not reliant on oxidative conditions would have been necessary to stabilize soft tissues and allow a slow thermally/time mediated mechanism of *in-situ* polymerization a chance to occur under the assumed geological conditions. AGE formation can function under anoxic conditions *via* the Maillard reaction mechanism to crosslink proteins, nucleic acids, and phospholipid head groups with carbohydrate monomers (Vistoli et al., 2013). Combined with the

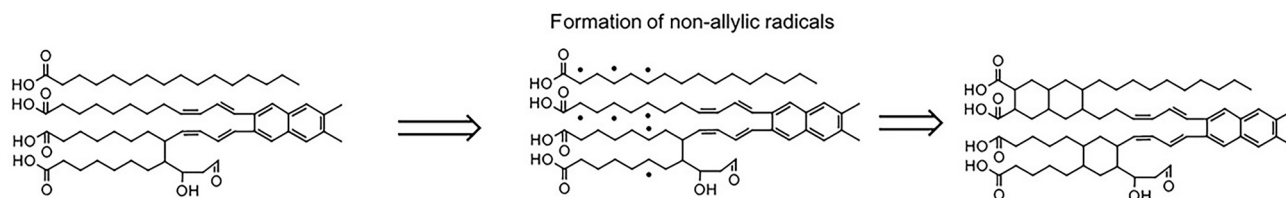


Fig. 6. Thermally/time mediated crosslinking of HLM/ITM. As time goes on and/or pressure and temperature increases with burial depth, non-allylic carbons can also form radicals and undergo thermally/time mediated crosslinking. This further crosslinks the original fatty acid chains. Environmental oxygen levels decrease as burial depth increases, limiting the potential for *in-situ* polymerization via oxidative reactions.

generally recalcitrant nature of lipids (Briggs et al., 2000; Schweitzer, 2011), this could stabilize soft tissues to some extent under anoxic conditions and allow thermally/time mediated *in-situ* polymerization to then take place. This would potentially account for reports of soft tissues from vertebrate specimens of anoxic, marine settings (Lindgren et al., 2011; Lindgren et al., 2017; Lindgren et al., 2018; Boskovic et al., 2021; Voegelé et al., 2022). However, further exploration of the nature of such a mechanism is outside the scope of this paper. Alternatively, anoxic depositional environments enriched in reduced sulfur could also initiate rapid *in-situ* polymerization of soft tissues and biomolecules. Specifically, hydrogen sulfide ions can function *via* addition reactions at ketones and alkene groups to form intra- and intermolecular crosslinks (Adam et al., 2000; Damsté et al., 2007). Such a mechanism, termed sulfurization, is well established to occur within sedimentary biomarkers (Koopmans et al., 1997; Adam et al., 2000; Damsté et al., 2007; McNamara et al., 2016), but evidence as to its significance within vertebrate soft tissues is limited (McNamara et al., 2016) and thus it is not further discussed herein.

Finally, complete desiccation of biological tissues shortly post-mortem would likely preserve them to the point that thermally/time-mediated *in-situ* polymerization could occur. Absent an aqueous environment, microbially-mediated enzymatic degradation, as well as degradative chemical reactions such as hydrolysis, cannot occur, and the result is the mummification of biological tissues (Lennartz et al., 2020). Tissues preserved *via* such a mechanism are known to persist indefinitely (Mustoe, 2018; Lennartz et al., 2020) even if initial oxidative *in-situ* polymerization and anaerobic AGE crosslinking reactions are limited. The addition of elevated temperature and pressure with increasing burial depth over time would then cause such preserved tissues to undergo thermally/time-mediated *in-situ* polymerization.

2.4. Carbonization

A chemical process often referred to as carbonization can occur to a limited extent alongside *in-situ* polymerization and contributes to the formation of HLMs/ITMs from labile biomolecules, in addition to crosslinking reactions (Harvey et al., 1983; Harvey and Boran, 1985). However, at higher temperatures and pressures and/or over longer timespans, carbonization-type reactions take place more extensively (Rouzaud et al., 2015). Extensive carbonization-type reactions within an HLM/ITM proceed beyond *in-situ* polymerization and result in the

conversion of the HLM/ITM to a KLM/MTM (Fig. 7). However, the exact point at which an HLM/ITM transitions to a KLM/MTM (or even at which humics are considered kerogens) on a structural/molecular basis is not well defined within the primary literature outside of operational definitions based on solubility (Vandenbroucke and Largeau, 2007).

During carbonization, the HLMs/ITMs of preserved cells and tissues undergo extensive conversion of aliphatic sp^3 orbitals to lower energy/more stable sp^2 orbitals of aromatic alkenes (Wei et al., 2005; Rouzaud et al., 2015; Agrawal and Sharma, 2018). Additionally, heteroatoms are removed from the polymer structure, resulting in a disorganized system of interconnected aromatic rings (polyaromatic hydrocarbons, *i.e.*, KLMs/MTMs) (Oberlin, 1984; Wei et al., 2005; Briggs and Summons, 2014; Rouzaud et al., 2015; Agrawal and Sharma, 2018), thus reducing structural variation within the macromolecules. The sp^2 orbitals of the carbon-carbon bonds within the aromatics are further stabilized by resonance of the conjugated π -bond system, resulting in electrochemical stability and low reactivity (Harvey, 1997; Lawal, 2017).

The chemical changes resulting in aromatization are likely due in part to elimination reactions, especially dehydrations (Bockisch et al., 2018) and dehydrogenations (Schimmel et al., 1999; Wei et al., 2005; Wang and Zhao, 2020). Dehydration favorability begins at lower temperatures and pressures, approximately 150 °C under experimental settings (Bockisch et al., 2018), and likely below ~50 °C under predicted geological conditions (Harvey et al., 1983; Harvey and Boran, 1985; Gupta, 2015). Dehydrogenations, in contrast, would be associated more closely with the onset of catagenesis (Schimmel et al., 1999; Wei et al., 2005; Gupta, 2015; Wang and Zhao, 2020). Under geologic settings, temperature is hypothesized to be the primary factor influencing the onset and rate of catagenesis (Stanov, 1981; Vandenbroucke and Largeau, 2007). Pressure during geologic catagenesis is hypothesized to fall within the range of ~300–800 bar (Stankiewicz et al., 2000; Vandenbroucke and Largeau, 2007), but currently this is not well studied.

This overall process of converting large, complex *in-situ* polymerization products, termed HLMs/ITMs, to aliphatic and aromatic macromolecules, termed KLMs/MTMs, occurs on a gradient, or maturity level, generally determined by applied heat and pressure (Hatcher et al., 1983; Oberlin, 1984; Stankiewicz et al., 2000; Rouzaud et al., 2015). Under laboratory conditions, one study reported that by 260 °C, many protein-related functional groups had been removed from kerogens and KLMs. Up to at least a temperature of 350 °C, the macromolecules still maintain a detectable aliphatic content (Stankiewicz et al., 2000). Other studies

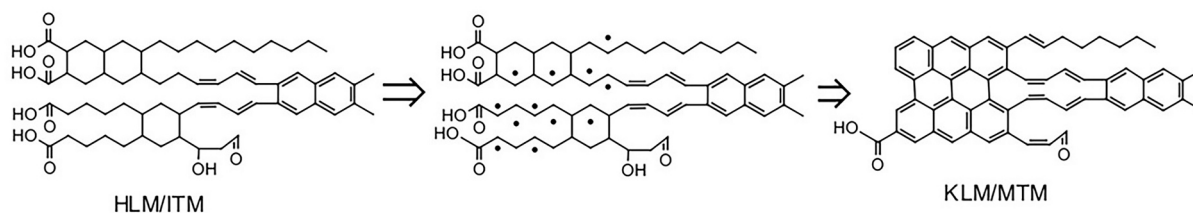


Fig. 7. Maturation via carbonization towards a KLM/MTM. Shown is a potential carbonization pathway for the product HLM/ITM of Fig. 6. Crosslinking via non-allylic radical intermediates continues under heat/pressure and anoxic conditions. Dehydrogenation reactions drive aromatization, and heteroatoms are removed from the structure; these reactions result in a KLM/MTM.

have likewise supported that the conversion of aliphatics to aromatics within kerogens and KLMS occurs gradually as temperatures increase from ~150 °C to ~400 °C (Ishiwatari and Machihara, 1982; Wei et al., 2005). When a temperature of ~435 °C is obtained, catagenesis begins in which the process of “cracking” releases some relatively small aliphatics and aromatics from the bound portions of kerogens and KLMS (Tissot and Welte, 1984; Vandenbroucke and Largeau, 2007; Buseck and Beyssac, 2014). These released molecules form the basis for oil and natural gas in geological sediments. In contrast, under geological conditions, catagenesis is hypothesized to have occurred between ~50–150 °C over an approximate timeframe of 10^6 – 10^8 years (Tissot and Welte, 1984; Vandenbroucke and Largeau, 2007; Gupta, 2015). At higher temperatures, up to 3000 °C for experimental conditions, kerogens and KLMS convert to highly aromatic structures with some potential small, interspersed aliphatic chains. Beyond 3000 °C, the carbon of mature kerogens and KLMS crystallizes through the process of graphitization. Exposure to high pressure can reduce the temperature threshold for graphitization, to 1000 °C (Vandenbroucke and Largeau, 2007; Buseck and Beyssac, 2014; Rouzaud et al., 2015). However, for fossil vertebrates of sedimentary strata, temperatures are generally hypothesized not to have exceeded 150 °C (Tissot and Welte, 1984; Vandenbroucke and Largeau, 2007) and graphitization generally need not be considered.

As described earlier in this paper, the many crosslinks that form during the *in-situ* polymerization process are proposed to “rigidify” a tissue and increase its structural integrity and resistance to physical deformation. This is akin to what occurs during tissue fixation with, for example, glutaraldehyde. The formed crosslinks “combine” multiple biomolecules into larger macromolecules, making chemical structure more difficult to displace (Talman and Boughner, 1995; Thavarajah et al., 2012; Costa, 2018; Singh et al., 2019). The process of carbonization can be, however, antagonistic to the stiffening of tissue that occurs from crosslinking. Both *in-situ* polymerization and carbonization can preserve soft tissue morphology to an extent, but carbonization can weaken the tissue's overall structural integrity. Examples of this are presented in the introduction of Petrenko et al. (2019). Post-carbonization, many biomaterials, including silk threads and plant xylem, despite maintaining their overall morphology and possessing increased chemical stability, lose structural integrity such that they collapse into powder upon handling (Petrenko et al., 2019). This is akin to the brittleness acquired by wood upon coalification (Mustoe, 2018).

The high carbon content observed in some fossil feathers, skin, and amber (Davis and Briggs, 1995; Ji et al., 2002; Mayr et al., 2002; Ji et al., 2006; Greenwalt et al., 2013; Moyer et al., 2014; Calede et al., 2018; Xing et al., 2020) using energy dispersive X-ray spectroscopy (EDS) analyses (Davis and Briggs, 1995; Vinther et al., 2008; Vinther et al., 2010) likely results from carbonization-type reactions. However, EDS is only capable of detecting the presence of elements down to a concentration of ~1000 ppm (0.1%) (Ngo, 1999; Nasrazadani and Hassani, 2016). Thus, EDS alone is not capable of distinguishing whether such films are truly carbonized, and thus considered KLMS/MTMS. As a result, the molecular composition of these carbonized remains is poorly explored for fossil vertebrate soft tissues. For example, examination of feathers purported to preserve as carbonized film revealed ultrastructure consistent with keratin protein and melanosomes (Moyer et al., 2014), suggesting total carbonization had not yet occurred. However, the actual molecular composition of such carbonized films, at least in fossil vertebrates, has not been fully explored.

The conversion of tissue biomolecules into ITMS and eventually MTMS has been examined widely within fossil invertebrates (Stankiewicz et al., 1997; Gupta et al., 2007c; Gupta et al., 2008; Gupta et al., 2009) and plants (Gupta et al., 2007a; Gupta et al., 2007b; Gupta et al., 2007d; You-Xing et al., 2007; Mustoe, 2018) but less so in fossil vertebrates (Manning et al., 2009; Gupta, 2014; Thiel et al., 2014; O'Reilly et al., 2017; Lindgren et al., 2018). One recent exception to this is an exceptionally preserved Jurassic ichthyosaur from the Holzmaden area

of Germany (Lindgren et al., 2018). Authigenic mineralization *via* phosphatization was reported in epidermis recovered from the specimen. However, demineralization and subsequent analysis with mass spectrometry revealed epidermal soft tissues consisting of high molecular weight aliphatic, and particularly, aromatic hydrocarbons; this is consistent with a preserved soft tissue structure/composition intermediate to ITMS and MTMS (Lindgren et al., 2018). Such macromolecules composing the ichthyosaur epidermal structure would be expected based on the framework mechanisms described for *in-situ* polymerization and some extent of carbonization-type reactions. This is consistent with previous hypotheses of low preservation potential of labile lipids common within organismal tissues, specifically the unaltered fatty acid moieties of phospholipids, and supports that such molecules are chemically transformed *via in-situ* polymerization in ancient vertebrates (Harvey et al., 1983; Briggs, 1999; O'Reilly et al., 2017; Lindgren et al., 2018).

Further, in a recent study on Oligocene whale bone of the El Cien Formation of Mexico (Thiel et al., 2014), extracts of ground bone were acquired and analyzed for their bound vs unbound fatty acid content. Substantially fewer fatty acids were recovered in the fossil whale bone than in the extant comparison. However, of those in a recoverable condition, anywhere from 21% to 70% (dependent on the specific fatty acid) were found to have undergone *in-situ* polymerization (with the rest preserved in an unbound, or labile, state), thus further supporting the occurrence of *in-situ* polymerization within ancient vertebrate tissues. With more time and/or elevated burial temperature and pressure, these remnant fatty acids would be hypothesized to increasingly skew in proportion towards the bound state. The authors of the study did add a caveat, however, that a microbial source for the fatty acids could not be ruled out with the data collected therein (Thiel et al., 2014). Furthermore, because bound *in-situ* polymerization products generally transition from aliphatic to aromatic in character as they mature *via* the process of carbonization (Oberlin, 1984; Wei et al., 2005; Rouzaud et al., 2015; Delarue et al., 2016; Agrawal and Sharma, 2018), these bound fatty acid moieties (of the El Cien whale) would be hypothesized to, over time, undergo transformation towards polyaromatic hydrocarbons, leading to a molecular composition closer to that of the Holzmaden ichthyosaur.

While further testing on a broader range of samples is necessary, these data and reasoning suggest that ITMS/MTMS could potentially serve a role as proxies of diagenetic history within ancient vertebrates. As the conversion of biomolecules into ITMS, and subsequently MTMS, proceeds, the quantity and quality of biomolecule preservation (dependent on type of biomolecule) is hypothesized to decrease. Molecular methods can quantify the extent to which original biomolecules have undergone *in-situ* polymerization and carbonization. This is similar to the fields of soil and petroleum science using sediment maturity, which is essentially the degree of *in-situ* polymerization and carbonization that has occurred within sedimentary organics, as a marker for the quality of oil and/or natural gas present at a given locality within a geologic formation (Thiel and Sjövall, 2011; Delarue et al., 2016; Ferralis et al., 2016; Schito et al., 2017; Lindgren et al., 2018; Sjövall et al., 2021). With fossil vertebrates however, instead of sediment maturity, soft tissue “maturity” can be measured and then correlated to quality or degree of biomolecule preservation. Establishing such correlations across a range of specimens spanning the fossil record would form the foundations of a chemical index with ITMS and MTMS as proxies of biomolecular preservation. Such an index could in turn be used to screen future specimens, from the Holocene to the Mesozoic, according to biomolecule/biomarker preservation potential, including for DNA and protein sequence information. Please see Anderson (2022) for an in-depth discussion of this potential future research direction (Anderson, 2022).

3. Biomolecule and biomarker preservation

The reactions described within this chemical framework, while potentially preservative for soft tissue morphology, are generally degradative of its constituent biomolecules. This is analogous to histological tissue fixation with chemicals such as glutaraldehyde (Srinivasan et al., 2002; Singh et al., 2019) or osmium tetroxide (Singh et al., 2019). Chemical fixation, depending on the fixative used, crosslinks tissue proteins, nucleic acids, and lipids, altering their chemical structure such that they are not amenable to degradation by most enzymes, including those of exogenous microbes (Singh et al., 2019). This preserves tissue morphology, but the alteration of chemical structure inherently degrades the crosslinked biomolecules. For example, histological fixation is known to inhibit DNA sequence recovery from fixed tissues (Srinivasan et al., 2002). Similarly, the chemical reactions presented within this chemical framework, while potentially preservative of soft tissue morphology, are predicted to gradually degrade soft tissue constituent biomolecules over time.

This raises the question of how reported biomolecule and biomarker preservation within early and pre-Pleistocene vertebrate remains is explained in context of this chemical framework. Preservation of sequence-able DNA has generally not been reported beyond the range of ~130–240 Ka outside of continuously frozen permafrost sediments and a few instances of cave deposits (see Figure 1 of Welker et al., 2019; Mitchell and Rawlence, 2021). This is consistent with the degradative nature of the chemistry described within this framework. Nucleic acids are hypothesized to be labile biomolecules (Lindahl, 1993; Briggs et al., 2000) and thus far have not been observed to persist deep into the fossil record, at least not in a sequence-able form.

For proteins, almost all sequences reported from early and pre-Pleistocene specimens originate from robust structural proteins, primarily type-1 collagen. Type-1 collagen, the biomineralized collagen of bone, consists of 3 tightly intertwined helical peptide chains. A repeating sequence of X-Y-Gly aids in forming tight helical turns (where the X and Y positions can be any amino acid but are often proline and hydroxyproline; Gly corresponds to glycine) (Lodish et al., 2000; Jenkins et al., 2003). Lysine residues in the collagen helix often undergo post-translational hydroxylation allowing the formation of inter- and intramolecular crosslinks between collagen fibrils, contributing to the stable nature of structures comprised of this protein (Yamauchi and Sricholpech, 2012). The stability of these crosslinked helices is such that spongin fibrils (the primary collagen of sponge “skeletons”) have been demonstrated to preserve structural integrity and nano-scale morphology even after carbonization at temperatures of 1200 °C (even if the fibrils are no longer recognizable chemically) (Petrenko et al., 2019). Biomineralization of this tightly interconnected matrix with (primarily) bioapatite (Ca₅(PO₄)₃(OH)) further enhances its structural stability and durability (Miles and Ghelashvili, 1999; Collins et al., 2000; Schweitzer et al., 2008; Wysokowski et al., 2020a; Wysokowski et al., 2020b).

These chemical features lead to high preservation potential for type-1 collagen which, when combined with *in-situ* polymerization, is hypothesized to contribute to the protein's preservation through deep time. Small segments of the collagen peptides that avoided transformation *via in-situ* polymerization (possibly within protected regions of the collagen molecule (San Antonio et al., 2008; Schweitzer et al., 2019)) may explain reported collagen sequences from Pliocene (Rybczynski et al., 2013; Buckley et al., 2019) and Mesozoic (Asara et al., 2007; San Antonio et al., 2008; Schweitzer et al., 2009; Schroeter et al., 2017) specimens. The close association of such sequences with precipitated mineral (whether endogenous or otherwise) would serve to stabilize them against chemical degradation, including *in-situ* polymerization, as proposed by multiple studies (Keil et al., 1994; Salmon et al., 2000; Salamon et al., 2005; Demarchi et al., 2016). The *in-situ* polymerization of surrounding biomolecules within the tissue (in regions more prone to *in-situ* polymerization) would further protect them by sterically

hindering access to microbes/microbial enzymes (Grabber et al., 1998; Wiemann et al., 2018; Singh et al., 2019).

Furthermore, sequences for proteins other than collagens have been reported from early (Cappellini et al., 2019; Welker et al., 2019; Welker et al., 2020) and pre-Pleistocene (Cleland et al., 2015b; Demarchi et al., 2016; Demarchi et al., 2022) specimens, but in almost all cases the proteins are likewise of a robust, structural nature and/or intimately associated with a highly mineralized matrix. Demarchi et al. (2016) recently reported the preferential preservation of aspartic acid mineral binding domains within the non-collagenous proteins Struthioalcalin-1 and Struthioalcalin-2 of Pliocene *S. camelus* eggshells. The aspartic acid-rich peptide -YSALDDDDYPKG- was predicted, based on chemical dynamics simulations, to be preferentially preserved when associated with its biogenic mineral matrix; the activation energy required for hydrolysis of such calcite-associated -DDDD- peptide domains was predicted by the simulations to be substantially higher than when solubilized in water. This prediction was supported through LC-MS/MS sequencing of ancient *S. camelus* eggshell specimens in which multiple peptides with -DDDD- motifs were reported. Thermal degradation experiments were also performed, and peptides with the motif were not detected when isolated from associated biogenic matrix (at 140 °C in water for 24 h) (Demarchi et al., 2016). More recently, Demarchi et al., 2022 reported the preservation of peptides with the -DDDD- motif in Miocene *S. camelus* eggshell from the Liushu formation of China (Demarchi et al., 2022).

Regarding lipids, their saturated hydrocarbon chains are generally unreactive under conditions of mild heat and/or pressure. Accordingly, saturated lipids have been shown to be relatively stable throughout time, albeit often in the form of *in-situ* polymerization products (Oberlin, 1984; Tegelaar et al., 1989; Briggs et al., 2000; Stankiewicz et al., 2000; Rouzaud et al., 2015; O'Reilly et al., 2017). Unaltered phospholipids and glycerides are rapidly hydrolyzed post-mortem, releasing their constituent fatty acids from glycerol (Kreps et al., 1981; Briggs et al., 2000; Schoenen and Schoenen, 2013). Intact phospholipids are generally not recovered even from permafrost frozen Pleistocene soft tissues (Kreps et al., 1981; Kostyukevich et al., 2018). Unbound, saturated fatty acids, on the other hand, can be recovered from permafrost specimens (Kreps et al., 1981; Kostyukevich et al., 2018). Volatile, unbound fatty acids have even been reported from whole bone extracts of Pliocene, Miocene, and Oligocene specimens (You-Xing et al., 2007; Shinmura and Sawada, 2010; Thiel et al., 2014). However, some researchers have hypothesized unbound fatty acid moieties to undergo *in-situ* polymerization relatively rapidly post-mortem (Briggs, 1999; Briggs et al., 2000; Briggs and Summons, 2014). The specific geological time frame for complete *in-situ* polymerization of fatty acids is unclear, but has potentially not occurred at least for some specimens of Oligocene strata (Solli et al., 1984; Thiel et al., 2014).

Other lipids, particularly steroids, sometimes remain unbound under thermally mild conditions but undergo NonP taphonomic alteration and are recoverable as sterane biomarkers (Mackenzie et al., 1981; Mackenzie et al., 1982; Briggs and Summons, 2014; O'Reilly et al., 2017). The saturated tetracyclic, hydrocarbon core of these sterane biomarkers has been shown to be relatively stable throughout geologic time (Mackenzie et al., 1982). Alternatively, under conditions of elevated heat and pressure, such biomarkers are often recovered as bound portions of sedimentary humics and kerogens (Mackenzie et al., 1981; Mackenzie et al., 1982; Briggs and Summons, 2014) or potentially are even degraded beyond recognition (Mackenzie et al., 1982). Under such conditions, the saturated tetracyclic core of steranes can be converted to a system of aromatics as a part of the carbonization process (Mackenzie et al., 1982). Other lipid biomarkers, including squalanes (corresponding to squalene, a biological steroid precursor) and related compounds, are known to persist into deep time as well (Ferrer et al., 2018). Despite their predicted stability, such lipid biomarkers, while present within ancient sedimentary plant and microbial matter, have not been commonly reported within ancient vertebrates, at least prior to Neogene

strata (Thiel et al., 2014; O'Reilly et al., 2017). One recent exception is the Holzmaden Jurassic ichthyosaur, in which C₂₇ cholestanes were reported to be preserved within the specimen's integument (Lindgren et al., 2018).

4. Conclusion

Individual hypotheses have been proposed in prior studies that partially explain the preservation of ancient vertebrate cells and other soft tissues, either *via* iron-mediated radical formation (Winterbourn, 1995; Schweitzer et al., 2014; Boatman et al., 2019) or AGE/ALE formation (Collins et al., 1998; Vistoli et al., 2013; Schweitzer et al., 2014; Wiemann et al., 2018). However, this review proposes a chemical framework that re-describes these hypotheses in the context of established chemistry across a diversity of scientific disciplines. These two pathways, while originally described separately (Collins et al., 1998; Vandenbroucke and Largeau, 2007; Schweitzer et al., 2014; Wiemann et al., 2018; Boatman et al., 2019), are now shown in many cases to form subsequent steps of a single oxidative *in-situ* polymerization pathway. A key example of this is the generation of radical biomolecules *via* oxidative *in-situ* polymerization that ultimately form the reactive carbonyl precursors for ALE/aerobic AGE formation (Wolff and Dean, 1987; Vistoli et al., 2013; Schweitzer et al., 2014).

To summarize this proposed framework, biomolecules of soft tissues (post-mortem), for which normal (microbial and other) decay pathways have been arrested, undergo a variety of NonP taphonomic alterations as well as *in-situ* polymerization/crosslinking reactions; these *in-situ* polymerization/crosslinking reactions are largely described by the oxidative *in-situ* polymerization (Schweitzer et al., 2014; Boatman et al., 2019) and anaerobic AGE formation (Collins et al., 1998; Wiemann et al., 2018) mechanisms. Such reactions lead to the formation of ITMs from the original biomolecules of cells and soft tissues, including those of vertebrates (Tegelaar et al., 1989; Stankiewicz et al., 1997; Stankiewicz et al., 2000; Gupta et al., 2007c; Gupta et al., 2007d; Gupta et al., 2008; Gupta et al., 2009; Lindgren et al., 2018). Preservation of segments of biomolecules, including protected, recalcitrant regions of proteins (Wiemann et al., 2018; Schweitzer et al., 2019), as untransformed portions of these ITMs may explain reported peptide sequences for Pliocene (Rybczynski et al., 2013; Demarchi et al., 2016; Buckley et al., 2019), Miocene (Demarchi et al., 2022), and Mesozoic (Asara et al., 2007; Schweitzer et al., 2009; Cleland et al., 2015b; Schroeter et al., 2017) fossils. Additionally, further chemical transformation of these ITMs, whether through heat, pressure, the passage of time, *etc.*, facilitates the removal of heteroatoms and the conversion of their carbon backbones from aliphatic to aromatic in character, resulting in MTMs (Tegelaar et al., 1989; Vandenbroucke and Largeau, 2007; Buseck and Beyssac, 2014; Rouzaud et al., 2015; Delarue et al., 2016).

The underlying chemical reactions of this framework explain observations by some authors (Schweitzer et al., 2014; Wiemann et al., 2018; Muscente et al., 2019) that oxidizing conditions may be favorable for soft tissue preservation. Free oxygen can promote the formation of radical species that contribute to the initiation and rapid propagation of *in-situ* polymerization (Wolff and Dean, 1987; Roberfroid and Calderon, 1995; Gryn'ova et al., 2011; Vistoli et al., 2013; Smith et al., 2018). This does not rule out, however, that soft tissue preservation can occur within anoxic depositional environments. Rather, such soft tissues must be well-stabilized against microbial degradation as *in-situ* polymerization is generally a slower process under anoxic conditions. Anaerobic AGE formation could contribute to this stabilization relatively shortly post-mortem (Vistoli et al., 2013), as could sulfurization in the presence of substantial hydrogen sulfide (Koopmans et al., 1997; Damsté et al., 2007; McNamara et al., 2016). This would account for the observation that specimens exhibiting cell and other soft tissue preservation can be recovered from anoxic depositional environments (Lindgren et al., 2011; Lindgren et al., 2017; Lindgren et al., 2018; Boskovic et al., 2021; Voegelé et al., 2022).

Finally, soft tissues of vertebrates from sediments of lower thermal/time-dependent maturity are predicted to possess less aromaticity and a higher heteroatom percentage relative to more matured fossils and to possess a greater potential to harbor endogenous biomolecules (Oberlin, 1984; Wei et al., 2005; Rouzaud et al., 2015; Delarue et al., 2016; Agrawal and Sharma, 2018). Such a prediction is in agreement with previously reported data for Cenozoic, Mesozoic, and Paleozoic invertebrates (Stankiewicz et al., 1997; Briggs et al., 2000; Gupta et al., 2007c; Gupta, 2014) and sedimentary organic matter (Wei et al., 2005; Rouzaud et al., 2015; Agrawal and Sharma, 2018). In fact, degree of vertebrate soft tissue molecular diagenesis may be usable as a proxy for informative biomolecule preservation, including for ancient DNA and proteins. This is akin to how the fields of soil and petroleum science use sediment maturity as a predictor of fossil fuel quality within geologic formations. Expanding the use of such a proxy for ancient vertebrate remains would require broader testing of Cenozoic and Mesozoic vertebrate soft tissue chemical compositions as this has only been done in limited studies. Inspiration for doing so can be drawn from methods currently used to study thermal maturity of humics and kerogens, including ToF-SIMS and Raman spectroscopy (Thiel and Sjövall, 2011; Delarue et al., 2016; Ferralis et al., 2016; Schito et al., 2017; Lindgren et al., 2018; Sjövall et al., 2021). Conclusions realized from such experiments (and compared against prior studies on invertebrates and sedimentary organic matter) would provide insight as to why soft tissues of some strata and/or depositional environments yield biomolecules while others do not; this would serve as the foundation for a chemical index for screening fossils according to biomolecule preservation potential. This has potential to broadly affect the focus of biomolecule recovery efforts within the fields of molecular archaeology, molecular palaeontology, ancient DNA research, and palaeoproteomics, and to create a more unified understanding of soft tissue and biomolecule preservation throughout the fossil record. Please see Anderson (2022) for a more in-depth discussion on the merits and potential of such a research direction.

Declaration of Competing Interest

The author has no relevant financial or non-financial interests to disclose.

Data availability

Data will be made available on request.

Acknowledgements

I would like to thank the journal co-editor-in-chief Dr. Asfawossen Asrat, Ph.D., the Journal Manager Mr. Parthiban Rajendran (Earth-Science Reviews) and the three anonymous reviewers for their time and efforts invested toward improving this manuscript. I would also like to thank my Ph.D. advisor, Dr. Mary H. Schweitzer, Ph.D. and Dr. Jonathan S. Lindsey, Ph.D., for their reading of the manuscript and the helpful feedback and suggestions they provided. Thank you to the Museum of the Rockies at Montana State University for access to specimens used in this research: MOR 2598. MOR 2598 was collected from lands managed by the Montana Department of Natural Resources and Conservation. Furthermore, thank you to both Dr. Cary Woodruff, Ph.D., for his collection of specimen GPDM 328, and to the Great Plains Dinosaur Museum & Field Station for allowing me access to specimen GPDM 328 for this research. I additionally want to recognize and thank the donors (to Dr. Mary H. Schweitzer) Lynn and Susan Packard Orr, and Vance and Gayle Mullis, for funding the article processing charge for this manuscript. Finally, thank you to my family and everyone who has supported me, as well as everyone who has contributed to my growth as an academic scholar and as a person over the years. Without the many efforts of these people in my life, the completion and publication of this

manuscript would not have been possible.

References

- Adam, P., Schneckenburger, P., Schaeffer, P., Albrecht, P., 2000. Clues to early diagenetic sulfurization processes from mild chemical cleavage of labile sulfur-rich geomacromolecules. *Geochim. Cosmochim. Acta* 64, 3485–3503.
- Agrawal, V., Sharma, S., 2018. Improved kerogen models for determining thermal maturity and hydrocarbon potential of shale. *Sci. Rep.* 8, 17465.
- Alberts, B., 1998. The cell as a collection of protein machines: preparing the next generation of molecular biologists. *Cell* 92, 291–294.
- Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., Walter, P., 2002. *Molecular Biology of the Cell*, 4th edition. Garland Science, New York.
- Alleon, J., Montagnac, G., Reynard, B., Brulé, T., Thoury, M., Gueriau, P., 2021. Pushing Raman spectroscopy over the edge: purported signatures of organic molecules in fossil animals are instrumental artefacts. *BioEssays* 43, 2000295.
- Anbar, M., Meyerstein, D., Neta, P., 1966. The reactivity of aromatic compounds toward hydroxyl radicals. *J. Phys. Chem.* 70, 2660–2662.
- Anderson, L.A., 2022. Biomolecular histology as a novel proxy for ancient DNA and protein sequence preservation. *Ecol. Evol.* 12, e9518.
- Armitage, M.H., Anderson, K.L., 2013. Soft sheets of fibrillar bone from a fossil of the supraorbital horn of the dinosaur *Triceratops horridus*. *Acta Histochem.* 115, 603–608.
- Asara, J.M., Schweitzer, M.H., Freimark, L.M., Phillips, M., Cantley, L.C., 2007. Protein sequences from mastodon and *Tyrannosaurus Rex* revealed by mass spectrometry. *Science* 316, 280–285.
- Bada, J.L., Wang, X.S., Hamilton, H., 1999. Preservation of key biomolecules in the fossil record: current knowledge and future challenges. *Phil. Trans. R. Soc. Lond. B* 354, 77–87.
- Bailleul, A.M., Zheng, W., Horner, J.R., Hall, B.K., Holliday, C.M., Schweitzer, M.H., 2020. Evidence of proteins, chromosomes and chemical markers of DNA in exceptionally preserved dinosaur cartilage. *Natl. Sci. Rev.* 7, 815–822.
- Boatman, E.M., Goodwin, M.B., Holman, H.-Y.N., Fakra, S., Zheng, W., Gronsky, R., Schweitzer, M.H., 2019. Mechanisms of soft tissue and protein preservation in *Tyrannosaurus rex*. *Sci. Rep.* 9, 15678.
- Bockisch, C., Lorance, E.D., Hartnett, H.E., Shock, E.L., Gould, I.R., 2018. Kinetics and mechanisms of dehydration of secondary alcohols under hydrothermal conditions. *ACS Earth Space Chem.* 2, 821–832.
- Boskovik, D.S., Vidal, U.L., Nick, K.E., Esperante, R., Brand, L.R., Wright, K.R., Sandberg, L.B., Siviero, B.C.T., 2021. Structural and protein preservation in fossil whale bones from the Pisco Formation (Middle-Upper Miocene), Peru. *Palaios* 36, 155–164.
- Briggs, D.E.G., 1999. Molecular taphonomy of animal and plant cuticles: selective preservation and diagenesis. *Phil. Trans. R. Soc. Lond. B* 354, 7–17.
- Briggs, D.E.G., Evershed, R.P., Lockheart, M.J., 2000. The biomolecular paleontology of continental fossils. *Paleobiology* 26, 169–193.
- Briggs, D.E.G., Summons, R.E., 2014. Ancient biomolecules: their origins, fossilization, and role in revealing the history of life. *BioEssays* 36, 482–490.
- Buckley, M., Lawless, C., Rycyzynski, N., 2019. Collagen sequence analysis of fossil camels, *Camelops* and c.f. *Paracamelus*, from the Arctic and sub-Arctic of Pliocene North America. *J. Prot.* 194, 218–225.
- Buseck, P.R., Beyssac, O., 2014. From organic matter to graphite: graphitization. *Elements* 10, 421–426.
- Butzbach, D.M., 2010. The influence of putrefaction and sample storage on post-mortem toxicology results. *Forensic Sci. Med. Pathol.* 6, 35–45.
- Cadena, E., 2016. Microscopical and elemental FESEM and Phenom ProX-SEM-EDS analysis of osteocyte- and blood vessel-like structures obtained from fossil vertebrates of the Eocene Messel Pit, Germany. *PeerJ* 4, e1618.
- Cadena, E., 2020. In situ SEM/EDS compositional characterization of osteocytes and blood vessels in fossil and extant turtles on untreated bone surfaces; different preservational pathways microns away. *PeerJ* 8, e9833.
- Calede, J.J.M., Orcutt, J.D., Kehl, W.A., Richards, B.D., 2018. The first tetrapod from the mid-Miocene *Clarkia lagerstätte* (Idaho, USA). *PeerJ* 6, e4880.
- Cappellini, E., Jensen, L.J., Szklarzyk, D., Ginolhac, A., da Fonseca, R.A.R., Stafford Jr., T.W., Hohen, S.R., Collins, M.J., Orlando, L., Willerslev, E., Gilbert, M.T.P., Olsen, J.V., 2012. Proteomic analysis of a Pleistocene mammoth femur reveals more than one hundred ancient bone proteins. *J. Proteome Res.* 11, 917–926.
- Cappellini, E., Welker, F., Pandolfi, L., Ramos-Madrugal, J., Samodova, D., Rütger, P.L., Fotakis, A.K., Lyon, D., Moreno-Mayer, J.V., Bukhsianidze, M., Jersie-Christensen, R., Mackie, M., Ginolhac, A., Ferrer, R., Tappen, M., Palkopoulou, E., Dickinson, M. R., Stafford Jr., T.W., Chan, Y.L., Götherström, A., Nathan, S.K.S.S., Heintzman, P.D., Kapp, J.D., Kirillova, I., Moodley, Y., Agusti, J., Kahlke, R.-D., Kiladze, G., Martínez-Navarro, B., Liu, S., Velasco, M.S., Sinding, M.-H. S., Kelstrup, C.D., Allentoft, M.E., Orlando, L., Penkman, K., Shapiro, B., Rook, L., Dalén, L., Gilbert, M.T.P., Olsen, J. V., Lordkipanidze, D., Willerslev, E., 2019. Early Pleistocene enamel proteome from Dmanisi resolves *Stephanorhinus* phylogeny. *Nature* 574, 103–107.
- Casares, D., Escribá, P.V., Rosselló, C.A., 2019. Membrane lipid composition: effect on membrane and organelle structure, function and compartmentalization and therapeutic avenues. *Int. J. Mol. Sci.* 20, 2167.
- Catalá, A., 2010. A synopsis of the process of lipid peroxidation since the discovery of the essential fatty acids. *Biochem. Biophys. Res. Commun.* 399, 318–323.
- Child, A.M., 1995. Towards an understanding of the microbial decomposition of archaeological bone in the burial environment. *J. Archaeol. Sci.* 22, 165–174.
- Cleland, T.P., Schroeter, E.R., Colleary, C., 2021. Diagenetiforms: a new term to explain protein changes as a result of diagenesis in paleoproteomics. *J. Prot.* 230, 103992.
- Cleland, T.P., Schroeter, E.R., Schweitzer, M.H., 2015a. Biologically and diagenetically derived peptide modifications in moa collagens. *Proc. R. Soc. B* 282, 20150015.
- Cleland, T.P., Schroeter, E.R., Zamborg, L., Zheng, W., Lee, J.E., Tran, J.C., Bern, M., Duncan, M.B., Lebleu, V.S., Ahlf, D.R., Thomas, P.M., Kalluri, R., Kelleher, N.L., Schweitzer, M.H., 2015b. Mass spectrometry and antibody-based characterization of blood vessels from *Brachyophosaurus canadensis*. *J. Proteome Res.* 14, 5252–5262.
- Cody, G.D., Gupta, N.S., Briggs, D.E.G., Killoyne, A.L.D., Summons, R.E., Kenig, F., Plotnick, R.E., Scott, A.C., 2011. Molecular signature of chitin-protein complex in Paleozoic arthropods. *Geology* 39, 255–258.
- Collins, M.J., Gernaey, A.M., Nielsen-Marsh, C.M., Vermeer, C., Westbroek, P., 2000. Slow rates of degradation of osteocalcin: green light for fossil bone protein? *Geology* 28, 1139–1142.
- Collins, M.J., Walton, D., King, A., 1998. The geochemical fate of proteins. In: Stankiewicz, B.A., van Bergen, P.F. (Eds.), *Nitrogen-Containing Macromolecules in the Bio- and Geosphere*. ACS Symposium Series. American Chemical Society, Washington D.C., pp. 74–87.
- Costa, P.M., 2018. Chapter 3 - Sample preparation. In: Costa, P.M. (Ed.), *The Handbook of Histopathological Practices in Aquatic Environments*. Academic Press, pp. 51–81.
- Dabney, J., Meyer, M., Pääbo, S., 2013. Ancient DNA damage. *Cold Spring Harb. Perspect. Biol.* 5, a012567.
- Damsté, J.S.S., Rijpstra, W.I.C., Coolen, M.J.L., Schouten, S., Volkman, J.K., 2007. Rapid sulfurisation of highly branched isoprenoid (HBI) alkenes in sulfidic Holocene sediments from Ellis Fjord, Antarctica. *Org. Geochem.* 38, 128–139.
- Davis, P.G., Briggs, D.E.G., 1995. Fossilization of feathers. *Geology* 23, 783–786.
- Delarue, F., Rouzau, J.-N., Derenne, S., Bourbin, M., Westall, F., Kremer, B., Sugitani, K., Deldicque, D., Robert, F., 2016. The Raman-derived carbonization continuum: a tool to select the best preserved molecular structures in archaic kerogens. *Astrobiology* 16, 407–417.
- Demarchi, B., Hall, S., Roncal-Herrero, T., Freeman, C.L., Woolley, J., Crisp, M.K., Wilson, J., Fotakis, A., Fischer, R., Kessler, B.M., Jersie-Christensen, R., Olsen, J.V., Haile, J., Thomas, J., Marean, C.W., Parkington, J., Presslee, S., Lee-Thorp, J., Ditchfield, P., Hamilton, J.F., Ward, M.W., Wang, C.M., Shaw, M.D., Harrison, T., Domínguez-Rodrigo, M., MacPhee, R.D.E., Kwekason, A., Ecker, M., Horwitz, L.K., Chazan, M., Kröger, R., Thomas-Oates, J., Harding, J.H., Cappellini, E., Penkman, K., Collins, M.J., 2016. Protein sequences bound to mineral surfaces persist into deep time. *eLife* 5, e17092.
- Demarchi, B., Mackie, M., Li, Z., Deng, T., Collins, M.J., Clarke, J., 2022. Survival of mineral-bound peptides into the Miocene. *eLife* 11, e82849.
- Dizdaroglu, M., Gajewski, E., Reddy, P., Margolis, S.A., 1989. Structure of a hydroxyl radical-induced DNA-protein crosslink involving thymine and tyrosine in nucleohistone. *Biochemistry* 28, 3625–3628.
- Dmitriev, L.F., Titov, V.N., 2010. Lipid peroxidation in relation to ageing and the role of endogenous aldehydes in diabetes and other age-related diseases. *Ageing Res. Rev.* 9, 200–210.
- Domínguez, R., Pateiro, M., Gagaoua, M., Barba, F.J., Zhang, W., Lorenzo, J.M., 2019. A comprehensive review on lipid oxidation in meat and meat products. *Antioxidants* 8, 429.
- Donaldson, A.E., Lamont, I.L., 2013. Biochemistry changes that occur after death: potential markers for determining post-mortem interval. *PLoS ONE* 8, e82011.
- Ehrlich, H., Rigby, J.K., Botting, J.P., Tsurkan, M.V., Werner, C., Schwill, P., Petrásek, Z., Písera, A., Simon, P., Sivkov, V.N., Vyalikh, D.V., Molodtsov, S.L., Kurek, D., Kammer, M., Hunoldt, S., Born, R., Stival, D., Steinhof, A., Bazhenov, V. V., Geisler, T., 2013. Discovery of 505-million-year old chitin in the basal demospore *Vauxia gracilenta*. *Sci. Rep.* 3, 3497.
- Evershed, R.P., Bland, H.A., van Bergen, P.F., Carter, J.F., Horton, M.C., Rowley-Conwy, P.A., 1997. Volatile compounds in archaeological plant remains and the Maillard reaction during decay of organic matter. *Science* 278, 432–433.
- Farrimond, P., Taylor, A., Telnaes, N., 1998. Biomarker maturity parameters: the role of generation and thermal degradation. *Org. Geochem.* 29, 1181–1197.
- Ferralis, N., Matys, E.D., Knoll, A.H., Hallmann, C., Summons, R.E., 2016. Rapid, direct and non-destructive assessment of fossil organic matter via microRaman spectroscopy. *Carbon* 108, 440–449.
- Ferrer, F.M., Bailey, J.V., Corsetti, F., Moldovan, J.M., Barbanti, S.M., Caron, D., Bryant-Huppert, J., 2018. Assessing biomarker syngeneity: an in situ approach using monoclonal antibodies. *Org. Geochem.* 124, 112–122.
- Ferrer, I., Santpere, G., Arzberger, T., Bell, J., Blanco, R., Boluda, S., Budka, H., Carmona, M., Giaccone, G., Krebs, B., Limido, L., Parchi, P., Puig, B., Strammiello, R., Ströbel, T., Kretzschmar, H., 2007. Brain protein preservation largely depends on the postmortem storage temperature: implications for study of proteins in human neurologic diseases and management of brain banks: a BrainNet Europe study. *J. Neuropathol. Exp. Neurol.* 66, 35–46.
- Gardner, H.W., 1986. Reactions of hydroperoxides—products of high molecular weight. In: Chan, H.W.-S. (Ed.), *Autoxidation of Unsaturated Lipids*. Academic Press, London, pp. 51–93.
- Gill, V., Kumar, V., Singh, K., Kumar, A., Kim, J.-J., 2019. Advanced glycation end products (AGEs) may be a striking link between modern diet and health. *Biomolecules* 9, 888.
- Goth, K., de Leeuw, J.W., Püttmann, W., Tegelaar, E.W., 1988. Origin of Messel Oil Shale kerogen. *Nature* 336, 759–761.
- Grabber, J.H., Ralph, J., Hatfield, R.D., 1998. Ferulate cross-links limit the enzymatic degradation of synthetically lignified primary walls of maize. *J. Agric. Food Chem.* 46, 2609–2614.
- Greenwalt, D.E., Goreva, Y.S., Siljeström, S.M., Rose, T., Harbach, R.E., 2013. Hemoglobin-derived porphyrins preserved in a Middle Eocene blood-engorged mosquito. *Proc. Natl. Acad. Sci.* 110, 18496–18500.

- Grice, K., Eiserbeck, C., 2014. 12.3 - the analysis and application of biomarkers. In: Holland, H.D., Turekian, K.K. (Eds.), *Treatise on Geochemistry*. Elsevier, pp. 47–78.
- Grynova, G., Hodgson, J.L., Coote, M.L., 2011. Revising the mechanism of polymer autooxidation. *Org. Biomol. Chem.* 9, 480–490.
- Gupta, N.S., 2014. *Biopolymers: a molecular paleontology approach*. Springer Science+Business Media Dordrecht.
- Gupta, N.S., 2015. Plant biopolymer-geopolymer: organic diagenesis and kerogen formation. *Front. Mat.* 2, 61.
- Gupta, N.S., Briggs, D.E.G., Collinson, M.E., Evershed, R.P., Michels, R., Jack, K.S., Pancost, R.D., 2007a. Evidence for the *in situ* polymerisation of labile aliphatic organic compounds during the preservation of fossil leaves: implications for organic matter preservation. *Org. Geochem.* 38, 499–522.
- Gupta, N.S., Briggs, D.E.G., Collinson, M.E., Evershed, R.P., Michels, R., Pancost, R.D., 2007b. Molecular preservation of plant and insect cuticles from the Oligocene Enspel Formation, Germany: evidence against derivation of aliphatic polymer from sediment. *Org. Geochem.* 38, 404–418.
- Gupta, N.S., Briggs, D.E.G., Landman, N.H., Tanabe, K., Summons, R.E., 2008. Molecular structure of organic components in cephalopods: evidence for oxidative cross linking in fossil marine invertebrates. *Org. Geochem.* 39, 1405–1414.
- Gupta, N.S., Cody, G.D., Tetlie, O.E., Briggs, D.E.G., Summons, R.E., 2009. Rapid incorporation of lipids into macromolecules during experimental decay of invertebrates: initiation of geopolymer formation. *Org. Geochem.* 40, 589–594.
- Gupta, N.S., Erik Tetlie, O., Briggs, D.E.G., Pancost, R.D., 2007c. The fossilization of eurypterids: a result of molecular transformation. *Palaios* 22, 439–447.
- Gupta, N.S., Michels, R., Briggs, D.E.G., Collinson, M.E., Evershed, R.P., Pancost, R.D., 2007d. Experimental evidence for the formation of geomacromolecules from plant leaf lipids. *Org. Geochem.* 38, 28–36.
- Harvey, G.R., Boran, D.A., Chesal, L.A., Tokar, J.M., 1983. The structure of marine fulvic and humic acids. *Mar. Chem.* 12, 119–132.
- Harvey, G.R., Boran, D.A., 1985. Geochemistry of humic substances in seawater. In: Aiken, G.R., McKnight, D.M., Wershaw, R.L., MacCarthy, P. (Eds.), *Humic Substances in Soil, Sediment, and Water: Geochemistry, Isolation and Characterization*. Wiley, New York, pp. 233–247.
- Harvey, R.G., 1997. In: *Polycyclic Aromatic Hydrocarbons: chemistry and carcinogenicity*. Wiley, New York, NY, p. 682.
- Hatcher, P.G., Spiker, E.C., Szeverenyi, N.M., Maciel, G.E., 1983. Selective preservation and origin of petroleum-forming aquatic kerogen. *Nature* 305, 498–501.
- Hawkins, C.L., Davies, M.J., 2001. Generation and propagation of radical reactions on proteins. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* 1504, 196–219.
- Hayashi, T., Namiki, M., 1981. On the mechanism of free radical formation during browning reaction of sugars with amino compounds. *Agric. Biol. Chem.* 45, 933–939.
- Hofreiter, M., Jaenicke, V., Serre, D., von Haeseler, A., Pääbo, S., 2001. DNA sequences from multiple amplifications reveal artifacts induced by cytosine deamination in ancient DNA. *Nucleic Acids Res.* 29, 4793–4799.
- Hong, S., Ding, H., Greenberg, M.M., 2006. Oxygen independent DNA interstrand cross-link formation by a nucleotide radical. *J. Am. Chem. Soc.* 128, 485–491.
- Hulbert, A.J., Kelly, M.A., Abbott, S.K., 2014. Polyunsaturated fats, membrane lipids and animal longevity. *J. Comp. Physiol. B* 184, 149–166.
- Ishiwatari, R., Machihara, T., 1982. Structural changes of young kerogen during laboratory heating as revealed by alkaline potassium permanganate oxidation. *Geochim. Cosmochim. Acta* 46, 825–831.
- Jenkins, C.L., Bretscher, L.E., Guzei, I.A., Raines, R.T., 2003. Effect of 3-hydroxyproline residues on collagen stability. *J. Am. Chem. Soc.* 125, 6422–6427.
- Jensen, A.M., Sheehan, G.W., 2014. Frozen conditions: preservation and excavation. In: Smith, C. (Ed.), *Encyclopedia of Global Archaeology*. Springer, New York, NY, pp. 2930–2935.
- Ji, Q., Luo, Z.-X., Yuan, C.-X., Tabrum, A.R., 2006. A swimming mammaliaform from the Middle Jurassic and ecomorphological diversification of early mammals. *Science* 311, 1123–1127.
- Ji, Q., Luo, Z.-X., Yuan, C.-X., Wible, J.R., Zhang, J.-P., Georgi, J.A., 2002. The earliest known eutherian mammal. *Nature* 416, 816–822.
- Jones, M.K., 1999. Introduction to molecular information and prehistory. A themed issue. *Phil. Trans. R. Soc. Lond. B* 354, 3–5.
- Kaye, T.G., Gaugler, G., Sawlowicz, Z., 2008. Dinosaurian soft tissues interpreted as bacterial biofilms. *PLoS ONE* 3, e2808.
- Kehrer, J.P., 2000. The Haber-Weiss reaction and mechanisms of toxicity. *Toxicology* 149, 43–50.
- Keil, R.G., Montlucon, D.B., Prahl, F.G., Hedges, J.L., 1994. Sorptive preservation of labile organic matter in marine sediments. *Nature* 370, 549–552.
- Kirschenbauer, H.G., 1960. In: *Fats and Oils - An Outline of Their Chemistry and Technology*. Reinhold Publishing Corporation, New York, p. 240.
- Koopmans, M.P., de Leeuw, J.W., Damsté, J.S.S., 1997. Novel cyclised and aromatised diagenetic products of β -carotene in the Green River Shale. *Org. Geochem.* 26, 451–466.
- Kostyukovich, Y., Bugrova, A., Chagovets, V., Brzhozovskiy, A., Indeykina, M., Vanyushkina, A., Zherebker, A., Mitina, A., Kononikhin, A., Popov, I., Khaitovich, P., Nikolaev, E., 2018. Proteomic and lipidomic analysis of mammoth bone by high-resolution tandem mass spectrometry coupled with liquid chromatography. *Eur. J. Mass Spectrom.* 24, 411–419.
- Kreps, E.M., Avrova, N.F., Chebotarëva, M.A., Chirkovskaya, E.V., Levitina, M.V., Pomazanskaya, L.F., 1981. Brain lipids in fossilized mammoths, *Mammuthus primigenius*. *Compar. Biochem. Physiol. Part B* 68, 135–140.
- Lawal, A.T., 2017. Polycyclic aromatic hydrocarbons. A review. *Cogent Environ. Sci.* 3, 1339841.
- Lee, Y.-C., Chiang, C.-C., Huang, P.-Y., Chung, C.-Y., Huang, T.D., Wang, C.-C., Chen, C.-I., Chang, R.-S., Liao, C.-H., Reisz, R.R., 2017. Evidence of preserved collagen in an early Jurassic sauropodomorph dinosaur revealed by synchrotron FTIR microspectroscopy. *Nat. Commun.* 8, 14220.
- Lees, S., 1989. Some characteristics of mineralised collagen. In: Hukins, D.W.L. (Ed.), *Calcified Tissue*. Palgrave, London, pp. 153–173.
- Lennartz, A., Hamilton, M.D., Weaver, R., 2020. Moisture content in decomposing, desiccated, and mummified human tissue. *Foren. Anthropol.* 3, 1–16.
- Lewis Jr., C.A., Crayle, J., Zhou, S., Swanstrom, R., Wolfenden, R., 2016. Cytosine deamination and the precipitous decline of spontaneous mutation during Earth's history. *Proc. Natl. Acad. Sci.* 113, 8194–8199.
- Lindahl, T., 1993. Instability and decay of the primary structure of DNA. *Nature* 362, 709–715.
- Lindgren, J., Kuriyama, T., Madsen, H., Sjövall, P., Zheng, W., Uvdal, P., Engdahl, A., Moyer, A.E., Gren, J.A., Kamezaki, N., Ueno, S., Schweitzer, M.H., 2017. Biochemistry and adaptive colouration of an exceptionally preserved juvenile fossil sea turtle. *Sci. Rep.* 7, 13324.
- Lindgren, J., Sjövall, P., Thiel, V., Zheng, W., Ito, S., Wakamatsu, K., Hauff, R., Kear, B.P., Engdahl, A., Alwmark, C., Eriksson, M.E., Jarenmark, M., Sachs, S., Ahlberg, P.E., Marone, F., Kuriyama, T., Gustafsson, O., Malmberg, P., Thomen, A., Rodríguez-Meizoso, I., Uvdal, P., Ojika, M., Schweitzer, M.H., 2018. Soft-tissue evidence for homeothermy and crypsis in a Jurassic ichthyosaur. *Nature* 564, 359–365.
- Lindgren, J., Uvdal, P., Engdahl, A., Lee, A.H., Alwmark, C., Bergquist, K.-E., Nilsson, E., Ekström, P., Rasmussen, M., Douglas, D.A., Polcyn, M.J., Jacobs, L.L., 2011. Microspectroscopic evidence of Cretaceous bone proteins. *PLoS ONE* 6, e19445.
- Lodish, H., Berk, A., Zipursky, S.L., 2000. Collagen: the fibrous proteins of the matrix. In: *Matsudaira, P., Baltimore, D., Darnell, J. (Eds.), Molecular Cell Biology*. W. H. Freeman, New York.
- Mackenzie, A.S., Brassell, S.C., Eglinton, G., Maxwell, J.R., 1982. Chemical fossils: the geological fate of steroids. *Science* 217, 491–504.
- Mackenzie, A.S., Lewis, C.A., Maxwell, J.R., 1981. Molecular parameters of maturation in the Toarcian shales, Paris Basin, France—IV. Laboratory thermal alteration studies. *Geochim. Cosmochim. Acta* 45, 2369–2376.
- Maillard, L.C., 1912a. Action des acides amines sur les sucres; formation des melanoidines par voie methodique. *C. R. Acad. Sci.* 154, 66–68.
- Maillard, L.C., 1912b. Formation d'humus et de combustibles minéraux sans intervention de l'oxygène atmosphérique, des microorganismes, des hautes températures, ou des fortes pressions. *C. R. Acad. Sci.* 155, 1554–1556.
- Maillard, L.C., 1916. Syntheses des matières humiques par action des acides amines sur les sucres reducteurs. *Ann. Chim.* 9, 258–317.
- Manini, P., La Pietra, P., Panzella, L., Napolitano, A., d'Ischia, M., 2006. Glyoxal formation by Fenton-induced degradation of carbohydrates and related compounds. *Carbohydr. Res.* 341, 1828–1833.
- Manning, P.L., Morris, P.M., McMahon, A., Jones, E., Gize, A., Macquaker, J.H.S., Wolff, G., Thompson, A., Marshall, J., Taylor, K.G., Lyson, T., Gaskell, S., Reamton, O., Sellers, W.I., van Dongen, B.E., Buckley, M., Wogelius, R.A., 2009. Mineralized soft-tissue structure and chemistry in a mummified hadrosaur from the Hell Creek Formation, North Dakota (USA). *Proc. R. Soc. B* 276, 3429–3437.
- Mayr, G., Peters, S.D., Plodowski, G., Vogel, O., 2002. Bristle-like integumentary structures at the tail of the horned dinosaur *Psittacosaurus*. *Naturwissenschaften* 89, 361–365.
- McNamara, M.E., van Dongen, B.E., Lockyer, N.P., Bull, I.D., Orr, P.J., 2016. Fossilization of melanosomes via sulfurization. *Palaeontology* 59, 337–350.
- Miles, C.A., Ghelashvili, M., 1999. Polymer-in-a-box mechanism for the thermal stabilization of collagen molecules in fibers. *Biophys. J.* 76, 3243–3252.
- Min, B., Ahn, D.U., 2005. Mechanism of lipid peroxidation in meat and meat products—a review. *Food Sci. Biotechnol.* 14, 152–163.
- Mitchell, K.J., Rawlence, N.J., 2021. Examining natural history through the lens of palaeogenomics. *Trends Ecol. Evol.* 36, 258–267.
- Moldogazieva, N.T., Mokhosoev, I.M., Mel'nikova, T.I., Porozov, Y.B., Terentiev, A.A., 2019. Oxidative stress and advanced lipoxidation and glycation end products (ALEs and AGEs) in aging and age-related diseases. *Oxidative Med. Cell. Longev.* 2019, 3085756.
- Moyer, A.E., Zheng, W., Johnson, E.A., Lamanna, M.C., Li, D.-Q., Lacovara, K.J., Schweitzer, M.H., 2014. Melanosomes or microbes: testing an alternative hypothesis for the origin of microbodies in fossil feathers. *Sci. Rep.* 4, 4233.
- Muscente, A.D., Martindale, R.C., Schiffbauer, J.D., Creighton, A.L., Bogan, B.A., 2019. Taphonomy of the lower Jurassic Konservat-Lagerstätte at Ya Ha Tinda (Alberta, Canada) and its significance for exceptional fossil preservation during oceanic anoxic events. *Palaios* 34, 515–541.
- Mustoe, G.E., 2018. Non-mineralized fossil wood. *Geosciences* 8, 223.
- Nackerdien, Z., Rao, G., Cacciuttolo, M.A., Gajewski, E., Dizdaroglu, M., 1991. Chemical nature of DNA-protein cross-links produced in mammalian chromatin by hydrogen peroxide in the presence of iron or copper ions. *Biochemistry* 30, 4873–4879.
- Nagaraj, R.H., Linetsky, M., Stitt, A.W., 2012. The pathogenic role of Maillard reaction in the aging eye. *Amino Acids* 42, 1205–1220.
- Namiki, M., Hayashi, T., 1983. A new mechanism of the Maillard reaction involving sugar fragmentation and free radical formation. In: Waller, G.R., Feather, M.S. (Eds.), *The Maillard Reaction in Foods and Nutrition*. American Chemical Society, pp. 21–46.
- Nasrazadani, S., Hassani, S., 2016. Chapter 2 - Modern analytical techniques in failure analysis of aerospace, chemical, and oil and gas industries. In: Makhlof, A.S.H., Aliofkhaezai, M. (Eds.), *Handbook of Materials Failure Analysis with Case Studies from the Oil and Gas Industry*. Butterworth-Heinemann, pp. 39–54.
- Nawar, W.W., 1978. Reaction mechanisms in the radiolysis of fats: a review. *J. Agr. Food Chem.* 26, 21–25.

- Nawar, W.W., 1984. Chemical changes in lipids produced by thermal processing. *J. Chem. Educ.* 61, 299–302.
- Ngo, P.D., 1999. Energy dispersive spectroscopy. In: Wagner, L.C. (Ed.), *Failure Analysis of Integrated Circuits: Tools and Techniques*. Springer Science+Business Media, New York, NY, pp. 205–215.
- Nguyen, K., Love, G.D., Zumbeke, J.A., Kelly, A.E., Owens, J.D., Rohrsen, M.K., Bates, S.M., Cai, C., Lyons, T.W., 2019. Absence of biomarker evidence for early eukaryotic life from the Mesoproterozoic Roper Group: searching across a marine redox gradient in mid-Proterozoic habitability. *Geobiology* 17, 247–260.
- Nolan, A.-N., Mead, R.J., Maker, G., Speers, S.J., 2020. A review of the biochemical products produced during mammalian decomposition with the purpose of determining the post-mortem interval. *Aust. J. Foren. Sci.* 52, 477–488.
- O'Reilly, S., Summons, R., Mayr, G., Vinther, J., 2017. Preservation of uropygial gland lipids in a 48-million-year-old bird. *Proc. R. Soc. B* 284, 20171050.
- Oberlin, A., 1984. Carbonization and graphitization. *Carbon* 22, 521–541.
- Orlando, L., Ginolhac, A., Zhang, G., Froese, D., Albrechtsen, A., Stiller, M., Schubert, M., Cappellini, E., Peterson, B., Moltke, I., Johnson, P.L.F., Fumagalli, M., Vilstrup, J.T., Raghavan, M., Korneliusen, T., Malaspina, A.-S., Vogt, J., Szklarczyk, D., Kelstrup, C.D., Vinther, J., Dolocan, A., Stenderup, J., Velazquez, A.M.V., Cahill, J., Rasmussen, M., Wang, X., Min, J., Zazula, G.D., Sequin-Orlando, A., Mortensen, C., Magnussen, K., Thompson, J.F., Weinstock, J., Gregersen, K., Røed, K.H., Eisenmann, V., Rubin, C.J., Miller, D.C., Antczak, D.F., Bertelsen, M.F., Brunak, S., Al-Rasheid, K.A.S., Ryder, O., Andersson, L., Mundy, J., Krogh, A., Gilbert, M.T.P., Kjær, K., Sicheritz-Ponten, T., Jensen, L.J., Olsen, J.V., Hofreiter, M., Nielsen, R., Shapiro, B., Wang, J., Willerslev, E., 2013. Recalibrating *Equus* evolution using the genome sequence of an early Middle Pleistocene horse. *Nature* 499, 74–78.
- Osés, G.L., Petri, S., Voltani, C.G., Prado, G.M.E.M., Galante, R., Rizzutto, M.A., Rudnizki, I.D., da Silva, E.P., Rodrigues, F., Rangel, E.C., Sucerquia, P.A., Pacheco, M.L.A.F., 2017. Deciphering pyritization-kerogenization gradient for fish soft-tissue preservation. *Sci. Rep.* 7, 1468.
- Petrenko, I., Summers, A.P., Simon, P., Żótkowska-Aksamitowska, S., Motylenko, M., Schimpf, C., Rafaja, D., Roth, F., Kummer, K., Brendler, E., Pokrovsky, O.S., Galli, R., Wysokowski, M., Meissner, H., Niederschlag, E., Joseph, Y., Molodtsov, S., Ereskovsky, A., Sivkov, V., Nekipelov, S., Petrova, O., Volkova, O., Bertau, M., Kraft, M., Rogalev, A., Kopani, M., Jesionowski, T., Ehrlich, H., 2019. Extreme biomimetics: preservation of molecular detail in centimeter-scale samples of biological meshes laid down by sponges. *Sci. Adv.* 5, eaax2805.
- Philp, R.P., Gilbert, T.D., 1987. A review of biomarkers in kerogens as determined by pyrolysis-gas chromatography and pyrolysis-gas chromatography-mass spectrometry. *J. Anal. Appl. Pyrolysis* 11, 93–108.
- Poinar, H.N., Hofreiter, M., Spaulding, W.G., Martin, P.S., Stankiewicz, B.A., Bland, H., Evershed, R.P., Possnert, G., Pääbo, S., 1998. Molecular coproscopy: dung and diet of the extinct ground sloth *Nothrotheriops shastensis*. *Science* 281, 402–406.
- Poinar, H.N., Stankiewicz, B.A., 1999. Protein preservation and DNA retrieval from ancient tissues. *Proc. Natl. Acad. Sci.* 96, 8426–8431.
- Prescott, S.C., 1912. The bacteriology of fermentation and putrefaction in relation to the conservation of foods. *Am. J. Public Health* 2, 834–839.
- Repetto, M.G., Ferrarotti, N.F., Boveris, A., 2010. The involvement of transition metal ions on iron-dependent lipid peroxidation. *Arch. Toxicol.* 84, 255–262.
- Roberfroid, M.B., Calderon, P.B., 1995. In: *Free Radicals and Oxidation Phenomena in Biological Systems*. Marcel Dekker Inc, New York, New York, p. 272.
- Rouzau, J.-N., Deldicque, D., Charon, E., Pageot, J., 2015. Carbons at the heart of questions on energy and environment: a nanostructural approach. *Compt. Rendus Geosci.* 347, 124–133.
- Rybczynski, N., Gosse, J.C., Harington, C.R., Wogelius, R.A., Hidy, A.J., Buckley, M., 2013. Mid-Pliocene warm-period deposits in the High Arctic yield insight into camel evolution. *Nat. Commun.* 4, 1550.
- Saitta, E.T., Liang, R., Lau, M.C.Y., Brown, C.M., Longrich, N.R., Kaye, T.G., Novak, B.J., Salzberg, S.L., Norell, M.A., Abbott, G.D., Dickinson, M.R., Vinther, J., Bull, L.D., Brooker, R.A., Martin, P., Donohoe, P., Knowles, T.D.J., Penkman, K.E.H., Onstott, T., 2019. Cretaceous dinosaur bone contains recent organic material and provides an environment conducive to microbial communities. *eLife* 8, e46205.
- Salamon, M., Tuross, N., Arensburg, B., Weiner, S., 2005. Relatively well preserved DNA is present in the crystal aggregates of fossil bones. *Proc. Natl. Acad. Sci.* 102, 13783–13788.
- Salmon, V., Derenne, S., Lallier-Vergès, E., Largeau, C., Beaudoin, B., 2000. Protection of organic matter by mineral matrix in a Cenomanian black shale. *Org. Geochem.* 31, 463–474.
- San Antonio, J.D., Schweitzer, M.H., Jensen, S.T., Kalluri, R., Buckley, M., Orgel, J.P.R.O., 2008. Dinosaur peptides suggest mechanisms of protein survival. *PLoS ONE* 6, e20381.
- Schafer, F.Q., Qian, S.Y., Buettner, G.R., 2000. Iron and free radical oxidations in cell membranes. *Cell. Mol. Biol.* 46, 657–662.
- Schiffner, H.A., 2011. 5.41 - Pharmaceutical proteins - structure, stability, and formulation. In: Moo-Young, M. (Ed.), *Comprehensive Biotechnology*, second edition. Academic Press, pp. 521–541.
- Schimmel, P.H.A., Ruttink, P.J.A., de Jong, B.H.W.S., 1999. *Ab Initio* calculations on hydroaromatics: hydrogen abstraction and dissociation reaction pathways. *J. Phys. Chem. B* 103, 10506–10516.
- Schito, A., Romano, C., Corrado, S., Grigo, D., Poe, B., 2017. Diagenetic thermal evolution of organic matter by Raman spectroscopy. *Org. Geochem.* 106, 57–67.
- Schoenen, D., Schoenen, H., 2013. Adipocere formation - the result of insufficient microbial degradation. *Forensic Sci. Int.* 226, 301.e1–301.e6.
- Schroeter, E.R., DeHart, C.J., Cleland, T.P., Zheng, W., Thomas, P.M., Kelleher, N.L., Bern, M., Schweitzer, M.H., 2017. Expansion for the *Brachylophosaurus canadensis* collagen I sequence and additional evidence of the preservation of Cretaceous protein. *J. Proteome Res.* 16, 920–932.
- Schweitzer, M.H., 2011. Soft tissue preservation in terrestrial Mesozoic vertebrates. *Annu. Rev. Earth Planet. Sci.* 39, 187–216.
- Schweitzer, M.H., Avci, R., Collier, T., Goodwin, M.B., 2008. Microscopic, chemical, and molecular methods for examining fossil preservation. *Compt. Rend. Palevol.* 7, 159–184.
- Schweitzer, M.H., Moyer, A.E., Zheng, W., 2016. Testing the hypothesis of biofilm as a source for soft tissue and cell-like structures preserved in dinosaur bone. *PLoS ONE* 11, e0150238.
- Schweitzer, M.H., Schroeter, E.R., Cleland, T.P., Zheng, W., 2019. Paleoproteomics of Mesozoic dinosaurs and other Mesozoic fossils. *Proteomics* 19, 1800251.
- Schweitzer, M.H., Wittmeyer, J.L., Horner, J.R., 2007. Soft tissue and cellular preservation in vertebrate skeletal elements from the Cretaceous to the present. *Proc. R. Soc. B* 274, 183–197.
- Schweitzer, M.H., Wittmeyer, J.L., Horner, J.R., Toporski, J.K., 2005. Soft-tissue vessels and cellular preservation in *Tyrannosaurus rex*. *Science* 307, 1952–1955.
- Schweitzer, M.H., Zheng, W., Cleland, T.P., Bern, M., 2013. Molecular analyses of dinosaur osteocytes support the presence of endogenous molecules. *Bone* 52, 414–423.
- Schweitzer, M.H., Zheng, W., Cleland, T.P., Goodwin, M.B., Boatman, E., Theil, E., Marcus, M.A., Fakra, S.C., 2014. A role for iron and oxygen chemistry in preserving soft tissues, cells and molecules from deep time. *Proc. R. Soc. B* 281, 20132741.
- Schweitzer, M.H., Zheng, W., Organ, C.L., Avci, R., Suo, Z., Freemark, L.M., Lebleu, V.S., Duncan, M.B., Vander Heiden, M.G., Neveu, J.M., Lane, W.S., Cottrell, J.S., Horner, J.R., Cantley, L.C., Kalluri, R., Asara, J.M., 2009. Biomolecular characterization and protein sequences of the Campanian hadrosaur *B. canadensis*. *Science* 324, 626–631.
- Shahidi, F., Zhong, Y., 2010. Lipid oxidation and improving the oxidative stability. *Chem. Soc. Rev.* 39, 4067–4079.
- Shimura, T., Sawada, K., 2010. Compositions and stable carbon isotope ratios of fatty acids and steroids in fossil bones of marine mammals —application to fossil diagenesis and palaeodietary analyses. *Chikyukagaku (Geochemistry)* 44, 17–29.
- Simic, M.G., Jovanovic, S.V., Niki, E., 1992. Mechanisms of lipid oxidative processes and their inhibition. In: Angelo, St. A.J. (Ed.), *Lipid Oxidation in Food*. American Chemical Society, Washington, D.C., pp. 14–32.
- Simoneit, B.R.T., 2002. Molecular indicators (biomarkers) of past life. *Anat. Rec.* 268, 186–195.
- Singh, H., Bishen, K.A., Garg, D., Sukhija, H., Sharma, D., Tomar, U., 2019. Fixation and fixatives: roles and functions—a short review. *Dental J. Adv. Stud.* 7, 51–55.
- Sjövall, P., Bake, K.D., Pomerantz, A.E., Lu, X., Mitra-Kirtley, S., Mullins, O.C., 2021. Analysis of kerogens and model compounds by time-of-flight secondary ion mass spectrometry (TOF-SIMS). *Fuel* 286, 119373.
- Smith, L.M., Aitken, H.M., Coote, M.L., 2018. The fate of the peroxy radical in autoxidation: how does polymer degradation really occur? *Acc. Chem. Res.* 51, 2006–2013.
- Smith, O., Dunshea, G., Sinding, M.-H.S., Fedorov, S., Germonpre, M., Bocherens, H., Gilbert, M.T.P., 2019. Ancient RNA from Late Pleistocene permafrost and historical canids shows tissue-specific transcriptome survival. *PLoS Biol.* 17, e3000166.
- Solli, H., van Graas, G., Leplat, P., Krane, J., 1984. Analysis of kerogens of Miocene shales in a homogenous sedimentary column. A study of maturation using flash pyrolysis techniques and carbon-13 CP-MAS NMR. *Org. Geochem.* 6, 351–358.
- Srinivasan, M., Sedmak, D., Jewell, S., 2002. Effect of fixatives and tissue processing on the content and integrity of nucleic acids. *Am. J. Pathol.* 161, 1961–1971.
- Stankiewicz, B.A., Briggs, D.E.G., Evershed, R.P., Flannery, M.B., Wuttke, M., 1997. Preservation of chitin in 25-million-year-old fossils. *Science* 276, 1541–1543.
- Stankiewicz, B.A., Briggs, D.E.G., Michels, R., Collinson, M.E., Flannery, M.B., Evershed, R.P., 2000. Alternative origin of aliphatic polymer in kerogen. *Geology* 28, 559–562.
- Stanov, V.V., 1981. Catagenesis of coals. *Int. Geol. Rev.* 23, 1076–1084.
- Stefanova, V., Disnar, J.R., 2000. Composition and early diagenesis of fatty acids in lacustrine sediments, lake Aydat (France). *Org. Geochem.* 31, 41–55.
- Strlic, M., Kolar, J., 2005. Ageing and stabilisation of paper. National and University Library, Ljubljana.
- Surmik, D., Boczarowski, A., Balin, K., Dulski, M., Szade, J., Kremer, B., Pawlicki, R., 2016. Spectroscopic studies on organic matter from Triassic reptile bones, Upper Silesia, Poland. *PLoS ONE* 11, e0151143.
- Sykes, G.A., Collins, M.J., Walton, D.I., 1995. The significance of a geochemically isolated intracrystalline organic fraction within biominerals. *Org. Geochem.* 23, 1059–1065.
- Talman, E.A., Boughner, D.R., 1995. Glutaraldehyde fixation alters the internal shear properties of porcine aortic heart valve tissue. *Ann. Thorac. Surg.* 60, S369–S373.
- Tegelaar, E.W., de Leeuw, J.W., Derenne, S., Largeau, C., 1989. A reappraisal of kerogen formation. *Geochim. Cosmochim. Acta* 53, 3103–3106.
- Tessier, F.J., 2010. The Maillard reaction in the human body. The main discoveries and factors that affect glycation. *Pathol. Biol.* 58, 214–219.
- Thavarajah, R., Mudimbaimannar, V.K., Elizabeth, J., Rao, U.K., Ranganathan, K., 2012. Chemical and physical basics of routine formaldehyde fixation. *J. Oral. Maxillofac. Pathol.* 16, 400–405.
- Thiel, V., Blumenberg, M., Kiel, S., Leefmann, T., Liebenau, K., Lindgren, J., Sjövall, P., Treude, T., Zilla, T., 2014. Occurrence and fate of fatty acyl biomarkers in an ancient whale bone (Oligocene, El Cien Formation, Mexico). *Org. Geochem.* 68, 71–81.
- Thiel, V., Sjövall, P., 2011. Using time-of-flight secondary ion mass spectrometry to study biomarkers. *Annu. Rev. Earth Planet. Sci.* 39, 125–156.

- Thornalley, P.J., Langborg, A., Minhas, H.S., 1999. Formation of glyoxal, methylglyoxal and 3-deoxyglucosone in the glycation of proteins by glucose. *Biochem. J.* 344, 109–116.
- Tian, X., 2013. Chemical factors affecting degradation processes of vegetable oils during frying. Rutgers, The State University of New Jersey, New Brunswick, New Jersey, 149 pp.
- Tissot, B.P., Welte, D.H., 1984. In: *Petroleum Formation and Occurrence*. Springer-Verlag, Berlin Heidelberg, p. 702.
- Toporski, J., Steele, A., 2002. The relevance of bacterial biomarkers in astrobiological research. In: *Proceedings of the Second European Workshop on Exo/Astrobiology*, 518, pp. 239–242.
- Trueman, C.N., Martill, D.M., 2002. The long-term survival of bone: the role of bioerosion. *Archaeometry* 44, 371–382.
- Tuross, N., 1989. Albumin preservation in the Taima-taima mastodon skeleton. *Appl. Geochem.* 4, 255–259.
- Vandenbroucke, M., Largeau, C., 2007. Kerogen origin, evolution and structure. *Org. Geochem.* 38, 719–833.
- Vercellotti, J.R., St. Angelo, A.J., Spanier, A.M., 1992. Lipid oxidation in foods: an overview. In: St. Angelo, A.J. (Ed.), *Lipid Oxidation in Food*, ACS Symposium Series. American Chemical Society, Washington, D.C., pp. 1–11.
- Vinther, J., Briggs, D.E.G., Clarke, J., Mayr, G., Prum, R.O., 2010. Structural coloration in a fossil feather. *Biol. Lett.* 6, 128–131.
- Vinther, J., Briggs, D.E.G., Prum, R.O., Saranathan, V., 2008. The colour of fossil feathers. *Biol. Lett.* 4, 522–525.
- Vistoli, G., De Maddis, D., Cipak, A., Zarkovic, N., Carini, M., Aldini, G., 2013. Advanced glycoxidation and lipoxidation end products (AGEs and ALEs): an overview of their mechanisms of formation. *Free Radic. Res.* 47, 3–27.
- Voegelé, K.K., Boles, Z.M., Ullmann, P.V., Schroeter, E.R., Zheng, W., Lacovara, K.J., 2022. Soft tissue and biomolecular preservation in vertebrate fossils from glauconitic, shallow marine sediments of the Hornerstown Formation, Edelman Fossil Park, New Jersey. *Biology* 11, 1161.
- Wadsworth, C., Buckley, M., 2014. Proteome degradation in fossils: investigating the longevity of protein survival in ancient bone. *Rapid Commun. Mass Spectrom.* 28, 605–615.
- Wang, X., Zhao, Y.-P., 2020. The time-temperature-maturity relationship: a chemical kinetic model of kerogen evolution based on a developed molecule-maturity index. *Fuel* 278, 118264.
- Wei, Z., Gao, X., Zhang, D., Da, J., 2005. Assessment of thermal evolution of kerogen geopolymers with their structural parameters measured by solid-state ¹³C NMR spectroscopy. *Energy Fuels* 19, 240–250.
- Welker, F., Ramos-Madrigo, J., Gutenbrunner, P., Mackie, M., Tiwary, S., Jersie-Christensen, R.R., Chiva, C., Dickinson, M.R., Kuhlwil, M., de Manuel, M., Gelabert, P., Martínón-Torres, M., Margvelashvili, A., Arsuaga, J.L., Carbonell, E., Marques-Bonet, T., Penkman, K., Sabidó, E., Cox, J., Olsen, J.V., Lordkipanidze, D., Racimo, F., Lalueza-Fox, C., de Castro, J.M.B., Willerslev, E., Cappellini, E., 2020. The dental proteome of *Homo antecessor*. *Nature* 580, 235–238.
- Manuel, M., Samodova, D., Mackie, M., Allentoft, M.E., Bacon, A.-M., Collins, M.J., Cox, J., Lalueza-Fox, C., Olsen, J.V., Demeter, F., Wang, W., Marques-Bonet, T., Cappellini, E., 2019. Enamel proteome shows that *Gigantopithecus* was an early diverging pongine. *Nature* 576, 262–265.
- Westbroek, P., van der Meide, P.H., van der Wey-Kloppers, J.S., van der Sluis, R.J., de Leeuw, J.W., de Jong, E.W., 1979. Fossil macromolecules from cephalopod shells: characterization, immunological response and diagenesis. *Paleobiology* 5, 151–167.
- Wiemann, J., Crawford, J.M., Briggs, D.E.G., 2020. Phylogenetic and physiological signals in metazoan fossil biomolecules. *Sci. Adv.* 6, eaba6883.
- Wiemann, J., Fabbri, M., Yang, T.-R., Stein, K., Sander, P.M., Norell, M.A., Briggs, D.E.G., 2018. Fossilization transforms vertebrate hard tissue proteins into N-heterocyclic polymers. *Nat. Commun.* 9, 4741.
- Winterbourn, C.C., 1995. Toxicity of iron and hydrogen peroxide: the Fenton reaction. *Toxicol. Lett.* 82–83, 969–974.
- Wolff, S.P., Dean, R.T., 1987. Glucose autooxidation and protein modification. The potential role of 'autooxidative glycosylation' in diabetes. *Biochem. J.* 245, 243–250.
- Wysokowski, M., Petrenko, I., Galli, R., Schimpf, C., Rafaja, D., Hubalkova, J., Aneziris, C.G., Dyshlovoy, S., von Amsberg, G., Meissner, H., Yakovlev, Y.M., Tabachnick, K.R., Stelling, A.L., Ehrlich, H., 2020a. Extreme biomineralization: the case of the hypermineralized ear bone of gray whale (*Eschrichtius robustus*). *Appl. Phys. A* 126, 727.
- Wysokowski, M., Zaslansky, P., Ehrlich, H., 2020b. Macrobiomineralogy: insights and enigmas in giant whale bones and perspectives for bioinspired materials science. *ACS Biomater. Sci. Eng.* 6, 5357–5367.
- Wysokowski, M., Zatoň, M., Bazhenov, V.V., Behm, T., Ehrlich, A., Stelling, A.L., Hog, M., Ehrlich, H., 2014. Identification of chitin in 200-million-year-old gastropod egg capsules. *Paleobiology* 40, 529–540.
- Xing, L., Niu, K., Taylor, R.S., Evans, S.E., 2020. Integumentary remains and abdominal contents in the Early Cretaceous Chinese lizard, *Yabeinosaurus* (Squamata), demonstrate colour banding and a diet including crayfish. *Cretac. Res.* 108, 104320.
- Yamagata, K., Nagai, K., Miyamoto, H., Anzai, M., Kato, H., Miyamoto, K., Kurosaka, S., Azuma, R., Kolodeznikov, I.L., Protopopov, A.V., Plotnikov, V.V., Kobayashi, H., Kawahara-Miki, R., Kono, T., Uchida, M., Shibata, Y., Handa, T., Kimura, H., Hosoi, Y., Mitani, T., Matsumoto, K., Iritani, A., 2019. Signs of biological activities of 28,000-year-old mammoth nuclei in mouse oocytes visualized by live-cell imaging. *Sci. Rep.* 9, 4050.
- Yamauchi, M., Sricholpech, M., 2012. Lysine post-translational modifications of collagen. *Essays Biochem.* 52, 113–133.
- You-Xing, Z., Cheng-Sen, L., Xiao-Dong, L., Lu, Z., Tie-Mei, Y., Jun, Z., 2007. Palaeophytochemical constituents from the Pliocene-fossil wood of *Tsuga dumosa* (Pinaceae). *Plant Divers.* 29, 367–370.