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Frontier article

Importance and challenges of environmental ligand binding and exchange: Introducing single molecule imaging as a model characterization technique

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ABSTRACT

Highly surface active nano-scale materials, when released into the natural environment, tend to adsorb geo- and bio-macromolecules and end up presenting a modified interface to biological species. Capped nanocrystals and polymer/surfactant modified nanomaterials also are known to undergo ligand exchange when exposed to natural systems. Thus, nano-bio interactions will primarily be governed by the adsorbed or exchanged natural macromolecules. To-date there has been no established technique determining the kinetics of ligand exchange or characterizing the bound geo-biomacromolecular corona in an environmental setting. Single-molecule imaging utilizing near-infra red spectrometry, and single-molecule imaging of fluorophore-tagged polymeric ligands can enable detailed characterization of biopolymeric corona. This perspective aims to highlight the importance of ligand exchange, identify roles of surface ligands on nano-bio interaction, and present initial evidence of macromolecular characterization on nanotube surfaces using single-molecule techniques. This commentary also aims to outline the challenges facing nano-environmental health and safety community on assessing biological interaction with complex nano-scale heterostructures in a realistic environmental matrix.

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1. Introduction

Physicochemical transformation of nanomaterials (NMs) is inevitable when released into the natural environment (Lowry et al. 2012), which strongly influences the interaction of these surface-active materials at the environmental and biological interfaces (Louie et al., 2016a). Geo- and bio-macromolecules present in natural waters will interact with NM surfaces and will adsorb either via direct binding onto pristine or covalently functionalized materials or by replacement of pre-existing engineered ligands of capped nanocrystals (Amal et al., 1992; Diegoli et al., 2008; Keller et al., 2010; Zhou and Keller, 2010; Zhang et al., 2009; Yang et al., 2009; Wang et al., 2010). Thus, biological systems and their membranes will interact with NMs first at the interface of the geo- or bio-molecular corona the NM has accrued prior to reaching the biological structure in question. Detailed characterization of this corona is essential for nano environmental health and safety (EHS) studies in complex but realistic natural environmental conditions.

Reduction in free energy by bringing in a monomer to a surface has been identified to be relatively large compared to its thermal energy, hence is known to drive macromolecular adsorption onto colloidal surfaces (De Gennes, 1987). Naturally occurring geo- and bio-macromolecules are subjected to solid-liquid distribution in geochemical systems due to their amphiphilicity. During adsorption process a net reduction in free energy is achieved by expulsion of water molecules; this is attained via a reduction in enthalpy (through elimination of van der Waals forces between water molecules and hydrophobic components), which is somewhat compromised by entropic loss (via dislodgement of oriented water molecules) (Lowry et al., 2012). Presence of high surface area NMs when suspended in natural waters (Tanford, 1980) enhances this adsorption process. NOM when at the vicinity of a surface introduces strong van der Waals interaction between the sorptive surface and the sorbing high molecular weight macromolecule (Schlautman and Morgan, 1994). Furthermore, hydrogen bonding between the NOM functional groups and surface moieties can drive macromolecules toward interfaces (Lau et al., 2013). Thus, preferential adsorption of NOM onto uncoated NM surfaces is a likely outcome, which will govern the interfacial interaction of these materials in the environment.

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Metal and metal oxide nanocrystals are mostly capped with polymers and polymeric surfactants. The interaction of these pre-existing ligands with naturally occurring macromolecules may undergo via partial or complete ligand exchange (Lowry et al., 2012). Such exchange will be dictated by ligand and macromolecular types and will be modulated by the surrounding environmental matrix. Energetic gain due to replacement of relatively smaller ligands with larger NOM (Lau et al., 2013) or reaction (Murphy et al., 1990; Parfitt et al., 1977) of humic functional moieties with those at the capping agents will determine the kinetics and extent of ligand exchange. Furthermore, NM physicochemical properties will also influence NOM adsorption and ligand exchange processes (Aich et al., 2014), where complexity in emergent nano-heterostructures present an additional degree of complexity (Aich et al., 2014; Plazas-Tuttle et al., 2015; Saleh et al., 2015; Saleh et al., 2014). These key factors of the ligand exchange process will eventually result in the evolution of NM surface chemistry and thus the eventual nano-bio interaction.

Irrespective of whether the NOM will bind to NM surfaces by adsorption or via ligand exchange, the corona formed on these surfaces necessitate detailed characterization. To-date, indirect approaches have been utilized in characterizing geo- and bio-macromolecules on NM surfaces; e.g., Tracking protein, surfactant, and polymer coatings has been accomplished via attenuated total reflection-FTIR, absorption, and bulk fluorescence techniques, which have evaluated desorption of these ligands from various nanomaterial surfaces (Mudunkotuwa and Grassian, 2015; Jain et al., 2015; Louie et al., 2016b; Tsai et al., 2011; Smith et al., 2015). Additionally, calorimetry has been used in conjunction with electrophoresis to measure protein adsorption on NM, and small angle neutron scattering experiments to measure nanomaterial-adsorbed polymers (Cosgrove et al., 1987; Cárdenas et al., 2005). Fourier transformed infrared (FTIR) spectroscopy (Karajanagi et al., 2004), interfacial force measurements (Treuel and Nienhaus, 2012), ellipsometry (Byrne et al., 2008), contact stylus instruments (Consiglio et al., 1998), are prominent experimental techniques while theoretical modeling (Phenrat et al., 2008) of bound NOM layers provide further insight into these complex corona. However, a robust technique is yet to be developed to directly characterize the conformation of the macromolecules on NM surfaces, kinetics of ligand exchange, and extent of macromolecular adsorption or exchange. Although many tools exist to characterize both NMs and the interfacial synthetic or biological coronas (Sapsford et al., 2011), these methods are currently unable to give a detailed picture of biomolecular structure at the nano-bio interface (Nel et al., 2009). As a result, the molecular basis for local electronic properties, bioavailability, toxicological effects, structure and conformation of biomolecules on NMs remain unclear (Shvedova et al., 2010; Hauck et al., 2008).

Single-molecule imaging, utilizing near-infra red spectrometry and visible fluorophores, can enable detailed characterization of optically dense biopolymeric corona (Beyene et al., 2009). Single molecule imaging is a powerful technique to study individual molecules and singular intermolecular interactions (Zhang et al., 2013; Bisker et al., 2016). In particular, single-molecule fluorescence microscopy can be used to study individual polymers and macromolecules and their interactions both in vitro (Kruss et al., 2014; Wong et al., 2016) and in biologically complex environments (Giraldo et al., 2015; Giraldo et al., 2014; Landry et al., 2014). Single-molecule total internal reflection fluorescence microscopy (smTIRF) can achieve sub-diffraction limited imaging resolution by imaging molecules within a ~100 nm-deep field of view that is excited by an evanescent field, thereby eliminating signal from out-of-focus fluorophores. smTIRF allows nanometer spatial resolution and millisecond temporal resolution of single fluorescently-labeled polymers. As we show here, we can extend this technique to also spatially and temporally resolve interactions of polymers, and ligands, both among themselves and with synthetic nano-scale materials (Jain et al., 2011).

The focus of this perspective is to introduce single molecule imaging as an effective technique for bio-corona characterization. Differences in NOM chemical composition based on variation in natural water compartments is characterized, and the role of corona composition on environmental and nano-bio interaction is also analyzed. This article also highlights the challenges associated with characterization of complex nano heterostructures. A brief description of single molecule imaging with smTIRF and preliminary corona characterization data are presented. Strategies are discussed for adoption and utilization of this technique to study the detailed kinetics, extent and conformation of natural macromolecule adsorption, and corona formation on NM surfaces.

1.1. Environmental macromolecules and ligand exchange

The chemistry of naturally occurring bio- and geo-macromolecules widely vary depending on the phase of origin, i.e., terrestrial vs. freshwater or marine aquatic environment, and other environmental parameters (Niederer et al., 2007). These natural macromolecules can be broadly classified as non-humified (that originate from minor alteration via decay of tissue from living organisms) and humified (that are decomposition products of non-humified constituents) substances (Mulder et al., 1994). Carbohydrates, amino acids, proteins, lignin, hormones and low molecular organic acids are the first degradation products or non-humified substances that decomposes further to humified humic acids, fulvic acids, and humins (the combination is known as NOM). Among these natural macromolecules, NOM and polysaccharides are the most ubiquitous, which also are composed of a wide variety of functional groups and can demonstrate anionic/cationic as well as hydrophilic/hydrophobic behavior, depending on their chemical structure (Vannote et al., 1980; Mao et al., 2000). Thus, appreciation of the complex and variable chemistry of the geo- and bio-macromolecules when studying NM interaction is critical.

In a natural water body, the organic components can either be dissolved or suspended as particulates. The non-humic substances originate from viable cells and are relatively amenable to degradation, which generate altered molecular structure of new aquatic organic substances (Jose, 2009). Among these non-humified substances, carbohydrates are the most ubiquitous. These can be present as simple monosaccharide structures (composed of 3 to 6 carbon atoms) (Aich et al., 2014) or more complex branched polysaccharides (generally composed of 40 to 3000 carbon atoms) (Aich et al., 2014). Among these carbohydrates, monosaccharides and disaccharides are the water soluble and biodegradable fractions (Pigman, 2012); whereas, starch and glycogen, the primary energy sources of plants and animals, respectively, are the most biodegradable fractions in polysaccharides (Pigman, 2012). The availability of primary and secondary hydroxyl groups in starch and glycogen makes these polysaccharides hydrophilic in nature (Lu et al., 2009). Other polysaccharides such as cellulose and chitins, on the other hand, are not readily biodegradable and neither are water soluble. These carbohydrates are comprised of uniform glucose structures which allow these to resist enzymatic breakdown (O'Sullivan, 1997).

Proteins with amino acids as the primary building block (with amines and carboxyl functional moieties) are another important macromolecule in the aquatic environment, that can be structurally complex and can exhibit variation in surface charge (cationic, anionic, or nonionic) (Goldenberg and Steinberg, 2010). Amino acids, such as cysteine and other proteins containing cys-residuals also possess thiol functional groups (Trivedi et al., 2009), which enhance the affinity of these bio macro-molecules for some metal and metals oxides surface that are reactive to such disulfide and thiol groups (Aryal et al., 2006). Based on the environmental conditions, these thiol-containing proteins can form disulfide bonds, which might entirely change the interaction with NMs. Another prominent source of these non-humic substances is EPS, which can be excreted by both unicellular and multicellular organisms. The chemical composition of EPS varies depending on the organism from which these are produced (Wotton, 2004; Decho, 1990; Flemming and Wingender, 2010). EPS is typically responsible for conditioning environmental surfaces to allow formation of biofilms on