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This Week's Citation Classic

Hedrick I L & Smith A J. Size and charge isomer separation and estimation of molecular weights of proteins by disc gel electrophoresis.
Arch. Biochem. Biophys. **126**:155-64, 1968.
[Department of Biochemistry and Biophysics, University of California, Davis, CA]

A simple method was devised relating the electrophoretic mobility of a protein determined by disc gel electrophoresis to its size and charge characteristics. The method is applicable to a single protein or to mixtures of proteins, provided a specific detection test is available. Knowing the relative size and charge of proteins is not only useful for their differential characterization but also as a predictive aid in their purification. [The *SCI*® indicates that this paper has been cited over 800 times since 1968.]

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"This paper is a specific application of the disc gel electrophoretic technique brilliantly developed by L. Ornstein and B.J. Davis in the late 1950s.[1,2] I was first introduced to the technique by Bob Metzenberg in 1961 while I was a graduate student at Wisconsin. I was very excited by its potential. When I arrived at the University of Washington, Seattle, in 1962 as a postdoctoral fellow, I rapidly applied the method to the study of the two forms of an enzyme. At that time, the isomeric forms of the enzyme were thought to differ predominantly in terms of size and minimally in terms of charge. However, the results I obtained were not consistent with the then accepted paradigm. Even though the validity of my observations could be accepted, the interpretation of them could not and, accordingly, I shelved this result till my first academic position at Davis.

"In 1966, I invited Al Smith to join me in Davis after I obtained my first federal grant. As I was a new independent investigator coincident with the beginning of the reduction in federal research funds, we had minimal resources with which to work. Our electrophoretic equipment was made of bits and pieces from discarded equipment and plasticware purchased at the local grocery store–carbon electrodes salvaged from dead flashlight batteries and baby blue 'Popeye' cereal bowls with a 'magic eye' for use as electrode reservoirs.

"With this crude but functional equipment, our approach to the problem was purely an empirical one, the usual case in method development. A very simple method of estimating the relative size and charge of a protein was eventually found by determining its electrophoretic mobility as a function of gel concentration. A log-linear plot of the data gave the sought-after straight line relation. The slope of the line was related to the size of the protein and the intercept, the charge.

"The excitement I felt about our discovery was heightened as this was my first independent creation as an assistant professor. The thrill was abruptly dampened when our paper was rejected by a leading biochemistry journal as being inappropriate. We submitted it to another journal where it was rapidly reviewed and accepted. We subsequently applied the method to the enzyme isomer problem and showed that the paradigm existing in 1962 was incorrect and extended the method to the case of proteins binding noncharged ligands.[3] This extension, in contrast to the original paper, has gone virtually unnoticed.

"I believe the paper has been popular because of its wide applicability and simplicity. The method itself is simple as are the interpretations of the results. Unfortunately, many of the recent putative theoretical attempts to mathematically relate mobility and the size and charge of a protein have neither simplified nor explained the fundamental principles of gel electrophoresis, but rather obfuscated them. The axiom relating simplicity and acceptability seems verified by the popularity of this paper."

1. **Ornstein L.** Disc electrophoresis. I. Background and theory. *Ann. NY Acad. Sci.* **121**:321-49, 1964.
2. **Davis B J.** Disc electrophoresis. II. Method and application to human serum proteins. *Ann. NY Acad. Sci.* **121**:404-27, 1964.
3. **Hedrick J L, Smith A J & Bruening G E.** Characterization of the aggregated states of glycogen phosphorylase by gel electrophoresis. *Biochemistry* **8**:4012-9, 1969.