The role of the outer membrane in the bacterial biouptake of mercury in aquatic systems

I. Why is mercury biouptake by bacteria important?

Each can of tuna that we eat comes with a tiny serving of mercury. Mercury is an extremely dangerous substance that has found its way into our aquatic systems due to human activities, particularly from our massive combustion of fossil fuels. It is estimated that 2480 tons of mercury was released into the environment globally as a result of human activities in 2006, and 65% of that was from stationary fuel combusting sources. The mercury becomes airborne and much of this ends up in our aquatic systems in an inorganic form (Figure 1). Bacteria in anoxic sediments of the water convert the mercury to the organic compound and neurotoxin methylmercury (CH$_3$Hg$^+$). Unlike inorganic mercury, methylmercury is lipophilic and bioaccumulates in fish and shellfish. As a result, the consumption of fish and shellfish contaminated with methylmercury is the primary route of human exposure to mercury.

II. What is already known about bacterial mercury biouptake?

Mercury’s biouptake is controlled by its chemical speciation (i.e., what it is bound to) in aqueous solution, which means understanding which mercury species are bioavailable is essential to understanding the biouptake process as a whole. Anthropogenically produced chelating ligands (e.g., EDTA, NTA, and SHMP) as well as naturally present ligands (e.g., cysteine and glutathione) will strongly bind Hg in solution but have drastically different effects on bacteria’s ability to uptake mercury.

All known mercury-methylating bacteria are Gram-negative, meaning they have two membranes: a cytoplasmic membrane and an outer membrane. The mercury must pass through both of these membranes to enter the bacteria. Mercury is thought to be transported across the inner cytoplasmic membrane through an active transport process (Figure 2). Since there are no known bacterial metal transporters that import mercury, it is also thought that the mercury is thus probably taken up accidentally by a transporter that evolved for another essential metal (like zinc). The mercury is thought to pass through the outer membrane by simply going through the membrane’s porins, which are small protein channels that enable the passing of small compounds such as nutrients into the cell. Although the transport of Hg through the outer membrane is a vital step in the Hg biouptake process, there is only one study that has noted the importance of the outer membrane in regulating mercury uptake. It finds that high concentrations of divalent base cations like calcium and magnesium (which are known to give the outer membrane its structure - keeping unwanted toxins and such outside of the cell) inhibit mercury uptake. Two large ligands have also been found to inhibit the uptake of mercury, and
I hypothesize they work like these divalent cations by preventing the mercury from being able to fit through the porins due to their sheer size.

**III. My research goal and timeline**

My project will study the influence of the outer membrane on mercury uptake in *E. coli* bacteria. This can be observed by removing the outer membrane and converting bacteria into “spheroplasts” to see how the bacteria’s uptake of mercury differs with and without an outer membrane.

Professor Gaillard’s lab is in possession of a genetically modified strain of *E. coli* that contains a mer-lux fusion gene (*i.e.*, a Hg biosensor). When exposed to mercury in solution, the biosensor will emit light at intensities proportional to the concentration of intracellular Hg. Luminescence is detected with a luminescence microplate reader, allowing for high-throughput quantification of Hg biouptake. Spheroplasts will be created from the Hg biosensor via a well-known procedure that uses the enzyme lysozyme to degrade the outer membrane. To confirm the presence of only sphere shaped cells, I will dye the cell suspension and image with an epifluorescence microscope. The dye is composed of two nucleic acid stains – SYTO9 and propidium iodide – and will serve two purposes: (1) providing a detectible image and (2) confirming that the cytoplasmic membrane remains intact after the lysozyme treatment. SYTO9 is able to pass through damaged and undamaged cytoplasmic membranes, while propidium iodide can only enter through damaged cytoplasmic membranes. Thus when excited, cells that contain only SYTO9 will fluoresce at green wavelengths, while cells containing both SYTO9 and propidium iodide (dead or damaged cells) fluoresce at red wavelengths. The experimental conditions to which spheroplasts and whole cells will be exposed include Hg strongly bound to different ligands known to control Hg biouptake in solution as well as different divalent cations that may inhibit the diffusion of Hg through the outer membrane. The following is the timeline:

- Obtain calibration curves for whole cells and spheroplasts in the presence of different concentrations of total mercury in solution (1-2 weeks).
- Determine the influence of strong ligands (*e.g.*, EDTA, cysteine, and glutathione) on the biouptake of Hg in whole cells and spheroplasts (3-4 weeks)
- Determine the influence of divalent base cations (*e.g.*, calcium and magnesium) on the biouptake of Hg in whole cells and spheroplasts (3-4 weeks)

If ligand size is indeed the causal factor in preventing mercury biouptake in these control examples, I hypothesize that these ligands will not inhibit the biouptake of mercury when the outer membrane is removed and porins no longer limit ligand size. Additionally, it is suspected that the divalent base cations that give the outer membrane its structure in whole cells will have no influence on mercury biouptake in spheroplasts.

**IV. My Qualifications**

I am working towards my Bachelor’s in Environmental Engineering and plan on pursuing a Master’s or PhD in the subject. I am very interested in the environment and am involved in many environmental clubs on campus. I have taken many relevant courses for this research including the full general chemistry and organic chemistry sequences (which also gave me laboratory experience), cell biology, and multiple environmental science classes. I was also a national finalist in the Chemistry Olympiad competition in 2012. Sara Thomas, a graduate student in Professor Gaillard’s lab whose research has paved the way for my project, will also help mentor me throughout the summer. I will be trained to operate the epifluorescence microscope, grow bacteria, create spheroplasts, and perform biosensor assays during spring quarter so I will be able to perform the research this summer.
Figure 1 – The geochemical cycle of mercury in the environment with the bacterial biouptake step highlighted. Inorganic forms of mercury from fossil fuels and some natural sources are converted into the dangerous neurotoxin methylmercury by anaerobic bacteria in the sediments. This methylmercury then bioaccumulates up the food chain.²

Figure 2 – Possible mechanisms for how inorganic mercury enters bacteria. The active transport model on the far right is most supported by recent research. Current research (as exemplified here) focuses on the entry through the cytoplasmic membrane, but ignores and makes assumptions about entry through the outer membrane.³


