

2D or not 2D

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2D gel electrophoresis is the technology that everyone loves to hate – it requires manual dexterity and precision to reproduce precisely and is thus not well-suited as a high-throughput technology. Although almost everyone would like to replace it, the resolution and sensitivity it offers are exquisite and unsurpassed if one wants a global view of cellular activity. There have been several recent developments, for example, the detection of low abundance proteins, and the resolution possible with narrow-range IPG gels.

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Abbreviations

| | |
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| 2DGE | two-dimensional gel electrophoresis |
| IPG | immobilised pH gradient |
| MALDI-TOF | matrix-assisted laser desorption-ionisation time-of-flight |
| MS | mass spectrometry |

Introduction

Two D, or not two D: that is the question:
Whether 'tis nobler in the mind to suffer
The streaks and blobs of intractable proteins
Or to take chips against a sea of genes
And by comparing, find them that
hold the bitter taste of disease and death.

The past year has been a watershed year for two-dimensional gel electrophoresis (2DGE) — it has become the most important technology for high-resolution separation of proteins for proteomics.

The question is — now that 2DGE is centre stage — will it evolve into the powerful tool that everyone needs or will it be replaced by another, higher-throughput technology? Some of the alternatives will be reviewed elsewhere in this issue and so the main drive of this article is to consider the capabilities of 2DGE combined with mass spectrometry (MS).

What does proteome analysis need to do?

In the ideal world, proteome analysis should characterise and quantitate all the proteins in a cell or biopsy at a particular time and under a particular set of environmental conditions. This must also include all the post-translational modifications, whether these are post-translational (such as phosphorylations, methylations or acetylations) or processing. Similar samples can then be compared to identify

the mechanisms that link the genotype (whether microorganism, plant or animal) and environment together into the phenotype.

In the real world, there is no technique that can accomplish this, but the technique that comes closest is the combination of 2DGE and MS. These two technologies take a 'snapshot' of the cell so that many proteins can be separated, quantitated and identified. Whether this is the majority of the proteins in the cell or not depends upon the system under analysis — for simpler organisms (such as mycoplasma, or bacteria) it is the majority, and for more complex ones (such as humans), probably it is not.

The reason why proteome analysis is causing so much excitement is that, in contrast to the genome, which is relatively static and essentially identical in every somatic cell of an organism, protein expression (the proteome) is in a state of dynamic flux — constantly changing and responding to stimuli (both internal and external). Thus, the proteome brings us much closer to the living processes of the cell — but this makes it much more difficult to study [1••]. Although 2DGE has been around for more than 25 years, the breakthrough in rapid protein-identification by MS has suddenly persuaded many that this technology has come of age — and in fact could be the source of big business [2].

On the basis of the central dogma, that the information flow is from DNA via mRNA to protein, it could be expected that mRNA expression would reflect exactly the same as the proteome. Thus, all the excellent high-throughput technologies that have been and continue to be developed to analyse DNA or RNA could be used to carry out proteome analysis. In the past year, there have been a number of new technologies for analysing transcription [3,4••] that will undoubtedly play a major role in increasing our understanding of cellular biology.

Obviously, mRNA expression is important and should not be dismissed, but at the present time we know too little about the cell to be able to predict when an increase in the expression of a particular species of mRNA will be realised by a corresponding increase in protein expression and functional activity. Some of the modulations in mRNA expression must be used to specify the 'general directions' for the cell activity, whereas the modulations in protein expression levels may often represent the fine tuning of the cellular apparatus, given the cell's environment. Therefore it is crucial to be able to quantitate expression of the proteins and their modifications.

New technologies

2DGE has been a technology that involves a great deal of expertise and hands-on time to execute reproducibly, but

this is changing. There have been a number of significant advances (for example with the introduction of immobilised pH gradient [IPG]) 2D technologies [5^{••},6[•]) that enable highly reproducible patterns to be obtained amongst different laboratories using different equipment and chemicals [7]. However, a standard set of running conditions has never been agreed upon and probably never will be implemented by the majority of scientists. This means that individual 2DGE patterns from different labs cannot be compared in detail and reliable deductions can only be made when the protein spot has been identified in both laboratories (luckily, this is becoming easier with the increasing user-friendliness of MS instruments). However, this single fact greatly reduces the value of the databases that have been published and has retarded progress in the field. This has spurred the search for a successor to the throne. Currently, there are two contenders that are worth mentioning.

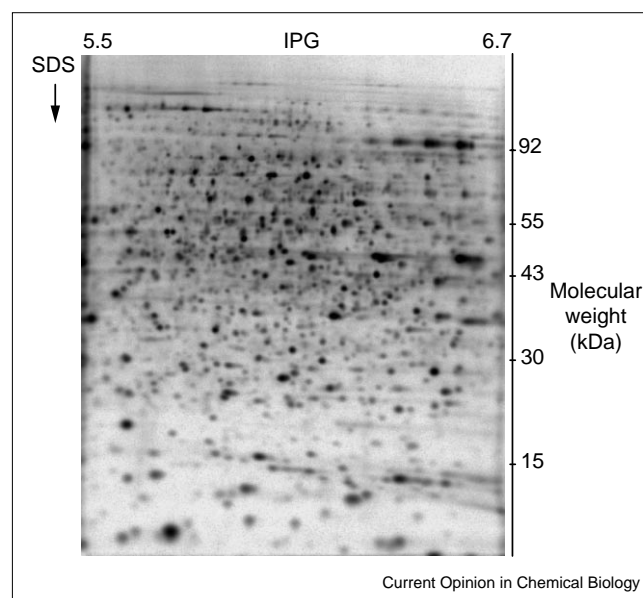
The first is the use of large-scale peptide or protein arrays [8^{••}]. These arrays are best suited, at present, to answer specific questions about particular proteins (for example the specific activities of single peptide or protein variants). One of the major drawbacks is maintaining protein function: proteins normally exist in complexes and the correct function of a particular protein may only be exhibited in that complex. The other possibility is to array living organisms (differing at each location by, for example, the expression of a different Gal4 activation domain open reading frame) so that it is possible to detect when particular genes are activated (and in doing so provide data similar to mRNA transcription data). Alternatively, human proteins can be expressed in a bacterial library immobilised in an array, and these can then be probed using antibodies [9]. The reverse is also possible: antibodies can be arrayed and probed with whole-cell lysates [10]. Although these approaches offer powerful, high-throughput answers to specific questions, none are suited to the global investigation of the modulation in cellular metabolism in response to stimulus or disease.

The second approach attempts to replace 2DGE. One very promising approach that is being developed uses capillary isoelectric focusing to separate the proteins. Protein detection can be carried out either by conventional UV [11], or by Fourier transform MS [12^{••}]. This technology has improved greatly in the past few years and can now be carried out quantitatively. It is very well suited to proteins of low molecular weight that are often overlooked by conventional 2DGE and could become very powerful as it can be fully automated. Other options involve using MS, either of proteins or selected peptides — but none currently have the resolution and sensitivity that is required.

Improvements in 2D technology

There have been many good reviews recently [5^{••},13^{••}] and so our aim will be to discuss critical aspects of the technology.

Figure 1



High-resolution separation of human proteins. 2DGE gel of [³⁵S]-methionine labelled HeLa cell proteins separated on a narrow range IPG gel covering the pH range 5.0–6.0.

Current limitations in the technology are the inability of 2DGE to resolve all the proteins present, their exceptionally wide range in expression levels and the extreme differences in their solubility. Two approaches are being explored to solve these drawbacks. The first is to run multiple gels that cover overlapping pH and/or molecular-weight regions (to increase the resolution of the 2DGE) and the second is to prefractionate the sample (to reduce the complexity of the sample).

Narrow range gels

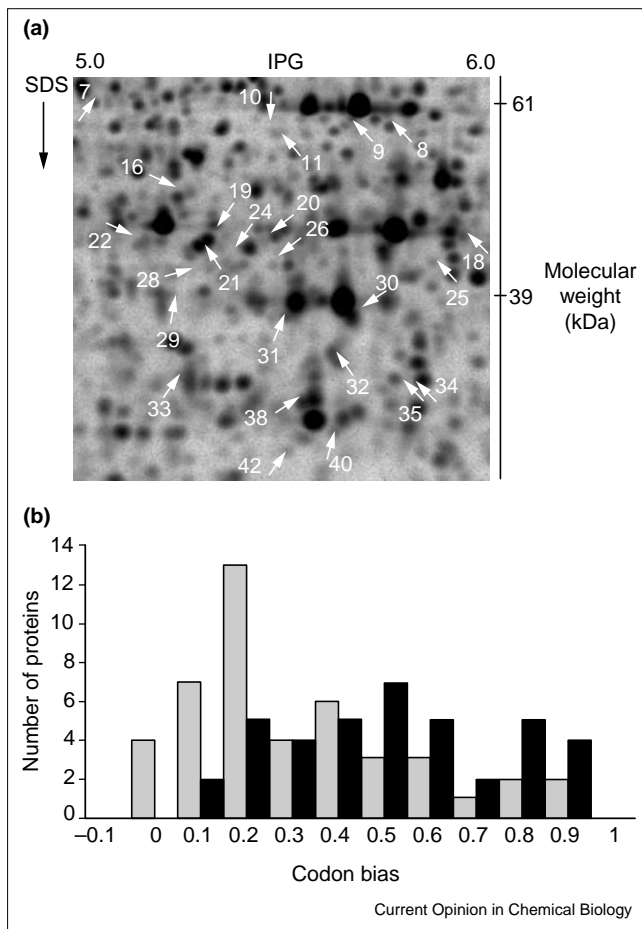
The best approach to increase the resolution on the crowded 2DGE gels is to run multiple gels that cover narrow pH ranges (e.g. covering 1 pH unit) [5^{••},14–16]. Using these gel systems, it is possible to resolve more than 10,000 proteins from a higher eukaryotic cell lysate (Figure 1).

Although effective, this technique requires more sample (which might be limiting, for example, from human biopsies) and it multiplies the amount of work to be done. Another limitation is that, as yet, no narrow-range gels are commercially available for the 'basic' ranges above pH 7.

Low-abundance proteins

One hotly debated issue concerns whether it is possible to detect and identify low-abundance proteins. This is an important group of proteins because it encompasses receptors, signal transduction and regulatory proteins. The debate has centred on yeast because of the correlation between codon bias and protein expression levels (genes that are destined to be highly expressed during mid-exponential growth use certain codons preferentially to ensure rapid, low-error translation). These observations have then

Figure 2



Detection of low-abundance proteins. (a) Close-up of a 2DGE gel of [^{35}S]-methionine-labelled *Saccharomyces cerevisiae* proteins (total protein load 70 μg) separated on an IPG gel covering the pH range 4–7. Arrows indicate some of the proteins identified by MS. (b) Codon bias of proteins identified above compared with those identified by Gygi *et al.* [19]. SDS, sodium dodecyl sulfate.

been used to say that either levels of mRNA and protein expression do [17••] or do not correlate [18]. The debate turns out to be much ado about nothing because the authors actually have very similar data. Codon bias has also been used to suggest that low-abundance proteins cannot be found by 2DGE-MS gel [19]. This hypothesis is based on two observations: firstly, that the authors could not identify any low-abundance proteins (despite using narrow-range gels and high loads); and secondly, that no low-abundance proteins are entered into any 2DGE databases. To take the second point first: groups who are building databases will obviously select high-abundance proteins first as they are the easiest to identify and so it is obvious that this will result in a strong bias. As to the first point, examining our own data, which has been derived from investigation of the function analysis of unknown genes (a region of the gel is shown in Figure 2a to illustrate the proteins identified) from yeast, revealed a preponderance of low-abundance proteins (codon bias below 0.2,

Figure 2b). This is despite the fact that narrow-range gels were not used and the protein loaded on the gels was nearly 10 times lower (70 μg). Thus, the authors conclusions appear to be based on their own technology and not a physical limitation in 2DGE-MS.

Sample prefractionation

There are also several groups that are now advocating sample prefractionation using differential solubilisation [20••,21] or by prefractionating the proteins by isoelectric focussing in solution prior to 2DGE [22••]. The main danger with sample prefractionation is, of course, that the reproducibility of the prefractionation is not perfect. Because the primary utility of proteome analysis is to quantitate cellular changes (and not merely to catalogue the proteins present), prefractionation might introduce additional variability into the results. Another potential drawback of this method is that if proteins are extracted with a particular detergent, then the detergent should be present throughout the electrophoresis. Obviously this will perturb the image obtained and thus images of different fractions cannot simply be added together, but in the days of rapid protein identification by MS, this is not a problem. Obviously, proteolysis must be prevented throughout all procedures. This alternative also has the disadvantage that it multiplies the amount of work that has to be done — but at least it should not consume significantly more sample.

Membrane proteins

One special example of prefractionation is the situation for membrane proteins. It has been recently estimated that 30% of proteins are membrane proteins [23]. These are notoriously difficult to work with and it has been reported that only about 1% of integral membrane proteins are actually resolved on current 2DGE gels (even when thio-urea is used in the lysis buffer) [24]. Considering their key roles in signal transduction, cell adhesion, metabolite and ion transport and the fact that they are often the primary target for drug interactions, it remains critical to solve this problem [25••]. One very promising approach is the use of specific detergents [26•,27,28••].

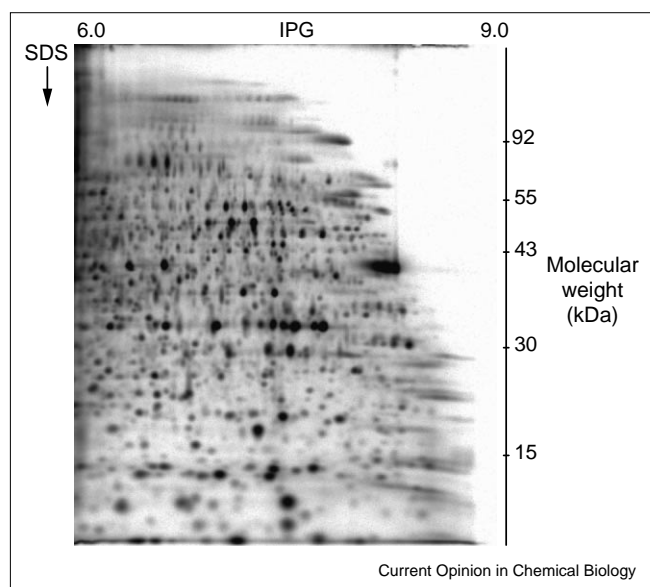
Basic proteins

In dramatic contrast to the concern with revealing as many proteins as possible by using narrow-range gels or sample prefractionation is that very few groups utilise specific gel systems for basic proteins despite the fact that approximately one third to one half of the cellular proteins have isoelectric points above pH 7. Part of the explanation could be that the resolution of the commercial basic IPG gels (pH ranges 6–9, 6–11 or 7–10) is not as good as the acidic gels (Figure 3) even though solutions have been published for some pH ranges [5••].

Protein visualisation

It has been known for many years that protein resolution by 2DGE increases as the amount of protein applied decreases and this is why radioisotopic detection of biosynthetically

Figure 3



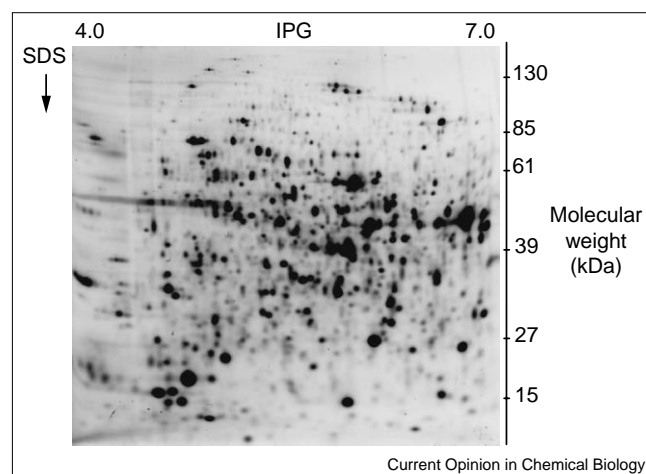
High-resolution separation of human proteins. 2DGE gel of [³⁵S]-methionine-labelled HeLa cell proteins separated on an IPG gel covering the pH range 6.0–9.0. Note the absence of proteins around pH 9.0. SDS, sodium dodecyl sulfate.

labelled proteins has been so successful. However, not all samples can be labelled this way. Also, cells in or from biopsies dedifferentiate during the labelling period (which is often from 4 to 20 hours) and thus do not accurately represent the *in vivo* proteome. Thus, rat hepatic stellate cells respond differently to the cells *in vivo* [29**]. Fortunately, there are several novel approaches to circumvent this problem. The first uses post-harvest alkylation [30*], in which the proteins are radio-iodinated after electrophoresis. This technique has the potential to become ultrasensitive if procedures are found to label all the proteins present. In addition, a wide variety of new fluorescent dyes have appeared that can react with proteins either before (e.g. bimane; Figure 4; [31]) or after 2DGE (e.g. Sypro ruby, red and orange; [32*]). Although none of these can match radioisotopic labelling, they are still a great improvement over (colloidal) Commassie blue[®] or silver staining in that they are sensitive (down to the nanogram range) and can be quantitated (dynamic range >10⁴).

Mass spectrometry

MS is now firmly entrenched as the first choice methodology for protein identification and characterisation. It is continuing to evolve rapidly and diversify into an array of technologies, with each variant adapted to specific applications. Currently, matrix-assisted laser desorption-ionisation time-of-flight (MALDI-TOF) MS is the preferred technique for protein identification and characterisation of post-translational modifications [33], whereas nanoelectrospray ionisation tandem MS is preferred for partial peptide sequencing when there is no entry in the available databases. MALDI-TOF is compatible with high-throughput

Figure 4



Protein detection using bimane. *Saccharomyces cerevisiae* proteins were labelled with bimane and then separated on a pH 4–7 IPG gel.

applications, whereas nanoelectrospray ionisation tandem MS is more sensitive. It is also becoming apparent that sample preparation is critical. Luckily, many new simple micro-procedures for purifying peptides [34**] or phosphopeptides [35] are emerging.

With a view towards high throughput, a molecular scanner has been described in which the proteins from a 2DGE gel are digested and electroblotted onto a membrane. This membrane is then scanned with a MALDI-TOF mass spectrometer to generate a fully annotated 2DGE image [36**]. If image blurring because of diffusion during transfer could be prevented, this technique would become very important.

Post-translational modifications are, sadly, often overlooked in proteome analysis despite their visibility on 2DGE gels (e.g. by isotope labelling of phosphate, myristylation, amino-terminal acetylation, palmitoylation or isoprenylation [37]). Because there may be, on average, more than five modification variants of each protein in humans, and because presumably all of these variants have specific roles, it is clear that they play significant roles in the regulation of cellular activity. Characterisation of these roles can be carried out using a combination of enzymes and MALDI-TOF to localise which residues are modified [38**] followed by site-directed mutagenesis to investigate the biological significance of the individual modifications.

New tools for proteome analysis

There are a number of tools emerging that, although not specifically for 2DGE, will greatly increase the utility of proteome analysis.

One problem that has confounded the cancer biopsy analysis is the simple fact that tumours are heterogeneous and often the majority of the tissue that is present

is normal [6•]. This has obviously made it much harder to identify tumour-specific differences. The introduction of laser-assisted microdissection has greatly helped in this field [39•,40].

Proteins do not exist in isolation in the cell: they take part in complexes and interact extensively. Although 2DGE, using interlink analysis, can reveal which proteins are co-regulated, an additional important task for proteome analysis is to characterise which, where, how, and when proteins interact with one another. There are two exciting approaches to these questions. The first is the two-hybrid system, which has developed into a highly automated means to identify protein–protein interactions on a proteome-wide scale [41••,42•]. The second is a means by which protein complexes can be purified using a tandem-affinity purification tag [43••].

Bioinformatics

By far the weakest link in the 2DGE-MS proteome analysis chain is, in fact, bioinformatics. Although bioinformatics already plays a very prominent role in proteome analysis, there are at least three levels where improved bioinformatics could significantly increase both the speed and the value of proteome analysis.

The first of these levels is in the analysis of the 2DGE gel images. There is no program that is remotely automatic when presented with complex 2DGE images containing thousands of protein spots, and most programs require often more than a day of user hands-on time to edit the image before it can be fully entered into the database. The problems lie in initial spot recognition and in the subsequent matching of spots between gels. Hopefully, advances such as those proposed for spot matching [44,45•] will greatly accelerate data collection. Data comparison tools are beginning to emerge so that the user can compare the expression of particular proteins on several gels [46].

The second level is that the databases which are being created in many different labs [47•], although they may be excellent repositories of spot identifications, rarely have extensive links to the literature (noticeable exceptions are the Expasy server (<http://www.expasy.ch/>), the YPD/worm databases (<http://www.proteome.com/databases/index.html>) [17••,48••] and the Microbial Proteome Database (<http://www.abdn.ac.uk/~mmb023/2dhome.htm>) [20••]). These databases often lack protein expression data (under given environmental growth conditions), data that will be invaluable to build up a working model to animate life in the cell. What is needed to interpret this type of data will be an electronic model of the cell so that protein expression levels, enzyme reaction rates, and metabolite concentrations can be entered into the model and so that the cells response to stimuli can be simulated in order to understand the complex changes that occur. Such a virtual cell model is being constructed and, as this becomes more

elaborate, will provide an increasingly powerful research tool (see URL: <http://e-cell.org/>) [49••].

The third level is that the source of the information that is presented in these protein databases should be made very clear. There are an increasing number of predictive tools (e.g. for protein modifications [50]) that can be used to annotate protein sequences, but if used indiscriminately they can lead to more confusion than benefit [51•].

Applications

There is a wealth of good applications for proteomics and so only a few examples are given to illustrate the scope of this technology.

In the world of microorganisms, there have been excellent basic cell-physiological studies of the regulation of central carbon pathways and phosphate-starvation-induced proteins in *Bacillus subtilis* [52••, 53••]. Stress response is often seen and, as such, often can be underappreciated. And yet the response is specific and has been shown to be different for different types of stress (in *Streptococcus mutans*) [54]. That unexpected relationships exist between cellular functions was demonstrated by the observation that a reduction in mRNA capping leads to hygromycin B resistance in *Candida albicans* [55••]. Comparative proteome analysis has led to the identification of vaccine candidates in *Helicobacter pylori* [56•] and one novel area for proteome analysis is the taxonomy of yeast strains [57].

In addition, complex cellular phenomena are being studied; for example, the proliferative response induced in primary hepatocytes by epidermal growth factor or nafenopin [58•] and the regulation of ubiquitin-conjugating enzymes by interferon α in human T cells [59]. Finally, proteome analysis is being used to study physiological processes; for example, during ovulation [60], in the prostate [61] and during embryogenesis [62] and a wide variety of diseases including diabetes [63,64], cancer [65•,66] and even schizophrenia [67].

Conclusions

The reasons why 2DGE is still around are obvious: it is a very sensitive technique (at least an order of magnitude more sensitive than current nucleic-acid-screening technologies); it has exquisite resolution (separating more than 10,000 different proteins and their modification products); and it allows an unbiased search for novel relationships at the global level. After all, all the world's a stage and all the proteins, merely players.

Acknowledgements

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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. Abbott A: **A post-genomic challenge: learning to read patterns of protein synthesis.** *Nature* 1999, **402**:715-720.
A good introduction to the field describing many of the key issues and competing technologies.
 2. Service RF: **Proteomics. Can Celera do it again?** *Science* 2000, **287**:2136-2138.
 3. Westin L, Xu X, Miller C, Wang L, Edman CF, Nerenberg M: **Anchored multiplex amplification on a microelectronic chip array.** *Nat Biotechnol* 2000, **18**:199-204.
 4. Brenner S, Johnson M, Bridgham J, Golda G, Lloyd DH, Johnson D, Luo S, McCurdy S, Foy M, Ewan M *et al.*: **Gene expression analysis by massively parallel signature sequencing (MPSS) on microbead arrays.** *Nat Biotechnol* 2000, **18**:630-634.
A refreshing and exciting new approach to nucleic-acid sequencing that gives an indication of the potential of thinking of radically different strategies.
 5. Gorg A, Obermaier C, Boguth G, Harder A, Scheibe B, Wildgruber R, Weiss W: **The current state of two-dimensional electrophoresis with immobilized pH gradients.** *Electrophoresis* 2000, **21**:1037-1053.
A well-written description of the state-of-the-art.
 6. Hanash SM: **Biomedical applications of two-dimensional electrophoresis using immobilized pH gradients: current status.** *Electrophoresis* 2000, **21**:1202-1209.
A well-balanced description of the strengths and weaknesses of the 2DGE technology and the requirements for its future application.
 7. Blomberg A, Blomberg L, Norbeck J, Fey SJ, Mose Larsen P, Roepstorff P, Degand H, Boutry M, Posch A, Görg A: **Interlaboratory reproducibility of yeast protein patterns analysed by immobilised pH gradient two-dimensional gel electrophoresis.** *Electrophoresis* 1995, **16**:1935-1945.
 8. Emili AQ, Cagney G: **Large-scale functional analysis using peptide or protein arrays.** *Nat Biotechnol* 2000, **18**:393-397.
A clear review of the potential and the limitations of peptide and protein arrays.
 9. Holt LJ, Bussow K, Walter G, Tomlinson IM: **By-passing selection: direct screening for antibody-antigen interactions using protein arrays.** *Nucleic Acids Res* 2000, **28**:E72.
 10. Borrebaeck CA: **Antibodies in diagnostics – from immunoassays to protein chips.** *Immunol Today* 2000, **21**:379-382.
 11. Shen Y, Berger SJ, Anderson GA, Smith RD: **High-efficiency capillary isoelectric focusing of peptides.** *Anal Chem* 2000, **72**:2154-2159.
 12. Jensen PK, Pasa-Tolic L, Peden KK, Martinovic S, Lipton MS, Anderson GA, Tolic N, Wong KK, Smith RD: **Mass spectrometric detection for capillary isoelectric focusing separations of complex protein mixtures.** *Electrophoresis* 2000, **21**:1372-1380.
A potential successor technology to 2DGE that requires sophisticated equipment.
 13. Harry JL, Wilkins MR, Herbert BR, Packer NH, Gooley AA, Williams KL: **Proteomics: capacity versus utility.** *Electrophoresis* 2000, **21**:1071-1081.
A comprehensive review of the current status of the proteomics field.
 14. Wildgruber R, Harder A, Obermaier C, Boguth G, Weiss W, Fey SJ, Larsen PM, Gorg A: **Towards higher resolution: two-dimensional electrophoresis of *Saccharomyces cerevisiae* proteins using overlapping narrow immobilized pH gradients.** *Electrophoresis* 2000, **21**:2610-2616.
 15. Corthals GL, Wasinger VC, Hochstrasser DF, Sanchez JC: **The dynamic range of protein expression: a challenge for proteomic research.** *Electrophoresis* 2000, **21**:1104-1115.
 16. Hoving S, Voshol H, van Oostrum J: **Towards high performance two-dimensional gel electrophoresis using ultrazoom gels.** *Electrophoresis* 2000, **21**:2617-2621.
Specific consideration of the most efficient way to increase protein resolution.
 17. Fitcher B, Latter GI, Monardo P, McLaughlin CS, Garrels JI: **A sampling of the yeast proteome.** *Mol Cell Biol* 1999, **19**:7357-7368.
A very solid discussion of many of the fundamental questions related to proteomics.
 18. Gygi SP, Rochon Y, Franz BR, Aebersold R: **Correlation between protein and mRNA abundance in yeast.** *Mol Cell Biol* 1999, **19**:1720-1730.
 19. Gygi SP, Corthals GL, Zhang Y, Rochon Y, Aebersold R: **Evaluation of two-dimensional gel electrophoresis-based proteome analysis technology.** *Proc Natl Acad Sci USA* 2000, **97**:9390-9395.
 20. Cordwell SJ, Nouwens AS, Verrills NM, Basseal DJ, Walsh BJ: **Subproteomics based upon protein cellular location and relative solubilities in conjunction with composite two-dimensional electrophoresis gels.** *Electrophoresis* 2000, **21**:1094-1103.
An ambitious attempt to reveal more proteins using the combination of sub-cellular fractionation and narrow pH range gels.
 21. Molloy MP, Herbert BR, Walsh BJ, Tyler MI, Traini M, Sanchez JC, Hochstrasser DF, Williams KL, Gooley AA: **Extraction of membrane proteins by differential solubilization for separation using two-dimensional gel electrophoresis.** *Electrophoresis* 1998, **19**:837-844.
 22. Zuo X, Speicher DW: **A method for global analysis of complex proteomes using sample prefractionation by solution isoelectrofocusing prior to two-dimensional electrophoresis.** *Anal Biochem* 2000, **284**:266-278.
An interesting alternative based on isoelectric focussing between membranes to fractionate proteins prior to 2DGE.
 23. Paulsen IT, Sliwinski MK, Nelissen B, Goffeau A, Saier MH Jr: **Unified inventory of established and putative transporters encoded within the complete genome of *Saccharomyces cerevisiae*.** *FEBS Lett* 1998, **430**:116-125.
 24. Garrels JI, McLaughlin CS, Warner JR, Fitcher B, Latter GI, Kobayashi R, Schwender B, Volpe T, Andersen DS, Mesquita-Fuentes R, Payne WE: **Proteome studies of *Saccharomyces cerevisiae*: identification and characterization of abundant proteins.** *Electrophoresis* 1997, **18**:1347-1360.
 25. Santoni V, Molloy M, Rabilloud T: **Membrane proteins and proteomics: un amour impossible?** *Electrophoresis* 2000, **21**:1054-1070.
In inimitable style, a description of loves labours lost?
 26. Rabilloud T, Blisnick T, Heller M, Luche S, Aebersold R, Lunardi J, Braun-Breton C: **Analysis of membrane proteins by two-dimensional electrophoresis: comparison of the proteins extracted from normal or *Plasmodium falciparum* – infected erythrocyte ghosts.** *Electrophoresis* 1999, **20**:3603-3610.
A demonstration of the effects of using different detergents to reveal membrane proteins involved in the life cycle of *Plasmodium falciparum*.
 27. Wissing J, Heim S, Flohe L, Billitewski U, Frank R: **Enrichment of hydrophobic proteins via Triton X-114 phase partitioning and hydroxyapatite column chromatography for mass spectrometry.** *Electrophoresis* 2000, **21**:2589-2593.
 28. Wasinger VC, Pollack JD, Humphery-Smith I: **The proteome of *Mycoplasma genitalium*. Chaps-soluble component.** *Eur J Biochem* 2000, **267**:1571-1582.
A description of the most comprehensive proteome to date.
 29. Bach Kristensen D, Kawada N, Imamura K, Miyamoto Y, Tateno C, Seki S, Kuroki T, Yoshizato K: **Proteome analysis of rat hepatic stellate cells.** *Hepatology* 2000, **32**:268-277.
This study demonstrates the clear danger of working with cells *ex vivo*.
 30. Vuong GL, Weiss SM, Kammer W, Priemer M, Vingron M, Nordheim A, Cahill MA: **Improved sensitivity proteomics by postharvest alkylation and radioactive labelling of proteins.** *Electrophoresis* 2000, **21**:2594-2605.
Detection of low-abundance proteins is one of the challenges in the field – this paper presents a method whereby proteins can be labelled with [³H] or [¹²⁵I] iodoacetamide in an attempt to increase the sensitivity of protein detection.
 31. Fey SJ, Nawrocki A, Larsen MR, Görg A, Roepstorff P, Skews GN, Williams R, Mose Larsen P: **Proteome analysis of *Saccharomyces cerevisiae* – a methodological outline.** *Electrophoresis* 1997, **18**:1361-1372.

32. Patton WF: **A thousand points of light: the application of fluorescence detection technologies to two-dimensional gel electrophoresis and proteomics.** *Electrophoresis* 2000, **21**:1123-1144.
- This is a comprehensive review of the currently available alternatives for fluorescent labelling of proteins. Fluorescence labelling is essential for proteins that cannot be isotopically labelled and will become more important as its sensitivity increases.
33. Roepstorff P: **MALDI-TOF mass spectrometry in protein chemistry.** *EXS* 2000, **88**:81-97.
34. Gobom J, Kraeuter KO, Persson R, Steen H, Roepstorff P, Ekman R: **Detection and quantification of neurotensin in human brain tissue by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.** *Anal Chem* 2000, **72**:3320-3326.
- A simple device for micropurification of minute amounts of peptides.
35. Larsen MR, Sørensen GL, Mose Larsen P, Fey SJ, Roepstorff P: **Phospho-proteomics: evaluation of the use of enzymatic de-phosphorylation and differential mass spectrometric peptide mass mapping for site specific phosphorylation assignment in proteins separated by gel electrophoresis.** *Electrophoresis* 2000, in press.
36. Binz PA, Muller M, Walther D, Bienvenut WV, Gras R, Hoogland C, Bouchet G, Gasteiger E, Fabbretti R, Gay S: **A molecular scanner to automate proteomic research and to display proteome images.** *Anal Chem* 1999, **71**:4981-4988.
- The third dimension?
37. Gromov PS, Madsen P, Celis JE: **Identification of isoprenyl modified proteins metabolically labeled with [3H]farnesyl- and [3H]geranylgeranyl-pyrophosphate.** *Electrophoresis* 1996, **17**:1728-1733.
38. Muller DR, Schindler P, Coulot M, Voshol H, van Oostrum J: **Mass spectrometric characterization of stathmin isoforms separated by 2D PAGE.** *J Mass Spectrom* 1999, **34**:336-345.
- The straightforward approach to characterisation of post-translational modifications.
39. Banks RE, Dunn MJ, Forbes MA, Stanley A, Pappin D, Naven T, Gough M, Harnden P, Selby PJ: **The potential use of laser capture microdissection to selectively obtain distinct populations of cells for proteomic analysis – preliminary findings.** *Electrophoresis* 1999, **20**:689-700.
- Proteomic studies of many tissues and tumours have been hampered by the fact that most are a complex mixture of cell types. This technology offers a way to pick the cells of interest.
40. Sirivatanauskorn Y, Drury R, Crnogorac-Jurcovic T, Sirivatanauskorn V, Lemoine NR: **Laser-assisted microdissection: applications in molecular pathology.** *J Pathol* 1999, **189**:150-154.
41. Legrain P, Selig L: **Genome-wide protein interaction maps using two-hybrid systems.** *FEBS Lett* 2000, **480**:32-36.
- An intriguing new approach to sequentially map protein-protein interactions.
42. Buckholz RG, Simmons CA, Stuart JM, Weiner MP: **Automation of yeast two-hybrid screening.** *J Mol Microbiol Biotechnol* 1999, **1**:135-140.
- One of the fundamental challenges to our understanding of proteomics is to identify which proteins interact with which. Automation of the yeast two-hybrid system offers to bring us a large step forward.
43. Rigaut G, Shevchenko A, Rutz B, Wilm M, Mann M, Seraphin B: **A generic protein purification method for protein complex characterization and proteome exploration.** *Nat Biotechnol* 1999, **17**:1030-1032.
- A very useful approach to work laterally and characterise all the components in selected molecular machines.
44. Pleißner K-P, Hoffmann F, Kriegel K, Wenk C, Wegner S, Sahlström A, Oswald H, Alt H, Fleck E: **New algorithmic approaches to protein spot detection and pattern matching in two-dimensional electrophoresis gel databases.** *Electrophoresis* 1999, **20**:755-765.
45. Panek J, Vohradsky J: **Point pattern matching in the analysis of two dimensional gel electropherograms.** *Electrophoresis* 1999, **20**:3483-3491.
- Image analysis remains one of the most intractable bottlenecks hindering the widespread application of proteomics. This paper describes a very reliable approach to matching protein patterns on 2DGE gels.
46. Lemkin PF, Thornwall G: **Flicker image comparison of 2-D gel images for putative protein identification using the 2DWG meta-database.** *Mol Biotechnol* 1999, **12**:159-172.
47. Oh JMC, Hanash SM, Teichroew D: **Mining protein data from two dimensional gels: tools for systematic post-planned analyses.** *Electrophoresis* 1999, **20**:766-774.
- Vast amounts of data are generated in proteomics, ranging from clinical data, sample preparation data, 2D gel images, protein expression data, mass spectrometric patterns and identification-associated search data. This has to be interpreted with respect to information available on the Internet and thus this requires data-mining tools that have not yet been developed. This paper presents a serious step towards this goal.
48. Costanzo MC, Hogan JD, Cusick ME, Davis BP, Fancher AM, Hodges PE, Kondu P, Lengjeza C, Lew-Smith JE, Lingner C *et al.*: **The yeast proteome database (YPD) and *Caenorhabditis elegans* proteome database (WormPD): comprehensive resources for the organization and comparison of model organism protein information.** *Nucleic Acids Res* 2000, **28**:73-76.
49. Tomita M, Hashimoto K, Takahashi K, Shimizu TS, Matsuzaki Y, Miyoshi F, Saito K, Tanida S, Yugi K, Venter JC, Hutchison CA III: **E-cell: software environment for whole-cell simulation.** *Bioinformatics* 1999, **15**:72-84.
- The beginnings of a very extensive project to simulate cellular life?
50. Wilkins MR, Gasteiger E, Gooley AA, Herbert BR, Molloy MP, Binz PA, Ou K, Sanchez JC, Bairoch A, Williams KL, Hochstrasser DF: **High-throughput mass spectrometric discovery of protein post-translational modifications.** *J Mol Biol* 1999, **289**:645-657.
51. Boguski MS: **Biosequence exegesis.** *Science* 1999, **286**:453-455.
- A nice discussion of the dilemma of bioinformatics – how to interpret the sea of data!
52. Tobisch S, Zuhlke D, Bernhardt J, Stulke J, Hecker M: **Role of CcpA in regulation of the central pathways of carbon catabolism in *Bacillus subtilis*.** *J Bacteriol* 1999, **181**:6996-7004.
- An example of the global application of proteome analysis.
53. Antelmann H, Scharf C, Hecker M: **Phosphate starvation-inducible proteins of *Bacillus subtilis*: proteomics and transcriptional analysis.** *J Bacteriol* 2000, **182**:4478-4490.
- Another example of the global application of proteome analysis.
54. Svensater G, Sjogreen B, Hamilton IR: **Multiple stress responses in *Streptococcus mutans* and the induction of general and stress-specific proteins.** *Microbiology* 2000, **146**:107-117.
55. De Backer MD, de Hoogt RA, Froyen G, Odds FC, Simons F, Contreras R, Luyten WH: **Single allele knock-out of *Candida albicans* CGT1 leads to unexpected resistance to hygromycin B and elevated temperature.** *Microbiology* 2000, **146**:353-365.
- An example that should warn caution in our interpretation of biological data.
56. Jungblut PR, Bumann D, Haas G, Zimny-Arndt U, Holland P, Lamer S, Siejak F, Aebischer A, Meyer TF: **Comparative proteome analysis of *Helicobacter pylori*.** *Mol Microbiol* 2000, **36**:710-725.
- A clear illustration of the power of proteome analysis to identify virulence or pathogenic antigens.
57. Joubert R, Brignon P, Lehmann C, Monribot C, Gendre F, Boucherie H: **Two-dimensional gel analysis of the proteome of lager brewing yeasts.** *Yeast* 2000, **16**:511-522.
58. Chevalier S, MacDonald N, Tonge R, Rayner S, Rowlinson R, Shaw J, Young J, Davison M, Roberts RA: **Proteomic analysis of differential protein expression in primary hepatocytes induced by EGF, tumour necrosis factor alpha or the peroxisome proliferator nafenopin.** *Eur J Biochem* 2000, **267**:4624-4634.
- This is a good descriptive proteome analysis of hepatocyte function that illustrates the complexity of cellular response when seen from the global viewpoint.
59. Nyman TA, Matikainen S, Sareneva T, Julkunen I, Kalkkinen N: **Proteome analysis reveals ubiquitin-conjugating enzymes to be a new family of interferon-alpha-regulated genes.** *Eur J Biochem* 2000, **267**:4011-4019.
60. Brockstedt E, Peters-Kottig M, Badock V, Hegele-Hartung C, Lessl M: **Luteinizing hormone induces mouse vas deferens protein expression in the murine ovary.** *Endocrinology* 2000, **141**:2574-2581.
61. Nelson PS, Han D, Rochon Y, Corthals GL, Lin B, Monson A, Nguyen V, Franza BR, Plymate SR, Aebersold R, Hood L: **Comprehensive analyses of prostate gene expression: convergence of expressed sequence tag databases, transcript profiling and proteomics.** *Electrophoresis* 2000, **21**:1823-1831.

62. Kanaya S, Ujiie Y, Hasegawa K, Sato T, Imada H, Kinouchi M, Kudo Y, Ogata T, Ohya H, Kamada H *et al.*: **Proteome analysis of *Oncorhynchus* species during embryogenesis.** *Electrophoresis* 2000, **21**:1907-1913.
63. Andersen HU, Mose Larsen P, Fey SJ, Karlens AE, Mandrup-Poulsen T, Nerup J: **Two dimensional gel electrophoresis of rat islet proteins. Interleukin-1 β induced changes in protein expression are reduced by L-arginine depletion and nicotinamide.** *Diabetes* 1995, **44**:400-407.
64. Mose Larsen P, Fey SJ, Larsen MR, Nawrocki A, Andersen HU, Kähler H, Heilmann C, Voss MC, Roepstorff P, Pociot F *et al.*: **Proteome analysis of IL-1 β induced changes in protein expression in rat islets of Langerhans.** *Diabetes* 2001, in press.
65. Celis JE, Wolf H, Ostergaard M: **Bladder squamous cell carcinoma • biomarkers derived from proteomics.** *Electrophoresis* 2000, **21**:2115-2121.
This study illustrates how much hard work has to go into a thorough study at the level of the proteome.
66. Unwin RD, Knowles MA, Selby PJ, Banks RE: **Urological malignancies and the proteomic-genomic interface.** *Electrophoresis* 1999, **20**:3629-3637
67. Edgar PF, Douglas JE, Cooper GJ, Dean B, Kydd R, Faull RL: **Comparative proteome analysis of the hippocampus implicates chromosome 6q in *schizophrenia*.** *Mol Psychiatry* 2000, **5**:85-90.