Source-Directed, pH-Triggered Bacteriophage to Prevent *Proteus mirabilis* Struvite Encrustation and Catheter Blockage

Project Summary / Abstract

Proteus mirabilis is the leading cause of infection-type (struvite/carbonate-apatite) urinary stones and of the encrustation and blockage that disable indwelling catheters in long-term catheterized patients. Its potent urease hydrolyzes urea, alkalinizing urine and driving precipitation of struvite and apatite crystals within a dense crystalline biofilm that occludes the catheter lumen and nucleates recurrent stones. These crystalline biofilms tolerate antibiotics and evade host immunity, so antimicrobials and catheter exchange remain stopgaps rather than solutions. Lytic bacteriophages are mechanistically matched to this problem: they self-amplify where their host is densest, kill antibiotic-tolerant biofilm cells independently of antibiotic-resistance status, and—by depleting the urease-producing population—act *upstream* of crystal formation, lowering urine pH at its source. Foundational work shows that phage can prevent encrustation and blockage of catheters by *P. mirabilis* in physiologically realistic bladder models (Nzakizwanayo 2016) and that a pH-responsive coating can release phage on cue as urine alkalinizes (Milo 2017); complementary studies define multi-phage cocktails that broaden host range and suppress resistance against *P. mirabilis* (Melo 2016; Mirzaei 2022). Yet the evidence base is entirely preclinical and largely non-US, and no indication-specific package integrates a contemporary US-relevant cocktail with smart delivery and a defined dosing window. This exploratory R21 will (Aim 1) assemble and genome-characterize a US-relevant, broad-host-range anti-*Proteus* cocktail and benchmark its suppression of urease, pH rise, and crystalline biofilm; (Aim 2) optimize a pH-triggered phage-release catheter coating and test it in closed-drainage bladder models; and (Aim 3) define the early-infection dosing window that governs prevention of blockage. Each aim carries a quantitative go/no-go milestone, and a reference cocktail de-risks Aims 2–3 against Aim 1. Outcomes will furnish the rigorous, US-relevant preclinical foundation required to justify a future IND-/eIND-enabled first-in-human study and would reposition catheter blockage from an expected complication to a preventable event.

Specific Aims

Catheter-associated *P. mirabilis* colonization predictably progresses to encrustation, luminal blockage, urinary retention, reflux, pyelonephritis, and urosepsis, and the same urease-driven chemistry seeds recurrent struvite stones after surgical clearance. Antibiotics fail because the crystalline biofilm is metabolically tolerant and physically shielded. Lytic phage can prevent encrustation and blockage of catheters by *P. mirabilis* in realistic bladder models, with the greatest benefit when delivered at early colonization (Nzakizwanayo 2016), and a pH-responsive coating can release phage as urine alkalinizes (Milo 2017). Defined cocktails broaden host coverage and suppress resistance against this species (Melo 2016; Mirzaei 2022). What does not yet exist is an indication-specific, US-relevant package linking a contemporary cocktail to smart delivery and a quantified dosing window. We will build that package.

Aim 1 — Assemble and characterize a US-relevant, broad-host-range anti-*Proteus* cocktail. Against a panel of contemporary US clinical *P. mirabilis* isolates we will curate lytic phages, define host range (spot assay, efficiency of plating) and lytic kinetics, screen genomes to exclude lysogeny/toxin/antibiotic-resistance determinants, and down-select a complementary, resistance-suppressing cocktail. Efficacy will be judged not by killing alone but by suppression of **urease activity, urine-pH rise, and crystalline-biofilm mass** in artificial urine, extending the cocktail logic of Melo (2016) and Mirzaei (2022). *Go/no-go:* a defined cocktail covering a majority of panel isolates [ILLUSTRATIVE: ≥70%] and suppressing pH rise and biofilm mass versus the best single phage by a pre-specified margin.

Aim 2 — Optimize a pH-triggered phage-release catheter coating. We will refine a coating that is inert at physiologic urine pH and releases a phage burst as pH rises with incipient colonization, extending the pH-triggered principle of Milo (2017). We will optimize phage loading, trigger threshold, release kinetics, retained infectivity, and coating integrity, then test top formulations on catheter segments in **closed-drainage bladder models**, with time-to-blockage and encrustation as primary readouts. *Go/no-go:* a formulation that is stable at physiologic pH, releases infective phage upon alkalinization, and significantly prolongs time-to-blockage versus uncoated and non-triggered controls.

Aim 3 — Define the early-infection dosing window. In closed-drainage models we will deliver the cocktail (instillation and via the Aim 2 coating) at staged time points spanning early colonization to established infection, mapping how timing governs prevention of blockage and measuring pH, urease, and biofilm, building on the timing dependence reported by Nzakizwanayo (2016). As a clearly *exploratory* extension we will pilot phage–antibiotic pairing for additive crystalline-biofilm control. *Go/no-go:* a quantified window confirming maximal benefit at early colonization, sufficient to specify a future clinical dosing protocol.

De-risking. Aims 2–3 use a validated **reference cocktail** as a fallback so they are not contingent on Aim 1 fully succeeding; the three aims are designed to be independently informative.

Impact. This project would deliver the first indication-specific, US-relevant preclinical foundation for a smart phage-releasing catheter and bladder-instillation strategy, directly enabling a future trial to prevent catheter blockage and struvite-stone recurrence.

Significance

Infection-type stones and catheter encrustation share one driver: urease-producing *P. mirabilis*. Urea hydrolysis alkalinizes urine and precipitates struvite and carbonate-apatite, which lock into a crystalline biofilm. In long-term catheterized patients this biofilm occludes the lumen, causing blockage, retention, reflux, pyelonephritis, and urosepsis; the same chemistry nucleates and regrows infection stones that recur after lithotomy. Because the crystalline biofilm is antibiotic-tolerant and immunologically shielded, antimicrobial therapy and scheduled catheter exchange manage but do not prevent the problem. This sits squarely within NIDDK's benign-urology and urinary-stone mission: preventing urease-driven infection stones, the recurrent obstruction they cause, and the repeat instrumentation and stone surgery that follow addresses a high-burden, underserved problem in a population—long-term catheterized and stone-forming patients—that is disproportionately elderly, disabled, and medically complex.

Lytic phage is mechanistically matched to this gap. Phage self-amplifies where its host is densest, can be matched to the colonizing strain, and kills dormant, antibiotic-tolerant biofilm cells regardless of the bacterium's resistance profile. Critically, by depleting the urease-producing population, phage acts *upstream* of crystal formation—curtailing pH rise and struvite/apatite deposition rather than merely clearing planktonic cells. Demonstrations that phage can prevent encrustation and blockage of catheters in realistic bladder models (Nzakizwanayo 2016) and that a pH-responsive coating can release phage on cue (Milo 2017), together with defined anti-*Proteus* cocktails (Melo 2016; Mirzaei 2022), establish feasibility. What gates translation is a rigorous, US-relevant, indication-specific package addressing host-range breadth, smart-coating optimization, and the early-infection dosing window. Delivering it would justify the first dedicated clinical trial in this indication.

Innovation

This proposal advances three innovations beyond the current preclinical literature.

- **Source-directed chemistry control.** We evaluate phage efficacy explicitly as suppression of urease activity and urine-pH rise—the proximal cause of crystal deposition—not as planktonic clearance alone. This reframes the endpoint from "kill bacteria" to "stop the chemistry that blocks catheters and builds stones."

- **A pH-responsive "smart" delivery system.** Rather than continuous elution, the coating senses the alkaline shift of incipient colonization and releases a therapeutic phage burst only when needed, conserving payload and matching dose to the early-infection window. This builds directly on the pH-triggered release principle of Milo (2017).
- **A US-relevant, broad-host-range, resistance-suppressing cocktail** assembled against contemporary domestic clinical isolates and designed for future strain-matching, extending the multi-phage strategies of Melo (2016) and Mirzaei (2022).

Consistent with the exploratory R21 mechanism, depolymerase-armed and phage–antibiotic-synergy approaches are scoped as clearly labeled extensions to inform later engineered-phage development, not as core deliverables. The exploratory scope is appropriate: the biology is promising, but the indication-specific, delivery-integrated, US-relevant package does not yet exist.

Approach

Rigor and Reproducibility (applies to all aims)

All quantitative endpoints (viable counts, urease activity, pH, crystalline-biofilm mass) use pre-specified ≥3 independent biological replicates with technical replicates, defined positive/negative and vehicle controls, and pre-registered analysis plans with effect sizes and confidence intervals rather than significance alone. Crystalline-biofilm and encrustation scoring in bladder models will be performed by an assessor blinded to treatment arm, with standardized inocula and randomized model assignment. **Key resource authentication:** clinical isolates will be species-confirmed and genotyped; phage stocks will be sequence-verified, purity- and endotoxin-checked, and titer-standardized before use. Artificial-urine composition and bladder-model flow parameters will be documented to support reproducibility.

Aim 1 — Assemble and characterize a US-relevant, broad-host-range anti-*Proteus* cocktail

Rationale. Cocktails broaden host range across clinical isolates and suppress resistance in *P. mirabilis* (Melo 2016; Mirzaei 2022). For struvite disease, efficacy should be judged by suppression of urease, pH, and crystalline biofilm—not killing alone.

Design. We will bank a panel of contemporary, de-identified US clinical *P. mirabilis* isolates [ILLUSTRATIVE: ~30 isolates spanning catheter-associated and stone sources] and curate lytic phages from environmental sources and existing collections. Each phage is characterized for host range (spot, efficiency of plating), one-step growth/lytic kinetics, and whole-genome screening to exclude integrase/lysogeny, toxin, and antibiotic-resistance genes. We down-select a complementary cocktail [ILLUSTRATIVE: 3 phages] maximizing coverage and resistance suppression, using time-kill assays with explicit monitoring for resistant regrowth and receptor-diversity selection. In artificial urine we quantify viable counts, urease activity, pH trajectory, and crystalline-biofilm mass/architecture.

Expected outcomes. A defined, genome-characterized cocktail covering a majority of US isolates [ILLUSTRATIVE: ≥70%] with demonstrable suppression of urease, pH rise, and biofilm versus the best single phage.

Pitfalls & alternatives. If resistance emerges, we reformulate with phages using distinct receptors and scope depolymerase-bearing candidates. If host-range gaps persist, we expand isolation and incorporate complementary phages from partner collections.

Aim 2 — Optimize a pH-triggered phage-release catheter coating

Rationale. Delivery is decisive. A coating that releases phage as urine alkalinizes matches dose to incipient colonization (Milo 2017); the cocktail from Aim 1—or the reference cocktail—provides the payload.

Design. Onto clinical-grade catheter segments we apply a pH-responsive layer loaded with the cocktail, varying polymer chemistry, phage loading, trigger threshold (targeting the alkaline shift of early colonization), and protective excipients. We characterize release kinetics across pH, retained phage infectivity over storage, and coating integrity. Top formulations advance to closed-drainage bladder models seeded with *P. mirabilis*; primary readouts are time-to-blockage, encrustation, and crystalline-biofilm burden against uncoated and non-triggered controls.

Expected outcomes. A coating inert at physiologic pH that releases an effective phage burst on alkalinization, prolonging time-to-blockage and reducing encrustation relative to controls.

Pitfalls & alternatives. If phage titer falls during coating/storage, we optimize excipients and lyophilization-compatible processing and define shelf-life. If pH-triggered release is too slow, we tune polymer composition or adopt a hydrogel reservoir providing a low-level release floor plus a pH-triggered burst.

Aim 3 — Define the early-infection dosing window

Rationale. Benefit depends on timing: phage prevents blockage most effectively at early colonization and only delays it once infection is established (Nzakizwanayo 2016). Defining this window is essential for a clinical protocol.

Design. In closed-drainage models we deliver the cocktail (instillation and via coating) at staged time points from early colonization to established infection, measuring time-to-blockage, pH, urease, and biofilm. We assess durability across multiple isolates and repeated/maintenance dosing. As an *exploratory* extension, we pilot phage–antibiotic pairing for additive crystalline-biofilm reduction.

Expected outcomes. A quantified dosing window confirming maximal benefit at early colonization, durability data across isolates, and preliminary combination signal—directly informing trial design.

Pitfalls & alternatives. If the window proves narrow, the pH-triggered coating (Aim 2) is the mitigation because it senses colonization automatically; instillation can be reserved for established stones. Model variability is addressed by adequate replication and standardized inocula.

Timeline

[ILLUSTRATIVE] **Months 1–9:** isolate banking; phage curation, genomics, and cocktail down-selection (Aim 1); reference-cocktail validation to de-risk Aims 2–3. **Months 6–15:** urease/pH/biofilm assays (Aim 1); coating formulation and release characterization (Aim 2). **Months 12–21:** bladder-model testing of coatings (Aim 2). **Months 15–24:** dosing-window, durability, and exploratory combination studies (Aim 3); integration toward an IND-/eIND-enabling package. Go/no-go milestones are evaluated at Months 9 (Aim 1), 15 (Aim 2 formulation), and 21 (Aim 2 in-model).

Budget Justification (modular)

[ILLUSTRATIVE] Requested as an R21 over two years within the modular direct-cost cap. **Personnel:** PI (urology/medical microbiology) [ILLUSTRATIVE ~1.8 cal months]; Co-I biomaterials/coating engineer; one postdoctoral phage biologist; one research technician (microbiology/bladder models). **Supplies:** de-identified clinical isolates and biobanking; phage isolation, purification, endotoxin testing, and whole-genome sequencing; artificial urine, clinical-grade catheters, coating polymers/excipients; closed-drainage bladder-model consumables. **Other:** sequencing/microscopy core fees; biosafety and regulatory-readiness consultation. No major equipment requested. Indirect costs per the institutional negotiated rate. Total direct costs within the R21 cap [ILLUSTRATIVE].

Vertebrate Animals

Not applicable. All studies use *in vitro* assays and physiologically realistic closed-drainage bladder models; no vertebrate animals are proposed. Should later work require *in vivo* models, a separate Vertebrate Animals section and IACUC approval would be obtained.

Human Subjects / Clinical Trial

This R21 is exclusively preclinical (*in vitro* and bladder-model) and is not expected to constitute human-subjects research: only de-identified, banked clinical *P. mirabilis* isolates are used, obtained under appropriate institutional biospecimen approvals/exemptions, with no intervention on or identifiable data from human participants. For completeness, the translational path: a future first-in-human study of investigational phage in this indication would proceed in the US under an FDA investigational new drug application—including the emergency/expanded-access IND (eIND) route for individual patients with refractory catheter blockage or infection stones—with IRB oversight, informed consent, and strain-matching of the cocktail to each patient's isolate. This R21 is designed to generate the characterization, delivery, and dosing-window data required to support such an application.

Investigators, Team, and Environment

This interdisciplinary project will be led by a US-based team; roles below are templates to be filled with named investigators and institutions.

- **Principal Investigator [NAME, INSTITUTION]** — urologist or medical microbiologist with expertise in catheter-associated UTI, infection stones, and bladder-model systems; overall direction and Aims 1/3.
- **Co-Investigator, Biomaterials/Coating [NAME, INSTITUTION]** — polymer/biomaterials engineer leading pH-responsive coating development (Aim 2).
- **Co-Investigator, Phage Biology [NAME, INSTITUTION]** — bacteriophage isolation, genomics, purification, and cocktail design.
- **Key collaborators [NAME, INSTITUTION]** — a US clinical microbiology laboratory providing contemporary de-identified isolate banking, and a regulatory consultant for IND/eIND strategy. The international groups that established this field (in the UK, Portugal, and Belgium) inform the evidence base and may serve as scientific advisors, but the core team and isolate sourcing are US-based to ensure domestic relevance.
- **Environment** — institutional microbiology and biomaterials laboratories with BSL-2 capacity, sequencing and microscopy cores, and established closed-drainage bladder-model capability sufficient to execute all aims.

Fit to NIDDK is direct: the proposal targets urease-driven infection stones and catheter encrustation within NIDDK's benign-urology and urinary-stone-disease mission. (Anti-infective and antimicrobial-resistance dimensions are noted only as secondary context.)

References

1. Nzakizwanayo J, Hanin A, Alves DR, et al. Bacteriophage Can Prevent Encrustation and Blockage of Urinary Catheters by *Proteus mirabilis*. *Antimicrob Agents Chemother.* 2016;60(3):1530–1536. https://pubmed.ncbi.nlm.nih.gov/26711744/
2. Milo S, Hathaway H, Nzakizwanayo J, et al. Prevention of encrustation and blockage of urinary catheters by *Proteus mirabilis* via pH-triggered release of bacteriophage. *J Mater Chem B.* 2017;5(27):5403–5411. https://doi.org/10.1039/c7tb01302g
3. Melo LDR, Veiga P, Cerca N, et al. Development of a Phage Cocktail to Control *Proteus mirabilis* Catheter-associated Urinary Tract Infections. *Front Microbiol.* 2016;7:1024. https://pubmed.ncbi.nlm.nih.gov/27446059/
4. Mirzaei A, Wagemans J, Nasr Esfahani B, Lavigne R, Moghim S. A Phage Cocktail To Control Surface Colonization by *Proteus mirabilis* in Catheter-Associated Urinary Tract Infections. *Microbiol Spectr.* 2022;10(5):e0209222. https://doi.org/10.1128/spectrum.02092-22