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Virus-Based Separation of Rare Earth Elements

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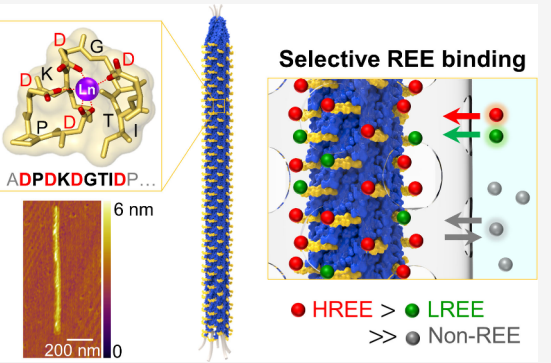
ABSTRACT: The utilization of biomaterials for the separation of rare earth elements (REEs) has attracted considerable interest due to their inherent advantages, including diverse molecular structures for selective binding and the use of eco-friendly materials for sustainable systems. We present a pioneering methodology for developing a safe virus to selectively bind REEs and facilitate their release through pH modulation. We engineered the major coat protein of M13 bacteriophage (phage) to incorporate a lanthanide-binding peptide. The engineered lanthanide-binding phage (LBPh), presenting ∼3300 copies of the peptide, serves as an effective biological template for REE separation. Our findings demonstrate the LBPh's preferential binding for heavy REEs over light REEs. Moreover, the LBPh exhibits remarkable robustness with excellent recyclability and stability across multiple cycles of separations. This study underscores the potential of genetically integrating virus templates with selective binding motifs for REE separation, offering a promising avenue for environmentally friendly and energy-efficient separation processes.



KEYWORDS: *Rare earth elements, lanthanide-binding phage, biological template, sustainable system*

Rare earth elements (REEs), particularly lanthanides, are in significant global demand due to their pivotal role in modern technologies, such as permanent magnets, batteries, lasers and displays.[1,2] The extraction and processing of REEs are restricted in many locations due to environmental issues mainly associated with the separation of REEs.[3−5] The lanthanides are all chemically similar, having the same outer electronic structure, differing only in their 4f orbitals.[6] As a result, they occur together geologically and require multiple stages of separations, first to separate the REEs from other, more abundant cations found in REE ores, and second to isolate one lanthanide from the others.[7] Methods such as ion exchange and solvent extraction using synthetic reactants, organic solvents, and stripping agents have potentially adverse environmental and health impacts and may not be effective for separations from associated impurities such as thorium and uranium.[7−9] To establish a robust supply chain for REEs to support a sustainable future, we need to develop environmentally benign and energy-efficient separation systems.

The integration of biological ligands into REE separations has received considerable attention due to the high selectivity of certain bioderived molecular coordinating agents for capturing REEs, and the potential to deploy them in environmentally friendly processes.[10−16] Relatively short peptides exhibiting strong binding affinities for REEs have been identified through high-throughput screening and analysis of naturally evolving microorganisms. For example, the peptides referred to as lanthanide-binding tag[17,18] and lanthanide ion mineralization peptide[19] were discovered through high-throughput screening using peptide and phage library screening processes, respectively. Also, Cotruvo et al. elucidated four 12mer-amino acid sequences showing picomolar binding affinities for trivalent lanthanide cations (denoted as Ln^{3+}) by analyzing proteins from *Methylobacterium extorquens* (*Mex*), a naturally occurring lanthanide-utilizing bacterium.[20−22] They named the protein Lanmodulin (LanM), and it has shown the strongest binding affinity for lanthanides to date.[20,22] These discoveries offer valuable biological codes for selectively capturing lanthanides over other metal ions, thereby facilitating the advancement of biomaterial-based, sustainable processes that can separate REEs from other cations. The next phase involves engineering the unique peptide sequences into biological templates that can be used for resilient separation via biosorption on a large scale.

The M13 bacteriophage (phage) is a filamentous bacterial virus that infects bacterial host cells. It is nonharmful and benign to humans. It presents various advantageous structural features as a smart biological template for chemical separation. It can self-replicate through bacterial host infection, which holds the potential for generating enough material for commercial scale operations. Phages replicate millions of

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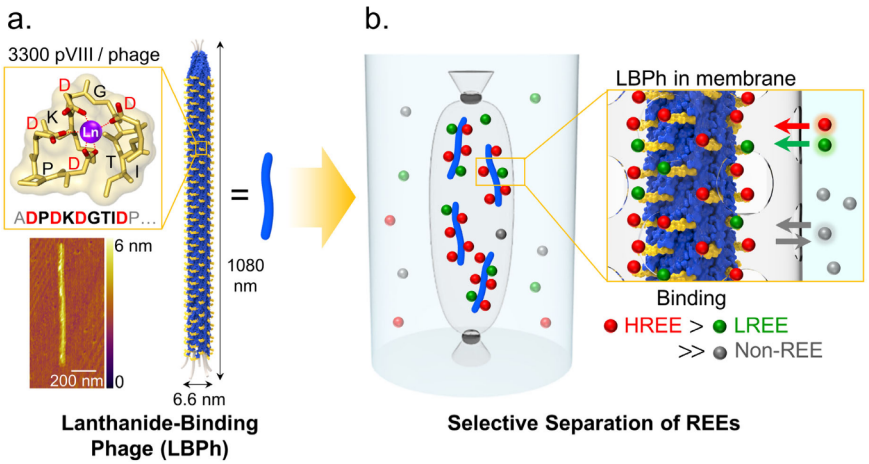

Figure 1. Schematics of genetically engineered LBPh and REE separation. (a) Engineered LBPh coated with ~3300 pVIII proteins and its topographic image. Each pVIII has the short EF1 peptide of *Mex*-LanM (DPDKDGTID; red: negatively charged amino acid) at the N-terminal. (b) Selective separation of REEs using LBPh. The LBPh inside the dialysis membrane selectively adsorbs REEs over non-REE cations, with greater uptake of HREEs than LREEs.

times within 4.5 h through infection of *Escherichia coli* (*E. coli*) host cells.[23] Wild-type (WT) phage has a single-stranded DNA encapsulated within ~2700 copies of the pVIII major coat protein along the 880 nm long and 6.6 nm thick individual unit.[24] Additionally, pIII and pIX minor proteins can be further engineered with additional functions. Genetic engineering of phage DNA enables modification of the desired peptide sequences on their coat proteins, thereby providing abundant binding sites for target species. Previously, engineered M13 phages have shown versatile ability to recognize inorganic, organic, and biomolecules.[25−30] In addition, phages exhibit high stability in aqueous solutions across a wide range of pH, temperature, and ionic strength, rendering them suitable for industrial applications.[31,32] Leveraging these unique advantages, M13 phage shows potential as a biological template for programming REE-binding peptides, thereby facilitating the development of a sustainable REE separation process.

In this study, we present the M13 phage as a novel biological template for the selective adsorption and separation of REEs. Through genetic engineering, we fused a lanthanide-binding peptide derived from LanM at the N-terminal region of pVIII major coat proteins to construct lanthanide-binding phage (LBPh; Figure 1). Circular dichroism (CD) and inductively coupled plasma optical emission spectroscopy (ICP-OES) analyses show that LBPh exhibits stronger binding affinities for heavy REEs (HREEs) than light REEs (LREEs). By separating the REE-enriched phage and then reducing the pH, we can release adsorbed REEs from the phage, thereby separating the REEs from the mixtures in which they were originally present. Our results demonstrate that the efficacy of LBPh in separating REEs remains consistent over multiple separation cycles. After multiple rounds of pH cycles and solution processing, the LBPh remains stable, demonstrating robust structural integrity. Furthermore, when we contacted LBPh with the acidic leachate of allanite ore, the LBPh was able to separate REEs from non-REEs. Our novel approach to exploiting viral particles as a template for selective REE adsorption holds great promise for the development of a large scale, sustainable separation process for REEs.

We genetically engineered phage with a lanthanide-binding peptide motif, the EF1 segment of *Mex*-LanM[20] inserted into the N-terminal of pVIII proteins (LBPh, Figure 1). The REE binding peptide segment (DPDKDGTID) originates from one of four REE binding motifs (EF1: DPDKDGTIDLKE) of LanM, which has been reported as the strongest lanthanide-binding protein identified to date.[20,22] The strong binding affinity for Ln^{3+} in an aqueous solution has been attributed to the molecular coordinates of negatively charged Asp (D) and a single Pro (P) at the second position.[33] The resulting LBPh measures 1080 ± 110 nm in length, corresponding to a molecular weight of 21.8 MDa. We estimated that approximately 3300 copies of the pVIII protein cover a single phage based on the length of LBPh. Owing to the large number of REE-binding motifs on its surface, LBPh is expected to exhibit a high binding capacity for REEs.

We characterized the LBPh's molecular interaction with REEs and binding capacity in an aqueous solution by observation of the secondary structure of the pVIII major coat proteins and quantitative measurement of equilibria under different conditions. The LBPh exhibits alteration in the secondary structure of pVIII major coat proteins via interaction with REEs, more markedly with HREEs than LREEs. Figure 2a-b presents the variation of CD spectra of LBPh caused by interaction with Tb^{3+} (a model HREE) and La^{3+} (a model LREE) in aqueous solution at pH 5 at different Ln^{3+}/pVIII ratios. The CD spectra for other REEs are provided in the Supporting Information (Figure S1). Because pVIII proteins of the M13 phage possess intrinsic α-helical structures, we characterized the α-helix content of phage by monitoring CD peaks through the interaction with different REEs. Above a molar ratio of Tb^{3+}/pVIII of 7.5, a notable reduction in the intensity of the α-helical peak (209 nm) is observed. The interaction with La^{3+} (LREE) was found to be less pronounced compared to Tb^{3+} (HREE), with no observable change in the pVIII secondary structure below a molar ratio of La^{3+}/pVIII of 20. Minimal change was detected with Al^{3+} (non-REE) under the same conditions, demonstrating that the alteration of LBPh-pVIII secondary structure was much less sensitive to this non-REE trivalent cation (Figure S1 in the Supporting Information).

Further investigations of the secondary structure change and dynamic light scattering (DLS) analyses of LBPh following the interaction with Ln^{3+} confirm that LBPh interacts preferentially with HREEs compared to LREEs. The reduction of the α-

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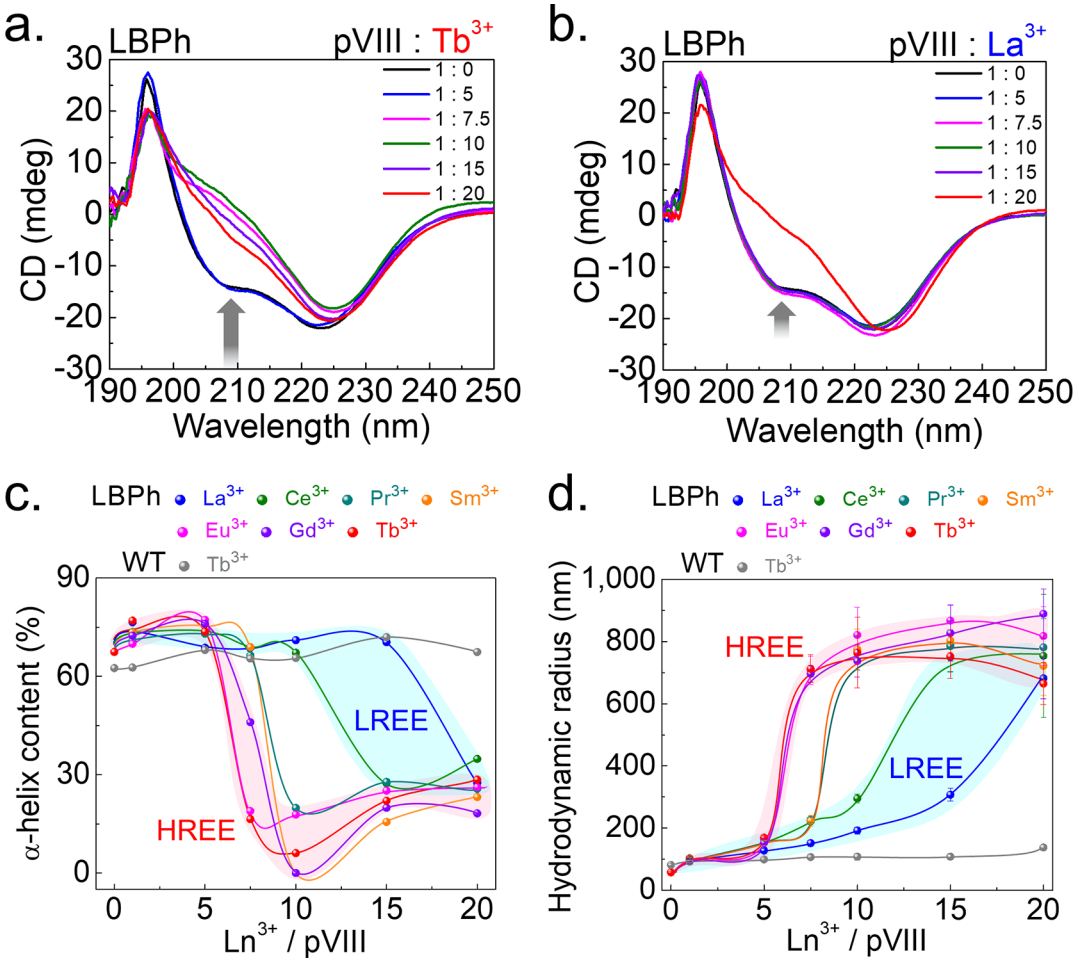

Figure 2. Secondary structure change and aggregation of LBPh induced by interaction with REEs. (a, b) The CD spectrum of LBPh, showing pVIII secondary structure changes induced by interaction with Tb^{3+} and La^{3+}, in an aqueous solution at pH 5. (c) α-helix content of LBPh and WT phage, obtained by fitting the CD spectrum, as a function of Ln^{3+}/pVIII (10 μM, phage 0.06 mg/mL) mole ratio. (d) Particle size of the phages resulting from aggregation induced by the interaction with Ln^{3+}, measured using DLS in aqueous solution.

helical structure occurs at significantly lower concentrations of HREEs (Tb^{3+}, Gd^{3+}, Eu^{3+}) than LREEs (La^{3+}, Ce^{3+}) as shown in Figure 2c. The α-helix contents were obtained by fitting CD spectra of phages between 195 and 250 nm (Figure S1 in the Supporting Information). Furthermore, DLS analyses of LBPh-Ln^{3+} show the formation of large aggregates at lower concentrations of HREEs compared to LREEs as shown in Figure 2d. The size of LBPh-Ln^{3+} complex sharply increases above the molar ratio (Ln^{3+}/pVIII) of 5 for HREEs, whereas gradual size increases are observed for LREEs at higher REE concentration ranges. These results indicate that HREEs exhibit a greater affinity than LREEs for LBPh, leading to a more sensitive conformational change in the pVIII protein (as observed in CD analysis) and a decrease in electrostatic repulsions between phages (as observed by DLS) in aqueous environments. No secondary structure change or aggregation is observed from WT phage-Ln^{3+} complexes (Figure 2c–d and Figure S2 in the Supporting Information). This demonstrates a specific interaction between LBPh and Ln^{3+} because the WT phage possesses the same net negative charge near the N-terminus of pVIII protein as LBPh (Figure S3 in the Supporting Information), but no WT-pVIII structure change occurred under identical conditions. Thus, we verified that the surface coat protein engineering of phage with *Mex*-LanM's EF1 segment enhances the phage's interaction with REEs, more preferentially with HREEs than LREEs.

It is worth noting that LBPh exhibits a different internal selectivity toward REEs compared to *Mex*-LanM. While LBPh transforms its pVIII secondary structure more sensitively with HREEs than LREEs, *Mex*-LanM transforms the structure of EF-hands more sensitively with LREEs than HREEs.[20] This variance likely stems from the inherent difference in protein conformations of LBPh-pVIII and *Mex*-LanM. LBPh-pVIII has α-helical structures whereas *Mex*-LanM has random coils in the absence of REEs in aqueous solutions. Upon introduction of REEs, LBPh-pVIII captures the REEs and the degree of α-helix in pVIII protein reduces as LBPhs aggregate in solution by the reduced electrostatic repulsion between phages. *Mex*-LanM's structural transition is the opposite, from random to helical by the interaction with REEs.

To investigate how the structural changes observed in LBPh influence the separation of REEs, we conducted a quantitative analysis of REE adsorption and desorption using LBPh. LBPh exhibits a preference for binding HREEs over LREEs (Figure 3a). REE adsorption was tested by immersing an LBPh-enclosed dialysis tube into a dialysis solvent containing 8 different REEs at pH 5. The REEs in the dialysis solvent diffused into the membrane and bound to LBPh. The decrease in REE concentrations in the solvent outside the membrane

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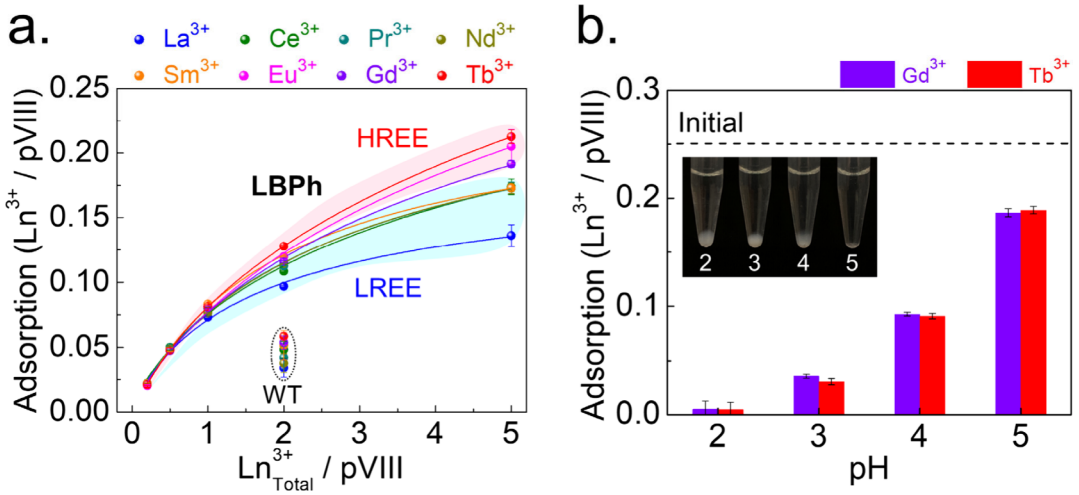

Figure 3. Adsorption of REEs to LBPh. (a) Adsorption of lanthanides onto LBPh and WT phage under varying Ln^{3+}_{Total}/pVIII mole ratios in pH 5 aqueous solution. Within a dialysis membrane, 3 mg of phage (pVIII: 0.5 μmol) in 1 mL solution is fixed, while various Ln^{3+}_{Total} concentrations (equal molar concentrations of each Ln^{3+}) in 30 mL solution outside the membrane are tested. Adsorption is indicated by the reduction in Ln^{3+}, expressed in μmol outside the membrane per 0.5 μmol of LBPh-pVIII. The mean and standard deviations were obtained from two measurements and the fitting lines were generated using the Hill Model.[37] (b) pH-dependent adsorption of Gd^{3+} and Tb^{3+} onto LBPh following the same dialysis method with 0.6 mg of LBPh (pVIII: 0.1 μmol). The dashed line (initial) indicates the initially mixed Ln^{3+}/pVIII mole ratio in the solution. The inset image shows the pH-dependent aggregations of LBPh followed by centrifugation. The mean and standard deviations were obtained from three measurements.

was measured after equilibration. The adsorption onto LBPh was quantified by difference, accounting for differences in the volumes of dialysis solvent and the dialysis tube, along with the concentration of LBPh and pVIII. Approximately 0.7 μmol of lanthanides adsorbed onto 0.5 μmol of pVIII at Ln^{3+}_{Total}/pVIII mole ratio of 5. Notably, the result shows stronger adsorption of HREEs than LREEs onto LBPh; for example, Tb^{3+} exhibits 56% more adsorption than La^{3+} at Ln^{3+}_{Total}/pVIII mole ratio of 5. This difference in adsorption between HREEs and LREEs increases with the mole ratio of Ln^{3+}_{Total}/pVIII, indicating that the preferential binding of HREEs over LREEs becomes more dominant at higher Ln^{3+}_{Total} concentrations where the different REEs compete for binding sites. The WT phage exhibits weaker REE binding compared to LBPh, as observed in Figure 3a, consistent with the CD and DLS analyses.

The LBPh demonstrates a high binding capacity for REEs (35 mg/g, REEs/phage) at Ln^{3+}_{Total}/pVIII mole ratio of 5. This binding capacity is comparable to that of Lanmodulin (40 mg/g, REEs/protein),[12] despite the significantly larger size of the engineered LBPh (21.8 MDa) compared to Lanmodulin (12.8 kDa). The large size and high binding capacity of LBPh offer several advantages for the development of efficient REE separation systems. For instance, it allows for the use of larger pore-size membranes, facilitating rapid REE separation via membrane-based methods. Also, it will reduce energy consumption for the separation of REEs from mixtures through centrifugation after binding. Several studies have reported the immobilization of REE-binding peptides onto synthetic polymers thorough cross-linking.[34−36] The genetically engineered LBPh inherently possesses ~3300 lanthanide-binding sites on its surface without requiring any chemical modification, showing potential advantages for developing REE separation systems on a larger scale.

The adsorption of Ln^{3+} with LBPh is dependent on pH (Figure 3b). Adsorption relies on Coulombic interaction between Ln^{3+} ions and recurring Asp (D) residues spaced along LBPh-pVIII. Lowering the pH increases the degree of protonation of the carboxylate groups in Asp (D), thereby weakening the Coulombic interaction between LBPh-pVIII and Ln^{3+}. The pK_a value of aspartate side chain carboxylic acid is approximately 3.9; thus, noticeable aggregation of LBPh and a decrease in the adsorption of Gd^{3+} and Tb^{3+} ions are observed below the pK_a. When we changed the pH of the dialysis solvent from 5 to 4, 3 and 2, the amount of REEs adsorbed by LBPh decreased by 50%, 80% and 97%, respectively (Figure 3b).

Although we established the pH dependence of adsorption of REEs onto LBPh, one cannot assume *a priori* that desorption is reversible, with no hysteresis, particularly given the complex structural changes associated with adsorption. Accordingly, we employed the diffusion dialysis method to quantitatively measure the desorption of bound REEs (Figure 4a and Figure S4 in the Supporting Information). We saturated LBPh at pH 5 using a dialysis solvent outside the membrane containing six metal ions [HREEs (Gd^{3+}, Tb^{3+}), LREEs (La^{3+}, Ce^{3+}) and non-REEs (Mn^{2+}, Zn^{2+}), 2.5 μM each], then transferred the dialysis tube containing the LBPh-metal complex to a fresh dialysis solvent at pH 2 to desorb the metal ions from LBPh. By measuring the concentration of metal ions outside the membrane using ICP-OES, we quantitatively analyzed the desorbed REEs. At pH 2, all the bound REEs were released. Interestingly, a higher recovery of HREEs was observed than that of LREEs; for instance, nearly twice the amount of Tb^{3+} ion was recovered compared to La^{3+}, consistent with the adsorption results. A minimal amount of non-REEs (Mn^{2+}, Zn^{2+}) was detected outside the membrane at pH 2 after the desorption, indicating their negligible adsorptions onto LBPh at pH 5. The pH-dependent adsorption/desorption is a necessary condition for application of this approach in real world conditions, but not sufficient—it would be essential that LBPh remains effective over multiple adsorption−desorption cycles, so that it mirrors the functionality of commercial solvent extraction reagents or ion exchange resins.

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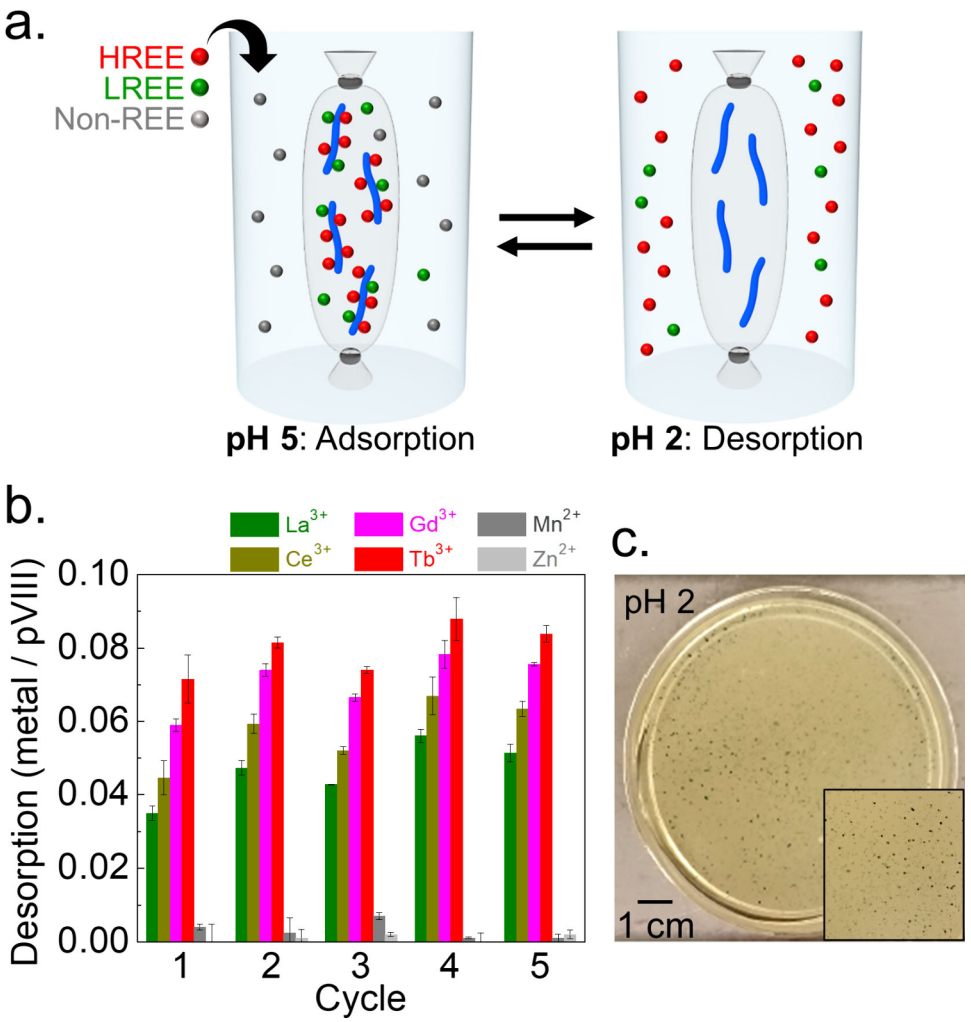

Figure 4. Recycling LBPh for REE separation. (a) Schematics of multiple REE separation cycles using LBPh within a dialysis membrane. The dialysis membrane, containing 2.5 mg of LBPh (pVIII: 0.42 μmol) in 1.4 mL solution, was submerged in the dialysis solvent containing 0.1 μmol of each La, Ce, Gd, Tb, Mn and Zn in 42 mL to initiate adsorption at pH 5. Once adsorption equilibrium was attained, the LBPh-enclosed dialysis tube was transferred to fresh solvent at pH 2 to release the bound REEs. (b) Separation of REEs from the dialysis solvent using LBPh across 5 cycles. Desorption indicates the recovered metal ions outside the membrane at pH 2, expressed in μmol per 0.42 μmol of LBPh-pVIII. The mean and standard deviations were derived from two measurements. (c) LBPh infection of *E. coli* after the lanthanide desorption at pH 2.

Figure 4b shows that LBPh does, indeed, shows consistent ability to adsorb REEs over five cycles. There was no decrease in separation efficiency, and consistent amounts of HREEs and LREEs were recovered. Furthermore, we confirmed that LBPh retained its infectivity after multiple cycles of REE solution exposure and pH changes during the adsorption and desorption process. The recovered phage exhibits blue plaque formation of XL10-Gold *E. coli* lawn on the X-Gal/IPTG plate (Figure 4c and Figure S5 in the Supporting Information) after the desorption of REEs at pH 2. The blue plaque formation and DNA sequencing confirmed the absence of degradation or mutation after separations within the pH range of 2−5, which confirms the stability of LBPh in acidic pH ranges and the REE separation process and suggests that the LBPh should retain its functionality over multiple cycles.

In summary, the pH-dependent adsorption of Ln^{3+} ions on LBPh and the efficacy over multiple cycles mirrors characteristics of cation-exchange solvent extractants and ion exchange resins, making LBPh suitable in function for separation and purification of REEs in real-world processing circuits. The precise pH values at which adsorption and desorption occur would align well with the pH values encountered using organic acids as leaching agents for rare-earth ores.

The studies described above used LBPh to adsorb REEs from solutions containing either single cations or idealized mixtures. In real-world hydrometallurgical operations, REEs must be separated from more complex solutions generated by leaching minerals, concentrates or ores. The efficacy of LBPh in more complex solutions was tested using acidic leachate from allanite, an epidote group mineral rich in REEs (La, Ce, Pr, Nd, Sm, Eu, etc.). Multiple cations are released during acid leaching of this allanite ore. Although the allanite was leached with 40% aqueous nitric acid, the leachate was diluted and neutralized to pH 5 to induce the adsorption of dissolved metal ions onto LBPh. The leachate contains varying concentrations of REEs including La, Ce, Pr, Nd, Sm and Eu, as well as a significant amount of non-REEs including Mg, Ca, Mn and Zn, as shown in Figure 5. The adsorption test, conducted using LBPh-enclosed in the dialysis membrane, shows LBPh's selective adsorption of REEs over non-REEs. Due to the selective adsorption inside the membrane, reduced concentrations of REEs were observed in the allanite leachate

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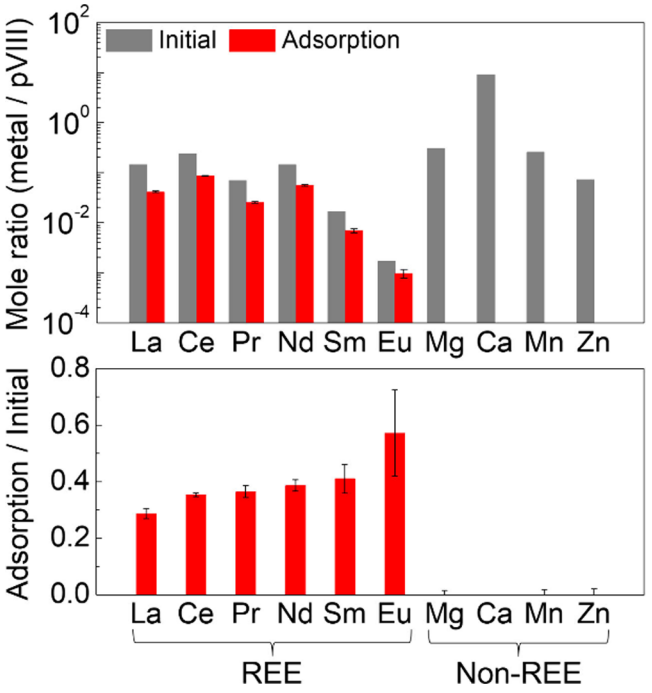

Figure 5. Selective separation of REEs from allanite ore using LBPh. Initial (gray) and adsorbed (red) mole ratios of metal/pVIII in aqueous solution at pH 5. The dialysis membrane, containing 8 mg of LBPh (pVIII: 1.33 μmol) in 1 mL solution, was submerged in the initial allanite solution containing REEs (La, Ce, Pr, Nd, Sm, Eu) and non-REEs (Mg, Ca, Mn, Zn) in 30 mL. The adsorption results reflect the reduced metal ions in the allanite leachate solution outside the dialysis membrane after the adsorption onto LBPh. The mean and standard deviations were obtained from two measurements.

solution outside the membrane. The ratio of adsorption to initial concentration of REEs shows the preferential adsorption of REEs onto LBPh compared to non-REEs. Despite the orders of magnitude higher concentration of Ca, LBPh selectively adsorbs REEs over non-REEs in the solution. Also, the normalized ratio (Adsorption/Initial) shows higher adsorption of heavier REEs (Nd, Sm, Eu) onto LBPh compared to lighter REEs (La, Ce, Pr). These results show the potential of engineered LBPh for selective separation of REEs from industrial ore, even in the presence of significantly higher concentrations of non-REEs. The phage's preferential adsorption of HREEs over LREEs, coupled with its recyclability and stability, offers promising prospects for the separation of REEs from industrial ore on a large scale.

LBPh, the newly engineered virus template, demonstrates very promising binding capacity, recyclability, and stability for selective separation of REEs in aqueous solutions. Through genetic engineering, we created a new M13 virus with the lanthanide-binding peptide from *Mex*-LanM fused into ~3300 copies of pVIII surface coat proteins. Due to its abundant binding sites and robustness across various acidic pHs, LBPh exhibits a high REE binding capacity (35 mg/g, REE/phage). We selectively separated REEs from non-REEs within allanite ore in an aqueous solution using LBPh. The modified pVIII proteins of LBPh show preferential binding to HREEs over LREEs, accompanied by alteration of their secondary structure. This demonstrates the notable potential of LBPh as a REE-binding resin in an ion exchange process for internal REE separations. Moreover, LBPh offers promise as an effective REE extracting agent, maintaining selective REE recovering performance across multiple separation cycles. With the unique characteristics of LBPh along with its inherent advantages as a viral particle, this study suggests the significant potential to develop an environmentally benign, highly efficient, and sustainable REE separation process for real-world applications.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.nanolett.4c02510.

Experimental section, genetic engineering of LBPh, full CD spectrum of phages, comparison between LBPh and WT phage, p-values, and titration (PDF)

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Author Contributions

I.C. and S.-W.L. conceived the idea. I.C. took charge of genetic engineering, amplification, and purification of phage. A.S. assisted with the amplification and purification of phage. I.C. conducted the characterization of the phage's secondary structure and hydrodynamic radius in the presence of metal ions. I.C. led the selective separation of REEs using LBPh. I.C., F.M.D., and S.-W.L. wrote the manuscript.

Notes

The authors declare no competing financial interest.

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