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RESEARCH ARTICLE

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A novel protein for bioremediation of gadolinium waste

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Abstract

Several hundreds of tons of gadolinium-based contrast agents (GBCAs) are being dumped into the environment every year. Although macrocyclic GBCAs exhibit superior stability compared to their linear counterparts, we have found that the structural integrity of chelates is susceptible to ultraviolet light, regardless of configuration. In this study, we present a synthetic protein termed GLamouR that binds and reports gadolinium in an intensiometric manner. We then explore the extraction of gadolinium from MRI patient urine as a preventative measure for gadolinium pollution and investigate the viability of employing cost-effective bioremediation techniques for treating contaminated water bodies. Based on promising results, we anticipate proteins such as GLamouR can be used for detecting and mining rare earth elements beyond gadolinium and hope to expand the biological toolbox for such applications.

KEYWORDS
binding, bioremediation, gadolinium, gadolinium waste, lanmodulin, protein, rare earth elements, rare earths

1 | INTRODUCTION

Many critical decisions in the clinic today rely on Magnetic Resonance Imaging (MRI). Due to its large magnetic moment and a long electronic relaxation time, gadolinium (^{64}Gd) is commonly used to enhance the contrast of the MR image (Caravan et al., 1999; Le Fur & Caravan, 2019). It is estimated that more than 30 million doses of gadolinium-based contrast agents (GBCAs) are administered to patients annually (Runge, 2015), which are then excreted into the environment. Any unused portions of each bottle/syringe are also discarded, totaling up to several hundred tons of GBCAs being emptied into our wastewater every year. The accumulation of gadolinium in our environment is being addressed with increasing urgency since the second decade of the 21st century; recent studies show anthropogenic gadolinium to be lurking in our

wastewaters, crops, tap water, and even marine wildlife (Brunjes & Hofmann, 2020; Ebrahimi & Barbieri, 2019).

One side effect of administering gadolinium-containing products in vivo would be the accumulation inside our bodies. It has been reported that repeated administration of GBCAs leads to gadolinium retention inside the skin, bones, brain, and muscle (Gulani et al., 2017). The species of which gadolinium exists (chelated, non-chelated, bound to other compounds such as phosphates, etc.) has yet to be proven with hard evidence. However, if gadolinium breaks free from its chelates, it would be possible for non-chelated, free gadolinium to bind to receptors that should otherwise be binding calcium (Sherry et al., 2009). This would hinder any biological functions requiring calcium, such as muscle contraction or neuronal activity – typically found in symptoms associated with Nephrogenic Systemic Fibrosis and Gadolinium Deposition Disease (Ersoy & Rybicki, 2007; Ramalho et al., 2017; Semelka et al., 2016). Accumulation of gadolinium in the environment has now been going on for more than 30 years since the introduction of gadopentetate in 1988, with increasing GBCA administration every year.

The current state-of-the-art for disposing of radioactive waste is to collect and store it for 10 half-lives or until the activity has decayed to less than 0.1% (Khan, 2010). Pharmaceutical waste is treated and incinerated at legally registered, licensed facilities (Windfeld & Brooks, 2015). GBCAs, on the other hand, do not have any regulations in terms of proper disposal – the rationale being that gadolinium is safe to use when properly chelated. Many GBCAs have been FDA-approved for use in the clinic for their safety during bodily circulation and the unparalleled contrast they provide for better healthcare. Although gadolinium retention inside the body has recently come to light, the benefits of GBCAs still undoubtedly outweigh the possible side effects of repeated administration in critical patients (Fraum et al., 2017). However, the long-term effects of GBCAs being disposed of into the environment (and, ultimately, the human body, which consumes products of the environment) have not been fully considered.

As a potential solution to mitigate Rare Earth Element (REE) pollution, several methylotrophic bacteria have been shown to uptake and store light lanthanides and are being proposed as a potential tool for bioremediation and biomining (Peplow, 2021; Skovran & Martinez-Gomez, 2015). Furthermore, we have recently developed a mutant of *Methylobacterium* (Leducq et al., 2022)/*Methylorubrum extorquens* AM1 that has acquired the ability of hyperaccumulating heavy lanthanides such as gadolinium (Good et al., 2022). The methylotrophic bacteria express a unique protein with the ability to bind lanthanides with picomolar affinity, termed lanmodulin (Cotruvo Jr. et al., 2018). Lanmodulin binds lanthanides via EF-hand motifs that are 12 amino acids long (Gutenthaler et al., 2022). Due to the similarity of these lanthanide-binding motifs to calcium-binding motifs, we used the backbone of a well-established genetically encoded calcium indicator in the GCaMP family (Chen et al., 2013) to construct a new synthetic protein termed Green Lanmodulin-based Reporter (GLamouR), which reports gadolinium binding through green fluorescence.

2 | RESULTS

2.1 | Protein design

Many genetically encoded calcium indicators incorporate two pieces of a circularly permuted fluorescent protein that come together to constitute a fully functioning state (Akerboom et al., 2012; Akerboom et al., 2013; Inoue et al., 2015; Kostyuk et al., 2019; Nakai et al., 2001; Shen et al., 2018; Tian et al., 2009; Yongxin Zhao et al., 2011). In such proteins, calmodulin is known to wrap around and interact with the M13/RS20 domain upon binding calcium, which effectively restricts water access to the chromophore, resulting in deprotonation to an anionic, fluorescent state (Akerboom et al., 2009). Due to the similarity in primary structure for calcium-binding motifs to lanthanide-binding motifs, it was hypothesized that a hybrid of lanmodulin and calmodulin would be compatible with the M13 while maintaining its selectivity for gadolinium over calcium. Figure 1a depicts the protein map on the left, with a 2-dimensional representation of conformational change on the right. After constructing the protein *in silico*, DNA was purchased and cloned into an expression vector for *E. coli*, which was transformed with the purified plasmid (See also supporting material and Materials and Methods). Expression and purification of the protein were subsequently carried out, followed by verification of the identity of the protein via LC/MS/MS (Figure S1) before assessing function and characteristics.

2.2 | Rapid detection and quantification of gadolinium

Gadolinium detection capabilities of GLamouR were assessed with fluorescence spectroscopy. Upon binding gadolinium, the protein increases its green fluorescence (Figure 1b). Ten readings were measured and averaged for each datapoint every 10 s, and the reaction was found to be within this window of time, suggesting rapid binding kinetics. As seen in Figure 1c, the fluorescence increases immediately after injection and does not increase as more time is given, with slight photobleaching resulting from repeated reads. TRIS buffer and calcium were also tested as negative controls, showing a slight decrease in fluorescence due to the lowered concentration of protein upon administering

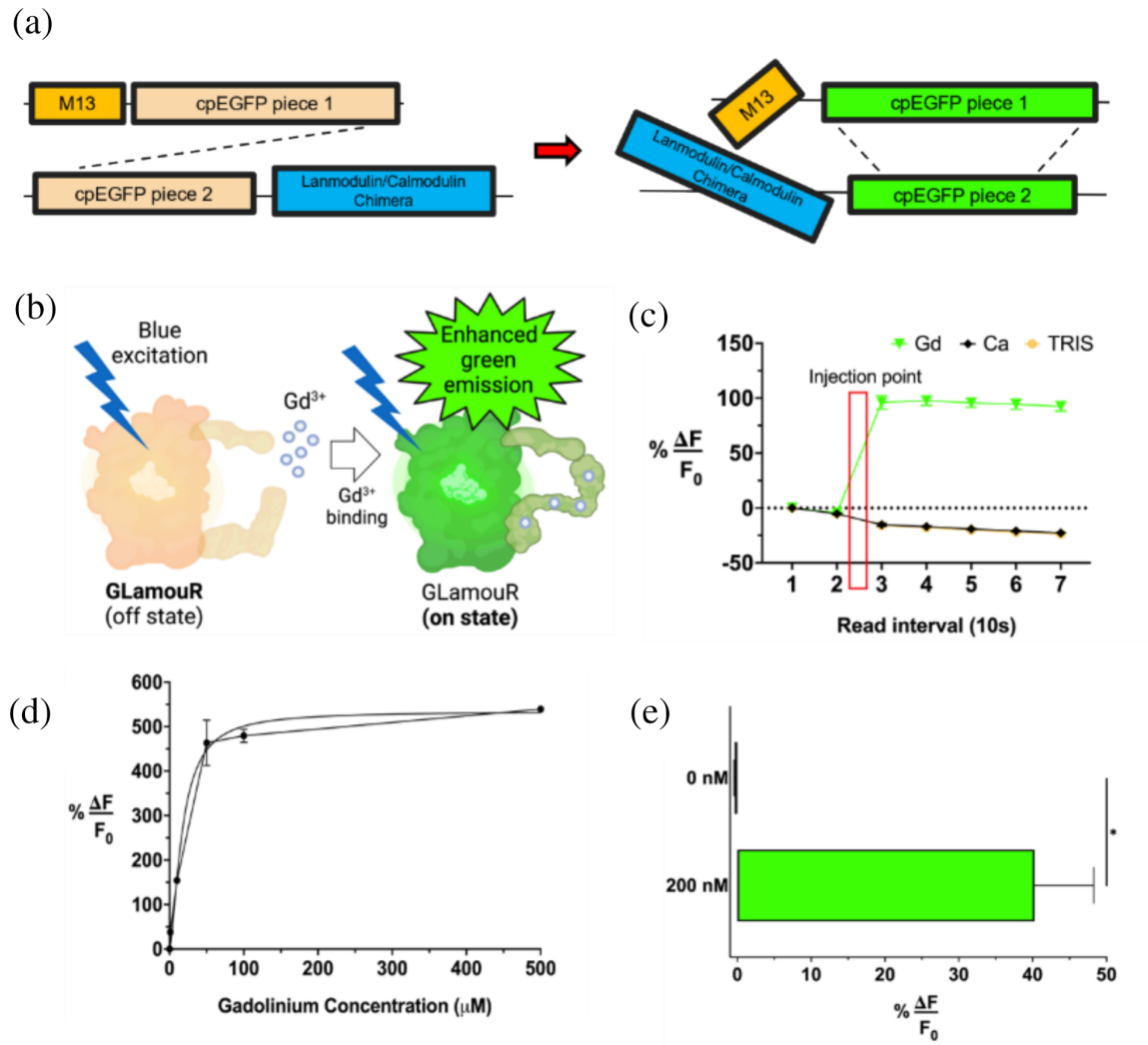

FIGURE 1 Fluorescence properties of GLamouR. (a) A map of the protein in its native state on the left, with the hypothesized compatibility of the lanmodulin/calmodulin chimera with M13 on the right. (b) Schematic representation of fluorescence and GLamouR-Gd^{3+} binding. GLamouR in its "on" state will possess enhanced green emission upon excitation with blue light. (c) Fluorescence kinetics of GLamouR with gadolinium (Gd), calcium (Ca), and TRIS buffer injection, which were administered after the second datapoint (10 reads per datapoint). (d) Fluorescence saturation curve for GLamouR with gadolinium shows a linear response up to 50 μM. (e) 200 nM concentrations of gadolinium were detectable with GLamouR with a 40% increase in fluorescence upon injection. Statistical significance was calculated by an unpaired *t*-test with Welch's correction, *p*-value = 0.0128 (two-tailed).

negative controls, followed by slight photobleaching of the chromophore over consecutive reads.

With increasing concentrations of gadolinium chloride hexahydrate in separate wells, the protein's saturation kinetics were observed. Figure 1d shows that GLamouR has a linear response up to 50 μM, suggesting its capability of quantifying gadolinium in the micromolar range. Furthermore, GLamouR is reliably detectable at 10 nM (Figure S2a) and is sensitive enough for 200 nM concentrations of gadolinium (Figure 1e). Gadolinium detection assays were demonstrated to be effective in the pH range of 6–8 (Figure S3), with the limit of detection calculated to be 98 nM and the limit of quantification being 425 nM. Saturation curves for other REEs are reported in Figure S4. GLamouR was also tested for its thermal/temporal stability by leaving a 20 μM solution of GLamouR at room temperature for a period of 1 week (Figure S2b). Although there was a slight decline in performance over time, GLamouR was able to maintain functionality for the entirety of the week. As it is with most proteins repetitive freeze/thaw cycles are likely to reduce protein activity and are not recommended.

2.3 | Verification of gadolinium binding via membrane filtration and MRI

Membrane filtration and MRI were used to verify that GLamouR was in fact capturing gadolinium and not just

reporting its presence in an indirect manner. After binding GLamouR with gadolinium, the solution was dialyzed to remove excess gadolinium and subsequently filtered with washed membranes to isolate the protein (Figure S5). The retentate would therefore hold GLamouR-Gd conjugates while the filtrate would be the surrounding buffer. T1 and T2 relaxation times are shown in Figure 2a,b, with their corresponding T1 and T2 maps (Figure 2c,d). Figure 2a–d show the filtrate containing insignificant levels of gadolinium after dialysis, whereas the protein was able to hold on to gadolinium at high levels of centrifugal force and possess MRI contrast. By plotting relaxation rates as a function of gadolinium concentration, we obtained the r1 and r2 relaxivities of GLamouR-Gd conjugates, which were 6.0 and 41.85 mM^{-1} s^{-1} respectively. These numbers represent the enhanced relaxivity of gadolinium upon binding GLamouR (Table S1) (Noebauer-Huhmann et al., 2010; Rohrer et al., 2005; Shen et al., 2015; Szomolanyi et al., 2019; Wahsner et al., 2019), which is likely to be resultant from the tumbling time shifting closer to the Larmor frequency as a conjugate, when compared to free gadolinium. To obtain higher concentrations of GLamouR-Gd conjugates, GLamouR was

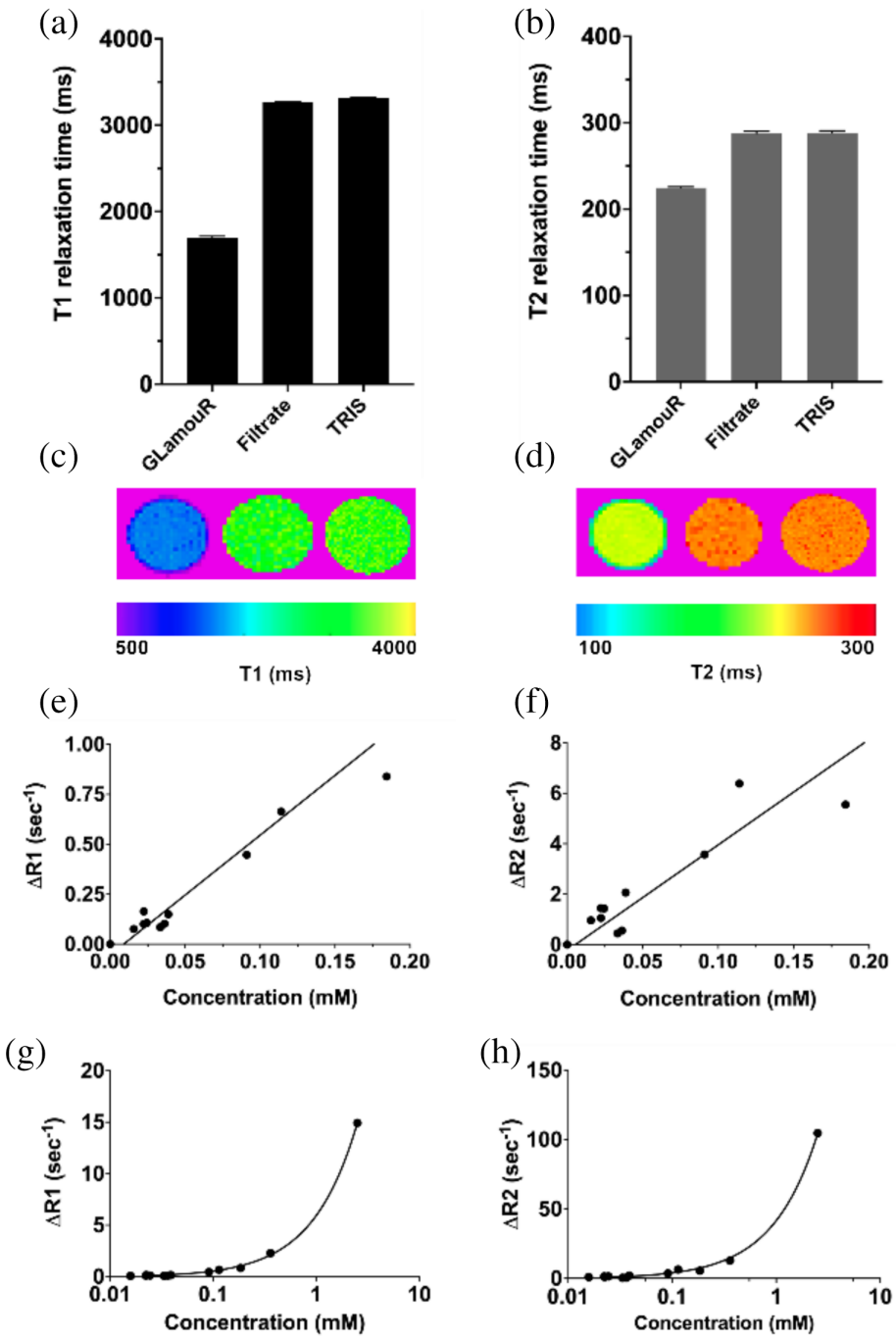

FIGURE 2 MRI data confirm protein-Gd binding. GLamouR was bound with gadolinium and dialyzed thoroughly prior to the separation of filtrate (surrounding buffer) and retentate (GLamouR-Gd conjugates). T1 (a) and T2 (b) relaxation times were compared against TRIS buffer by averaging the ROIs of their corresponding T1 (c) and T2 (d) maps. r1 and r2 relaxivities (per-gadolinium) were derived from 13 dilutions across six different batches of GLamouR. Linear regression for R1 and R2 datapoints as a function of gadolinium concentration is shown in linear (e, f) and logarithmic scale (g, h), with $p < 0.00001$ and $r^2 = 0.999$.

lyophilized, reconstituted at a lower volume with DDIW, and dialyzed with fresh TRIS buffer at 25 mM to match the salt concentrations before initiating gadolinium binding. Compatibility with centrifugal force, lyophilization, and long dialysis times demonstrates the durability and stability of GLamouR. Thermal properties were also assessed with a thermal shift assay suggesting tighter folding at temperatures closer to 45°C, but minimal variance in performance was found due to temperature (Figure S6).

2.4 | GBCAs used in the clinic today are susceptible to breakdown from UV irradiation regardless of configuration

Macrocyclic chelates have been proven to be more stable than linear chelates and thus are considered safer to use (Morcos, 2008). Although GBCAs are used immediately upon opening in clinical practice, it is not uncommon for researchers to keep bottles of GBCAs for prolonged periods of time for in vitro studies.

In Figure 3a, various contrast agents that had been open for an unknown period of time were tested for their stability. GLamouR was used to detect transmetalation from unstable chelates that had released gadolinium. As expected, macrocyclic agents were more stable than linear configurations, with Gadopentetate Dimeglumine triggering the highest fluorescence increase (which is currently discontinued for use in the clinic). However, upon 24 h of UV irradiation, the GBCAs had similar amounts of free gadolinium in solution regardless of configuration (with the exception of Gadopentetate Dimeglumine, which was much more susceptible to UV breakdown than other chelates). Figure S7 shows LC-ESI-MS data confirming the breakdown of Gadopentetate Dimeglumine under UV irradiation.

To emulate urine samples from GBCA-administered MRI patients, artificial urine was spiked with the most stable GBCA of the group (Gadoterate Meglumine) to a concentration of 2.25 mM, equivalent to a dose for a 50 kg patient with 90% of GBCA eliminated in 2 L (Figure 3b) for conservative calculation. Samples were then pre-treated by removing phosphates, which compete with GLamouR for gadolinium binding, prior to UV irradiation. Once gadolinium had been released from its chelates, GLamouR was introduced to the solution for

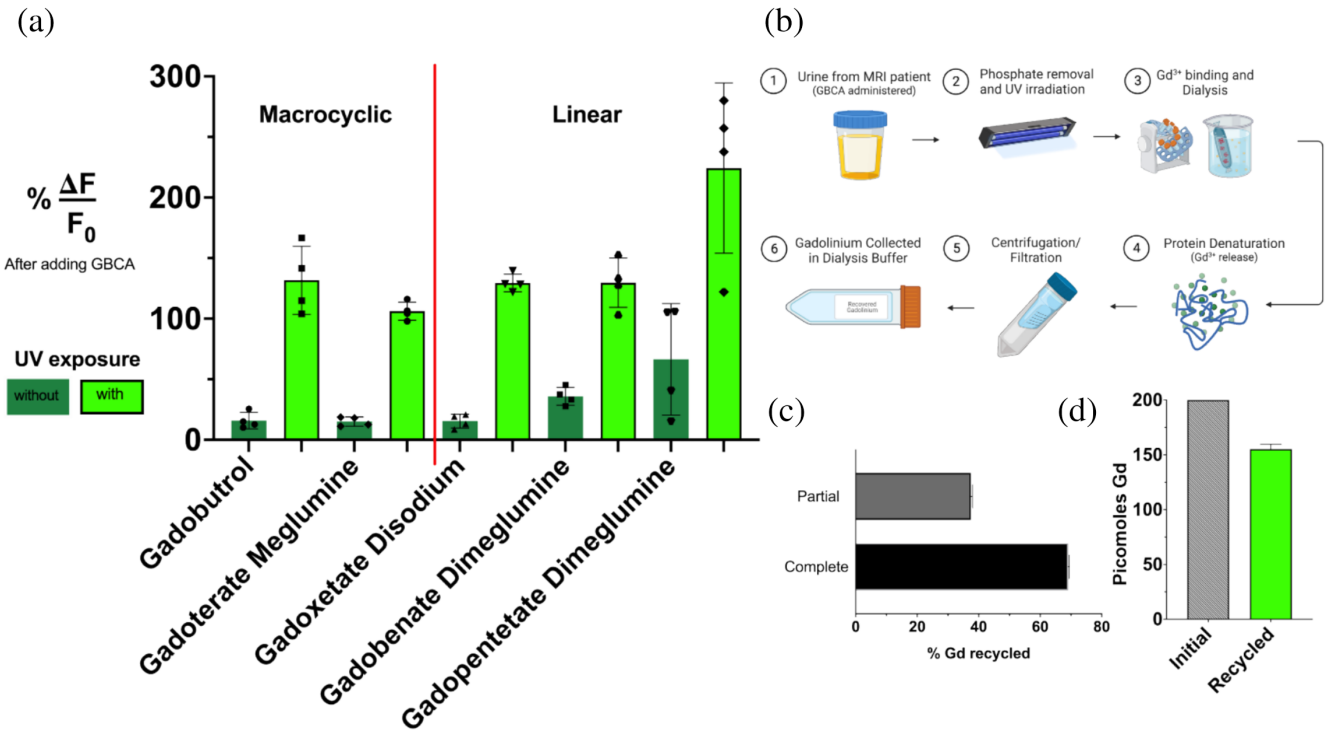

FIGURE 3 Exploring the collection of gadolinium from clinical GBCAs. (a) Clinical GBCAs were tested for unchelated gadolinium ions with GLamouR, before and after UV irradiation. (b) Schematic showing the proposed procedure for collecting gadolinium from MRI patient urine samples, which was substituted for Gadoterate Meglumine-spiked artificial urine in this study. (c) Recycled gadolinium after partial removal and complete removal of phosphates from Gadoterate Meglumine-spiked artificial urine samples. For complete removal, Gadoterate Meglumine was added after passing artificial urine through phosphate removal resin, whereas partial removal was emulated by adding Gadoterate Meglumine before passing through the column with a lower resin ratio. (d) Gadolinium scavenging capabilities at 200 pM. 200 picomoles of gadolinium chloride hexahydrate in 1 L TRIS were gathered and subsequently concentrated down to nanomolar concentration while retaining 75% of total gadolinium mass.

binding the released ions, which was then subsequently dialyzed to exclusively obtain gadolinium-protein conjugates. After the protein is denatured to release gadolinium, the solution can then be filtered, so the final product is gadolinium in TRIS buffer. Figure 3c shows that 37% of total gadolinium was bound to GLamouR with partial removal of phosphates, whereas 69% was bound when they were removed more efficiently, showing that pre-treatment of samples is an important factor for effective extraction.

GLamouR was also assessed for its ability to upcycle anthropogenic gadolinium at environmentally relevant concentrations. Anthropogenic gadolinium has been reported to exist at the picomolar scale in various polluted bodies of water, even including tap water (Ebrahimi & Barbieri, 2019). Gadolinium was therefore introduced into TRIS buffer to achieve a final concentration of 200 pM with a volume of 1 L. GLamouR was then used to collect the gadolinium, which was subsequently concentrated down to 100 μL. The final gadolinium concentration was measured via ICP-MS, and the total number of moles was calculated to be 150.07 picomoles, equivalent to 75% of the initial number of moles of gadolinium. Proper pre-treatment is indeed important to obtain a high percentage of collection, as seen in Figure 3c. However, it is demonstrated in Figure 3d that low concentrations of gadolinium do not pose a hurdle in collecting or filtering anthropogenic gadolinium from water.

Based on data represented in Figure 3a, we have found that gadolinium is most likely released from its chelates once GBCAs are excreted into the environment, especially in circumstances involving prolonged UV irradiation from the sun. However, we have also demonstrated that gadolinium can potentially be collected from GBCA-administered MRI patient urine samples before it gets excreted into the environment, which can be a preventative measure for such pollution.

2.5 | Recycling gadolinium waste with affordable technology

Figure S8a depicts a device setup in which the sample flows through a pre-treatment chamber for removing competing compounds such as phosphates, which may then also include UV irradiation to break any pre-existing bonds that could interfere with gadolinium collection. The GLamouR column would be placed inside the detection unit, where an increase in fluorescence would indicate remaining gadolinium, and saturation of fluorescence would indicate the need for a fresh column. The flow of the sample would be continuously repeating until fresh columns do not increase in fluorescence, and the remaining waste could then be redirected for disposal. The existing columns could then be further processed to release gadolinium, as depicted in Figure 3b, steps 4–6. The advantage of this system would be that high volumes of gadolinium-containing waste in low concentrations could be concentrated to high concentrations and subsequently eluted for isolating gadolinium.

A prototype device (Figure 4a) was constructed with a budget of less than 500 USD. The outer shell was 3D-printed, with the bulk of the cost coming from the Arduino microcontroller and Hamamatsu spectrometer. Samples can be pumped in and out of the device by creating a pressure differential inside a bottle containing the sample by utilizing an air pump inflator typically found on sphygmomanometers. Figure 4b shows data collected from the device, where gadolinium concentration was brought up to 100 μM by injecting a 1 mM solution of gadolinium equaling 1/10th of the final volume into the sample chamber. In addition to this "chromatography" method, which would require the development of a GLamouR-immobilized column or hydrogel that would remain inside the filtration unit under a pressure differential, a different collection system can be implemented where the protein remains inside a dialysis device such as tubing, pouch, or conical tube membrane unit. This device can then be placed inside a sample of large volume to collect gadolinium overnight and be recovered the next day. Figure 4c shows a dialysis pouch tested with gadolinium in TRIS buffer, with the pouch before dialysis on the left and the pouch after dialysis on the right, showing increased fluorescence with the corresponding histogram shown in Figure S8b, which validated the feasibility of such a collection method.

A conical tube dialysis device was used to collect gadolinium from a pre-treated MRI patient (Australian shepherd, 6 years old, female) urine sample. In Figure 4d,e the GLamouR solution that had collected gadolinium possessed striking MRI contrast in comparison to GLamouR that had been dialyzed with artificial urine without gadolinium. The T1 relaxation time of GLamouR-Gd was also lower than that of the urine itself, which had been diluted and pre-treated in the same manner, suggesting a higher gadolinium concentration and/or increased per-gadolinium relaxivity upon binding GLamouR, as demonstrated in Figure 2. Figure 4f shows that the GLamouR mixture also increased in fluorescence, as it did for Figure 4c.

In essence, GLamouR can be lyophilized and packaged into dialysis devices such as the pouch in Figure 4c and used as a "reverse" teabag to collect REEs from samples of various volumes simply by dropping them in solution and harvesting when the mixture inside has saturated fluorescence. As it is with the chromatography method, the process can be repeated until fresh pouches do not increase in fluorescence, indicating an REE "depleted" sample. Based on the results in Figure 4c–f, it is anticipated that this collection method can be implemented in a

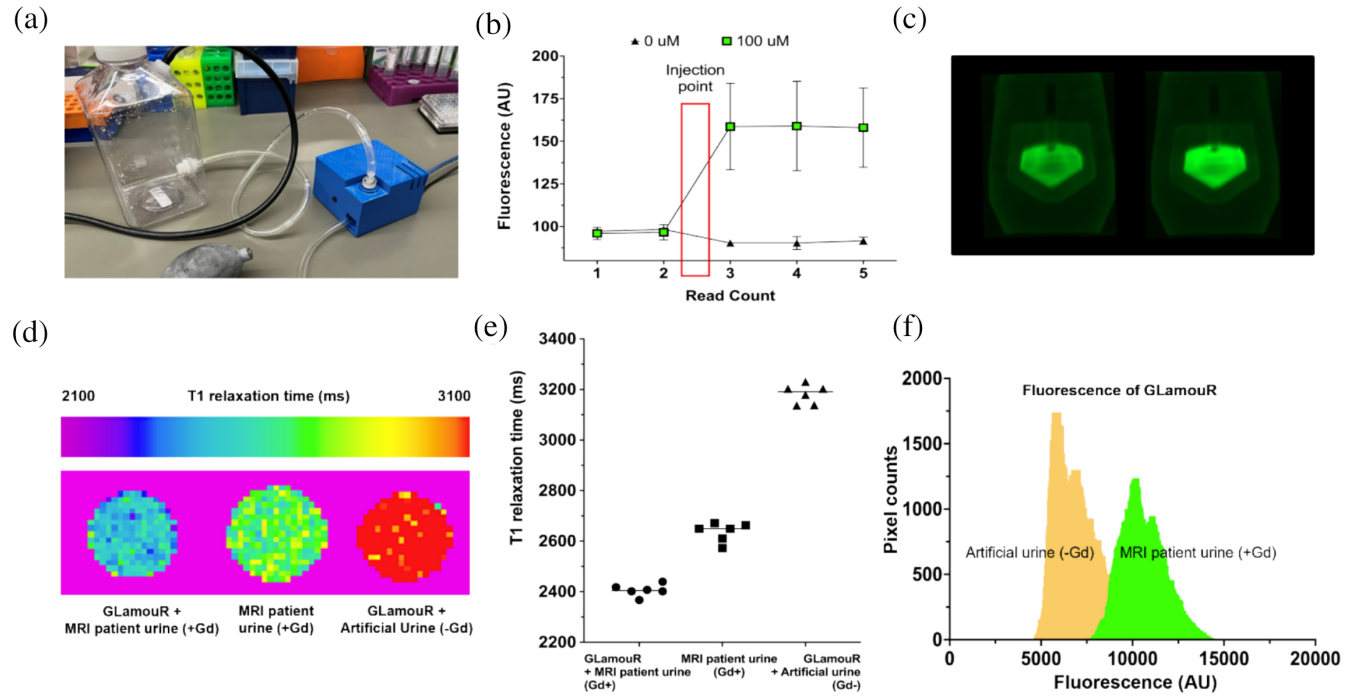

FIGURE 4 Prototype devices for collecting gadolinium from MRI patient urine samples (a) Picture of the prototype device constructed for employing a chromatography-style collection system. (b) Data produced with the prototype device for gadolinium detection show reliable detection on the micromolar scale with a production cost of less than 500 USD. (c) Fluorescence image of a different collection system utilizing a dialysis device. On the left is a dialysis pouch before initiating dialysis with gadolinium, with after dialysis on the right. (d) Gadolinium extraction from an MRI patient urine sample using a GLamouR-filled conical tube dialysis unit represented as a T1 map, with (e) average T1 relaxation times represented on a graph. T1 for <GLamouR+MRI patient urine (+Gd) > sample is thought to be lowest due to higher gadolinium concentration and/or increased per-gadolinium relaxivity. (f) Histogram representing a fluorescence image taken of the GLamouR-filled conical tube dialysis unit dialyzed against artificial urine without gadolinium versus MRI patient urine (with gadolinium).

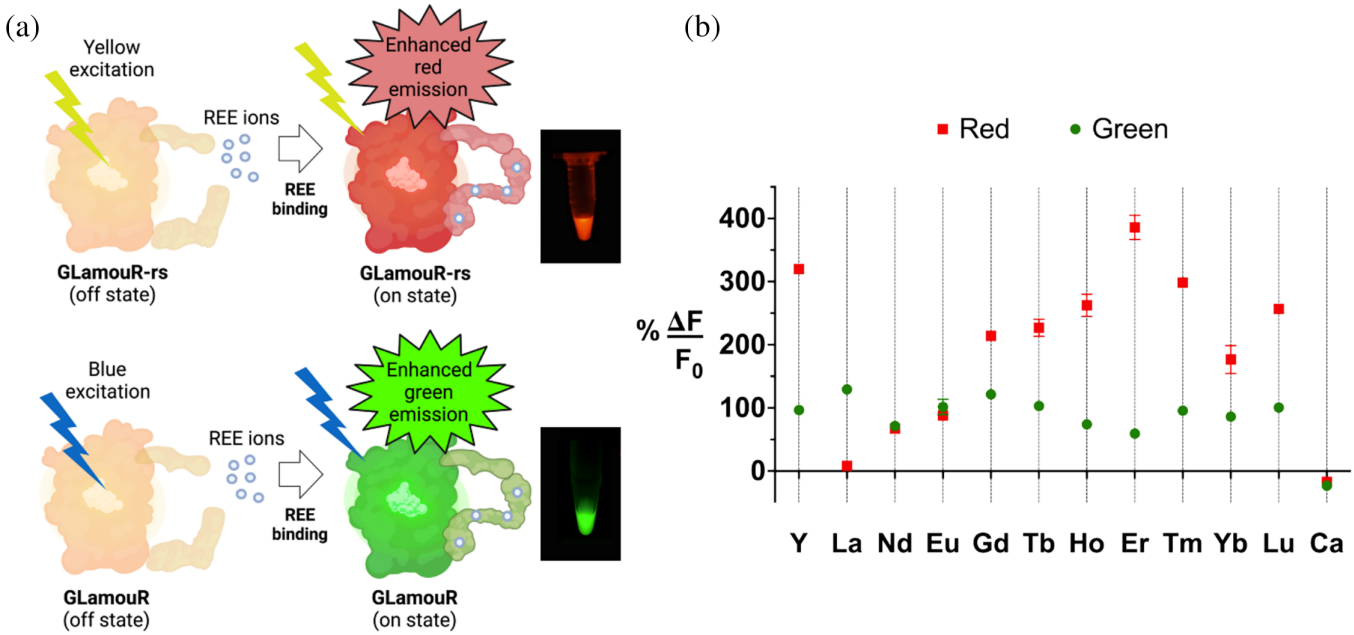

FIGURE 5 REE binding with GLamouR-rs and GLamouR. (a) Schematic of the red-shifted GLamouR (GLamouR-rs) and original GLamouR binding REEs, with their corresponding fluorescence images to the right. (b) Fluorescence increase of green and red-shifted GLamouR upon addition of 11 different REEs show a difference in their preference for certain REEs such as lanthanum (with calcium as negative control) or erbium. Non-saturating levels (25uL of 1 mM solutions in TRIS buffer) of REEs/non-REEs were added to wells containing protein. Due to brightness differences between the two proteins ($n = 4$ wells), GLamouR concentration was 100 nM while GLamouR-rs was at 10 uM so that their corresponding AUs would be similar values well within the dynamic range of the multimode plate reader.

widespread and inexpensive manner with low levels of required technical expertise.

2.6 | Identification and collection of REEs beyond gadolinium

Based on Figure 14, GLamouR is a novel candidate protein for the detection, quantification, and recycling of gadolinium waste. However, gadolinium is not the sole REE pollution resulting from industrial activities (Gwenzi et al., 2018), and thus further experiments were carried out to investigate the protein's responses to different REEs. As seen in Figure 5b, GLamouR was effective in detecting 11 REEs and distinguishing them from calcium. Change in fluorescence was not particularly unique for different REEs, as most of them lingered around the 100% region.

A red-shifted GLamouR was then created (Figure S9) to see if REEs could be identified due to unique REE responses when used in conjunction with green. While the GLamouR-rs has a much lower brightness in both its on and off states than the original green version, it produces a much higher delta across all REEs with the exception of Lanthanum (Figure 5), where the response was recorded to be very minimal (+8.26%). In this case, green fluorescence could be measured to quantify the REE, while the lack of fluorescence increase in red could specify the REE to be Lanthanum.

The creation of GLamouR proteins with unique responses to different REEs suggests the possibility of future GLamouR variants that bind with a stronger affinity or react more efficiently to specific REEs. This would make it feasible to utilize the systems depicted in Figure 4 for collecting and isolating specific REEs from environmental samples that contain a mixture of several REEs, as it is often the case in nature. Even with moderate specificities, low concentration mixtures could be accumulated to high enough concentrations for downstream separation to achieve higher yields for cost-effectiveness with minimum impurities of undesirable REEs.

In addition to mining and recycling specific REEs, it is hoped that using a series of GLamouR variants will allow users to determine compositions of REE mixtures and identify REE-rich sources in a rapid, affordable, and simplistic manner. Although urine samples from GBCA-administered patients have a pre-defined matrix with a guaranteed range of gadolinium concentration, this would not be the case for samples with diverse and unique compositions for every batch, such as electronic waste or samples obtained from the environment.

3 | DISCUSSION

REEs are an essential resource for modern technology – anything with a screen, lens, glass, lights, magnets, steel alloys, or batteries requires the use of REEs, as well as conventional "non-electric" vehicles that run on a form of fossil fuel (which also require a catalytic converter) (Balaram, 2019). Current methods for mining REEs require extensive use of harsh chemicals and intense labor, not to mention low yields and excessive byproducts (Liu & Chen, 2021). Moreover, not only do REEs exist on Earth in a finite amount, but their distribution is alarmingly reliant on REE-rich countries with affordable labor costs and looser regulations on environmental pollution arising from industrial practices (Abraham, 2015). On the bright side, efforts are being made to develop new technologies for mining REEs at a lower cost and for recycling them (Jowitt et al., 2018). The market for recycling REEs was evaluated at 248 million USD in 2021, and it is projected to grow at a rapid pace with a CAGR of 11.2%, reaching 422 million USD by 2026 (Markets and Markets, 2022).

It would thus be beneficial for nations to develop technology for recycling REEs, preferably at higher yields, cheaper prices, and hopefully with environmentally friendlier methods. In addition to utilizing microbes, cutting-edge technology involving the use of peptides and proteins for biomining is being developed by researchers as well (Deblonde et al., 2020; Featherston et al., 2021; Gao et al., 2021; Lederer et al., 2017; Mattocks et al., 2019; Mattocks & Cotruvo, 2020; Park et al., 2016). A combination of both technologies (Figure S10) could also be envisioned, where GLamouR is utilized as a genetically encoded indicator for monitoring the microbial (Good et al., 2022) collection of lanthanum.

Similarly, there are some studies that examine aspects related to those investigated in this work; a novel protein termed LaMP1 developed by Mattocks et al. (Mattocks et al., 2019) is a ratiometric reporter for REEs that utilizes Förster Resonance Energy Transfer between two fluorescent proteins of different emission wavelengths. In addition, lanmodulin has been incorporated into a device in the form of a chromatography column and is currently in development to be used as an effective means of REE separation (Deblonde et al., 2020; Mattocks et al., 2023). Another group has demonstrated the detection of linear GBCAs in urine (Pallares et al., 2020), which relies on a chelator to extract Gd^{3+} from the linear GBCAs, where adding extra Eu^{3+} elicits binding to the chelator to indicate empty chelators that have not extracted Gd^{3+}, which in turn correlates to lower gadolinium levels.

To our knowledge, this study is the first to have proved the irreversible increase of free gadolinium upon UV irradiation of GBCAs with a fluorescent protein-based reporter, undergone the technological development in the removal of gadolinium from MRI patient urine, and utilized the intensiometric fluorescence of a newly developed protein to monitor column/pouch saturation, all at an affordable price. We have

also concentrated gadolinium from picomolar to nanomolar while retaining roughly 70% of total gadolinium mass. These findings suggest that not only can gadolinium pollution be prevented, but also potentially reversed from its current state, which has been reported to reach up to nanomolar concentrations in natural waters. Aspects to consider for industrializing the application of such technologies would be the identification of reliable REE sources, performance and reproducibility across various sample types, and cost-effectiveness.

Once established on Earth, it is anticipated that GLamouR-like proteins could be used in outer space for extraterrestrial biomining. The near side of the Earth's moon is reported to possess massive reserves composed of a mixture of Potassium, REEs, and Phosphorus (KREEP), estimated to be 220 million cubic kilometers (McLeod & Krekeler, 2017). A recent study by Cockell et al. demonstrated extraterrestrial biomining of REEs where microorganisms were tested for their mining capabilities from basaltic rock in various micro-g environments on the international space station (Cockell et al., 2020). As it is for any type of travel but especially air and space travel, reducing cargo weight is essential for increasing distance per unit fuel. Compatibility with lyophilization to reduce weight during takeoff, simple mining procedures with light and affordable equipment, and instant feedback during REE collection could be desirable characteristics of GLamouR for REE mining in outer space.

The use of biological tools to carry out functions otherwise achieved through chemical, electrical, or mechanical means is often met with higher performance and cheaper production costs, such as the modern development and production of recombinant human insulin in *E. coli* (Johnson, 1983). Furthermore, biological therapies are the best-selling drugs on the market today (Urquhart, 2020), and new products that mimic (biosimilars) and even improve upon the precision and efficacy of traditional medications (biobetters) are being developed as well (Beck, 2011). Such technologies involving biological tools are expected to be tailored for their respective applications in each field, whether it be industrial practices, agriculture, precision medicine, or even unearthing new scientific discoveries.

AUTHOR CONTRIBUTIONS

Harvey D. Lee: Conceptualization; data curation; formal analysis; visualization; writing – original draft; writing – review and editing; project administration; investigation; methodology; validation; funding acquisition; resources; supervision; software. **Connor J. Grady:** Conceptualization; data curation; investigation; methodology; formal analysis; visualization. **Katie Krell:** Investigation; data curation; formal analysis; visualization. **Cooper Strebeck:** Investigation. **Aimen Al-Hilfi:** Investigation. **Brianna Ricker:** Investigation. **Melanie Linn:** Investigation; resources. **Nicole Y. Xin:** Investigation. **Nathan M. Good:** Supervision; writing – review and editing. **N. Cecilia Martinez-Gomez:** Supervision; writing – review and editing. **Assaf A. Gilad:** Writing – review and editing; project administration; resources; funding acquisition; validation; supervision; conceptualization; methodology.

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DATA AVAILABILITY STATEMENT

All data are available in the manuscript or the supplementary materials.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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