A look back: how the proteome got its spots

Even as it passes its 40th birthday[1], polyacrylamide gel electrophoresis (PAGE) remains one of the most potent tools for protein analysis. Nonetheless, researchers conducting broader experiments—say, examination of global protein modification in a given tissue—may be hard pressed to decode the nasty smears that can appear on a one-dimensional gel.

Graduate student Patrick O'Farrell faced this problem in 1974, in his efforts to study changes in protein expression during *Volvox carteri* development. Two-dimensional electrophoresis (2-DE) techniques, wherein complex protein mixtures are sequentially separated by two unrelated physical properties, had previously been described (ref. 2), but were largely impractical for generalized studies. The most promising strategy involved using isoelectric focusing (IEF) to separate proteins by charge, followed by size-separation via sodium dodecyl sulfate (SDS)-PAGE. O'Farrell improved this system and further enhanced the quality of his findings by using urea and nonionic detergent to thoroughly solubilize his proteins, developing an optimized pH gradient for the first dimension and radiolabeling rather than staining his proteins. His first 2-DE gels displayed an astonishing 1,100 *Escherichia coli* proteins, laying the groundwork for protein studies with an unprecedented level of detail.

O'Farrell's findings spread quickly, and he was soon leading workshops to teach his new method, even as he worked to finish his thesis and write up his findings, though this last part proved surprisingly difficult. "[The paper] got all kinds of grief," recalls O'Farrell, "I mean, it got rejected when we first submitted it. I remember one of the lines quite vividly... I had 'grossly overinterpreted my results and overstated the importance of the technique'. And it was really quite bizarre, because... there was no interpretation of results, except describing the characteristics of the method. And as far as overstating the importance of the technique...!"

His paper finally saw print in 1975 (ref. 3) and was quickly followed by publications from groups in Germany and New York that had independently developed 2-DE approaches of their own[4,5]. This indicated that rather than overstating the technique's importance, O'Farrell was in fact at the vanguard of a number of researchers who recognized the need for techniques suitable for more detailed analysis of what would eventually be dubbed the 'proteome'.

Jim Garrels, who pioneered the computerized analysis of 2-DE results[6], was amazed by O'Farrell's early results: "In those days, everyone who saw those was just blown away by the number of proteins that you could see on one gel... we thought it was going to change biology." This level of detail also deterred many investigators, who were ill–prepared to tackle the sort of large-scale analyses that 2-DE enabled. After showing his early results, recalls O'Farrell, "the response from most people was, 'There [are] too many spots! How can you deal with so many spots?'" Technical complications further impeded the spread of 2-DE. For many years, the identification of individual spots was a major chore, if not an impossibility. Additionally, the early days of 2-DE were plagued with inter-gel heterogeneity resulting from subtle gradient variations in the IEF dimension, confounding comparative analyses.

Over time, many of these problems would be effectively solved. Immobilized pH gradient strips[7] eliminated much of the variability from the IEF procedure, making 2-DE experiments more reproducible. Computerized spot analysis (refs. 6 and 8) would spare researchers a lot of the measuring and migraines accompanying 2-DE gel analysis. Perhaps most important of all was the development of mass spectroscopy[9], which finally enabled researchers to identify virtually any spot on a given 2-DE gel.

Nonetheless, even its staunchest admirers testify that 2-DE still requires considerable skill and patience. "With 2-D gels," says Garrels, "you've got the chemistry, the vagaries of the gel, the solubilization conditions... it's a lot of variables." He and O'Farrell agree that this complexity has been a key obstacle that has kept 2-DE out of the mainstream-biology toolbox.

Nevertheless, the rise of 2-DE contributed immensely to the birth of proteomics, offering the capability to create protein maps at a level of detail that, even 30 years later, remains largely unsurpassed. And O'Farrell, who admits to having largely soured on proteomics research since those early days, still remains proud of the method: "It's remarkably effective, [and] there's an enormous amount, when you've had some experience with the technique, which you can read off of the gel."

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