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Critical Review: Impacts of Macromolecular Coatings on Critical Physicochemical Processes Controlling Environmental Fate of Nanomaterials

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Nanoimpact statement

Macromolecular coatings, including natural organic matter, greatly affect the environmental fate of engineered nanomaterials (ENMs). However, understanding of interactions between natural macromolecules and ENMs at the nanoscale is elusive due to the complexity and heterogeneity of natural macromolecules and inadequate characterization methods. We briefly review relevant theory from colloid and polymer science and critically review the literature to identify advantages and limitations of different approaches to correlate ENM behavior with macromolecular coating properties, and we identify further research needs to improve our understanding of the effects of natural macromolecules on ENMs and to bridge mechanistic studies with empirical correlations.

Abstract

Attachment of engineered and naturally occurring macromolecules greatly affects the environmental fate and toxicity of engineered nanomaterials (ENMs). A better understanding of macromolecule-ENM interactions at the nanoscale will improve the ability to predict the effects of macromolecular coatings, e.g. natural organic matter (NOM), on ENM fate, reactivity, and toxicity. This review briefly discusses relevant theory from colloid and polymer science for highly idealized polymers on surfaces that can be used to describe ENM environmental behaviors and introduces classes of macromolecules of interest in the field of environmental nanotechnology. Methods to characterize adsorbed macromolecules on ENMs are presented along with their limitations for ENMs in natural systems. Finally, the current state of knowledge regarding the effects of attached organic macromolecules, both engineered and incidental, on the environmental fate and reactivity of ENMs is critically reviewed. These concepts in whole are synthesized to identify the fundamental gaps in understanding and metrology that must be addressed to improve our mechanistic understanding of the effects of organic macromolecules on ENM environmental fate, and approaches to correlate the properties of coated ENMs to their environmental fate are discussed. We postulate that a first principles approach to modeling ENM-macromolecule interactions is not warranted, particularly for complex and heterogeneous natural macromolecules. On the other hand, a mechanistic understanding is needed to inform parameter selection for empirical correlations, which may offer tractable alternatives to predicting the behavior of macromolecule-coated ENMs. Development of these empirical correlations is currently hampered by incomplete characterization of the adsorbed macromolecule layer properties and their evolution over time in natural systems.

1. Introduction

Elucidating the interactions of engineered nanomaterials (ENMs) with macromolecules is fundamental to understanding their fate and effects. Many ENMs are designed with macromolecular coatings, such as polymers, proteins, and DNA, that will determine their environmental behavior and fate. All ENMs, coated or uncoated, will also interact with natural macromolecules, such as natural organic matter (NOM) or humic substances, proteins, and biological exudates, when introduced into a natural aqueous environment or uptaken by an organism.[1-10] Myriad studies have demonstrated the often dramatic effects of macromolecular coatings on aggregation, deposition, removal in water and wastewater treatment plants, and reactivity.[1-6, 11-14] The important role of macromolecular coatings on the biological uptake, pharmacokinetics, and toxicity potential of ENMs is well-recognized, and reviews of these effects have been published in the biomedical literature,[7-10, 15] as well as the ecotoxicological literature for silver nanoparticles.[16] Reviews of the macroscopic effects of natural organic matter on the environmental behavior of ENMs have also been published.[17, 18] To our knowledge, there has not yet been a review of the environmental fate literature that synthesizes the breadth of mechanisms by which natural macromolecules affect the environmental fate of ENMs. Here, we review the relevant literature from a broad range of systems that have been studied, focusing in large part on NOM coatings and supplementing the discussion with relevant examples from polymer and biomacromolecule studies.

This article begins with a brief review of relevant theory from the closely related fields of colloid and polymer science that can be used to describe ENM environmental behaviors (Section 2), introduction of classes of macromolecules of interest in the field of environmental nanotechnology (Section 3); and discussion of methods to characterize adsorbed macromolecules on ENMs (Section 4). We present various approaches to experimentally probe the effects of coatings and correlate coating properties to ENM behavior (Section 5). The available literature are then assessed to highlight the advantages and limitations of these approaches (Section 6), and the results are also discussed in context of what is already known in this area from decades of colloid science research. Finally, in Section 7, we identify future research directions that must be addressed to improve our mechanistic understanding of the effects of organic macromolecules on ENM environmental fate.

2. Prior Knowledge from Colloid Science

This section presents a brief overview of the theory describing the adsorption of macromolecules onto colloids and ENMs and the surface forces they impart, which will affect ENM behavior in the environment. This is addressed only briefly to orient the reader because these forces have been reviewed recently[14] and discussed at length elsewhere.[19]

2.1. Adsorption of macromolecules to solid surfaces

Chemisorption (in which a chemical bond is formed) or physisorption (in which only non-covalent forces are involved) of macromolecules to nanoparticles in water can result is a variety of adsorbed layer conformations. The conformation of an adsorbed polymer chain is generally depicted as in Figure 1(a) and includes trains, loops, and tails, where trains are segments adsorbed to the substrate, loops are non-adsorbed segments between trains, and tails are the non-adsorbed ends of the macromolecule.[19] These conformations are produced by linear homopolymers (i.e., polymers composed of only one type of monomer) or random copolymers (linear chains comprising more than one type of monomer but with no bias in their distribution) where no particular section of the polymer will have a higher or lower affinity to adsorb to the ENM. Block copolymers are also typically used to stabilize ENMs, where contrasting polymer segments are chosen to adsorb to the ENM surface or extend into solution. The flatness or extension of the layer will be determined by the strength of interaction between polymer segments and the particle surface, the solvent quality for the polymer, the charge on polyelectrolytes, the density of the adsorbed polymer, and the persistence length of the polymer segments.

Other more ordered conformations can be achieved [20] e.g., by grafting *from* or *to* the ENM surface . The conformation of these grafted layers will depend on the grafting density. At low grafting density, “mushroom” or “pancake” conformations are attained (depending on the solvent quality and interaction with the surface). At high grafting density, relatively uniform “brush” coatings are formed (Figure 1(b)).[21] Under good solvent conditions, chains in brushes are highly stretched and the layer thickness may even approach the contour length for polyelectrolytes grafted at high densities.

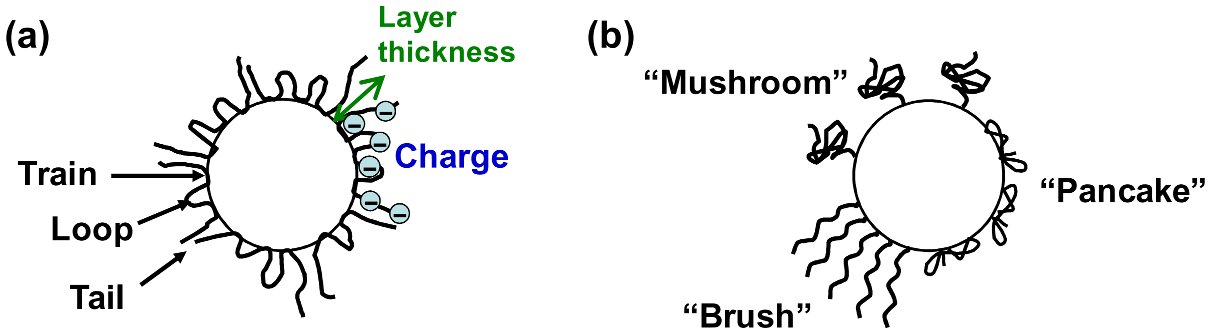

Figure 1. Basic conformations of an adsorbed macromolecule on an engineered nanomaterial (ENM). Physisorbed macromolecules can form trains, loops, and tails (a); end-grafted polymers can form “mushroom” or “pancake” layers at low grafting density, or “brush” layers at high grafting density (b). Adsorbed layer properties of interest include the adsorbed mass (not labeled), layer thickness, charge (or charge density), and segment density or permeability of the adsorbed layer to the solvent (not depicted). The effect of nanoparticle size (i.e., high surface curvature) on the adsorbed layer conformation is illustrated in Figure 3.

It is emphasized that some of the more complex macromolecules that may come to exist on ENMs, either by design or by default, can deviate significantly from the standard models depicted in Figure 1. For example, nonlinear (branched) macromolecules, such as humic acids and dendrimers, can exhibit conformations other than the loop-train-tail conformation depicted in Figure 1. Incomplete surface coverage and the effects of ENM surface curvature on the adsorbed layer conformation should also be considered. These considerations are discussed in Section 2.3.

2.2. Interactions of coated particles

Fundamental colloid science informs much of our understanding of the surface properties and forces that dictate ENM interactions in the environment. Reviews of these Derjaguin-Landau-Verwey-Overbeek (DLVO) forces for uncoated particles in an environmental context have been provided elsewhere.[14, 22, 23] Adsorbed macromolecules will change the DLVO forces and impart additional forces of interaction between two particles or between a particle and a surface. These "extended DLVO" forces including steric and electrosteric forces, Lewis acid-base interactions (including "hydrophilic" forces), and hydrophobic interactions.[24, 25] have also been reviewed.[14] For the reader with limited background in this area, we provide a brief primer on both DLVO and extended DLVO forces in the supporting information.

2.3. Unique considerations for nanoparticles, and limitations of extended DLVO models

The small size and high surface curvature of nanoparticles present additional challenges to typical models for macromolecule adsorption and coated particle interactions. First, the conformation of the coating (e.g., the layer thickness and segment density profile around the particle) will depend on the surface curvature. For brushes, "blob" models for neutral polymers and polyelectrolytes at high ionic strength (where charges are screened) predict a decrease in layer thickness with surface curvature (Figure 2(a)) For polymers that adsorb in train-loop-tail configurations, simple geometric scaling suggests that a thinner layer will be produced as surface curvature increases[26], assuming the same adsorbed mass and volume occupied per macromolecule as on a flat surface (Figure 2(b)). , Experimental studies have demonstrated that further conformational changes on a high curvature surface can result in an even greater effect on layer thickness than geometrically expected.[27] In addition, edge effects for nanocrystals can result in disorder in coatings of even small molecules (alkane thiols) that typically produce self-assembled monolayers on flat surfaces.[28] For large adsorbed polymers, non-uniform radial segment distributions are expected even on flat surfaces, and can produce a “mushroom” region consisting of a sparse number of extended loops or tails when adsorbed onto smaller particles[29] (Figure 2(c)).

Macromolecules with limited conformational flexibility, such as globular or fibrillar proteins, can exhibit significantly different behavior from linear polymers. They do not adopt train-loop-tail conformations on surfaces. Although they normally experience some degree of conformational change after adsorption, in which case the conformational entropy of adsorption can be favorable, they typically remain globular and retain significant secondary or tertiary structural order after adsorption. Experimental studies have demonstrated that globular proteins experience more significant conformational change when adsorbing to larger particles and maintain more of their native structure when adsorbing to small (high curvature) particles[30-32] (Figure 2(d)). This has been attributed to various factors, and remains an active area of research. Trends are only starting to emerge for model systems (e.g., single component protein solutions adsorbing to silica ENMs[30-32]), but the effect of nanoparticle size on adsorbed protein conformational change can be attributed to the formation of more protein-surface contacts on the larger particles.[30, 32] The actual contact area is smaller than the projected area of the protein on the surface, and when the particle radius of curvature is comparable to that of the protein, fewer segmental contacts can be made.

More extreme asymmetries may be attained when the nanoparticle is smaller than the macromolecule. In this scenario, the conceptual model of an organic coating surrounding the particle may be inaccurate, and other morphologies should be considered, e.g., a “nanoparticle-decorated macromolecule” or a “nanoparticle-macromolecule complex.” Stiff macromolecules will not wrap around the nanoparticle, leading to bridging of multiple nanoparticles by stiff polyelectrolytes[33] or polysaccharides[34] (Figure 2(e)).

These studies emphasize that fundamental theories for synthetic homopolymers or block copolymers are not appropriate for more complex macromolecules, such as humic substances or proteins. Perhaps because only small layer thicknesses are needed to overcome van der Waals attraction between nanoparticles, stabilization by globular proteins has in some cases been observed to be comparable to that provided by adsorbed block copolymers and superior to that provided by linear polymers[35]. However, the understanding gained using well characterized homopolymers or block co-polymers is essential for the development of mechanistically based empirical model for behaviors of more heterogeneous macromolecules, like NOM on nanoparticle surfaces.

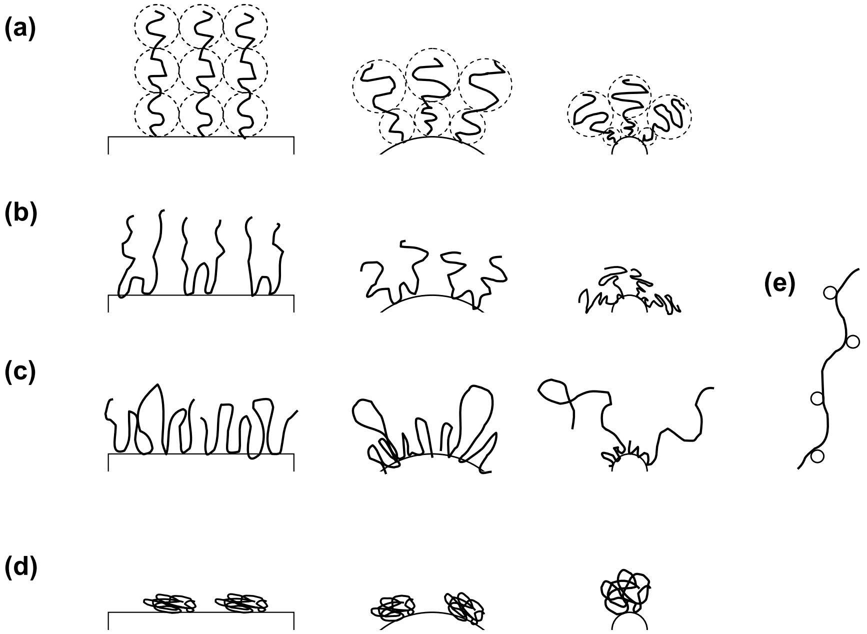

Figure 2. Effects of surface curvature (nanoparticle size) on the conformation of the adsorbed layer for end-grafted, neutral polymers (a); physisorbed homopolymers, following geometric considerations (b) or loop density models (c); and globular proteins (d). Nanoparticles can also be bridged by polymer (e). Diagrams are redrawn based on figures presented elsewhere for (a), flat[21] and curved[36] surfaces; (b)[37]; (c)[29]; and (d).[30-32]

Additional challenges for extended DLVO computations include their limited ability to incorporate non-uniform segment distributions and inability to capture effects from non-uniform or unsaturated surface coverage (laterally around the particle). The use of uniform segment distributions in DLVO calculations typically results in an overprediction of the steric interaction for high curvature nanoparticles.[38]

Saturation amounts for physisorption are typically one to a few milligrams per square meter for uncharged homopolymers or a few tenths of a milligram per square meter for polyelectrolytes[19] so portions of the particle surface are likely to be exposed. For example, polyelectrolytes are known to form inhomogeneous, “patchy” coatings on oppositely charged particles, resulting in attractive forces between oppositely charged patches[39, 40] (Figure 3).

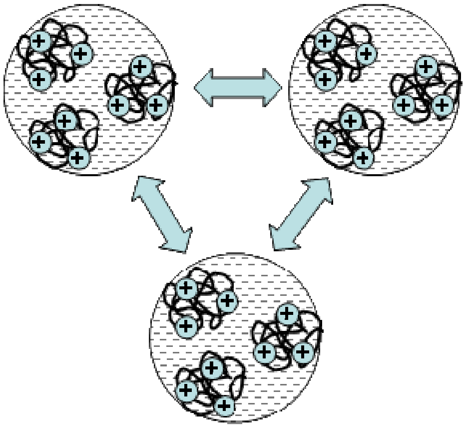

Figure 3. Patch-charge attraction for polyelectrolytes on oppositely-charged particles. Other conformations are also possible (e.g., wrapping of the polyelectrolyte around the particle).

Although forces such as patch-charge attraction can be included in an extended DLVO model, application of these models to real systems would require adsorbed layer characterization at a level of detail far beyond that which is typically achievable. In general, the constraints or assumptions of most analytical solutions to the extended DLVO models are not met for most real ENM-macromolecule systems, limiting their use to qualitative explanations of behaviors, rather than quantitative or predictive explanations. Therefore, empirical correlations developed using extensive experimental data will likely be needed to predict attachment and transport parameters[41] needed to estimate exposure concentrations for coated ENMs.

3. Macromolecules of interest for environmental nanotechnology

The scope of the environmental nanotechnology discipline is broad, spanning the period of the ENM life cycle from its release into the natural environment during manufacture or application (e.g. in consumer products or biomedical applications) to transport of the nanomaterial in the environment and possible uptake by and toxicity to organisms. Macromolecules will play an important role in ENM behavior in all of these processes. We focus this review primarily on natural macromolecules such as humic substances that are likely to be encountered from release of the ENM into the environment until uptake by an organism. Other macromolecules of interest include synthetic polymers that may be applied during ENM production and biomacromolecules that will be encountered after biouptake and control the ENM pharmacokinetics and toxicity.[7-10, 15] Because fundamental principles of ENM-macromolecule interactions can apply across all classes of macromolecules, we supplement the discussion with examples from the polymer and protein literature where useful to highlight mechanisms of coating effects. Here we provide a brief overview of the types of macromolecules that may be encountered to provide context for later sections of the review.

3.1. Intentionally applied macromolecules

Macromolecular coatings that are used in industry span the breadth of synthetic polymers (e.g., poly(ethylene glycol) (PEG), poly(vinylpyrrolidone) (PVP), poly(acrylic acid) (PAA)), naturally derived polymers (e.g., dextran, chitosan, carboxymethylcellulose (CMC)), and biomacromolecules (e.g., proteins, DNA). Although these coatings can be relatively simple with respect to the known chemical structure of the polymer, we emphasize that polydispersity in molecular weight (MW) can affect adsorption kinetics and the adsorbed layer characteristics,[19] thereby changing the coated ENM behavior (e.g. stability against aggregation).[42, 43] Thus, reporting MW distributions of the polymers used, in addition to the type of polymer and its average MW, is needed to compare behaviors across studies.

Engineered macromolecular coatings are often used to stabilize ENMs against aggregation[20, 44-47] when stable, homogeneous suspensions are required for the product functionality (e.g., in paints or sunscreens) or for improved delivery of the ENM (e.g., in drug delivery or for subsurface remediation of environmental contaminants).[41, 48, 49] The coating may also provide other functionalities such as biocompatibility or targeting of specific cells or organs for biomedical applications.[46, 50-55] The coating can be used to tune reactivity of the ENM, e.g., for catalysis.[45, 56] ENMs may also be embedded into polymeric matrices or thin films to form nanocomposite materials, e.g., for water treatment membranes,[57] food packaging,[58] or medical devices;[59] any ENM released from these materials will likely have polymeric material attached. ENMs with highly specialized, engineered coatings such as those for drug delivery may be of interest for future studies if they impart unique behavior that results in a greater risk for exposure or toxicity.

3.2. Incidentally obtained, environmental macromolecules

ENMs will also encounter geochemical or biological macromolecules in the environment or upon uptake by an organism.[4, 5, 11] The most common geochemical macromolecule is natural organic matter (NOM) which is derived from organisms, either via exudation of biomacromolecules or decay of biomass. Dissolved organic carbon is an important component of NOM and is found in concentrations in natural waters ranging from 0.5 to over 30 ppm.[60] .

Higher concentrations of biological exudates are likely to be encountered when an ENM comes into close proximity with an organism (e.g., bacteria, algae, and plant roots) or is exposed to an environment with high concentrations of biota (e.g. the secondary treatment process of a wastewater treatment plant[61]). Release of biomacromolecules can also be stimulated upon exposure of organisms to stressors, including ENMs.[62-65] These exudates include extracellular polymeric substances (EPS) and mucilage. EPS and mucilage are comprised primarily of carbohydrates (including high molecular weight polysaccharides) and proteins or amino acids, as well as lipids, nucleic acids, and organic acids.[66-68] For example, in bacterial EPS, the polysaccharide, protein, and lipid content can range from 40 to 95%, 1 to 60%, and <1 to 40%, respectively.[67] The molecular weights of different components of EPS can vary considerably: for example, alginate in the EPS from *Pseudomonas aeruginosa* can have molecular weights of 1 to 2 million Da,[69] whereas protein molecular weights are typically in the range of 10 to 100 kDa. The extreme size difference was illustrated by Flemming and Wingender and is reprinted in Figure 4.[67]

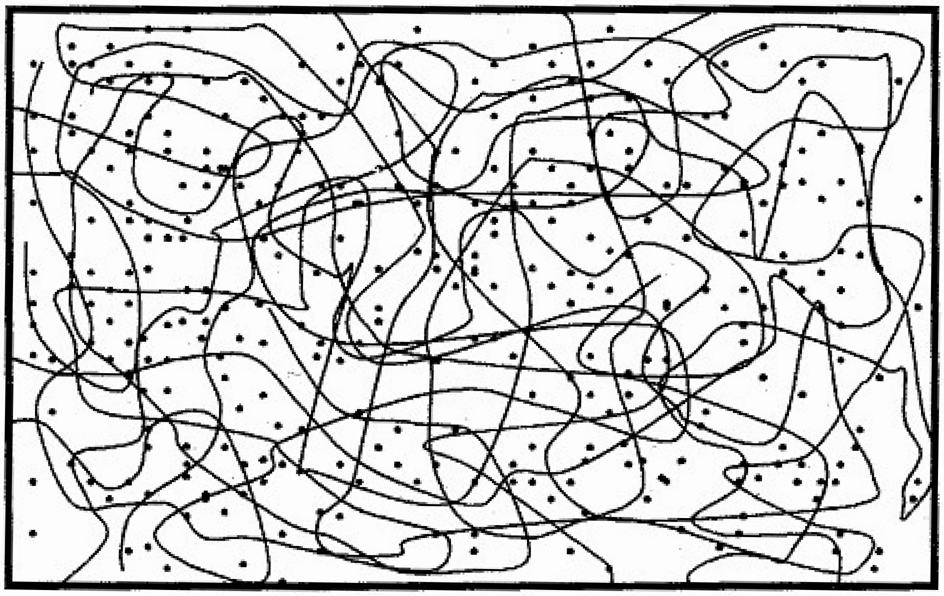

Figure 4. Representation of 10 molecules of alginate (2 million Da) and 300 molecules of protein (30 kDa) in an area and thickness of 1 μm^2 and 1 nm, respectively. Reproduced from Flemming, H. C.; Wingender, J. (2001) Relevance of microbial extracellular polymeric substances (EPSs) - Part I: Structural and ecological aspects. *Water Sci. Technol.* **43** (6), 1-8,[67] with permission from the copyright holders, IWA Publishing.

Simple carbohydrates, proteins, and lipids tend to be labile (i.e., rapidly degraded);[60, 70, 71] hence, in natural waters, refractory (degradation-resistant) humic and fulvic substances accumulate[72, 73] and can account for 60-80% of the total dissolved organic carbon.[71] Humic and fulvic substances (and NOM in general) have been described as “supermixtures” because of their broad heterogeneity with respect to the number and variety of different molecules present, the polydispersity in molecular weight, and the dynamic equilibrium state in which supramolecular assemblies may associate or dissociate depending on the solution conditions.[74] No truly pure component of “humic substances” can be isolated.[74]

Size ranges of humic and fulvic acids and other organic components of natural organic matter are depicted in Figure 5. Measuring and predicting possible interactions of nanoparticles with the mixture of biomass decay products represented in NOM will be more difficult than with simpler exudates or synthetic polymers because of the structural complexity of the NOM (i.e., lack of repeating monomeric units), the heterogeneous mixture of components in NOM, and the spatial and temporal variability of the NOM in the environment. Variability among samples of natural macromolecules that are collected or extracted from specific environments or from an organism should be acknowledged when comparing laboratory studies or extrapolating findings. Alternatively, purer samples may be purchased or synthetic analogs can be used, e.g., poly(acrylic acid) as an NOM analog,[75] alginate as a representative EPS,[76] or bovine serum albumin or human serum albumin as representative proteins. Effects of heterogeneity and advantages or disadvantages afforded by the use of heterogeneous versus purified substances are discussed later in this review.

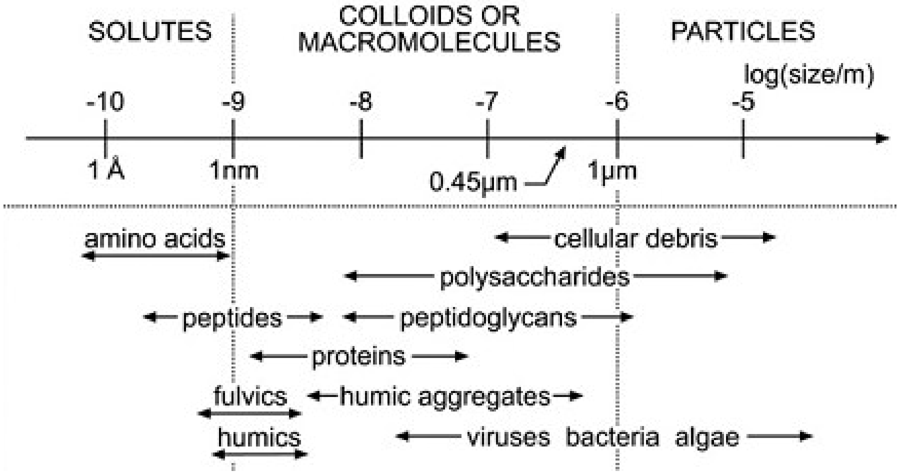

Figure 5. Classes of components and size ranges of natural organic matter. Note that each class itself (e.g., "humics") is heterogeneous. Figure adapted from Wilkinson, K. J. and Lead, J. R. (2007) *Environmental colloids and particles: Behaviour, separation and characterization*, with permission from John Wiley and Sons.

The conformation of any particular macromolecule adsorbed to an ENM will also depend on the type of ENM and the solution conditions.[77] Although qualitative conclusions can be made regarding the effects of macromolecules on ENM fate and toxicity, mechanistic and quantitative studies will require thorough characterization of the coated ENM in the medium of interest, or development of a correlation between the macromolecule and solution properties and the adsorbed layer conformation.

4. Characterization approaches to enable mechanistic inferences of ENM-macromolecule interactions and effects

Important physicochemical properties of the adsorbed layer include the composition of the layer, the adsorbed mass, the distribution or surface coverage around the ENM surface, and the conformation of the macromolecules on the ENM (i.e., the layer thickness, charge, permeability or segment density profile, location of moieties that adsorb to the ENM surface, and orientation of the macromolecule extending into the solution). These properties will affect the electrostatic and steric interactions between the nanoparticles and other surfaces that are commonly used to explain ENM behaviors. Specific interactions can also be imparted, e.g., binding of the macromolecule coating to biological receptors for cellular recognition and uptake of the coated nanoparticle. Thorough characterization of the coated ENM will be required to identify the mechanisms contributing to the ENM interfacial behavior.

4.1. Characterization methods and applications

Characterization methods to measure adsorbed layer properties on nanoparticles are summarized in Table 1. The use of multiple methods to probe different properties of the coated ENM is necessary to gain a complete understanding of the adsorbed layer properties needed to predict behaviors. Most of these methods can be categorized as follows:

(1) microscopy techniques to visualize the coated ENM, e.g. transmission electron microscopy (TEM), scanning TEM (STEM), and scanning electron microscopy (SEM), possibly coupled with spectroscopic analyses for spatially resolved elemental composition;

(2) light and neutron scattering methods to determine the size or structure of the coated ENMs or ENM aggregates, e.g. dynamic light scattering (DLS), static or multi-angle light scattering (SLS, MALS), and small angle neutron scattering (SANS);

(3) size separation methods to determine the hydrodynamic size distribution of the coated ENMs, e.g. asymmetric flow field-flow fractionation (FFF or AF4) or disc centrifugal sedimentation (DCS) coupled with online detectors to assess concentration, light scattering, absorbance, or other properties of the eluting ENMs;

(4) optical, spectroscopic, or spectrometric techniques to assess the composition, conformation, or chemistry of the coating, e.g. fluorescence spectroscopy or excitation-emission matrices (EEM), Fourier transform infrared (FTIR) spectroscopy, x-ray photoelectron spectroscopy (XPS), energy-dispersive x-ray (EDX) spectroscopy, low energy ion scattering spectroscopy (LEIS), secondary ion mass spectrometry (SIMS), sum frequency generation (SFG), circular dichroism (CD), and hyperspectral imaging (HSI);

(5) charge characterization or electrokinetic methods to assess the charge or electron transfer behavior of the coated ENM, e.g. titration, electrophoretic mobility, capillary electrophoresis, and voltammetry; and

(6) mass measurements (e.g., thermogravimetric analysis) or solution-depletion based methods (i.e., deducing the quantity or types of macromolecules adsorbed to ENMs by quantifying unbound macromolecule concentrations before and after exposure to ENMs).

Advantages and limitations for these methods are listed in Table 1. We do not intend to review all of the ENM-macromolecule characterization literature; rather, a few examples demonstrating each method are specified, highlighting environmental studies where available. It is noted that a larger and more detailed set of characterization studies is generally available for ENMs coated with well-defined synthetic polymer or protein coatings than for natural organic matter coatings, which are more difficult to characterize due to their heterogeneity.

Table 1. Methods to characterize macromolecular coatings on nanoparticles

Method	Coating properties that can be assessed	Advantages	Technical limitations	Applications in coated ENM studies (environmental studies, where available)
Transmission electron microscopy (TEM)	Presence of layer Morphology	High resolution Embedding in hydrophilic resins or use of cryogenic TEM or hydrated conditions in environmental TEM (ETEM) may preserve structure of coatings	Poor sensitivity to thin, low electron density organic coatings without staining[78] Artifacts due to sample preparation (e.g. drying, staining)	Adsorption of NOM and morphology of NOM-coated ENM[78-80]
Scanning TEM (STEM)	Presence of layer Morphology	High resolution Can be performed under hydrated conditions (ESTEM) Can couple with energy dispersive X-ray (EDX) or electron energy loss spectroscopy (EELS) analysis for elemental composition	Possible sample damage under electron beam[81]	Spatially resolved analysis of lipid coatings on ENMs[81]
Scanning electron microscopy (SEM)	Presence of layer Morphology	Can be performed under hydrated conditions for environmental SEM (ESEM) Can couple with EDX spectroscopy for elemental analysis	Lower resolution than TEM	Adsorption of NOM and morphology of coated ENM / ENM aggregates[82]

Method	**Coating properties that can be assessed**	**Advantages**	**Technical limitations**	**Applications in coated ENM studies (environmental studies, where available)**
Atomic force microscopy (AFM)	Presence of layer Morphology Interaction forces Layer thickness	High sensitivity to the adsorbed layer Ability to measure forces with varied AFM tip materials or coatings Liquid cells can be used for *in situ* measurements on hydrated particles adsorbed to a substrate	Artifacts due to sample preparation, AFM tip size, or disturbance of the layer during measurement[78, 83]	Adsorption of NOM and morphology of coated ENM[78] Measurement of (electro)steric forces or attractive patch-charge forces due to polymer layers[38, 39, 84] Measurement of humic acid layer thickness[85]
Dynamic light scattering (DLS)	Hydrodynamic layer thickness	Ease of operation and availability of equipment Provides *in situ* measurements on liquid suspensions	Adsorbed layer thickness may be difficult to resolve for broadly polydisperse samples, especially considering bias toward large ENMs or aggregates[86-89]	Measurement of NOM layer thickness to explain coated ENM deposition[90, 91]
Nanoparticle tracking analysis (NTA)	Hydrodynamic layer thickness	Provides *in situ* measurements on liquid suspensions	Reliance on subtraction of coated and uncoated particle sizes Small ENMs may not be detected[88, 89]	
Asymmetric flow field-flow fractionation (FFF or AF4)	Hydrodynamic layer thickness	Provides *in situ* measurements on liquid suspensions Fractionation allows separation of ENMs from unbound macromolecules and improves confidence in ENM size distributions obtained Ability to add online detectors for further characterization	Possibility for aggregation during measurement[88] or artifacts due to interactions with the FFF membrane or between different ENM components[92] Adsorbed layer thickness may be difficult to resolve for broadly polydisperse samples	Adsorption of NOM onto ENMs[65, 93, 94]

Method	Coating properties that can be assessed	Advantages	Technical limitations	Applications in coated ENM studies (environmental studies, where available)
Disc centrifugal sedimentation (DCS) or analytical ultracentrifugation (AUC)	Layer thickness	Provides *in situ* measurements on liquid suspensions Fractionation improves size resolution and confidence in size distributions obtained	Assumptions of adsorbed layer density required to calculate layer thickness Adsorbed layer thickness may be difficult to resolve for broadly polydisperse samples	Adsorbed layer thickness of proteins and DNA[95, 96]
Small angle neutron scattering (SANS)	Layer thickness Segment density or volume fraction profile[97]	Provides *in situ* measurements on liquid suspensions Contrast matching of ENMs can be used to improve analysis of adsorbed coatings	Models required to fit layer parameters may not be available for complex coating conformations	Aggregation behavior and size/structure of natural colloids with NOM[98, 99]
Thermogravimetric analysis (TGA)	Adsorbed mass and composition	High sensitivity to coating mass loss Possibility to distinguish coating materials via temperature-resolved profiles	Large quantities of dried ENMs required (mg scale) Selectivity may be poor	Quantification of adsorbed mass of polymer[100] Assessment of exchange of small molecule and polymer coatings[101]
Differential scanning calorimetry (DSC); microcalorimetry	Presence of layer Structural changes of macromolecule upon adsorption	Provides *in situ* measurements on liquid suspensions Possibility to distinguish free vs. adsorbed macromolecules	Will not detect adsorption of macromolecules that do not undergo conformational changes resulting in thermal phase transitions	Adsorption and denaturation of proteins on ENMs[102, 103] Structure of polymer-grafted coating on ENMs[104]
UV-vis spectroscopy	Presence of adsorbed layer	Provides *in situ* measurements on liquid suspensions	Interferences due to ENM aggregation	Identification of adsorbed protein on ENMs due to change in localized surface plasmon resonance[105]

Method	Coating properties that can be assessed	Advantages	Technical limitations	Applications in coated ENM studies (environmental studies, where available)
Fluorescence spectroscopy	Presence of layer and changes in composition	Provides *in situ* measurements on liquid suspensions High sensitivity is possible, especially for fluorescent tags with high quantum yields	Macromolecule must fluoresce Interpretation of fluorescence intensity or spectral changes may not be straightforward due to interactions between the fluorophore, nanomaterial, and matrix components Fluorescence spectrum and intensity (e.g. quenching) can depend on distance of fluorescing moiety from ENM surface	Estimation of protein binding constants[105, 106] Identification of bound proteins on gold ENMs (sensor applications)[107]
Proton neutron magnetic resonance (^{1}H NMR) spectroscopy	Attachment/confinement of molecules on ENM surface Exchange of adsorbates	Can distinguish free vs. adsorbed molecules	Sample preparation may perturb coating, e.g. if lyophilization is used to exchange from H_2O into D_2O	Adsorption and exchange of humic acid[108] or polymers[109] on ENMs
X-ray photoelectron spectroscopy (XPS)	Elemental composition and speciation Layer thickness (< 10 nm)	Selective for near-surface region; quantitative analysis possible[110]	Possible sample damage; vacuum required[110] Detailed models required for accurate analysis of layer thickness on nanoparticles[110, 111]	Evaluation of changes in polymer adsorption during ENM transformation processes[112] Layer thickness of carbon in adsorbed NOM on ENMs[113] Elemental composition and layer thickness of self-assembled monolayers on gold ENMs[111, 114]

Method	Coating properties that can be assessed	Advantages	Technical limitations	Applications in coated ENM studies (environmental studies, where available)
Secondary ion mass spectrometry (SIMS)	Presence of surface coatings Information on functional groups	High sensitivity; selective to outer surface of coated ENM	Sample damage during ion sputtering[110]	Surface chemistry of self-assembled monolayers on gold ENMs[111]
Low-energy ion scattering (LEIS)	Presence of ultra-thin coatings Layer thickness	Highly sensitive to outer atomic layer	Sensitive to contamination; vacuum required[110]	Layer thickness of self-assembled monolayers on gold ENMs[114]
Hyperspectral imaging (HSI)	Layer composition	Provides *in situ* measurements in liquid Selective for molecules near the particle surface that affect the ENM scattering spectrum	Interferences due to ENM aggregation Library of known coated particle spectra is required to identify unknown layer composition	Adsorption of macromolecules in wastewater samples[115]
Surface-enhanced Raman spectroscopy (SERS)	Layer composition	Provides *in situ* measurements in liquid Selective for surface-bound molecules	Only relevant for ENMs with strong localized surface plasmon resonance (e.g., gold, silver ENMs) Library of known spectra is required to identify unknown layer composition	Binding of proteins to gold ENMs[105] Sensing of biomolecules[116] Analysis of humic acid structure[117]
Sum frequency generation (SFG), including second harmonic generation (SHG) and SFG-vibrational spectroscopy (SFG-VS)	Adsorption of molecules to ENM surface Chemical identity and ligand binding for SFG-VS	Selective for the ENM-solvent interface	Modeling required[110]	Adsorption of organic molecules to gold ENMs[118]

Method	Coating properties that can be assessed	Advantages	Technical limitations	Applications in coated ENM studies (environmental studies, where available)
Circular dichroism (CD)	Adsorbed mass Structural conformation of macromolecule	Provides *in situ* measurements on liquid suspensions High selectivity, e.g. for proteins Can provide quantitative information	Will only detect optically active, chiral molecules	Binding and conformation of proteins to ENMs[119, 120]
Attenuated total reflectance - Fourier transform infrared (ATR-FTIR) spectroscopy	Attachment or exchange of functional groups	Can provides *in situ* measurements when a liquid cell is used Selective for surface-bound molecules (i.e., deposited near the FTIR substrate)	High material quantities may be needed due to sensitivity limitations	Adsorption and displacement of ligands, polymers, and NOM on ENMs[121-123]
Isothermal titration calorimetry (ITC)	Energy of adsorption; binding constants	Sensitivity to small changes in energy absorbed or released during macromolecule adsorption	Prone to interferences due to interactions that may not be of interest, e.g. entropy change upon dilution of injected sample High ENM concentrations may be required Requirement for high pH buffer concentrations may induce ENM aggregation	Adsorbed amount and binding energy of proteins to ENMs[124, 125] Assessment of depletion and bridging flocculation by polymers[126]

Method	Coating properties that can be assessed	Advantages	Technical limitations	Applications in coated ENM studies (environmental studies, where available)
Charge titration	Charge	Not sensitive to adsorbed layer hydrodynamics (as opposed to electrokinetic methods)	Charges must be titratable by acid/base (i.e., strong polyelectrolytes will not be titratable) Model fitting required (e.g. assuming two classes of acidic functional groups such as carboxyl and phenolic groups[127])	Charge of bacteria with adsorbed humic acid (and effect on ENM deposition)[128]
Electrophoretic mobility (EPM); electrokinetic modeling	Layer thickness Charge density Permeability Segment density profile	Ease of operation and equipment availability Can be insensitive to particle aggregation and polydispersity	EPM cannot be converted directly to zeta potential or charge for coated ENMs due to dependence on hydrodynamic properties of the coating Statistical uncertainty inherent in modeling approaches to obtain layer properties from EPM measurements[129]	Estimation of adsorbed layer charge and thickness for polymers and NOM to assess (electro)steric interactions for coated ENMs[41, 49, 84, 128]
Electrophoretic separations (e.g. capillary and gel electrophoresis)	Similar information as EPM	ENMs and excess macromolecules can be separated Variation in capillary electrophoresis experimental setup possible to probe different interactions between macromolecules and ENMs	Potential for undesired interactions with the capillary or gel, e.g., ENM adsorption	Interactions of NOM with ENMs[130]

Method	Coating properties that can be assessed	Advantages	Technical limitations	Applications in coated ENM studies (environmental studies, where available)
Electrospray Differential Mobility Analysis (ES-DMA)	Dry layer thickness (from electrical mobility of aerosolized particles)	Surface coverage can be deduced by comparison with hydrodynamic diameter (e.g., by DLS)	Artifacts due to required drying of ENM suspension Adsorbates must have distinguishable size difference to assess exchange or competitive adsorption	Adsorption and displacement of polymers and ligands on ENMs[122, 131]
Adsorption/binding of small molecules to the adsorbed layer	Hydrophobicity Adsorbed mass	Ease of detection and quantification due to features of molecular probe selected (e.g. fluorescence)	Artifacts due to incomplete reaction with or sorption to coatings Interferences due to the ENM	Hydrophobicity of polymer-coated ENMs[132] Quantification of adsorbed protein on ENMs[133]
Solution depletion methods	Adsorbed mass Composition of adsorbed layer	Ease of operation and equipment availability	May not be sensitive enough to assess removal of macromolecules from solution, particularly if ratio of ENM to macromolecule concentration is low	Adsorbed mass of NOM, polymer, and protein coatings to explain ENM aggregation and deposition behavior[43, 77]
Removal of adsorbed macromolecules from ENMs (e.g., by ENM dissolution or cleavage or displacement of adsorbates)	Adsorbed mass Composition of adsorbed layer	Removal of ENM allows for broader range of methods to quantify and characterize macromolecules	Possibility for incomplete removal of adsorbed macromolecules or modifications during the treatment process	Identification of proteins adsorbed to ENMs[96]

4.2. Challenges and limitations for adsorbed layer characterization

Each method for adsorbed layer characterization has inherent limitations that can preclude its ability to provide accurate or reliable adsorbed layer properties. These limitations may be exacerbated by the nature of the ENM sample. In particular, ENM polydispersity or aggregation will limit the usability of many sizing methods to determine adsorbed layer thicknesses, especially when the method is significantly biased toward larger particles, as in DLS.[86, 134] Methods that provide size separation followed by detection (e.g. FFF, DCS) can provide more accurate size distributions,[87-89] but adsorbed layer thicknesses that are small relative to the width of the size distribution of the uncoated ENM core will still be difficult to distinguish with certainty.

Another possible problem is the introduction of artifacts due to the sample preparation method required. For example, any method that requires drying (e.g. SEM/TEM) can perturb the physical conformation of the macromolecular layer, precluding the measurement of the hydrated layer thickness. Baer et al. have provided a thorough discussion of artifacts in coating characterization, including damage under high-energy irradiation as in XPS, effects of drying (or incomplete drying), and modification of coatings upon sample purification or storage.[110]

Other challenges arise from the complexity of the adsorbed layer or a lack of suitable methods to measure the layer properties of interest on particles in suspension. For example, characterization methods may not have sufficient sensitivity to determine low adsorbed masses, or sufficient selectivity to distinguish different components in an adsorbed layer. Some of the methods listed in Table 1 will require further testing to assess the feasibility of their application for ENMs coated with complex environmental macromolecules such as NOM. For example, fluorescence spectroscopy has high sensitivity, but this method has been demonstrated primarily for well-defined systems of one or a few types of fluorophore-tagged polymers or proteins interacting with ENMs or for specialized applications (e.g. sensing) in which the ENM is designed with a coating that can bind or react with specific compounds or proteins.[116, 135] Even in these relatively well-controlled systems, spectroscopic methods are also highly prone to artifacts such as quenching or enhancement of signals due to interactions of the detected moieties with the ENM.

The conformation of a macromolecule on the ENM surface (e.g., volume fraction profile of macromolecule around the ENM) and the layer homogeneity (i.e., patchiness of the coating) can have important effects on ENM behavior but are difficult to probe at the nanoscale. This information may be obtained by SANS[97, 136] or deduced from AFM force measurements on larger particles.[39] Comparison of orthogonal methods may provide more complete characterization of the macromolecular layer: for example, hydrodynamic sizing methods such as DLS will be sensitive to long tails of attached macromolecules (even at low densities), whereas a mass sensitive method (e.g., SANS) will give smaller sizes representing the more densely packed loop region near the ENM surface rather than the sparser, extended loops and tails.[19, 137, 138] It has been shown that the aggregation of coated ENMs is sensitive to even a small proportion of extended macromolecule tails,[137] so selection of the most appropriate characterization methods will be important in order to explain the coated ENM behavior.

4.3. Improvements in characterization and data reporting

Some possible improvements are suggested for the implementation of characterization methods in environmental nanotechnology research,. First, studies seeking to compare ENM behavior with different coatings should provide characterization of coatings in addition to the minimal characterization recommendations for uncoated ENMs.[139] Many studies provide only size and electrophoretic mobility (EPM), but these measurements are not sufficient to determine important adsorbed layer properties such as layer thickness. It is also noted that ionic strength and pH must be reported for EPM measurements or other coating characterization to be meaningful because the value of EPM and the coating conformation are sensitive to the solution chemistry. Hence, EPMs measured in controlled solutions may not be comparable to those obtained in environmental milieu.

Interpretation of data can also be improved. For example, EPM measurements are often compared for coated and uncoated ENMs and interpreted as differences in zeta potential or surface charge, using Henry's equation (or the Smoluchowski or Hückel approximations) for hard spheres to estimate zeta potential from EPM. However, for particles coated with a “soft” (i.e. hydrodynamically permeable) layer, the shear plane is not well defined and hence a “zeta potential” is not strictly defined. More importantly, the EPM will be affected not only by the diffuse distribution of charge throughout the layer but also by hydrodynamic effects due to the drag imparted by the layer (a function of layer thickness and permeability) and by Donnan equilibrium effects on the distribution of counterion density and potential. For example, the magnitude of the EPM for a charged particle coated with a like-charged macromolecule can *decrease* if the layer produces enough drag to overcompensate for the force imparted by the charges on the adsorbed macromolecules. Interpretation of this EPM as a decrease in charge (and, ultimately, electrostatic force between the coated ENM and another surface) would then be inaccurate and potentially misleading. A discussion of appropriate interpretation of zeta-potentials for coated nanoparticles has been provided by Doane et al.[140]

A tendency often observed in laboratory conditions for adsorbed macromolecular layers to reside in persistent non-equilibrium states can also be problematic, particularly regarding the suitability of thermodynamic models.[141, 142] The extent to which these states persist in adsorbed layers on ENM in the environment is not known. Ideally, characterization of the adsorbed layer would be performed over the duration of the experiment for all studies using coated ENMs, including fate and toxicity studies. Such characterization is rarely provided, in part because of the lack of simple analytical techniques to assess adsorbed layer transformations.

Here, we focused on characterization methods that provide information on the adsorbed layer on the ENM. It is noted that additional characterization of the free macromolecule would also be useful, because these characteristics will affect the conformation and properties of the adsorbed layer on the ENM and hence may be useful to predict ENM behavior even if complete characterization of the resultant coated ENM is unavailable. Lack of macromolecule characterization (e.g. molecular weight and polydispersity) is a shortcoming in many published ENM studies; similar issues have been discussed specifically regarding NOM characterization in environmental studies.[71] In practice, better characterization of the free macromolecule, including molecular weight distribution, chemical composition, and charge density, will likely be easier to address than adsorbed layer characterization because methods have been developed in the polymer science and NOM research communities to determine these characteristics.[60, 143]

4.4. Utility of thorough characterization to explain coated ENM behavior

Studies on the environmental behavior of coated ENMs often overlook the importance of coating characterization. Thorough characterization of coatings could significantly improve the ability of many studies to fully explain the observed ENM fate or toxicity. For example, Tiraferri and Borkovec have recently reviewed the application of light scattering techniques to investigate macromolecule adsorption to ENMs and determine properties such as adsorbed layer thickness that influence particle attachment behavior.[134]

We also highlight a study by Pelaz et al. in which highly detailed characterization of the binding of proteins to PEG-coated ENMs was required to explain the cellular uptake of the ENMs.[144] Pelaz et al. designed iron-platinum (FePt) ENMs with a fluorescently-labeled polymer coating, to which amine-terminated PEGs were covalently grafted to form a dense PEG coating. Increases in the coated ENM size measured by fluorescence correlation spectroscopy indicated adsorption of human serum albumin (HSA) and fibrinogen onto both PEGylated and non-PEGylated ENMs. Despite the occurrence of protein adsorption, Pelaz et al. found that cellular uptake of the PEG-coated FePT ENMs in protein-containing serum was still significantly reduced compared to that of non-PEGylated ENMs. Further characterization of the nature of the protein adsorption helped the authors resolve this apparent contradiction. First, the authors determined that the size increase upon protein adsorption was small relative to either monolayer formation of HSA or the large size of fibrinogen, suggesting that the proteins may have penetrated into the PEG layer. Fluorescence lifetime experiments provided more direct evidence that the proteins resided near the underlying fluorophore-tagged ENM surface. Therefore, the extended PEG layer could minimize interactions of the embedded proteins with the surface of the cells for uptake. This study thus demonstrates that characterization of both the composition and conformation of adsorbed layers may be needed to explain ENM biouptake. These considerations should also be applied to studies of ENM attachment and reactivity in the natural environment.

5. Approaches for quantitative correlations and experimental design to predict coated ENM behavior

Quantitative correlations are highly desirable to predict the transport and fate of coated ENMs for risk assessment. However, questions remain regarding the properties of the coatings that must be characterized and the best approach to incorporate these properties into correlations of coated ENM behavior. In Section 5.1, we provide a high-level overview of two different approaches to correlate coating properties to ENM behavior.

Development of quantitative correlations is expected to be particularly difficult for complex natural macromolecular mixtures such as NOM where a highly mechanistic understanding of the coating may be intractable (as discussed in Section 2). Therefore, we specifically discuss experimental approaches for the use of NOM in ENM studies in Section 5.2, and we highlight the advantages and limitations of these approaches to inform models of ENM behavior.

In Section 6, we delve into a survey of the effects of coatings on ENM attachment behavior and reactivity. These studies are discussed in context of theoretical expectations for coating effects, as described in Section 2. Where available, case studies from the literature that provide quantitative correlations between coated ENM properties and behavior are highlighted and placed in context of the frameworks presented here in Section 5.

5.1. Development of correlations for coated ENM behavior

Two approaches to correlate properties to coated ENM behavior are presented in Figure 6. These approaches are distinguished by whether the coated ENM is treated as a single entity (i.e., the adsorbed layer properties are measured) or as the sum of its components (i.e., the properties of the ENM core and the macromolecule are separately used in the correlation). Here, we describe the expected advantages and limitations of each approach.

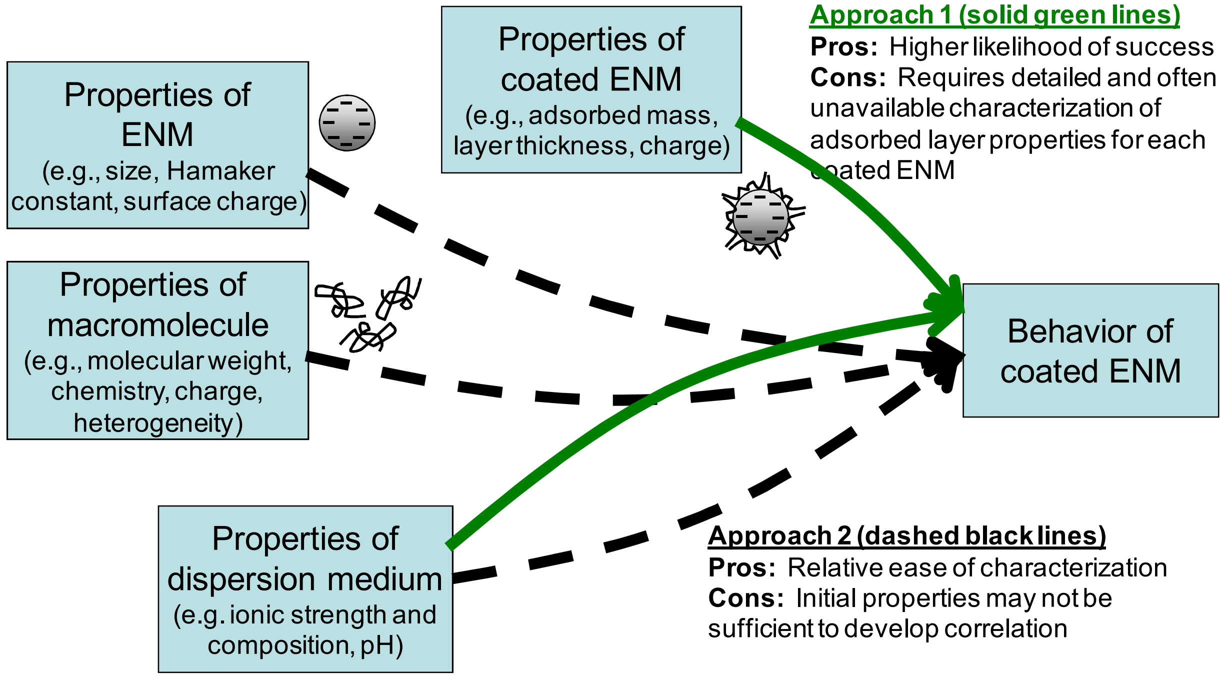

Figure 6. Possible approaches to correlate the properties of coated ENMs to their behavior. In Approach 1 (solid green lines), the properties of the coated ENM are measured and, along with the properties of the dispersion medium, correlated to the coated ENM behavior (e.g., aggregation, deposition). In Approach 2 (dashed black lines), the properties of the uncoated ENM and free macromolecule are used to develop the correlation.

In one approach (Figure 6, Approach 1), ENM behavior can be correlated to the properties of the coated ENM (e.g., the overall charge or potential at the edge of the adsorbed layer, layer thickness, adsorbed mass) and the dispersion medium (e.g., ionic strength). This approach is likely to produce the most accurate predictions of coated ENM behavior because it provides the properties of the "final" coated ENM product, which should directly determine its behavior. However, characterization of the adsorbed layer properties will present a significant analytical challenge, as described previously. Not all published studies will provide sufficient characterization information, so a limited data set is available to develop correlations.

In the alternative approach, one may characterize the initially manufactured ENM and the free macromolecule (e.g., NOM), then correlate these properties to the ENM behavior of interest (Figure 6, Approach 2). The advantage to this approach is that the properties of the free macromolecule (e.g. molecular weight) are often easier to characterize than the properties of the adsorbed layer on the ENM. Furthermore, systematic variation of these properties can be relatively easy to perform (e.g., using ultrafiltration to control the molecular weight distribution). However, it is not yet known whether the interaction between the properties of the coating, ENM, and dispersion medium will be too complex to predict using a simple quantitative correlation. Examples and discussion of studies applying Approach 1 and Approach 2 are presented in Section 6.

5.2. Experimental design to assess the effects of heterogeneous natural macromolecules

The heterogeneity of "supermixtures" of macromolecules, such as NOM, can present a significant challenge in either of the two correlation approaches described in Section 5.1. Here, we provide perspective on various approaches for the utilization and analysis of NOM (Figure 7) and the advantages and limitations of these approaches to explain the effects of NOM on ENM behavior.

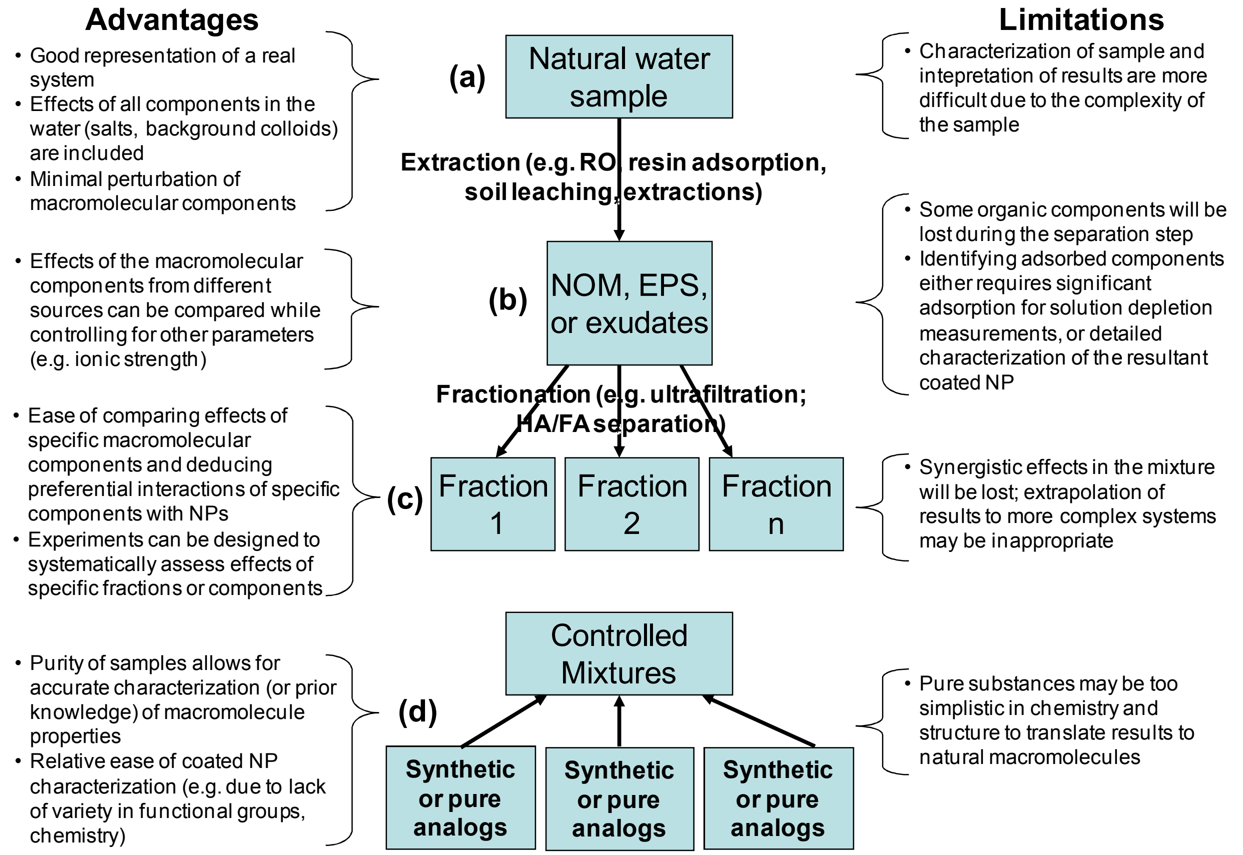

Figure 7. Experimental considerations and challenges presented by the heterogeneity of natural macromolecular "supermixtures" such as NOM. Sample heterogeneity decreases from top to bottom. Advantages and limitations are listed for the use of complex and realistic samples (a), bulk extracts (b), and fractionated extracts (c). Synthetic analogs for NOM (d) may also be used for highly mechanistic studies.

Figure 7 lists advantages and limitations of using NOM samples that are collected or extracted at various stages. Raw water samples used as collected (Figure 7(a)) can provide the most realistic representation of ENM behavior in a particular environment, but collection of a suitably large set of natural samples and elucidation of the interplay of several variables (e.g., NOM concentration, ionic strength, and natural colloid concentrations) can be difficult. The use of NOM extracts (Figure 7(b)) or "standardized" NOM isolates, such as the humic and fulvic acid standards provided by the IHSS, allows for preparation of better controlled samples to focus on the effects of the NOM. However, the influence of the heterogeneous composition of these NOM isolates is not often considered. Further fractionation (Figure 7(c)) allows for elucidation of the importance of different components of NOM, but possible interactions between components in the heterogeneous mixtures may no longer be observable. Lessons learned from several case studies in the literature on ENM aggregation in the presence of NOM isolates of varying complexity (Figure 7(a) to (c)) are discussed in Section 6.

6. Effects of adsorbed macromolecules on critical physicochemical processes affecting ENM environmental behavior

In this section, we review the current state of knowledge about two categories of processes that are strongly affected by the adsorbed macromolecules: attachment of ENMs to surfaces (i.e., aggregation and deposition), and ENM reactivity (including dissolution, photoreactivity, and oxidation-reduction reactivity). We aim to identify important mechanisms that dictate the macromolecule-ENM interactions and behavior in the environment, as well as the role of key variables affecting these processes.

6.1. Attachment of ENMs to surfaces (aggregation and deposition)

Macromolecular coatings are well known to modify the attachment behavior of colloids and ENMs to surfaces, i.e., their likelihood to homoaggregate, heteroaggregate with other suspended particles, or be removed from suspension due to deposition onto mineral or biological surfaces (Figure 8). Many studies have been published regarding ENM aggregation and deposition, as summarized by Petosa et al. for ENMs in general[14] and Philippe and Schaumann for ENMs interacting with NOM.[18] Overall, these studies typically demonstrate the ability of coatings to reduce ENM aggregation or deposition[4, 5, 11] or disagglomerate ENMs[145, 146] by imparting electrostatic, steric, or electrosteric repulsion. Alternatively, enhanced attachment may occur due to charge neutralization (if the macromolecule and ENM are oppositely charged)[93, 145, 147, 148] or bridging.[34, 75, 80, 149, 150]

Here, we survey the literature to identify the effects of key macromolecule properties (e.g., concentration, chemistry, and molecular weight) on ENM attachment behavior. We begin with studies that assess general qualitative effects of macromolecules on ENM attachment. We then discuss studies that provide a more thorough assessment of the effects of specific properties of the macromolecules, allowing greater mechanistic insight into their effects on ENM behavior. Where available, we highlight systematic studies that demonstrate the various approaches described in Section 5. Throughout the survey, we provide critical evaluation of the experimental results from the literature in terms of the theory presented in the Background, and we note further data needs and questions to address in future studies.

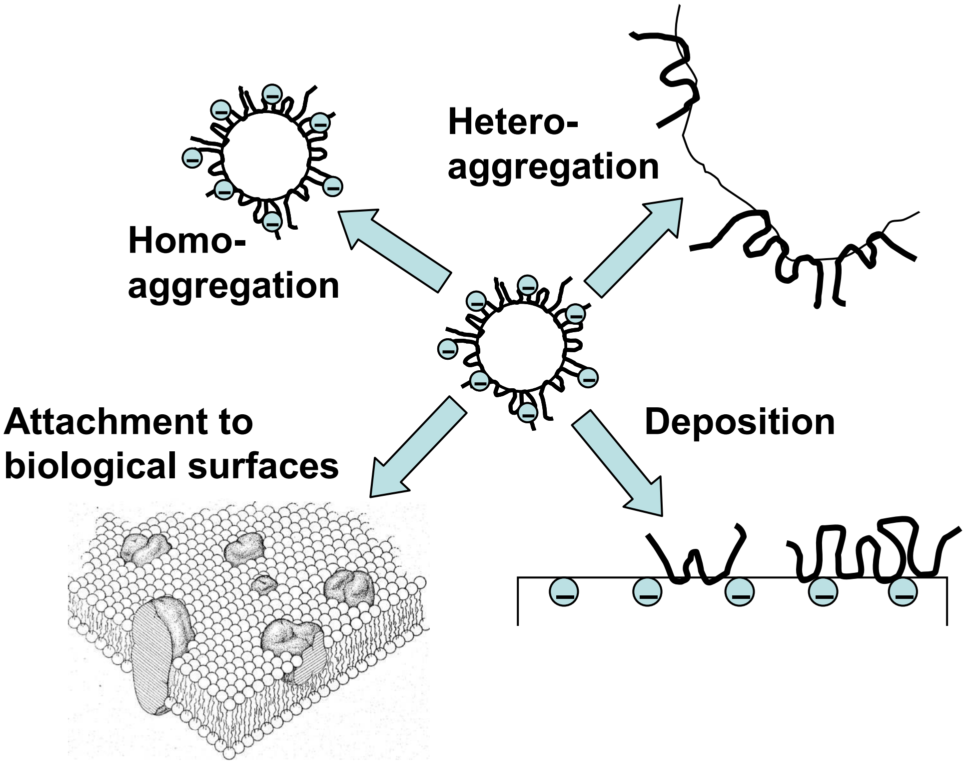

Figure 8. Attachment processes for ENMs in natural environments, including homoaggregation, heteroaggregation, deposition to mineral surfaces, and attachment to biological surfaces. Adsorbed macromolecules on the ENM will modify its surface properties and hence its interactions with other surfaces. Other surfaces in the environment can also be coated with macromolecules, e.g. humic substances or polysaccharides. Cartoons of particles and macromolecules are drawn at arbitrary scale. Cell membrane drawing from Singer et al. (1972). The fluid mosaic model of the structure of cell membranes. *Science* **175**: 720-731.[151] Reprinted with permission from AAAS.

6.1.1. General effects of macromolecular coatings on ENM attachment

The effects of bulk system properties such as macromolecule concentration and ionic strength on ENM attachment behavior have now been well studied using functional assays such as homoaggregation or deposition studies. Here, we highlight systematic studies that broadly demonstrate the effects of NOM and other macromolecular coatings. In one study, Keller et al.

evaluated the homoaggregation of metal oxide ENMs (TiO_2, ZnO, and CeO_2) in natural waters covering a wide range of solution chemistries.[152] In another study, Ottofuelling et al. investigated TiO_2 homoaggregation in systematically varied synthetic test matrices and used these results to predict aggregation in natural water samples.[153] Finally, Phenrat et al. used statistical analysis of deposition data for a variety of ENM and coating types (NOM and synthetic polymers) to identify key coating properties to explain the ENM behavior.[41] These three studies align most closely with Approach 1 in Figure 6 and correlate the properties of the coated ENMs (e.g., surface charge and adsorbed layer properties) to attachment behavior.

In the studies by Keller et al. and Ottofuelling et al., improved ENM stability against aggregation is observed with increasing NOM or total organic carbon (TOC) concentration and decreasing ionic strength. Keller et al. attributed this behavior to adsorption of negatively-charged NOM, resulting in more negative electrophoretic mobilities for the coated ENMs and higher electrostatic repulsion between the ENMs. Ottofuelling et al. also observed trends between NOM concentration, electrophoretic mobility, and colloidal stability of TiO_2 ENMs. Hence, they reached a similar conclusion to Keller et al. and proposed that ENM aggregation in natural waters could be predicted from assays in synthetic test waters given knowledge of the pH, divalent electrolyte concentration, and NOM concentration as represented in Stiff diagrams.

In contrast to these studies, which focused primarily on electrostatic forces imparted by the adsorbed NOM, Phenrat et al. found that incorporation of other adsorbed layer properties, including layer thickness and adsorbed mass resulted in significant improvement of empirical correlations to predict ENM deposition, compared to correlations only including electrostatic interactions.[41] These layer properties are expected to be important based on extended DLVO theories for electrosteric interactions. Thus, the study by Phenrat et al. provides further refinement of the correlations by incorporating coating effects.

For the approach used by these three studies, NOM was used either without modification in raw collected waters or as prepared from standardized humic or fulvic acid stocks (Figure 7(a,b)). Aside from the inclusion of average molecular weight in the correlation by Phenrat et al., the properties or composition of the macromolecules were not considered in detail. The following sections explore these effects in more detail for NOM coatings on ENMs.

6.1.2. Effects of the bulk properties of NOM

NOM is often used as a bulk material in ENM studies (Figure 7(a,b)) and characterized by averaged or ensemble measurements, e.g., UV absorptivity of the bulk solution. Here, we highlight a study by Deonarine et al. that provided a quantitative correlation of nanoparticle growth and aggregation with these averaged properties for nine different NOM isolates.[154] The stabilization of zinc sulfide nanoparticles against growth and aggregation correlated primarily with the aromaticity and average molecular weight of the NOM, as opposed to other properties such as elemental composition (including sulfur content), carboxyl content, and the electrophoretic mobility of the coated particles.[154] These results are corroborated by other studies that compared the effects of humic acids and fulvic acids on ENM aggregation[155, 156] or deposition[157] and showed that humic acids, which typically have higher molecular weight and aromaticity, result in lower ENM attachment efficiency.

The postulated mechanism for these effects is that NOM with higher aromaticity or molecular weight can have higher adsorption affinity and adsorbed mass[158] (consistent with polymer adsorption theory) or will produce a thicker adsorbed layer, resulting in stronger (electro)steric repulsion. This steric or electrosteric repulsion explanation is typically used when lesser aggregation or deposition of ENMs coated with humic acids versus fulvic acids cannot be explained solely by electrostatic effects (i.e., attachment behavior does not correlate with the measured electrophoretic mobility of the coated ENMs).[91, 155, 159] Other phenomena beyond electrosteric repulsion have also been invoked when unexpected behavior of coated ENMs is encountered. For example, hydrophobicity was suggested to contribute to the enhanced aggregation of citrate-stabilized Au ENMs in the presence of Pony Lake fulvic acid at high concentrations.[156]

6.1.3. Effects of heterogeneity and polydispersity

Because NOM is a complex “supermixture”[74] and different components in the mixture can have different chemistries, bulk or averaged properties may not be sufficient to fully describe the interactions of NOM with ENMs. The use of more highly fractionated samples can aid in elucidating the role of different components.

Here, we highlight studies by Louie et al. that assessed quantitative correlations of gold ENMs with the properties of six different NOM isolates, each fractionated into two molecular weight fractions to assess the effect of NOM polydispersity.[160, 161] For most of the NOM isolates, the highest MW fraction (> 100 kDa) was generally found to provide significantly better stabilization of gold ENMs than the bulk of the NOM (< 100 kDa) at high ionic strength (100 mM NaCl). The stabilizing effect of the high MW NOM was also observed when comparing the effect of unfractionated NOM and the < 100 kDa filtrate, even when less than 6% of the NOM was removed by filtration. This result was reflected in the better quantitative correlation between gold ENM aggregation rates and weight-averaged MW, as opposed to the geometric mean MW. Notably, aggregation rates were also found to correlate better with MW than aromaticity for the gold ENMs studied, and this distinction was only possible upon fractionation of the NOM.

These results agree with fundamental polymer adsorption theory, in which higher MW macromolecules are expected to adsorb preferentially and could potentially displace smaller molecules.[19] Other studies have also shown preferential adsorption of higher MW components of NOM onto multi-walled carbon nanotubes (MWCNT),[158] iron oxide colloids or ENMs,[162, 163] and aluminum oxide colloids.[164] Similar to the results for gold ENMs by Louie et al.,[160, 161] NOM fractions containing higher MW components produced significantly lower deposition of hematite colloids in column experiments[165] and minimized the homoaggregation of aluminum oxide,[85] silver[166] and fullerene ENMs.[167] However, high MW components have also been shown to induce bridging, depending on solution conditions,[168] e.g., in the presence of high concentrations of Ca^{2+}.[167]

In the correlation study by Louie et al., one NOM isolate, Pony Lake Fulvic Acid (PLFA), was found to provide anomalously strong stability of gold ENMs despite its low MW; this effect was also observed in other studies for gold and silver ENMs.[156, 169] Louie et al. and Gunsolus et al. postulated that the high sulfur and nitrogen contents of the PLFA could result in higher adsorption of PLFA to the gold and silver ENMs and hence a stronger stabilizing effect relative to other NOM isolates.[160, 169] This result highlights that preferential adsorption of high MW components should only be assumed if the chemistry of different components are similar. Higher chemical affinity of low MW components may explain the results of studies showing preferential adsorption of lower molecular weight components in NOM, e.g., for soil-derived dissolved organic matter on silver nanoparticles,[170] purified Aldrich humic acid on hematite,[171] Suwannee River humic acid onto iron oxide,[172] and peat humic acid on metal (hydr)oxide-coated sands.[173] Higher binding of low MW components may also explain studies showing no significant difference in ENM stabilization with humic versus fulvic acids,[174, 175] although other factors (such as the type of ENM and solution conditions) may also be involved. These studies again emphasize the need for more thorough characterization (e.g., of the adsorbed layer composition) to fully explain the mechanisms by which heterogeneous coatings affect ENM behavior.

6.1.4. Comparisons across correlation studies

Here, we holistically consider the results of the studies described in Sections 6.1.1, 6.1.2, and 6.1.3. First, we contrast the conclusions of the studies by Keller et al.[152] and Ottofuelling et al.[153] (using natural water samples or synthetic waters prepared with a single type of NOM and varied electrolyte compositions) with those by Deonarine et al.[154] and Louie et al.[160] (using a variety of NOM isolates and MW fractions in controlled electrolyte solution). Notably, Keller et al. and Ottofuelling et al. concluded that electrostatic effects determined the ENM attachment behavior, whereas Deonarine et al. and Louie et al. concluded that steric or electrosteric effects were more important than electrostatic effects.

Two explanations for the contrasting results are considered. First, it is possible that electrostatic effects are dominant when considering ENM aggregation across a wide variety of NOM concentrations and water chemistries, as opposed to controlled solutions prepared in the laboratory. This explanation highlights a limitation of highly focused, mechanistic studies of NOM-ENM interactions that may not consider broader variability in environmental conditions. A contrasting explanation is that electrosteric effects imparted by NOM did indeed contribute in the systems studied by Keller et al. and Ottofuelling et al., but that insufficient characterization of the NOM was provided to identify these effects. For example, in the study by Keller et al., ENM attachment efficiency correlates best to zeta-potential only at extremes of the zeta-potential range, whereas poor correlation is observed at intermediate zeta-potentials. It is possible that consideration of the composition and MW of the NOM in the different water samples could improve the correlation in the intermediate zeta-potential region. Likewise, Ottofuelling et al. acknowledged in their study that differences in NOM composition may contribute to discrepancies observed when comparing ENM aggregation in some natural waters to synthetic waters prepared using Suwannee River NOM.

Two studies by Nason et al.,[156] and Shen et al.[167] help to bridge the gap between the aforementioned studies by varying both NOM properties and the background electrolyte concentration to determine the CCC of the NOM-coated ENMs. These studies compared different NOM isolates[156] and different MW fractions of NOM.[167] The results of these studies were consistent with the results of Deonarine et al. and Louie et al.; i.e., higher MW NOM generally shifted the CCC to higher electrolyte concentrations than lower MW NOM (with exceptions for PLFA[156] and for high MW NOM that bridged ENMs at very high Ca^{2+} concentrations.)[167] These results suggest that electrosteric effects imparted by NOM can be significant regardless of water chemistry, and they demonstrate the value of expanding the range of environmental conditions assessed in mechanistic studies.

Following this trend, further research is needed that connects the approaches shown in Figure 7. Studies assessing ENM attachment in natural waters (Figure 7(a)) should provide more detailed characterization of the NOM in their samples. On the other hand, mechanistic studies using fractionated NOM (Figure 7(c)) should be broadened to explore the effects of NOM concentration and mixtures of NOM fractions, along with the effects of water chemistry. The complementary application of all approaches in Figure 7 will enable a better understanding of the relative importance of different NOM coating properties (e.g., charge and MW) across a wider range of environmental conditions.

We now compare the two quantitative correlation approaches depicted in Figure 6. The aforementioned studies by Deonarine et al.[154] and Louie et al.[160] followed Approach 2 to correlate NOM properties such as aromaticity and MW to ENM attachment behavior. However, only a single type of nanomaterial (i.e., ZnS or Au nanoparticles) and a single background electrolyte solution was used in each correlation. The data presented by Shen et al.[167] could potentially be used to incorporate the effects of ionic strength and composition to predict ENM aggregation rates, but further analysis of the data is required to determine if such a correlation can successfully be made. Incorporating data across a variety of ENM types (metals, metal oxides, etc.), will require a much larger data set and present a significant challenge.

With this consideration, characterization of the coated ENM as a single entity (Figure 6, Approach 1) has clear advantages for the development of a generalized correlation to predict coated ENM attachment behavior across a variety of ENM and coating materials. To our knowledge, only the aforementioned study by Phenrat et al.[41] has quantitatively attempted this approach with inclusion of adsorbed layer properties. As described in Section 5.1, a significant challenge for implementation of this approach is the difficulty in measuring adsorbed layer properties on ENMs in suspension. Another potential problem is the difficulty in obtaining tractable coated ENM samples where adsorbed layer properties can be systematically varied, particularly when using natural macromolecules such as NOM. Without controlled variation of the coating properties to identify mechanistic effects, empirical correlations will likely be needed, as demonstrated by Phenrat et al. Moving forward, Approaches 1 and 2 should be applied complementarily; i.e., the mechanistic knowledge obtained from Approach 2 should inform the appropriate selection of coating properties for the development of empirical correlations in Approach 1.

Finally, we again emphasize the need for thorough characterization regardless of the approach used to develop correlations for coated ENM behavior. This problem was highlighted in a study by Hotze et al. in which deposition behavior was assessed for three ENMs (silver, titanium dioxide, and fullerene), either uncoated or coated with one of three macromolecules (poly(acrylic acid), humic acid, and bovine serum albumin).[77] This study tested the hypothesis that the macromolecular coating would control the deposition of the ENM to silica surfaces, such that the behavior of different ENMs could be predicted solely from the macromolecule's properties (a simplification of Approach 2 in Figure 6). However, this hypothesis was disproven, suggesting that the same macromolecule forms different adsorbed layers on different ENMs. Then, correlations for the coated ENM deposition behavior were attempted using the coated ENM properties (size, electrophoretic mobility, adsorbed mass, and layer thickness from electrokinetic modeling), as in Approach 1 (Figure 6), but these properties were also insufficient to explain the observed deposition. These results demonstrate that more detailed or accurate characterization of adsorbed coatings will be required to develop a robust model to predict coated ENM behavior.

6.1.5. Further questions: Kinetics, synergistic effects, and specific interactions with divalent cations

While significant progress has clearly been made in correlating ENM attachment behavior to coating properties, further questions remain. Here, we highlight research gaps regarding the kinetics of the formation and transformation of adsorbed layers (depicted in Figure 9) and synergistic effects among heterogeneous macromolecules. We also highlight interactions of adsorbed layers with divalent cations, which are often studied but poorly predictable.

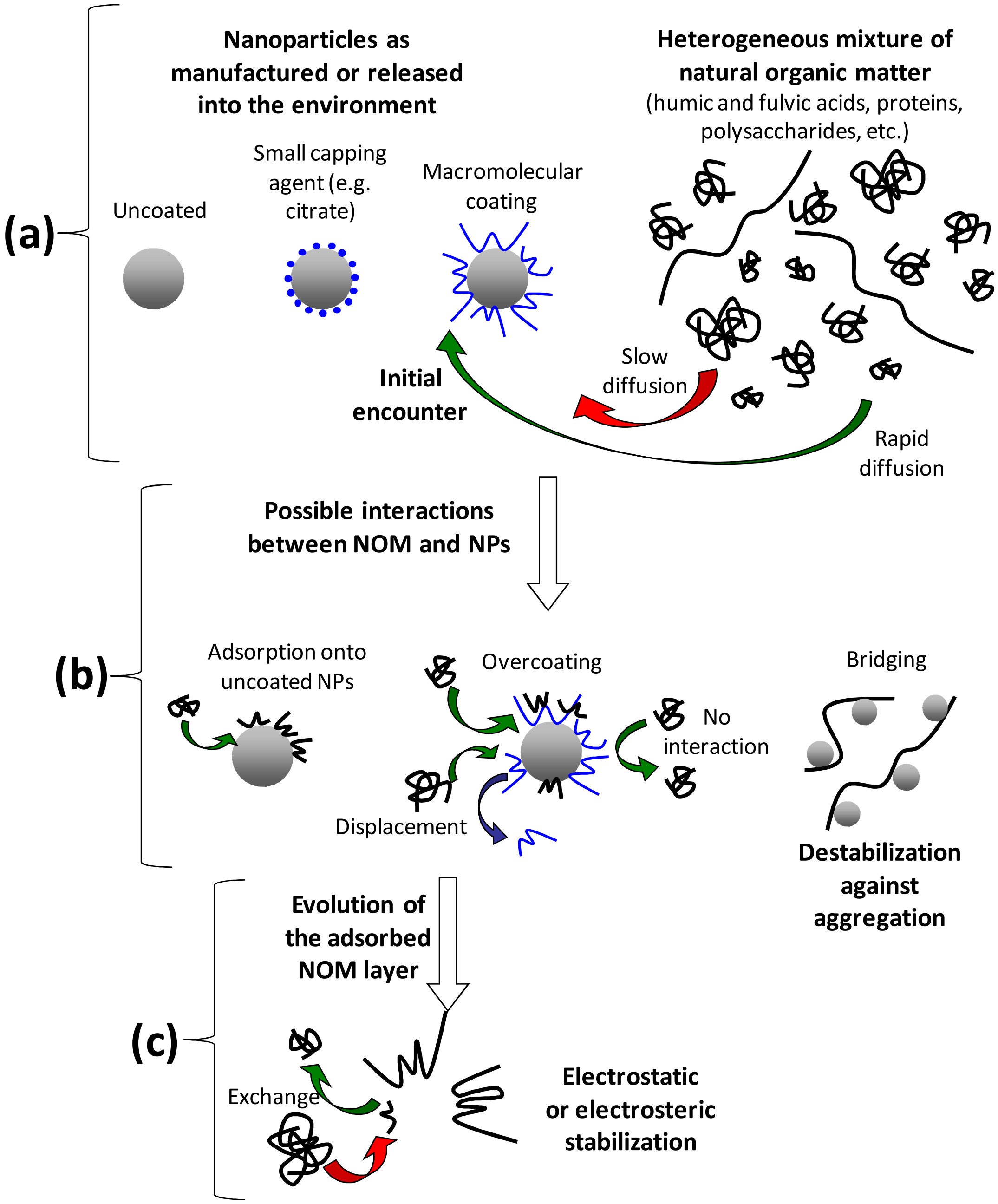

Figure 9. Schematic of the sequence of interactions between an ENM and NOM or any heterogeneous macromolecule mixture, including the kinetics of the diffusion of various components to the ENM surface (a), interaction of the macromolecule with the ENM surface or any initial surface coatings (b), and exchange of adsorbed components over time (c). The ability to assess these interactions will rely on the development of sensitive and selective detection or characterization methods for adsorbed coatings. Cartoons of particles and macromolecules are drawn at arbitrary scale.

Thermodynamic affinity is not the only parameter that must be considered for macromolecule-ENM interactions, because these systems are not typically at equilibrium with their surroundings. As such, rate-limited processes such as adsorption and exchange kinetics, as well as the order of exposure to macromolecules, will also be important (Figure 9(a)). That is, macromolecules with lower adsorption affinities may still adsorb to the ENM if they adsorb more quickly. Therefore, ENM behavior in a mixture of macromolecules is not necessarily predictable solely by assessing its interactions with individual components. For example, low molecular weight molecules can diffuse more quickly to the ENM surface and occupy surface sites to prevent bridging by slower-diffusing, high molecular weight components (Figure 10).[43]

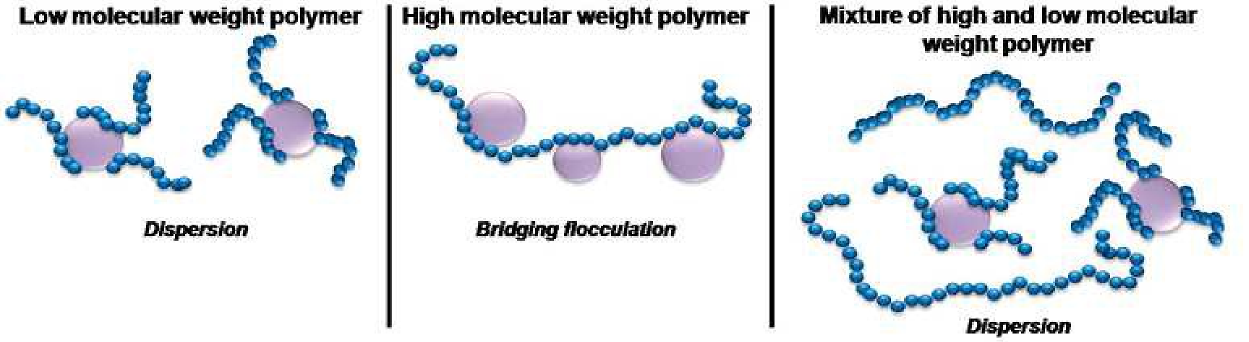

Figure 10. Interactions of nanoparticles with polymers of different MW and polydispersity. The presence of low MW polymers can prevent bridging by higher MW polymers by occupying surface sites more quickly. Figure reprinted with permission from Golas, P. L., et al. Comparative study of polymeric stabilizers for magnetite nanoparticles using ATRP. *Langmuir* **2010**, *26* (22), 16890-16900.[43] Copyright 2010 American Chemical Society.

After a macromolecule comes into contact with the ENM, the affinity between the macromolecule and ENM surface will determine its adsorption behavior (Figure 9(b)). Notably, interactions between initial stabilizing ligands or polymers and subsequently encountered macromolecules such as NOM are still poorly understood, with contradictory results found in the literaturee. Stankus et al. compared the effect of humic acid on the aggregation of gold nanoparticles initially coated with four different capping agents of different charges (neutral, positive, and negative) and found that humic acid generally produced the same response (reduced aggregation in monovalent electrolyte, enhanced aggregation in Ca^{2+} and Mg^{2+}) regardless of the initial coating.[176] On the other hand, Liu et al. found that NOM stabilized citrate-capped ENMs but enhanced the aggregation of mercaptoundecanoic acid-capped gold ENMs in the presence of Ca^{2+}.[177] Yin et al. recently investigated the aggregation of bare, citrate-, and polyvinylpyrrolidone (PVP)-coated silver ENMs in different MW fractions of NOM and found that the stabilizing or destabilizing effect of the NOM depended on both the initial coating on the ENMs and the MW of the NOM.[178]

A better mechanistic understanding of NOM interactions with the initial ENM coatings is clearly needed to explain the wide variety of results. However, characterization of these interactions will be difficult and require the application of sensitive techniques. For example, no interaction of humic acid with PVP-coated gold ENMs was detectable from measurements of UV-vis absorbance (surface plasmon resonance), size, ENM aggregation behavior, or surface chemistry.[179] However, solution depletion studies[180] and sensitive Raman spectroscopy methods[108] were useful to demonstrate interaction of humic acid with PVP-coated silver ENMs, which is consistent with the NOM-PVP interactions suggested in the aforementioned aggregation study by Yin et al.[178] Therefore, application of several complementary methods may be needed to fully characterize coating interactions.

After the initial adsorption step, exchange of lower affinity components for higher affinity components can occur (Figure 9(c)), as demonstrated in NOM adsorption studies on large particles or bulk surfaces. For example, Joo et al. observed an initial phase of rapid adsorption of lower MW fractions onto FeO(OH)-coated or Al_2O_3-coated sand within 15 min by specific interactions (e.g., ligand exchange between carboxylic and phenolic acid groups and metal oxide surfaces), followed by exchange with higher MW fractions over 4 hours due to hydrophobic interactions.[173] Hur and Schlautman observed similar behavior, but exchange continued over 120 hr.[171] While exchange kinetics on ENMs are known to be of high importance for protein mixtures in the context of ENM pharmacokinetics and toxicity,[9, 96] little attention has been given to this topic for the exchange of NOM, and further study is needed.

Finally, synergistic interactions between different macromolecules should be considered in all adsorption processes. Again, analogous studies from the biomacromolecule literature can be considered. For protein stabilization of ENMs, two- or three-component mixtures of proteins from fetal bovine serum (FBS) provided better stability than any single protein alone;[181] in this study, the co-sorption of three components was required in order to obtain similar stability to the complete FBS mixture. It is unknown if this synergistic effect resulted from the kinetic effects described above, or from the formation of a mixed-component adsorbed layer that provides better ENM stability, e.g. by co-sorption of components to yield a more extended or more uniform (less patchy) mixed coating around the ENM. Similar mechanisms were suggested to explain the improved stability of gold ENMs in unfractionated NOM compared to the separated NOM components,[160, 161] but further studies demonstrating direct measurement of interactions between NOM components are needed.

Additional studies are also needed to assess interactions among a broader variety of chemical classes of macromolecules in the environment, including humic substances, polysaccharides, and proteins. Buffle et al. proposed that humic and fulvic substances tend to stabilize colloids, whereas rigid biopolysaccharides can bridge or destabilize them,[34] leading to enhanced aggregation and deposition.[182] Saleh et al. demonstrated that a protein, bovine serum albumin (BSA), provided the best stabilization of single walled carbon nanotubes (SWCNT), followed by humic acid, a cell culture medium, and alginate (which induced aggregation at high Ca^{2+} concentrations).[183] It was suggested that BSA produced the greatest steric repulsion due to its globular structure and hence thicker adsorbed layer. Further work is needed to assess co-adsorption or sequential interactions of these varied macromolecules with ENMs.

In addition to interactions between macromolecules, specific interactions with other dissolved constituents, particularly divalent cations, are not easy to predict based on current knowledge. Divalent ions such as Ca^{2+} can induce aggregation not only by charge screening but also by specific complexation or bridging interactions with NOM or polysaccharides such as alginate.[76, 149, 150, 176, 183-185] These interactions may help to explain contrasting effects of NOM and biomacromolecules reported in the literature, such as enhanced aggregation of Ag ENMs by algal exudates in Ca^{2+}-containing medium[186] but disaggregation of TiO_2 ENMs in other exposure media.[63] Although Ca^{2+} typically enhances aggregation, contradictory results have been shown as well: Schwyzer et al. observed *enhanced* stability of carbon nanotubes in the presence of NOM within a certain Ca^{2+} concentration range.[82] This result was postulated to be attributable to either floating of the ENMs aggregated in loose network structures of NOM, or multilayer adsorption of NOM due to Ca^{2+} complexation. A better mechanistic understanding of the relationship between NOM or biomacromolecule composition and their interactions with Ca^{2+} is needed to predict these effects.

6.1.6. Additional considerations for heteroaggregation and deposition

Prediction of the heteroaggregation or deposition behavior of coated ENMs will require more information than prediction of homoaggregation behavior because the surface chemistry of a second surface must also be considered. For example, if the macromolecular coating on an ENM has an affinity to attach to the uncoated substrate, it can enhance attachment due to bridging.[187] However, if the substrate is also coated (or if free macromolecules are included in the background solution and allowed to adsorb before and during the experiment),[90, 128, 159, 165, 175, 188, 189] steric repulsion will be imparted and deposition will be prevented.[187] This phenomenon was directly demonstrated by Chen and Elimelech[190] and Furman et al[155] and recently incorporated into an ENM transport model by Becker et al.[191] Hydrophobic or hydrophilic effects may also affect deposition: Song et al. demonstrated that the deposition of coated silver ENMs onto hydrophobic or hydrophilic surfaces correlated with the hydrophobicity of the macromolecular coating on the ENMs.[192] Further work on heteroaggregation and deposition is needed to assess the potential importance of surface heterogeneity and to incorporate these effects into transport models for ENMs.

6.2. Solubility and reactivity

Adsorbed macromolecules can modify the reactivity and physicochemical transformations of the underlying inorganic ENM (Figure 11). The surface atoms on the ENM can be directly modified via chemical binding (chemisorption) of the macromolecule. More generally, adsorbed macromolecules can modify the availability of ENM surface sites for adsorption and reaction, the interactions of the ENM with light (and hence its photoactivity), and the flux of reactants to or from the ENM surface. We note that the approaches outlined in Figures 6 and 7 are in principle applicable to ENM reactivity studies. However, the reactivity of coated ENMs will likely be more difficult to predict than their attachment behavior because detailed information on the chemical reactivity and physical conformation of the coating may be needed. Here, we review studies that elucidate mechanisms by which adsorbed macromolecules affect ENM reactivity. Further mechanistic studies such as these will be required before general correlations can be developed to predict the effects of coatings on ENM reactivity.

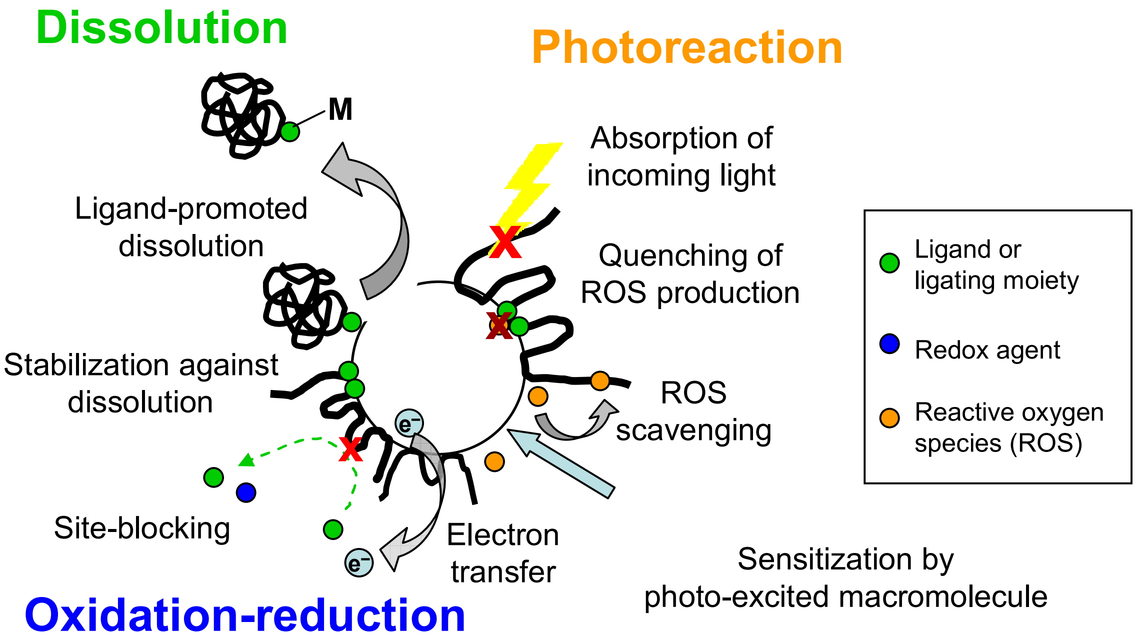

Figure 11. Possible effects of adsorbed macromolecules on ENM dissolution, oxidation-reduction reactions, and photoactivity. For strongly binding (e.g., chemisorbing) macromolecules, the inherent chemistry or surface energy of the ENM can be modified; the chemistry of binding will dictate whether reactivity is enhanced or reduced. Weakly binding (physisorbing) macromolecules will not change the inherent solubility or reactivity of the ENM,

but can participate in light absorption, electron transfer, and binding or scavenging of chemical species of interest (e.g., redox agents and ROS). A photo-excited macromolecule can also transfer energy to the ENM to sensitize its photoreaction. Cartoons of particles and macromolecules are drawn at arbitrary scale.

6.2.1. Solubility

The solubility of many metal and metal oxide ENMs is of interest in environmental contexts because dissolved metal species, such as Ag^+ or Cd^{2+}, are toxic to a variety of aquatic species and humans. The solubility of the ENMs, as well as the rate of dissolution, will determine the persistence of the ENMs in the environment and the potential for localized delivery of dissolved species from deposited or attached ENMs (e.g. to bacteria).

Adsorbed organic coatings that chemically bind to the ENM surface can in some cases change the equilibrium solubility of the ENMs or their rate of dissolution. General mechanisms by which organic molecules can promote the dissolution of some metals and metal oxides have been determined for bulk materials. These same mechanisms can apply to ENMs. Dissolution can be enhanced by both protons (i.e. acids) and inorganic or organic ligands in solution; simple rate laws for these two dissolution mechanisms for mineral dissolution were presented by Fürrer and Stumm.[193] Alternatively, complexation of the dissolved metal ions in solution by some macromolecules can act as a sink for dissolved metal ions to promote ENM dissolution. In addition, oxidation or reduction reactions, e.g. oxidation of silver or reduction of iron oxides,[194] can promote dissolution. For macromolecules interacting with ENMs, both the ligation mechanisms and the effects of the adsorbed macromolecule layer on redox reactions will be relevant.

Important strong ligating moieties for metals and metal oxide ENMs include carboxylate and thiol groups. The formation of complexes that are both mononuclear (i.e., binding between a single ligand moiety and a single metal atom) and bi- or polydentate (i.e., two or more ligands binding to a single metal atom) can enhance dissolution by weakening the bonds between the surface metal atom and the bulk metal or metal oxide.[193] For example, siderophores and small organic acids excreted by organisms promote the dissolution of iron oxides.[195-197] On the other hand, polynuclear surface complexes can be formed by large macromolecules that bind to multiple metal atoms; these coatings may inhibit dissolution due to the high energy barrier for simultaneous detachment of multiple surface atoms.

Currently available data in environmental nanotechnology generally support these trends. In toxicological studies, small organic acids were shown to enhance CeO_2 ENM dissolution,[198] and humic acid was shown to enhance ZnO ENM dissolution.[199] More rigorously controlled dissolution studies were performed by Mudunkotuwa et al[200]. and Bian et al.,[147] in which pH was controlled (thereby controlling for proton-mediated dissolution) and dissolution over time was measured. These studies demonstrated enhanced ZnO ENM dissolution by citrate,[200] but no significant difference in either the dissolution rate or solubility of ZnO ENMs for humic acid.[147] Reduced sulfur ligands have a particularly high affinity for ENMs made from soft metals such as Ag, Au, and Zn. For organic thiol ligands, cysteine was shown to enhance the dissolution of citrate-stabilized silver ENMs (i.e. higher concentrations of dissolved silver were observed after 50 hours of dissolution than for citrate-stabilized silver in ligand-free water), whereas serine (the OH analog to cysteine) showed no effect.[201] However, cysteine was found to reduce the dissolution of silver ENMs in another study;[202] further study is needed to determine why contrasting results were obtained.

While a fairly extensive literature exists on the effects of small molecule organic acids on mineral dissolution, additional work is needed to determine if the enhanced dissolution observed for small molecules and/or bulk minerals also holds for large macromolecules interacting with small ENMs with high surface curvature. Comparing between ENMs and bulk materials, the adsorbed macromolecule could take a different conformation on the surface, which could affect the nature of the binding (e.g. mononuclear versus polynuclear) and hence the enhancement or reduction of dissolution. This will likely depend on the density and spatial distribution of the ligating moieties on the macromolecule and the steric effects between macromolecules adsorbing to the ENM surface. The pH of the system will affect the protonation or deprotonation of functional groups on the macromolecule and thereby change its binding to the ENM surface, as proposed by Ochs to explain pH-dependent effects of humic substances on mineral dissolution.[203]

In contrast to macromolecules with functional groups that act as strong complexing ligands, physisorbed or weakly adsorbed macromolecules do not appear to change the equilibrium solubility of the ENMs, as demonstrated by Ma et al. when comparing poly(vinylpyrrolidone) (PVP) and gum arabic coated silver ENMs.[204] Similarly, Gondikas et al. found that PVP coatings on silver ENMs did not significantly change their solubility compared to citrate-stabilized silver ENMs (in the presence of cysteine and serine).[201]

On the other hand, the kinetics of ENM dissolution are modified by both chemisorbed and physisorbed coatings. Coatings can reduce the flux of oxidants or reactants or change the availability of surface sites. For example, PVP coatings on silver ENMs decreased the dissolution rate compared to citrate stabilizers (a small molecule), both in the presence of cysteine[201] or under UV irradiation.[205] The surface coverage or conformation of the macromolecule around the ENM can be very important for site blocking. Schulz et al. compared the stability of gold ENMs against etching by cyanide, a process involving complexation of CN^- with Au and oxidation of Au(0) to Au(I).[206] In their study, thiolated PEG_{2000} coatings were compared, varying only the spacer used between the thiol end group and the PEG tail. PEG conjugated with mercaptoundeanoate (MUA) stabilized the ENMs against chemical etching significantly better than either mercaptoproprionate (MPA) or mercaptophenylethanoate (MPAA). The stability imparted by the MUA coating was attributed to the packing of the MUA to form a thick and dense (highly ordered) hydrophobic layer around the gold ENM, protecting it against cyanide etching. Differences in aggregation state were proposed to contribute to the faster dissolution of silver ENMs coated in Tween 80 surfactant, compared to citrate-stabilized or bare silver ENMs.[207] Further research is also needed to apply the mechanistic knowledge from these studies to assess the effects of NOM on ENM dissolution rates.

6.2.2. Oxidation-reduction reactions

ENMs can undergo oxidation-reduction (redox) reactions with other species in the environment. These reactions can be utilized beneficially for contaminant remediation, e.g., reduction of trichloroethylene (TCE) by nanoscale zerovalent iron (NZVI) or Fe(II) produced by microbial processes that reduce bulk or nanoscale ferric materials.[208, 209] Alternatively, redox active ENMs can impose a toxicity hazard to organisms, either by direct oxidation or reduction of biological components or the formation of hazardous species, such as reactive oxygen speices (ROS).[10, 210]

Adsorbed macromolecules can affect the ENM redox activity by directly participating in electron transfer processes. For example, Kang et al. found that NOM adsorbed to NZVI enhanced electron transfer to dissolved oxygen to form hydrogen peroxide and hydroxyl radical, thereby enhancing the degradation of 4-chlorophenol.[211] Niu et al. also observed that humic acid resulted in faster degradation of sulfathiazole by a Fenton-like reaction with Fe(II) in magnetite and hydrogen peroxide.[212] The enhanced degradation was attributed to reduction of Fe(III) to Fe(II) by humic acid, enhancing the production of hydroxyl radical.

Adsorbed macromolecules can also indirectly affect ENM reactivity by blocking active sites on the ENM or acting as a physical barrier to electron transfer. Niu et al. showed that, in contrast to humic acid, adsorbed PEG, PAA, and CMC coatings reduced the rate of sulfathiazole degradation by magnetite, which was attributed to site blocking effects.[212] Saleh et al.,[213] Phenrat et al.,[214] and Wang et al.[215] also found that adsorbed polymers reduced the rate of reductive TCE dechlorination by NZVI (which is oxidized); the results were consistent with site blocking effects as well as partitioning of TCE to the adsorbed polymer (reducing its flux to the reactive NZVI surface). Corredor et al. found that the catalysis of electron transfer between sodium borohydride and methylene blue by gold nanoparticles was reduced for ENMs coated with synthetic polymers and tannic acid, compared to the uncoated ENM.[216] However, in more complex systems in which the coating and ENM interact with multiple species, the effect of the coating can differ. For example, when studying the competitive reduction of TCE, chromate, and nitrate by CMC-coated versus uncoated NZVI, Kaifas et al. observed higher reduction of TCE with the coated ENMs because the negatively-charged coating repelled the competing, negatively-charged chromate and nitrate ions.[217]

The effects of NOM coatings on redox activity will be particularly difficult to predict because of the ability of NOM to participate in redox reactions while also acting as a physical barrier around the ENM. Xie et al. found that humic acid reduced the rate of bromate reduction by NZVI and attributed this behavior to site blocking as well as complexation of reactive $Fe(II)_{(aq)}$ species.[218] However, they also found that these effects were moderated by the reduction of Fe(III) to Fe(II) by humic acid, which could encourage bromate reduction. Many other studies have observed that NOM decreases the rate of reduction of organic contaminants by NZVI and iron oxides.[219-222]

Most environmental studies on the effects of coatings on ENM redox activity have focused on iron or iron oxide ENMs for environmental remediation. Redox transformations of other ENMs (e.g., oxidation of silver ENMs) are of interest for environmental implications, but relatively little is known about the effects of adsorbed macromolecular coatings on these processes.[13]

6.2.3. Photoactivity

Under illumination (e.g., sunlight or UV irradiation), photoreactive ENMs can act as photocatalysts to degrade organic compounds via oxidation or reduction reactions, or they can produce reactive oxygen species (ROS) in water which can react with organic compounds or biota. Photoreactive ENMs include anatase TiO_2, ZnO, and single-walled carbon nanotubes, fullerenes, and fullerols. The development of doped or composite nanomaterials with enhanced photoactivity, such as gold-TiO_2 nanocomposites, is also a large and active field of research. The interactions of adsorbed macromolecules with the ENM itself or with incoming light, produced reactive oxygen species, or the compound or organism of interest can affect the photoactivity of the ENM and its environmental effects. These interactions can either quench or sensitize the ENM photoreaction. Most environmental studies to date that assess the effects of macromolecules on ENM photoreactivity are focused on NOM, often in the context of ENM toxicity [207, 223-229] or the utilization of ENMs for contaminant photodegradation.[230]

Adsorbed macromolecules can directly change the photoactivity of the underlying ENM by forming a complex with the ENM material and changing its photochemical properties prior to photoexcitation. Alternatively, energy transfer during collisions between the ENM and free macromolecule can change the effective photoactivity of the ENM. Kong et al. suggested that these two mechanisms could result in the contrasting fullerene quenching versus fullerol sensitization that they observed in NOM.[231] The fullerene quenching was attributed to formation of an NOM-fullerene complex (reducing the number of photoactive sites) rather than energy transfer from the excited fullerene to dissolved NOM molecules. On the other hand, fullerol has lesser association with NOM, and NOM was observed to sensitize its photoactivity, which was attributed to energy transfer from excited NOM molecules that collided with the fullerol.

More indirectly, macromolecules (either free or adsorbed) can absorb incoming light, reducing the energy reaching the ENM surface and hence reducing the photoreaction.[232] Macromolecules can also scavenge the produced ROS,[223] or they can act as a physical barrier around the ENM surface, hindering interactions of ROS produced at the ENM surface with organisms or species of interest, e.g., contaminants. Alternatively, sorption of small molecules in an adsorbed layer of macromolecules can result in higher concentrations of contaminants near the ENM surface and hence enhanced interaction with the produced ROS.[232] Finally, adsorbed macromolecules can change the ENM aggregation state, which has been shown to affect ROS production and quenching. [233, 234]

Currently, few studies are available that compare the effects of different types of adsorbed coatings on ENM photoactivity. For synthetic polymers with simple and known chemistries, the effect of the coating may ultimately be predictable from the photochemistry of the polymer and the interaction of its functional groups with the ENM surface. For NOM, the heterogeneity of the NOM and its potential to either quench or sensitize photoreaction in different scenarios results in a complex system that may not be easily predictable. Further study is required to assess the role of various types of NOM across various types of ENM to determine a correlation between material properties and quenching or sensitization behavior.

7. Future Directions: Assessment of coatings on nanoparticles challenged in realistic environmental scenarios

In many recent studies,[1-5, 10] as well as the traditional colloid and polymer science literature, it is apparent that adsorbed macromolecules will significantly affect the fate and transport behavior, as well as the toxicity, of ENMs. Much of the mechanistic work to date on the effects of adsorbed macromolecules has been performed in simple, well-controlled systems. However, ENMs released into complex environments will be exposed to conditions that challenge the stability or persistence of the macromolecular coating; that is, the coating is likely to be transformed. Transformations of the inorganic nanoparticle core (e.g. sulfidation) have been studied in realistic systems (e.g., pilot wastewater treatment plants, environmental mesocosms) and can have significant effects on their fate and toxicity.[13, 235-238] Analogous studies on the transformations of organic coatings are needed, e.g. on the ability of the coating to degrade, desorb, or be displaced under environmentally relevant conditions.[239]

Challenging environmental conditions include exposure to new macromolecules (e.g., in a wastewater treatment plant), interactions with organisms (e.g. bio-degradation), exposure to sunlight, and changing pH, ionic strength, or redox conditions as the nanoparticle moves among various environmental compartments. Under these conditions, an adsorbed coating can change conformation, be displaced by other adsorbing species, or be degraded (Figure 12).

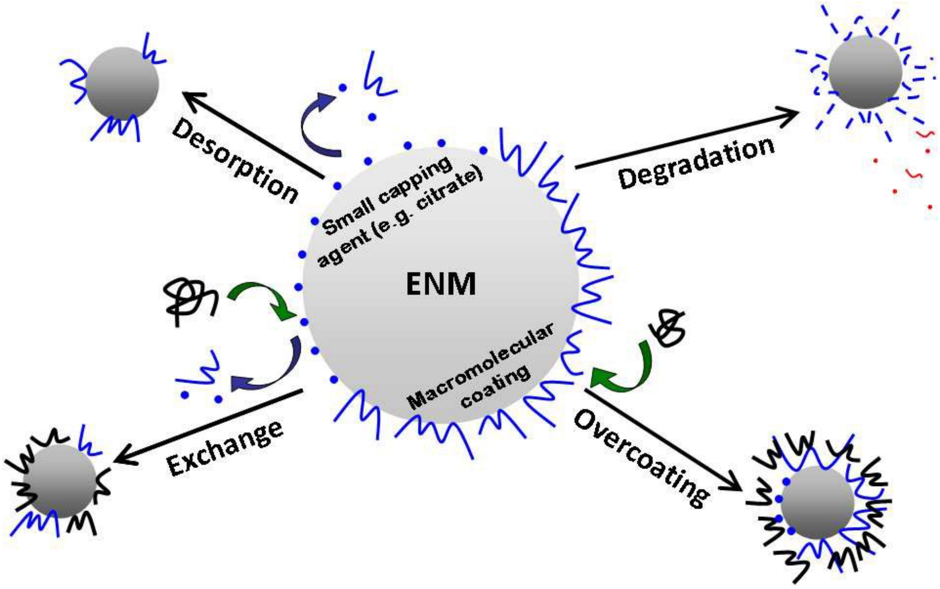

Figure 12. Possible transformations of adsorbed coatings on an ENM. Experimental studies of exchange and overcoating processes are prevalent in the biological literature, but further work is needed for environmental macromolecules. Few studies on the desorption or degradation of coatings have been performed to date, but these studies will be essential to predict the long-term fate of coated ENMs. Cartoons of particles and macromolecules are drawn at arbitrary scale.

As discussed in Section 6.1.5, analogous studies in biological systems have demonstrated the importance of protein adsorption and exchange,[9, 10, 240] and these processes are also beginning to be probed for synthetic coatings interacting with natural organic matter.[62, 65, 108, 176, 177] Much less work has been performed to date to examine the loss of coatings by desorption[239] or degradation, e.g., by exposure to bacterial biodegradation.[241] Characterization of these coating loss processes and the conditions under which they occur will be necessary to predict the ultimate fate of coated ENMs.

Finally, additional studies are needed that describe the kinetics of macromolecule adsorption or transformations processes on ENMs in a very broad environmental context, i.e., compared to the rates of other environmental processes and the residence time of the nanoparticle in different environmental compartments. The ability of NOM to disaggregate nanoparticles has not been well characterized, although recent studies have demonstrated the ability of NOM to disaggregate TiO_2 nanoparticles.[145, 146] Reactions that transform the nanoparticle surface chemistry, such as oxidation or sulfidation, will also change the affinity of macromolecular components; vice versa, adsorbed layers can change the rate of reaction of the nanoparticle. The kinetics of adsorption, heteroaggregation, deposition, or chemical transformation will depend on the concentration of macromolecules, suspended solids, or reactants, as well as the properties of the system (e.g., flowrate and ionic strength). Finally, a wide range of time scales (hours to years) and potentially drastic spatial or temporal changes in system conditions must be considered when assessing ENM fate and transport across its lifetime in the environment, as it moves among different compartments of interest (e.g., wastewater treatment plants, soils, surface waters, groundwater and sediments, and estuaries). Comparison of the rates of multiple transformation processes across multiple environmental compartments will be crucial to predict the likely sequence of ENM transformations and improve predictions of ENM behavior in realistic environments.

8. Conclusions

We now summarize conclusions drawn from a comparison of fundamental colloid and polymer science to the current literature on the effects of natural macromolecular coatings on the environmental fate of ENMs. Furthermore, we summarize approaches that will be valuable to enable prediction of the effects of coatings, and we identify research needs to improve upon these approaches.

Highly detailed characterization and modeling of the adsorption of macromolecules onto colloids and the effects of the adsorbed layer on interfacial forces are possible for homogeneous macromolecules. However, the literature demonstrates that interactions with heterogeneous natural macromolecules such as NOM can be highly complex. Detailed properties of the NOM (e.g., MW distribution and chemistry) in addition to system properties (e.g., ionic strength and composition) and ENM properties will all contribute to the fate and toxicity of the coated ENM. In this scenario, a first-principles approach to predict coated ENM behavior may not be appropriate, so more thorough and direct characterization of adsorbed layer properties will be needed.

The current literature suggests that quantitative prediction of the attachment behavior of coated ENMs can be feasible using empirical correlations that are informed by mechanistic studies. Moving forward, both the empirical and mechanistic approaches can be merged or extended to improve correlations for ENM attachment. Specifically, highly empirical approaches should provide thorough coating characterization to ensure that important adsorbed layer properties affecting ENM behavior can be more thoroughly accounted for. Alternatively, detailed mechanistic studies focusing on specific coating effects should expand their scope to assess a range of water chemistries and ENM types to develop more generalized correlations. In contrast to ENM attachment, prediction of the effects of coatings on ENM reactivity is expected to be much more difficult. Systematic studies are needed to demonstrate how specific adsorbed layer properties affect ENM reactivity.

Future directions for research include the assessment of the environmental transformations of coatings on ENMs and big-picture evaluations of the effects of coatings in context of the broad range of physicochemical processes that ENMs can undergo in the environment. This thorough understanding of the effects of ENM coatings will be challenging to attain but crucial for assessment of the exposure and toxicity risks of ENMs.

Acknowledgments

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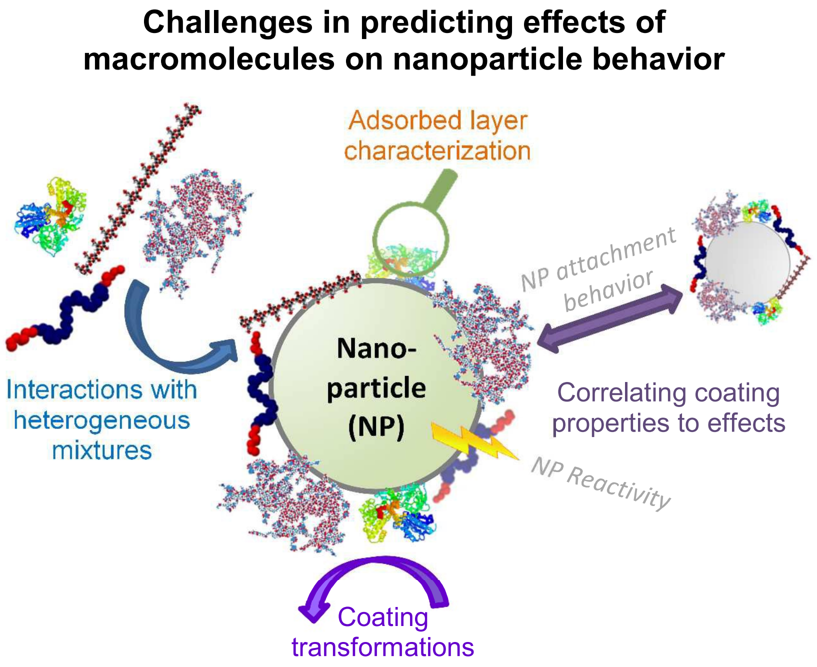
Challenges in predicting effects of macromolecules on nanoparticle behavior
Adsorbed layer characterization
NP attachment behavior
Nano-particle (NP)
Interactions with heterogeneous mixtures
Correlating coating properties to effects
NP Reactivity
Coating transformations