# Enabling Biology by Design with Cell-Free Expression System and Bacteriophages

Presented by Yan Zhang

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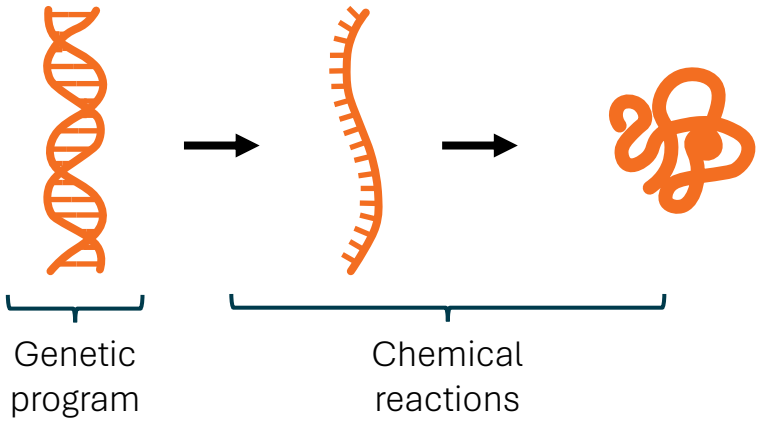
Caltech

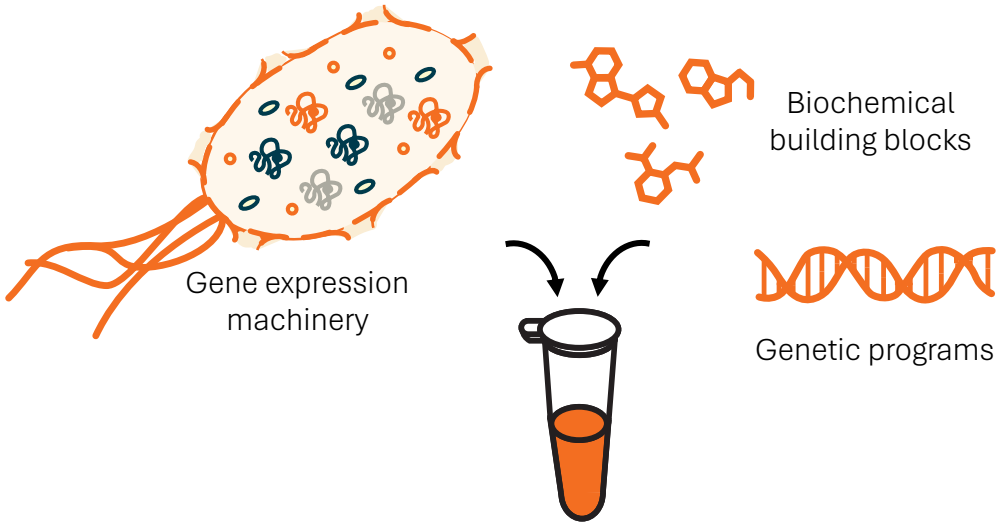
# Cell-Free gene expression system provides a powerful platform to enable biology by design

Living systems are templated by genetic programs and assembled through a series of chemical reactions




With the ability to compose the genetic program and recreate the chemical reactions, we can synthesize “living systems”

Cell-free expression systems harness living cell’s gene expression machinery to enable gene expression *in vitro*




Custom gene expression programs can now be executed in a test tube reaction

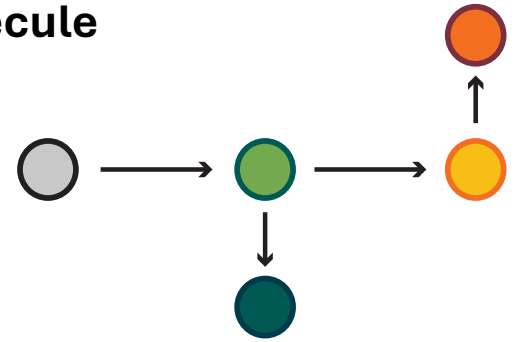
# The complexity of genetic programs in cell-free systems

**Make a protein**

1+ genes

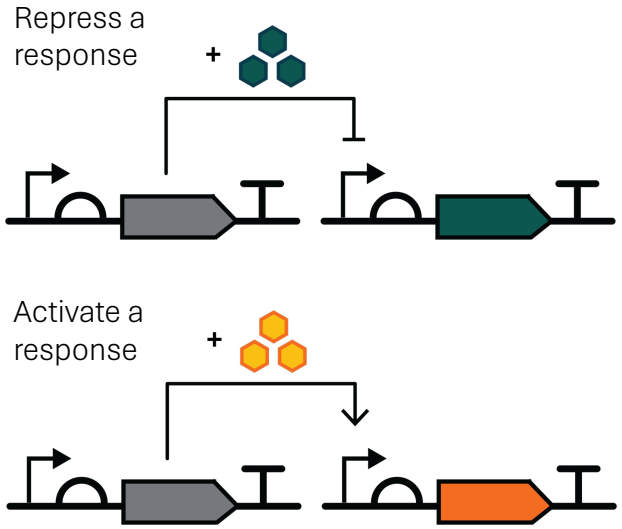

**Make a macromolecule**

3+ genes



**Make a decision**

2+ genes

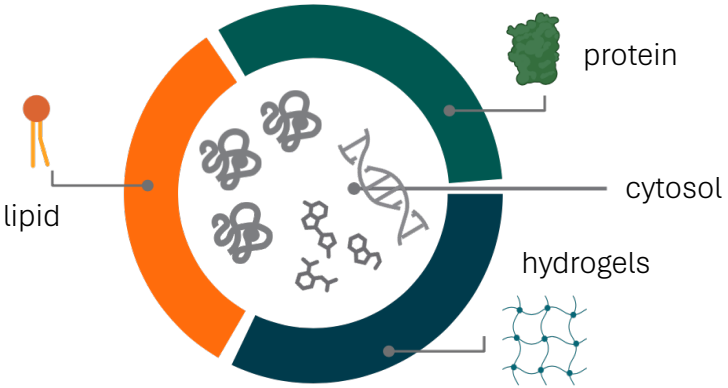



**Can we make a cell?**

>90 genes

At minimum

- 1 TX factor
- 31 TL factors
- 4 Eng. Regen.
- 1 Ribosome

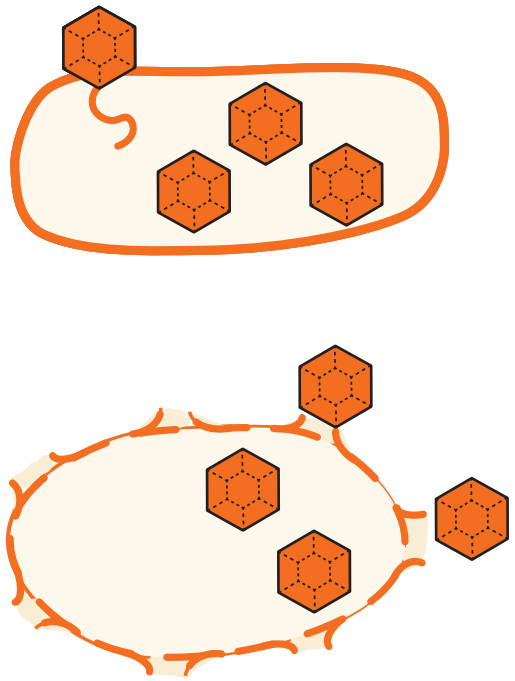





Parts of figure created with BioRender

# Bacteriophages – viruses infecting bacteria – can fill this complexity gap

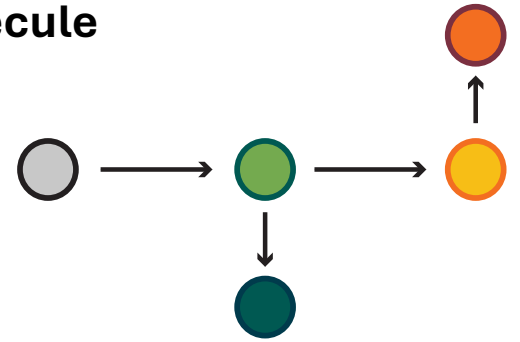
**Make a Bacteriophage?**



10-fold complexity jump

**Make a macromolecule**

3+ genes



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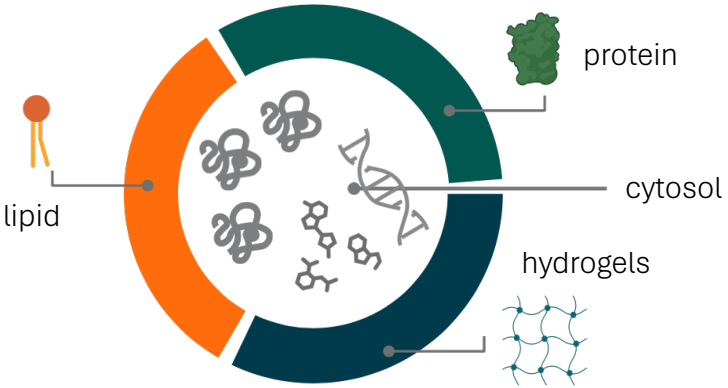

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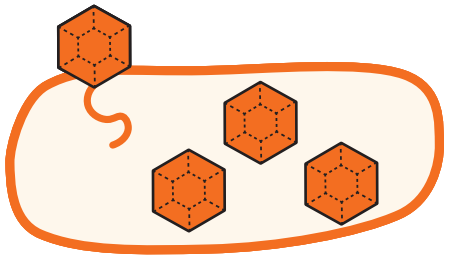
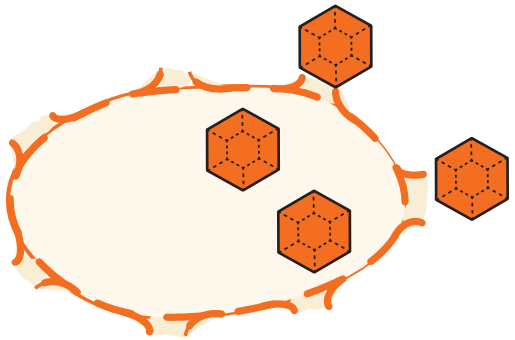
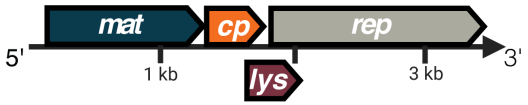
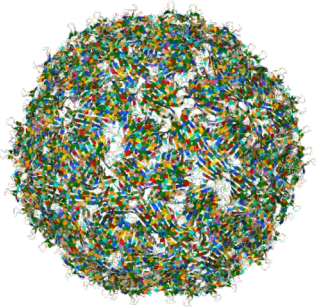




They can be simple



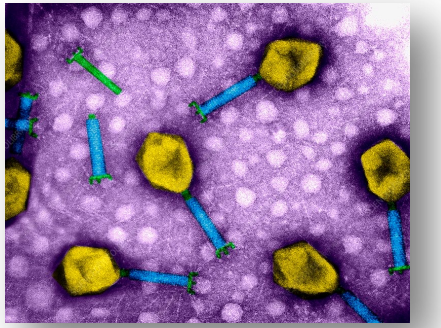

MS2 genome



MS2 particle
PDB 2MS2

Or just as complex

**T4 Coliphage**
170 kbp dsDNA genome
289 genes



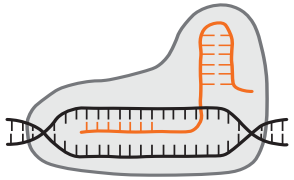
Dennis Kunkel Microscopy
@ Science Photo Library

# Significance and opportunities in cell-free bacteriophage production and design

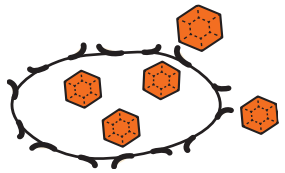
Bacteriophages have been a treasure trove powering biotechnological and biomedical innovations

**Phage display** to evolve antibodies

**CRISPR-Cas9** for gene editing

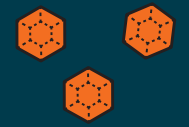


**Phage Therapy** against AMR pathogens



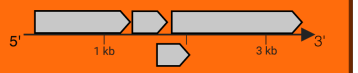
## Critical Gaps

### Producing diverse bacteriophages at large enough quantities



- Use cell-free systems to produce infectious phages from genome templates.

### Engineering phage therapeutics without sequence entanglement

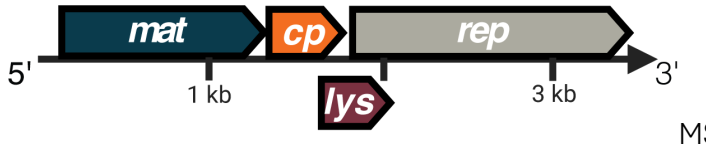



- Decompress phage genome using *in vitro* genome assembly to remove sequence entanglement.

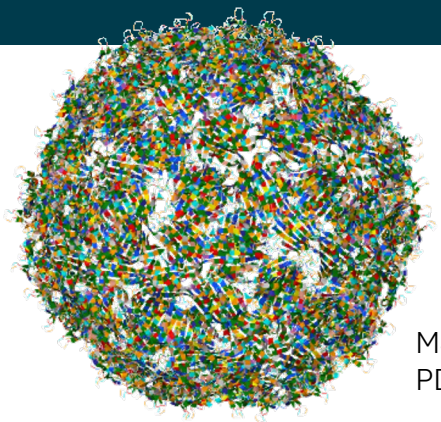
# Establishing cell-free production of MS2 bacteriophages

**Can cell-free systems translate and assemble bacteriophages?**

Start simple
The MS2 ssRNA coliphage

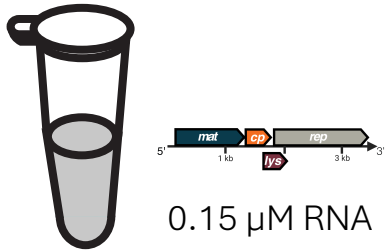
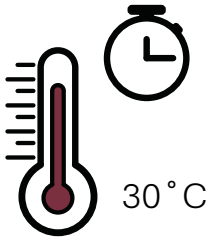
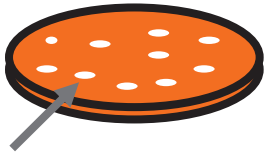



MS2 genome



MS2 particle
PDB 2MS2

- It takes 180 coat protein (cp) and 1 maturase (mat) to assemble a phage particle
- How many infectious phage particles can be produced in a cell-free reaction?

| Assemble reaction with MS2 genome | Incubate overnight | Mix phages with *E. coli* C-3000 | Plate with molten agar | Check plaque formation |
|---|---|---|---|---|
|  0.15 µM RNA |  30˚C |  |  |  Plaques – area without bacteria growth means active phage lysis |

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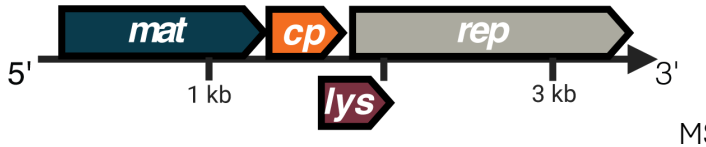


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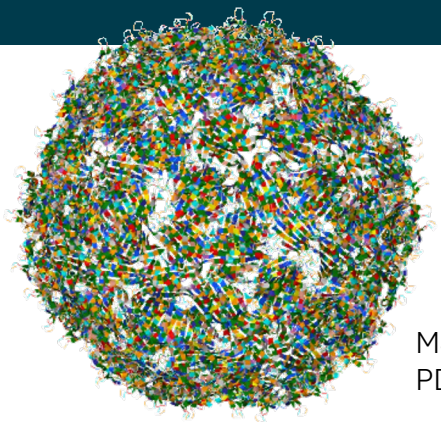

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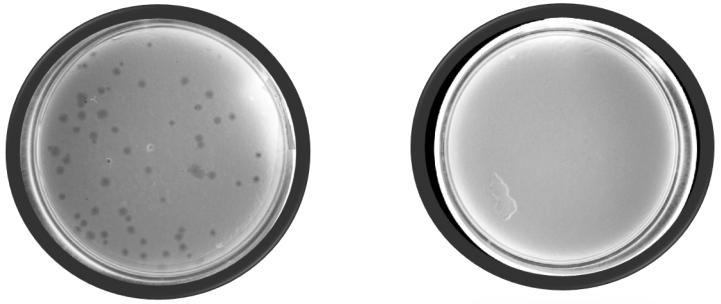
|---|---|---|---|---|

**Success!**

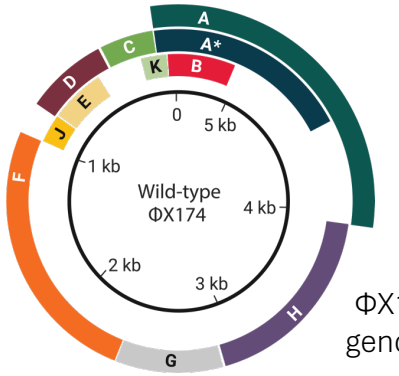
- Left - $10^3$ plaque-forming units/mL of reactions (+) MS2 genome
- Right -No plaques for reactions (-) MS2 genome



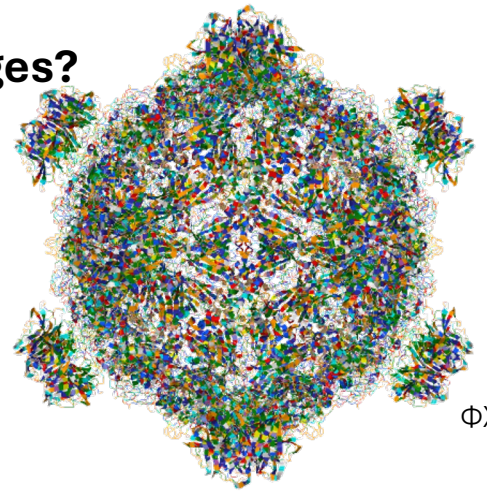
# Extending cell-free phage production to ssDNA coliphage

**Can cell-free systems transcribe, translate, and assemble bacteriophages?**

Increasing complexity ΦX174 ssDNA coliphage
- 11 sequence-entangled genes
- Multiple coat proteins for capsid (B, D, F, G)

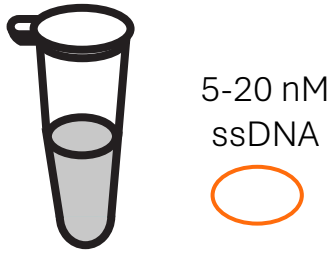



ΦX174 genome

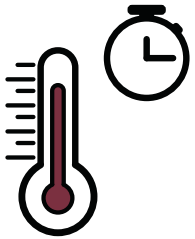


ΦX174 particle
PDB 2BPA

| Assemble reaction with ΦX174 genome | Incubate overnight | Mix phages with *E. coli* HF4704 | Plate with molten agar | Check plaque formation |
|---|---|---|---|---|



5-20 nM ssDNA



Initial results – no plaques what so ever!!!

# Extending cell-free phage production to ssDNA coliphage

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Increasing complexity ΦX174 ssDNA coliphage

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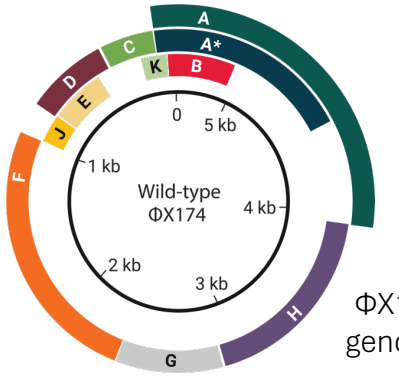
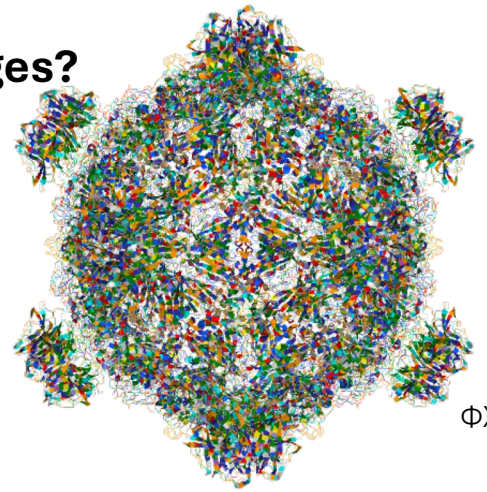


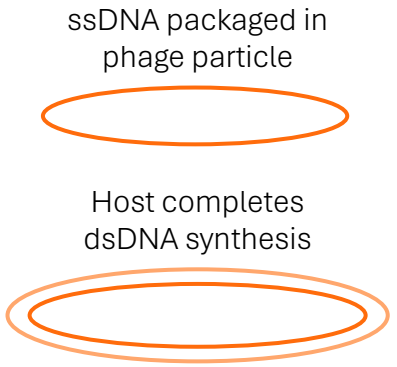

ΦX174 genome



ΦX174 particle
PDB 2BPA

## It turns out that…

1. ΦX174 ssDNA enters the host
2. Host DNA polymerase synthesizes the complementary (-) strand of ΦX174 genome
3. The double-stranded, replicative form of the ΦX174 genome is the gene expression template



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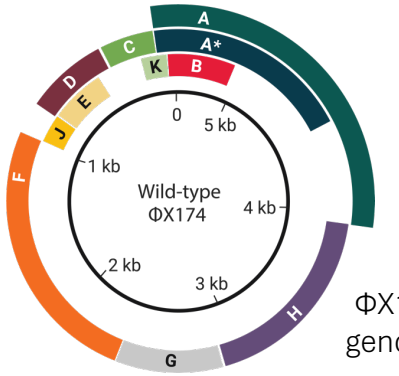
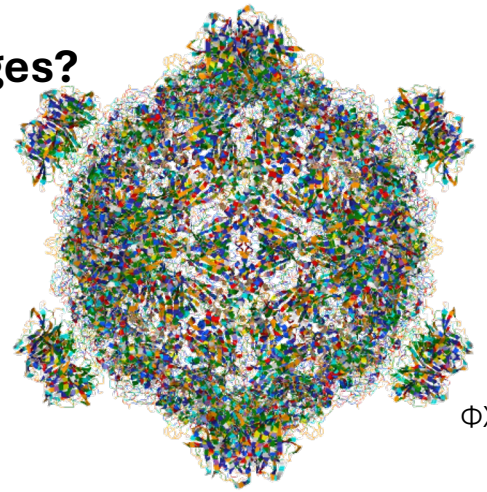


ΦX174 genome


ΦX174 particle
PDB 2BPA

**Attempt #2 with RF dsDNA**

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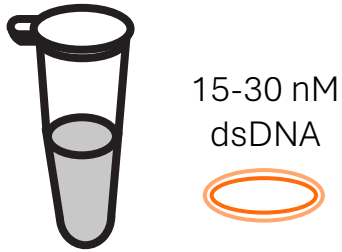
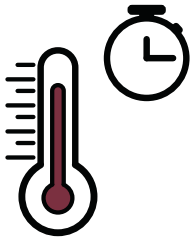
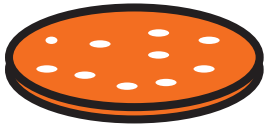
|---|---|---|---|---|





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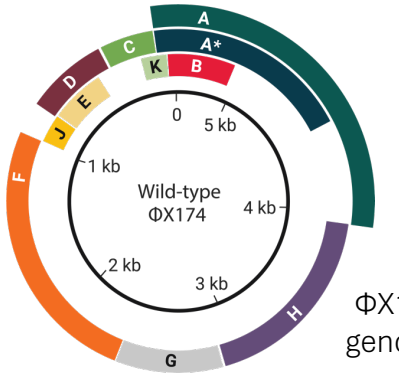
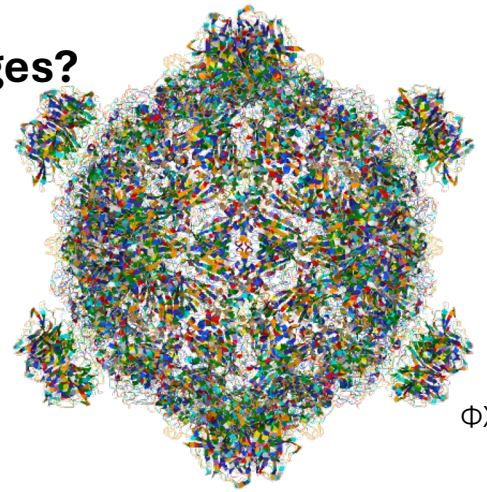


ΦX174 genome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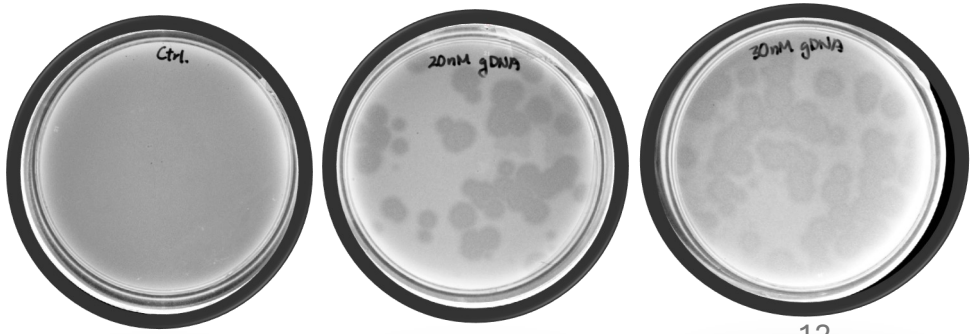
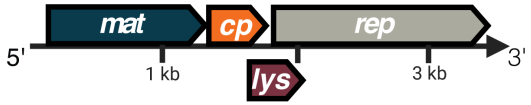
- Mid, Right - $10^3$ plaque-forming units/mL of reactions (+) genome

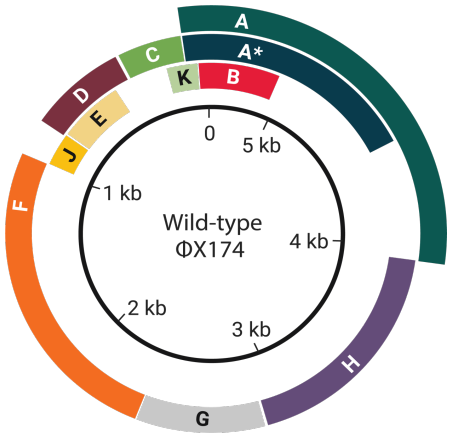


# Composing bacteriophage genome free of sequence entanglements

Sequence entanglement is prevalent on natural phage genome



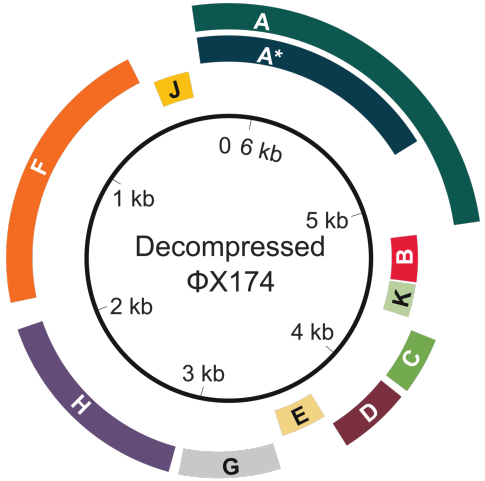

MS2 genome




ΦX174 genome


Genome decompression will expand the genome size by 15% and interfere with effective packaging

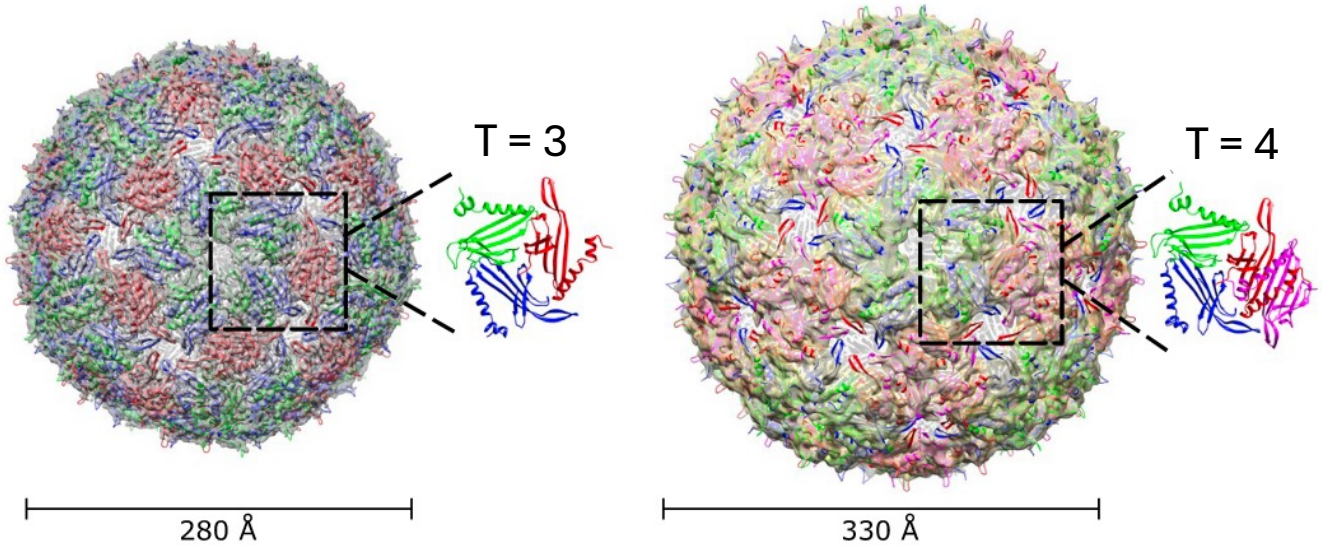

Decompressed MS2 genome




Decompressed ΦX174 genome

# This is now becoming a DNA packaging and protein engineering problem…

MS2 has a natural variant with a bigger volume

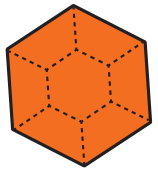



de Martín Garrido, N., et. al., Mol Microbiol 2020, 113 (1), 143–152.

- How do we get T = 4 to happen more frequently?
- How do we engineer MS2 coat protein to give us T = 4?
- Would love to chat more and get your thoughts

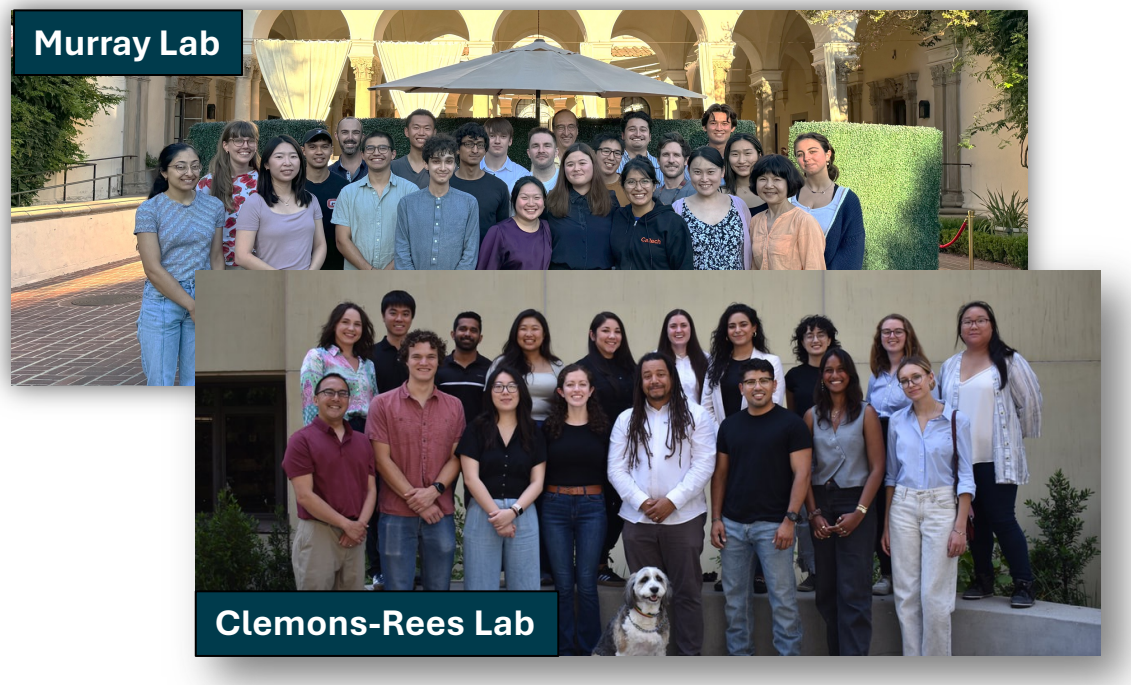
# Takeaways, Next Steps, and Acknowledgement

- Composing bacteriophages provides **a simpler yet just as impactful approach** to enable biology by design



- Cell-free expression of simple coliphages

- Decompressing the phage genome introduces a packaging problem




Slide deck for this talk:
https://yzhang952.github.io
/files/CEMI2025.pdf

Caltech cemi
Center for Environmental Microbial Interactions

NIH National Institute of Biomedical Imaging and Bioengineering
Grant #: 1K99EB036553