Escherichia coli Based Biotemplating
Team Minnesota

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ABSTRACT
The objective of our project is the construction of a light-inducible system in which E. coli is engineered to express silicatein in order to create a biotemplating system. This system has many potential uses, such as the creation of precise nano-structures or biomimetic bone. The colloid light-inducible system was assembled by cloning a fusion protein of Cph1 and EnvZ, called Cph8, from a previous iGEM group, and isolating and cloning the two other genes (Heme Oxygenase and PcyA) required for functionality of the system and putting all the parts into a vector. Then the silicatein alpha gene isolated from Suberites domuncula was fused with E. coli outer membrane protein A (OmpA) and ice nucleation protein (INP) and inserted in the vector under control of the colloid system.

INTRODUCTION – BIOPOLYMERIZATION
• Organisms have evolved diverse and complex methods of biomineralization.
• Many biopolymers are valuable in industry, medicine, and the environment.

SILICATEIN
• Protein responsible for spicule formation in sponges
• Nucleates silica polymerization and metal crystallization
• Can cause the formation of metal sheets when expressed in E. coli

LIGHT INDUCTION SYSTEM
• Light induction system designed by University of Texas at Austin and UCSF iGEM team in 2004
• Bacteria take a chemical image – essentially a photograph
• Colloid system available in the iGEM parts registry

OBJECTIVES
1) Achieve E. coli cell surface display of functional silicatein.
2) Assemble Colloid system from parts registry.
3) Couple Colloid system to surface-displayed silicatein to achieve light-induced silica encapsulation of cells.

STRATEGY
• surface display of silicatein:
  - obtain gene for silicatein from Suberites domuncula
  - create fusions with outer membrane protein A (OmpA) and Ice Nucleation Protein (INP)
  - assess surface display through microscopy and silicatein functional assay

• colloid control of silicatein expression:
  - assemble colloid system from parts registry was unsuccessful
  - reassemble the colloid system using plasmids from Voigt lab
  - insert silicatein fusions behind the pomC promoter
  - test light-controlled expression of silicatein

RESULTS
• The silicatein standard curve was generated by diluting the initial TMOS concentration
• There is good linear agreement among replicates allowing absorbance data to be correlated to the fitted curve

CONCLUSIONS
1) Cloning of silicatein with surface display components has resulted in E. coli expressing fully functional silicatein. These parts have been submitted to the parts registry (BBa_K632001 and BBa_K632002)
2) The colloid light-sensitive system has been successfully assembled, and has been submitted to the parts registry (BBa_K632003).
3) Future directions include coupling the light-sensitive system to surface-displayed silicatein to achieve light-induced silica encapsulation of cells.

FOOTNOTES
3. A generous gift from Dr. Garnier of the Institute of Physiological Chemistry, University of Giessen University, Giessen, Germany.