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Antibody microarray analysis of directly labelled complex proteomes

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In recent years, the antibody microarray technology has made significant progress, going from proof-of-concept designs to established high-performing technology platforms capable of targeting non-fractionated complex proteomes. In these cross-disciplinary efforts, a particular focus has lately been placed on two key technological issues: the sample and data handling. To this end, robust protocols have been designed for direct labelling of whole proteomes compatible with a sensitive fluorescent-based sensing. Tagging of the proteins with biotin in a single-colour approach has, in many cases, proven to be the preferred approach. Furthermore, based on modified approaches, adopted from the DNA microarray field, the first bioinformatic standards for performing the antibody microarray data analysis have emerged, though general standard operating procedure(s) remains to be implemented.

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Introduction

Proteomics — the large-scale analysis of all proteins — is a key discipline for generating detailed protein expression profiles, or protein atlases, of human body fluids in both health and disease [1–3]. However, the need for novel technologies capable of targeting non-fractionated complex proteomes in rapid and multiplexed manner while displaying high sensitivity, selectivity and specificity is significant. To this end, antibody-based microarrays have emerged as a strong candidate proteomic technology [4–8]. Miniaturised microarrays (<1 cm²) can be printed with numerous individual antibodies (<2000 antibodies/cm²) in discrete positions (about 200-μm-sized spots) in an ordered pattern, a microarray, onto a solid support where they will act as specific catcher molecules, or probes, for

the target proteins. The microarray is then incubated with minute amount (μl scale) of labelled sample, where later specifically bound analytes are detected and quantified, using mainly fluorescent-based sensing. The read-out generates semi-quantitative microarray images that can be converted into protein expression profiles, revealing the detailed composition of the sample.

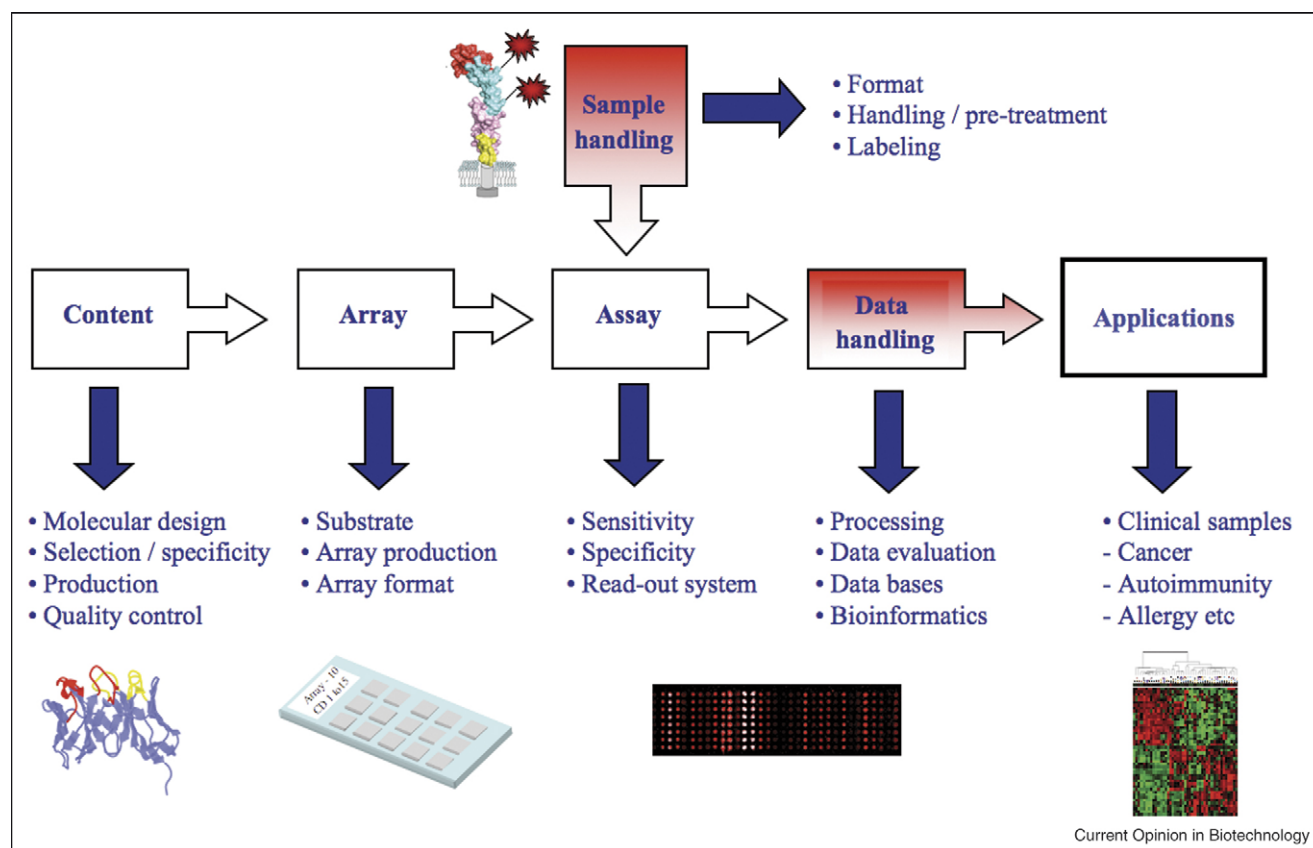
In recent years, the antibody microarray technology has made significant progress. In fact, the high-end technology platforms are now capable of specifically and selectively targeting also low-abundant protein analytes (pM to fM range) in directly labelled whole proteomes, such as human serum [9[•],10[•],11[•]]. This progress has only been made possible by adopting a multidisciplinary approach, and in parallel addressing all of the key technological bottlenecks required to design a state-of-the-art antibody microarray platform (Figure 1) [12], including: first, content; second, solid support; third, array design; fourth, array fabrication; fifth, sample handling; sixth, analytical principle and seventh, data handling. In this review, we will focus on two of these key technological issues, namely sample and data handling (Figure 1), with a particular emphasis on advances achieved during the past two years.

Sample handling — fractionated versus non-fractionated proteomes

The by far dominating microarray format is based on direct labelling of the samples interfaced with a fluorescent-based read-out system [4–7,13]. Although competing layouts, such as sandwich-based arrays [14,15], display some attractive features, for example, high sensitivity and specificity, they are not technically, logistically and economically compatible with high-density array set-ups [7,8,10[•]]. In addition, microarrays interfaced with label-free sensing poses a promising option [16–20], though issues regarding scaling-up and sensitivity remains to be explored further [8]. At an early point, concerns were, however, raised that the threshold for limit of detection (LOD) for layouts targeting directly labelled complex proteomes would be insufficient, and that a 100 ng/ml range cut-off might be the limit. But as the sample format has received appropriate attention, in particular in the past few years, the possibilities have changed dramatically [12].

Targeting complex proteomes, composed of thousands of different proteins, present at a dynamic range of up to 10 orders of magnitude, where a few high-abundant species may mask many of the low-abundant variants [3], is indeed technologically very challenging. Inspired by

Figure 1



Schematic overview of the key technological issues to consider when designing high-performance antibody microarrays.

traditional proteomic-based approaches [21–24] (for more recent reviews see [25,26]), Ingvarsson *et al.* first presented a set-up based on a simple one-step fractionation (based on size) of a proteome, to reduce sample complexity (>98% of the original protein content was removed) [27]. The LOD was significantly boosted, and also low-abundant (sub-pg/ml range), low-molecular weight (<50 kDa) analytes could now readily be detected [27]. Albeit successful, in the sense that the analytes could be assayed, issues regarding yield and reproducibility were associated with this approach, as with any other pre-fractionation and/or depletion strategies commonly applied within traditional proteomics [25,26].

In the next generations of antibody microarrays, where a single or a few of the key array issues (Figure 1) had been optimised further, the ability to target directly labelled complete proteomes was gradually improved, displaying moderate to high assay sensitivities [28–33,34^{••},35–40]. The breakthrough(s) came after meticulously optimising the sample format with respect to labelling conditions (e.g. choice of dye and labelling ratio), sample/assay buffers, etc., in parallel with addressing the other key technological array issues (Figure 1) [9^{••},10^{••},11^{••}].

Robust and reproducible labelling protocols for direct labelling of complex proteomes, for example, serum, plasma and cell expression supernatants are now at hand [9^{••},10^{••},11^{••}], paving the way for sensitive (pM to fM range) profiling of low-abundant protein analytes in non-fractionated proteomes [9^{••},10^{••},11^{••},41]. It is also in this concentration range that many tentative biomarker candidates is expected to be found, further highlighting the applicability of antibody microarrays in disease proteomics for screening, profiling and diagnostics.

Performing clinical studies, additional key sample parameters remains to be optimised and standardised, including first, the format of the clinical sample, for example, serum sample versus plasma sample and second, sample handling and storage, for example, frozen directly or stored at room temperature before freezing [7,8,42,43].

One-colour versus two-colour labelling approach

Both single-colour [9^{••},11^{••},37,41] and dual-colour [28–33,34^{••},35–37] labelling approaches (Table 1) have been successfully used for a broad set of applications,

Table 1

General overview of selected dyes and reagents commonly used for direct labelling of samples for antibody microarray analysis and selected applications thereof based on single-colour and dual-colour labelling approaches

Dye/reagent		Protein residue targets
Cy-dyes: Cy3 and Cy5		Primary amines (lysines)
Alexa-dyes: Alexa-647 and Alexa-555		Primary amines (lysines)
ULS-dyes: ULS-fluorescein and ULS-biotin		Methionine, histidine and cysteine
Biotin: NHS-biotin		Primary amines (lysines)
Digoxigenin: NHS-digoxigenin		Primary amines (lysines)

Approach	Application	Reference
Single-colour NHS-biotin	Tissue protein profiling of gastric adenoma carcinoma and <i>Helicobacter pylori</i> infection	[41]
	Serum protein profiling of complement deficient clinical samples	[9**]
Dual-colour Cy3/Cy5	Protein profiling of endothelial supplement (angiogenesis)	[28]
	Protein expression profiling of cell supplement (mantle cell lymphoma)	[31]
	Serum protein profiling of cystic fibrosis	[36]
	Tissue extract profiling of lung cancer	
NHS-biotin/NHS-digoxigenin	Plasma protein profiling of intestinal cancer	[32]
	Serum protein profiling of bladder cancer	[34**]
	Serum protein profiling of lung cancer	[30]
	Serum protein profiling of pancreatic cancer	[33]
	Serum protein profiling of prostate cancer	[35]

such as differential protein expression profiling and onco-proteomics (for review see [4,5,7,8,13]). Although a two-colour labelling approach is intuitively attractive when the expression profiles of two samples are to be compared, it is also associated with a set of serious concerns [7,8,11**]. First, recent studies have clearly shown that the signal intensities observed for a protein separately labelled with either of the two dyes of a matching pair (e.g. Cy3 versus Cy5, Alexa-647 versus Alexa-555, Universal Linkage Systems (ULS)-fluorescein versus ULS-biotin) differed significantly (Figure 2) [11**]. These findings clearly indicated on inherent differences in labelling efficiency or 'transmitted signal intensities' of the matching dyes. Hence, when adopting a dual-colour labelling approach it is vital to ensure that any observed differences are true differences and not reflecting technical issues. Second, this latter problem can be addressed by adopting criss-cross labelling, in which each sample is labelled with both dyes and analysed on separate arrays [29,31,36,38]. Although feasible, this means that twice as many arrays will have to be analysed, posing a major logistic bottleneck for large-scale studies. Further, as the least performing dye often gives very low signal intensities, it could in some cases be like running a one-colour approach [11**]. Third, mixing two samples labelled with different dyes means that the differentially labelled analytes will be competing for the same set of probes available on the array. Compared to a single-colour approach, this will then result in decreased signal intensities for each dye and a risk for a decreased sensitivity. Taken together, there is currently a clear edge in adopting a

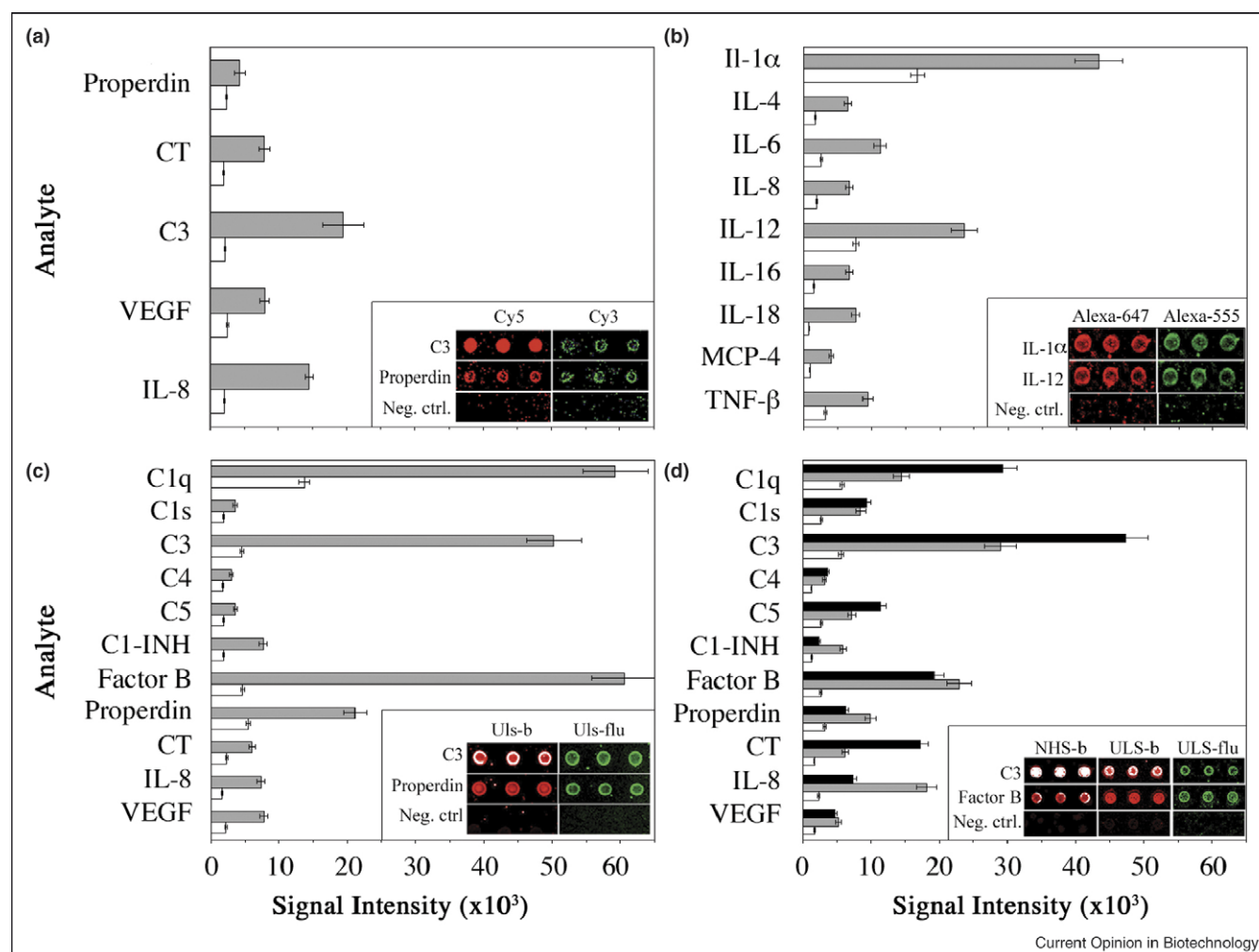
single-colour approach over a dual-colour set-up when analysing directly labelled samples using antibody microarrays.

Choice of dye

A variety of dyes are available (Table 1), providing different physico-chemical properties and coupling chemistries. Depending on the choice of dye, different labelling strategies have been implemented [5,7,8]. The protein analytes can be directly tagged with a fluorescent dye, for example, Cy-dyes, Alexa-dyes and ULS-dyes, or indirectly by first tagging the proteins with a small hapten, such as biotin (ULS-biotin or *N*-hydroxysulfosuccinimide ester group (NHS)-biotin) and digoxigenin. In the latter case, the arrays are then visualised by adding fluorescently labelled streptavidin or anti-biotin and anti-digoxigenin, respectively. Recently, compelling evidence has been presented showing that indirect tagging of the analytes with fluorescent dyes via biotin outperformed the direct tagging strategies with respect to assay performances, for example, increased sensitivity and lowered non-specific background binding [10**,11**]. These observations could most probably be explained by differences in first, labelling efficiency; second, dye stickiness (as displayed by a lowered non-specific background binding) and third, our ability to remove non-reacted dyes. In the latter case, any remaining traces of fluorescent dyes will have a direct impact on the background signals, while this might not be the case for biotin. It has been proposed that any non-specifically bound biotin (small hapten) may not be readily accessible by the labelled secondary

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Figure 2



Comparison of one-colouring and two-colouring labelling of complex proteomes for recombinant antibody microarray analysis performed on various solid supports. **(a)** Cy5 (grey bars) and Cy3 (open bars) labelled human serum analysed on Xenoslide N slides. **(b)** Alexa-647 (grey bars) and Alexa-555 (open bars) labelled cell expression supernatants analysed on Xenoslide N slides. **(c)** ULS-biotin (grey bars) and ULS-fluorescein (open bars) labelled human serum analysed on FAST slides (new formula). **(d)** NHS-biotin (black bars), ULS-biotin (grey bars) and ULS-fluorescein (open bars) labelled human serum analysed on black polymer Maxisorb slides. Microarray images from a selected set of analytes are shown. The array signal intensities shown represent the mean value of eight replicates after subtracting local background and negative control, that is, meaning that the signals should reflect specific signals. Similar results were obtained whether the analyses were performed as two-colour assays or one-colour assays. NHS-b: NHS-biotin; ULS-b: ULS-biotin; Neg. ctrl.: negative control. The figure is adopted from Ref. [11^{••}] and reproduced with permission.

reagent, streptavidin or anti-biotin antibody (large proteins), because of sterical hindrances [10^{••}].

Hence, the precise choice of dye is dependent on several interconnected parameters, including first, the choice of solid support, for example, how well any non-specific binding can be blocked; second, the demands on assay sensitivity, for example, whether high-abundant and/or low-abundant analytes will be analysed; third, whether one-colour or two-colour labelling approaches are adopted and fourth, the choice of tagging chemistries (Table 1).

The latter is important, as the position(s) of the tag molecule(s) on the target protein is vital in order to maintain immunoreactivity. In this context, it should be noted that both NHS-biotin and ULS-biotin has proven to be strong candidates for labelling of whole, complex proteomes, in particular when targeting rare protein analytes [8,9^{••}–11^{••}].

Once the dye has been specified, the degree of labelling is as crucial in order to maintain immunoreactivity [9^{••},11^{••}]. Although too low ratios will impair the assay

sensitivity, too high ratios can result in masking of the epitope and subsequently a lowered reactivity and thus decreased LODs [9^{••},11^{••}]. As for example, when tagging crude serum proteomes with biotin, a labelling molar ratio of biotin to protein of 15:1 was recently found to be the preferred choice using an optimised recombinant antibody microarray platform based on black polymer Maixsorb slides [9^{••},11^{••}]. Similar results were observed whether high-abundant or low-abundant analytes were targeted [9^{••},11^{••}]. From a logistical point of view, this is a very attractive feature, indicating that one labelling protocol may be sufficient even when analytes spanning a large dynamic range present in crude proteomes, such as serum and plasma, are targeted. In comparison, other set-ups have used lower labelling ratios, commonly in the order of 4:1 [30,33,34^{••}]. This difference in labelling ratios could be explained by the fact that a different array set-up was used, for example, a solid support with different blocking capabilities, and targeting of mainly high-abundant analytes (μ M range).

Data handling

As of today, no standardised procedure(s) have yet been established for antibody microarray data handling, including quantification, normalisation and data analysis. This feature is likely to change in the coming years, as the technology becomes more and more established, and the focus is shifted from technology development to more applicative efforts. As outlined below, the first standards

have also begun to emerge, often based on modified, but validated procedures and softwares readily adopted from the more mature field of DNA microarrays.

The scanned microarrays can be quantified using a set of established softwares available from several different vendors. Next, the data has to be normalised to enable data generated on different arrays to be readily compared. This is, however, a challenging key step that remains to be validated. Using a set of house-keeping proteins for normalisation, equivalent to how house-keeping genes are used for DNA microarray normalisation, will be much more difficult, if at all possible. Finding suitable candidate house-keeping proteins will be challenging, though, for example, tubulin or actin are frequently used house-keeping proteins for western blots. Till date, a set of different normalisation strategies have been evaluated for antibody (protein) microarrays, of which five major fundamentally different approaches are outlined in Table 2 [9^{••},41,44,45[•],46]. So far, the use of spike-in reference proteins and semi-global normalisation approaches has been found to work best [8,9^{••},45[•]]. As these methods all display different pros and cons (Table 2), additional work will be required before any leading methodology/-ies can be pin-pointed.

Having normalised the microarray data, the bioinformatic analysis of the antibody array data, including efforts, such as identification of differentially expressed analytes and

Table 2

General overview of five major normalisation methods evaluated for antibody (protein) microarrays

Normalisation method	Comments/pros and cons	Reference
Algorithm-based	An internally normalised ratio algorithm based on a dual-colour labelling approach Requires a dual-colour labelling methodology to be adopted	[44]
Antibody-based	The amount of spotted antibody is used for normalisation Does not normalise for differences in handling and labelling, etc.	[46]
Single analyte	A single analyte or a group of analytes for which the sample concentration have been determined <i>a priori</i> using an alternative method (e.g. ELISA) is used for normalisation Have been found to work well for some analytes, while others perform poorly	[9 ^{••} ,45 [•]]
Spike-in	One or several references protein(s) normally not present in the sample is spiked in at a known concentration before the labelling. Antibodies against the spike-in protein(s) is included on the array and the observed signals are used for normalisation Have been found to work well Requires access to spike-in protein(s) and matching antibody(ies)	[9 ^{••} ,41,45 [•]]
Semi-global approach	Based on the quantified array data, the coefficient of variation (CV) is first calculated for each analyte (i.e. antibody) over all samples and ranked. The fraction (10–15%) of the analytes displaying the lowest CV-values over all samples are identified, and used to calculate a chip-to-chip normalisation factor Similar to the procedure used for DNA microarrays Requires at least semi-dense arrays to be used, to have sufficient number of antibodies on the chip and the design should preferentially include antibodies directed against a set of analytes that could be expected to be present at rather constant levels	Carlsson <i>et al.</i> [47] and Ingvarsson <i>et al.</i> (unpublished data)

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candidate biomarker signatures, can be approached by using validated approaches and softwares adopted from the DNA microarray world. It is crucial to point out the importance of adopting appropriate study designs. Preferentially, a sufficiently large number of samples should be included to enable the data set to be split into a training set, used to find a candidate signature and an independent test set, which is used to validate the signature. If this is not possible because of sample limitations, leave-one out cross-validation approaches could be adopted to allow stringent statistical analysis of the data sets. A range of softwares/approaches has been successfully used, including Cluster, Genespring, Spotfire, Principle Component Analysis, Receiver Operating Characteristics (ROC) curves, Sammon maps, significance of microarray analysis (SAM), Support Vector Machine (SVM), TreeView, etc. In addition, work is currently on-going to extend the BioArray Software Environment (BASE) software package with a module especially designed for protein microarray data analysis.

When publishing antibody microarray data, no public repositories for data deposition, similar to the protein data bank (PDB) for protein X-ray crystallography data and the MIAME system (minimum information about a microarray) for DNA microarrays have yet been established. This feature is also likely to change in the coming years, as the technology becomes more mature and the number of clinical application increases.

Conclusions

The sample and data handling are two key issues in the quest of developing high-performing antibody microarrays that have received significant interest in the past few years. To this end, robust labelling protocols for direct labelling of non-fractionated, complex proteomes have been designed. Tagging the proteomes with biotin in a one-colour labelling approach has been found to be among the most promising designs, resulting in very sensitive set-ups. Further, the first standards for antibody microarray data handling has only recently begun to emerge and significant efforts remain before more standardised operating procedures will be established and implemented. In future efforts, we believe that one-colour labelling methodologies will become even more established, based on its high performances, compatibility with high-density arrays and logistics (i.e. keeping the required number of slides to a minimum). The field of antibody microarray data handling is also expected to make rapid progress, going from preliminary protocols towards more and more standardised operating procedures.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Borrebaeck CAK: **Antibody microarray-based oncoproteomics**. *Expert Opin Biol Ther* 2006, **6**:833-838.
2. Cho WC: **Contribution of oncoproteomics to cancer biomarker discovery**. *Mol Cancer* 2007, **6**:25.
3. Hu S, Loo JA, Wong DT: **Human body fluid proteome analysis**. *Proteomics* 2006, **6**:6326-6353.
4. Haab BB: **Applications of antibody array platforms**. *Curr Opin Biotechnol* 2006, **17**:415-421.
5. Kingsmore SF: **Multiplexed protein measurement: technologies and applications of protein and antibody arrays**. *Nat Rev Drug Discov* 2006, **5**:310-320.
6. Wingren C, Borrebaeck CAK: **Progress in miniaturization of protein arrays — a step closer to high-density nanoarrays**. *Drug Discov Today* 2007, **12**:813-819.
7. Wingren C, Borrebaeck CAK: **Antibody microarrays: current status and key technological advances**. *Omics* 2006, **10**:411-427.
8. Wingren C, Borrebaeck CAK: **High-throughput proteomic using antibody microarrays — an update**. *Expert Rev Mol Diagn* 2007, **7**:673-686.
9. Ingvarsson J, Larsson A, Sjöholm AG, Truedsson L, Jansson B, Borrebaeck CAK, Wingren C: **Design of recombinant antibody microarrays for serum protein profiling: targeting of complement proteins**. *J Proteome Res* 2007, **6**:3527-3536.
- In this paper, the authors demonstrate tagging of serum and plasma proteins with biotin to provide a sensitive recombinant antibody microarray technology platform.
10. Kusnezow W, Banzon V, Schroder C, Schaal R, Hoheisel JD, Ruffer S, Luft P, Duschl A, Sygailo YV: **Antibody microarray-based profiling of complex specimens: systematic evaluation of labeling strategies**. *Proteomics* 2007, **7**:1786-1799.
- In this paper, the authors demonstrate that indirect tagging of proteomes with fluorescent dyes via biotin to outperform direct tagging approaches.
11. Wingren C, Ingvarsson J, Dexlin L, Szul D, Borrebaeck CAK: **Design of recombinant antibody microarrays for complex proteome analysis: choice of sample labeling-tag and solid support**. *Proteomics* 2007, **7**:3055-3065.
- In this paper, the authors present a state-of-the-art recombinant antibody microarray technology platform based on black polymer Maxisorb slides and one-colour labelling (biotinylation) of complex proteomes.
12. Wingren C, Borrebaeck CAK: **Recombinant antibody microarrays**. *Screen Trends Drug Discov* 2006, **2**:13-15.
13. Angenendt P: **Progress in protein and antibody microarray technology**. *Drug Discov Today* 2005, **10**:503-511.
14. Duffy HS, Iacobas I, Hotchkiss K, Hirst-Jensen BJ, Bosco A, Dandachi N, Dermietzel R, Sorgen PL, Spray DC: **The gap junction protein connexin32 interacts with the Src homology 3/Hook domain of discs large homolog 1**. *J Biol Chem* 2007, **282**:9789-9796.
15. Vazquez-Martin A, Colomer R, Menendez JA: **Protein array technology to detect HER2 (erbB-2)-induced 'cytokine signature' in breast cancer**. *Eur J Cancer* 2007, **43**:1117-1124.
16. Finnskog D, Jaras K, Ressine A, Malm J, Marko-Varga G, Lilja H, Laurell T: **High-speed biomarker identification utilizing porous silicon nanovial arrays and MALDI-TOF mass spectrometry**. *Electrophoresis* 2006, **27**:1093-1103.
17. Koga H, Kyo M, Usui-Aoki K, Inamori K: **A chip-based miniaturized format for protein-expression profiling: the exploitation of comprehensively produced antibodies**. *Electrophoresis* 2006, **27**:3676-3683.
18. Kyo M, Usui-Aoki K, Koga H: **Label-free detection of proteins in crude cell lysate with antibody arrays by a surface**

- plasmon resonance imaging technique.** *Anal Chem* 2005, **77**:7115-7121.
19. Larsson C, Bramfeldt H, Wingren C, Borrebaeck CAK, Hook F: **Gravimetric antigen detection utilizing antibody-modified lipid bilayers.** *Anal Biochem* 2005, **345**:72-80.
 20. Zheng G, Patolsky F, Cui Y, Wang WU, Lieber CM: **Multiplexed electrical detection of cancer markers with nanowire sensor arrays.** *Nat Biotechnol* 2005, **23**:1294-1301.
 21. Adkins JN, Varnum SM, Auberry KJ, Moore RJ, Angell NH, Smith RD, Springer DL, Pounds JG: **Toward a human blood serum proteome: analysis by multidimensional separation coupled with mass spectrometry.** *Mol Cell Proteomics* 2002, **1**:947-955.
 22. Pieper R, Gatlin CL, Makusky AJ, Russo PS, Schatz CR, Miller SS, Su Q, McGrath AM, Estock MA, Parmar PP *et al.*: **The human serum proteome: display of nearly 3700 chromatographically separated protein spots on two-dimensional electrophoresis gels and identification of 325 distinct proteins.** *Proteomics* 2003, **3**:1345-1364.
 23. Righetti PG, Castagna A, Herbert B, Reymond F, Rossier JS: **Prefractionation techniques in proteome analysis.** *Proteomics* 2003, **3**:1397-1407.
 24. Tirumalai RS, Chan KC, Prieto DA, Issaq HJ, Conrads TP, Veenstra TD: **Characterization of the low molecular weight human serum proteome.** *Mol Cell Proteomics* 2003, **2**:1096-1103.
 25. Canas B, Pineiro C, Calvo E, Lopez-Ferrer D, Gallardo JM: **Trends in sample preparation for classical and second generation proteomics.** *J Chromatogr A* 2007, **1153**:235-258.
 26. Luque-Garcia JL, Neubert TA: **Sample preparation for serum/plasma profiling and biomarker identification by mass spectrometry.** *J Chromatogr A* 2007, **1153**:259-276.
 27. Ingvarsson J, Lindstedt M, Borrebaeck CAK, Wingren C: **One-step fractionation of complex proteomes enables detection of low abundant analytes using antibody-based microarrays.** *J Proteome Res* 2006, **5**:170-176.
 28. Ahn EH, Kang DK, Chang SI, Kang CS, Han MH, Kang IC: **Profiling of differential protein expression in angiogenin-induced HUVECs using antibody-arrayed ProteoChip.** *Proteomics* 2006, **6**:1104-1109.
 29. Bartling B, Hofmann HS, Boettger T, Hansen G, Burdach S, Silber RE, Simm A: **Comparative application of antibody and gene array for expression profiling in human squamous cell lung carcinoma.** *Lung Cancer* 2005, **49**:145-154.
 30. Gao WM, Kuick R, Orzechowski RP, Misek DE, Qiu J, Greenberg AK, Rom WN, Brenner DE, Omenn GS, Haab BB *et al.*: **Distinctive serum protein profiles involving abundant proteins in lung cancer patients based upon antibody microarray analysis.** *BMC Cancer* 2005, **5**:110.
 31. Ghobrial IM, McCormick DJ, Kaufmann SH, Leontovich AA, Loegering DA, Dai NT, Krajnik KL, Stenson MJ, Melhem MF, Novak AJ *et al.*: **Proteomic analysis of mantle-cell lymphoma by protein microarray.** *Blood* 2005, **105**:3722-3730.
 32. Hung KE, Kho AT, Sarracino D, Richard LG, Krastins B, Forrester S, Haab BB, Kohane IS, Kucherlapati R: **Mass spectrometry-based study of the plasma proteome in a mouse intestinal tumor model.** *J Proteome Res* 2006, **5**:1866-1878.
 33. Orzechowski R, Hamelinck D, Li L, Gliwa E, vanBrocklin M, Marrero JA, Vande Woude GF, Feng Z, Brand R, Haab BB: **Antibody microarray profiling reveals individual and combined serum proteins associated with pancreatic cancer.** *Cancer Res* 2005, **65**:11193-11202.
 34. Sanchez-Carbajo M, Socci ND, Lozano JJ, Haab BB, Cordon-Cardo C: **Profiling bladder cancer using targeted antibody arrays.** *Am J Pathol* 2006, **168**:93-103.
- In this paper, the authors describe the first attempt to define a predictor biomarker profile in bladder cancer.
35. Shafer MW, Mangold L, Partin AW, Haab BB: **Antibody array profiling reveals serum TSP-1 as a marker to distinguish benign from malignant prostatic disease.** *Prostate* 2007, **67**:255-267.
 36. Srivastava M, Eidelman O, Jozwik C, Paweletz C, Huang W, Zeitlin PL, Pollard HB: **Serum proteomic signature for cystic fibrosis using an antibody microarray platform.** *Mol Genet Metab* 2006, **87**:303-310.
 37. Wingren C, Steinhauer C, Ingvarsson J, Persson E, Larsson K, Borrebaeck CAK: **Microarrays based on affinity-tagged single-chain Fv antibodies: sensitive detection of analyte in complex proteomes.** *Proteomics* 2005, **5**:1281-1291.
 38. Smith L, Watson MB, O'Kane SL, Drew PJ, Lind MJ, Cawkwell L: **The analysis of doxorubicin resistance in human breast cancer cells using antibody microarrays.** *Mol Cancer Ther* 2006, **5**:2115-2120.
 39. Berczki E, Gonda S, Csont T, Korpos E, Zvara A, Ferdinandy P, Santha M: **Overexpression of biglycan in the heart of transgenic mice: an antibody microarray study.** *J Proteome Res* 2007, **6**:854-861.
 40. Rucker VC, Havenstrite KL, Herr AE: **Antibody microarrays for native toxin detection.** *Anal Biochem* 2005, **339**:262-270.
 41. Ellmark P, Ingvarsson J, Carlsson A, Lundin BS, Wingren C, Borrebaeck CAK: **Identification of protein expression signatures associated with *Helicobacter pylori* infection and gastric adenocarcinoma using recombinant antibody microarrays.** *Mol Cell Proteomics* 2006, **5**:1638-1646.
 42. Haab BB, Geierstanger BH, Michailidis G, Vitzthum F, Forrester S, Okon R, Saviranta P, Brinker A, Sorette M, Perlee L *et al.*: **Immunoassay and antibody microarray analysis of the HUPO Plasma Proteome Project reference specimens: systematic variation between sample types and calibration of mass spectrometry data.** *Proteomics* 2005, **5**:3278-3291.
 43. Wilkins MR, Appel RD, Van Eyk JE, Chung MC, Gorg A, Hecker M, Huber LA, Langen H, Link AJ, Paik YK *et al.*: **Guidelines for the next 10 years of proteomics.** *Proteomics* 2006, **6**:4-8.
 44. Andersson O, Kozlowski M, Garachtchenko T, Nikoloff C, Lew N, Litman DJ, Chaga G: **Determination of relative protein abundance by internally normalized ratio algorithm with antibody arrays.** *J Proteome Res* 2005, **4**:758-767.
 45. Hamelinck D, Zhou H, Li L, Verweij C, Dillon D, Feng Z, Costa J, Haab BB: **Optimized normalization for antibody microarrays and application to serum-protein profiling.** *Mol Cell Proteomics* 2005, **4**:773-784.
- The first study in detail addressing the key issue of normalisation antibody (proteome) microarray data.
46. Olle EW, Sreekumar A, Warner RL, McClintock SD, Chinnaiyan AM, Bleavins MR, Anderson TD, Johnson KJ: **Development of an internally controlled antibody microarray.** *Mol Cell Proteomics* 2005, **4**:1664-1672.
 47. Carlsson A, Wingren C, Ingvarsson J, Ellmark P, Baldertorp, Fernö M, Olsson H, Borrebaeck CAK: **Serum protein profiling of metastatic breast cancer using recombinant antibody microarrays.** *Eur J Cancer* 2008, in press.