INNOVATIVE BIOSENSOR PLATFORM BASED ON A PHAGE DISPLAY SANDWICH BIOTECHNOLOGY

Most of the recent analytical methodologies for the detection of cell systems are mainly based on indirect detection systems which, although sensitive, suffer from the quite laborious laboratory steps for sample preparation and detection, the consequent need for specialized centralized labs, long turnaround times, expensive costs. As such, sensitive, inexpensive and direct readout analytical systems, that can be implemented for Point-Of-Care Technologies, are required. In this context, the phage display technology can be exploited to create innovative, sensitive, economical and robust detection systems. Herein we present a new analytical method for the direct detection of cells and proteins, by means of a "molecular sandwich" of capture and detection with probes based on engineered phages. In particular, the capture probe consists in magnetic microbeads (MNP) bioconjugated with engineered M13 phages, which expose fusion peptides capable of selectively binding the analyte. These peptides, in fusion with the major coat protein pVIII of the phage can be identified by phage display for the selective recognition of molecular interactors present on the surface of eukaryotic cells, microorganisms, viruses or other biological macromolecules of interest. The engineered phages allow the capture of the analyte, and subsequent enrichment due to MNP-mediated magnetic immobilization. For detection of the analyte, a second engineered M13 phage constitutes the signal transducing element. In particular, the five-copies of the minor coat pIII protein of this reporter phage are genetically fused to a selective targeting peptide/protein/antibody, displayed in multivalence on the phage tip. At the same time, the capisd of the reporter phage is chemically conjugated with hundreds of reporter dyes, providing a truly orthogonal signal transduction scheme. The successful capture and identification of the analyte can been detected by optical or electronic sensors, reading out the signal from the reporter phage. As a proof-of concept, a prototype of the platform has been engineered, able to detect the presence of pathogenic Pseudomans aerugionosa bacteria.
Finally, the possibility of decorating the phages with different recognition element exposed allows to implement multiplexing detection for the integrated analysis of analytes simultaneously.
This method is of particular relevance to direct detection of infectious microorganisms such as SARS-CoV-2, but is also extendable to eukaryotic cells. In particular, this new platform could be used for liquid biopsy with the possibility of capture, concentration and direct detection of cancer cells by phages engineered to recognize specific tumor markers exposed on the cell surface.

Keywords
Engineered M13 phages, major coat protein pVIII, minor coat protein pIII, molecular sandwich, biosensor, microorganism and protein detection.