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AN ARCHAEOMETRIC SYNTHESIS OF PREHISTORIC ROCK-ART BINDERS

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Chapter 1: Introduction: The Overlooked Component

Prehistoric rock art represents one of the most vivid testimonies of human symbolic expression and remains central to archaeological inquiry across continents. Its study has long revolved around the materials used to produce motifs, especially the inorganic pigments that survived well over time, such as hematite, goethite, manganese oxides, and charcoal. These substances lend themselves to analysis by standard mineralogical and spectroscopic methods, from optical microscopy and X-ray diffraction to Raman spectroscopy (Hernanz et al., 2008). In contrast, the organic components of paints binders made of lipids, proteins, plant exudates, or resins were rarely studied due to their fragility, degradation, and the lack of suitable non-destructive methods (Colombini & Modugno, 2009).

This methodological imbalance produced what has become known as the pigment-centric bias. Research traditions across Europe (Chieli et al., 2022; Gomes et al., 2024; Hernanz et al., 2008; Roldán et al., 2018), Africa (Prinsloo et al., 2008; Tournié et al., 2011), South America (Brook et al., 2018; Fiore et al., 2008; Gomes et al., 2019), Australia (Cole & Watchman, 1992; Huntley et al., 2015), and the Americas often treated pigments as the sole material evidence, relegating binders to speculation. As a result, binders were sidelined in favor of pigments, a tendency that spread across regions as archaeometric approaches became standardized internationally. Yet this oversight restricts technological reconstructions, reduces interpretive depth concerning cultural practices, and undermines conservation strategies. The persistence of this bias is best understood by reviewing its regional development.

1.1 Defining the Problem: The Pigment-Centric Bias

1.1.1 Historical reasons for focusing on inorganic pigments

The dominance of inorganic pigments in archaeometric research on rock art is best understood as a product of historical constraints in instrumentation and methodology. When archaeologists first began applying scientific techniques to prehistoric paintings in the mid-twentieth century, the available tools were overwhelmingly mineralogical. X-ray diffraction, optical and polarized light microscopy, and later scanning electron microscopy with energy-dispersive X-ray analysis were designed to characterize crystalline solids. These methods were extremely effective in detecting iron oxides, manganese compounds, and carbon-based pigments, which preserved their structural integrity over archaeological timescales (Hernanz et al., 2008). Because they delivered clear, reproducible identifications from very small samples, they rapidly became the standard for pigment studies.

Organic binders, by contrast, presented far greater analytical challenges. Their molecular structures degrade quickly under exposure to moisture, oxygen, and microbial activity, often leaving only trace residues that are chemically transformed. Unlike minerals, they lack crystalline signatures that can be detected non-invasively. To identify them requires extraction and separation through chromatography, typically coupled with mass spectrometry, or immunological assays in the case of proteins (Colombini & Modugno, 2009). These methods were not only technically demanding but also destructive, requiring relatively large quantities of material that could rarely be spared from fragile rock art panels. The contrast was stark: minerals could be studied with portable, non-destructive, or micro-sampling techniques, while organics required invasive and uncertain laboratory procedures.

This asymmetry was compounded by the development of portable instruments in the late twentieth and early twenty-first centuries. Portable X-ray fluorescence and Raman spectrometers allowed researchers to conduct analyses directly on painted surfaces without removing samples. These instruments became central to heritage science because they were rapid, non-invasive, and often provided results in the field (Huntley et al., 2015). Yet their effectiveness was almost entirely limited to inorganic materials, further entrenching the pigment-focused paradigm. The very success of these portable tools reinforced the marginalization of binders, as research agendas increasingly gravitated toward what could be measured quickly and reliably. Preservation also played a role in shaping expectations. The inorganic fraction of paint is often what survives visibly on the rock surface, sometimes accompanied by alteration products such as oxalates or gypsum crusts (Brook et al., 2018; Hernanz et al., 2014; Ruiz et al., 2012). In contrast, any organic components were either invisible or suspected to have degraded completely. This fostered an assumption that even if binders had once been used, they were unlikely to survive in detectable form. Institutional and ethical factors further reinforced this trajectory. Sampling from rock art has always been highly restricted, with permissions often allowing only microscopic fragments to be taken (Chieli et al., 2022; Domingo & Chieli, 2021a; Roldán et al., 2018; Wang et al., 2025). In this context, researchers preferred to apply methods that could yield reliable results from minimal material. Inorganic-focused analyses were well suited to these constraints, while organic detection usually required larger samples and more complex pretreatments (Dayet et al., 2022; Di Gianvincenzo et al., 2022; Domingo & Chieli, 2021a; Livingston et al., 2009; Martín-Ramos et al., 2023; Roldán et al., 2018; Rousaki et al., 2017). The conservation imperative to minimize intervention thus acted as a practical barrier against systematic study of binders.

The interpretive frameworks of archaeology aligned more easily with mineral evidence. Provenance studies of ochres provided insights into mobility, exchange networks, and technological choices, while the identification of heating practices could be linked to pyrotechnology and color symbolism (Domingo & Chieli, 2021a). Inorganic pigments could therefore be integrated smoothly into prevailing archaeological narratives, whereas degraded organics, even if detected, raised complex questions of authenticity, contamination, and interpretive value (Garcês et al., 2019; Gomes et al., 2024; Li et al., 2012).

These instrumental, methodological, and institutional conditions explain why research into rock art became historically centered on pigments. The preference for inorganic analysis was not a reflection of prehistoric practices but rather the result of analytical reliability, preservation visibility, and conservation ethics that systematically privileged minerals over organics. Only with the recent development of multiproxy approaches, combining spectroscopic mapping with chromatographic and proteomic analyses, has this imbalance begun to be questioned. Studies such as the identification of fatty acids in Brazilian pigments (Gomes et al., 2019), plant-based organics identified in prehistoric pigments in Spain (Garcês et al., 2022) and the experimental creation of spectral libraries for pigment–binder mixtures (Garcês et al., 2019) demonstrate that organic binders can survive and be studied. Their findings underscore that the historical neglect of organics was not inevitable but a product of the methodological environment in which rock art research developed. Analyses focused on ochre characterization, such as studies documenting the composition and application of mineral pigments have offered substantial insights into prehistoric material procurement and processing (Froment et al., 2008; Villa et al., 2015; Zipkin et al., 2017); however, these investigations often omit the critical step of how pigment powders were transformed into durable paints.

1.1.2 The consequences: incomplete understanding of technology, symbolism, and conservation needs

The historical concentration on inorganic pigments has shaped not only the methods applied to rock art but also the kinds of knowledge produced. By privileging minerals, archaeologists reconstructed the technological and cultural significance of painting in ways that are necessarily partial. The technological process of paint-making involves more than the selection and grinding of minerals; it requires binding agents to transform powders into a workable medium, to ensure adhesion to rock, and to enhance durability (Casoli, 2021). When research restricts itself to pigments alone, the *chaîne opératoire* of paint production is left incomplete. Analyses focused on ochre characterization have offered substantial insights into prehistoric material procurement and processing; however, these investigations often omit the critical step of how pigment powders were transformed into durable paints (Dubiel et al., 2010). The neglect of organic components therefore creates a technological gap that obscures prehistoric skill in recipe formulation, mixture control, and surface preparation.

The consequences extend beyond technology into the realm of symbolism. Pigments are visually striking, but binders often carry cultural meanings of their own. Ethnographic and experimental evidence suggests that substances such as animal fat, blood, milk, or plant resins were not only functional adhesives but also elements charged with ritual or cosmological significance (Arocena et al., 2008). By overlooking binders, researchers risk reducing paintings to their visible mineral hues, stripping away layers of meaning that may have been embedded in their material composition. In this sense, the pigment-centric bias not only narrows the scope of technological interpretation but also impoverishes the symbolic reading of prehistoric art.

The implications are equally significant for conservation. Preservation strategies are generally developed on the basis of material composition (Domingo Sanz et al., 2021; Pérez-Gandarillas et al., 2024; Russ et al., 1999b), yet when only pigments are considered, treatments may fail to account for the presence of degraded organics (Casoli, 2021; Cather, 1991; Gelzo et al., 2019). Proteins and lipids follow different pathways of chemical alteration than minerals: they may become cross-linked, oxidized, or microbially consumed in ways that require specialized stabilization (Cather, 1991; Di Gianvincenzo et al., 2022; López-Miras et al., 2013). Conservation protocols tailored solely to inorganic stability such as surface consolidation or cleaning may inadvertently accelerate the degradation of surviving organics or erase residues that could otherwise be analyzed (Cather, 1991). A comprehensive conservation strategy therefore requires a full appreciation of both mineral and organic components. By focusing on pigments alone, the field has risked implementing incomplete or even counterproductive preservation measures (Colombini & Modugno, 2009).

The cumulative effect of these omissions is a distorted picture of prehistoric painting. Technologies appear simpler than they were, symbolism appears less rich, and conservation frameworks appear more secure than they truly are. The pigment-centric bias thus shapes not only what we know, but also what we fail to see. Only by systematically incorporating binders into the study of rock art can archaeologists hope to reconstruct the full complexity of prehistoric painting practices and develop preservation strategies that do justice to the fragile materials that once held pigments in place.

1.2 Research Aim and Objectives

1.2.1 Aim

The primary aim of this thesis is to provide a comprehensive and critical synthesis of recent studies on the analysis and identification of organic binders in prehistoric rock art pigments. While the study of pigments has long dominated archaeometric research, the detection and interpretation of binders whether animal- or plant-based substances used to prepare, apply, or stabilize. This research will critically evaluate the potential and the limitations of current analytical approaches through discussing current methodological advances and case studies.

By integrating results from diverse archaeometric techniques (Barberena et al., 2009; Batiashvili et al., 2023; Bersani & Lottici, 2016; Casoli, 2021; Gomes et al., 2019, 2024), ethnographic studies (Cilli et al., 2010), and experimental reconstructions (Garcês et al., 2019; Santos Da Rosa et al., 2023), this research aims to evaluate how reliably organic compounds can be detected in prehistoric rock art contexts and how these findings contribute to broader questions of technological choice, cultural practice, and symbolic expression in prehistory. The dissertation therefore aims not only to document the state of knowledge on organic binders, but also to highlight the methodological constraints, issues of contamination and preservation, and interpretive uncertainties that continue to shape the field.

1.2.2 Objectives

This thesis is coordinated around a series of clearly defined objectives that address both the empirical evidence and the methodological challenges of binder research. The first objective is to catalogue and categorize the organic substances proposed or identified as binders in prehistoric rock art, establishing a typology that incorporates chemical identifications, ethnographic analogies, and experimental reconstructions, and that is further applied to the regional case. The second objective is to review, compare, and critically evaluate the analytical techniques employed for binder detection, ranging from portable *in-situ* instruments to laboratory-based molecular and separation methods, with particular attention to their respective strengths, limitations, and interpretive risks and revisited in the synthesis of results. The third objective is to evaluate the influence of taphonomy and contamination on the survival and detectability of organic residues, considering microbial alteration, mineral crust formation, and sampling constraints. The fourth objective is to integrate the available evidence in order to examine what the presence or absence of specific binders can reveal about prehistoric technological knowledge, resource management, and cultural or symbolic practices.

1.3 Scope and Limitations

1.3.1 Scope

This thesis undertakes a critical, literature-based archaeometric synthesis of organic binders in prehistoric rock-art paints. It evaluates what has actually been detected, how those detections were produced, and what they plausibly mean for technology, practice, and conservation.

The geographical scope is global in review but uneven in evidentiary depth. Detailed synthesis is anchored in regions where the literature provides replicable analyses of organics associated with paint layers, especially the Iberian Peninsula, parts of South America, and Eurasian contexts with micro-destructive or micro-imaging results. For example, Levantine Iberian panels where proteomics isolated casein peptides from hematite paints provide one of the strongest positive signals for a specific binder (Roldán et al., 2018); Brazilian panels where lipid biomarkers were reported add comparative evidence for fatty substances; and micro-sampled Urals material illustrates how organics, minerals, and biofilms co-occur at micrometric scales (Roldán et al., 2018; Gomes et al., 2019; Kiseleva et al., 2023). These regional emphases reflect the distribution of available data rather than an a priori spatial focus and are made explicit to avoid overgeneralization.

The chronological scope follows the published attributions of the original studies and remains tied to prehistoric contexts as defined by each author. No attempt is made here to recalibrate cultural chronologies. Where dates are relevant to interpretations of organics, they are treated cautiously and in context with the authors' own reservations about alteration, mobility of molecules, and contamination. Inferences are kept within the limits that the published data allow (Roldán et al., 2018).

The material scope centers on binding media such as, animal fats and proteins (Domingo & Chieli, 2021a), plant exudates such as gums and resins (Arocena et al., 2008; Loubser, 1992; Prinsloo et al., 2013), plant oils and waxes (Kakoulli & Balonis, 2023). Claims considered here require one or more of the following: laboratory identifications of diagnostic lipid or peptide markers; *in-situ* or micro-analytical spectra that plausibly indicate organics when interpreted with appropriate controls; or experimental/ethnographic correlates used as supportive, not determinative, evidence (see 2.1–2.3). It is applied consistently across the analysis to maintain comparability (Colombini & Modugno, 2009; Garcês et al., 2019; Bonneau et al., 2017).

Methodologically, the review covers both non-invasive screening and micro-destructive laboratory techniques as defined in Chapter 3. Portable optical microscopy, *in-situ* FTIR, and Raman are treated as contextual tools that guide or constrain sampling; GC-MS for lipids and LC-MS-based paleoproteomics for proteins are treated as the principal confirmatory approaches when appropriate sampling, blanks, and controls are reported. The chapter on fundamental obstacles is integral to scope: it sets out why even the “gold standards” can fail when preservation is poor or contamination is unmanaged (Hernanz et al., 2008; Colombini & Modugno, 2009; Huntley et al., 2015; Roldán et al., 2018; Kiseleva et al., 2023).

1.3.2 Limitations

Concerning the limitations, several explicit limitations follow from this research. First, because the research synthesizes published work, it inherits the uneven reporting practices and sampling strategies of that work. Many studies lack full metadata on sampling context, cleaning steps, or negative controls; others provide spectra or chromatograms without sufficient discussion of degradation pathways. These gaps limit the strength of cross-study comparison and are acknowledged in Chapter 4 when weighing positive and negative results.

Second, contamination and taphonomic overprinting place hard limits on what can be claimed about binders from legacy surfaces. For example, in Valltorta proteomic dataset shows how human keratins can dominate spectra even under sterile protocols, and why casein signals must be evaluated against environmental histories and rates of modification such as deamidation (Roldán et al., 2018). Urals micro-samples demonstrate that microbial aggregates and mineral accretions can be embedded within single flakes, confounding signal assignment to the original paint. These constraints are not incidental; they structure the inference space for organics in rock art. (Roldán et al., 2018; Kiseleva et al., 2023).

Third, non-invasive tools are treated in this thesis as screening methods rather than final arbiters for organics. They are essential for mapping stratigraphy, guiding sampling, and minimizing damage, but their diagnostic power for degraded binders is limited relative to separation and mass spectrometry (Ma et al., 2025). Interpretations that rely only on field instruments are flagged as provisional. This stance aligns with the methodological chapters and prevents overstatement of evidence where functional group overlap or substrate effects are likely. (Hernanz et al., 2008; Huntley et al., 2015).

Finally, no direct radiocarbon dating of purified organic fractions is undertaken in this study. The potential of compound-specific dating is acknowledged in the discussion and future-work framework, but its feasibility is assessed against the sampling, contamination, and yield constraints documented in Chapters 3-4. Any chronological claims tied to binders in this thesis therefore remain interpretive and second-order, not new determinations.

1.4 Thesis Structure

This thesis is organized into six chapters, each of which addresses a distinct stage in the research. Chapter 1 establishes the research problem by outlining the pigment-centric bias in archaeometric studies of rock art. It explains why pigments have historically dominated analytical approaches, examines the consequences of neglecting binders for technological, symbolic, and conservation interpretations, and defines the research aim, objectives, scope, and limitations.

Chapter 2 presents a typology of organic substances that could function as binders. It integrates three strands of evidence: chemical identifications from archaeometric studies, ethnographic analogies documenting traditional paint preparation, and experimental archaeology testing the performance of reconstructed paint recipes. This chapter distinguishes animal-based binders such as fats, marrow, casein, and blood, from plant-based substances including gums, resins, oils, and waxes, highlighting both their technical and cultural significance.

Chapter 3 evaluates the methodological frameworks applied to the detection of organic media. It first considers fundamental obstacles, including taphonomic degradation and contamination risks linked to micro-sampling. It then reviews non-invasive approaches such as optical microscopy, portable FTIR, and Raman spectroscopy, followed by laboratory-based techniques such as GC–MS for lipids, LC–MS for proteins, and SEM–EDS for microstructural analysis. Each method is assessed in terms of strengths, limitations, and susceptibility to false positives.

Chapter 4 consolidates the empirical record from published analyses. It compiles the results of a large dataset of samples, showing the consistent identification of pigments but the rarity of secure organic detections. The chapter revisits “classic” case studies where early claims of binders were later challenged, and contrasts these with more recent successful identifications in the Iberian Peninsula, South America, and Africa. It also emphasizes the interpretive importance of negative results, demonstrating how absence, ambiguity, and methodological limits shape reconstructions of prehistoric paint recipes.

Chapter 5 develops a critical interpretation of the evidence. It assesses what binder choices reveal about technological knowledge, explores how resource availability, symbolism, and ritual influenced selection, and considers how binder chemistry determines preservation outcomes. It also evaluates the prospects and limitations of radiocarbon dating of purified organic compounds. This discussion situates binders as both functional and cultural components of paint making, embedded in wider subsistence and symbolic practices.

Chapter 6 draws together the findings of the study. It begins with a summary of results, stressing the asymmetry between secure pigment identifications and rare but decisive binder detections. It then identifies persistent gaps and methodological limitations that continue to constrain the field, such as contamination, degradation, and uneven reporting standards. The conclusion emphasizes that prehistoric painting was a compound practice of mineral and organic materials, and that binders must be integrated into analytical and interpretive frameworks to reconstruct the full *chaîne opératoire* of rock art and to design effective strategies for its conservation and chronological study.

Chapter 2: The Palette of Possibilities: A Typology of Potential Binders

2.1. Sources of Evidence

2.1.1 Analysis with chemistry

The identification of binding media in prehistoric rock art rests on three complementary lines of evidence: chemical analysis, ethnographic analogues, and experimental archaeology. Each of these approaches has its own strengths and limitations, and together they form the basis for reconstructing the range of organic substances that may have been used to prepare paints.

From a chemical perspective, archaeometric advances in chromatography, spectroscopy, and mass spectrometry have provided tools capable of detecting degraded biomolecules even at trace levels. Gas chromatography–mass spectrometry (GC–MS), for example, has been applied to rock art samples to identify lipid degradation products such as palmitic and stearic acids, which may derive from animal fats or plant oils (Colombini & Modugno, 2009). More recent work has expanded to liquid chromatography coupled with mass spectrometry (LC–MS) for peptide analysis, which can in principle identify proteinaceous binders such as casein, albumin, or collagen, though such results remain rare in rock art contexts (Bonneau, Moyle, et al., 2017). These chemical studies demonstrate the feasibility of detecting binders, but they are also hindered by taphonomic transformation, contamination, and sampling constraints.

2.1.2 Ethnographic Analogues

Ethnographic analogues provide an equally important avenue of insight. Studies of traditional painting practices among First-Nation people communities have shown that pigments were often combined with animal fats, blood, milk, eggs, gums, or resins to create paints that adhered to rock or body surfaces (Arocena et al., 2008; Loubser, 1992; Prinsloo et al., 2013). Ethnographic records of San painters in southern Africa describe mixtures of ochre with fat or blood, conferring both durability and symbolic resonance (Loubser, 1992). Similar practices have been documented in Australian Aboriginal traditions, where ochres were bound with resins, plant exudates, or animal products (Cole & Watchman, 1992). While ethnographic parallels cannot be assumed to replicate prehistoric techniques directly, they highlight plausible technological options and underscore the cultural significance of binder choice (Cole & Watchman, 1992).

2.1.3 Experimental Archaeology

Experimental archaeology complements these approaches by testing the physical and chemical properties of paint recipes reconstructed from plausible raw materials. Experimental mixtures of ochre with animal fat, egg yolk, plant gums, or resins have been created and applied to rock substrates, demonstrating differences in workability, color stability, and long-term adhesion. Garcês and colleagues, for example, prepared ATR-FTIR reference spectra of ochre mixed with organic binders such as egg, fat, and saliva, showing that diagnostic vibrational markers can persist even after accelerated aging (Garcês et al., 2019; Santos Da Rosa et al., 2023; Vandenabeele et al., 2000). Such experimental frameworks not only validate analytical detection but also reconstruct the experiential knowledge of prehistoric painters.

2.2 Animal-Based Binders

Animal products are among the most frequently discussed organic binders in prehistoric rock art. animal fats and proteins leave molecular traces that, under the right conditions, can survive long enough to be detected. Animal fats have been identified in several sites through lipid biomarker analysis. The most common compounds preserved are palmitic acid (C16:0) and stearic acid (C18:0) (Vázquez et al., 2008). These saturated fatty acids are more stable than unsaturated ones and tend to remain in archaeological residues. The ratio of palmitic to stearic acid, together with the presence of odd-carbon-number fatty acids such as C15:0 and C17:0, can indicate the origin of the fat. A dominance of palmitic acid with smaller amounts of these odd-chain compounds often points to ruminant sources (Kiseleva et al., 2023). In the Ignatievskaya Cave in the Urals, chromatographic analysis revealed a lipid profile dominated by palmitic acid with significant stearic acid and traces of C15:0 and C17:0. This pattern was interpreted as degraded ruminant fat, possibly marrow fat from wild cervids (Kiseleva et al., 2023). In South America, samples from Alero Hornillos 2 in Argentina showed similar results. GC–MS analysis identified palmitic and stearic acids together with minor odd-chain fatty acids, consistent with ruminant fats. The lipid concentrations were high, reaching up to 36,780 $\mu g\ g^{-1}$ in one sample, supporting the conclusion that fat was deliberately added as a binder (Vázquez et al., 2008). The archaeological context, which included camelid bone remains processed for marrow extraction, reinforces this interpretation (Kakoulli & Balonis, 2023). Rendering processes may have included heating bone marrow or adipose tissue to produce fat with adhesive qualities (Kakoulli & Balonis, 2023). In some cases, bitumen-like steranes and n-alkanes were also detected in residues, though their interpretation is debated, since they may come from natural gypsum deposits or other environmental sources (Vázquez et al., 2008). Nevertheless, the consistent presence of fatty acids in pigment layers points to intentional use of animal fats. Some examples of sites are the Palaeolithic sites of Tito Bustillo (Navarro Gascón & Gómez González, 2003), some Levantine paintings of la Saltadora site (Roldan et al. 2018) or the more recent art of the dolmen of Dombate, where the use of milk fat and butter was suggested (Carrera Ramírez & Bello Diéguez, 1997).

Casein and milk proteins represent an important group of animal-derived binders. Proteomic analysis has allowed the detection of specific peptides from bovine αS1-casein in Levantine rock art at La Saltadora in Spain (Roldán et al., 2018). The identification of these peptides is significant because it suggests that milk was used as a binder in the Neolithic Iberian context (Roldán et al., 2018). The presence of casein links rock art to broader economic and symbolic practices. Using milk in paint preparation may have been a practical choice, since milk proteins provide excellent adhesion (Villa et al., 2015). It may also reflect symbolic meanings connected with domestication and subsistence. Experimental reconstructions confirm that mixtures of ochre and milk produce paints with good consistency and durability (Roldán et al., 2018). Similar casein signals have been reported in other contexts, raising the possibility that milk was a common binder in many regions, though the risk of modern contamination remains a challenge (Domingo & Chieli, 2021a).

Animal fats have been securely identified in Iberian Peninsula (Domingo & Chieli, 2021), South America (Gomes et al., 2019; Vázquez et al., 2008), in the Urals of Russia (Kiseleva et al., 2023), and in the Caucasus (Golovanova et al., 2024). Lipid residues were most often linked to black and red figures, where high concentrations of fatty acids were preserved. Casein peptides were identified in red Levantine motifs. These patterns show that different animal-based products were chosen depending on context and availability.

This is important to mention that fatty acids can also come from microbial activity in oxalate crusts, leading to false positives (Spades & Russ, 2005). Proteins are often detected in small amounts and raise doubts about contamination (Roldán et al., 2018). Despite these issues, animal fats and casein remain among the best documented binders. Their presence expands the understanding of prehistoric technology, showing that artists combined mineral pigments with complex organic media.

2.3 Plant-Based Binders

Plant-derived substances form a distinct typological class of potential binders in prehistoric rock art. Plant-based binders include polysaccharide gums, terpenoid resins, and lipid-rich materials such as oils and waxes. Each of these groups has specific physical properties that could improve the workability and durability of paints. Gums are soluble in water and can act as stabilizers. While resins are sticky and hydrophobic, hardening when exposed to air. Oils and waxes give fluidity, gloss, and water resistance (Kakoulli & Balonis, 2023). Their archaeological identification, however, is challenging. They degrade quickly and often leave only faint molecular traces. In addition, they are easily confused with environmental contaminants such as microbial polysaccharides or natural waxes in mineral crusts (Kakoulli & Balonis, 2023).

Gums are complex polysaccharides, while resins are dominated by terpenoid compounds (Kakoulli & Balonis, 2023). Their functional role as binders is plausible because they are adhesive and widely available. They are also known from later painting traditions, including ancient Egyptian and medieval manuscripts, where gum arabic was a standard medium (Kakoulli & Balonis, 2023). In rock art contexts, their presence has been more difficult to prove. Detection usually depends on pyrolysis or gas chromatography coupled with mass spectrometry, which can reveal sugar derivatives or resin acids (Lluveras-Tenorio et al., 2012; Kakoulli & Balonis, 2023).

At Serra da Capivara in Brazil, multiproxy analyses of red paintings reported organic residues alongside pigments. The main compounds were lipids, but saccharide-like signals also suggested the possible presence of plant gums (Gomes et al., 2019). The interpretation is not definitive, since contamination from oxalates could not be ruled out, yet the findings point to a carbohydrate component that may have functioned as a binder. In Spain, at the Les Dogues shelter, SEM analysis of black charcoal-based motifs revealed plant cell fragments and obstructed vessels. These structures suggested that a viscous binder was added to make charcoal workable as paint. The authors proposed honey or gum as the most likely candidate (López-Montalvo et al., 2017). Although the identification was indirect, the study demonstrates how microscopic and contextual evidence can point to gum-like materials.

At Gode Roriso in Ethiopia, GC–MS analysis of white paintings identified beeswax, a lipid-based wax of plant and insect origin (Gomes et al., 2019). Beeswax is chemically stable due to its long-chain esters and hydrocarbons, making it one of the few plant-derived binders that can survive in archaeological contexts. Its presence shows that prehistoric artists used natural waxy substances to create durable paints. Resinous materials have also been noted in comparative studies of Aboriginal artefacts in Australia, where ATR-FTIR and GC–MS identified native plant resins used as adhesives and paint media (Matheson & McCollum, 2014, Kakoulli & Balonis, 2023). These comparative data support the plausibility of resins as prehistoric paint binders, even if direct examples in rock art remain rare.

Oils are mainly triglycerides from seeds and fruits, while waxes are long-chain esters that remain solid at room temperature. Both add hydrophobic properties to paints. Their survival is limited, since unsaturated fatty acids oxidize quickly. However, some saturated compounds can persist. Archaeological detection usually focuses on the presence of long-chain fatty acids such as palmitic (C16:0) and stearic (C18:0), though these compounds overlap with animal fats and complicate interpretation (Kakoulli & Balonis, 2023).

At Serra da Capivara, fatty acids were found in association with pigments. These may have come from animal fats, but a plant origin could not be excluded without isotopic analysis (Gomes et al., 2019). At Les Dogues, the proposed viscous binder may also have included plant oils, since experiments showed that mixtures of charcoal and plant-derived fluids produced paints similar to the archaeological samples (López-Montalvo et al., 2017). At Gode Roriso, the identification of beeswax again demonstrates the persistence of waxy binders under favorable conditions (Gomes et al., 2013). These examples show the variability of plant lipids in rock art contexts, but also the difficulty of distinguishing their sources.

The interpretation of plant-based binders is further complicated by contamination. Microorganisms can produce polysaccharides and fatty acids that mimic those expected from plant gums or oils. This was seen in the Lower Pecos, Texas, where fatty acids detected in black paints were shown to derive from microbial oxalate crusts rather than original binders (Spades & Russ, 2005). Modern infiltration of sugars or waxes from the environment is another risk, especially in open-air shelters (Domingo & Chieli, 2021). For this reason, the identification of gums, resins, oils, or waxes requires caution.

Despite these challenges, plant-based binders remain an important part of the typology of potential binding agents. They represent a range of natural substances that could alter the texture, adhesion, and durability of paints. Although their identification in prehistoric rock art is less common than animal products, the cases from Brazil (Steelman et al., 2002), Spain (Chieli et al., 2022), and Ethiopia (Lofrumento et al., 2012) demonstrate their possible use. The combination of mineral pigments with gums, resins, oils, or waxes reflects an understanding of plant resources and their properties.

Chapter 3: The Analytical Challenge: Methodologies for Binder Identification

3.1 Fundamental Obstacles

3.1.1 Taphonomy and Degradation: The effects of time, light, moisture, and microbes

Analytical work on prehistoric binders is critically limited by taphonomic processes. In the Uralian sites, mineralogical and biological alterations were observed directly within the pictographs. Hematite pigments showed structural disorder, which was linked to mechanical grinding, heat treatment, and subsequent weathering (Kiseleva et al., 2023). Secondary mineral formations, including authigenic gypsum and phosphate encrustations, developed as a consequence of long-term chemical interaction with water. Calcium oxalate crusts were also identified, and these were associated with microbial colonization, indicating that biological agents contributed to the modification of the painted surfaces (Kiseleva et al., 2023). Such post-depositional accretions obscure the original pigment-binder mixture and complicate the recognition of organic compounds.

The role of microbial activity is particularly visible in Levantine rock art. High-throughput sequencing of samples from La Saltadora revealed that *Firmicutes, Enterococcus,* and *Thermicanus* dominated the bacterial community, while cyanobacteria prevailed on the surrounding natural rocks (Roldán et al., 2018). These taxa are not passive occupants. Some bacterial groups are capable of producing oxalic acid, which can reinforce a protective calcium oxalate patina (Di Bonaventura et al., 1999). Others, such as Flavobacterium, solubilize calcium carbonate and destabilize these patinas (Rinaldi, 2006). The equilibrium between producers and degraders of oxalates determines whether microbial action has a protective or destructive outcome. This balance was visible in the ratio of Bacilli to Flavobacteria, which suggested a predominantly protective microbiome in the Valltorta samples (Roldán et al., 2018). Yet this protective role is neither universal nor stable, and shifts in microbial community structure can rapidly alter preservation trajectories.

Experimental reconstructions of pigment recipes demonstrated that the stability of mixtures depends not only on binder choice but also on the substrate and exposure. When red ochre was mixed with vegetable oil, the paint was homogeneous on sandstone but unstable on granite. Conversely, the mixture of ochre and water showed better adherence on granite but faded more rapidly (Garcês et al., 2019). Water runoff and substrate porosity thereby emerge as major taphonomic factors in pigment degradation (Bednarik, 1994). Heating of ochres altered their color from bright red to darker tones, a transformation that may reflect both intentional preparation and subsequent oxidation (Barnett et al., 2006). Such changes complicate interpretation, since present hues may be products of alteration rather than original artistic decisions.

Taphonomy affects organic detection at the molecular scale. Proteomic analysis of Levantine pigments identified casein peptides, but the study noted post-translational modifications such as deamidation. Deamidation has been proposed as a marker of protein antiquity (Solazzo et al., 2014; van Doorn et al., 2012), but the authors cautioned that rates are highly dependent on environmental conditions and cannot be used uncritically (Schroeter & Cleland, 2016). The persistence of casein could therefore represent either genuine Neolithic use of milk as a binder or later contamination, a distinction that taphonomic alteration makes difficult to resolve (Roldán et al., 2018).

These studies demonstrate that time, light, water, and microbial action combine to reshape prehistoric pigments. Alteration may protect, transform, or obliterate the organic traces that scholars seek to identify. The resulting ambiguity forms a fundamental obstacle to binder analysis. Any interpretation of organic compounds must therefore consider layers of chemical accretion, microbial activity, and environmental weathering that obscure the original paint composition.

3.1.2 Micro-sampling and Contamination: The paramount importance of field and lab protocols

The analysis of binders in prehistoric rock art is attached from the problem of sampling. Often rock paintings survive as fragile surface films, and the recovery of organic traces need careful removal of only microscopic fragments. In the Valltorta case, less than 15–20 mg of material could be collected from painted motifs. This quantity had to be divided between metagenomic and proteomic approaches, pushing both techniques to their detection limits (Roldán et al., 2018). Despite careful preparation, proteomic data were dominated by human keratins, a common indicator of laboratory contamination (Leo et al., 2009; Roldán et al., 2018). The limited recovery of non-keratin proteins underscores the precarious balance between destructive sampling and the preservation of analytical reliability. Samples were collected with scalpels treated to avoid protease activity, handled with gloves, and compared against unpigmented controls from the same shelters (Roldán et al., 2018). Nevertheless, the detection of casein peptides raised the possibility that the signals might derive from modern contamination rather than prehistoric binders. Similar findings have been reported in other studies, where casein appeared repeatedly in rock art samples worldwide, but its attribution to original binders or later intrusion remained uncertain (Villa et al., 2015). This ambiguity illustrates how contamination is not incidental but structural to the methodological framework. Kiseleva et al. (2023), approached micro-sampling with a different set of techniques, including SEM-EDS, Raman spectroscopy, and GC-MS. Their analysis relied on sub-millimetric fragments, which provided detailed characterization while limiting destruction (Kiseleva et al., 2023). Yet even with such precision, the authors observed microbial aggregates within the micro-samples. These aggregates contained cyanobacteria and other taxa, showing that each fragment encompassed both pigment and later biological accretions (Kiseleva et al., 2023). The problem, therefore, was not only modern contamination introduced during collection but also ancient contamination from post-depositional colonization. Distinguishing between authentic binders and intrusive organics demanded strict comparison across samples and contexts.

Experimental reference work also demonstrates the importance of contamination-aware design. Garcês et al. produced a database of ATR-FTIR signatures from experimental paints, combining ochres with substances such as animal fat, egg, blood, and plant saps. All samples were prepared under sterile conditions, and micro-sampling was performed with sterilized scalpels (Garcês et al., 2019). This procedure ensured that reference spectra would not contain modern contaminants that could later mislead archaeological interpretation. Without such precision, experimental spectra risk embedding extraneous signatures into comparative databases, thereby compounding errors in archaeological studies.

The evidence across these studies highlights that contamination arises at multiple stages. Modern laboratory intrusions, ancient microbial colonization, and interpretive crossovers all contribute to uncertainty. The paramount importance of rigorous micro-sampling lies in designing protocols that include sterile handling, comparative unpigmented controls, and documentation of every intervention. Only under such conditions can researchers approach the fragile task of identifying prehistoric binders without conflating them with later intrusions.

3.2 Non-Invasive and *In-Situ* Techniques

3.2.1 Portable Optical Microscopy and Digital Microscopy

In-situ optical microscopy (OM) in prehistoric rock-art research refers to the use of portable, high-magnification imaging systems to examine micro-textures, pigment grain clusters, micro-laminations, brush or drag stroke markers, crusts, and micro-reliefs directly on the rock surface. Such non-destructive documentation enables stratigraphic reading, assessment of pigment layering and deterioration, and guidance for non-invasive chemical or targeted micro sampling (Dayet et al., 2022; Domingo & Chieli, 2021a; Hernanz et al., 2008; Lahlil et al., 2012). In El

Castillo cave, Cantabria, Spain, Dayet et al. (2022) used a Hirox VCR-800 digital microscope alongside *in-situ* pXRF to examine red and yellow disk paintings. The high-magnification images revealed surface heterogeneity including secondary crusts and pigment distribution, which in turn explained why pXRF readings were influenced more by environmental deposits than by pigment composition. García-Alonso et al. (2022) applied a Dino-Lite Edge AM7115MZTL microscope (100× magnification) in El Buxu Cave, Asturias, Spain, to resolve superimpositions among red painted strokes. The micro-stratigraphic view provided definitive sequencing of overlapping elements in contexts where macroscopic inspection was inconclusive, thereby clarifying relative painting chronology (García-Alonso et al., 2022). In the Sierra de las Cuerdas open-air Levantine shelters (Cuenca, Spain), Hernanz et al (2008) utilized *in-situ* optical microscopy to pre-document micro-textures such as lamination structures, coatings, and pigment morphology. The optical documentation improved contextual understanding of subsequent Raman spectra and guided laboratory-based evaluations (Hernanz et al., 2008). Guagnin et al. (2023) investigated potential prehistoric cave art in the central Mediterranean and employed a digital microscope to photograph all sampled areas prior to further analysis (Guagnin et al., 2023). The OM photographs helped determine whether pigment traces were anthropogenic in origin and documented relationships between surface weathering, crusts, and possible paint layers, organic matters. Optical or digital microscopy alone is not sufficient to detect binders or organic matter in a sample; however, it can identify the microstratigraphy of paintings, which can then be analyzed through other methods to determine their exact molecular composition.

3.2.2 In-situ Spectroscopy Portable Fourier-Transform Infrared Spectroscopy (FTIR)

In-situ spectroscopy designates a set of analytical methods capable of obtaining molecular or elemental information directly from the surface of an object without destructive intervention. Among these methods, Fourier-Transform Infrared (FT-IR) spectroscopy has become a principal tool. It operates by recording the absorption of infrared radiation by molecular bonds, producing spectra characteristic of both inorganic and organic compounds (Genestar & Pons, 2005). This allows the identification of mineral phases such as carbonates, oxalates, and silicates, as well as organic substances including proteins and lipids. The use of attenuated total reflectance (ATR-FTIR) is particularly advantageous in fieldwork, since it enables measurements on uneven surfaces with limited preparation (Brunetti et al., 2017). Hernanz et al. (2014) applied *in-situ* diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) for the spectroscopic characterization of crusts interstratified in open-air rock art shelters of the

Iberian Peninsula. The infrared spectra were acquired with a 4100 ExoScan hand-held FTIR spectrometer equipped with a DRIFTS sampling head. *In-situ* DRIFTS spectra from the painting panel of the Cova dels Rossegadors show the typical profile of calcite from a rough porous or fractured surface. Spectra from the ochre crust display bands of whewellite and gypsum. Both spectra attributable to α-quartz and clay minerals (Hernanz et al., 2014). Information about the presence of binder or organic matter is present in the study.

Portable Raman spectroscopy provides a complementary technique based on the inelastic scattering of monochromatic light. It yields highly specific vibrational spectra that are especially useful for characterizing crystalline pigments such as hematite, goethite, and manganese oxides. The development of portable Raman systems has allowed its application in caves and open-air contexts, although the frequent occurrence of fluorescence can reduce sensitivity to organic phases (Hernanz et al., 2008). Examples of this approach are the *in-situ* Raman spectroscopic study of San rock art in South Africa (Tournié et al., 2011); the *in-situ* non-destructive analysis, by making use of a suite of three different portable instruments, for carbon screening before sampling for dating prehistoric rock paintings in the Rouffignac-Saint-Cernin and Villars caves, both of them located in Dordogne, France (Beck et al., 2013; Lahlil et al., 2012).

In-situ spectroscopy, while indispensable for the study of immovable or fragile artworks, is critically constrained by a series of limitations that stem from both instrumental miniaturization and the intrinsic complexity of cultural heritage materials (Beck et al., 2013). Portable systems inevitably sacrifice optical path length, spectral range, and resolution compared to laboratory instruments, and their compact design makes them more susceptible to environmental disturbances such as ambient light or mechanical vibrations during fieldwork. Reflection FTIR exemplifies these drawbacks: spectra are distorted by the unpredictable balance of specular and diffuse reflection, producing derivative-like band profiles and, in some cases, inversion of fundamental absorptions, which renders interpretation difficult and often ambiguous. The problem is compounded in the near-IR range, where the dominance of overtone and combination bands limits specificity, reducing the technique’s discriminating power. Portable Raman spectroscopy presents analogous weaknesses: fluorescence from organic constituents or restoration materials frequently overwhelms the weak Raman signal, and the absence of confocal optics in mobile devices increases matrix effects and hampers depth resolution. Moreover, the necessity to strictly control laser power to avoid thermal or photochemical alteration of delicate surfaces restricts its operational flexibility, while the use of higher-wavelength excitation reduces fluorescence but simultaneously diminishes sensitivity due to the λ^{-4} dependence of Raman scattering. Although these methods can generate extensive datasets directly from painted surfaces, their interpretive reliability is undermined by spectral distortions, fluorescence interference, and instrumental compromises, making them heavily reliant on preparatory laboratory calibrations, spectral reference libraries, and the integration of complementary approaches to compensate for their intrinsic shortcomings (Brunetti et al., 2017).

3.3 Micro-Destructive Techniques: Laboratory-Based Analysis

3.3.1 Molecular Spectroscopy

3.3.1.1 Fourier-transform infrared (FTIR) Microscopy

Fourier-transform infrared spectroscopy (FTIR) has been accepted as essential in the analysis of prehistoric rock-art pigments and binders due to its ability to identify both crystalline and amorphous compounds, including mineral pigments, alteration products and certain organic residues. The method detects molecular vibrations by measuring the absorption of infrared light at characteristic wavelengths, allowing differentiation between chemically similar substances and the detection of functional groups indicative of binders such as proteins, lipids, or polysaccharides. In western Iberia, ATR-FTIR has been used to disentangle overlapping signals from pigments and alteration crusts. At Benquerencia, FTIR spectra confirmed hematite pigments by identifying Fe–O stretching bands while also revealing absorption features consistent with kaolinite and quartz extenders, as well as sharp sulphate peaks corresponding to gypsum efflorescence that had formed post-depositionally (Rosina et al., 2019). The Maltravieso Cave investigation similarly used FTIR to distinguish between pigment minerals and carbonate-rich alteration layers, enabling a clearer understanding of the sequence of wall accretions over stenciled motifs (Rosina, Collado, Garcês, et al., 2023,).

Comparable approaches have been applied to other contexts, where FTIR has proved especially valuable in detecting non-mineral components. In Spanish Levantine rock art, FTIR contributed to the identification of trace organic compounds alongside mineral pigments, data that were integrated with proteomic and metagenomic results to assess the likelihood of ancient binder use (Gomes et al., 2024).

3.3.1.2 Raman Microscopy

Raman spectroscopy has become a cornerstone in the archaeometric analysis of rock-art pigments and alteration layers, valued for its ability to identify crystalline and amorphous phases at micrometer scale with minimal intervention. The method operates by directing a monochromatic laser onto a target point and detecting inelastically scattered light, producing a spectrum in which peak positions and relative intensities are diagnostic of specific mineral structures (for theory and instrumentation see: Candeias & Madariaga, 2019; Ruello et al., 2022; Smith & Clark, 2004). This allows for clear discrimination between visually similar pigments, for example, separating hematite, with characteristic peaks near 225, 292 and 410 cm^{-1}, from goethite, which presents broader bands around 300–400 cm^{-1}, or from manganese oxides, which exhibit different spectral features altogether. In addition to pigment identification, Raman spectroscopy can detect alteration products such as gypsum, calcite and calcium oxalates, as well as carbon-based materials, where the disordered (D) and graphitic (G) bands occur near 1350 and 1580 cm^{-1} respectively.

Applications to schematic and Levantine rock art in the Iberian Peninsula have demonstrated the method's versatility. At the Abrigo del Águila shelter, micro-Raman confirmed the use of hematite in red motifs and amorphous carbon in black ones, while also detecting whewellite and gypsum in surface crusts, findings cross-checked with FTIR and SEM–EDS to separate original paint from post-depositional films (Rosina et al., 2018). In the Benquerencia shelter, *in-situ* Raman analyses revealed hematite-based reds and charcoal-derived blacks, alongside alteration crusts composed primarily of calcium sulphates, information that informed conservation assessments by identifying soluble salt risks (Rosina et al., 2019). The Maltravieso Cave study applied Raman to hand stencils, confirming hematite pigments and mapping gypsum overgrowths stratigraphically associated with wall microtopography, an approach that contextualized the preservation state of the motifs (Rosina, Collado, Garces, et al., 2023). In the Kimberley region of Australia, portable Raman combined with pXRF was used directly on painted panels to detect both hematite and maghemite phases within the same red motifs, revealing that some pigments had been heat-modified to alter their optical and physical properties, a technological choice supported by experimental replication (Huntley et al., 2015). In Spanish Levantine rock art, Raman spectroscopy was employed alongside molecular techniques to explore potential organic binders, detecting faint carbonaceous signals interwoven with mineral peaks, a result later evaluated through proteomic screening (Roldán et al., 2018, pp. 3–4). These cases illustrate how Raman data can be cross-referenced with complementary methods to strengthen pigment identifications, reconstruct layer sequences and characterize alteration processes.

3.3.2 Separation and Mass Spectrometry Techniques

3.3.2.1 Gas Chromatography-Mass Spectrometry (GC-MS)

Gas Chromatography–Mass Spectrometry (GC-MS) has emerged as a preeminent technique for the molecular characterization of organic binders in prehistoric rock art, chiefly identifying lipid residues such as fats, waxes, and plant resins due to their hydrophobic stability. In research on Urals cave paintings, GC-MS enabled differentiation of fatty acids within colorant microsamples, elucidating both pigment additives and potential organic binder components (Kiseleva et al., 2023). Earlier studies of rock paintings in Texas (Lower Pecos region) applied GC-MS to lipid fractions and revealed trace fatty acid levels similar in painted and unpainted rock, suggesting either minimal use or extensive degradation of fat-based binders (Spades & Russ, 2005). GC-MS analysis of red paint samples from the Dolmen de Dombate detected animal fat, with diagnostic fatty acids (myristic and palmitoleic) indicating the intentional use of cow butter as a binder. Experimental tests confirmed that butter improved plaster cohesion and reduced cracking, supporting its deliberate addition (Rivas & Carreras, 2010).

3.3.2.2 Liquid Chromatography-Mass Spectrometry (LC-MS):

Liquid Chromatography–Mass Spectrometry (LC-MS has become central to paleoproteomic investigations, offering sensitive separation and sequencing of degraded ancient proteins. One pioneering application targeted Levantine rock art in the Valltorta ravine (Castellón, Spain), where proteomic analysis of microsamples revealed potential proteinaceous binders within the paint matrix. This study also employed high-throughput sequencing to characterize microbial patina communities (dominated by Firmicutes) that may modulate protein preservation (Roldán et al., 2018).

3.3.3 Other Techniques

3.3.3.1 Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM-EDS/EDX)

Scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM–EDS) has become a fundamental technique for studying prehistoric rock-art pigments at the microstructural and elemental levels. SEM provides high-magnification imaging of pigment particles, surface textures and microstratigraphic relationships, while EDS detects and quantifies the elemental composition of targeted areas. Together, these methods can identify pigment mineralogy, detect extenders and inclusions, characterize alteration products and reveal textural evidence of pigment preparation such as grinding marks or agglomeration patterns. In painted quartzite shelters of western Iberia, SEM–EDS has been used to complement Raman and FTIR analyses, offering a deeper view of pigment microstructure (Gomes et al., 2014). The Abrigo del Águila, SEM-EDS analyses on red samples revealed the presence of Al and Si (Rosina et al., 2018). In the Kimberley rock art complex, SEM analysis revealed that while the RRS ochre source contained distinctive clusters of disc-shaped hematite (Sepúlveda et al., 2012) confined to compact clay-banded aggregates and atypical Y-coated zircon crystals, the K1 rock art pigment instead exhibited large layered Fe-sheet structures with variably sized Fe grains dispersed throughout a P-rich matrix in which Fe was consistently present (Huntley et al., 2015). In Spanish Levantine sites, SEM–EDS has been integrated with proteomic analysis to study binder preservation, detecting mineral matrices rich in iron, silica and aluminum that may have facilitated the protection of organic residues over millennia (Roldán et al., 2018).

3.3.3.2 X-ray microfluorescence (μ-XRF)

X-ray microfluorescence (μ-XRF) has become one of the most widely applied non-invasive methods for elemental characterization of prehistoric rock-art pigments, valued for its ability to collect spatially resolved chemical data without sampling. The technique operates by directing a focused X-ray beam often at spot sizes below 50μm onto a target area, causing atoms in the sample to emit secondary (fluorescent) X-rays at energies characteristic of their constituent elements. This enables the detection and quantification of major, minor and some trace elements, which can then be used to infer pigment mineralogy, detect compositional heterogeneity within a motif and differentiate between paint layers, preparation coats and alteration products (Heimler et al., 2023). In the Iberian Peninsula, μ-XRF has been used alongside Raman and FTIR to cross-validate pigment identifications and assess material variability across panels (Gomes et al., 2024). At the Benquerencia shelter, spot analyses revealed high iron counts in red motifs consistent with hematite, along with silicon and aluminum peaks linked to clay extenders and minor titanium suggestive of accessory minerals in the raw ochre source (Rosina et al., 2019). In Maltravieso Cave confirmed consistent hematite signatures across multiple stencils while detecting localized variations in silicon and potassium that may relate to substrate preparation or pigment admixtures (Rosina, Collado, Garcês, et al., 2023). μ-XRF has demonstrated its utility in identifying technological patterns and sourcing raw materials. At El Castillo Cave, combined μ-XRF, Raman and portable XRD analyses characterized yellow and red motifs as goethite- and hematite-based respectively, with elemental data showing consistent iron-to-aluminum ratios indicative of a shared geological source for different colors (Dayet et al., 2022). In the Kimberley rock art complex, μ-XRF confirmed elevated manganese levels in certain black motifs, differentiating them from charcoal-based paintings and allowing researchers to distinguish between stylistic phases linked to distinct pigment recipes (Huntley et al., 2015).

3.3.3.3 X-ray diffraction (XRD)

X-ray diffraction (XRD) analysis method used to identify the crystalline mineral phases present in rock-art pigments and alteration layers, complementing spectroscopic methods by providing unambiguous structural characteristics. The technique operates by directing X-rays onto powdered or micro-sampled material, where the resulting diffraction pattern corresponds to the atomic arrangement within the sample. This structural specificity allows XRD to resolve ambiguities in pigment identification, differentiating hematite from other iron oxides or hydroxides that may yield overlapping peaks in Raman or FTIR spectra and confirming the presence of accessory minerals such as quartz, feldspar, or clay components that may influence pigment behavior and preservation (Beck et al., 2012). In the Abrigo Remacha rock shelter (Villaseca, Segovia, Spain), XRD data reinforced Raman identifications of hematite and revealed gypsum crystallization within overlying alteration crusts, which correlated with surface efflorescence patterns observed during conservation assessment (Hernanz et al., 2013). In Maltravieso Cave, XRD analysis was conducted to confirm hematite in stencil pigments while quantifying calcite and gypsum in mineral accretions, helping to contextualize the role of wall microtopography in crust formation (Rosina, Collado, Garcês, et al., 2023). Integrated XRD applications have been crucial in cases where pigments display mixed mineralogy or have undergone post-depositional alteration. In the Kimberley region, XRD complemented Raman and pXRF analyses by distinguishing heat-altered iron oxides (hematite and maghemite) from naturally occurring ochres, supporting interpretations of intentional thermal processing in pigment preparation (Huntley et al., 2015). Portable XRD has also been successfully used *in-situ* at sites such as El Castillo Cave, where combined pXRD, pXRF and Raman analyses identified goethite and hematite in yellow and red motifs and verified the presence of clays and quartz as natural inclusions in the pigment matrix, reflecting geological sourcing patterns (Dayet et al., 2022).

Chapter 4: Results and Synthesizing the Evidence: Case Studies from the Literature

4.1 Overall Results

The results from literatures show that pigments can be identified with good confidence. Hematite is the most common mineral, while other pigments are less frequent. The detection of binders and organic matter, however, is more difficult. Many samples did not show clear traces of binders. Even when organics were present, interpretation was often problematic.

Hematite appears in almost two fifths of all samples. It was usually found together with clays, goethite, or gypsum. Its identification was consistent across methods such as Raman, XRF, and XRD (Rosina et al., 2018; Huntley et al., 2015). Other minerals, such as magnetite or ferrihydrite, were detected in a smaller number of cases. Rare pigments include jarosite, which was used in Kimberley rock art (Huntley et al., 2015), and bone black, found at Zmiev Kamen' in the Urals (Kiseleva et al., 2023). Manganese oxides were used in both European cave art and Texan pictographs (Beck et al., 2013; Spades & Russ, 2005). These identifications are solid because mineral structures leave clear signals in Raman spectra, XRD patterns, and SEM–EDS data.

Binders and organics are harder to identify. They often appear as degraded. Examples include the black camelid motifs at Alero Hornillos 2 in Argentina, where TXRF, FTIR, and GC–MS revealed ruminant fat (Vázquez et al., 2008). Similar fatty residues were found at Serra da Capivara in Brazil (Gomes et al., 2019) and in Ural sites (Kiseleva et al., 2023). Proteins, especially casein from milk, were identified with proteomics in Levantine art at La Saltadora, Spain (Roldán et al., 2018). This was the first solid proof of milk as a binder in prehistoric rock art. Other reports include blood (Brook, 2008; Golovanova et al., 2024), saliva and honey suggested by experiments at Les Dogues (López-Montalvo et al., 2017), and possible plant gums in South African contexts (Prinsloo, 2013; Tournié et al., 2011).

The main challenge is that organic matter degrades fast and can be contaminated. Several cases highlight this problem. In the Lower Pecos, Texas, Spades and Russ (2005) found fatty acids in paint samples, but the same compounds were also in oxalate crusts. This means the lipids were from microbial growth in the crust, not from binders in the paint. Similar warnings have been given in other studies. Domingo and Chieli (2021) argued that proteins or lipids found in open-air shelters may come from the environment. At Les Dogues, López-Montalvo et al.

(2017) saw blocked plant cells under SEM, which suggested a viscous binder. But no chemical proof was found.

Different methods show different strengths and limits. Portable Raman and XRF are excellent for pigment detection, but they cannot detect organic residues. FTIR sometimes shows lipid or protein groups, but the signals often overlap with those of gypsum or carbonates (Hernanz et al., 2014). GC–MS is the main tool for lipids, but interpretation depends on separating ancient residues from later contamination. Some progress has been made by looking at sterane biomarkers and specific fatty acid ratios, as in the Alero Hornillos study (Vázquez et al., 2008). Newer work uses proteomics to detect peptides with high accuracy, such as casein in Spain (Roldán et al., 2018). Lipidomics can now tell apart ruminant fats from monogastric fats or marine oils (Kiseleva et al., 2023). These methods give more certainty, but they require destructive sampling and are vulnerable to modern contamination.

Negative results are also important. The fact that hematite samples rarely show binders may not mean that binders were absent. Organics may have degraded or escaped detection. Some studies confirm this. At Idrisovskaya II in the Urals, no binder was found because the sample was too small (Kiseleva et al., 2023). At Serra da Capivara, the association of organics with oxalates raised doubts about their origin (Gomes et al., 2019). The absence of organics in many hematite paints could also reflect bias in methods. Iron-rich surfaces are poor at preserving organics, and most tests used (Raman, XRF) target minerals, not organics.

Where binders are confirmed, their meaning is significant. Animal fats in South America and Eurasia show that prehistoric artists used animal products for paint preparation. Casein peptides in Iberia link rock art to the exploitation of milk in early farming societies. Honey and saliva at Les Dogues may reflect opportunistic choices of materials. These identifications demonstrate knowledge of paint preparation and use of resources from daily life. They also show that a variety of binders was used, each giving the paint different properties.

The results show both progress and limits in rock art research. Pigments are well documented, with hematite as the clear favorite. Binders are more elusive. Positive cases such as ruminant fat, casein, blood is rare but highly valuable, as they reveal cultural practices. Most samples, however, give no clear answer, which reflects both decay of organics and the limits of methods.

Table 1: Positive detection of binder evidence across the regions

Sample	Site/Region	Raman	ATR-FTIR	GC-MS	SEM-EDS	Remark	Reference
Mzm-2016 (Sample 1, bone pendant)	Mezmaiskaya Cave, Russia	-	Bitumen, Proteinaceous glue (Amide I, Amide II), Red bolus/kaolin	Modern contaminants only (phthalates, sunscreen)	Increased Si, Al, Fe; consistent with red bolus/kaolin; Ca/P ratio 2.35	Black pigment residues; composite paint with bitumen, red bolus/kaolin, proteinaceous binder (animal glue); earliest direct evidence of animal glue boiling (~31–27.5 ka BP).	Golovanova et al., 2024
Mzm-2002 (Sample 2, mammoth tusk stripe-bead)	Mezmaiskaya Cave, Russia	-	Bitumen, Proteinaceous glue (Amide I, Amide II), Red bolus/kaolin	Modern contamination; no ancient organics identified	Elevated Si, Al, Fe; Ca/P ratio 1.90; red bolus signature	Black pigment residues; composite paint with bitumen, red bolus/kaolin, proteinaceous binder (animal glue); significant for Upper Paleolithic (~25.5–23 ka BP).	Golovanova et al., 2024
Ignatievskaya Cave – Red pigment (cruciform/horse)	Ignatievskaya Cave, Russia	-	-	Ruminant animal fat (palmitic, stearic, odd-chain fatty acids, bone marrow signature)	Hematite, clayey extender	Red pigment; hematite, clay. Binder identified: ruminant fat (bone marrow). Groundbreaking first lipid-based binder in Uralian rock art.	Kiseleva et al., 2023
Zmiev Kamen' – Red pigment	Zmiev Kamen', Russia	-	-	Monogastric animal fat (palmitic, stearic, myristic, oleic, iso-C15:0, iso-C16:0)	Hematite, bone black (calcined bone carbon), clay, gypsum/apatite	Red pigment; hematite with bone black. Binder identified: monogastric fat (pig/boar lard). First lipid evidence for fat binder in Ural open-air pictographs.	Kiseleva et al., 2023
Buitres_3	Los Buitres 1 Shelter, Spain	Ochre	Ochre; fatty acids.	-	-	Dark-red fingerprints; ochre, fatty acids; possible organic binder (animal or plant origin).	Garcês et al., 2022
BSQM-2	Benquerencia, Spain	Hematite, Quartz	Clay, Organics	Organic matter (herbs, plant origin)	-	Red fingerprints; hematite with clay, organics; binder evidence (vegetal).	Rosina et al., 2019
BSQM-4	Benquerencia, Spain	Hematite, Magnetite	Clay, Organics (pyridine-like)	Organic matter (pyridine-like, plant root)	-	Red figure; hematite, clay, pyridine-type organics; binder evidence.	Rosina et al., 2019
BSQM-6	Benquerencia, Spain	Hematite	Red ochre (clay), Nitrogen substance	Organic matter (N, P substances)	-	Large red figure; hematite, red ochre, nitrogenous substance; binder evidence.	Rosina et al., 2019
CSI-01 (Goat motif)	La Saltadora, Spain	Hematite	-	-	-	Proteomics identified casein peptides (bovine milk). Groundbreaking evidence for milk as a binder in Levantine rock art.	Roldán et al., 2018

Sample	Site/Region	Raman	ATR-FTIR	GC-MS	SEM-EDS	Remark	Reference
CSV-01 (Deer motif)	La Saltadora, Spain	Hematite	-	-	-	Proteomics identified casein peptides (bovine milk).	Roldán et al., 2018
CSV-02 (Archer motif)	La Saltadora, Spain	Hematite	-	-	-	Casein peptides (milk) strongly enriched vs controls. First direct proteomic evidence of organic binder in Levantine art.	Roldán et al., 2018
Paint 1 (dark red)	San rock art, South Africa	Specularite (hematite)	Fat, Egg proteins	Egg proteins, fatty acids (palmitic, stearic, oleic)	-	Dark red paint; specularite pigment; fat, egg binder strongly present.	Prinsloo et al., 2013
Paint 2 (yellow)	San rock art, South Africa	Goethite (yellow ochre)	Egg proteins	Egg proteins	-	Yellow paint; ochre pigment; egg binder confirmed.	Prinsloo et al., 2013
Paint 3 (white)	San rock art, South Africa	Raptor faeces (uric acid)	Raptor faeces	Egg white obscured by uric acid	-	White paint; pigment raptor faeces; saliva/water not detectable; egg masked.	Prinsloo et al., 2013
Paint 4 (white)	San rock art, South Africa	Bone white (phosphate)	Egg proteins	Egg proteins, fatty acids (low intensity)	-	White paint; bone white pigment; egg binder faint but detectable.	Prinsloo et al., 2013
Red pigment (detailed)	Dolmen de Dombate	-	Animal fat detected	Animal fat detected; fatty acids (myristic, palmitoleic) → cow butter	Fe confirmed; C, S, P detected	Clear case of intentional binder (cow butter) added to reduce cracking; experimental reproduction confirmed	Rivas & Carrera 2010
Plaster (revoco)	Dolmen de Dombate	-	Animal fat detected	Animal fat detected; cow butter indicated	SEM shows stratification; C, S, P detected	Evidence of butter addition also in revoco, improving resistance to cracking	Rivas & Carrera 2010
VQ1-1 (reddish dots)	Patagonia, Argentina	Hematite; Animal fat (binder)	-	-	Fe-rich areas >91% Fe2O3; Ca–Si–Al matrix;	20–40 µm paint layer; animal fat identified by Raman 532 nm; calcium oxalate present.	Brook et al., 2008
M10 (black camelid leg, Gley 3/1)	Alero Hornillos 2, Argentina	-	lipids (KBr pellet)	Animal fat (lipid binder): degraded ruminant fat	-	Gypsum abundant. Lipid extract ~962 µg g^{-1}. Minor n-alkanes (C22–C25) and steranes reported. Binder = animal fat (ruminant).	Vázquez et al., 2008
M15 (black camelid leg, Gley 4/1)	Alero Hornillos 2, Argentina	-	lipids (KBr pellet)	Animal fat (lipid binder): degraded ruminant fat	-	Gypsum abundant. Lipid extract ~36,780 µg g^{-1}. Minor n-alkanes and cholestane observed. Binder = animal fat (ruminant).	Vázquez et al., 2008

4.2 The "Classic" Case Studies: Early Claims and Their Re-evaluation

Efforts to identify organic binders in prehistoric rock art have long been driven by two motivations: to understand the technological choices of ancient painters and to secure datable carbon for direct radiocarbon analysis. The case of *La Casa de Las Golondrinas* in the Guatemalan Highlands exemplifies how these objectives could converge in early research and, ultimately, how re-evaluation forced scholars to reconsider claims that seemed promising but were in fact methodologically flawed.

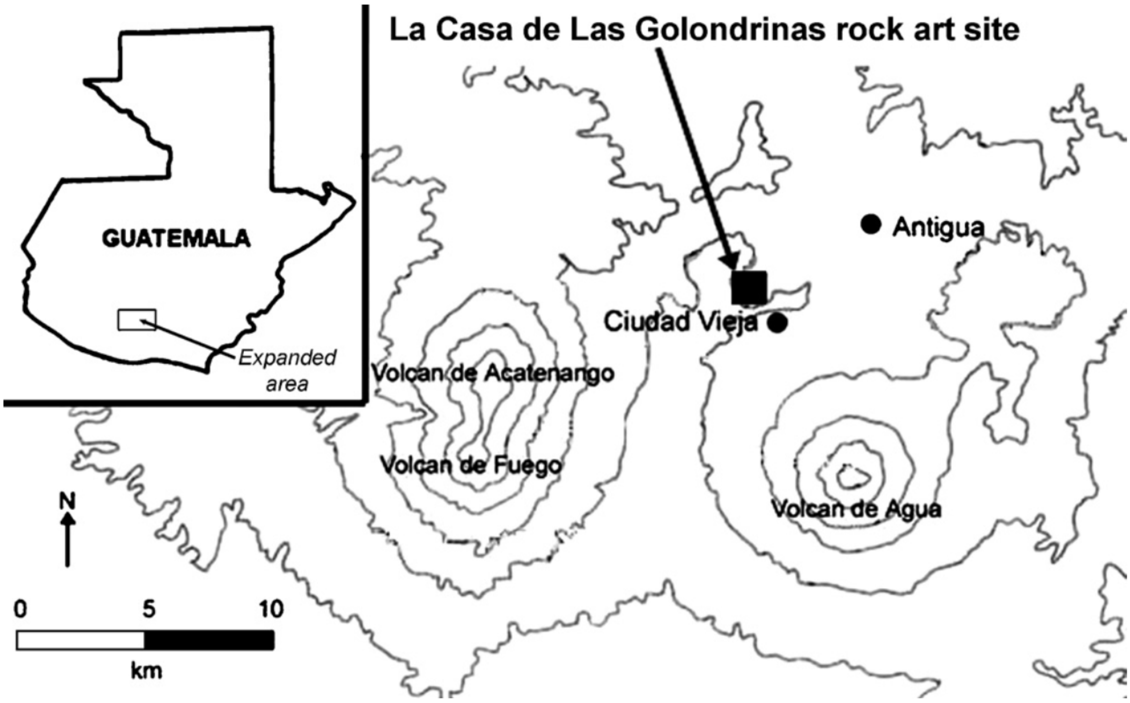

Figure 1: Approximate location of La Casa de Las Golondrinas in the Guatemalan Highlands (Livingston et al., 2009).

At La Casa de Las Golondrinas in the Guatemalan Highlands, the question of whether pigments were mixed with organic binders has been central to attempts at direct dating. The broader tradition of such research stretches back to the late 1970s and 1980s, when accelerator mass spectrometry (AMS) was first applied to small organic samples in order to bypass the destructive sampling required by conventional radiocarbon methods (Bennett et al., 1977; Nelson et al., 1977). Early successes came with charcoal pigments, rich in inherent carbon, such as in South Africa and later Paleolithic France (Clottes et al., 1992; Van der Merwe et al., 1987). In North America, AMS was applied to charcoal-based paints in the early 1990s, but these also demonstrated the so-called “old charcoal” problem, where the age of the pigment material itself could pre-date its artistic use (Farrell & Burton, 1992; Geib & Fairley, 1992).

When pigments other than charcoal were encountered, other organics, such as beeswax or plant fibers were at times identified as binding agents and targeted for dating (Cole & Watchman, 1992; Nelson et al., 1995; Watchman & Cole, 1993). The development of plasma-chemical oxidation (PCO) by Russ et al., offered a way to isolate the tiny amounts of carbon presumed to derive from a binder or vehicle in ochre paintings (Russ et al., 1990, 1991). PCO–AMS was applied in Texas and then widely elsewhere, becoming a cornerstone for direct dating of non-charcoal paintings (Armitage et al., 2001; Chaffee et al., 1993). Yet this method soon faced criticism because it oxidized all organic carbon present, whether associated with the paint or introduced from extraneous sources such as humic acids, lichens, or human activity (Chaffee et al., 1993; Hyman & Rowe, 1997).

Within this trajectory, La Casa de Las Golondrinas represented an ambitious attempt to secure dates from the largest known rock art site in the Highlands, with over 225 figures executed on volcanic tuff walls. The site, located in the Antigua Valley, is embedded in a landscape of volcanoes, springs, and lakes, and human presence there is documented for at least six millennia (Livingston et al., 2009). Its imagery ranges from Late Postclassic Mayan motifs to undatable forms, making direct AMS analysis particularly attractive (Livingston et al., 2009). Samples were collected under sterile conditions in 2003, targeting both painted flakes and, where possible, adjacent unpainted substrate for comparative purposes. Two samples produced measurable radiocarbon ages: one around 1500–900 cal B.C. (Sample 4) and another around 6250–5550 cal B.C. (Sample 8) (Livingston et al., 2009). At first sight, these ages seemed consistent with the long sequence of occupation in the valley and gave the impression that organic binders preserved in the pigments could indeed provide chronologically meaningful results (Livingston et al., 2009).

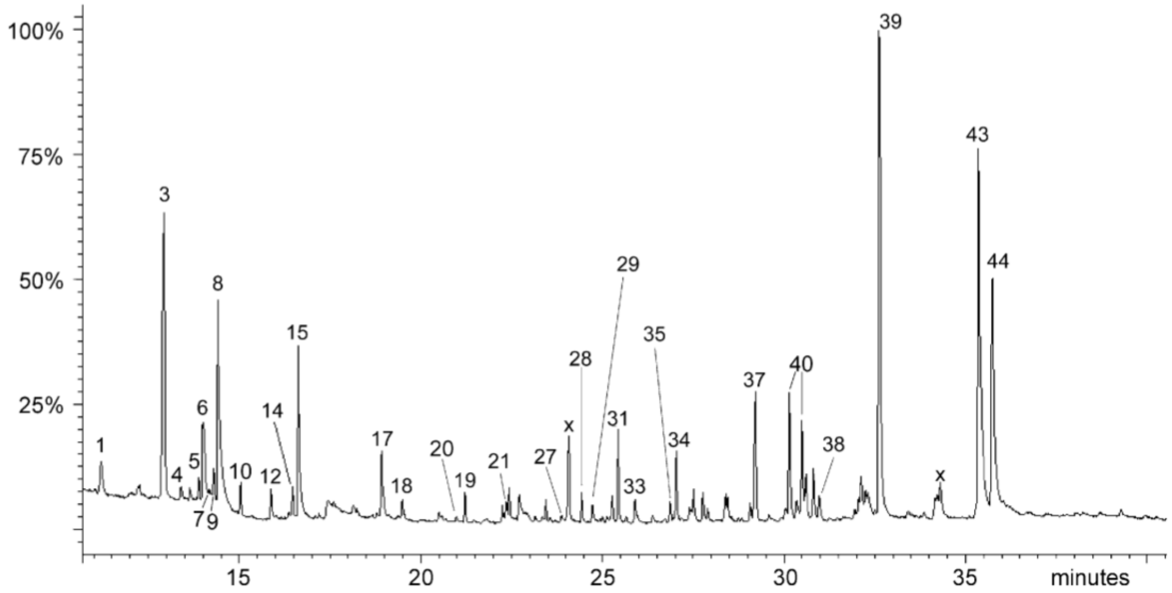

Figure 2: Total ion chromatogram for paint Sample #8, which yielded a date of 6890 ± 160 uncalibrated BP ((Livingston et al., 2009).

The subsequent chemical characterization of these same samples, however, raised significant questions. Thermally assisted hydrolysis/methylation–gas chromatography–mass spectrometry (THM–GC–MS) was applied to both paint and substrate. The unpainted tuff was found to yield substantial carbon on its own, and chromatograms revealed fatty acids and other organic compounds that were indistinguishable from those present in the paint layers (Livingston et al., 2009). This echoed earlier warnings by Spades and Russ (2005), who had shown that lipid concentrations in Texas rock paintings were no higher than in unpainted limestone, suggesting that their presence was not diagnostic of binders (Spades & Russ, 2005). At La Casa de Las Golondrinas, fatty acids, carbohydrates, and nitrogen-containing compounds appeared sporadically, but in patterns consistent with environmental contamination or microbial activity rather than a distinct anthropogenic medium (Livingston et al., 2009). Sample 8, in particular, showed abundant phenolic derivatives characteristic of humic acids, even after alkaline pretreatment designed to remove them. When compared with humic fractions extracted from a gourd cache at the site, the chemical profiles matched closely, pointing to soil-derived organics as the likely source of the carbon that produced the radiocarbon date (Livingston et al., 2009). Sample #4 contained some carbohydrates and long-chain fatty acids that might, under other circumstances, be read as traces of a binder, but without substrate controls from the same panel, these signals could not be distinguished from background contamination (Livingston et al., 2009). Other samples produced negligible yields, confirming the difficulties of extracting meaningful organics from such contexts.

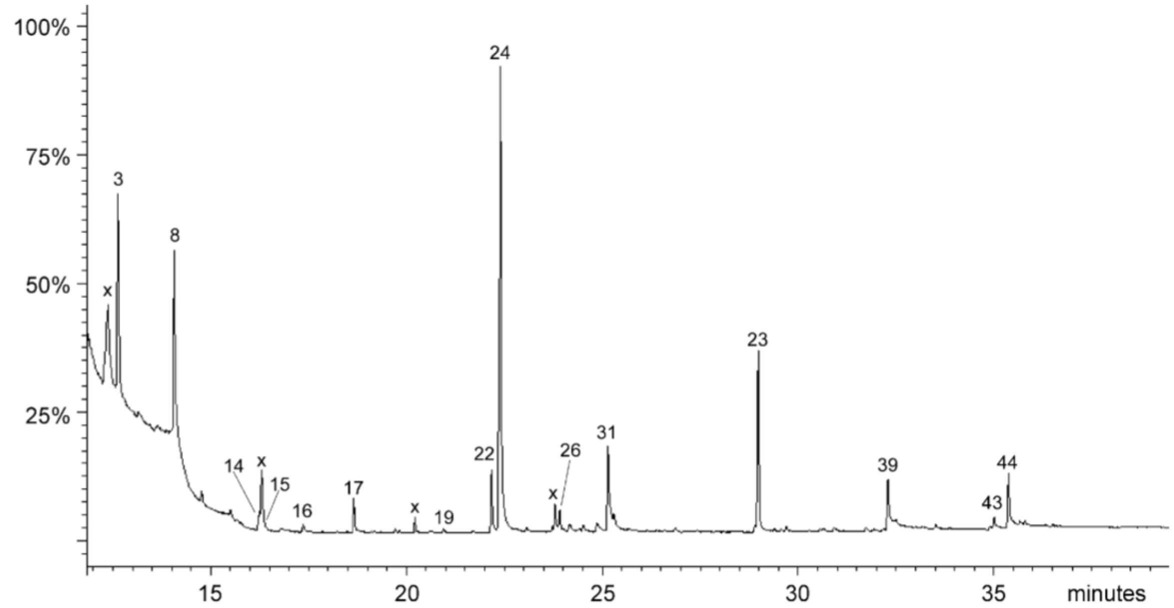

Figure 3: Total ion chromatogram for paint Sample #4, which yielded a date of 3010 ± 90 uncalibrated BP (Livingston et al., 2009).

The case therefore highlights a critical issue in the study of prehistoric binders: the difficulty of discriminating genuine anthropogenic additives from environmental organics that infiltrate rock surfaces over time. Earlier studies employing gel electrophoresis, Raman and FTIR spectroscopy, or even DNA amplification had suggested binders in other traditions, such as the Pecos River paintings, but these claims were later reevaluated by gas chromatography and DNA reanalysis, which cast doubt on the initial identifications (Edwards et al., 1998; Mawk et al., 2002; Reese et al., 1996; Russ et al., 1993) Similarly, controversies in Australia, including work at Laurie Creek, exposed how easily contamination could obscure interpretation (Gillespie, 1997; Loy, 1994; Nelson, 1993). Against this backdrop, La Casa de Las Golondrinas illustrates how optimistic readings of organic signals as binders and carriers of chronological information could not withstand rigorous chemical comparison between paint and substrate. The presence of humic acids and background lipids proved that organic matter in such samples cannot be assumed to reflect painting technology, and that direct radiocarbon ages based on these signals may lack anthropological meaning. This realization has reinforced the necessity of pairing chemical characterization with dating protocols and of treating every claim of binder identification with caution.

4.3 Successful Identifications: A Thematic Review

4.3.1 Case Study 1: The Los Buitres 1 rock shelter

The Los Buitres 1 rock shelter lies in the municipality of Capilla in northeastern Badajoz, Spain, on quartzite walls that open toward the southeast and overlook the Zújar River corridor linking La Serena's plains with the Guadalquivir Valley; the cavity is about 11.2 m deep with ceiling heights exceeding 15 m, and conservation issues include biological activity, natural coatings, and minor damage from nesting vultures (Garcês et al., 2022). Its schematic rock art, anthropomorphs, zoomorphs, sun figures, circular and geometric motifs, appears in red from light to dark tones and occasionally in black, with superimpositions suggesting multiple phases; in the Iberian tradition, such schematic imagery is broadly framed from the Early Neolithic through the Late Bronze Age and is anchored historically by foundational studies from Breuil and subsequent syntheses and typological work that set the style's formal and thematic rules (Acosta, 1968; Breuil, 1933; Collado Giraldo & García Arranz, 2013; Collado, 2006; Garcês et al., 2022; Hernández Pérez, 2006; Ruiz et al., 2012).

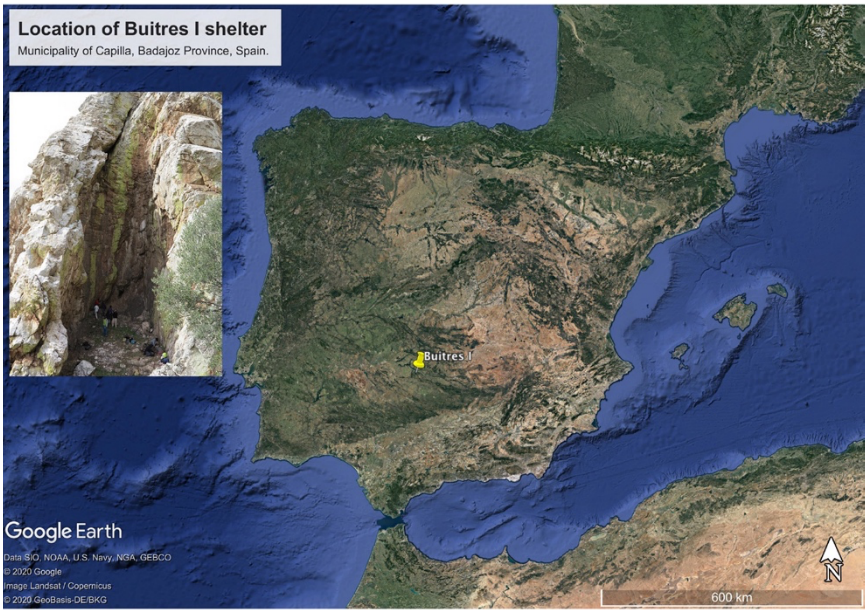

Figure 4: Location of the Buitres 1 rock shelter (Garcês et al., 2022).

To investigate pigment technology and, critically, the presence of organic matter within the paint, the study selected four tiny pigment samples (three red, one dark red) from distinct motifs, plus a bedrock control; micro-Raman spectroscopy (LabRam HR800, 632.82 nm excitation) was used to establish mineralogy, while ATR-FTIR (Bruker Alpha, diamond ATR, 4000–400 cm^{-1}, 4 cm^{-1} resolution) targeted both mineral and organic functional groups, noting that microstratigraphy was not possible due to sample size (Garcês et al., 2022). Raman and FTIR results show that samples 1 and 2 from red figures are hematite-based ochres with clay signatures attributable to montmorillonite, a combination well attested for schematic red paints (Gomes, 2015; Mas et al., 2013). The key finding concerns sample 3 from dark-red fingerprint motifs: ATR-FTIR recorded bands at 2924 and 2853 cm^{-1} diagnostic of CH_3/CH_2 stretching in lipids alongside ochre-related peaks near 1007, 911, 778, 518, and 451 cm^{-1}, indicating the presence of fatty acids mixed with red ochre; the absence of phosphorus and nitrogen reduces the likelihood that these organics derive from bacterial or fungal residues, although the authors cautiously note alternative sources such as plant materials, a possibility also entertained in regional parallels (Ch'ng et al., 2016a; Kooli et al., 2018; Mastandrea et al., 2011; Omotoso & Ajagum, 2016). Comparative spectra from nearby Benquerencia La Serena (Cueva Media) fingerprint figures show strikingly similar organic signatures, raising the prospect, still tentative, of a shared pigment "recipe" for fingerprint motifs within the same regional and chronological framework (Rosina et al., 2019). Sample 4 from a zoomorph is consistent with burnt umber, inferred from iron-oxide bands (hematite) together with features near 1030 and 470–555 cm^{-1} and interpreted as heat-modified umber, suggesting thermal processing to obtain darker reddish-brown tones (Garcês et al., 2022; Genestar & Pons, 2005).

Figure 5: *In-situ* samples and digital enhancement with DStretch (Garcês et al., 2022).

In context, the range of red hues at Los Buitres 1 may reflect technical control over hematite-based pigments and perhaps symbolic choices in color shading, consonant with wider Iberian practices from the Late Upper Paleolithic through early farming communities (Domingo et al., 2012; Gomes, 2015; Hernanz et al., 2012; C. J. S. Oliveira et al., 2017, 2019).

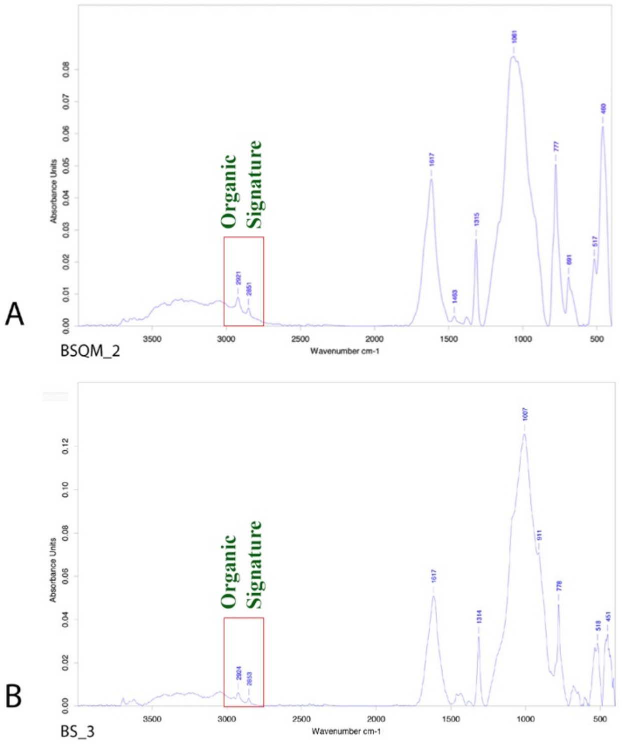

Figure 6: the presence of organic signature in the sample 3 (BS_3) in compare with Benquerencia (BSQM_2), detected by ATR-FTIR (Garcês et al., 2022).

While the ATR-FTIR evidence for fatty acids in sample 3 constitutes a successful identification of organic matter in a prehistoric paint, an uncommon outcome given volatilization, sampling limits, and instrumental constraints, the authors treat the attribution with due caution, emphasize the preliminary nature of FTIR-based lipid assignments, and note that multiproxy biomolecular methods such as GC-MS and LC-MS have yielded higher-confidence binder identifications elsewhere; nonetheless, the convergence of lipid bands in the Los Buitres fingerprint paint with the Benquerencia comparison (See: Figure 6) provides a coherent, conservative argument for intentional organic addition to pigment in the regional schematic tradition, inviting targeted follow-up with complementary techniques and a broader sampling strategy to confirm binder identity and distribution (Brook et al., 2018; Garcês et al., 2022; Rivas & Carreras, 2010; Roldán et al., 2018; Vázquez et al., 2008).

4.3.2 Case Study 2: Viuda Quenzana, Patagonia, Agentina

Viuda Quenzana lies on the southern Deseado Massif of central-southern Patagonia, Argentina, in a shallow canyon about 4 km long that trends northeast to southwest and drains via an ephemeral channel to the intermittent Seco River, with abundant rock shelters developed in cream-white Jurassic ignimbrites of the Chon Aike Formation (Panza & Marin, 1998). The local climate at Gobernador Gregores is cold desert (BWk) with mean annual precipitation near

185 mm, an average 55 precipitation days per year, and a mean annual temperature around 8.5 °C, conditions that favor thin spalling of ignimbrite walls and the accumulation of fallen painted flakes on shelves and floors (Brook et al., 2018). Survey documented 44 sites with rock art, dominated by painted motifs including multicolored hand negatives, dots, zoomorphs, and geometric figures; engravings occur but are uncommon, and there is no macroscopic evidence of lichens or smoke staining on shelter walls and ceilings today, a detail that constrains potential sources of organic films (Acevedo, 2017; Fiore & Acevedo, 2016; Brook et al., 2018).

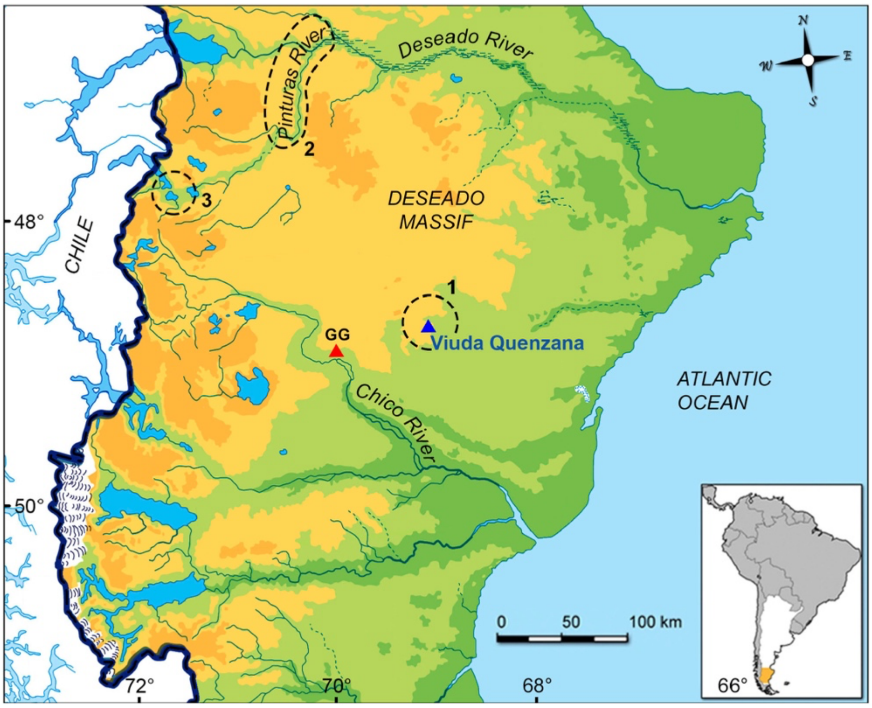

Figure 7: Location of the Viuda Quenzana (VQ) rock shelter (Brook et al., 2018).

Brook et al. (2018) studied two motifs from two shelters separated by roughly 80 m to rigorously test for binders: a reddish dotted motif fragment from VQ1 (sample VQ1-1) and a pink negative hand fragment from VQ2 (sample VQ2-2), both on friable ignimbrite (Brook et al., 2018). VQ1 is a north-northwest–facing cave about 17 m wide, 7 m deep, and 6 m high with multiple panels (See: Figure 8-a); the studied fragment bears one complete dot 1.5 cm in diameter and part of another and was found on a rock shelf beneath a panel of dotted red motifs consistent in hue and design with the fragment's paint (See: Figure 9-a) implying detachment from that panel (Acevedo, 2017; Brook et al., 2018;). VQ2 is a northwest-facing cave about 22 m wide, 10 m deep, and 7 m high; the studied piece is a 2 × 2 cm flake from a deteriorated pink negative hand panel (See: Figure 8-b) that also preserves two other hand negatives and several pink stains, sampled along natural fissures to minimize impact (Acevedo, 2017; Brook et al., 2018).

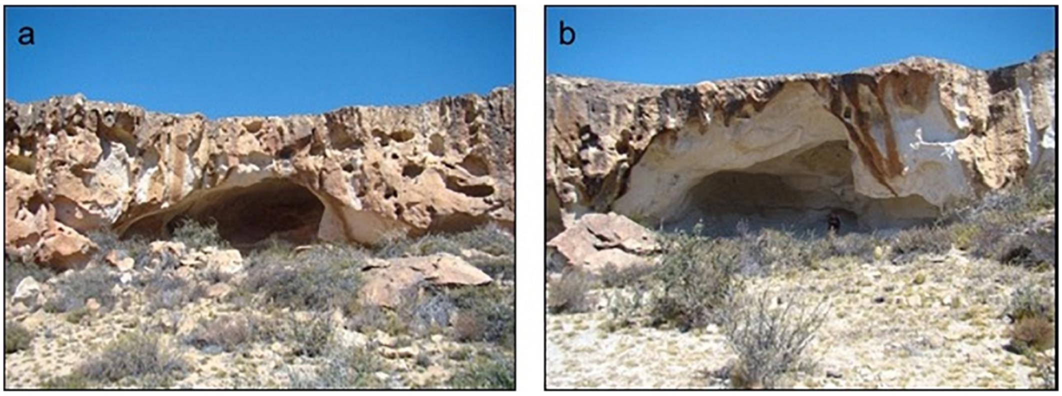

Figure 8: View of VQ1(a) and VQ2 (b) rock shelter. (Source: Brook et al., 2018).

Collection avoided direct contact with painted surfaces, bagged samples in clean plastic, and maintained low-temperature dark storage to suppress microbial growth, a chain of custody that matters for organic detection (Brook et al., 2018).

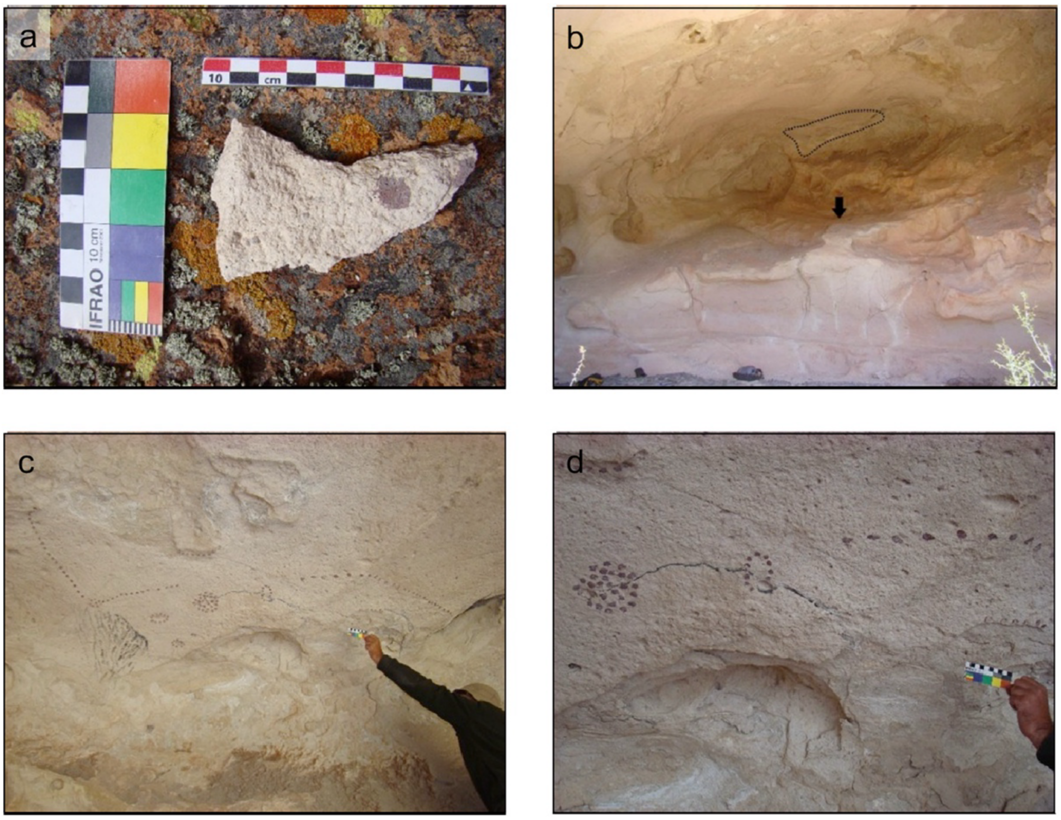

Figure 9: Sample VQ1-1 (a) Detached rock fragment (VQ1-1) from the shelter wall bearing one complete and one partial reddish dot on the right. (b) Arrow indicates the find spot of the fragment, while the dotted line marks its probable original position within the panel. (c) Overview of the full panel showing reddish dots arranged in straight lines, curved lines, and circular geometric motifs (VQ1). (d) Detail illustrating the advanced degradation of both the paintings and the underlying rock surface (VQ1) (Brook et al., 2018).

Analytical design prioritized the chemical identification of organics at the paint surface and the segregation of any environmental carbon from true binders before any chronological inference. Powder X-ray diffraction (Bruker D8 Advance, Co-Kα, 40 mA, 40 kV, 3–80° 2θ at 0.1 s per step) characterized substrate mineralogy several millimeters below exposed faces (Brook et al., 2018). Micro-Raman spectroscopy used two systems: a Renishaw InVia at 785 nm with about 2 cm^{-1} resolution, Si 521 cm^{-1} calibration, and 50–200× optics, and a 532 nm probe-head Kaiser/Verdi-V5 setup with 20 s acquisition time, 0.1–0.5 W laser power, millimeter-scale spot, and about 2 cm^{-1} resolution; spectra were baseline-corrected and cosmic spikes removed, permitting confident assignment of mineral and organic bands (Brook et al., 2018). High-resolution SEM/EDS used an FEI Teneo FE-SEM (10 kV imaging, carbon coat) and an Oxford X-MAXN 150 mm detector at 20 keV to map paint microstratigraphy and elemental associations, especially Ca and Fe (Brook et al., 2018). AMS radiocarbon sampled with a rotary Dremel diamond bit, unavoidably mixing paint with the uppermost substrate; pretreatment used 1 N HCl at 80 °C for 1 h to remove carbonates and calcium oxalates following protocols shown to be effective by FTIR testing in prior work; combustion proceeded at 900 °C, CO_2 was purified and graphitized per Cherkinsky et al. (2010), and measurement used a 0.5 MeV AMS with size-matched standards and backgrounds to control small-sample effects (Bonneau et al., 2011, 2016; Cherkinsky et al., 2010, 2013). Radiocarbon ages used the 5568-year half-life, $\delta^{13}C$ corrections to −25‰, and calibration with CALIB 7.0 and SHCal13 to 2σ ranges and median probability ages, ensuring transparent reporting of chronological uncertainty (Hogg et al., 2013; Reimer et al., 2013; Stuiver & Reimer, 1993).

Raman spectra of VQ1-1 substrate revealed calcium oxalate monohydrate (whewellite) via its diagnostic doublet just below 1500 cm^{-1} along with gypsum and broad bands between 1200 and 1500 cm^{-1} (See: Figure 10) that the authors interpret as organics while recognizing possible contributions from disordered aluminosilicates noted in other studies, a necessary ambiguity in porous ignimbrite (Bonneau, Pearce, et al., 2017; Frost & Weier, 2003). The presence of whewellite and diffuse organics several millimeters below the surface indicates the post-exposure ingress of non-native substances into the rock matrix, a taphonomic reality that complicates the attribution of any generic organic band to a paint binder without surface-specific confirmation (Brook et al., 2018).

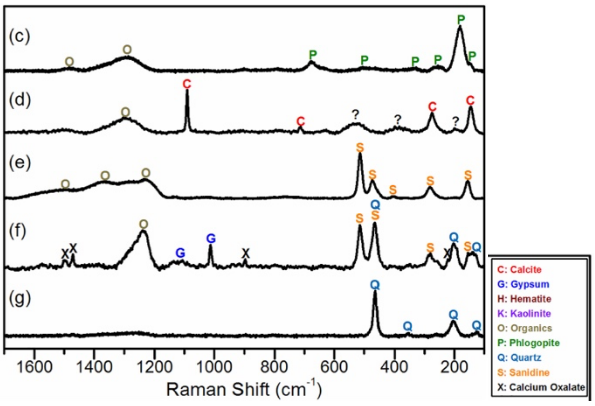

Figure 10: Micro-Raman spectroscopy of individual grains in the rock substrate from sample VQ1-1: black grain (c), black grain (d), light brown grain (e), white grain (f), and light grey grain (g) (Brook et al., 2018).

Pigment identification is unambiguous: α-Fe_2O_3 occurs in both motifs, and in the VQ1-1 dot paint SEM/EDS maps reveal heterogeneous zones with local Fe_2O_3 contents above 90% surrounded by Ca-, Si-, and Al-bearing matrices, while backscattered SEM images show 0.5–2 μm platelets diagnostic of microplaty hematite, a distinctive morphology also recognized in other rock-art contexts but here functioning mainly as a textural marker of pigment preparation (Brook et al., 2018). Calcium is enriched around Fe-rich particles in the dot paint, reaching approximately 27% CaO in mapped areas, a spatial association that invites but does not prove a link between Ca-bearing phases and paint organics (Brook et al., 2018).

The key binder result rests on surface-focused Raman spectroscopy at 532 nm. On the painted surface of VQ1-1, spectra exhibit bands near 1600, 1400, 1350, 1300, and 1240 cm^{-1} that the authors assign to vibrations of unsaturated triglycerides, a pattern reported for animal fats in controlled simulations, pure fat references, and archaeological paints, and cited accordingly (Boyacı et al., 2014; Maier et al., 2005; Prinsloo et al., 2008). That match supports a specific identification of animal fat as an organic binder in the VQ1-1 dot paint, a result the study treats as decisive because it derives from the paint surface itself rather than from the bulk substrate or mixed powders (Brook et al., 2018). Equivalent 532 nm measurements on the VQ2-2 hand paint do not show lipid bands, and the authors therefore report no resolved binder for that motif, noting the discontinuous, thin paint consistent with spray application that may not require or preserve a grease binder at detectable levels (Brook et al., 2018).

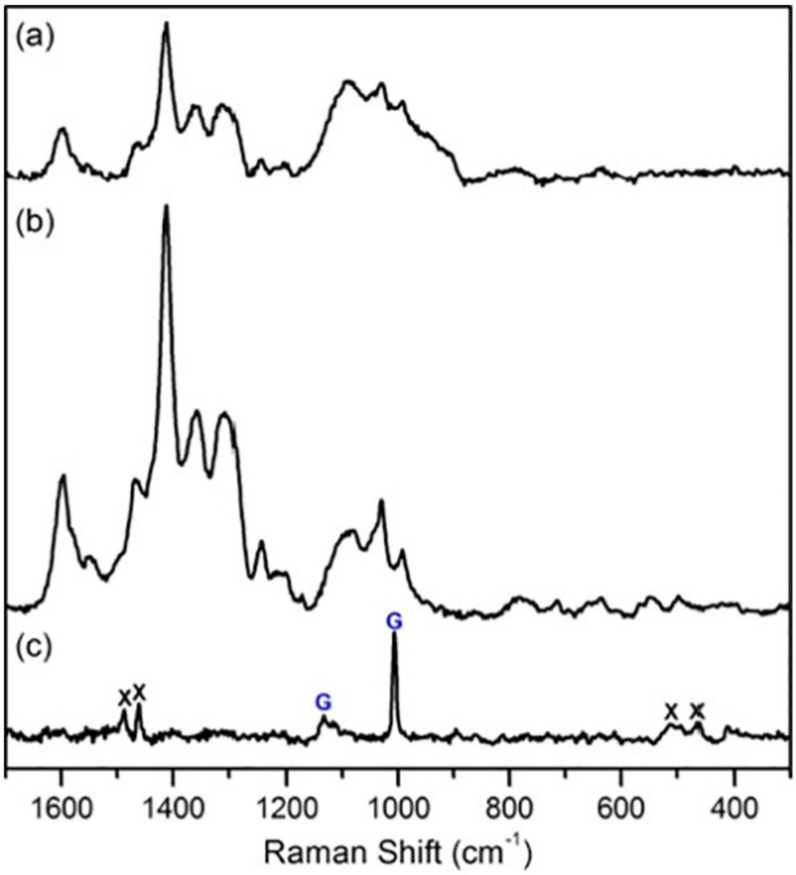

Figure 11: Raman spectra from sample VQ1-1 (532 nm laser): two colored areas (a, b) and a substrate area (c) (Brook et al., 2018).

Broader organic signals at 785 nm occur in both paints and substrates as ill-defined 1200–1400 cm^{-1} envelopes, but the authors explicitly refuse to treat those as evidence of a binder because similar bands appear below the paint and may arise from environmental inputs or aluminosilicate disorder; only the 532 nm lipid pattern underpins the binder identification in the dotted motif (Bonneau, Pearce, et al., 2017). This methodological caution echoes published warnings that dating or interpreting “unidentified organics” in rock-art contexts is hazardous because carbon can derive from oxalates, micro-organisms, humics, or soot deposited long after painting, and the authors cite those cautions to frame their approach (Watchman, 1993; Livingston et al., 2009).

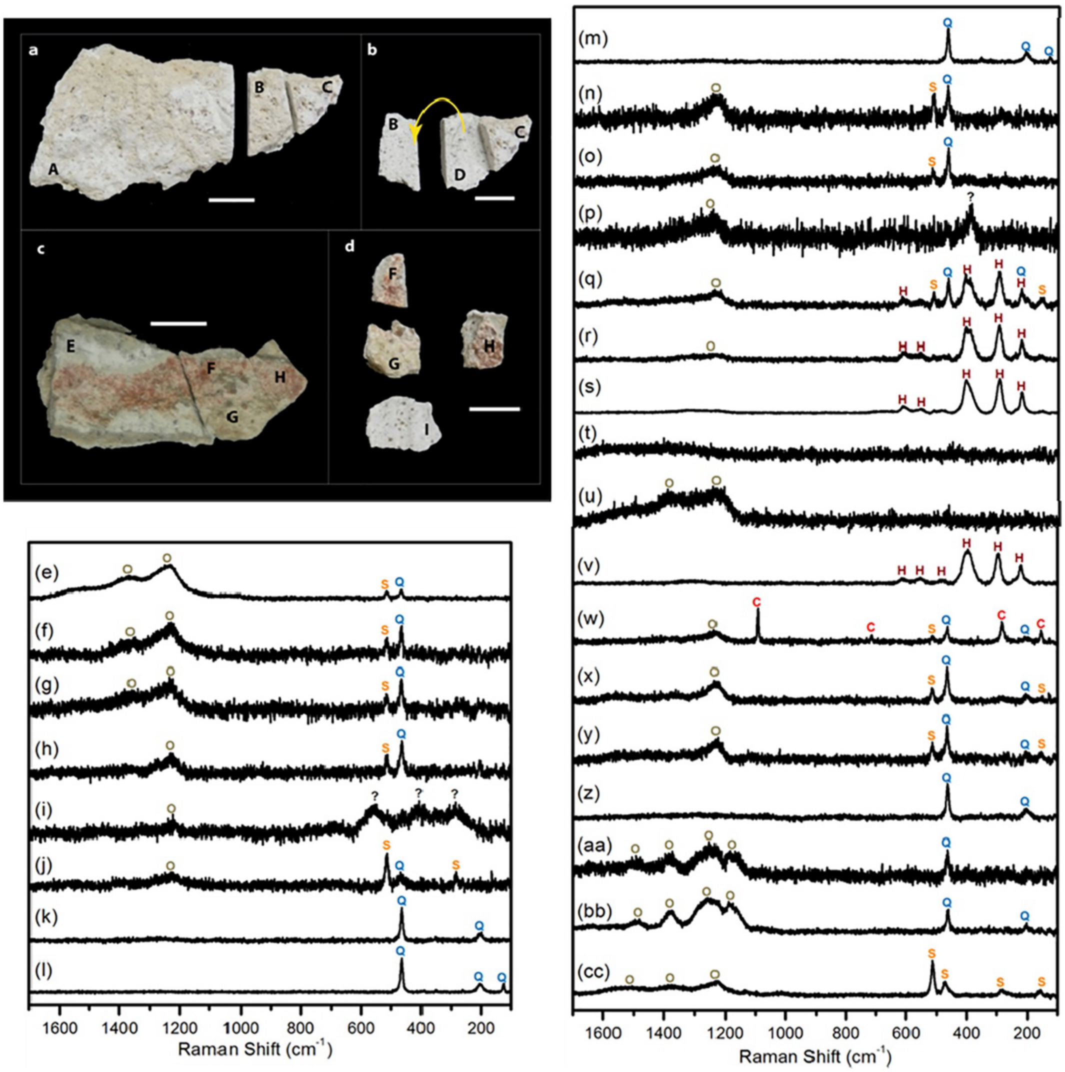

Figure 12: Top left panel: Raman spectra of samples VQ1-1(a, b) and VQ2-2 (c, d) for substrate characterization. Lower left panel: Raman spectra (785 nm laser) of VQ1-1 fragment B; ss spectra (e) through (i) are from smooth areas of the cut surface whereas spectra (j) through (l) are from rough areas. Right panel: Raman spectra of VQ2-2. Spectra (m) through (p) are from the painted surface of fragment F, whereas spectra (q) through (s) are from rough areas on the surface where paint was removed. Spectra (t) and (u) are ps spectra from the roughened upper surface of fragment G. Spectra (v) through (z) are ss spectra from smooth cut surfaces of fragment I, whereas spectra (aa) through (cc) are from roughened areas. [Labels are defined in the legend of Figure 10] (Brook et al., 2018).

Radiocarbon measurements align with the spectroscopic picture and the realities of friable ignimbrite sampling. After HCl pretreatment to remove carbonates and oxalates, the VQ1-1 dot paint yielded 31 µg of carbon and an age of 3050 ± 50 ^{14}C BP, which calibrates to 3007–3357 cal BP at 2σ with a median probability age of 3194 cal BP, while adjacent unpainted substrate gave 3440 ± 60 ^{14}C BP (3481–3829 cal BP, MPA 3648 cal BP), a sensible older result given inevitable inclusion of substrate in the paint micro-sample (Brook et al., 2018). The VQ2-2 hand paint yielded 42 µg of carbon and an age of 520 ± 50 ^{14}C BP, calibrating to 341–624 cal

BP or 1326–1609 CE at 2σ with an MPA of 518 cal BP (1432 CE), consistent with Late Holocene production of hand stencils (Brook et al., 2018). The reported small yields, size-matched standards, and explicit calibration choices are integral to interpretation because dating is used only as context for the binder result and not as proof of binder presence (Cherkinsky et al., 2013; Hogg et al., 2013; Stuiver & Reimer, 1993; Reimer et al., 2013).

Methodologically, the success of binder identification at VQ joints on three linked practices that the authors emphasize. First, they rely on surface Raman at 532 nm to look for lipid-specific bands rather than treating broad "organic" envelopes as meaningful, a distinction grounded in comparative spectra from experimental and archaeological fats (Boyacı et al., 2014; Maier et al., 2005; Prinsloo et al., 2008). Second, they document microstratigraphy in detail, including paint thickness, continuity, and dust mantles, because thick continuous layers can shield and concentrate organics whereas thin spray deposits may not, a structural control that their data illustrate clearly (Brook et al., 2018). Third, they treat radiocarbon with caution by pairing paint dates with nearby unpainted substrate dates when possible and by using acid pretreatment shown to remove carbonates and oxalates, a strategy designed to quantify and limit environmental carbon contributions without over-interpreting ages (Bonneau et al., 2011, 2016; Brook et al., 2018). These practices respond directly to published warnings about the risks of dating unidentified organics in rock-art contexts, which the authors cite in framing their protocol (Watchman, 1993; Livingston et al., 2009).

Within this framework, only the VQ1-1 dots produce a positive, motif-specific identification of a binder. The dot paint is thick and continuous, displays microplaty hematite with Ca-rich matrices, and yields a 532 nm Raman lipid pattern characteristic of animal fats; its radiocarbon age is younger than the directly adjacent unpainted substrate, as expected given sampling realities, and its $\delta^{13}C$ value lies within the C_3 domain compatible with animal fat, all of which align without overdetermining the interpretation (Brook et al., 2018). The VQ2-2 hand lacks a lipid signal under the same surface Raman conditions, and its thin, discontinuous, likely mouth-sprayed paint and young age are consistent with either no binder or a binder now below detection, a negative result that the authors report without extrapolation (Brook et al., 2018).

The study's constraints are explicit and central to its credibility. Whewellite's origin remains unresolved between plant additives and microbiological pathways, and the authors refuse to use oxalate presence to bolster binder claims or as a dating proxy, a conservative stance sustained by spectroscopic and EDS mapping data (Brook et al., 2018; Franceschi & Nakata, 2005; Gadd, 2006). $\delta^{13}C$ values do not isolate a species source for the fat and cannot discriminate among C_3-domain contributors in mixed micro-samples; they are used only for plausibility checks (Brook et al., 2018). Ignimbrite friability forces paint-plus-substrate sampling for AMS, so paint ages act as maximum-age estimates under inevitable mixing, a limitation the authors quantify by dating adjacent unpainted substrate at VQ1-1 and discussing the logical outcome for VQ2-2 (Brook et al., 2018). A thin K–Si dust mantle blankets both motifs and may affect surface chemistry and detection efficiency; the study acknowledges this layer and integrates it into microstratigraphic interpretation rather than ignoring it (Brook et al., 2018).

In regional perspective, the Viuda Quenzana results are significant because they document an *in-situ* lipid binder signature in a Patagonian rock-art paint and separate it from environmental organics through wavelength-specific Raman, SEM/EDS microstratigraphy, and disciplined radiocarbon practice, while avoiding claims beyond the measurements (Brook et al., 2018). The authors note that other Patagonian sites often lack binder evidence *in-situ* or show proteinaceous or lipid traces in excavated painted fragments, and they present those studies as context rather than as direct analogs, keeping the VQ binder identification strictly local and motif-specific (Wainwright et al., 2002; Boschín et al., 2002; Fiore et al., 2008). The conclusion is straightforward: the VQ1-1 dotted motif paint contains animal fat; the VQ2-2 hand does not yield a detectable binder; and the analytical pathway that leads to those outcomes is reproducible in other South American contexts if microstratigraphy, surface-specific spectroscopy, and conservative AMS protocols are respected (Brook et al., 2018).

This case study establishes a successful identification of organic binder material in prehistoric South American rock art by combining site-scale taphonomic awareness with laboratory rigor. The canyon's arid, windy setting produces dust mantles and promotes spalling, so the team collected pieces about to detach, preserved chain of custody, and documented shelter walls free of visible colonizers, all to control contamination pathways before analysis (Brook et al., 2018). The laboratory sequence then discriminated pigment, substrate, and organics by pairing 785 nm Raman for mineral and generic organic screening with 532 nm Raman for lipid specificity, while SEM/EDS established paint continuity, thickness, and Ca-Fe associations that matter for preservation and interpretation (Brook et al., 2018). AMS dating was used cautiously, with acid pretreatment that removes carbonates and oxalates, tiny carbon yields, size-matched standards, and explicit calibration against SHCal13, and with an unpainted substrate date to constrain background carbon, all steps designed to avoid over-reading numerical ages and to keep chronology in a supporting role (Bonneau et al., 2011, 2016; Hogg et al., 2013; Cherkinsky et al., 2013; Brook et al., 2018). The result is robust: animal fat was added to the hematite paint for the VQ1-1 dots; no binder was resolved for the VQ2-2 hand; and oxalate presence is treated as environmental or ambiguous rather than evidentiary for binder, a distinction that preserves analytical integrity (Brook et al., 2018).

This technical clarity is why the VQ1-1 dots serve as a model of successful binder identification. The lipid bands are where they should be: on the paint surface, at a wavelength that resolves triglyceride features, matched to published references and archaeological analogs, and not confounded by substrate organics that also occur at shallow depths (Prinsloo et al., 2008; Maier et al., 2005; Boyacı et al., 2014; Brook et al., 2018). The microstratigraphy explains preservation: a 20–40 μm continuous layer applied by direct contact shields residues, whereas a thin, discontinuous spray layer does not, which is exactly what the negative hand demonstrates (Fiore & Acevedo, 2016; Brook et al., 2018). The radiocarbon pairing with unpainted substrate quantifies the background, and the isotope values remain within a broad C_3 domain without being used to over-specify the fat source, a methodological humility that prevents category errors that have troubled other studies of "unidentified organics" in rock art (Watchman, 1993; Livingston et al., 2009; Brook et al., 2018).

4.3.3 Case Study 3: The uKhahlamba-Drakensberg Park, South Africa

The uKhahlamba-Drakensberg Park in KwaZulu-Natal, South Africa, a UNESCO World Heritage Site, encompasses the Giant's Castle sector where San rock art survives in exceptional density and quality on Clarens Formation sandstone cliffs (Prinsloo et al., 2008). Nearly 30,000 individual images in close to 600 shelters span a broad timeframe from approximately 100 to 4000 years before present (Prinsloo et al., 2008).

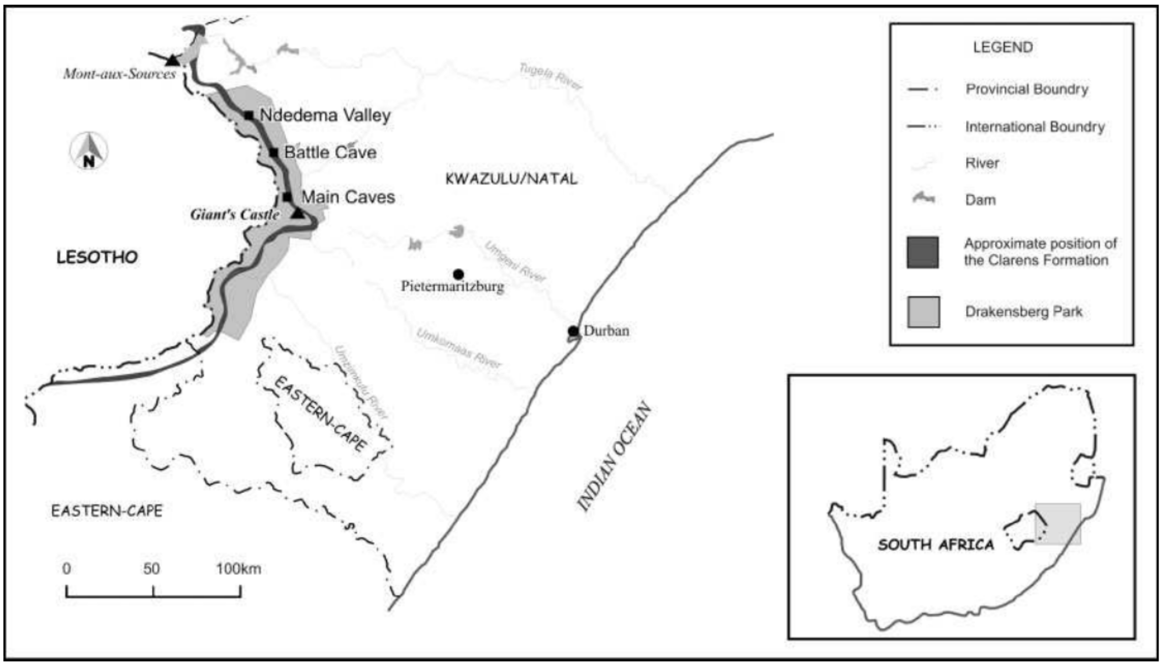

Figure 13: Location od uKhahlamba Drakensberg Park (Prinsloo et al., 2008).

The paintings exhibit fine polychrome figures of eland, therianthropes, hunters, dancers, and other animals, often rendered with great delicacy and in dynamic poses (Prinsloo et al., 2008). Ethnographic analogies with Kalahari San emphasize the role of trance experiences and the symbolic potency of the eland, whose blood and fat were associated with spiritual power and believed to confer potency on the pigments (Lewis-Williams, 1985; Lewis-Williams & Dowson, 1990). To test paint composition and the feasibility of spectroscopic analysis under field conditions, investigations began with laboratory study of a detached fragment from Barnes' Shelter that fortuitously preserved red and white paint on its surface, and a tiny (<1 mm^2) scrap of black pigment from Wilcox Shelter, along with reference ochre samples collected nearby (Prinsloo et al., 2008). The study applied a combination of micro-Raman spectroscopy using 514.5 nm Ar+ and 568 nm Kr+ excitation with a Dilor XY confocal system, a handheld DeltaNu portable Raman with a 785 nm diode laser, Fourier transform infrared spectroscopy (FTIR) on powdered subsamples pressed in KBr pellets, and powder X-ray diffraction (XRD) for phase identification (Prinsloo et al., 2008). The subsequent phase extended the work to *in-situ* analysis at Main Caves (Giant's Castle) and RSA BUF1 (Eastern Cape) using a Horiba Jobin-Yvon LabRAM Infinity portable micro-Raman equipped with a 532 nm frequency-doubled Nd:YAG laser and a fiber-optic probe. The instrument, mounted on a tripod and fitted with a 50× long-working-distance microscope objective, allowed spot analyses on painted panels (Tournié et al., 2011). The authors detailed procedures for focusing, adjusting laser power between 1 and 30 mW depending on the substrate, and separating paint signals from those of the sandstone or alteration layers, as well as probing stratigraphy by gradually increasing power (Tournié et al., 2011).

The laboratory analyses confirmed hematite as the principal red pigment, recognizable by Raman bands at 225, 245, 292, 409, 497, and 608 cm^{-1} (Prinsloo et al., 2008). Amorphous carbon, with broad D and G bands at ~1350 and 1600 cm^{-1}, was identified in the black pigment (Prinsloo et al., 2008). The white paint yielded α-quartz with bands at 126, 203, and 462 cm^{-1}, and titanium dioxide polymorphs anatase (143, 395, 513, 636 cm^{-1}) and rutile (230, 445, 606 cm^{-1}) (Prinsloo et al., 2008).

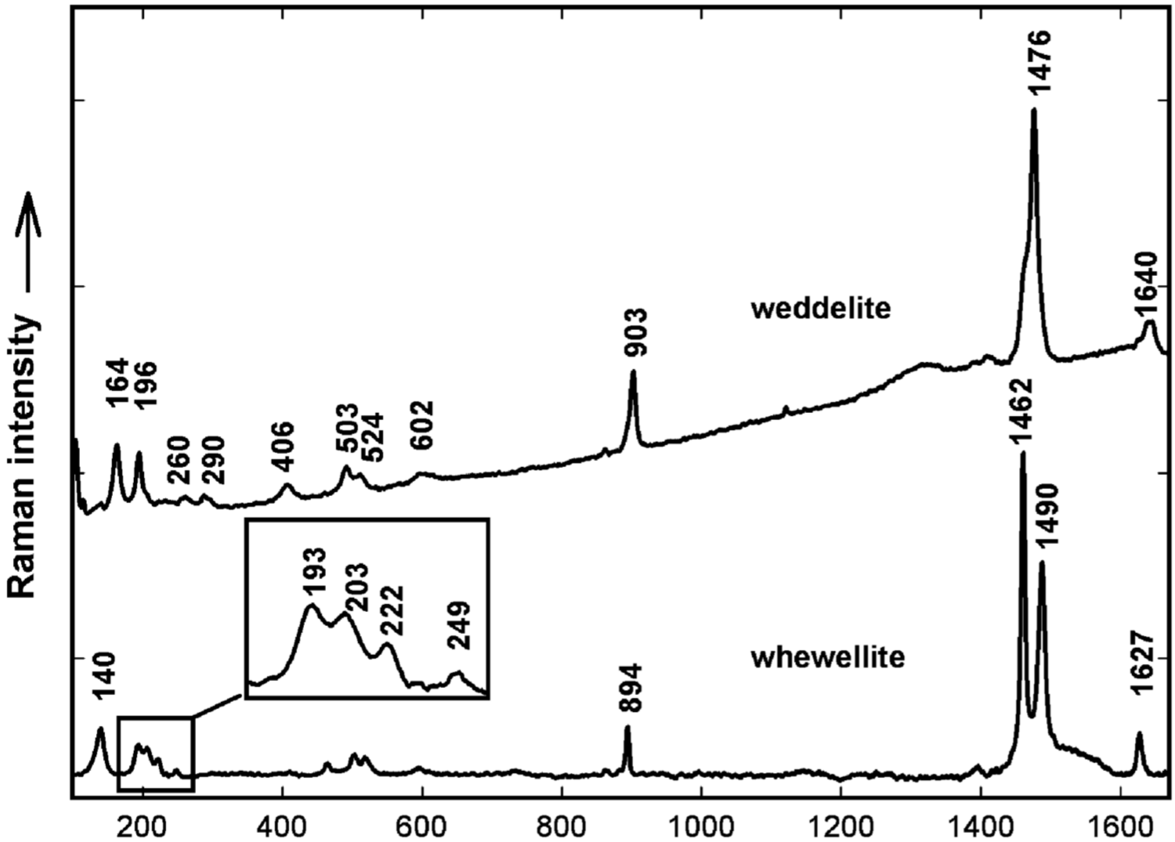

Figure 14: Raman analysis recorded on the red pigment with the 514.5 nm laser line showing the hydrate phases of calcium oxalate (Prinsloo et al., 2008).

Across red, white, and black pigments, as well as the back of the fragment, calcium oxalates, whewellite and weddellite, were recorded, with characteristic Raman bands at 1462 and 1490 cm^{-1} (whewellite) and 1476 cm^{-1} (weddellite), along with C–C stretching at 894 and 903 cm^{-1} (See: Figure 14) (Prinsloo et al., 2008). These oxalates, though clearly detected in paint layers, were shown to concentrate particularly on outer surfaces, suggesting they post-dated the painting and derived from biological colonization or shelter microclimate, though their presence raised the prospect of radiocarbon dating when pigments are encapsulated between oxalate films, as reported in Spain and Texas (Hernanz et al., 2007; Russ et al., 1999, cited in Prinsloo et al., 2008).

The most significant outcome was the detection of organic phases. Using the 785 nm portable Raman, spectra were recorded that matched the diagnostic profile of animal fat (Figure 15) : a carbonyl (C=O) stretch at ~1738 cm^{-1}, a C=C stretch at 1628 cm^{-1}, CH vibrations at 1419 cm^{-1}, in-phase methylene twisting near 1300 cm^{-1}, and CH deformation at 1237 cm^{-1} (Prinsloo et al., 2008).

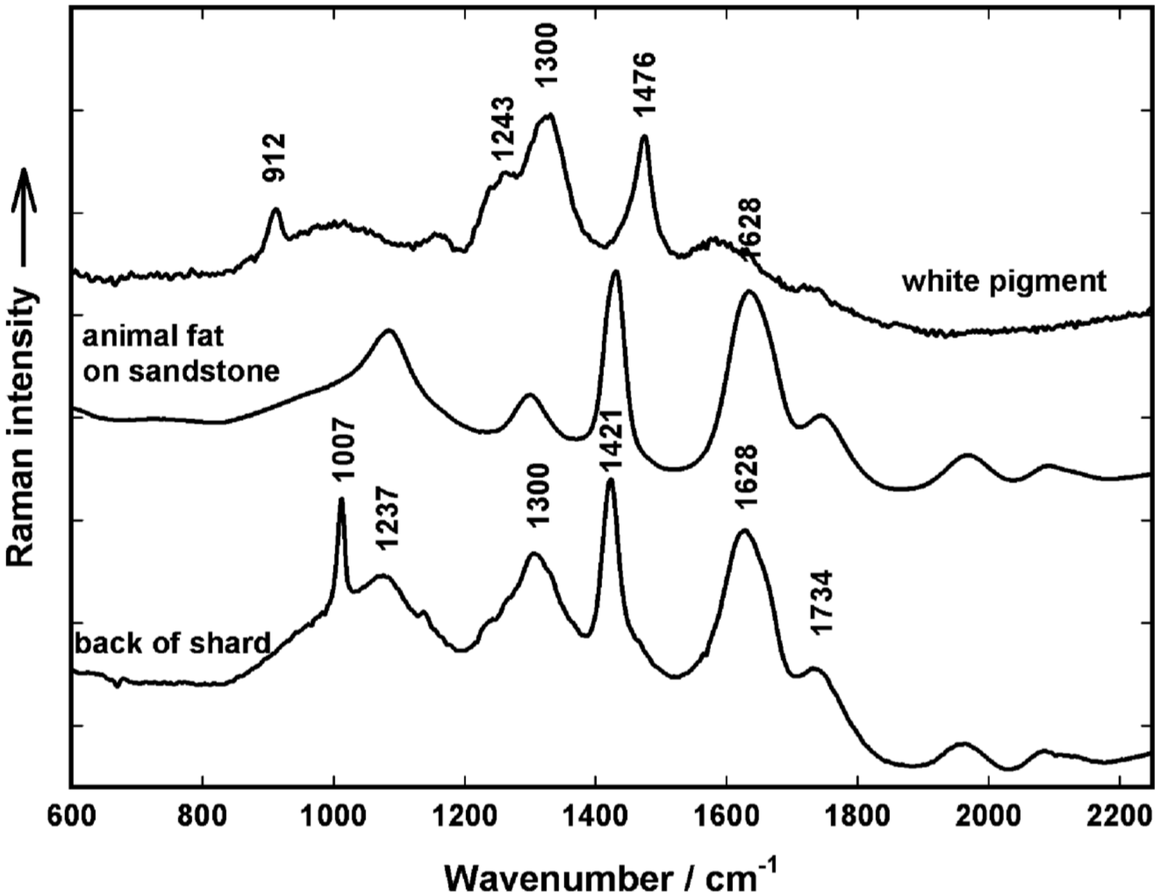

Figure 15: Spectra recorded animal fat on sandstone (middle) and the white pigment (top) with the 785 nm portable Raman instrument (Prinsloo et al., 2008).

These signals, when compared with spectra of beef fat smeared on sandstone, corresponded closely, confirming the presence of lipid material (Prinsloo et al., 2008). This animal fat signal was identified on the red and white paints, on the adjacent rock surface, and in the strongest concentration on the back of the fragment (Prinsloo et al., 2008). FTIR spectra of the red and white pigment confirmed whewellite but also showed additional absorptions, including a band at 1655 cm⁻¹ on the white paint, pointing to another organic phase (Figure 16) (Prinsloo et al., 2008).

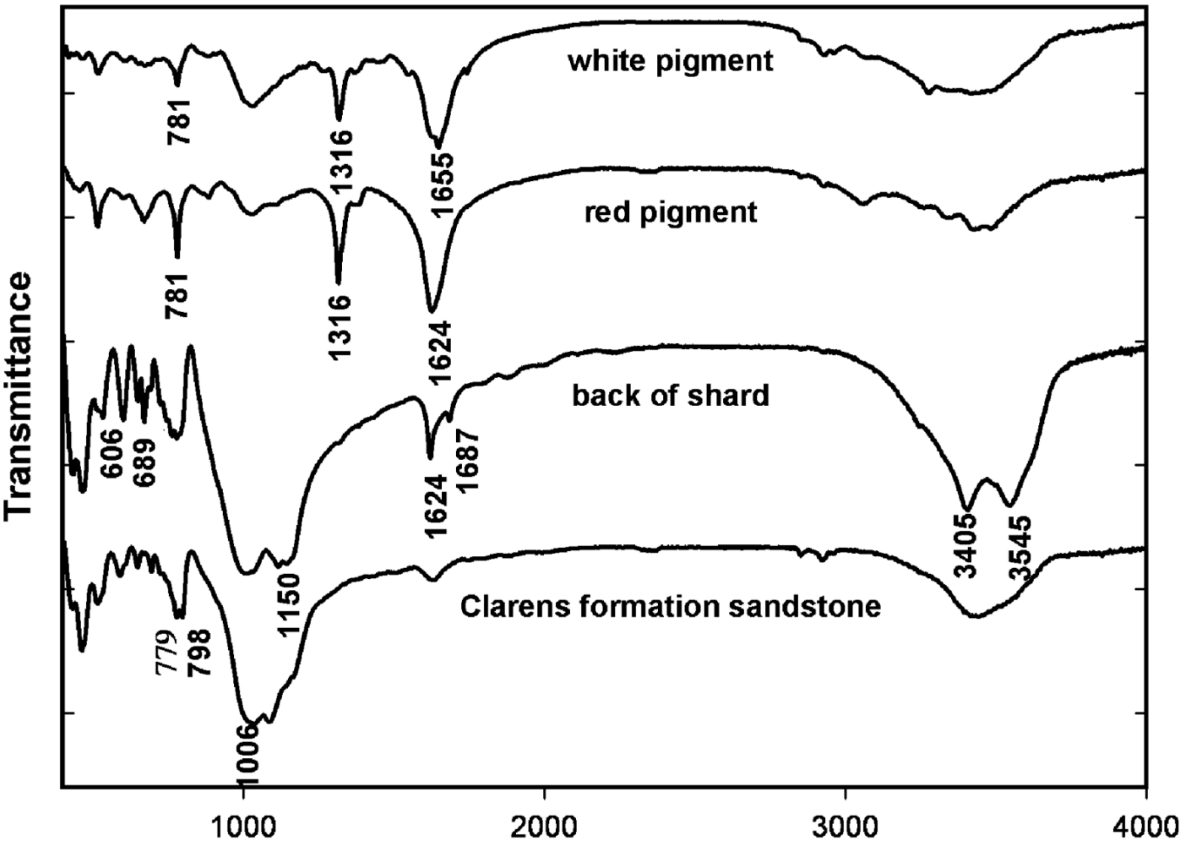

Figure 16: FTIR spectra of white pigment, red pigment, back of the shard, and clarens formation sandstone (Prinsloo et al., 2008).

Resonance Raman at 514.5 nm on the white pigment detected spectral features consistent with carotenoid pigments, with strong bands resembling lutein from egg yolk (Figure 17), long used as a tempera binder, though the authors noted that carotenoids could equally derive from lichens or plant material (Prinsloo et al., 2008). In the black pigment, resonance Raman revealed a carotenoid with C=C stretching at 1503 cm⁻¹ and C–C stretching at 1148 cm⁻¹, interpreted as a methyl-substituted carotenoid with a polyacetylenic backbone, structurally similar to bacterioruberin produced by halobacteria (Prinsloo et al., 2008).

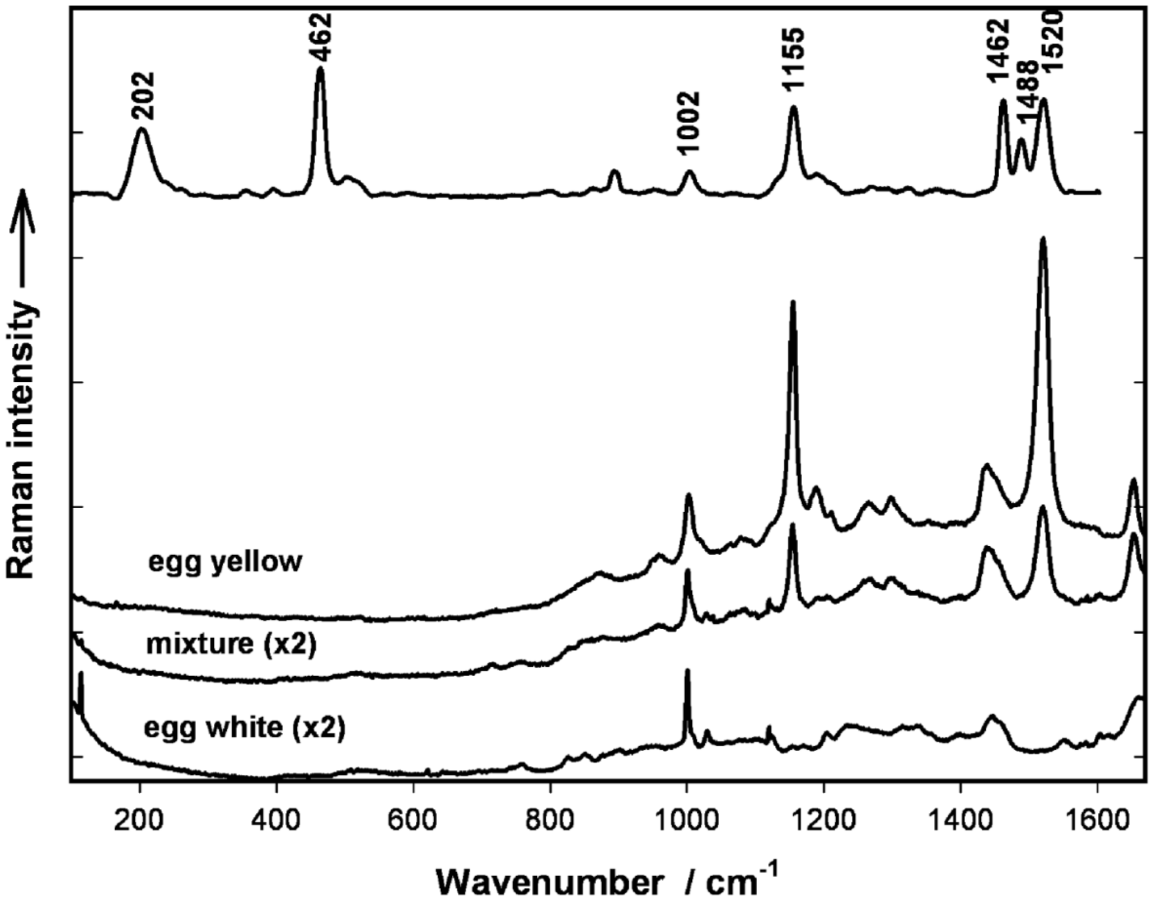

Figure 17: Organic phase in white pigment detected through 514.5 nm Raman in comparison with spectra recorded from egg white, the yolk and a mixture (Prinsloo et al., 2008).

The field campaign in contrast emphasized inorganic pigments and alteration layers. Red paints yielded hematite spectra, while white motifs occasionally showed calcite (Tournié et al., 2011). Gypsum and anhydrite were ubiquitous surface salts (Tournié et al., 2011). Anatase occurred in some yellow pigments, while α-quartz dominated the substrate (Tournié et al., 2011). By cautiously raising laser power, the researchers observed stratigraphic layering in which gypsum and anhydrite formed a crust overlying mixtures of sulfates, pigment, and oxalates, with hematite detectable beneath (Tournié et al., 2011). Weddellite and whewellite were again ubiquitous (Tournié et al., 2011). However, at the 532 nm excitation employed, no organic compounds were detected, and the authors emphasized that near-infrared excitation such as 785 nm, which had proven effective in the laboratory, would likely be necessary to recover organic signals *in-situ* (Tournié et al., 2011).

The interpretation carefully weighed the possible origins of both oxalates and fat. Oxalic acid could have been introduced by lichens, fungi, or shelter fauna such as rock hyrax, which produce oxalic metabolites, or through plant sap if used as a binder (Prinsloo et al., 2008).

Similarly, the fatty layer could represent deliberate surface preparation with hot fat to prevent paint absorption into porous sandstone, as suggested by the high concentration on the back of the shard, but it could also have resulted from later contamination due to human cooking activities or animal occupation (Prinsloo et al., 2008). The authors explicitly acknowledged these uncertainties and stressed that further systematic analyses would be required at multiple sites to confirm whether fat was consistently employed as a binder (Prinsloo et al., 2008)

Taken as a whole, the Giant's Castle case demonstrates a technological system in which hematite, carbon, and mineral whites formed the primary pigments, with oxalates and sulfates developing as alteration layers over time (Prinsloo et al., 2008; Tournié et al., 2011). Within this framework, the laboratory identification of animal fat alongside carotenoid signals represents a rare and significant positive detection of organic material in San rock art (Prinsloo et al., 2008). At the same time, the field spectroscopy established the feasibility of *in-situ* Raman mapping of pigments and alteration products but underlined the current limitations of green excitation for organics (Tournié et al., 2011). The authors concluded that these results mark important progress toward understanding the composition and preservation of San paintings, while stressing that the organic signals, though robust spectroscopically, must be interpreted cautiously in light of possible alternative sources (Prinsloo et al., 2008; Tournié et al., 2011).

4.4 The Significance of Negative Results

While Pigments are easier to detect, binders are more elusive. Minerals such as hematite or gypsum are stable and leave clear signals in Raman spectra, XRF patterns, or XRD analyses. In contrast, binders are fragile, easy to contaminate, and often invisible to common techniques. The table below summarizes cases across regions with positive pigment identification and negative results for organic matter. The following table demonstrates the results of different analyses across regions that yielded negative binder detection.

Table 2: Negative binder detection

Sample	Site/Region	Raman	ATR-FTIR	GC-MS	SEM-EDS	XRF / EDXRF	Remark	Reference
Puerto_01	Puerto Roque Shelter, Spain	Hematite	Heated clay	-	-	Ca, Fe	Dark red digit paintings. hematite pigment with heated clay traces; no binder detected.	Gomes et al., 2024
Puerto_03	Puerto Roque Shelter, Spain	Hematite	Heated clay	-	-	Si, Fe	Dark red linear figures; hematite pigment; heated clay detected.	Gomes et al., 2024
Puerto_04	Puerto Roque Shelter, Spain	Hematite, Carbon black, Red ochre	-	-	-	Ca, Fe	Visible plant motif; hematite mixed with carbon black and red ochre; no binder evidence.	Gomes et al., 2024
Puerto_05	Puerto Roque Shelter, Spain	Hematite	Heated clay	-	-	Si, Ca, Fe, Sr	Accumulated pigment near human figures; hematite pigment with heated clay; no binder.	Gomes et al., 2024
Puerto_06	Puerto Roque Shelter, Spain	Hematite	-	-	-	Ca, Fe	Superimposed pigments; hematite pigment only; no binder detected.	Gomes et al., 2024
Puerto_07	Puerto Roque Shelter, Spain	Hematite	Hematite, Quartz, incrustations	-	-	Ca, Fe	Eroded figure; hematite with incrustations; no binder detected.	Gomes et al., 2024
Puerto_08	Puerto Roque Shelter, Spain	Goethite, Hematite	Quartz (substrate)	-	-	Ca, Fe	Different hue, pasty texture; goethite with hematite; no binder.	Gomes et al., 2024
Puerto_09	Puerto Roque Shelter, Spain	Hematite	Quartz, Hematite	-	-	Si, Fe	Superimposed figures, good quality pigment; hematite with quartz; no binder.	Gomes et al., 2024
Puerto_10	Puerto Roque Shelter, Spain	Hematite	-	-	-	-	Orange figures impacted by sun; hematite pigment; no binder.	Gomes et al., 2024
Puerto_11	Puerto Roque Shelter, Spain	Hematite	Clay	-	-	Ca, Fe	Difficult to see; hematite pigment with clay; no binder.	Gomes et al., 2024
Puerto_12	Puerto Roque Shelter, Spain	Hematite	-	-	Al, P, K, Ca, Fe	Al, Si, P, K, Fe	Finger painting, splashing; hematite pigment with Al, P, K, Fe matrix; no binder.	Gomes et al., 2024
Puerto_13	Puerto Roque Shelter, Spain	-	Quartz (substrate)	-	-	Si, Ca, Fe	Faded figure; quartz substrate signal only; no pigment detected.	Gomes et al., 2024

Sample	Site/Region	Raman	ATR-FTIR	GC-MS	SEM-EDS	XRF / EDXRF	Remark	Reference
Puerto_14	Puerto Roque Shelter, Spain	Hematite, Magnetite	Quartz, Hematite	-	-	Ca, Fe	Linear figures made from small dots; hematite and magnetite detected.	Gomes et al., 2024
Puerto_15	Puerto Roque Shelter, Spain	Hematite	-	-	-	Si, Fe	Blurred orange figures; hematite pigment; no binder evidence.	Gomes et al., 2024
Puerto_16	Puerto Roque Shelter, Spain	Hematite	Quartz, Hematite	-	-	Si, Ca, Fe	Small red dots; hematite pigment with quartz; no binder.	Gomes et al., 2024
Puerto_17	Puerto Roque Shelter, Spain	Goethite	Goethite	-	-	Ca, Fe	Local raw ochre; goethite composition.	Gomes et al., 2024
Puerto_18	Puerto Roque Shelter, Spain	Hematite	Red Earth (Brown Ochre)	-	-	Ca, Fe	Local raw ochre; hematite pigment; brown ochre.	Gomes et al., 2024
Puerto_19	Puerto Roque Shelter, Spain	Goethite, Hematite	Goethite	-	-	Ca, Fe	Local raw ochre; goethite, hematite detected; possible fungi contamination (CH3/NH3 peak).	Gomes et al., 2024
Mzm-2018 (Sample 3, caprid tooth pendant)	Mezmaiskaya Cave, Russia	-	Bitumen, Red bolus/kaolin; weak Amide I (1630 cm⁻¹)	Modern contamination only	High Mn, Fe, Al, F, S in red areas; Ca/P ratio 2.00	-	Red pigment residues; mixture of bitumen, red bolus/kaolin; weak Amide I band suggests possible but not definitive binder.	Golovanova et al., 2024
Mzm-2018 (Sample 4, mammoth tusk stripe-bead)	Mezmaiskaya Cave, Russia	-	Bitumen, Red bolus/kaolin; wide band near Amide I (1630 cm⁻¹)	Modern contamination only	Slightly elevated Si; Ca/P ratio 2.35	-	Red and black pigment residues; mixture of bitumen, red bolus/kaolin; binder evidence weaker than in samples 1 & 2.	Golovanova et al., 2024
Ignatievskaya Cave – Black pigment (arrow sign)	Ignatievskaya Cave, Russia	-	-	-	Charcoal (carbon black), gypsum crystals	-	Black pigment; charcoal (vegetal origin). Gypsum encrustations secondary. No binder detected.	Kiseleva et al., 2023
Idrisovskaya II – Red pigment	Idrisovskaya II, Russia	-	-	-	Hematite, clayey extender; oxalates in cracks	-	Red pigment; hematite layered with clay. Sample too small for binder analysis. No binder identified.	Kiseleva et al., 2023

Sample	Site/Region	Raman	ATR-FTIR	GC-MS	SEM-EDS	XRF / EDXRF	Remark	Reference
MALT 1 (MALT-2020-CII-3)	Maltravieso Cave, Spain	-	Hematite, Magnetite, Goethite, Chalk	-	-	-	Brown hand stencil; earth pigment (iron oxides); no binder detected.	Rosina et al., 2023
MALT 2 (MALT-2020-CIV-1)	Maltravieso Cave, Spain	-	Kaolinite, Hematite, Goethite, Metallic oxides, Chalk	-	-	-	Red horse figure; kaolin-based ochre; no binder detected.	Rosina et al., 2023
MALT 3 (MALT-2020-CV-1)	Maltravieso Cave, Spain	Carbon black	Aluminium-silicates, Chalk	-	-	-	Black bull; charcoal pigment confirmed by Raman; no binder.	Rosina et al., 2023
MALT 5 (MALT-2020-PVIII-1)	Maltravieso Cave, Spain	-	Manganese oxide, Hematite, Magnetite, Goethite, Chalk	-	-	-	Red hand stencil; manganese, iron oxides; earth pigment; no binder.	Rosina et al., 2023
MALT 10 (MALT-2020-CHIV-6)	Maltravieso Cave, Spain	-	Manganese oxide, Hematite, Chalk	-	-	-	Brown lines; manganese-rich ochre (wads); no binder.	Rosina et al., 2023
MALT 12 (MALT 20 GSIII-1)	Maltravieso Cave, Spain	-	Celadonite-type mica, Hematite, Metallic oxides, Chalk	-	-	-	Red hand stencil; celadonite, iron oxides; no binder detected.	Rosina et al., 2023
MALT 13 (MALT2021-14)	Maltravieso Cave, Spain	Calcite only (tiny dark particles not identified)	Silicates, Hematite, Magnetite, Metallic oxides, Chalk	-	-	-	Black lines; ambiguous; Raman shows calcite; ATR-FTIR iron oxides; no binder.	Rosina et al., 2023
MALT 15 (MALT 20 GSV-3)	Maltravieso Cave, Spain	-	Celadonite-type mica, Hematite, Metallic oxides, Chalk	-	-	-	Red hand stencil; celadonite, iron oxides; no binder detected.	Rosina et al., 2023

Sample	Site/Region	Raman	ATR-FTIR	GC-MS	SEM-EDS	XRF / EDXRF	Remark	Reference
Buitres_1	Los Buitres 1 Shelter, Spain	Hematite, Montmorillonite	Hematite, Montmorillonite, Quartz	-	-	-	Red circular figure; hematite, montmorillonite; no binder identified.	Garcês et al., 2022
Buitres_2	Los Buitres 1 Shelter, Spain	Hematite, Montmorillonite	Hematite, Montmorillonite, Quartz	-	-	-	Red line figure; hematite, montmorillonite; no binder identified.	Garcês et al., 2022
Buitres_4	Los Buitres 1 Shelter, Spain	Burnt Umber, Hematite, MnO2, goethite	Hematite, Quartz	-	-	-	Zoomorphic figure; burnt umber identified; no binder evidence.	Garcês et al., 2022
BPF1 (grey)	Boqueirão da Pedra Furada, Brazil	No clear identification	-	Plant fatty acids; even-numbered n-alcohols	Fe, K, Si; traces Al	Fe, K, Si; traces Al	Binder evidence from HT-GC-MS; very low concentration; phthalates present; tentative plant origin.	Gomes et al., 2019
BPF2 (white)	Boqueirão da Pedra Furada, Brazil	Gypsum; Kaolin	-	Plant fatty acids; even-numbered n-alcohols	Gypsum; Kaolin	Ca, S; Al, Si	Binder evidence from HT-GC-MS; low concentration; phthalates present; tentative plant origin.	Gomes et al., 2019
BPF3 (red)	Boqueirão da Pedra Furada, Brazil	Hematite	-	-	Al, Ca, P	Fe; Al, Ca, P	Phosphorus association noted; no binder reported.	Gomes et al., 2019
TP01 (red)	Toca do Paraguaio, Brazil	Hematite	-	-	-	Si, K	Red pigment; elements likely linked to substrate/clay; no binder reported.	Gomes et al., 2019
TP02 (red)	Toca do Paraguaio, Brazil	Hematite	-	-	-	Aluminosilicates; concretions noted	Red pigment; multiple elements likely from substrate/concretions; no binder reported.	Gomes et al., 2019
TP03 (red)	Toca do Paraguaio, Brazil	Hematite	-	-	-	Ca (significant)	High Ca likely from concretion; no oxalate peaks reported; no binder reported.	Gomes et al., 2019
TP04 (red)	Toca do Paraguaio, Brazil	Hematite	-	-	-	Ca (dominant)	Very high Ca (concretion suspected); oxalate not detected; no binder reported.	Gomes et al., 2019

Sample	Site/Region	Raman	ATR-FTIR	GC-MS	SEM-EDS	XRF / EDXRF	Remark	Reference
BSQM-1	Benquerencia, Spain	Hematite	Quartz (substrate)	-	-	-	Red eye-shaped idol; hematite pigment; no organics.	Rosina et al., 2019
BSQM-3	Benquerencia, Spain	Hematite	Impurity (substrate)	-	-	-	Red fingerprint; hematite pigment; ATR-FTIR only substrate impurity.	Rosina et al., 2019
BSQM-5	Benquerencia, Spain	Hematite	Quartz (substrate)	-	-	-	Thick red figure; hematite pigment; no organics.	Rosina et al., 2019
BSQC-1	Benquerencia, Spain	Amorphous carbon (charcoal)	-	-	-	-	Black fingerprints; charcoal pigment; no binder.	Rosina et al., 2019
BSQC-2	Benquerencia, Spain	Amorphous carbon (charcoal)	-	-	-	-	Black fingerprints; charcoal pigment.	Rosina et al., 2019
BSQC-3	Benquerencia, Spain	Amorphous carbon (charcoal)	-	-	-	-	Black lines; charcoal pigment.	Rosina et al., 2019
AGU_01	Abrigo del Águila, Spain	Anatase	-	-	Titanium, Barium	-	White pigment; non-representative figure (parallel lines); anatase with Ti, Ba detected.	Rosina et al., 2018
AGU_02	Abrigo del Águila, Spain	Hematite	-	-	-	-	Red pigment; pectiniform figure; hematite detected.	Rosina et al., 2018
AGU_03	Abrigo del Águila, Spain	Hematite	-	-	Silicium	-	Red pigment; Eye Idol figure; hematite with Si signal.	Rosina et al., 2018
AGU_04	Abrigo del Águila, Spain	Hematite, Calcite	-	-	-	-	White pigment; non-representative figure (parallel lines); hematite with calcite.	Rosina et al., 2018
AGU_05	Abrigo del Águila, Spain	Hematite	-	-	Iron, Titanium	-	Red pigment; anthropomorphic figure; hematite with Fe, Ti present.	Rosina et al., 2018
AGU_06	Abrigo del Águila, Spain	-	-	-	-	-	Red pigment; red figure.	Rosina et al., 2018
AGU_07	Abrigo del Águila, Spain	Hematite	-	-	-	-	Red pigment; pectiniform figure; hematite identified.	Rosina et al., 2018

Sample	Site/Region	Raman	ATR-FTIR	GC-MS	SEM-EDS	XRF / EDXRF	Remark	Reference
AGU_08	Abrigo del Águila, Spain	Amorphous carbon	-	-	Barium	-	Black pigment; non-representative figure (parallel lines); amorphous carbon with Ba detected.	Rosina et al., 2018
AGU_09	Abrigo del Águila, Spain	Hematite, Organic compound	-	Organic compound (undefined)	Iron, Arsenic, Barium	-	Dark red pigment; soliform figure; hematite plus undefined organic matter; Fe, As, Ba detected.	Rosina et al., 2018
DOGUES_1 (Cavity I – archer)	Les Dogues rock shelter, Spain	Charcoal (amorphous carbon; plant-derived organic matter)	-	-	Charcoal layer embedded between calcite/gypsum; plant tissue fragments observed	S, Ca, Fe	Organic matter: charcoal (plant-derived carbon). Experiments suggest a viscous binder (fat/honey) could explain cell obstruction, but this was not chemically identified.	López-Montalvo et al., 2017
DOGUES_2 (Cavity I – battle scene)	Les Dogues rock shelter, Spain	-	-	-	Angiosperm vessel; obstructed pits; calcium oxalates present	S, Ca, Fe	Organic matter: charcoal (plant-derived carbon). Experimental replication indicates a viscous binder plausible, but no molecular identification reported.	López-Montalvo et al., 2017
DOGUES_3 (Cavity I – battle scene)	Les Dogues rock shelter, Spain	-	-	-	Conifer charcoal (tracheids, ray cells); obstructed cells; oxalates present	S, Ca, Fe	Black pigment interpreted as charcoal from plant cell remains. Organic matter: charcoal (plant-derived carbon). Binder: not detected	López-Montalvo et al., 2017
DOGUES_4 (Cavity II – archer)	Les Dogues rock shelter, Spain	Charcoal (amorphous carbon; plant-derived organic matter)	-	-	Charcoal layer with calcite/gypsum accretions; masked cells	S, Ca, Fe	Organic matter: charcoal (plant-derived carbon). Fluid paint with viscous binder favored by experiments, but not chemically identified *in-situ*.	López-Montalvo et al., 2017

Sample	Site/Region	Raman	ATR-FTIR	GC-MS	SEM-EDS	XRF / EDXRF	Remark	Reference
MM20 (Mambi Gwion, Reindeer Cave)	Kimberley, Australia	-	-	-	Al phosphate, quartz, minor montmorillonite, Mg–Cu sulphide, rostitie	Fe, Al, P	Mulberry paint; hematite confirmed. No binder.	Huntley et al., 2015
RRS (Ochre source, Reindeer Cave)	Kimberley, Australia	-	-	-	Quartz, illite, phlogopite mica; distinctive Fe-disc clusters	Fe, Si, Al	Anthropogenically exploited mulberry ochre quarry. No binder. first documented mulberry pigment quarry in Kimberley.	Huntley et al., 2015
K1 (Legacy sample, Drysdale River NP)	Kimberley, Australia	-	-	-	P-rich matrix, quartz, alumina silicates	Fe, S, P	Gwion motif pigment; jarosite confirmed as mulberry pigment mineral. No binder. rejects earlier sap/blood binder speculations.	Huntley et al., 2015
KSMA (Mineral accretion, Malauwarra – Kangaroo Shelter)	Kimberley, Australia	-	-	-	Gypsum, scawtite, syngenite, minor loweite, bassanite, dawsonite	-	not binders. Phosphates as taphonomic, not organic.	Huntley et al., 2015
Villars – Torch mark (Recoin du Balcon 1)	Villars Cave, France	Carbon (charcoal/soot)	-	-	-	-	Torch residue; organic carbon detected (charcoal/soot).	Beck et al., 2013
Villars – Torch mark (Recoin du Balcon 2)	Villars Cave, France	Carbon (charcoal/soot)	-	-	-	-	Torch residue; charcoal/soot organic matter.	Beck et al., 2013
Villars – Torch mark (Narrow passage)	Villars Cave, France	Carbon (charcoal/soot)	-	-	-	-	Torch residue; organic carbon binder (soot).	Beck et al., 2013
Villars – Torch mark (Carrefour 1)	Villars Cave, France	Carbon (charcoal/soot)	-	-	-	-	Torch residue; charcoal/soot organic matter.	Beck et al., 2013

Sample	Site/Region	Raman	ATR-FTIR	GC-MS	SEM-EDS	XRF / EDXRF	Remark	Reference
Villars – Torch mark (Carrefour 2)	Villars Cave, France	Carbon (charcoal/soot)	-	-	-	-	Torch residue; charcoal/soot organic matter.	Beck et al., 2013
Rouffignac – Mammoth #66	Rouffignac Cave, France	Carbon (charcoal/soot), Manganese oxides	-	-	-	-	Line shows carbon overlay (charcoal/soot); likely modern but organic carbon confirmed.	Beck et al., 2013
Rouffignac – Mammoth #180	Rouffignac Cave, France	Manganese oxides, Carbon particles	-	-	-	-	Carbon particles mixed with Mn oxides; charcoal-like residues detected.	Beck et al., 2013
Rouffignac – Rhino/Mammoth friezes	Rouffignac Cave, France	Manganese oxides, Weak carotenoid-type organics	-	-	-	-	Trace carotenoid organics (C=C, C–C stretching); uncertain origin; very low concentration.	Beck et al., 2013
Main Caves, Therianthrope (brown nose)	uKhahlamba-Drakensberg Park, South Africa	Gypsum, Hydromagnesite, Whewellite, Haematite (?)	-	-	-	-	Brown paint; alteration products gypsum and whewellite; weak haematite signal; hydromagnesite detected.	Tournié et al., 2011
Main Caves, Therianthrope (cream eyelid)	uKhahlamba-Drakensberg Park, South Africa	Gypsum, Hydromagnesite, Whewellite, Haematite (?)	-	-	-	-	Cream pigment; gypsum, hydromagnesite, whewellite; possible haematite contamination.	Tournié et al., 2011
Main Caves, Therianthrope (cream nose)	uKhahlamba-Drakensberg Park, South Africa	Whewellite, Haematite (?)	-	-	-	-	Whewellite oxalate layer dominates; weak haematite; possible substrate contamination.	Tournié et al., 2011
Main Caves, Eland (yellow)	uKhahlamba-Drakensberg Park, South Africa	Anatase, Gypsum, Haematite (?)	-	-	-	-	Yellow paint; anatase identified; gypsum alteration; weak haematite signal.	Tournié et al., 2011

Sample	Site/Region	Raman	ATR-FTIR	GC-MS	SEM-EDS	XRF / EDXRF	Remark	Reference
RSA BUF1, Man (orange)	RSA BUF1 Shelter, South Africa	Haematite, Magnetite, Gypsum	-	-	-	-	Orange paint; haematite with magnetite; gypsum alteration.	Tournié et al., 2011
RSA BUF1, Red Hartebeest	RSA BUF1 Shelter, South Africa	Haematite, Gypsum	-	-	-	-	Red paint; haematite detected; gypsum alteration.	Tournié et al., 2011
RSA BUF1, Rhebuck (white)	RSA BUF1 Shelter, South Africa	Gypsum, Calcite	-	-	-	-	White pigment; gypsum alteration; calcite likely pigment source (ostridge egg shell).	Tournié et al., 2011
RSA BUF1, Bicolour cow (white)	RSA BUF1 Shelter, South Africa	Gypsum, Calcite	-	-	-	-	White paint; gypsum alteration; calcite pigment identified.	Tournié et al., 2011
RSA BUF1, Bicolour cow (yellow)	RSA BUF1 Shelter, South Africa	Gypsum, Haematite (?)	-	-	-	-	Yellow paint; gypsum dominates; weak haematite signal.	Tournié et al., 2011
RSA BUF1, Eland (yellow)	RSA BUF1 Shelter, South Africa	Gypsum	-	-	-	-	Yellow paint; gypsum only, likely alteration product.	Tournié et al., 2011
RSA BUF1, Leaping Lion (white)	RSA BUF1 Shelter, South Africa	Gypsum	-	-	-	-	White pigment; gypsum only detected, probably alteration.	Tournié et al., 2011
RSA BUF1, Leaping Lion (yellow)	RSA BUF1 Shelter, South Africa	Gypsum	-	-	-	-	Yellow pigment; gypsum only detected, probably alteration.	Tournié et al., 2011
Red pigment	NW Iberia, Spain		-	-	Fe frequently dominant; C, S, P traces in some samples	-	Red pigments show homogeneity; hematite consistently identified as coloring agent	Rivas & Carrera 2010
Black pigment	NW Iberia, Spain	-	-	-	Carbon only	-	Identified as charcoal	Rivas & Carrera 2010

Sample	**Site/Region**	**Raman**	**ATR-FTIR**	**GC-MS**	**SEM-EDS**	**XRF / EDXRF**	**Remark**	**Reference**
Plaster (revoco)	Galicia, Spain	-	-	-	C, S, P traces detected	-	Revocos consistently show clay, quartz base; organic traces often attributed to biological contamination	Rivas & Carrera 2010
3B	La Casa de Las Golondrinas, Guatemala	-	-	Lipids detected, but similar to substrate; no clear binder.	-	-	Red pigment; no evidence of binder beyond contamination.	Livingston et al, 2009
13	La Casa de Las Golondrinas, Guatemala	-	-	Lipids present but non-diagnostic; composition like substrate.	-	-	Weathered red pigment; possible water as vehicle.	Livingston et al, 2009
4	La Casa de Las Golondrinas, Guatemala	-	-	Carbohydrates, long-chain fatty acids; potential plant/animal binder.	-	-	White/red pigment; possible binder but uncertain without substrate comparison.	Livingston et al, 2009
8	La Casa de Las Golondrinas, Guatemala	-	-	Fatty acids and phenolic compounds linked to humic acids (soil).	-	-	Pink pigment; date influenced by humic contamination, not binder.	Livingston et al, 2009
7	La Casa de Las Golondrinas, Guatemala	-	-	Trace lipids, carbohydrate, nitrogen compounds;	-	-	Red pigment; no conclusive binder evidence.	Livingston et al, 2009
12	La Casa de Las Golondrinas, Guatemala	-	-	Mainly fatty acids at low concentrations.	-	-	Red pigment; no binder identified.	Livingston et al, 2009
5	La Casa de Las Golondrinas, Guatemala	-	-	Long-chain and unsaturated lipids detected, could suggest binder.	-	-	Red pigment; possible binder but not confirmed.	Livingston et al, 2009
6	La Casa de Las Golondrinas, Guatemala	-	-	No significant signals (very small flake).	-	-	Red pigment; no binder detected.	Livingston et al, 2009

Sample	Site/Region	Raman	ATR-FTIR	GC-MS	SEM-EDS	XRF / EDXRF	Remark	Reference
VQ2-2 (pink negative hand)	Patagonia, Argentina	Hematite; No binder detected	-	-	Sparse α-Fe2O3 grains on substrate; K–Si sanidine dust layer at surface	-	<10 µm discontinuous paint layer consistent with sprayed stencil; calcium oxalate present; organics in substrate noted.	Brook et al., 2008
41VV75-3a (Red paint)	Lower Pecos Region,USA (site 41VV75)	-	-	Fatty acids detected but indistinguishable from oxalate coating;	-	-	GC–MS chromatograms of paint match oxalate crust; C16:0 & C18:0 identical to crust. Lipids attributed to microbial oxalate coating; no binder detected.	Spades & Russ, 2005
41VV75-3 (Red paint)	Lower Pecos Region,USA (site 41VV75)	-	-	Fatty acids detected but indistinguishable from oxalate coating;	-	-	Paint lipids native to oxalate patina; no binder detected. Authors conclude organic matter derives from crust, not paint. Fatty acids in oxalate coatings can mimic original paint organics.	Spades & Russ, 2005
41VV75-2 (Black paint)	Lower Pecos Region,USA (site 41VV75)	-	-	Fatty acids detected but indistinguishable from oxalate coating;	-	-	Chromatograms identical to crust; no binder detected. Organic matter interpreted as microbial origin from crust, not paint.	Spades & Russ, 2005

One explanation for negative results could be degradation. Lipids, proteins, and gums decay far faster than minerals. They oxidize, hydrolyze, or are consumed by microorganisms. Even in caves with stable climates, organics can vanish over millennia. Fatty acids can migrate into crusts or break down into forms no longer detectable. Proteins are even more fragile. Casein or animal glue lose their peptide sequences quickly. In many hematite samples of the dataset, no organics are present. It is unlikely that prehistoric painters avoided binders entirely. It is more likely that the binders have been lost through time. This idea is supported by rare survivals. At La Saltadora in Spain, casein peptides were detected through proteomics, proving the use of milk as a binder (Roldán et al., 2018). At Alero Hornillos 2 in Argentina, lipid analysis revealed ruminant fat associated with black camelid motifs (Vázquez et al., 2008). At Zmiev Kamen' in the Urals, GC–MS identified monogastric fat in red pictographs (Kiseleva et al., 2023). These rare findings highlight the exceptional nature of organic survival. The majority of paints, in contrast, show no residue. Negative results in these cases most likely reflect the natural loss of organics.

Another reason for negative results is methodological limits. Many techniques are suited to minerals, not organics. Portable Raman and XRF can identify hematite, magnetite, or manganese oxides with ease. They cannot detect degraded fats or proteins. FTIR has the potential to identify lipids or gums, but its spectra are often masked by gypsum, oxalates, or carbonates (Hernanz et al., 2014). GC–MS is the most widely used tool for lipid analysis, but it brings problems. Fatty acids may be ancient, or they may come from modern contamination. In the Lower Pecos, Texas, GC–MS detected fatty acids in black paints. However, the same compounds were found in oxalate crusts that covered the paintings. The authors concluded that the lipids came from microbial activity in the crust, not from the paint binder (Spades & Russ, 2005). This shows that detection does not always mean cultural use. Other cases demonstrate similar risks. At Serra da Capivara in Brazil, lipids were detected, but their link to the paint layer remained unclear due to oxalate associations (Gomes et al., 2019). At Les Dogues in Spain, microscopy revealed blocked plant cells that suggested the use of a viscous binder, but no direct chemical proof was found (López-Montalvo et al., 2017). These examples show that negative results may simply reflect the weaknesses of the tools applied.

The dataset confirms this bias. Out of 60 hematite samples, 52 show no binders. Yet the majority of these were tested only with Raman, XRF, or other mineral-focused techniques. It is not surprising that no organics were detected. In contrast, where GC–MS or proteomics were used, more positives were found. At La Saltadora, peptides diagnostic of casein was confirmed (Roldán et al., 2018). At Alero Hornillos, fatty acid profiles matched ruminant fat (Vázquez et al., 2008). In the Urals, degraded bone marrow fat was identified at Ignatievskaya Cave (Kiseleva et al., 2023). These examples highlight the methodological gap. Negative results often mean that sensitive tests were not used, rather than that binders were absent.

A third explanation is that some paints were prepared with water alone. This is a simple but effective method. Ethnographic examples show that mineral powders can be mixed with water to form workable paints. Hematite is especially suited for this. When finely ground, it suspends well in water and binds directly to porous rock. Many hematite paints in the dataset may have been prepared in this way. Their negative results may reflect true recipes that did not rely on organics. This is consistent with cases where sensitive analysis still failed to detect binders. At Serra da Capivara, some red paints showed no organics despite GC–MS analysis (Gomes et al., 2019). At Idrisovskaya II in the Urals, sample size was too small to recover any binders (Kiseleva et al., 2023). These results suggest that in some cases water was indeed the only medium.

Negative results represent that absence of evidence is not evidence of absence. Organics are fragile and may vanish. However, they show that methods matter. Reliance on non-invasive spectroscopy leads to an image of rock art dominated by pigments and empty of binders. Moreover, they open the possibility that many prehistoric artists used water as their medium. At the same time, positive identifications highlight what is possible when organics survive or when sensitive methods are applied. Lipids at Alero Hornillos 2 (Vázquez et al., 2008), casein at La Saltadora (Roldán et al., 2018), and fats at Zmiev Kamen' (Kiseleva et al., 2023) show that binders were sometimes used deliberately. Blood at Viuda Quenzana in Patagonia (Brook, 2008) and Mezmaiskaya Cave in Russia (Golovanova et al., 2024) shows that symbolic choices also mattered. These cases contrast with the majority of negative results.

The negative results for binders in prehistoric rock art may reflect different factors such as: the decay of organics, the limits of current methods, and the possible use of water-based paints, etc. On the contrary, these factors force to think critically about what has been lost, what cannot be detected, and what may never have been there. Hematite dominates, but most hematite paints are binder-negative. It is part of the story of prehistoric painting technology. Negative results are therefore not an absence of evidence but a different kind of evidence, one that points to both the fragility of organics and the diversity of human craftsmanship.

Chapter 5: Discussion: Interpreting the Binder Beyond Technology

The identification of binders in prehistoric rock art provides crucial information about technological knowledge. Analytical results demonstrate that the preparation of paint was not a simple matter of applying ground pigment to rock but involved specific decisions about organic media (Chapter 2; Chapter 4). Gas chromatography–mass spectrometry at Ignatievskaya Cave revealed lipid profiles characterized by palmitic and stearic acids, with odd-chain fatty acids indicative of degraded ruminant marrow fat (Kiseleva et al., 2023). Comparable evidence at Alero Hornillos 2 in Argentina showed similarly high concentrations of fatty acids, reaching up to 36,780 $\mu g\ g^{-1}$ in one sample, again consistent with ruminant fats (Vázquez et al., 2008). These results confirm that animal products were deliberately added to pigments and not introduced accidentally. The presence of lipids in significant concentrations demonstrates knowledge of their adhesive and film-forming qualities. Proteomic analysis at La Saltadora in Spain identified peptides diagnostic of bovine αS1-casein (Roldán et al., 2018). The use of milk proteins as a binding medium cannot be explained by chance; it implies familiarity with the properties of dairy products, which provide high viscosity and durable adhesion. The evidence links painting technology directly to the management of domesticated animals, since casein is not accessible without dairying (Roldán et al., 2018). Experimental reconstructions further support the technological validity of these results, since paints prepared with milk produce workable and persistent coatings (Roldán et al., 2018; see also Chapter 2).

Plant-derived substances also show deliberate knowledge of resources. At Serra da Capivara, lipid residues included even-numbered n-alcohols and fatty acids consistent with degraded plant oils or gums (Gomes et al., 2019). At Les Dogues, scanning electron microscopy revealed plant tissue remains obstructed by viscous material, interpreted as gum or honey (López-Montalvo et al., 2017). The findings suggest that painters exploited local vegetation to create binders that adjusted the viscosity of charcoal paints, enabling application where water alone would fail. Similarly, beeswax identified at Gode Roriso demonstrates recognition of its hydrophobic and stabilizing properties (Gomes et al., 2019). These examples point to the consistent use of plant and insect products to alter the mechanical behavior of paints.

The recurrence of these identifications across different region indicates that prehistoric communities understood not only pigment procurement but also the chemistry of adhesion. The evidence from lipid, protein, and plant-derived binders shows that paint recipes were not accidental mixtures. They were products of technological steps embedded in a *chaîne opératoire* that included grinding, mixing, testing, and applying with knowledge of how organic and inorganic materials interact (Chapter 3.3).

Although technical effectiveness explains part of binder choice, it does not account for all patterns observed in the dataset. The archaeological evidence indicates that functionality, availability, and symbolism interacted in determining which substances were employed. Fat residues at Alero Hornillos 2 occur in association with camelid bone remains processed for marrow, showing that painting technology was linked to subsistence practices (Vázquez et al., 2008). Lipids in the Urals point to marrow extraction from wild cervids, again-situating binder production within broader food-processing routines (Kiseleva et al., 2023). Such examples show that availability structured technological decisions, as binders were drawn from resources already circulating in subsistence economies. However, some results suggest that symbolic or ritual factors may have played a role. Casein at La Saltadora links painting to milk exploitation, a product with both nutritional and cultural dimensions in early Neolithic Iberia (Roldán et al., 2018). The use of blood as a binder has been proposed in Patagonia and Mezmaiskaya Cave (Brook, 2008; Golovanova et al., 2024). While contamination risks remain, the repeated suggestion of blood across contexts points to its symbolic resonance. Blood carries associations of vitality, sacrifice, and social identity, making its inclusion in paints more than functional. Similarly, gums and resins are not only adhesives but also substances with strong sensory properties, such as smell and gloss, which could have had cultural or ritual significance (López-Montalvo et al., 2017; Gomes et al., 2019).

Ethnographic analogies cited in Chapter 2 confirm that in many traditional societies paint media were selected not solely for performance but also for meaning (Arocena et al., 2008; Loubser, 1992; Cole & Watchman, 1992). Mixtures of ochre with fat or blood among San communities in southern Africa, or with resins in Australian Aboriginal contexts, demonstrate the dual role of binders as technical and symbolic agents. The archaeological evidence reviewed in Chapter 4 suggests that prehistoric painters operated within similar frameworks. Binder choices were embedded in subsistence, environment, and cultural values, producing paints that functioned both materially and symbolically.

Different binders degrade along distinct chemical pathways, and this variability explains much of the uneven preservation observed across the research (Chapter 3.1). Proteins are particularly vulnerable. At La Saltadora, casein peptides were identified, but the study noted extensive deamidation, which complicates both preservation and interpretation (Roldán et al., 2018). Lipids are more stable than proteins but remain subject to oxidation and microbial alteration, as shown in the Lower Pecos where fatty acids were indistinguishable from those produced in oxalate crusts (Spades & Russ, 2005). Plant gums and polysaccharides are highly soluble, making them susceptible to rapid loss in humid conditions (Gomes et al., 2019). Beeswax, by contrast, survives better due to its long-chain esters, which explains its rare but secure identification in Ethiopia (Gomes et al., 2019). Microbial activity further complicates preservation. In Levantine art, sequencing revealed that Firmicutes dominated painted surfaces, while other taxa solubilized carbonates, producing differential effects on paint stability (Roldán et al., 2018). Calcium oxalate patinas may protect pigments in some cases but can also entrap secondary organics, creating false signals (Roldán et al., 2018; Spades & Russ, 2005). Experimental studies confirmed that substrate porosity and water overflow strongly affect how paints endure, with oil-based paints proving stable on sandstone but less so on granite (Garcês et al., 2019). These observations demonstrate that preservation is not uniform but contingent on binder chemistry, microbial ecology, and substrate properties. Negative results in the analysis cannot automatically be interpreted as absence of binders. They reflect the combined effects of degradation and methodological limits (Hernanz et al., 2014; Gomes et al., 2019). For conservation, this variability is critical. Protein-based binders require protection from microbial activity, while lipid-based binders are more stable but vulnerable to oxidation. Plant-derived binders are fragile and rarely persist without special conditions. Conservation strategies must therefore be tailored to the likely chemistry of binders, not only to the mineral pigments that dominate analytical results (Colombini & Modugno, 2009).

The possibility of extracting chronological information from binders has been central to rock art research since the introduction of accelerator mass spectrometry (AMS) for small samples. Early attempts, such as at La Casa de Las Golondrinas, illustrate both the promise and the risk. Radiocarbon ages derived from presumed binder residues initially appeared consistent with the cultural sequence of the site, but subsequent chemical analysis revealed that the carbon originated from humic acids in the volcanic tuff substrate rather than from paint media (Livingston et al., 2009). This case demonstrates that radiocarbon ages obtained without rigorous molecular characterization cannot be assumed to date the paintings themselves. At the Lower Pecos, fatty acids detected in paints were chemically identical to those present in oxalate crusts, which proved to be microbial in origin (Spades & Russ, 2005). In such cases, AMS dating of bulk organic fractions would yield spurious ages, since the carbon does not derive from the original binder. Even when authentic residues are present, the quantities are often extremely small, typically in the microgram range, and prone to contamination from modern handling or infiltration (Roldán et al., 2018). Proteomic investigations at La Saltadora highlighted this difficulty, as casein peptides were identified but accompanied by keratin contamination, complicating any attempt to isolate pure ancient proteins (Roldán et al., 2018). Despite these challenges, the potential remains significant. Compound-specific radiocarbon dating of purified lipids or peptides could, in principle, provide direct ages for rock paintings. The successful isolation of ruminant fats at Ignatievskaya and Zmiev Kamen' (Kiseleva et al., 2023) or casein peptides at La Saltadora (Roldán et al., 2018) shows that organic fractions can survive in datable form. Similarly, beeswax at Gode Roriso demonstrates the persistence of long-chain hydrocarbons that could yield measurable radiocarbon signals (Gomes et al., 2019). However, the feasibility of dating depends on rigorous pretreatment protocols capable of excluding contaminants such as humic acids, microbial carbonates, or modern conservation materials. Without such controls, the chronological significance of radiocarbon ages remains uncertain.

The comparative analysis of binders across regions and analytical methods demonstrates that prehistoric painting practices cannot be fully interpreted on the basis of pigments alone. The technological evidence demonstrates that binders were deliberately selected and prepared, while the symbolic dimension indicates that their meanings extended beyond function. At the same time, the extreme fragility of organic compounds explains why most identifications are negative and absence of detection cannot be likened with absence of use. Positive identifications prove that organic media can survive and be studied, but only under severe protocols. They also demonstrate that painting practices were linked with subsistence, ritual, and environmental knowledge. The failures of past approaches underline the consequences of the pigment-centric bias, which distorted technological reconstructions and underestimated cultural complexity. The critical lesson is that binders are not supplementary; they are integral to both the *chaîne opératoire* of paint-making and the anthropological interpretation of rock art.

Chapter 6: Conclusion and Future Directions

6.1 Summary of Findings

The review shows a persistent asymmetry between how securely pigments are identified and how rarely binders are confirmed. The compiled results of archaeometric analysis confirms the dominance of iron oxides for red paints and carbon for black paints, with anatase, jarosite, gypsum, and clays present in specific contexts (Rosina et al., 2018; Rosina et al., 2019; Huntley et al., 2015; Gomes et al., 2019). The difficulty is isolating organics from complex mineral matrices that have been altered by water, oxalates, gypsum, phosphates, and microbial communities over the time (Kiseleva et al., 2023; Roldán et al., 2018). Only a part of analyzed samples yielded organic signals, and many of those signals are ambiguous because substrates and accretions carry comparable compounds (Spades & Russ, 2005; Livingston et al., 2009).

Across the literature, lipids are the most frequently reported organic residues, while proteins and polysaccharides are reported more rarely and with more caveats (Gomes et al., 2019; Vázquez et al., 2008; Roldán et al., 2018). Secure identifications include casein peptides at La Saltadora, beeswax at Gode Roriso, and coherent fatty-acid profiles from selected motifs at Serra da Capivara (Roldán et al., 2018; Gomes et al., 2019). Other reports document fatty acids at Alero Hornillos 2 and lipid signals in Ural pictographs, but these remain non-diagnostic because the same compounds occur in unpainted substrates and secondary crusts (Vázquez et al., 2008; Kiseleva et al., 2023; Livingston et al., 2009). At Les Dogues, microscopy and experiments point to a viscous medium, plausibly honey or gum, for making charcoal workable, yet no molecular marker was recovered (López-Montalvo et al., 2017).

Pigment identifications are vigorous because mineral lattices yield distinctive Raman and X-ray responses. Hematite dominates red palettes; charcoal dominates many blacks; anatase and jarosite appear in specific white and mulberry contexts; quartz, micas, and clays are frequent associates or substrates (Rosina et al., 2018; Rosina et al., 2019; Huntley et al., 2015; Gomes et al., 2019). These mineral baselines frame how organic traces are interpreted and help separate pigment recipes from later accretions (Rosina et al., 2018; Gomes et al., 2019). Binder signals vary with color and recipe. Coherent lipid profiles occur more often in whites and grays than in hematite-rich reds, which often lack organics consistent with mineral paste application (Gomes et al., 2019). The Gode Roriso white paints contain beeswax, a chemically stable wax with long-chain esters (Gomes et al., 2019). The La Saltadora reds include casein peptides, which suggest deliberate protein addition despite contamination risks (Roldán et al., 2018). The

Les Dogues charcoal paints show microstructural evidence for a viscous additive that improved flow and adhesion even though chemistry remained silent (López-Montalvo et al., 2017).

Methodological comparisons explain many inconsistencies. Raman, µEDXRF/EDS, and micro-Raman define pigment phases and rule out some hypothesized binders when oxalates or carbonates are absent (Gomes et al., 2019). GC–MS and HT-GC target fatty acids and alcohols but cannot on their own separate ancient media from modern phthalates, humics, and microbial inputs without strict controls and substrates (Livingston et al., 2009; Gomes et al., 2019). Proteomics can detect specific proteins such as casein, yet keratins and other contaminants often co-occur and must be modeled with field and lab blanks (Roldán et al., 2018). Degradation and matrix effects further reduce detectability: proteins hydrolyze, unsaturated lipids oxidize, and polysaccharides dissolve or are mimicked by microbial products (Kakoulli & Balonis, 2023; Spades & Russ, 2005).

In Ural case studies, hematite shows structural disorder from grinding and heat; gypsum and phosphates form authigenically; oxalates and microbial colonization reshape paint layers and trap exogenous organics (Kiseleva et al., 2023). In Levantine panels, bacterial communities differ between paintings and surrounding rock; some taxa solubilize carbonates and alter surface chemistry (Roldán et al., 2018). In the Lower Pecos, fatty acids in paints match those in oxalate crusts, demonstrating that microbial activity can generate binder-like signatures (Spades & Russ, 2005). These observations show why negative results cannot be read as absence of binders and why positive results require matched controls and multi-method confirmation (Hernanz et al., 2014; Gomes et al., 2019).

Author interpretations converge on selective and knowledgeable use of media. At Serra da Capivara, the contrast between mineral reds and organic-bearing whites and grays implies recipe variation and choice (Gomes et al., 2019). At Les Dogues, a viscous plant-derived medium is inferred from microstructures and experimental replication, pointing to intentional rheology control rather than incidental contamination (López-Montalvo et al., 2017). In the Kimberley, jarosite and mineral evidence reject earlier proposals of sap or blood for "mulberry" paints, underscoring how mineral and contextual data can overturn persistent myths (Huntley et al., 2015). Together, these readings indicate technological knowledge in selecting media for adhesion, opacity, and durability, with choices that vary by motif, color, and support (Gomes et al., 2019; Rosina et al., 2018).

Strong inferences arise when mineralogy, microstratigraphy, and organics align across independent tests and when substrates and accretions are analyzed alongside paints (Gomes et al., 2019; Livingston et al., 2009). Weak or equivocal signals recur where single methods are used, where controls are absent, or where taphonomy dominates the organic fraction (Spades & Russ, 2005; Kiseleva et al., 2023). This pattern explains why secure binder identifications remain exceptional even in well-studied regions (Roldán et al., 2018; Gomes et al., 2019).

Direct dating provides cautionary lessons and future potential. Bulk or whole-fraction approaches such as PCO–AMS pick up any carbon present and can yield ages driven by humics or microbial inputs, as demonstrated at La Casa de Las Golondrinas (Livingston et al., 2009). Case studies in Texas further show that lipid yields in paints can equal those in unpainted limestone and oxalate crusts, undermining binder attributions and any dates derived from them (Spades & Russ, 2005). The presence of beeswax or well-preserved lipid series in specific motifs suggests a path forward if purified compounds can be isolated at microgram scale for compound-specific radiocarbon analysis with rigorous pretreatment (Gomes et al., 2019).

The evidence supports a conservative synthesis. Mineral pigments are well characterized; organic media are detected sporadically and require converging lines of proof. Secure cases indicate casein, beeswax, and plant lipids in targeted contexts, with recipe and color covariation that imply informed technical choices (Roldán et al., 2018; Gomes et al., 2019). Many apparent organics reflect substrate and accretion chemistry or microbial overprints rather than paint technology (Livingston et al., 2009; Spades & Russ, 2005; Kiseleva et al., 2023). The most reliable interpretations combine pigment mineralogy, microstructural context, and targeted biomolecular assays, with strict controls and explicit taphonomic models (Gomes et al., 2019; Hernanz et al., 2014). This logic underpins the findings laid out in Chapter 4 and frames the methodological and interpretive recommendations that follow.

6.2 Persistent Gaps and Limitations in Current Knowledge

Evidence for organic binders in prehistoric rock paintings remains uncertain. Most signals attributed to binders are weak, non-diagnostic, or indistinguishable from alteration products and environmental inputs. Oxalate crusts, humic substances, and microbial residues can produce fatty acids and other compounds that mimic paint media. Several case studies warn against assigning such compounds to the paint layer without matched controls and microstratigraphic confirmation (Spades & Russ, 2005; Livingston et al., 2009). This pattern appears across different regions and analytical approaches. It defines a persistent gap between what can be detected and what can be securely interpreted as anthropogenic binder.

In-situ spectroscopy cannot resolve this gap on its own. Portable FTIR and Raman systems suffer from short optical paths, reduced resolution, and strong matrix effects. Reflection geometries distort band shapes and can invert absorptions. Portable Raman often fails in the presence of fluorescence from organics or alteration products. Field measurements therefore depend on laboratory reference spectra, careful calibration, and corroboration by complementary methods. Even then, organic phases are rarely identified with confidence on rock surfaces that are rough, porous, and chemically heterogeneous (Brunetti et al., 2017; Hernanz et al., 2014; Beck et al., 2013; Tournié et al., 2011). This limitation constrains many published “positive” identifications to cautious language and to functional group assignments, not compound-specific identifications.

Laboratory analyses face their own limits. Thermally assisted GC–MS, HT-GC-MS, and related workflows extract complex mixtures where endogenous and exogenous inputs co-elute. Without matched substrate controls, non-paint accretions can dominate the chromatograms. At La Casa de Las Golondrinas, unpainted tuff yielded carbon and lipid profiles similar to the painted flakes. Humic fractions further matched environmental inputs from nearby features. The radiocarbon ages obtained from bulk oxidation targeted all carbon indiscriminately. The authors concluded that the dated carbon was not demonstrably paint binder. These results emphasise prior warnings from the Lower Pecos, where fatty acids in oxalate coatings were identical to those measured in the paint layer (Livingston et al., 2009; Spades & Russ, 2005). The implication is clear. Bulk chemical detection, without strict controls, cannot distinguish binder from crust or soil organics.

Taphonomy complicates detection at molecular scale. Proteomic work on Levantine pigments recovered casein peptides, yet the same study emphasized deamidation variability and the risk of over-interpreting protein aging markers. Keratin contamination dominated several datasets, despite sterile collection and processing. The authors framed casein either as a genuine Neolithic addition or as later intrusion. They could not decide between these alternatives from the proteomic data alone. This uncertainty is structural rather than incidental. It arises from the small mass of recoverable paint, the mixed microstratigraphy of shelter walls, and the long exposure of painted surfaces to water, microbes, and salts (Roldán et al., 2018).

Micro-sampling reveals the same structural problem. Sub-millimetric fragments contain pigment particles intergrown with microbial aggregates and alteration minerals. Cyanobacteria and other taxa occur within the analyzed chips. These inputs introduce lipids and polysaccharides that can mask or resemble ancient media. Even when SEM-EDS, Raman, and GC–MS are combined, the chemical signals record a palimpsest rather than a pure paint film. Analytical confidence rises with strict comparison across painted and unpainted controls. Yet such controls are not always available or adequately reported (Kiseleva et al., 2023).

Color does not resolve the problem. Red paintings usually rely on hematite. Black often comes from charcoal. These pigments are widely confirmed. But their associated organics are rarely specific. Heating of ochres changes color and phase. Later oxidation and crust growth further alter visible hue. Present colors may therefore reflect alteration rather than original recipes. In several *in-situ* Raman studies, gypsum and other alteration minerals dominate the spectra of "white" and "yellow" motifs. These patterns limit any straightforward link between hue and binder choice (Tournié et al., 2011; Barnett et al., 2006).

Experimental reference libraries improve interpretation but do not close the gap. Controlled mixtures of ochres with egg yolk, plant saps, saliva, animal fats, and blood define spectral and chromatographic targets. They also show how substrate, grain size, and heating change signals. These datasets help to reject over-confident claims. They also expose overlaps among binders that cannot be resolved at low concentration or after aging. In the field casework compiled here, several Iberian samples show hematite with clay and traces of organics suggestive of vegetal inputs, yet many adjacent samples show hematite with no organics at all. The heterogeneity within single sites indicates variable recipes, partial preservation, or both. Experimental data constrain hypotheses but cannot substitute for microstratigraphic proof of association between organics and pigment particles (Garcês et al., 2019; Rosina et al., 2019).

Regional case studies reinforce these limits. In Brazil, most analyzed panels yielded hematite with high calcium from concretions and no firm evidence for binders. A single panel showed low-level plant-type signals with phthalates, which are consistent with contamination. In Iberia, several motifs show clear earth pigments and frequent absence of organics. When organics appear, they are sparse and chemically equivocal. In the Urals, a lipid profile matched monogastric fat and was interpreted as a binder. Yet even here, the microstratigraphic and environmental context remains central to confidence. These examples show that isolated "positives" exist but are not yet common or generalizable across sites and regions (Gomes et al., 2019; Rosina et al., 2019; Kiseleva et al., 2023).

Direct radiocarbon dating remains constrained by chemistry. Plasma-chemical oxidation with AMS provided early results, but it oxidizes all organics, including humics, microbial residues, and modern contaminants. Charcoal pigments avoid some of these problems but introduce "old wood" issues that pre-date painting. Where pigments are iron oxides, compound-specific isolation of authentic binder molecules is needed. The reviewed casework shows how bulk carbon measurements can show persuasive narratives while drawing on exogenous carbon. Until paint-specific compounds can be purified and dated, many "direct" ages will remain provisional (Livingston et al., 2009).

Standards of reporting and control are uneven. Several studies lack paired unpainted controls from the same microenvironment. Others do not report detection limits, extraction blanks, or contamination checks. Portable measurements are sometimes presented without subsequent laboratory confirmation. Even when controls exist, the stratigraphic relation between organics and pigment is not always demonstrated. This heterogeneity prevents robust meta-analysis and weakens cross-site comparison.

These limits shape interpretation of technological knowledge. Many authors propose plant gums, saps, or animal fats because these materials are plausible and ethnographically attested. Yet the compiled results show that such claims often rest on low-abundance markers, ambiguous lipid profiles, or functional group assignments without compound specificity. Casein is an instructive example. It appears in proteomic screens, but the same datasets flag keratin and stress contamination risks. Deamidation does not rescue the signal because rates are environment-dependent. As a result, casein remains a hypothesis for some Levantine panels, not a secure identification of milk as binder (Roldán et al., 2018).

Symbolic interpretations require firmer chemical ground. Experimental libraries include human blood for reference, but the field cases summarized here do not demonstrate blood as a common or confirmed binder. Lipid or protein signals that might invite symbolic readings can be matched by alteration pathways or contamination. Without stratigraphic linkage of organics to pigment grains, and without exclusion of crust and substrate sources, symbolic arguments rest on insecure evidence. The same caution applies to claims that specific colors required specific binders. Present color and mineralogy reflect both ancient preparation and post-depositional change (Garcês et al., 2019; Tournié et al., 2011).

The scope of this thesis foregrounds these persistent limits. The synthesis relies on published datasets with uneven protocols, variable control designs, and heterogeneous reporting. The comparative tables demonstrate that confident binder identifications are rare relative to pigment identifications. Positive results are exceptional and context-dependent. Negative or equivocal results are common, even within the same site or motif class. This pattern is stable across different analytical approaches and regions represented in the corpus. It supports a conservative reading of technological choices. It also shows how interpretive claims can outpace the security of the chemical evidence when controls are incomplete.

The central outcome is therefore twofold. First, the available evidence does not yet support broad, cross-regional generalizations about specific binders in prehistoric rock art. Second, the most robust path forward requires compound-specific identification tied to microstratigraphy, strict paired controls, and transparent reporting of blanks and detection limits. Where radiocarbon is pursued, compounds must be isolated from crust and substrate inputs before dating. Until such protocols become standard, the field will continue to face the same limits identified here: ambiguous organics, strong taphonomic overprint, and interpretive claims that exceed what the chemistry can bear (Livingston et al., 2009; Spades & Russ, 2005; Roldán et al., 2018).

This persistent gap is not a failure of method but a signature of the materials under study. Thin paint films on porous stone sit at the boundary between culture and environment. They record use, weathering, and microbial colonization. Analytical success therefore depends on establishing association, not only detection. The literature summarized here shows progress toward that goal but has not yet achieved it at scale.

6.3 Final Conclusion

The synthesis presented in this thesis demonstrates a persistent asymmetry between the secure identification of pigments and the fragile, often ambiguous detection of binders. Pigments such as hematite, goethite, manganese oxides, and charcoal are consistently confirmed across methods including Raman, XRD, and XRF, making their technological role clear in red, yellow, black, and occasionally white motifs (Rosina et al., 2018; Huntley et al., 2015; Hernanz et al., 2013; Gomes et al., 2019). By contrast, binders are seldom identified with confidence, and when detected, their signals are frequently compromised by degradation, contamination, or overlap with environmental inputs (Livingston et al., 2009; Spades & Russ, 2005; Kiseleva et al., 2023).

Where organic media are securely recognized, the results are decisive. Proteomics at La Saltadora revealed bovine casein peptides, proving the intentional use of milk proteins in Levantine red motifs (Roldán et al., 2018). Lipid analysis at Alero Hornillos 2 showed degraded ruminant fats in black camelid figures, supported by archaeological association with marrow extraction (Vázquez et al., 2008). GC–MS data from the Urals confirmed monogastric fat binders alongside hematite and bone black pigments (Kiseleva et al., 2023). At Gode Roriso, beeswax was identified in white motifs, its chemical stability explaining its survival in contexts where proteins and plant gums fail (Gomes et al., 2019). Microscopy at Les Dogues further demonstrated viscous additives that improved the workability of charcoal paints, even where chemistry remained silent (López-Montalvo et al., 2017). These examples show that binders were deliberately selected, prepared, and used as integral components of paint recipes.

Negative and equivocal results, however, dominate the dataset. Fatty acids indistinguishable from microbial oxalates in the Lower Pecos highlight the problem of false positives when substrates and crusts are not controlled (Spades & Russ, 2005). Humic fractions at La Casa de Las Golondrinas produced radiocarbon ages that proved to be unrelated to painting events, showing how environmental inputs can undermine chronological claims (Livingston et al., 2009). Proteomic analyses repeatedly detected keratins, demonstrating how modern contamination obscures low-abundance ancient proteins (Roldán et al., 2018). These outcomes reveal the structural obstacles to identifying organics and underscore the necessity of rigorous sampling strategies, paired controls, and multiproxy approaches.

The comparative evidence indicates that binder choices were embedded in technological knowledge, subsistence practices, and cultural values. Lipids appear in association with animal processing, such as marrow extraction in South America and Eurasia (Vázquez et al., 2008; Kiseleva et al., 2023). Casein links painting to Neolithic dairying in Iberia, situating paint preparation within emerging agro-pastoral economies (Roldán et al., 2018). Plant gums, waxes, and honey were exploited to adjust paint viscosity and durability, as shown by experimental reference libraries and microscopic traces (Garcês et al., 2019; López-Montalvo et al., 2017; Gomes et al., 2019). These findings illustrate that paint-making involved informed choices grounded in environmental knowledge, with binders contributing both to functional performance and to the cultural significance of the imagery.

Methodological lessons follow directly. Reliable identifications arise only when mineral phases, microstratigraphy, and biomolecular signals converge under strict control. Portable spectroscopy can establish pigments, but compound-specific identifications require GC–MS or proteomics with rigorous blanks. Even then, results are valid only when stratigraphically associated with pigments and clearly distinguished from crusts and substrates (Roldán et al., 2018; Kiseleva et al., 2023). For conservation, the chemistry of binders matters: proteins degrade differently from lipids or waxes, and each demands tailored preservation strategies (Colombini & Modugno, 2009). For chronology, compound-specific radiocarbon dating remains a future priority, but bulk carbon ages drawn from crusts or humics must be rejected as non-informative (Livingston et al., 2009).

The central outcome of this thesis is that binders are not supplementary but integral to understanding prehistoric painting. Secure identifications are rare, yet they demonstrate technological sophistication, resource knowledge, and cultural choice. Negative results are equally significant and represent the fragility of organic matter and the methodological limits of current approaches. Together, they show that prehistoric painting was a compound practice where mineral and organic materials interacted, and that its study demands equal attention to both. Only by embedding binders within analytical protocols and interpretive frameworks can archaeometry reconstruct the full chaîne opératoire of rock art and recover the cultural complexity it encodes.

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This thesis made limited use of OpenAI's ChatGPT and Google's Gemini for language refinement and organizational support. The tools were used strictly under the supervision of the author and in full compliance with the **IMQP Guidelines for the Use of Generative Artificial Intelligence**. Its application was confined to drafting assistance, while all critical analysis, interpretation of data, and final conclusions were carried out exclusively by the author.

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