Supporting Information

Bridging Hydrometallurgy and Biochemistry: A Protein-based Process for Recovery and Separation of Rare Earth Elements

Ziye Dong¹, Joseph A. Mattocks², Gauthier J.-P. Deblonde¹,³, Dehong Hu⁴, Yongqin Jiao,¹ Joseph A. Cotruvo, Jr.²*, Dan M. Park¹*

¹Physical and Life Sciences Directorate, Lawrence Livermore National Laboratory, Livermore, California 94550, United States
²Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802, United States
³Glenn T. Seaborg Institute, Lawrence Livermore National Laboratory, Livermore, California 94550, United States
⁴Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, Washington 99354, United States

Corresponding authors: juc96@psu.edu (J.A.C.); park36@llnl.gov (D.M.P.)

This file contains methods, supplementary results, supplementary Tables S1-8, supplementary Figures S1-6, and supplementary References.
METHODS

Note: No unexpected or unusually high safety hazards were encountered.

Chemicals and materials

REE chloride salts (> 99.9%), solvents, and buffers were purchased from Millipore Sigma. Amine-functionalized agarose beads were purchased from Nanocs Inc. N-Succinimidyl 4-(maleimidomethyl) cyclohexane-1-carboxylate (SMCC) was purchased from Chem-Impex International, Inc. and used without further purification. NHS-Fluorescein (5/6-carboxyfluorescein succinimidyl ester), mixed isomer, was purchased from ThermoFisher Scientific.

Preparation of LanM

The plasmid containing the gene encoding for LanM-GSGC was obtained from Twist Bioscience (pET-29b(+) - LanM-GSGC). The gene sequence consisted of the codon-optimized wt-LanM sequence with (ggcagcggctgc) inserted immediately 5' to the stop codon.\(^1\) The protein was overexpressed in *E. coli* BL21(DE3) cells (NEB) at 37 °C after induction with 0.2 mM IPTG at an OD\(_{600nm}\) of 0.6. Purification was carried out as described except all buffers contained 5 mM TCEP.\(^2\) Briefly, cells were lysed and loaded to a 25-mL (2.5 × 5 cm) Q-Sepharose Fast Flow column and eluted using a 0.01 – 1 M NaCl gradient. LanM-GSGC containing fractions were purified further using gel filtration chromatography (HiLoad 16/600 Superdex 75 pg column in 30 mM MOPS, 100 mM KCl, 5 mM CaCl\(_2\), 5% glycerol, 5 mM TCEP, pH 7.0). LanM-GSGC-containing fractions were exchanged by FPLC into 20 mM acetate, 10 mM EDTA, 100 mM NaCl, 5% glycerol, pH 4.0, concentrated to ~3 mM, and frozen in liquid N\(_2\).
**Spectrofluorometric titrations of wt-LanM**

A solution of 20 μM Chelex-treated LanM was prepared in 20 mM acetate, 100 mM KCl, pH 5.0. In experiments that began with metalated LanM, 40 μM (2 equivalents) of metal was also added. The protein solution (600 μL) was placed in a 10-mm quartz spectrofluorometry cuvette (Starna Cells, 18F-Q-10-GL14-S) and assayed using a PerkinElmer fluorescence spectrometer FL 6500 in kinetic mode (80 kW power, 278 nm excitation, 2.5 nm excitation slit width, 307 nm emission, 5 nm emission slit width). Titrations were carried out through addition of at least 0.6 μL of titrant (either 10 mM metal or 1 mM citrate, pH 5.0) followed by ~1 min of signal equilibration. For each data point, 10 s of signal were averaged after equilibration. These values were corrected for dilution and normalized to $F_{307\text{nm}} = 1$ for the apoprotein. Each experiment was performed in triplicate.

**Maleimide functionalization of agarose beads**

Amine-functionalized agarose microbeads (1.2 mL) were transferred into a 5 mL Eppendorf tube and washed with pH 7.4 phosphate-buffered saline (PBS) three times and resuspended at a final volume of ~ 1.7 mL (1.2 mL microbeads and 0.5 mL PBS supernatant). SMCC (0.15 g) was dissolved in 3.4 mL DMSO and combined with the microbeads. After 2.5 h incubation on a rocker mixer at room temperature, the functionalized agarose microbeads were washed with DMSO three times to remove unreacted SMCC and then washed with coupling buffer (50 mM HEPES, 50 mM KCl, and 10 mM ethylenediaminetetraacetic acid (EDTA), pH 7) three times to remove DMSO solvent. The maleimide-microbeads were then used for LanM immobilization within 2 h.
**LanM immobilization**

LanM immobilization was carried out using a thiol-maleimide conjugation reaction. Specifically, immediately before immobilization, LanM-GSGC was exchanged into the coupling buffer using VivaSpin® 2 centrifugal concentrators (molecular weight cut-off 3,000 Da, GE Healthcare), which yielded a final protein concentration of ~ 2 mM. Then 2 mL of LanM solution was combined with 1 mL of maleimide-microbeads and the conjugation reaction was run for 16 h at room temperature. Unconjugated LanM was removed by washing with coupling buffer and the LanM-microbeads were stored in coupling buffer for subsequent tests. The maleimide-microbeads were also incubated with coupling buffer without LanM protein as an unconjugated control sample. To quantify LanM immobilization yield, Pierce™ BCA Protein Assay (ThermoFisher Scientific) was used to determine the LanM concentration in the reaction solution before and after the conjugation reaction. Briefly, a working solution was made by combining Reagent A and Reagent B at a 1:8 ratio. Working solution (200 µL) and 10 µL of sample (protein) were added to each well, respectively. Samples containing more than 2000 µg/mL protein were pre-diluted with coupling buffer. The 96-well plate was then shaken at 300 rpm for 30 s on a plate shaker, followed by 30 min incubation at 37 °C. After cooling the plate to room temperature, absorbance at 562 nm was measured and compared to BSA standards. In control experiments, it was shown that 2.0 mg/mL LanM gives the same assay response as 1.0 mg/mL BSA. Similarly, LanM immobilization kinetics were determined by monitoring the depletion of LanM protein in the reaction solution. The LanM concentration in the reaction solution at 6 min, 1 h, 3 h and 15 h after the start of conjugation reaction was measured in triplicate.
Fourier transform infrared (FTIR)

Fourier transform infrared (FTIR) spectra were recorded with a Cary 630 FTIR spectrometer (Agilent Technologies, USA) using a diamond-ATR sample module. Each acquisition was the average of 64 scans (650 - 4000 cm\(^{-1}\)) and was background corrected.

LanM labelling with fluorescein

LanM (0.3 mL, 2 mM) was exchanged into 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (10 mM; pH 7) with 100 mM KCl and 8 mM Nd\(^{3+}\) using a spin column (Zeba™ Spin Desalting Columns, 7K MWCO, ThermoFisher Scientific). Fluorescein NHS ester (1 mg) was dissolved in dimethyl sulfoxide (DMSO, 30 µL) and added into the LanM solution at ~ 3 mg/mL final concentration. After a 3 h incubation at room temperature, the untagged dyes were removed using a spin column (Zeba™ Spin Desalting Columns, 7K MWCO, ThermoFisher Scientific).

Confocal Microscopy

For confocal microscopy, fluorescein labeled LanM was used for immobilization on agarose microbeads. The microbeads were then dropped onto a microscope glass coverslip. The samples were imaged by a Zeiss LSM 710 confocal microscope equipped with a 10X NA 0.3 objective. A 488 nm wavelength laser was used to excite the fluorescein in the microbeads. 3-dimensional fluorescence images and bright-field images were acquired simultaneously.

Breakthrough column experiments

Econo-Column glass chromatography columns (Bio-Rad; 5 cm × 0.5 cm) were filled with MilliQ water (18.2 MΩ cm\(^{-1}\)) and then LanM-microbeads were added gravimetrically. Columns were washed with 25 mM HCl, MilliQ water, and conditioned with buffer solution (same buffer for
breakthrough experiment, see below) before conducting breakthrough experiments. REE stock solutions were prepared by dissolving individual REE chloride salts in 1 mM HCl. The stock solutions were diluted either in 10 mM buffer (pH 5: Homopiperazine-1,4-bis(2-ethanesulfonic acid, Homo-PIPES); pH 4: acetate acid; pH 3.5 – 2.2: glycine) or HCl at the desired pH. The REE solutions were pumped at 0.5 mL/min unless otherwise specified and the column effluent was collected in 1.0 mL aliquots. A washing step with 5 bed volumes of MilliQ water was included before performing desorption experiments. For single REE ion solutions, REE ion concentrations were quantified by Arsenazo III assay. Specifically, 40 µL of sample was combined with 40 µL of 12.5 wt.% trichloroacetic acid (TCA) and then added to 120 µL of filtered 0.1 wt.% Arsenazo in 6.25 wt.% TCA. Absorbance at 652 nm was measured and compared to standards to determine the REE metal ion concentrations. The accuracy of the colorimetric assay was also confirmed in our previous work by ICP-MS. For experiments with REE mixtures or leachates, the metal ion concentrations were determined by ICP-MS.

For the REE pair separation experiments, the metal ion purity is defined as:

\[
Purity_{REE1} = \frac{C_{REE1}}{C_{REE1} + C_{REE2}}
\]

where \(C_{REE1}\) and \(C_{REE2}\) are molar concentration of REE1 and REE2, respectively.

The purification factor \(\beta_{ij}\) is defined as:

\[
\beta_{ij} = \frac{Y_i/Y_j}{X_i/X_j}
\]

where \(Y_i\) and \(Y_j\) are molar concentration of metal ions i and j in eluent, respectively, and \(X_i\) and \(X_j\) are molar concentration of metal ions i and j in feed, respectively.
Inductively coupled plasma mass spectrometry (ICP-MS)

The elemental composition of solutions was measured by ICP-MS as previously described.\textsuperscript{4, 5} The elemental composition of the multi-element (non-REE and Nd; Nd/Y) synthetic solutions and leachate solutions were determined using an Thermo Fisher Scientific iCAP RQ ICP-MS. In the case of Dy/Nd binary synthetic solution, ICP-MS analyses were performed on a Thermo X series II ICP-MS. In the case of PRB leachate breakthrough and non-selective desorption, the elemental composition was determined using an Agilent 7900 ICP-MS. All samples and calibration standards were acidified using concentrated nitric acid (70 wt%, purity $\geq 99.999\%$ trace metals basis, Sigma-Aldrich) to 2\% (v/v), and spiked with internal standards of In, Rh, and Bi to adjust for shifts in signal intensity during analysis. Samples were analyzed in both hydrogen (for Ca and Si) and helium (for all other elements) reaction gas mode to reduce doubly charged and polyatomic interferences. Due to the complex matrix of coal ash leachate, it was necessary to use correction equations to adjust for mass interferences with of $^{45}$Sc and $^{153}$Eu. A single replicate was used for the column breakthrough tests given the high sampling frequency throughout the breakthrough curves. All feed solutions were performed in triplicate. To describe the experimental uncertainty of metal ion quantification in complex synthetic solutions and leachate solutions, a coefficient of variation for each metal ion concentration was determined based on measurements made in triplicate with feed solutions.

Leaching and pH adjustment of PRB coal ash

Leaching and pH adjustment of PRB coal ash was performed as described previously.\textsuperscript{5, 6} Briefly, a PRB coal fly ash sample collected in 2017 from a pulverized coal-fired power plant was leached in 40 mL of a 1 M HCl solution for 4 h at 85 °C at a pulp density of 100 g/L. The leachate was cooled to room temperature and centrifuged at 3000 $\times$g for 15 min to remove any undissolved
particles. The supernatant of the leachate solution was then collected and 10 M NaOH was added dropwise to adjust the pH from 3.8 to 5 (as monitored by a combination pH electrode) to reduce the Fe content via precipitation. The pH-adjusted leachate was centrifuged again to remove precipitates formed during pH adjustment and then filtered using a 0.2 μm polypropylene filter.
SUPPLEMENTAL RESULTS

FT-IR

As shown in the Figure 1, the 1060 cm⁻¹ peak was assigned to a glycosidic bond that forms the backbone of the agarose polymer. In the SMCC spectrum, the peaks located at 1200 cm⁻¹ and 1706 cm⁻¹ were ascribed to succinimide and maleimide groups, respectively. After reaction of amine-functionalized agarose with SMCC, the presence of a 1706 cm⁻¹ peak and the lack of a 1200 cm⁻¹ peak indicates successful maleimide functionalization. Lastly, after the thiol-maleimide click chemistry, the LanM-agarose spectrum showed two peaks at 1650 cm⁻¹ and 1520 cm⁻¹, which correspond to Amide I and Amide II bands from the protein backbone, respectively, supporting immobilization of LanM.
### SUPPLEMENTAL TABLES AND FIGURES

#### Table S1. Metal ion concentration of synthetic feed solution (mM) in Figure 3.

|  | Average | Standard deviation | Coefficient of variation (%) |
|---|---------|-------------------|-----------------------------|
| Na | 14.01   | 0.14              | 1.00                        |
| Mg | 3.33    | 0.07              | 2.00                        |
| Al | 9.18    | 0.09              | 1.00                        |
| Ca | 2.32    | 0.19              | 8.12                        |
| Co | 1.93    | 0.01              | 0.77                        |
| Ni | 1.95    | 0.02              | 0.87                        |
| Cu | 1.82    | 0.02              | 1.00                        |
| Zn | 2.13    | 0.02              | 1.07                        |
| Nd | 0.19    | 0.00              | 0.86                        |

#### Table S2. Metal ion concentration of synthetic feed solution (mM) in Figure S2.

|  | Average | Standard deviation | Coefficient of variation (%) |
|---|---------|-------------------|-----------------------------|
| Fe | 0.94    | 0.01              | 0.86                        |
| Nd | 0.20    | 0.00              | 0.90                        |

#### Table S3. Metal ion concentration of synthetic feed solution (µM) in Figure 4 and Figure S3-5.

|  | 50/50 Feed |  | 95/5 Feed |  | Collected 56/44 Feed |  |  |
|---|------------|---|------------|---|----------------------|---|---|
| Nd | Average | Standard deviation | Coefficient of variation (%) | Average | Standard deviation | Coefficient of variation (%) | Average | Standard deviation | Coefficient of variation (%) |
|   |          |                        |                          |          |                        |                          |          |                        |                          |
| Nd | 178.92   | 0.59                    | 0.33                     | 337.52   | 0.92                    | 0.27                     | 26.76   | 0.23                    | 0.85                     |
| Dy | 179.37   | 0.92                    | 0.51                     | 17.63    | 0.07                    | 0.40                     | 20.93   | 0.14                    | 0.67                     |
| Nd | 20.93    | 0.14                    | 0.67                     |          |                        |                          |          |                        |                          |
| Dy | 20.93    | 0.14                    | 0.67                     |          |                        |                          |          |                        |                          |
Table S4. Comparison between the LanM column and reported methods for Nd/Dy separation

| Type                   | Method                              | Solvent or mobile phase | Dy purity Feed | Dy purity Product | Yield<sup>a</sup> | Experimental conditions | Process requirements and applications                                                                 | Ref. |
|------------------------|-------------------------------------|-------------------------|----------------|-------------------|-------------------|-------------------------|-----------------------------------------------------------------------------------------------------------------|------|
| Liquid-liquid extraction | Cyanex® 272                         | Kerosene                | 6.7%           | 47.1%             | 92.3%             | Phase ratio 1:1=Aqueous/organic, single stage | Currently used for mass production of REE; compatible with non-REE impurities (may require extra stage with different type of organic ligand); | 7    |
|                        | PC88A                               | Kerosene                | 16.4%          | 74.5%             | 99%               | Phase ratio 1:1=Aqueous/organic, three stages |                                                                                                                  | 8    |
|                        | PC88A                               | Kerosene                | 16.7%          | 94.6%             | 99%               | Phase ratio 1:1.5=Aqueous/organic, four stages |                                                                                                                  | 8    |
| Crystallization         | Chelating tripodal ligand RE(TriNOx) | Benzene                 | 50%            | 94.6%             | 90%               | Precipitation in benzene | Limited to batch operation; unknown compatibility with non-REE impurities.                                      | 9    |
|                        | Borate crystallization               | Aqueous                 | 50%            | 99.0%             | >99.9%            | 200 °C incubation for 5 days following by cooling for 2 days |                                                                                                                  | 10   |
| Ion exchange            | Chelation ion chromatography         | HNO<sub>3</sub> aqueous | 13.4%          | >99.9%            | >99%              | Elution ion exchange | Limited to analytical chemistry or small-scale REE purification.                                               | 11   |
|                        | Ligand-assisted displacement chromatography | EDTA+Cu              | 45%            | 99.2%             | 70%               | Displacement ion exchange | Comparable productivity to liquid-liquid extraction; unknown compatibility with non-REE impurities.             | 12   |
| Solid-phase extraction  | Solvent-impregnated resin            | HNO<sub>3</sub> aqueous | 16.7%          | 99.9%             | 66%               | Pure Dy solution applied as scrubbing process to upgrade product Dy purity | Pre-treatment required for removal of non-REE impurities.                                                        | 13   |
|                        | LanM column                          | HCl aqueous             | 50%            | 99.9%             | 76.2%             | Two pH-step desorption | Compatible with complex non-REE impurities; adaptable to current adsorption process for continuous operation (i.e. column rotation); improved loading capacity required for scale-up. | This work |
|                        |                                     | HCl aqueous             | 5%             | 46.1%             | 88.6%             | Two pH-step desorption |                                                                                                                 |      |
|                        |                                     | Citrate+HCl aqueous     | 50%            | 99.1%             | 94.2%             | Citrate-pH step desorption |                                                                                                                 |      |
|                        |                                     | Citrate+HCl aqueous     | 5%             | 50.3%             | 73.9%             | Citrate-pH step desorption |                                                                                                                 |      |

<sup>a</sup> Yield calculations do not take into account feed recycling.

<sup>b</sup> Only a Nd/Dy separation step (Zone II column A) was used for direct comparison.
Table S5. Metal ion concentration of synthetic feed solution (µM) in Figure 5.

| Metal Ion | Average | Standard deviation | Coefficient of variation (%) |
|-----------|---------|--------------------|-----------------------------|
| Y         | 74.81   | 0.97               | 1.30                        |
| Nd        | 255.74  | 2.36               | 0.92                        |

Table S6. Metal ion concentration in PRB feed solution (µM) at pH 5.

| Metal Ion | Average   | Standard deviation | Coefficient of variation (%) |
|-----------|-----------|--------------------|-----------------------------|
| Li        | 203.010   | 9.856              | 4.855                       |
| Be        | 0.963     | 0.325              | 33.706                      |
| Na        | 61626.204 | 989.812            | 1.606                       |
| Mg        | 80210.044 | 1178.194           | 1.469                       |
| Al        | 858.709   | 16.698             | 1.945                       |
| Si        | 8.503     | 0.594              | 6.980                       |
| K         | 1739.691  | 32.054             | 1.843                       |
| Ca        | 273173.440| 1477.705           | 0.541                       |
| Sc        | 0.069     | 0.009              | 12.586                      |
| V         | 0.021     | 0.005              | 23.876                      |
| Mn        | 157.649   | 1.738              | 1.103                       |
| Fe        | 3.990     | 0.546              | 13.675                      |
| Co        | 27.054    | 0.165              | 0.610                       |
| Ni        | 50.702    | 0.421              | 0.830                       |
| Cu        | 94.046    | 0.577              | 0.613                       |
| Zn        | 127.133   | 0.324              | 0.255                       |
| Se        | 0.151     | 0.015              | 10.066                      |
| Rb        | 4.176     | 0.002              | 0.043                       |
| Sr        | 2057.752  | 9.659              | 0.469                       |
| Y         | 35.326    | 0.136              | 0.384                       |
| Ba        | 11.503    | 0.067              | 0.582                       |
| La        | 23.636    | 0.060              | 0.253                       |
| Ce        | 46.504    | 0.156              | 0.336                       |
| Pr        | 6.300     | 0.027              | 0.424                       |
| Nd        | 24.453    | 0.114              | 0.467                       |
| Sm        | 4.467     | 0.040              | 0.892                       |
| Eu        | 0.987     | 0.003              | 0.267                       |
| Gd        | 4.188     | 0.016              | 0.379                       |
| Tb        | 0.600     | 0.004              | 0.692                       |
| Dy        | 3.448     | 0.020              | 0.580                       |
| Ho        | 0.648     | 0.005              | 0.749                       |
| Er        | 1.780     | 0.016              | 0.916                       |
| Tm        | 0.224     | 0.001              | 0.279                       |
| Yb        | 1.256     | 0.020              | 1.585                       |
| Lu        | 0.185     | 0.004              | 1.927                       |
| Pb        | 2.443     | 0.004              | 0.151                       |
| Th        | 0.000     | 0.000              | 0.000                       |
| U         | 0.030     | 0.023              | 77.765                      |
Table S7. Metal ion concentration (µM) in the combined three most concentrated fractions of the PRB desorption solution. The uncertainty for each element was assumed based on the triplicate test from Table S5. Note: Compared with the feed composition in Table S5, HREEs (Tb-Lu, Y) are less enriched than LREEs (La-Gd) due to the column being run past the breakthrough point for the presentation of Figure 5A.

| Element | Average | Standard deviation | Coefficient of variation (%) |
|---------|---------|--------------------|-----------------------------|
| Li      | 0.011   | 0.000              | 0.000                       |
| Be      | 0.000   | 0.000              | 0.000                       |
| Na      | 199.627 | 4.997              | 2.503                       |
| Mg      | 19.309  | 0.155              | 0.804                       |
| Al      | 21.718  | 0.178              | 0.821                       |
| Si      | 12.628  | 0.124              | 0.979                       |
| K       | 148.153 | 2.448              | 1.653                       |
| Ca      | 74.565  | 0.204              | 0.273                       |
| Sc      | 0.339   | 0.001              | 0.356                       |
| V       | 0.000   | 0.000              | 0.000                       |
| Mn      | 1.487   | 0.007              | 0.445                       |
| Fe      | 5.206   | 0.162              | 3.115                       |
| Co      | 0.000   | 0.000              | 0.000                       |
| Ni      | 0.000   | 0.000              | 0.000                       |
| Cu      | 0.272   | 0.000              | 0.000                       |
| Zn      | 6.660   | 0.005              | 0.082                       |
| Se      | 0.092   | 0.004              | 3.854                       |
| Rb      | 0.023   | 0.000              | 0.030                       |
| Sr      | 0.339   | 0.001              | 0.229                       |
| Y       | 10.956  | 0.026              | 0.234                       |
| Ba      | 0.000   | 0.000              | 0.000                       |
| La      | 177.838 | 0.564              | 0.317                       |
| Ce      | 448.001 | 6.465              | 1.443                       |
| Pr      | 63.554  | 0.116              | 0.183                       |
| Nd      | 247.153 | 1.892              | 0.765                       |
| Sm      | 44.894  | 0.119              | 0.266                       |
| Eu      | 9.838   | 0.007              | 0.075                       |
| Gd      | 36.405  | 0.063              | 0.173                       |
| Tb      | 4.307   | 0.013              | 0.309                       |
| Dy      | 14.679  | 0.044              | 0.302                       |
| Ho      | 1.200   | 0.005              | 0.420                       |
| Er      | 1.506   | 0.007              | 0.487                       |
| Tm      | 0.067   | 0.000              | 0.169                       |
| Yb      | 0.346   | 0.003              | 0.874                       |
| Lu      | 0.050   | 0.000              | 0.943                       |
| Pb      | 0.000   | 0.000              | 0.000                       |
| Th      | 0.000   | 0.000              | 0.000                       |
| U       | 0.028   | 0.005              | 19.369                      |
Table S8. Metal ion concentration (µM) in the PRB leachate feed, and three desorption zones in Figure 5E and 5F.

|       | Feed Average | Standard deviation | Coefficient of variation (%) | Front | Middle | Rear |
|-------|--------------|--------------------|------------------------------|-------|--------|------|
| Li    | 221.220      | 8.278              | 3.742                        |       |        |      |
| Be    | 1.247        | 0.057              | 4.530                        |       |        |      |
| Na    | 83262.588    | 2974.199           | 3.572                        |       |        |      |
| Mg    | 84478.993    | 2756.163           | 3.263                        | 5.246 | 0.743  | 0.044|
| Al    | 981.596      | 22.688             | 2.311                        | 10.474| 2.076  | 1.725|
| K     | 1910.022     | 55.005             | 3.572                        |       |        |      |
| Ca    | 126773.564   | 3260.582           | 2.572                        | 7.645 | 1.500  | 1.030|
| Sc    | 0.021        | 0.000              | 1.089                        | 0.012 | 0.009  | 0.045|
| Ti    | 546.018      | 13.212             | 2.420                        | 0.318 | 0.290  | 0.291|
| V     | 0.009        | 0.000              | 1.530                        |       |        |      |
| Cr    | 0.028        | 0.001              | 3.869                        | 0.015 | 0.015  | 0.100|
| Mn    | 165.665      | 3.303              | 1.994                        | 0.044 | 0.024  | 0.019|
| Fe    | 3.011        | 0.128              | 4.244                        | 1.103 | 0.644  | 0.889|
| Co    | 26.208       | 0.119              | 0.455                        | 0.003 | 0.000  | 0.001|
| Ni    | 48.771       | 0.689              | 1.413                        | 0.375 | 0.012  | 0.043|
| Cu    | 82.732       | 1.478              | 1.786                        | 1.977 | 0.122  | 0.108|
| Zn    | 107.892      | 1.259              | 1.167                        | 0.226 | 0.214  | 0.236|
| Se    | 2.799        | 0.050              | 1.784                        | 1.977 | 3.010  | 7.355|
| Rb    | 4.641        | 0.009              | 0.193                        | 0.120 | 0.033  | 0.002|
| Sr    | 1929.365     | 4.871              | 0.252                        | 0.684 | 0.076  | 0.032|
| Y     | 34.502       | 0.176              | 0.510                        | 77.459| 13.651 | 0.152|
| Cd    | 0.755        | 0.001              | 0.157                        | 0.000 |        | 0.001|
| Cs    | 0.218        | 0.002              | 1.021                        | 0.000 |        | 0.000|
| Ba    | 11.581       | 0.101              | 0.868                        | 0.007 | 0.011  | 0.005|
| La    | 25.551       | 0.087              | 0.341                        | 16.677| 23.929 | 41.284|
| Ce    | 49.069       | 0.229              | 0.468                        | 11.680| 17.585 | 115.726|
| Pr    | 6.230        | 0.023              | 0.375                        | 0.860 | 1.316  | 15.781|
| Nd    | 21.673       | 0.110              | 0.508                        | 2.696 | 4.208  | 55.799|
| Sm    | 4.328        | 0.036              | 0.828                        | 0.334 | 0.556  | 10.439|
| Eu    | 1.145        | 0.005              | 0.447                        | 0.173 | 0.283  | 2.445|
| Gd    | 4.266        | 0.016              | 0.370                        | 2.425 | 3.680  | 6.761|
| Tb    | 0.645        | 0.003              | 0.409                        | 0.606 | 0.794  | 0.701|
| Dy    | 3.490        | 0.020              | 0.559                        | 4.891 | 4.736  | 1.738|
| Ho    | 0.690        | 0.004              | 0.560                        | 1.311 | 0.773  | 0.084|
| Er    | 1.835        | 0.008              | 0.460                        | 3.951 | 1.257  | 0.071|
| Tm    | 0.235        | 0.002              | 0.665                        | 0.579 | 0.078  | 0.001|
| Yb    | 1.243        | 0.005              | 0.428                        | 2.943 | 2.111  | 0.016|
| Lu    | 0.189        | 0.003              | 1.410                        | 0.457 | 0.013  | 0.004|
| Pb    | 2.165        | 0.024              | 1.113                        | 0.749 | 0.004  | 0.002|
| U     | 0.024        | 0.000              | 1.049                        | 0.057 | 0.002  | 0.002|
**Figure S1.** Stoichiometric titrations of LanM-Cys with La(III) demonstrate binding of 3 equiv. of REEs. A) Competitive titration using xylenol orange as an indicator, performed in Chelex-treated 20 mM MES, 100 mM KCl, pH 6.0. B) The conformational response of LanM to La(III) can be tracked as a shift in the A$_{285\text{nm}}$. Titration performed in Chelex-treated 30 mM MOPS, 100 mM KCl, pH 7.0.

**Figure S2.** Nd breakthrough curves in the presence and absence of Fe$^{3+}$. Feed: Nd+Citrate: 0.2 mM Nd, 20 mM citrate, pH 3; Nd+Fe+Citrate: 0.2 mM Nd, 0.9 mM Fe, 20 mM citrate, pH 3.
Figure S3. (A) Accumulative desorption profiles of single-element independently loaded columns using a stepwise citrate concentration (pH 5) scheme; (B) A feedstock comprising a 22:78 mixture of Y:Nd was loaded to 75% column saturation and then subjected to a two-step desorption scheme using 10 mM citrate (pH 5, bed volumes 1-10) followed by pH 1.7. The values above each panel indicate the purity of REE over each elution zone divided by vertical dot lines. The detailed feed composition and measurement uncertainty are listed in Tables S3 and S5. 1 bed volume = 0.94 mL.
Figure S4. Dy/Nd separation using a two-pH and pH-citrate desorption scheme. The values above each panel indicate the purity of REE over each elution zone divided by vertical dotted lines (yellow). The duration of each pH step is depicted by the gray dashed line. Experimental conditions: REE solutions at pH 3 were used to load the column to 75% saturation; Feed composition: (A) and (C) Dy:Nd=50:50, (B) and (D) Dy:Nd=5:95. Desorption condition: (A) pH 2.1 and pH 1.7, (B) pH 2.2 and pH 1.7, (C) 15 mM citrate (pH 5, bed volumes 1-10) and pH 1.7, and (D) 10 mM citrate (pH 5, bed volumes 1-10) and pH 1.7. The detailed feed composition and measurement uncertainties are listed in Table S3 and Table S5. 1 bed volume = 0.94 mL.
In order to perform a coupled two-step desorption (Figure 4E and F), a feedstock comprising a 5:95 mixture of Dy:Nd was subjected to 5 cycles of a two-step pH desorption scheme. The individual Nd and Dy concentrations for cycle 1 were measured by ICP-MS as shown in Figure 4E. The total REE (Nd+Dy) concentrations for each cycle were quantified using an Arsenazo III assay and plotted here. 1 bed volume = 1 mL. The collected desorption profiles are almost identical, suggesting the high reproducibility of the column experiments.

Desorption profiles of a Powder River Basin (PRB) fly ash leachate using the LanM agarose column. Experimental conditions: 35 bed volumes of PRB fly ash leachate was pumped through a 0.8 mL column, followed by a washing step with 5 bed volumes of pH 3.5 water. Desorption was performed with 10 bed volumes of pH 1.5 HCl.
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