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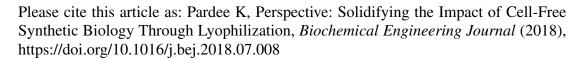
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ACCEPTED MANUSCRIPT

Perspective: Solidifying the Impact of Cell-Free Synthetic Biology Through Lyophilization

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Highlights

- Review of freeze-dried cell-free (FD-CF) systems
- FD-CF applications in sensing and portable biomanufacturing
- Challenges and opportunities ahead for the field

Abstract

Cell-free synthetic biology is an exciting and new branch in the field of synthetic biology. Based on in vitro transcription and translation systems, this application-focused domain builds on decades of cell-free biochemistry and protein expression to operate synthetic gene networks outside of cellular environments. This has brought new and perhaps even unexpected advantages. Chief among these is the ability to operate genetically encoded tools in a sterile and abiotic format. Recent work has extended this advantage by freezedrying these cell-free systems into dried pellets or embedded paper-based reactions. Taken together, these new ideas have solved the longstanding challenge of how to deploy poised synthetic gene networks in a biosafe mode outside of the laboratory. There is significant excitement in the potential of this newfound venue and the community has begun to extend proof-of-concept demonstrations in important and creative ways. Here I explore these new efforts and provide my thoughts on the challenges and opportunities ahead for freeze-dried, cell-free synthetic biology.

Keywords: Cell-free synthetic biology, paper-based, sensors, diagnostics, portable therapeutic manufacturing, freezedried cell-free reactions

Introduction

The field of cell-free synthetic biology has undergone tremendous growth in the past few years and is on track to become an important domain of application-based synthetic biology. This builds on years of pioneering efforts by many such as, Swartz, Ueda, Noireaux and Jewett, who established the fundamental "operating systems" for a diverse range of proof-of-concept applications [1–7]. As part of this applied effort, during my postdoctoral studies with Jim Collins, we showed that cell-free systems can be freeze-dried and hosted in porous matrices such as paper [8]. This, along with the work of others [9], has introduced the exciting possibility of deploying poised synthetic gene networks outside of the laboratory in a biosafe mode. These freeze-dried cell-free (FD-CF) reactions also have the important advantage of allowing for distribution and storage at room temperature, and thus avoid the need for a cold chain. In the time since these ideas were first reported, work from us and from across the community has extended this concept to other exciting applications [8,10–16], and there is a growing interest in using FD-CF reactions for the delivery of synthetic biology and biotechnology to new environments.

From my perspective, what makes FD-CF systems so compelling is their potential to extend access to healthcare through the development of freeze-dried platforms for de-centralized diagnostics [10] and the portable manufacturing of protein-based therapeutics [11]. A similar case could be made for many other applications where portable sensing and manufacturing could enhance real world capabilities (e.g. agriculture, national security). Research in general stands to benefit greatly from the potential for FD-CF to enable rapid prototyping of genetic constructs and to make on-demand, small-batch custom molecular reagents broadly accessible [11,15,17]. Below, I will highlight a subset of the most exciting work to date in cell-free synthetic biology and

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