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Group Separation of Rare Earth Elements by Protein-Functionalized Magnetic Nanoparticles

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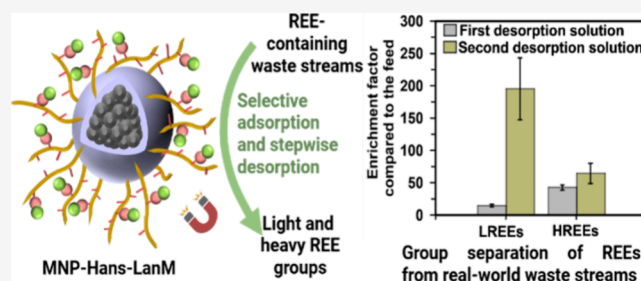
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ABSTRACT: Rare earth elements (REEs) are essential for low-carbon technologies and a wide range of consumer products, yet intra-REE separation remains a critical challenge due to their similar physicochemical properties and co-occurrence in waste streams. Here, we developed a novel biosorbent, termed MNP-Hans-LanM, by immobilizing *Hanschlegelia quercus*-derived Lanmodulin (Hans-LanM) on the surface of SpyTag-functionalized magnetic nanoparticles (MNPs) for the separation of REEs into light and heavy groups from waste streams. The biosorbent exhibited an adsorption activity of $5.6 \pm 0.8 \mu\text{mol-neodymium/g-sorbent}$, and the adsorbed REEs could be recovered with $>90\%$ efficiency. The MNP-Hans-LanM could effectively separate representative light and heavy REE pairs and maintained $>90\%$ of activity over five adsorption–desorption cycles. Importantly, when applied to a low-grade course coal refuse natural leachate, the biosorbent selectively captured REEs and enabled enrichment of bound REEs into light and heavy REE groups without the use of hazardous solvents, resulting in 195- and 64-fold enrichment, respectively, relative to the initial REE feedstock. Results from this study contribute to the development of innovative biosorptive technologies for sustainable intra-REE separation from complex waste streams.

KEYWORDS: rare earth elements, intra-REE separation, protein, nanomaterials, waste streams



1. INTRODUCTION

Rare earth elements (REEs), including lanthanide (Ln) series, scandium, and yttrium (Y), are indispensable for clean energy technologies and manufacturing consumer products (e.g., computers and smartphones).^{1,2} Each REE exhibits distinct electronic, magnetic, and optical properties, making them uniquely suitable for a wide range of industrial applications.³ For example, neodymium (Nd) is critical for producing high-performance magnets used in electric vehicles, while Y is widely used in phosphors for displaying and lighting technologies.^{4,5} While significant progress has been made in separating REEs from non-REEs (i.e., REE extraction) during REE mining and recycling,^{6–9} intra-REE separation remains a major challenge. The difficulty arises from the similar physical and chemical properties of REEs and the frequent co-occurrence in natural ores and waste streams.^{10,11} Current methods for intra-REE separation mainly rely on solvent extraction and ion exchange chromatography, both of which have some limitations. Solvent extraction involves the use of organic solvents containing selective extractants to partition REEs, but achieving high-purity intra-REE separation often requires dozens to hundreds of extraction stages, which results in a large process footprint, high capital investment, and the generation of substantial secondary liquid waste.^{12,13} Ion exchange chromatography suffers from its limited capacity

(e.g., <3 mequiv/g-resin) and relatively high operational costs.^{14,15} Furthermore, both methods are inefficient for processing low-grade and diluted REE feedstocks (REEs $< 1\%$) containing high concentrations of non-REE impurities due to limited selectivity for REEs.¹⁶ Pretreatment steps are needed to remove interfering substances before REE separation.¹⁷ Therefore, it is critically needed to develop effective and environmentally sustainable technologies for intra-REE separation from waste streams.

Protein-based biosorption has emerged as a green-chemistry alternative to conventional methods for effective REE extraction and separation due to its high affinity and selectivity, low energy/chemical requirements, and minimal waste generation. Lanmodulin, a highly selective REE-binding protein isolated from *Methylobacterium extorquens* (Mex-LanM),¹⁸ has been used for efficient REE extraction from synthetic REE-containing solutions or solid waste leachates.^{8,19–21} Recent studies further demonstrated that immobi-

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lized *Mex*-LanM on agarose beads enabled effective separation of REE pairs (e.g., Nd/Dy) and light/heavy REE groups by leveraging the protein's preferential response to light REEs (La–Gd).^{22,23} More recently, a newly identified LanM homologue, *Hans*-LanM, was isolated from *Hansschlegelia quercus* and exhibited a 10^3 – 10^6 -fold higher selectivity for REEs (apparent dissociation constant, $K_{d,app}$ in the pM to nM range) over Ca^{2+} ($K_{d,app} = 60 \mu M$), representing another highly selective REE-binding protein.²⁴ The bound REEs could be easily desorbed using a weak chelator (e.g., malonate) or an acid solution (pH < 2), enabling the efficient recovery of REEs. Notably, *Hans*-LanM demonstrated improved light/heavy REE separation due to a distinct metal coordination mechanism, wherein its oligomeric state responds sensitively to rare-earth ionic radius, compared to *Mex*-LanM.²⁴ Structure-guided mutagenesis further enhanced its performance, resulting in the *Hans*-LanM R100 K variant with greater selectivity for Nd/Dy pair separation compared to the wild type.²⁴ These properties position *Hans*-LanM R100 K as a promising candidate for intra-REE separation; however, its performance in complex REE-containing waste streams remains unexplored.

For cost-effective intra-REE separation, it is essential that *Hans*-LanM-based biosorbents maintain high functionality, stability, and reusability. Using free *Hans*-LanM suffers from short protein lifetime, nonreusability, and high single-use costs.²⁵ Protein immobilization onto solid supports offers a viable strategy to improve protein stability and reusability.²⁶ Recently, we developed a novel nanobio platform for protein immobilization based on SpyTag-SpyCatcher chemistry (Spy chemistry).²⁷ This approach enables stable and efficient immobilization of target proteins onto magnetic nanoparticles (MNPs) through the formation of a covalent iso-peptide bond between SpyTag-functionalized MNPs and SpyCatcher-fused proteins. Based on this platform, we previously constructed a *Mex*-LanM-functionalized biosorbent (MNP-LanM) for selective REE extraction from real-world coal fly ash leachate.¹⁹ Our results demonstrated that immobilization of *Mex*-LanM on MNPs considerably enhanced protein stability while preserving its REE-binding functionality.¹⁹ The *Mex*-LanM-immobilized MNPs also retained colloidal stability and magnetic responsiveness, enabling an easy magnetic separation. Additionally, the covalent attachment enabled the MNP-LanM to endure multiple adsorption–desorption cycles under harsh conditions such as high ionic strengths and low-pH treatments.¹⁹ Building on these advantages, we envision leveraging our nanobio platform to engineer a *Hans*-LanM-based biosorbent for effective intra-REE separation from waste streams.

In this study, we developed a novel biosorbent (MNP-*Hans*-LanM) by immobilizing *Hans*-LanM-SpyCatcher on the surface of SpyTag-functionalized MNPs for intra-REE separation. We evaluated REE-binding and desorption activity, demonstrating that MNP-*Hans*-LanM enabled effective separation of representative REE pairs using stepwise desorption conditions. The biosorbent also maintained its functionality for REE pair separation over five adsorption–desorption cycles. Furthermore, we tested group separation of REEs under real-world conditions, where the MNP-*Hans*-LanM selectively captured REEs from the natural coarse coal refuse leachate and enriched bound REEs into light and heavy REE groups without using hazardous organic solvents. To the best of our knowledge, this is the first study to demonstrate that *Hans*-LanM could achieve group enrichment of REEs

from real-world waste streams, representing an important step toward practical intra-REE separation. The engineered biosorbent showed great potential to enable effective and sustainable intra-REE separation applications, which will advance green-chemistry strategies for a circular economy of REEs.

2. MATERIALS AND METHODS

2.1. Chemicals, Strains, and Plasmids. REE chloride salts (>99.9%) were purchased from ThermoFisher Scientific (Waltham, MA), and buffer reagents were obtained from Sigma-Aldrich (St. Louis, MO). Restriction enzymes and molecular reagents for cloning were purchased from New England Biolabs (Beverly, MA), while primers for cloning were synthesized by Integrated DNA Technologies (Coralville, IA). *Escherichia coli* T OP10 strain and BL21 (DE3) were used for gene cloning and for recombinant protein expression and purification, respectively. *E. coli* cells were cultured in Luria–Bertani (LB) medium with 100 $\mu g/mL$ ampicillin at 37 °C.

The strains, plasmids, and gene fragments used in this study are listed in Table S1. The Lanmodulin gene from *Hansschlegelia quercus*²⁴ with a single mutation of R100 K was synthesized by Integrated DNA Technology with codon optimization for *E. coli* expression. To construct pET21b(+)-*Hans*-LanM(R100 K)-SpyCatcher (Figure S1), the *Hans*-LanM(R100 K) gene fragment was digested with 5' *Nde*I and 3' *Bam*HI and ligated to the plasmid pET21b(+)-LanM-SpyCatcher¹⁹ digested with the same restriction enzymes. The recombinant plasmid was then transformed into *E. coli* TOP10 competent cells, which were selected on LB agar plates supplemented with 100 $\mu g/mL$ ampicillin. The plasmid was extracted using a ZymoPURE Plasmid Miniprep Kit (Zymo Research, Irvine, CA) and confirmed by Sanger sequencing.

2.2. Production of *Hans*-LanM(R100 K)-SpyCatcher and SpyTag-Functionalized MNPs. The expression and production of *Hans*-LanM(R100 K)-SpyCatcher (hereafter *Hans*-LanM) followed the previously published protocol,²⁴ and described in detail in the Supporting Information. SpyTag-functionalized MNPs were also synthesized as previously described,²⁷ with details in the Supporting Information.

2.3. Construction and Characterization of MNP-*Hans*-LanM Biosorbent. The MNP-*Hans*-LanM biosorbent was constructed by incubating *Hans*-LanM with SpyTag-functionalized MNPs in a pH 7.0 Tris-buffered saline (TBS) buffer at room temperature with rotary shaking at 30 rpm for 1 h. SpyTag:SpyCatcher molar ratio was controlled at 1:1, an optimal condition for immobilizing SpyCatcher-fused proteins onto SpyTag-functionalized MNPs.^{19,28} After immobilization, MNP-*Hans*-LanM was washed three times with TBS buffer supplemented with 150 mM imidazole and 0.05% (v/v) Tween 20 to remove nonspecifically bound proteins, and the protein concentration was measured using the detergent-compatible Bradford assay (Thermo Scientific, USA). The loading of *Hans*-LanM onto the MNPs was calculated by deducting the protein quantity present in both the conjugation and washed solutions from the initial amount used for immobilization. To confirm the successful immobilization, *Hans*-LanM was labeled with fluorescein isothiocyanate (FITC)²² and incubated with the MNPs for immobilization, and the resulting MNP-*Hans*-LanM was characterized by a Zeiss fluorescent microscope, with details in Supporting Information.

2.4. Intra-REE Separation Experiments. Five mg of MNP-Hans-LanM was incubated with ~5 ppm of REE pairs (Nd/Dy, La/Dy) or a synthetic REE mixture at specified molar ratios in 1 mL of 10 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer (pH 5.0) at room temperature with rotary shaking (30 rpm) for 60 min. After adsorption, the MNP-Hans-LanM was washed twice with 1.7 mL of TBS buffer containing 0.01% Tween 20 to remove unbound REEs, then resuspended in 1.8 mL of sodium malonate buffer (70–90 mM, pH 5.0) for 60 min. For the second desorption step, the MNP-Hans-LanM was washed twice, dispersed in 1.8 mL of pH 1.7 or 1.5 HCl solution, and incubated with rotary shaking (30 rpm) for 60 min.

REE separation by MNP-Hans-LanM was also tested in the natural leachate. The leachate was collected from a coarse coal refuse plant located in western Kentucky.²⁹ Adsorption experiments were conducted in 15 mL centrifuge tubes containing 20 mg of MNP-LanM and 3 mL of leachate (pH 5.0) for 60 min. After two washing steps, sequential desorption was carried out using 3 mL of 80 mM sodium malonate buffer (pH 5.0), followed by 8 mL of pH 1.5 HCl solution for group enrichment and separation of REEs. For each test, SpyTag-functionalized MNPs without immobilized *Hans-LanM* served as a negative control. After each adsorption/desorption step, the sample was centrifuged (13,000 rpm, 3 min), and the supernatant was collected for REE analysis. Adsorption efficiency (%) was calculated as the percentage of REEs adsorbed relative to the initial amount, while desorption efficiency (%) was the percentage of REEs recovered relative to the amount adsorbed. All experiments were conducted in triplicate.

For the REE pair separation experiments, the metal ion purity and yield were defined as

$$\text{REE1 purity} = \frac{C_{\text{REE1}}}{C_{\text{REE1}} + C_{\text{REE2}}}$$

$$\text{REE1 yield} = \frac{\text{REE1 recovered}}{\text{REE1 adsorbed}}$$

where C_{REE1} and C_{REE2} are the molar concentration of REE1 and REE2 in the desorption solutions, respectively.

For group separation of REEs from the natural leachate, the REE purity and enrichment factor were defined as

$$\text{LREEs purity} = \frac{\text{CLREEs}}{\text{CLREEs} + \text{CHREEs} + \text{C}_{\text{non-REEs}}}$$

$$\text{Enrichment factor} = \frac{\text{REE purity in desorption solution}}{\text{REE purity in feed solution}}$$

where C_{LREEs} , C_{HREEs} , and $C_{\text{non-REEs}}$ are the molar concentration of LREEs, HREEs, and non-REEs in the desorption solutions, respectively.

2.5. Analytical Methods. REE and non-REE concentrations were quantified using a NexION 300X ICP-MS instrument (PerkinElmer, Waltham, MA) operating in a standard mode. Samples were introduced via an ESI PFA ST nebulizer at a flow rate of 120 $\mu\text{L}/\text{min}$. Calibration was performed using a commercial standard solution containing equal concentrations of each REE or non-REE, with indium as the internal standard. Before analysis, all calibration and supernatant samples (collected after adsorption and/or desorption) were acidified to 2% (v/v) with concentrated nitric acid (70 wt%, trace metal grade, Sigma-Aldrich).

Calcium concentrations were determined separately using an Optima 8300 ICP-OES (PerkinElmer, Waltham, MA). Significant difference in Dy desorption efficiency between 80 and 90 mM malonate treatment was performed using two-sample *t* tests.

3. RESULTS AND DISCUSSION

3.1. Successful Immobilization of *Hans-LanM* onto MNPs. The MNP-Hans-LanM biosorbent was engineered by immobilizing the *Hans-LanM* protein onto SpyTag-functionalized MNPs using highly efficient Spy chemistry. The *Hans-LanM* protein was expressed and purified, and its purity was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis, which showed a single band of the expected molecular weight of ~25 kDa (Figure S2). The protein could react with cysteine-modified SpyTag in TBS buffer solution by forming a stable complex resistant to boiling, confirming that the recombinant protein that we purified has a functional SpyCatcher domain (Figure S2). Meanwhile, SpyTag-functionalized MNPs were synthesized following our previously established procedure.²⁷ Consistent with our MNP design, the synthesized MNPs possessed a core-shell-corona structure under transmission electron microscopy (Figure S3A). Dynamic light scattering analysis revealed that the MNPs had excellent dispersibility with a hydrodynamic size of 833 nm (Figure S3B). The MNPs also demonstrated high magnetic responsiveness, reaching nearly 100% within 10 min (Figure S3C). The amount of SpyTag incorporated onto each MNP was quantified to be 3.6 wt% using thermogravimetric analysis and fluorescamine assay (Figure S3D, S3E). To construct the MNP-Hans-LanM biosorbent, the purified *Hans-LanM* was incubated with SpyTag-functionalized MNPs in TBS buffer at a 1:1 molar ratio of SpyCatcher to SpyTag. The simple mixing step enabled covalent iso-peptide bond formation between SpyCatcher and SpyTag,³⁰ resulting in stable protein immobilization onto the MNPs. Notably, the immobilization process was completed within 10 min, highlighting its high efficiency.

The successful immobilization of *Hans-LanM* onto the MNP surface was confirmed through fluorescence imaging and activity characterization. To visualize protein localization on MNPs, *Hans-LanM* was labeled with FITC, a green fluorescent dye, and conjugated to SpyTag-functionalized MNPs. Under fluorescence microscopy, the resulting MNP-Hans-LanM-FITC particles displayed a bright-green shell surrounding a dark magnetic core (Figure 1A). These MNPs also showed a strong green fluorescence intensity (825.7 ± 12.2 au), while the MNPs without SpyTag functionalization (as a negative control) incubated with *Hans-LanM*-FITC exhibited background-level fluorescence intensity similar to that of PBS buffer (Figure 1B). Additionally, the adsorption activity of the MNP-Hans-LanM was evaluated using Nd as a representative REE. The MNP-Hans-LanM biosorbent, with an immobilized protein density of 2.6 ± 0.6 $\mu\text{mol-Hans-LanM}/\text{g-MNP}$, adsorbed 5.6 ± 0.8 $\mu\text{mol-Nd}/\text{g-sorbent}$. In contrast, the empty MNPs without *Hans-LanM* immobilization showed no detectable Nd adsorption (Figure 1C). The molar ratio of adsorbed Nd to the immobilized protein was 2.2 ± 0.4 , aligning with the ~2:1 REE: protein stoichiometry observed in previous studies with free and immobilized *Hans-LanM* on agarose beads.²⁴ This result suggests that the metal-binding functionality (e.g., binding affinity) of *Hans-LanM* was largely

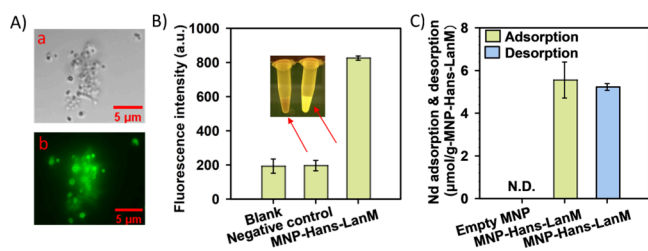


Figure 1. Spectroscopy and activity characterization of the MNP-Hans-LanM biosorbent. (A) Bright-field (a) and fluorescence (b) micrographs of SpyTag-functionalized magnetic nanoparticles (MNPs) immobilized with fluorescein isothiocyanate (FITC)-labeled *Hans-LanM*. (B) Fluorescence intensity of MNPs without and with SpyTag functionalization after immobilization with FITC-labeled *Hans-LanM*. Nonfunctionalized MNPs serve as a negative control. Inset: Digital images of MNPs without (left) and with (right) SpyTag functionalization after immobilization with FITC-labeled *Hans-LanM* under UV illumination. (C) Neodymium (Nd) adsorption and desorption by SpyTag-functionalized MNPs without LanM immobilization (control) and the MNP-Hans-LanM. N.D. indicates not detectable.

preserved after immobilization onto MNPs. Furthermore, over 90% of the adsorbed Nd could be efficiently desorbed using a pH 1.7 HCl solution (Figure 1C). Together, these results confirmed the functional immobilization of *Hans-LanM* onto the MNPs.

3.2. Effective Separation between REE Pairs. We next investigated the potential of the MNP-Hans-LanM biosorbent for REE pair separation by using a stepwise desorption strategy. Previous studies have shown that the HREE complex of *Hans-LanM* was more easily destabilized by chelators like malonate compared to the LREE complex, providing a mechanistic basis for selective desorption.²⁴ We first tested the separation of Nd/Dy, a REE pair commonly found in NdFeB magnet-bearing E-waste, by using a synthetic Nd/Dy solution (~95:5 molar ratio) designed to mimic the LREE/HREE composition.^{31,32} The MNP-Hans-LanM biosorbent achieved ~75% adsorption efficiency, with Nd and Dy representing $97.3 \pm 0.1\%$ and $2.7 \pm 0.1\%$ of the adsorbed REEs, respectively. The increased proportion of Nd on the biosorbent relative to the feed indicates preferential Nd adsorption. This result aligns with the behavior of free *Hans-LanM*, which binds higher equivalents of Nd than Dy.²⁴ To separate Dy from Nd, the first-step desorption mediated by malonate is essential, as Dy is more readily destabilized by malonate than Nd.²⁴ Therefore, we evaluated the effects of malonate concentrations (70, 80, and 90 mM) on the recovery efficiency of Nd and Dy and product purity in the first desorption solution. Increasing malonate from 70 to 80 mM improved Dy recovery efficiency from $53.1 \pm 2.8\%$ to $70.0 \pm 5.7\%$, while Nd recovery efficiency remained unchanged (Table S2). Correspondingly, Dy purity in the first desorption solution increased from $30.5 \pm 2.6\%$ to $41.7 \pm 2.0\%$ (Figure 2A,B). 90 mM malonate did not further improve Dy recovery efficiency (two-sample *t*-test, $P > 0.05$) compared to 80 mM malonate, but increased Nd recovery efficiency to $8.6 \pm 0.4\%$ from $2.7 \pm 0.4\%$ (Table S2). The unintended increase in Nd release reduced the Dy purity in the first desorption solution to $18.3 \pm 0.3\%$ (Figure 2C). The corresponding mass balance of REEs for adsorption and desorption processes, shown in Figure 2, is provided in Table S3. The results showed that REE recovery was approximately 85–95% after two desorption

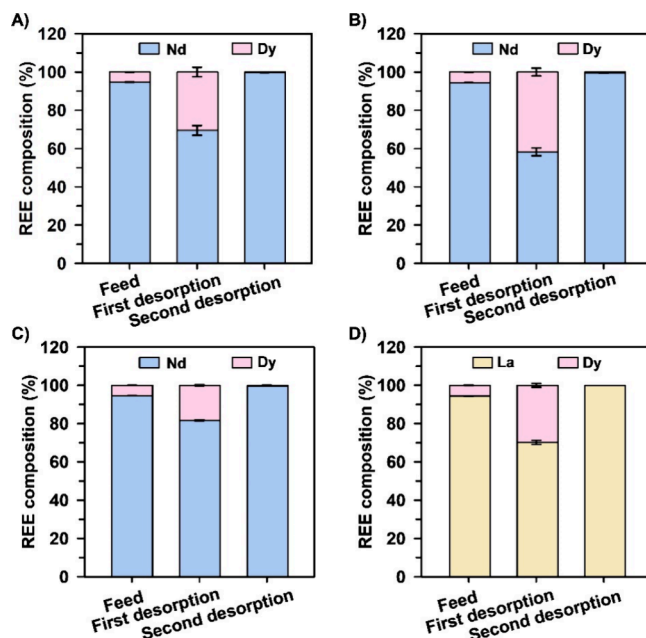


Figure 2. Separation of rare earth element (REE) pairs from synthetic feed solutions. (A) Distribution of neodymium (Nd) and dysprosium (Dy) in the feed solution, the first desorption solution (70 mM malonate), and the second desorption solution (pH 1.7 HCl). (B) Distribution of Nd and Dy in the feed solution, the first desorption solution (80 mM malonate), and the second desorption solution (pH 1.7 HCl). (C) Distribution of Nd and Dy in the feed solution, the first desorption solution (90 mM malonate), and the second desorption solution (pH 1.7 HCl). (D) Distribution of lanthanum (La) and Dy in the feed solution, the first desorption solution (80 mM malonate), and the second desorption solution (pH 1.7 HCl).

steps, indicating that most REEs adsorbed on MNP-Hans-LanM were effectively recovered. Based on these results, 80 mM malonate was selected for optimal HREE desorption in the following experiments. After the first-step desorption, Dy purity was enriched to $41.7 \pm 2.0\%$, representing an 8-fold increase compared to ~5% Dy purity in the feed solution (Figure 2B). Notably, our biosorption approach provides a greener alternative to traditional solvent extraction methods that rely on hazardous organic solvents. For example, a prior study used bis(2,4,4-trimethylpentyl) phosphinic acid dissolved in kerosene to enrich Dy purity from ~6.7% to ~47%, whereas our method achieved comparable Dy enrichment without the use of toxic solvents.³³ A second-step desorption using pH 1.7 HCl yielded a high-purity Nd fraction ($99.5 \pm 0.004\%$) with a recovery efficiency of $85.0 \pm 4.8\%$ (Figure 2B). The 99.5% Nd purity met the threshold for commercial salability, which enables the reintegration of high-purity neodymium oxide products into the magnet manufacturing supply chain.³⁴ Similarly, a previous study using a *Mex-LanM*-based column achieved 46.1–50.3% Dy purity and 98.7–99.8% of Nd purity when processing a 95%Nd:5%Dy feed solution.²² Although a direct comparison with *Mex-LanM* under identical conditions was not conducted in this study, previous work has shown that *Hans-LanM* immobilized on agarose beads exhibits greater intra-REE selectivity between representative LREE/HREE pairs (e.g., Nd/Dy) than *Mex-LanM* under comparable conditions.²⁴ We therefore anticipate that integrating *Hans-LanM* into our nanobio platform will provide improved intra-REE separation performance compared

to Mex-LanM under the same conditions, though further study on direct comparison by parallel experiments is needed in future research.

Considering the relatively low Dy purity of $41.7 \pm 2.0\%$, we investigated whether an additional malonate desorption step could enhance the Dy enrichment. To evaluate this approach, we prepared a synthetic feed solution with a $\sim 55:45$ molar ratio of Nd to Dy that mimics the Nd/Dy composition of the malonate desorption solution obtained from a $\sim 95\text{Nd}:5\text{Dy}$ feed. During desorption, 80 mM malonate released $76.8 \pm 7.3\%$ of Dy (2.4 ± 0.05 nmol from 3.2 ± 0.3 nmol) with an improved purity of $84.6 \pm 1.9\%$, and the subsequent pH 1.7 desorption recovered $77.0 \pm 3.2\%$ of Nd (5.0 ± 0.08 nmol from 6.5 ± 0.3 nmol) at $93.4 \pm 0.2\%$ purity (Figure S4 and Table S3). The results suggested that incorporating extra malonate desorption steps could further improve the separation efficiency, yielding high-purity Dy from Nd. From a practical standpoint, the malonate carried over from the first desorption step may influence the readsorption behavior of Nd and Dy on MNP-Hans-LanM. Before the adsorption cycle, the malonate can be removed by pH adjustment to dissociate the chelates, followed by anion exchange resin treatment.³⁵

Additionally, we evaluated the separation of La/Dy, a representative LREE/HREE pair that frequently occurred in coal byproduct leachates, by the MNP-Hans-LanM biosorbent. A synthetic solution containing $\sim 95\%$ La and $\sim 5\%$ Dy was used to mimic the typical LREE/HREE composition.^{36,37} The first desorption step with 80 mM malonate upgraded Dy purity to $29.8 \pm 1.1\%$ with $80.0 \pm 3.6\%$ recovery efficiency, while the subsequent pH 1.7 HCl achieved $77.3 \pm 1.5\%$ La recovery efficiency with nearly 100% purity (Figure 2D). The corresponding La and Dy mass balance for adsorption and desorption processes was provided in Table S3. Dimerization is a unique structural feature of *Hans-LanM* that has been shown to contribute to its ability to discriminate between LREEs and HREEs in solution.²⁴ In our MNP system, *Hans-LanM* was covalently attached to the MNP surface via Spy chemistry, which could spatially restrict rotational freedom compared with free protein in solution. However, the high density of SpyTag peptides incorporated into the polymers on MNPs, along with the excellent water dispersity provided by the surface-charged polymers,²⁷ may facilitate the close spatial arrangement of immobilized *Hans-LanM* to allow functional dimer formation. In addition, the preferential binding of *Hans-LanM* for light REEs is largely an intrinsic property of its structural features, including binding sites optimized for the ionic radii and electronic configurations of light REEs.^{24,38} From this perspective, the immobilization on MNPs is expected to have a minimal impact on the binding selectivity between light and heavy REEs, as evidenced by the effective separation between representative LREE/HREE pairs (Figure 2). Together, these results indicated that the MNP-Hans-LanM biosorbent could effectively enrich and separate Nd/Dy and La/Dy pairs, even when starting from feed solutions with low Dy content, typical of REE-containing E-waste and coal-derived leachates. The recovered high-purity Nd and La could be recycled for use in magnet and hybrid-car battery manufacturing,³⁹ contributing to a more sustainable REE supply chain.

3.3. Reusability of MNP-Hans-LanM Biosorbent for REE Pair Separation. A key motivation for immobilizing proteins onto MNPs is to enable the reuse of proteins for cost-effective applications. Therefore, we examined the reusability

of the MNP-Hans-LanM biosorbent for REE pair separation by monitoring the REE adsorption activity over multiple adsorption–desorption cycles. The Nd/Dy pair ($\sim 95:5$ molar ratio) was used for the adsorption experiments, followed by stepwise desorption using 80 mM malonate and a pH 1.7 HCl solution. In each cycle, the adsorption capacity for Nd and Dy was measured to evaluate the biosorbent activity retention (Figure S5). The Nd and Dy compositions after each desorption step were analyzed to evaluate the intra-REE selectivity during reuse (Table S4). After five cycles of Nd/Dy pair separation, the biosorbent retained $91.2 \pm 2.4\%$ of its initial adsorption activity (Figure 3), indicating protein stability

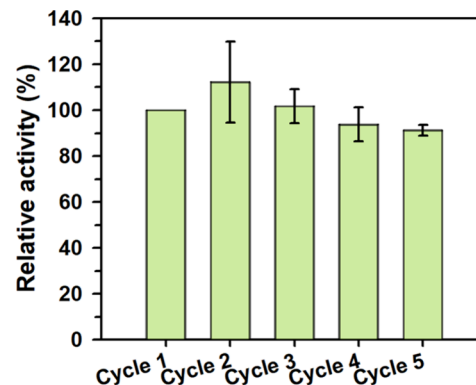


Figure 3. Reusability of MNP-Hans-LanM for neodymium (Nd)/dysprosium (Dy) pair separation. Experiments were conducted by adsorption of the Nd/Dy pair ($\sim 95:5$ molar ratio), followed by stepwise desorption processes using 80 mM malonate and a pH 1.7 HCl solution. The adsorption capacity for Nd and Dy (defined as the amount of adsorbed Nd and Dy (μmol) per gram of sorbent) was determined and normalized with the first cycle as the relative activity.

during repeated REE pair separation. The Nd/Dy compositions after the first and second desorption steps remained approximately 55%:45% and 99.5%:0.5%, respectively, up to the fourth cycle (Table S4), indicating that intra-REE selectivity was maintained during reuse. Considering the stable covalent bond between *Hans-LanM* and SpyTag on the MNPs, the observed activity loss ($\sim 10\%$) and altered Nd/Dy composition ($\sim 65\%:35\%$) after the fifth cycle are likely attributed to partial inactivation of *Hans-LanM* from repeated exposure to low-pH desorption conditions. Notably, this work is the first to examine the stability of *Hans-LanM* under repeated low-pH treatments. The demonstrated stability enables repeated reuse of the *Hans-LanM*-functionalized biosorbent for intra-REE separation, which could help reduce operational costs. These results highlight the potential of the MNP-Hans-LanM biosorbent for repeated uses in REE pair separation with maintained activity.

3.4. Group Separation of REEs from a Complex REE Solution. Having demonstrated the separation of representative LREE/HREE pairs (Nd/Dy, La/Dy), we further investigated whether the MNP-Hans-LanM could achieve effective group separation of LREEs and HREEs from a complex REE solution by using a stepwise desorption strategy. Group separation of REEs is an important early step in the REE mining and recovery process, particularly considering that real-world REE sources often contain complex mixtures with highly unbalanced LREE/HREE compositions.^{22,40} To this end, we conducted group separation experiments using a synthetic feed solution ($\sim 96\%$ LREE:4%HREE) (Table S5)

designed to mimic the LREE/HREE distributions commonly found in coal combustion products^{41,42} and mine tailings.⁴³ The biosorbent effectively adsorbed REEs with $\sim 70\%$ efficiency, with LREEs and HREEs comprising $97.0\% \pm 0.1\%$ and $3.0\% \pm 0.1\%$ of the adsorbed REEs, respectively. During desorption, 80 mM malonate eluted $64.0\% \pm 4.9\%$ HREEs at a purity of $18.5\% \pm 0.8\%$, representing a 5-fold increase in the purity of HREEs compared to that of the feed solution ($3.7\% \pm 0.01\%$ HREEs), while a subsequent pH desorption step recovered $82.6\% \pm 3.5\%$ of LREEs with $\sim 99\%$ purity and $26.2\% \pm 2.2\%$ of HREEs (Figure 4A, Table S6).

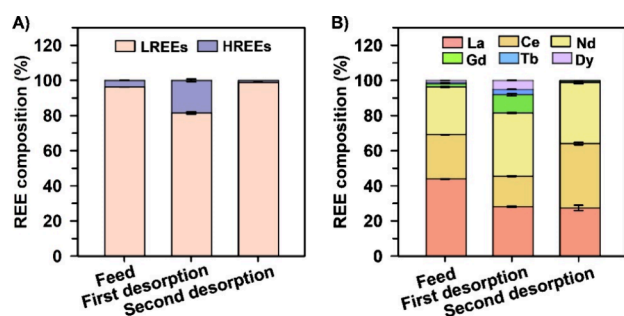


Figure 4. Group separation of rare earth elements (REEs) from a complex REE solution. (A) Distribution of light REEs (LREEs) and heavy REEs (HREEs) in the feed solution, the first desorption solution (80 mM malonate), and the second desorption solution (pH 1.5 HCl). (B) Distribution of individual REE (La: lanthanum, Ce: cerium, Nd: neodymium, Gd: gadolinium, Tb: terbium, Dy: dysprosium) in the feed solution, the first desorption solution (80 mM malonate), and the second desorption solution (pH 1.5 HCl).

Specifically, the purities of Gd, Tb, and Dy in the first desorption step increased by 5.6-, 3.3-, and 2.6-fold, respectively, relative to their initial proportion in the feed solution (Figure 4B). Similarly, a previous study using *Mex-LanM*-based column enriched HREEs (Gd–Lu) from $\sim 5\%$ to 15–25% when processing a synthetic REE mixture.²³ The 2.5- to 6-fold enrichment of HREEs would reduce the number of downstream intra-REE separation stages needed to produce high-purity individual REEs compared to that of processing the feed solution. These results highlighted the potential of the MNP-Hans-LanM biosorbent for effective LREE/HREE enrichment and separation from complex REE mixtures.

3.5. Separation of REEs under Environmentally Relevant Conditions. To assess the feasibility of MNP-Hans-LanM for group separation of REEs from real-world feedstocks, we tested its adsorption and desorption performance with natural leachate. Natural leachate, generated by acid leaching within the coarse coal refuse pile containing pyrite, is enriched in REEs at the level of parts per million (ppm) and has emerged as a promising yet underutilized source for REE recovery.²⁹ Using such leachate not only supports diversification of the REE supply chain but also contributes to the management of acidic waste streams.^{43,44} The leachate used in this study was naturally generated from coarse coal waste generated from the processing of Baker seam coal and contained approximately $26 \mu\text{M}$ (~ 3.5 ppm) of REEs, along with 1–3 order-of-magnitude higher concentrations of competing ions, including Mg^{2+} , Ca^{2+} , Mn^{2+} , Zn^{2+} , Al^{3+} , Fe^{3+} (Table S7), making REE molar percentage to be 0.3%. Despite the low REE concentration, the MNP-Hans-LanM could effectively adsorb most REEs from the leachate with $\sim 65\%$

efficiency, except for Sc (Figure S6). Notably, the MNP-Hans-LanM showed high selectivity for REEs in the leachate, with minimal interaction with the major competing non-REEs such as Ca^{2+} , Al^{3+} , and Fe^{3+} (Figure 5A, Table S8). The high

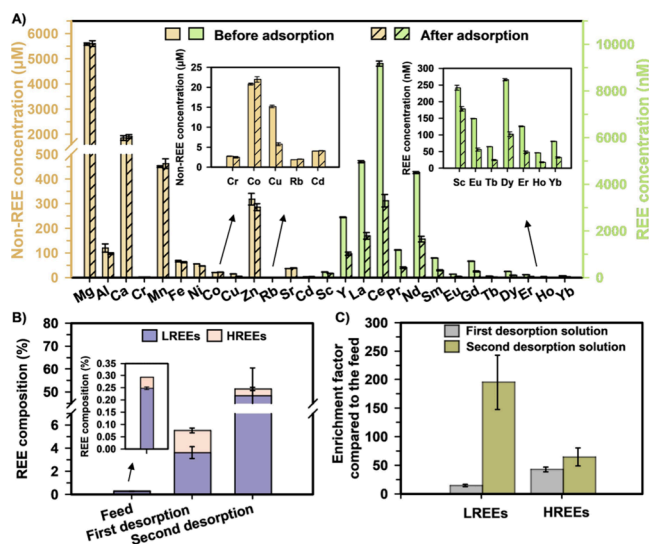


Figure 5. Performance of MNP-Hans-LanM in the group separation of rare earth elements (REEs) from the natural leachate. (A) Concentrations of REEs and non-REEs in the natural leachate before and after adsorption. Adsorption was conducted in 3 mL of natural leachate (pH 5.0) using 20 mg MNP-Hans-LanM. (B) Distribution of light REEs (LREEs) and heavy REEs (HREEs) in the feed solution, the first desorption solution (80 mM malonate), and the second desorption solution (pH 1.5 HCl). (C) Enrichment factor of LREEs and HREEs, defined as the fold increase in REE purity after desorption, in the two desorption solutions relative to the feed solution.

selectivity for REEs over non-REEs is attributed to multiple key amino acid residues (Asn83, Asp85, Asp87, Glu91, Glu94) in *Hans-LanM* as well as a backbone carbonyl group (Thr89), which together form a strong coordination with REEs involving nine or ten coordination sites.²⁴ Notably, this selectivity presents a key advantage over traditional solvent extraction, which is prone to interference from Al^{3+} and Ca^{2+} . Specifically, Al^{3+} can reduce REE purity and promote emulsions or third-phase formation via gelatinous hydroxide precipitation, while Ca^{2+} may contribute to fouling through gypsum formation, both of which complicate solvent extraction.^{17,45} Together, the results demonstrated that MNP-Hans-LanM could effectively and selectively extract REEs from complex, low-grade natural leachate, highlighting its potential for REE recovery under environmentally relevant conditions.

After demonstrating the effective removal of most non-REEs during adsorption, we next evaluated the capability of MNP-Hans-LanM to separate LREEs (La–Eu, $84.6 \pm 0.03\%$) from HREEs (Gd–Lu, Y, $15.0 \pm 0.02\%$) using the established two-step desorption scheme. In the first step, $63.4 \pm 1.8\%$ of HREEs were eluted with a purity of $1.9 \pm 0.2\%$, along with $\sim 20\%$ of LREEs recovered at $3.6 \pm 0.5\%$ purity (Figure 5A). The purity is calculated as the molar percentage of HREEs or LREEs relative to the total amounts of REEs and non-REEs in the desorption solution. A subsequent desorption with pH 1.5 HCl recovered $63.5 \pm 6.9\%$ of LREEs with considerably improved purity ($48.4 \pm 12.0\%$), along with $\sim 28\%$ of HREEs

at $2.9 \pm 0.7\%$ purity (Figure S5B). Analysis of non-REEs in both desorption solutions identified Zn^{2+} , Cu^{2+} , and Ni^{2+} as the primary impurities (Table S9), which contributed to the relatively low REE purity in the desorption solutions. These impurities might result from their unintended binding to the His_6 tag on the *Hans-LanM* protein.⁴⁶ This tag contains histidine residues that can coordinate with transition metals like Ni^{2+} or Cu^{2+} , a principle exploited by commercial Ni- or Cu-nitrilotriacetic acid (NTA) agarose resins commonly used for His-tagged protein purification.⁴⁷ A prewash step with imidazole to strip Zn^{2+} , Cu^{2+} , and Ni^{2+} bound to the His_6 tag, or removal of the His_6 tag prior to immobilizing *Hans-LanM* onto MNPs, could be a potential approach to further improve REE purity.⁴⁸ To further quantify separation performance, we calculated the enrichment factor, defined as the fold increase in REE purity during desorption compared to that of the original feed solution. In the first desorption step, the enrichment factors were 43- and 15-fold for HREEs and LREEs, respectively (Figure S5B). In the second step, the enrichment was further increased to 195-fold for LREEs and 64-fold for HREE (Figure S5B). The results suggested that the first desorption preferentially enriched HREEs, while the second step more effectively enriched LREEs. Collectively, these results suggested that MNP-*Hans-LanM* could enable effective enrichment of REEs into LREE and HREE groups from real-world REE sources such as coal refuse leachate.

4. CONCLUSIONS

This study created a novel biosorbent by immobilizing *Hans-LanM* onto the surface of SpyTag-functionalized MNPs and demonstrated effective group separation of REEs from dilute solutions and low-grade waste streams. The MNP-*Hans-LanM* biosorbent effectively enriched and separated representative LREE/HREE pairs without relying on hazardous organic solvents, offering a green alternative to traditional solvent extraction methods. Notably, the biosorbent could be reused for multiple cycles while retaining its activity under repeated low-pH regeneration. Using a stepwise separation strategy, the biosorbent achieved group-specific enrichment of HREE and LREE groups from a complex synthetic REE mixture. Furthermore, the MNP-*Hans-LanM* efficiently and selectively captured REEs from the real-world natural leachate and enriched light and heavy groups from the adsorbed REEs. In summary, these results suggest MNP-*Hans-LanM* as a promising biosorptive platform for developing green-chemistry technologies for intra-REE separation from waste streams, which will contribute to the circular economy of the critical materials and sustainable management of REE-containing waste streams.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsestengg.5c00508>.

Chemicals, protein production, MNP synthesis, protein storage stability, and fluorescent microscopy characterization (Text S1–S4), plasmid construction materials, dysprosium desorption by different concentrations of malonate, mass balance of REEs during REE pair separation, Nd and Dy composition during biosorbent reuse, REE composition in synthetic solution, mass balance of LREEs and HREEs, metal ions in the natural

leachate, metal composition in the natural leachate before and after adsorption, metal composition in desorption solutions (Tables S1–S9), recombinant plasmid map, protein purification, synthesis of MNPs, Nd/Dy separation from a synthetic solution with a ~55:45 molar ratio, REE adsorption efficiency in the natural leachate (Figures S1–S6) (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) Hazel, G. *Rare earth elements: critical resources for high technology*; US Department of the Interior, US Geological Survey: 2002; Vol. 87.
- (2) Stegen, K. S. Heavy rare earths, permanent magnets, and renewable energies: An imminent crisis. *Energy Policy* **2015**, *79*, 1–8.
- (3) Jha, A. R. *Rare earth materials: properties and applications*; CRC Press, 2014.
- (4) Dent, P. C. Rare earth elements and permanent magnets. *J. Appl. Phys.* **2012**, *111* (7), No. 07A721.
- (5) Scarangella, A.; Fabbri, F.; Reitano, R.; Rossi, F.; Priolo, F.; Miritello, M. Visible emission from bismuth-doped yttrium oxide thin films for lighting and display applications. *Sci. Rep.* **2017**, *7* (1), 17325.
- (6) Rocha, R. A.; Alexandrov, K.; Scott, C. Rare earth elements in biology: From biochemical curiosity to solutions for extractive industries. *Microb. Biotechnol.* **2024**, *17* (6), No. e14503.
- (7) Ye, Q.; Wang, D.; Wei, N. Engineering biomaterials for the recovery of rare earth elements. *Trends Biotechnol.* **2024**, *42* (5), 575–590.
- (8) Xie, X.; Yang, K.; Lu, Y.; Li, Y.; Yan, J.; Huang, J.; Xu, L.; Yang, M.; Yan, Y. Broad-spectrum and effective rare earth enriching via Lanmodulin-displayed *Yarrowia lipolytica*. *Journal of Hazardous Materials* **2022**, *438*, No. 129561.
- (9) Stoy, L.; Diaz, V.; Huang, C. H. Preferential Recovery of Rare-Earth Elements from Coal Fly Ash Using a Recyclable Ionic Liquid. *Environ. Sci. Technol.* **2021**, *55* (13), 9209–9220.
- (10) Chen, Z.; Li, Z.; Chen, J.; Kallem, P.; Banat, F.; Qiu, H. Recent advances in selective separation technologies of rare earth elements: a review. *Journal of Environmental Chemical Engineering* **2022**, *10* (1), No. 107104.
- (11) Li, Z.; Diaz, L. A.; Yang, Z.; Jin, H.; Lister, T. E.; Vahidi, E.; Zhao, F. Comparative life cycle analysis for value recovery of precious metals and rare earth elements from electronic waste. *Resources, conservation and recycling* **2019**, *149*, 20–30.
- (12) Xie, F.; Zhang, T. A.; Dreisinger, D.; Doyle, F. A critical review on solvent extraction of rare earths from aqueous solutions. *Minerals Engineering* **2014**, *56*, 10–28.
- (13) Gergoric, M.; Ekberg, C.; Steenari, B.-M.; Retegan, T. Separation of heavy rare-earth elements from light rare-earth elements via solvent extraction from a neodymium magnet leachate and the effects of diluents. *Journal of Sustainable Metallurgy* **2017**, *3*, 601–610.
- (14) El Ouardi, Y.; Virolainen, S.; Mouele, E. S. M.; Laatikainen, M.; Repo, E.; Laatikainen, K. The recent progress of ion exchange for the separation of rare earths from secondary resources—A review. *Hydrometallurgy* **2023**, *218*, No. 106047.
- (15) José, L. B.; Silva, G. C.; Ladeira, A. C. Q. Pre-concentration and partial fractionation of rare earth elements by ion exchange. *Min. Eng.* **2024**, *205*, No. 108477.
- (16) Mwewa, B.; Tadie, M.; Ndlovu, S.; Simate, G. S.; Matinde, E. Recovery of rare earth elements from acid mine drainage: A review of the extraction methods. *Journal of environmental chemical engineering* **2022**, *10* (3), No. 107704.
- (17) Judge, W. D.; Azimi, G. Recent progress in impurity removal during rare earth element processing: A review. *Hydrometallurgy* **2020**, *196*, No. 105435.
- (18) Cotruvo, J. A., Jr.; Featherston, E. R.; Mattocks, J. A.; Ho, J. V.; Laremore, T. N. Lanmodulin: A Highly Selective Lanthanide-Binding Protein from a Lanthanide-Utilizing Bacterium. *J. Am. Chem. Soc.* **2018**, *140* (44), 15056–15061.
- (19) Ye, Q.; Jin, X.; Zhu, B.; Gao, H.; Wei, N. Lanmodulin-functionalized magnetic nanoparticles as a highly selective biosorbent for recovery of rare earth elements. *Environ. Sci. Technol.* **2023**, *57* (10), 4276–4285.
- (20) Hussain, Z.; Kim, S.; Cho, J.; Sim, G.; Park, Y.; Kwon, I. Repeated Recovery of Rare Earth Elements Using a Highly Selective and Thermo-Responsive Genetically Encoded Polypeptide. *Adv. Funct. Mater.* **2021**, *32* (13), No. 2109158.
- (21) Deblonde, G. J.; Mattocks, J. A.; Park, D. M.; Reed, D. W.; Cotruvo, J. A. Jr.; Jiao, Y. Selective and Efficient Biomacromolecular Extraction of Rare-Earth Elements using Lanmodulin. *Inorg. Chem.* **2020**, *59* (17), 11855–11867.
- (22) Dong, Z.; Mattocks, J. A.; Deblonde, G. J.; Hu, D.; Jiao, Y.; Cotruvo, J. A., Jr.; Park, D. M. Bridging Hydrometallurgy and Biochemistry: A Protein-Based Process for Recovery and Separation of Rare Earth Elements. *ACS Cent. Sci.* **2021**, *7* (11), 1798–1808.
- (23) Dong, Z.; Mattocks, J. A.; Seidel, J. A.; Cotruvo, J. A., Jr.; Park, D. M. Protein-Based approach for high-purity Sc, Y, and grouped lanthanide separation. *Sep. Purif. Technol.* **2024**, *333*, No. 125919.
- (24) Mattocks, J. A.; Jung, J. J.; Lin, C.-Y.; Dong, Z.; Yennawar, N. H.; Featherston, E. R.; Kang-Yun, C. S.; Hamilton, T. A.; Park, D. M.; Boal, A. K. Enhanced rare-earth separation with a metal-sensitive Lanmodulin dimer. *Nature* **2023**, *618*, 87–93.
- (25) Hutchison, J. M.; Mayer, B. K.; Vega, M.; Chacha, W. E.; Zilles, J. L. Making Waves: Biocatalysis and Biosorption: Opportunities and Challenges Associated with a New Protein-Based Toolbox for Water and Wastewater Treatment. *Water Research X* **2021**, *12*, No. 100112.
- (26) Ansari, S. A.; Husain, Q. Potential applications of enzymes immobilized on/in nano materials: A review. *Biotechnology advances* **2012**, *30* (3), 512–523.
- (27) Jin, X.; Ye, Q.; Wang, C.-W.; Wu, Y.; Ma, K.; Yu, S.; Wei, N.; Gao, H. Magnetic nanoplatforms for covalent protein immobilization based on spy chemistry. *ACS Appl. Mater. Interfaces* **2021**, *13* (37), 44147–44156.
- (28) Ye, Q.; Jin, X.; Gao, H.; Wei, N. Site-specific and tunable co-immobilization of proteins onto magnetic nanoparticles via spy chemistry. *ACS Applied Bio Materials* **2022**, *5* (12), S665–S674.
- (29) Zhang, W.; Honaker, R. Q. Rare earth elements recovery using staged precipitation from a leachate generated from coarse coal refuse. *International Journal of Coal Geology* **2018**, *195*, 189–199.
- (30) Banerjee, A.; Howarth, M. Nanoteamwork: covalent protein assembly beyond duets towards protein ensembles and orchestras. *Curr. Opin Biotechnol.* **2018**, *51*, 16–23.
- (31) Ding, Y.; Harvey, D.; Wang, N.-H. L. Two-zone ligand-assisted displacement chromatography for producing high-purity praseodymium, neodymium, and dysprosium with high yield and high productivity from crude mixtures derived from waste magnets. *Green Chem.* **2020**, *22* (12), 3769–3783.
- (32) Yang, Y.; Walton, A.; Sheridan, R.; Güth, K.; Gauß, R.; Gutfleisch, O.; Buchert, M.; Steenari, B.-M.; Van Gerven, T.; Jones, P. T.; Binnemans, K. REE Recovery from End-of-Life NdFeB Permanent Magnet Scrap: A Critical Review. *Journal of Sustainable Metallurgy* **2017**, *3* (1), 122–149.
- (33) Sun, P.-P.; Kim, D.-H.; Cho, S.-Y. Separation of neodymium and dysprosium from nitrate solutions by solvent extraction with Cyanex272. *minerals Engineering* **2018**, *118*, 9–15.
- (34) Ghorbani, Y.; Ilankoon, L.; Dushyantha, N.; Nwaila, G. T. Rare earth permanent magnets for the green energy transition: Bottlenecks, current developments and cleaner production solutions. *Resources, Conservation and Recycling* **2025**, *212*, No. 107966.
- (35) Inui, A.; Hama, C.; Katsuragawa, T.; Iwata, S.; Yuchi, A. Divalent/monovalent selectivities and secondary interactions of multibasic acids on anion exchange resins. *Ind. Eng. Chem. Res.* **2013**, *52* (47), 16880–16886.
- (36) Hussain, R.; Luo, K. Geochemical evaluation of enrichment of rare-earth and critical elements in coal wastes from Jurassic and

Permo-Carboniferous coals in Ordos Basin. *China. Natural Resources Research* **2020**, *29*, 1731–1754.

(37) Middleton, A.; Park, D. M.; Jiao, Y.; Hsu-Kim, H. Major element composition controls rare earth element solubility during leaching of coal fly ash and coal by-products. *International Journal of Coal Geology* **2020**, *227*, No. 103532.

(38) Wang, Q.; Yu, J.; Zhong, Z.; Hui, C.; Zhang, Y.; Yang, L.; et al. Alanmodulin-based fluorescent assay for the rapid and sensitive detection of rare earth elements. *Analyst* **2025**, *150* (4), 750–759.

(39) Cheilas, P.; Christou, T.; Karkalakos, S.; Kottaridi, C.; Michaelides, P. G. Rare earth elements and the US renewable economy: A causality exploration between critical materials and clean energy. *Resources Policy* **2025**, *101*, No. 105491.

(40) Taggart, R. K.; Hower, J. C.; Dwyer, G. S.; Hsu-Kim, H. Trends in the rare earth element content of US-based coal combustion fly ashes. *Environ. Sci. Technol.* **2016**, *50* (11), 5919–5926.

(41) Dai, S.; Graham, I. T.; Ward, C. R. A review of anomalous rare earth elements and yttrium in coal. *International journal of coal geology* **2016**, *159*, 82–95.

(42) Zhang, W.; Honaker, R. Characterization and recovery of rare earth elements and other critical metals (Co, Cr, Li, Mn, Sr, and V) from the calcination products of a coal refuse sample. *Fuel* **2020**, *267*, No. 117236.

(43) Zhang, W.; Honaker, R. Process development for the recovery of rare earth elements and critical metals from an acid mine leachate. *Minerals Engineering* **2020**, *153*, No. 106382.

(44) Honaker, R. Q.; Zhang, W.; Yang, X.; Rezaee, M. Conception of an integrated flowsheet for rare earth elements recovery from coal coarse refuse. *Minerals Engineering* **2018**, *122*, 233–240.

(45) Ye, Q.; Li, G.; Deng, B.; Luo, J.; Rao, M.; Peng, Z.; Zhang, Y.; Jiang, T. Solvent extraction behavior of metal ions and selective separation Sc³⁺ in phosphoric acid medium using P204. *Sep. Purif. Technol.* **2019**, *209*, 175–181.

(46) Wątył, J.; Hecel, A.; Rowińska-Żyrek, M.; Kozłowski, H. Impact of histidine spacing on modified polyhistidine tag–Metal ion interactions. *Inorg. Chim. Acta* **2018**, *472*, 119–126.

(47) Khan, F.; He, M.; Taussig, M. J. Double-hexahistidine tag with high-affinity binding for protein immobilization, purification, and detection on Ni– nitrilotriacetic acid surfaces. *Analytical chemistry* **2006**, *78* (9), 3072–3079.

(48) Haglin, E. R.; Yang, W.; Briegel, A.; Thompson, L. K. His-tag-mediated dimerization of chemoreceptors leads to assembly of functional nanoarrays. *Biochemistry* **2017**, *56* (44), 5874–5885.