Lipid Corona Formation from Nanoparticle Interactions with Bilayers and Membrane-Specific Biological Outcomes


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ABSTRACT. While mixing nanoparticles with certain biological molecules can result in coronas that afford some control over how engineered nanomaterials interact with living systems, corona formation mechanisms remain enigmatic. Here, we report spontaneous lipid corona formation, i.e. without active mixing, upon attachment to stationary lipid bilayer model membranes and bacterial cell envelopes, and present ribosome-specific outcomes for multi-cellular organisms. Experiments show that polycation-wrapped particles disrupt the tails of zwitterionic lipids, increase bilayer fluidity, and leave the membrane with reduced ζ-potentials. Computer simulations show contact ion pairing between the lipid headgroups and the polycations’ ammonium groups leads to the formation of stable, albeit fragmented, lipid bilayer coronas, while microscopy shows fragmented bilayers around nanoparticles after interacting with Shewanella oneidensis. Our mechanistic insight can be used to improve control over nano-bio interactions and to help understand why some nanomaterial/ligand combinations are detrimental to organisms, like Daphnia magna, while others are not.

Main Text. The propensity of biological species to form coronas around nanoparticles\textsuperscript{1-4} has been used for preparing engineered nanomaterials that can be distributed in biological systems with some control\textsuperscript{5-10}. While protein coronas in particular have been studied extensively\textsuperscript{1-3,5,6}, our understanding of lipid coronas is now just beginning to emerge\textsuperscript{11,12}, especially of those formed upon unintended nanoparticle contacts with living cells. The protein and lipid corona formation mechanisms appear to differ substantially, as “hard” and “soft” coronas\textsuperscript{13}, typical for the former, have not been described for the latter\textsuperscript{3}. While pulmonary surfactants can lead to lipid corona formation\textsuperscript{3,14-16}, it is unclear whether the process can also occur in the more general case of lipid bilayers, which lack the considerable dipole potential carried by their monolayers counterparts\textsuperscript{17}. Some precedent for lipid corona formation from cellular bilayer membranes exists in the budding of viruses, which do not
possess the machinery to produce their own lipids but instead use charged patches on proteins for sheathing their RNA with a membrane scavenged from the host cell membranes\textsuperscript{18,19}. Likewise, computer simulations indicate coronas of certain lipids may be stable on certain particles\textsuperscript{20-22}, but the roles of specific functionalization patterns or charge remain poorly understood\textsuperscript{23}.

Here, we ask whether lipid coronas can form spontaneously, i.e. without active mixing, around nanoparticles when they interact with immobilized lipid bilayer membranes, such as those surrounding cells fixed within the extracellular matrix of a living organism. To test whether corona formation differs for particles having diameters close to or larger than the bilayer thickness, we examine spherical gold metal nanoparticles (AuNPs) having diameters of 4 and 15 nm, chosen as the methods to synthesize, functionalize, and characterize them are well established\textsuperscript{24}. Complementary experiments using 15-nm diameter nanodiamond particles inform on the generality of the interactions across some varied range of core compositions.

We first coordinate advanced imaging and spectroscopy with molecular dynamics simulations\textsuperscript{25} to probe the lipid corona at an unprecedented level of detail over a wide spectrum of model systems ranging from supported to suspended lipid bilayers to \textit{Shewanella oneidensis}, an important earth dwelling bacterium. We then connect our findings to mitochondria-specific third-generation sequencing data obtained after exposing the water flea \textit{Daphnia magna}, chosen as a well-characterized eukaryote, to the same particle formulations used in our bilayer and bacterial models.

Our study focuses largely on particles wrapped in the cationic encapsulation polyelectrolyte poly(allylamine hydrochloride) (PAH). Particles functionalized with the anionic ligand mercaptopropionic acid (MPA) are probed as well but show generally little interaction with bilayers and biological systems surveyed. As assessed by fluorescence\textsuperscript{26}, double centrifugation, the method used to prepare the particles used in our vibrational sum frequency
generation (SFG) studies, resulted in a fraction of 0.27 ± 0.04 for the free PAH mass concentration when compared to the mass concentration of cationic AuNPs in solution. Special attention was therefore given to the role of unbound ligands on the systems surveyed. Our idealized model systems focus on particle interactions with supported and suspended lipid bilayers composed of the lipid 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), given the direct relevance of the zwitterionic PC headgroup to biological membranes²⁷,²⁸. Additional work probed bilayers formed from DMPC mixed with 10% lipids containing negatively charged headgroups in the form of 1,2-dimyristoyl-sn-glycero-3-phospho-1'-rac-glycerol (DMPG). PC and PG lipid from pulmonary surfactant monolayers have also been reported to be preferentially taken up by carboxylated carbon nanotubes in pulmonary exposure routes.¹⁶ Other lipid combinations we surveyed²⁹-³¹ did not show the effects reported herein.

**Results and Discussion.**

**Lipid tail disruption in zwitterionic lipid bilayers.** Vibrational SFG spectra of lipid bilayers report on molecular structure and order within them³²-³⁴, in our case the molecular environment of the lipid's C–H oscillators. Indeed, the lipid alkyl tails within the supported bilayers produce strong signals (Fig. 1a, top spectrum) near 2870, 2920, and 2970 cm⁻¹, consistent with the presence of well-formed bilayers. In contrast, the ligands on the AuNPs used in this study are spectroscopically silent (see Supplementary Section 2C), indicating they are likely to be highly disordered under our experimental conditions.

Following exposure of the bilayer at 21±1 °C to 10 nM solutions of the 4-nm sized anionic gold particles, we find only negligible changes in the spectral lineshapes and intensities (Fig. 1a, bottom spectrum). In contrast, exposure of the supported lipid bilayers to the cationic particles coincides with the vanishing of the sharp vibrational features from the lipids (Fig. 1b). A new, spectrally broad signal is observed that is reminiscent of non-resonant sum
frequency responses produced by thin gold films\textsuperscript{35}, albeit with much smaller intensities, indicating the presence of gold cores at the interface. This outcome is observed for particle concentrations as low as 1 nM. Experiments using bilayers formed from pure DMPC (Supplementary Figure 2a) indicate negatively charged DMPG lipids are not necessarily needed to promote the effect. Moreover, the outcome appears to be invariant with particle size or core type over the range surveyed, as PAH-wrapped 15-nm sized gold and nanodiamond particles show qualitatively the same result (Fig. 1c), albeit with a smaller response from the gold cores than for the 4-nm sized AuNPs. This outcome is consistent with the fourfold smaller mass gain for the larger vs. the smaller particles (\textit{vide infra}). Controls (see Supplementary Figures 2b and 2c) indicate the loss of bilayer structure and order upon exposure to the cationic particles does not depend on ionic strength within the range surveyed. Likewise, controls using free PAH do not show altered spectral responses from the bilayer unless PAH is present in a 100-fold excess over the nanoparticle concentrations surveyed here (Fig. 1d). Other free polycations surveyed also disrupt bilayer structure at comparably high cation concentrations (Supplementary Fig. 2d and 2e).

**High coverage of cationic nanoparticles.** To quantify the amount of cationic AuNPs on the membranes, we applied quartz crystal microbalance with dissipation (QCMD) monitoring to estimate mass gains (Fig. 2a) and resonantly enhanced second harmonic generation (SHG) measurements to specifically probe the gold cores (Fig. 2b). Mass gains were not detectable above the \(\sim 2 \text{ ng \cdot cm}^{-2}\) limit of detection of the instrument for the anionic particles interacting with supported lipid bilayers rich in DMPC. This outcome is consistent with result reported for supported lipid bilayers formed from pure DOPC\textsuperscript{30} as well as pure POPC\textsuperscript{31} exposed to MPA-functionalized AuNPs. Yet, the SHG measurements provided evidence for the presence of some particles\textsuperscript{36}, albeit presumably at much smaller surface coverages than what is observed for the cationic particles. We conclude that the 4-nm sized anionic particles interact
weakly, if at all, with the bilayers studied under these experimental conditions.

In contrast to the anionic particles, the QCM-D experiments revealed considerable acoustic mass gains when the bilayers were exposed to cationic AuNPs (Fig. 2a). Attributing the surface mass gain solely to the AuNPs, this mass would correspond to roughly $1.9 \pm 0.2 \times 10^{12}$ particles·cm$^{-2}$. Because the acoustic mass includes hydrodynamically coupled solvent, the number of particles per cm$^2$ may be less. As shown in Supplementary Fig. 5, the 15-nm diameter cationic particles led to mass gains corresponding to $5.0 \pm 0.4 \times 10^{11}$ particles·cm$^{-2}$ if the acoustic mass were attributed solely to the nanoparticles. Rinsing the supported lipid bilayers with nanoparticle-free solution after attachment of PAH-AuNPs resulted in decreases in energy dissipation and small decreases in mass, which may indicate release of PAH-AuNPs, a small fraction of the bilayer, or some combination of the two, from the surface (see Supplementary Fig. 5).

These results, together with the spectroscopic observations presented earlier, point towards an interaction mechanism that is specific to a combination of bilayer membranes containing lipids having transition temperatures close to room temperature and nm-sized nanoparticles wrapped with polycations, as opposed to with just the free polymer wrapping.

**Nanoparticles leaving membrane carry lower $\zeta$-potential.** Motivated by the lines of evidence pointing to the association of membrane lipids with the cationic nanoparticles, we proceeded to collect 4- and 15-nm core PAH-AuNPs after interacting them with the supported lipid bilayers prepared from a 9:1 mix of DMPC:DMPG, measured their electrophoretic mobilities, and calculated their apparent $\zeta$-potentials using the Smoluchowski approximation. PAH-AuNPs that had passed over bare SiO$_2$-coated sensors were used as controls. Fig. 2c shows the $\zeta$-potentials of the 4- and 15-nm cationic particles measured in the eluent of the QCM-D flow cell (100 mM salt, pH 7.4, 10 mM Tris buffer) decreased from $+32 \pm 2$ mV and $+36 \pm 1$ mV, respectively, in the absence of lipids, to $+24 \pm 2$ mV and $+26 \pm$
1 mV, respectively, upon rinsing from the supported lipid bilayers. These results are consistent with the notion that the nanoparticles collected following interaction with the supported lipid bilayer acquired negatively charged species, likely DMPG, as further supported by calculations (vide infra).

**Nanoparticles increase bilayer fluidity 3-4 fold.** Single molecule fluorescence microscopy, which we used to track the trajectories of individual phospholipid molecules (Fig. 3a-b), showed a significant increase in the local diffusion coefficients of individual lipids, from an average of $0.0095 \pm 0.0062$ to $0.035 \pm 0.011 \, \mu m^2 \, s^{-1}$, after interaction with the cationic particles. A histogram analysis shows that the average diffusion coefficients increase substantially because of the presence of a large new lipid population, possibly co-localized with the bound particles, that is distinct from the one having coefficients equivalent to the control (Fig. 3c).

The observed increase in the molecular diffusion coefficients indicates an increase in the local fluidity of the gel-phase 9:1 DMPC:DMPG bilayer. We rationalize this outcome by the change in the tilt angle of the electric dipole of the choline headgroup induced by interaction with a cationic amine in the coating\textsuperscript{37}, increasing the area per lipid head group and thereby decreasing lipid packing density. This interaction represents an initial step in the process of lipid extraction. Removal of lipids from the bilayer would increase the free area per lipid in the remaining bilayer\textsuperscript{38}, consistent with the observed increase in diffusivity.

**Computer simulations show stable bilayers form on nanoparticles wrapped in cationic polyelectrolytes.** Molecular dynamics simulations using coarse-grained models resulted in the formation of a lipid corona (Fig. 4a) and provided molecular-level information regarding its structure and composition (Fig. 4b). The structure of the coarse-grained PAH-AuNP was validated using atomistic simulations (Fig. 4c). Microsecond-long simulations show significant bilayer bending upon nanoparticle attachment (see Supplementary Note V and
Given that potential of mean force calculations show lipid extraction by PAH models to be associated with a significant free energy penalty (see Supplementary Fig. 14), the extraction process of multiple lipids for an entire PAH-wrapped particle interacting with the bilayer is difficult to sample. Therefore, we examined lipid corona formation by starting from a random distribution around a PAH-wrapped 4-nm sized gold particle. The lipid molecules are observed to quickly self-assemble into ribbons and micelles, which then attach to the nanoparticle during ~100 ns (see Supplementary Fig. 15).

This result is comparable to what has been reported for the hypothetical case of a bare (ligand free) gold particle interacting with DPPC but, unlike in that work, shows the lipid bilayer is not uniformly distributed around the particle but instead patchy. An analysis of ion/lipid distributions around the nanoparticle indicates that the cationic PAH polymer plays the key role of attracting lipids by contact ion pairing between the ammonium groups and phosphate and glycerol groups (Supplementary Table 1). Besides DMPC, the positive charges also recruit anionic lipids (DMPG), leading to a higher fraction of DMPG (32% and 43% for the two simulated systems, respectively) in the lipid corona when compared to the bulk concentration (10%). The dimensionality of the lipid corona model is consistent with observations from our electron microscopy analysis and measured changes in the hydrodynamic radii of the PAH-wrapped nanoparticles following corona formation described below.

**Nanoparticle-vesicle suspensions form aggregated superstructures featuring lipid headgroup association with particle wrapping moieties.** To investigate whether the cationic AuNPs also interact with suspended, as opposed to supported, lipid bilayers, we exposed suspended vesicles to PAH-AuNPs. $^1$H NMR spectra of vesicles formed from a 9:1 mixture of DMPC and DMPG show that the ammonium headgroup protons (−N−CH$_3$, 3.16 ppm) disappear upon addition of 4 nm PAH-AuNPs (Fig. 5a). The hydrocarbon proton
resonances are broadened beyond detection because the lipids exist in the lipid ordered phase at the laboratory temperature at which the experiment was run (20 °C, below the transition temperatures of DMPC (24 °C) and DMPG (23 °C)). The disappearance of the headgroup proton resonance can best be explained by attachment of the headgroup to another species, presumably PAH, which would immobilize the protons and broaden the resonance into the baseline. Indeed, the PAH-AuNP protons shift significantly upfield after interaction with the lipid vesicles, suggesting that the PAH layer is now sandwiched between the gold surface and an additional species. Taken together, the NMR spectra support the conclusions from the supported lipid bilayer studies that lipids strongly associate with the surface of the cationic AuNPs.

Additional experiments using dynamic light scattering and fluorescence microscopy of the nanoparticle-vesicle suspensions reveal the formation of aggregated superstructures: the hydrodynamic diameters of the vesicles double upon addition of the cationic particles to 200 ± 10 nm and further increase in the subsequent two hours to >1 µm. Spinning disk confocal fluorescence microscopy experiments show the rapid build-up of sub- and super-µm-sized agglomerates that are immobilized at the solution/glass interface of the imaging cell (Fig. 5b), indicating the newly formed structures are considerably stickier than the PAH-wrapped nanoparticles or the lipid vesicles themselves. These microscopy images also show that the particles that produce fluorescence signals have the labels far enough from the gold core that the fluorescence is not quenched. Yet, we find that many of the fluorescent features are seemingly co-localized with similarly sized features observed in the bright field (Supplementary Fig. 8), which visualizes particles that are agglomerated to µm-sized structures. This effect is seen for many of the sub-micron sized features. These features are not formed when PAH-wrapped nanoparticles are brought in contact with vesicles formed from a 9:1 mix of DOPC and DOTAP (Supplementary Fig. 6), when 9:1 DMPC:DMPG
vesicles are exposed to 10 nM free PAH, or when 9:1 DMPC:DMPG vesicles are brought in contact with anionic, MPA-functionalized AuNPs (Supplementary Figure 7).

**Molecular connections to decreased bacterial survival and respiration rates and to changes in ribosomal gene expression in eukaryotes.** To evaluate biological consequences of lipid corona formation, we considered the major motifs in cell surface chemistry presented by different types of organisms. Specifically, we exposed single cell organisms, namely the gram-negative bacteria *Shewanella oneidensis*, and the aquatic multicellular organism *Daphnia magna*, to PAH-AuNPs. For the former, TEM micrographs show clear evidence of cell lysis (Fig. 6a-c) and particle clustering near spilled, membrane-free cytoplasmic content from cells with deformed cell walls. Closer inspection of these nanoparticle clusters at higher magnification reveals multiple segments of lipid bilayer-like structures (Fig. 6b-c), that are 4–10 nm thick, consistent with the expected thickness of two pieces of lipid bilayer stuck back to back. These TEM images provide compelling visual evidence that the cationic particles studied here acquire fragmented bilayer structures, akin to the lipid corona results obtained in the coarse-grain simulations (Fig. 4). We hypothesize that these results may be related to observed dose-dependent decreases in the viability of *Shewanella* that cannot be accounted for using the free PAH ligand at particle-equivalent concentrations alone.

Our findings of lipid corona formation around the cationic particles can also be viewed in light of our previously reported work on the consequences of exposing *Daphnia magna* to the same anionic and cationic nanoparticle formulations used in the supported lipid bilayer model studies presented in this work. *Daphnia* have been shown to be affected by PAH-AuNPs, while there is no impact of the PAH polymer alone even at concentration 100 times higher, indicating a nanoparticle specific impact. Even at concentrations as low as 1 µg/L daphnids have a reduced survival rate and reproduction where neither the free PAH polymer nor anionic nanoparticles have an impact. These same PAH-AuNPs have been shown to
cause oxidative stress in daphnid tissues and alter the expression of individual genes, in the form of RNA production, specifically those associated with metabolism, reproduction, and growth.

To further explore whether the interaction between the nanoparticles and organism cell membranes, as seen in the lipid bilayer experiments, is one possible cause for a decrease in daphnid reproduction, we analyzed here *Daphnia* chronically exposed to PAH-AuNPs for 21 days using third-generation Pacific Biosciences RSII sequencing. Global gene expression patterns revealed the up regulation of a number of pathways that differed significantly from those of just the PAH polymer alone (Fig. 6d). This finding indicates particle-membrane interactions specific to Daphnia’s mitochondria, including its transmembrane proteins, electron transport, and cytochrome C proteins/components. PAH ligand controls, in contrast, show up regulation of different genes associated more with ion balance and energy metabolism, namely triglyceride storage, ribosomal proteins, and muscle function, further emphasizing the importance of studying not just the particle but also the free ligands and wrappings surrounding them.

**Conclusions**

Our work presents unprecedented views of lipid coronas forming spontaneously over a broad spectrum of biological membrane systems that range from idealized lipid bilayer models to bacterial cell envelopes to eukaryotic cytoplasmic membranes. We “zoom” out from atomistic information derived from idealized model studies, which indicate contact ion pair formation as a key lipid corona formation mechanism, all the way to biological outcomes obtained from Third-gen sequencing. As summarized in the table shown in Fig. 6, lipid coronas were shown to form spontaneously when polycation-wrapped 4- and 15-nm diameter nanoparticles, be their cores made of gold or nanodiamond, interact with a variety of bilayer membranes, be they immobilized, suspended, or biologic in nature. Molecular connections to
laboratory observations of decreased survival and respiration rates and changes in ribosomal
gene expression were made, all the while paying careful attention to free ligand studies to
identify the particle-specific effects.

Taken together, the results described above provide several concrete, as opposed to
hypothetical, lines of evidence regarding nanoparticle-lipid interactions that may help us
understand and predict, from a molecular level, why some nanomaterial/ligand combinations
are detrimental to cellular organisms while others are not. We caution that the implications of
our results are limited to the nanoparticle formulations and lipid compositions surveyed here.
Yet, initial experiments with two environmentally relevant organisms support the notion that
lipid corona formation may also be critically important in even more complex biological
systems. We believe that our approach, where consideration of both the nanomaterial and the
biological membrane are on an equal footing, will increase our ability to predict the impact
that the increasingly widespread use of engineered nanomaterials in industrial applications
and consumer products has on the fate of these materials once they enter the environment and
the food chain, which many of them may eventually do.

3105 of 3000 Words Max for Intro, Results, Discussion

Methods

Materials, nanoparticle synthesis, bilayer preparation, and characterization. The
synthesis, functionalization, and characterization of the nanoparticles and bilayers studied
here have been described in our prior work and are discussed in Supplementary Note 1.

Single molecule trajectories. Supported lipid bilayers were formed within 35/22 mm #1.5
glass bottom dishes (Willco Wells). Dishes were rinsed with ultrapure water (18 MΩ·cm;
MilliQ Advantage A10, Millipore), dried with N₂, and cleaned in a UV/Ozone chamber (PSD
Pro Series, Novascan) for 20 min. Cleaned dishes were equilibrated with a solution
containing 0.1 M NaCl buffered to pH 7.4 with 0.010 M Tris for at least 1 h. Suspensions of
small unilamellar vesicles (SUVs) (0.0625 mg·mL⁻¹ 9:1 DMPC:DMPG with 0.0001 mol% fluorescent Atto 647N DOPE (Atto-Tec; λ_ex = 642 nm; λ_em = 667 nm) in the same buffered solution used to equilibrate the dishes) were introduced to the dish. After bilayer formation, the solution in the dish was exchanged five times with 2 mL aliquots of the buffered solution. Single molecule fluorescence imaging of supported lipid bilayers was conducted before and after introduction of 1 nM PAH-AuNPs. Imaging was conducted on an Olympus IX71 inverted microscope with a UPlanSApo 100× 1.4 NA oil-immersion objective. Fluorescence emission was acquired with an Andor iXon Ultra EMCCD operated at 6.9 Hz. The excitation source was a 643 nm pumped laser (CL-2000, Crystal Laser). A series of at least 500 frames was collected at three spots (250 × 250 pixels, 40 × 40 µm) before and after 1 nM PAH-AuNP addition, and this was repeated on three different supported lipid bilayers. MATLAB (R2015a, MathWorks) was used to determine the trajectories of individual lipid molecules and lateral lipid diffusion coefficients. Individual fluorescent molecules were identified as unique, diffraction limited points with the expected fluorescence intensity of single dye molecules, as predetermined by spin-coating the dye molecules on a glass coverslip and imaging them using the same settings. These points were tracked from one frame to the next to reconstruct the trajectories of individual lipid molecules. The lateral diffusion coefficient, $D_L$, was determined from the mean squared displacement, MSD, and the time between frames, $t$, using the relation $MSD = 4D_Lt$.

**Computer Simulations.** To understand how lipid corona is formed around gold nanoparticles wrapped with positively charged PAH polymers, we use molecular dynamics simulations with the POL-MARTINI coarse-grained force field⁴⁸,⁴⁹. The gold nanoparticle is constructed as a 4-nm diameter sphere with fcc lattice structure, where all beads are treated as the C1 type (the most hydrophobic in the MARTINI force field) with 4.7 Å as the van der Waals radius; a similar model for the gold nanoparticle has been employed successfully in
previous coarse-grained molecular dynamics simulations of functionalized nanoparticles and their interactions with lipid membranes\textsuperscript{50}. All beads beyond 1.8 nm from the center of the nanoparticle are assigned with a negative charge of -1, qualitatively mimicking the effect of surface passivation by citric acids. The force field of PAH polymer is established by combining the carbon backbones of polystyrene\textsuperscript{51} and the top bead of the lysine side chain from MARTINI\textsuperscript{48}. Two PAH models that contain 160 and 200 monomers, respectively, are constructed, since exploratory simulations indicate that these chain lengths lead to a significant degree of surface coverage of the nanoparticle once it is wrapped by the PAH polymer. All monomers are assumed to be positively charged, which likely leads to an overestimated charge of PAH\textsuperscript{52}. The interfacial electrostatic potential of the nanoparticle in salt solution is about +100 mV at the van der Waals surface of the PAH polymer that wraps the gold nanoparticle, thus the \( \zeta \)-potential is expected to be substantially lower, in the range of 50 mV, which can be compared to the experimental measured value of +30 mV\textsuperscript{29}. Thus, we expect that the current model is appropriate for exploring the qualitative nature of the lipid corona. The calibration of the coarse-grained model using an all-atom representation of the PAH interacting with the gold nanoparticle is described in Supplementary Section 5.

To study the organization of lipids near the PAH-wrapped gold nanoparticle, self-assembly simulations are carried out by randomly placing lipids around a gold nanoparticle wrapped with a PAH chain that contains 160 or 200 monomers. The solution contains 423 copies of DMPC molecules and 47 copies of DMPG molecules, and 0.02 M NaCl solution with MARTINI polarizable water model. The dimension of the simulation box is $15 \times 15 \times 15$ nm\textsuperscript{3}. The time step of the simulations is 20 fs. Electrostatic interactions are treated with the particle mesh Ewald (PME) method\textsuperscript{53}, with a Fourier spacing of 0.12 nm. Van der Waals interactions are treated by the shift scheme, with a cut-off distance of 1.2 nm and the switching function turned on at 0.9 nm. Isothermal-isobaric (NpT) simulations are carried
out at 300 K and 1.0 bar using the Berendsen thermostat and pressure coupling (compressibility of $3 \times 10^{-5}$ 1/bar)$^{54}$, calculations are repeated using the semi-isotropic pressure coupling and the qualitative results remain the same. The system is subject to 20,000 steps of steepest descendant energy minimization before 1 ns of NVT equilibration and 400 ns of production simulations. Several independent simulations are carried out to ensure the robustness of the qualitative trends.

**Biological studies.** As described in Supplementary Note 6, *Shewanella oneidensis* cells suspended in a HEPES buffer (2 mM HEPES and 25 mM NaCl at pH 7.40) were exposed to 0.5 mg·L$^{-1}$ PAH-AuNPs for 10 minutes, fixed with 2.5% glutaldehyde in 0.1 M cacodylate buffer, dehydrated in a graded ethanol series, and embedded in an epoxy resin. The thin-sectioned resin sample (~60-nm-thick) was stained with uranyl acetate and lead citrate prior to TEM imaging on a Tecnai T12 electron microscope operating at 120 kV.

Standard Pacific Biosciences IsoSeq cDNA synthesis and library preparation were performed with the addition of adding PacBio barcodes during cDNA synthesis. cDNA was synthesized using the SMARTer PCR cDNA Synthesis Kit (ClonTech) and amplified by PCR. All barcoded cDNAs were pooled equally by mass. Following BluePippin (Sage Science) size selection, pooled amplified cDNA was divided into four size bins: 1-2kb, 2-3kb, 3-6kb. Size selected cDNA was further PCR amplified, enzymatically repaired and ligated to a PacBio adapter to form the SMRTbell Template. Templates were annealed to sequencing primer, bound to polymerase, and then bound to PacBio Mag - Beads and SMRTcell sequenced.

Data was primarily analyzed through barcoded use of the Pacific Biosciences IsoSeq pipeline, in which asymmetric barcodes were employed where the reverse barcode was of ClonTech design. Execution of IsoSeq was performed through SMRTanalysis version 2.3, where only isoforms possessing at least 2 full passes around the SMRTbell adapters were
permitted to pass the initial consensus sequencing correction step (e.g. CCS1 or Reads-of-Insert). The resulting isoforms were then submitted to the ICE and Quiver algorithms to produce polished, high-quality isoforms clustered into putative gene families. IsoSeq, by method design, relies on a strategy of size-fractioning genes into three size-bins: 1-2 kb, 2-3 kb, and 3+ kb. The resulting sets of genes were aligned against a composite transcriptomic reference composed of *D. magna, D. pulex, C. elegans,* and *D. melanogaster* via BLASTX; these alignment results were used to construct a *Daphnia* reference consisting of per-size-bin, per-cluster gene identifiers. This reference was then relationally joined with per-isoform barcoding results to generate lists of annotated genes expressed within each experimental sample.

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References


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**Author Contributions**

LLO, JMT, AV, ESM, SL, TRK, IG, ACM, MD, TL, SRW, SL, HO, MH, MDT, VF, KRH, JD, JB, and GD performed the experiments. LZ, JH, GC, RH, and QC performed the computational work. The data was analyzed and the manuscript was written with substantial contributions from all authors.

**Additional information**

Supplementary material accompanies this paper at http://www.nature.com/

**Competing financial interests:** The authors declare no competing financial interests.
List of Figures.

Figure 1 | Molecular order within lipid bilayers changes upon interaction with PAH-wrapped particles. (a) *ssp*-Polarized SFG spectra obtained from supported lipid bilayers prepared from a 9:1 mixture of DMPC:DMPG held at 100 mM NaCl and pH 7.4 (10 mM Tris buffer) before (top) and after (bottom) introduction of MPA-coated 4-nm sized gold metal nanoparticles. (b) SFG responses obtained from supported lipid bilayers prepared from a 9:1 mixture of DMPC:DMPG held at 100 mM NaCl and pH 7.4 (10 mM Tris buffer) following exposure to 4-nm sized PAH-wrapped particles at concentrations indicated. (c) SFG responses obtained from supported lipid bilayers prepared from a 9:1 mixture of DMPC:DMPG held at 100 mM NaCl and pH 7.4 (10 mM Tris buffer) following exposure to PAH-wrapped gold and nanodiamond particles having the indicated core diameters. (d) SFG responses obtained from supported lipid bilayers prepared from a 9:1 mixture of DMPC:DMPG held at 100 mM NaCl and pH 7.4 (10 mM Tris buffer) following exposure to free ligand at concentrations indicated.

Figure 2 | PAH-wrapped nanoparticles form large surface coverages on supported lipid bilayers and lower their ζ Potentials. (a) Acoustic mass gains, determined from QCM-D measurements, of 10 nM solutions of PAH-(blue) and MPA-(red) coated 4-nm AuNPs before, during (20 min), and after contact with supported lipid bilayers composed of 9:1 DMPC:DMPG maintained at pH 7.4, 10 mM Tris buffer, and 100 mM salt. (b) Fractional SHG signal gain as a function of concentration of PAH-(blue) and MPA-(red) coated 4-nm AuNPs referenced to the SHG signal intensity obtained from supported lipid bilayer composed of 9:1 DMPC:DMPG maintained at pH 7.4, 10 mM Tris buffer, and 100 mM salt. (c) ζ Potentials of PAH-AuNPs prior to contact with and after rinsing from 9:1 DMPC:DMPG bilayers. Solutions were pH 7.4 (10 mM Tris) and contained 100 mM NaCl.
Figure 3 | Lipid diffusion is altered by interaction with PAH-wrapped nanoparticles. Trajectories of individual Atto 647N DOPE lipid molecules in a 9:1 DMPC:DMPG bilayer. Reconstructed lipid trajectories before (a) and after (b) the addition of 1 nM PAH-AuNPs. Colors indicate lateral diffusion coefficients \( [D_L, \mu m^2 s^{-1}] \) for individual lipid molecules. Lateral diffusion coefficients of all labeled lipid molecules (three spots on each bilayer and three bilayer replicates) were averaged to determine the mean lateral diffusion coefficients presented in (e). The error bars correspond to one standard deviation. (c) Histograms of molecular diffusion coefficients shown in (a) and (b) before (green) and after (blue) interaction of bilayers with the PAH-wrapped nanoparticles.

Figure 4 | Coarse grain molecular dynamics simulations show stable bilayer membrane fragments surrounding PAH-wrapped nanoparticles. The final snapshot after 400 ns of simulations using the POL-MARTINI coarse-grained model from a self-assembly simulation of lipids corona formation around a gold metal nanoparticle wrapped in a single PAH polymer having 160 monomers (a) and zoomed in view of just the phosphate (orange), PG head group glycerol (red) and choline (green) groups within 6 Å of the cationic side chain of PAH (ice blue/gray) or Au (yellow) (b). Phosphates are colored in dark blue, lipid tails are shown as while/purple lines. Smaller yellow and light blue spheres indicate sodium and chloride ions (0.1 M). Coarse-grained water molecules omitted for clarity. (c) Final snapshot after 52.5 ns of an all-atom simulation of PAH deposition on a citrate-AuNP. Counterions are omitted for clarity.

Figure 5 | PAH-wrapped nanoparticles alter bilayers in suspended vesicles, which then form sticky, macroscopic superstructures. (a) Normalized proton NMR spectra of (i) vesicles formed from a 9:1 mixture of DMPC:DMPG, (ii) PAH-AuNPs interacting with 9:1 DMPC:DMPG vesicles, (iii) PAH-AuNPs alone, and (iv) unbound PAH. The green dotted line tracks the chemical shift of the lipid headgroup protons; the blue, purple, and grey dotted
lines track the chemical shifts of the PAH protons. All measurements made at 20 °C and in 0.1 M NaCl and at pH 7.4 (0.01 M Tris) in D$_2$O, for 12 nM vesicles and 10 nM particles after 2 h of incubation. z-Stack fluorescence image obtained from a glass surface in contact with a solution formed from mixing a 5 nM solution of PAH-wrapped 15-nm sized gold metal nanoparticles under conditions of 100 mM NaCl and 10 mM Tris buffer with a solution containing (b) 0.0625 mg/mL and (c) 0.00625 mg/mL vesicles (100 nm diameter) formed from a 9:1 mixture of DMPC:DMPG and 0.1% TopFluor-labeled PC. The liquid/solid boundary is visualized by the presence of micron-sized features and marked by the black rectangle in each image. Scale bars are 5 µm.

**Figure 6 | PAH-wrapped nanoparticles acquire bacterial cell envelope fragments in *Shewanella* while *Daphnia* responds by up-regulating several mitochondrial pathways.**

(a) Transmission electron micrographs of sectioned *Shewanella oneidensis* cells incubated with 0.5 mg L$^{-1}$ PAH-AuNPs for 10 min. Magnified boxed areas, top in (b) and bottom in (c) show PAH-AuNP clusters associated with cell debris and lipid bilayer-like structures, indicated by red arrows. Scale bars are 200 nm in (a), and 50 nm in (b) and (c). (d) Outcomes from Third-Gen sequencing of daphnids exposed to free PAH ligand (left) and PAH-wrapped gold metal nanoparticles (right). (e) Experimental design probing positive (+) and negative (-) outcomes regarding the interaction of supported lipid bilayers and suspended vesicles with anionic and cationic nanoparticles.
Fig. 1.
Fig. 2.
Fig. 3.
Fig. 4.
Fig. 5.
Fig. 6.

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### Free PAH Polymer impacts

Energy and metabolism
1. Ribosomal function
2. Triglyceride storage
3. RNA metabolism
4. Muscle function

### PAH-Au Nanoparticles impact

Mitochondrial Function
1. Cyt C oxidase
2. Electron transport
3. Mitochondrial transmembrane proteins
4. Apoptosis

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All particle concentrations at 10 nM except where indicated. Outcome determined *by SFG; ‡ by ¹H NMR; § by QCM-D and ¹H NMR from ref. 29; ¶ by molecular dynamics simulations; † by QCM-D; ‡ by SFG for 10 nM PAH-equivalent concentration; ¶ by SFG for 1 μM PAH-equivalent concentration; ¶ by spinning disk confocal microscopy; † by QCM-D from ref. 30 and by QCM-D, SHG, and cell sorting from ref. 31; * by TEM.
Supporting Information For

Lipid Corona Formation from Nanoparticle Interactions with Bilayers and Membrane-Specific Biological Outcomes


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Supplementary Note 1: General Information

A. Nanoparticle synthesis, bilayer preparation, and characterization. The 15-nm gold and diamond nanoparticles were prepared and wrapped in PAH ligand following our previously described procedure. For gold particles, 1 L of 0.8 mM HAuCl₄ was heated to a rolling boil, followed by addition of 16 mL of 5% (w/v) sodium citrate. The deep red solution was stirred and boiled for 30 minutes to yield citrate-capped particles. The particles were centrifuged at 8000g for 40 minutes, and the combined pellets were redispersed in 1 L of 1 mM NaCl and stirred overnight with 50 mL of PAH solution (10 mg·mL⁻¹ of 15,000 g·mol⁻¹ PAH (Sigma-Aldrich) in 1 mM NaCl). The PAH-wrapped particles were purified by either tangential flow filtration (MWCO 50K) or centrifugation.

Diamond nanoparticles (DNPs) were obtained from Microdiamant (Smithfield, PA). Synthetic diamond was synthesized by high-temperature high-pressure (HPHT) synthesis and subsequently milled and sized to achieve 15 nm diameter. The particles were oxidized by reflux in a mixture of 3:1 sulfuric and nitric acid overnight, isolated by centrifugation, refluxed again overnight in a fresh acid mixture, washed by centrifugation, and finally resuspended in NanoPure water until reaching a circumneutral pH. PAH functionalization was carried out in a manner similar to that for Au nanoparticles, by mixing concentrated diamond nanoparticle stock (~1 mg·mL⁻¹ by gravimetric analysis) with 1 mg·mL⁻¹ PAH in 1 mM NaCl, sonicating overnight. The particles were then cleaned by dialysis (50 Da MWCO, Spectrum Labs) through at least 12 L of water.

Functionalized particle sizes and wrapping were confirmed by transmission electron microscopy, dynamic light scattering and laser Doppler microelectrophoresis as described in the Supporting Information.

The amount of unbound PAH polymer in PAH-AuNPs purified by double centrifugation was determined following a fluorescence assay reported before using amine-reacting
Briefly, a buffer composed of 10 mM HEPES and 100 mM NaCl adjusted to pH 7.4 was used instead of water to dissolve free PAH polymers to obtain calibration curves, and the same buffer was used to disperse PAH-AuNPs prior to centrifugation to obtain supernatant that contained free PAH ligand. After the sample was mixed with borate buffer and fluorescamine, the solution was incubated at room temperature for 15 minutes, followed by fluorescence readout with excitation/emission wavelengths of 425/480 nm. In the data analysis of the calibration curves, second-order polynomial fitting was used instead of linear fitting to better fit the data points ($R^2 > 0.99$). Average of ten technical replicates from two material replicates were used to calculate the final concentration of free PAH ligand.

The nonlinear optical, QCM-D, and NMR procedures to study lipid bilayer-nanoparticle interactions have been described in our earlier work. Solutions used in these experiments were pH 7.4, contained 0.01 M Tris and 0.1 M NaCl, and were prepared in ultrapure water (18.2 M$\Omega$·cm). Experiments using free PAH ligands were conducted with poly(allylamine hydrochloride), Sigma-Aldrich, average $M_r$ ~15,000 g·mol$^{-1}$) dissolved in ultrapure water and diluted to the desired concentration without further purification. After forming a supported lipid bilayer on the surface of fused silica windows as described previously and rinsing with CaCl$_2$-free buffer to remove excess vesicles, we injected the desired solution into the flow cell, allowed the solution to interact with the bilayer for 20 min, collected an SFG spectrum (not shown), then rinsed with our buffer solution and collected the rinsed SFG spectrum. All spectra shown in this work are those collected after rinsing the surface.

**B. Materials.** Buffer solution refers to a 0.01 M tris(hydroxymethyl)aminomethane (Tris) (Sigma-Aldrich, 99.8%), 0.1 M NaCl (VWR, 99.0%), pH 7.4 aqueous solution. Tris and NaCl were used as received. 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC); 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC); 1,2-dimyristoyl-sn-glycero-3-phospho-(1'-
rac-glycerol) (DMPG); and 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) were purchased from Avanti Polar Lipids, Inc. and used without further purification.

C. Lipid Vesicle Preparation. Lipid vesicles were prepared via the vesicle fusion method\textsuperscript{3,4} as outlined in Supporting Information for Troiano et al.\textsuperscript{1}

D. Lipid Vesicle Characterization. Size and ζ-potentials of the lipid vesicles were determined using dynamic light scattering (DLS) and laser Doppler micro-electrophoresis using a Zetasizer Nano ZS according to Supporting Information of Troiano et al.\textsuperscript{1}

E. Gold Nanoparticle Characterization. AuNPs were characterized by UV-Vis absorption spectroscopy, transmission electron microscopy (TEM), DLS and laser-Doppler micro-electrophoresis explained in the Supporting Information for Troiano et al.\textsuperscript{1}

Supplementary Note 2: Sum Frequency Generation (SFG)

A. Preparation of Optical Cell and Substrate. Fused silica windows (ISP Optics, QI-W-25-3) were soaked overnight in NOCHROMIX® (Godax Laboratories) cleaning solution. The windows were then removed from the NOCHROMIX® solution, rinsed with water (18 MΩ-cm; Millipore) and dried under a flow of N\textsubscript{2} and placed in an oven (110°C) for a maximum of 3 hours. Immediately before use, the windows were removed from the oven and plasma cleaned for 8 minutes. The custom built Teflon cell was sonicated in methanol for 30 min, rinsed with water, dried under a flow of N\textsubscript{2} and plasma cleaned for 12 minutes immediately before use. SFG spectra were taken of the window before each experiment to ensure a starting surface free of C-H stretches.

B. Experimental Setup.

1. Laser System. As previously described,\textsuperscript{5,6} SFG experiments were conducted using a regeneratively amplified Ti:Sapphire system producing 800 nm light with femtosecond (~120 fs) pulses (Spitfire Pro, Spectra Physics, 1 kHz repetition rate). Using a 50/50 beam splitter,
half of the 800 nm light was pumped into an optical parametric amplifier (OPA-800CF, difference frequency mixing option, Spectra Physics) to produce an infrared (IR) beam in the C–H stretching frequency region (~3.4 μm, 140 cm\(^{-1}\) fwhm). The remaining 800 nm beam and the IR beam produced in the OPA were temporally and spatially overlapped at the lipid bilayer/buffer solution/silica interface. The SFG signal generated at the sample was detected with a liquid nitrogen chilled charged coupled device (CCD) camera (Roper Scientific, 1340 × 100 pixels\(^2\)) combined with 0.5 m Acton Research spectrograph. All SFG data reported here were obtained using near total internal reflection. Each SFG spectrum shown is composed of an average of 5 acquisitions with an integration time of 4 min, and is \(ssp\)-polarized (\(s\)-polarized SFG, \(s\)-polarized 800 nm light, \(p\)-polarized IR light). During analysis, each SFG spectrum was background-subtracted, calibrated to the 2850 cm\(^{-1}\) and 3060 cm\(^{-1}\) peaks of polystyrene, and normalized to the nonresonant sum frequency signal of a gold coated silica window plus one. This gold normalization procedure accounts for the distribution of IR power across the frequency region of interest. The averaged, normalized spectra taken at 2 or 3 different IR-centered wavelengths are then summed in the hybrid scanning/broadband method pioneered by Esenturk and Walker to maintain IR power over the region of interest.\(^7\)

2. Bilayer Experiments. As described in the main text as well as in Troiano \textit{et al.},\(^1\) we formed the lipid bilayer on the optical window using the vesicle fusion method. After the lipid bilayer was formed, we injected a 10 mL solution of 12.8 nM nanoparticles in buffer (10 mM Tris, 100 mM NaCl, pH 7.4) into the flow cell (max volume 3.5 mL), allowed the nanoparticles to interact for 40 minutes, collected an SFG spectrum (not shown), then rinsed with 30 mL of our buffer solution and collected the rinsed SFG spectrum.
C. Control Studies.

Control experiments indicate that neither the positively charged particles nor the free PAH polyelectrolyte attach to the bare supporting substrate in our flow cells (no bilayer present).

1. Tris Buffer. SFG control studies of solutions of (a) D$_2$O and 110 mM NaCl and (b) 10 mM Tris, 100 mM NaCl, in D$_2$O with pD between 7 and 8 are explained in the Supporting Information of Troiano et al. These studies show that no SFG signal is present in either of these solutions on a fused silica window. Additional studies shown in Troiano et al. support the conclusion that SFG signal from the Tris buffer solution in H$_2$O stems from the onset of the OH-stretch continuum.

2. Free Ligands. As explained in the main text, the concentration of PAH relevant to the PAH-AuNP experiments (i.e. approximately 10 nM) does not cause a loss of the characteristic peaks of the bilayer formed from a mixture of 9:1 DMPC/DMPG. This result of no observable change in the SFG signal of the bilayer after interaction with an experimentally-relevant concentration of free PAH is also true for the MPA ligand and is shown in the Supporting Information of Troiano et al.

3. Nanoparticles. SFG control studies illustrated in Troiano et al. show that the PAH-AuNPs and MPA-AuNPs do not yield SFG signal at the fused silica interface in D$_2$O, Tris buffer solution, without the presence of the bilayer. In addition, we conducted a control study to test if PAH-ND contributes to the SFG signal. We recorded the ssp-polarized SFG spectrum of the plain fused silica/Tris buffer interface containing 0.012 M PAH-ND, 0.01 M Tris buffer, 0.1 M NaCl and at pD 7 using D$_2$O as the solvent rather than H$_2$O. Supplementary Figure 1 indicates a negligible SFG intensity of the particles.
D. Bilayer Interaction Studies. As explained in the main text, interaction of a supported lipid bilayer formed from a 9:1 mixture of DMPC/DMPG with 4 nm PAH-AuNPs in a 10 mM Tris, 100 mM NaCl, pH = 7.4 buffer solution cause a loss of the characteristic 3-peaks in the SFG signal of the bilayer. Supplementary Figure 2 shows that this loss also occurs after interaction of these materials in 1 mM NaCl buffer solution.

Supplementary Figure 1 | SFG control study of IR-grade fused silica window/Tris buffer solution interface containing 0.0128 M PAH-ND, 0.01 M Tris buffer, 0.1 M NaCl in D2O with pD between 7 and 8.
Supplementary Figure 2 | (a) ssp-Polarized SFG spectra of a supported lipid bilayer formed from pure DMPC before (top) and after (bottom) introduction of a 12.8 nM solution of PAH-AuNP with core diameter of 4 nm in 100 mM NaCl and 10 mM Tris buffer. (b) ssp-Polarized SFG spectra of a supported lipid bilayer formed from a 9:1 mixture of DMPC:DMPG before (top) and after (bottom) introduction of a 12.8 nM solution of PAH-AuNP with core diameter of 4 nm at 1 mM NaCl and 10 mM Tris buffer. (c) Same as in (b) but with 15 nm-sized PAH-AuNPs (right). (d and e) Same as in (b) but at 100 mM salt and using the polycations polydiallyldimethylammonium chloride (400-500kDa) at 100 nM polymer concentration (d) and polyvinylamine at 600 nM polymer concentration (e).
Supplementary Note 3: Second Harmonic Generation (SHG)

A. Preparation of Optical Cell and Substrate. Fused silica hemispheres (ISP Optics, QU-HS-25) were covered with NOCHROMIX® (Godax Laboratories) cleaning solution for 1 h and rinsed with water (18 MΩ·cm; Millipore). Next, the hemispheres were sonicated in methanol for 6 minutes, rinsed with methanol and water, and dried with N₂. Finally, the hemispheres were plasma cleaned for 2 minutes and stored in ultrapure water until use. The custom built Teflon cell was sonicated in methanol for 10 min and rinsed with methanol and water prior to each experiment.

B. Experimental Setup.

1. Laser System. Following our previously published approaches,⁶⁻¹⁰ the second harmonic generation (SHG) experiments were conducted using a regeneratively amplified Ti:Sapphire system (Hurricane, Spectra Physics, kHz repetition rate, 120 fs pulses) pumping an optical parametric amplifier (OPA-CF, Spectra-Physics) tuned to a fundamental frequency (ω) between 610 and 615 nm. Using a variable density filter, the pulse energy was attenuated to 0.40 ± 0.05 µJ, corresponding to a power below the damage threshold of the sample, as determined in a previous study.¹¹ At an angle just below total internal reflection, the p-polarized visible beam was directed through the silica hemisphere and focused to a diameter of ca. 30 µm at the silica/aqueous interface where the lipid bilayer was formed. The beam exiting the sample was passed through a Schott filter to remove the fundamental light signal, and the second harmonic signal (2ω) was directed into a monochromator set at 2ω, sent into a photomultiplier tube, amplified, and collected using a gated single-photon detection system allowing for all polarizations.

2. Bilayer Experiments. Tris buffer solution was introduced into the flow cell first, and the SHG response was recorded until a steady signal was attained for at least 15 min. Next, the vesicles were introduced into the cell and allowed to self-assemble into a lipid bilayer on the
silica substrate for 30 min. We included 0.005 M CaCl$_2$ (Sigma-Aldrich, 99.0%) in the buffer solution when forming the bilayers to facilitate the formation of charged bilayers on the silica surface.$^{12-14}$ Following bilayer formation, Tris buffer, without Ca$^{2+}$, was flushed through the cell to remove any excess vesicles. Following the rinse with buffer, increasing concentrations of gold nanoparticles were introduced into the cell and the SHG signal was recorded until a steady signal was attained for at least 15 min.

C. Control Studies.

1. **Tris Buffer.** Under the conditions of these experiments, the bilayers have been shown by SHG spectroscopy to contain adsorbed Tris buffer,$^1$ which, however, is too disordered to produce molecularly sharp features in SFG controls.

2. **Free Ligands.** SHG data detailed in our earlier work show that neither the MPA nor the PAH free ligand adsorbs to the supported bilayer formed from a mixture of 9:1 DMPC/DMPG at 10 mM NaCl for concentrations studied between $10^{-10}$ to $10^{-3}$ M.$^1$

3. **Nanoparticles.** Our earlier work shows that the MPA-AuNPs and PAH-AuNPs are not surface active at the silica-buffer interface in the absence of a bilayer.$^1$ In addition, control experiments explored the silica-buffer solution interface after addition of PAH-ND at varying concentrations. Supplementary Figure 3 shows minimal adsorption of PAH-ND to bare fused silica in SHG experiments conducted at 300 +/- 3 nm.
Supplementary Figure 3 | SHG adsorption isotherms indicate that PAH-ND with core diameter of 15 nm does not adsorb to bare fused silica (no bilayer present) at either 1 mM (A) or 100 mM NaCl (B).

D. Bilayer Interaction Studies. As shown in Supplementary Figure 4, the SHG intensity increases near 306 nm when the bilayers are exposed to PAH-AuNPs at concentrations as low as $1 \times 10^{-14}$ M, suggesting resonance enhancement of the SHG signal upon particle adsorption to the interface as we have reported previously,\textsuperscript{11} indicating particle attachment to the SLB.

Supplementary Figure 4 | SHG intensity as a function of nanoparticle concentration for a supported lipid bilayer formed from a 9:1 mixture of DMPC and DMPG upon exposure to positively charged PAH-AuNPs. $p$-in/all-out polarization combination, $\lambda_{\text{SHG}} = 306$ nm, 0.1 M
NaCl and 0.01 M Tris buffer. Uncertainties on each SHG E-field is below 1% as given by the Poisson statistics of photon counting. Reproducibility of the experiments is assessed by performing the experiments in triplicate.

**D. Quartz Crystal Microbalance Studies.**

Upon addition of a 12.8 nM solution of PAH-ND in buffer to a bilayer formed from a 9:1 mixture of DMPC/DMPG, QCM-D indicates significant irreversibility in terms of particle attachment to the bilayer (Supplementary Figure 5).

![Supplementary Figure 5](image-url)

**Supplementary Figure 5** | Frequency and dissipation traces for the attachment of 4 nm and 15 nm PAH-AuNPs (12.8 nM) to supported lipid bilayers formed from vesicles composed of a 9:1 mixture of DMPC and DMPG. Experiments were conducted in 0.1 M NaCl buffered to pH 7.4 with 0.01 M Tris. Data are reported for the 5th harmonic.

**Supplementary Note 4: Fluorescence Microscopy**

**A. Experimental Conditions.** For spinning disk fluorescence microscopy (Leica Spinning Disk AF), suspensions of SUVs (0.0625 or 0.00625 mg·mL⁻¹) formed from 9:1 mixtures of DMPC and DMPG with 0.1 mol% TopFluor PC (Avanti Polar Lipids) in 100 mM NaCl maintained at pH 7.4 using a Tris buffer (10 mM) were analyzed with and without the presence of 4 nm or 15 nm PAH-AuNPs. For experiments in the presence of the PAH-
AuNPs, a 5 nM solution of the AuNPs (0.1 M NaCl, 0.01 M Tris, pH 7.4) was gently mixed with the vesicle solution by using the aspiration and injection action of a 1 mL volume syringe two times for 10 seconds. After allowing the solution to sit for 2 hours, 3 mL of the solution containing the vesicles or the vesicles with the nanoparticles was placed into a dish (35 × 10mm, PELCO® Clear Wall Glass Bottom Dishes) and imaged. Prior to use, the dishes were rinsed with water and methanol, dried with N2, and placed in a plasma cleaner for 5 min.

**B. Vesicle Interaction Studies.** As described in the main text, suspensions of SUVs (0.0625 or 0.00625 mg·mL$^{-1}$) in 0.1 M NaCl and 0.01 M Tris at pH 7.4 were analyzed by confocal fluorescence microscopy (Leica Spinning Disk AF) with and without the presence of 5nM 4 nm or 15 nm PAH-AuNPs, as well as 4 nm sized anionic MPA-functionalized AuNPs. Here we present experiments conducted with vesicles formed from a 9:1 mixture of DOPC and DOTAP with 0.1 mol% TopFluorPC. After exposure to a 5 nM concentration of 15 nm PAH-AuNPs, the lipid-NP structures present for the experiments with DMPC rich vesicles are not observed. (Supplementary Figures 6 and 7a)
Supplementary Figure 6 | Bright field (A-C) and corresponding fluorescence (D-F) images of vesicles formed from a 9:1 mixture of DOPC and DOTAP with 0.1 mol% TopFluorPC and 5 nM 15 nm PAH-AuNPs after interaction for 2 hours.

Supplementary Figure 7 | (A) Confocal fluorescence microscopy z-stack obtained from a glass surface in contact with a solution formed from mixing a 5 nM solution of PAH-wrapped
15-nm sized gold metal nanoparticles under conditions of 100 mM NaCl and 10 mM Tris buffer with a solution containing 0.0625 mg/mL of 100 nm-sized vesicles formed from a 9:1 mixture of DOPC and DOTAP with 0.1 mol % TopFluor-labeled PC. Image size is approximately 50 x 50 x 5 \( \mu m \). (B) Same as in (A) but with 4-nm sized MPA-functionalized particles brought into contact with vesicles formed from a 9:1 mix of DMPC:DMPG at 100 mM salt and 10 mM Tris buffer.

To further investigate the nature of the changed surface affinity of the PAH-coated particles upon contact with the DMPC-containing bilayers, we exposed suspended vesicles formed from a 9:1 mixture of DMPC and DMPG containing 0.1% TopFluor-labeled PC to PAH-AuNPs, followed by confocal fluorescence imaging. Supplementary Figures 8 shows the presence of three-dimensional fluorescent structures extending from the liquid/solid boundary, which is visualized by the presence of micron-sized features, into the aqueous bulk when PAH-wrapped nanoparticles are brought in contact with vesicles rich in DMPC. These data also show that the particles that produce fluorescence signals have the labels far enough from the gold core that the fluorescence is not quenched. Yet, we find that many of the fluorescent features are seemingly co-localized with similarly sized features observed in the bright field (Supplementary Figure 8c), which visualizes particles that are agglomerated to \( \mu m \)-sized structures in 0.1 M NaCl. This effect is seen for many of the sub-micron sized features, one of which is shown in Supplementary Figure 8d-g. Such features are not formed when PAH-wrapped nanoparticles are brought in contact with vesicles rich in DOPC (Fig. S7) or when 9:1 DMPC:DMPG vesicles are exposed to 10 nM free PAH. While zeta potentials obtained for the agglomerated features are indistinguishable from those obtained for plain PAH-wrapped gold metal nanoparticles for up to two hours, the hydrodynamic diameters of the agglomerated features are twice that of the plain lipid vesicles or
nanoparticles upon initiating mixing (200 ± 10 nm) and increase in the subsequent two hours to more than 1 µm.

**Supplementary Figure 8** | Confocal fluorescence microscopy z-stack obtained from a glass surface in contact with a solution formed from mixing a 5 nM solution of PAH-wrapped 15-nm sized gold metal nanoparticles under conditions of 100 mM NaCl and 10 mM Tris buffer with a solution containing 0.0625 mg/mL (A) and 0.00625 mg/mL (B) of vesicles (100 nm diameter) formed from a 9:1 mixture of DMPC:DMPG and 0.1% TopFluor-labeled PC. The liquid/solid boundary is visualized by the presence of micron-sized features and marked by a black rectangle in each image. (C) Overlay of fluorescence (green) and bright field (red) image at cross section indicated in grey. (D) Confocal fluorescence microscopy z-stack of a submicron-sized feature, fluorescence (E) and bright field (F) images of indicated slice, and blended image of E and F (G).
Supplementary Note 5: Computer Simulations

**Methodology for all-atom simulations.** The PAH-AuNP model at atomistic resolution with explicit citrate was constructed using the OPLS-UA force field\(^\text{15}\) for the carbon backbones of citrate\(^3\) and PAH, and the OPLS–AA force field\(^\text{16}\) for charged terminal groups and counterions.\(^\text{15}\) The all-atom simulations were carried out using LAMMPS.\(^\text{17}\) The surface of the 4-nm AuNP was covered with 90 citrate\(^3\) molecules to match the corresponding experimentally observed density of \(2.8 \times 10^{10}\) mol cm\(^{-2}\).\(^\text{18}\) The central carboxylate groups of the 90 citrate\(^3\) molecules were fixed at uniformly chosen points on the AuNP surface. Sodium (270 for 90 citrate\(^3\)) and chloride (200 per PAH-200mer) ions were included explicitly. A box size of 28.5x13.5x13.5 nm\(^3\) was chosen so that the counterion concentration is 0.1 M when adding the first PAH. To model implicit solvent, we use a Langevin thermostat, a relative permittivity of 80.1, and the particle-particle particle-mesh method to compute electrostatic interactions. The homogeneous dielectric model likely overestimates the effect of solvent screening at short distances; a more complete comparison of different implicit solvent models and explicit solvent simulations for PAH and PAH-AuNP interactions will be reported separately.

A PAH chain of 200 monomers was first solvated in TIP3P water with counterions and equilibrated for 50 ns. The equilibrate structure was then deposited 8 Å away from the citrate-coated AuNP in an implicit solvent. These were held fixed while the salt ions and the carboxylate groups of the citrate molecules were equilibrated through 2.5 ns of propagation by Langevin dynamics. Subsequently, five independent molecular dynamics simulations were run in implicit solvent with Langevin dynamics at 300 K and a damping constant of 10 ps\(^{-1}\) — using a different PAH conformation and citrate distribution for each simulation — until all monomers were seen to relax within 4 nm of the nanoparticle’s center of mass. The structures were then inspected visually and wrapping of the particle by PAH was confirmed in each
The five trajectories sampled all reached this point at times ranging within 28-48 ns. An additional 5 ns were simulated as further equilibration and another 1 ns for data collection of the equilibrium structure.

As with the coarse-grained simulations, PAH covered the citrate-coated AuNP upon relaxation from starting positions (Figure 4c in the main text) in which PAH was in the vicinity but not absorbed onto the nanoparticle. The radial distribution function $g(r)$ for the nitrogen atoms of PAH relative to the nanoparticle’s center of mass was obtained for the equilibrium configurations as the nitrogen atoms act as reporters of the positions of the monomers. Histograms with bin sizes of 1 Å were used to calculate the $g(r)$ shown in Supplementary Figure 9. The charged groups of PAH with 200 monomers separate into two dense layers 0.6 nm apart, approximately 2.2 and 2.8 nm away from the nanoparticle center of mass, once adsorbed onto the citrate-coated AuNP surface. This result is in qualitative agreement with the coarse-grained simulations in which PAH is seen to coat the AuNP but with several monomers outside of the first shell.

Supplementary Figure 9 | Radial distribution function, $g(r)$, between nitrogen atoms of
Calibration of the coarse-grained model for PAH and PAH-AuNPs. As a benchmark, we calculate the radius of gyration ($R_g$) for PAH polymers of different length in 100 mM NaCl solution with our coarse-grained (CG) model; all simulations are run for 200 ns and $R_g$ values are averaged over the last 100 ns. The scaling exponent of $R_g$ with respect to the chain length, denoted as $\varphi$, is computed and compared with available experimental data\textsuperscript{19} and all-atom simulations of Hoda and Larson\textsuperscript{20}. As shown in Supplementary Table 2, our CG model predicts a $\varphi$ value about 0.85, as expected for a poly-electrolyte, and close to the experimental value of 0.81. Interestingly, previous all-atom model simulations\textsuperscript{20} led to a $\varphi$ value of 0.6, suggesting that PAH behaves as a polymer in a good solvent with the all-atom model. Thus it appears that our CG model provides a description for the chain properties in better agreement with available experimental data than the all-atom model of Hoda and Larson\textsuperscript{20}.

To consider this question further, complementary all-atom simulations of the citrate-coated gold nanoparticle interacting with a PAH molecule with 200 monomers were also performed. The coarse-grained PAH-AuNP model reproduces the separation of charged monomers of PAH into two layers normal to the nanoparticle surface observed in the atomistic simulations, (Figs 4 and Supplementary Figure 9). Our all-atom simulations of PAH deposition on the citrate-coated AuNP reveal that PAH only covers a hemisphere of the nanoparticle illustrated in Fig. 4c, suggesting that an additional PAH can be adsorbed onto the AuNP. The possibility of adsorbing an additional PAH and develop higher surface-charge density also suggests a greater propensity of PAH-AuNPs to extract lipids from the bilayer and may account for the slight discrepancy with the CG model which did not attempt to include additional PAH.
Three-Microsecond Long Computer Simulations of Nanoparticle Binding to Bilayer. To probe the binding of a cationic nanoparticle to a lipid bilayer and the subsequent impact on membrane stability, we conducted microsecond-long coarse-grained simulations of a PAH-wrapped gold nanoparticle (AuNP) bound to a 9:1 DMPC:DMPG lipid bilayer. To properly describe potential bending of the bilayer upon nanoparticle binding, a rather large system of 40 nm×40 nm×15 nm was established. The simulation was carried out using the POL-MARTINI model as described in the main text and contained 150 mM NaCl. The nanoparticle was initially placed 5 nm above the bilayer and the system was equilibrated for 100 ns with an integration time step of 10 fs. Then a production run for 3 µs was conducted with a larger integration time step of 20 fs. Both equilibration and the production runs were carried out in the NPT ensemble with $T = 300$ K and $p = 1$ atm, using Berendsen temperature coupling and Berendsen semi-isotropic pressure coupling.

In our analysis, we used the distance between the center of mass (COM) of the AuNP and the COM of the phosphate bead of the lipids located within 3 nm of the AuNP along the $z$ axis to monitor the extent of binding. As shown in Supplementary Figure 10, the NP approaches the bilayer spontaneously and becomes bound to the lipid surface (note that the radius of the AuNP is about 18.5 Å, thus $dZ < 20$ is classified as “bound”) rather quickly, although it detaches frequently, indicating a fairly weak association. A snapshot is shown in Supplementary Figure 11, which illustrates a tightly bound configuration that leads to notable membrane bending; the change in area-per-lipid, however, is found to be rather modest (data not included).
Supplementary Figure 10 | Center of Mass (COM) distance between the PAH wrapped gold nanoparticle (AuNP) and phosphate groups in the 9:1 DMPC:DMPG lipid bilayer (left). Considering the size of AuNP, dZ < 20 Å is classified as bound. Horizontal blue line at dZ=20 indicates upper estimates of particle-bilayer distances that classify "bound" states. The corresponding histogram is shown on the right, indicating close association.

Supplementary Figure 11 | A snapshot of a PAH-wrapped gold nanoparticle bound at the surface of a 9:1 DMPC:DMPG lipid bilayer (shown in transparent mode). Due to binding, there is considerable membrane bending. For clarity, water and ions are not shown.
To gain more quantitative insights into the degree of membrane bending induced by the binding of AuNP, we computed the principal and Gaussian curvatures of the upper monolayer,\textsuperscript{21} i.e. the membrane leaflet proximal to the PAH-AuNP. Examples of the two curvature components for a snapshot with the AuNP bound to the bilayer are shown in Supplementary Figure 12, which illustrates that the bending is rather local in nature.

![Supplementary Figure 12](image_url)

**Supplementary Figure 12** | Principal (left) and Gaussian (right) curvature of the membrane for a snapshot that features a PAH-wrapped gold nanoparticle tightly bound to the 9:1 DMPC:DMPG bilayer.

To further evaluate the degree of membrane bending, we computed the membrane bending free energy by integrating the curvature over the entire surface in the simulation shell;\textsuperscript{21} for the calculation, a membrane bending modulus of $0.57 \times 10^{-19} \text{J}$ estimated from recent simulations was used (X. Gu et al., unpublished). As shown in Supplementary Figure 13, the bending energy is on the order of several kcal/mol throughout the 3 microseconds simulation, suggesting again that the degree of membrane bending induced by AuNP binding is modest and that corona formation requires additional activation step(s).
Supplementary Figure 13 | Membrane bending energy computed along the 3 microseconds of coarse-grained simulation of a PAH-wrapped gold nanoparticle near a 9:1 DMPC:DMPG lipid bilayer. The left panel shows the time evolution of the bending energy, and the right panel shows the histogram.

During the 3 microseconds of production runs, no spontaneous insertion or lipid extraction was observed, which strongly suggests that lipid corona formation requires overcoming considerable barrier(s). This behavior is in contrast to situations observed for the interaction of highly hydrophobic materials (e.g., graphene) with lipid membranes, where spontaneous lipid extraction was observed at the nanosecond time scale.22

Advanced simulations such as metadynamics along carefully designed collective variables are needed to explore the corona formation pathways(s), which we leave as future studies. In the meantime, we proceeded to study lipid corona formation using the approach described in the main text. Additional details and results regarding coordination numbers within the lipid corona are presented in the following section.

To further confirm the free energy penalty associated with lipid extraction at the atomistic level, free energy simulations using the CHARMM36 lipid force field were carried out to
extract a DOPC molecule from either a DOPC bilayer or a 9:1 DOPC:DOPG bilayer. The potential of mean force calculations were carried out using the standard umbrella sampling technique, with 40 umbrella windows and each window sampled for 20 ns. As shown in Supplementary Fig. 14, lipid extraction from both lipid bilayers costs more than 10 kcal/mol, explaining the difficulty of observing spontaneous corona formation in unbiased molecular dynamics simulations.

**Supplementary Figure 14** | Computed Potential of Mean Force (PMF) for extracting a DOPC lipid molecule from either a DOPC bilayer (top) or a 9:1 DOPC:DOPG bilayer (bottom) using atomistic simulations.
Computer Studies of Coordination Number. Supplementary Figure 15 shows the coordination numbers of selected groups as functions of simulation time. Comparing the solid and dotted blue lines helps characterize the organization of the lipids: it shows that lipid corona formation occurs rapidly on the time scale of nanoseconds, while lipid adsorption to the nanoparticle reaches a stable phase after about 100 ns.

Supplementary Figure 15 | Coordination numbers of selected groups as functions of simulation time during corona formation. Black and red lines indicate the number of PC and PG head groups within 10 Å of the PAH-gold nanoparticle, respectively. The solid blue line indicates the number of phosphate group within 25 Å of any lipid tail C3B beads; the dotted lines represent limiting values for different lipid organizations.
Supplementary Table 1 | The average numbers of groups within 10 Å of either Au in the nanoparticle (NP) or PAH from coarse-grained (POL-MARTINI) molecular dynamics simulations of the lipid corona.  

<table>
<thead>
<tr>
<th>Group</th>
<th>PAH160-NP&lt;sup&gt;b&lt;/sup&gt;</th>
<th>PAH200-NP&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Au&lt;sup&gt;c&lt;/sup&gt;</td>
<td>PAH</td>
</tr>
<tr>
<td>Na&lt;sup&gt;+&lt;/sup&gt;</td>
<td>19.9±1.1</td>
<td>17.7±1.5</td>
</tr>
<tr>
<td>Cl&lt;sup&gt;−&lt;/sup&gt;</td>
<td>0.8±0.9</td>
<td>10.3±2.2</td>
</tr>
<tr>
<td>Phosphate-DMPC</td>
<td>20.7±2.0</td>
<td>43.6±4.0</td>
</tr>
<tr>
<td>Phosphate-DMPG</td>
<td>0.3±0.5</td>
<td>15.6±2.7</td>
</tr>
<tr>
<td>Glycerol of DMPG</td>
<td>1.9±1.2</td>
<td>15.9±2.5</td>
</tr>
</tbody>
</table>

<sup>a</sup>All averages are calculated from the last 200 ns of the production run. <sup>b</sup> PAH160/PAH200 indicates PAH with 160/200 monomers. <sup>c</sup>The coarse-grained Au particles are negatively charged to mimic the passivation of the gold nanoparticle with citric acids.
### Supplementary Table 2

The dependence of $R_g$ (in nm) on chain length ($N$) from experiments, all-atom (AA) simulations and current coarse-grained (CG) simulations for PAH polymers in 100 mM NaCl solution. The underlined numbers are extrapolated values based on the corresponding $\phi$ value.

<table>
<thead>
<tr>
<th>$N$</th>
<th>Experiment$^{19}$</th>
<th>All atom (Hoda et al.$^{20}$)</th>
<th>CG (current work)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.66</td>
<td>0.62±0.01</td>
<td>0.71±0.02</td>
</tr>
<tr>
<td>20</td>
<td>1.06</td>
<td>0.94±0.04</td>
<td>1.25±0.04</td>
</tr>
<tr>
<td>30</td>
<td>1.54</td>
<td>/</td>
<td>1.81±0.06</td>
</tr>
<tr>
<td>780</td>
<td>21.5</td>
<td>/</td>
<td>28.4</td>
</tr>
<tr>
<td>$\phi$</td>
<td>0.81</td>
<td>0.60</td>
<td>0.85</td>
</tr>
</tbody>
</table>

### Supplementary Note 6: TEM Analysis of S. Oneidensis

For TEM analysis, *S. Oneidensis* MR-1 was cultured in LB broth overnight, and pelleted by centrifugation at 750 x g for 10 min. The pellet was washed with D-PBS buffer and transferred into HEPES buffer (2 mM HEPES, 25 mM NaCl at pH 7.4) ahead of exposure to 0.5 µg/mL PAH-AuNP. After incubating with nanoparticles for 10 minutes, the cells were centrifuged and washed twice in 0.1 M cacodylate buffer. The pellets were then fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer for 40 minutes, followed by three rinses with cacodylate buffer. The cells were then dehydrated in a graded series of ethanol solutions followed by propylene oxide. Resin infiltration and embedding was carried out in graded Epon resin/propylene oxide mixtures of 33% (2 h), 50% (overnight) and 100% (overnight). The resin-embedded cells were then polymerized for 24 h at 40°C and 48 h at 60°C. After microtome sectioning of the resin block into 60-nm-thick samples with a diamond knife, the
sectioned samples were stained with uranyl acetate and lead citrate, and imaged on a Tecnai T12 electron microscope at 120 kV.
References:


