

### 63. Identification of genomic elements required for uranium resistance by *Caulobacter crescentus*

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**Project Goals:** Uranium (U), particularly in its water soluble form U(VI), has been shown to be a significant environmental toxin and a major contaminant at DOE legacy sites. One potential method to remediate U contamination is to use microbes that are highly tolerant of U and are able to decrease its mobility in the environment. *Caulobacter crescentus* is an aerobic, aquatic bacterium that is highly tolerant of U and has the potential to be used for U bioremediation especially in oxic zones. The overall aim of our studies is to understand the mechanisms by which *Caulobacter crescentus* tolerates and immobilizes U in the environment using a combination of genetic, biochemical, and omic approaches.

*Caulobacter crescentus* is a ubiquitous, aerobic bacterium known to survive in nutrient limited environments and to tolerate high levels of U(VI). The detoxification mechanisms by which *C. crescentus* resists U are poorly understood. Here, we show that *C. crescentus* is able to facilitate U (VI) biomineralization through the formation of U-Pi precipitates via its native alkaline phosphatase activity. The U-Pi precipitates, deposited on the cell surface in the form of meta-autunite structures, have a lower U/Pi ratio compared to U precipitates formed abiotically. The enzyme that is responsible for the phosphatase activity and thus the biomineralization process is identified as PhoY, a periplasmic, alkaline phosphatase with broad substrate specificity. PhoY is shown to confer a survival and growth advantage to *C. crescentus* under U (VI) stress. These results highlight U(VI) biomineralization as a resistance mechanism in aerobic microbes with potential applications for bioremediation in the environment.

In order to gain a further understanding of how *C. crescentus* resist U, we employed a transposon mutagenesis screening approach (Tn-seq) to identify essential genomic elements that are required for U resistance. In our method, highly saturated transposon (Tn) mutagenesis was first performed to generate a library of 106 mutants. The library was then grown on solid agar plates containing U, Cd, or no metal control. Mutants surviving each exposure were subsequently sequenced via high-throughput Illumina sequencing at JGI to identify Tn insertion sites. Genes that accumulated fewer Tn insertions under U stress compared to the Cd or no stress controls were identified as genes specific for U tolerance. Using this method, we identified 15 genes potentially involved in U tolerance, which were subsequently tested through mutational analysis. Genes identified to be involved in U tolerance include those for TolC-like transporters RsaFa and RsaFb, previously identified to be involved in S-layer protein transport, and stress factors CztR and CztA. These key genes provide important insight into the various resistance pathways employed by *C. crescentus* for survival under U stress.