CC/NUMBER 22
JUNE 2, 1980

This Week's Citation Classic

Cuatrecasas P. Protein purification by affinity chromatography: derivatizations of agarose and polyacryfamide beads. *J. Biol. Chem.* **245**:3059-65, 1970. [Lab. Chem. Biol., Nat. Inst. Arthritis and Metabolic Diseases, NIH, Bethesda, MD]

The preparation of agarose and polyacrylamide bead derivatives for the purification of a variety of proteins and enzymes is described. The methodologies described permit the attachment of ligands directly or through extended hydrocarbon chains to immobilized supports. [The *SCI*® indicates that this paper has been cited over 1,580 times since 1970.]

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May 14, 1980

"One of the more gratifying aspects of scientific work is the knowledge that one's own contributions have helped and influenced other scientists and thus furthered the overall progress of science. The manner by which this paper may have had an impact and the reason for its frequent citation are difficult to assess, but the methods and suggested applications may have spurred interest and encouragement. This was one of the first papers in this field, which has since seen literally thousands of publications.

"The term 'affinity chromatography' was first christened in 1968 when my colleagues, M. Wilchek and C.B. Anfinsen, and I used biospecific adsorption to purify several enzymes.[1] The basic concepts described in that and the present paper were, as in virtually all scientific discoveries, heavily based on previous knowledge. The fundamental ideas were simple and rational and surely had been in the minds of others. As is frequently the case, the time was probably ripe for more formally promulgating the principles and procedures in the simplest of terms in order to help establish the generali-ty of the method.

"The impetus for this work arose from studies of the active site of micrococcal nuclease by affinity labeling with specific inhibitors. The reasoning was simply that if an inhibitor could be directed irreversibly to the *active site*, then why couldn't the enzyme be made to bind by its active site to an inhibitor irreversibly bound to a solid polymer or support. It is on this historical note that the idea and term 'affinity chromatography' were conceived.[1]

"Although excellent examples of the basic concepts existed prior to this work, these had apparently been considered by others as isolated and unique examples. The paper under consideration attempted to formalize the approach and methodology in order to focus attention on its general applicability and feasibility. By detailing the concepts and techniques, and by describing specific chemical manipulations of possible general utility, it was hoped that others would also perceive that the purification of biologically active macromolecules by biospecific adsorption was a potentially valuable tool that could be approached *systematically* in various fields of biochemistry and biology. The fact that affinity chromatography is now common nomenclature in biochemistry, without need for citation, attests to the validity and inherent obviousness of the concepts championed in our early publications.[2]

"The paper tried to describe the basic procedural principles in practical terms, and it detailed numerous simple chemical strategies for derivatizing ligands and solid supports for use as specific adsorbents. It described the importance of interposing spacers between the matrix backbone and the ligand. The general feasibility of deliberately designing insoluble supports of virtually any ligand was stressed. Illustrative examples were presented to provoke interest; in addition to enzymes, the applicability to hormone receptors, cyclic nucleotidebinding proteins, SH-group-containing proteins and intact cells was described.

"Since this publication, much progress and improvements have been made both in technology and in specific applications; the methods are now routine for purifying receptors, binding proteins, and for cell separations."[2]

1. **Cuatrecasas P, Willchek M & Anfimeen C B.** Selective enzyme purification by affinity chromatography.*Proc. Nat. Acad. Sci. US* **61**:636-43, 1968.
2. **Jakeby W B & M, eds.** Affinity techniques. Enzyme purification: part B. *Methods Enzymol.* **34**:3-610,1974.