Silver NanoParticles

A nanoparticle is defined as a small particle that behaves as a single unit with a size ranging from one to one hundred nanometers. Due to their high surface area to size ratio, nanoparticles typically have unique physical and chemical properties not present at the macro level. For example, silver nanoparticles exhibit a range of unique optical, electrical, and anti-microbial properties not present in elemental silver. As a result, in recent years there's been a steady increase in the incorporation of silver nanoparticles into commercial goods such as: semi-conductors, wound dressings, and even household appliances. Despite their useful applications, there's been growing concern about the detrimental effects silver nanoparticles can have on both human and environmental health. Harmful effects include:

- **Liver damage** due to the tendency of colloidal silver (a popular alternative medicine that uses AgNP's) to **accumulate to toxic concentrations in the liver**.
- **Argyria** – A **permanent gray-blue discoloration** of the skin caused by AgNP deposition in deep tissues.
- Both **stem cell and sperm cell toxicity** at high concentrations. (>10ug/ml)
- 22% **reduced rate of plant growth** in soil saturated with AgNP due to death of soil microbes.

Most of these detrimental effects spring from the tendency of silver nanoparticles to generate reactive oxygen species which can damage both cell parts and DNA. In spite of the growing public anxiety over the effects of nanoparticle pollution much is still unknown.

Biosensors/Engineering Goals

Biosensors are devices used to detect and measure the presence of biologically important substances such as metabolites, nutrients, or, in this case, toxins. Standard biosensors are typically composed of a mix between biological parts, used for detecting the biological "signal", and electrical parts used to amplify the signal and produce a visual output. As a result of our research into the growing problem of nanoparticle pollution, NCSSM iGEM sought to create an inexpensive and modular silver nanoparticle biosensor using existing and new biological parts in *Escherichia coli.* With this in mind we came up with the following Experimental and Engineering Goals:

1. To determine a simple, inexpensive, method for creating silver nanoparticles for use in our experimental assays.
2. To determine whether nanoparticles can be detected by the same regulatory elements that detect macro scale particles.
3. To design an inexpensive and highly modular gene network for detection of nanoparticle pollution.
4. To experimentally characterize our gene network in terms of detection threshold for silver nanoparticles.
5. To convert both our new biological parts and AgNP detecting gene network into bio-brick format and to submit them to the Registry of Biological Parts.

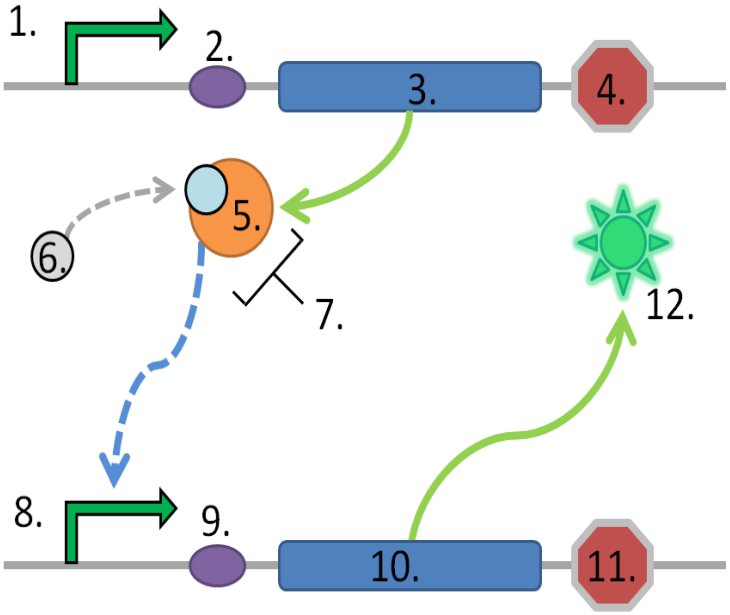
Biological Parts and Network Design

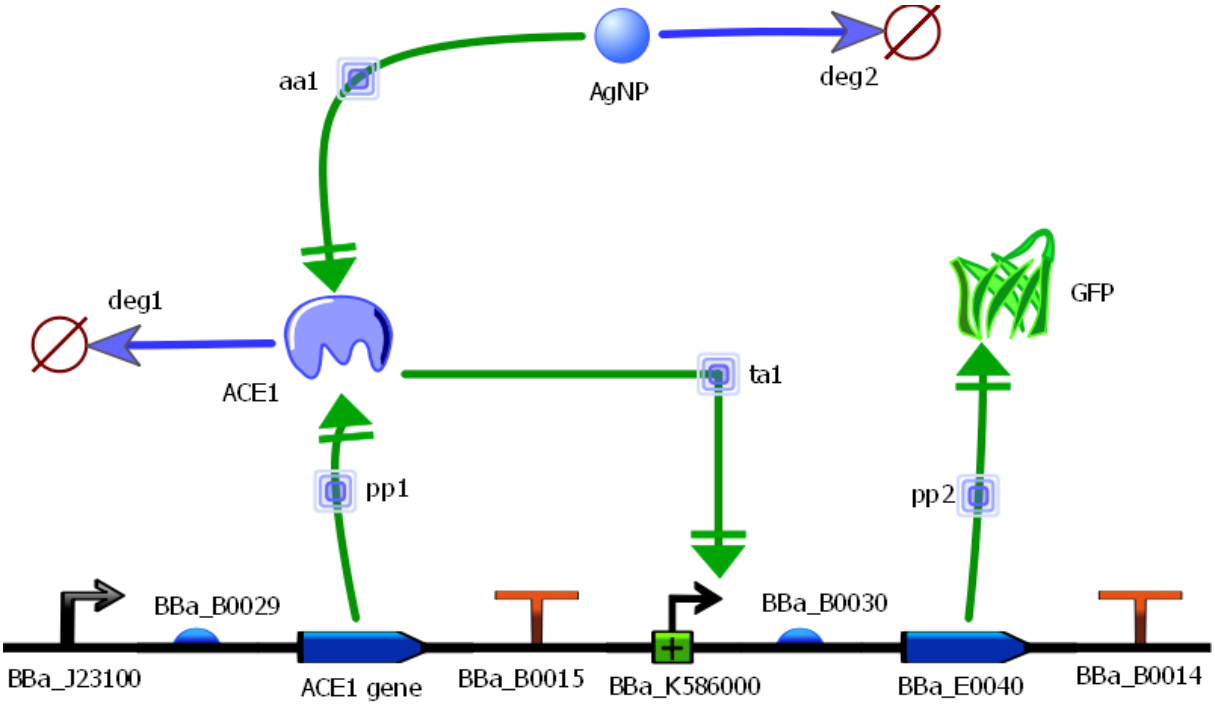
We incorporated a diverse selection of existing and new biological parts into our silver nanoparticle biosensor. Existing parts were selected from the Registry of Biological Parts based on both reliability (user experience) and strength. On the other hand, we identified our new biological part, the yeast transcription factor ACE1, by using BLASTn to search numerous yeast genome databases for the DNA sequence coding for ACE1. Afterwards we used the network modeling program Tinker Cell to design our

network and the program ApE (A plasmid Editor) to assemble the plasmid that would contain our gene network (pictured below).

Biological Parts

1. BBa_J23100 - Constitutive Promoter
2. Bba_B0029 - RBS Community Collection
3. ACE1 gene - aka CUP2
4. BBa_B0015 - Double Terminator
5. ACE1 Transcription factor (Activator)
6. AgNP - silver nanoparticle
7. AgNP/ACE1 Protein Ligand Complex
8. BBa_K586000 - CUP1 Promoter
9. BBa_B0030 - RBS Community Collection
10. BBa_E0040 - GFP Gene
11. Bba_B0014 - Double Terminator
12. GFP





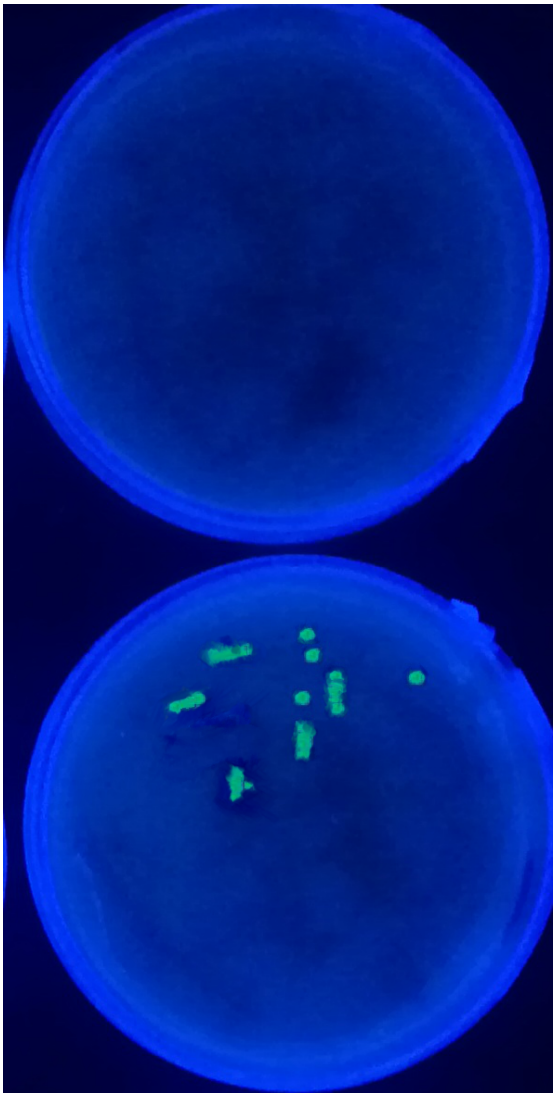
In this biosensor network, a constitutive promoter (1.) continuously produces the transcriptional activator ACE1 (5). This activator only functions in the presence of silver (6.) or copper and activates transcription at the CUP1 Promoter (8). CUP1 is placed upstream of a Green Fluorescent Protein (10) gene. As a result, in the presence of silver, our network begins producing GFP causing the *E. coli* to glow green under UV light.

Results:

After computationally designing our biosensor network we synthesized the network plasmids *de novo* through Gene Oracle (sponsor). Network plasmids contained the ampicillin resistance gene and arrived already transformed into *E. coli* in the form of bacterial stabs. Network success was tested *in vivo* through a simple fluorescence based assay of our design. In order to create the silver nanoparticles necessary for the assay, silver nitrate (AgNO3) was oxidized using a 1% sodium citrate solution. Directly following filtration, 50 uL of silver nanoparticle solution was spread on each of three experimental plates of LB Agar Medium supplemented with ampicillin. Biosensor Bacteria were plated using sterile technique on each of the three experimental plates as well as on three control plates containing ampicillin but no silver nanoparticles. Plates were incubated over night at 37 degrees Celsius. In order to observe fluorescence, plates were placed under UV light.

After overnight incubation, all three control plates showed bacterial growth indicating that the gene network had successfully been transplanted into the bacteria. On the other hand, only one of the experimental plates showed bacterial growth, and only in a certain region of the plate. The most likely explanation is that the bacteria on the experimental plates showing no growth had died out due to high concentrations of the highly antimicrobial silver nanoparticles. However, due to uneven spreading, the silver nanoparticles in certain regions of the experimental plate with growth had been low enough to allow for bacterial growth. Our theories were confirmed after the plates were examined under UV light. The experimental plates with no bacteria showed no fluorescence as expected. In addition the control plates (top picture) also showed no fluorescence indicating that the biosensor would not produce GFP without the presence of silver. Finally the experimental plate with bacterial growth (bottom picture) showed fluorescence indicating that the bacteria were able to detect the presence of the silver nanoparticles and produce GFP in response.

After testing our silver nanoparticle biosensor we mini-prepped out our network plasmid and converted it into biobrick format using a series of restriction and ligation digests.



Discussion:

While the AgNP Biosensor has now been shown to work, we are curious as to why the network and bacteria were only active in one of the three experimental plates. Our current hypothesis is that the AgNP's antimicrobial effects must be taken into account after a certain concentration threshold. Correspondingly, our biosensor network should be responsive for a specific range of AgNP concentrations. In our experiment, it is very likely that the bacteria grew on a portion of one of the plates due to uneven spreading of the AgNPs - the bacteria grew in an area of reduced AgNP concentration. Although our biosensor bacteria may be extremely sensitive to high concentrations of AgNP's we've demonstrated that they can both survive and sense low concentrations of AgNP's. Once we determine this sensitivity we can begin construction of a simplified assay which should allow us to determine the approximate AgNP concentration by diluting water samples until the bacteria is able to both survive and detect the AgNP's. In addition, our biosensor constitutes a major first step toward both creating inexpensive biosensors for nanoparticle pollution and addressing a growing public health concern.

Also, we are currently in the process of producing and analyzing accurate computational models (using

TinkerCell, which generates a system of coupled differential equations to describe our network and can also provide deterministic and stochastic models of gene expression). We plan to present these results alongside our biological results for comparison at the 2012 iGEM jamboree.

Future Work:

As mentioned before, we are currently in the process of producing computational models for our network in silico. However, we also want to further experiment with our biosensor in vivo to better characterize our part and to clearly define the sensitivity of the biosensor. We plan to do this by testing the gene expression of the bacterial gene network on agar plates with only 20, 30, and 40 ul of AgNP solution (same concentration as used before). We also want to get a better understanding of the microbial effects of AgNPs.

We would also like to test the modularity of our biosensor network by cutting out the ACE1 gene and CUP1 promoter and replacing them with other transcription factor - promoter combinations for the detection of metal nanoparticles.

Conclusion:

The 2012 NCSSM iGEM team set out with a goal to engineer an inexpensive biosensor for harmful silver nanoparticles using a highly modular gene network. We also sought to experimentally characterize the network *in vivo* using AgNPs we harvested in our own lab. We have largely addressed these goals and are continuing to work towards some of them. We have successfully shown that our network does work but have found that the network is extremely sensitive to the concentration of the AgNPs present on agar plates. We have also successfully produced and submitted our own biobricks to the registry of biological parts and eagerly look forward to the 2012 HS iGEM jamboree! Our silver nanoparticle biosensor is a crucial first step in addressing the relatively unacknowledged problem of silver nanoparticle pollution and its social implications.