Supporting Information

Understanding Nanoparticle Toxicity Mechanisms to Inform Redesign Strategies to Reduce Environmental Impact

Joseph T. Buchman[§], Natalie V. Hudson-Smith[§], Kaitlin M. Landy[§], Christy L. Haynes[§]*

[§]Department of Chemistry, University of Minnesota, Minneapolis, MN 55455, United States

*Corresponding author: Prof. Christy Haynes, chaynes@umn.edu

S1. Binding of NPs to the Bacterial Cell Surface

Investigating nanoparticle binding to bacteria, Jacobson et al. showed that the interaction with Gram-negative bacteria is dominated by binding to the lipopolysaccharides (LPS), a negatively charged surface moiety.[1] In this work, the importance of LPS for nanoparticle binding was shown in both supported lipid bilayers (SLBs) with varying amounts of incorporated LPS as well as the bacterium, *Shewanella oneidensis* MR-1. Binding of positively charged and negatively charged AuNPs to SLBs was monitored with a quartz crystal microbalance in dissipation mode (QCM-D). SLBs with LPS exhibited higher binding with cationic NPs than those without LPS, and that in an ionic strength of 25 mM, an increasing trend in binding amount was seen with amount of incorporated LPS into the bilayer. To assess association with *S. oneidensis*, flow cytometry, dark field microscopy, and hyperspectral imaging were employed on bacteria with native and depleted LPS levels. More bacteria were bound with cationic AuNPs when they had native LPS levels than the bacteria with reduced LPS. A 50% depletion in wild type LPS led to a ~70% decrease in bacteria-associated nanoparticles. Anionic AuNPs were not found to appreciably bind to the SLBs or bacteria.

An analogous moiety for Gram-positive bacteria is under investigation with the hypothesis that teichoic acids may be the critical mediator for nanoparticle-bacteria interactions. Even though the important site for nanoparticle binding on the Gram-positive bacterial surface is unknown, it is clear that NPs do bind to their surface.[2,3]

Lai et al. showed that the association of quantum dots (QDs) to *Escherichia coli* and model membranes correlated with toxicity and membrane damage.[4] Cysteamine-coated (positively charged) and mercaptopropionic acid-coated (negatively charged) QDs were used, with effects only seen with positively charged QDs. There was notable inhibition of *E. coli* upon exposure to cysteamine-coated QDs (CA-QDs) as well as increased attachment. Monitoring lysis activity by sodium dodecyl sulfate treatment revealed that there was increased membrane permeability for *E. coli* exposed to CA-QDs. Model membrane studies, which showed an increase in membrane fluidity and increased liposome leakage after exposure to CA-QDs, supported these findings. Similar work by Williams et al. showed greater liposome disruption and toxicity to bacteria by cadmium-based QDs (CdSe and CdSe/ZnS core/shell structures) compared to cadmium-free QDs (ZnSe and ZnSe/ZnS).[5]

S2. Dissolution is a Major Toxicity Mechanism for AgNPs

Further examples of dissolution as a major toxicity mechanism come from work by Xiu et al., which demonstrates the toxicity of AgNPs is dictated by the release of Ag^+ ions.[6] In the study, they take advantage of the fact that AgNPs do not dissolve to release Ag^+ ions under anaerobic conditions. Exposing *E. coli* to AgNPs in aerobic and anaerobic conditions, they found significant toxicity in oxygenated test atmospheres and no toxicity under anaerobic conditions. To probe further, they exposed AgNPs to aerobic conditions, measured released Ag^+ ion concentration, and then moved the nanoparticles to anaerobic conditions to prevent further dissolution and compared the toxicity observed with toxicity from Ag^+ dosed via $AgNO_3$. Toxicity between the AgNP doses and equivalent Ag^+ doses was indistinguishable, indicating the importance of Ag^+ release. Similarly, XANES/EXAFS analysis showed that the silver inside of *Bacillus subtilis* after AgNP exposure was in the form Ag_2O, suggesting that Ag^+ ions penetrated the cell wall and were oxidized by internal cell machinery.[7]

S3. Toxicity of QDs and QD-polymer Composites

Mahendra et al. investigated the effects of weathered CdSe-based QDs on three different bacteria.[8] They monitored bacterial growth by taking optical density measurements and noted that weathered CdSe-based QDs were toxic to all three bacteria, while unweathered QDs were minimally toxic. They attribute the toxicity to the release of Cd^{2+} and SeO_3^{2-} ions by introducing the bacteria to Cd^{2+} or to Cd^{2+} and SeO_3^{2-} simultaneously. Only when both ions were dosed simultaneously did the observed toxicity match the QD toxicity. The addition of chelating agents such as oxalate or EDTA reduced the QD dissolution to both Cd^{2+} and SeO_3^{2-} ions. Consequently, bacteria exposed to these co-introduced QDs with chelating agents experienced decreased toxicity from the QDs.

Similarly, the toxicity of weathered QD-polymer composites to the bacterium, *S. oneidensis* MR-1, was investigated by Gallagher, et al.[9] Tracking the toxicity after various weathering times of the QD-PMMA nanocomposites (210.5, 336, and 504 hr) indicated that toxicity increased with weathering time. This is likely because the polymer was degrading to small QD-containing fragments and over time, these polymer fragments were being further degraded to smaller sizes. Since these smaller sizes are more likely to associate with the bacterial surface, they would likely release high local concentrations of toxic Cd^{2+} ions.

S4. ROS Production by Titania and Zinc Oxide NPs

The generation of ROS by different metal oxide species after photoillumination was investigated by Wang et al.[10] All nanoparticles tested generated superoxide radical, whereas hydroxyl radical and hydrogen peroxide were only detected from TiO_2 and ZnO NPs. The toxicity of the materials to *Photobacterium phosphoreum* matched the trend seen in superoxide production, with those materials generating more superoxide exhibiting the highest toxicity. To further implicate the importance of superoxide in the toxicity, superoxide dismutase was added to scavenge superoxide, which reduced toxicity. However, when isopropanol was added to scavenge hydroxyl radical or hydrogen peroxide was directly added, no change in toxicity was observed.

S5. Toxicity of SiQDs is Impacted by Boron- and Phosphorus-doping

In this Account, SiQDs are introduced as an alternative to cadmium-based QDs, and they demonstrate a lack a toxicity to both *S. oneidensis* and *Bacillus subtilis*. However, in a follow-up study investigating boron- and phosphorus-doped SiQDs,[11] it was shown that both dopants caused the otherwise nontoxic SiQDs to exhibit some toxicity to bacteria, with the most highly doped phosphorus-doped SiQDs being most toxic. This correlated with significantly increased ROS production by phosphorus-doped SiQDs and a slight increase by boron-doped SiQDs.

Interestingly, boron-doped SiQDs were observed to more significantly bind to the surface of *S. oneidensis* than phosphorus-doped SiQDs, yet showed less damage, revealing that not all toxicity mechanisms have the same level of bacterial impact.

S6. Increased Stability of Iron-doped ZnO NPs Reduces Toxicity

There are other studies beyond those presented in the main text of this Account that have taken advantage of increased nanoparticle stability to reduce NP toxicity. In work by Xia et al., the toxicity of ZnO nanoparticles to zebrafish, mice, and rats was noted to be due to the release of toxic Zn^{2+} ions.[12] Iron was doped into ZnO nanoparticles by mixing zinc naphthenate with iron naphthenate at desired weight percents and then synthesizing nanoparticles by flame spray pyrolysis. Zinc dissolution was reduced for nanoparticles with increasing iron content, and therefore the hatching rate of zebrafish was found to increase with increasing iron content of the nanoparticles. Increasing the iron content also benefitted the mice and rats by reducing pulmonary inflammation.

S7. Use of Novel Metal Nanoclusters to Reduce Toxicity

For nanomaterial applications where the use of nanoclusters that are comprised of merely dozens or hundreds of atoms would have the same desired functionality, using nanomaterials of this regime could present another method for redesigning nanoparticles. These novel materials already find use in several applications, with speculation of further applications that could benefit from the use of nanoclusters.[13,14] Due to the nature of these ultrasmall materials, even upon potential particle dissolution, only a small amount of ions would be released into the environment. Nanoclusters have found uses in biomedical applications as well as biological sensors/imaging agents due to their low biological toxicity;[15] they also have a demonstrated low environmental toxicity and are therefore used for different environmental applications.[16,17]

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