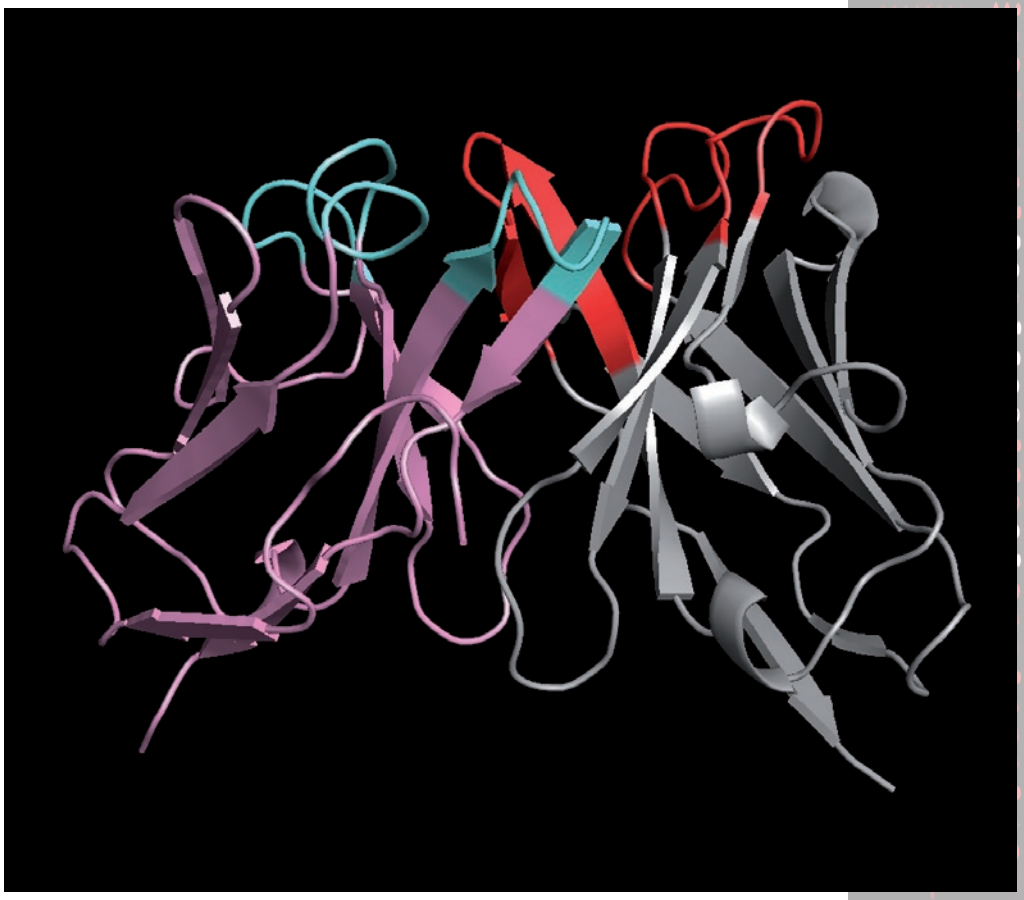
Recombinant Antibody Microarrays

Key Technological Issues

Affinity protein microarrays, and in particular recombinant antibody microarrays, are among the novel, rapidly evolving technologies that hold great promise in high-throughput proteomics. The technology will provide miniaturized, ultra-sensitive set-ups capable of targeting numerous protein analytes in a multiplex manner. However, the process of developing high-performing recombinant antibody microarray technology platforms, as well as protein arrays in general, has proven to be challenging. In this review, we discuss the key technological issues that must be resolved in order to overcome these hurdles.



What Can Antibody Microarrays Deliver?

Entering the postgenomic area, proteomics will play a major role within biomedicine [1–3]. As there are very few effective methodologies available for high-throughput proteomics, the novel recombinant antibody microarray technology has raised great expectations [1, 3, 4]. Miniaturized arrays can be printed with thousands of antibodies carrying the desired specificities, and with biological sample (e.g. an entire proteome) added, virtually any specifically bound analytes can be detected (fig. 1). The microarray patterns generated can then be transformed into proteomic maps, or detailed molecular fingerprints, revealing the composition of the proteome. Thus, protein expression profiling and global proteome analysis using this tool will offer new opportunities for drug target and biomarker discovery, disease diagnostics, and insights into disease biology [1].

In our quest of designing high-performing recombinant antibody microarrays, we have identified five key technological issues that must be considered, including the i) content, ii) array design, iii) sample format, iv) assay design and v) data processing (fig. 2).

The Content

The choice and molecular design of the content are the most critical factors when developing affinity protein microarrays. While a variety of antibody mimics, such as affibodies, trinectins, ankyrins, and aptamers, have been evaluated as potential probes, antibodies are still the obvious choice of content since they are the most well-documented binding molecules [1, 5].

In the case of antibodies, molecular designs ranging from intact polyclonal and monoclonal antibodies to small scFv antibodies have been successfully used [1, 4–6]. However, in recent years it has become evident that using recombinant antibody libraries as probe source is the way to go, at least for large-scale set-ups [1, 5]. By adopting this probe format most, if not all, of the inherent limitations

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associated with the monoclonal and polyclonal probe formats, including availability (no. of antibodies, range of specificities/affinities), scaling-up (costs, logistics), molecular properties (on-chip specificity-functionality-stability) will be resolved [1, 5], since molecular designs can be made that address particular technological challenges.

In our laboratory, we have shown that our human recombinant scFv phage display library [7], genetically constructed around one framework (VH-3-23/VL-1-47) and composed of $2x10^{10}$ clones, has been an excellent probe source [1, 8, 9]. Briefly, these probes, microarray adapted by molecular design, were shown to display a remarkable on-chip functionality, stability (up to 16 month), specificity and sensitivity (pM to fM range) [1, 8, 9].

Array Design

Despite the large repertoire of solid supports available, the precise choice is still not obvious [1]. The optimal support should display (i) high biocompatibility, (ii) high, selective and orientated probe binding capacity, (iii) low non-specific binding. In other words, it should be possible for crude probe preparations to be purified, coupled, enriched and specifically orientated in a one-step procedure directly on the chip, at high density. Clearly, additional surface engineering efforts will be needed. Meanwhile, using pure probes, we have found FAST-slides and Nexterion slide H to perform well. Black Maxisorb slides, in particular, demonstrated an excellent signal-to-noise ratio and has been used by us in a number of clinical studies.

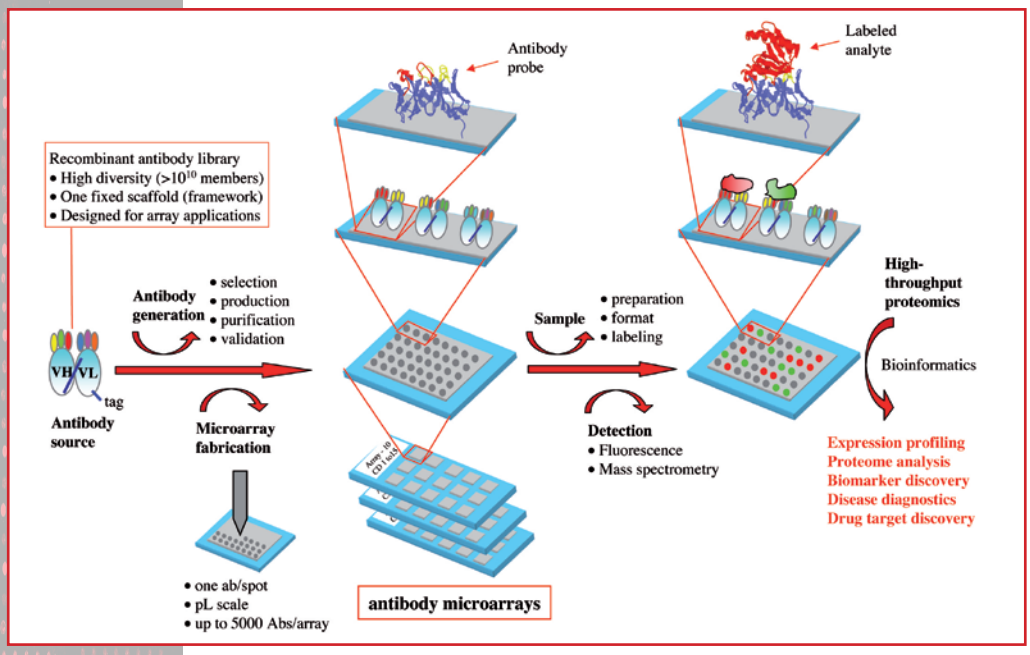

Fig. 1: Schematic illustration of the recombinant antibody microarray technology platform

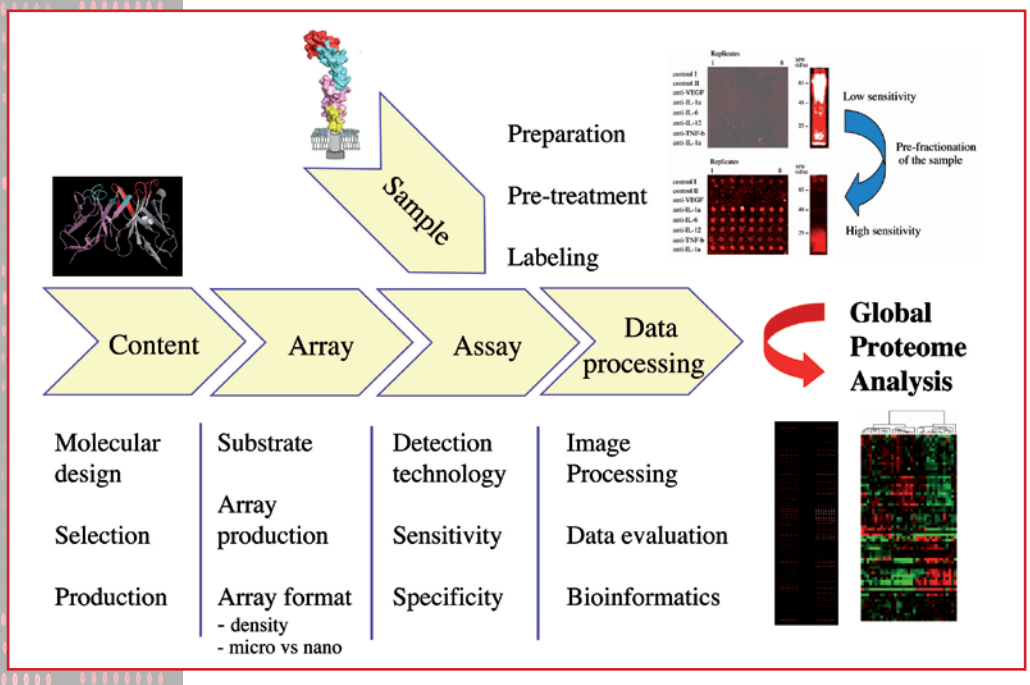

Fig. 2: Key technological issues to consider when designing recombinant antibody arrays

Of note, recent work has outlined a new way of fabricating protein arrays. While mainly non-contact printers have so far been used to deposit the probes one by one, this new concept is based on self-addressing. Each probe will carry a unique zipcode tag that will direct the probes to their unique spot on the chip, i.e. the probes can simply be poured onto the chip and they will find the way to their spot on their own.

High-density arrays will be required in order to perform global proteome analysis [10]. To this end, the first steps to expand the microarray format of today (~1,000 spots/cm^2), to the array format of tomorrow, megadense nanoarrays (~100,000 spots/mm^2), by adopting nanotechnology strategies has been taken [10]. This area will undoubtedly be the focus of significant attention during the coming years.

Sample Format

All samples generated in a soluble format can be analyzed by antibody microarrays. However, to be able to generate complete maps of entire proteomes, technology platforms targeting not only water-soluble proteins, but also membrane proteins must be designed [1]. In fact, the first monoclonal antibody microarray set-up targeting membrane proteins in the format of intact cells was only recently developed. In this context, we have added a clear technological edge by designing the very first recombinant antibody array against membrane proteins.

The tremendous complexity of a proteome may impair the assay by making it hard to label the sample in a representative manner, and/or by causing a high non-specific binding. But by optimizing

the sample format (e.g. the labeling protocol) and the array design (e.g. choice of solid support, blocking reagents), we have successfully designed a state-of-the-art recombinant antibody array technology platform capable of analyzing complex proteomes in a highly specific and sensitive manner [1]. In analogy to traditional proteomics, we have also designed a protocol for pre-fractionation of the proteome to reduce the sample complexity prior to array analysis.

Assay Design

To date, most array platforms rely on tagging the analytes and using a fluorescent-based read-out system. So far, a limit of detection in the pM to fM range has been reported for the best-performing set-ups [1]. Notably, we have shown that similar sensitivities could be obtained even when analyzing directly labeled human whole serum representing one of the most complex proteomes. In fact, sub-zeptomole amounts of the analyte may be sufficient for detection.

In recent years, the first efforts to implement label-free detection have been made to eliminate all problems associated with protein labeling. Several techniques, including MS, SPR, resonance light scattering, nanomechanical cantilevers, and QCM-d, are currently being evaluated [1, 5]. These approaches are promising, but have so far only been used for analyzing small prospective arrays.

Data Processing

In analogy to DNA microarray, recombinant antibody arrays have the capacity of generating tremendous amounts of data that will require advanced bioinformatics. While this field is rapidly evolving, validated approaches and software adopted from the DNA microarray field may very well be used. Still, critical issues such as data normalization (e.g. chip-to-chip) and the possibility to compare data generated on different antibody microarray platforms remains to be resolved. In the end, stringent and standardized procedures should, as in the case of DNA microarrays, be implemented.

Future Outlook

Recombinant antibody-based microarrays will undoubtedly provide a key proteomic research tool, that combined with parallel strategies can be used to generated high-quality integrated data sets suitable for almost any type of proteomic-based investigations. Based on current platforms, we and others have already demonstrated the potential of the technology for rapid and sensitive analysis, ranging from focused assays to semi-proteome scale analysis with applications within mainly cancer research. By successfully addressing the key issues outlined here, the technology has the potential to provide truly proteome-wide analysis in the years to come, with numerous applications within disease proteomics.

Acknowledgements

This study was supported by grants from the BioArray program of SWEGENE, the Swedish National Science Council (VR-NT), Åke Wiberg Foundation, the Crafoord Foundation, the Swedish Medical Association (the National Board of Health and Welfare) and BioInvent International AB.

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Further references are available from the authors.

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