A cell-free polymer material platform: DNA-encoded smart materials for sensing and decontamination

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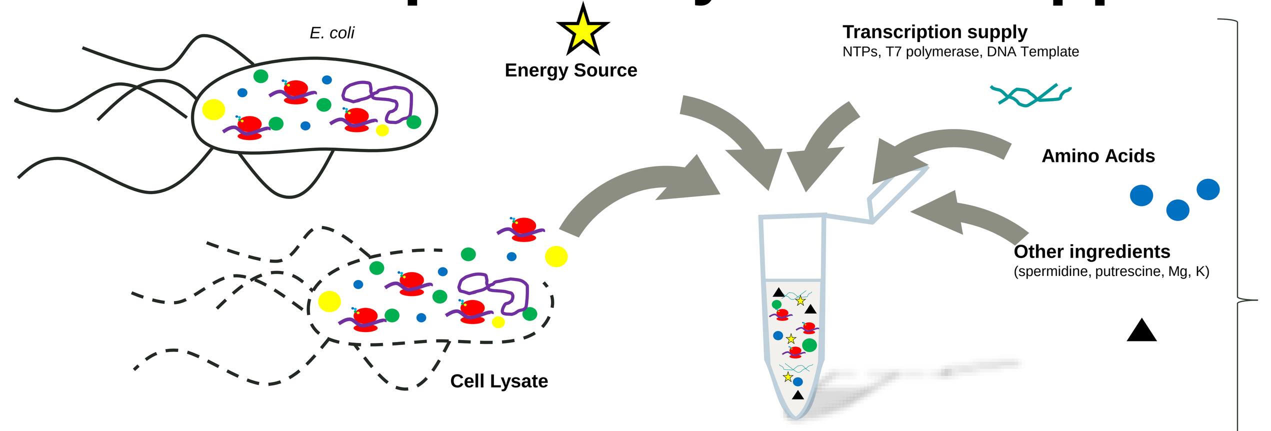
Abstract

Synthetic biology has yielded advanced capabilities to design biological functions including sensing, biomolecule or material synthesis, and catalysis, all controlled by DNA-encoded instructions. A conventional approach to bioengineering requires delivering new DNA instructions to a living cell and relying on that organism to perform the desired function while also maintaining cell growth and viability. That approach poses challenges for the stability of the system outside of the laboratory and incurs regulatory limitations surrounding release of genetically modified organisms. An alternative to the cell-based approach is using cell-free systems. These non-living bio-reactions are capable of complex functions like sensing and molecule synthesis without being packaged in a cell. We have found that dried cell-free systems are remarkably stable to both high temperatures and exposure to organic solvents, enabling the delivery of sophisticated and dynamic bio-reactions to synthetic polymer materials. We demonstrate that cell-free polymer composites can perform protein synthesis, sensing, and production of a functional antimicrobial. The ability to cast bio-functionalized polymers with solvents and heat greatly expands the types of materials and form factors that could benefit from bio-activity. We envision future applications in multi-functional coatings, fibers, or objects embedded with sensing, decontamination, or material modifying functions.

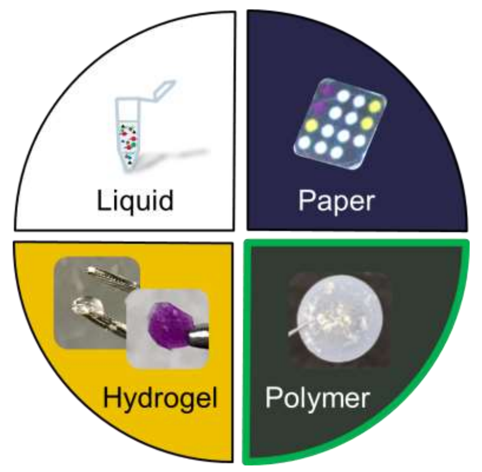
Cell-free protein synthesis: applications in materials



Features
- No cell viability limits
- Fast DBT cycle
- **May store dried – no cold chain**

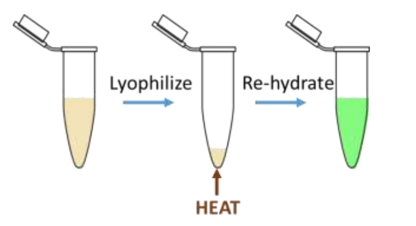
Applications
- Genetic prototyping
- Point-of-use biosynthesis
- Sensing

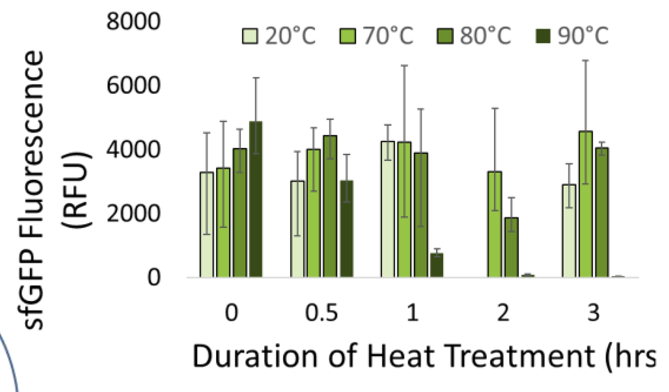
Freeze-dried CFPS

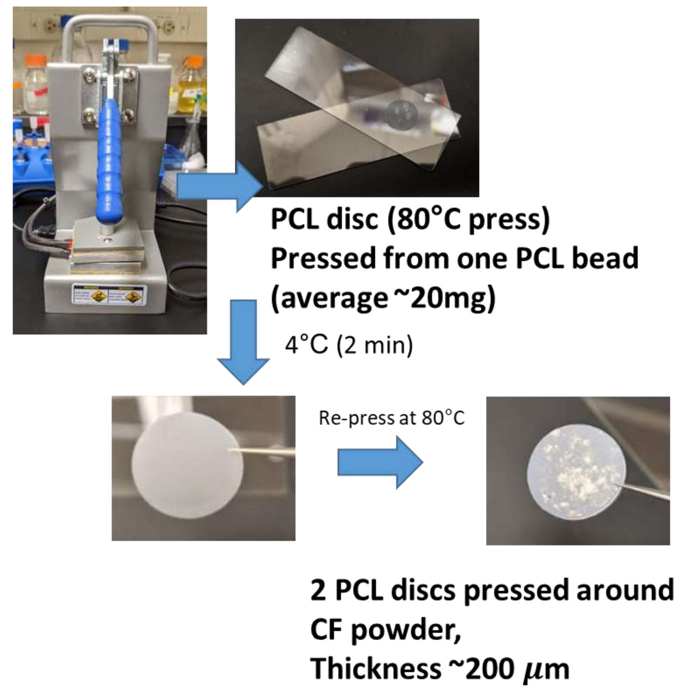


CFPS Materials

Stability of Dried CFPS: Heat







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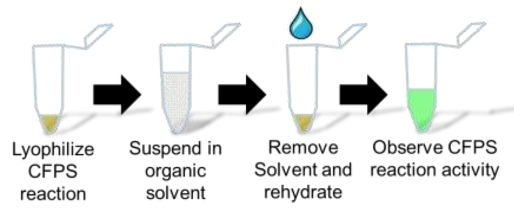
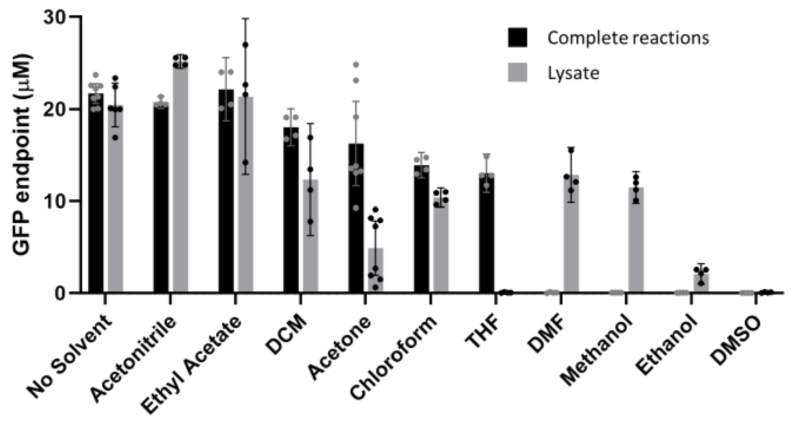
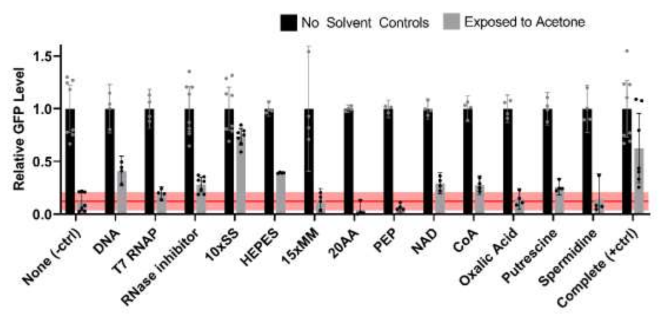
Stability of Dried CFPS: Organic Solvents

- Solvent stability well-studied for single enzymes
- However, this is the first demonstration of solvent tolerance for a complex multi-component bio-reaction.
- Excipients in CFPS recipes are important for solvent tolerance.

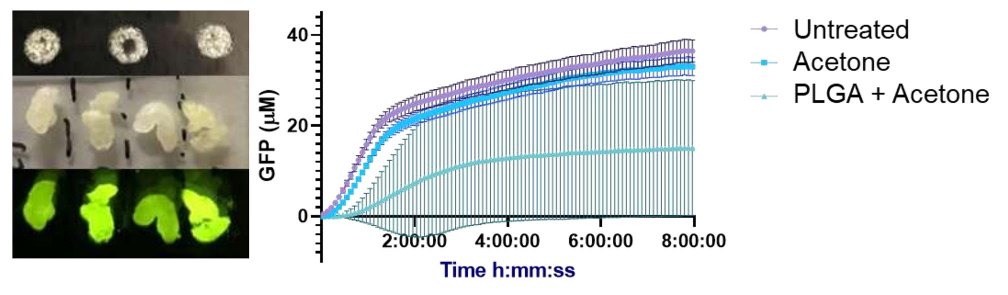




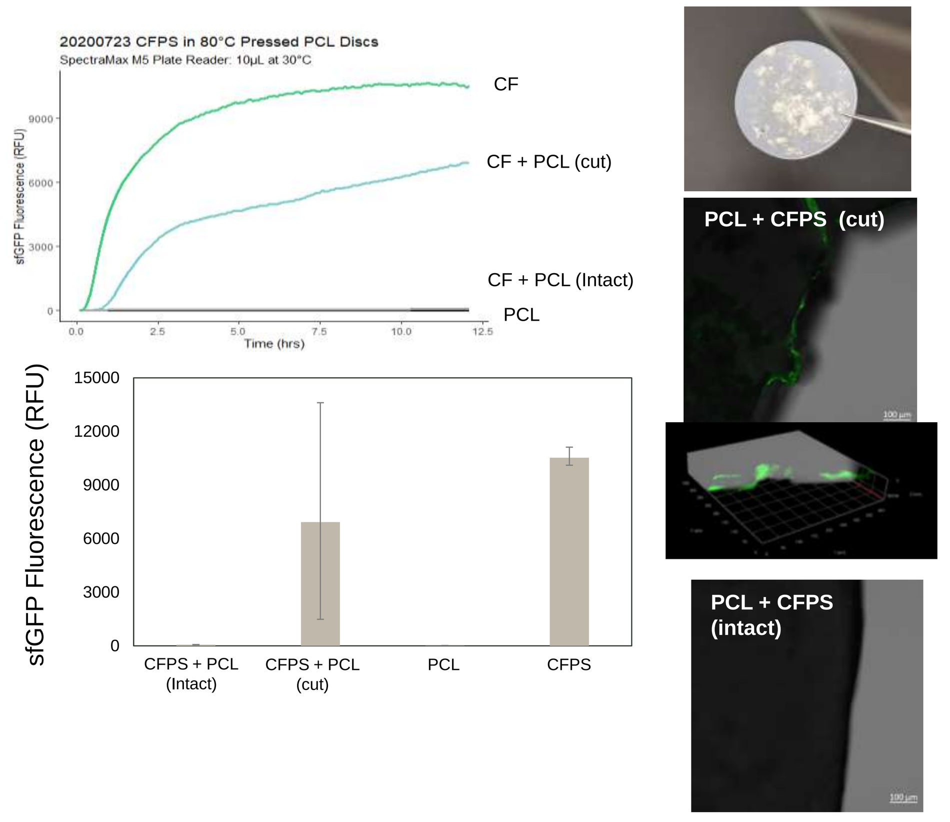


CFPS activity characteristics in different polymers

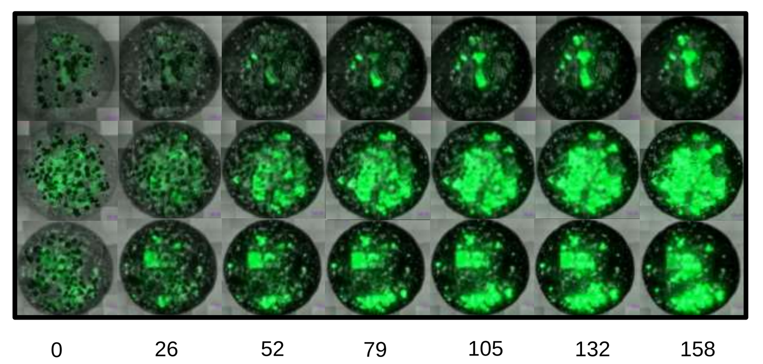
Acetone-cast poly lactic-co-glycolic acid (PLGA) with embedded CFPS powder



Heat-pressed Polycaprolactone (PCL)



- GFP production is exhibited in both PLGA and PCL films
- While PLGA quickly absorbs water – clouding the film and activating GFP production throughout, PCL does not readily absorb water.
- In PCL, GFP production only occurs in damaged (cut) films, at the cut site
- Variability of CFPS performance observed in polymer films might be improved by improving powder dispersion and uniformity in casting.

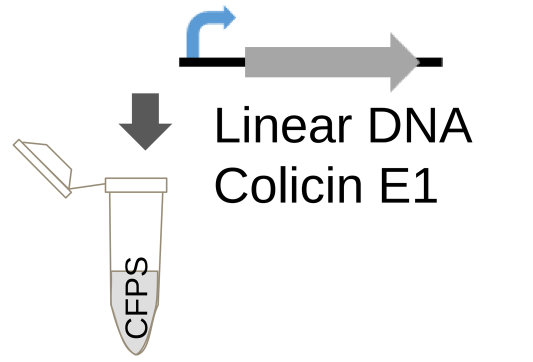


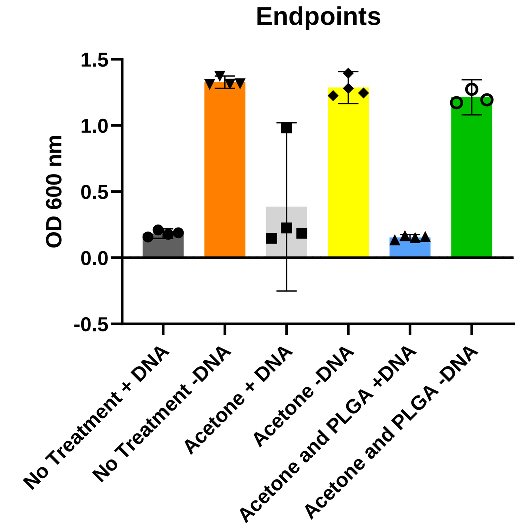
Conclusions

- We demonstrate that protein synthesis activity is recovered after solvent or heat casting with PLGA or PCL polymers.
- This expanded perspective on dried CFPS stability has broad implications for developing materials with bio-activity and other processing steps compatible with this complex bio-system
- Differences in polymer properties and geometry lead to varying characteristics in the reactivation of cast CFPS reactions – supporting the hypothesis that a polymer composite may be designed to gradually and continually hydrate reaction material
- This potentially enables the development of **coatings**, **films**, **fibers**, or **objects** that can perform bio-functions, including **sensing**, **decontamination**, or **molecule synthesis.**

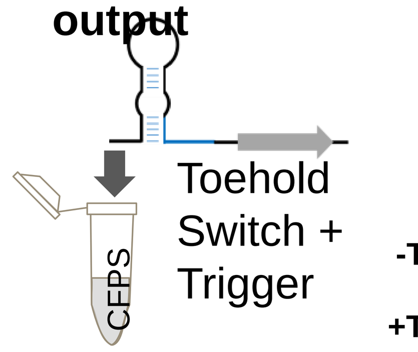
Decontamination, Biosensing, and Localization in Solvent-cast Polymer

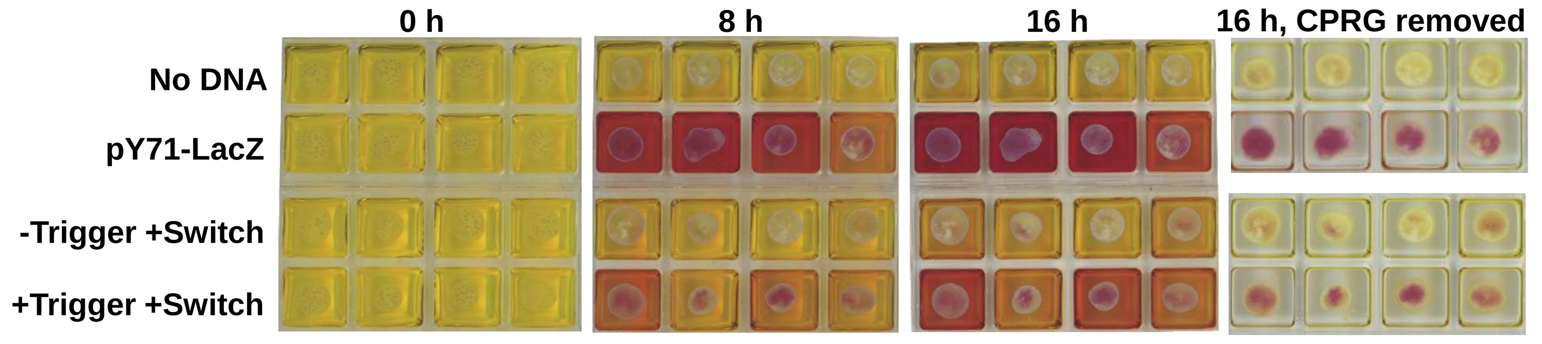
- **Colicin production inhibits E. coli growth in CFPS reactions treated with acetone or embedded in acetone-cast PLGA**



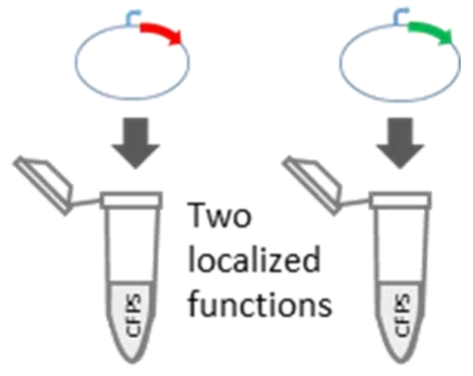


- **An RNA-activated toehold switch sensor functions in polymer to produce a color change output**

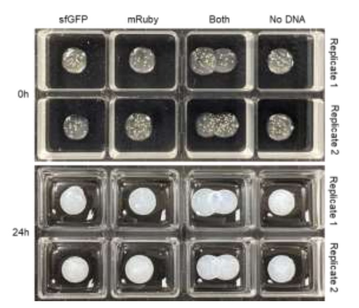


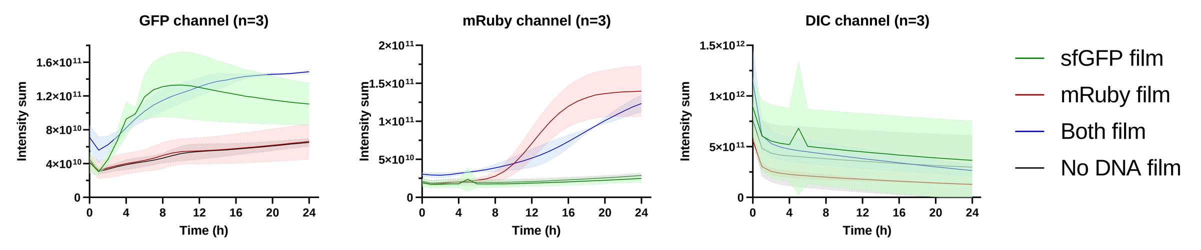


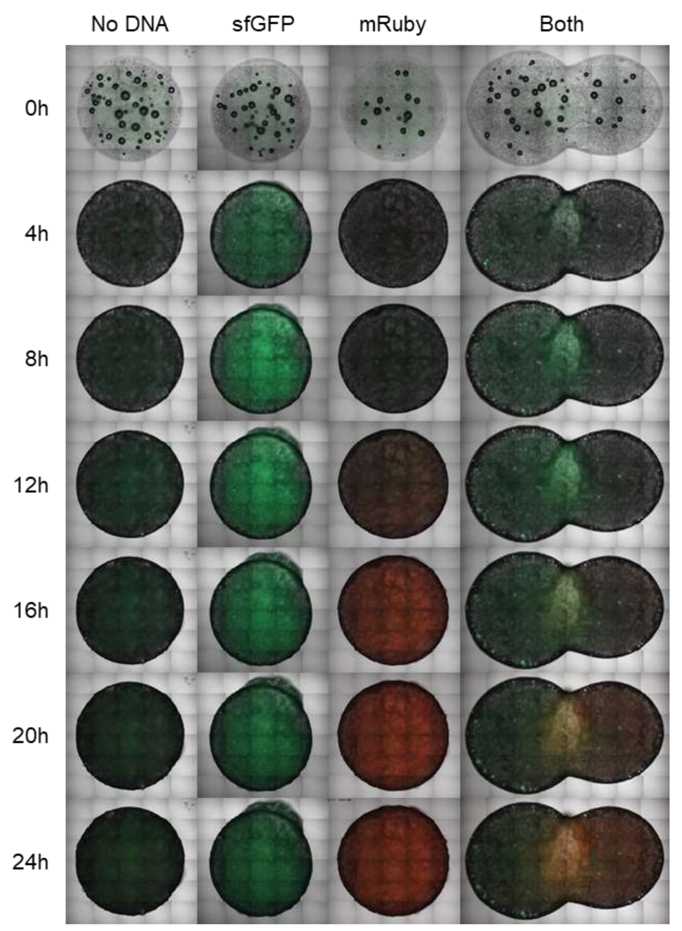
- **Separate functions are localized to different areas of a PLGA film**



PLGA films before and after rehydration







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