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Identification of perrhenate-binding peptides by phage display

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Pertechnetate is the most stable and highly environmentally mobile form of the radioactive element technetium. To explore the ability of peptides to remove this molecule from aqueous solutions, a phage display library was biopanned against immobilized perrhenate, a nonradioactive analog of pertechnetate. Six unique peptides were identified from the screen and their ability to bind perrhenate, both free in solution and immobilized on a solid support, was explored. It was found that the peptides, particularly when immobilized, were able to remove perrhenate from aqueous solutions, and despite not being screened for selectivity, demonstrated some preference for perrhenate over other anions such as chromate and nitrite. These results demonstrate the feasibility of using engineered biological systems for remediation of pertechnetate in the environment.

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Introduction

99-Technetium is a radioactive nuclide present in nuclear waste streams with a relatively long half-life of 2.13×10^5 years. The most stable chemical form is the pertechnetate anion (TcO_4^-), which is highly soluble in water and environmentally mobile, raising environmental and ecological concerns should it be released from those streams. Additionally, pertechnetate is volatile enough that only a fraction is incorporated during vitrification to immobilize nuclear waste into borosilicate glass.[1] These properties make technetium a priority for remediation and containment.

Rhenium, a non-radioactive element, exhibits very similar chemical behavior to technetium, and perrhenate (ReO_4^-) is widely employed as a surrogate for TcO_4^- in laboratory studies. Recent advances in TcO_4^- or ReO_4^- remediation have yielded materials such as pyridinium-based polymers,[2] bipyridine-polymers,[3] and cationic metal–organic frameworks[4] that have binding capacities of up to 1000 mg ReO_4^- per g of material and function over a broad range of pHs present in nuclear waste. Other approaches to immobilize pertechnetate involve materials such as organic polymers,[5–7] covalent organic frameworks,[8] and layered double hydroxides,[9] among others, or reduction of pertechnetate to a much less soluble form of technetium.[10,11] While these approaches to TcO_4^- immobilization are likely to greatly impact our treatment of nuclear waste streams, the importance of remediating this pollutant warrants developing alternative and complementary approaches.

Bioremediation, which uses plants and microorganisms to detoxify or immobilize pollutants in the environment, could be one such complementary approach.[12] For example, stimulating a microbial community lowered the presence of uranium in ground water at the U.S. Department of Energy in Oak Ridge, TN, presumably by enriching microbes that are able to reduce uranium(VI) to the sparingly soluble uranium(IV).[13] In lab based studies, bacteria and plants have been engineered to detoxify arsenite by methylating it *via* a naturally occurring arsenite methyl transferase enzyme,[14] detoxify methylmercury *via* a naturally occurring organomercurial lyase enzyme,[15] and immobilize cadmium using engineered surface proteins[16] among many other studies.[17] While there are no known proteins or peptides that bind TcO_4^- or ReO_4^-, engineering systems to do so could allow for the bioremediation of TcO_4^-.

A number of short amino acid motifs that bind anions such as phosphate and sulfate have been identified in proteins.[18] These include nests,[19] structural P-loops,[20] cups,[21] and $C^{\alpha}NN$ motifs.[22] These motifs range in length from three to eight amino acid residues and largely interact with the anions through hydrogen bonds with the amide backbone. While these motifs bind anions in the context of larger proteins that will reduce their flexibility and potentially enforce certain conformations, shorter peptides based on these motifs are able to bind phosphate outside of the context of a protein.[23,24] These phosphate and sulfate binding amino acid motifs raise the possibility of identifying motifs that are able to bind TcO_4^- or ReO_4^-.

Phage display is a technique that was originally developed for affinity maturation of antibodies.[25] While multiple platforms have been developed to anchor proteins or peptides to the

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surface of phage particles, a common approach is to genetically fuse genes encoding the library members to the gene encoding protein III of the M13 phage. This technique allows up to five copies of the same protein or peptide to be anchored to the surface of a phage particle. The phage particle also contains the DNA that encoded the library member, attaching the protein or peptide to the genetic information that encoded it. Phage are then mixed with the target, target binders are then pulled down, and the protein or peptide that mediates the interaction between the target and the phage can be identified by sequencing the genetic material that encoded it. This process allows for the screening of up to 10^9 different amino acids sequences for the ability to bind to a target. Phage display has been used to identify peptides with the ability to bind inorganic matter such as GaAs,[26] ZnS,[27] SiO_2,[28] TiO_2,[29] and many other examples.[30]

Here we report the identification of short peptides that can bind ReO_4^- using phage display and demonstrate their ability to remove the anion from aqueous solution when immobilized on resin.

Materials and methods

Commercial materials

Luria-Bertani (LB) Broth Miller, LB Agar Miller, and glycerol were purchased from EMD Biosciences (Darmstadt, Germany). Isopropyl-*b*-D-thiogalactopyranoside (IPTG), 5-bromo-4-chloro-3-indolyl-beta-D-galacto-pyranoside (X-gal), tris(hydroxymethyl) aminomethane hydrochloride (Tris–HCl), sodium chloride, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and tetracycline, were purchased from Fisher Scientific (Pittsburgh, United States). Dowex 1X8 200–400 (Cl), phosphate buffered saline, and sodium perrhenate were purchased from Sigma-Aldrich (St. Louis, United States). All 20 amino acids were purchased from Combi-Blocks, Inc. (San Diego, CA). All solvents were purchased from VWR International, LLC (Radnor, United States), and the organic solvents used were of analytical grade. The Phage Displayed Peptide library (Ph.D. 7) was procured from New England Biolabs (Ipswich, United States). UV-vis data were recorded on Cary 60 UV-vis spectrometer (Agilent Technologies, Santa Clara, United States). Purification was done using DIONEX UltiMate 3000 HPLC (Thermo Fisher Scientific, Waltham, United States).

Perrhenate immobilization

Experiments were performed as previously described.[31] 20 mg Dowex 1X8 200–400 (Cl) was packed in a glass pipette stoppered with cotton wool. 10 mL of normal saline was passed through the resin column followed by 5 mL of water. To determine the loading capacity of the packed column, sodium perrhenate stock solution of 10 mg mL^{-1} was prepared. 2 mg of $NaReO_4$ was loaded onto the resin. Three buffers (HEPES, HEPES saline, and phosphate buffer saline; 20 mM, pH 7.4) were used to determine the ideal buffer for the experiment. Each of these buffers were passed over a $NaReO_4$ loaded resin column and the amount of perrhenate displaced was determined using a Cary 60 UV spectrophotometer. Absorbances at 225 nm were compared to a standard curve to determine the quantity of perrhenate present in the eluent.

Panning of phage library

Biopanning experiments were performed according to manufacturer's protocol. Briefly, approximately 5.76×10^{12} phage were passed over a $NaReO_4$ loaded resin column. The column was washed with 5 mL of wash buffer (20 mM of HEPES buffer at pH 7.4). 1 mL of 10 mg mL^{-1} $NaReO_4$ in wash buffer was used to elute the retained phage. The eluted phage were amplified by adding them to a 20 mL culture in a 250 mL Erlenmeyer flask inoculated with 200 μL of an overnight culture of *E. coli* K12 ER2738 (New England Biolabs) culture. This culture was grown at 37 °C with shaking at 250 rpm for 4–5 h. Cells were removed by centrifugation at 4500×*g* for 10 min and the supernatant was transferred to a fresh tube. 16 mL of supernatant was transferred to a new tube and 4 mL of 2.5 M NaCl/20% PEG-8000 (w/v) was added and briefly mixed. The phage were precipitated overnight at 4 °C. The phage were collected by centrifugation at 12 000×*g* for 15 min at 4 °C. The supernatant was discarded, and the pellet was resuspended in 1 mL of Tris buffered saline at pH 8.0. The resuspended phage were transferred to an Eppendorf tube and centrifuged briefly to remove any cell debris. The supernatant was transferred to a fresh tube and the phage were precipitated a second time by adding 200 μL of 2.5 M NaCl/20% PEG-8000 and incubating on ice for 15–60 min. The phage were collected by centrifugation at 16 000×*g* in a benchtop centrifuge at room temperature for 10 min. The supernatant was discarded, the tube was centrifuged briefly, and the remaining supernatant was removed with pipette and the pellet was resuspended in 200 μL Tris buffer saline pH of 8.0. These amplified phages were subjected to another round of biopanning and the process was repeated for a total of six rounds of biopanning.

Tittering of eluted phage

Tittering of the phage in the eluate was performed according to the manufacturer's protocol.[32] Briefly, a colony of *E. coli* ER2738* cells grown overnight at 37 °C on an LB plate supplemented with 20 μg mL^{-1} of tetracycline was used to inoculate 5 mL of LB that was subsequently incubated with shaking for 4–8 hours until the culture reached mid-log phase ($OD_{600} \sim 0.5$). At the same time, serial dilutions from 1 : 10 to 1 : 10 000 of the phage containing eluate were prepared. 10 μL of each serial dilution was added to 200 μL of the *E. coli* culture, mixed, and incubated at room temperature for 1–5 min. These mixtures were added to molten top agar, mixed, and poured onto LB plates containing 50 μg mL^{-1} of IPTG and 40 μg mL^{-1} of Xgal. The plates were gently tilted and rotated to spread the top agar, allowed to cool at room temperature for 5 min, and then incubated overnight at 37 °C. Plates that had approximately 100 plaques were counted and the plaque-forming units per volume were determined.

Phagemid sequencing

After the final round of biopanning, 20 plaques were randomly picked and amplified. The resulting phage pellet was suspended thoroughly in 100 μL of iodide buffer (10 mM Tris–HCl, 1 mM EDTA, 4 M sodium iodide, pH 8.0) by vigorously tapping the tube. Single-stranded phage DNA was precipitated by the addition of ethanol (250 μL) followed by incubation for 10–20 minutes at room temperature. Precipitated DNA was collected by centrifugation at 20 000×*g* for 10 minutes. The pellet was washed with 0.5 mL of cold (−20 °C) 70% ethanol, re-centrifuged, discarded the supernatant, and the pellet was briefly dried under vacuum. The dried pellet was dissolved in 30 μL of TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0) and analyzed by Sanger sequencing (Azenta Life Sciences, South Plainfield, United States) using the -96gIII sequencing primer (5′-CCCTCATAGTTAGCGTAACG-3′).

Synthesis of free peptides

Peptides were synthesized using the solid-phase peptide synthesis (SPPS) method and the Fmoc strategy.[33] As the identified peptides were anchored to the phage at the C-terminal end (*i.e.* did not have a free c-terminus), the peptides were synthesized with a c-terminal amide. 155 mg of Rink Amide resin was used for a scale of 0.1 mmol scale synthesis. The resin was transferred to a peptide reactor (Biotage LLC, Uppsala, Sweden) and resin was swollen in 3–5 mL DCM for 30 minutes with agitation. The resin was washed three times with DMF prior to the Fmoc cleavage from the resin using 40% piperidine in DMF for 10 minutes. Based on the amount of resin, 4 : 3.6 : 8 equivalents of the amino acids, HBTU and DIPEA respectively were used to achieve 100% loading of the resin. Amino acids were activated by the addition of HBTU and DIPEA in a glass scintillation vial in 2 mL of DMF. After cleavage of the Fmoc from the resin was complete, a vacuum pump was used to drain the 40% piperidine. The resin was washed three times with DMF and the amino acid/HBTU/DIPEA solution was added to the resin and rocked at room temperature for 1 hour. After 1 hour, a vacuum pump was used to drain the amino acid solution. The Fmoc group on the N-termini was cleaved using 40% piperidine in DMF and the loaded resin was washed with DCM three times and DMF three times. This cycle was repeated for the remaining amino acids. After the last amino acid was coupled and Fmoc deprotected, the loaded resin was dried under high vacuum overnight. The dried resin was transferred into a falcon tube and 95 : 2.5 : 2.5 TFA : H_2O : TIPS were added to the resin and left to rock for 1.5–2 hours. The resulting solution was concentrated by blowing air over TFA solution, and the peptides were precipitated with cold anhydrous ethyl ether. The peptides were collected by centrifugation (8000×*g* for 3 minutes), the supernatant was decanted, and the pelleted precipitate was left to dry. The dried precipitate was dissolved in 50 : 50 ACN/H_2O and the resin was removed by filtration. The filtrate was frozen using liquid nitrogen and lyophilized using the FreeZone Lyophilizer (Labconco Corporation, Kansas City, United States). The dried crude peptide was purified using the 1260 Infinity II HPLC (Agilent Technologies). The peptides were purified using a Macherey-Nagel (Duren, Germany) C18 column with dimensions of 250 mm × 10.0 mm and 10 μm particle size. A gradient from 98% water with 0.1% TFA and 2% acetonitrile with 0.1% TFA to 100% acetonitrile with 0.1% TFA over 60 min was used to elute the peptides. Fractions containing the desired peptides were identified by mass spectrometry with a Waters QDa single quadrupole mass spectrometer (Waters Corporation, Milford, United States).

Synthesis of immobilized peptides

Immobilized peptides were synthesized using Tentagel S NH_2 (VWR) as solid support, using Fmoc SPPS protocol. 200 mg of Tentagel S NH_2 resin was used to achieve a loading capacity of 0.04 mmol scale for the synthesis. The resin was added to a peptide reactor. The resin was swollen in DCM for 30 min and washed three times with 5 mL of DMF. The Fmoc was cleaved from the resin three times with 5 mL of 40% piperidine in DMF for 10 minutes. The activation of the amino acids was carried out as described for the free peptides. After the first amino acid was coupled to the resin, the remaining free amines on the resin were capped using 10% acetic anhydride in DMF and DIPEA. The Fmoc on the N-termini of the first coupled amino acid was deprotected using 40% piperidine in DMF and washed with DCM 3× and DMF 3×. The cycle was repeated for the remaining amino acids and the acid labile protecting groups were deprotected using 95 : 2.5 : 2.5 TFA : H_2O : TIPS respectively for 1.5–2 hours. The loaded resin was dried overnight using a high vacuum and the mass of the peptide was determined. The resin contained approximately 1.1 μmol peptide per mg of resin.

NMR titrations

Experiments were performed as previously described.[34] All of the reagents were weighed separately on an analytical balance (readability to 0.0001 mg) in 1.5 mL Eppendorf tubes. D_2O-d_2 was used as the solvent for NMR titration. A solution of 2 mM peptide was prepared for the NMR analysis. 200 mM solution of $NaReO_4$ in 2 mM peptide solution was prepared to avoid diluting the peptide during the titration. The titration was performed by adding aliquots (20 μL) of the perrhenate solution to the peptide solution (400 μL) and recording the ^{1}H NMR spectrum following each addition of perrhenate solution. The temperature inside the NMR probe was held constant at 25 °C. Association constants were calculated based on the changes in the chemical shifts of the most affected protons of the peptide.

Retention of metal anions by immobilized peptides

The mass of corresponding to 4.3 mg of each resin-bound peptide was loaded in a 1 mL disposable syringe plugged with cotton to retain beads. The resin was washed five times with 1 mL of wash buffer (HEPES buffer, 20 mM, pH 7.4) to swell and equilibrate the resin as well as wash off any remaining impurities. Solutions of 2 mg mL^{-1} $NaReO_4$, $NaNO_2$, Na_2CrO_4, and NaI were prepared in the wash buffer. 500 μL of the metal anion solutions were incubated with the resin-bound peptide column for 15 minutes with shaking at room temperature. The remaining solution was drained into scintillation vial for UV-vis

analysis. The resin column was washed five times with 1 mL of Milli-Q water. These washes were collected to assess metal loss during washing.

Competition with other anions

Perrhenate was bound to the resin-bound peptide columns as described above. 500 μL of NaCl, Na_2SO_4, or Na_3PO_4 (2 mg mL^{-1} of the respective salts in HEPES, 20 mM, pH 7.4) was incubated with the resin containing the retained $NaReO_4$ for 15 minutes with shaking. The solution was drained after 15 minutes to assess the displaced $NaReO_4$.

Quantification by UV-vis spectrometry

Metal anion concentrations in the flowthrough and wash fractions were quantified using UV-visible spectroscopy. Absorbance measurements were recorded using a Cary 60 UV-vis spectrometer at the appropriate wavelength for each metal anion (372 nm for chromate, 230 nm for perrhenate and nitrate, 235 nm for iodide and nitrite). Standard curves were generated from serial dilutions of metal salt solutions prepared in the wash buffer, with linearity confirmed over the relevant concentration range. The concentration of metal anions in each fraction was determined by interpolating from the standard curve. The % retention of metal ions by the peptide-functionalized resin was calculated using the following equation:

$$\%\ \text{retention} = \frac{C_{\text{input}} - C_{\text{eluate}}}{C_{\text{input}}} \times 100$$

where C_{input} is the concentration of metal in the input solution, and C_{eluate} is the combined concentration of metal in the flowthrough and wash fractions. All measurements were performed in triplicate.

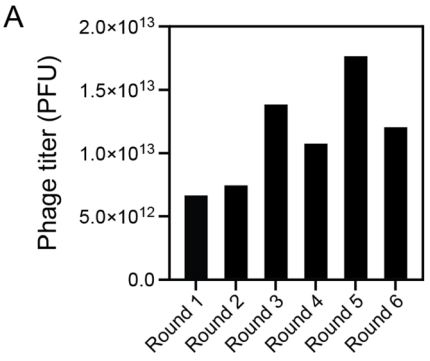
Results and discussion

Screening of phage library

We selected the NEB PhD-7 phage library, with seven variable amino acid positions, to mimic the length of phosphate and sulfate binding motifs present in proteins. ReO_4^- was immobilized on positively charged Dowex 1X8 anion exchange resin that has been reported to retain it.[31] Each round of biopanning was carried out by incubating the phage with this ReO_4^- loaded resin, followed by washing, and finally elution with free ReO_4^-.

An increase in the number of phage eluted was observed after the third round of biopanning, but sequencing select phages did not reveal an enrichment in any particular peptide sequences (SI Table S1). However, all of the identified peptides had a net neutral or positive charge, suggesting binding to the anion exchange resin was not being selected for. Therefore, three more rounds of biopanning were carried out.

Phagemids from 20 randomly selected plaques were sequenced, with 12 of those clones providing unambiguous sequence data. These 12 clones represented 9 unique peptide sequences (Fig. 1). Two of these peptide sequences were identified following the third round of biopanning, suggesting that, had deeper sequencing been performed following that round, enrichment of specific sequences may have been observed. Again, none of the identified peptides contained a negatively charged residue, further supporting that the phage were not being enriched for the ability to bind to the anion exchange resin. Furthermore, all of the peptides contained at least one positively charged residue, which could be important for binding to the perrhenate anion.



B

Name	Sequence	Counts
ST1	KMISTIS	3
ST2	KMISTLS	1
ST3	KMIYTIS	1
ST4	KMISILW	1
ST5	KMISTLW	1
ST6	GKSIGSW	2

Fig. 1 Biopanning against immobilized perrhenate. (A) Titer of phages eluted from column with immobilized perrhenate. (B) Peptide sequences identified after 6 rounds of selection. Counts are the number of times the gene encoding the given peptide was sequenced.

These peptides do not match any amino acid motifs that bind phosphate in proteins,[18] but they do contain residues that are prevalent in these motifs, G, S, T, and K. Out of the 9 unique peptides, 5 began with the same sequence (KMI). These five peptides were selected for further characterization (ST1–ST5) as well as another peptide that appeared twice among the sequenced clones (ST6).

Perrhenate binding

To confirm that the peptides identified by biopanning bound ReO_4^-, these peptides were synthesized (SI Fig. S1–S6) and examined by ^1H NMR with increasing concentrations of ReO_4^- (SI Fig. S7–S12). The spectra of ST1, ST2, ST3, and ST5 in D_2O revealed a downfield shift of the protons of the thioether methyl group of the methionine side chain as the concentration of ReO_4^- increased. The spectra of ST4 revealed the appearance of a new peak and disappearance of the methionine peaks as the concentration of ReO_4^- increased. These changes in the spectra of the peptides may indicate complex formation, however, the peptides could not be saturated by increasing concentrations of perrhenate.

Interestingly, as ST4 was incubated with ReO_4^-, a precipitate formed. As precipitation of ReO_4^- could be a potential approach

for its remediation, further characterization revealed that out of the 1.2 mg of the precipitate submitted for ICP-MS analysis, 0.37 mg was Re. This corresponds to approximately 1.5 moles of ReO_4^- per mole of peptide in the precipitate or two peptides interacting with three ReO_4^- molecules. This stoichiometry of the precipitate suggests that rather than interacting with individual peptide chains, there could be multidentate interactions between ReO_4^- and the peptides.

To determine if the precipitation of ST4 was due to nonspecific interactions with functional groups on the peptide, a scrambled peptide with the sequence LMIWKSI was synthesized and incubated with ReO_4^-. This scrambled peptide did not result in the formation of a precipitate, suggesting that the sequence of ST4, not simply the presence of the same functional groups, is responsible for the formation of the precipitate. Additionally, truncated peptides with the sequences KMIS (SI Fig. S13) and KMIY (SI Fig. S14) were synthesized and incubated with ReO_4^-. Again, no precipitation formed, suggesting the c-terminal residues of ST4 play a role in the formation of the precipitate.

While we were unable to accurately determine association constants for the individual peptides binding to ReO_4^-, in the phage display system that was used, the multiple copies of the displayed peptides are located in close proximity, raising the possibility that more than one peptide chain participated in the interaction with ReO_4^- during the screening process. This organization of the displayed peptides and the stoichiometry of the ST4 precipitate suggest a multivalent interaction by be responsible for the phage binding to the immobilized ReO_4^-.

Binding and selectivity of immobilized peptides

To test whether more closely packed peptides that mimic the environment for peptides displayed on phage could take part in polydentate interactions with ReO_4^- we sought to establish whether peptides immobilized on a solid support were able to remove ReO_4^- from solution. Approximately 5 μmol of each peptide synthesized on Tentagel resin were incubated with approximately 3.7 μmol of ReO_4^- in HEPES buffer solution for 15 minutes. The immobilized peptides were able to remove between 86% and 93% of the ReO_4^- from the buffer solution (Fig. 2). ST5 bound the largest fraction of ReO_4^-, at 93%, while ST1 retained the least, with 86%. The Tentagel resin, when free amines were capped with acetic anhydride retained less than 1% of the loaded ReO_4^-, suggesting the resin itself is not responsible for the retention of ReO_4^-. Furthermore, resin with peptides of an arbitrary sequence that included a negatively charged amino acid (ST.b1 and ST.b2) retained approximately 3% of the loaded ReO_4^-, suggesting any arbitrary peptide would not retain ReO_4^-. The ability of these peptides to bind ReO_4^- when they are immobilized on a resin provides further evidence that the phage display screen selected for avidity-based interactions.

To examine the impact of the sequence of the peptides on the retention of ReO_4^- we synthesized scrambled variants of all six peptides. The amount of ReO_4^- retained by the scrambled versions of each peptide was lower than the peptide itself, but the scrambled peptides did retain between 42% and 74% of the loaded ReO_4^-, suggesting non-specific interactions between ReO_4^- and peptides do occur, but that the sequence of the peptide plays a role as well.

To explore the ability of the peptides to maintain an interaction with ReO_4^- in the presence of other ions which are present in soil and low activity nuclear waste, they were washed with buffer containing 2 mg mL^{-1} of sodium chloride, sodium

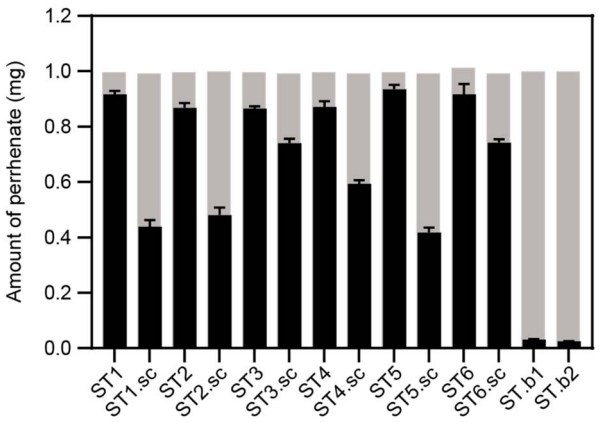


Fig. 2 Retention of ReO_4^- by immobilized peptides. The amount of ReO_4^-, retained by peptides immobilized on resin (black bars) was determined by the difference between the amount of ReO_4^- loaded on the column (grey bars) and the amount present in the flow through and a wash with water. The error bars represent the standard deviation of the results from loading three separate aliquots of resin.

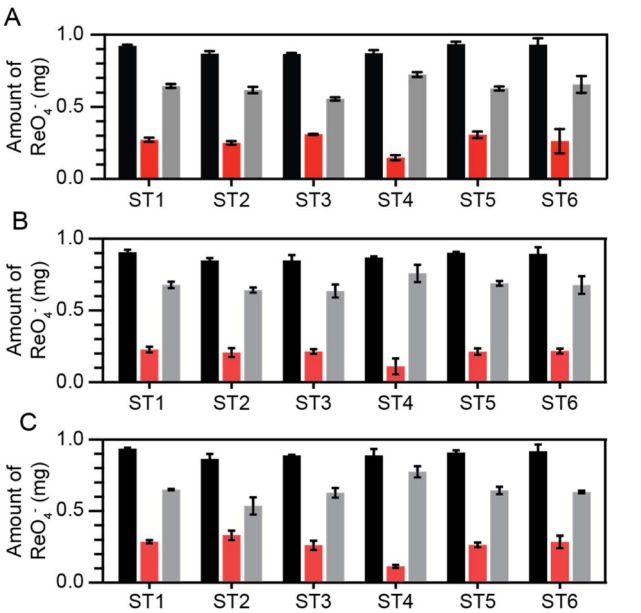


Fig. 3 Displacement of ReO_4^- by select anions. The amount of ReO_4^- retained by the immobilized peptides (black), compared to the amount of ReO_4^-eluted from the resin following incubation with sodium chloride (A), sodium phosphate (B), and sodium sulfate (C) (red) and the amount of ReO_4^- remaining on the resin following incubation (grey). The error bars represent the standard deviation of the results from loading three separate aliquots of resin.

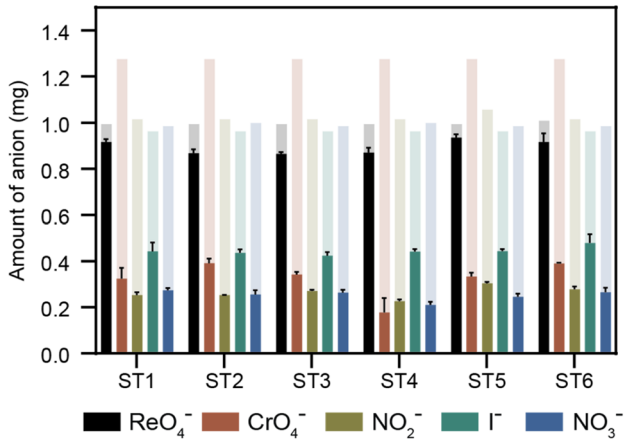


Fig. 4 Retention of other anions by peptides. The amount of other anions that were retained by the immobilized peptides (dark colors) compared to the amount of those anions that were loaded onto the resin (light colors). The error bars represent the standard deviation of the results from loading three separate aliquots of resin.

phosphate, or ammonium nitrate after being loaded with ReO_4^-(Fig. 3). These washes removed between 13% and 38% of the ReO_4^- that had been retained on the column. ST4 lost the lowest amount of ReO_4^- in the presence of the other anions, 17% in the presence of chloride, and 13% in the presence of both sulfate and phosphate. The ability of ST4 to retain ReO_4^- may be associated with its precipitating in the presence of ReO_4^-. Different peptides lost the most ReO_4^- in the presence of other anions with ST5 losing 33% in the presence of chloride, ST2 losing 38% in the presence of sulfate, and ST1 losing 25% in the presence of phosphate. These data potentially suggest different selectivities for anions between the identified peptides.

To further explore different selectivities for anions between the identified peptides, their ability to bind other anions was explored. The immobilized peptides were incubated with chromate, nitrite, nitrate, and iodide. All of the peptides were able to bind these anions to some extent (Fig. 4). The peptides retained the most iodide, retaining between 42% and 48% of what was loaded. The peptides retained similar amounts of nitrate and nitrite, between 21% and 30% of what was loaded. Chromate retention fell in the middle with 24% to 31% of what was loaded being retained. ST4 retained the least of the other ions, except for iodide. The fairly narrow ranges of the other anions suggest that there are not large differences in anion selectivity between the different peptides, but the differences in retention of different anions suggests that the peptides may have some selectivity for ReO_4^- despite not being counter screened against the ability to bind other anions.

Conclusions

In this study we sought to establish a proof of concept for using biological systems for the remediation of TcO_4^- from the environment. We used phage display to identify several peptides that were able to interact with ReO_4^-, a nonradioactive surrogate. All of the identified peptides contained a positively charged residue, and we hypothesize that the interaction between the peptide and ReO_4^- is due to ionic interactions between that residue and possibly hydrogen bonding with other residues or the amide backbone of the peptide, in a manner analogous to how proteins interact with phosphate.

While these peptides on their own had exceedingly weak affinity for ReO_4^-, when they are immobilized on resin, they were able to reduce the level of ReO_4^- in buffered solution. Additionally, the interaction between these peptides and ReO_4^- was not abolished by the presence of other anions such as chloride, sulfate, and phosphate. However, these peptides are limited in that they are not strongly selective for ReO_4^-. Further optimization of ReO_4^- binding peptides, such as constraining their conformational space by embedding them in a larger protein or other approaches such as macrocyclization may help increase the affinity and selectivity of peptides for ReO_4^-

While these peptides only bind approximately 0.15 mg of ReO_4^- per g of resin, dramatically below the capacity of current materials, they can, unlike inorganic materials, be incorporated into organisms. These results demonstrate that it is possible to identify biological material that is capable of interacting with ReO_4^-, and are a first step to designing a bacteria or plant that could bind and immobilize TcO_4^- in the environment, which while not competing directly with current state of the art materials, may allow for deployment and use in situations that are not ideal for those materials.

Author contributions

B. G. and M. C. W. conceptualized the study. S. T., M. A. M. F., and S. D. S. performed the investigation. S. T. and M. C. W. wrote the original draft of the manuscript. All authors reviewed and edited the manuscript.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of the supplementary information (SI). Supplementary information: the sequences of peptides identified during the screening, characterization of the soluble peptides used, and NMR spectra of the soluble peptides in the presence of perrhenate. See DOI: https://doi.org/10.1039/d5ra08091f.

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