

Thermoresponsive Virus-Based Separation of Heavy Rare-Earth Elements

Christine Yuna Jang¹, Taehun Kim^{1,2}, Seung-Wuk Lee^{1,2}

¹ University of California, Berkeley, Berkeley, California, United States

² Biological Systems and Engineering, Lawrence Berkeley National Laboratory, Berkeley, California, United States



Abstract

Rare-earth elements (REEs) are essential for clean energy, defense, and advanced electronics, but their separation is challenging because of their nearly identical chemical properties. We engineered a bifunctional bacteriophage, LBPh-ELP, displaying a lanthanide-binding peptide (LBP) and a thermoresponsive elastin-like peptide (ELP) on its pVIII coat protein. LBP selectively captures heavy REEs, while ELP enables temperature-triggered phase separation for mild aqueous recovery. REEs are released by pH adjustment, allowing phage regeneration and reuse. This recyclable platform provides a sustainable alternative to conventional solvent extraction.

Background

Why REEs matter

Heavy REEs (Dy, Tb, Lu) are critical for clean energy and advanced electronics but are difficult to separate because of their nearly identical chemical properties.

Why current refining is challenging

Conventional refining requires acid leaching and multistage solvent extraction, generating hazardous waste and consuming large amounts of chemicals.

Why bacteriophage?

M13 bacteriophage enables high-density peptide display on pVIII coat proteins, providing a sustainable platform for selective REE recovery.

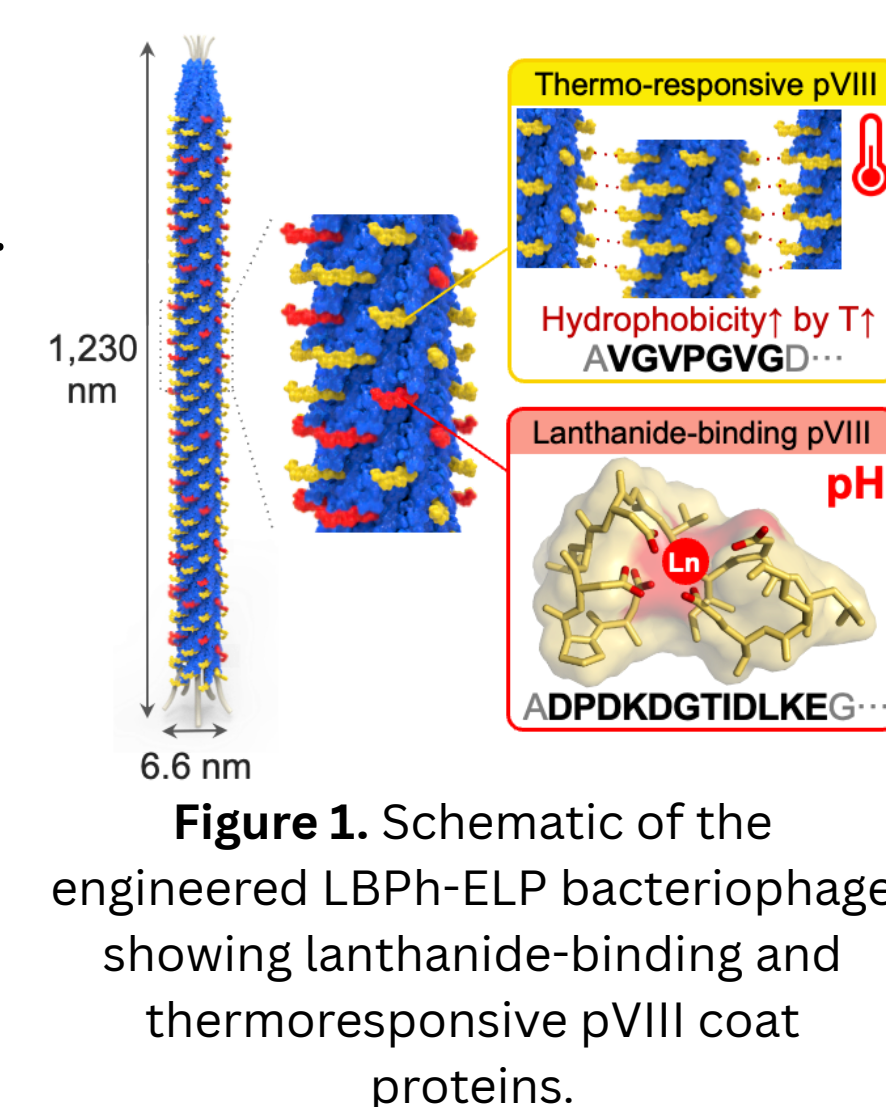


Figure 1. Schematic of the engineered LBPh-ELP bacteriophage showing lanthanide-binding and thermoresponsive pVIII coat proteins.

Methodology

1. Genetic Engineering & Sequence Verification

The lanthanide-binding peptide (LBP) and thermoresponsive elastin-like peptide (ELP) were genetically fused to the N-terminus of the pVIII major coat protein of filamentous M13 bacteriophage. Following phage amplification, DNA was extracted and sequenced to verify successful insertion of both peptide-encoding sequences and confirm the correct construct prior to downstream experiments.

2. Phage Amplification

The engineered LBPh-ELP bacteriophage was amplified by infecting Escherichia coli host cells and incubating overnight with shaking at 37°C. During bacterial growth, filamentous phages were continuously secreted into the culture supernatant without lysing the host cells.

3. Phage Purification

Bacterial cells were removed by centrifugation, and the phage-containing supernatant was collected. Phage particles were precipitated using polyethylene glycol (PEG)/NaCl and recovered by centrifugation. The purified phage pellet was resuspended in TBS buffer to obtain purified LBPh-ELP for subsequent characterization and precipitation experiments.

4. Thermoresponsive Phase Separation

Purified LBPh-ELP samples were diluted to 100 µg/mL (500 µL total volume). Ammonium sulfate was added to final concentrations of 2, 5, 20, 50, and 200 mM to evaluate salt-dependent phase transition, followed by optimization at 500 mM. Samples were incubated overnight at 50°C to induce ELP-mediated thermoresponsive aggregation. After incubation, precipitated phage was separated from the supernatant by centrifugation.

5. Concentration Measurement

The concentration of purified and thermoresponsively concentrated phage samples was determined using UV-Vis spectroscopy. Measurements were used to evaluate phage recovery after purification and phase separation.

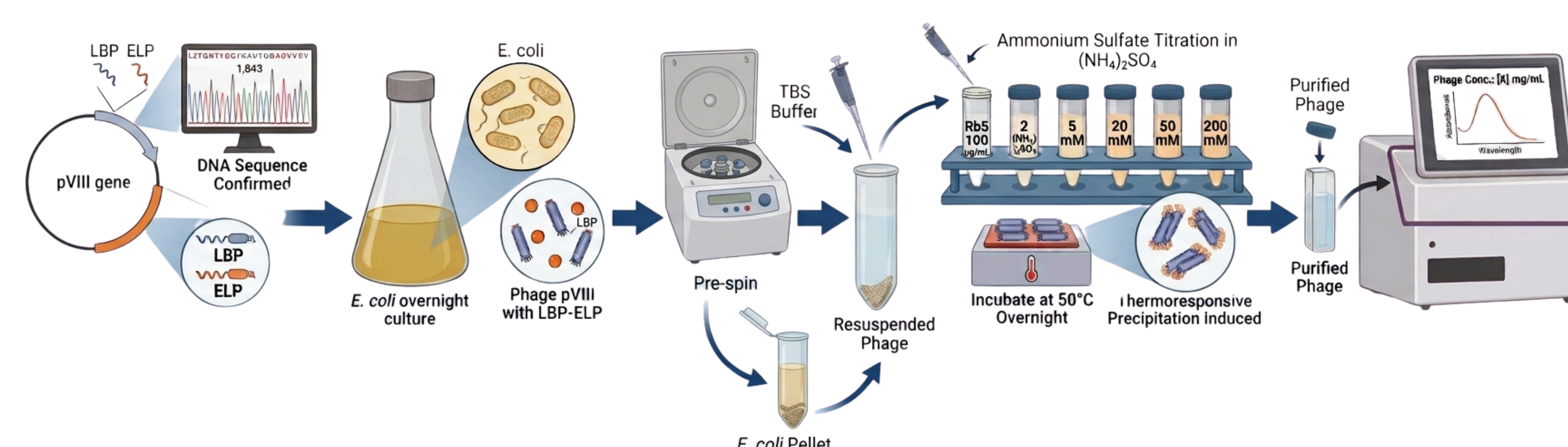


Figure 2. Methodology for Phage Engineering, Purification, and Thermoresponsive Concentration.

Results

Sequence verification of engineered peptide motifs

Sanger sequencing confirmed successful insertion of the lanthanide-binding peptide (LBP) and elastin-like peptide (ELP) into the engineered LBPh-ELP bacteriophage. Both peptide motifs (DPDKDGTID and VPGVG) were detected at the expected positions.

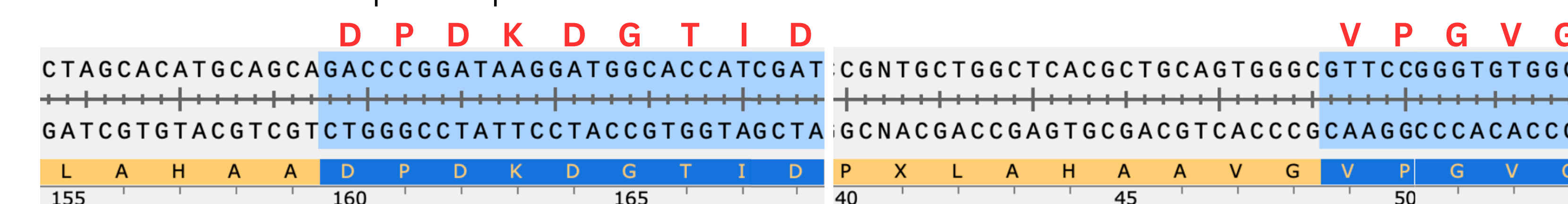


Figure 3. Sequence verification of engineered peptide motifs. (a) Identification of the lanthanide-binding peptide (LBP) motif, DPDKDGTID. (b) Identification of the elastin-like peptide (ELP) motif, VPGVG, confirming successful genetic insertion into the engineered LBPh-ELP phage.

Ammonium sulfate precipitation and recovery of LBPh-ELP

Ammonium sulfate was added to purified LBPh-ELP samples to induce precipitation through the salting-out effect. Following overnight incubation, visible precipitates were observed, confirming successful aggregation of the engineered phage. The precipitated phage was resuspended, and recovery was quantified by UV-Vis spectroscopy. Recovery (%) was calculated relative to the initial phage concentration (0.208 mg/mL) measured before ammonium sulfate treatment. Recovery increased with ammonium sulfate concentration, reaching 56%, 69%, 63%, 88%, and 88% at 2, 5, 20, 50, and 200 mM, respectively. The highest recovery was obtained at 50–200 mM, indicating more efficient precipitation of LBPh-ELP under these conditions.

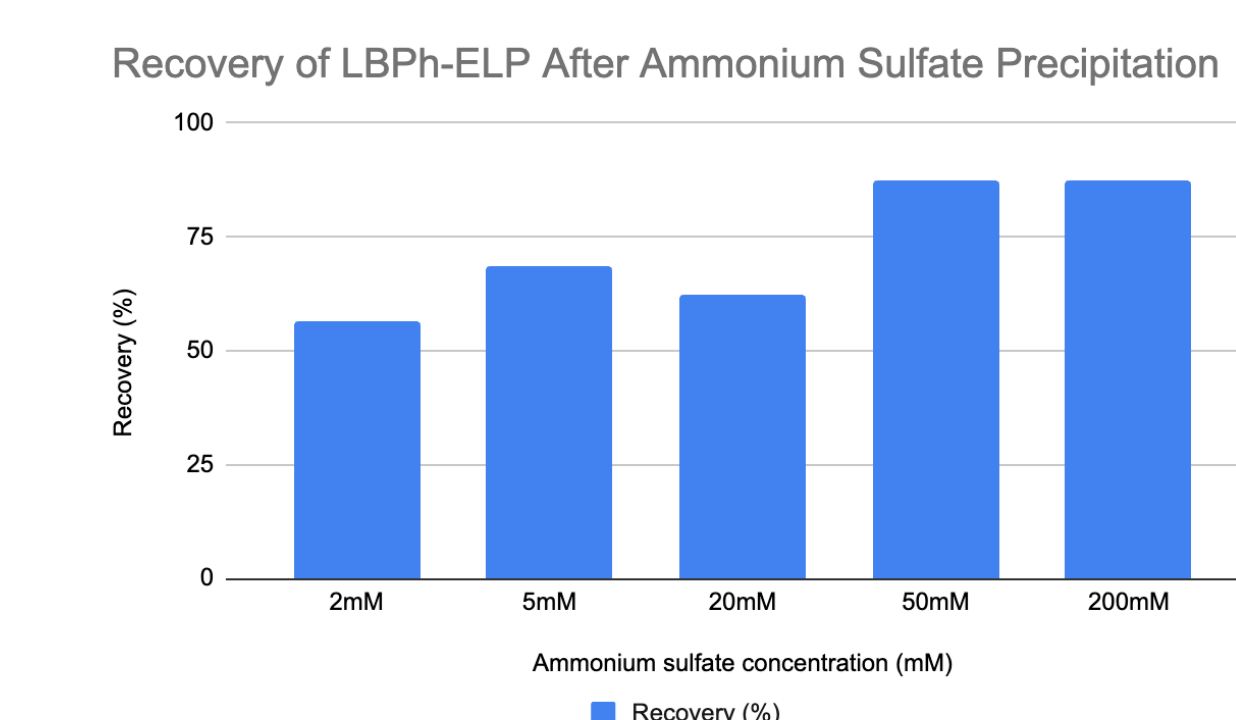
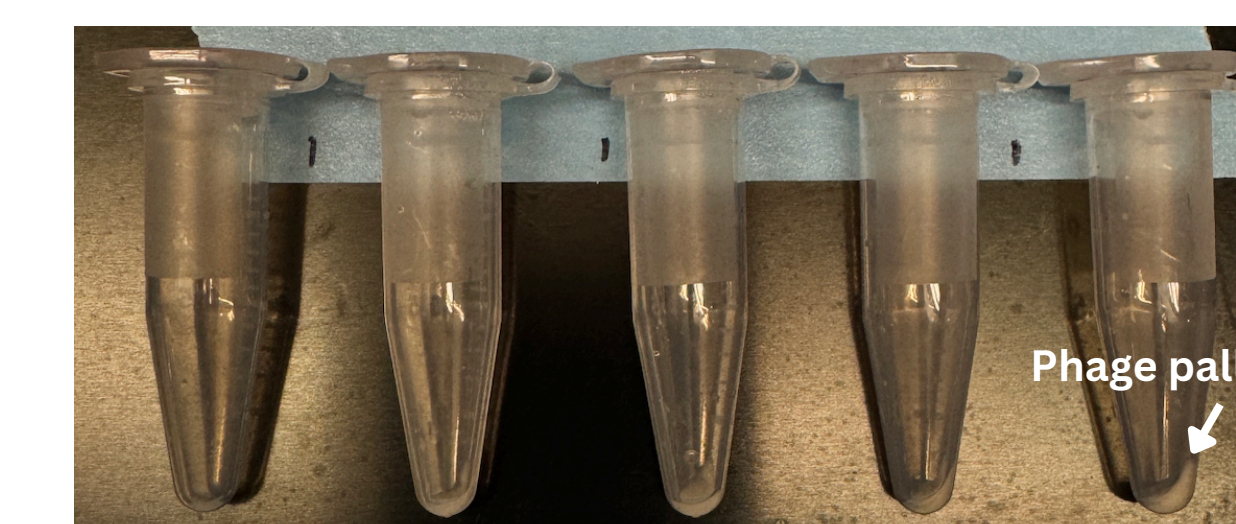


Figure 4. Ammonium sulfate precipitation and recovery of LBPh-ELP. (a) Visible precipitation after treatment with 2, 5, 20, 50, and 200 mM ammonium sulfate. (b) Recovery of resuspended LBPh-ELP, calculated relative to the initial phage concentration (0.208 mg/mL).

Conclusion & Future Work

The engineered LBPh-ELP bacteriophage was successfully constructed and verified by Sanger sequencing. A reproducible purification and ammonium sulfate precipitation workflow was established, enabling phage recovery and quantification by UV-Vis spectroscopy. Future work will optimize LBPh-ELP production and precipitation conditions to improve yield and recovery, followed by evaluation of REE binding and selective separation.

References

- [1] Chae I. et al. Virus-Based Thermoresponsive Separation of Rare-Earth Elements. Nano Lett. 2025, 25, 15754–15760.
- [2] Chae I. et al. Supporting Information for Virus-Based Thermoresponsive Separation of Rare-Earth Elements. Nano Lett. 2025.