I'll write this grant proposal. Let me note one correction I need to honor: the task specifies an R21 mechanism, but the structure template says "modular R01 sketch" for the budget — I'll write an R21-appropriate modular budget since R21s use $275K direct over two years, which is the credible framing.

Bacteriophage Cocktail Therapy for Intrinsically Resistant *Stenotrophomonas maltophilia*: De-Risking a Biofilm-Penetrating Salvage Strategy

Project Summary / Abstract

Stenotrophomonas maltophilia is a Gram-negative, non-fermenting opportunistic pathogen and a leading cause of difficult-to-treat nosocomial infection in immunocompromised, ICU, cystic fibrosis (CF), and ECMO/ventilated patients. It is intrinsically multidrug-resistant: chromosomally encoded L1 metallo-β-lactamase and L2 serine β-lactamase inactivate nearly all β-lactams and carbapenems, while abundant RND-family efflux pumps and avid biofilm formation leave only a handful of eroding options (trimethoprim-sulfamethoxazole, minocycline, levofloxacin, cefiderocol, ceftazidime-avibactam/aztreonam). Strictly lytic bacteriophages kill through a mechanism entirely independent of these β-lactamases and efflux pumps, self-amplify at the infection site, and—when armed with depolymerases—degrade and penetrate the biofilm that defines *S. maltophilia* persistence. This R21 will de-risk a defined, biofilm-penetrating three-phage cocktail before a full R01. We will (1) assemble and genomically characterize a complementary-receptor cocktail and map host range across a diverse clinical-isolate panel; (2) quantify suppression of phage-resistant regrowth and phage–antibiotic synergy (PAS) against planktonic cells and mature biofilm; and (3) test efficacy in a murine *S. maltophilia* pneumonia model. The work directly addresses the failure mode seen in the only detailed clinical report to date—a TAILOR-program ECMO case in which IV/intra-abdominal phage cleared bloodstream infection but biofilm-laden collections stayed culture-positive (Cullen et al., 2024). Expected outcomes are a genomically defined, biofilm-active cocktail with measured PAS and in vivo proof-of-concept, providing the preclinical package needed for an eventual FDA expanded-access/eIND-enabled trial and positioning phage therapy for this intrinsically untreatable organism as a standardized, regulated salvage option rather than a heroic last resort.

Specific Aims

Conventional antibiotics fail against *S. maltophilia* because intrinsic L1/L2 β-lactamases and RND efflux pumps neutralize most agents, and biofilm shields the organism on catheters, lungs, and abscess walls. Lytic phages are mechanistically orthogonal to these defenses, self-amplify, and can carry matrix-degrading depolymerases—making them a rational salvage and adjunctive strategy. Yet the evidence base is thin: no RCTs exist, clinical experience is limited to compassionate-use case reports, and the central unsolved problem is biofilm/anatomical sanctuary, which defeated phage in the best-documented case (Cullen et al., 2024). This exploratory R21 will generate the missing preclinical data.

Aim 1. Assemble and genomically characterize a complementary-receptor three-phage cocktail and define host range. We will combine genetically distinct, strictly lytic phages recognizing different surface receptors (LPS, outer-membrane proteins, pili), sequence and annotate genomes (confirming lytic lifestyle, absence of toxin/AMR/lysogeny genes, and depolymerase content), and screen lysis across a panel of clinical isolates. *Hypothesis:* a rationally combined cocktail covers substantially more isolates than any single phage (cf. XAN_XB1 ~56%).

Aim 2. Quantify resistance suppression and phage–antibiotic synergy against planktonic cells and biofilm. Using kinetic profiling (adsorption rate, burst size) and time-kill/regrowth assays, we will test whether mixing phages with differing kinetics suppresses regrowth better than single phages, and whether sub-lethal standard-of-care antibiotics (TMP-SMX, levofloxacin, minocycline) potentiate biofilm killing (PAS). *Hypothesis:* the cocktail plus a sub-lethal antibiotic maximally reduces biofilm viability and resistant regrowth.

Aim 3. Establish in vivo proof-of-concept in murine *S. maltophilia* pneumonia. We will test cocktail efficacy (survival, pulmonary CFU, inflammatory markers IL-6/procalcitonin) alone and with antibiotic, benchmarked to single-phage data (XAN_XB1: ~30% survival gain, ~2-log CFU reduction).

Impact. Success yields a genomically defined, biofilm-active, synergy-characterized cocktail with in vivo proof-of-concept—the de-risking package required to justify an R01 and an FDA eIND-enabled salvage protocol for an organism with vanishing therapeutic options.

Significance

S. maltophilia has emerged as a sentinel of the intrinsic-resistance problem. Unlike acquired resistance, its defenses are hardwired: the L1 metallo-β-lactamase and L2 serine β-lactamase together inactivate essentially all β-lactams and carbapenems, RND efflux pumps expel structurally diverse

drugs, and the organism forms tenacious biofilm on indwelling devices and in the CF airway (McCutcheon & Dennis, 2021). The remaining agents—TMP-SMX, minocycline, levofloxacin, cefiderocol, ceftazidime-avibactam/aztreonam—are few, increasingly compromised by resistance, and often poorly active against biofilm-embedded cells. Patients at highest risk (ICU, ECMO/ventilated, CF, neutropenic) are precisely those who tolerate treatment failure least.

Phage therapy is significant here because its killing mechanism is *orthogonal* to every major *S. maltophilia* resistance pathway: lytic phages adsorb to surface receptors and lyse from within, so β-lactamases and efflux pumps are irrelevant to their action (McCutcheon & Dennis, 2021). Phages self-amplify at the infection focus and, critically, many *S. maltophilia* phages encode depolymerases/lysins that erode the exopolysaccharide matrix—targeting the biofilm mode of persistence that antibiotics struggle to reach. The clinical signal is real but immature: the Baylor TAILOR case (Cullen et al., 2024) showed a personalized IV phage cocktail safely cleared *S. maltophilia* bacteremia in an ECMO patient, yet biofilm-laden intra-abdominal collections remained culture-positive and the patient died of multiorgan failure—pinpointing biofilm and anatomical sanctuaries as *the* limiting variable. Closing that gap with rigorous preclinical data is the high-impact opportunity this proposal addresses.

Innovation

This project is innovative in four respects. First, it is **mechanism-matched to intrinsic resistance**: rather than chasing another small molecule that efflux pumps and L1/L2 will defeat, it exploits a killing modality those determinants cannot touch. Second, it makes **biofilm the primary design criterion**, deliberately selecting depolymerase-armed phages and testing mature-biofilm killing—directly targeting the failure mode of the Cullen et al. (2024) case rather than optimizing only planktonic kill. Third, it applies **rational kinetic cocktail design**: combining phages with deliberately divergent adsorption rates and burst sizes to suppress resistant regrowth, extending the Monsibais/Whiteson three-phage strategy (ANB28, KB824, SBP2-φ2) that outperformed any single phage (Monsibais et al., 2025). Fourth, it **front-loads phage–antibiotic synergy** with the exact standard-of-care agents a clinician would co-administer, so results translate to how phages are actually deployed at TAILOR and IPATH—alongside antibiotics—rather than as a standalone product.

Approach

Aim 1 — Cocktail assembly, genomic characterization, and host range

Rationale. Any single *S. maltophilia* phage covers only a fraction of this heterogeneous species (XAN_XB1 lysed ~56% of clinical isolates; Yang et al., 2026). Complementary-receptor cocktails

broaden coverage and raise the genetic barrier to resistance (Monsibais et al., 2025; McCutcheon & Dennis, 2021).

Experimental design. We will assemble three strictly lytic phages selected for distinct receptor usage (LPS, outer-membrane proteins, pili). Each will undergo whole-genome sequencing and annotation to confirm a lytic lifestyle and the absence of integrase/lysogeny, antibiotic-resistance, and toxin genes, and to catalog depolymerase/lysin genes. Host range will be screened by spot and efficiency-of-plating assays across a diverse panel of clinical *S. maltophilia* isolates [ILLUSTRATIVE: ~50 isolates], scoring single-phage versus cocktail coverage.

Expected outcomes. A genomically defined, safety-screened cocktail whose combined host range materially exceeds any constituent phage, with documented depolymerase content supporting biofilm activity.

Potential pitfalls & alternative approaches. If coverage is inadequate, we will swap in additional receptor-distinct phages from collaborating banks (TAILOR, IPATH, Dennis Lab) and broaden host range via host-range expansion on resistant isolates. If a candidate carries undesirable genes, it is excluded.

Aim 2 — Resistance suppression and phage–antibiotic synergy

Rationale. Mixing phages with different adsorption rates and burst sizes suppressed regrowth far better than single phages (Monsibais et al., 2025), and sub-lethal antibiotics can sensitize cells to phage (PAS) (McCutcheon & Dennis, 2021)—relevant to biofilm, the dominant persistence mode.

Experimental design. We will measure adsorption rate and burst size (one-step growth) for each phage and the cocktail, then run time-kill/regrowth assays on planktonic cells and on mature biofilm (microtiter and, where feasible, surface/CF-relevant models). PAS will be assessed by checkerboard-style combinations of the cocktail with sub-lethal TMP-SMX, levofloxacin, and minocycline, quantifying biofilm biomass and viable CFU and emergence of phage-resistant mutants.

Expected outcomes. Demonstration that the kinetically diverse cocktail suppresses regrowth better than single phages and that cocktail-plus-sub-lethal-antibiotic maximizes biofilm killing—defining the lead combination for Aim 3.

Potential pitfalls & alternative approaches. If phage resistance emerges in biofilm, we will test depolymerase-forward phages and sequence resistant mutants to identify receptor changes; if a given antibiotic antagonizes phage, we will down-select to synergistic pairs.

Aim 3 — In vivo proof-of-concept (murine pneumonia)

Rationale. A single phage (XAN_XB1) improved survival ~30% and reduced pulmonary CFU ~2 logs with lower IL-6/procalcitonin in mice (Yang et al., 2026); a kinetically optimized cocktail should match or exceed this.

Experimental design. Using an established murine *S. maltophilia* pneumonia model [ILLUSTRATIVE], mice will receive cocktail, cocktail+antibiotic, antibiotic alone, or vehicle. Endpoints: survival, pulmonary CFU, and IL-6/procalcitonin. Group sizes powered to detect a CFU difference comparable to prior work [ILLUSTRATIVE: n≈10–12/group].

Expected outcomes. Significant CFU reduction and survival benefit for cocktail (and greater for cocktail+antibiotic), with attenuated inflammatory markers—establishing translational proof-of-concept.

Potential pitfalls & alternative approaches. If monotherapy underperforms, the combination arm provides the primary readout; dosing/route (including nebulized delivery) will be optimized. Endotoxin will be minimized in phage preps to avoid confounding inflammation.

Timeline

[ILLUSTRATIVE] **Months 1–12:** Aim 1 (assembly, sequencing, host range) and initiation of Aim 2 kinetics. **Months 10–18:** Aim 2 biofilm/PAS assays completed; lead combination selected. **Months 16–24:** Aim 3 in vivo studies, analysis, and manuscript/R01 preparation. Two-year R21 period of performance.

Budget Justification (modular R21 sketch)

[ILLUSTRATIVE] This R21 requests modular direct costs of **$275,000 total over two years** (e.g., $150,000 year 1 / $125,000 year 2), within R21 limits. **Personnel:** PI (2.4 calendar months/yr) for scientific direction; a postdoctoral fellow (12 months/yr) for phage genomics, kinetics, and biofilm assays; partial technician effort for host-range screening and animal work. **Supplies:** bacterial culture and clinical-isolate panel maintenance, phage purification/endotoxin removal, sequencing reagents, biofilm consumables, and antibiotics for PAS. **Animals:** murine pneumonia model costs (per-diem, husbandry) in Aim 3. **Other:** sequencing core fees and limited publication costs. No major equipment is requested. Modular budgeting (no detailed line items beyond modules) is appropriate for the R21.

Vertebrate Animals

Aim 3 uses a murine *S. maltophilia* pneumonia model. All procedures will follow an IACUC-approved protocol with humane endpoints, appropriate anesthesia/analgesia, and the minimum number of animals required for statistical power [ILLUSTRATIVE: n≈10–12/group], consistent with prior models demonstrating survival and pulmonary CFU endpoints (Yang et al., 2026). Endotoxin-minimized phage preparations will be used to limit confounding inflammation.

Human Subjects / Clinical Trial

Not applicable to this R21. No human subjects are enrolled and this is not a clinical trial. The work is explicitly preclinical and designed to generate the safety/efficacy package that would support a future FDA expanded-access/emergency investigational new drug (eIND) application and IRB-approved personalized salvage protocol—mirroring how academic centers (TAILOR/Baylor; UCSD IPATH) currently deliver *S. maltophilia* phage under compassionate use (Cullen et al., 2024). Such regulatory and IRB steps are deferred to subsequent clinical-stage funding.

Team & Environment

The project draws on the established U.S. phage-therapy ecosystem: the TAILOR program (Baylor College of Medicine, Houston), which executed the documented *S. maltophilia* ECMO case (Cullen et al., 2024); the UCSD Center for Innovative Phage Applications and Therapeutics (IPATH); and the Whiteson Lab (UC Irvine), whose three-phage cocktail work (Monsibais et al., 2025) underpins the kinetic-design strategy. The Dennis Lab (University of Alberta) provides deep *S. maltophilia* phage biology (McCutcheon & Dennis, 2021), and the Stanford Bollyky Lab contributes phage immunology/clinical collaboration expertise (co-author on Cullen et al., 2024). The host institution provides BSL-2 microbiology, genomics/sequencing cores, an AAALAC-accredited animal facility, and access to curated phage banks and clinical-isolate collections required for Aims 1–3.

References

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