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Journal of Extracellular Biology

MEETING REPORT OPEN ACCESS

Biomolecular Corona of Extracellular Vesicles: Report on Isev Workshop

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Edit Buzás and Annalisa Radeghieri are co-last authors.

[Correction added on 18 August 2026, after first online publication: the statement for co-last authors have been updated.]

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Received: 25 February 2026 | **Revised:** 11 June 2026 | **Accepted:** 5 July 2026

ABSTRACT

The concept of the "biomolecular corona" (BC) was first introduced to describe the spontaneous absorption of molecules, mainly proteins, onto the surface of synthetic nanoparticles upon exposure to biological fluids. More recently, the same term has been used to describe biomolecules that dynamically interact with the surface of extracellular vesicles (EVs) during their biogenesis, secretion, or upon exposure to intracellular or extracellular milieus, including biological fluids. The EV biomolecular corona is a key determinant of EV identity, shaping their cellular uptake, biodistribution, and functional outcomes in both physiological and pathological contexts. EVs, in contrast to synthetic nanoparticles, introduce distinct challenges and perspectives due to their biological origin, innate heterogeneity, and complex combination of physicochemical and biological properties. To address this evolving topic, the International Society for Extracellular Vesicles (ISEV) organized a dedicated workshop in Brescia, Italy, on 27th to 28th February, 2025. The event gathered selected participants from academia and industry, with expertise spanning synthetic nanomaterials, EVs, biophysics, and translational nanomedicine. The program included keynote lectures, oral presentations, posters, and a series of interactive roundtables structured around fundamentals of the biomolecular corona, EV corona biogenesis and composition, analytical and separation tools and clinical and diagnostic potential applications. This report summarizes the key scientific insights, methodological challenges, conceptual debates, and future perspectives that emerged from the workshop.

1 | Introduction

Originally conceptualized in the context of synthetic nanoparticles (Wilkins and Myers 2026; Cedervall et al. 2007; Walkey and Chan, 2012; Dawson and Yan, 2021) (SNPs), the biomolecular corona (BC) refers to the progressive and selective adsorption of proteins and other biomolecules (such as lipids, nucleic acids, and metabolites) onto the surface of nanostructures upon exposure to biological fluids (Lundqvist et al. 2008; Monopoli et al. 2012; Mohammad-Beigi et al., 2020). This process profoundly reshapes the surface properties of nanoscale entities at the single particle level, thereby conferring new biological identities that govern their interactions with surrounding biological systems. The BC influences bionanoscale recognition events, uptake pathways, biodistribution, and ultimately the biological fate and safety of SNPs (Monopoli et al. 2012).

A recent growing body of scientific literature demonstrated, for the first time, that a BC also forms on extracellular vesicles (EVs), emerging from an interplay between EV biogenesis, environmental exposure and dynamic remodeling, positioning the BC as an integrated and context dependent functional interface (Buzás et al. 2018; Buzas, 2022; Cominal et al. 2025; Liam-Or et al., 2024; Wolf et al. 2022). Importantly, emerging evidence also indicates that EVs naturally associate with other extracellular nanoparticles (ENPs) naturally present in biofluids, such as lipoproteins (Busatto et al., 2020; Busatto et al. 2022), further broadening our understanding of the complexity and dynamics of EV–environment interactions.

Only in recent years, to describe this phenomenon, the EV field adopted the term BC (Wolf et al. 2022; Tóth et al., 2021), since the interactions at the surface of EVs (EV-BC) exhibit multiple parallels with those observed in SNP-BC.

For these reasons, the ISEV Workshop (WS) on the Biomolecular Corona of Extracellular Vesicles, held in Brescia (Italy) on 27th to 28th February, 2025 brought together invited BC experts from the SNPs (both solid state and soft nanoparticles) field and researchers working on EV-BC. The event aimed to foster cross-disciplinary dialogue and promote the exchange of methodological tools to advance research on the complex and dynamic BC that forms around EVs. The discussion indeed took advantage of prior experience gained in the field of SNPs that provided valuable insights and brought diverse perspectives to the discussion. The workshop featured eight invited keynote lectures, twelve talks selected from abstracts, and twenty poster presentations. During the workshop, participants engaged in both structured roundtable discussions and informal on-the-spot surveys aimed at exploring shared perspectives, open questions, and conceptual tensions around the BC of EVs. The combined insights from these activities provide a snapshot of the field's

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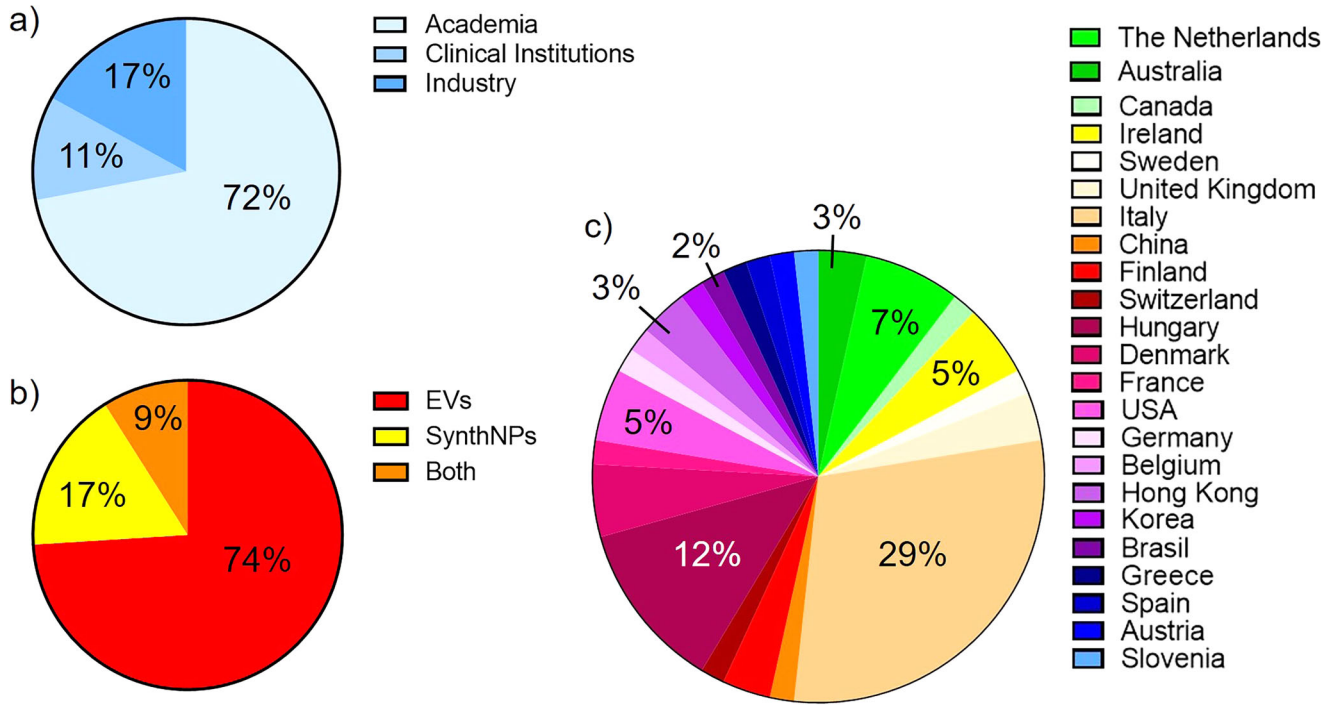

FIGURE 1 | Graphs of participant sub-division and percentage of each group on the total number of attendees (comprising IOC, introductory speakers and scientists with selected abstracts). Attendees depicted according to: (a) affiliation: Academia, Clinical Institutions, Industry (b) background of expertise: Extracellular Vesicles (EVs), Synthetic Nanoparticles (SNPs), Both fields (Both); (c) geographical representation. Percentage of attendees of the most represented countries is indicated in the graph.

current state, characterized by emerging consensus in some areas and lively debate in others. This report aims to present the topics discussed during the workshop, highlight areas of consensus, and outline the remaining open issues, with the goal of increasing awareness within the EV community of the BC concept and its relevance to EV research.

2 | Workshop Features

2.1 | Topics

The scientific program focused on four main interconnected themes bridging the synthetic and biological NP fields:

1. Fundamentals of Biomolecular Corona: origin, thermodynamics and colloidal properties: exploring the thermodynamic, colloidal and physicochemical mechanisms of BC formation both on SNPs and ENPs.
2. EV biomolecular corona biogenesis, biological significance and molecular composition: addressing the biological origin, acquisition mechanisms, and molecular makeup of BC on EVs.
3. Biogenic nanoparticle corona separation and characterization: highlighting techniques to separate, visualize, and characterize the BC and its dynamics on EVs.
4. Potential applications of the Biomolecular Corona: examining how BC knowledge could translate into therapeutic delivery, biomarker discovery, or immunomodulation.

2.2 | Participants

The workshop hosted approximately 70 participants, including researchers from academia (72%), clinicians (11%), and industry stakeholders (17%) (Figure 1a). Attendees were selected based on research area, submitted abstracts, and motivation statements. The group reflected a mix of expertise spanning from extracellular vesicles (74% of the participants) to synthetic nanomaterials (9%) and both fields (17%) (Figure 1b). Participants came from 23 countries across Europe, North America, Asia, South America, and Oceania, underscoring the global relevance of the topic. Italy accounted for the largest share of attendees (29%), followed by Hungary (12%) and the Netherlands (7%), with additional representation from Ireland (5%), the USA (5%), Hong Kong (3%), Australia, (3%), Brazil (2%), and others (Figure 1c). This broad geographic distribution enriched the scientific dialogue and enabled cross-disciplinary and cross-cultural exchange throughout the event.

3 | Emerging Insights Raised During the Workshop

This section summarizes the most significant questions discussed during the oral presentations and round-table sessions on the four topics outlined in 2.1. It is built on the well-established concept of the SNP-BC and extends it to EVs, where the idea is more complex and still evolving. It introduces major challenges and potential strategies for overcoming obstacles in studying the EV-BC and its potential applications in the clinical field. Themes that received large consensus are detailed in the text, while open questions

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among the WS participants are presented and discussed at the end of each topic. Many of these challenges were common across all four key topics of the meeting.

A key point that emerged during the discussions was that most participants (64%) believe the EV community is not fully familiar with the concept of BC and how it relates to EVs. The concept of a BC forming around EVs was first conceptualized in 2021[15] although the idea of association of different molecules and nanoparticles with EVs was already demonstrated by different groups (Buzás et al. 2018; Lima et al. 2021; Tassoni et al., 2025). One of the main goals of this WS and the resulting report is to raise awareness of this relatively novel concept, that is marking a paradigm shift in the field, moving from viewing EV associated molecules and nanoparticles as contaminants to recognizing them as integral to EV biological and physicochemical identity and function.

3.1 | Topic 1: Fundamentals of Biomolecular Corona: origin, Thermodynamics and Colloidal Properties & Topic 2: EV Corona Biogenesis, Biological Significance and Molecular Composition

A key outcome of the workshop was the recognition that Topics 1 and 2, originally conceived as separate (or at least separable at an initial level of treatment) are in fact tightly interlinked. The lively discussion during the meeting and throughout the manuscript preparation helped to integrate them into the rational framework presented in this section.

The framework can be set by contrasting the EV-BC versus the SNP-BC, that we may refer to as the "classic" BC (Buzas, 2022; Radeghieri and Bergese, 2023; Latreille et al. 2022). Compared to this classic paradigm, EVs pose new challenges and perspectives due to an intricate blend of physicochemical and biological features, namely: (1) their membranous, complex structure, (2) their innate heterogeneity, (3) their biological origin, and (4) their evolved interaction and synergy with the BC.

The BC modulates the physicochemical properties, and in turn the biological identity of any nanoparticle, therefore including SNPs and EVs.

From a physicochemical perspective, the BC phenomenon is governed by the dynamic interplay between the chemical and physical traits of the nanoparticles and of the surrounding biofluid, such as composition, size, structure, pH, ionic strength, and temperature. BC formation changes the colloidal properties of the nanoparticles, such as surface energy, surface charge, hydrodynamic radius, and aggregation/stability. On the flip side, interaction with the nanoparticle surface can induce structural and conformational changes of the proteins confined in the BC (Latreille et al. 2022).

Research regarding the physicochemical mechanisms of the BC formation to date has been primarily focused on solid-state SNPs. The established model for SNPs posits a stable inner layer of irreversibly physisorbed biomolecules (the 'hard corona') interfacing with one or more outer layers of loosely bound, dynamically exchanging molecules (the 'soft corona') (Mohammad-Beigi et al., 2020; Latreille et al. 2022). Yet, for these SNPs, a true predictive and control-level understanding remains elusive, and fundamental questions remain open.

Far fewer studies have been performed on EVs. However, key differences between EV-BC with respect to SNP-BC can be already outlined, with important implications.

From thermodynamic arguments, it can be shown that the elastic energy required to form an EV and the energy required to form its BC are of the same order of magnitude (Manno et al. 2024; Bergese et al. 2022). In addition, not only the energy but also the types of intermolecular interactions that mediate protein adsorption are similar to those responsible for EV assembly. This thermodynamically connects the process of EV biogenesis with the phenomenon of BC acquisition, suggesting that some proteins acquired through BC formation are energetically allowed to become components of the EV itself, including that a BC can form intracellularly during EV biogenesis (Manno et al. 2024).

EVs are characterized by a higher heterogeneity than SNPs, beyond the separation and production processing, at different length scales and levels. Single EVs may have physicochemical traits very different to the average, determined at a collective level and ascribed to the bulk. This heterogeneity includes both inter-subpopulation heterogeneity (differences amongst EVs from various sources) and intra-subpopulation heterogeneity (differences in EVs within subpopulations derived from the same source, and even cell). For example, EVs from the same cell have different sizes as those separated from plasma (Nikoloff et al. 2021; Jeppesen et al. 2023; Buzas, 2023; Ahmadi et al. 2024; Carney et al. 2025). In addition, some evidence suggests that the membrane of EVs can present spatial (and probably also temporal) variations of the lipid composition, resembling lipid rafts (Skotland et al., 2017; Pfrieger and Vitale, 2018; Sapoń et al. 2023). All these characteristics can influence BC formation, stability and structural organization depending on the EV's origin and corresponding surface properties, thereby adding an additional layer of heterogeneity among EVs (Musicò et al. 2024).

A key distinction between EV- and nanoparticle-associated BCs is that EVs display a native protein landscape, so their BC largely arises from protein–protein interactions rather than simple protein–lipid surface adsorption (Zendrini et al. 2022). This intrinsic complexity both enhances the biological roles of the EV-BC as well as posing significant challenges for its experimental characterization, as many interactions are transient and/or dynamic (Tóth et al., 2021; Tassoni et al., 2025).

Altogether, the above arguments suggest that EV-BC architecture and dynamics may differ fundamentally from those typically described for solid-state NPs. Recent work shows that the BC on EVs does not self-organize in a simple layer-by-layer process but instead can accumulate into discrete patches of protruding protein aggregates (Tóth et al., 2021; Musicò et al. 2024).

The BC determines nanoparticle biological identity and recognition at the nanoscale, and therefore its function, which has been demonstrated for SNPs (Mahmoudi et al., 2023) and, more recently, for EVs (Buzas, 2022; Liam-Or et al., 2024; Wolf et al. 2022; Tóth et al., 2021; Dietz et al. 2023).

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A main turning point here lies in the fact that the BC forms upon exposure to a biological environment, yet the EV itself has a biological origin and may be secreted with a BC already acquired during biogenesis (Buzas 2022). Consequently, the concept of BC becomes intrinsically linked also to its biogenesis. This perspective leads to an evolution of the BC paradigm set for SNPs, from being seen as an adsorbed biomolecular layer on an artificial surface to representing an integral part of the EV intrinsic structural organization. As laid out above, this perspective also has a physicochemical foundation.

In a wider biological context, EVs are part of the nanostructured secretome of cells, a colloidal solution of extracellular nanoparticles that also includes protein aggregates, exomeres, lipoproteins and midbody remnants (Busatto et al. 2020; Zhang et al. 2021; Rai et al., 2021; Lozano-Andrés et al. 2023). There is growing and compelling evidence that, under physiological conditions, they undergo dynamic interactions not only with soluble macromolecules, leading to the formation of BC, but also with other components of the cell secretome, resulting, for example in EV-lipoprotein aggregates and fusion. This prompts a broader definition of the BC, one that embraces these complex interactions and suggests that the BC may represent just one facet of the intricate and dynamic metabolism of extracellular nanoparticles (Lozano-Andrés et al. 2023; Busatto et al. 2022; Sódar et al., 2016; Ghebosu et al. 2024; Ghebosu et al. 2025, Musicò et al. 2025).

Overall, these arguments posit a fundamental shift: whereas the BC on SNPs is typically an unintended or poorly controlled byproduct, for EVs it is an inherent, evolutionarily conserved, and likely functional feature.

The points discussed above were presented and debated during the workshop, leading to broad consensus while also bringing unresolved issues to the forefront.

3.2 | Topic 1 & 2 Open Issues

3.2.1 | Innate vs. acquired EV-BC, viz. is the BC Part of the EV or a Separate Entity?

The conceptual distinction between an innate (endogenous/intracellularly acquired) and an extracellularly acquired (exogenous) BC was considered useful but difficult to establish experimentally. A potential method based on spatially resolved proteomics has been proposed at the workshop and very recently published (Menaceur Vandenbroucke et al., 2026) (see also Topic 3). Participants noted that molecules adsorbed on the membrane during EV biogenesis (e.g., via endosomal pathways) and those adsorbed after secretion may both influence functionality.

Since no consensus has emerged, either conceptually or experimentally, on tools that allow a clear distinction between innate and acquired BC, it has been proposed that definitions of BC should prioritize binding dynamics and biologically meaningful residence time over molecular origin. Given the fact that EVs acquire a BC both intracellularly and extracellularly, does it make sense to talk about "bare" EVs (without considering BC)? In general, the idea of a "bare EV" was seen as theoretical, and participants generally agreed that from a structural and functional point of view, the BC cannot be ignored.

A further point arising from these discussions, and agreed upon by all participants, is that intrinsic membrane components, such as transmembrane proteins, cannot be considered part of the BC (Figure 2).

3.2.2 | How Is the EV–BC Functional?

Building on the intertwined physicochemical and biological origins of the EV-BC, its molecular composition is expected to be both diverse and context-dependent (Tóth et al., 2021), encompassing proteins (Caponnetto et al. 2023), nucleic acids (Tutanov et al., 2022, Sedej et al. 2022, Tsering et al., 2024), lipids (Lozano-Andrés et al. 2023), and glycoconjugates (Hallal et al. 2022; Radeghieri et al. 2022) (Figure 2). These components may modulate EV stability, trafficking, and recognition by recipient cells, for example by masking or altering the exposure of surface ligands involved in uptake (Liam-Or et al., 2024; Dietz et al. 2023), yet such effects vary markedly across cell types and environments. This variability, combined with the blurred boundary between molecules acquired during biogenesis and those adsorbed post-secretion, complicates efforts to ascribe universal functions to specific BC constituents or to adopt a unified definition of the BC itself. Although accumulating evidence suggests that BC composition can influence receptor engagement (Liu et al., 2023) and downstream responses, particularly in nanomedicine (Wolf et al. 2022) contexts, direct causal links between distinct BC signatures and biological or pathological outcomes remain limited. Advancing this field will require more in vivo studies and analytical strategies capable of resolving which BC features are mechanistically relevant. Altogether, the EV-BC emerges as a dynamic, environment-responsive interface that contributes in fundamental ways to EV fate and function (Tóth et al., 2021).

3.3 | Topic 3: Tools for the Separation and Characterization of Biogenic Nanoparticle Corona

Accurate characterization of the BC remains a major technical challenge (Mahmoudi, 2022). Most studies of SNP-BC and EV-BC interactions have mainly relied on *ex-situ* analytical techniques, which typically require multiple sample-preparation steps to separate the NPs from the biological environment to further analyze the BC.

However, as previously demonstrated for SNPs, common separation procedures, such as high-shear microfluidic processing or centrifugation–resuspension protocols, can substantially alter the BC (Palchetti et al. 2017; Li et al. 2021, Hoang et al. 2022; Daramy et al. 2024). Conventional nanoparticle purification methods tend to preferentially retain the hard corona, while the soft corona is largely lost during processing. Current efforts therefore focus on developing methodologies capable of preserving both layers, thereby enabling a more faithful representation of the BC as it exists in biological environments (Mohammad-Beigi et al., 2020; Weber et al., 2018; Baimanov et al. 2022; Chetwynd et al., 2019).

This challenge is even more pronounced for EV-BC systems, as the delicate and complex physicochemical properties of EVs make

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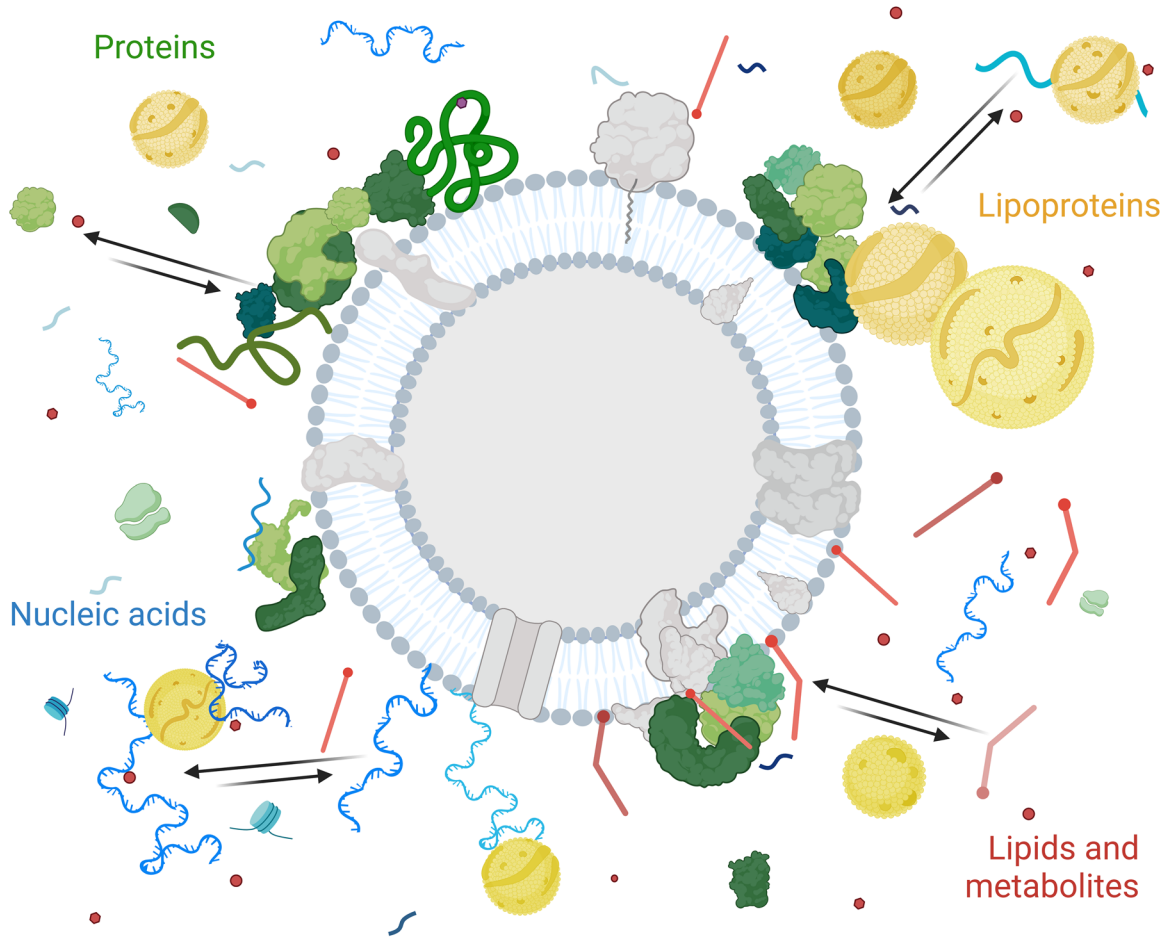

FIGURE 2 | Schematic of the EV-BC dynamic interface. EV intrinsic membrane proteins, including membrane integral proteins and lipid-anchored proteins, are represented in grey. BC components are in color: proteins in green; lipoproteins in yellow, lipids and metabolites in red; nucleic acids in blue. Created in BioRender. Tassoni, S. (2026) https://BioRender.com/sb6gffd.

it difficult to separate the BC from EVs without compromising their native state (and vice versa) (Tassoni et al., 2025; Manno et al. 2024) even if some successful attempts have been made (Singh et al. 2020; Försönits et al., 2025).

During the discussion, it emerged that the most commonly used protocols for EV separation in BC studies, such as ultracentrifugation (UC) (Tóth et al., 2021; Palviainen et al. 2020) and size-exclusion chromatography (SEC) often yield different BC protein profiles. This variability is likely due to differences in how well the BC is preserved during the different separation steps. SEC is generally considered less disruptive to BC-EV structure, yet still not non-invasive. The influence of the separation method on EV-BC has also been reported for other techniques (e.g., TFF, Wolf et al. 2022; Kawai-Harada et al., 2024), immunoaffinity capture (Ströhle et al. 2022). However, as with SNPs, no consensus exists on an optimal method, as each approach presents its own advantages and limitations (Tassoni et al., 2025).

Most participants agreed that raising awareness of how separation protocols can influence the EV-BC is an important first step for the EV field (Poupardin et al. 2024). Equally essential is ensuring that these protocols are applied in a reproducible and consistent manner. Promoting transparent reporting of experimental conditions (sample origin, biofluid type including batch-to-batch variation and donor variability, separation procedures, incubation times, and characterization methods) is viewed as essential for ensuring data comparability, supporting reproducibility, and ultimately accelerating both discovery and clinical translation. Furthermore, functional readouts following EV-BC manipulation (e.g., uptake, immune activation, or downstream signaling) should be systematically integrated as complementary validation tools, particularly when stripping or reconstitution strategies are applied.

Characterizing the BC also requires sophisticated and multimodal analytical approaches (Varga et al., 2020). Techniques such as single-molecule localization microscopy (SMLM (Wolf et al. 2022; Tóth et al., 2021; Ghanam et al. 2023), that is, Stochastic Optical Reconstruction Microscopy (STORM)), Photoactivated localization microscopy (PALM), atomic force microscopy (AFM (Wolf et al. 2022)), have revealed important BC features, including dynamic protein adsorption, structural heterogeneity, and specific interactions with growth factors (Shelke et al. 2019), lipoproteins (Busatto et al. 2022, Ghebosu et al. 2024; Musicò et al. 2025, Haddad et al., 2023) and antimicrobial peptides (Singh et al. 2023; Sonallya et al., 2025).

To overcome the limitations of introducing separation steps, more recent in situ approaches, such as differential dynamic

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microscopy (DDM) (Tóth et al., 2021; Shelke et al. 2019; Haddad et al., 2023), fluorescence correlation spectroscopy, Fluorescence cross correlation spectroscopy (FCS (Musicò et al. 2025), FCCS (Ghebosu et al. 2025, Li et al. 2023) have been introduced, in order to capture BC dynamics in "physiological" environments.

Among these methods, proximity labeling strategies (Li et al. 2023) (for example, via enzymes conjugated to ligands or antibodies targeting EV markers) represent a powerful tool to map BC components in situ more comprehensively and contextually, while minimizing disruption of EV-BC complexes and enabling downstream multi-omics analysis (Min et al., 2024; Fredolini et al. 2022; Branon et al. 2018; Qin et al., 2023). Furthermore, another recently developed method, based on spatially resolved proteomics, which exploits EV membrane proteins to study BC profiles with EV marker specificity can be applied to study BC composition also within EV subpopulations (Menaceur Vandenbroucke et al., 2026).

3.4 | Topic 3 Open Issue

3.4.1 | Purity vs Function

The concept of "purity" in the context of EVs needs to be reconsidered (Paolini et al. 2022) after introducing the concept of the BC. The concept of "pure EVs" was associated with the idea of a preparation of EVs that do not contain any biomolecule that does not belong to the EV lumen or EV membrane, assuming that native EVs are created in this form and that this form determines their function. Now it is clear that EVs naturally carry associated molecules and nanoparticles (the BC), that are or become an integral part of the EVs and are critical for their function. Supporting this perspective, another strong point of consensus (reported by 85% of participants) is that the biological function of EV formulations takes precedence over the blurry analytical definition of purity.

Notably this topic has also been described as a key challenge in clinical translation of mesenchymal stem cell (MSC)-EVs (Gimona et al., 2021; Giebel and Lim, 2025) and has been circulating within the EV community for several years (Branon et al. 2018) and first introduced in the most recent MISEV guidelines (Welsh et al. 2024). Given the dynamic nature of the BC and its dependence on environmental conditions, absolute purity of EVs may be both unrealistic and biologically irrelevant. Lessons from the SNP field suggest a practical approach: first define the functional outcomes using any suitable method, ensure these outcomes are reproducible, and then investigate the factors that influence the function.

Within this framework, a key challenge becomes translating function-first insights into comparable and interpretable measurements across experimental contexts. This raises the question of how standardized metrics and readout panels, spanning composition, dynamics, topology, and functional impact can be defined to compare EV-BC characterized using ex situ, in situ, and in vivo approaches, and to link these readouts to measured biological functions.

3.5 | Topic 4: Biomolecular Corona Potential Applications (e.g., Clinics/Diagnostics)

As discussed so far, the BC has important implications for the translational application of SNPs and EVs (Wiklander et al., 2019), influencing biodistribution, uptake, function, clearance, and therapeutic targeting. BC composition can significantly alter the fate of administered SNPs (Mohammad-Beigi et al., 2020; Bertrand et al. 2017) or EVs (Lara et al., 2017; Esmaeili et al. 2025), giving rise to unexpected results and therefore must be taken into account (Heidarzadeh et al. 2023). These effects are cell-type specific and may be driven by differential expression of surface receptors for common BC proteins (Lara et al., 2017).

On the other hand, it has recently become evident that modulation of the BC, whether through pre-incubation of NPs with selected BC components or through surface engineering strategies that control BC adsorption, can be exploited to enhance nanoparticle targeting and functionality (Dietz et al. 2023; Heidarzadeh et al. 2023). Similarly, engineering the BC via controlled exposure to specific proteins or genetic surface modifications can improve biological performance; for example, albumin-enriched coronas have been shown to redirect EV uptake from macrophages toward hepatocytes and endothelial cells (Liam-Or et al., 2024).

Importantly, the BC also shapes immune interactions. Endogenous EVs likely evolved to evade immune detection through their native BC, while exogenously engineered EVs may lack such properties, contributing to their rapid clearance. Understanding these differences will be crucial for the design of next-generation EV-based therapeutics.

Regarding the application of BC in diagnostics, 92% of participant believe that the EV-BC is a relevant entity to explore in terms of biomarkers (Radeghieri et al. 2022) as already explored for SNPs (Blume et al., 2020; Corbo et al. 2021). However, the EV-BC presents both a challenge and an opportunity (Carney et al. 2025). On one hand, the BC may mask surface biomarkers and reduce the specificity of EV-based diagnostics. On the other, BC itself may carry systemic signatures of disease (Tassoni et al., 2025). The enrichment or binding of certain BC elements on the EV surface may show higher abundance compared to their free forms in biological fluids, reflecting changes in the extracellular environment and providing unique diagnostic or prognostic values. In addition to SNPs, EVs can be used as biomarkers from a dual perspective: valuable information can be obtained by studying both their cargo and surface components. Interest in BC profiling is growing, particularly in the development of BC-based biomarker panels and liquid biopsy platforms.

As discussed in paragraph 3.3 reproducibility is a key parameter also for the translation of EV-BC both in therapeutics and diagnostics, to enhance regulatory compliance in future clinical applications.

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3.6 | Topic 4 Open Issues

3.6.1 | BC in Diagnostics

The dynamic and variable nature of the BC presents challenges in diagnostics. It remains unclear how factors such as pre-analytical variables (e.g., pre- and post-prandial plasma states), biological variables (e.g., immune status, metabolic state, or disease-specific microenvironments and circadian rhythms), may substantially reshape EV-BC composition and function, complicating standardization and translational efforts.

Reporting these variables is essential for reliably translating EV-BC as a diagnostic tool.

4 | Conclusions and Recommendations

The workshop fostered a rich interdisciplinary exchange on the emerging field of the EV-associated BC, highlighting that standardization should be guided by functional relevance and methodological transparency rather than rigid definitions. Although the complexity and variability of BC formation still preclude the establishment of universally applicable Standard Operating Procedures, participants strongly valued the collective efforts initiated through the workshop and emphasized the need for disseminating educational resources and developing practical recommendations, an approach endorsed by both the EV and SNP communities. A key outcome was the recognition that many open questions and technical challenges are shared across synthetic and biological nanoparticles, underscoring the importance of synergizing expertise and learning from one another. One of the strengths of the workshop was precisely the opportunity for these communities to discuss side by side, revealing how much common ground exists and how each field can benefit from the insights of the other. Together, these elements point toward the urgent need for coherent terminology, reproducible protocols, and sustained interdisciplinary collaboration to advance understanding of the biological and translational impact of the EV-BC and to shape a more coordinated and impactful research agenda. As of today, the main current limitation in BC research (also for SNP) is the heterogeneity of protocols (preparation, biological fluids, incubation protocol, isolation, work up, analysis, etc.) (Boselli et al., 2024). Such heterogeneity affects the reliability of BC data and calls the need of shared approaches.

In addition to standardization and analytical advances, future progress in the EV-BC field will require the systematic integration of functional validation frameworks. Correlating BC composition with biological outcomes across different physiological and pathological contexts will be essential to move beyond descriptive classifications and toward predictive and translational relevance.

5 | Workshop Takeaways

Theme	Community Consensus
Terminology	"Biomolecular Corona" remains useful, but may need further discussion in the EV field
Structural identity	The BC is functionally part of the EV, but structurally debated
Function vs. purity	Function is the priority, especially for clinical applications
Innate vs. acquired BC	Conceptually useful, but experimentally difficult to distinguish
Non-disruptive characterization	Technically challenging but essential; promising tools are emerging such as stripping or in situ methods
Standardization	Strong need for transparent protocol reporting rather than rigid definitions
Functional/ mechanistic perspective	Shift from static composition to dynamic, context-based functional characterization
Broad perspective	The theoretical "bare" EVs retain cell origin characteristics, but BC reflects the intracellular or extracellular microenvironment

Author Contributions

Lucia Paolini: conceptualization, Writing – original draft, Writing – review and editing. **Selene Tassoni**: conceptualization, writing – original draft, writing – review and editing. **Khuloud T. Al-jamal**: conceptualization, writing – review and editing. **Xavier Banquy**: conceptualization, writing – review and editing. **Tamás Beke-somfai**: conceptualization, writing – review and editing. **Paolo Bergese**: conceptualization, writing – original draft, writing – review and editing. **Fanni Annamária Boros**: conceptualization, writing – review and editing. **Massimo Bottini**: conceptualization, writing – review and editing. **Ben Boyd**: conceptualization, writing – review and editing. **Sara Busatto**: conceptualization, writing – review and editing. **Benedetta Bussolati**: conceptualization, writing – review and editing. **Ester Canepa**: conceptualization, writing – review and editing. **Valentina Castagnola**: conceptualization, writing – review and editing. **Aled Clayton**: conceptualization, writing – review and editing. **Kenneth Dawson**: conceptualization, writing – review and editing. **Samir El-andaloussi**: conceptualization, writing – review and editing. **András Försönits**: conceptualization, writing – review and editing. **Orazio Fortunato**: conceptualization, writing – review and editing. **Roberto Frigerio**: conceptualization, writing – review and editing. **Martina Gabrielli**: conceptualization, writing – review and editing. **Cristina Grange**: conceptualization, writing – review

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Acknowledgements

The workshop success was possible thanks to the active participation of all attendees. The workshop was supported by: University of Brescia under "Funds for international activities" from the Italian Ministry of University and Research; Italian Society for Extracellular Vesicles (EVIta); International Society for Extracellular Vesicles (ISEV); Project ORGANO_CAR ID PE_00000019 Heal Italia- Spoke 5- UNIMIB- Next Gen Therapeutics Italian Ministry of University and Research; Martina Gabrielli was supported by a BBSRC Fellowship UKRI896; Fanni Annamária Boros was supported by Interdisciplinary Center for Clinical Research (Interdisziplinäres Zentrum für Klinische Forschung; IZKF, Erlangen) Junior Research Program J109; Maria Sancho Albero thanks the AECC for postdoctoral research fellowship funding (POST234966SANC), "Ayuda a Talento AECC 2023" and the financial support from Catedra SAMCA (NAIVE project) and the Spanish Ministry of Science, Innovation and Universities from the Spanish Government for a Ramón y Cajal Fellowship (RYC2024-050017-I).

Workshop Ioc

Annalisa Radeghieri (Chair); Edit Buzas (Chair); Sara Busatto; Aled Clayton; Metka Lenassi; Andreas Möller, Lucia Paolini, Ganesh Vilas Shelke.

Conflicts of Interest

Massimo Bottini, Benedetta Bussolati, Mỹ G. Mahoney, and Annalisa Radeghieri serve as Associate Editors and/or Guest Editors of the Journal of Extracellular Biology. They were not involved in the peer review process of this manuscript. All other authors declare no conflicts of interest.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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