The rise of the nanomaterial metabolite corona, and emergence of the complete corona

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Bio–nano interactions are a promising area of research, focused particularly on the interactions between proteins and nanomaterials (NMs). These interactions lead to the formation of the protein corona, a layer of proteins adsorbed to the surface of NMs; this corona determines the way in which cells within organisms “recognise” and interact with NMs. Corona formation has proven critical for cellular uptake, intracellular localization and toxicity arising from NMs. However, a major class of biological chemicals have been overlooked in the efforts to understand and predict the interactions of NMs with living organisms, namely metabolites, which are also involved in signaling cascades and toxic responses in biological systems. To date, very few studies have investigated this aspect of the NM corona, with initial work focusing upon the use of carbon nanotubes to clean persistent organic pollutants from contaminated air or water. As the protein corona field grew, limited interest in the metabolite corona began to emerge with investigations into the lipid composition of the corona around inhaled NMs and eventually more holistic analyses of the metabolite corona. We suggest that the metabolite corona co-exists with the protein one, since these smaller molecules can fit in between proteins, and indeed are often bound into protein complexes. We hypothesize that metabolites influence NMs impacts on molecular signaling and adverse outcome pathways. We show initial evidence that the metabolite corona is complementary to protein coronas, following similar rules of adsorption based on abundance and affinity leading to metabolite fingerprints akin to protein fingerprints. This work aims to highlight the metabolite corona as an important, but as yet greatly under represented, area of bio–nano interaction research, and encourage researchers to explore this aspect of the interactions between NMs and their surrounding biological environments.

Environmental significance

The protein corona has been extensively studied over the past decade; the adsorbed proteins confer a biological identity to nanomaterials and influence their uptake by cells and organisms. However, this protein centric view of the corona overlooks a significant group of molecules in environmental matrices, namely metabolites, which greatly outnumber proteins. These coronal metabolites are beginning to garner interest from the bio–nano community, although not always discussed in terms of the corona or bio–nano interface. This review encompasses early studies using carbon nanomaterials for the removal of persistent organic pollutants from water through to metabolomics style analysis of the metabolite corona acquired by nanomaterials in contact with the model organism Daphnia magna. The co-existence of metabolites and proteins in the corona is also discussed, leading to the concept of the complete corona.

1.0 Introduction

The protein corona has been an intense field of research over the past decade since its potential significance for toxicity, cellular entry and trafficking were realized. As a result, a wide range of studies have investigated its formation, effects on cellular localization, toxicological consequences and how various physico/chemico properties of NMs and biological matrices can influence its qualitative and quantitative protein constituents. The number and breadth of publications in this field have now enabled models to be constructed to predict the formation and composition of the corona and the interactions that the NM–corona complexes have in their local environs. However, one aspect of the corona that has been largely ignored to date, is the metabolite corona. Metabolites are orders of magnitude smaller than proteins, typically below 1000 Da whereas proteins are measured on the kDa scale, and metabolites are typically reactants, intermediaries and products of enzymatic activity. While the idea of interactions between metabolites and NMs is not a new one, as this is one of the applications of surface enhanced Raman spectroscopy (SERS) and several approaches to “clean” samples prior to analyses, the
Our bodies maintain a diverse and sophisticated network of biochemical pathways, which play critical roles in metabolism, energy production, and cellular signaling. These pathways not only drive fundamental metabolic functions like glucose breakdown and oxygen use, but are also critical for the development and maintenance of tissues, organs, and even whole organisms. Metabolites, the small molecules produced or used in these reactions, are essential for nearly every aspect of life.

Metabolites are defined as small molecules, typically less than 5000 Da, which are primarily involved in metabolic processes. They can be organic compounds, such as carbohydrates, lipids, and amino acids, or inorganic compounds like ions and gases. These molecules are crucial for the body’s ability to maintain homeostasis and respond to environmental changes.

The metabolic pathways in which metabolites participate are incredibly diverse, ranging from simple reactions like the conversion of glucose to pyruvate, which is then further metabolized by the Krebs cycle or glycolysis, to complex processes like photosynthesis in plants or oxidation of fatty acids in animals. These pathways are not only fundamental to life, but also provide a rich resource for studying the interactions between nanomaterials (NMs) and biological systems.

NMs can interact with metabolites in a variety of ways, from physical adsorption to chemical modification. These interactions can alter the activity or localization of metabolites, potentially affecting their distribution within the body. For example, some metabolites may be more likely to be found in the lung surfactant, while others may be more concentrated in the liver. Non-polar metabolites, such as phospholipids, are more likely to be found in adipose tissue, while polar metabolites may be more prevalent in extracellular fluids.

Understanding how NMs interact with metabolites is essential for predicting the potential adverse effects of these materials. The formation of the biomolecule corona, which can include both endogenous and exogenous metabolites, can influence the way NMs are processed by cells. This corona can affect the binding affinity of NMs to specific receptors or enzymes, potentially altering their activity or distribution within the body.

The diversity of metabolites in the environment is vast, with tens of thousands of metabolites present in just humans alone. This diversity is critical for understanding how NMs can interact with metabolites in different environments, from the gut to the lungs to the bloodstream. The interactions between NMs and metabolites are complex and often not fully understood, making it challenging to predict the potential impacts of NMs on human health.

Despite these challenges, research in this area is rapidly advancing, with a growing number of studies exploring the interactions between NMs and metabolites. This research is critical for understanding the impacts of NMs on biological systems and for developing strategies to mitigate any potential adverse effects.
likely that the recruitment of the protein corona is influenced by the presence of metabolites particularly given that many NMs are produced and purchased in solutions stabilized by citrate, a metabolite from the citric acid cycle, while others are stabilized with tannic acid, glutamic acid, oleic acid, salicylic acid, or trichloroacetic acid.42,43 This also means that data about metabolite–protein interactions in the corona has already been collected serendipitously in many studies but the significance of this information has been overlooked to date. The reverse is also a distinct possibility as many proteins are known to interact with both endogenous metabolites or drugs,31,43 and as such the binding of proteins onto NMs is likely to attract metabolites also. Exploring and understanding the role of the metabolites in the NM bio/eco-corona is an exciting prospect as many metabolites, either naturally occurring in organisms such as amino acids, sugars and lipids or unnatural contaminants from human activity such as cosmetics, pharmaceuticals and agricultural waste can act as signaling molecules or toxicants.31 Thus, understanding the contribution of metabolites to the NM corona, elucidating the fraction of the total corona that constitutes metabolites, and potentially uncovering new metabolite driven mechanisms behind NM uptake, distribution and toxicity that have, to date, eluded researchers due to the lack of available information on the metabolites components of the corona, offers important new research directions. The various potential mechanisms of corona formation and interactions between metabolites and proteins and between the corona and NMs are illustrated schematically in Fig. 1, and include metabolite recruitment of other metabolites or proteins, or protein-induced recruitment of metabolites as has been established for protein-induced recruitment of other proteins. Although the evidence for the metabolite corona is quite sparse to date, there are tantalizing hints that similar patterns of interaction exist for metabolites as for proteins, including abundance driven initial binding and differential binding of metabolites based on their affinities for the NMs surface leading to NM metabolomic fingerprints, which we highlight in the sections below where relevant. Thus, we present here a conceptual framework for the metabolite corona, complementary to and indeed interwoven with, that of the protein corona. It will only be once a full understanding of all aspects of the complete bio/eco-corona is achieved that the field can hope to expand into the tightly regulated world of medicine to deliver on the promise of novel nanomedicines and indeed novel agrochemicals.

In much the same way that nanoscientists have leveraged the success of proteomics in the analysis of the protein corona, researchers will need to embrace metabolomics, a much younger omics science, to uncover the secrets of the metabolite corona. Indeed, it will be necessary to bridge both proteomics and metabolomics in order to begin to unwind the interactions between metabolites and proteins during the formation and evolution of the complete NM corona and interactions of the combined biomolecule corona with living organisms and the environment. In some cases, this will require additional instrumentation and expertise, particularly for researchers coming from the environmental background, however, researchers already involved in protein corona characterisation already possess the necessary equipment to investigate the metabolite corona. As such it may be possible to assess both in parallel and collect twice as much data from the same sample, and a workflow for this, including some of the relevant points to consider is presented, that captures in brief some of the main challenges and pitfalls to watch out for. Future instrument optimization may allow for simultaneous analysis of the complete corona.

This work aims to position the metabolite corona as a new and greatly underappreciated aspect of the bio–nano interface, which sits alongside and complements the protein corona. We introduce the metabolite corona as a key constituent of the complete corona, by discussing current research in this emerging field and looking ahead to the future whereby this aspect of the corona may unlock further understanding of NM uptake, distribution, toxicity and drug/ agrochemical delivery.43 It is clear from the current research that there are key similarities between the metabolite corona

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**Fig. 1** Schematic illustration of hypothetical mechanisms for formation of the complete corona around NMs. A wide range of possibilities exist for the corona to acquire metabolites and proteins. (a) The direct adsorption of proteins from the environment is the predominant model at the moment, however protein-protein interactions (b), metabolite adsorption (c), metabolite induced adsorption of proteins (d), protein induced recruitment of metabolites (e), metabolite induced recruitment of further metabolites (f) or more likely a complex combination of all aforementioned methods with elements such as metabolite induced conformational changes in proteins (g).
and protein corona fingerprints, which this review aims to spotlight. These key properties of the metabolite corona, such as preferential binding based on surface affinities and evolution based on available metabolites and competition from other biomolecules, which shadow those of the protein corona are highlighted throughout. Key directions in which to focus future research are identified, including development of predictive models of the complete corona.

2.0 Targeted analysis of persistent organic pollutant/xenometabolites binding to NMs

While not presented as corona formation, the ability of small molecule pollutants, which are often referred to as xenometabolites, to interact with NMs has been investigated for at least 12 years, with initial studies investigating the sequestering of persistent organic pollutants (POPs) by carbon nanotubes (CNTs). Due to the potential applications of carbon NMs in environmental remediation for removal of POPs, several studies modelling the binding characteristics of dioxins, polyhalogenated compounds and poly aromatic hydrocarbons (PAH) have been carried out. It is worth noting that in real environmental systems larger molecules such as humic acid would also compete for the NM surface, and indeed natural organic matter (NOM) is a known sink for pollutants, such that co-binding of pollutants and humics could occur.46–49

The first paper to report the adsorption of xenometabolites to NMs was Long et al. in 2001 (ref. 50) who found that dioxins exhibited strong π–π interactions between the hexagonal arrays of atoms in the CNT and the benzene rings in dioxins. Since then a wide array of POPs have been found to adsorb to carbon NMs including polyhalogentaed51–53 and PAH54–56 compounds and that this adsorption is highly pH dependent,51–53 an important factor also for protein corona formation. As with the protein corona, several models have also been developed to investigate computationally how these POPs interact with NMs and demonstrated that the adsorption of PAHs correlated with CNT surface area, micropore volume, volume ratio between the mesopores versus micropores and planar versus non-planar structures.55,56 These surface-specific interactions are analogous to those reported previously for proteins, where mesoporous silica NMs resulted in different proteins binding compared to smooth silica NMs, and differential protein binding to different pore volumes.57

While these investigations were not explicitly focused on endogenous metabolites they highlight the fact that xenometabolite adsorption to NMs may play a critical role in biological and environmental systems. Furthermore, these studies highlighted the importance of characterizing the adsorption characteristics of these compounds due to the potential for increased toxicity to humans and the wider environment through so-called Trojan horse effects, as seen in the protein corona, whereby they are carried into organisms as the NMs are taken up.58–60 Due to the ubiquitous nature of both POPs and NMs in the natural environment it is highly likely that these NM–POP complexes are to be found in all cases where both are present. Thus, a thorough understanding of the (xeno)metabolite corona and how it interacts with biological systems is vital to comprehend the potential harm caused by the release of NMs into the ecosystem.

3.0 Moving towards understanding the role of endogenous metabolites in the metabolite corona

An important application areas for NMs in the 21st century is their role as nanomedicines. Researchers have already begun to investigate the potential of the NM protein corona as a tool to diagnose patients and to understand the distribution and uptake of nanomedicines and its role in determining the efficacy of such medications.61–63 While proteins play vital roles in the signaling pathways determining NM uptake and biodistribution, metabolites are also important in these signaling cascades, such as eicosanoids in inflammation, and in a wide range of metabolic processes. As such, characterizing the endogenous metabolite corona could unlock the potential of nanomedicines while also informing studies into human and ecological NM toxicity.

3.1 Lipid corona

The first class of chemicals to be investigated in terms of the NM metabolite corona were lipids. The lipid constituents of the corona are of particular interest as they represent an initial point of contact between NMs and living organisms in the form of the lipid bilayer in cell membranes64 or in lung surfactant for inhaled NMs.65,66 Lung surfactant is comprised of 90% lipids and just 10% proteins.66,67 While the abundance of compounds doesn’t necessarily reflect the abundance in the corona, it is important to consider the fact that in cases of NM inhalation, the predominant class of molecule present in the lungs are lipids not proteins and that 25% of human NM exposure is via inhalation.68 This makes the metabolite corona potentially of huge significance in the lungs particularly if there is evidence that it affects the uptake of NMs. The lipid portion of lung fluid is comprised of phospholipids and cholesterol while there are 4 predominant proteins, namely surfactant protein-A (SP-A), SP-B, SP-C and SP-D. SP-A and SP-D, which are large hydrophobic proteins that play a role in innate host defense and bind to inhaled NMs and pathogens.69 SP-B and SP-C are smaller hydrophilic membrane proteins which reduce alveolar surface tension.69 These properties and functions of the protein surfactant are vital, which the metabolite corona may influence thus affecting alveolar surface tension and NM uptake by alveolar epithelial cells and macrophages.66
As with the environmental contaminants discussed above, several studies of endogenous metabolites were assessed as smaller well controlled groups of metabolites rather than in the complex mixtures that NMs are exposed to in reality. In 2012, Ruge et al., investigated the interplay between lung surfactant lipids and proteins and its effect on macrophage clearance of NMs. Here magnetite nanoparticles (mNPs) exposed to SP-A and SP-D significantly increased alveolar macrophage uptake. When exposed to the vesicles constructed from surfactant lipids in the absence of the surfactant proteins, creating a lipid corona, cellular binding of starch coated mNPs greatly increased compared to pristine mNPs, indicating that lipids in the corona are key for internalisation. However, phosphatidylcholine-coated mNPs did not show increased internalization, due to their already lipid like surface coating. Interestingly surfactant lipids diminished the effect of SP-A on mNP–macrophage interactions, potentially due to the role surfactant lipids play in the stimulation of immune cells; surfactant lipids and SP-A may also competitively bind to the surface of NMs leading to this reduced effect, demonstrating the importance of generating competitive binding data to enable accurate binding kinetics to be modelled.

Computational modelling has also been used to investigate the inhalation route of exposure to silver and polystyrene NMs. Here coarse-grained molecular dynamic models determined that the lipids and proteins of the alveolar fluid immediately coat the NMs upon exposure. This immediate formation of a complex lipid–protein corona reflects the rapid formation of the protein corona, though it remains unclear if the metabolite corona also evolves over time in the same way the protein corona. It remains to be seen if the metabolite corona has not yet been thoroughly investigated with just a single study focusing on the impact of NM composition on metabolite corona composition. Gold NMs with a core diameter of 14 nm were functionalized with either cationic or anionic surface modifications. Anionic modifications were made by coating the AuNPs with citrate (anionic) whereas two cationic coatings (16-mercaptohexadecyl)-trimethylammonium bromide (MTAB) or poly(allylamine)hydrochloride (PAC)) were investigated. These NPs were exposed to 4 different vesicles made up of either 100% dipalmitoylphosphatidylcholine (DOPC), 9:1 DOPC:1,2-dioleoyl-sn-glycero-3-phospho-1-serine (DOPS), 9:1 DOPC:1,2-dioleoyl-sn-glycero-3-phospho-1-rac-glycerol, (DOPG) and 9:1 DOPC:1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) (Fig. 2). Previously a study using quartz crystal microbalance (QCM) measurements showed that DMPC and DOPC interact with the same gold NPs when exposed to lipid bilayers. Interestingly hydrodynamic size was only significantly increased for the PAC-AuNPs upon vesicle exposure suggesting the NMs had agglomerated. The zeta potential inverted when the AuNPs were introduced into the 9:1 DOPC:DOPS and 9:1 DOPC:DOPG vesicles, suggesting that the anionic lipids had interacted with the cationic AuNPs. However, for the 100% DOPC and 9:1 DOPC:DOPE vesicle exposures no change in zeta potential was observed, likely due to the zwitterionic nature of DOPC and DOPE maintaining a positive surface despite lipid binding. When exposed to DOPC the AuNPs bound 80% of the available lipids with PAC and MTAB modified NMs retuning very similar lipid coronas. When co-exposed to DOPS and DOPC the PAC modified AuNPs bound 95% of both lipids compared to <65% for the MTAB modified NPs. This suggests that surface modifications influence corona formation in complex mixtures and that metabolites may aid the recruitment of others and vice versa (as suggested in Fig. 1d and f). This co-recruitment of metabolites (or proteins) is an important consideration as past exposures are going to have significant implications in the evolution of the complete corona; co-adsorption of toxins (xenometabolites) to the corona is important for potential Trojan horse effects.

The disparity between PAC and MTAB is due to differences in chemical structures; MTAB contains a quaternary ammonium head group and is less prone to forming hydrogen bonds, PAC is comprised of C16 chains more favorable for hydrophobic interactions with the lipid backbones. This confirms that surface modification/ligand binding plays a significant role in the formation of the metabolite corona. The anionic AuNPs only weakly interacted with the lipids with <3% adsorbing to the NMs. These findings suggest that manipulation of the NM physico/chemico properties influences the composition of the metabolite corona in much the same way it affects the protein corona. It remains to be seen
how these properties affect interactions between the proteins and metabolites in the complete corona (see schematic in Fig. 1). However, it does potentially open the door for “designer” coronas through manipulation of NM properties with the goal of targeting specific tissues or having specific functions.

A significant recent publication quantified both the protein and lipid corona of polystyrene NMs while highlighting that a full characterization of the complete corona is likely to be key in future safe clinical applications of nanomedicines. Interestingly, cholesterol levels in the corona of polystyrene NMs were independent of the surface area but correlated with particle size. Furthermore, over a 24 h time period cholesterol levels decreased in the corona potentially due to lipid dissociation. Conversely, triglyceride concentrations in the corona had a correlation with surface area of the NMs. As with cholesterol there was a decrease in the concentration of triglycerides over time, this however was related to a decrease in apolipoprotein E which has a role in transporting lipids around the body. The authors noted the intriguing point that the binding mechanism between lipoproteins and NMs are not understood and that whether the lipids were present on the lipoprotein when it bound to the NM, or whether the lipids were adsorbed to the NM first which then attracted the lipoprotein, is not yet known. This perfectly highlights the need to characterise the metabolites within the corona as they have the potential to be key driving factors in the formation of the complete corona.

3.2 Targeted plasma metabolites

The blood is the most common biological matrix used for protein corona characterisation, as once within the body it’s likely that NMs will become exposed to blood either after inhalation, ingestion or topical contact and thereby be systemically delivered around the body. Blood also represents one of the most commonly analysed biofluids in bioanalysis for drug development and biomarker discovery using proteomic and metabolomic approaches. As such, databases have been developed detailing the proteomic and metabonomic composition of human (and animal e.g. calf, rabbit etc.) blood making it an ideal candidate for coronal analysis.

A biological surface adsorption index (BSAI) has been proposed as a method to characterise competitive interactions between NMs and a selection of (xeno)metabolites. One such study proposed that the major factors driving adsorption of (xeno)metabolites to MWCNTs and 12 other NMs were primarily the lipophilicity of the (xeno)metabolites, their hydrogen bond basicity and their dipolarity/polarizability. Furthermore, this study demonstrated a strong binding capacity of 7 steroid hormones, progesterone, testosterone, estradiol, hydrocortisone, aldosterone, l-thyroxine and ethinyl estradiol, which play vital roles in regulating metabolism and act as important signaling molecules in biological systems, with ethinyl estradiol being the principal component in the contraceptive pill, an important endocrine disrupting chemical in the environment. Furthermore, the model predicted that DNA bases would also bind to NMs with a descending order of preference of adenine, thymine, guanine and cytosine, implying that NMs may interact with DNA, potentially being a source of mutations. The BSAI model builds upon the early binding studies of PAHs and is the first to model the binding properties of endogenous metabolites.

The presence of metabolites adsorbed to the surface of NMs was found to induce protein conformational changes that facilitated recognition and cellular uptake of the NMs. This study provides a critical insight into the significance of metabolites in the protein corona and leaves the door open for a host of follow-up work to begin to develop a full understanding of the roles that both proteins and metabolites play in the recruitment of each other into the corona and how they interact to bring about physiological effects in the body.

3.3 Dietary corona

Although very much an overlooked exposure route, dietary sources of NMs make up approximately 16% of human...
exposures to NMs, compared to 58% and 25% for topical and inhalation exposure, respectively.\textsuperscript{68,81} The high dietary exposure is typically through silica NMs which are used as a food additive (E551) and act as an anticaking agent.\textsuperscript{81} Furthermore, nano silver is being used as an antimicrobial and in some cases as a (supposed) supplement in health foods, nano selenium is used as an additive in green tea products, nano iron is used to treat contaminated water or as a supplement, and a variety of metal oxides are used as colorants or may elute from packaging materials.\textsuperscript{82} Due to the diverse compositions of diets around the world each meal or item of food represents a complex mixture of a vast array of biomolecules such as proteins and metabolites from a range of plant and animal species, making the dietary-corona potentially the most complex and diverse corona relevant for human exposure. Indeed the diversity of the food metabolome has been reviewed extensively.\textsuperscript{83–85} Despite the potential significance of this, just a single study has been performed thus far investigating interaction of NMs with dietary saccharides from honey and lipids from olive oil.\textsuperscript{81}

Silica NM exposure to honey revealed a correlation between exposure time and honey concentration with more negative zeta potentials, however temperature appeared to have no effect on zeta potential. When run as mixtures of pure glucose, fructose, sucrose and maltose the zeta potential did not vary and the hydrodynamic size actually decreased suggesting other biomolecules in honey are responsible for the changes in DLS and for stabilizing the NMs. In honey, glucose and fructose interacted with the silica NMs in a concentration dependent manner at 25 °C, however zeta potential and hydrodynamic diameter were not affected by time but increased with increasing temperature. Sucrose and maltose were outcompeted by glucose and fructose (due to their higher concentration in honey) in the honey and showed no interaction, however when incubated with the NMs individually no preference for particular saccharides was observed.\textsuperscript{81} Upon exposure to olive oil DLS and zeta potential measurements were not possible due to the colour of the oil (which is excessively light scattering) and the fact that it lacks electrical conductivity. Instead, GC-MS was used to characterize the adsorption of lipids, which was found to be minimal and not correlated with temperature or exposure time. The poor interaction between lipids and the silica NMs was ascribed to the hydrophilic nature of the silica NMs generating repulsion between the lipids and silica.\textsuperscript{81} These findings highlight the importance of determining binding affinities for metabolites (as well as for proteins) individually and under competitive conditions, and assessing the nature of the binding, in order to facilitate the development of predictive models of the compete corona (proteins and metabolites).

The link between NMs taken up by edible plants and dietary exposure to the NMs has yet to be explored, although evidence is emerging of bioaccumulation in edible plants.\textsuperscript{86} The role of the metabolite corona in facilitating NM uptake into plants, their transformation \textit{in situ}, and the impact of NMs on the metabolites themselves are all areas of active research currently linked to nano-enabled agriculture.

4.0 The application of untargeted analysis of the NM metabolite corona

Only \textit{via} a full understanding of the protein and metabolite corona can their impact be understood and harnessed for better informed policies regarding NM use, disposal, safe-by-design NMs, and to exploit their full potential in nanomedicine. As such, a more holistic approach to analyzing the complete NM corona is required, with approaches to characterize and quantify the metabolite corona following in much the same manner as the protein corona is currently analyzed. In the case of the metabolite corona the field of metabolomics has a lot to offer,\textsuperscript{87} whereby a global analysis of metabolite (<1500 Da) are analyzed in an unbiased a fashion as possible.

4.1 Isolation of the metabolite corona

The first issue to arise when analyzing the metabolite corona is the generation of the sample for analysis following the incubation of the NMs in the desired matrix. There are two very different approaches to this: the first is a passive approach whereby the matrix is characterised prior to NM exposure and then again once the NMs have been pelleted (by centrifugation) and removed. Upon the second characterisation of the matrix following exposure it can be inferred that any drop in metabolite level is a result of adsorption to the NM, particularly if suitable controls are run to demonstrate that the metabolites do not adsorb to the reaction vessel. This technique will work very well for initial investigations using known standards for metabolites, either singly or as mixtures, and also offers the possibility to properly quantify the adsorption. However, in complex biological matrices where metabolite concentrations are dynamic this approach becomes more difficult, though would still work and drops in metabolite concentrations may still be detectable, however quantification of all metabolites then becomes unfeasible. This approach is also of no use in realistic exposure monitoring conditions as it would be impossible to analyse the matrix before and after exposure. The second, more direct, approach is to elute the metabolites from the NMs, as is done for protein corona analysis.\textsuperscript{25,88} There are a number of possible ways to do this, many of which have been applied in the metabolomics community to isolate metabolites from tissues or biofluids.\textsuperscript{89–91} The most likely to work are successive washes in solvents ranging from the polar, such as water, to the less polar solvents such as methanol and hexane.\textsuperscript{35} The chosen solvents would also be very much dependent upon the type of metabolite being isolated, for example the lipid corona will be more suited to an isopropanol or chloroform elution\textsuperscript{92} whereas the more polar fractions would be better suited to water and/or methanol.\textsuperscript{35} In cases where both the polar and non-polar
portions of the corona need to be characterized then a biphasic extraction, such as the Bligh–Dyer,93 Folch94 and Matyash95 methods or their derivatives, where phase separation occurs between water and chloroform/MTBE with polar metabolites in the aqueous phase and lipids in the organic phase is preferred.91 These aforementioned methods also create a protein layer either at the solvent interface or at the bottom of the biphas, thus opening up the potential to be able to isolate the protein, polar metabolite and apolar metabolite portions of the complex corona. It is also likely that varying the pH of the solvent would have a significant effect on the elution of metabolites, either through hydrolysis of any bonds or changing the charge status of either the metabolite, NM or NM surface ligands thus releasing the metabolites.88 It may also be possible to dissolve the NM if the right pH and material is being used.96 Each NM and surface modified NM will require optimization of the metabolite corona isolation method, although general rules may emerge. As such it is recommended that future studies perform adequate method development to ensure that the full metabolite corona is being analysed rather than a small subset of the NM-bound metabolites. The aspects that require significant input will be which solvents and isolation techniques provide the most comprehensive overview of the metabolite corona, what steps are most prone to contamination and determination of whether any of the metabolites are adsorbed to the reaction or incubation vessels. A summary of these two approaches can be found in Table 1. It is also pertinent to point out that multi-omic sample preparation techniques are increasing in popularity in recent years with a number of studies developing and validating methods that isolate both the proteins and metabolites of a given sample in similar ways as described above.97–100 While it is likely that slight modifications may need to be made to these approaches to ensure the NMs themselves are also isolated to avoid injection into the analytical platforms, this is unlikely to be a major roadblock to the implementation to such approaches. Once the samples are prepared it is vital that they are correctly stored in order to prevent metabolite degradation. A general rule is to store metabolite samples at the lowest possible temperature available as many metabolites such as lipids degrade at room temperature or even in a fridge.101 While many methods are available for the analysis of metabolites in the literature it is vital that the NM bionano interactions and corona community follow the reporting guidelines published by the metabolomics community102 alongside those already in place in the NM community103–105 to ensure that high quality data is generated and to enable the data to be re-used by computational modelers to model the formation and interactions of the metabolite and eventually the complete corona.

4.2 Instrumentation for metabolomics
A wide range of analytical techniques have been implemented in metabolomics to date, in several cases these technologies will be new to most nanoscientists, however some have seen extensive long-term use in several nanoscience labs. As such, several labs already contain the relevant instruments to characterise the metabolite corona. Here we provide an overview of the main approaches, the advantages and limitations of each approach, and the types of metabolites each is optimized for (Table 2).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>A comparison of passive and active isolation of the metabolite corona</th>
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<tbody>
<tr>
<td>Passive isolation</td>
<td>Active isolation</td>
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<tr>
<td><strong>Pros</strong></td>
<td>• Straightforward approach</td>
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<tr>
<td></td>
<td>• Any biofluids can be assessed</td>
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<tr>
<td></td>
<td>• No recovery study required in addition to a NP blank</td>
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<td></td>
<td>• Same process for all metabolite class and NMs</td>
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<tr>
<td><strong>Cons</strong></td>
<td>• Can be applied to real world applications such as NPs found in the environment</td>
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<tr>
<td></td>
<td>• Requires few injections as a before and after analysis isn’t required</td>
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<tr>
<td></td>
<td>• A recovery study for each material and metabolite class is required</td>
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<tr>
<td></td>
<td>• Multiple wash steps are likely to be required to fully recover corona</td>
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Table 2 Analytical techniques for metabolite corona characterisation highlighting their advantages, disadvantages and target metabolites

<table>
<thead>
<tr>
<th>Analytical technique</th>
<th>Pros</th>
<th>Cons</th>
<th>Metabolites suitable for</th>
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<tr>
<td>LC-MS – hydrophilic interaction liquid chromatography (HILIC) – reverse-phase (RP)</td>
<td>Most popular method for metabolomics; wide range of different separation types possible (polar metabolites to lipids); common in many protein corona characterization labs</td>
<td>Limited databases for metabolite identification as yet</td>
<td>HILIC for polar metabolites such as amino acids, nucleotides, sugars, neurotransmitters, environmental contaminants</td>
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<tr>
<td>GC-MS</td>
<td>Sensitive analysis; very reproducible; number of databases to identify metabolites; good for polar and volatile metabolites</td>
<td>Non-volatile metabolites require derivatisation which is time consuming and adds expense</td>
<td>Volatile metabolites such as amino acids, nucleotides, PAHs, environmental contaminants</td>
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<tr>
<td>CE-MS</td>
<td>Highly sensitive due to low flow rates; well suited for high polar and highly charged metabolites can inject from very small sample values &lt;5 μL</td>
<td>Relatively expensive compared to LC-MS and GC-MS; fewer researchers with CE-MS experience; same limited databases for metabolite identification as LC-MS</td>
<td>Well suited for highly charged polar metabolites such as amino acids, nucleotides, sugars, organic acids, neurotransmitters</td>
</tr>
<tr>
<td>DMS (direct-infusion mass spectrometry)</td>
<td>Very high throughput</td>
<td>No definitive identification of metabolites</td>
<td>There are no specific classes of metabolites this suits, however those present at high concentrations are preferentially analysed</td>
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<tr>
<td>NMR</td>
<td>Ability to identify metabolites and their isomers; high throughput; good spectral databases</td>
<td>Poor sensitivity when compared to mass spectrometry methods</td>
<td>High abundance metabolites such as amino acids, sugars etc. however its poor with lipids in general</td>
</tr>
</tbody>
</table>

have equivalent databases, making the identification of metabolites much more complex. However, a number of databases are emerging such as the Human Metabolome Database,77 Metlin110 and mycompoundID,111 which include accurate mass measurements and fragmentation patterns. Each database offers slightly different information as different types of fragmentation and/or different mass spectrometers were used, so there is still some work needed to integrate or compare across them.

Conveniently, many nanoscientists already have access to LC-MS, as nano liquid chromatography-mass spectrometry (nLC-MS) is also used to characterise the protein corona.3,112 While nLC-MS is not yet widely adopted for metabolomics it is growing in popularity11,113–115 and has proven to not only be highly reproducible but also very sensitive.32,118–120 Indeed, this technique has already had some success with determination of the lipid corona around nanofibrils, TiO2 and PS NMs.92

4.2.2 Gas chromatography-mass spectrometry (GC-MS). GC-MS is a reasonably common technique in the metabolomics fields and has been extensively described in previous reviews.121–123 This technique is used to separate volatile chemicals which are resistant to thermal degradation. These are separated using an inert carrier gas, typically helium, which flows through a GC column.12 Analytes are retained on the GC column and are eluted by changing the mass transfer between stationary phase and mobile phase in a temperature dependent manner. Over the course of the analyses the temperature increases in a similar way to the hydrophobicity of the mobile phase in LC-MS. Once eluted, the analytes enter a mass spectrometer that is usually equipped with electron ionization. This is a hard ionization technique and due to the high ionization energy applied, 70 eV, the analytes become heavily fragmented, often to such an extent that the parent ion is depleted.12 This high degree of fragmentation is critical to the identification of the analyte as each compound fragments in predictable patterns which can be screened against a number of well-established databases.124 To date, this approach has been used once for the metabolite corona around 4 metal oxide NMs.88

4.2.3 Capillary electrophoresis-mass spectrometry (CE-MS). CE is a technology that has been used in colloid, particle and nanoscience labs for around 3 decades to characterise the size, surface charge and zeta potential of (nano)particles and has started to find a role in protein corona characterisation.87,125 When coupled to a mass spectrometer CE-MS is a valuable tool for determination and quantification of metabolomics, offering an orthogonal separation to both GC-MS and LC-MS.126–128 In CE separation, metabolites are separated based upon their size and charge over a fused glass capillary filled with a buffer solution under a high potential difference.87 This technique is particularly suited for small highly polar metabolites and is capable of injecting from a very small sample volume (<5 μL) and is extensively reviewed for metabolomics analysis.129–131 As with LC-MS, ionization is via ESI enabling metabolite database searches to be performed via the same (emerging) databases used for LC-MS based metabolomics.

4.2.4 Nuclear magnetic resonance (NMR). NMR has been used for thousands of metabolomics studies and was initially the most widely applied technique due to its ability to analyse complex mixtures, to quantify metabolites, to elucidate chemical structures and the fact that it is extremely reproducible.132,133 However, the key limitation to
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the use of NMR is a lack of sensitivity which, to some extent, can be alleviated by larger magnetic field instruments or use of miniaturized or cryo-probes. A lack of NMR has been used for NM characterization and to identify apolipoproteins and conformational changes in the proteins of the corona, it is still a relatively rare technique in NM studies and has not yet been directly applied to NM metabolite corona assessment, despite the relative abundance of NMR instrumentation in academia and industry. As with the other analytical techniques discussed, NMR based metabolomics has been extensively reviewed.

4.3 Data analysis

Data analysis remains a bottleneck in untargeted metabolomics analysis, particularly with ESI-MS based metabolomics databases and extensive computational requirements. A number of open source software have been developed for chromatogram deconvolution, metabolite annotation and statistical analysis. These include XCMS and mzMine for deconvolution, normalization and visualization of the datasets with annotation packages possible to linked to them. Metaboanalyst is an online tool or can be accessed through R which will perform data transformation, normalization, imputation and univariate/multivariate statistical analysis of metabolomics datasets. More recently an open access end to end platform has been developed to encompass everything from peak picking to normalization, to missing value imputation, metabolite annotation and statistical analysis which offers a potential solution to this bottleneck. Experimental design and the research question are vital in order to perform the correct data analysis. To compare metabolite coronas between different NMs or matrices a classical metabolomics study can be performed with multivariate data analysis to highlight the metabolites that vary significantly between coronas and matrices. The multivariate analysis for metabolomics in these cases has already been extensively described and reviewed. However, a study just to identify what is present in the corona compared to a blank is more complex as missing values skew multivariate analysis meaning a peak picking and deconvolution step can be performed and metabolite annotation carried out (with adequate manual confirmation of peaks) or a known mixture of standards could be used to form a corona, which would allow for a targeted approach to be taken where specific masses are searched in the mass spectra.

4.4 Untargeted analysis of the metabolite corona

NMs have already started to find a niche in assisting in various metabolomics experiments in which they selectively bind to metabolites and thus concentrate them facilitating identification, as has been extensively reviewed by Zhang

| Table 3 Published metabolite corona studies including inadvertent ones where the NMs were utilized to “clean up” samples to reduce interference with target analytes

<table>
<thead>
<tr>
<th>NM</th>
<th>Biological matrix</th>
<th>NM isolation method</th>
<th>Corona isolation method</th>
<th>Method for metabolite detection</th>
<th>Metabolites found</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWCNT</td>
<td>Bronchoalveolar lavage fluid</td>
<td>50% sucrose gradient</td>
<td>Folch method</td>
<td>-</td>
<td>5 PC, 4 PG, 3 PE, 2 PI, 2 PS, SM</td>
<td>65</td>
</tr>
<tr>
<td>TiO₂, PS, CNF</td>
<td>Serum and heavy cream</td>
<td>Centrifugation</td>
<td>Modified Folch method CA: particle digestion using HCl and methanol</td>
<td>PC: 80% methanol</td>
<td>6 LPC, 12 PC, 2 LPE, 6 PE, 13 DAG, 22 TAG</td>
<td>92</td>
</tr>
<tr>
<td>Carbonate apatite, PS</td>
<td>Serum, saliva, urine, FBS</td>
<td>Centrifugation</td>
<td>LC-MS</td>
<td>670 metabolites including: acyl carnitines, amino acids, aromatic compounds, bile acids, sugars, eicosanoids, glycerolipids, GPC, MGC, peptides, pyridines, fatty acids, sphingolipids, steroids and vitamins</td>
<td>147</td>
<td></td>
</tr>
<tr>
<td>CuO, ZnO</td>
<td>Cell media and simulated lung fluid</td>
<td>Centrifugation</td>
<td>Heating in HCl, mercaptoethanol and phenol</td>
<td>GC-MS</td>
<td>&gt;200 metabolites including: carboxylic acids, primary aliphatic amines, amino acids and derivatives, aliphatic acyclic compounds, polyamines, phenols and derivatives and aromatic homomonocyclic compounds 1-Methylhistidine, 1-methylguanidine, betaine, carnitine, creatinine, dimethylamine, dimethylglycine, lysine, trigonelline, trimethylamine N-oxide and arginine</td>
<td>88</td>
</tr>
<tr>
<td>Anionic and cationic silica</td>
<td>Urine</td>
<td>N/A analysed in situ</td>
<td>N/A analysed in situ</td>
<td>NMR</td>
<td>102 in +ve ESI 182 in −ve ESI including: amino acids, carbohydrates, fatty acids, glycolipids, nucleic acids and steroids</td>
<td>35</td>
</tr>
<tr>
<td>Amino modified PS</td>
<td>Conditioned Daphnia magna culture medium</td>
<td>Centrifugation</td>
<td>2 × water wash, 1 × 2.5 : 1 MeOH : water</td>
<td>Direct infusion mass spectrometry</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

et al. Here, the use of NMs to carry out SERS, solid phase extraction (SPE) metabolite enrichment and for enhancing MALDI-MS ionization of metabolites are discussed in detail. An overview of all the untreated studies using NMs for selective enrichment, including methods used and metabolites identified can be found in Table 3.

4.4.1 Lipid corona. Lipids have again been a class of high interest molecules for global analysis of NMs coronas. Initial work exposed SWCNT to mouse lung surfactant and the lipid corona was characterized using LC-MS, in addition to investigating the effect of the lipid corona on SWCNT phagocytosis by macrophages. LC-MS analysis of the corona identified phosphatidylcholines (PC), phosphatidylglycerols (PGs), phosphatidylinositols (PI), phosphatidylethanolamine (PE) and a sphingomyelin in the lipid corona however, they were found at different abundances to the lung surfactant itself. This reflects a well-known characteristic of the protein corona, that high affinity metabolites and proteins comprise the corona rather than the high abundance ones, however this implies that a similar evolution to the protein corona may also apply whereby initially high abundance lipids adsorb to be latter displaced by higher affinity lipids. Computational modelling of the binding mechanism suggested that the acyl chains of the phospholipids interact via hydrogen bonding with the SWCNT, with the polar head groups facing outwards into the biofluid (Fig. 3). In the absence of PG or PS or without both PS and PG macrophage internalization was reduced by 4, 9 and 18% respectively indicating that not only are phospholipids important for macrophage uptake of SWCNTs but the presence of lipid mixtures is synergistic, suggesting that the absence of one in the corona could prevent another from binding. This highlights a significant issue with the current protein centric view of the corona as currently the literature does not take into account the role of metabolites in cellular uptake. The synergistic effects seen between lipids is a particularly interesting property and opens up the field to investigate the possible synergistic effects of proteins and metabolites in NM uptake and distribution.

A subsequent lipid corona investigation examined the adsorption of lipids to cellulose nanofibrils, titania and polystyrene NMs from serum and heavy cream to represent exposures to humans directly via blood and food products, respectively. A modified Folch method was applied to isolate the lipid corona with good recoveries being found across all NMs (>50%) when validated using a mixture of lipid standards. The lipid coronas isolated from the two matrices varied significantly with the serum coronas for all 3 NMs being comprised of 75% triacylglycerides (TAGs), 15–17% phospholipids and 7.5–11% diacylglycerides (DAGs) whereas for the heavy cream coronas for polystyrene and cellulose nanofibrils TAGs and DAGs made up 59% and 13–14% of the lipid corona with phospholipids accounting for a further 25% and lysophospholipids the remaining 2–3%. However, on titania the corona was comprised of 51% TAGs, 14% DAGs, 26% phospholipids and 9% lysophospholipids. These differences are thought to be due to the much more hydrophilic nature of the titania enabling up to 3 times more lysophospholipids to adsorb. While no explanation was given for these differences in TiO₂ NM corona composition between cream and blood, it is possible that lysophospholipids are present in higher concentration in heavy cream than in blood and out-compete the other lipids for the hydrophilic titania NMs surface.

4.4.2 Metabolite corona. The first metabolite corona study to use the term metabolomics was performed by Martel et al. in 2016. Here the corona acquired by an endogenous mineralo-organic NM, carbonate apatite, was investigated. These particles were exposed to a range of biofluids including plasma, saliva and urine all with a foetal bovine serum (FBS) concentration of 10%. The NMs were isolated and dissolved in acid, pelleted and dried and the metabolites solubilized for LC-MS analysis in 80% methanol. In total, 670 potential metabolites were uncovered from the analysis of the metabolite corona, with 377 being putatively identified based upon accurate mass matching with the human metabolome database. The metabolites putatively identified spanned a broad range of chemical classes including amino acids, bile acids, sugars, lipids, steroids, vitamins and carnitines. There were clear differences seen in concentrations between the different biofluids suggesting that the metabolite corona is biofluid dependent. This is a property of the metabolite corona which reflects the behavior of the protein corona, although it is too early days for analysis of whether trends in metabolite and protein coronas co-vary, although our expectation is that they will, given the multiple metabolite protein interactions inherent in biology, and the potential modes of complete corona formation shown in Fig. 1.

Pink et al. carried out extensive method development to isolate, derivatise, separate and detect 200 metabolite features from CuO, ZrO₂, TiO₂, ZnO and carbon black NMs incubated with cell culture medium and simulated lung fluid. Of the 200 features detected, 69% could be annotated based upon their retention index, mass and fragmentation patterns. Hierarchical clustering analysis (HCA) of these data primarily clustered NMs based upon the exposure media further strengthening the commonalities between the metabolite and protein coronas. However, the CuO and ZnO...
clustering only according to the NM composition suggesting that the physico-chemico properties of the NM are just as significant in corona formation as the biochemicals they are exposed too. The first principal component of the PCA scores plot, discriminated metabolite coronas based upon the presence of carboxylic acid, primary aliphatic amines, poly amines, plus other NH₂ or COOH containing metabolites. The second component separation was driven by aromatic homo and monocyclic compounds, phenols and their derivatives, and pyrimidines. Interestingly both the Pink et al. and Martel et al., studies demonstrated that nucleotides adsorb into the NM corona which confirms BASI modelling predictions. This suggests that the nucleotides should adsorb strongly to NMs. This further raises the possibility that DNA and RNA may also bind, thus creating a DNA/RNA corona which could be of interest for novel biologics drugs or opening up the possibility that NMs could potentially act as a transfer mechanism of DNA via connection to other higher order nucleic acid polymers.  

Non-ferrous charged silica NMs were added to urine to enhance the quality of a urine NMR metabolomics analysis. The adsorption of metabolites to the anionic NMs eliminated several overlapping peaks from cationic metabolites including arginine which interferes with detection of lysine, histidine and glucose. As a result, the NMR analysis gained sensitivity, reduced peak broadening and reduced ambiguous metabolite identification. These studies demonstrated that nucleotides adsorb strongly to NMs. This further raises the possibility that DNA and RNA may also bind, thus creating a DNA/RNA corona which could be of interest for novel biologics drugs or opening up the possibility that NMs could potentially act as a transfer mechanism of DNA sequences between organisms.

5.0 Future perspectives – metabolite and complete NM coronas

The current knowledge on the metabolite corona supports the notion that this is a vital area of research that is yet to be exploited by nanoscientists for applications of NMs in the environment, agriculture and medicine, as well as for nanosafety assessment. It is clear that many of the NM properties that are known to influence the composition of the protein corona are also of significant importance to the formation of the metabolite corona, with properties such as NM elemental composition, surface charge, surface modification and biological environment being highlighted thus far.

However, there is still a long way to go to fully understand the implications and effects of metabolite adsorption to NMs, and especially in terms of the interactions and cooperativity between metabolites and proteins. It will also become increasingly important to understand the relative contributions of proteins and metabolites to the corona, and how each is bound and recruited into the corona (i.e. direct interaction with the surface or via intramolecular interactions). To begin to do this, truly quantitative methods would be required for both the metabolite and protein coronas beginning with more simplistic standard solutions before progressing to the complex complete corona. These data may then be used to develop truly representative models of corona formation. We envision that the metabolite corona will follow a similar path as the protein corona in terms of interest and impact upon the nanoscience community, whereby in less than 10 years the number of papers increased exponentially from 35 published papers in 2009 to 234 in 2018 (PubMed "protein corona"). Factors such as NM curvature and structure have yet to be investigated from the metabolite perspective in addition to the time resolved evolution of the metabolite corona. From the protein corona we are well aware that proteins adsorb almost immediately to
the surface of NMs following the introduction of biological matrices. These initial proteins are slowly exchanged for proteins with higher affinities for the NM surface, a significant direction for corona research would be to investigate the time evolution of the metabolites in the corona to inform corona kinetics models. Perhaps the most important consideration for future research is the co-variance of interactions between proteins and metabolites which are yet to be investigated to understand how each influence the other in the formation of the overall corona (Fig. 1), its interactions with organisms and its evolution as the NMs move through ecosystems and organisms. The combination of the metabolite and protein corona, or the complete corona, is likely the key to predicting NMs uptake, localization and impacts on molecular signaling and eventual adverse outcome pathways. It is likely also to be of vital importance for optimization of personalized medicine. Similarly, increased understanding of the protein and metabolite constituents of NOM or dissolved organic matter (DOM) is needed for characterization of environmental coronas; NM papers using NOM or DOM to date do not chemically characterise the NOM to identify the proteins and metabolites present in them, focusing only the role of NOM as a dispersant and characterising changes in NM diameter, dissolution or zeta potential. A key recommendation for the future is that researchers utilizing NOM/DOM provide chemical characterization of the protein and metabolite constituents as well as the humics.

Once the methods have been fully developed and validated for the characterisation of the metabolite corona around all types of NMs, we would expect that any studies which characterise the protein corona in the future will also look into the metabolite corona in parallel, given the similarity of approaches utilized and their likely co-variance. Knowledge of the complete corona will advance NMs applications in the clinical domain and facilitate applications of NMs for environmental remediation, as well as accelerating understanding of the implications of both aspects of the corona in terms of driving NM organism and cellular uptake, distribution, toxicity, bioaccumulation and dissolution. All of these metabolite corona data could then be used to build models to predict metabolite corona formation and predict its effect on biological systems in much the same way as models have been developed for the protein corona in an attempt to produce safe-by-design NM. Indeed, the real predictive power is likely to emerge from models of the complete corona, combining understanding of the presence and cooperativity of binding of both metabolites and proteins.

The ideal solution would be to characterise the protein and metabolite coronas of the same NMs for each exposure, as it would mean that no additional exposures would be required and twice as much data could be collected per sample. This would apply to both in vitro and in vivo studies, and to human and environmental samples. A proposed work flow based on current state of the art in metabolomics and proteomics is given below (Fig. 4) for initial progress going forward, allowing investigation of the two coronas (metabolite and protein) in parallel until such time as a method can be developed to isolate the protein and metabolites corona from the same NMs in the same vial. Among the challenges that need to be overcome in order to
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This is to develop sample preparation and isolation methods that are compatible for both approaches; issues to be resolved include use of additional chemicals for reduction and alkylation of proteins that may displace metabolites in the corona for example, whereas washing the metabolite corona off with organic solvents may inadvertently cause a mis-characterization of the protein corona by selectively removing proteins such as albumin. Additionally, as new sample preparation techniques and analytical platforms develop it may become possible to simultaneously characterize the small and large biomolecules in the complete corona thus greatly increasing the data value from a single analysis. One potential solution to this are the biphasic extractions such as the Bligh and Dyer and the Matyash methods previously discussed which form a bilayer between polar and apolar metabolites but also forms a protein layer as an interface between the solvents or as a layer below the biphasic. Once the metabolite phases have been removed for analysis this would leave a protein layer which could then be digested and analysed for the protein corona. While similar approaches have been used in proteomics and metabolomics analyses from the same sample this would require significant work to validate to ensure that the NMs are not mixed in with either biphasic or the protein layer. As such the Bligh and Dyer approach may be the optimal starting point as the proteins are suspended between the two immiscible solvents with the NMs potentially sedimenting following the centrifugation step. Once these methods have been developed and suitably validated across a broad range of NMs then rapid complete corona analysis may be possible using high throughput methods which will enable high quality data to inform the fields of environmental nanoscience, nanomedicine and corona modelling.

6.0 Summary

To date, research on the metabolite corona is sparse, focusing primarily on the lipid corona acquired by NMs during inhalation by humans, or interactions with xenometabolites either for remediation or to investigate potential changes in their bioavailability through binding to NMs. However, the studies that have been performed suggest that there are many similarities with the well-established protein corona in terms of specificity of binding and evolution over time and as a result of changes in the available biomolecules. As such, continued and increasing research on the metabolite corona and complete corona acquired by NMs has the potential to fill missing gaps in understanding of bio-nano interactions and the role of the corona and eco-corona in driving NMs interactions with biota. As knowledge develops, future regulatory guidelines on the use of NMs in agriculture and medicine, and broadly for environmental risk assessment, will increasingly require characterization of the complete corona as opposed to focusing on the single class of biological molecules that has dominated research to date, i.e., proteins. Metabolite corona research offers a rich vein of information to be tapped into to accelerate clinical and environmental applications and regulatory oversight of NMs in the future.

Conflicts of interest

There are no conflicts to declare.

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