Amino Acid Composition Analysis Enables Dose-Accurate MIC Evaluation of Colistin–M13PAB Phage Conjugates

Yanxi Yang et al. Nucleic Acids Research (2025) 53: gkaf984 | DOI: 10.1093/nar/gkaf984

Our role: Amino acid composition analysis to quantify colistin loading on M13PAB, establishing drug-to-phage stoichiometry for colistin-equivalent MIC dosing.

Background & Challenge

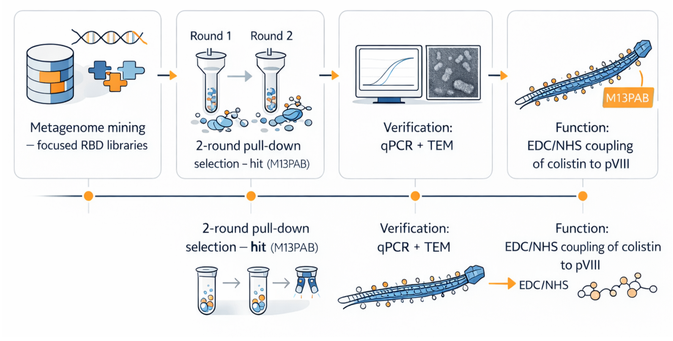
- **AMR pressure:** Gram-negative pathogens (e.g., P. aeruginosa) carry layered resistance mechanisms.
- **Phage bottleneck:** natural phages are highly strain-specific, driving slow isolate-by-isolate screening.
- **Engineering goal:** a focused, screenable library that shortens the path from clinical isolate → targeted hit.

Study Objective

Engineer the non-lytic M13 scaffold with metagenome-mined inovirus RBDs to enable rapid binder selection and “controlled phage therapy” via antibiotic conjugation.

Workflow (Bind → Verify → Function)

- **Metagenome mining:** ~10k inovirus-like genomes → focused RBD libraries
- **2-round selection:** pull-down enrichment on clinical isolates → hit identification (M13PAB)
- **Verification:** qPCR quantification + TEM visualization
- **Function:** EDC/NHS coupling of colistin to pVIII



Why this matters

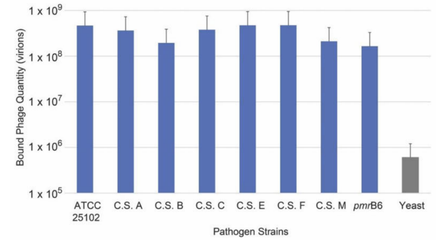
A short selection cycle accelerates strain-relevant targeting, while quantitative payload loading is the bridge between conjugation chemistry and defensible potency benchmarking.

Case Study Highlights

Metagenome Mining → Targeted Binders

RBD libraries produced binders across multiple Gram-negative pathogens (E. coli, K. pneumoniae, A. baumannii).

Lead hit M13PAB showed >100× higher binding to multiple P. aeruginosa clinical isolates vs. negative control.



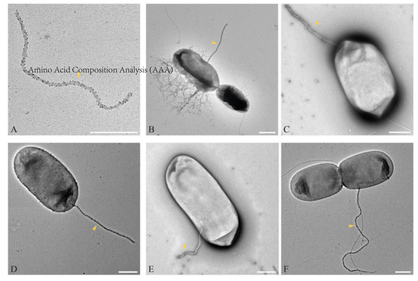
M13PAB binds P. aeruginosa clinical isolates >100× vs. control (qPCR)

Quantitative Drug Loading

Amino acid composition analysis using Thr/Leu signatures to quantify colistin payload.

Loading: ~2.5 colistin/pVIII → ~6,750 colistin/virion

Conversion: 1.3×10^{-11} µg colistin/virion (colistin-equivalent dosing)



TEM confirms pIII-terminus binding and intact filamentous virion.

Potency + Reported Safety Signals

MIC reduction: 31–125× across P. aeruginosa strains (reported)

Resistant strain PAKpmrB6: 63× MIC reduction (reported)

Safety (reported): <0.4% hemolysis; no detectable cytotoxicity in HEK-293 up to 2×10^{10} virions/mL

Species	Strain	MIC_{MP} (virions/mL)	MIC_{col} (µg/mL)	MIC_{CMP} (virions/mL)	MIC_{CMP} (µg/mL)[a]	$\frac{MIC_{col}}{MIC_{CMP}}$ (n-fold reduction in MIC)
E. coli	ATCC 25922		0.5			
P. aeruginosa	ATCC 25102	$> 1 \times 10^{12}$	1	1.25×10^{9}	0.016	63
P. aeruginosa	PAKpmrB6	$> 1 \times 10^{12}$	64	8×10^{10}	1.036	63
P. aeruginosa	Clinical Strain A	$> 1 \times 10^{12}$	0.5	1.25×10^{9}	0.016	31
P. aeruginosa	Clinical Strain B	$> 1 \times 10^{12}$	0.5	6.25×10^{8}	0.008	63
P. aeruginosa	Clinical Strain C	$> 1 \times 10^{12}$	0.5	6.25×10^{8}	0.008	63
P. aeruginosa	Clinical Strain E	$> 1 \times 10^{12}$	0.5	6.25×10^{8}	0.008	63
P. aeruginosa	Clinical Strain F	$> 1 \times 10^{12}$	0.5	6.25×10^{8}	0.008	63
P. aeruginosa	Clinical Strain G	$> 1 \times 10^{12}$	0.5	6.25×10^{8}	0.008	63
P. aeruginosa	Clinical Strain L	$> 1 \times 10^{12}$	0.5	1.25×10^{9}	0.016	31
P. aeruginosa	Clinical Strain 320	$> 1 \times 10^{12}$	0.5	6.25×10^{8}	0.008	125

Colistin–M13PAB lowers MIC 31–125×; 63× in resistant PAKpmrB6.

Our Contribution (Creative Proteomics)

Creative Proteomics quantified colistin payload loading on colistin–M13PAB by **Amino Acid Composition Analysis,** using Thr/Leu signatures to derive a defensible drug-to-phage ratio and drug-equivalent dosing factor for potency benchmarking.

Analytical Challenge

- High background matrix: ~2,700 copies of pVIII per virion amplify the protein signal.
- Dose must be quantitative: potency claims require a defensible drug-to-carrier ratio.
- Clear conversion needed: translate particle concentration into drug-equivalent dosing for MIC/MBC comparisons.

What We Delivered

- Amino acid composition table (absolute / normalized as applicable)
- Mole% profile with QC checks
- Payload loading factor calculation (assumptions stated, auditable)
- Summary-ready numbers for publication/poster reporting

Why Choose Creative Proteomics

For complex bioconjugates like phage-drug complexes, standard UV-Vis quantification is insufficient. Our amino acid composition analysis platform provides gold-standard stoichiometric accuracy:

- Full Coverage: 20 amino acid profile per sample for absolute quantification.
- Rigor: 5–7 point calibration options with internal-standard support to ensure linearity.
- Quality: ≥2 QC checks/run (e.g., blank + standard recovery) with documented criteria.
- Confidence: n=2–3 technical replicates available for higher-confidence loading factor determination.
- Transparency: Deliverables include composition tables → mole% → loading factor with fully auditable assumptions.

Need Precise Stoichiometry to Validate Your Research?

Whether you are developing next-generation ADCs, viral-based delivery systems, or functionalized nanoparticles, Creative Proteomics delivers the gold-standard Amino Acid Composition Analysis required to support your potency claims and high-impact publications. Let our experts help you build a robust analytical plan for your complex bioconjugates.

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