Simplified Cells as New Tools for Synthetic Biology and Biological Sensing

David C. Garcia[1,2] Bryan Gerber[2], Rory Williams[2], Charles Sanfiorenzo[2], Matthew Lux[1], Kaihang, Wang[2], Richard Murray[2]

[1]Combat Capabilities Development Command Chemical Biological Center, Aberdeen Proving Ground, MD, [2] California Institute of Technology, Pasadena, CA

Abstract

A set of simplified cells (SIM cells) capable of controllably transitioning between growth and growth arrest have been developed to function as a new platform for biosensing and biomanufacturing applications. Using a synthetic auxotrophy for a non-canonical amino acid(ncAA), Boc-Lysine (BOC), a recoded strain of Escherichia coli has been developed capable of transitioning between unperturbed growth in the presence of BOC and a dormant state in its absence. Interestingly, the dormant SIM cell's machinery remains active allowing them to process genetic programs, react to external stimuli, and regenerate new SIM cells even after weeks of dormancy. We show that a variety of SIM cells can be generated through the introduction of stop codons into essential proteins using multiplex automated genome engineering (MAGE) and that the control of escape frequency makes them a potential safe agent for biological sensing and bioremediation efforts. This technology showcases a set of tools for reprogramming cellular design and the construction of biological chassis with smart systems ideal for environmental, healthcare, biomanufacturing applications.

Introduction

Engineering robust biological frameworks requires specialized systems capable of reliably performing researcher-designed tasks while maintaining the basic functions required for survival. As a result, the incentives to evolve, adapt, and reproduce often stand in contrast to the needs of synthetic biology as the inherent complexity and variability in cells and genetic networks can obfuscate and impede the function of genetic circuitry. Simplified cells function as a novel solution for synthetic biology as they do not follow the traditional rules for living organisms, instead existing in a simplified state wherein their growth and evolution are impeded, but their cellular machinery remains active[1]. Sim cells straddle the divide between living and nonliving organisms making them ideal tools to generate insights into the fundamental requirements for life as well as bioproduction and biosensing platforms in non-traditional environments. The advantage of not replicating allows sim cells to function within the human body and natural environments without the dangers of unregulated growth[2]. We foresee the ability to reversibly control cellular growth will allow for significant improvements to biological chassis, and enable novel functions like cryptographically protected bioproduction strains and long-term dormant biosensors.

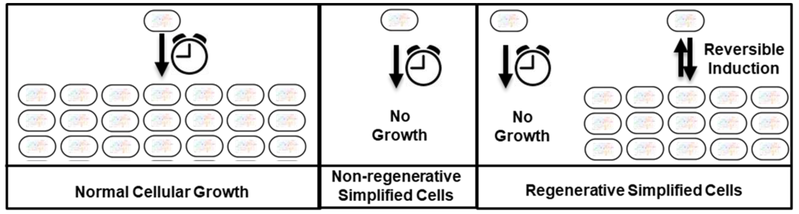

Figure 1. Various growth regimes for biological systems. **A.** Normal cellular exponential growth over time. **B.** SIM cell state wherein regrowth of biomass is not possible. **C.** SIM cell state where cells can transition between growth and non-growth phenotypes.

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Methods

Gene	ftsL	murD	ftsZ	dnaE	dnaX	mukB	murJ	zipA	dnaG	dnaA	dnaB	mreB
Function	Cell Division	Cell Wall	Cell Division	DNA Replication	DNA Replication	Cell Division	Cell Wall	Cell Division	Cell Division	Cell Division	Cell Division	Rod shape-determining

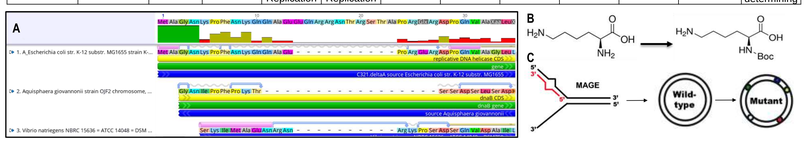


Figure 2. Addition of amber suppression codons through MAGE was selected based on the essentiality of genes and permissiveness of residues within proteins. **Table .** subset of genes known to control various aspects of division and replication. **A.** Homology analysis based on multiple protein homologs indicates permissive residues based on tertiary structure. **B.** Boc-lysine was chosen due to ease of use and price. **C.** MAGE uses separate oligos to simultaneously introduce multiple mutations along a bacterial genome[3].

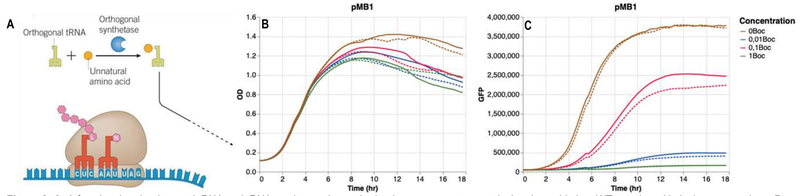


Figure 3. A. A functional and orthogonal tRNA and tRNA synthetase is required to incorporate non-canonical amino acids into WT proteins with Amber stop codons. **B.** The pMB1 orthogonal tRNA and tRNA synthetase pair was shown to not cause growth defects in WT cells. **C.** Effective Boc incorporation was shown using the pMB1 pair through the expression of GFP with an Amber mutation.

Results

Cultures are Enriched for SIM cells using Antibiotic Treatments

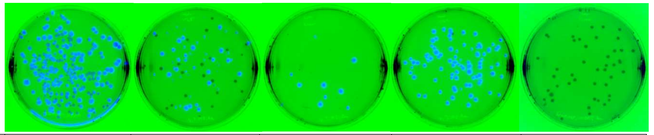


Figure 5. WT LUX+ cells were co-cultured with SIM cells. Enrichment was tested using antibiotic treatments while starving before plating on BOC.

Treatment	No BOC	Carb	Riff	H2O 1:10k	Kan
Sim Cell %	0	29	100	1	100

SIM cell state is Active and Reversible

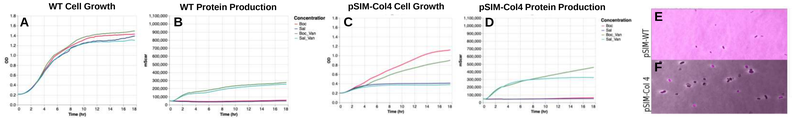
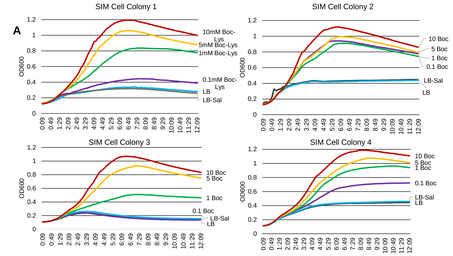


Figure 6. Starved SIM cells can recover from a growth impediment caused the addition of amber stop codons. **A/B.** WT cells grow normally under BOC. **C/D.**SIM cells are capable of growing on BOC and produce protein in the simplified state. **E.** Microscopy images of induced WT cells. **F.** Sim cells induced for mScarlet production.

Varying ncAA Requirements are Based on Genotype



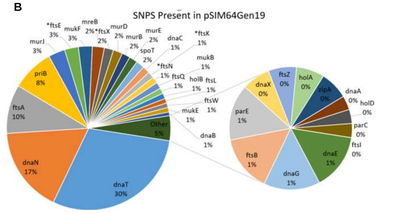
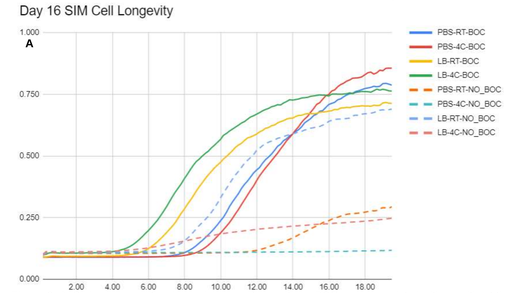


Figure 7. A. Amino acid requirements for regeneration of SIM cells changed based on the genotype of the cell. Sequencing data reveals a preference for specific genes being more amenable to recombineering during MAGE.

Simplified Cells Maintain Metabolic Activity



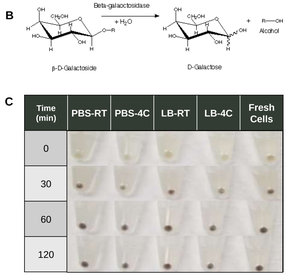


Figure 8. A. SIM cells were stored in various conditions with no BOC and reinoculated with an without the addition of BOC. **B.** The breakdown of CPRG causes a red precipitate to form and accumulate in cells. **C.** β-galactosidase test used as test for metabolic activity indicating cells can transfer and process ions and sugars following 26 days starvation in multiple conditions.

Conclusions

- Boc Lysine can be incorporated into essential proteins to create reversible auxotrophies.
- Simplified cells can maintain a dormant state using reversible auxotrophies.
- MAGE cultures can be enriched with SIM cells using antibiotic treatments.
- Reversible auxotrophies are long lasting, but not unbreakable depending on conditions.
- Dormant SIM cell allows genetic programs and metabolic functions to be carried out.

References

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