

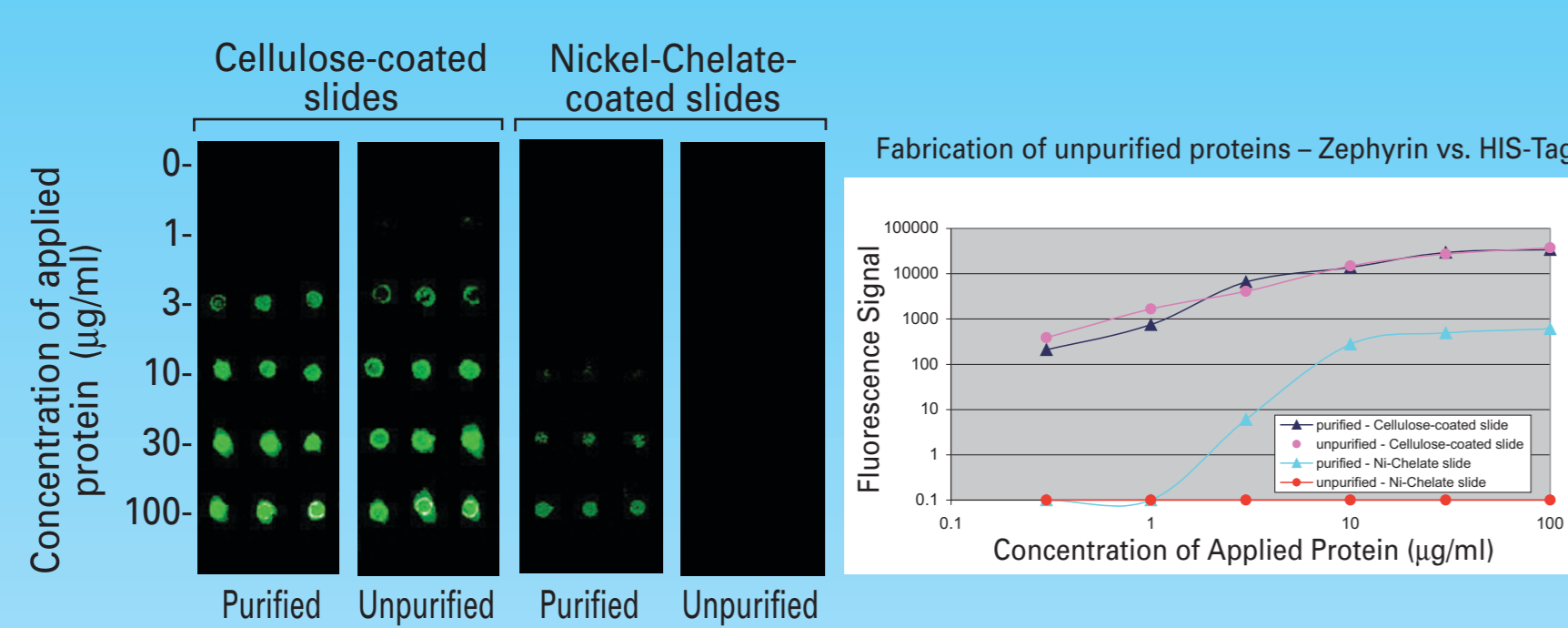
# Zephyrin-based microarray - A novel and versatile platform for protein microarray

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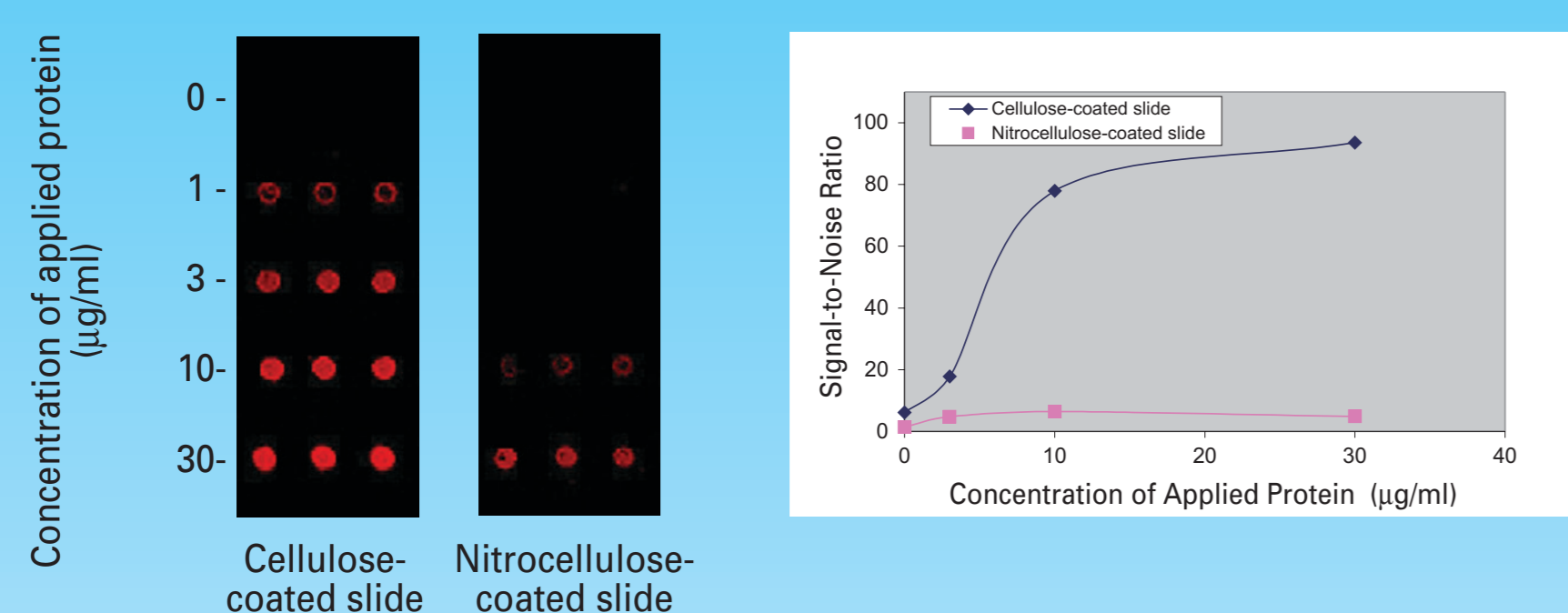
## Properties and Advantages

### Simple fabrication of purified and unpurified proteins



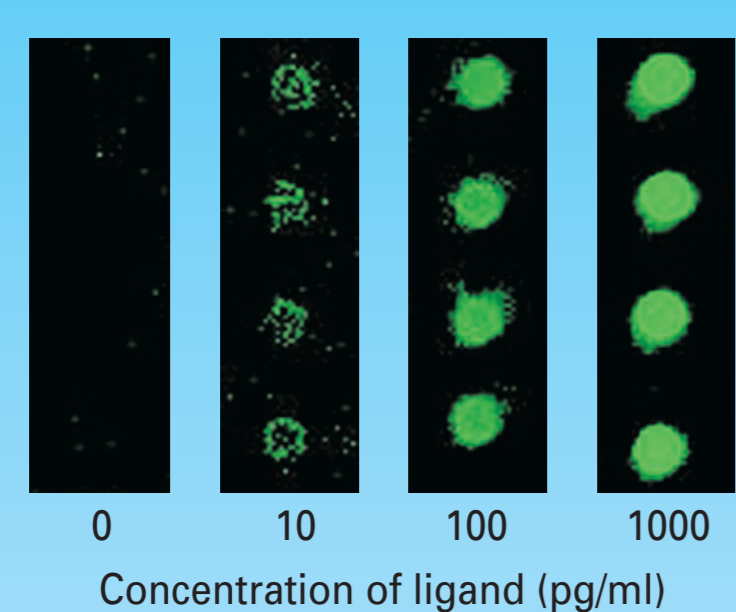
For conventional protein microarray systems, sources of large numbers of purified proteins and their immobilization represent a major technical challenge. Zephyrin-based technology offers simple noncovalent fabrication of unpurified proteins. Zephyrin-fused proteins can be fabricated directly and efficiently without further purification compared to other affinity-tagged based microarray. To demonstrate this feature, purified HIS-tag-Zephyrin-ZZ (modified *Staphylococcal* protein A, SpA domain) was spiked and serially diluted either into rabbit reticulocyte lysate (unpurified) or PBS (purified). The samples were fabricated on cellulose-coated glass slides and on commercial nickel-chelate-coated slides and allowed to interact with fluorescence-labeled rabbit IgG.

### Superior signals and signal-to-noise ratios



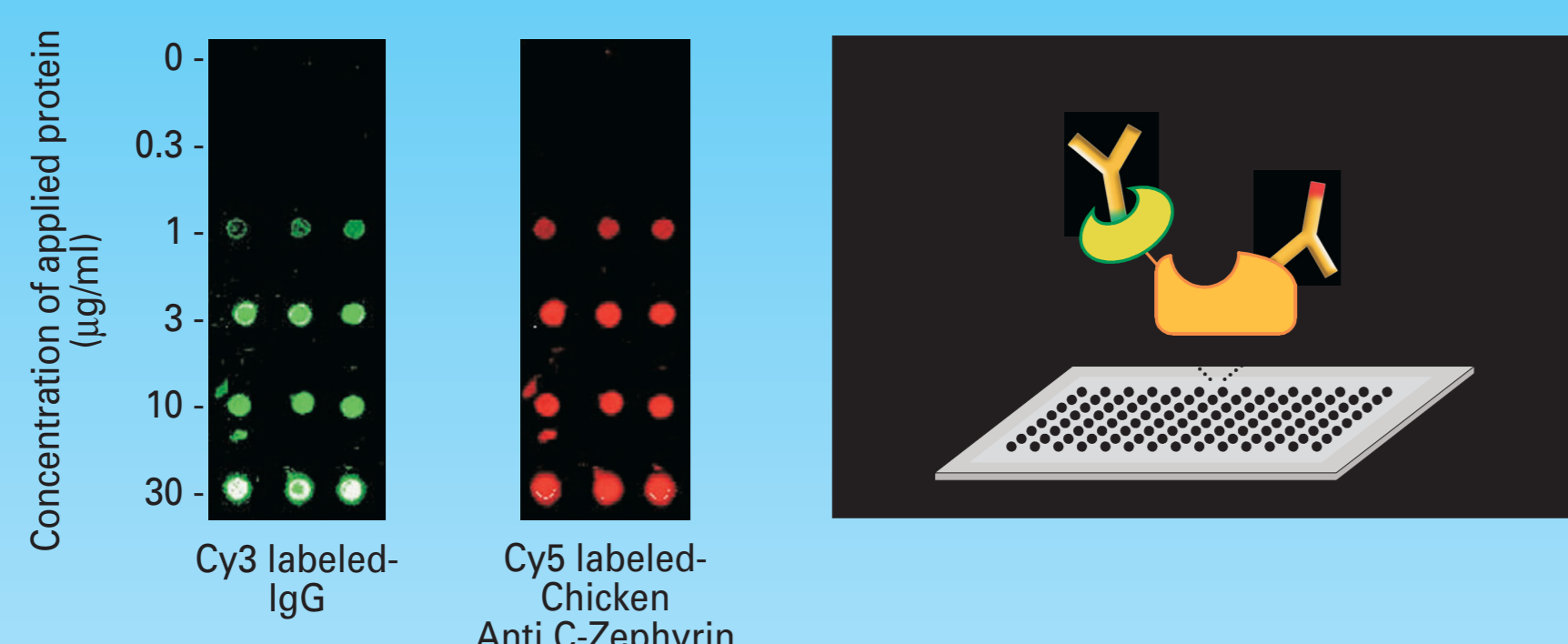
Microarray technologies, based on immobilization to three-dimensional polymers, usually generate high signal intensities. However, the high signals are often accompanied by high background, thus rendering the apparent advantage of strong absolute signals rather useless. In contrast, the cellulose-coated slides employed in Zephyrin-based technology benefit from both extremely high signal and exceptionally high signal-to-noise ratio. In this example, serially diluted Zephyrin-ZZ (SpA domain) was fabricated on cellulose-coated and nitrocellulose-coated glass slides and allowed to interact with rabbit IgG, followed by incubation with fluorescence-labeled chicken-anti rabbit antibodies.

### Extremely low range detection limit



The lowest detection limit is one of the basic features of protein microarray technology. Zephyrin-based microarray technology offers low detection limits of analytes in the range of ng/ml. This detection limit can be accomplished in remarkably short assay times (5-10 min). However, detection levels of attomole quantities of analytes, in the range of pg/ml, is attainable by extending the incubation time to about 2 hr. In this example, Zephyrin-ZZ (SpA domain) was fabricated on cellulose-coated glass slide and allowed to interact (~120 min) with fluorescence-labeled rabbit IgG (detection limit ~ pg/ml analyte).

### Unique quality control monitoring for the end-user



Quality control testing (QC) is crucial when massive numbers of proteins are implemented in a single biological device such as for protein microarrays. Zephyrin-based technology offers a simple, yet accurate, method for QC tests for the end-user utilizing fluorescence-labeled anti-Zephyrin antibodies. The QC test can be accomplished simultaneously in any experiment. In the following example, serially diluted Zephyrin-ZZ (SpA domain) was fabricated on cellulose-coated glass slides and allowed to interact concurrently with Cy3-labeled rabbit IgG and Cy5-labeled anti-Zephyrin antibodies.

## Introduction

The field of protein microarrays has undergone significant progress since their introduction in the late 20th century. However, the production of protein microarrays represents major technological challenges, regarding approaches for immobilization and generation of a large diversity of purified proteins. Currently, two well-established immobilization strategies are widely employed: noncovalent adsorption to 3D membrane (e.g., nitrocellulose) and immobilization of proteins to chemically activated surfaces. These approaches suffer from high background, low detection limits, and low signal intensities, and share two critical drawbacks: (1) the prerequisite for generation of thousands of pure proteins for efficient immobilization, and (2) the non-oriented and uncontrollable immobilization of proteins, which impairs their functionality.

Here we describe a novel affinity-based protein microarray technology that diminishes the above-mentioned drawbacks of existing protein microarray technologies. The proposed technology is based on a potent cellulose-binding molecule termed Zephyrin and on its ability to bind in an irreversible and specific manner 3D microporous cellulosic-based supports.

### Zephyrin - a powerful display molecule for microarray

Zephyrin, a universal display molecule for protein microarray, is the major cellulose-binding domain from the cellulosome of the bacterium *Clostridium thermocellum*. Zephyrin is one of several scaffoldin-borne domains involved in integrating into the cellulosome complex enzymes that catalyze the degradation of cellulose and other plant cell wall polysaccharides. The Zephyrin molecule has been successfully exploited as an affinity tag for expression and purification of a variety of proteins.

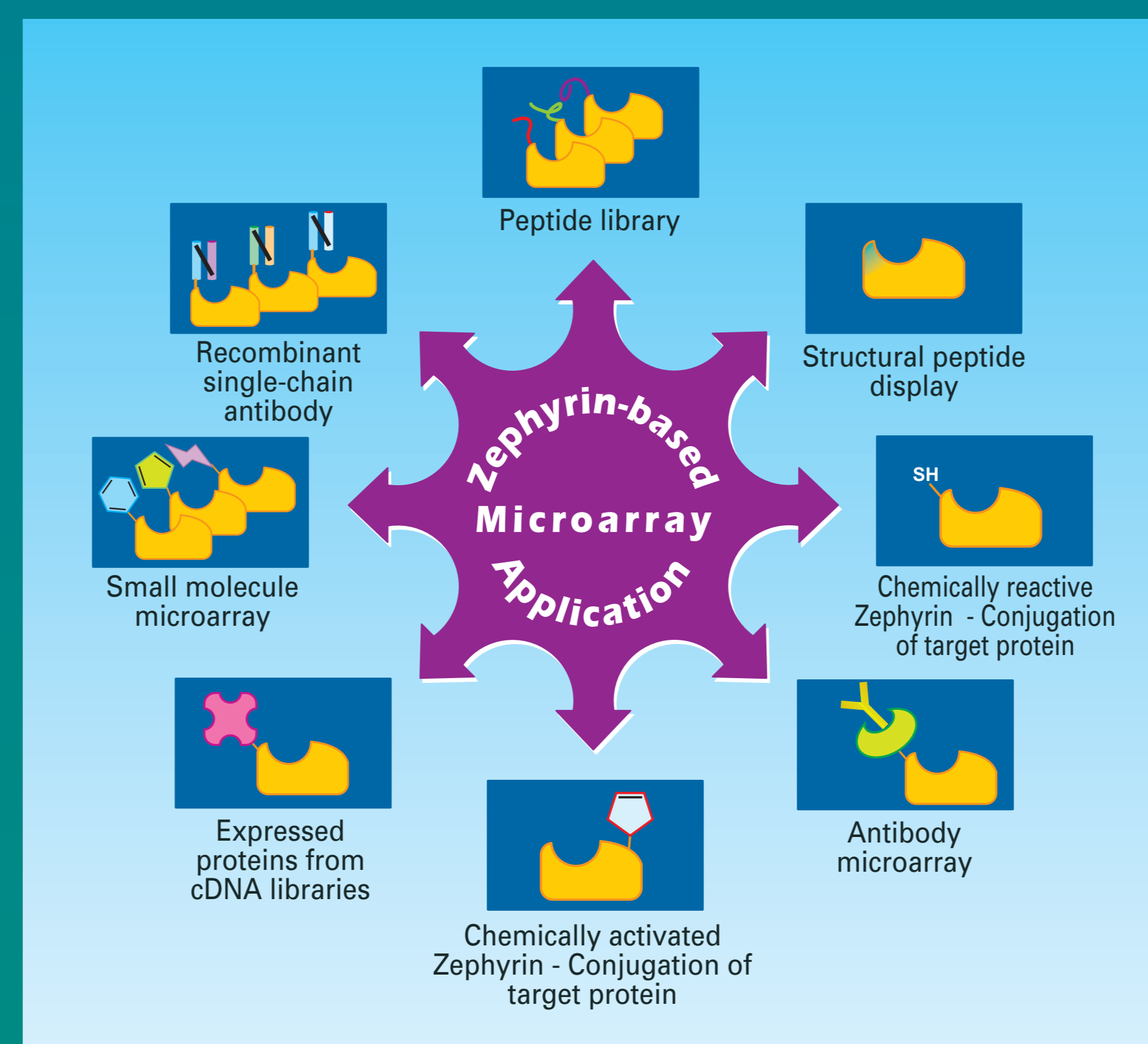


### Cellulose - an alternative surface support for microarray technology

The solid support of Zephyrin-based microarray technology consists of cellulose, an insoluble polymer that is the major constituent of plant matter. Cellulose is remarkably stable, consisting of a linear polymer of  $\beta$ -1,4-linked glucose (repeating units called cellobiose). The individual cellulose chains contain from about 50 to more than 5000 cellobiose units, packed in parallel, three-dimensional fashion into microfibrils.

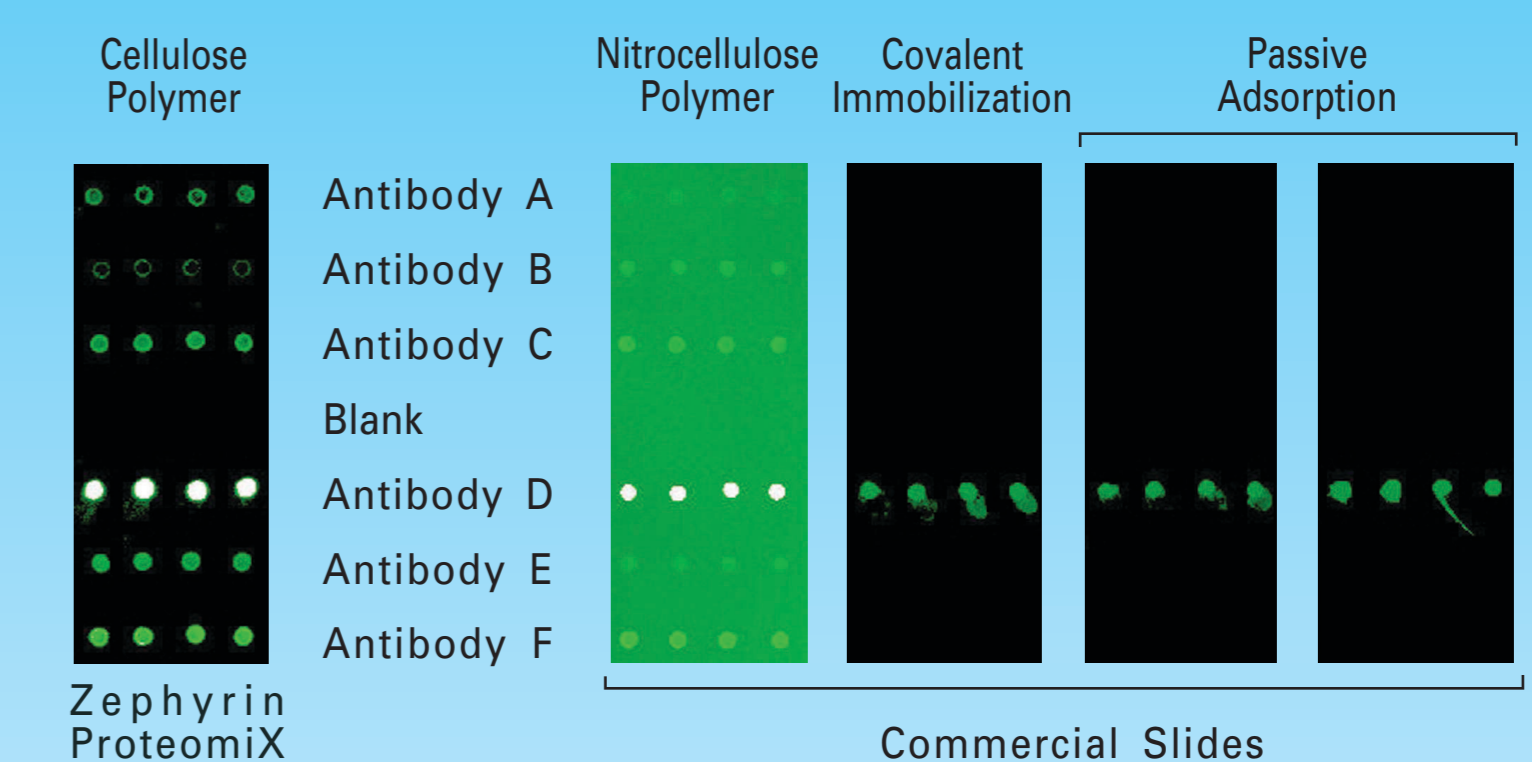
### The Zephyrin-based microarray offers

- Simple and low-cost production
- High flexibility and versatility
- Fabrication of non-purified protein
- Detection levels at attomole quantities
- Extremely high signal-to-noise ratio
- Fully quality-control monitoring for the end-user



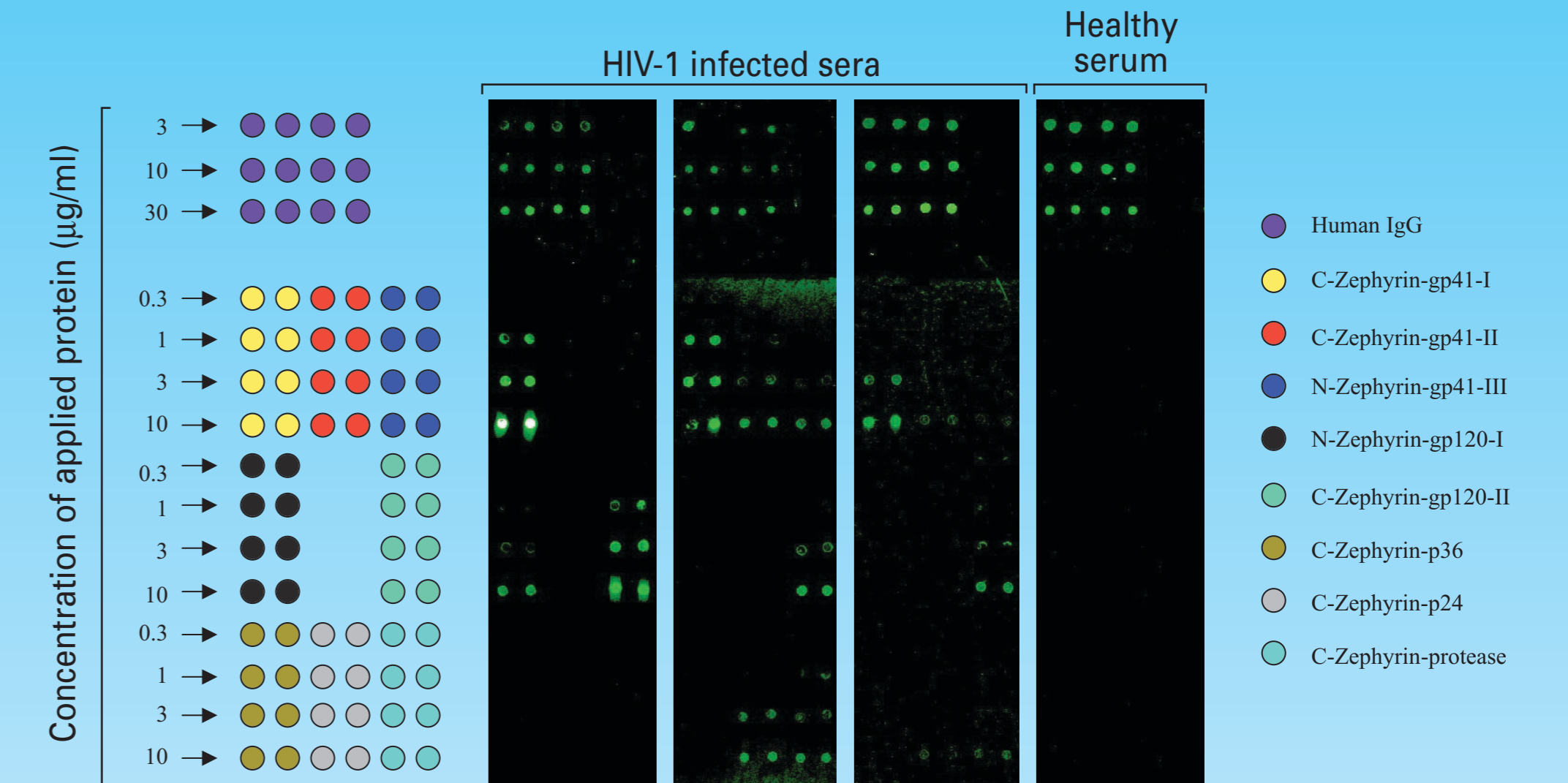
## Applications

### Zephyrin-based vs. conventional antibody microarray



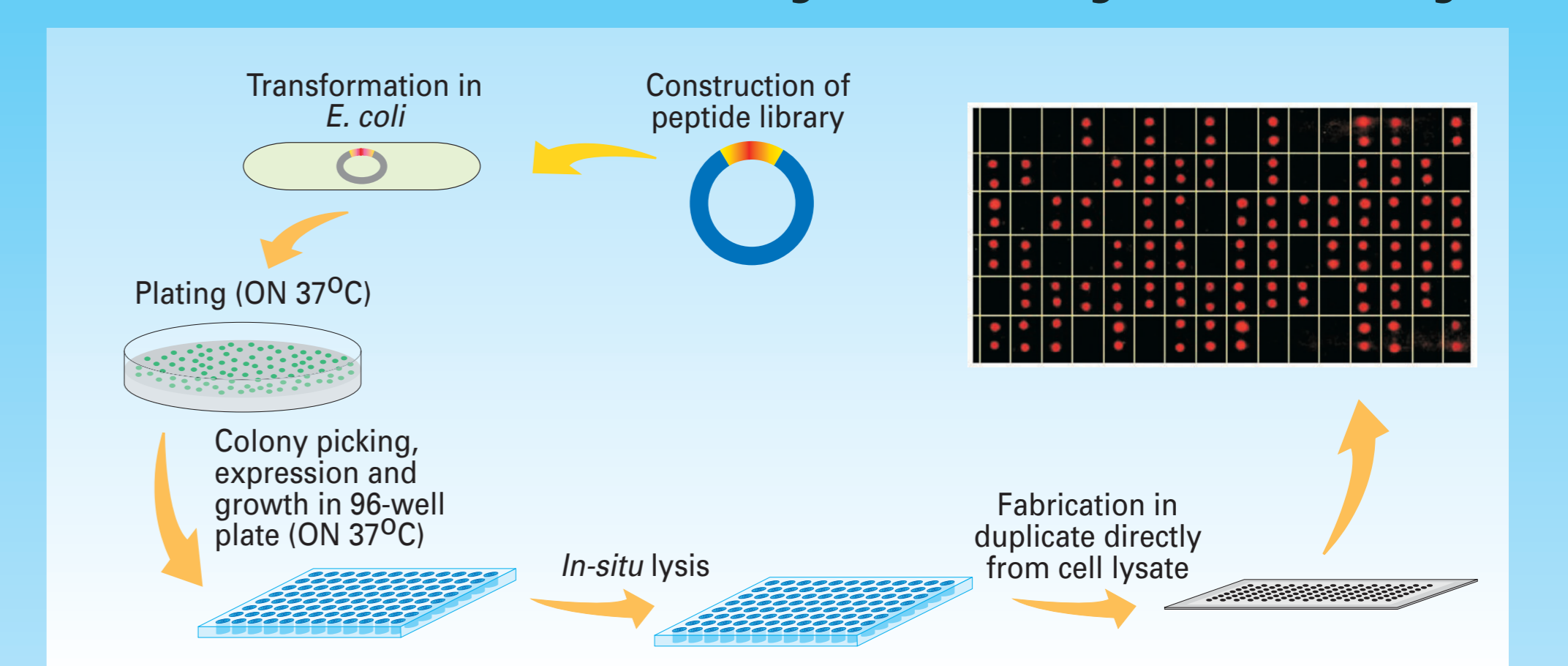
Antibody microarrays have had an enormous impact on the analysis of the human proteome and might eventually become an indispensable tool in disease diagnosis and monitoring. In this example, the sensitivity and specificity of six different antibodies were tested using commercial slides and were compared to Zephyrin-based antibody microarray. Antibodies were immobilized according to the manufacturer's instruction or via Zephyrin-ZZ (SpA domain), followed by incubation with a mixture of six different fluorescence labeled-antigens.

### Serodiagnosis of HIV-1 in human sera - A test model



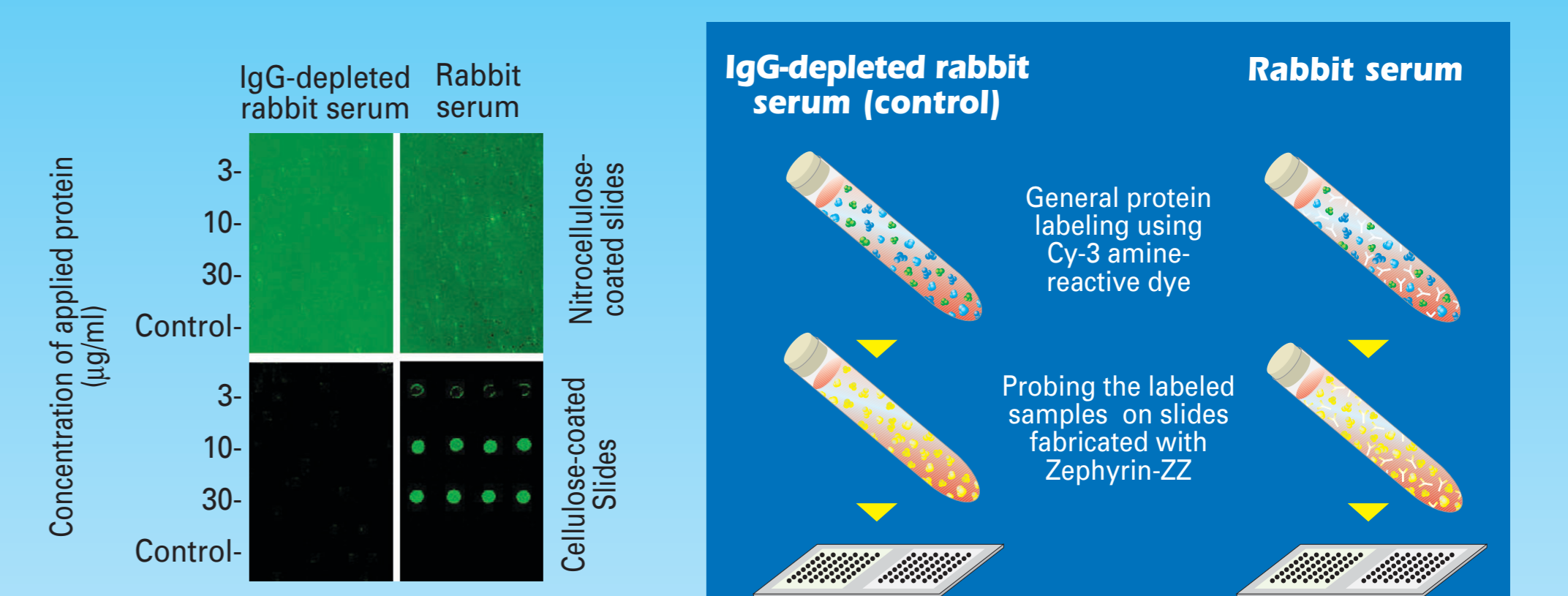
HIV-1 diagnosis is currently performed in ELISA formats using recombinant proteins or immunogenic synthetic peptides. In this example, a Zephyrin-based peptide microarray has been exploited for serodiagnosis of HIV-1 in human sera. For this purpose, immunogenic HIV peptides were fused to the Zephyrin molecule, expressed in *E. coli* and fabricated on cellulose-coated slides. An internal calibration curve was generated by fabrication of human IgG on nitrocellulose membranes. The slides were incubated with sera from either HIV-1-infected or healthy individuals, followed by incubation with fluorescence-labeled rabbit anti-human antibodies.

### Zephyrin-based peptide library microarray - Evaluation and feasibility of library microarrays



Protein and peptide libraries are collections of very large numbers of random recombinant entities. In many cases, fabrication of such libraries may be impossible to accomplish, since hundreds of thousands of proteins or peptides need to be purified prior to the fabrication step. The following example demonstrates a small-scale recombinant display of a random peptide library fused to the C-terminus of the Zephyrin molecule via a short linker and hinge region. The overall strategy is uniquely appropriate for the production of library microarrays for high-throughput formats.

### Differential identification of unknown bio-markers - Feasibility test



The search for proteins associated with disease is an ambitious challenge. Recent advances in protein microarray technologies are beginning to offer an alternative to classical differential approaches. However, several technological hurdles, such as the immobilization of large collections of protein, low sensitivity, and non-specific signals must be resolved before such an approach can succeed. The following example and the overall features shown in this presentation demonstrate the potential of Zephyrin-based microarray technology as an alternative approach for the pursuit of proteins associated with disease.



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