



Tracing the Forensic Fate of Glycans: Environmental Transformation, Persistence, and Biological Trace Interpretation

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Abstract

Glycans are a relatively underexplored class of structurally diverse and biologically informative molecules in forensic biology. Glycan structures are generated through complex enzymatic pathways and can vary by cellular origin, tissue type, biological state, and individual characteristics unlike DNA and proteins. Their possible forensic significance is not restricted to their occurrence in biological matrices, since environmental conditions after deposition may influence their persistence, degradation and structural changes. This review examines the forensic journey of glycans from their biological source to deposition, via environmental exposure, molecular transformation, analytical recovery and forensic interpretation. Special emphasis is put on glycan containing components in blood, saliva, seminal material and other biological materials of possible relevance, and on the chemical, enzymatic, environmental and substrate related mechanisms that may affect their stability. Review of current analytical strategies including glycan release, derivatization, mass spectrometry, chromatography, electrophoresis, lectin-based approaches and emerging high-sensitivity technologies in the setting of aged and low-template forensic evidence. The review also considers whether the persistence or transformation of glycan signatures may contribute to biological trace identification, evidence stability, environmental exposure history, time-related interpretation, and trace transfer evaluation. Here we present a framework for glycan-based forensic trace interpretation that incorporates source, deposition, exposure, transformation, recovery and bounded interpretation. The main obstacles are the absence of forensic-specific experimental studies, the lack of longitudinal and environmental models, inter-individual variability, analytical reproducibility, the absence of validated reference datasets, and unresolved evidential interpretation. Future research should focus on controlled exposure studies, multimolecular integration, computational approaches, standardized reference resources, and rigorous validation. Glycans should in general be regarded as an emerging additional molecular dimension, not as a well-established standalone forensic marker.

Keywords: Forensic Glycobiology; Glycans; Glycosylation; Biological Evidence; Glycan Persistence; Post-Deposition Transformation; Forensic Trace Interpretation; Glycomics; Forensic Serology; Glycoproteomics

Abbreviations

CE: Capillary Electrophoresis; DNA: Deoxyribonucleic Acid; HILIC: Hydrophilic Interaction Liquid Chromatography; IgG: Immunoglobulin G; LC-MS: Liquid Chromatography–Mass Spectrometry; MALDI-MS: Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry; MS: Mass Spectrometry; MS/MS: Tandem Mass Spectrometry; MUC: Mucin; N-glycan: N-linked glycan; O-glycan: O-linked glycan; RNA: Ribonucleic Acid; UHPLC: Ultra-High-Performance Liquid Chromatography; UV: Ultraviolet

Introduction

Biological evidence as a dynamic molecular system

Biological evidence is a vital source of information in forensic investigations. It comprises blood, saliva, semen, epithelial cells, tissues and trace biological material, which can be used for individual identification, characterization of body fluids and reconstruction of events. These materials are composed of various molecular components, such as DNA, RNA, proteins, lipids, and carbohydrates, with extremely different chemical properties and environmental stabilities. Once deposited outside the body a biological trace is removed from the regulated physiological conditions

which originally maintained its molecular composition. Its evidence characteristics may then be affected by external factors, leading to gradual changes in molecular integrity and analytical recoverability [1, 2]. Thus, the material analyzed in a forensic laboratory may not only be from its original biological source, but from the cumulative impact of post-depositional exposure and persistence.

The fate of a biological trace is controlled by complex interactions of environmental and substrate-related factors. Temperature, humidity, ultraviolet radiation, exposure to water, microbial activity, chemical agents, and the nature of the surface on which material is deposited can affect the persistence and recovery of biological components [1, 3, 4]. DNA and RNA may degrade differently in dry and humid conditions as shown in experimental studies [2]. Ultraviolet and solar radiation may degrade DNA in bloodstains [5]. The substrate properties may also play a role in the collection and persistence of biological material [6]. These observations demonstrate that molecular change does not follow one single universal pathway. In contrast, each biological trace follows a context-dependent process depending on its molecular composition and the environment within which it exists.

The dynamic behavior has important implications for forensic interpretation. Molecular degradation is often considered a disadvantage, as it may reduce the quantity or quality of material available for analysis. However, studies with DNA, RNA and proteins increasingly show that degradation and persistence may themselves create detectable patterns of possible forensic interest [7-9]. Such observations support a more general view of biological evidence as a dynamic molecular system rather than as a static repository of identifying information. Glycans and glycosylated molecules form an additional, relatively uncharted molecular layer in this context. The conceptual basis for investigating the possible role of biological traces in forensic biological trace interpretation is an understanding of the extent to which the persistence (or alteration) of biological traces can retain information relating to the history of a biological trace.

The current molecular landscape of forensic biological evidence

on the persistence and the transformation of biological traces after deposition. The field of forensic biology has developed from conventional serological testing to more complex molecular techniques. Conventional methods of identification of biological materials include presumptive and confirmatory tests, microscopy, immunological techniques and blood group analysis. However, these methods are still useful and molecular technologies have greatly increased the information that can be derived from biological traces. DNA profiling is still the main method of identifying individuals, and developments in the analysis of RNA, DNA methylation, proteomics, metabolomics and microbial profiling have opened up new possibilities for determining the nature, origin and biological characteristics of forensic evidence [10-12].

Among these molecular approaches, RNA-based analysis has been most thoroughly studied for body-fluid identification, because messenger RNA and other RNA molecules can demonstrate tissue- and fluid-specific expression patterns [13, 14]. Forensic epigenetics has examined DNA methylation signatures for applications such as age estimation, tissue identification, and other biological inference applications [15, 16]. Proteomic approaches have also shown promise for the identification of biological fluids based on characteristic protein profiles, particularly where conventional serological tests or nucleic

acid-based methods may be limited [17, 18]. These developments suggest a slow but steady shift from the sole analysis of biological evidence for donor identification to the retrieval of multiple levels of biological information from the same trace.

These advances notwithstanding, the molecular landscape of forensic biology is not uniformly charted. While DNA, RNA, proteins and epigenetic markers have been studied extensively, the myriad of other biomolecular features that distinguish cellular identity, molecular stability and cell interactions with the surrounding environment are relatively underrepresented in forensic research. One such molecular layer is glycans. The glycosylation of carbohydrates contributes to the structural and functional diversity of proteins and lipids and can differ between cell types, tissues and biological states [19, 20]. Therefore, the investigation of this neglected field could enlarge the molecular basis of forensic biology and provide new knowledge.

Why glycans have remained largely overlooked

Although molecular approaches have rapidly expanded in forensic biology, glycans have received considerably less attention as potential forensic markers. Part of this imbalance is due to the inherent complexity of glycan biology. However, unlike DNA and proteins, glycans are not synthesized from a direct template and their structures are generated through coordinated enzymatic processes that can generate extensive structural diversity [21, 22]. The different monosaccharide composition, linkage position, anomeric configuration and branching can result in a large number of structurally related molecules, including isomers, which may be difficult to distinguish analytically [23, 24]. Thus, translation of glycan analysis to routine forensic settings poses challenges that are fundamentally different from those of conventional nucleic-acid or protein-based methods.

The comparatively slow progress of forensic glycomics has also been constrained by analytical limitations. Comprehensive glycan characterization usually requires multiple steps of sample preparation, glycan release, purification, derivatization, chromatographic separation and mass spectrometric analysis [25, 26]. Structural isomers and microheterogeneity further complicate interpretation, especially as some glycan structures cannot be fully resolved on a single analytical platform [24, 27]. Separation technologies, ion mobility, tandem mass spectrometry and computational analysis have led to great advances in structural characterization, but glycomic workflows can still necessitate specialized instrumentation and technical expertise [26-28]. Such requirements may have limited their uptake in forensic laboratories, where methods ultimately need to demonstrate robustness, sensitivity, reproducibility and practical applicability to small or degraded samples.

But the limited forensic application of glycans should not be seen as a lack of potential for evidence. A remarkable study demonstrated that N-glycan profiles derived from dried bloodstains could be correlated to chronological age, providing an example of the fact that glycan information can be preserved measurable in biological material after deposition and storage [29]. At the same time, progress in modern glycomics is steadily increasing the sensitivity and structural resolution accessible for complex biological samples [26-28]. These advances provide a timely opportunity to consider glycans from a forensic perspective, extending beyond simple marker identification. Their structural variety, biological specificity and propensity for persistence or transformation may add a molecular

dimension to the understanding of the origin and post-depositional history of biological traces.

From glycan identity to glycan fate

Conventional studies on glycans have been mainly focused on glycan structural determination and deciphering their biological roles such as cellular recognition, protein folding, molecular stability and disease-related modifications. However, for forensic biological evidence, the question of interest goes beyond which glycans are present at the time of deposition. It is also about how these structures behave when biological material is taken out of its physiological environment. Glycans can be attached to proteins and lipids or be part of larger glycoconjugate systems, so their persistence may not only depend on the intrinsic properties of carbohydrate structures but also on the stability of the surrounding molecular matrix [30, 31]. In this way, a deposited biological trace can be progressively altered at the molecular scale by a series of interlinked processes including protein degradation, enzymatic activity, hydrolysis, oxidation and other environmental influences [32, 33].

Thus, the idea of glycan fate offers a wider context within which to investigate post-deposition molecular change. Instead of assuming that glycan profiles are forever fixed, or simply disappear with degrading biological material, we need to consider the possibility of selective persistence and structural transformation. Glycosylation can affect protein conformation and stability, and molecules containing glycans may be degraded in a highly specific manner under biological conditions [31, 34]. However, outside the body, the pathways that control these changes may be influenced by temperature, moisture, microorganisms, radiation, exposure to oxygen and the physical characteristics of the substrate. These interacting factors may alter the relative abundance of particular glycan structures or the molecular context in which they are detected, yielding patterns that do not reflect the original deposited profile.

Such a view is of forensic importance as informative N-glycan profiles can be obtained from dried bloodstains that have been stored for many years [35]. In this study, bloodstains gathered from hundreds of people and kept for approximately six to nine years still had sufficient glycan information to build a model to predict age [35]. Although this finding does not demonstrate that glycan transformation can now determine the age or environmental history of each biological trace, it does demonstrate the persistence and analytical recoverability of glycan signatures in aged forensic material. When taken with known information about stability and degradation of molecules, this finding supports a new forensic question, namely whether patterns of persistence, loss or change of glycans might ultimately give information about the post-deposition history of biological evidence.

Research gap, rationale, and objectives of the review

Glycosylation has been extensively studied in Biochemistry, Medicine and Molecular Biology but its potential in Forensic Biology is relatively less explored. So far, forensic molecular research has been mainly limited to DNA profiling, RNA-based body-fluid identification, epigenetic markers, and protein-based approaches, while glycan structures have been only sparsely studied systematically [10, 13, 15, 17]. Available evidence suggests that glycans exhibit substantial structural diversity and are able to retain biologically informative patterns in some forensic materials including dried bloodstains [29]. However, in the current literature, there is no comprehensive forensic

framework describing the persistence, degradation or transformation of glycans after deposition of biological traces under different environmental conditions.

This is a particularly important gap as post-deposition processes are a major hurdle in forensic interpretation. The molecular composition of biological evidence is affected by temperature, humidity, radiation, microbial activity, substrate characteristics and duration of exposure [1-6]. Such effects are well recognized for nucleic acids and increasingly for proteins, but far less is known regarding their effect on glycan structures. Glycobiology principles have revealed that glycosylation can impact protein conformation, molecular recognition and stability [30, 34], but these biological rules have not yet been systematically used in the forensic context. As such, it remains unknown if particular glycans demonstrate preferential persistence, predictable loss, or structurally-related environmental changes after deposition.

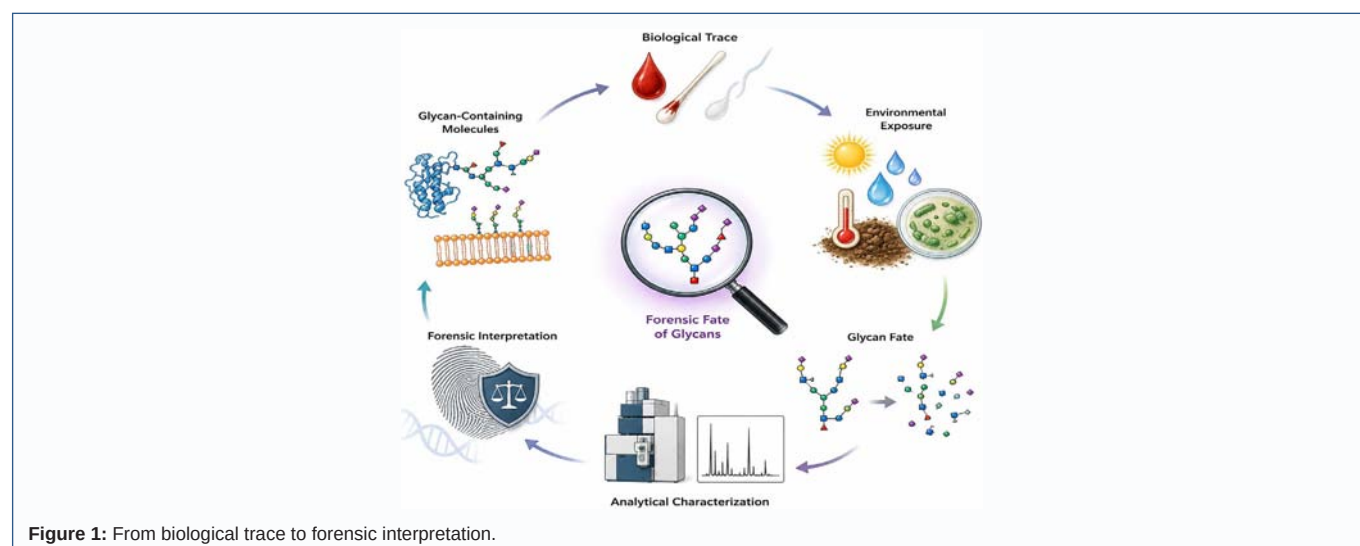
Therefore, this review aims to provide an overview of the forensic fate of glycans, by merging knowledge from forensic biology, glycoscience, molecular degradation and analytical chemistry. This review seeks to evaluate the potential for persistence and transformation of glycans in biological evidence, identify environmental and biological factors that may affect glycan profiles, assess available and emerging analytical approaches for their characterization, and explore their potential contribution to biological trace interpretation. This review attempts to provide a conceptual basis for an emerging area of forensic molecular research and to identify the key experimental questions needed for its future development by focusing on the transition from glycan identity to postdepositional glycan fate. This review attempts to provide a conceptual basis for an emerging area of forensic molecular research and to identify the key experimental questions needed for its future development by focusing on the transition from glycan identity to postdepositional glycan fate, as illustrated in Figure 1.

Glycans and the Biological Information They Carry

Fundamentals of glycans and glycosylation

Glycans are carbohydrate molecules consisting of monosaccharide units linked together by glycosidic bonds. They may be present as free oligosaccharides, or, more often in biological systems, as constituents conjugated with proteins and lipids. Their information content is obtained from a number of structural features such as monosaccharide composition, linkage position, anomeric configuration, branching, chain length and terminal modifications. Sequence information is derived from a relatively simple template-based system in nucleic acids, whereas glycan structures are generated via complex interconnected biosynthetic pathways involving many enzymes and cellular processes [25, 26]. This biosynthetic organization allows a single protein or lipid scaffold to be expressed in multiple glycosylated forms, greatly increasing molecular diversity and generating complex biological signatures.

Glycosylation is the enzymatic process of covalently linking glycans to proteins, lipids, or other biomolecules. Glycosyltransferases participate in glycan assembly and glycosidases and other processing enzymes participate in glycan modification and turnover [36, 37]. Protein glycosylation can be classified into two major types: O-linked and N-linked glycosylation. N-glycans are attached to asparagine residues and are usually processed through the endoplasmic reticulum



and Golgi apparatus to give high-mannose, hybrid or complex structures [38]. O-linked glycans are commonly attached to serine or threonine residues and comprise several structurally distinct types, with mucin-type O-glycosylation being a major class [39]. Thus, the final glycan profile reflects not only the underlying biomolecule but also the cellular environment and the enzymatic machinery involved in its synthesis and processing.

This distinction is particularly relevant if one considers glycans as carriers of biological information. The same protein can exist in several glycoforms as the extent and nature of glycosylation can differ between sites of attachment, cell types, developmental stages and physiological conditions [25, 40]. Glycan structures can affect protein folding, stability, trafficking, molecular recognition and cell-cell and cell-environment interactions [40]. Thus, the glycan component provides a further layer of molecular complexity beyond genomic or proteomic information alone. This is especially true in forensic biology, because biological traces are not just a collection of DNA molecules, but have several molecular layers, whose composition can provide clues regarding their biological origin and subsequently undergo changes after deposition. Therefore, it is necessary to understand the structural principles and biosynthetic basis of glycans before exploring the variation, persistence or transformation of glycan signatures in forensically relevant materials.

Major classes of glycoconjugates

In biological systems glycans are hardly ever found to act as independent molecular entities. Instead, they are generally present as components of larger structures, called glycoconjugates, in which carbohydrate chains are covalently linked to proteins or lipids. The three main classes that are of most interest to this discussion are glycoproteins, glycolipids and proteoglycans. All contain carbohydrate components but they differ markedly in the molecular organization, biological distribution and function. Such differences are important because persistence and transformation of a glycan after deposition may depend in part on the molecular framework to which it is attached. The following sections review each of the major classes of glycoconjugates as possible carriers of biologically informative glycan signatures.

Glycoproteins: Glycoproteins are proteins with covalently attached carbohydrate chains and are among the most widespread

types of glycoconjugates in biological systems. Their glycans may be N-linked or O-linked glycosylated and vary widely in composition, branching and terminal modifications [38, 40]. Glycosylation can play a role in protein folding, stability, intracellular trafficking, molecular recognition, and interaction with other biological molecules [34, 40]. Importantly, one protein can exist in different glycoforms, since glycosylation of individual attachment sites can be variable depending on cellular and physiological conditions [40, 41]. This structural heterogeneity significantly increases the information content of glycoproteins beyond their amino acid sequence.

Glycoproteins are found widely in forensically relevant biological materials such as blood, saliva and other body fluids. Many secreted and membrane-associated proteins contain complex glycan structures and these contribute to their biological properties and interactions [42, 43]. Thus, serum proteins, immunoglobulins, mucins and other glycoproteins may contain molecular information that is both protein-specific and glycan-specific. This dual molecular architecture is of particular importance from a forensic perspective as the protein component and its attached glycans may not necessarily exhibit the same patterns of persistence post deposition. Changes to the protein backbone, loss of specific glycans or favored survival of glycosylated structures could plausibly explain the molecular profile obtained from a biological trace.

Thus, for the analytical study of glycoproteins, both the identity of the protein and the structural features of the attached glycans need to be considered. Site-specific glycosylation can lead to a high degree of microheterogeneity, *i.e.*, the same protein can have different glycan structures at different sites or in different biological contexts [44]. This complexity is increasingly addressed by modern glycoproteomic approaches combining mass spectrometry with chromatographic separation and computational analysis [44, 45]. These technologies were developed primarily for biomedical research, but they offer a critical conceptual and analytical foundation for the future forensic analysis of glycoprotein-related glycan signatures.

Glycolipids: Glycolipids are membrane-bound glycoconjugates in which carbohydrate structures are covalently attached to lipid molecules. They are especially abundant on the outer surface of the cell membranes where their glycan moieties participate in cell recognition, cell-to-cell communication, membrane organization,

and interactions with proteins, pathogens and the external environment. The structural diversity of these molecules is due to the diversity of both the lipid backbone and the attached carbohydrate chains, resulting in different molecular patterns in different cell types and tissues [46, 47]. Glycosphingolipids are among the most important glycolipid groups as they participate in many biological recognition processes and form part of the molecular identity of cellular surfaces [48].

Biological systems do not have a uniform distribution of glycolipids. Different cells express different glycosphingolipid profiles depending on tissue type, developmental stage and physiological state [47, 48]. Changes in glycolipid composition have also been linked to cellular differentiation and disease-related processes, showing that the glycan structures on membrane lipids can reflect underlying biological features [49]. This variability is relevant to forensic science as cellular material deposited in biological traces may retain molecular features related to its original cellular source. The forensic utility of glycolipid signatures is still at its infancy but their cell-associated distribution suggests that they could constitute an extra level of information to DNA, proteins and other well-established biomarkers.

Analytically, glycolipids are challenging because their characterization requires the consideration of both the glycan and the lipid part. The advancement of modern mass spectrometry based lipidomics and glycomics has improved the capacity to identify and quantify complex glycolipid species including structurally related glycosphingolipids [50, 51]. However, the stability of these molecules following deposition and the behavior of their glycan structures under environmental exposure conditions are poorly understood. A key open question concerns the selective persistence, degradation or transformation of specific glycolipids after deposition. Future studies of this aspect may help to determine whether membrane-associated glycans may contribute to molecular interpretation of biological evidence.

Proteoglycans: Proteoglycans are a unique class of glycoconjugates that have a core protein that is covalently modified with one or more glycosaminoglycan (GAG) chains. Compared to many conventional glycoproteins, the polysaccharide chains of proteoglycans are long, often linear, and highly charged. These polysaccharide chains are chondroitin sulfate, dermatan sulfate, heparan sulfate, keratan sulfate, and related structures. These molecules are important components of the extracellular matrix and cell surfaces where they are involved in tissue organization, hydration, molecular signaling and cell-environment interactions [52, 53]. Their structure is therefore representative of the protein core and the type, length, sulfation and spatial arrangement of the attached glycosaminoglycan chains.

Proteoglycans are biologically diverse and their composition is tissue type, cellular origin, developmental stage and physiological conditions dependent. Changes in glycosaminoglycan synthesis and sulfation can affect molecular interactions and regulate processes such as growth factor signaling, cell adhesion, and extracellular matrix organization [53, 54]. Such heterogeneity associated with tissue is especially pertinent to the more general notion of biological information encoded in glycans. DNA offers a relatively stable genetic scaffold. The carbohydrate structures of proteoglycans may reflect the particular biochemical environment in which they were synthesized. Their molecular profiles may therefore contain information related to cell and tissue context, that is not directly represented by nucleic-acid sequence alone.

From a forensic perspective, proteoglycans have been little addressed directly as molecular sources of trace information. However, due to their abundance in extracellular matrices and their close relationship to tissue architecture, they are relevant for the study of biological evidence, in particular if tissue fragments, cellular material or degraded biological traces are considered. The longevity of proteoglycan components may vary depending on the stability of the core protein and the vulnerability of glycosaminoglycan chains to enzymatic and chemical degradation [55]. Progress in glycosaminoglycan analysis, including chromatographic and mass spectrometric approaches, are enhancing the ability to characterize these structurally complex molecules [56]. Thus far unexplored is the extent to which post-deposition processes preserve tissue-associated proteoglycan signatures to the extent that they provide forensic information and this is a possible future avenue of investigation.

Biological variability of glycan signatures: Glycosylation is not a simple template driven process, leading to a high biological variability of glycan signatures. Rather, the final glycan profile is determined by the concerted actions of glycosyltransferases, glycosidases, availability of substrates, cellular arrangements and access to specific glycosylation sites on the underlying proteins or lipids [25, 40, 57]. Thus, the same molecular backbone can be modified by different glycan structures when produced by different cells or under different biological conditions. The variability is modulated by age, sex, genetic background and physiological state [58-60]. Large-scale glycomic studies have shown that circulating N-glycan profiles are different according to age and sex. Genetic studies have identified loci associated with variation in human glycosylation patterns. Hence, glycans constitute a dynamic layer of molecules that mirror the manner in which genetics and biological processes interact.

This variation offers potential value but also significant challenges for forensic interpretation. In contrast, differences in glycan composition may carry information related to the biological characteristics, physiological state or cellular source of an individual from which a trace originated. The capacity to assess chronological age from N-glycan profiles obtained from dried bloodstains presents an important example of the informational potential preserved in glycan signatures [29, 61, 62]. However, changes related to disease, environmental exposure, sample degradation or post-deposition transformation must be distinguished from natural biological variation. So, a difference observed in a forensic sample could reflect the original biological state of the donor, or subsequent molecular change, or both. Understanding these interacting sources of variation and establishing baseline patterns will be important before glycan signatures can be reliably interpreted as forensic evidence.

Tissue-, cell-, and body-fluid-associated glycosylation

Glycosylation patterns are intimately linked to cellular identity, as different sets of glycosyltransferases, glycosidases, transporters and other elements of glycan biosynthesis and processing are expressed in different tissues and cell types [40, 57]. Thus, the same glycoprotein or lipid may have different glycan structures, depending on the cell or tissue where it is made. Tissue-specific glycosylation has been observed in a wide range of biological systems and is influenced by cellular differentiation, secretory activity and the local biochemical environment [63, 64]. This diversity is especially relevant to forensic biology as biological traces stem from various cellular origins and body fluids, all of which have characteristic molecular compositions. Thus, glycan patterns can provide complementary information to

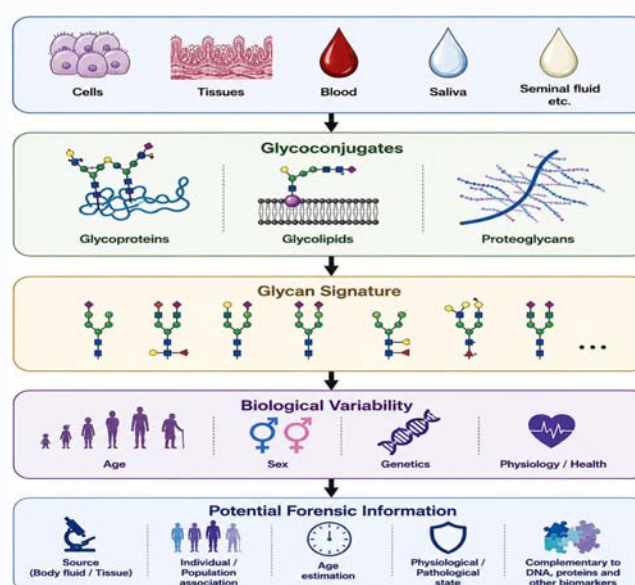


Figure 2: Glycan signatures as a source of biological information for forensic interpretation.

standard body-fluid identification methods based on proteins, RNA or DNA methylation.

Also, the glycosylated molecular profiles of body fluids differ. Saliva is especially rich in heavily glycosylated mucins and other secreted glycoproteins [43, 65], whereas blood is rich in numerous circulating glycoproteins and immunoglobulins. The seminal plasma contains a complex mixture of glycoproteins, whose glycosylation is affected by the male reproductive system and the associated secretory tissues [66]. These biological differences suggest that glycan signatures could be used to discriminate the molecular origin of forensic material. However, source-related variation needs to be evaluated systematically in terms of individual differences and post-deposition changes before reliable forensic interpretation can be attained. Therefore, the establishment of reproducible glycan patterns for tissues, cells and body fluids is a crucial step in determining whether glycosylation can deliver meaningful information for biological trace characterization.

The glycan layer as potential forensic information

The biological variability and source-associated distribution of glycans suggests that the glycan layer may contain information that is relevant for forensic interpretation. Unlike DNA, which encodes primarily information about genetic identity, glycan patterns can be a marker for the biological processes occurring in specific cells, tissues and physiological environments [25, 40, 57]. Their structural diversity might therefore provide a further molecular dimension to the study of biological evidence. Differences in glycosylation have been linked to age, gender, genetic regulation, tissue origin, and physiological or pathological state [59-62]. Importantly, the recovery of informative N-glycan profiles from aged bloodstains shows that at least some glycan signatures can remain analytically accessible after deposition of biological material [29]. To summarize, these findings support the possibility that glycans can complement existing forensic biomarkers, providing information about the biological source and characteristics of a trace.

Nevertheless, the forensic value of glycans remains to be demonstrated and needs to be systematically validated before a

practical interpretation can be established. Future applications will need to be able to discriminate between inherent biological variation and changes induced by environmental exposure, microbial activity, degradation or analytical procedures. Changes in a recovered profile could be due to modification of the glycan, degradation of the carrier molecule, or both [34, 40], since the molecular context in which glycans occur also influences glycan structures. However, advances in glycomic and glycoproteomic techniques are beginning to allow the detection of complex molecular signatures from limited biological material [26, 44, 45]. The glycan layer should not therefore be seen as an immediate replacement of existing forensic methods but as a potentially informative molecular dimension whose persistence, variability and transformation need dedicated investigation. Understanding these relationships is critical to assessing the eventual contribution of glycan signatures to biological source characterization and broader forensic trace interpretation, as illustrated in Figure 2.

The Glycan Landscape of Forensically Relevant Biological Materials

Blood and blood-derived material

Blood is one of the most abundant biological materials encountered in forensic investigations, and is also one of the best characterized human sources from a glycomic perspective. The glycan landscape comprises circulating plasma and serum glycoproteins, immunoglobulins and cell associated glycoconjugates, resulting in a complex mixture of N-linked and O-linked carbohydrate structures. Glycosylation can be highly heterogeneous for major blood proteins, and the circulating N-glycan profile has been shown to change according to biological factors such as age and sex [59, 60]. Additionally, there is measurable variation in human glycosylation patterns due to genetic regulation [61]. These observations suggest that blood does not have a single glycan composition, but that its glycan profile is a biologically variable molecular landscape influenced by the proteins and cells present in the sample.

Blood-derived material provides the most compelling direct evidence, to date, of the forensic utility of glycan analysis. Gudelj et al. showed that N-glycan profiles can be recovered from dried

bloodstains stored for several years and used to model chronological age, supporting the persistence of measurable glycan information in deposited forensic material [29]. More generally, the analysis of the human plasma and serum N-glycome revealed that blood glycosylation is an integration of the contributions of numerous circulating glycoproteins and can be modulated by genetic, physiological, metabolic and pathological factors [67, 68]. This complexity is important for forensic interpretation: a recovered bloodstain glycan profile may contain biologically informative variation, but this cannot be automatically attributed to a single factor. Thus, it will be necessary to establish the interaction between donor related variability, sample composition and post deposition changes in an effort to determine which of the blood associated glycan features can be reliably translated into forensic information.

Saliva and oral biological traces

Saliva is a complex biological material of forensic relevance, whose glycan composition is strongly influenced by secreted glycoproteins, such as mucins. O-glycosylation is widespread in the major salivary mucins MUC5B and MUC7, and their carbohydrate structures are involved in lubrication, microbial interactions and protection of the oral environment [43, 63]. Saliva also contains many other glycoproteins, as well as proteins and cellular material from oral epithelial cells [65]. Therefore, an oral bio-trace will not have a single glycan profile, but a composite molecular signature that depends on the secretions from salivary glands, oral cells, and biological conditions at the local level. This complexity may be of special relevance for forensic traces such as saliva deposits, bite marks, cigaret butts, drinking vessels or other items with oral biological material.

Factors present in the oral environment may also influence the interpretation of salivary glycan signatures. Salivary glycans participate in interactions with the oral microbiota and microorganisms producing glycosidases may alter glycoconjugates or use carbohydrate structures in the oral environment [69, 70]. Moreover, the differences between samples could be due to differences in the composition of salivary proteins, secretory activity, and individual biological features. These factors indicate that a glycan pattern obtained from an oral trace may be representative of the original biological material, as well as processes taking place within the oral environment before and after deposition. Glycan based identification of saliva has not yet been established as a routine forensic method, but the abundance of highly glycosylated molecules and the unique nature of oral secretions, make saliva an important material for investigating the persistence and forensic interpretation of glycan signatures.

Seminal and reproductive biological material

Seminal material, also relevant in forensic contexts, presents a complex glycan landscape. Seminal plasma is a complex mixture of proteins and glycoproteins derived from the testes and accessory glands (seminal vesicles, prostate and epididymis). These secretions contribute different molecular components to the final seminal profile, while the sperm cells themselves have associated with their surface glycoconjugates involved in maturation, recognition and interactions in the reproductive tract. Glycosylation is thus distributed across cellular and fluid components of semen generating a potentially informative molecular profile, rather than a single uniform glycan signature. The extensive proteomic characterization of seminal plasma provides an important molecular basis for exploring this diversity [66].

Modern glycomic and glycoproteomic studies have indicated

that seminal plasma and spermatozoa display common and specific glycosylation features. Detailed studies have described complex, high-mannose, fucosylated and sialylated glycans including abundant Lewis-type structures on seminal plasma glycoproteins [69-72]. Recent site-specific analyses have also demonstrated the extensive presence of N- and O-glycosylation among proteins found in human semen, highlighting a marked structural heterogeneity between sperm cells and the surrounding seminal plasma [73-75]. Also, the sperm glycocalyx is modulated during maturation and interaction with reproductive fluids, suggesting that reproductive glycan signatures are biologically dynamic, not static [76]. From a forensic perspective this complexity may be a potential source of trace information although its stability post deposition is poorly understood. Future studies will need to determine if characteristic seminal glycan features persist in aged or environmentally exposed stains, and whether they can be reliably distinguished from normal donor-to-donor and physiological variation.

Other potentially relevant body fluids and tissues

Other biological materials such as saliva and seminal material besides blood, can contain glycan signatures of potential forensic relevance, but are much less extensively characterized from a forensic point of view. Mucins and other highly glycosylated molecules are present in vaginal and cervical secretions and are organized for epithelial protection and interactions with the reproductive microbiota. Menstrual blood is also a complex mixture of peripheral blood, endometrial tissue, cellular debris, and reproductive tract secretions and may provide a glycan profile that is distinct from peripheral blood alone. Glycoproteins or glycoconjugates also present in other samples such as urine, tears, sweat and tissue fragments are characteristic in their composition of the tissue or secretory source. These differences support the broader potential of glycans to assist in molecular characterization of various biological traces.

The interpretation of these materials must, however, take into account both compositions related to the source and biological variation. For instance, urinary glycoproteins are probably derived from plasma filtration, renal tissue and the urogenital tract [77], and tear fluid contains a unique secretory proteome with many glycosylated components [78]. Secretions from the mucosa and tissue associated glycoconjugates may also serve as indicators of local cellular activity and tissue specific glycosylation [40, 43, 57]. Although glycosylation signatures characteristic for some of these biological materials have been identified in glycosmic investigations, their forensic persistence and discriminative value are poorly understood. This is an important research gap, especially for body fluids which are hard to identify by conventional methods. Systematic studies of their baseline glycan signatures, inter-individual variation and post-deposition stability could establish whether these less-studied materials provide additional molecular information for the interpretation of forensic biological traces.

Inter-individual and intra-individual glycan variation

Glycosylation is genetically regulated as well as by age, sex, physiology and other biological influences, and the glycan landscape of a biological trace can vary considerably between individuals. Large scale studies of human plasma glycosylation have revealed large inter-individual differences, and genome-wide studies have identified genetic loci associated with variation in specific glycan traits [59-61]. These results demonstrate that two individuals can exhibit different glycan profiles even when analyzing the same type of biological

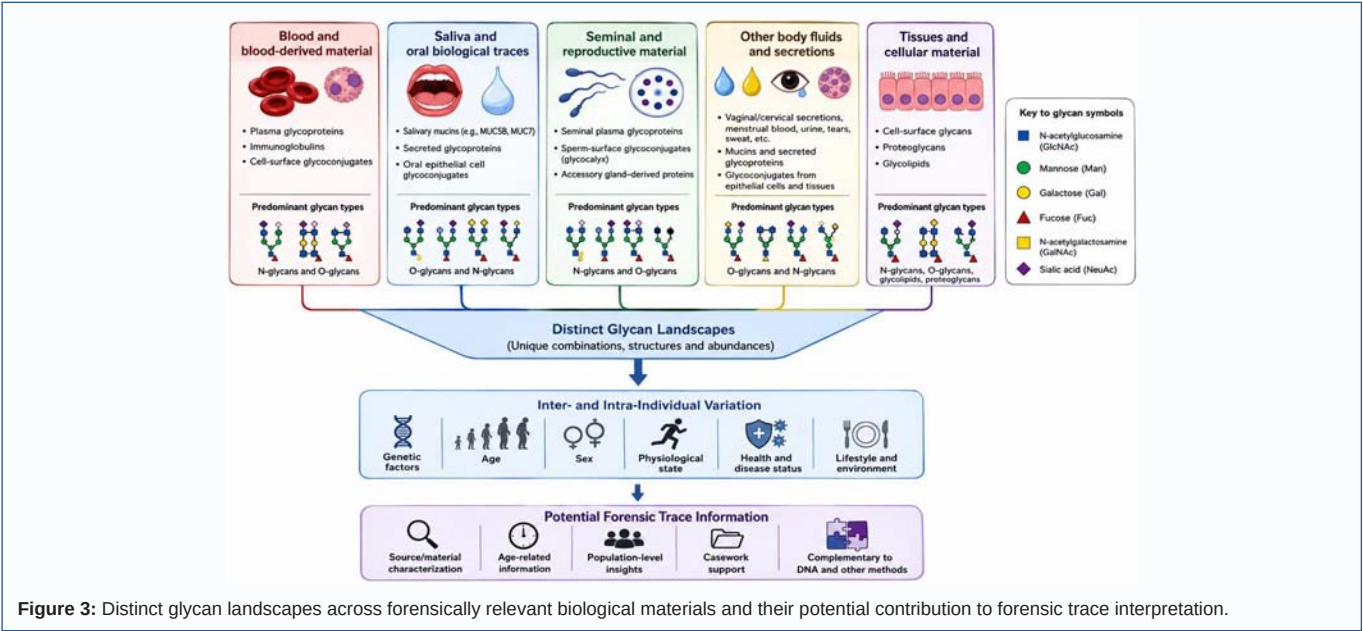


Figure 3: Distinct glycan landscapes across forensically relevant biological materials and their potential contribution to forensic trace interpretation.

Table 1: Overview of glycan-associated features and potential forensic relevance of biological materials.

Biological material	Major glycan-associated components	Key source of variability	Potential forensic relevance
Blood	Plasma glycoproteins, immunoglobulins, cell-surface glycoconjugates	Age, sex, genetics, physiology	Biological characterization; potential age-related information
Saliva	Mucins, secreted glycoproteins, oral epithelial glycoconjugates	Secretory activity, oral environment, individual variation	Oral trace characterization
Seminal material	Seminal plasma glycoproteins, sperm-surface glycoconjugates	Accessory gland contribution, individual and physiological variation	Characterization of seminal biological material
Vaginal/cervical material	Mucins and reproductive tract glycoproteins	Hormonal and physiological state	Potential differentiation of reproductive biological traces
Urine	Urinary glycoproteins and glycoconjugates	Renal and urogenital contributions	Potential molecular characterization
Tears/sweat/other secretions	Secreted glycoproteins and glycoconjugates	Tissue origin and physiological state	Emerging or unexplored forensic potential
Tissues/cellular material	Cell-surface glycans, proteoglycans, glycolipids	Cell type, tissue origin, differentiation	Tissue and cellular source information

Abbreviations: IgG: Immunoglobulin G; MUC: Mucin; N-glycan: N-linked glycan; O-glycan: O-linked glycan; DNA: Deoxyribonucleic Acid

material. Such variation does not, however, equate to individual identification and should not be taken to replace DNA profiling at present. Rather, inter-individual glycan variation is an additional level of biological diversity that may eventually contribute to trace characterization alongside other molecular evidence [62, 67].

Intra-individual variation is another further complication, as glycan profiles may change over time or in response to physiological and pathological processes within the same individual. Age-related alterations are among the best characterized examples, with quantifiable changes in glycosylation associated with plasma and immunoglobulins during the human lifespan [29, 59, 60]. Glycan signatures can also be affected by changes in cellular activity, inflammation, hormonal status and other biological conditions [57, 58]. Thus, for forensic purposes, it will be important to distinguish between stable donor-associated features and those that fluctuate naturally. Before glycan profiles can be evaluated as potential forensic information, reliable understanding of inter- and intra-individual variation is required. This baseline knowledge will also set the stage for later sections on whether observed changes are due to the original biology of the donor or to environmental transformation after deposition, as illustrated in Figure 3. An overview of the major glycan-associated features and their potential forensic relevance across different biological materials is provided in Table 1.

From Deposition to Transformation: The Environmental Fate of Glycans

The moment of deposition: transition from biological system to environmental trace

The deposition of biological material is a key alteration of the molecular environment of a forensic trace. Prior to deposition, glycans and their associated proteins or lipids are part of a regulated biological system under controlled temperature, hydration, pH, enzymatic activity, and ongoing cellular or physiological processes. When blood, saliva, semen or other biological material is deposited on an external surface, this regulation is lost and the molecular components are exposed to environmental conditions that may progressively alter their original state [40, 57]. The trace thus produced is thus a dynamic system, not a chemical record of the biological material at the time of deposition. Dehydration, altered enzymatic activity, oxidation or interactions with the surrounding surface and atmosphere may immediately induce changes [32, 55]. For glycans, the effects of this transition may depend on both their intrinsic structural properties and the stability of the glycoproteins, glycolipids or proteoglycans to which they are attached.

This transition is especially important because any glycan profile obtained during forensic investigation may result from overlapping sources of variability: the original biological signature of the donor and

the molecular changes that occur after deposition. As environmental conditions change over time, glycan-containing molecules may be retained, selectively degraded, or structurally modified [26, 34]. However, direct evidence for these processes in forensic traces, in particular, is still limited. Much of the present understanding must be inferred from wider research into glycan stability, degradation of glycoconjugates and molecular responses to environmental stress [31, 32, 55]. This gap provides the central rationale for examining glycan fate as a distinct forensic concept. It is important to understand the transition from a living biological system to an environmentally exposed trace so that it can be determined whether or not recovered glycan signatures are reflective of their original biological source, subsequent environmental transformation or a combination of the two.

Temperature and thermal stress

Temperature is among the most important environmental variables affecting the stability of biological traces after deposition. Temperature changes can affect molecular mobility, reaction kinetics, protein conformation and enzymatic activity, and thus can have an impact on glycans and glycoconjugates [31, 34, 40]. At higher temperatures, proteins may denature or aggregate, which can change the molecular environment that protects or presents attached glycans. Temperature can also affect hydrolytic and oxidative reactions, but the susceptibility of individual glycan structures is dependent on their composition, linkage and molecular context. Thermal stress is therefore unlikely to affect all glycans within deposited forensic material in the same way. Selective changes may occur within complex populations of glycans and the relative abundance of structures originally present in the trace may be altered.

The role of temperature must also be considered as part of the molecular carrier and not as a structure in isolation. It has been shown in experimental studies that glycosylation may play a role in protein conformational and thermal stability, whereas removal or alteration of carbohydrate moieties may lead to increased susceptibility to thermal denaturation and aggregation [40, 79-82]. Exposure to severe heat in certain chemical conditions, however, may cause the release of glycan or detectable alterations in the glycoform distributions [83, 84]. However, most of the available studies were performed on purified glycoproteins or controlled experimental systems rather than on environmentally aged biological traces and therefore these results cannot yet be directly translated into predictions for forensic stains. However, they provide a mechanistic basis for investigating whether temperature produces reproducible shifts in glycan persistence or composition following deposition. Therefore, controlled forensic studies over realistic temperature ranges and exposure periods are necessary to separate stable glycan features from thermally induced molecular change.

Humidity and water-mediated change

The post-deposition behavior of biological traces can be markedly affected by the humidity because the presence or absence of water influences molecular mobility, chemical reactions, enzymatic activity and microbial growth. Progressive drying at early stages post-deposition may reduce the water availability and change activity of endogenous enzymes, whereas contact with humid conditions or repeated wetting-drying cycles may partially reactivate biochemical processes and increase opportunities for hydrolytic change [32, 55, 57]. For glycan containing molecules water mediated effects may be direct, via hydrolysis under suitable conditions, or indirect, via

structural changes in the associated glycoprotein, glycolipid or proteoglycan matrix. The magnitude of these changes will likely depend on temperature, pH, duration of exposure and physical properties of the deposited material. Thus, humidity should be considered a dynamic factor which may differentially influence glycan persistence across forensic substrates and biological sources.

Water availability may also influence glycan fate by affecting the general degradation environment of a biological trace. Higher moisture content can facilitate colonization by microbes and enzymatic activity including that of glycosidases which can cleave certain carbohydrate bonds. Conversely, dry conditions may inhibit many biological degradation processes without necessarily inhibiting chemical transformation [31, 32]. Molecular framework and attachment to carrier molecules also influence glycan stability, so some glycoconjugates may be relatively protected while others become more vulnerable as the surrounding matrix breaks down [34, 55]. There are very few direct studies to date examining humidity-driven changes in glycan profiles of forensic stains. This lack of evidence constitutes a major research gap and controlled studies comparing dry, humid and cyclic wet-dry conditions could help determine whether moisture produces reproducible changes in glycan abundance or structural composition after deposition.

Ultraviolet radiation and photochemical transformation

UV radiation is another environmental stressor that can affect the molecular composition of deposited biological traces. There is little direct evidence on changes to forensic glycan profiles due to UV-exposure, but it is known that UV radiation causes photochemical reactions and leads to the formation of reactive species that can modify biological molecules. In systems with glycans, irradiation effects may be due to direct effects on the carbohydrate moieties and, importantly, damage to the associated proteins, lipids or other surrounding molecular components [40, 81]. Photooxidative processes can modify the chemical environment of a trace and possibly affect the stability and recoverability of individual glycoforms. They are likely to occur to varying extents depending on the wavelength of radiation, length of exposure, availability of oxygen, moisture and composition of the biological material.

Experimental and mechanistic studies indicate that carbohydrates and molecules containing carbohydrate moieties can undergo light-driven chemical transformations when sufficient energy is available or appropriate photoactive conditions are met. High-energy UV irradiation has been used to initiate radical and electron-transfer reactions involving carbohydrate derivatives, and more recent work has shown that light-mediated chemistry can induce structural transformations at anomeric and non-anomeric positions [85, 86]. Photooxidative processes can result from reactive surface environments in natural carbohydrates upon UV exposure [87]. These results do not establish a particular, universal UV-degradation pathway for glycans in forensic stains, but rather provide mechanistic evidence that carbohydrate structures and glycoconjugate systems can be chemically altered under appropriate photochemical conditions. In real casework, therefore, the UV exposure may be affected by oxygen, moisture, surface chemistry, and stability of associated proteins or lipids, leading to selective changes rather than complete and uniform glycan loss.

Oxidative and chemical stress

Oxidative and chemical stress can change the molecular composition of deposited biological traces by reacting with oxygen,

reactive oxygen species, pollutants, cleaning agents, acids, bases, and other environmental chemicals. For glycans, these effects may result from direct modification of carbohydrate residues or indirectly through damage to the glycoproteins, glycolipids, and other molecular frameworks associated with glycans [34, 40]. Oxidative processes are especially relevant because biological traces deposited in the air can be exposed to oxygen and reactive species over long periods of time. The resultant molecular changes may not involve complete degradation, but rather selective oxidation, cleavage or alteration of particular structures could modify the relative glycan profile recovered from a trace.

This process may be further modified by the chemical environment of a trace element. Experimental and mechanistic studies show that reactive oxygen and nitrogen species can fragment or depolymerize carbohydrate-rich macromolecules including polysaccharides and glycosaminoglycans [88, 89]. Chemical oxidation can modify particular carbohydrate residues, especially structures with vulnerable hydroxyl arrangements, providing proof that glycans can act as chemically reactive sites within larger glycoconjugates [90]. From a forensic point of view, such mechanisms may be relevant when biological traces are exposed to oxidizing agents, atmospheric pollutants, cleaning chemicals, or reactive surfaces. However, the effects of these exposures on complex glycan profiles in true forensic stains are largely unknown. Thus, controlled studies are necessary to determine whether reproducible structural changes occur due to oxidative and chemical stress that could both confound glycan-based interpretation, or possibly provide information about the environmental history of a trace.

Microbial interaction and enzymatic glycan modification

Microbial activity could be one of the most important biological drivers of glycan transformation following deposition of a forensic trace. Many microorganisms possess enzymes capable of recognizing, cleaving, modifying, or metabolizing carbohydrate structures, including glycosidases that act on specific glycosidic linkages [69, 70]. Microbial communities from the biological material, surface, air or soil may interact with glycoproteins, glycolipids and other glycoconjugates of the biological trace once it is exposed to the outside environment. How much they interact depends on the environmental conditions including temperature, humidity, nutrients, and how long they are exposed to the conditions. Microbial activity may therefore selectively modify some glycan structures and not the other, which may change the molecular profile recovered from an aged biological trace rather than a uniform degradation.

Enzymatic modification of glycans is of particular importance as microbial carbohydrate processing systems can exhibit significant substrate specificity. Microorganisms possess a range of glycosidases and other carbohydrate-active enzymes that can release terminal monosaccharides, degrade complex glycans or utilize released carbohydrates as nutrients [91, 92]. In principle, such activity could convert an original donor-associated glycan signature in deposited forensic material into a profile dominated more and more by post-deposition microbial processes. However, direct studies of the microbial modification of glycans in forensic biological stains are very limited. This is a significant knowledge gap and a major research opportunity for the present subject. Future controlled studies combining microbial profiling with longitudinal glycomic analysis may help in distinguishing glycan characteristics derived from the donor from enzymatic changes during exposure to the environment.

Surface and substrate effects

The properties of the surface onto which a biological material is deposited can influence the subsequent molecular fate of the material by the effects of drying, adsorption, retention, accessibility and exposure to environmental stressors. Porous substrates like cotton, paper, wood, and untreated fabrics might soak up biological fluids into their interior, potentially impacting the distribution and recovery of glycan-containing molecules. Material on non-porous surfaces such as glass, metal or plastic is generally present closer to the surface where it may be more exposed to oxygen, light, cleaning agents or physical disturbance. Interactions of glycoconjugates with surfaces may also influence the molecular conformation and stability, in particular because proteins and macromolecules containing carbohydrates may adsorb differently depending on the surface chemistry, charge and hydrophobicity [34, 55, 93].

Surface effects are of particular importance in forensic glycan analysis as a recovered molecular profile may reflect not only biological and environmental change but also selective retention or loss during deposition and recovery. Glycoproteins and other glycoconjugates can bind solid materials via electrostatic forces, hydrogen bonding, hydrophobic interactions and adsorption which can affect the molecular components remaining available for subsequent extraction [94, 95]. A porous substrate may protect some components from direct environmental exposure but make full recovery more difficult, whereas a non-porous surface may help recovery but provide less physical protection. Currently, the exact effect of forensic substrates on persistence of glycans remains largely unknown. Thus, comparative studies on the same biological samples deposited on different materials could determine if substrate-dependent glycan changes are an important source of variation in the interpretation of forensic traces.

Combined environmental exposures

In real forensic cases biological traces are rarely exposed to only one single environmental factor. A bloodstain, saliva deposit or seminal trace can be exposed to changes in temperature and humidity, ultraviolet radiation, atmospheric oxygen, microbial activity and interaction with the underlying substrate simultaneously. These factors may work together to create situations in which the total molecular response cannot be predicted simply by examining individual stressors. For example, increased humidity can be beneficial for molecular mobility and microbial activity and temperature can accelerate chemical and enzymatic reactions [31, 32, 55]. Photooxidative processes can also be enhanced by UV exposure, especially in the presence of oxygen and reactive surfaces [85, 87]. Thus, the glycan profile from an environmentally exposed trace may be a cumulative effect of several interacting transformation processes rather than a simple linear pattern of degradation.

One of the greatest challenges to understanding the forensic fate of glycans is this combined-exposure scenario. Individual laboratory studies can isolate the effects of temperature, moisture, oxidation or microbial activity, but conditions in real casework are complex and involve continuously changing environmental histories. Depending on the biological material, substrate and duration of the exposure, these interactions can generate synergistic, antagonistic or protective effects [88, 89, 93]. There is currently little direct evidence for how complete glycan profiles change under realistic multi-factorial forensic conditions. Future research should, therefore, involve controlled experimental designs in which identical biological traces

Table 2: Environmental factors potentially influencing the post-deposition fate of glycans in forensic biological traces.

Environmental factor	Potential mechanism	Possible effect on glycan-containing material	Current forensic evidence
Temperature	Thermal stress and altered reaction rates	Changes in molecular stability and glycoconjugate degradation	Limited
Humidity	Water-mediated reactions and increased molecular mobility	Hydrolytic change and altered degradation processes	Limited
UV radiation	Photochemical and photooxidative reactions	Possible structural modification of glycans or carrier molecules	Very limited
Oxidative/chemical stress	Reactive species and chemical exposure	Oxidation, cleavage, or modification	Limited
Microbial activity	Glycosidases and carbohydrate metabolism	Selective enzymatic removal or modification of glycans	Emerging
Surface/substrate	Adsorption, retention, and differential exposure	Selective preservation, loss, or recovery differences	Largely unexplored
Combined exposures	Interaction of multiple environmental factors	Complex and potentially non-linear transformation patterns	Major research gap

Abbreviations: UV: Ultraviolet

are exposed to combinations of environmental parameters for defined periods of time. Then, longitudinal glycomic analysis might reveal which glycan features are stable, which are predictably changed, and which are no longer reliable for forensic interpretation. The major environmental factors that may influence the post-deposition fate of glycans in forensic biological traces are summarized in Table 2.

Mechanisms of Glycan Persistence and Transformation

Chemical stability of glycosidic structures

The chemical stability of glycosidic linkages is of fundamental importance for the persistence of a glycan after deposition. The susceptibility of glycosidic bonds to cleavage is dependent on monosaccharide composition, anomeric configuration, linkage position, branching and the chemical environment surrounding the molecule [57, 55]. Many glycosidic structures are stable for long periods under neutral and relatively dry conditions, but acid or alkaline conditions, high temperatures, and reactive chemical species can increase the likelihood of hydrolysis or structural modification [34, 88]. The molecular context is also important as glycans are often attached to proteins or lipids and the stability of these carrier molecules can influence the accessibility and protection of the attached carbohydrate structures. Therefore, persistence of glycans is not expected to be a uniform property, and different individual structures may respond to the same environmental conditions.

Chemical transformation can also take place without complete destruction of the glycan. Partial hydrolysis can remove single monosaccharide residues or susceptible linkages while oxidation and other reactions can alter specific carbohydrate residues and change the initial structural profile [89, 90]. These processes might gradually alter the relative abundance of individual glycoforms in a deposited biological trace. Thus, an environmentally aged sample may still contain detectable glycans, but perhaps not the exact molecular composition of the time of deposition. This is particularly important for forensic interpretation as persistence does not necessarily imply structural preservation. Although glycan chemistry currently provides a mechanistic basis for understanding such processes, systematic studies evaluating the stability of complex glycan profiles under realistic forensic conditions remain scarce. Thus, it will be important to identify chemically stable and environmentally sensitive glycan structures to determine their future forensic applications.

Enzymatic degradation and glycosidase activity

Enzymatic activity is a major mechanism for the progressive alteration of glycan structures after biological material has left its original physiological environment. Glycosidases are enzymes that

are capable of recognizing and cleaving specific glycosidic bonds and their activity may derive from endogenous enzymes already present in the biological material or from introduced microorganisms, before or after deposition [91, 92]. Depending on their substrate specificity, these enzymes can cleave terminal monosaccharides or specific internal linkages and may cause stepwise changes rather than complete destruction of an entire glycan structure. The implications for a deposited biological trace can therefore be dependent on the original glycan composition, enzyme availability, environmental conditions and accessibility of individual carbohydrate residues. Enzymatic activity can be further modulated by temperature, moisture and pH, thus linking glycan degradation to the wider post-deposition environment discussed in Section 4 [31, 32].

The enzymatic conversion of glycans may also produce molecular patterns systematically different from the original biological profile. Selective removal of terminal residues, such as sialic acid or fucose, can change glycan branching patterns, molecular recognition properties, and relative abundance of detectable glycoforms [57, 91]. In microbial systems, carbohydrate-active enzymes are often clustered into complex degradation pathways, enabling the microorganisms to degrade a variety of glycans as possible nutrient sources [92]. However, the identity and activity of relevant endogenous and microbial glycosidases are ill-characterized in forensic traces. That makes a key difference between theoretical susceptibility and demonstrable post-depositional change. Thus, future research should investigate specific biological traces longitudinally, while also assessing microbial or enzymatic activity and the resultant changes in glycan profiles. Such approaches may provide insight into whether enzymatic modification occurs along reproducible patterns that may ultimately aid rather than merely hinder forensic trace interpretation.

Glycoprotein degradation versus glycan modification

Glycoprotein degradation and glycan modification are two related but different molecular processes. A deposited biological trace may be proteolytically degraded along the protein backbone while parts of the associated glycan are structurally intact. Or the glycan can be enzymatically or chemically modified without destroying the carrier protein [40, 57]. This distinction is important because analytical methods may detect changes in the abundance or accessibility of glycopeptides that reflect carrier degradation rather than direct loss of the glycan per se. In addition, proteolysis may reveal otherwise inaccessible glycosylation sites or release glycopeptide fragments into the surrounding matrix. Therefore, an apparent change in a recovered glycan profile may be due to altered carrier stability, direct carbohydrate modification, or both.

The molecular outcome depends on the type of glycoconjugate

and the environment in which the trace is deposited. Glycosylation can influence protein folding, solubility and resistance to proteolytic degradation, and attached glycans may either contribute to persistence of the carrier molecule or become differently accessible upon breakdown of the protein structure [81, 82]. Meanwhile, degradation of proteins can alter the recovery and analytical detection of their attached glycans, especially when the analysis relies on intact glycoproteins or site-specific glycopeptides. For forensic applications, it will be critical to disentangle these mechanisms as the loss of a glycoprotein signal should not be interpreted as the complete loss of glycan information. Future studies should compare intact proteins, released glycans, and glycopeptide profiles from the same environmentally aged samples to determine which molecular components remain and how degradation of one component affects the interpretation of another.

Selective persistence of glycan structures

Not all glycan structures are expected to be equally persistent post-deposition. Structural features such as monosaccharide composition, linkage type, branching, terminal modifications and association with a protein, lipid or extracellular matrix component may determine their susceptibility to environmental or biochemical change [55, 57]. Terminal residues may be especially vulnerable when they are accessible to particular enzymes or chemical reactions, whereas glycans ensconced in a stable molecular setting may be relatively immune. The glycosylation itself can also affect the stability of the carrier molecule's structure, which can affect the persistence of the attached carbohydrate structures [81]. The post-deposition fate of a glycan must therefore be considered as a selective process in which some molecular features are preserved and remain detectable, whereas others are gradually lost or changed.

Selective persistence has important implications for forensic interpretation. With the loss of environmentally sensitive structures relatively stable glycans may become proportionally more prominent in the recovered profile yielding a composition different from the original biological material but not indicative of complete degradation. Thus, changes in relative abundance need to be interpreted with care, as an apparent increase in one glycan might be due to preferential loss of another, rather than production of new molecules. Complex glycan populations have been demonstrated to be structurally diverse and differentially susceptible to enzymatic and chemical processes [34, 91, 92]. But direct longitudinal evidence about which glycan structures persist consistently in forensic biological traces is still scarce. Establishing persistence hierarchies under controlled environmental conditions could thus help to identify robust glycan markers and to distinguish stable biological information from environmentally sensitive features.

Fragmentation, loss, and structural remodelling

Exposure to the environment does not necessarily mean a simple progression from a complete glycan structure to complete molecular loss. Instead, degradation can be achieved through intermediate steps involving cleavage of individual glycosidic bonds, removal of terminal monosaccharides, fragmentation of larger glycoconjugates or other structural modifications [55, 57, 89]. These processes can produce partially modified glycan structures which remain detectable but are no longer representative of the original molecular profile. The other way in which fragmentation can occur is indirectly, when the protein or other carrier molecule to which the glycan is attached is degraded to smaller glycopeptides or the glycans are released into the

surrounding matrix [40, 81]. Thus, the molecular composition of an aged biological trace can be composed of intact structures but also of partially modified forms and degradation products, resulting in a complex transformation pattern instead of a uniform loss of signal.

Thus, structural remodeling may be of particular importance for the interpretation of glycan profiles of environmentally exposed forensic samples. Enzymatic activity can lead to selective removal of certain monosaccharide residues while oxidative or chemical processes can lead to modifications of vulnerable carbohydrate groups, resulting in changes of the analytical behavior of the resulting structures [88-92]. Such changes may alter the relative abundance of glycoforms and may lead to recurrent transformation patterns under certain exposure conditions. However, we need to be cautious not to assume that every difference in structure observed reflects active biological remodeling, because post-deposition changes may be a result of selective degradation or differential recovery of molecular components. There are still few longitudinal studies directly following intact glycans and their transformation products in forensic biological traces. Future work combining time-resolved glycomic and glycoproteomic analysis may be able to distinguish fragmentation, selective loss and true structural modification, and thus improve interpretation of the molecular history preserved within a trace.

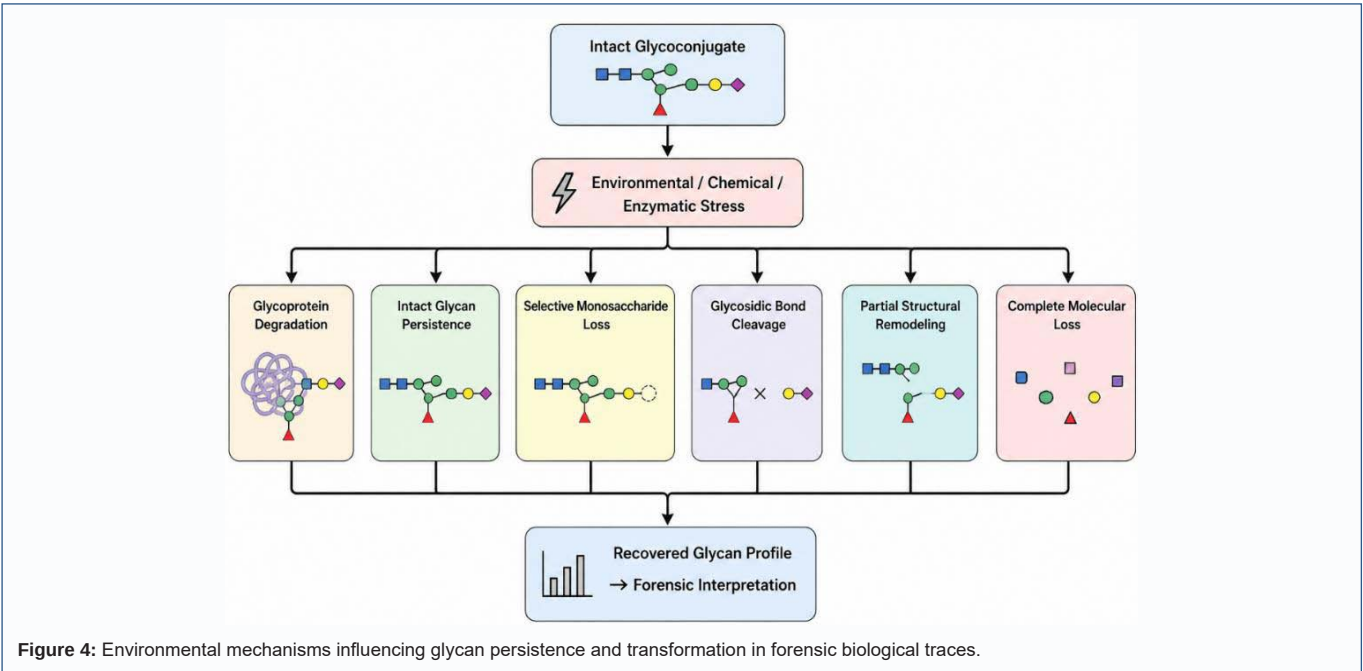
Factors influencing inter-sample variability

It is unlikely that glycans are preserved and converted in a uniform manner on biological traces, since each sample is defined by a different initial molecular composition and a potentially different post-deposition history. Variability may arise from the biological source, initial abundance and structural diversity of glycans, donor-related features, and composition of the surrounding biological matrix [59-62, 67]. Blood, saliva, seminal material and other biological fluids have different populations of glycoproteins, glycolipids and other glycoconjugates. These may respond differently to environmental stress. Furthermore, the concentration of deposited biological material and its distribution on a surface can affect drying behavior, molecular accessibility and exposure to chemical or microbial processes [31, 32]. Thus, two traces of the same body fluid may not necessarily show the same patterns of glycan persistence when subjected to seemingly similar conditions.

Environmental history adds another level of inter-sample variability. Differences in temperature, humidity, ultraviolet exposure, microbial activity, substrate, and time of exposure can affect degradation pathways and the relative abundance of recovered glycan structures [85, 87-89, 91-95]. Analytical variation must also be considered since extraction efficiency, procedures for the release of glycans, sample quantity and instrumental sensitivity can all affect the final profile obtained from a degraded trace. Thus, future forensic applications will need to discriminate real post-deposition transformation from biological and analytical variability. Reproducible patterns will require careful experimental design with standardized biological samples, replicate exposures, and longitudinal analysis. Understanding these sources of variability will ultimately determine whether specific glycan features can be considered robust forensic markers, or whether their interpretation must remain limited to broader patterns of biological trace characterization. The major mechanisms influencing glycan persistence and transformation in deposited biological traces are summarized in Table 3. The potential pathways through which glycans may persist, degrade, or undergo structural transformation following environmental exposure are

Table 3: Major mechanisms influencing glycan persistence and transformation in deposited biological traces.

Mechanism	Primary process	Potential molecular outcome	Possible forensic implication
Chemical instability	Hydrolysis or chemical modification of glycosidic structures	Bond cleavage or structural alteration	Loss or alteration of original glycan information
Enzymatic degradation	Endogenous or microbial glycosidase activity	Removal of terminal or internal sugar residues	Progressive modification of glycan profiles
Carrier degradation	Breakdown of glycoproteins or other glycoconjugates	Glycan release, altered accessibility, or molecular loss	Changes in recovery and interpretation
Selective persistence	Differential stability of specific structures	Some glycans remain detectable longer than others	Identification of potentially stable glycan markers
Fragmentation and remodeling	Partial cleavage or structural modification	New fragments or altered glycan patterns	Environmental transformation signatures
Inter-sample variability	Differences in source, environment, substrate, and exposure history	Variable persistence between traces	Need for cautious interpretation and validation



illustrated in Figure 4.

Recovering the Glycan Signal: Analytical Challenges in Forensic Evidence

Sampling and preservation of glycan-containing evidence

Recovery of glycan information from forensic material begins at the time of sampling, where collection and preservation procedures may affect the quantity and condition of the molecular signal available for subsequent analysis. Glycans are often present as part of glycoproteins, glycolipids and proteoglycans and not as free molecules; therefore, preservation has to consider the stability of the entire glycoconjugate system [40, 55]. Biological traces collected at crime scenes may already have undergone drying, environmental exposure or partial degradation before being analyzed in the laboratory. Other changes during handling and storage can take place because of moisture exposure, temperature changes, microbial contamination or repeated processing. Therefore, appropriate sampling strategies should aim to minimize unnecessary molecular disturbance while retaining sufficient material for both glycan analysis and conventional forensic examinations.

Preservation is especially difficult because the best conditions for keeping glycans intact may depend on the biological sample and on the analytical method used. Storage that limits enzymatic

and microbial activity may help to limit further post-collection modification, whilst tight control of temperature and moisture can reduce further degradation processes [31, 32, 91]. Forensic evidence is often available in small quantities, which means that destructive analytical procedures must be carefully planned to avoid unnecessary consumption of sample. Sampling from porous or environmentally contaminated substrates may present additional challenges because glycoconjugates may interact with the surface and become unevenly distributed or difficult to recover [93-95]. Reproducible glycan-based analysis cannot be transferred into forensic practice until systematic studies of collection methods, storage conditions and preservation intervals are conducted, as standardized workflows for glycan-containing forensic evidence have not yet been established.

Extraction from aged and environmentally exposed traces

Extraction is a critical step in recovery of glycan associated information from forensic biological evidence as deposited material may be present in low amounts, partially degraded and strongly bound to the substrate on which it was deposited. Recovery may be further complicated by environmental exposure which can change physical and chemical properties of glycoconjugates or lead to degradation of their protein, lipid or polysaccharide components [34, 55]. In aged traces the recovered material may consequently be only a part of the original molecular composition. Extraction

procedures should be designed with consideration of the biological source, substrate characteristics and the intended analytical method, as conditions that efficiently recover intact glycoproteins may be different from those optimized for released glycans or glycopeptides [67]. Thus, the extraction process itself may affect the observed glycan profile and should be considered an important potential source of analytical variation.

Adsorption, uneven distribution and selective retention of molecular components on different surfaces [93-95] further complicate a recovery from environmentally exposed traces. Mild conditions may preserve the molecular integrity but leave some of the available material unrecovered, while severe extraction conditions may improve the total recovery but also increase the risk of protein degradation or chemical modification. These trade-offs are particularly relevant in the analysis of low-template forensic evidence where repeated extraction or loss of sample may not be possible. Various approaches have been developed in glycomics workflows for the isolation and enrichment of glycan-containing molecules from complex biological matrices; however, these methods have been mostly optimized for clinical or research samples rather than aged forensic traces [96, 97]. Comparative studies using environmentally aged biological stains on representative forensic substrates are therefore needed to determine which extraction strategies provide the best balance between recovery, structural preservation and reproducible glycan profiling.

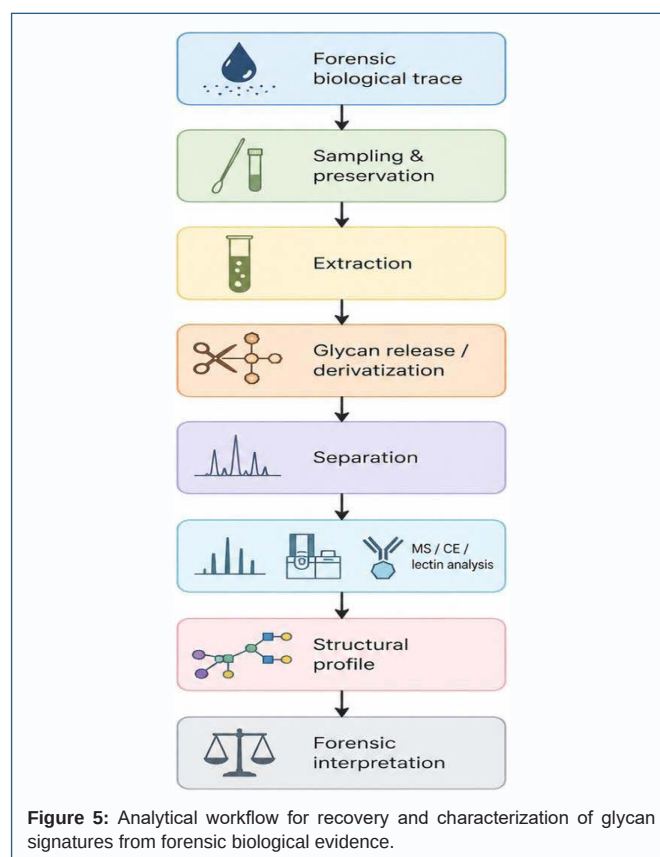
Glycan release and derivatization strategies

Many analytical platforms cannot effectively characterize glycans unless the carbohydrate structures are first released from their parent glycoconjugates and prepared in a form that is suitable for detection. Peptide-N-glycosidases are the most commonly used enzymes for enzymatic release of N-linked glycans. For O-linked glycans, a chemical or other enzymatic approach is usually required, as the structure and linkage types are too diverse to allow a universal O-glycan release [98, 99]. The method of release can affect the final analytical profile, especially in cases of scarce or partially degraded samples. This is especially important in forensic material where harsh conditions may cause additional structural changes and poor release may lead to selective loss of low abundance glycans and an incomplete representation of the original molecular composition.

Glycan detection may be improved by derivatization and labeling strategies that enhance fluorescence, chromatographic separation, ionization efficiency or structural discrimination in mass spectrometric analysis. Derivatization methods including reducing-end labeling and others are used to increase analytical sensitivity and enable comparison between samples [100, 101]. However, each additional preparation step introduces the possibility of sample loss, incomplete reaction or analytical bias, which may be of particular concern when dealing with low-template or environmentally exposed forensic traces. Thus, the analytical workflow has to balance sensitivity against preservation of structural information. Future forensic glycomics protocols will have to be validated on small, degraded and substrate associated samples to test if glycan release and derivatization procedures can reproducibly recover informative profiles without introducing transformations that could be mistaken for genuine post-deposition changes.

Mass spectrometry-based glycan analysis

Mass Spectrometry (MS) is one of the most powerful methods to characterize glycan structures, owing to its high sensitivity and



capacity to provide molecular-level information from complex biological samples. Glycan compositions and their relative abundances can be determined using techniques such as Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-MS) and Liquid Chromatography coupled with electrospray ionization Mass Spectrometry (LC-MS). When these methods are coupled with tandem Mass Spectrometry (MS/MS), structural aspects, *e.g.*, monosaccharide composition and fragmentation patterns, can be determined [102, 103]. Such capabilities are particularly relevant to forensic evidence, where the sample in question may be composed of only small amounts of heterogeneous biological material. Thus, MS-based glycomics can provide a broad molecular profile instead of a single targeted marker.

However, interpretation of glycan data obtained by MS can be challenging, particularly in the case of aged or environmentally exposed samples. Many glycans are isomeric, meaning they have the same masses but different linkages or branching patterns, which necessitates further separation, fragmentation or complementary analytical methods for reliable characterization [22, 104, 105]. Low-abundance samples can also be affected by ion suppression, contamination, incomplete glycan release and preferential detection of certain structures. These analytical limitations may overlap with real environmental transformation in degraded forensic traces and it is difficult to distinguish a real biological change from a technical artifact. Standardized workflows that include appropriate controls, replicate analysis, and orthogonal confirmation will therefore be essential if MS-based glycan profiling is to be reliably applied to forensic biological evidence. The major steps involved in the recovery and analytical characterization of glycan signatures from forensic biological evidence are illustrated in Figure 5.

Chromatographic and electrophoretic approaches

Chromatographic and electrophoretic methods are important and complementary approaches for the separation of complex glycan mixtures prior to and in conjunction with structural analysis. Hydrophilic Interaction Liquid Chromatography (HILIC) is a common method used for glycan profiling as it is capable of separating released glycans according to their hydrophilic properties and is compatible with fluorescence and mass spectrometric detection [97, 100]. Additional resolution can be achieved with reversed-phase and porous graphitized carbon-based separations, particularly for structurally related or isomeric glycans. Capillary electrophoresis is another very sensitive technique based on differences in charge-to-size ratio and may be especially useful for the separation of small amounts of labeled glycans [106]. Such separation methods could be applicable to forensic samples where direct analysis is challenging due to complex biological backgrounds or environmental contaminants.

Coupling separation-based methods with glycan analysis has the major advantage of reducing sample complexity and improving discrimination between structures that may not be distinguishable by mass alone. However, chromatographic retention and electrophoretic migration are affected by derivatization, sample preparation, and instrument conditions, which must be standardized carefully for meaningful comparison between forensic samples [104, 107]. Such approaches can also necessitate relatively extensive preparation and sufficient recovery from the original trace, which can be difficult when evidence is aged or only available in minute quantities. However, recent developments in high sensitivity HILIC, ultra-performance liquid chromatography and capillary electrophoresis form a solid analytical basis for future forensic glycomics. The greatest value of such methods may be in combination with mass spectrometry or other orthogonal methods, yielding better structural confidence while retaining as much information as possible from limited biological evidence.

Lectin- and affinity-based methods

Lectin and affinity approaches offer an alternative to full structural glycomics by selectively recognizing specific glycan motifs or classes. Lectins are proteins that bind carbohydrates and can recognize features such as mannose, fucose, galactose, N-acetylglucosamine or sialylated structures depending on their binding specificity [108, 109]. These interactions can be applied in lectin arrays, affinity enrichment, blotting or other targeted analytical platforms. Such approaches may provide a relatively rapid means to screen glycan associated patterns in biological traces for forensic applications prior to more detailed structural characterization. Instead of identifying the precise identity of each glycan, a lectin-based assay might provide a more general pattern of glycan characteristics that can help to distinguish between biological materials or to detect environmentally induced changes.

A major shortcoming, however, is that lectin binding does not always lead to unambiguous structural identification. Individual lectins can recognize many related motifs and the binding behavior is also affected by steric effects, glycan density and accessibility [109, 110]. The environmental degradation of the carrier molecule may also be altered if a glycan is accessible to a lectin even if the carbohydrate structure itself remains present. Thus, affinity-based methods are best viewed as complementary rather than stand-alone approaches for forensic glycan analysis. Their strength might be in targeted enrichment combined with high-throughput screening, especially if sample quantity is a problem. Lectin or other affinity-based

recognition coupled with mass spectrometry could enable selective enrichment with better structural confirmation. This could be especially useful in the case of complex or environmentally exposed forensic traces.

Emerging high-sensitivity and microscale technologies

The growing need to characterize glycans from very small biological samples has prompted the development of microscale and high-sensitivity analytical technologies. Conventional glycomics workflows can consume considerable amounts of sample during extraction, purification, labeling and separation, which is problematic for forensic evidence that is limited to minute or partially degraded biological traces. Miniaturized approaches, such as nanoflow liquid chromatography, capillary electrophoresis, microchip electrophoresis, and microfluidic separation, can reduce sample requirements while improving concentration efficiency, separation performance, and analytical sensitivity [111, 112]. Microfluidic platforms can integrate multiple steps of the analytical workflow such as glycoprotein capture, glycan release, separation and detection, which can minimize sample losses between each individual processing step [112]. These features make microscale technologies especially attractive for future forensic glycomics, though their application to real forensic traces remains to be explored extensively.

Recent advances demonstrate the large increase in sensitivity that is happening in glycan analysis. Using high sensitivity label-free capillary electrophoresis-mass spectrometry, the N-glycome has been profiled at the single-cell level and the glycan profiles have been characterized from nanogram quantities of blood-derived proteins and plasma isolates [113]. Emerging mass-spectrometric workflows are also incorporating improved ionization, fragmentation, quantitative strategies, computational analysis, and machine-learning approaches to improve structural coverage and interpretation capabilities [114]. These developments suggest that future forensic workflows could potentially analyze glycan signatures from evidence amounts that would be analytically challenging in the past. But increased sensitivity does not necessarily mean forensic reliability. Highly sensitive methods can also reveal contaminants, minor molecular populations or artifacts associated with preparation, and thus stringent controls, contamination assessment, reproducibility studies and validated reference materials are imperative before these technologies can be transferred from research laboratories to forensic casework.

Challenges associated with low-template and degraded samples

Glycan profiling is particularly difficult in low-template and degraded biological evidence where the amount of recoverable molecular material can be very low and its composition may have been modified by environmental exposure. Stochastic variation, incomplete extraction, selective glycan recovery, ion suppression, and instrumental sensitivity can all significantly impact the final analytical profile of such samples [97, 102-105]. Degradation of the protein or lipid carrier can further decrease the accessibility of associated glycans. Some degree of partial cleavage of carbohydrate structures may result in a mixture of intact and modified molecules. These effects are particularly significant when only a small portion of the original trace is available for analysis, as any additional preparation or purification steps can lead to disproportionate molecular losses. Therefore, analytical sensitivity should be considered as a performance parameter that is not independent but should be regarded together with structural preservation and recovery efficiency.

Table 4: Analytical approaches for recovering and characterizing glycan signatures from forensic biological evidence.

Analytical approach	Main purpose	Major strengths	Key limitations	Forensic applicability
Glycan extraction/release	Recover glycans from glycoconjugates	Enables downstream analysis	Sample loss, incomplete release	Foundational
Derivatization/labeling	Improve detection and separation	Higher sensitivity	Additional preparation steps	High potential
Mass spectrometry	Composition and structural analysis	High sensitivity and molecular specificity	Isomers, matrix effects, instrumentation	High potential
Chromatography	Separate glycan populations	Excellent resolving capability	Time and sample requirements	High potential
Electrophoresis	High-resolution separation	Sensitive and efficient	Specialized instrumentation	Emerging
Lectin/affinity methods	Target specific glycan motifs	Rapid, selective screening	Limited structural specificity	Screening potential
Microscale technologies	Analyse very small samples	Low sample requirement	Limited forensic validation	Emerging
Integrated approaches	Combine complementary methods	Greater structural confidence	Complex workflows	Future direction

Abbreviations: MS: Mass Spectrometry; CE: Capillary Electrophoresis; HILIC: Hydrophilic Interaction Liquid Chromatography; UHPLC: Ultra-High-Performance Liquid Chromatography

A further major challenge is to distinguish between genuine biological or environmental variability and analytical artifacts. A decrease in glycan signal in an aged trace may be caused by true molecular degradation, inefficient release, substrate adsorption, incomplete derivatization, or preferential ionization of particular structures during mass spectrometric analysis [96, 100, 103, 104]. Likewise, the interpretation of highly sensitive analytical platforms may be hindered by the presence of low abundant contaminants or background glycans when the target material is present at very low concentrations [111-114]. Thus, reliable forensic application will require rigorous negative controls, process blanks, replicate measurements, recovery experiments, and standardized reporting of analytical uncertainty. Reference materials from various biological sources and degradation states will also be needed to assess which glycan features are reproducible and which are too sensitive to handling or environmental history to be reliably interpreted. These limitations currently highlight that glycan profiling of low-template forensic evidence is an emerging research capability and not a validated stand-alone forensic method. The major analytical approaches for recovering and characterizing glycan signatures from forensic biological evidence are summarized in Table 4.

What Can a Changed Glycan Signature Tell Us?

Glycan signatures and biological trace identification

The potential value of glycan signatures to forensic biology begins with their ability to reflect differences in the biological material from which they originated. Distinct populations of glycoproteins, glycolipids, proteoglycans and associated carbohydrate structures are present in blood, saliva, seminal material and tissues, resulting in molecular profiles that are influenced by tissue-specific biosynthetic pathways and cellular activity [40, 43, 52, 57]. In principle, these differences could enable glycan profiling to contribute to biological trace characterization in addition to the established serological, RNA-based, proteomic and DNA-based approaches [10, 13, 17, 23]. Glycomic analysis may be able to assess patterns across multiple structures rather than a single biomarker-based test. Such multicomponent profiles may be of particular use when individual glycan features are of limited specificity but combinations of structures provide a more representative molecular pattern of the originating biological material.

Direct forensic evidence is still limited, but what is known is that glycan information can survive in deposited biological material and retain measurable biological associations. In a striking study,

N-glycan profiles from dried bloodstains were well enough preserved to be used for age-related modeling, showing that informative glycan patterns can be detected even after deposition and long-term storage [29]. However, a measurable glycan profile that persists is not alone sufficient to reliably identify the source. The profile obtained from a trace can be affected by several factors, including, inter-individual variability, biological state, environmental exposure, microbial activity and analytical recovery [59-62]. Thus, the future potential forensic use of glycan signatures will rely on the possibility to establish reproducible source-associated patterns in sufficiently large reference populations and to assess the effect of environmental transformation on their discriminating power. Until this evidence is available, glycan profiling should be seen as a potential complementary approach and not as a substitute for validated DNA or traditional biological identification methods.

Persistence as a marker of evidence stability

Persistence of a glycan signature describes the extent to which key structures of a glycan signature remain detectable and interpretable after removal of biological material from its original physiological environment. Persistence does not mean that there is still some carbohydrate material present. Persistence is about whether the molecular features that were originally present are still sufficiently intact to preserve meaningful biological information. The monosaccharide composition, glycosidic linkage, branching pattern, terminal residues, and association with a protein or lipid carrier of a glycan can affect its stability [34, 55, 57]. Glycosylation itself may also influence the stability of associated proteins, providing a connection between the longevity of the glycan and the longevity of the molecular framework hosting it [40, 81]. Therefore, a biological trace could keep glycans detectable but slowly changing in composition.

The distinction is significant in a forensic context because analytical detectability is not the same as evidential stability. N-glycan profiles have been obtained from dried bloodstains that had been stored for several years, demonstrating that at least some glycan-associated information can survive long-term storage and can be measured [29]. However, the persistence of selected structures may differ from that of the overall glycome, especially when environmental factors selectively affect susceptible linkages or carrier molecules [31, 32, 55]. Therefore, a potentially useful forensic marker would need to demonstrate not only long-term detectability but also reproducible behavior across different environmental conditions, substrates, biological sources and storage intervals. The establishment of such persistence profiles may help to identify glycan structures that are relatively stable compared to molecular features which are highly

susceptible to post-deposition transformation.

Environmental transformation as a potential indicator of exposure history

The exposure to the environment could induce subtle changes in the molecular composition of a biological trace, thus raising the possibility that changes in the glycan signature might encode information about the post-depositional environment. The stability of glycoconjugates can be affected by temperature, humidity, UV radiation, oxidation, microbial activity and interactions with the substrate, resulting in selective cleavage, modification or loss of particular glycan structures [31, 32, 55, 85, 87]. Different environmental factors may affect molecular components by different mechanisms and therefore the resultant glycan profile may not be a simple marker of whether or not a sample is degraded but may be informative of the nature and extent of its exposure. In that case, one would have to find reproducible molecular changes associated with defined environmental conditions, rather than assuming any difference in the amount of glycan implies a specific type of exposure.

The idea is especially appealing as conventional forensic analysis tends to view environmental degradation as an obstacle to evidence recovery, rather than a potential source of information. In theory, a glycan profile that predictably changes under controlled exposure could give an additional molecular record of the history of a biological trace. However, this is still a hypothesis and not a proven forensic capability. Environmental factors frequently co-occur and similar molecular changes can arise through different degradation pathways [88, 89, 91-95]. Moreover, the initial biological composition of the trace and donor-related variation might mask comparably subtle environmental effects [59-62]. Future work should therefore combine controlled environmental experiments with longitudinal measurements of the glycome to determine whether specific glycan changes can be reliably associated with specific exposure conditions or histories of exposure.

Potential contribution to time-related interpretation

Interpretation of time in forensic biology There are several different concepts related to time in forensic biology that should not be confused. Chronological age of the donor, time since deposition of the biological trace and molecular age or degradation state of the evidence deal with different biological and forensic questions. Studies of glycans have already demonstrated that glycan profiles in human blood can harbor age-related information with measurable change throughout the lifespan [29, 59, 60, 62]. However, these findings are not related to the length of time a biological stain has been present at a crime scene, but rather to the characteristics of the donor. When considering whether glycans can contribute to temporal interpretation, it is important to differentiate these concepts, as a glycan profile may represent biological aging without carrying any information about when the corresponding trace was deposited.

A more challenging prospect is whether progressive changes in glycan composition post-deposition might eventually yield information related to time since deposition. This possibility is theoretically supported by the susceptibility of glycoconjugates to environmental, enzymatic, oxidative and hydrolytic processes that may modify molecular profiles over time [31, 32, 55, 88, 89]. If defined glycan structures can be shown to reproducibly change under controlled environmental conditions, then their relative abundance or structural state might be potentially integrated into a molecular

model for temporal interpretation. However, such an approach would be substantially confounded by temperature, humidity, substrate, microbial activity, initial glycan composition and donor variability [59-62, 91-95]. Thus, currently, the evidence for glycan-based age estimation is stronger than for time-since-deposition estimation, and the latter should be considered an important but unvalidated research direction.

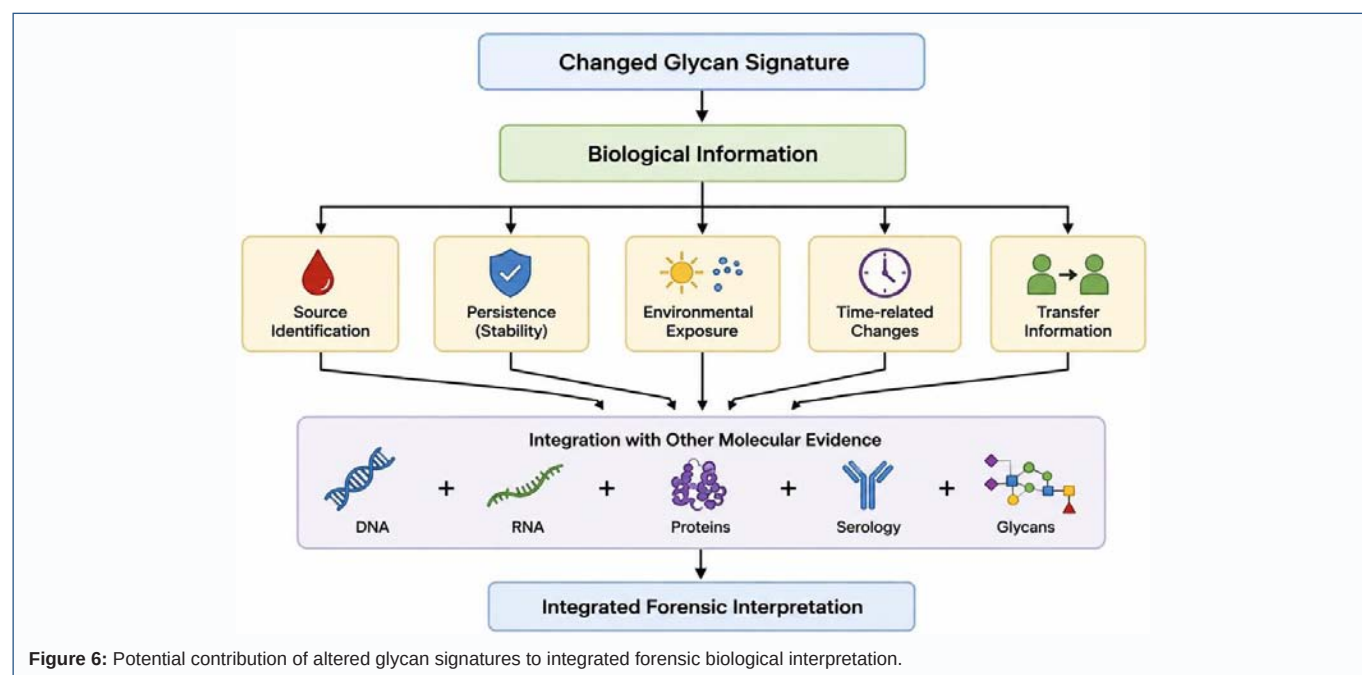
Glycan information and biological trace transfer

Biological material can be transferred from one surface or individual to another, and this is an important consideration in forensic interpretation, as the molecular composition of a recovered trace may be representative of both the original source and the circumstances of deposition. Studies of DNA transfer have demonstrated that biological material may be deposited by direct contact, secondary transfer and repeated contact, and transfer efficiency depends on the quantity and type of material, surface characteristics, conditions of contact and environmental factors [115, 116]. Glycan-containing molecules may have different properties than cellular DNA, since they can exist as components of extracellular proteins, or of membrane-associated glyco-conjugates, or of secretions or other biological matrices. Therefore, their transfer can depend on the physical form of the original material, the molecular carrier and the interactions at the surface on contact. These differences indicate that glycan signatures may offer information that is complementary to traditional DNA-based interpretation.

A major outstanding question is if glycan profiles are sufficiently source-associated after direct or secondary transfer to contain meaningful biological information. Such transfer can result in selective deposition of some glycoproteins or extracellular components, and subsequent surface interactions may further influence which molecular features remain detectable [93-95]. Also, repeated transfer could gradually reduce the abundance of source associated material, which could potentially change the relative distribution of glycan structures. However, direct evidence is currently lacking to establish whether glycan transfer patterns are reproducible or whether they can distinguish primary from secondary deposition. A controlled experiment comparing direct, indirect and sequential transfer of glycan containing biological material might provide an important basis for the assessment of this possibility. Such studies would also establish whether glycans are a complementary trace level marker or merely another molecular component subject to the same interpretive uncertainties as other transferred biological materials.

Complementing DNA, RNA, protein, and conventional serological evidence

The value of glycan analysis is primarily as an additional molecular layer of analysis, not as an alternative to established forensic methods. Although DNA profiling is still the main method for identification of individuals, RNA-based methods can help to characterize body fluids and protein-based methods can give information about the biological source of a trace [10, 13, 17]. Conventional serological methods are still important, as they can provide rapid information about the presence or possible origin of biological material. Unlike these markers, glycans are defined by enzymatic biosynthesis, cellular context and molecular interactions, and can encode information not directly captured by DNA sequence or protein abundance alone [25, 40, 57]. Thus, a combined analytical strategy can provide complementary information from different molecular layers in the same biological trace.



However, integration demands careful consideration of the unique strengths and limitations of each molecular approach. DNA can offer highly discriminating individual information, but does not always provide the biological context or activity associated with a trace. RNA and proteins may offer additional source-related information, but may be affected by degradation and environmental exposure [11-18]. Glycan signatures could potentially yield information about biological source, molecular persistence or post-deposition transformation, but their interpretation is currently hampered by the absence of forensic datasets and incomplete knowledge of environmental behavior [29, 59-62]. Instead of proposing a forensic test based solely on the glycan profile, a more realistic future model is to combine the glycan profile with DNA, RNA, protein and traditional serological results in an integrated evidential framework. This kind of multimolecular analysis could allow us to better characterize biological traces and to identify and study inconsistencies between molecular layers. The potential integration of changed glycan signatures with other molecular evidence for forensic interpretation is illustrated in Figure 6.

Towards a Framework for Glycan-Based Forensic Trace Interpretation

In the previous sections, we have shown that the biological source of a biological specimen, its molecular organization, its environment exposure, the degradation pathways, the analytical recovery and the biological variability of the specimen itself can influence the glycan profile of a biological specimen. These factors should not be evaluated independently when determining the forensic meaning of a recovered glycan profile. Instead, they can be embedded in a sequential framework following the molecular history of a biological trace from its original biological source through deposition, environmental exposure, transformation, recovery and final interpretation. This is especially true for glycans, since their structures may be bearing biological information, which is also under selective modification after deposition [29, 34, 40, 55]. The framework we propose thus considers the recovered glycan profile as a consequence of both source information and post-depositional molecular history, rather

than considering each detected feature as an unaltered feature of the biological material of origin.

The framework starts with Source, which points to the biological material from which the glycan profile was obtained and then Deposition, which indicates the transition from a regulated biological environment to an external trace. Exposure considers the environmental conditions encountered after deposition including temperature, moisture, radiation, chemical stress, microbial activity and substrate interactions [31, 32, 85, 91, 93]. Transformation is about the persistence, degradation, selective loss and structural remodeling of glycan-containing molecules [88-92]. The next step, recovery, assesses whether the residual molecular information can be sampled, extracted, released, and analytically characterized without introducing significant further bias [96-105]. Finally, Interpretation assesses what conclusions can be reasonably supported by the recovered profile, and, equally important, what conclusions cannot be justified. The last distinction is important, because a forensic glycan framework needs to incorporate uncertainty, biological variation, environmental effects and analytical limitations rather than treating glycan patterns as inherently definitive evidence.

Source

The Source stage sets the biological context in which a glycan signature is derived. Blood, saliva, seminal material, epithelial cells, tissues and other biological materials contain different combinations of glycoproteins, glycolipids, proteoglycans and free or carrier-associated glycans [40, 43, 52, 67]. Thus, the first glycan landscape is a mixture of tissue- and cell-associated biosynthesis, secretion, molecular abundance, and donor-specific biological variation [59-62]. This starting profile is essential to be created, as all further changes need to be understood in terms of the original molecular composition. A glycan feature that seems unique in a recovered trace may otherwise be misassigned to environmental transformation when it actually reflects normal biological variation between individuals or between different biological materials.

Therefore, for forensic purposes, source assessment should

differentiate between source-specific, donor-associated and non-specific glycan features. A good useful source marker would be consistently enriched in a defined biological material, and sufficiently stable to survive relevant post-deposition conditions. However, the existence of different glycan landscapes in blood, saliva, seminal material and tissues provides a rationale for further investigation [29, 67-76]. So far, the current evidence does not offer such a complete set of validated glycan source markers. The source stage should therefore be considered as the baseline with which subsequent molecular changes are compared, rather than an immediate identifying end point.

Deposition

Deposition is the transition from a biological system under physiological control to the external environment. Before deposition, glycans are present in cellular or extracellular systems, and factors including temperature, hydration, *pH*, enzymatic activity and molecular turnover control their. These conditions can change rapidly after deposition and drying, temperature change, exposure to atmospheric oxygen and interaction with the receiving surface become relevant [31, 32, 55]. The subsequent molecular fate may depend also on the physical distribution of the deposited material, especially if a porous substrate is penetrated by a biological fluid or the fluid is unevenly distributed over a surface [93-95].

Thus, the deposition event is not only a transfer of material, but also the beginning of the post-deposition molecular history of the trace. Initial environmental conditions, the surface to which the glycan is attached, the physical form of the trace, and the amount of biological material deposited may influence subsequent glycan persistence and accessibility. The deposition stage adds an important layer of uncertainty to subsequent interpretation because these variables may not be fully known in casework. As such, a forensic framework should recognize deposition as a transition point in which the original biological glycan profile begins to interact with the environment rather than an assumption that the recovered profile is molecularly identical to the source material.

Exposure

After deposition, the glycan-containing trace is subject to environmental conditions that can affect molecular stability and transformation. Temperature, humidity, ultraviolet radiation, oxidative conditions, microbial activity, chemical exposure, and substrate properties can act individually or in concert [31, 32, 85, 87, 91, 93]. These factors can affect the glycan structures as well as the associated proteins, lipids or extracellular matrices [34, 40, 55, 81]. Critically, environmental exposure is seldom constant over the lifetime of a forensic trace. Temperature and humidity can shift, light exposure can fluctuate during the day, and microbial activity can increase as moisture and nutrient availability change.

That is why exposure should be considered as an environmental history that depends on time, and not as a single variable. Depending on whether it is exposed indoors or outdoors, under dry or humid conditions, on porous or non-porous surfaces, or in the presence of chemical contaminants, the same biological material can be subject to wildly different molecular stresses. Direct forensic glycan datasets for these combinations are currently limited. Thus, the proposed framework views environmental exposure as a potential source of molecular transformation, the effects of which must be experimentally characterized before specific glycan changes can be interpreted as reliable indicators of specific environmental conditions.

Transformation

The Transformation stage describes the molecular changes that occur after deposition. These may include glycosidic bond cleavage, enzymatic removal of monosaccharide residues, oxidation, hydrolysis, fragmentation of glycoconjugates, selective persistence and structural remodeling [55, 88-92]. Importantly, transformation does not have to result in a complete loss of glycan information. Some structures may remain relatively stable and others are preferentially lost or modified, so that the composition of the recovered glycome does not match the original profile [34, 57, 81]. Additional alterations in glycan accessibility may result from degradation of the protein or lipid carrier, thus resulting in complex interactions between carrier stability and carbohydrate persistence.

This stage is crucial in the proposed forensic framework, since transformation can be both a source of uncertainty and a potential source of information. If particular glycan structures exhibit reproducible changes under defined environmental conditions, then their transformation could in theory provide information about exposure history or molecular stability. Erratic transformation, however, would compromise their value as forensic markers. Current evidence is insufficient to establish general transformation trajectories for forensic glycans. Therefore, longitudinal studies that quantify glycan structures at multiple time points under controlled conditions will be important to establish whether the transformation occurs in reproducible patterns distinguishable from normal biological variability.

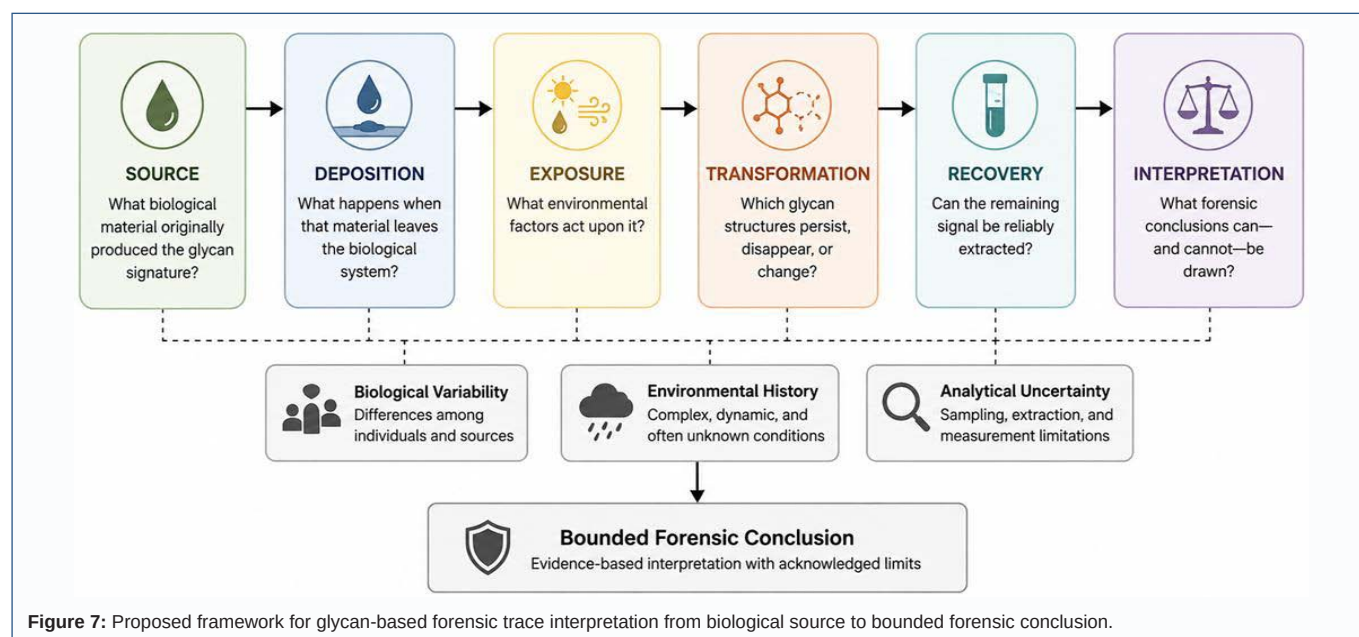
Recovery

The Recovery stage tackles the practical question of whether the molecular information left behind after environmental exposure can actually be recovered and measured. Losses or biases may be introduced by sampling, storage, extraction, glycan release, derivatization, separation and instrumental detection [96-105]. These effects may be particularly important for low-template traces where even small processing losses can significantly change the observed profile. Further, interactions with substrates that retain glycoproteins or other glycoconjugates on surfaces may complicate recovery and environmentally damaged molecules may behave differently under extraction conditions than intact material [93-95].

Thus, successful analytical detection does not necessarily mean that the profile recovered is an accurate representation of the trace at the time of deposition. However, a forensic recovery workflow should be evaluated for reproducibility, structural preservation, recovery efficiency and vulnerability to preparation related artifacts. Emerging microscale and high-sensitivity platforms may reduce sample requirements and improve detection capabilities [111-114]; however, increased analytical sensitivity also increases the importance of contamination controls and reference materials. The framework thus positions recovery between molecular transformation and forensic interpretation, stressing that analytical processing itself is a potential source of variation to be accounted for before recovered glycan profiles are assigned evidential significance.

Interpretation

The last stage is Interpretation. Here, the recovered glycan profile is translated into a forensic conclusion. At this point it should be made clear what is experimentally demonstrated, supported by emerging evidence, and hypothetical. For instance, age-related glycan patterns have been demonstrated in blood and dried bloodstains [29,



59, 60, 62], while the utility of post-deposition glycan transformation for inferring environmental history or time since deposition remains unvalidated. Furthermore, glycan signatures may be a useful adjunct to DNA, RNA, protein and traditional serological evidence, but should not be offered at this time as independent substitutes for established forensic identification methods [10, 13, 17, 23].

Moreover, a scientifically defensible interpretation must take into account the uncertainty associated with the variability of donors, the composition of the source, environmental history, microbial activity, substrate effects, extraction efficiency, analytical sensitivity, and the limited knowledge of glycan transformation pathways [59-62, 91-95]. Thus, the proposed framework emphasizes bounded interpretation: a glycan result should be used only to support conclusions that are supported by validated data and must explicitly account for uncertainty when evidence is insufficient. In this respect, the greatest value of forensic glycans may not be in providing one definitive answer, but rather, in providing an additional molecular dimension that can be combined with other independent forms of biological evidence. proposed framework integrating source, deposition, environmental exposure, transformation, recovery, and bounded forensic interpretation is summarized in Figure 7.

Current Evidence Gaps and Barriers to Forensic Translation

Limited forensic-specific experimental studies

One of the major hurdles to the implementation of glycan analysis in forensic practice is the limited number of studies that have directly analyzed glycans from authentic or simulated forensic biological evidence. The best example remains the study of Gudelj *et al.*, who analyzed the N-glycan profiles from bloodstains obtained from 526 individuals and showed an association between glycan patterns and chronological age, with a prediction error of around 9 years [29]. The study is an important proof of concept, but is a rather narrow proof of concept demonstration, and only addresses the issue of dried bloodstains and their age estimation, rather than the broader forensic issues of glycan persistence, source identification, environmental transformation and transfer. Later on, glycomics

studies have expanded substantially in biomedical and analytical applications, but such developments do not automatically translate into forensic validation, given the wide variations in experimental conditions, sample types and analytical targets [26, 44, 114].

The recent emergence of dedicated reviews on glycomics in forensic serology suggests growing awareness of the field, but also highlights continuing requirements for validated protocols and standardization [117]. So, there is a huge imbalance in the current evidence base between glycoscience knowledge and direct forensic experimental evidence. Many of the mechanisms involved in the proposed forensic fate of glycans including glycan stability, enzymatic modification, environmental transformation and analytical recovery are based largely on biochemical and biomedical studies rather than controlled forensic experiments [31, 34, 55, 91]. This distinction is important to the current review because mechanistic plausibility should not be presented as a demonstrated forensic ability. Before glycan signatures are considered reliable forensic evidence, dedicated experiments are necessary with authentic biological traces, realistic substrates, controlled environmental exposures, longitudinal sampling and validated analytical workflows.

Lack of standardized environmental exposure models

A major challenge in evaluating the postdeposition fate of glycans is the absence of standardized experimental models that mimic the environmental conditions to which forensic biological evidence is subjected. Biological trace persistence studies usually focus on a single variable (*e.g.*, temperature, humidity, ultraviolet radiation, water exposure), and frequently use different sample types, substrates, exposure durations and analytical endpoints [1-6]. Another complication in glycan research is that structural responses may depend on the composition of the original glycan population and the molecular carrier to which the carbohydrate is attached [34, 40, 55]. Thus, it is difficult to compare results from one experimental condition with those from another study. Without harmonized protocols to define exposure intensity, duration, environmental cycling, substrate, biological matrix and sampling intervals, it is difficult to determine whether the glycan changes observed are a general molecular behavior or specific to individual experimental

systems.

The problem becomes more serious in realistic forensic conditions where environmental factors are acting simultaneously and they may also interact in a non-linear fashion. Temperature can influence reaction rates, humidity can influence hydration and microbial activity, UV radiation can stimulate photochemical reactions, and substrate properties can influence molecular retention and accessibility [31, 32, 85, 87, 93-95]. However, most of the laboratory designs simplify these conditions by studying factors separately, which may be not sufficient to represent the molecular history of real traces. A useful standardized model would therefore necessitate both controlled single-factor experiments and increasingly complex combined-exposure designs including temperature and humidity cycling, realistic UV exposure, microbial challenge, and representative porous and non-porous substrates. Such harmonization would allow comparison of datasets between laboratories and provide the experimental basis for recognition of reproducible patterns of glycan transformation with potential forensic significance.

Insufficient longitudinal persistence studies

Longitudinal studies are critical to determine if glycan signatures are stable, progressively change, or become undetectable as biological evidence ages after deposition. Recent studies demonstrated the capability of detecting glycan profiles in dried bloodstains stored for several years, with the N-glycan patterns containing enough information for age-related modeling [29]. The environmental history of an actual crime scene trace is not necessarily reproduced by long-term storage. Importantly, few studies have serially analyzed the same biological sample from the point of deposition over defined intervals of environmental exposure. Without such time-resolved observations, it is unclear if particular glycan structures follow predictable trajectories of persistence, selective loss or structural transformation.

This limitation is especially important for the proposed use of glycans as time-related or environmental proxies. Research on nucleic acids has demonstrated complex, molecule specific persistence of biological molecules under different environmental conditions [1, 2] and glycan studies have shown that the structural stability is dependent on the molecular composition, the glycosidic linkage, the carrier association and chemistry of the environment [34, 55]. These results provide a mechanistic basis for the expectation of time-dependent glycan changes but do not constitute a validated molecular clock for forensic stains. Therefore, well designed longitudinal experiments should look at fresh biological material over multiple time points and at the same time record temperature, humidity, radiation, microbial activity, substrate, and so on. Repeated glycomic and glycoproteomic measurements might then disclose stable structures, progressive transformation patterns and crucial stages at which a glycan signature loses forensic reliability.

Inter-individual biological variability

Inter-individual biological variability is a major challenge for the evaluation of glycans as potential forensic markers, since the composition of glycans is affected by genetic, physiological and environmental factors. Research on the human glycome has revealed substantial differences in the glycan profiles of plasma and serum between individuals, and genome-wide studies have identified genetic loci associated with variation in specific glycosylation traits [59-61]. Age-related changes have been well described and several studies have

shown systematic changes in the circulating N-glycan profiles across the human lifespan [60, 62]. Sex-related differences have also been reported for human serum N-glycomes [59]. The results show that the glycan profile derived from forensic evidence may vary naturally between donors even when analyzing the same type of biological material. Hence, it will be critical to be able to differentiate true source-associated characteristics from normal biological variation prior to glycan patterns being ascribed a reliable forensic significance.

Another complication is the intra-individual variation since the glycan profiles can vary within the same individual over time and in response to physiological and pathological conditions [57, 58]. Baseline biological trace profile of an individual can be modified by changes in age, inflammation, metabolism, hormones, disease and life style. Hence, a glycan feature that is discriminative for a particular reference population may not be equally informative for other populations or biological states. This means that forensic validation will need large and diverse reference datasets covering relevant age groups, sexes, populations and physiological conditions. The probability that an observed glycan profile is associated with a given biological source also requires statistical models to account for natural within-person variation.

Analytical sensitivity and reproducibility

The analytical sensitivity is an important aspect in forensic glycan analysis, as biological traces may only contain very small quantities of glycan-bearing molecules, particularly after environmental exposure, degradation or incomplete recovery. Recent developments of mass spectrometric and separation-based glycomic approaches have greatly enhanced detection capabilities. High-sensitivity workflows are now able to profile glycans from very small biological samples [97, 103, 111-114]. But increased sensitivity does not eliminate analytical bias. The observed glycan profile can be affected by factors such as matrix effects, ion suppression, incomplete glycan release, derivatization efficiency, chromatographic recovery, preferential ionization and differences in instrument response [24, 26, 102-105]. These issues are especially important when comparing low template forensic samples where small differences in analysis can lead to seemingly meaningful changes in relative glycan abundance.

A related but separate challenge is reproducibility, as complex glycan structures can give rise to different analytical outcomes, depending on sample preparation, instrumentation, structural interpretation, and data-processing procedures. Isomeric glycans may have identical nominal or exact mass but differ in their branching or linkage and therefore require complementary separation and fragmentation strategies for reliable identification [104, 105]. Thus, even nominally identical samples may be analyzed differently in different laboratories. For forensic applications, analytical workflows will need to incorporate standardized sample-processing procedures, validated quality-control materials, technical replicates, suitable blanks, and well-defined acceptance criteria. Inter-laboratory studies will also be required to determine if glycan profiles can be replicated independently using different analytical platforms. Glycan measurements should be considered exploratory molecular information and not independent definitive forensic evidence until such validation has been established.

Lack of validated forensic reference databases

Adequate reference populations are necessary for reliable forensic interpretation of glycan profiles to which an unknown sample can be

compared. To date, such datasets are far less developed for glycans than for well-established forensic genetic markers. Large-scale glycomic studies have pointed to significant differences in human glycosylation based on age, sex, genetics and physiology [59-62, 67]. However, these studies were designed mainly for biomedical or population research rather than forensic source attribution. Thus, the biological material, analytical platform, glycan annotation, sample preparation and reporting standards of the available datasets may vary. The absence of harmonized forensic reference collections prevents the determination of normal glycan variation ranges in specific body fluids or tissues, and limits the ability to determine whether a recovered profile is unusual, source-associated or representative of natural biological diversity.

An ideal forensic glycan database would include samples from a broad spectrum of individuals, with detailed documentation of variables known to influence glycosylation, such as age, sex, ancestry, physiological state, medication, lifestyle and relevant environmental exposure history. It would also require standardized analytical protocols and consistent structural annotation so that profiles generated by different laboratories could be meaningfully compared [26, 44, 97]. It is important that reference datasets are not limited to fresh biological material, but should also contain experimentally aged samples at different temperatures, humidity, substrates, radiation and other post-deposition conditions. These types of datasets may allow the isolation of intrinsic biological variation from that induced by the environment and provide the statistical basis for future likelihood-based interpretation. Therefore, the establishment of standardized, publicly available and quality-controlled forensic glycan databases should be considered a major prerequisite for the translation of glycomic research into reliable forensic practice.

Interpretation and evidential limitations

The final hurdle for forensic translation is not the ability to detect or characterize glycans, but to translate a measured glycan pattern into a scientifically defensible forensic conclusion. The fact that there is a molecular difference between two samples does not automatically establish biological source, individual association, environmental exposure or time since deposition. Natural biological variation, sample composition, environmental history, enzymatic activity, substrate interactions, extraction efficiency and analytical procedures can influence glycan profiles [29, 59-62, 91-95]. Interpretation must therefore allow for the possibility that an observed molecular pattern reflects several contributing processes simultaneously. This is especially important for the proposed strategy of post-deposition glycan transformation, as similar structural changes may also occur under different environmental conditions. Without validated data sets and properly designed statistical models, qualitative differences in glycan profiles should not be considered definitive evidence of a specific source or exposure history.

For forensic use, the performance characteristics of glycan evidence would have to be well established, including sensitivity, specificity, reproducibility, error rates, population variability and robustness under relevant environmental conditions. Interpretation should also distinguish source-level information, activity or exposure-related information and temporal information, as these are different evidential propositions that require different validation strategies. The current forensic evidence increasingly suggests a probabilistic interpretation rather than categorical conclusions and any future glycan-based approach would have to follow the same principle

[115, 116]. The most defensible role for glycan analysis at present is therefore as a potentially complementary molecular layer which can provide additional information together with DNA, RNA, protein and serological findings. The future evidential value of this work will depend on rigorous validation, transparent estimates of uncertainty, and the ability to show that the proposed molecular signatures are interpretable under realistic forensic conditions.

Future Research Priorities

Controlled environmental and time-series studies

Among the most important objectives in the advancement of forensic glycan research is establishing controlled experiments of environmental exposure simulating circumstances under which biological traces may be present after deposition. Experimental designs should assess temperature, humidity, ultraviolet radiation, oxidative stress, microbial activity, substrate characteristics and repeated environmental fluctuations, independently and jointly [31, 32, 85, 87, 91, 93-95]. Future experiments need to measure changes in individual glycan structures, glycoform distributions, glycan-to-glycoprotein relationships and overall molecular profiles instead of just the detectability of glycan material. Standardization of biological material, quantity deposited, substrate, conditions of exposure, sampling intervals and analytical procedures would allow meaningful comparison between studies and would help in identifying structural features that are particularly resistant or susceptible to certain environmental influences.

These environmental models should be complemented by time-series experiments, whereby the same biological trace is analyzed repeatedly from the point of deposition to predetermined stages of aging. Such longitudinal designs would contribute to the distinction between immediate molecular changes and progressive transformation and could show if certain glycan structures follow reproducible persistence or degradation trajectories [29, 55]. Continuous environmental monitoring would also allow the molecular changes to be quantitatively related to cumulative exposure rather than just elapsed time. These studies may provide the scientific basis for assessing the potential utility of glycan profiles as a marker of molecular stability, environmental history, or, in the future, time-dependent features of forensic evidence. Importantly, temporal interpretations should only be considered appropriate for forensic application after the models generated have been tested on independent samples and diverse substrates.

Complex biological evidence and multimolecular integration

Future studies need to move beyond simple single-fluid samples and explore the glycan composition of more complex biological evidence representative of actual forensic casework. Stains that are mixed, containing blood, saliva, seminal material, menstrual material, or other biological materials, can have overlapping glycan populations and the relative contribution of each source can be further altered by environmental exposure [29, 43, 67, 69-76]. Experimental studies should therefore assess the potential for glycan profiles to discriminate between individual components in mixed traces, and whether source specific patterns survive the formation of the mixture, dilution, aging or exposure to the environment. It would be especially significant to determine the behavior of glycans in complex samples, as forensic interpretation is rarely conducted under idealized laboratory conditions.

A further important avenue is integration with existing molecular approaches. DNA profiling can provide highly discriminating individual information, and RNA, proteins, epigenetic markers and traditional serology can provide information regarding a biological source and trace characteristics [10, 13, 15-18]. Glycan analysis may complement these molecular layers with information on biological state, glycosylation-associated source characteristics or post-deposition transformation. Future research should therefore not aim at developing an isolated glycan test but investigate multimolecular workflows where glycan, DNA, RNA, protein and serological findings are interpreted together. Such integration could enhance the characterization of biological traces, while also providing internal cross-validation between independent molecular measurements.

Computational interpretation and forensic glycan reference resources

The growing complexity of glycomic data sets strongly argues for the development of computational approaches capable of identifying patterns that may not be readily apparent from conventional one-variable comparisons. Machine-learning and other statistical approaches could be used to analyze multidimensional glycan profiles and to evaluate relationships with biological source, age, sex, physiological state, environmental exposure and post-deposition transformation [117-119]. The computational performance, however, will highly depend on the quality and representativeness of the underlying data. Models trained on limited biomedical datasets may not generalize to forensic samples collected under different conditions. Therefore, machine learning development should be accompanied by rigorous feature validation, independent testing, transparent reporting of models and evaluation of uncertainty.

This requirement emphasizes the need for standardized forensic glycan reference datasets with samples from different biological sources and demographic groups that are sufficiently heterogeneous. Ideally, such resources would comprise of both fresh samples as well as traces aged under controlled conditions, exposed to environmental influences, different substrates and mixed sample conditions [59-62, 67]. Inter-laboratory consistency in sample preparation, glycan nomenclature, structural annotation, analytical metadata, and quality-control procedures would be required for interoperability. Publicly available or appropriately managed reference resources could allow researchers to compare results across platforms and populations and reduce duplication of effort. Integration of high-quality reference datasets with validated computational methods could ultimately enable probabilistic interpretation rather than simple pattern matching and would represent a major step forward in practical forensic glycomics.

Validation, reproducibility, and courtroom translation

Before glycan analysis can be considered for routine forensic use, analytical methods must progress from exploratory demonstrations to rigorous validation. Validation should establish sensitivity, specificity, precision, accuracy, repeatability, reproducibility, robustness to environmental exposure, and resistance to common sources of contamination and analytical interference [102-105, 111-114]. Inter-laboratory studies will be particularly important because glycomic workflows can vary substantially with extraction procedures, glycan-release methods, derivatization chemistry, chromatography, mass spectrometry, and structural annotation. Standardized reference materials, quality-control samples, process blanks, replicate analysis, and predefined acceptance criteria should therefore become integral

components of future forensic glycan workflows.

Courtroom translation presents an even higher evidential threshold because the relevant question is not simply whether a glycan pattern can be measured, but whether its evidential meaning can be communicated with scientifically defensible uncertainty. Any future forensic application would need validated population frequencies, error estimates, appropriate statistical frameworks, transparent reporting standards, and clearly defined boundaries on interpretation. Glycan evidence should also be evaluated within the broader context of existing DNA, RNA, protein, and serological findings rather than treated as inherently definitive. The path toward courtroom relevance will therefore depend on demonstrating reproducibility across laboratories, establishing validated reference populations, quantifying uncertainty, and showing that the conclusions derived from glycan profiles remain reliable under realistic casework conditions. Until these requirements are met, glycan analysis should remain a promising research direction rather than a validated standalone evidential method.

Conclusion

Glycans represent a largely unexplored molecular dimension of forensic biological evidence. DNA, RNA, proteins and epigenetic markers have been used increasingly to characterize biological traces. However, glycans provide a unique level of information that results from intricate glycosylation patterns within cells, tissues and body fluids. There is evidence that glycan profiles can contain biologically informative variation and importantly that measurable glycan signatures can persist in deposited biological material such as dried bloodstains [29, 59-62]. But the forensic importance of glycans is more than just their biological composition. Once deposited, the glycan landscape of a biological trace can be affected by temperature, humidity, radiation, oxidation, microbial activity, substrate interactions, molecular degradation, and analytical processing [31, 32, 55, 85, 91]. The resulting profile may therefore be a mixture of source derived information and of the molecular history after deposition.

The concept of the forensic fate of glycans proposed in this review highlights this dynamic relationship. Each stage in the process, from biological source and deposition through environmental exposure, molecular transformation, recovery, and interpretation, can ultimately affect the information available from a forensic trace. However, the evidence base remains inadequate to characterize glycan transformation as a validated indicator of environmental history, time since deposition, transfer or other activity-level propositions. The main challenges are the lack of forensic-specific studies, limited numbers of longitudinal experiments, lack of standardized exposure models, natural biological variability, analytical challenges and the lack of comprehensive forensic reference databases. These limitations should not be viewed simply as limitations but as clearly defined priorities for future research.

Future progress will depend on multidisciplinary studies that integrate controlled environmental experiments, longitudinal glycomic profiling, advanced analytical technologies, computational interpretation, standardized reference resources, and multimolecular strategies that combine glycan information with DNA, RNA, protein, and classical serological evidence. In this way, the most realistic application of forensic glycans in the near term is as a complementary molecular layer, not as a substitute for established forensic methods. Ultimately, the extent to which glycan signatures can move from a

developing research idea to a dependable part of forensic biological evidence will depend on rigorous validation, reproducibility, probabilistic interpretation and transparent assessment of uncertainty. The proposed framework provides a foundation for the study of this transition and highlights the potential of future forensic science to interpret biological traces not only based on the molecular information they contain, but also on how this information changes after deposition.

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