Designing the Cell-Free Gene Expression Environment with the One-Pot PURE System

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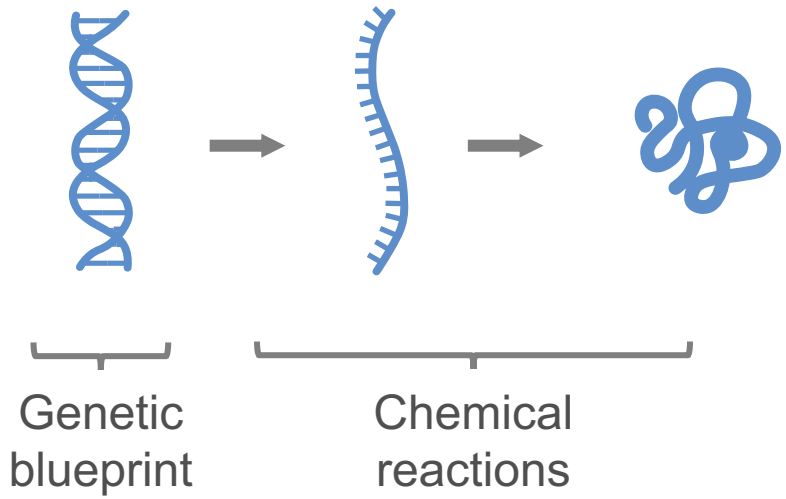
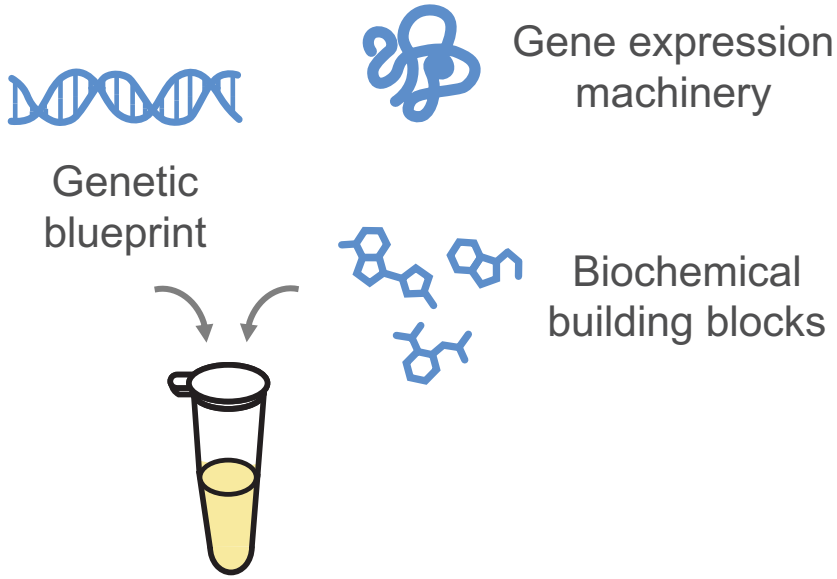
[2] Imperial College London

Cell-free systems recreate the chemical reactions of life

Living systems are assembled through a series of chemical reactions

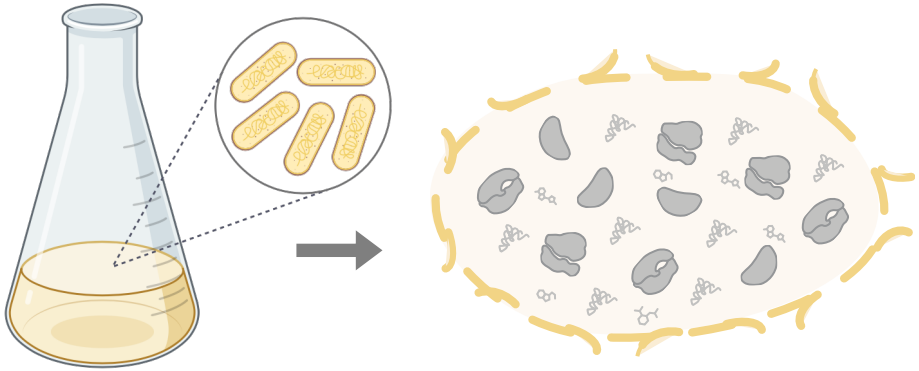


"Cell-free" systems recreate the chemical reactions of life in a test tube



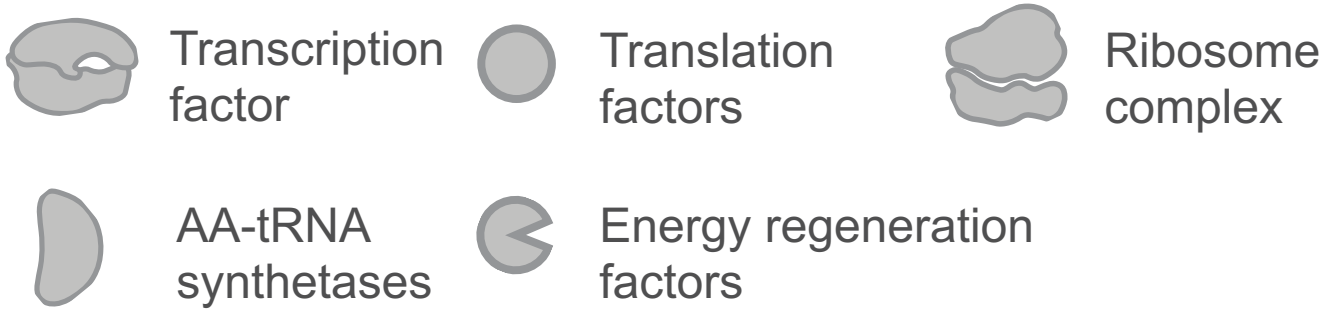
Cell-free systems come in varying degrees of freedom

Crude Cell Lysate



Easy to prepare
Recapitulate the native cell proteome

Protein synthesis Using Recombinant Elements (PURE) system

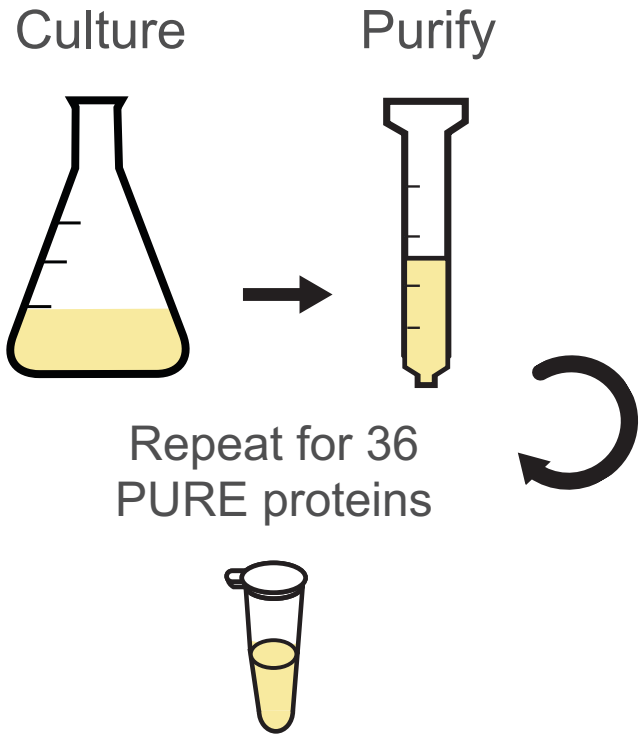


A nightmare to prepare
Full control over the reaction environment

Preparing PURE system is highly labor-intensive

Traditional PURE

Shimizu *et al.*, 2001



Protein synthesis Using Recombinant Elements (PURE) system


Transcription factor **x1**


Translation factors **x10**


Ribosome complex


AA-tRNA synthetases **x21**

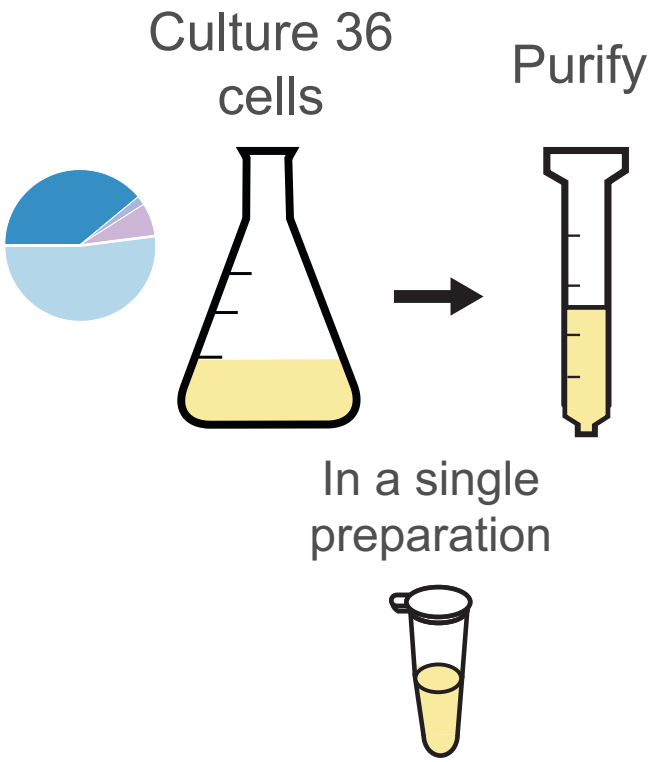

Energy regeneration factors **x4**

A nightmare to prepare
Full control over the reaction environment

One-Pot co-culture offers a streamlined approach to PURE

One-Pot PURE

Lavickova *et al.*, 2019



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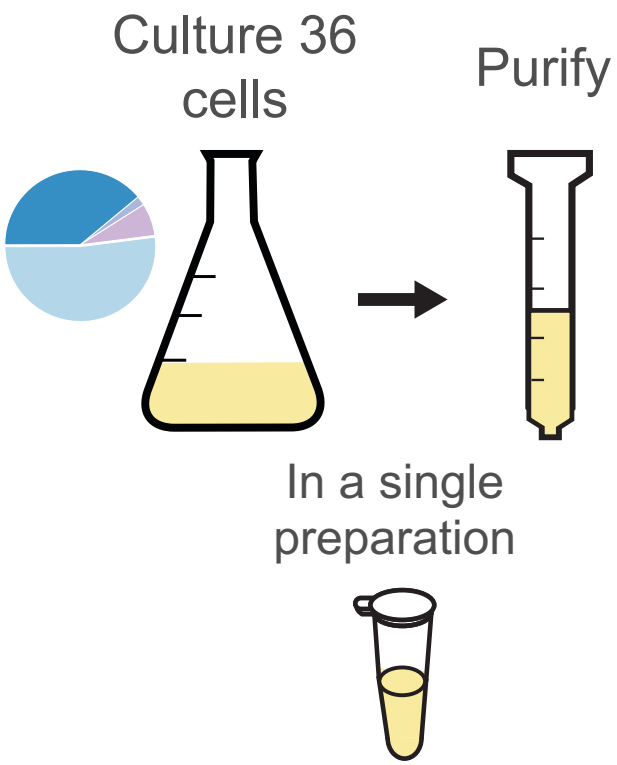
A nightmare to prepare
Full control over the reaction environment

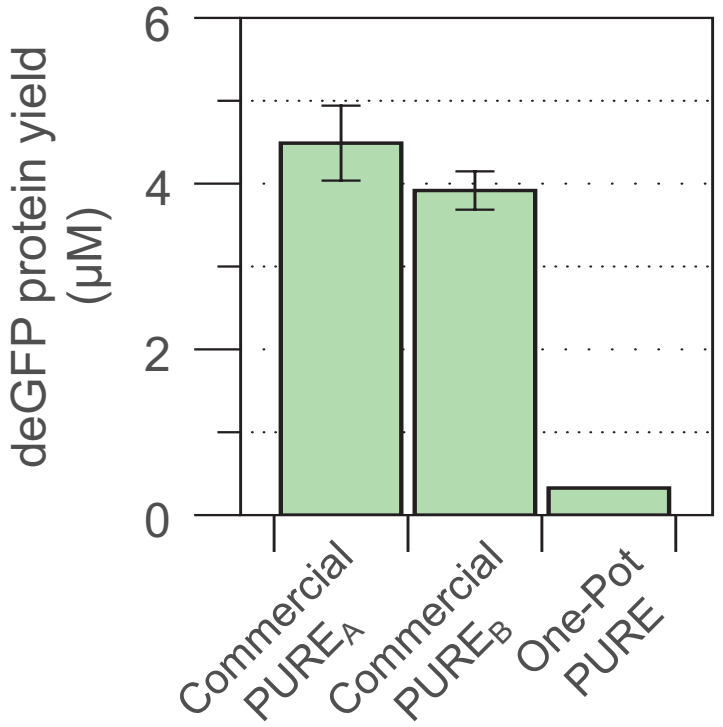
But One-Pot PURE productivity can be a hit-or-miss

One-Pot PURE

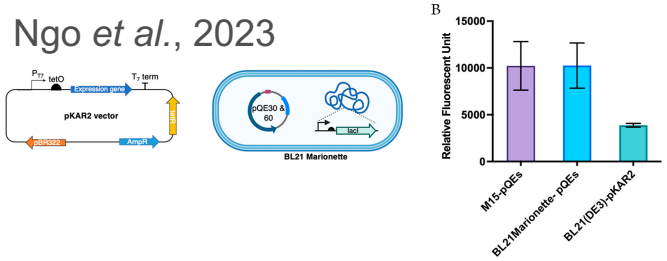
Lavickova *et al.*, 2019



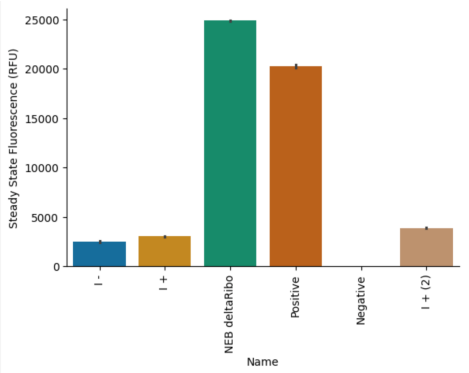
This work: Zhang *et al.*, 2025



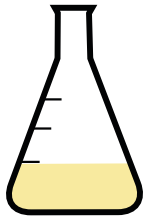
Ngo *et al.*, 2023



Bnext.bio



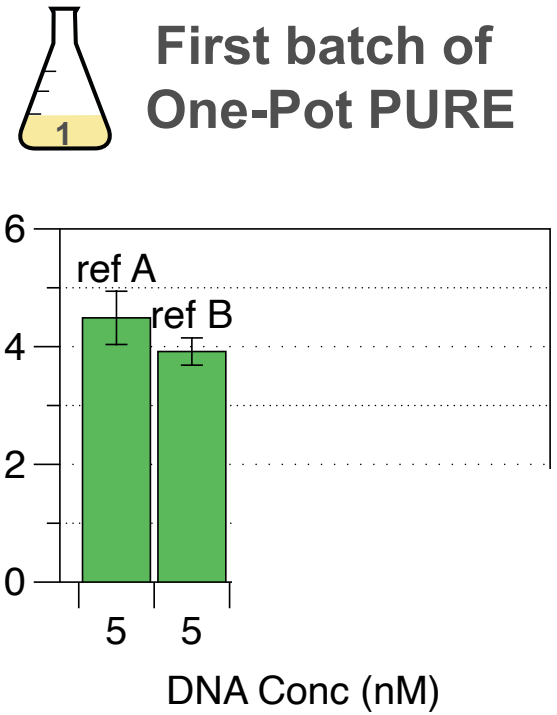
Overview of this talk

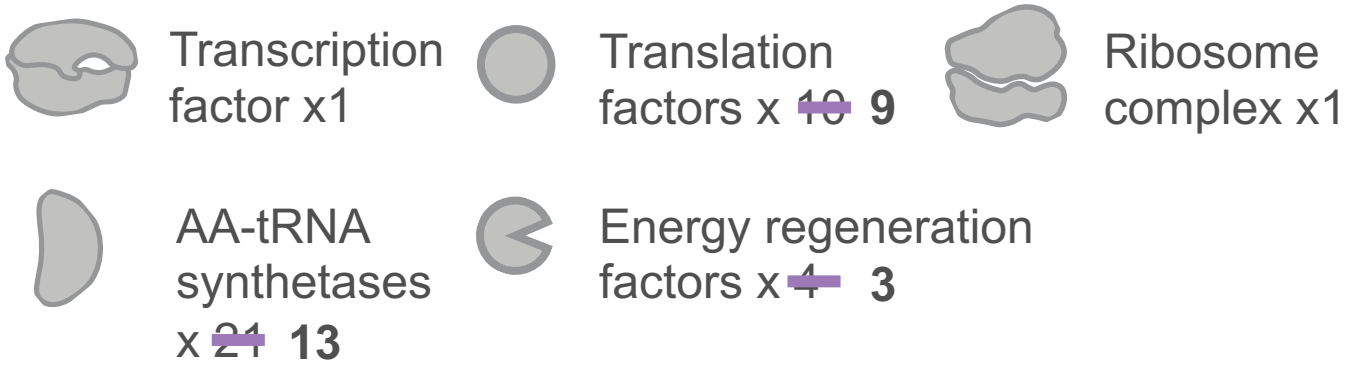


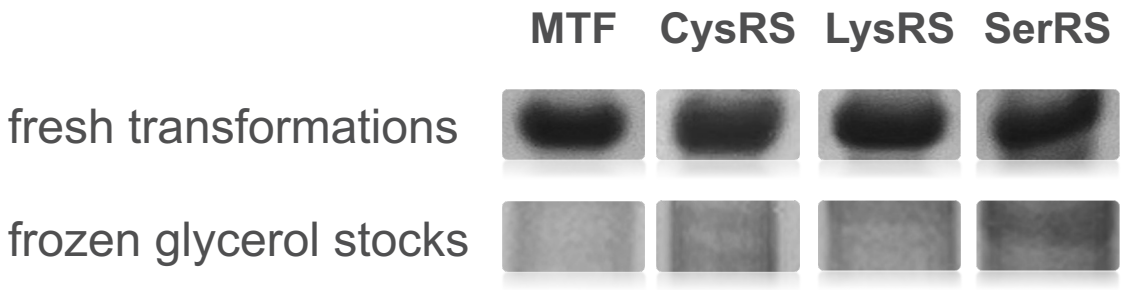
Preventing PURE protein “dropouts” is important for a productive system

Transfer RNA (tRNA) pool is an underappreciated complexity

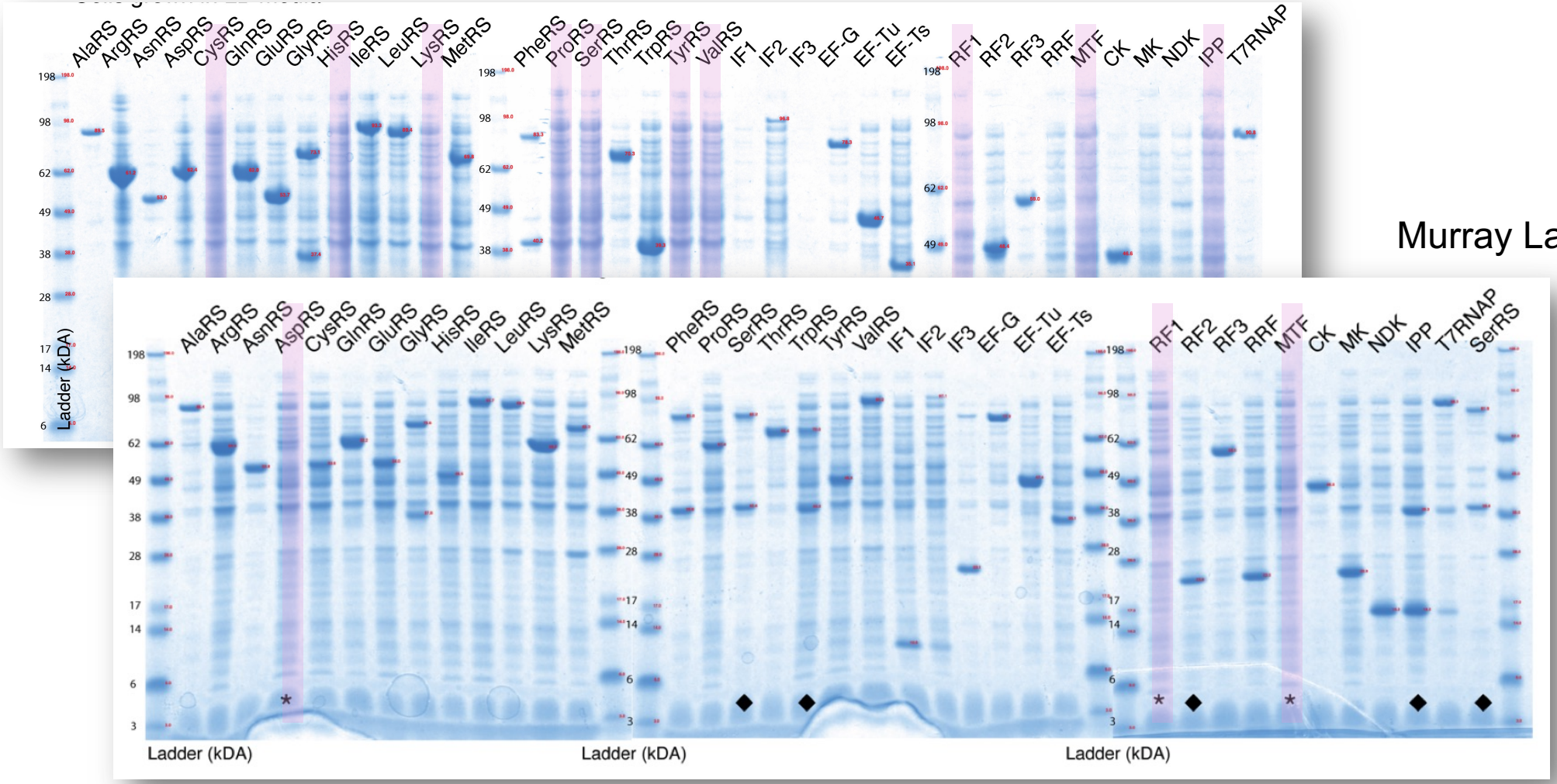
Low One-Pot PURE productivity comes from PURE protein dropouts







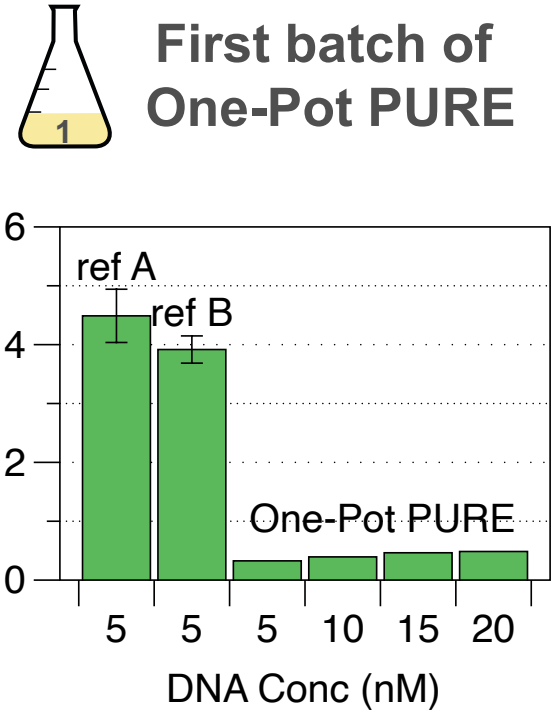
PURE protein dropouts in coculture is a frequent and stochastic event



Murray Lab, Caltech

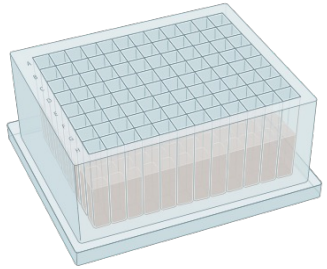
Freemont Lab, Imperial College London

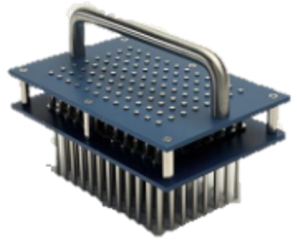
Low One-Pot PURE productivity comes from PURE protein dropouts



One-Pot PURE Workflow

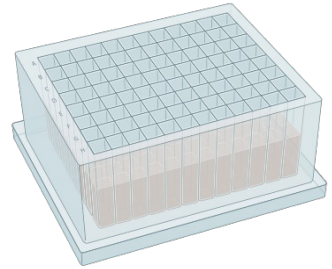
36 frozen glycerol stock





Cryo-replicator

Inoculate overnight culture

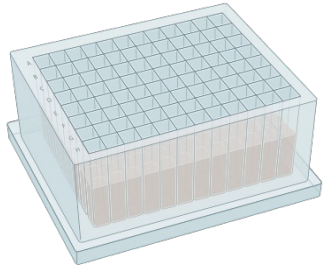


I refuse to do 36 fresh transformations every time!

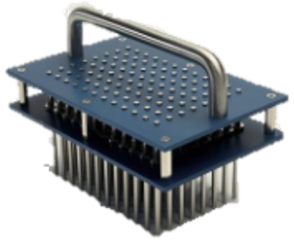
PURE protein dropouts arise from cell growth burden

One-Pot PURE Workflow

36 frozen glycerol stock



-80˚C



Cryo-replicator

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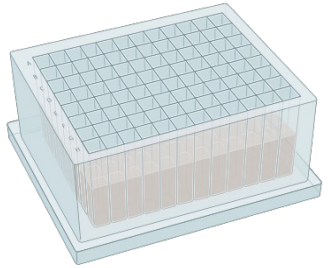
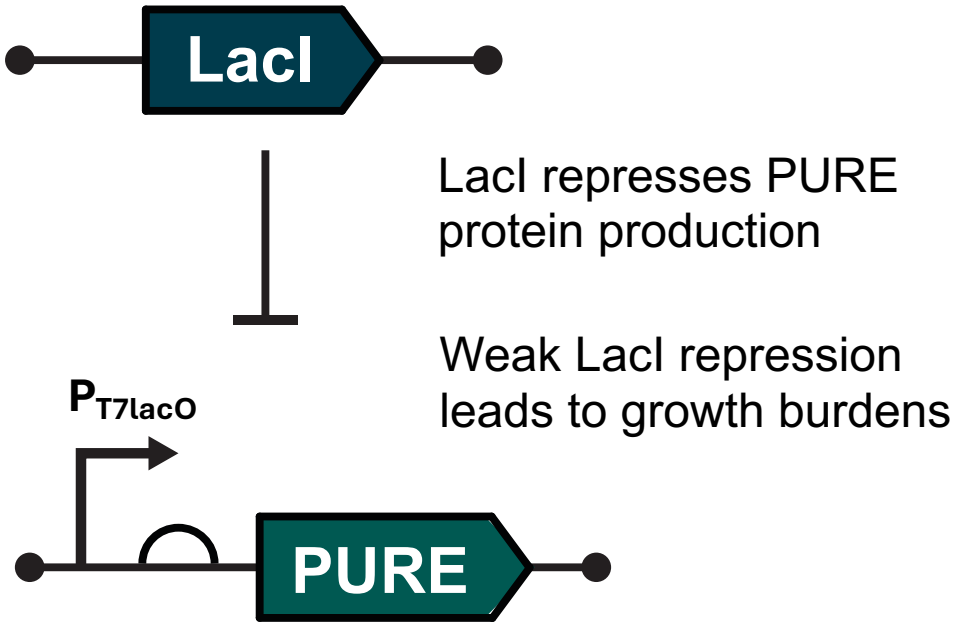
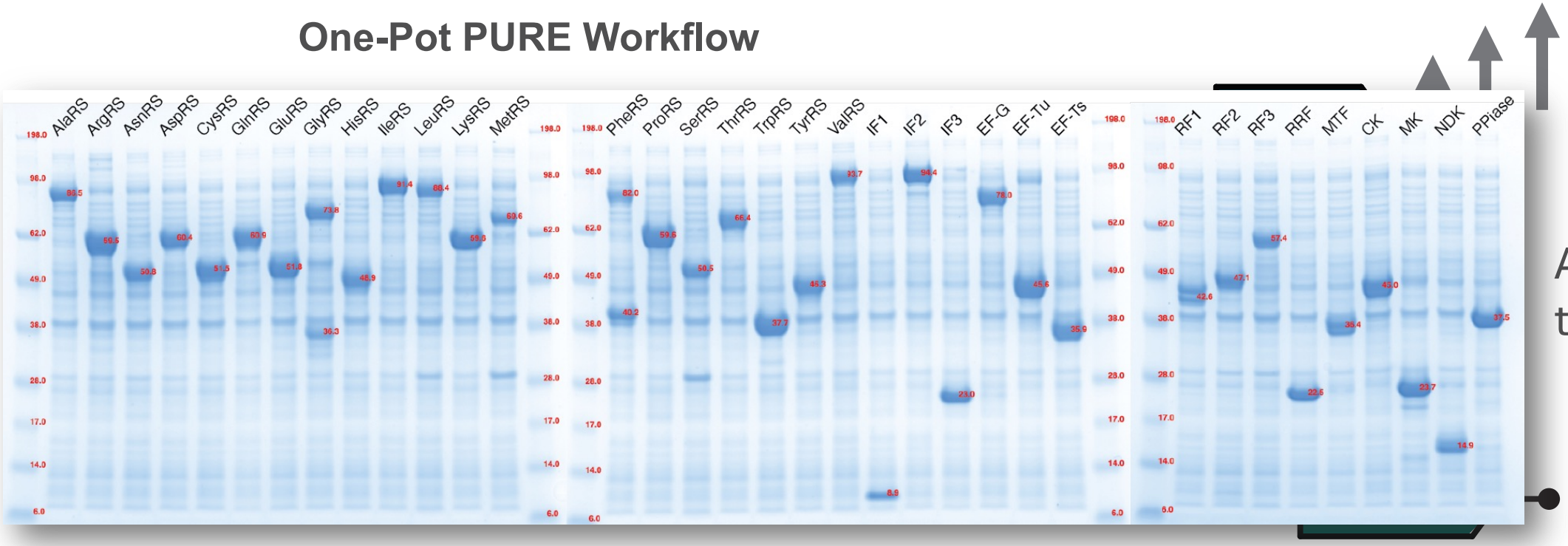


37˚C



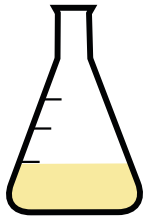
Glucose catabolite repression increase intracellular LacI

One-Pot PURE Workflow



Add glucose to media!

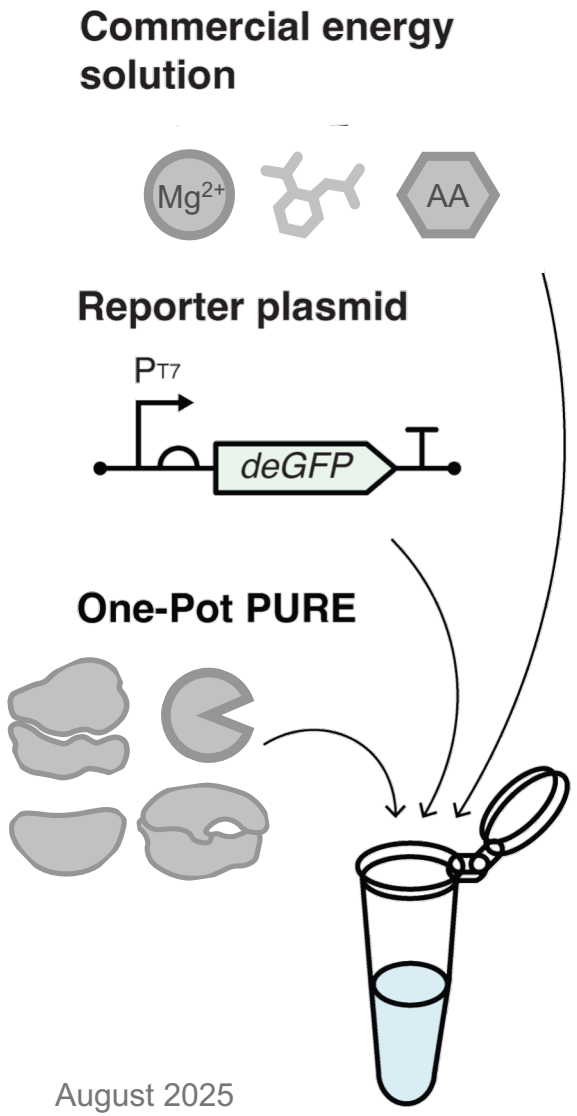
Overview of this talk

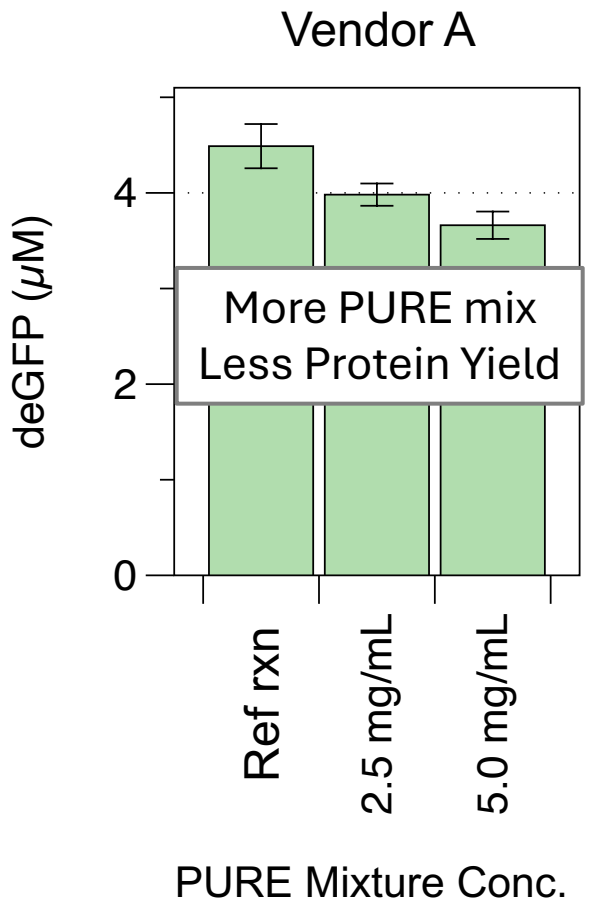


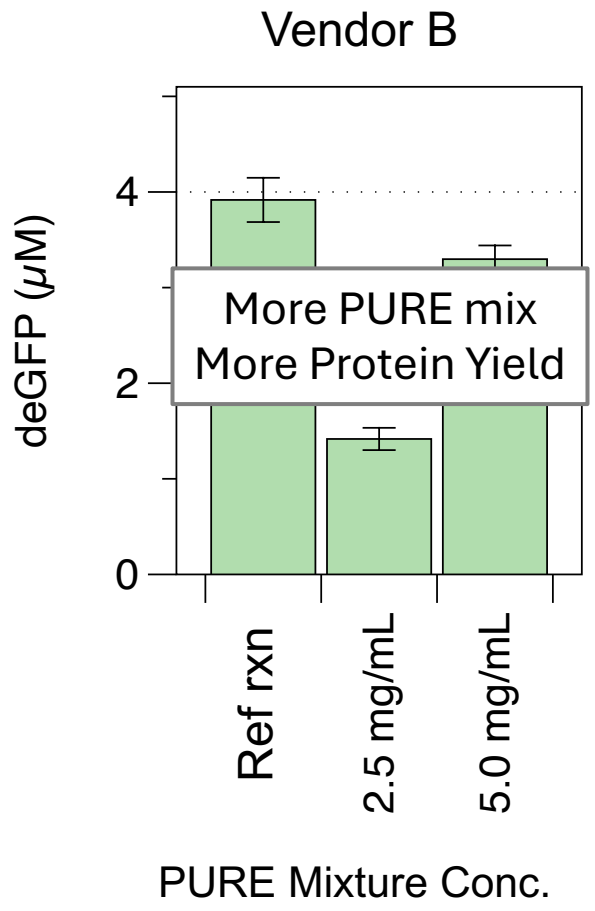
Preventing PURE protein “dropouts” is important for a productive system

Transfer RNA (tRNA) pool is an underappreciated complexity

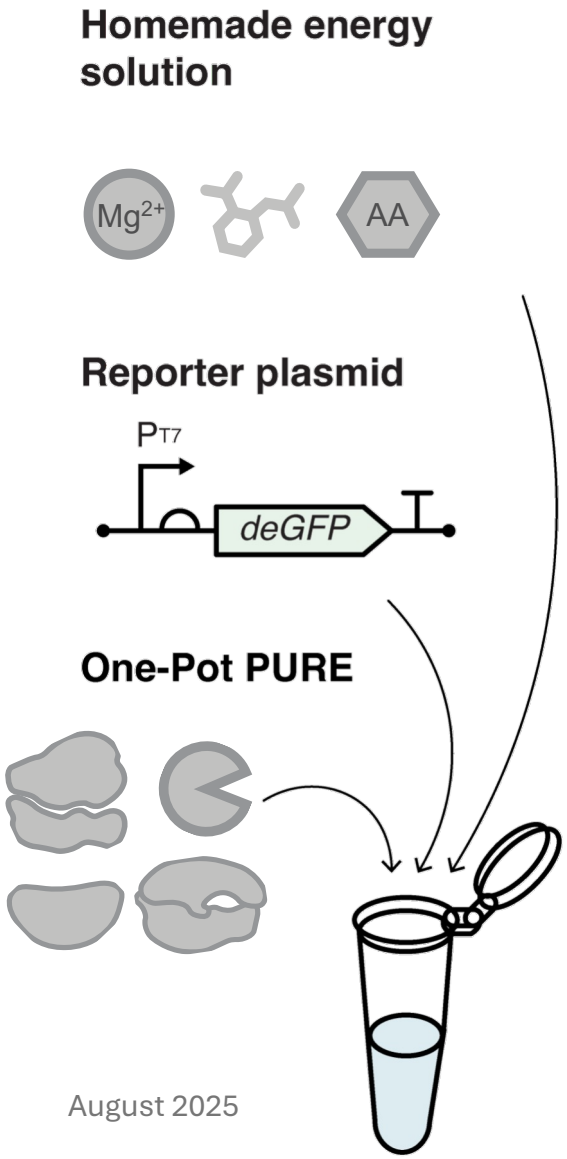
Same One-Pot PURE proteins, different behaviors in two energy mixes





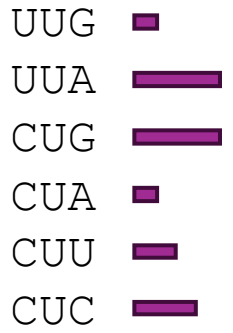


Homemade tRNA solution revealed hidden complexity of tRNA pool

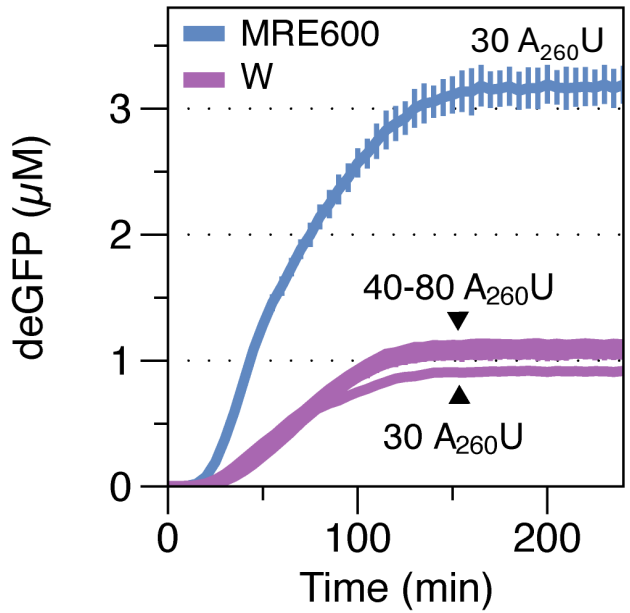


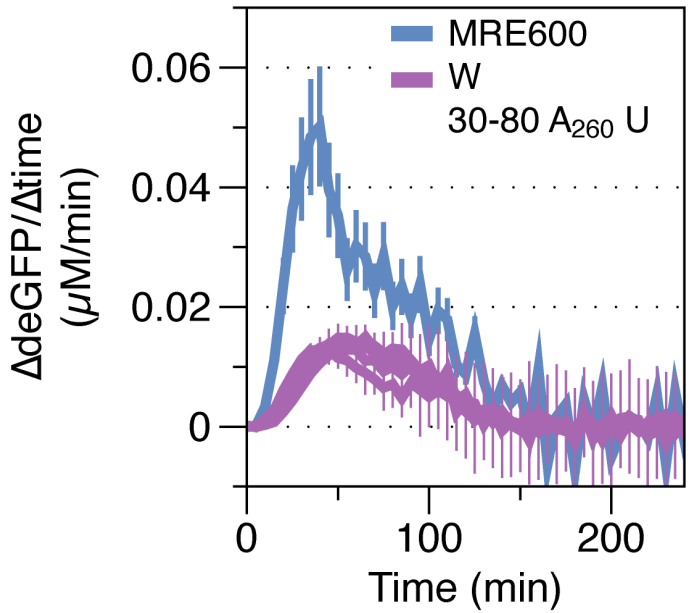
Two sources of *E. coli* tRNAs*

Leucine



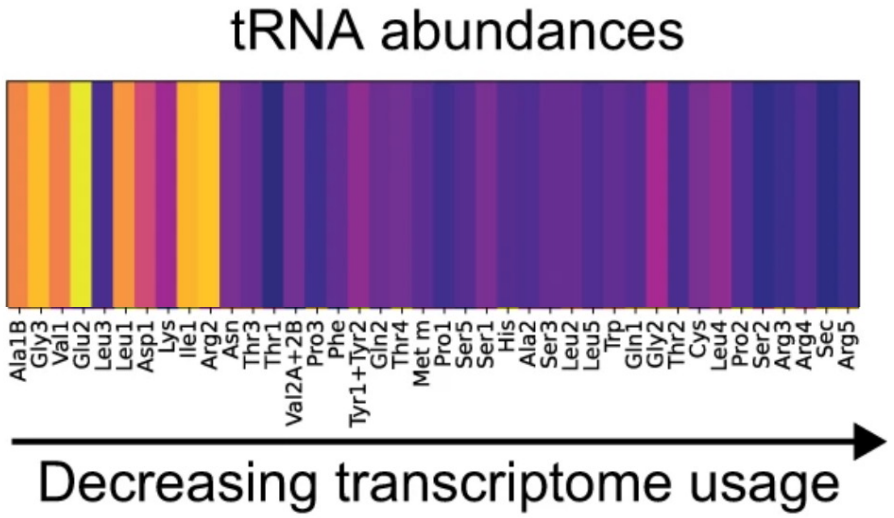
Synonymous codons are not the same!





*both tRNAs are discontinued for purchase

Building synthetic biosystems require systems-level knowledge

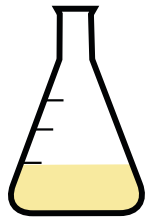


1 codon per amino acid

Design gene expression to match the tRNA pool

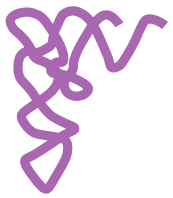
Design minimal tRNA pool with known composition

In summary

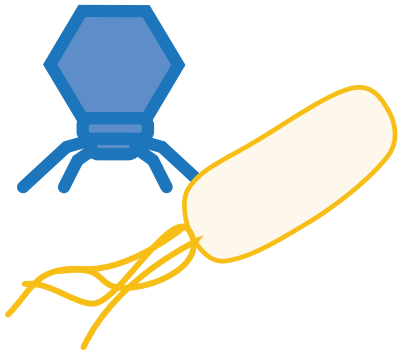


PURE protein dropouts during One-Pot coculture can be mitigated with media optimization

Protein design



Protein coding sequence needs to match the tRNA pool for productive expression



Synthetic biosystems design

People who made this work possible

Murray Lab Members




Paul Freemont Lab, Imperial College London
- Matas Deveikis

Caltech PEL
- Prof. Tsuifen Chou
- Dr. Yanping Qiu

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yzhang952.github.io/files/ACS2025.pdf

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