The EuroBiotech Journal
REVIEW
Medical Biotechnology

Biophysical reasons for the variability in detecting cancer biomarkers: the overlooked role of protein–lipid interactions

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Abstract

Background: Circulating protein biomarkers are central to oncology diagnostics. They exist in plasma as heterogeneous molecular populations, and immunoassays measure an analytically accessible fraction of a target biomarker rather than necessarily its total abundance. This accessibility can depend on chemical modification, conformation, and intermolecular association. Post-translational modifications such as glycosylation and glycation can remodel protein surfaces and influence their interactions with the surrounding environment. The plasma environment itself contains diverse lipid-rich particles that differ in composition and biological origin. These features may therefore shape interactions between circulating biomarkers and lipid-rich interfaces, but their potential contribution to biomarker measurement has rarely been considered together.

Purpose and scope: This perspective examines the biological and biophysical basis of a potential link between post-translational modification, lipid-particle association, and biomarker detectability, with emphasis on the molecular interactions underlying this relationship. It evaluates the evidence supporting this potential link and considers complementary approaches needed to determine whether such interactions contribute to variability in biomarker measurement.

Synthesis: Post-translational modifications, including glycosylation and glycation, can remodel protein surfaces and alter their physicochemical and recognition properties, potentially influencing interactions with lipid-rich interfaces. The dynamic and heterogeneous plasma environment provides lipid-rich interfaces whose composition can vary with physiological and pathological states, including nutritional status and tumour-associated metabolic remodelling. These variations are distinct from the well-established analytical effects of lipemia. Evidence from soluble tumour-associated and other plasma proteins indicates that protein–lipid association can occur, while simplified biophysical systems provide controlled models of these interactions. Such associations could reduce the analytical accessibility of circulating biomarkers by partially masking them from antibody recognition.

Conclusion: Existing biological and biophysical evidence is sufficient to justify testing whether protein–lipid interactions contribute to what is measured by immunoassays. Addressing this question requires complementary investigation across controlled biophysical systems, native biological samples, and immunological measurements.

Keywords: biomarkers; post-translational modifications; protein-lipid interactions; immunoassay interference; epitope accessibility

Introduction

Protein biomarkers are central to modern clinical diagnostics, particularly in oncology. Many circulating protein biomarkers undergo post-translational modifications (PTMs), with glycosylation being particularly prevalent among extracellular and secreted proteins (1–4). Such circulating biomarkers include prostate-specific antigen (PSA), cancer antigen 125 (CA-125/MUC16), and alpha-fetoprotein (AFP), which are important targets for diagnostics since their measured concentrations are routinely interpreted as indicators of tumour burden, disease progression, or treatment response. However, circulating biomarkers do not exist as chemically uniform populations. Differences in PTM state can generate molecularly distinct forms of the same protein, with potentially different physicochemical properties, molecular interactions, and accessibility to analytical reagents.

This molecular heterogeneity is very significant for the techniques used to measure biomarkers. An immunoassay does not necessarily measure the total amount of a target protein present in a sample. Instead, the measured signal depends on the fraction that is accessible to the capture and detection reagents under the conditions of the assay. The

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DOI: 10.2478/ebtj-2026-0025

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molecular state of the target, including its post-translational modifications, conformation, and intermolecular associations, can therefore influence its analytical accessibility and ultimately the concentration reported by the assay (5–8).

Among the factors that may contribute to this heterogeneity, enzymatic glycosylation and non-enzymatic glycation are of particular interest because they can remodel the physicochemical properties of protein surfaces, including their charge, hydration, steric accessibility, conformational dynamics, and hydrophobicity (9). These changes can influence molecular recognition and alter how modified proteins interact with other components of the circulating environment. This provides a potential mechanistic link between PTM-dependent molecular heterogeneity and the behavior of circulating proteins in their extracellular environment.

The relevance of this possibility becomes clear when considering the physical environment in which circulating biomarkers exist. Human plasma and serum are complex and compositionally heterogeneous biological fluids in which abundant soluble proteins coexist with diverse lipid-rich particles, including chylomicrons, very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL), high-density lipoproteins (HDL), and lipoprotein(a) [Lp(a)] (10–13). They also contain extracellular vesicles (EVs) and other membrane-derived particles and fragments originating from circulating and tissue cells (13). These particles differ substantially in size, lipid composition, surface properties, protein composition, and biological origin. Their abundance and composition are also influenced by nutritional status, inflammation, metabolic state, and disease-associated processes (14–16). Consequently, substantial differences in the composition of circulating lipid particles may exist between samples from individuals with different physiological or pathological conditions, even when the samples do not appear lipemic.

Evidence that soluble proteins, including circulating biomarkers, can associate with lipoproteins and other lipid-rich particles is already known in the literature (7, 17–19). However, the available studies focus mainly on individual proteins or specific biological contexts, making it difficult to determine whether common principles govern these interactions across different biomarkers and particle environments. A better understanding of these interactions may help clarify when protein–particle association is simply part of biomarker biology and when it might become relevant to biomarker accessibility and measurement.

In our view, these observations point to a potential link between post-translational modification, lipid-particle association, and biomarker detectability that has received comparatively little attention for clinical biomarker measurement, particularly from the perspective of the underlying biophysical interactions. This conceptual framework is summarized in Figure 1. This possibility does not necessarily require overt lipemia, as differences in the composition and properties of circulating lipid particles may occur between samples without being evident from visual inspection. The aim of this Perspective is therefore to examine the biological and biophysical basis of a potential link between post-translational modification, lipid-particle association, and biomarker detectability, with particular emphasis on the molecular interactions that may underlie this relationship. We first consider how glycosylation and glycation remodel protein surfaces and influence molecular recognition and intermolecular interactions. We then describe the lipid-rich compartments encountered by circulating biomarkers and the factors that determine their composition. Next, we examine

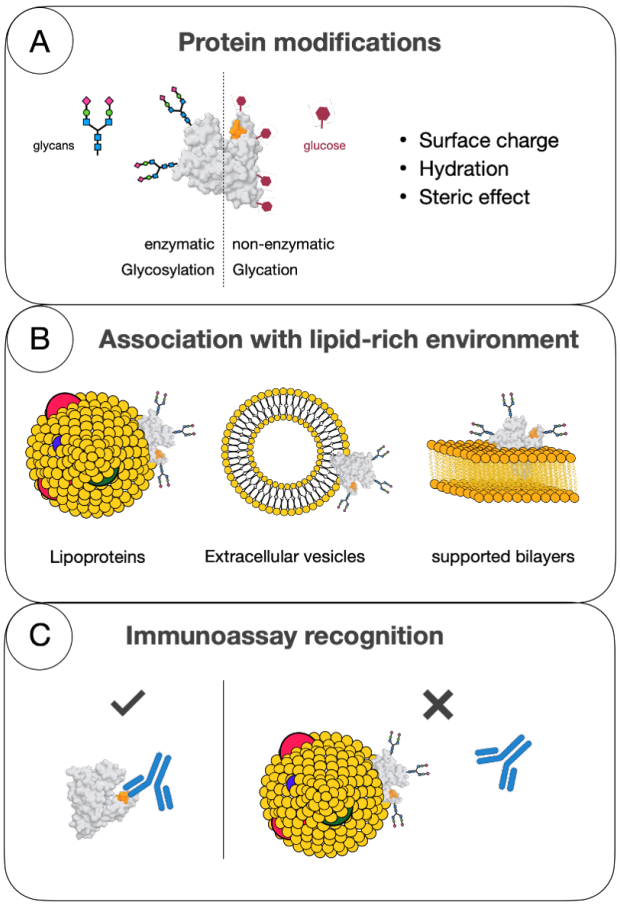


Figure 1. Potential link between post-translational modification, lipid-particle association, and biomarker detectability. (A) Protein modifications, including enzymatic glycosylation and non-enzymatic glycation, can alter the surface properties of a circulating protein biomarker, including surface charge, hydration and steric accessibility. (B) These changes may influence the association of the biomarker with lipid-rich components of plasma or serum, such as lipoproteins, extracellular vesicles (EVs) or lipid membranes. Complementary biological and biophysical approaches, using both biological fluids and controlled simplified systems such as supported lipid bilayers, can help identify and characterize these interactions. (C) Protein modifications and association with lipid-rich particles may alter the exposure, orientation or steric accessibility of antibody-recognized epitopes, as well as the kinetics of antibody binding, thereby potentially affecting detection in an immunoassay.

examples of protein–lipid-particle interactions and consider how these associations could affect biomarker detectability. Finally, we discuss complementary biophysical, biological, and analytical approaches that could determine whether such interactions contribute to variability in biomarker measurement and how their analytical consequences might be understood and, where appropriate, minimized.

2. Glycosylation and glycation: modification of protein surfaces and molecular recognition

Glycosylation and glycation both introduce carbohydrate-derived chemical complexity into proteins, but they arise through fundamentally different mechanisms. Glycosylation is an enzymatically regulated process that occurs primarily in the endoplasmic reticulum and Golgi apparatus. N-linked glycans are attached to asparagine residues, usually within an Asn-X-Ser/Thr sequence, whereas mucin-type O-linked glycans are initiated on serine or threonine residues. Subsequent processing generates high-mannose, hybrid, or complex structures with variable branching, galactosylation, fucosylation, and terminal sialylation. Differences in glycan site occupancy and processing can therefore generate glycoforms with identical amino-acid sequences but distinct molecular surfaces (20,21).

This structural diversity can translate into differences in the physical properties of a protein. Terminal sialic acids introduce negatively charged groups at physiological pH and can therefore alter local electrostatic potentials. Glycan chains also form extensive hydration shells through hydrogen bonding with water, modifying the interfacial properties of the protein surface. Their size, flexibility, and branching increase the effective steric volume of the protein and can restrict access to neighboring peptide regions. Depending on their location and structure, glycans may stabilize particular conformations, influence domain motion, or modify the energetic cost of approaching another macromolecule or interface (22,23). These effects are context-dependent rather than uniformly directional. An added glycan may shield one epitope while exposing another, reduce hydrophobic interactions while introducing new carbohydrate-mediated contacts, or alter molecular orientation without substantially changing the overall protein conformation. Glycosylation should therefore be viewed not simply as the addition of a carbohydrate moiety, but as a modification capable of reshaping the molecular surface presented by a protein.

Most tumour biomarkers are glycosylated, including PD-L1, for which the consequences of glycosylation have been illustrated by studies. Indeed, N-linked glycans on the extracellular domain of PD-L1 can interfere with recognition by antibodies used in tissue-based assays. The removal of these glycans moieties has been shown to enhance the detection of PD-L1 and, in some experimental settings, to alter the apparent classification of samples based on the measured abundance of the protein (24,5). This example is useful because it directly illustrates how glycosylation can mask antibody recognition and alter the analytical signal obtained from a protein target. It therefore provides a mechanistic precedent for considering whether similar effects could arise for circulating biomarkers whose glycosylation state varies between molecular forms.

Highly glycosylated circulating biomarkers provide further examples of how molecular heterogeneity can influence biomarker recognition. Among these biomarkers, Cancer Antigen 125 (CA125), also referred to as Carbohydrate Antigen 125, has played the most significant role in screening, detecting, and managing ovarian cancer for the last four decades. This antigen can be shed and quantified in serum samples from patients, and levels are elevated in 50% of early-stage tumours. Tumour-associated changes in glycosyltransferase activity can generate altered glycan motifs, including Tn and sialyl-Tn structures. Such molecular differences motivate approaches aimed at detecting selected CA-125 glycoforms rather than considering the antigen as a single homogeneous population (1,25). Similarly, AFP-L3, which is defined by a core-fucosylated glycoform of AFP, is increased in hepatocellular carcinoma and is used clinically as an informative fraction of total AFP (26). These examples show that glycoform composition can carry biological information and can be selectively recognised or quantified. Furthermore, they reinforce the idea that the molecular population represented by a clinical biomarker measurement may comprise distinct molecular forms with different structural and recognition properties.

The consequences of glycosylation are not restricted to interactions with antibodies. Because glycans modify the physical surface of proteins, they can also influence how proteins interact with molecular interfaces. Molecular-dynamics simulations of the CD2 ectodomain, for example, showed that glycosylation and membrane composition act together to influence ectodomain orientation. In this model, glycans contributed to maintaining a binding-competent presentation relative to the membrane, while cholesterol and sphingomyelin altered the interfacial environment (27). This provides a useful mechanistic example of how glycosylation can affect the spatial presentation of a protein at a lipid interface. Similarly, molecular-dynamics simulations of apolipoprotein D showed that N-glycosylation can alter hydration, adsorption energetics, and orientation at polymer-coated gold nanoparticles (28). Although the interface in this system differs from a biological membrane, the study illustrates how glycan-dependent changes in surface properties can influence protein–interface interactions. Collectively, these studies indicate that glycosylation may influence not only molecular recognition but also the presentation of proteins at, and their interaction with, heterogeneous interfaces.

An additional source of protein heterogeneity may be attributable to glycation through a fundamentally different chemical process. Glycation is a non-enzymatic reaction between reducing sugars or reactive carbonyl compounds, such as methylglyoxal, and amino groups on proteins. Initial Schiff-base and Amadori products can undergo further oxidation, rearrangement, and cross-linking to generate advanced glycation end-products (AGEs), which modify lysine and

arginine residues and alter local electrostatics, conformational properties, and surface hydrophobicity (29). For example, glycated bovine serum albumin has been reported to associate more strongly with specific negatively charged lipid bilayers than its non-glycated counterpart, indicating that glycation can alter the balance of interactions governing protein–membrane association (30). This provides a mechanistic rationale for investigating whether glycation-related changes in biomarker surface properties can influence their association with lipid-rich environments in native plasma. However, such supported bilayers are simplified model systems and do not fully reproduce the heterogeneous lipid particles and competing interactions present in plasma.

These examples highlight how enzymatic and non-enzymatic modifications can generate molecularly distinct protein forms with altered recognition and interfacial properties. The next question is therefore what types of lipid-rich interfaces circulating biomarkers encounter in plasma.

3. The lipid environment encountered by circulating biomarkers

Circulating proteins encounter a complex and dynamic lipid environment in plasma. This environment includes lipoproteins such as high-density lipoproteins (HDL), low-density lipoproteins (LDL), very-low-density lipoproteins (VLDL), chylomicrons, and lipoprotein(a) [Lp(a)], together with extracellular vesicles (EVs) and other membrane-derived particles (10–13). These particle classes differ in lipid composition, protein content, size, and surface organization. Lipoproteins themselves are not static structures, because lipids and associated proteins can be exchanged between particles and with the surrounding plasma, resulting in continuous particle remodelling (31). The composition of this environment can also vary with physiological conditions, including nutritional status. In the postprandial state, for instance, the blood contains elevated levels of triglyceride-rich lipoproteins and their remnants, along with alterations in the composition of other lipoprotein groups (32). Therefore, the lipid environment encountered by a circulating protein at the time of sampling is not necessarily equivalent between individuals or between fasting and non-fasting conditions.

These differences are relevant because the particles present in plasma provide distinct molecular interfaces. HDL is relatively small and protein-rich, whereas LDL contains a single ApoB-100 molecule that wraps around and stabilizes its cholesterol-rich lipid core. VLDL and chylomicrons are larger, triglyceride-rich particles, while Lp(a) retains an LDL-like structure with Apo(A) covalently attached to ApoB-100 (10). EVs and membrane-derived particles exhibit a different type of interface, characterized by the retention of lipid bilayers along with membrane proteins, lipids, glycans, and other surface components (33). Such differences may influence the physicochemical interactions available to a soluble protein, including electrostatic and hydrophobic interactions, steric compatibility, and the accessibility of specific surface regions.

Additionally, the composition of this extracellular environment may be further altered by disease in cancer. Indeed, tumour cells frequently undergo extensive metabolic reprogramming, changes in fatty-acid and cholesterol metabolism. Of interest, these alterations may influence lipid availability in blood as well as the production and molecular composition of extracellular vesicles. Cancer-derived EVs can carry molecular signatures that reflect the state of their cells of origin, including alterations in lipid composition, making them a potential source of heterogeneity in the circulating particle environment (34). Prostate cancer provides a relevant example, since lipid metabolic reprogramming and altered cholesterol handling have been linked to tumour progression and therapy resistance (16). The disease-related changes that occur in this type of cancer may be a contributing factor to the observed variability in the lipid environment encountered by circulating PSA between individuals.

These variations should be considered alongside the well-established analytical effects of lipemia. Lipemia refers to the accumulation of lipid-rich particles, particularly triglyceride-rich lipoproteins, that can increase sample turbidity and interfere with clinical measurements through matrix effects. Such interference is well documented in immunochemical and other laboratory assays and can result in assay-dependent analytical bias (35,36). The mechanism considered in this perspective is related to, but distinct from these conventional effects. Biomarker association with lipid-rich particles might not be associated with lipemia but may result in the masking of antibody recognition even in visually clear samples.

What matters, therefore, may be not only how much lipid is present, but also which particles and interfaces are available to a biomarker. The complexity of these environments, together with chemical modifications of proteins, may shape their interactions. The next section examines existing examples of such interactions and considers their potential relevance to biomarker behavior and detectability.

4. Protein–lipid interactions and consequences for detectability

The heterogeneous lipid environment described above provides numerous potential interfaces for proteins in blood. Evidence that proteins, and particularly soluble ones, can associate with lipoproteins and other lipid-rich particles is already known in the literature, however, further research is required to establish the nature and specificity of these associations. Proteomic studies have identified numerous non-apolipoprotein proteins in HDL-associated fractions, indicating that lipoprotein particles can carry a broader protein repertoire than their canonical apolipoproteins alone (37,38). Moreover, the composition and glycosylation of HDL-associated proteins can vary between clinical groups, suggesting that the protein cargo of lipoproteins is itself dynamic and potentially influenced by disease state (18). These observations raise the question of whether soluble proteins, including tumour-associated biomarkers, can associate with lipid-rich particles in blood and

whether such association affects their recognition.

Direct evidence for such an association has been reported for soluble Vascular Endothelial-cadherin (sVE), the shed extracellular domain of the endothelial adhesion protein VE-cadherin. sVE was first described in patients with human glioblastoma (39). It is a highly glycosylated protein and has been detected in lipid-containing serum fractions, where it preferentially associates with HDL-enriched material. Co-immunoprecipitation experiments have demonstrated an association with apolipoprotein A1. In a complementary biomimetic lipid-bilayer system, sVE also adsorbed to the membrane, demonstrating that its interaction with a lipid interface could be reproduced in a simplified experimental setting (7). Notably, detergent treatment increased the immunodetection of soluble VE-cadherin by Western blot. This finding is consistent with a change in analytical accessibility after disruption of the lipid-associated state. Because of its extensive glycosylation, sVE provides a compelling context in which PTM-dependent surface properties might influence lipid association, a hypothesis that requires further investigation.

A second example is alpha-fetoprotein (AFP), an established glycosylated biomarker for hepatocellular carcinoma (HCC). Recent work investigated its interactions with planar lipid membranes using neutron reflectometry, quartz-crystal microbalance with dissipation monitoring, and atomistic molecular-dynamics simulations. AFP showed markedly different interactions depending on membrane composition and surface charge, ranging from weak association with zwitterionic membranes to stronger adsorption and membrane perturbation in negatively or positively charged and lipid-domain-containing systems (19). As in the previously presented study of glycated albumin, these controlled model systems are simplified representations of the lipid interfaces encountered by circulating proteins and do not fully represent native plasma. However, they show that the same biomarker can exhibit markedly different interfacial behavior depending on the properties of the lipid environment. They therefore support the possibility that AFP association with lipid-rich particles in plasma may depend on local lipid composition, although direct evidence in patient samples is required.

A complementary example is β2-glycoprotein I (β2GPI), an abundant soluble plasma glycoprotein that interacts with negatively charged phospholipids. The interactions of the protein with phosphatidylserine and bacterial lipopolysaccharide were characterized using complementary biophysical approaches, showing stronger binding to phosphatidylserine than to lipopolysaccharide, while association with lipopolysaccharide promoted a more multilamellar organization of the aggregates and increased the phase-transition temperature of their lipid acyl chains (40). This system illustrates how specific interactions between soluble plasma proteins and charged lipid surfaces can influence the organization and physical properties of lipid interfaces.

Further biological precedents come from membrane and viral glycoproteins that recognize lipid-rich particles. The scavenger receptor CLA-1/SR-BI, for example, is an N-glycosylated membrane protein that recognizes several classes of lipoproteins (41), while CD36 interacts with modified lipoproteins and anionic lipid ligands (42). Similarly, the hepatitis C virus E2 glycoprotein interacts with HDL and Scavenger Receptor BI (SR-BI), and this interaction has been linked to changes in the exposure of antibody-recognized regions (43). Together with the examples above, these systems illustrate that protein–lipid interactions can depend on molecular surface properties and can influence how proteins are organized and presented at lipid-rich interfaces.

These interactions become relevant to biomarker detectability because particle association could alter both the molecular presentation of the protein and assay kinetics. First, association with a lipid-rich interface could reduce antibody access through steric shielding or changes in the orientation of the protein at the particle surface. Second, interaction with the lipid interface could alter conformation and thereby affect antibody recognition. Third, association with a lipoprotein or membrane-derived particle could increase the hydrodynamic radius of the biomarker, potentially slowing its diffusion and affecting its transport to the capture surface and subsequent binding kinetics. According to the Stokes–Einstein relationship, the diffusion coefficient decreases as hydrodynamic radius increases, assuming constant temperature and medium viscosity (44). Consistent with the relevance of transport to assay performance, antigen–antibody binding in microspot immunoassays has been shown to be strongly limited by mass transport (45). Although this study did not investigate lipid-particle-associated biomarkers, it illustrates how transport can influence immunoassay kinetics. The magnitude and direction of these effects are likely to depend on the molecular state of the biomarker, the properties of the particle surface, and the antibody pair and conditions used for detection.

Other soluble proteins, including albumin, can also interact with lipid interfaces, depending on their composition (19). This raises the question of whether interactions observed in complex biological samples reflect properties specific to the biomarker or more general protein–lipid interactions. Controlled biophysical studies using purified biomarkers and defined lipid interfaces, with appropriate controls and albumin as a comparator, could help determine whether these interactions depend on the molecular properties of the biomarker and the composition of the lipid interface. Studies in native plasma would then be needed to assess the relevance of these interactions in a physiological environment. Comparing immunoassay measurements with an independent measure of total biomarker abundance could further help determine whether particle association alters analytical accessibility.

While direct evidence for clinically relevant immunoassay bias remains limited, these mechanisms provide a plausible basis for considering protein–lipid association as a potential source of variability in biomarker accessibility and measurement.

Conclusion and outlook

The purpose of bringing these observations together was to highlight that the available biological and biophysical evidence is sufficient to raise the question of whether protein–lipid interactions may contribute to what is measured by immunoassays in human blood. Addressing this question will require complementary experimental approaches that combine simplified and controlled biophysical systems with studies in biological samples and immunological assays. Controlled studies using purified proteins, defined lipid compositions, and biomimetic membranes may therefore establish the molecular determinants of protein–lipid association, while studies in biological samples and immunological assays will determine whether these interactions persist under native conditions and influence biomarker accessibility.

In order to address these questions, a pragmatic experimental sequence could entail the characterization of the interaction between defined biomarker forms and selected lipid interfaces. Subsequently, the comparison of free and particle-associated forms in plasma could be determined. These experiments could then be coupled to parallel quantification by immunoassay and orthogonal methods such as mass spectrometry to determine whether particle association produces a measurable difference in reported concentration. Where an effect is identified, controlled disruption or release of the particle-associated fraction, for example using detergent, could be evaluated to determine whether disruption of the particle-associated state alters analytical accessibility without altering the biomarker or its antibody-recognized epitopes.

In addition, a low-cost first step would be to systematically record fasting status and time of blood collection and, where feasible, keep these conditions consistent across serial samples. Measuring a routine lipid profile alongside the biomarker could help assess whether variation in immunoassay readouts is associated with sampling conditions or circulating lipid levels. These observations could help identify preanalytical variables that warrant further investigation and potential standardization in subsequent studies.

If reproducible effects are demonstrated, these findings could inform biomarker-specific preanalytical and analytical protocols. Such protocols could include standardization of relevant sampling conditions for serial monitoring, characterization of particle-associated biomarker fractions, validated sample-treatment procedures where appropriate, or orthogonal confirmation when immunoassay results are unexpected or discordant with other measures. The aim would not be to replace established immunoassays, but to improve their robustness and minimize sources of variability arising from the molecular and physicochemical environment of the biomarker.

The ultimate goal will be to integrate biophysical studies with biological validation and immunological assay development. This will help to establish when protein–lipid association is simply part of biomarker biology and when it becomes an analytical variable.

Acknowledgements

This work was funded by an ERC Advanced Grant (N°101142533, Energion) granted by the European Research Council Executive Agency (ERCEA) under the powers delegated by the European Commission. Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the European Union or ERCEA. Neither the European Union nor the granting authority can be held responsible for them

Author contributions

CRediT statements for the authors. B.B.: Conceptualization, Writing – Original Draft; D.K.M.: Funding acquisition; B.B., I.V., M.M., D.K.M.: Writing – Review & Editing.

Ethical Approval

Not applicable.

Conflict of interest statement

The authors declare no conflict of interest.

References

1. Giamougiannis P, Martin-Hirsch PL, Martin FL. The evolving role of MUC16 (CA125) in the transformation of ovarian cells and the progression of neoplasia. Carcinogenesis 2021; 42(3): 327-343.
2. Piñero F, Dirchwolf M, Pessôa MG. Biomarkers in hepatocellular carcinoma: diagnosis, prognosis and treatment response assessment. Cells 2020; 9(6): 1370.
3. Adamczyk B, Tharmalingam T, Rudd PM. Glycans as cancer biomarkers. Biochim Biophys Acta 2012; 1820(9): 1347-1353.
4. Silsirivanit A. Glycosylation markers in cancer. Adv Clin Chem 2019; 89: 189-213.
5. Lee HH, Wang YN, Xia W, Chen CH, Rau KM, Ye L, Wei Y, Chou CK, Wang SC, Yan M, Tu CY, Hsia TC, Chiang SF, Chao KSC, Wistuba II, Hsu JL, Hortobagyi GN, Hung MC. Removal of N-linked glycosylation enhances PD-L1 detection and predicts anti-PD-1/PD-L1 therapeutic efficacy. Cancer Cell 2019; 36(2): 168-178.e4.
6. Dunbar C, Kushnir MM, Yang YK. Glycosylation profiling of the neoplastic biomarker alpha fetoprotein through intact mass protein analysis. J Proteome Res 2023; 22(1): 226-234.
7. Khalil-Mgharbel A, Polena H, Dembélé PK, Hasan Sohag MM, Alcaraz JP, Martin DK, Vilgrain I. A biomimetic lipid membrane device reveals the interaction of cancer biomarkers with human serum lipidic moieties. Biotechnol J 2018; 13(12): e1800463.
8. Pearce OMT. Cancer glycan epitopes: biosynthesis, structure and function. Glycobiology 2018; 28(9): 670-696.

9. Reily C, Stewart TJ, Renfrow MB, Novak J. Glycosylation in health and disease. Nat Rev Nephrol 2019; 15(6): 346-366.
10. Feingold KR. Introduction to lipids and lipoproteins. In: Feingold KR, Adler RA, Ahmed SF, Anawalt B, Blackman MR, Chrousos G, Corpas E, de Herder WW, Dhatariya K, Dungan K, Hamilton E, Hofland J, Jan de Beur S, Kalra S, Kaltsas G, Kapoor N, Kim M, Koch C, Kopp P, Korbonits M, Kovacs CS, Kuohung W, Laferrère B, Levy M, McGee EA, McLachlan R, Muzumdar R, Purnell J, Rey R, Sahay R, Shah AS, Sperling MA, Stratakis CA, Trence DL, Wilson DP, editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000–. Updated 2024 Jan 14.
11. Ghebosu RE, Pendiuk Goncalves J, Wolfram J. Extracellular vesicle and lipoprotein interactions. Nano Lett 2024; 24(1): 1-8.
12. Quehenberger O, Armando AM, Brown AH, Milne SB, Myers DS, Merrill AH, Bandyopadhyay S, Jones KN, Kelly S, Shaner RL, Sullards CM, Wang E, Murphy RC, Barkley RM, Leiker TJ, Raetz CR, Guan Z, Laird GM, Six DA, Russell DW, McDonald JG, Subramaniam S, Fahy E, Dennis EA. Lipidomics reveals a remarkable diversity of lipids in human plasma. J Lipid Res 2010; 51(11): 3299-3305.
13. Nieuwland R, Siljander PRM. A beginner's guide to study extracellular vesicles in human blood plasma and serum. J Extracell Vesicles 2024; 13(1): e12400.
14. Nordestgaard BG, Langsted A, Mora S, Kolovou G, Baum H, Bruckert E, Watts GF, Sypniewska G, Wiklund O, Borén J, Chapman MJ, Cobbaert C, Descamps OS, von Eckardstein A, Kamstrup PR, Pulkki K, Kronenberg F, Remaley AT, Rifai N, Ros E, Langlois M; European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine joint consensus initiative. Fasting is not routinely required for determination of a lipid profile: clinical and laboratory implications including flagging at desirable concentration cut-points—a joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine. Eur Heart J 2016; 37(25): 1944-1958.
15. Rosenson RS, Brewer HB Jr, Ansell BJ, Barter P, Chapman MJ, Heinecke JW, Kontush A, Tall AR, Webb NR. Dysfunctional HDL and atherosclerotic cardiovascular disease. Nat Rev Cardiol 2016; 13(1): 48-60.
16. Zhang Z, Wang W, Kong P, Feng K, Liu C, Sun T, Sang Y, Duan X, Tao Z, Liu W. New insights into lipid metabolism and prostate cancer. Int J Oncol 2023; 62(6): 74.
17. Romo EZ, Zivkovic AM. Glycosylation of HDL-associated proteins and its implications in cardiovascular disease diagnosis, metabolism and function. Front Cardiovasc Med 2022; 9: 928566.
18. Krishnan S, Shimoda M, Sacchi R, Kailemia MJ, Luxardi G, Kaysen GA, Parikh AN, Ngassam VN, Johansen K, Chertow GM, Grimes B, Smilowitz JT, Maverakis E, Lebrilla CB, Zivkovic AM. HDL glycoprotein composition and site-specific glycosylation differentiates between clinical groups and affects IL-6 secretion in lipopolysaccharide-stimulated monocytes. Sci Rep 2017; 7: 43728.
19. Barletti B, König M, Paracini N, Fragneto G, Alcaraz JP, Nelson A, Vilgrain I, Martin DK, Lolicato F, Maccarini M. How lipid composition shapes the nanostructural interaction of tumor biomarker alpha-fetoprotein and bovine serum albumin with model membranes. J Colloid Interface Sci 2026; 708: 139753.
20. Moremen KW, Tiemeyer M, Nairn AV. Vertebrate protein glycosylation: diversity, synthesis and function. Nat Rev Mol Cell Biol 2012; 13(7): 448-462.
21. Čaval T, Heck AJR, Reiding KR. Meta-heterogeneity: evaluating and describing the diversity in glycosylation between sites on the same glycoprotein. Mol Cell Proteomics 2021; 20: 100010.
22. Woods RJ. Predicting the structures of glycans, glycoproteins, and their complexes. Chem Rev 2018; 118(17): 8005-8024.
23. Petrescu AJ, Milac AL, Petrescu SM, Dwek RA, Wormald MR. Statistical analysis of the protein environment of N-glycosylation sites: implications for occupancy, structure, and folding. Glycobiology 2004; 14(2): 103-114.
24. Wang YN, Lee HH, Hsu JL, Yu D, Hung MC. The impact of PD-L1 N-linked glycosylation on cancer therapy and clinical diagnosis. J Biomed Sci 2020; 27(1): 77.
25. Napoletano C, Steentoft C, Battisti F, Ye Z, Rahimi H, Zizzari IG, Dionisi M, Cerbelli B, Tomao F, French D, d'Amati G, Panici PB, Vakhrushev S, Clausen H, Nuti M, Rughetti A. Investigating patterns of immune interaction in ovarian cancer: probing the O-glycoproteome by the macrophage galactose-like C-type lectin (MGL). Cancers (Basel) 2020; 12(10): 2841.
26. Hanif H, Ali MJ, Susheela AT, Khan IW, Luna-Cuadros MA, Khan MM, Lau DTY. Update on the applications and limitations of alpha-fetoprotein for hepatocellular carcinoma. World J Gastroenterol 2022; 28(2): 216-229.
27. Polley A, Orłowski A, Danne R, Gurtovenko AA, Bernardino de la Serna J, Eggeling C, Davis SJ, Róg T, Vattulainen I. Glycosylation and lipids working in concert direct CD2 ectodomain orientation and presentation. J Phys Chem Lett 2017; 8(5): 1060-1066.
28. Li XL, Yan ZS, Ma YQ, Ding HM. Impact of glycosylation of apolipoprotein D on its interaction with gold nanoparticles: insights from molecular dynamics simulations. ACS Appl Mater Interfaces 2025; 17(3): 4490-4501.
29. Rabbani N, Ashour A, Thornalley PJ. Mass spectrometric determination of early and advanced glycation in biology. Glycoconj J 2016; 33(4): 553-568.
30. Barletti B, Paracini N, Fragneto G, Alcaraz JP, Nelson A, Vilgrain I, Martin DK, Maccarini M. Glycation enhances protein association with lipid bilayer membranes. Langmuir 2025; 41(46): 31169-31178.
31. Masson D, Jiang XC, Lagrost L, Tall AR. The role of plasma

lipid transfer proteins in lipoprotein metabolism and atherogenesis. J Lipid Res 2009; 50(Supp1): S201-S206.
32. Ginsberg HN, Packard CJ, Chapman MJ, Borén J, Aguilar-Salinas CA, Averna M, Ference BA, Gaudet D, Hegele RA, Kersten S, Lewis GF, Lichtenstein AH, Moulin P, Nordestgaard BG, Remaley AT, Staels B, Stroes ESG, Taskinen MR, Tokgözoğlu LS, Tybjaerg-Hansen A, Stock JK, Catapano AL. Triglyceride-rich lipoproteins and their remnants: metabolic insights, role in atherosclerotic cardiovascular disease, and emerging therapeutic strategies—a consensus statement from the European Atherosclerosis Society. Eur Heart J 2021; 42(47): 4791-4806.
33. Hallal S, Tűzesi Á, Grau GE, Buckland ME, Alexander KL. Understanding the extracellular vesicle surface for clinical molecular biology. J Extracell Vesicles 2022; 11(10): e12260.
34. Sagini K, Urbanelli L, Buratta S, Emiliani C, Llorente A. Lipid biomarkers in liquid biopsies: novel opportunities for cancer diagnosis. Pharmaceutics 2023; 15(2): 437.
35. Nikolac N. Lipemia: causes, interference mechanisms, detection and management. Biochem Med (Zagreb) 2014; 24(1): 57-67.
36. Saracevic A, Dukic L, Simundic AM. Haemolysis and lipemia interfere with resistin and myeloperoxidase BioVendor ELISA assays. Biochem Med (Zagreb) 2019; 29(2): 020703.
37. Vaisar T, Pennathur S, Green PS, Gharib SA, Hoofnagle AN, Cheung MC, Byun J, Vuletic S, Kassim S, Singh P, Chea H, Knopp RH, Brunzell J, Geary R, Chait A, Zhao XQ, Elkon K, Marcovina S, Ridker P, Oram JF, Heinecke JW. Shotgun proteomics implicates protease inhibition and complement activation in the antiinflammatory properties of HDL. J Clin Invest 2007; 117(3): 746-756.
38. Ronsein GE, Vaisar T. Deepening our understanding of HDL proteome. Expert Rev Proteomics 2019; 16(9): 749-760.
39. Vilgrain I, Sidibé A, Polena H, Cand F, Mannic T, Arboleas M, Boccard S, Baudet A, Gulino-Debrac D, Bouillet L, Quesada JL, Mendoza C, Lebas JF, Pelletier L, Berger F. Evidence for post-translational processing of vascular endothelial (VE)-cadherin in brain tumors: towards a candidate biomarker. PLoS One 2013; 8(12): e80056.
40. Gries A, Prassl R, Fukuoka S, Rössle M, Kaconis Y, Heinbockel L, Gutsmann T, Brandenburg K. Biophysical analysis of the interaction of the serum protein human β2GPI with bacterial lipopolysaccharide. FEBS Open Bio 2014; 4: 432-440.
41. Calvo D, Gómez-Coronado D, Lasunción MA, Vega MA. CLA-1 is an 85-kD plasma membrane glycoprotein that acts as a high-affinity receptor for both native (HDL, LDL, and VLDL) and modified (OxLDL and AcLDL) lipoproteins. Arterioscler Thromb Vasc Biol 1997; 17(11): 2341-2349.
42. Park YM. CD36, a scavenger receptor implicated in atherosclerosis. Exp Mol Med 2014; 46(6): e99.
43. Bartosch B, Verney G, Dreux M, Donot P, Morice Y, Penin F, Pawlotsky JM, Lavillette D, Cosset FL. An interplay between hypervariable region 1 of the hepatitis C virus E2 glycoprotein, the scavenger receptor BI, and high-density lipoprotein promotes both enhancement of infection and protection against neutralizing antibodies. J Virol 2005; 79(13): 8217-8229.
44. Macchioni A, Ciancaleoni G, Zuccaccia C, Zuccaccia D. Determining accurate molecular sizes in solution through NMR diffusion spectroscopy. Chem Soc Rev 2008; 37(3): 479-489.
45. Kusnezow W, Syagailo YV, Rüffer S, Klenin K, Sebald W, Hoheisel JD, Gauer C, Goychuk I. Kinetics of antigen binding to antibody microspots: strong limitation by mass transport to the surface. Proteomics 2006; 6(3): 794-803.