Establishing a Cell-free *Vibrio natriegens* Expression System

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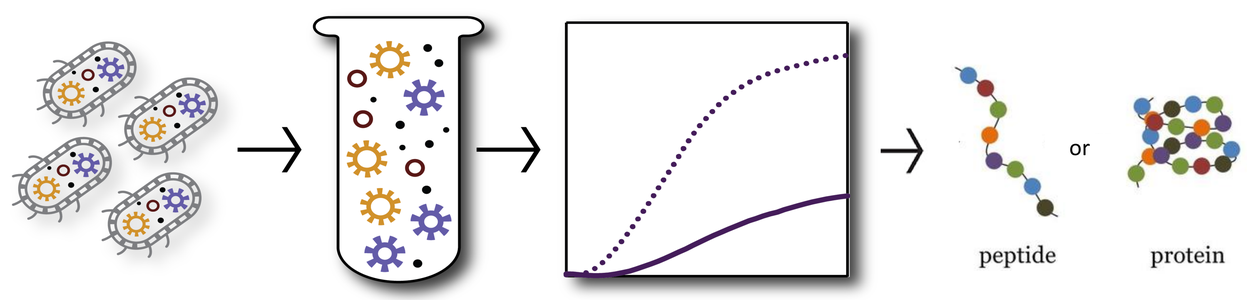
Abstract

The marine bacterium *Vibrio natriegens* has garnered considerable attention as an emerging microbial host for biotechnology due to its ultra fast growth rate. Utilizing its productive cellular components as an *in vitro* cell-free expression system is exceedingly advantageous to those interested in rapid, cost-efficient protein or peptide production in a high-throughput format. Here, we report the development of a *V. natriegens* cell-free expression system. We devised a simplified crude extract preparation protocol and achieved >260 µg/mL of superfolder GFP (sfGFP) in a small-scale batch reaction after 3 hours. Culturing conditions, including growth media and cell density, significantly affect translation kinetics and protein yield of extracts. We observed maximal protein yield at incubation temperatures of 26 or 30 °C, and show improved yield by tuning ions crucial for ribosomal stability. This work establishes an initial *V. natriegens* cell-free expression system, enables probing of *V. natriegens* biology, and will serve as a platform to accelerate peptide therapeutics and synthetic biology applications.

Technical Highlights

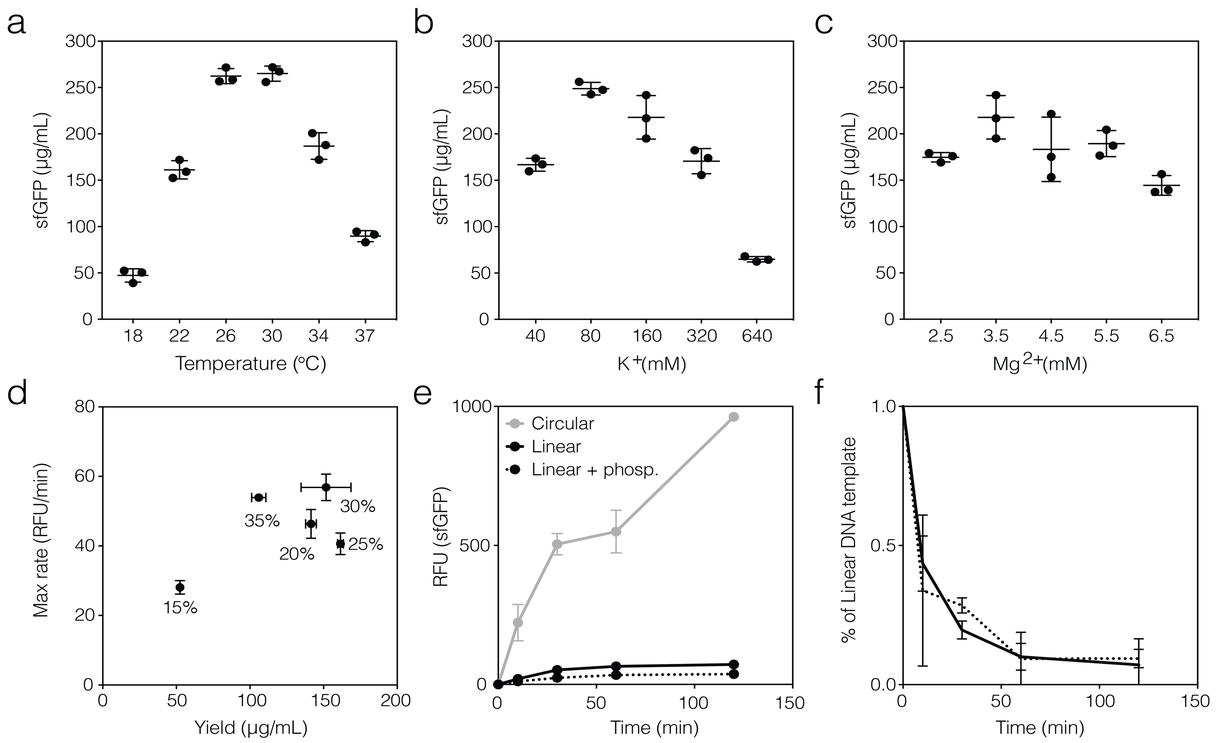


Vibrio natriegens Cell Extract Cell-free Reaction Product

- Cell-free expression reactions using wild-type *V. natriegens* crude extract
- Robust & reproducible cell lysis method via pulse sonication & centrifugation
- Multiple template types: plasmid DNA, linear DNA, and mRNA template
- Highly parallelizable, cost efficient, and easily integrated into current pipelines
- High yielding peptide or protein synthesis in approximately 3 hours for <$2/rxn

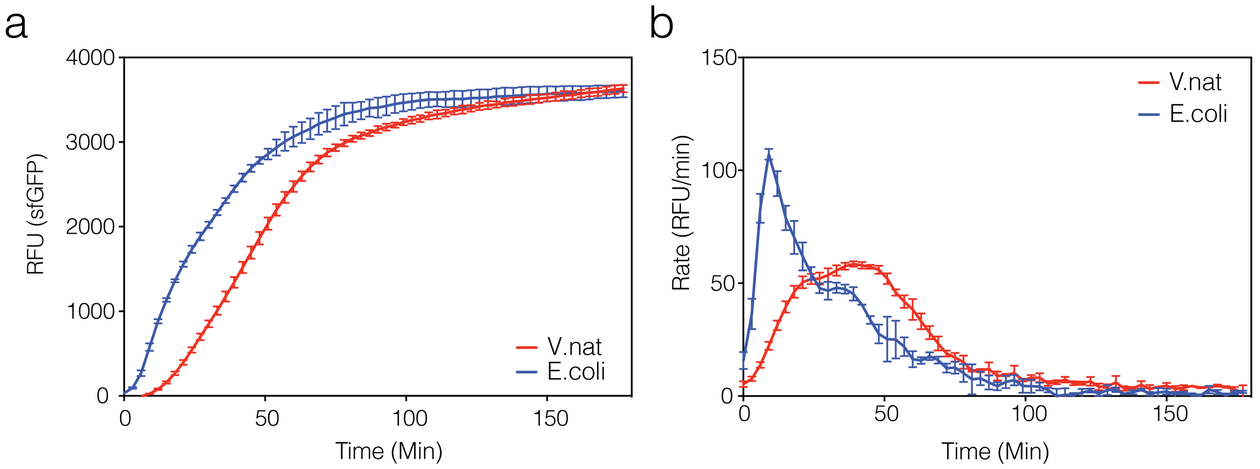
Results

Optimization of *V. natriegens* cell-free reactions



(a) cell-free incubation temperature; **(b)** supplemented potassium ions in reaction buffer; **(c)** supplemented magnesium ions in reaction buffer; **(d)** percent of extract used relative to total reaction volume; **(e)** template DNA provided in the cell-free reaction. Equimolar amount of circular plasmid (gray), linear DNA (PCR product, black solid line), or linear DNA with two phosphorothioated bonds on each end (PCR product, black dotted line) was used as a template for cell-free expression of sfGFP. **(f)** Degradation of linear DNA template. Fluorescence of AlexaFluor 594−5-dUTP labeled DNA template was monitored over 2 h. Linear template with (dotted) or without (solid) two phosphorothioated bonds on each end was used. Unless otherwise indicated, all experiments were performed using *V. natriegens* crude cell extract incubated at 26 °C for 3 h, supplemented with 80 mM K-glutamate and 3.5 mM Mg-glutamate. The mean and standard deviations are shown (N = 3).

Cell-free expression profiles *V. natriegens* and *E. coli* A19



(a) kinetic measurement of sfGFP in *E. coli* (blue, 37°C, 160 mM K-glutamate, 2.5 mM Mg-glutamate) and *V. natriegens* (red, 26°C, 80 mM K-glutamate, 3.5 mM Mg-glutamate) extracts. **(b)** rate of sfGFP expression in extracts. E. coli cultures were grown in 2xYTP media at 37°C and harvested at OD600 = 1.0. All experiments were performed using 500 ng plasmid DNA (pJL1). The mean and standard deviations are shown (N=3).

Conclusions

- cell-free system for proteins or peptides with WT *V. natriegens* established
- system will help facilitate rapid prototyping and high-throughput screens
- reaction conditions such as temperature, ion concentration, etc. optimized
- *V. natriegens* cell-free expression on par with engineered *E. coli* A19 strain
- provides the foundation for further improvements to *V. natriegens* cell-free
- worthy addition to repertoire of non-model organism cell-free systems
- a platform to accelerate peptide therapeutics & synthetic biology research

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