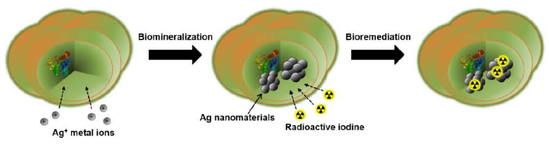
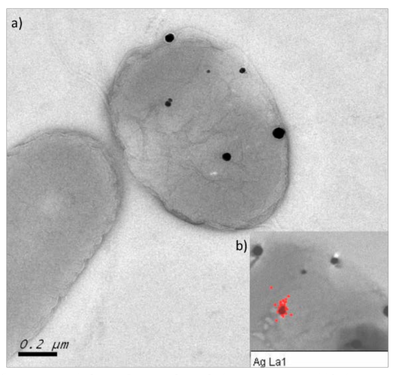
DNA-Binding of Unphosphorylated Response Regulator AgrR From *Cupriavidus metallidurans* Involved in Silver Resistance (Ag-NPs formation)

Md Muntasir Ali[1,2], Ann Provoost[2], Kristel Mijnendonckx[2], Rob Van Houdt[2], Daniel Charlier[1]
[1]Research Group of Microbiology, Department of Bioengineering Sciences, Vrije Universiteit Brussel, Brussels, Belgium
[2]Microbiology Unit, Interdisciplinary Biosciences, Belgian Nuclear Research Centre (SCK CEN), Mol, Belgium
E-mail: md.muntasir.ali@sckcen.be

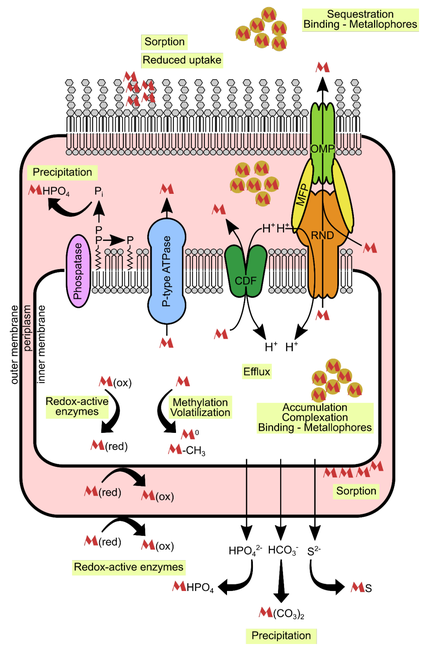
Introduction



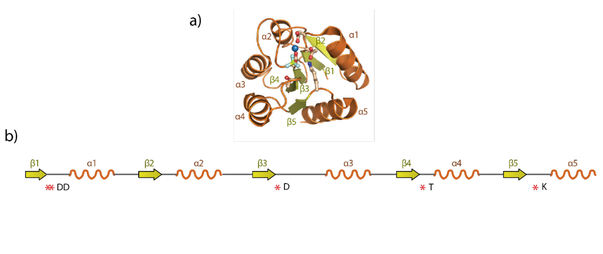
Ag Nanoparticles (Ag-NPs) can be used to bind radioactive elements for Bioremediation[1]



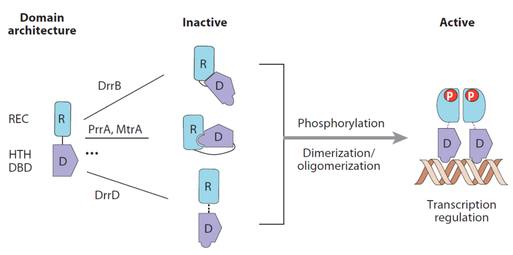
Ag-NPs formed in *Cupriavidus metallidurans* NA4S



Different mechanism of bacteria for heavy metal resistance



Signaling protein of Bacteria responsible for activating /deactivating certain mechanisms



Canonical pathway showing the need of phosphorylation for DNA-protein interaction[2]

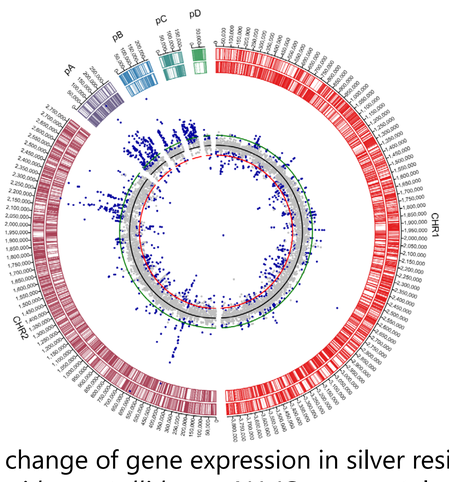
Methods

The metal resistant bacterium *Cupriavidus metallidurans* were subjected to high silver ion concentration to generate a laboratory evolved strain with higher resistance to silver ions. This strain known as *C. metallidurnas* NA4S was analyzed to find out that they form nanoparticles around them. This novel finding initiated us to find more about the pathway it uses to generate these nanoparticles. Expression of genes specific to this resistance strain forming nanoparticle was identified through microarray and RNA-seq. The gene *agrR* translating to regulatory protein were cloned and overexpressed in *E. coli*. This protein was mutated to change the amino acid responsible for signaling into a dephosphomimetic and a phosphomimetic amino acid. AgrR protein binding to DNA was shown with Electrophotretic mobility shift assay (EMSA). The bound DNA location was analyzed by DNase I and in gel copper-phenanthroline footprinting. AgrR-DNA complexes were analyzed to see the high resolution base-specific contact map of AgrR-DNA interactions using premodification binding interference techniques.

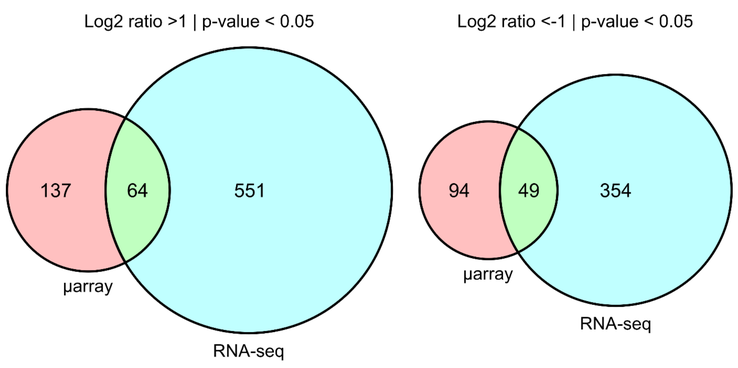
Objectives

- Investigate further the formation of Ag-NPs in *C. metallidurans*
- To pinpoint the previously unknown regulation of this novel silver resistance mechanism leading to silver nanoparticle formation.
- DNA-protein high resolution interaction map

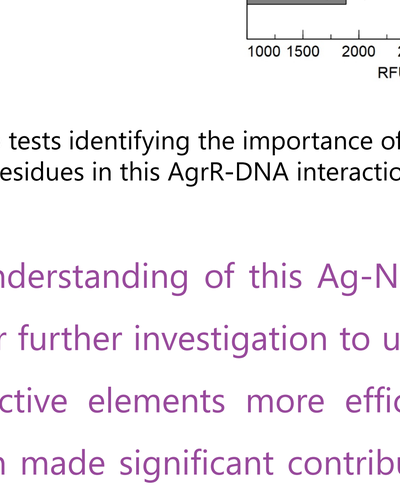
Results



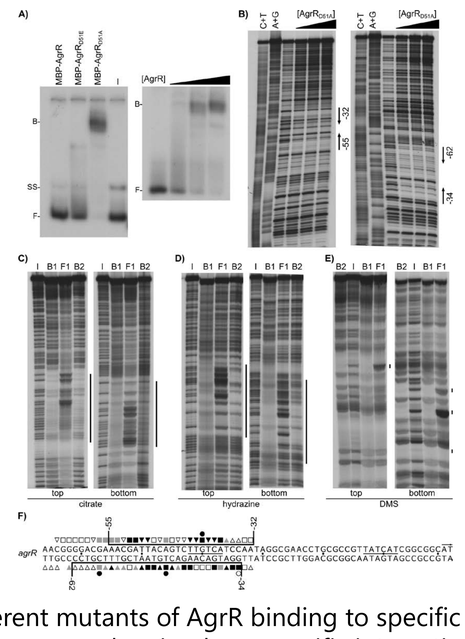
Global change of gene expression in silver resistant *Cupriavidus metallidurans* NA4S vs parental strain



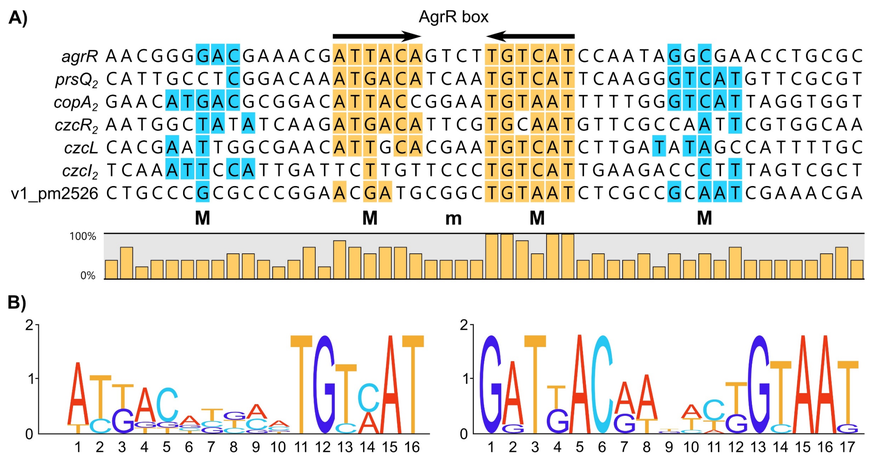
Overlapping genes in microarray and RNA-seq



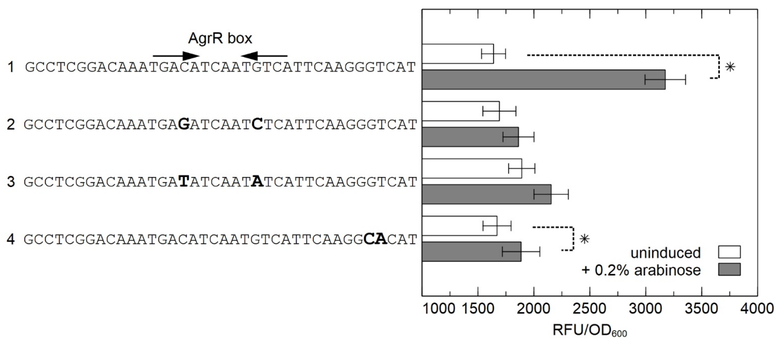
Mutant of different genes confirming the role of specific regulator *agrR* and some other genes



Different mutants of AgrR binding to specific DNA sequences showing base specific interactions[3]

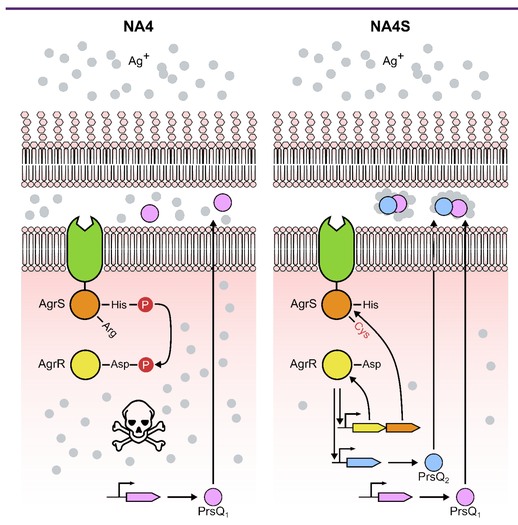


AgrR-DNA interaction revealed a pattern in the DNA sequence which it recognizes and binds effectively[3]



In-vivo tests identifying the importance of specific residues in this AgrR-DNA interaction[3]

Conclusion



Revealing the complexity of novel silver resistance mechanism leading to Ag-NPs formation

This research paved way for the understanding of this Ag-NPs forming pathway that is a stepping stone for further investigation to use the bio-remediation techniques for radioactive elements more efficiently and effectively. This research publication made significant contribution to fill the knowledge gap existed in this field. A fundamental understanding of the mechanism is needed. In this publication, we have provided the most fundamental understanding of the silver nanoparticle formation pathway. This unlocked prospect of using bioremediation techniques for radioactive elements from waste by Bacteria.

Exploring a better tomorrow
www.sckcen.be