Designing Predictive Cell-Free Systems with One-Pot PURE

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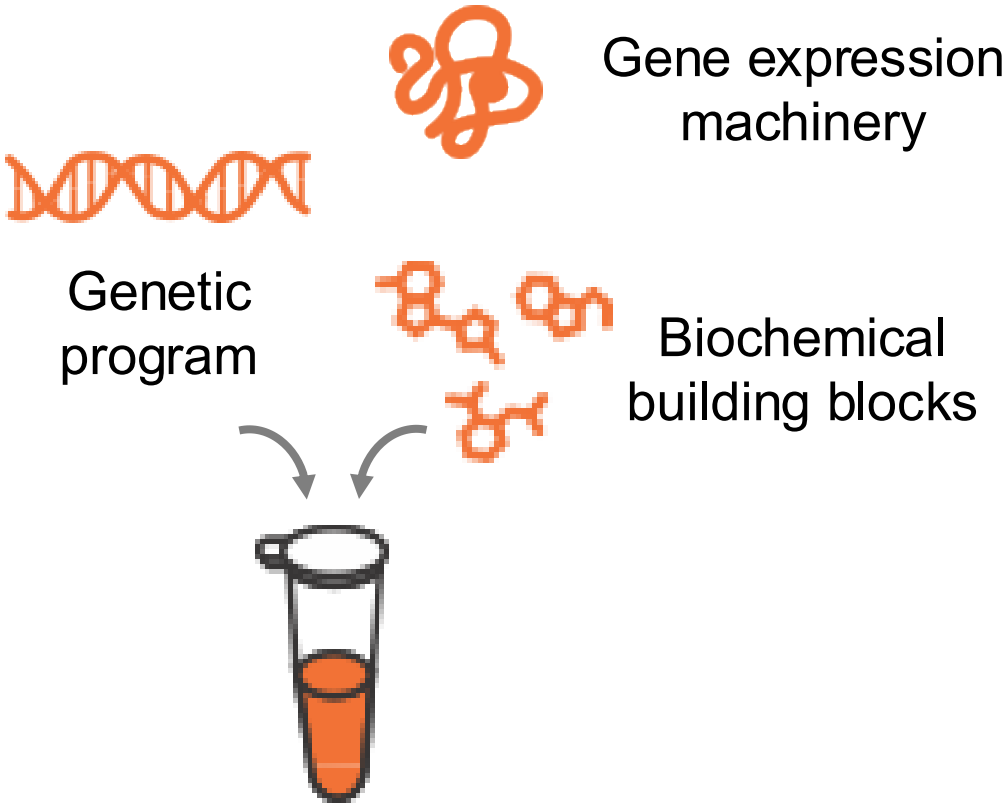
Caltech

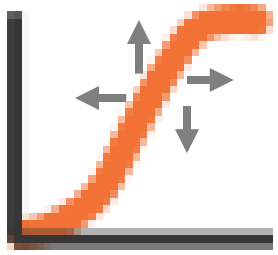
Cell-free gene expression takes biology outside living cells



Design gene program

Empirical
Not Generalizable
Major time sink

Tune desired outputs



What if cell-free gene expression can look something like this?

Parameter input:

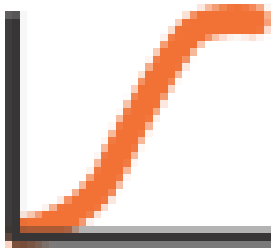
Make **X protein**,
at **Y amount**, in **Z time**.

***In silico* Design:**

Synthesize **X plasmid** for
Y system expression.

Guaranteed Outcome:

Built and done.



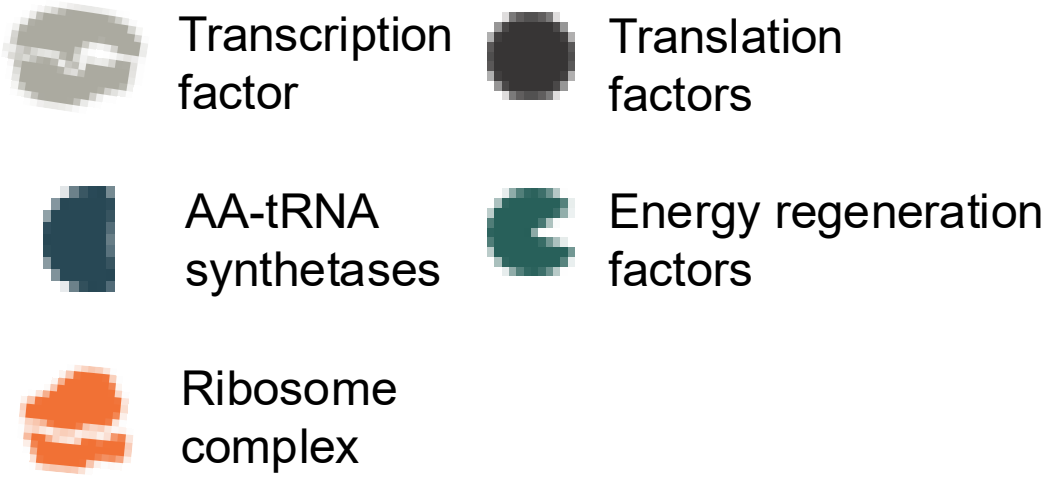
Unlocking this future requires system-level design

Building big by starting small, with a defined PURE system

Traditional Crude Cell Extract has too much going on

Recapitulate living cell

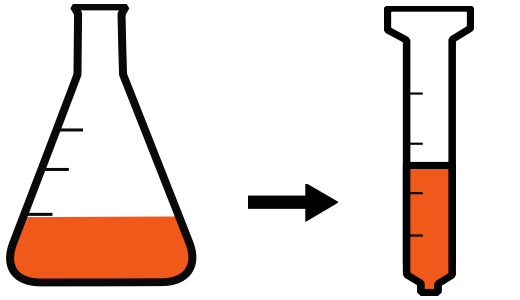
One-Pot PURE offers a controlled and defined system



Full system control

Building big by starting small, with a defined PURE system

Culture x36 **Purify**



In a single preparation

Lavickova and Maerkl., 2019

One-Pot PURE offers a controlled and defined system

Transcription factor

Translation factors

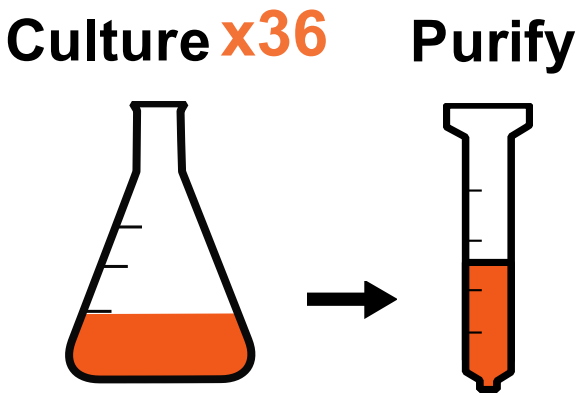
AA-tRNA synthetases

Energy regeneration factors

Ribosome complex

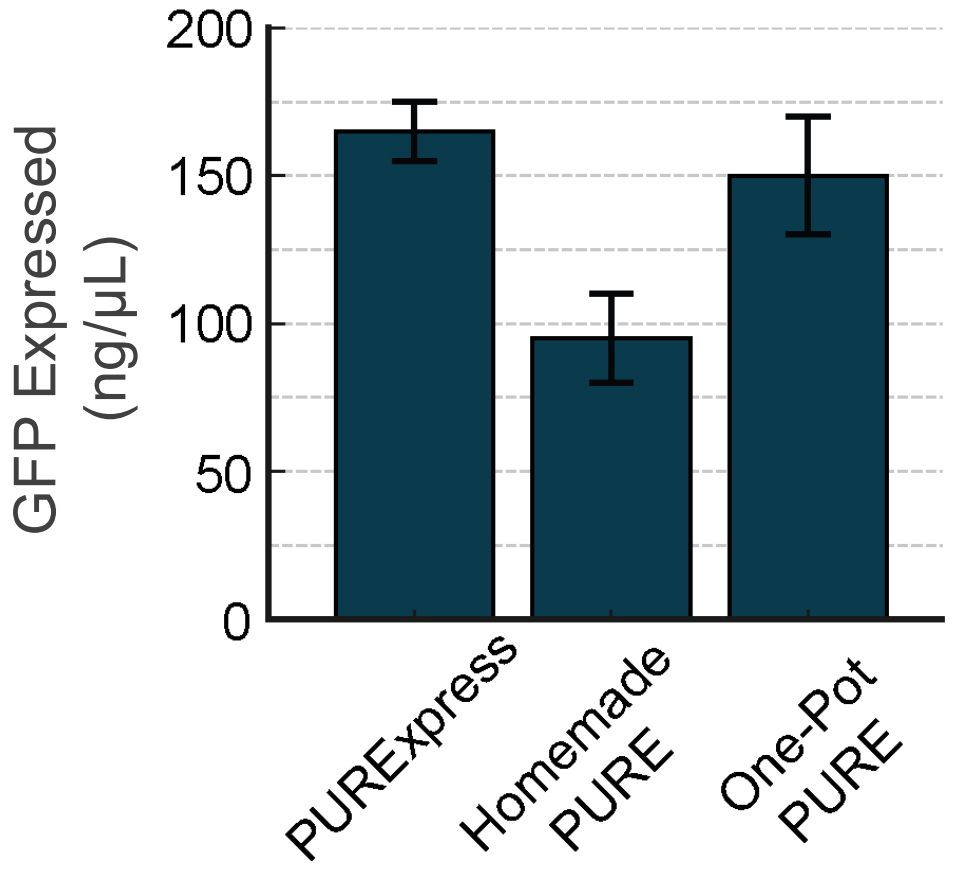
Full system control

Building big by starting small, with a defined PURE system

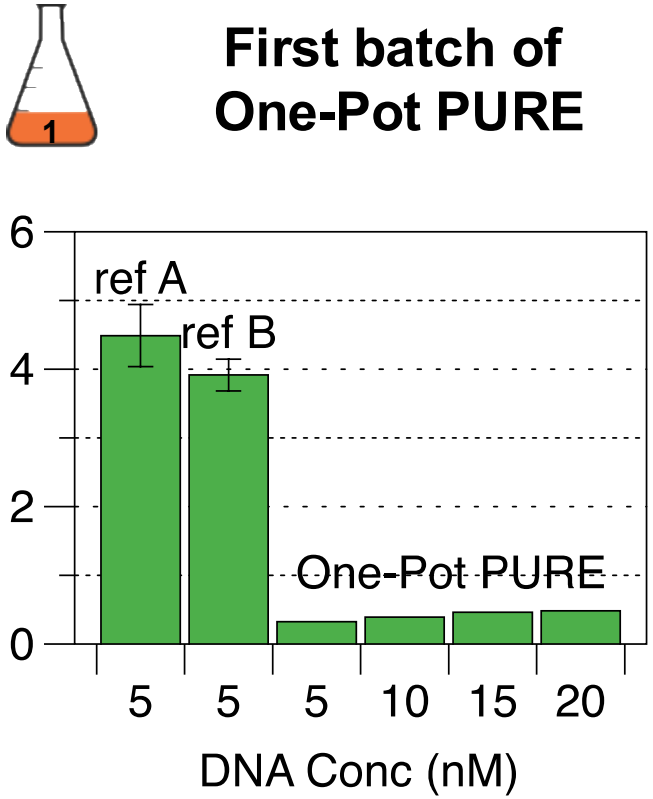


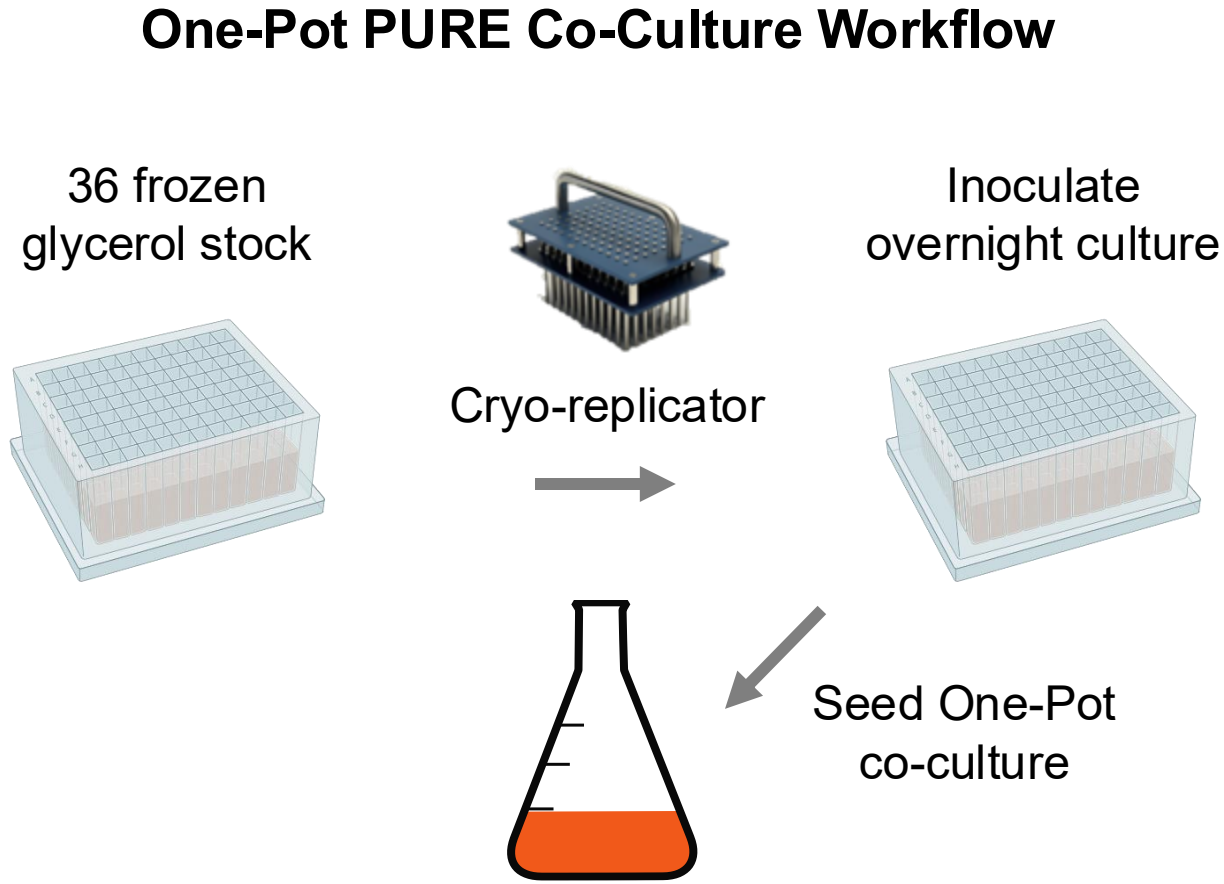
In a single preparation

Lavickova and Maerkl., 2019

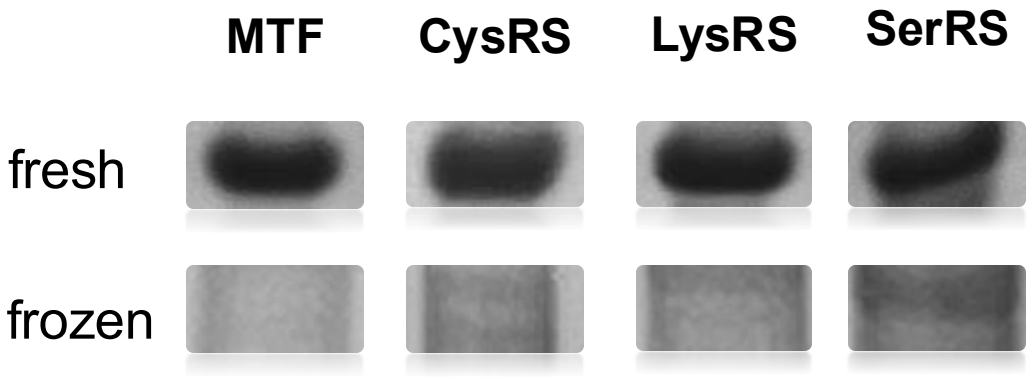


I need a working One-Pot PURE to build a predictive platform



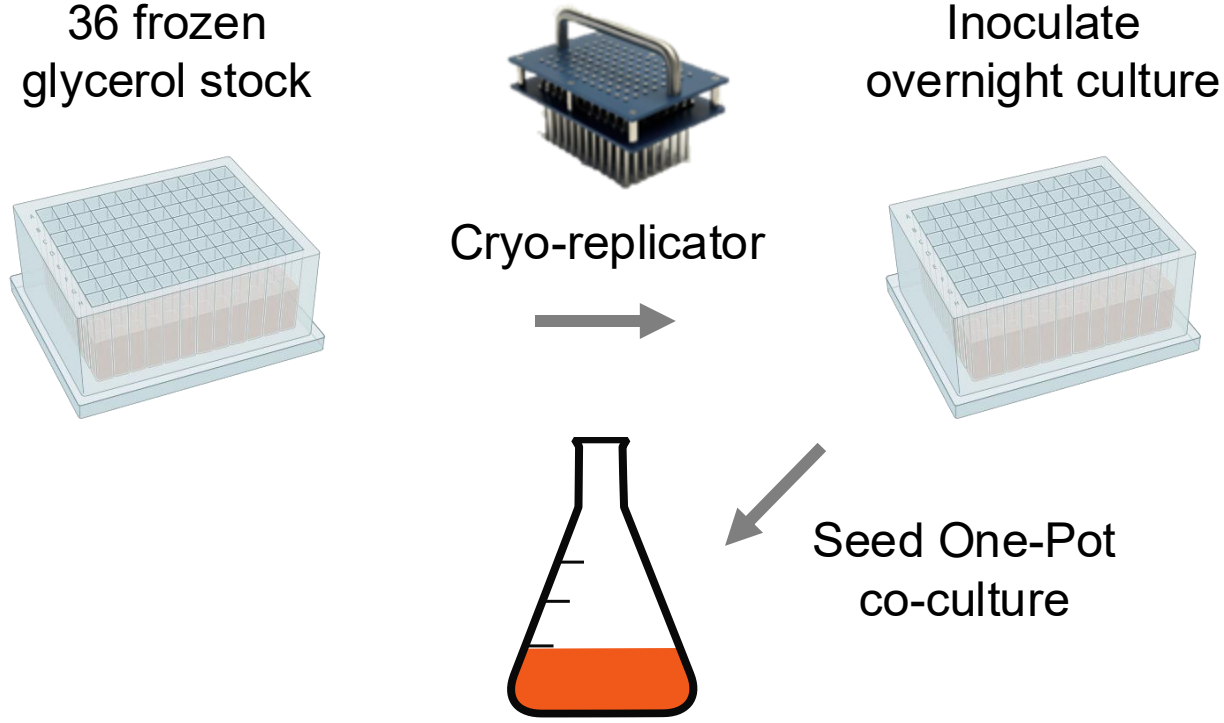


Leaky background expression causes PURE protein dropouts

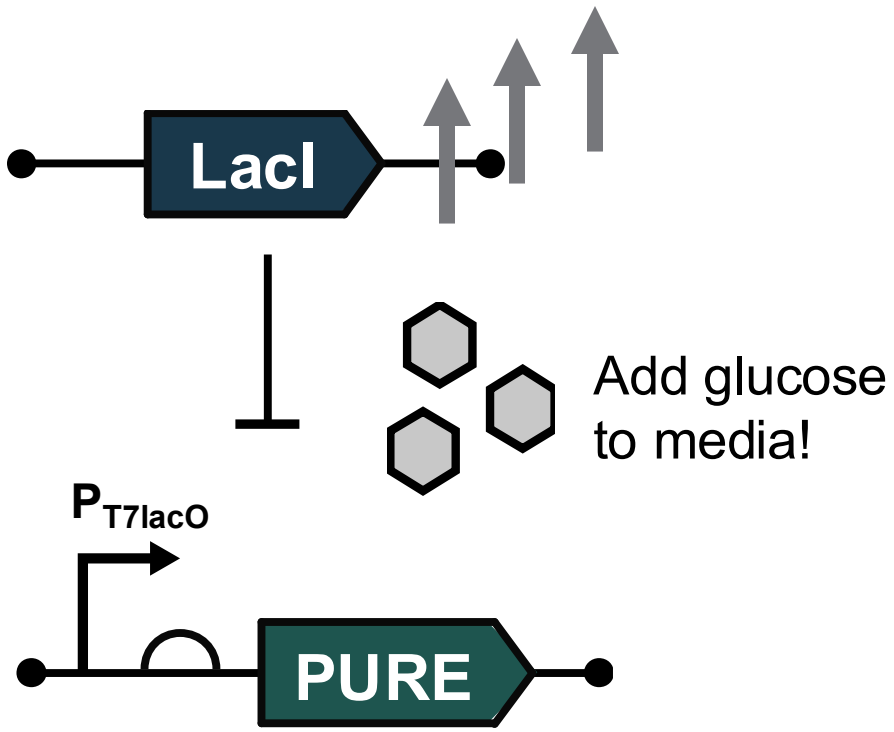


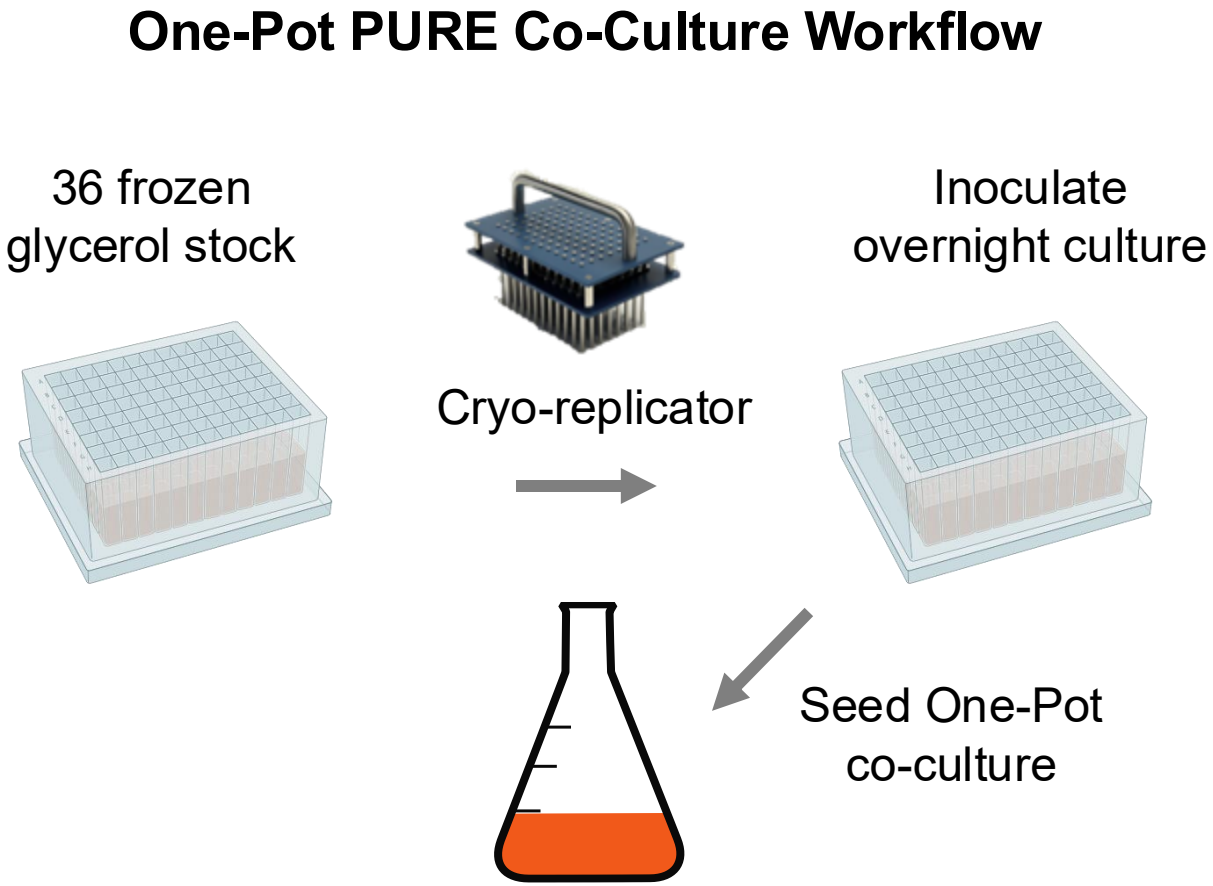
I had 0.3-Pot PURE, not One-Pot PURE

One-Pot PURE Co-Culture Workflow

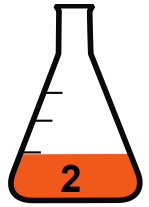


Adding glucose to media increased LacI repression

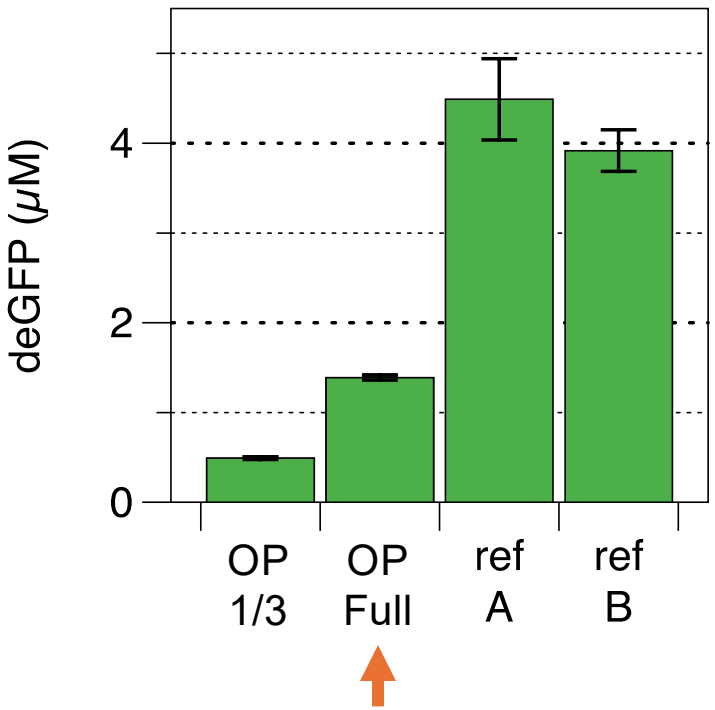


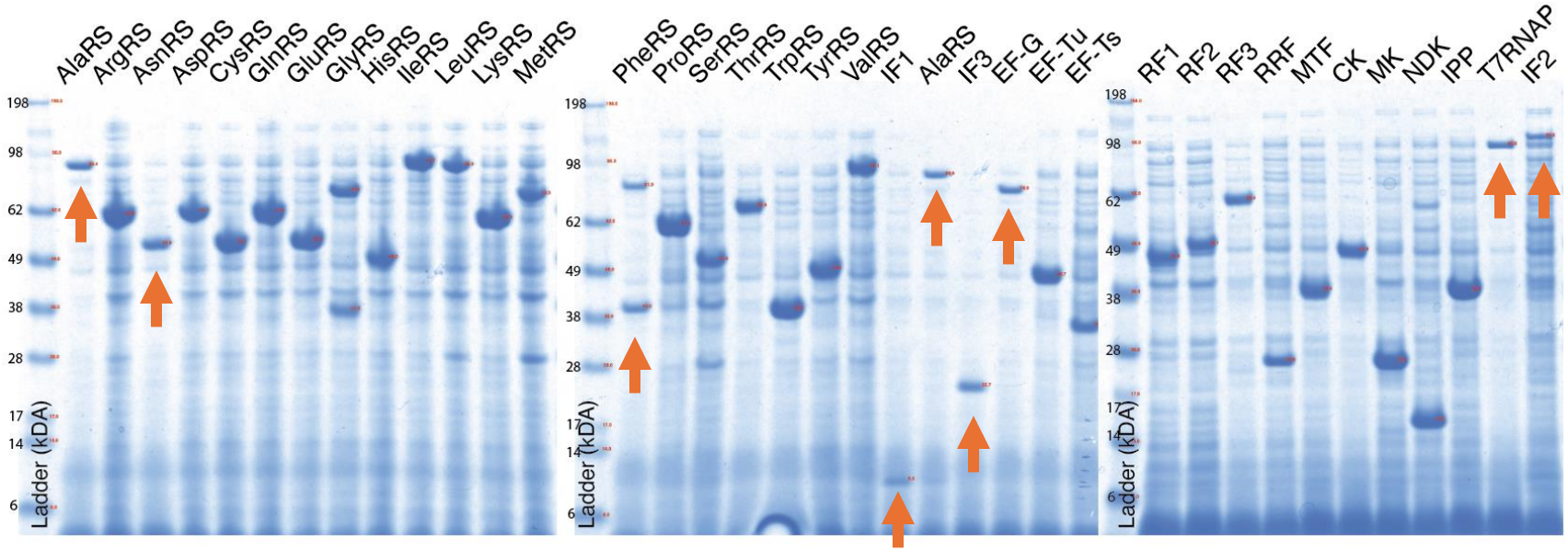


Protein expression level matters

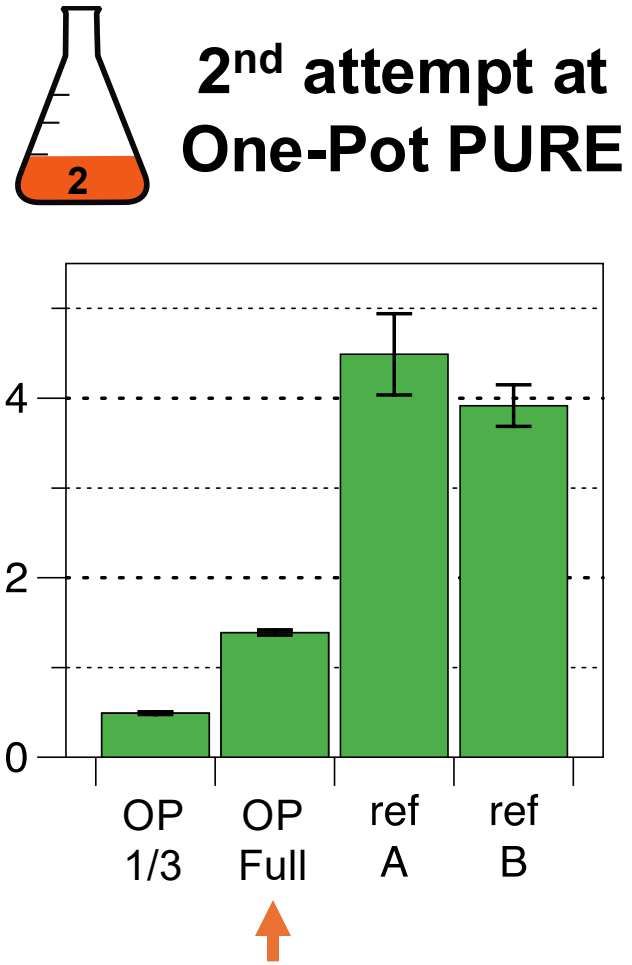


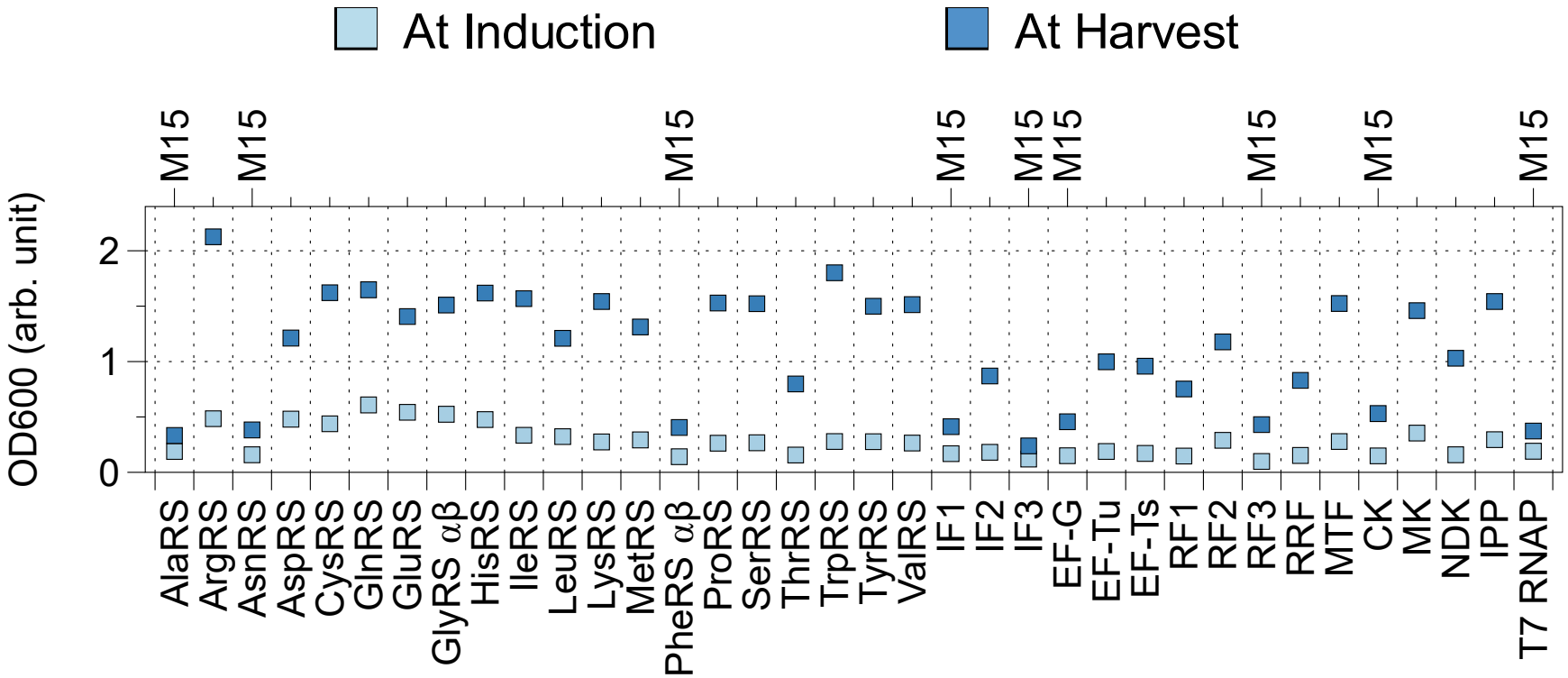
2nd attempt at One-Pot PURE



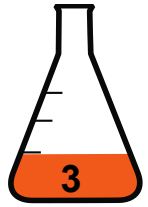


Dual-strain culturing approach creates coculture problems





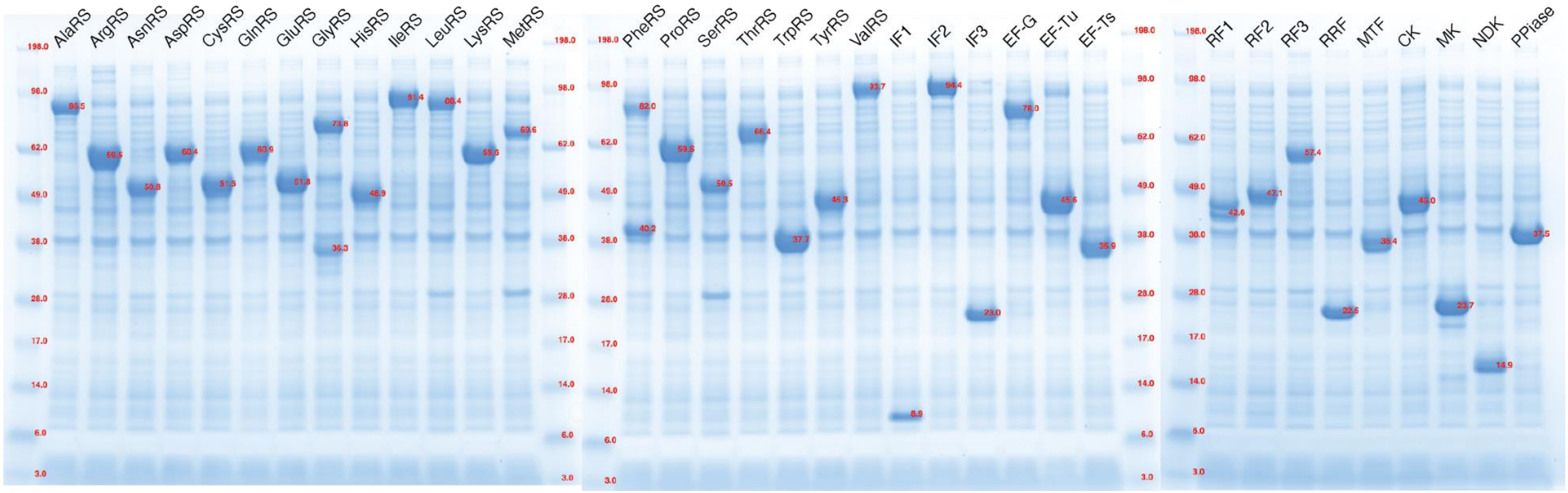
Switching to a single expression strain solved coculture problem



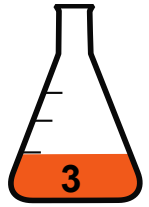
3rd attempt at One-Pot PURE

At Induction

At Harvest



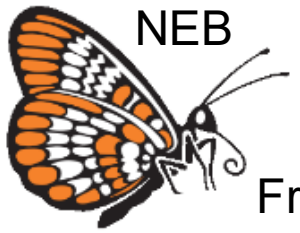
Same One-Pot PURE proteins, different behaviors in energy mixes



3rd attempt at One-Pot PURE

My protein mix

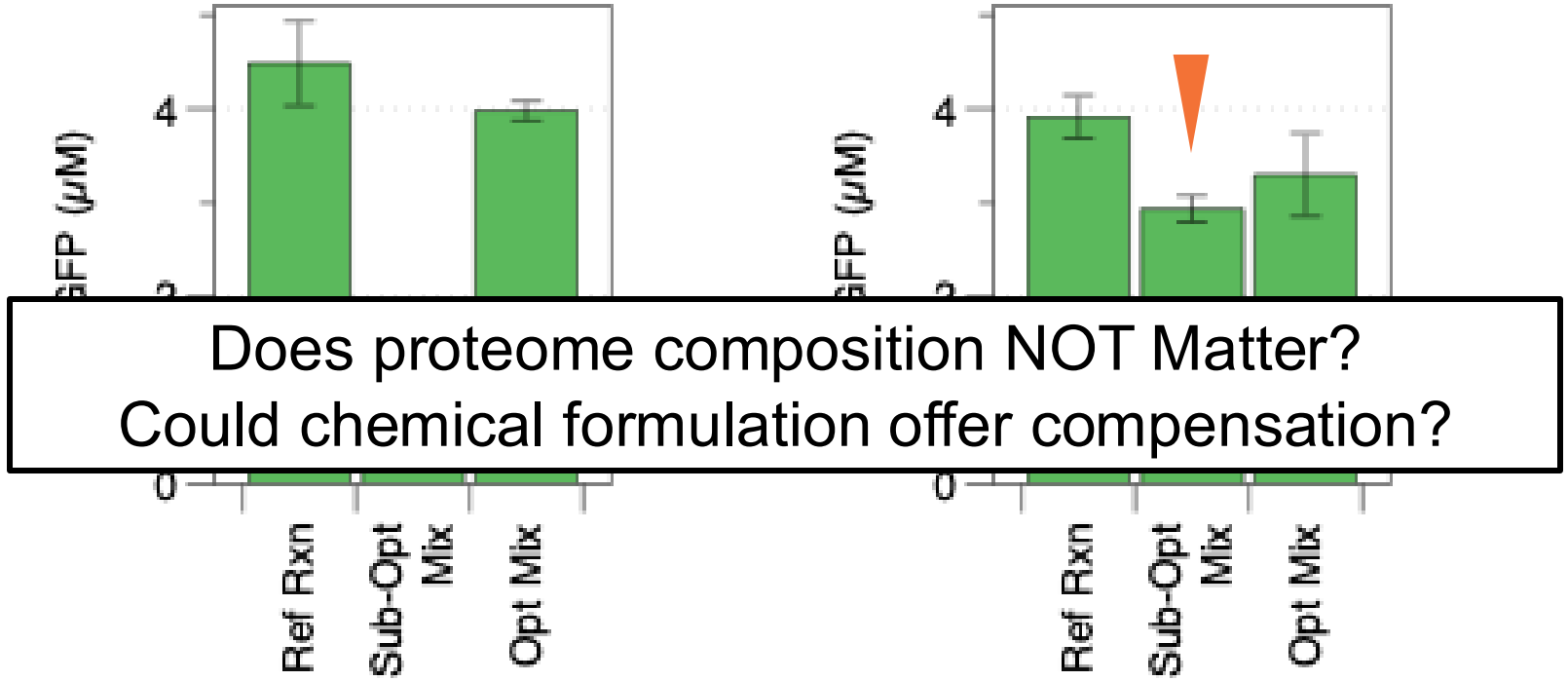
NEB



Gene Frontier

Formulation A

Formulation B

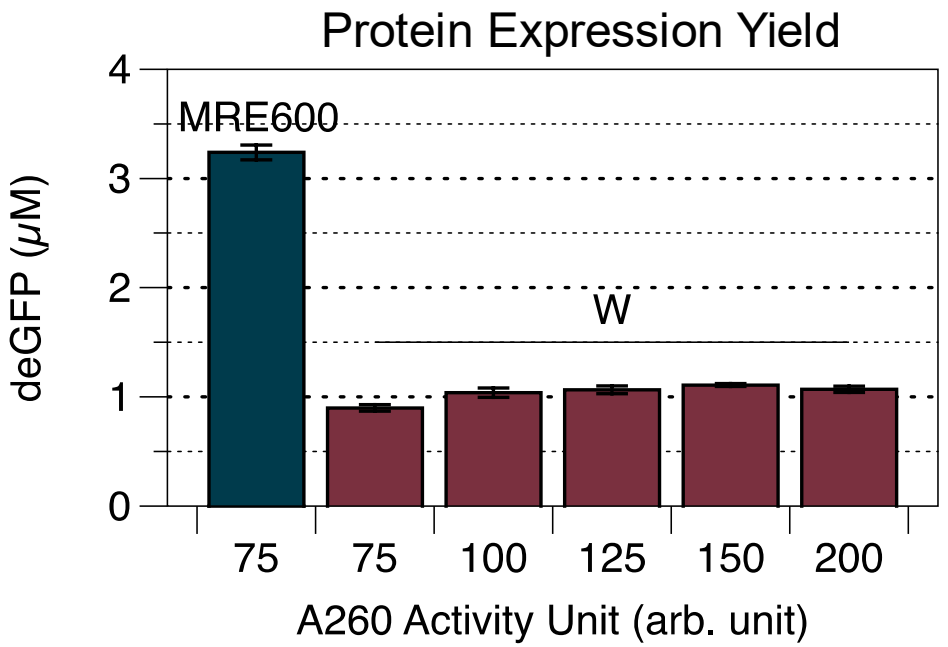


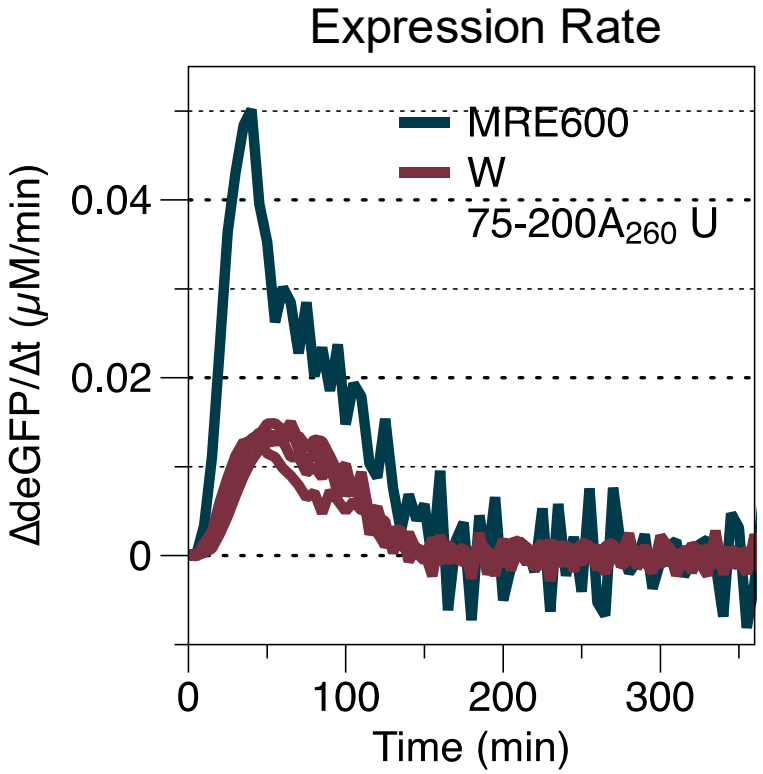
Does proteome composition NOT Matter?
Could chemical formulation offer compensation?

Homemade energy mix revealed hidden tRNA complexity

Roche
MRE600
tRNA

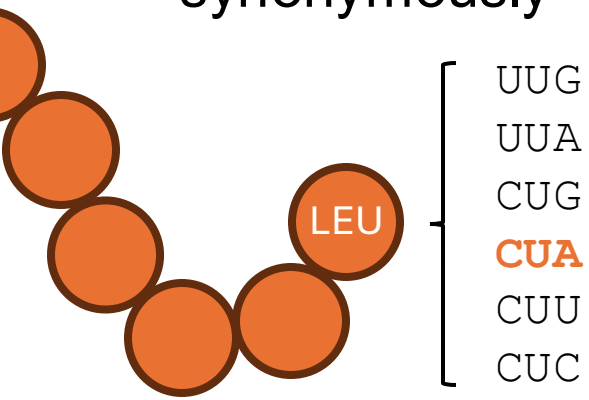
Sigma
W
tRNA





Homemade energy mix revealed hidden tRNA complexity

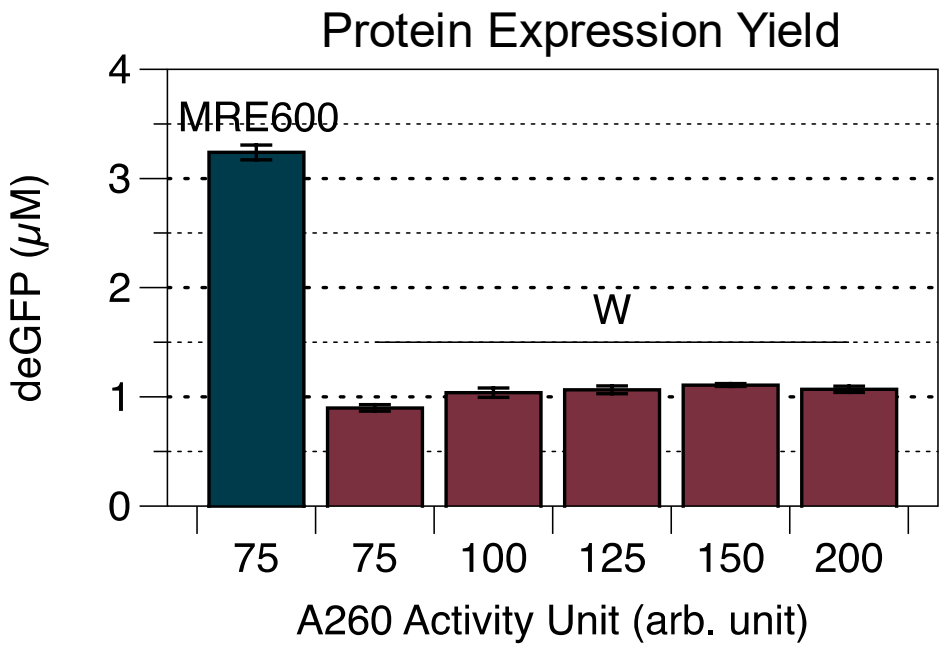
Proteins can be coded synonymously

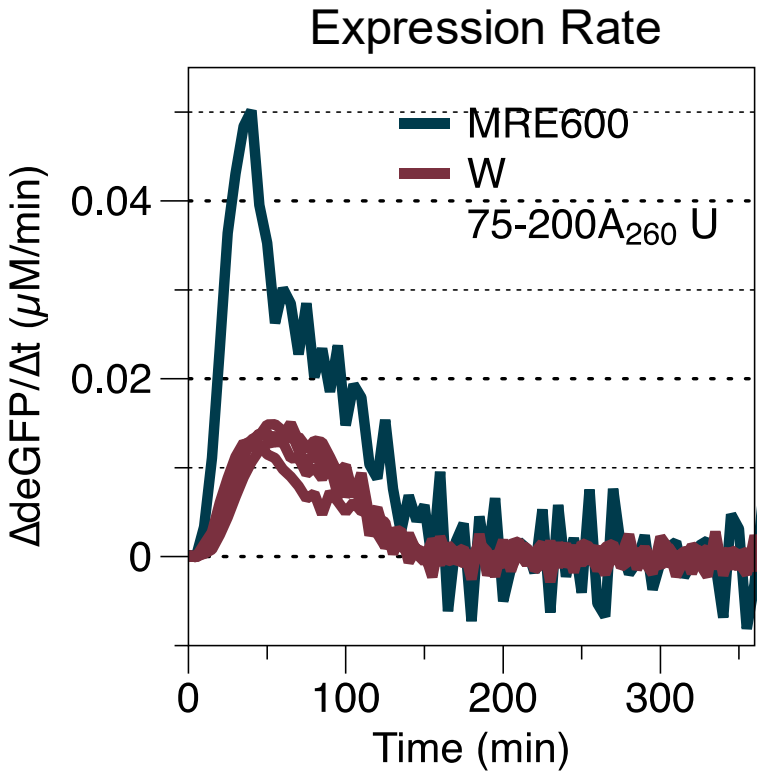


As long as there are corresponding tRNAs

Roche
MRE600
tRNA

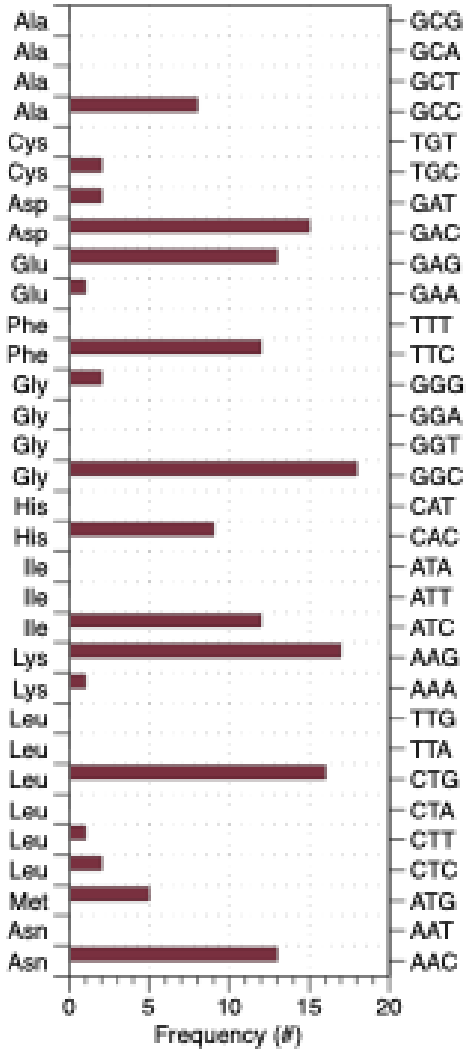
Sigma
W
tRNA

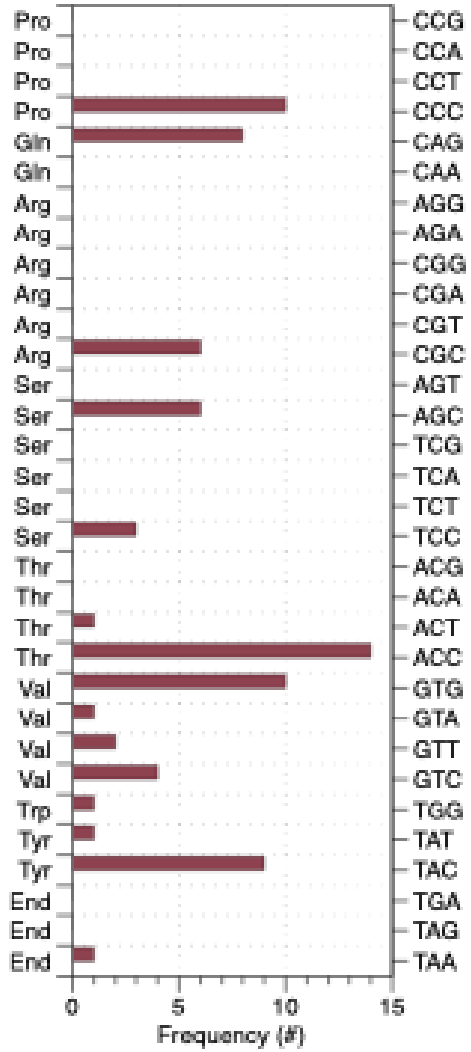




Synonymous proteins are not the same

GFP
clustered codons

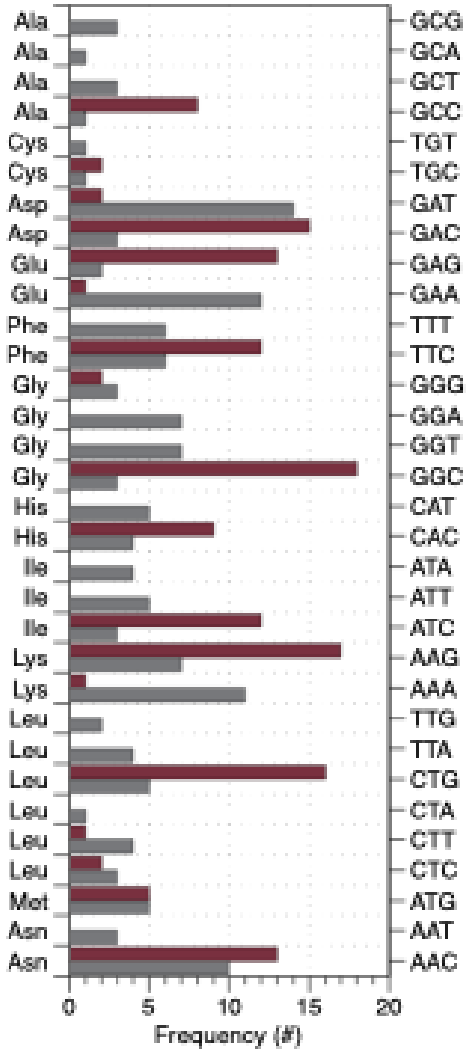


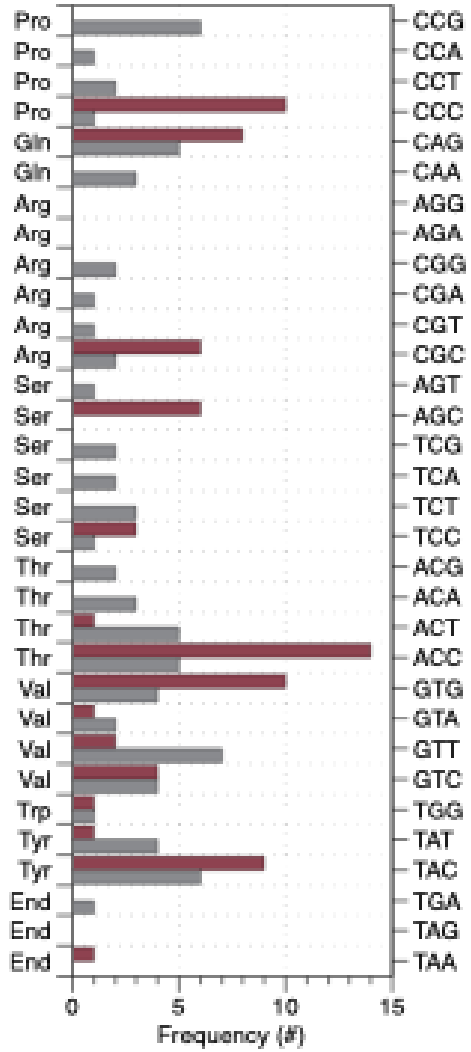


Synonymous proteins are not the same

GFP
clustered codons

GFP
spreadout codons





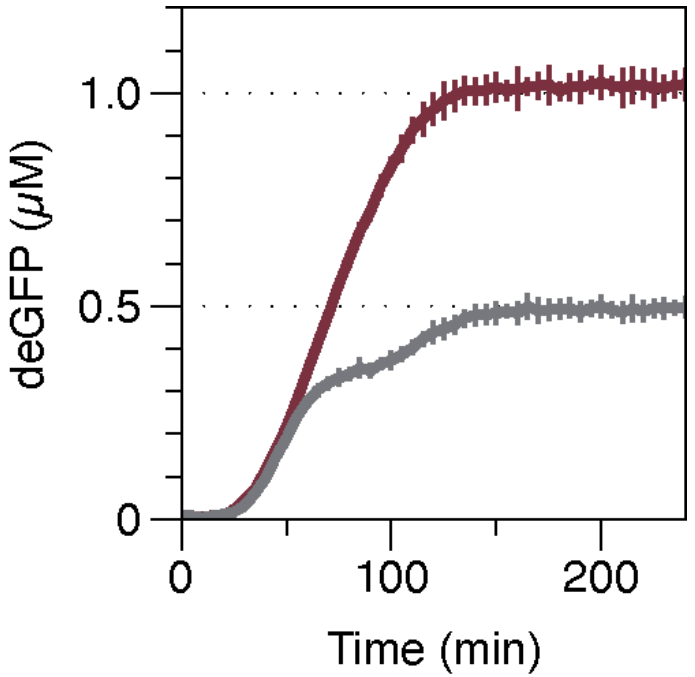
Synonymous proteins are not the same

In Sigma
W tRNA

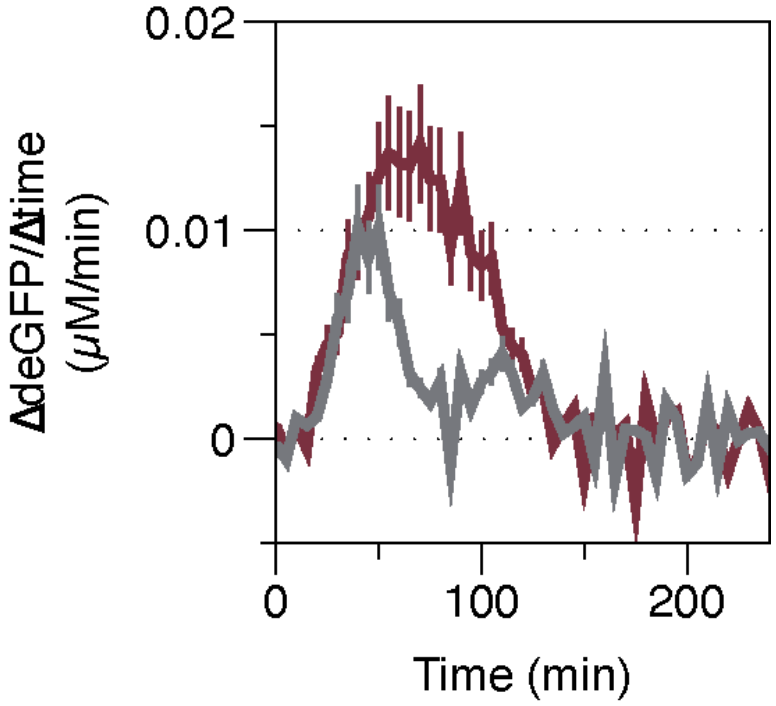
GFP
clustered codons

GFP
spreadout codons

Yield



Rate



We have **no idea what our tRNA pool looks like!**

So, how far am I from realizing *design, build, work*?

Parameter Input:

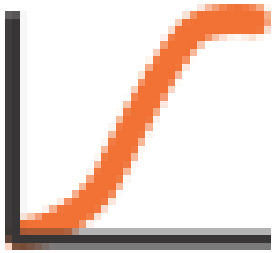
Make **X protein**,
at **Y amount**, in **Z time**.

***In silico* Design:**

Synthesize **X plasmid**
for **Y system** expression.

Guaranteed Outcome:

Built and **done.**



Blind spots identified:

- Predictive cell-free expression requires **system-level** engineering
- We need **data at the proteomic and biochemical level** (tRNAs) to inform design
- We could also **define proteomic and biochemical environments** to serve our design

People who made this work possible

Murray Lab Members

Collaborators

Paul Freemont Lab, Imperial College London

- Matas Deveikis

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UKRI Biotechnology and Biological Sciences Research Council

Manuscript for this work available at:
10.1021/acssynbio.4c00779

Slide deck for this talk available at:
yzhang952.github.io/files/AIChE2025.pdf