A New Foundation for Engineered Cell-Cell Adhesion

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Introduction

Multicellular systems rely on **cell–cell adhesion** to organize individual cells into coordinated structures. Engineering synthetic adhesion systems enables the controlled assembly of multicellular microbial communities, expanding the capabilities of synthetic biology in areas such as metabolic cooperation, environmental remediation, and living therapeutics.

Current synthetic adhesion strategies have key **limitations** [1]. As it stands, synthetic adhesion can either be **controllable but weak** via noncovalent bonding or **strong but irreversible** via covalent bonding [2, 3].

To address these gaps, we developed a **modular toolkit for programmable cell–cell adhesion,** leveraging surface-displayed SpyTag, SpyCatcher, and TEV protease proteins. The SpyTag-SpyCatcher system is a pair of proteins that was engineered to spontaneously form an isopeptide bond with each other [4]. The TEV protease is a virus-derived protein commonly used to cleave peptide bonds [5]. The Spy system creates covalent bonding across cells, and the TEV protease allows for breakage of those bonds.

This system led us to **achieve the first engineered covalent bond across domains of life**.

Potential Applications

- Improving the Retention of Live Microbial Therapies in the Intestinal Tract
- Optimizing Metabolite Diffusion for Biosynthesis Through Engineered Proximity
- Improving Microbial Wastewater Treatment Through Enhanced Symbiotic Relationships

Future Works

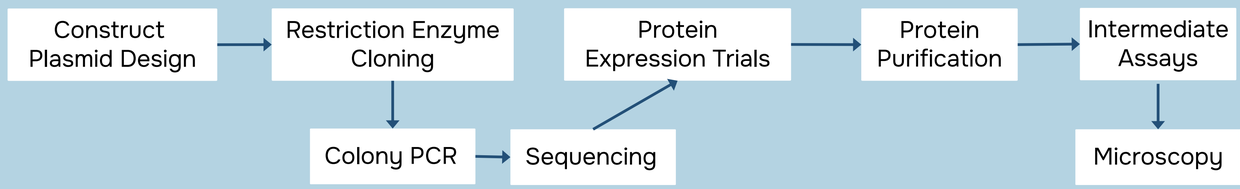
- Image samples during induction to monitor aggregate formation kinetics in real time.
- Perform finer IPTG titrations and varied induction times to quantify relationships between inducer concentration, induction time, and aggregate size.
- Expand the system into more diverse organisms.

Methods

Restriction-Ligation Cloning of Designed Constructs:
- Seven constructs were assembled via restriction-ligation cloning and transformed into *E. coli* DH5α/*S. cerevisiae*
- Cloning was confirmed by colony PCR, diagnostic digest, and whole-plasmid sequencing.

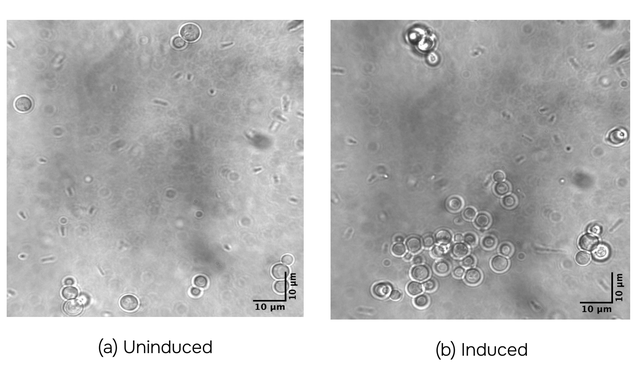
Protein Expression & Purification:
- Proteins of interest were expressed in BL21 under optimized IPTG induction conditions, purified by nickel bead affinity chromatography, and quantified by SDS-PAGE and NanoDrop.

Functional Validation:
- Bond formation, cleavage, and inter-species adhesion were assessed by fluorescence microscopy.



Results

(1) Yeast-Bacteria Aggregation



(a) Uninduced (b) Induced

Figure 1: Bright-field microscopy images of *S. cerevisiae-E. coli* clicking assay; 100x magnification.

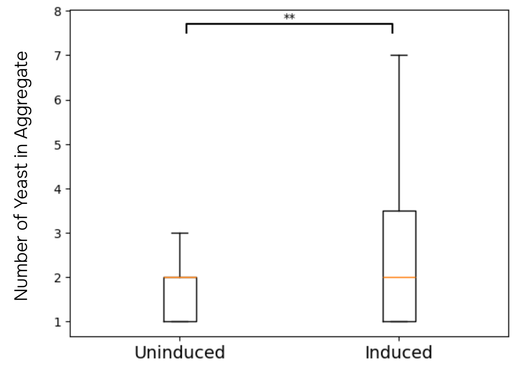
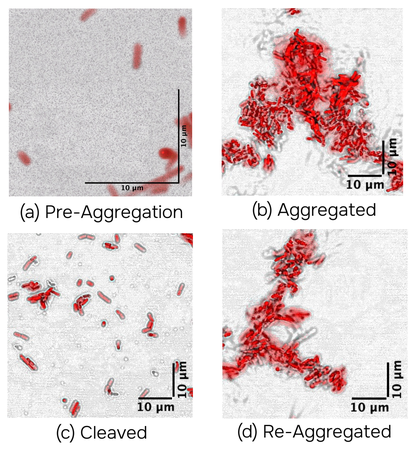


Figure 2: Comparison of Cell Count Per Aggregate

(2) Bacterial Aggregate Reformation



(a) Pre-Aggregation (b) Aggregated
(c) Cleaved (d) Re-Aggregated

Figure 3: Composite red fluorescence and bright-field microscopy image of aggregate reformation; 100× magnification.

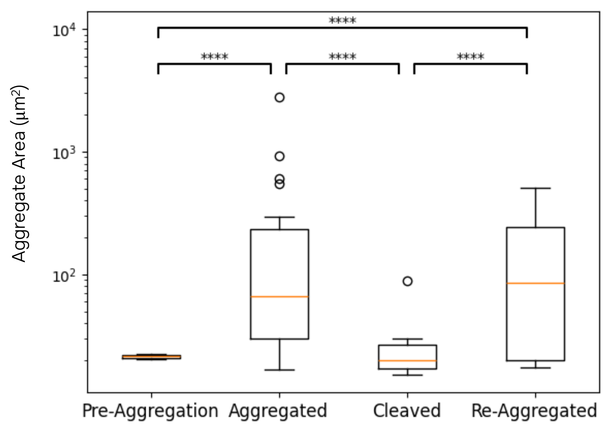


Figure 4: Comparison of Aggregate Area Under Different Induction Conditions

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