Microbial Cell Factories

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Phage display technology in ecotoxicology: phage display derived unique peptide for copper identification in aquatic samples

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Abstract

Background Ecotoxicology is essential for the evaluation and comprehension of the effects of emergency pollutants (EP) such as heavy metal ions on the natural environment. EPs pose a substantial threat to the health of humans and the proper functioning of the global ecosystem. The primary concern is the exposure of humans and animals to heavy metal ions through contaminated water. The presence of heavy metal ions in drinking water ought to be monitored in accordance with World Health Organization regulations. Among the numerous harmful metal ions, copper ions are responsible for a variety of human diseases.

Results This study investigates the application of phage display as a screening method for heavy metal toxicological targets, with copper served as the main focus. To identify a variety of Cu-binding M13 phage clones with unique peptides and to assess their affinity for metal ions, the study utilized *Escherichia coli* as a factories producing recombinant bacteriophages, modified biopanning procedure and an ELISA assay. The research highlights the increasing importance of phage display as a screening tool in ecotoxicology. We synthesized and modified the selected peptide to enable the rapid optical detection of Cu(II) ions in aqueous solutions. By incorporating the dansyl group into a designated peptide sequence, we implemented fluorescence detection assays for real-time measurements. The Cu^{2+}- binding peptide's efficacy was confirmed through spectroscopic measurements, which allowed for real-time detection with rapid response times with high selectivity.

Conclusions The phage display technique was successfully applied to develop the fluorescent peptide-based chemosensor that exhibited high selectivity and sensitivity for Cu^{2+}.

Keywords Phage display technology, Ecotoxicology, Copper(II) ions chemosensor, Fluorescent peptide-based sensors

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Background

The study of the effects of toxic chemicals on organisms, particularly at the population, community, ecosystem, and biosphere levels, is known as ecotoxicology [1]. In order to properly monitor emergency pollutants (EP), the multidisciplinary area of ecotoxicology which combines toxicology, molecular biology, and ecology has become crucial in recent years. One of the primary obstacles to modern human society is environmental pollution and contamination. A severe environmental concern within EPs is metal pollution, which is caused by the release of heavy metal ions into the natural environment [2]. Human activities, including metal mining, agriculture, and industrial processes, have significantly contributed to the increase in metal pollution in the air, water, and soil [3].

Contaminated water with heavy metal ions like chromium (Cr (VI)), cadmium (Cd (II)), lead (Pb (II)), arsenic (As (V and III)), mercury (Hg (II)), nickel (Ni (II)), and copper (Cu (II)) is responsible for several health issues in humans and other organisms [3, 4]. The World Health Organization (WHO) suggests that the concentration of copper in drinking water should be less than 31 μM [5].

Copper(II) ions (Cu^{2+}) are essential for the regulation of gene expression, electron transport, and enzyme catalysis, among other biological processes [6]. Copper is a critical trace element that is essential for the proper functioning of numerous enzymes, including cytochrome c oxidase, superoxide dismutase, and tyrosinase. Critical processes such as energy production, antioxidant defense, and melanin synthesis are facilitated by these enzymes. Nevertheless, the homeostasis of copper is crucial, despite its necessity [6]. The toxicity of copper is a result of its capacity to participate in redox reactions, which can produce reactive oxygen species (ROS) that possess the potential to induce oxidative stress, which can result in the destruction of cellular components, including DNA, proteins, and lipids [7, 8]. This oxidative damage is linked to a variety of pathological conditions, such as cardiovascular diseases, liver disorders such as Wilson's disease, and neuro-degenerative diseases like Alzheimer's and Parkinson's [7, 9–13].

In addition, even the presence of extremely low concentrations of Cu^{2+} ions can have a detrimental impact on specific organisms, resulting in a variety of disorders, including anemia and a low white cell count. Furthermore, excessive copper consumption can result in nausea, vomiting, abdominal pain and cramps, headache, dizziness, weakness, and diarrhea. Worth of note is the impact of copper ions on fish and other aquatic organisms that consequently can be toxic element in the ecosystem when exposed to higher copper concentrations [6, 14].

It is advisable to develop methods for the selective and sensitive detection of Cu^{2+} ions in aqueous media and living cells, as the excess of Cu^{2+} ions damages the entire food chain and the significance of Cu^{2+} in biological activity [15].

The high specificity and sensitivity of biodegradable peptides as ligands for the detection of heavy metal ions in aqueous systems have attracted significant attention within last decade [16]. Peptides are short chains of amino acids that can be engineered to bind specific target molecules, such as heavy metal ions, with high affinity [17, 18]. This renders them highly promising for the development of sensors, particularly in the areas of environmental monitoring and water quality assessment [18–20]. One of the methods that allows for the specific identification of unique amino acid sequences (peptides) towards the detection of heavy metal ions is phage display technology [21, 22].

Phage display is employed to identify peptides that have a specific affinity for a variety of targets, including chemical compounds, antigens, and proteins [23, 24]. It necessitates the utilization of phage libraries, which are comprised of a variety of unique phages with unique peptides or proteins on their surfaces. Bacteriophages need bacterial cells that act as cell factories in order to replicate. By utilizing the host's metabolic machinery, bacteriophages turn bacteria into factories that produce new viral particles, which can be used to identify selective sequences in the form of peptides. Phages that exhibit a high affinity for a specific compound can be isolated through an affinity selection procedure (biopanning) [24, 25]. Identifying the peptides displayed on these selected phages can be achieved via sequencing the gene that encodes the peptide [24]. Initially phage display technology has been extensively employed in the field of immunology. Nevertheless, since 1985 it has been implemented in a variety of research fields, such as drug discovery [26], protein engineering, and biotechnology [27–29]. In certain fields, such as ecotoxicology, phage display remains neglected, despite its potential. The potency of phage display can be advantageous for the toxicological assessment of chemical compounds by employing it as a screening tool to identify the primary toxicological targets [28, 30–35].

The potential of phage display as a screening tool for the primary toxicological targets of heavy metals was investigated in this research, with copper serving as a case study. The results that have been presented indicate that the peptide-based fluorescent chemosensor was highly selective and sensitive to Cu^{2+} ions in comparison to other metal ions.

Results

Phage display technology for identification of selective unique amino acid sequences for copper (II) ion detection

This study developed a biopanning procedure and modified microtiter plates coated with various metal ions to identify selective, unique amino acid sequences for copper(II) ion detection. Initially, the immobilization of metal ions on maleic anhydride-activated microtiter plates was achieved by functionalizing the surface with *N*,*N*-Bis(carboxymethyl)-L-Lysine Hydrate (BCML) and subsequently modifying it with various 10 mM metal salts. (Fig. 1) The microtiter plate was modified, and a subsequent biopanning procedure was conducted using five distinct metal ions: mercury (Hg (II)), copper (Cu (II)), lead (Pb (II)), nickel (Ni (II)), and manganese (Mn (II)).

Moreover, the standard biopanning procedure was modified to identify selective, unique amino acid sequences for copper(II) ion detection. A negative selection step was incorporated into the procedure to eliminate nonspecific phage clones from the library that bound to metal ions other than copper. The first microplate was coated with Ni (II) ions, and then a diluted phage library (1×10^{11} plaque-forming unit) was added. Phage clones that were not bound were transferred to a second microplate that was subsequently coated with Hg (II) ions. Microplates modified with Mn (II) and Cd (II) were used to propagate two additional negative selection steps. Three rounds of biopanning were conducted with the target metal ion (Cu (II)) following negative selection, as per the manufacturer's instructions (Fig. 2).

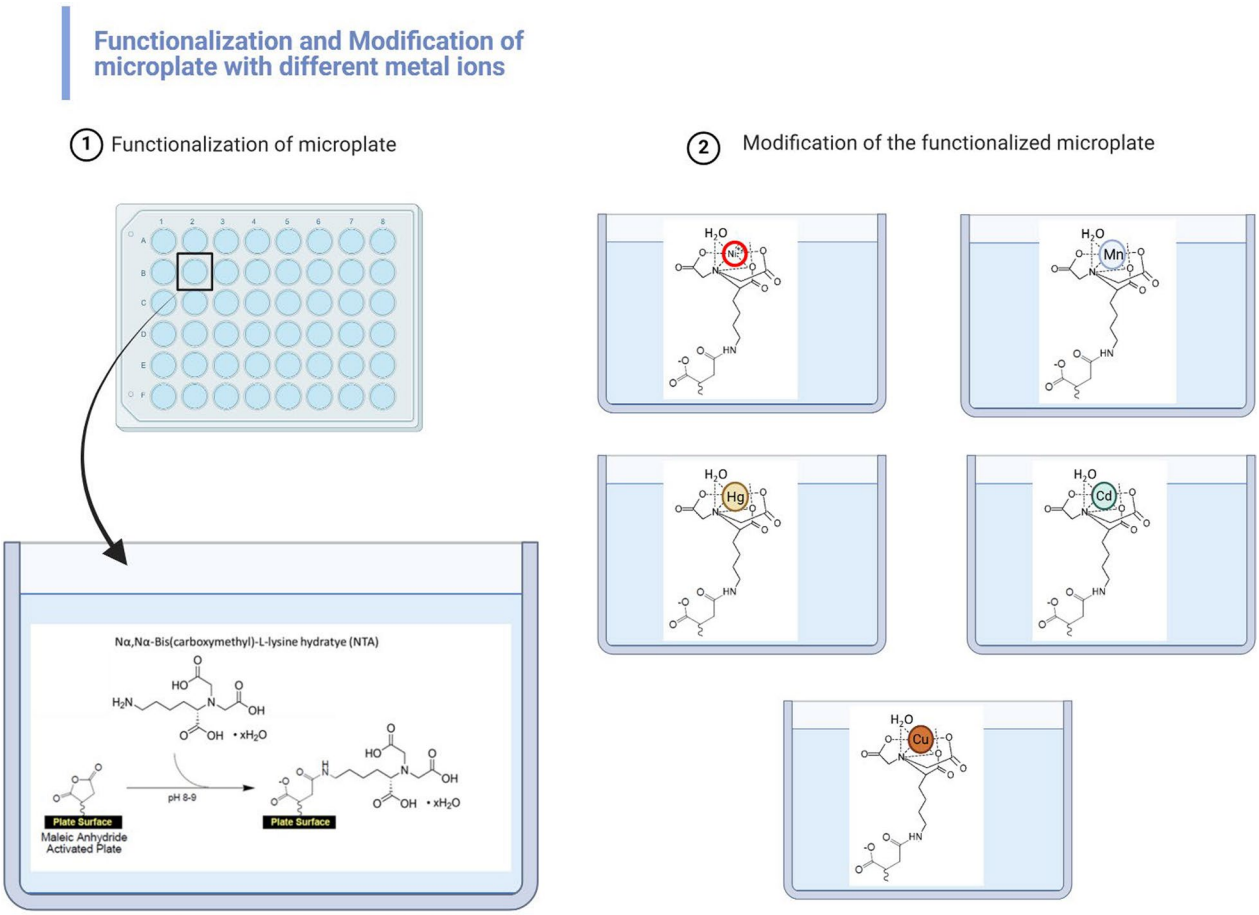


Fig. 1 Functionalization and modification of microplate with different metal ions for biopannig procedure. Schematic representation of functionalization and modification of microplate with different metal ions for biopannig procedure. The Nα,Nα-Bis(carboxymethyl)-L-lysine hydrate was added to each well of a maleic anhydride-activated microtiter plate and incubated over-night at room temperature. The plate was then washed three times with 0.05% TBST buffer and blocked with 3% BSA in 0.05% TBST buffer. Finnaly after washing step the plate was incubated with 10 mM metal salts for proper modification

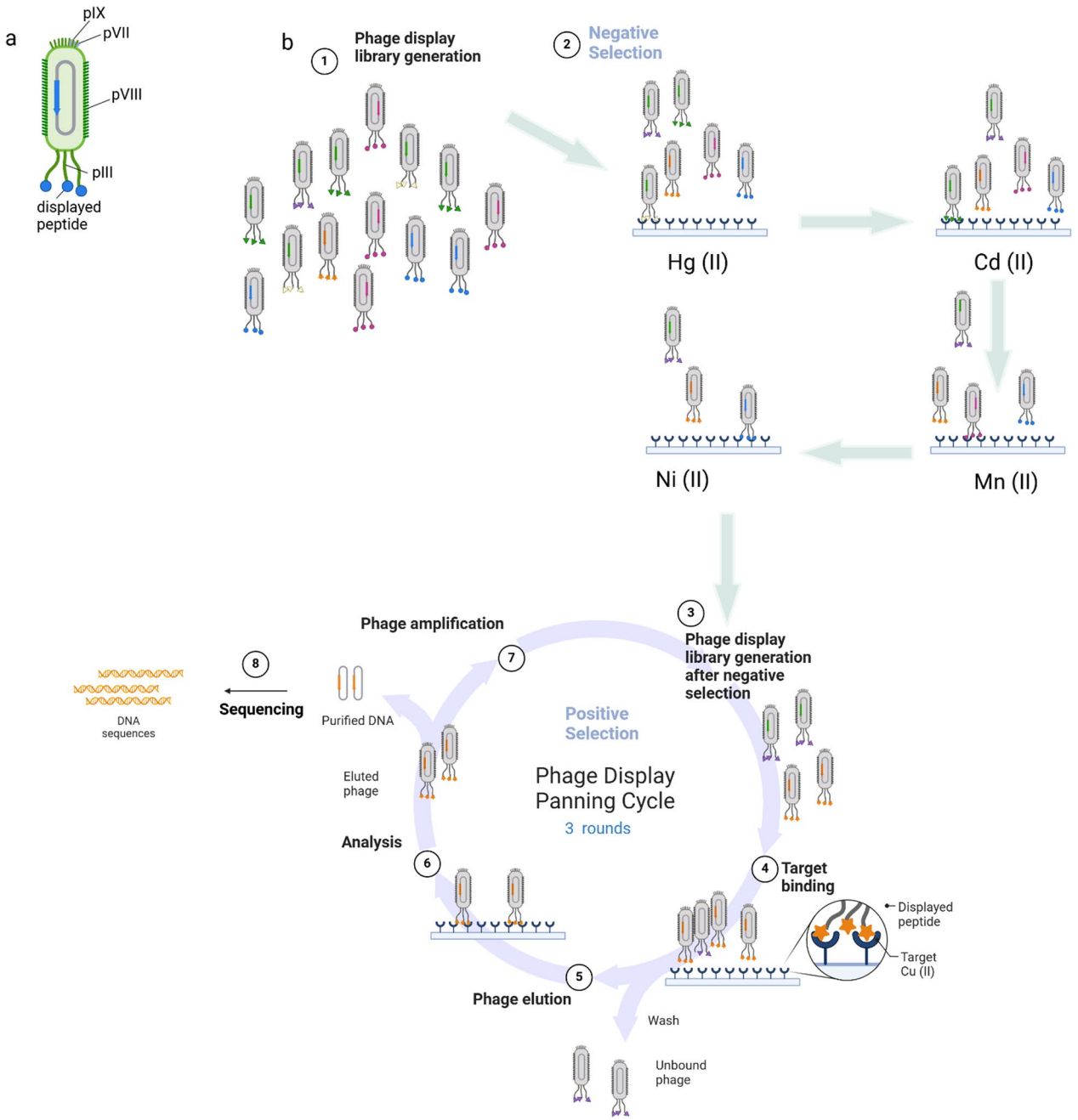
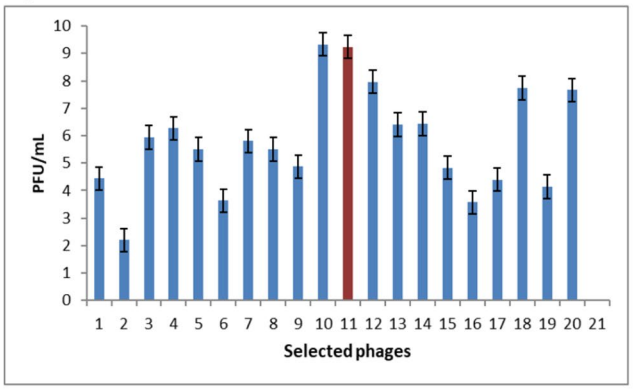


Fig. 2 Modified biopanning procedure. Schematic representation of modified biopanning procedure. The phage library kit Ph.D.™-7 Phage Display Peptide was used to conduct an overnight experiment in LB medium at 37 °C with the host bacterial strain *Escherichia coli* (*E. coli*) K12 ER 2738. The protocol was modified to include negative selection steps in order to eliminate nonspecific phage clones that bound to metal ions other than copper from the library. In conclusion, 1×10^{11} PFU of the library was diluted in 100 µL of 0.05% TBST buffer and subsequently added to a plate that had been coated with a 10 mM nontarget metal ion. Unbound phage clones were pipetted into wells that were coated with an additional negative target following incubation. Nickel, manganese, cadmium, and mercury were subjected to negative selection. Three rounds of biopanning were conducted with the target metal ion following negative selection. Following the third round of biopanning, M13 phage plaques were selected from titration plates to amplify phage clones for DNA sequencing. *PFU* plaque-forming unit

After the last round of panning, 240 single plaques were isolated from upper agar plates for phage amplification. Further, DNA from amplified clones was isolated and sequenced to reveal the structure of the displayed peptides. A total of 20 individual clones harboring unique peptides were identified from picked plaques (Fig. 3). An ELISA

a



b

No.	Peptide Sequence	Frequencies of Histidine residue
P-1	FPYRSTP	0
P-2	GVKMHTH	2
P-3	ITGTTSW	0
P-4	NLIHKHS	2
P-5	WSLGYTG	0
P-6	NWSSLLQ	0
P-7	DYSVRLI	0
P-8	SDDIRRN	0
P-9	GFLPRDT	0
P-10	DTAHGTW	1
P-11	MHIVPHE	2
P-12	DRSWVTS	0
P-13	SFDNLEP	0
P-14	GMQKSLP	0
P-15	LPVLETR	0
P-16	SVVTARQ	0
P-17	FSMKAEL	0
P-18	ASKGVGL	0
P-19	HLTSPML	1
P-20	EARAPGS	0

Fig. 3 Selection of M13 phage clones with Cu (II)-binding peptides. **a** Identification of M13 phages selective to Cu (II) ions in ELISA assay. Functionalyzied and modified with Cu (II) ions microtiter plate were incubated with 100 µL of 10^9 PFU selected clones per well. Nonbounded phages were discarded and plate was washed 10 times. For phage elution 100 µL of 0.2 M Glycine–HCl (pH 2.2) was used. Eluted phages were titered according to the method described in The Ph.D.™-7 Phage Display Peptide Library Kit manual. Binding specificity is presented as the phage titer eluted from wells coated with Cu metal ions. Three independent experiments were conducted and the mean is presented. *PFU* plaque-forming unit. **b** Representation of peptides sequences with the highest affinity to copper ions

assay was conducted using Cu (II)-modified microplates to ascertain the binding affinity of the 20 selected peptides to Cu (II) ions (Fig. 3a). The biopanning procedure was effective, as evidenced by the enhanced binding affinity of the phage clones bearing Cu (II)-binding peptides to Cu (II). P-10, P-11, P-12, P-18, and P-20 exhibited the highest affinity. A comparison and sequence analysis of the respective amino acid sequences indicated that P-12 (DRSWVTS), P-18 (ASKGVGL), and P-20 (EARAPGS) lacked histidine residues. In contrast, P-10 (DTAHGTW) contained a single histidine at the center of the sequence, while P-11 (MHIVPHE) had two histidine at both ends of the peptide, which may be more advantageous for the coordination of the peptide association with copper ions. In conclusion, we selected P-11 as the candidate peptide for further investigation due to its MHIVPHE sequence.

Cross reactivity assays were carried out with four additional metal ions (Ni, Cd, Hg, and Mn) to perform additional selectivity studies. The selective phage clone that displaces the MHIVPHE peptide exhibited a high affinity for copper ions and a very low affinity for other metal ions, including Ni, Cd, Hg, and Mn (Fig. 4). We conducted the nonparametric Mann–Whitney U test, which is employed for small sample sizes, as was the case in this study. The results suggested that there were significant differences between the means of Cu and Hg or Cd (assuming p = approx. 0.1).

Synthesis, modification and characterization of selective unique amino acid sequences for copper (II) ion fluorescence detection

In order to achieve specific optic functions, the unique selective native peptide sequence Met-His-Ile-Val-Pro-His-Glu, which was selected from Phage Display experiments, was modified. In order to eliminate the necessity for labeling reactions, the fluorophore (dansyl chloride) and tryptophan as a donor were conveniently attached during solid-phase peptide synthesis (SPPS). Dansyl (DNS) was introduced at the N-terminal side of the peptide, and a tryptophan residue was inserted at the C-terminus. In order to enhance the solubility of the peptide, reduce steric hindrance, and guarantee the fluorophore's proper positioning, a flexible linker, such as a lysine residue, was inserted between the native sequence and the donor fluorophore (DNS-L9) (Fig. 5a). The fluorescence spectroscopic response of the obtained DNS-L9 peptide (10 µM) toward metal ions (Cu^{2+}, Hg^{2+}, Pb^{2*}, Cd^{2*}, Ni^{2*}, Mn^{2*}, Cr^{3+}, As^{3+}-, Na^{+}, and K^{+}) was evaluated in HEPES buffer solutions (50 mM, pH 7.41) (Fig. 5b). Separate solutions of each metal ion prepared at concentrations of 10 µM were individually added to the DNS-L9 solutions, and fluorescence emission spectra were recoded with excitation at 330 nm. Comparing the fluorescence response of DNS-L9 in the presence of Cu^{2+} ions and other selected metal ions in the tested concentration, it was found that DNS-L9 exhibited selectivity for Cu^{2+} in aqueous solution and no fluorescent response to other metal ions. Conversely, after adding 1.0 equiv. Cu^{2+} ions, the emission intensity of

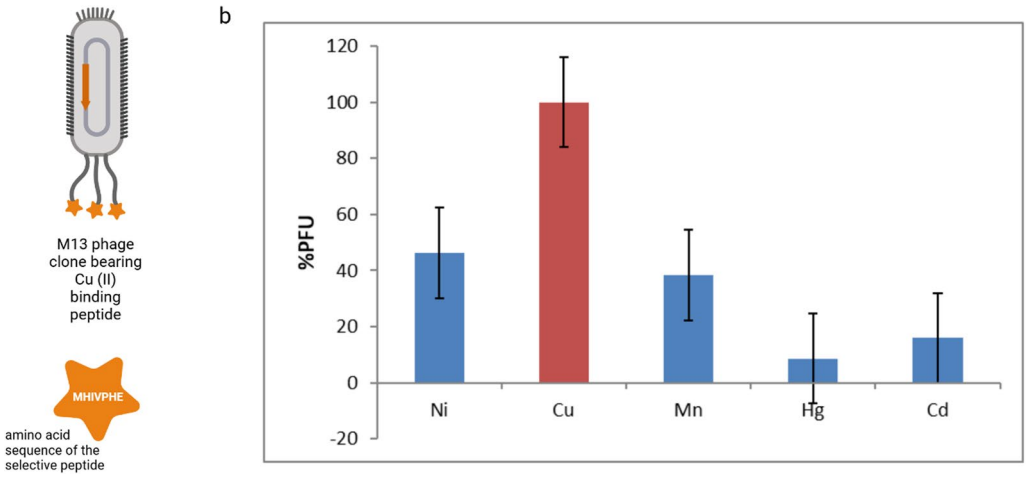


Fig. 4 Selectivity analysis of phage clone bearing MHIVPHE peptide in cross-reactivity ELISA assay. **a** Schematic representation of M13—phage clone bearing Cu (II) peptide. **b** Cross-reactivity assay of M13-Cu (II)-peptide. The microtiter plate was functionalized and modified with Cu (II), Mn (II), Hg (II), Ni (II), Pb(II) ions, and 100 µL of 10^9 PFU selected clones were incubated per well. The plate was washed ten times, and nonbounded phages were discarded. 100 µL of 0.2 M Glycine–HCl (pH 2.2) was used for phage elution. Eluted phages were titrated in accordance with the procedure outlined in the manual for The Ph.D.™-7 Phage Display Peptide Library Kit. As the phage titer is eluted from wells coated with different metal ions, binding cross-reactivity and specificity is demonstrated. The mean of three independent experiments is presented; *PFU* plaque-forming unit

DNS-L9 was significantly reduced with the fluorescence quenching rate for Cu^{2+} of about 58.5% (Fig. 5b).

The outcomes demonstrate that the fluorescent peptide has a discernible sensitivity and selectivity towards Cu(II) ions.

Discussion

Environmental pollution is responsible for 22% of global diseases and 23% of fatalities [36]. As the population grows, environmental analysis becomes crucial to protect the environment from contamination and mitigate its impact [19, 37]. Sensors are a valuable analytical tool for monitoring and screening environmental pollutants, offering cost-effective and user-friendly solutions [19, 37]. Heavy metals pose a significant threat to aquatic animals and human health, causing nephrotoxicity, poisoning, cardiovascular diseases and cancer [38, 39]. It is essential to detect these ions on-site, and bio-resistant devices can serve as sensors [38]. Electrochemical and optical detection provide high efficiency and sensitivity; however, certain sensors may not immediately identify all pollutants [19]. Nevertheless, there is the potential to create cost-effective, high-quality sensors that have a minimal environmental impact. Various transduction principles and biological elements influence factors such as specificity, affinity, response time, dynamic range, and lifetime, which significantly enhance the understanding and exploration of the environment by sensor technology [37].

Copper, a major contributor to global water resource pollution, poses a significant risk to human health and aquatic ecosystems [40]. The development of sustainable, feasible, and low-cost wastewater removal technologies is crucial due to copper concentrations in wastewater ranging from 2.5 to 10,000 mg/L [41]. Copper(II) is discharged into wastewater streams daily by industries like electroplating, paints, dyes, petroleum refining, fertilizers, mining, metallurgy, explosives, pesticides, and steel. Health diseases like headaches, cirrhosis, kidney failure, and cancer have been associated with copper mining activities [40–43]. Detection methods for Cu^{2+} have been developed using fluorimetric, colorimetric, and dual techniques, but challenges remain, including sensitivity, selectivity, response time, preparation, toxicity, and water solubility. Further research is needed to create Cu^{2+} sensors that are selective and highly sensitive for biological and analytical applications [20].

It is pivotal to develop a new fluorophore as well as receptors that can be achieved by using phage display technology. Since its inception, the phage display selection technology has been employed to identify specific peptides that selectively bind to cancer cells [44], proteins [45], nanoparticles and metal ions such as Cd (II) [46], Ni (II) [47], Pb (II) [48], Cu (II) [49], Cr (III) [34], As (III) [50].

The standard biopanning in phage display technology was modified to identify a unique M13 phage clone that is effective in detecting copper (II) ions. A negative selection step was implemented to eliminate nonspecific

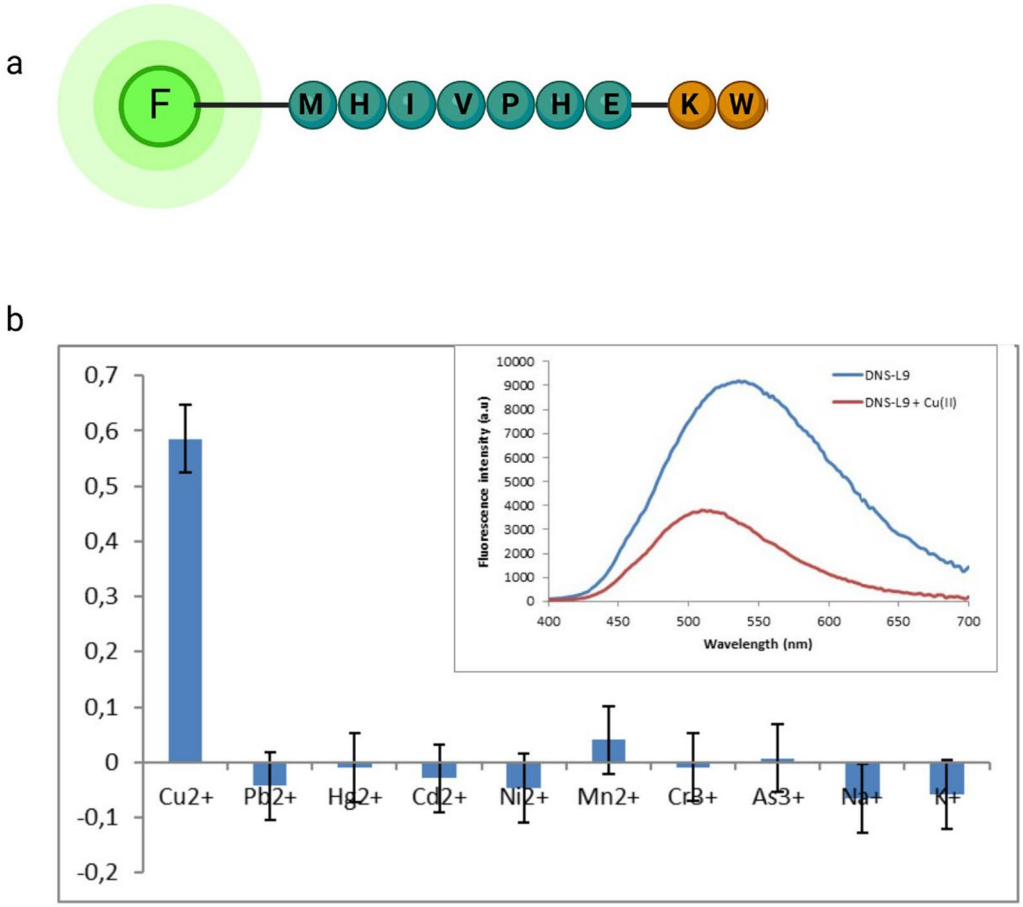


Fig. 5 Analysis of peptide based fluorescent chemosensor for copper(II) ion detection. **a** Schematic representation of design peptide based chemosensor (DNS-L9). **b** Sensitivity and selectivity of DNS-L9 chemosensor in the presence of various metal ions (1 equiv.|). DNS-L9, a fluorescent peptide that is highly soluble, was dissolved in double distilled water to produce a DNS-L9 solution (2 mM). The metal ions solutions were generated from $Hg(NO_3)_2$, $Pb(NO_3)_2$, $MnCl_2$, $ZnSO_4$, $CuSO_4$, and $NiCl_2$. Cd standard solution, Cr standard solution, $NaAsO_2$, KCl, and NaCl in double distilled water at a concentration of 10 mM. The Multimode Microplate Reader Synergy H1MG was utilized to measure the fluorescence spectra on a 96-well plate with an F-bottom Greiner Bio-one. The excitation wavelength was set to 330 nm. F0 and F were the fluorescence intensities of DNS-L9 in the absence and presence of various metal ions (1 equiv.). The mean of three independent experiments is presented. Inset represent fluorescence emission spectra of DNS-L9 (10 μM) with addition of Cu^{2+} (1.0 equiv.) in HEPES buffer solutions

phage clones that bound to metal ions other than copper. The majority of the previous results have either described the biopanning procedure without the negative selection steps [30, 33], or outlined negative selection after the positive selection [35]. Additionally, some have described negative selection with blanc resin [34]. The methodology of functionalization and modification of microplates for biopanning, which facilitates negative selection with other metal ions, is reported for the first time in this report.

A total of 20 individual clones containing distinctive peptides were identified from selected 240 monoclonal Cu (II)-binding M13 virus plaques. The biopanning procedure proved effective, demonstrated by the increased binding affinity of phage clones with Cu (II)-binding peptides to Cu (II). P-10, P-11, P-12, P-18, and P-20 demonstrated the greatest affinity.

Peptides rich in histidine are highly sought after as ligands for Cu^{2+} ions, as histidine residues can function as the binding site as well as peptides with MH motifs [51]. We have chosen P-11 as the candidate peptide for further examination based on its MHIVPHE sequence.

Cross-reactivity assays were conducted with four supplementary metal ions (Ni, Cd, Hg, and Mn) to enhance selectivity investigations. The selective Cu (II)-binding M13 phage clone that displaces the MHIVPHE peptide demonstrated a strong affinity for copper ions while exhibiting minimal affinity for other metal ions, such as Ni, Cd, Hg, and Mn. This result is of great importance, as the selectivity of the chemosensor for

specific ions is crucial. The incorporation of fluorophores and the precise control over peptide sequences have been made possible by advanced techniques in peptide synthesis, such as solid-phase peptide synthesis [52]. These developments have the potential to further improve the properties of peptide-based materials. It is possible to develop materials with specific chemical and biological properties that are specifically tailored to specific applications by customizing the sequence and structure of peptides.

Conclusion

Presented results clearly show that the phage display technology can be utilize to identify peptides that recognize inorganic analytes such as metal ions in aquatic solutions. Moreover, successful identification of phage clones able to bind copper ions as well as a synthetic modified peptide could be of great importance in the search for an alternative solutions for optical detection and recovery of copper ions in contaminated water.

Materials and methods

Phage library and bacterial strain

The Ph.D.™-7 Phage Display Peptide Library Kit was purchased from NEB (New Eng-land Biolabs GmbH, Frankfurt am Main, Germany). The host strain *Escherichia coli* b K12 ER 2738 (NEB) was grown overnight at 37 °C in LB medium with 20 µg/mL tetracycline (stock solution 20 mg/mL in 1:1 water/ethanol, stored at −20 °C) and stored in 100 µL aliquots each in PCR tubes until use at −80 °C. The strain was maintained during the ongoing experiments on LB agar plates (10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl, 15 g/L agar with 20 µgm–1L tetracycline).

Immobilization of copper ions on plates

100 µL of 10 mM Nα,Nα-Bis(carboxymethyl)-L-lysine hydrate (Sigma Aldrich Cat# 14580) in 0.1 M NaPO4, pH 8, was added to each well of a maleic anhydride-activated microtiter plate (Thermo Scientific Cat#15100) and incubated over-night at room temperature. The plate was then washed three times with 300 µL of 0.05% TBST buffer. The plate was blocked by incubating with 3% BSA in 0.05% TBST buffer for 2 h at room temperature. The plate was then washed three times with 300 µL of 0.05% TBST buffer. The plate was then incubated with 10 mM metal salts for 20 min at room temperature. Such modified plates were used for biopanning.

Surface panning procedure

The host bacterial strain *E. coli* K12 ER 2738 (NEB) and the phage library kit Ph.D.™-7 Phage Display Peptide Library Kit (New England Biolabs GmbH, Frankfurt am Main, Germany) were used in this study. The culture was conducted overnight at 37 °C in LB medium containing 20 µg/mL tetracycline and stored in PCR tubes in aliquots of 100 µL each until its subsequent use at −80 °C. The strain was maintained on LB agar plates in the course of the ongoing experiments. The plates contained tryptone 10 g/L, yeast extract 5 g/L, NaCl 5 g/L, and agar 15 g/L with 20 mg/1 L tetracycline. The phage display library was screened for metallospecific peptides in accordance with the manufacturer's instructions, with only minor modifications. In order to eliminate nonspecific phage clones from the library that bound to metal ions other than copper, a negative selection step was incorporated into the protocol. In summary, 1×10^{11} PFU (plaque-forming units) of the library was diluted in 100 µL of 0.05% TBST buffer and subsequently added to a plate that had been coated with a 10 mM nontarget metal ion. After incubating at room temperature for 60 min with a plate shaker at 100 rpm, unbound phage clones were pipetted into wells coated with an additional negative target and incubated for an additional 60 min at room temperature with a plate shaker at 100 rpm. All clones underwent the negative selection procedure. Negative selection were conducted against nickel, manganese, cadmium, and mercury. Following negative selection, the manufacturer's instructions were followed to conduct three rounds of biopanning with the target metal ion (Fig. 2). 240 plaques were selected from IPTG/X-gal LB titration plates following the third round to amplify phage clones for DNA sequencing. Following this, the 7-amino acid copper ion-binding sequences were isolated by sequencing individual phages.

Binding specificity assay

100 µL of 10 mM Nα,Nα-Bis(carboxymethyl)-L-lysine hydrate (Sigma Aldrich Cat# 14580) in 0.1 M $NaPO_4$, pH 8, was added to each well of a maleic anhydride-activated microtiter plate (Thermo Scientific Cat#15100) and incubated over-night at room temperature. The plate was then washed three times with 300 µL of 0.05% TBST buffer. The plate was blocked by incubating with 3% BSA in 0.05% TBST buffer for 2 h at room temperature. The plate was then washed three times with 300 µL of 0.05% TBST buffer. The plate was then incubated with 20 µM metal salts for 20 min at room temperature. Plates coated with metal ions were incubated with 100 µL of 10^9, 10^8, 10^7 PFU selected clones in TBS buffer per well. Plates were incubtaed 60 min in room temperature with 100 rpm shaking on plate shaker. Nonbounded phages were discarded and plate was washed 10 times with 300 µL 0.05% TBST buffer. For phage elution 100 µL of 0.2 M Glycine–HCl (pH 2.2) was used and after 15 min of incubation in room temperature 15 µL of 1 M Tris–HCl, pH 9.1

was added. Eluted phages were titered according to the method described in The Ph.D.™-7 Phage Display Peptide Library Kit manual using LB/IPTG/Xgal plates. Binding specificity is presented as the percentage of the phage titer eluted from wells coated with selected metal relative to the copper ions, for which a fixed value of 100% was assumed. Error bars represent the standard deviations of the results from three independent experiments.

Peptide synthesis, modification and characterization

The peptide with sequence of Dansyl-Met-His-Ile-Val-Pro-His-Glu-Lys-Trp-NH_2 was synthesized on Rink Amide resin (0.1 mmol) by microwave-assisted Fmoc solid-phase peptide synthesis (SPPS) [52]. Peptide chain elongation was carried out using an Initiator + Alstra™ (Biotage, Sweden) automated microwave peptide synthesizer. Couplings were performed twice for 5 min at 75 °C using Fmoc-amino acid (5 equiv.), *N,N′*-Diisopropylcarbodiimide (DIC) (5 equiv.), and Oxyma (5 equiv.) in DMF. The deprotection of Fmoc group involved 20% piperidine solution in Dimethylformamide (DMF) at room temperature (1 × 3 min., 1 × 10 min.). Dansyl (DNS) was introduced at the N-terminal side of peptide in separate step. For this purpose, DNS 5 equiv.) and TEA (3 equiv.) were added to the peptidyl resin in the dark for 4 h. DNS-labeled peptide was cleaved from Rink Amide resin using the mixture cleavage solution TFA/TIS/H2O (95:2,5:2,5) for 2 h, precipitated with anhydrous, cold diethyl ether and lyophilized. The obtained product was characterized using an analytical reverse-phase HPLC Shimadzu system (Prominence-i LC-2030C Plus, Shimadzu, Japan) with a Jupiter 4 µm Proteo, 90 Å, 4.6 × 250 mm column, with UV detection at λ = 224 nm, using linear gradient method from 5 to 95% solvent B for 60 min at a flow rate of 1 mL/min., where solvent A was water and B was acetonitrile as eluents containing 0.1% Trifluoroacetic acid (TFA). Electrospray ionization mass spectrometry (ESI MS) in positive ion mode (+) was performed using a single quadrupole mass spectrometer (LCMS 2020 Shimadzu, Japan). Isocratic elution, 60% B, where eluent A consisted of water and 0.1% formic acid (LCMS grade) and eluent B consisted of acetonitrile (LCMS grade) containing 0.1% formic acid as eluents containing 0.1% formic acid (FA), at a flow rate of 1.5 mL/min.

General spectroscopy measurements

DNS-L9, a fluorescent peptide that is highly soluble, was dissolved in double distilled water to produce a DNS-L9 solution (2 mM) that was stored at 4°C. The metal ions solutions were generated from $Hg(NO_3)_2$, $Pb(NO_3)_2$, $MnCl_2$, $ZnSO_4$, $CuSO_4$, and $NiCl_2$. Cd standard solution, Cr standard solution, $NaAsO_2$, KCl, and NaCl in double distilled water at a concentration of 10 mM. After the proper dilution, the resulting stock solution was employed for all spectral measurements. The Multimode Microplate Reader Synergy H1MG (BioTek Instruments, United States) was utilized to measure the fluorescence spectra on a 96-well plate with an F-bottom Greiner Bio-one. The excitation wavelength was set to 330 nm.

Statistical analysis

Collected data were analyzed using Excel (Microsoft, Redmond, WA, USA) and the calculations using the SciPy library in the Python3 environment. The analysis was carried out with the use of nonparametric tests due to the limited number of samples, which were insufficient to confirm the normal distribution of obtained results. Statistical analysis of the cross reactivity of monoclonal Cu (II)-binding M13 phage (assuming p = about 0.1) shows significant differences between means for Cu and Hg or Cd.

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Author contributions

Conceptualization, B.G. and M.S.; methodology, M.S., T.Ł., B.G.; formal analysis, B.G., M.S..; investigation, M.S., T.Ł., B.G.,. resources, B.G.; writing-original draft preparation, B.G., M.S.; writing-reviewing and editing, M.S., B.G.; visualization, M.S., B.G.; supervision, B.G.; project administration, B.G., M.O.; funding acquisition, B.G. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

No datasets were generated or analysed during the current study.

Declarations

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