Number 45 November 7, 1977

Citation Classics

Katz A M, Dreyer W J & Anfinsen C B, Jr. Peptide separation by two-dimensional chromatography and electrophoresis. *Journal of Biological Chemistry* **234**:2897-900, 1959.

The authors describe a modified method for the separation of peptides on filter paper that utilizes chromatography followed by high voltage electrophoresis cooled by a non-explosive organic solvent. [The *SCI*® indicates that this paper was cited 619 times in the period 1961-1975.]

Dr. Arnold Martin Katz
University of Connecticut
Medical Center
Farmington, Connecticut 06232

February 28,1977

..."I am somewhat amused to find this paper listed amongst the 'most cited articles ever published' as this contribution is primarily meth-odological in scope, and the method wasn't even original to us! The value of this detailed description of a modification of the peptide 'fingerprinting' method used earlier by V.M. Ingram probably lies primarily in our use of 'varsol' in plastic, instead of toluene in glass, for cooling during the high voltage electrophoresis of $18^{1/4}$ by $22^{1/2}$ inch filter paper chromatograms. This modification was necessitated by the configuration of our NIH lab, where the hood (in which we did electrophoresis) was adjacent to the only exit. The thought of a spark– generated by the 2000 volt, 250 milliamp power supply– igniting 10 gallons of toluene in a huge and fragile glass jar served as a most effective stimulus to this paper. Repeated demonstrations to visiting scientists (and girlfriends) of the explosive response of a few drops of toluene to a lighted match provided a vivid reminder of the hazards of the Ingram method. A number of us spent much time looking for other organic solvents, especially those which wouldn't permeate or dissolve plastics, which could be used in these peptide separations. As I recall, the use of varsol (a light petroleum fraction with a flash point well above room temperature) stemmed from several visits to the National Bureau of Standards and one to a local gas station.

"The remainder of this method was relatively standard; the use of n-butanolacetic acid-water for paper chromatography was well established in Dr. Anfinsen's laboratory, and high voltage paper electrophoresis had been employed with considerable elegance by many, including Sanger's group. The combination of these principles, which separated peptides in two dimensions by methods based on different properties of each peptide, had, as indicated earlier, been used by Ingram to identify specific amino acid substitutions in abnormal human hemoglobins. By performing the chromatography first, unlike Ingram who did the electrophoresis first, we were able to improve the resolution of the 'fingerprints' by taking advantage of the percolation of the buffer-applied to dried paper chromatograms– towards the origin, which tended to 'sharpen' the bands.

"At the onset, I indicated with some chagrin that this methodological paper, and not one of my more recent conceptual articles, has become a 'best seller'. Yet history's judgement may not be wholly inappropriate. This paper was, in fact, the only full-length paper to have come from my two-year tenure as Research Associate in Dr. Anfinsen's laboratory at the NIH. I was, at that time, most jealous of my friends who were able to grind out large numbers of papers laden with data while I struggled for almost two years with methodology. In retrospect, however, I have come to appreciate the opportunity I had to learn many different techniques that proved invaluable in my later work. It is for this reason that the paper I wrote with Bill Dreyer and Chris Anfinsen not only calls up vivid memories of my scientific youth, but also serves as a reminder of the value of methodology in scientific observations."