Influence of natural organic matter (NOM) coatings on nanoparticle adsorption onto supported lipid bilayers

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HIGHLIGHTS

\begin{itemize}
  \item Interaction of Ag and C\textsubscript{60} nanoparticles with charged lipid membranes was studied.
  \item Quartz crystal microbalance experiments measured the adsorption kinetics.
  \item Natural organic matter (NOM) either inhibited or promoted nanoparticle adsorption.
  \item Adsorption profile depended on nanoparticle type, electrolyte condition, and NOM.
\end{itemize}

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ABSTRACT

As the worldwide usage of nanoparticles in commercial products continues to increase, there is growing concern about the environmental risks that nanoparticles pose to biological systems, including potential damage to cellular membranes. A detailed understanding of how different types of nanoparticles behave in environmentally relevant conditions is imperative for predicting and mitigating potential membrane-associated toxicities. Herein, we investigated the adsorption of two popular nanoparticles (silver and buckminsterfullerene) onto biomimetic supported lipid bilayers of varying membrane charge (positive and negative). The quartz crystal microbalance-dissipation (QCM-D) measurement technique was employed to track the adsorption kinetics. Particular attention was focused on understanding how natural organic matter (NOM) coatings affect nanoparticle-bilayer interactions. Both types of nanoparticles preferentially adsorbed onto the positively charged bilayers, although NOM coatings on the nanoparticle and lipid bilayer surfaces could either inhibit or promote adsorption in certain electrolyte conditions. While past findings showed that NOM coatings inhibit membrane adhesion, our findings demonstrate that the effects of NOM coatings are more nuanced depending on the type of nanoparticle and electrolyte condition. Taken together, the results demonstrate that NOM coatings can modulate the lipid membrane interactions of various nanoparticles, suggesting a possible way to improve the environmental safety of nanoparticles.

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1. Introduction

Ongoing advances in nanoscience and nanotechnology have heralded a new age of innovation across a wide range of fields, including food science, environmental remediation, cosmetics, textiles, paints, and pharmaceuticals [1]. Subsequently, these advances have led to a significant increase in the usage of nanotechnology-inspired products (e.g., batteries [2], face creams [3], food storage bins [4] and bandages [5]) based on engineered nanoparticles (NPs). Among different types of NPs, silver nanoparticles (AgNPs) and derivatives of water-stable fullerene nanoparticle aggregates (C\textsubscript{60}NPs) have been widely investigated as models for inorganic and organic, carbon-based nanomaterials, respectively. AgNPs are found in many commercial products and have demonstrated toxic-
ities against numerous organisms and cell lines, inspiring extensive research into the environmental impact of AgNPs and transformation products in the environment [6,7]. Likewise, C60 NPs have low toxicity but can remain in soil and other deposits for extended periods due to poor bioavailability [8]. To date, the majority of related studies have focused on human health implications of nanoparticles, although recent literature has also increasingly focused on the ecological implications of nanoparticles with respect to their fate and toxicity [9–11]. The interaction of nanoparticles with environmental systems has been principally studied with respect to bioaccumulation and biotoxicity [12–22]. Understanding how the physicochemical properties of nanoparticles and their surface functionalization influences these activities is a key objective.

The interactions between nanoparticles and cell membranes largely determine the degree of toxicity for a particular class of nanoparticle. When nanoparticles interact with a cell membrane, they can adsorb onto the membrane surface and, in some cases, penetrate the membrane, resulting in cellular uptake and/or membrane damage [23–26]. To systematically characterize the interactions between nanoparticles and cell membranes, various kinds of biomimetic cell membrane platforms have been experimentally utilized, including lipid vesicle suspensions [27], black lipid membranes [28], mercury droplet membranes [29], giant unilamellar vesicles [30], tethered lipid bilayers, and supported lipid bilayers (SLBs) [31]. Due to their versatility, SLB platforms are one of the most commonly used model membranes for characterizing the interactions of molecules with cell membranes [32], and have been utilized to mimic human and bacterial cell membranes [33,34]. Indeed, SLB platforms of different lipid compositions can be fabricated on hydrophilic substrates by utilizing the solvent-assisted lipid bilayer (SALB) formation method [35,36] and a wide range of surface-sensitive measurement techniques such as the quartz crystal microbalance-dissipation (QCM-D), atomic force microscopy (AFM), and electrochemical impedance spectroscopy (EIS), can be used to monitor SLB formation and nanoparticle-membrane interactions [37]. Previous studies showed that nanoparticles can adsorb onto SLBs and either simply adsorb without damage or cause different types of membrane disruption such as pore formation and lipid solubilization depending on the system [38–40]. So far, the corresponding measurements have been typically conducted in pristine conditions and extending such measurements to include additional environmental factors would improve our knowledge about how nanoparticle-membrane interactions might occur in the environment.

Nanoparticles can undergo physical and chemical transformations depending on environmental conditions such as sunlight and temperature [41], as well as due to interactions with natural organic matter (NOM) that is ubiquitous in aquatic environments [42–45]. To study how environmental factors affect solution-phase nanoparticles, simplified experimental conditions that mimic aquatic environmental conditions have been utilized based on modulating NOM and electrolyte components. In general, it is understood that higher ionic strength conditions cause nanoparticle aggregation that further depends on the ion species [46,47]. Moreover, NOM is a particularly important determinant of nanoparticle fate in environmental systems. As the byproduct of decomposed organisms, NOM contains a diversity of hydrophobic lipids and proteins in addition to high molecular weight humic and fulvic acids, and the resulting mixture of molecular compounds contains a wide range of hydroxyl, alcohol, carbonyl, methoxy, carboxyl and phenolic functional groups [48–50]. As a result, NOM can easily coat nanoparticle surfaces [47–51] and replace weakly bound coating agents (e.g., noncovalently attached surfactants) to form heterogeneous molecular coating layers [50]. To our knowledge, there is no previous report detailing the effects of NOM on the interaction between nanoparticles and cell membranes.

In this study, we have systematically investigated the interaction of two well-studied nanoparticles, AgNPs and C60 NPs, with SLB platforms in the presence and absence of NOM and in different monovalent and divalent electrolyte solutions (NaCl or CaCl2). SLBs were fabricated with either positive or negative surface charges by using the SALB fabrication method. The QCM-D measurement technique was utilized to monitor the kinetics of nanoparticle–membrane interactions in a label-free format that does not require chemical labeling of either the SLB or nanoparticles [51,52]. Furthermore, we employed two different experimental scenarios to monitor the membrane–NP interactions in the presence of NOM, as outlined in Fig. 1. In the first scenario, the SLB was initially coated with NOM and then NPs were injected in order to mimic the situation when pristine NPs are first introduced into the environment. In the second scenario, NOM and NPs were initially mixed and subsequently added to the SLB platform, thereby more closely mimicking situations where both the cellular membranes and NPs have been present in the environment for some time.

2. Materials and methods

2.1. Materials

1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-sn-glycero-3-ethylphosphocholine (chloride salt) (DOEPC), and 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho(1'-rac-glycerol) (sodium salt) (POPG) were obtained in lyophilized powder form from Avanti Polar Lipids (Alabaster, AL). The lipid powders were stored at ~20 °C and dissolved in appropriate organic solvents immediately prior to SALB bilayer formation experiments. Bovine serum albumin (BSA) was obtained from Sigma-Aldrich (St. Louis, MO). Two different nanoparticles (NPs) were used in the experiments: polyvinylpyrrolidone (PVP)-coated AgNOS (>99.5%) NPs were purchased from Shanghai Huzheng Nano Technology Co., Ltd (Shanghai, China) and fullerene (C60) NPs (99.9% purified through sublimation) were obtained from the MCR Corporation (Tucson, AZ). The PVP-coated AgNOS NPs (denoted as AgNPs) are a stabilized form that is recommended for situations where chronic exposure is anticipated [53]. Suwannee River Natural Organic Matter (SRNOM; defined as NOM in this work) (RO isolation, 2R101N) was received from the International Humic Substance Society (St. Paul, MN). All solutions were prepared with Milli-Q-treated water (>18 MΩ cm) (Millipore, Billerica, MA).

2.2. Preparation and characterization of C60 aggregates and AgNPs

To emulate the fate of fullerenes upon release into the environment, water-soluble C60 aggregates (denoted as C60 NPs) were prepared by stirring C60 NPs in ultrapure water for six months in dark storage, as previously described [54]. Dynamic light scattering (DLS) was used to measure the size distributions of nanoparticle samples based on the diffusion properties of the nanoparticles in solution. A 90Plus particle size analyzer (Brookhaven Instruments, Holtsville, NY) with a 658.0 nm monochromatic laser was employed for all measurements, which were taken at the fixed scattering angle of 90°. The intensity autocorrelation function was determined in order to calculate the intensity-weighted size distribution of nanoparticles. The size distributions of nanoparticles were reported based on the average effective hydrodynamic diameter and polydispersity in water, as well as in 10 mM NaCl or CaCl2 aqueous solutions that contained varying concentrations of NOM (0–10 ppm). In natural freshwaters, NaCl or CaCl2 are the most prevalent monovalent and divalent salts and hence were chosen as the electrolyte conditions for this study [55]. In physiological envi-
Fig. 1. Schematic illustration of experimental scenarios to investigate how NOM influences membrane-nanoparticle interactions. Scenario 1: Precoating of SLB platform with NOM components to mimic an environmentally relevant situation. Scenario 2: Preincubation of nanoparticles with NOM components, prior to adding nanoparticles + NOM to the SLB platform.

environments, the Ca\textsuperscript{2+} concentration is typically 5 mM or lower and we chose a moderately higher concentration for direct comparison with monovalent salt conditions, as done in past studies [55] and in the range of tested concentrations [56,57]. To be in the optimal concentration range for DLS measurements, the tested nanoparticle concentrations were 1:10 dilutions of the stock solutions (0.25 ppm AgNPs or 45 nM nC\textsubscript{60}NPs), and prepared samples were incubated for 30 min before experiment. Electrophoretic mobility measurements were conducted using a Delsa Nano C particle analyzer (Beckman Coulter, Brea, CA) in order to determine the zeta potential of nanoparticle suspensions. Each sample was measured 10 times for determining the zeta potential value (mean ± standard deviation). The experimental temperature was maintained at 25 °C for all measurements.

2.3. Quartz crystal microbalance-dissipation (QCM-D)

All QCM-D experiments were performed with a Q-Sense E4 instrument (Biolin Scientific AB, Gothenburg, Sweden) to monitor the interaction between nanoparticles (AgNPs and nC\textsubscript{60}NPs) and an SLB platform in the presence and absence of NOM at varying concentrations. The QCM-D technique records changes in the resonance frequency (Δf) and energy dissipation (ΔD) of an oscillating silica-coated quartz crystal sensor chip (model no. QSX303) as a function of time, which reflect the acoustic mass and viscoelastic properties of an adsorbate on the sensor surface, respectively. In the present experiments, the SLB platform is coated on the QCM-D sensor surface such that nanoparticle adsorption onto the SLB can be measured by tracking the frequency shift. Specifically, the QCM-D technique enables measurement of the nanoparticle uptake...
(amount of adsorbed mass) and the kinetics of the adsorption process.

Before experiment, the sensor chips were sequentially cleaned with 2% SDS, water and ethanol, dried with nitrogen gas, and then subjected to oxygen plasma treatment for 2 min with an Expanded Plasma Cleaner (model no.PDC-002, Harrick Plasma, Ithaca, NY). Two SLB platforms with different membrane surface charges were created by using the SALB fabrication procedure. DOPC (zwitterionic) and DOPEPC (cationic) lipid powders were dissolved in isopropanol at a final concentration of 10 mg/ml. POPG (anionic) lipid powder was dissolved in ethanol at a final concentration of 5 mg/ml and incubated at 40 °C in order to aid lipid solubilization. Lipid solutions were mixed to a ratio of 70 mol% DOPC and 30 mol% DOPEPC for preparation of positively charged SLB platforms and 70 mol% DOPC and 30 mol% POPG for preparation of negatively charged SLB platforms, and then diluted with isopropanol to a final concentration of 0.5 mg/ml.

As part of the SALB experimental procedure, a measurement baseline was first recorded in aqueous buffer solution (10 mM Tris [pH 7.5] with 150 mM NaCl), followed by exchange with isopropanol solution. Then, 0.5 mg/ml lipid in isopropanol was deposited onto the silicon oxide-coated substrate and allowed to equilibrate for approximately 10 min, followed by exchange with aqueous buffer solution that completed the SLB fabrication process. Next, 0.1 mg/ml BSA was added in order to estimate the fraction of SLB surface coverage as well as to passivate any exposed regions on the silica sensor surface and therefore concentrate on probing lipid membrane interactions. After the SLB formation procedure was completed, the running buffer solution was exchanged with distilled water supplemented with either 10 mM NaCl or 10 mM CaCl₂, and then the nanoparticles or NOM in the appropriate aqueous solution were injected. All QCM-D measurements were conducted under continuous flow conditions, with the flow rate chosen as 100 µL/min for all steps in the bilayer formation and washing processes and 41.8 µL/min for nanoparticles or NOM addition as controlled by a Reglo Digital peristaltic pump (Ismatec, Glattbrugg, Switzerland). During the experiments, the temperature in the measurement cell was maintained at 25.0 ± 0.5 °C. The QCM-D measurement data were collected at the 3rd, 5th, 7th, 9th, and 11th overtone, and the reported QCM-D data were obtained at the 5th overtone and normalized (Δf₀ = Δf/Δf₀). Data processing was performed in the QTools (Q-Sense AB) and OriginPro 8.5 (OriginLab, Northampton, MA) software packages. Where applicable, the measurements were conducted in triplicate and reported as the mean ± standard deviation (s.d.).

3. Results and discussion

3.1. Morphological and zeta potential characterization of nanoparticles

We first characterized the effect of NOM on the size distribution of the NOM-treated nanoparticles in aqueous 10 mM NaCl or 10 mM CaCl₂ salt conditions, as presented in Figs. S1–S6. The intensity-weighted average hydrodynamic diameters of AgNPs alone were 62.6 nm in water, 74.3 nm in NaCl solution, and 111.9 nm in CaCl₂ solution. The presence of NOM in NaCl solution slightly increased the intensity-weighted average hydrodynamic diameters, confirming AgNP aggregation and the ability of NOM to inhibit aggregation. Higher concentrations of NOM decreased the intensity-weighted average hydrodynamic diameters of AgNPs from 94.4 nm (2.5 ppm) to 78.6 nm (10 ppm). In CaCl₂-containing solutions, increasing concentrations of NOM led to a decrease in the intensity-weighted average hydrodynamic diameters of AgNPs from 111.9 nm (0 ppm) down to 77.8 nm (2.5 ppm) and 53.6 nm (10 ppm). While NOM had a moderate effect on inhibiting AgNP aggregation in NaCl solutions, the effect was more pronounced in CaCl₂ solutions wherein significant reductions in AgNP aggregation were observed.

On the other hand, the intensity-weighted average hydrodynamic diameters for nC₆₀NPs were 336.1 nm in water, 310.6 nm in NaCl solution, and 850.1 nm in CaCl₂ solution. A high degree of aggregation is expected for nC₆₀NPs produced by extensive stirring in CaCl₂-containing water, hence confirming past findings [58]. In NaCl solution, the presence of NOM slightly decreased the intensity-weighted average hydrodynamic diameters from 310.6 nm (0 ppm) to 282.0 nm (2.5 ppm) and 237.3 nm (10 ppm). This finding is supported by previous observations that adsorption of NOM onto nC₆₀NPs stabilized nC₆₀NPs by promoting ionic hindrance between nanoparticles [59,60]. As such, NOM was observed to promote disaggregation of nC₆₀NPs in a concentration-dependent manner as indicated by the decrease in particle size with increasing NOM concentration. Similar concentration-dependent disaggregation of nC₆₀NPs was also observed in CaCl₂ solutions, as increasing concentrations of NOM led to decreasing intensity-weighted average hydrodynamic diameters from 850.1 nm (0 ppm) to 806.4 nm (2.5 ppm) and 603.7 nm (10 ppm).

As evidenced by the data above, both types of nanoparticles aggregated in the presence of NaCl and CaCl₂ media, indicating that both monovalent and divalent electrolytes promote the aggregation of nanoparticles. In the case of monovalent electrolytes, this is most likely due to the screening of the electrostatic double-layer that reduces electrostatic repulsion between nanoparticles. Similar effects are also observed with divalent electrolytes and the divalent cations in particular likely also promote aggregation through more specific interactions with anionic functional groups. Following this line, the data further show that NOM can disaggregate AgNPs and nC₆₀NPs and reduce their size in NaCl and CaCl₂ media, as reported in previous studies [58–63]. In the case of nC₆₀NPs, NOM binding likely occurs to aromatic groups on the NP surface [64]. Hence, NOM appears to influence the surface properties of the two types of nanoparticles.

To further characterize the two nanoparticle systems, the pH-dependent zeta potential values of the AgNPs and nC₆₀NPs in the absence and presence of NOM were measured, as reported in Fig. S7. The data indicate that the surfaces of AgNPs and CaCl₂ are negatively charged, and the latter’s surface is more negatively-charged than the former. With increasing pH, the zeta potential values of nC₆₀NPs tended to become more negative down to approximately −43 mV, while the zeta potential values of AgNPs remained similar at around −15 mV across all tested pH conditions. Interestingly, the effect of NOM on the zeta potential values of AgNPs and nC₆₀NPs was quite distinct. For nC₆₀NPs, the NOM coating had negligible effect on the zeta potential values recorded at the different pH conditions. By contrast, the NOM coating increased the negative zeta potential values for AgNPs down to around −30 mV at the different pH conditions. As such, our initial characterization experiments identify that both the size and zeta potential values of the anionic AgNPs and nC₆₀NPs depend on the presence of NOM in the different electrolyte conditions and establish trends for interpreting the QCM-D measurement data.

3.2. Characterization of SLB platform for membrane-nanoparticle investigations

In order to monitor the adsorption kinetics of AgNPs and nC₆₀NPs onto lipid membranes, SLB platforms with different surface charges were first fabricated on silicon oxide substrates by using the SALB technique. Mixtures of 70 mol% DOPC and 30 mol% DOPEPC were used to prepare positively charged SLBs, and mixtures of 70 mol% DOPC and 30 mol% anionic POPG were used to prepare negatively charged SLBs. The QCM-D technique was utilized
in order to characterize the SLB formation processes. Representative QCM-D sensorgrams of the resonance frequency and energy dissipation signals during the SLB formation process are shown in Fig. 2A,B. The measurement baselines were initially recorded in aqueous buffer solution (10 mM Tris, 150 mM NaCl, pH 7.5). After establishing stable measurement signals, isopropanol solution was injected into the measurement cell (Step 1) which led to significant changes in the frequency and energy dissipation due to differences in the bulk density and viscosity of the aqueous buffer versus isopropanol. Then, a freshly prepared 0.5 mg/mL lipid solution composed of either DOPC:DOPEC 70:30 dissolved in isopropanol or DOPC:POPG 70:30 dissolved in isopropanol was injected (Step 2). At the final step of the SLB method, the running isopropanol solution was gradually exchanged with buffer solution (Step 3), resulting in either a negatively or positively charged SLB platform with final frequency and energy dissipation shifts of −25.6 ± 2.0 Hz and 0.6 ± 0.3 × 10⁻⁶, respectively, which are in good agreement with earlier reported values [65–67] indicating successful SLB formation. By applying the Sauerbrey equation that relates the change in resonance frequency to adsorbed mass, the calculated mass of the SLBs was found to range between 400 and 450 ng/cm², which is consistent with SLB formation (Fig. 2C). Moreover, to estimate the surface coverage of the SLB coating on the sensor surface, nonspecific BSA adsorption onto bare and SLB-coated silicon oxides was measured and indicated that the SLBs had greater than 90% surface coverage (Fig. 2D).

3.3. Effect of membrane surface charge on nanoparticle adsorption

Using the SLB platform, the adsorption kinetics of AgNPs and nC₆₀NPs onto positively and negatively charged membranes were investigated. After SLB formation was completed, the running buffer was exchanged with a distilled water solution supplemented with 10 mM NaCl or 10 mM CaCl₂ and the QCM-D measurement responses were normalized to zero and then either 2.5 ppm AgNPs or 450 mM nC₆₀NPs were added in the presence of NaCl or CaCl₂ depending on the experiment. There was a general decrease in frequency shifts associated with the adsorption of nanoparticles onto the positively charged SLBs, indicating nanoparticle adsorption (Fig. 3A,B). There was significantly more adsorption of AgNPs onto positively charged SLBs in the presence of CaCl₂, as indicated by larger final frequency shifts (−17.9 ± 0.5 Hz) compared to the final frequency shifts in the presence of NaCl (−6.0 ± 0.3 Hz). By contrast, the adsorption of nC₆₀NPs onto positively charged SLB platforms was largely independent of the presence of NaCl or CaCl₂, with values around −18 Hz in both cases. These findings are consistent with nC₆₀NP attachment on the surface of the SLB platform [68,69]. In addition, the adsorption kinetics of nC₆₀NPs onto positively charged bilayers were appreciably slower than those of AgNPs. For example, adsorption of nC₆₀NPs reached equilibrium within 40 min while AgNPs reached equilibrium in less than 20 min. Considering that nanoparticle adsorption is diffusion-limited, this difference is likely due to the smaller size of the AgNPs which facilitates greater bulk diffusion (larger diffusion coefficient) and hence quicker adsorption uptake. A direct comparison of the absolute frequency shifts for AgNP and nC₆₀NP adsorption equilibrium is presented according to the different NP type and electrolyte composition (Fig. 3C). Similar absolute frequency shifts were obtained due to adsorption of both AgNPs and nC₆₀NPs in the presence of CaCl₂. By contrast, the absolute frequency shifts for nC₆₀NP adsorption were higher than those of AgNPs in the presence of NaCl. Furthermore, it was observed that both types of nanoparticles had minimal adsorption onto negatively charged bilayers in the presence of NaCl or CaCl₂, indicating that membrane association is likely mediated by attractive electrostatic forces (Fig. 3D,E). Hence, as
summarized in Fig. 3F, there were no effects of NaCl versus CaCl₂ on the absolute frequency shifts for nanoparticle adsorption onto negatively charged SLBs.

3.4. Effect of NOM-coated SLBs on nanoparticle adsorption

Fig. 4 presents the QCM-D measurement data for AgNP and nC₆₀NPs adsorption onto NOM-coated, positively charged SLBs, as outlined in Scenario 1. The SLB platforms were first coated with different concentrations of NOM, and then the nanoparticles were added to the NOM-coated SLBs. In NaCl conditions, NOM adsorbed onto the positively charged SLBs largely independent of NOM concentration, with absolute frequency shifts around −5.1 ± 1.3 Hz (Fig. 4A,B). Interestingly, the presence of NOM coatings on the positively charged SLB platforms prevented subsequent adsorption of both AgNPs and nC₆₀NPs. There was no further change in the measurement responses when NOM was reintroduced into the system. On the other hand, when NOM was added in the presence of CaCl₂, the frequency shifts depended on the NOM concentration (Fig. 4C,D). For 2.5 ppm NOM concentration, there was a final frequency shift around −5.3 ± 0.5 Hz, whereas the frequency shift increased to around −14.3 ± 7.0 Hz at 10 ppm NOM concentration. As in the NaCl case, the NOM coating inhibited AgNP adsorption although to a lesser extent. Indeed, while AgNP adsorption in the absence of NOM yielded frequency shifts around −18 Hz, AgNP adsorption was significantly reduced by almost 73% on NOM-coated SLBs, with frequency shifts around −5.7 Hz (Fig. 4C). By contrast, NOM-coated SLBs appeared to inhibit nC₆₀NP adsorption in the presence of CaCl₂. In this particular case, positive frequency shifts were observed, indicating that nC₆₀NPs interacted with NOM components and removed them from the SLB surface (Fig. 4D). Subsequent addition of NOM led to negative frequency shifts that support nC₆₀NPs removed some of the SLB-attached NOM components in the preceding step. Collectively, the data support that NOM adsorbs onto positively charged SLBs in both NaCl and CaCl₂ conditions, and NOM-coated SLBs can partially or fully inhibit NP adsorption. It was also observed that nC₆₀NPs, but not AgNPs, had a specific interaction with NOM components, as indicated by the removal of NOM components from the SLB surface.

In marked contrast, NOM adsorption onto negatively charged SLBs was negligible in NaCl conditions and, as a result, subsequent NP adsorption onto the SLB surfaces was also minimal (Fig. 5A,B). In the presence of CaCl₂, there was some NOM adsorption, especially at the 10 ppm NOM concentration, down to an approximately −4 Hz shift (Fig. 5C,D). While the NOM coating did not promote AgNP adsorption onto the negatively charged SLBs, it did promote nC₆₀NP adsorption down to around −7 Hz under CaCl₂ conditions. These data further support that there is a particularly strong attraction between NOM components and nC₆₀NPs, and that attached NOM components under CaCl₂ conditions can promote nC₆₀NP adsorption while CaCl₂ conditions alone are insufficient to do so.

3.5. Effects of NOM-coated nanoparticles on nanoparticle adsorption

Fig. 6 presents the QCM-D measurement data for the adsorption of NOM-coated AgNP and nC₆₀NPs adsorption onto positively charged SLBs, as outlined in Scenario 2. Prior to the experiment, AgNPs or nC₆₀NPs were mixed with NOM in the presence of 10 mM NaCl or CaCl₂ and subsequently added to the SLB platforms. In the NaCl condition, the adsorption of NOM-treated AgNPs led to a frequency shift of around −7 Hz and the magnitude was largely independent of the NOM concentration (Fig. 6A). By contrast, there was minimal adsorption of the NOM-treated nC₆₀NPs under the same condition (Fig. 6B). In the CaCl₂ condition, there was significantly greater adsorption of NOM-treated AgNPs and the magnitude of the frequency shift depended on the NOM concentration. The final frequency shift for NOM-treated
AgNP adsorption was around $-17.9 \pm 0.5$ Hz and $-39.1 \pm 6.2$ Hz at 2.5 ppm and 10 ppm NOM concentrations, respectively (Fig. 6C). Slight adsorption of the NOM-treated nC$_{60}$NPs was observed in the CaCl$_2$ condition, with minor frequency shifts around $-4$ Hz (Fig. 6D). By contrast, NOM-treated AgNPs and nC$_{60}$NPs did not adsorb onto negatively charged SLBs (Fig. 7). Taken together, the findings sup-
Fig. 6. Adsorption of NOM-coated AgNPs and nC60 NPs onto positively charged SLB platforms. QCM-D measurements track resonance frequency shifts. 2.5 or 10 ppm NOM + 2.5 ppm AgNPs or 450 nM nC60 NPs were added to the SLB platform. Addition of NOM-coated (A) AgNPs or (B) nC60 NPs in 10 mM NaCl. Addition of (C) AgNPs or (D) nC60 NPs in 10 mM CaCl₂.

Fig. 7. Adsorption of NOM-coated AgNPs and nC60 NPs onto negatively charged SLB platforms. QCM-D measurements track resonance frequency shifts. 2.5 or 10 ppm NOM + 2.5 ppm AgNPs or 450 nM nC60 NPs were added to the SLB platform. Addition of NOM-coated (A) AgNPs or (B) nC60 NPs in 10 mM NaCl. Addition of (C) AgNPs or (D) nC60 NPs in 10 mM CaCl₂.
port that NOM has a minor effect on the interaction between AgNPs and positively charged SLBs, whereas NOM has a stronger influence on the interaction between nC60NPs and positively charged SLBs. On the other hand, the interaction between the two types of NPs and negatively charged SLBs was negligible.

While NOM has been shown to prevent iron and silver nanoparticles from adhering to cell membranes and hence limited toxicity in certain contexts, our findings provide a more complete mechanistic picture of how NOM can either promote or inhibit membrane-nanoparticle interactions depending on the membrane surface charge and electrolyte conditions [70,71]. Indeed, the influence of NOM on nanoparticle attachment was only previously studied under a limited set of monovalent salt conditions. As discovered herein, under certain conditions, NOM can increase nanoparticle adsorption onto lipid membranes and the specific effects depend on the type of nanoparticle and lipid membrane under consideration. Looking forward, there is exciting potential to further understand how NOM influences the biological activities of nanoparticles under different electrolyte conditions and to connect the physiochemical insights obtained herein with broader environmental consequences.

4. Conclusion

As presented herein, we investigated how membrane surface charge influences the adsorption of AgNPs and nC60NPs onto SLB platforms under different electrolyte concentrations, and consequently how NOM influences these membrane-NP interactions. While the electrolyte conditions are simplified models of complex environmental systems, they offer insight into how monovalent and divalent cations influence NP adsorption onto charged lipid membranes. It was observed that bare, negatively charged AgNPs and nC60NPs adsorb onto positively charged SLBs, whereas they do not adsorb onto negatively charged lipid bilayers—a finding consistent with attractive electrostatic forces mediating membrane association. For AgNPs, membrane adsorption onto positively charged SLBs was enhanced in the presence of divalent cations and NOM had differential effects on the membrane-AgNP interaction under these conditions. Pretreatment of SLBs with NOM partially inhibited subsequent AgNP adsorption, while NOM-coated AgNPs exhibited appreciably greater adsorption. On the other hand, the extent of nC60NP adsorption onto positively charged SLBs was similar in the presence of monovalent or divalent cations. Strikingly, NOM treatment of SLBs or nC60NPs almost fully inhibited membrane adsorption in monovalent conditions, as did NOM of nC60NPs in divalent conditions. Overall, the data suggest that nC60NPs have a particularly strong interaction with NOM components that can either impede adsorption (via NOM-NP interaction) or promote adsorption (via NOM-SLB interaction), whereas the effects of NOM on AgNP adsorption are relatively minor. It should be noted that the ionic strength conditions used in this study were relatively low, and charge shielding under higher ionic strength conditions would reduce the strength of electrostatic forces alone. Considering that NOM is inherently present in many important environmental systems, the experimental scenarios explored in this work provide insight into how NOM may influence membrane-nanoparticle interactions and suggest that these effects are linked to the affinity of NOM components to lipid membrane interfaces and the particular type of nanoparticle being considered.

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