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Multiplex localization of sequential peptide epitopes by use of a planar microbead chip

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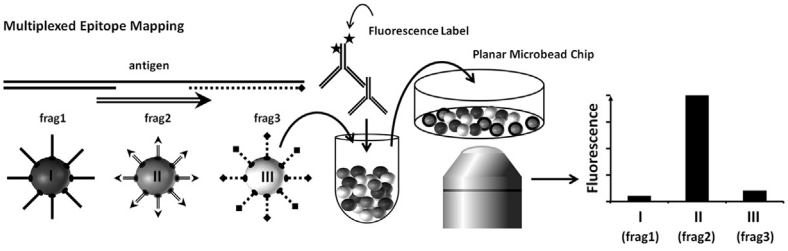
HIGHLIGHTS

- The epitopes of antibodies were successfully mapped with the novel multiparametric imaging platform.
- Biotinylated peptides were not chemically synthesized but manufactured recombinantly in *Escherichia coli*.
- Incubating crude *E. coli* lysates with streptavidin coupled microbeads were enough for peptide immobilization.
- The analysis can be completed within three weeks starting from the delivery of the oligonucleotides for cloning.

GRAPHICAL ABSTRACT



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ABSTRACT

Epitope mapping is crucial for the characterization of protein-specific antibodies. Commonly, small overlapping peptides are chemically synthesized and immobilized to determine the specific peptide sequence. In this study, we report the use of a fast and inexpensive planar microbead chip for epitope mapping. We developed a generic strategy for expressing recombinant peptide libraries instead of using expensive synthetic peptide libraries. A biotin moiety was introduced *in vivo* at a defined peptide position using biotin ligase. Peptides in crude *Escherichia coli* lysate were coupled onto streptavidin-coated microbeads by incubation, thereby avoiding tedious purification procedures. For read-out we used a multiplex planar microbead chip with size- and fluorescence-encoded microbead populations. For epitope mapping, up to 18 populations of peptide-loaded microbeads (at least 20 microbeads per peptide) displaying the primary sequence of a protein were analyzed simultaneously. If an epitope was recognized by an antibody, a secondary fluorescence-labeled antibody generated a signal that was quantified, and the mean value of all microbeads in the population was calculated. We mapped the epitopes for rabbit anti-PA28γ (proteasome activator 28γ) polyclonal serum, for a murine monoclonal antibody against PA28γ, and for a murine monoclonal antibody against the hamster polyoma virus major capsid protein VP1 as models. In each case, the identification of one distinct peptide sequence out of up

Abbreviations: GST, glutathione S-transferase from *S. japonicum*; VP1, hamster polyoma virus major capsid protein VP1; PA28γ, human proteasome activator protein 28γ; frag, fragment.

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to 18 sequences was possible. Using this approach, an epitope can be mapped multiparametrically within three weeks.

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1. Introduction

Epitope mapping is an important step in the improvement of antibody based assays, the understanding of autoimmune diseases and the development of effective and safe vaccines. Several epitope mapping methods are available as reviewed by Gershoni et al. [1]. Among them are sophisticated strategies such as determining the molecular structure of antibody–antigen complexes using X-ray analysis [2] or nuclear magnetic resonance, protein foot printing [3] and mutational approaches. In the latter, the antigen is systematically or randomly mutated leading to the identification of all amino acids contributing to the antibody's binding [4]. These methods determine both linear and conformational epitopes, but they are not suitable for routine high-throughput applications or multiplexed probing. Investigations with peptides often fail to detect conformational epitopes, but the ability to multiplex yields faster results for epitopes. Screening of so-called peptide display libraries can be applied to identify epitope sequences [5,6]. Probably the most prominent approach is the peptide scanning method introduced by Kazim et al. [7]. Therein, the antigen is divided into a panel of overlapping peptides that are then probed with the antibody individually. This method can include an ELISA wherein the peptides are immobilized in microtiter plates followed by antibody detection directly in the same well [8,9]. Peptides can also be immobilized on other solid surfaces such as peptide array chips [10,11]. However, ELISA requires more sample compared to peptide arrays and is limited in multiplexing [12]. Peptide arrays require sophisticated spotting facilities and are therefore expensive.

Multiplex detection refers to the simultaneous determination of multiple analytes per reaction tube. This reduces the amount of sample material, reagents and working steps compared to assays where analytes are processed separately [13]. One multiplexing strategy uses microbead assays, e.g., suspension microbead assays [14]. However, such assays require a complex and cost-intensive flow cytometry system for analysis.

Our epitope mapping assay is a microbead-based peptide-scanning method using the VideoScan technology [15]. Briefly, the method was developed for multiplex detection of proteins, nucleic acids and cells [16–19]. It is based on size- and fluorescence-encoded microbead populations, which can be coupled with different capture probes such as nucleotides, proteins or putative antigen epitopes. Fig. 1 illustrates our proposed workflow for the epitope mapping. Each microbead population presents one putative epitope on the surface. The microbeads are pooled prior to incubation with an antibody solution or serum. Epitope recognition by a specific microbead population is then detected with a fluorescence-labeled secondary antibody. To quantify the fluorescence, microbead images are captured by the fully programmed fluorescence microscope technology VideoScan. The pictures are evaluated automatically using the *FastFluoScan* imaging processing software, which assigns microbeads to their population and quantifies their surface fluorescence intensity.

Efficient peptide immobilization is achieved by binding biotinylated peptides onto microbeads coated with streptavidin. To avoid expensive and time-consuming chemical peptide synthesis, we took advantage of an *Escherichia coli* strain, AVB101, that expresses biotin ligase (Avidity Inc., CO, USA). This system enables the overexpression of site-directed biotinylated proteins and peptides harboring an AviTag™ sequence [20]. This 15 amino acid tag is fused to either the N-terminus or C-terminus of a protein/peptide to provide a substrate for the biotin ligase. A 15-mer peptide can be generated for approximately 70 €, whereas purchasing such a peptide manufactured by chemical synthesis entails 10-fold higher costs.

To demonstrate the practicability of our new method, we

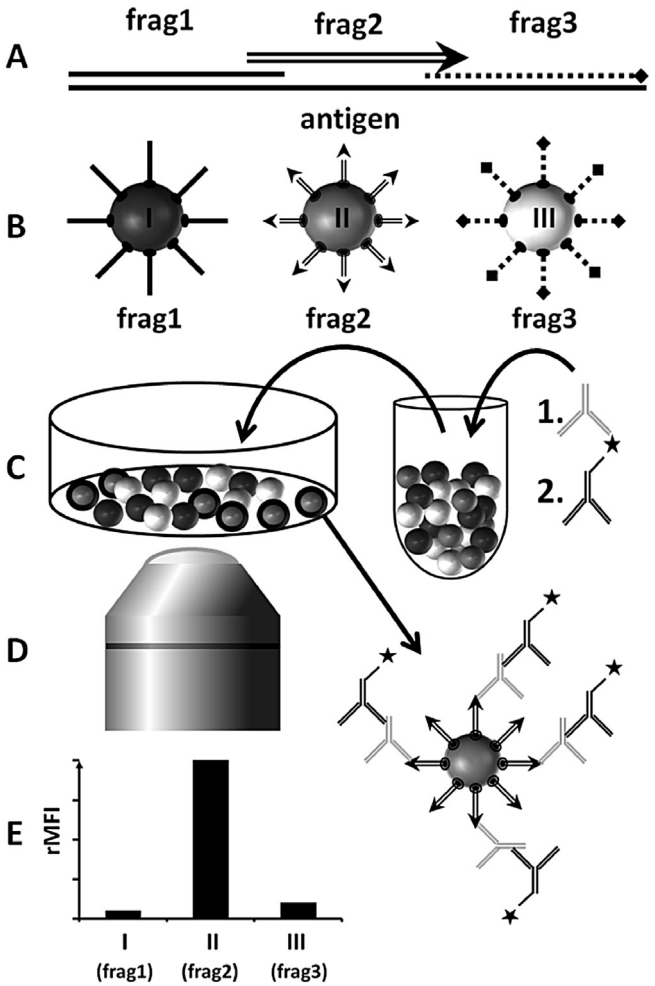

Fig. 1. Principle of the multiplex epitope mapping procedure using VideoScan technology (A) An antigen is subdivided into three overlapping fragments (frag1, frag2, frag3). (B) These fragments are coupled to three microbead populations that are fluorescence- and size-encoded. (C) The microbead populations are mixed and incubated with an antibody whose epitope is to be determined. After a further incubation with a secondary antibody labeled with a fluorescent dye, the microbeads are transferred into a well of a 96-well-plate, where they settle downward. (D) A fluorescence microscope is used to take photos of the bottom of the plate. Special imaging software analyzes the photos, groups the microbeads into populations and measures their surface fluorescent intensity. (E) Finally, for each population the (referenced) mean fluorescence intensity rMFI is calculated. The population that presents the fragment with the epitope will have the highest surface fluorescence (black circles around some microbeads in C).

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