Selection of exclusively binding peptides to copper ion by chromatographic biopanning

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Biopanning based on phage display has been known as a powerful tool enabling the screening of peptide with specific affinity to various target substrates. However, conventional biopanning is a methodology using batch adsorption equilibrium between phage and target so it might require excessively repeated screening step. Recently, chromatographic biopanning based on multiple stage phage-binding equilibrium was reported. This study is an application of the chromatographic biopanning, adopting a monolithic column in order to use insoluble phage particles, to the screening of copper binding phage peptides. Copper was first bound to the matrix of IDA-monolithic column through which commercially available phage display library was passed. After several rounds of positive panning, the isolated phage binders to Cu was passed through Fe-IDA-monolithic column and Pb-IDA-monolithic column in order to exclude copper binders crossly binding to Fe and Pb. From ssDNA sequencing analysis of randomly chosen 126 phage clones, the peptide sequence of the highest frequency, Asn-Ala-Lys-His-His-Pro-Arg('NAKHHPR'), was determined as the strongest binders to Cu.