Nature-Inspired Biomineralization Strategies for Sustainability

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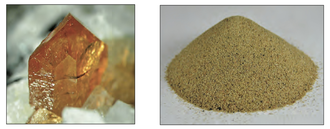
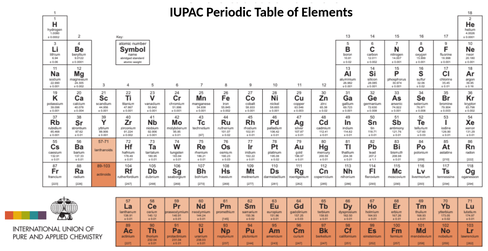
Background

Rare Earth Elements are ubiquitous in modern technology and with the electrification of our economy, demand is increasing. In 2022, $160 million of REEs were imported to the United States.[1]



Bastnaesite (left) and monazite (right) are minerals containing lanthanum and cerium. Images from ref. 2.

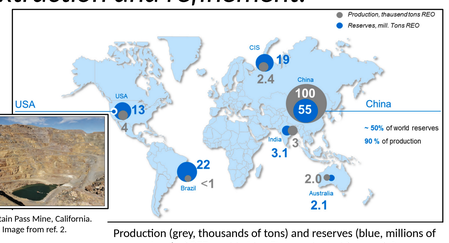
REEs are in our phones, cameras, cars, and more!



China controls up to 95% of REE extraction and refinement.[2]

To take advantage of the remaining 5% of REE deposits, there is a need for effective REE extraction. Current hydrometallurgical techniques require harsh solvents and high temperatures yet have low efficiency.

Mountain Pass Mine, California. Image from ref. 2.



Production (grey, thousands of tons) and reserves (blue, millions of tons) of REE worldwide. Figure adapted from ref. 3.

REE Extraction with Synthetic Biology

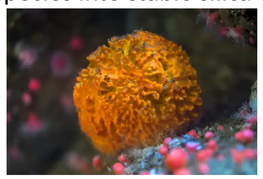
Rare Earth Elements are difficult to identify and extract.

Acid mine drainage. Image from ref. 2.

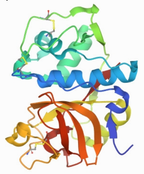
Mining can only identify large REE deposits and yields hazardous waste streams. Smaller amounts can only be identified with specialized techniques such as inductively coupled plasma optical emission spectrometry.

With synthetic biology and protein engineering we can target trace amounts of REEs.

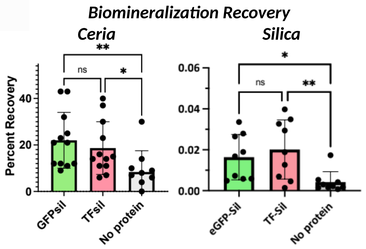
Silicatein is an enzyme found in marine sponges that mineralizes free silica species into stable silica oxide, and other inorganic ions to stable oxides.

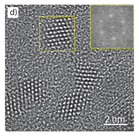


Silicatein was originally identified in *Tethya aurantia*, the orange puffball sponge.
Image source: Monterey Bay Aquarium

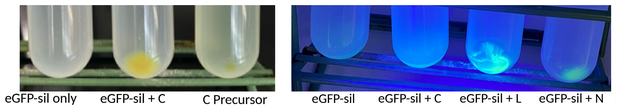


Crystal structure of recombinant silicatein.
PDB 6ZQ3





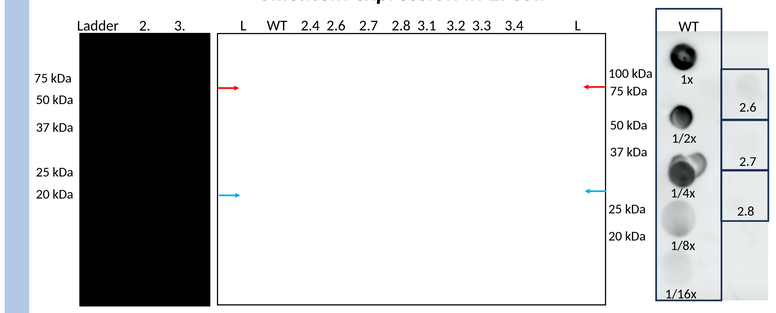
Mineralized ceria nanoparticles. Image from ref. 4.



Directed Evolution with Silicatein

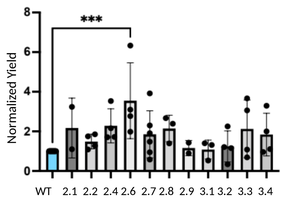
Recombinant expression of silicatein in E. coli requires the addition of a protein fusion tag to support protein solubility. Here, we seek to increase solubility, stability, and enzyme kinetics via directed evolution.

Silicatein expression in E. coli



Left: Nickel affinity chromatography for purified silicatein fusions 2) eGFP-silicatein anticipated MW 50 kDa, 3) TF-silicatein anticipated MW 75 kDa. Significant band at 26 kDa in lane 2 has been identified as DnaK chaperone protein. Figure from ref. 5. Middle: SDS PAGE with whole cell lysate library samples, including WT TF-sil, 2.4, 2.6, 2.7, 2.8, 3.1, 3.2, 3.3, and 3.4. Anticipated molecular weight of TF-silicatein and mutants is 75 kDa, indicated by red arrow. Anticipated molecular weight of DnaK chaperone protein is 26 kDa indicated by blue arrow. Figure from ref. 7. Right: Immunoblot with library whole cell lysate samples WT TF-silcation, 2.6, 2.7, 2.8. WT TF-silicatein dilutions are shown compared to 1x of mutants 2.6, 2.7, and 2.8. Figure from ref. 6.

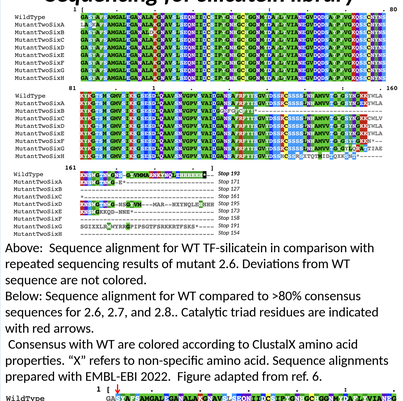
Ceria Mineralization



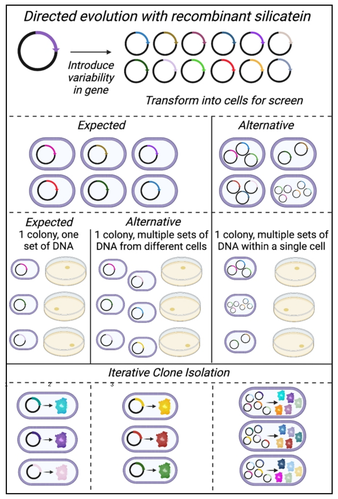
Ceria mineralization of wild-type and mutant TF-silicateins in whole cell lysates, normalized to the production of WT. Each sample normalized by total protein concentration. Two-way ANOVA, n > 2, *** P<0.001. Figure from ref. 6.

Secondary screening of library mutants shows significantly greater ceria mineralization with mutant 2.6. This is in stark contrast with the immunoblot results suggesting that each mutant has much less expression than the WT, despite all samples normalized by total protein concentration within lysate. Presumably, the sequence of mutant 2.6 confers greater biomineralization activity than the WT. Mutants 2.6, 2.7, and 2.8 were sequenced repeatedly, however results varied each time, which is consistent with multiple mutant proteins being co-expressed within the same sample.

Sequencing for silicatein library



Above: Sequence alignment for WT TF-silicatein in comparison with repeated sequencing results of mutant 2.6. Deviations from WT sequence are not colored.
Below: Sequence alignment for WT compared to >80% consensus sequences for 2.6, 2.7, and 2.8.. Catalytic triad residues are indicated with red arrows.
Consensus with WT are colored according to ClustalX amino acid properties. "X" refers to non-specific amino acid. Sequence alignments prepared with EMBL-EBI 2022. Figure adapted from ref. 6.



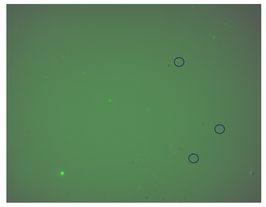
Expected and alternative outcomes of the experimental procedure surrounding directed evolution with recombinant silicatein are shown. The three potential outcomes following interative clone isolation are shown, with 1 and 2 being one set of DNA associated with each colony, and 3 showing multiple sets of DNA coinciding. Figure made with Biorender. Figure from ref. 6.

Collaboration with USAFA

Cyanobacteria can grow and thrive on Mars. Furthermore, cyanobacteria can function as a food source for E. coli or for oxygen generation in life support systems.[7,8] *Here, we explore silicatein genetic engineering in cyanobacteria and microbially induced calcite precipitation combined with biosilicification for in situ resource utilization. This work relies on cyanobacterial expertise and infrastructure at USAFA, in addition to targeting space applications of DoD interest.*

eGFP-silicatein expression in S. elongatus PCC 7942

Cyanobacteria consume CO_2 via photosynthesis. Expression of silicatein in cyanobacteria could provide carbon-negative biomineralization activity. Furthermore, utilizing cyanobacteria for recombinant protein expression includes the added benefit of post-translational modifications, which occur in marine sponges, but not in E. coli. We are also pursuing silicatein expression in S. elongatus UTEX 2973 and *Anabaena* PCC 7120.

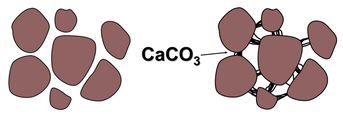


Biosilicification to enhance biocementation in austere environments

Martian regolith as seen by NASA Curiosity rover.
Image source: NASA

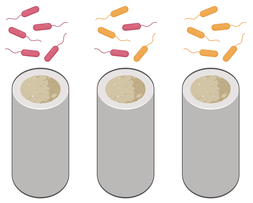
Biocementation with microbially induced calcite precipitation by S. pasteurii has been widely studied. Here, we hypothesize that the addition of biosilicification by silicatein will confer greater mechanical strength and resistance to UV degradation.

$$H_2N\text{-}CO\text{-}NH_2 + 2H_2O + Ca^{2+} \xrightarrow{\text{Urease}} 2NH_4^+ + CaCO_3$$



S. pasteurii has significant urease activity.

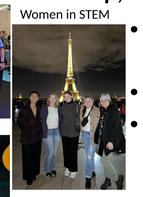
Here, we surface display silicatein on *E. coli* for *in vivo* use with bricks



S. pasteurii | *S. Pasteurii + E. coli INP-sil* | *E. coli INP-sil*

Preliminary experiments show that S. pasteurii sand-based bricks can withstand approximately 140 lbs of pressure. In future experiments, the mechanical strength of bricks treated with silicatein will be evaluated.

Leadership, Mentorship, and Educational Benefits



Women in STEM

USAFA iGEM

- Understanding the role of biotechnology and research in defense strategies
- Mentoring and women in STEM
- Attended conference "Biotech 4 Defense" with researchers from AFRL, ARL, NRL, and other DoD research agencies

DoD research with AFRL and Space Force exposure

The results of this collaborative research with USAFA and AFRL are expected to lead to a joint publication in a peer-reviewed journal, with a manuscript under preparation for Frontiers special issue: "Systems Microbiology in Biomanufacturing, and Industrial Scale-Up"

Future and Ongoing Work

- *Evaluate in vivo biomineralization with INP-silicatein for mine and e-waste water applications.*
- *Explore cyanobacterial silicatein expression (both internal and surface-displayed) for biomineralization activity and carbon sequestration.*
- *Examine S. pasteurii and E. coli INP-silicatein biocementation bricks for mechanical and optical strength, including evaluation of UV degradation. Strategize for applications in austere environments.*
- *Assess the potential for silicatein biomineralization in REE extraction in space.*
- *Identify alternative biomineralization players for critical mineral extraction.*

References and Acknowledgements

1. Survey, U. S. G. *Rare Earths - Mineral Commodity Summaries*; 2022. **2.** Van Gosen, B.S., *et al.* "The rare-earth elements – vital to modern technologies and lifestyles," *USGS*, 2014. **3.** Jaroni, M.S., *et al.* "Economical feasibility of rare earth mining outside China," *Minerals*, 2019. **4.** Curran, C.D., *et al.* "Direct single-enzyme biomineralization of catalytically active ceria and ceria-zirconia nanocrystals," *ACS Nano*, 2017. **5.** Vigil, T.N. *et al.* "Understanding the relationships between solubility, stability, and activity of silicatein," *Material Advances*, 2023. **6.** Vigil, T.N. *et al.* "Directed Evolution of Silicatein Reveals Biomineralization Synergism Between Protein Sequences," *Chemical Biology*, under review. **7.** Mapstone, J.L. *et al.* "Cyanobacteria and microalgae in supporting human habitation on Mars," *Biotechnology Advances*, 2022. **8.** Verseux, C. *et al.* "A low-pressure, N_2/CO_2 atmosphere is suitable for cyanobacterium-based life-support systems on Mars," *Frontiers in Microbiology*, 2021.

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