CC/NUMBER 51
DECEMBER 20, 1982

This Week's Citation Classic

O'Farrell P H. High resolution two-dimensional electrophoresis of proteins.
J. Biol. Chem. **250**:4007-21, 1975.
[Dept. Molecular, Cellular and Developmental Biology, Univ. Colorado, Boulder, CO]

This paper described a method for high resolution analytical separation of proteins by two-dimensional gel electrophoresis. Proteins are separated in the first dimension according to their isoelectric points, and in the second dimension according to their molecular weights. [The *SCI*® indicates that this paper has been cited in over 2,870 publications since 1975.]

Patrick H. O'Farrell
Department of Biochemistry and Biophysics
School of Medicine
University of California
San Francisco, CA 94143

November 4, 1982

"As a graduate student in Boulder, Colorado, in 1972, I had isolated a series of mutations affecting development of the colonial algae, *Volvox*, and had visions of defining the normal pattern of gene expression during development and the effects that mutations had on this regulatory program. This had proved to be a powerful approach in analyses defining the mechanisms regulating gene expression in bacteriophage. Through interactions with my adviser, Jacques Pêne, and another faculty member, Larry Gold, I became familiar with these bacteriophage systems. It was apparent that the reason for the success of the experimental approach was that sodium dodecylsulfate gel electrophoresis[1] provided a method capable of separating, identifying, and quantitating the majority of the bacteriophage proteins. To adapt this approach to the study of development in a eukaryote, such as *Volvox* (which has roughly 100 times the genetic complexity of a bacteriophage), I needed a separation system with much higher resolution. Although an adequate separation procedure did not exist, the use of two-dimensional methods for increased resolution was a well-established approach for chromatography and electrophoresis. Consequently, I set out to combine the two most powerful electrophoresis methods available.

"I had some early indications that this work was going to have considerable impact. Based on little more than a preliminary autoradiogram, the National Science Foundation awarded us a small grant for the development of the technique, and a number of investigators began to inquire whether the method was ready to be applied. Pêne left Boulder at this time; I stayed on to complete the work and my degree under the sponsorship of David Hirsh.

"While I was preparing my thesis, Gold and Hirsh organized a course that helped to introduce the method and contributed to the recognition of the paper. Scientists from institutions representing various areas of the country were invited to Boulder, where I presented a four-day course on the techniques and their applications. The two-dimensional electrophoresis method eventually played an important role in the research programs of several of the course participants, such as Fotis Kafatos and Peter Geiduschek.

"After the course, I defended my thesis, submitted the manuscript to the *Journal of Biological Chemistry*, and moved to the department of biochemistry and biophysics, University of California, San Francisco, to pursue postdoctoral studies with Gordon Tomkins. I was more than a little surprised when a few months later I received two unfavorable reviews and a letter rejecting my manuscript; it had been reviewed by half a dozen scientists prior to submission. The reviews from the journal concluded that the manuscript appeared to be 'highly speculative in places and to be extrapolated in terms of usefulness far beyond what the author has any reason to expect.' With the cooperation and help of members of the journal's editorial board, the initial rejection decision was reversed.

"This paper has been frequently cited because the method has found a wide range of applications. Although I never returned to examine patterns of gene expression in *Volvox*, many workers have now applied the method to examine the changing patterns of proteins made during development. Additionally, the method has played a major role in diverse developments extending from the identification of the bacterial recA protein (thereby assisting in the elucidation of recombinational mechanisms and the induction of these functions by DNA damage) to the detection of the phosphorylation targets of viral transforming proteins. Although other techniques are now in the forefront of advances in molecular biology, refinements of electrophoretic separation methods[2] have continued to extend their usefulness."

1. **Laemmli U K.** Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**:680-5, 1970.
2. **O'Farrell P Z, Goodman H M & O'Farrell P H.** High resolution two-dimensional electrophoresis of basic as well as acidic proteins. *Cell* **12**:1133-42, 1977.
[The *SCI* indicates that this paper has been cited in over 480 publications since 1977.]