Curli Fiber-Carbonic Anhydrase Fusion Proteins for Microplastic Capture and Reuse

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Abstract

Microplastics are becoming increasingly prevalent, with an estimated 5 to 12 million tons of plastic entering the oceans annually (Jambeck et al., 2015). Existing remediation efforts often consist of wastewater treatment or disposal in landfills, unsustainable solutions which eventually lead to microplastics leaching back into the environment. At the same time, the U.S. Geological Survey reports that one billion tons of limestone are quarried annually in the United States alone. Our team aims to address these issues by engineering *E. coli* to co-express plastic-binding curli fibers and a biomineralization pathway, thereby capturing microplastics in in situ limestone formations. We experimentally characterized modified curli-carbonic anhydrase fusion proteins to evaluate plastic binding and biomineralization capability, with computational modeling revealing structural constraints that limited proper curli fiber formation. We also developed a proof-of-concept using *S. pasteurii*, using its natural urea hydrolysis to produce biomineralized aggregates via induced calcite precipitation and designed molds to test the tensile and compressive strength of our biomineralized material. Together, our work demonstrates the feasibility of integrating microplastic trapping systems with biomineralization, offering a sustainable pathway for environmental remediation.

Microplastic Pollution

Microplastics are increasingly widespread and are now found in the environment, the food chain, and the human body. Jambeck et al. find that 5 to 12 million tons of plastic enter the ocean yearly, decomposing into microplastics and harming both the environment and humans. An estimated 7.2 trillion microplastic particles enter San Francisco Bay annually through urban stormwater runoff, which is equivalent to enough particles to carpet the Golden Gate Bridge three times over each year (Ross et al.). Efforts that reclaim plastics from the environment often end up in landfills and wastewater treatments, where the microplastics leak back into the environment (Landfills as a Major Point Source for Microplastic Pollution, n.d.).

Microplastics in the environment have a drastic impact. Zhu et al. (2025) find that current exposure to microplastics is responsible for a 7 to 12% reduction in photosynthesis in terrestrial plants globally. Microplastics travel up the food chain, ultimately ending up in the human body. “Plastics love fats, or lipids, so one theory is that plastics are hijacking their way with the fats we eat which are then delivered to the organs that really like lipids — the brain is top among those,” says Matthew Campen, Professor of Pharmaceutical Sciences at the University of New Mexico in Albuquerque. A 2024 study he coauthored found that across 12 autopsy samples, microplastics made up an average of 0.48% brain mass, 50% higher than samples taken earlier in 2016 (Nihart et al., 2025). Although the full extent of the impacts of microplastics is unknown, research indicates that they increase the likelihood of heart attack, stroke, or death. The need to remove microplastics from the environment has never been more urgent.

Current Solutions Addressing Microplastic Pollution

A number of solutions exist for microplastic capture, a few of which we describe here. “Microcleaners,” developed by Haeleen Hong et al. at NC State, are comprised of soft dendritic colloids (SDCs) and can be used as an efficient method for finding, collecting, and removing microplastics from oceans. The microcleaners concentrate into “pellet” agglomerates which have the ability to self-propel via releasing surface active compounds. The differential surface tension between released compounds and the water body induces propulsion described by the Marangoni effect. While traveling across the water, the microcleaners coil then disperse the SDCs, which sediment and entrap the microplastics, and the two combined perform transverse active motion and float up to the surface.

A number of companies even commercialized microplastic binding solutions, such as Polygone Systems, which uses such methods for water filtration, and Seabin, which runs on funding from company donors. A common thread is that while a number of companies are able to capture microplastics, few companies describe solutions to handle microplastics after their capture.

Currently the best solutions for handling plastics after collection tend to involve some kind of plastic degradation or recycling. For instance, Yip et al. describe microbial solutions to break down polyethylene terephthalate (PET), one of the most common plastics used today. There are microbes and insects that are able to metabolize plastics initially believed to be non-biodegradable, albeit at a slow rate. Solutions to recycle individual plastics, such as PET and HDPE, do exist and are often used Almack (2023).

One big issue is the purity of the plastics to be recycled in these systems. Yip et al. note that PET is more susceptible to biodegradation than most plastics due to hydrolysable ester bonds. The same problem exists with recycling. While certain kinds of pure plastics, such PET and HDPE, can be recycled, mixed plastics are rarely, if ever, recycled (Almack).

This creates a disconnect between systems that collect mixed plastics, like Seabin and Polygone, and forms of plastics disposal. Our project provides a possible solution to bridge these gaps.

The Sand Shortage and Current Solutions

The global demand for construction aggregates has grown at a fast rate, and estimates suggest that between 40-50 billion metric tons of sand and gravel are mined every year, outpacing the natural rate at which natural sediment is replenished (UNEP, 2019; Torres et al., 2017). Furthermore, riverine and coastal sand mining has been found to impact sediment transport, cause instability in riverbanks, decrease the groundwater table, and increase delta and coastal erosion (Koehnken & Rintoul, 2018). With the increasing scarcity of high-quality natural sand, there has been a shift towards using crushed stone and manufactured aggregates. In the United States alone, 1.5 billion tons of crushed stone were produced in 2023, with limestone being the dominant type of crushed stone (Willett & USGS, 2024). However, quarrying and processing crushed stone is energy-intensive, as well as causes significant land disturbance and produces carbon emissions. The use of recycled construction aggregates currently meets only a small portion of the total demand due to contamination, variability in mechanical properties, and regulatory issues (UNEP).

Our Solution

Our team aims to address both of these issues by engineering *E. coli* to co-express plastic-binding curli fibers and a biomineralization pathway, thereby capturing microplastics in *in situ* limestone formations. In particular, we have tested two different microbially induced calcite precipitation (MICP) pathways for biomineralization: a carbonic anhydrase-catalyzed pathway and a urease-catalyzed pathway. Our resulting aggregate could be utilized to both immobilize harmful microplastics and replace non-renewable resources like sand for construction and backfilling projects.

Our goal was to design bacteria that would simultaneously produce a sticky biofilm and induce biomineralization, capturing microplastics and forming an aggregate usable in construction. We chose to use curli fibers, a key component of *E. coli* biofilms, because they exhibit high adhesive properties and strong tensile mechanical properties (Zhang et al., 2017). Examining pathways involved in microbially induced calcite precipitation (MICP), we found that bacterial strains utilizing the urea hydrolysis pathway showed more calcite precipitation (~20–80%) in comparison with other metabolic pathways (Castro-Alonso et al., 2019). This pathway is primarily driven by the enzymes urease and carbonic anhydrase (CA). To simultaneously produce curli fibers and induce biomineralization, we decided to emulate the fusion protein of curli fiber and amylase that Neel Joshi's Biologically Fabricated Materials Lab designed (Birnbaum et al., 2021). Since carbonic anhydrase is a monomeric enzyme in the urea hydrolysis pathway, it was natural to design and experiment with a curli fiber and carbonic anhydrase fusion protein. We chose to use the strain *E. coli* K12 to express our fusion protein, as *E. coli* K12 can, under the right conditions, produce both curli fiber and carbonic anhydrase.

Our second goal was to create concrete biomineralized products. We chose to use *S. pasteurii*, which naturally has a urea hydrolysis pathway that induces calcium carbonate precipitation. We used sand to act as a substrate and scaffold for mineralization.

We divided our experimentation into two parts: characterizing expression of modified Curli-CA proteins and developing a proof-of-concept with *S. pasteurii*.

Characterizing Expression of Curli Fibers

Design and Insertion

We aimed to simultaneously produce curli fiber and induce biomineralization using one fusion protein. The 2023 iGEM team MSP-Maastricht's biomineralization carbonic anhydrase module inspired us to use sazCA, which is the fastest carbonic anhydrase to date (Zhu et al., 2022). Simply, we sought to engineer and produce a csgA-sazCA fusion protein.

To simultaneously produce curli fiber and induce biomineralization, we designed a part BBa_25A8Q07G that could produce and export a fusion protein of csgA, the primary curli monomer, and sazCA, the fastest carbonic anhydrase to date. Our designed curli fusion protein emulates the curli fusion proteins from Neel Joshi's Lab (Birnbaum et al.), connecting csgA to sazCA via a flexible linker. We inserted our composite part BBa_25A8Q07G into the Twist vector pET blank with ampicillin resistance, creating the plasmid BBa_25J7AMCD.

After transforming *E. coli* K12 with our curli-CA plasmid, we confirmed our results using colony PCR and sequencing.

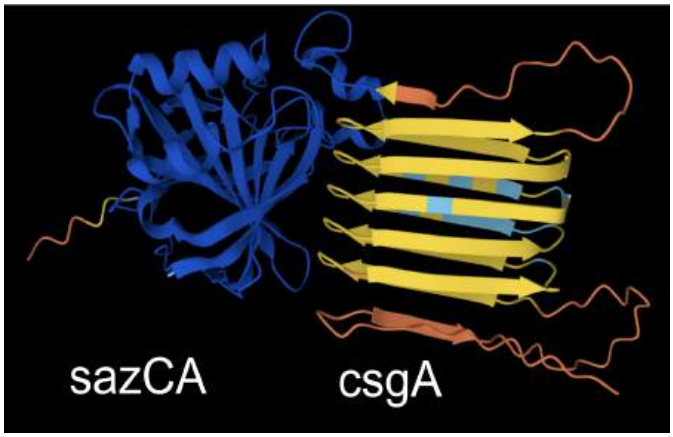
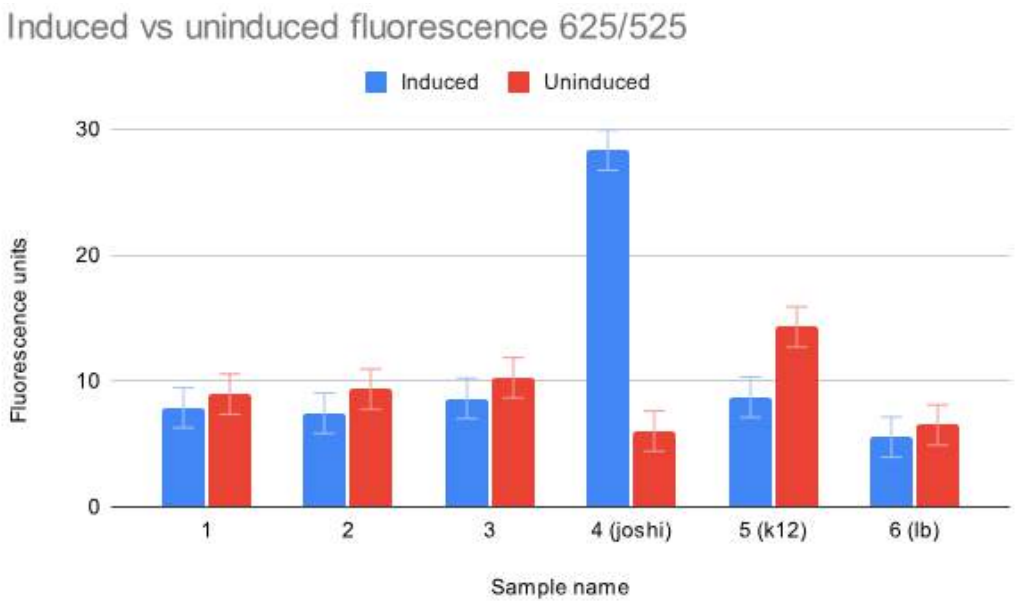


Image of our csgA-sazCA fusion protein, generated by AlphaFold

Checking Protein Expression

We tested a range of IPTG concentrations to find the optimal concentration for inducing protein expression. To test protein expression, we used a congo red fluorescence assay inspired by Kan et al. (2019) as well as an absorbance-based spin-down assay from the 2014 iGEM Delft-Leiden team. From these results, we decided to proceed with an inducer concentration of 0.2 mM. We found the fluorescence assay more reliable than the absorbance assay and used it for future experiments.

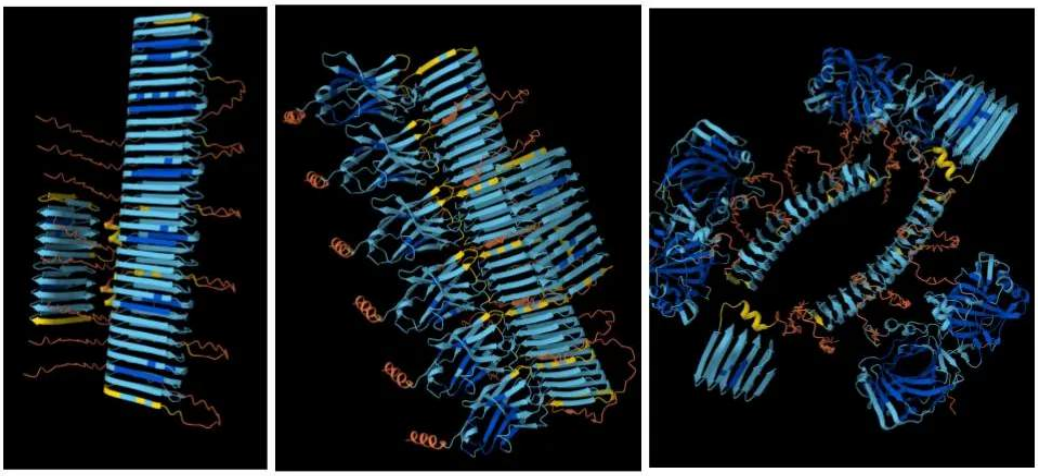
We then performed a congo red fluorescence assay in quadruplicate to test for the presence of curli fibers. Congo red binds to amyloid fibrils like those found in curli fibers, producing more fluorescence at 525 nm excitation and 625 nm emission when bound to these fibrils.



Induced vs uninduced fluorescence readings, with data in quadruplicate. Samples 1,2, and 3 are transformant cultures. Joshi is a curli expressing strain we received from the Joshi lab, intended as a positive control. Lb is a negative control of LB broth.

This assay supported our positive control expressing curli, but indicated to us that our transformants were struggling to express curli. Given that our transformants were confirmed by sequencing, we suspected that our protein had issues folding.

Using AlphaFold 3, we modeled the oligomerization of our modified curli fiber as compared to our positive control, and saw significant differences in folding.

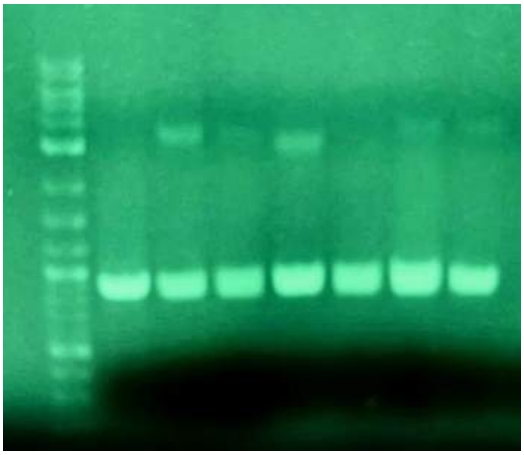


From left to right: Curli fiber monomers polymerizing normally, positive control Joshi lab strain curli fiber polymerization, our modified curli fiber polymerization. Note the pore-like structure instead of the expected fiber formation.

Checking RNA Expression

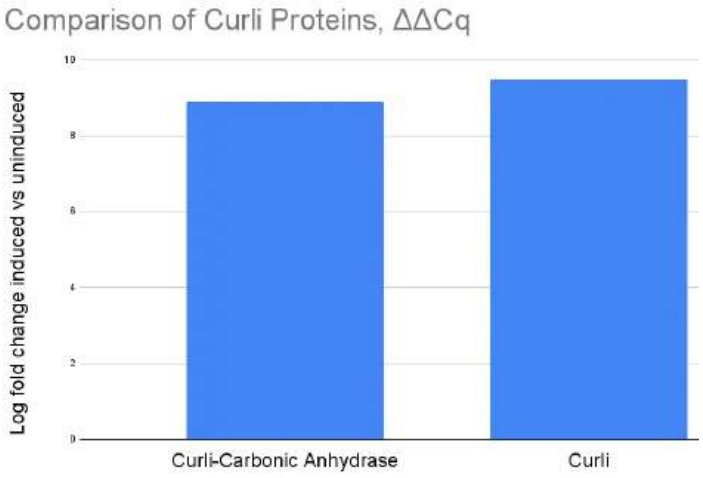
To determine whether the issue in expression occurred post-transcriptionally, we assayed RNA expression. We hypothesized based on the AlphaFold modeling that the issue occurred after transcription (e.g. translationally).

Induro RT was utilized to create cDNA of existing mRNA transcripts in induced samples. The RT-PCR gel showed mRNA expression of curli was present in both our modified protein as well as positive control, indicating the problem was likely related to protein folding further down the line. The bright lines below 1 kb show transcription of the housekeeping gene in all samples. The lines between 3 and 4 kb show curli expression.



Gel of RT results. From left to right: Ladder, K12, Joshi, Joshi induced, Curli, Curli induced, Curli-CA, Curli-CA induced.

To further quantify RNA expression of our genes relative to the rssA housekeeping gene, we ran RT-qPCR utilizing Bioneer's accupower greenstar qPCR mix. ΔΔCq values were plotted, and the results showed highly similar induction across csg-sazCA gene cluster (BBa_25A8Q07G) and the unmodified curli operon (BBa_25A80I7T). This further supports that lack of expression resulted from issues that were post-transcriptional.

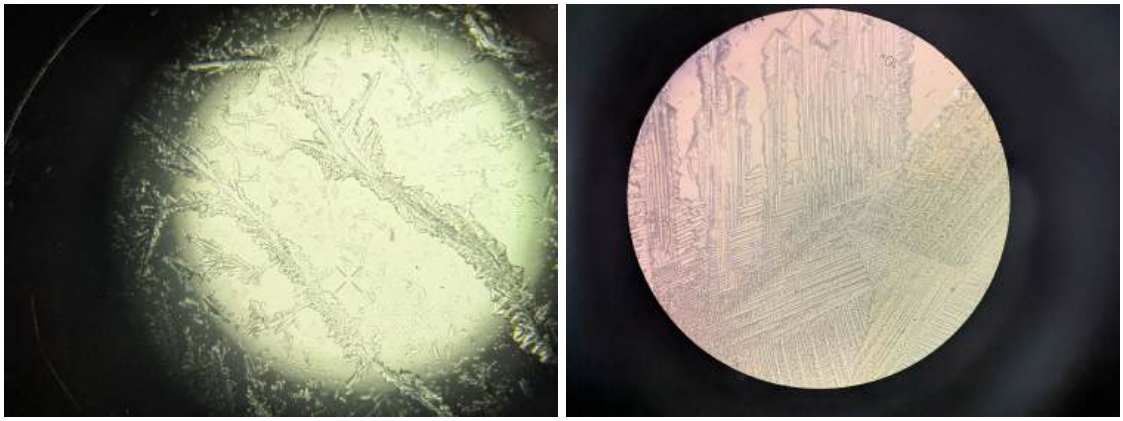


Graph of delta-delta Cq of curli-carbonic anhydrase expression vs plain curli expression. Note the relatively small, around log fold two difference in expression, indicating that RNA transcription does occur for the Curli-Carbonic Anhydrase fusion protein.

Proof-of-Concept

In parallel with our main experimental work, we sought to create a biomineralized product using *S. pasteurii*'s natural biomineralization capabilities. *S. pasteurii* uses the urea hydrolysis pathway, which converts urea into ammonia and bicarbonate. When Ca^{2+} ions are added to the solution, calcium carbonate mineralizes around the bacteria. The resulting product serves as a proof-of-concept of the formation, strength, and appearance of a biomineralized material.

We procured *S. pasteurii* culture, appropriate growth media, and the necessary protocol—Troyer et al., 2017—from Kelsey De Frates Ph.D., from the Hernandez Lab at UCSF. For one month, we conducted daily interchange between *S. pasteurii* culture and precipitation media to induce calcite precipitation. This interchange was performed in three molds, one which was our PDMS mold and two other normal 3D molds, all filled with sand to act as nucleation sites.



From left to right: S. pasteurii culture in CMM- under 400x magnification, S. pasteurii culture in CMM- under 100x magnification.

To estimate the strength of our biomineralized material, we created molds following ASTM standards for tensile/compressive strength testing, but with scaled down dimensions. Specifically, we built the PDMS mold from a 3D printed PETG inverse based on our CAD model, following our protocol "Manufacturing PDMS Channel-Pore Microfluidic Devices." The resulting material had a cross-sectional area of 2 mm^2, allowing us to estimate tensile strength. Samples were then taken out of molds and sterilized as per our sterilization protocol. We were able to confirm that our protocol successfully sterilized our samples via a swab test.Our project integrated computational modeling at every stage, from initial design to final system validation. We began with Internal Modeling to shape our project's design, and this foundational work led to the development of our primary contribution: a powerful, outward-facing External Modeling pipeline designed to quantify and predict the dynamics of engineered biological systems, which we present as a reusable tool for the iGEM community.

Image of our sterilized samples. Note that the right most sample, prepared in a PDMS mold, performed the best, breaking into 3 clean pieces on removal.

Computational Modeling

Part 1: Internal Modeling

To accelerate our project's design phase, we used a suite of in silico tools to make critical decisions before beginning wet lab work. This approach allowed us to evaluate potential candidates, rule out non-viable paths, and accelerate our conceptualization process, saving valuable time and resources.

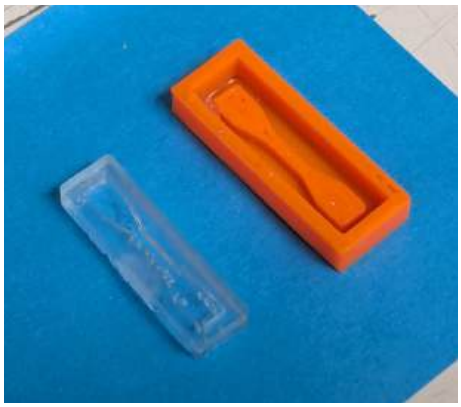


Image of PDMS casted from a 3D printed PETG inverse. The PDMS mold is then used to create a biomineralized sample in the appropriate shape for strength testing.

When we struggled to identify a core protein for our system, we turned to computational tools, such as AlphaFold to predict the 3D structure of our bioengineered protein.

We then used CAD to design physical hardware for our system, such as the mold which we in turn used to cast the final device from Polydimethylsiloxane (PDMS), a biocompatible polymer.

Part 2: Reusable Modeling Pipeline

To quantify and predict the performance of our system, we developed a comprehensive software pipeline. This tool automates the entire workflow from raw experimental data to a sophisticated system simulation with uncertainty analysis, and we have packaged it as a robust, reusable tool for the

iGEM community. We followed a three-stage pipeline described below:

Stage 1: Assay

This stage is the wet lab to dry lab conversion step, where we take dual-wavelength spectrophotometer absorbance traces (≈560/435 nm) by converting them to pH via the phenol-red ratio and the Henderson–Hasselbalch relationship.

Stage 2: Fit

We aggregate per-run initial rates v_0 from Stage 1 and pair each with its corresponding substrate concentration (([S])) from run_meta.csv. For each enzyme, we fit the Michaelis–Menten model by nonlinear least squares whenever at least three distinct substrate concentrations are available.

Stage 3: Simulate

Our software uses these experimentally-derived parameters to power a system of Ordinary Differential Equations (ODEs) that forecasts the system's behavior over time.

Conclusion

As the problem of plastic pollution increases in magnitude, solutions must continue to innovate. Existing processes, which focus only on filtering plastics out of the environment and disposing of them in landfills, are expensive and fail to address issues of redegradation of captured particles. Our new process, which immobilizes the microplastics in place and creates a calcium carbonate matrix around them, simplifies the process by removing the need for landfill space for captured plastics. The end product of the process, a rock composed mostly of calcium carbonate, can be used for many situations calcium carbonate would ordinarily be used in, such as infilling construction projects, helping offset costs of capture. Finally, infilling has the benefit of preventing microplastics from entering the ecosystem by burying them under several feet of inert material. Our resulting aggregate simultaneously immobilizes plastic and creates a new material usable in construction, effectively solving two problems at once.

Future work on potential leakage of microplastics and strength testing still need to be conducted before potential use. We hope our work provides an iterable basis from which further research can be conducted, contributing to making a more sustainable world.

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