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Originally published:

August 2017

Solid State Phenomena 262(2017), 443-446

DOI: https://doi.org/10.4028/www.scientific.net/SSP.262.443

Phage display – a Promising Tool for the Recovery of Valuable Metals from Primary and Secondary Resources

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Keywords: bacteriophage, biosorption, bioflotation, fluorescent phosphors, Ga-, Co-, Ni-ions, heterologous peptide expression

Abstract. The development of effective and ecofriendly processes for the recovery of critical elements poses a challenge for scientists all over the world. A novel approach is the generation of highly specific peptides that bind with high affinity to individual elements of interest. The peptides are selected by phage surface display (PSD) technology. In this study, PSD technology has been applied in two different approaches. The focus of the first approach was the identification of peptides that bind specifically to special particles of interest that are part of electronic scrap aiming towards the development of new recycling processes. In the second approach, metal ion binding peptides were isolated via PSD to use them for the targeted removal and enrichment of these elements from complex leaching solutions or from industrial waters. To address the economic production of peptides, the development of a new expression system is also part of this study.

Phage surface display

Phage surface display is a powerful tool to identify biomolecules with an extraordinary affinity and specificity for the chosen material of interest. PSD technology was originally developed to identify peptides with high binding specificity for biological molecules such as viruses, antibodies or fusion proteins. Medical and pharmaceutical applications have been the focus of this approach [1]. Later the technology was successfully applied to inorganic targets as well. PSD technology was used for a high number of different materials, for example copper or gold to identify material specific peptides [2, 3]. Other groups used the PSD technology to isolate peptides with semiconductor specificity for directed nanocrystal assembly [4]. Dunbar and Curtis identified peptides via PSD that were able to differentiate between chalcopyrite and enargite, making the peptides highly interesting for mineral separation [5].

The biopanning process uses phage peptide libraries with a diversity of 10^9 different expressed peptide motifs. This pool of random peptide motifs binds directly to the pure material of interest but with different affinities depending on the specific binding structures. While strong binding peptides attach to the material surface, loosely bound phage particles will be washed away. The strong binding phage particles are eluted and amplified. Amplified phage are cyclically screened with the desired material with increasingly stringent binding condition to select the strongest binding phage. Only those phage linked peptides that hold a strong affinity and specificity towards the material of interest remain attached to the material surface. Within three to five biopanning rounds those phage particles bearing strong binding peptide motifs were identified and later used for individual binding tests. Future applications of these binding peptides need to be phage-free which makes their handling easier and less expensive to produce in large amounts. Moreover, using whole phage may result in particular legislative constraints due to their effect on the environment. The phage-free peptides can be produced chemically or via heterologous expression. Peptides that selectively bind specific metal ions or other critical elements may be used for biosorption, bioflotation and bioremediation, allowing completely new biomining approaches [5, 6].

The application of PSD technology and peptides for recycling processes and the removal and enrichment of critical elements is a new and challenging approach (Fig. 1). Beside needing to be compatible with the environment, new products and techniques have to be highly specific for target materials and economically applicable. Critical elements focused in this study are rare earth elements as part of a multitude of modern electronic devices, gallium ions as part of industrial waste waters and nickel and cobalt ions from copper black shale leachates.

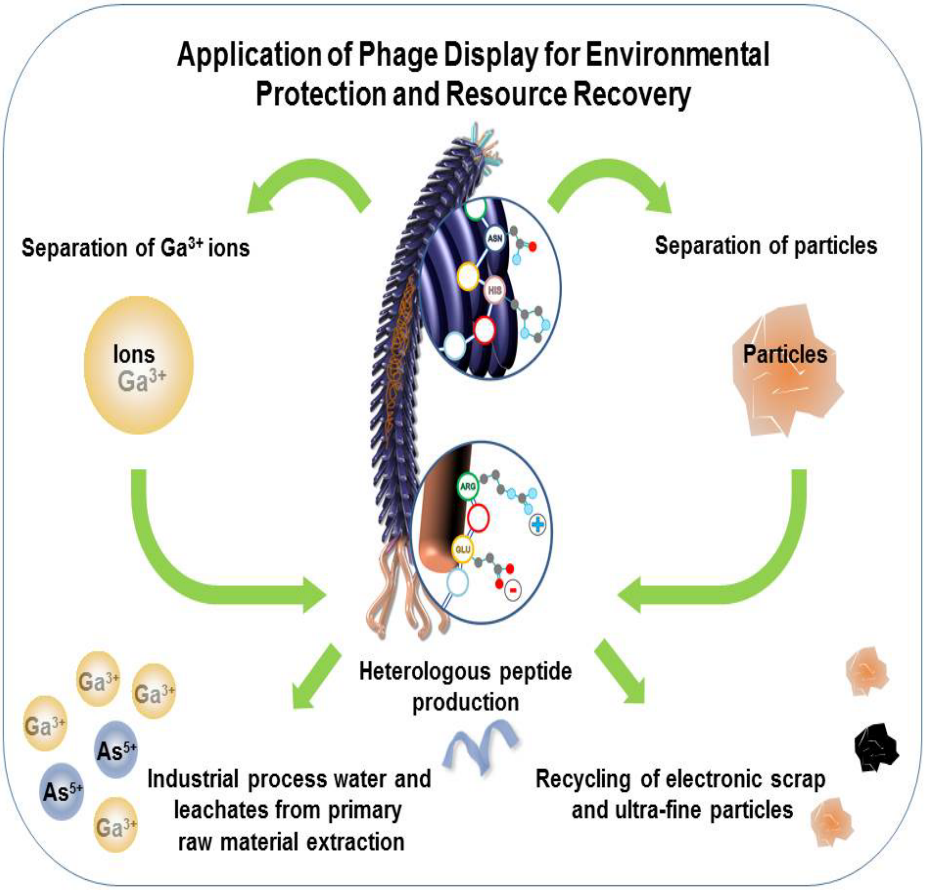

Figure 1. Genetically modified phage particles are the central tool of the PSD technology. Phage peptide libraries enable the identification of peptides with high binding affinities for the ions or particles of interest. The efficient heterologous peptide expression makes their application in recycling, enrichment and removal processes economically applicable.

Development of rare earth recycling processes.

The MinePep project (Marie Curie IOF, European Union) was focused on the establishment of a novel technology platform for bio-based mineral processing. The proof-of-principle for the development of peptides as agents for the separation of rare earth minerals via bioflotation was started with four different fluorescent phosphor components that are part of fluorescent light bulbs. Three different phage libraries that express peptides at the major coat protein pVIII of bacteriophage were used to identify a small number of specific binding peptides via PSD. Compared to the more commonly used pIII phage libraries, each of the randomly modified pVIII phage clones expresses the same peptide motif up to 150 times along the phage body making the screening process more efficient in case of complex materials. Using the f88.4 phage libraries Cys4 that expresses 14-mer peptide motives ($X_4CX_4CX_4$), Cys6 (Creative Biolabs) that expresses 16-mer peptide motives ($X_4CX_6CX_4$) and LX-4 (kindly provided by Dr. Jamie Scott) that expresses 8-mer peptide motives (XCX_4CX), two highly specific $LaPO_4$:Ce,Tb (LAP) binding peptides (8-mer and 16-mer) and four $CeMgAl_{11}O_{19}$:Tb (CAT) specific peptides (14-mer) were isolated. The CAT binding peptides were characterized by lysine-rich amino acid compositions. However, the newly identified peptides differ strongly in their amino acid composition and binding behavior. Binding properties can be improved by substitution of individual amino acids by more effective amino acids [6]. The application of peptides in bioflotation processes is now being tested as an efficient separation process for rare earth minerals. Phage particles that express the peptide motive RCQYPLCS have shown very good LAP agglomeration results in small scale experiments.

Identification of gallium binding peptides.

Gallium is used in the semiconductor compounds GaAs, GaN or GaP for high-potential future technologies. The resulting rapidly growing demand for gallium should not be exclusively met by the mining of new primary raw material sources. The EcoGaIn project aims, inter alia, to purify gallium from industrial wastes using biotechnological approaches.Biosorptive recycling of gallium from waste waters from the semiconductor industry is a promising and innovative contribution for establishing an economic and clean zero waste technology. Peptides are suitable ligands for the biosorptive complexation of gallium ions in aqueous solutions due to the variability in their amino acid sequence and robust properties. A very effective method has been established for the selection of different phage display libraries. Gallium ions immobilized on a monolithic ion exchanger are made accessible for biopanning in a Fast Protein Liquid Chromatography (FPLC) system. This chromatopanning allows the selective enrichment of gallium-binding clone variants under strictly controlled process conditions.In the present study, we report the enrichment, identification and characterization of several gallium-binding motifs. A total number of 92 unique putative gallium binding peptides was identified up to now. More detailed information regarding these experiments are given in another contribution to the IBS proceedings. The corresponding peptide sequences can be synthesized or heterologously expressed and used in subsequent experiments to develop biosorptive materials for selective gallium recovery from industrial waste waters.

Identification of nickel and cobalt binding peptides.

In the BMBF-funded German-French project "EcoMetals", we focused on novel innovative biological methods for the extraction of copper and accompanying elements from complex copper-containing ores or tailings. The selective separation of individual industrially relevant chemical elements from complex copper-containing leaching solutions represents a particular challenge. Currently, metal-binding peptides are regarded as particularly promising candidates to separate such elements effectively. For the isolation and characterization of nickel- and cobalt-specific peptides, we used the bacteriophage library Ph.D-C7C (New England Biolabs, Inc.). In this library the minor coat protein pIII is genetically modified leading to the expression of five copies of a phage tail protein containing a foreign heptapeptide loop flanked by a disulfide bridge. From a phage pool of

10^9 different peptide motifs, 24 peptide motif bearing phage for nickel and 18 for cobalt were isolated in a three-round-panning procedure as described in general above. Thereby, the nickel or cobalt ions were immobilized on sol-gel material with ion exchange capacity. Interestingly, the commonly good nickel or cobalt binding amino acid histidine is not dominant. The binding strength of the isolated phage clones was compared with the wildtype phage peptide by competitive binding assay on metal loaded agarose beads (PureCube Ni and Co-NTA agarose, Cube Biotech), where the amount of bound phage of each clone was determined. Cross binding tests revealed that most of the nickel binding phage would also bind to cobalt and vice versa. The peptide motifs of the most prominent binding phage will be synthesized afterwards for future application oriented experiments.

Effective heterologous expression of metal binding peptides.

In this study we describe the development of a fast and reliable cloning and expression system for metal binding peptides, allowing the transfer of pre-identified peptide sequences into small peptides. Peptide sequences were taken from ongoing PSD experiments. The commercially available IMPACT™ Kit (New England Biolabs) was combined with Gibson Assembly [7, 8] to simplify the method and avoid restriction enzyme-based cloning. The developed system can be rapidly adapted to other phage display libraries, making it a versatile tool for characterization of the interactions of many phage display derived peptide sequences.

Summary. Phage surface display is a promising tool for the development of highly specific binding peptides. The development of ecofriendly but specific collectors that can be applied in bioflotation, bioremediation and separation processes or for biosorption has the potential to revolutionize traditional metal recovery techniques.

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