Inspired by how it works the protein L in the presence or absence of Dnaj chaperons, and optimized the fitness + *E. coli* is a common bacterium. It could be used not for the therapy of phages for medicine, but also for Food Security/avoiding *E. coli* producing Biofilms (?

Made by: Ana Gomez | BIOPUNK

Main Goals:

1. Higher toxicity of lysis protein (hard)

→ Improve a faster lysis and robust (accelerate or reduce variants for *E. coli* lysis)

2. Higher titers (Medium)

→ Optimizing timing of lysis/and maximizing titers/fitness

1-Page Proposal: (from idea 2)

Title: Optimizing Phage Yield by Tuning MS2-L Lysis Timing for Higher Titers

Background:

- The MS2 bacteriophage triggers host lysis using a single small protein, L.
- The host physiological state (including temperature and chaperone activity) can influence how efficiently and when lysis occurs.
- While faster lysis can increase toxicity, premature lysis may reduce phage yield if virion assembly and packaging are not complete.

(Chamakura et al., 2017); (Barron, 2022); (Mezhyrova et al., 2020); (Kim et al., 2025)

Hypothesis:

MS2-L contains sequence features that act as a timing/threshold control module. Conservative, stability-preserving mutations (and/or modest timing-shifts) can produce L variants that delay lysis slightly and consistently, improving phage assembly completion and boosting titers.

Specific Objective:

We will computationally design and prioritize MS2-L variants predicted to:

a. Maintain core functional features (especially the hydrophobic/transmembrane region).
b. Avoid destabilizing changes that cause loss-of-function.

c. Shift lysis timing toward a more “assembly-friendly” window (slightly delayed, less variable across conditions).

Tools / Approaches (From Recitation) source: HTGAA TOOLs

(A) Sequence mining and conservation mapping

BLAST + Multiple Sequence Alignment (e.g., Clustal Omega)

- Collect L homologs and map conserved vs variable positions.
- Conserved residues likely encode essential function; variable regions may tolerate tuning mutations.

(B) Protein Language Models for conservative mutagenesis

ESM-2 / ESM-3

- Generate “plausible” mutant candidates that remain evolutionarily consistent.
- Focus on conservative substitutions (stability-preserving) rather than aggressive redesign.
- PLMs help propose mutations that preserve fold/function signals.

(C) Rapid structure screening for stability

ESMFold (or monomer AlphaFold if available)

- Predict structures for candidate variants quickly.
- Filter variants that show major structural collapse or loss of helix integrity.
- Higher titers require functional L, so stability screening prevents wasted candidates.

(Optional, if time) Complex check for timing modulation

AlphaFold-Multimer or Boltz-1 (L + DnaJ)

- Only for a small subset of top candidates.
- Evaluate whether variants slightly strengthen/retain regulatory interactions that could support “delayed but reliable” lysis.
- We do not need perfect accuracy—just a directional screen.

Potential Pitfalls

1. Trade-off uncertainty: Slightly delaying lysis might improve assembly time, but too much delay could reduce spread or lead to degradation of infected cells.

2. Model limitations for small/membrane-associated proteins: Structural predictions may be less reliable in transmembrane regions and flexible termini, making fine timing predictions approximate.

Pipeline:

1) Retrieve MS2-L sequence → 2) Homolog search (BLAST) → 3) Multiple sequence alignment + conservation map (Clustal Omega) → 4) Generate conservative variant library (ESM-2 / ESM-3) → 5) Rapid stability filter (ESMFold / monomer AF) →

6) Rank variants by:

- preserved TM helix integrity
- minimal destabilization risk
- "timing-tuning" substitutions (conservative)

7) Output: top candidates predicted to yield slightly delayed + consistent lysis timing → higher assembly completion → higher titers

References:

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Sources: HTGAA TOOLs