Affinity Chromatography: Methods and Protocols

METHODS IN MOLECULAR BIOLOGY™

Affinity Chromatography

Methods and Protocols

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Cover design by Patricia F. Cleary.

Cover illustration: Phage display technology *(1)* has enabled the limits of molecular diversity and biorecognition to be tested well beyond anyone's imagination when affinity chromatography was first discovered by Cuatrecasas, Wilchek, and Anfinsen in 1968 *(2)*. Like affinity chromatography (*left panel*), phage display (*right panel*) requires a library of peptides/proteins that are incubated with an immobilized target such as a soluble receptor, like the IL-2 receptor *(3,4)*, or with a monoclonal antibody, like humanized antiTac *(5)*. Noninteracting peptides/proteins are then washed away, and adsorbed peptides/proteins can be specifically or nonspecifically eluted. (For references and additional discussion, *see* Editor's Note, p. vi.)

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Preface

Affinity chromatography, with its exquisite specificity, is based upon molecular recognition. It is a powerful tool for the purification of biomolecules. In recent years, numerous new applications and modified techniques have been derived from group-specific interactions and biological recognition principles. An up-to-date review of the past, current, and future applications of affinity chromatography has been presented in the introductory chapter by Meir Wilchek and Irwin Chaiken.

Though many of these new applications and techniques are well documented in the literature, it is often difficult to find methods that are written with the intent of helping new practitioners of affinity chromatography. This volume on *Affinity Chromatography: Methods and Protocols* is intended for the novice, as well as for experts in the field. The protocols are written by experts who have developed and/or successfully employed these methods in their laboratories. Each chapter describes a specific technique, and since the book is intended to help the beginner, each technique is described simply and clearly, making sure that all relevant steps are included, assuming no previous knowledge.

Each chapter contains an introduction describing the principles involved, followed by a Materials and Methods section, which lays the groundwork for the reader to conduct experiments step-by-step, in an orderly fashion. The following Notes section, which describes many of the problems that can occur, makes suggestions for overcoming them, and provides alternate procedures. These are precisely the sort of important, practical details that never seem to appear in the published literature.

The exemplary separations detailed in *Affinity Chromatography: Methods and Protocols* range from small molecules, like haptens, to protein ligands, to supramolecular structures, as in phage display. It is our fervent hope that the reader will develop a better understanding and a deeper appreciation of the power and range of affinity interactions. We acknowledge that owing to lack of space, we are not able to include every affinity chromatography method in this particular volume.

We wish to express our heartfelt thanks to all the authors who are the acknowledged experts in their respective fields for their dedication and cooperation. We are grateful to Dr. Sittichoke Saisanit for his assistance in the handling of electronic documents. It was a great pleasure working with the editorial staff of Humana Press Inc.

Pascal Bailon
George K. Ehrlich
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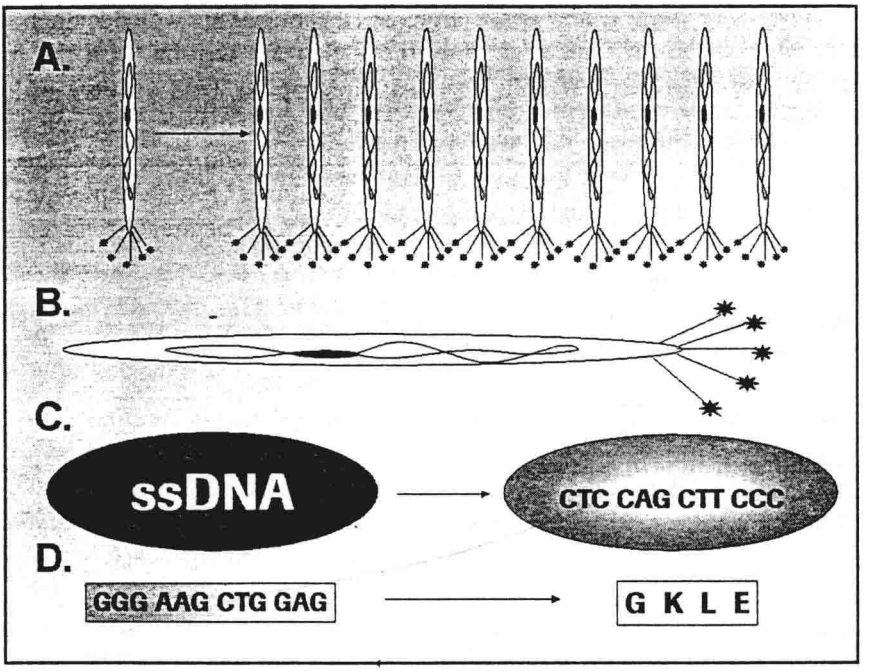
Editor's Note

Following elution of interacting peptides/proteins in phage display (*see* Cover, *bottom, right and center*, and "Cover illustration [p. iv]"), their copy number can be increased by bacterial infection since these peptides/proteins are displayed on bacteriophage (**A,** illustration on this page). This *screening/amplification* process can be repeated as necessary to obtain higher-affinity phage display peptides/proteins. The high affinity phage display peptides/proteins can be isolated (**B,** this page) in order to purify and sequence its coding ssDNA (**C**). In turn, these codons can be translated to determine the amino acid sequences of the high affinity phage-derived peptides/proteins (**D**). As Disney would say, "Maybe a better mousetrap, but always the same Mickey." (Cover illustration and Editor's Note figure, courtesy of Gia K. Luhr, Karolinska Institute, Stockholm, Sweden, and George K. Ehrlich.)



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Contents

1

An Overview of Affinity Chromatography

Meir Wilchek and Irwin Chaiken

1. Introduction

Affinity chromatography is pervasively accepted and used as a tool in biomedical research and biotechnology; yet its origins only 30 years ago sometimes seem dimmed in history. However, the potential of this technology continues to stimulate continued development and new applications. Having a new book on this methodology is eminently appropriate today. And being able to introduce this book is our pleasure.

Affinity chromatography as it is known today was introduced in 1968 by Pedro Cuatrecasas, Chris Anfinsen, and Meir Wilchek, one of the authors of this chapter. Though few related methods were described earlier, the concept and immense power of biorecognition as a means of purification was introduced first in that 1968 paper *(1)* entitled "Affinity Chromatography."

If you examine the Medline Database for how many times "affinity chromatography" has appeared in the title of scientific papers, you will find almost 30,000 papers cited. This means that, over the past 30 years, three published papers per day have featured this technology. Moreover, 300 patents have been granted during the last 2 years alone. In a recent review *(2)*, Chris Lowe stated that affinity chromatography is a technique used in 60% of all purification protocols. So what exactly is affinity chromatography—the technique to which this book is devoted?

2. Affinity Chromatography and Its Applications for Purification

Affinity chromatography is based on molecular recognition. It is a relatively simple procedure. Any given biomolecule that one wishes to purify usually has an inherent recognition site through which it can recognize a natural or artificial molecule. If one of these recognition partners is immobilized on a poly-

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Table 1
Biomolecules Purified by Affinity Chromatography

1. Antibodies and antigens	9. Lectins and glycoproteins
2. Enzymes and inhibitors	10. RNA and DNA (genes)
3. Regulatory enzymes	11. Bacteria
4. Dehydrogenases	12. Viruses and phages
5. Transaminases	13. Cells
6. Hormone-binding proteins	14. Genetically engineered proteins
7. Vitamin-binding proteins	15. Others
8. Receptors	

meric carrier, it can be used to capture selectively the biomolecule by simply passing an appropriate cell extract containing the latter through the column. The desired biomolecule can then be eluted by changing external conditions, e.g., pH, ionic strength, solvents, and temperature, so that the complex between the biomolecule and its partner will no longer be stable, and the desired molecule will be eluted in a purified form.

Numerous books and reviews on the application and theory of affinity chromatography have appeared in recent years *(3)*. Here, we simply list classes of compounds purified by this method (*see* **Table 1**).

3. Techniques that Stem from Affinity Chromatography

The broad scope of the various applications of affinity chromatography has generated the development of subspecialty adaptations, many of which are now recognized by their own nomenclature as an expression of their generality and uniqueness. Because some of these applications have a chapter of their own in this volume, we only summarize them in **Table 2**.

As this book shows, some of the subcategories have become generally accepted as useful techniques. Among the most popular of these affinity-derived techniques is immunoaffinity chromatography, which utilizes antibody columns to purify antigens, or antigen columns to purify antibodies. Immunoaffinity chromatography is, in fact, used in most biological studies. Other methods, such as metal–chelate affinity chromatography, apply site-directed mutagenesis to introduce various affinity tags or tails to the biomolecule to be purified. For example, the His-Tag is used both in metal–chelate chromatography and as an antigen in immunoaffinity chromatography. More recently, the use of combinatorial libraries has become increasingly popular for developing new affinity ligands.

4. Carriers

It is interesting that in all these developments the carriers used were polysaccharides, modified polysaccharides, silica and to a lesser extent polystyrene.

Table 2
Various Techniques Derived from Affinity Chromatography

1. Immunoaffinity chromatography
2. Hydrophobic chromatography
3. High performance affinity chromatography
4. Lectin affinity chromatography
5. Metal-chelate affinity chromatography
6. Covalent affinity chromatography
7. Affinity electrophoresis
8. Affinity capillary electrophoresis
9. Dye-ligand affinity chromatography
10. Affinity partitioning
11. Filter affinity transfer chromatography
12. Affinity precipitation
13. Affinity density perturbation
14. Perfusion affinity chromatography
15. Centrifuged affinity chromatography
16. Affinity repulsion chromatography
17. Affinity tails chromatography
18. Theophilic chromatography
19. Membrane-based affinity chromatography
20. Weak affinity chromatography
21. Receptor affinity chromatography
22. Avidin-biotin immobilized system
23. Molecular imprinting affinity
24. Library-derived affinity ligands

Even today, 95% of all affinity purification methods involve Agarose-Sepharose, the carrier that was originally introduced in the first paper on affinity chromatography.

5. Activation and Coupling

In this book, most of the chapters deal with application and not with methodology for the preparation of the affinity columns. Indeed, the methodology is well documented and widely used *(4)*. Here we describe only briefly some of the procedures used to prepare an affinity column.

Affinity chromatography is a five-step process, which consists of activation of the matrix, followed by coupling of ligands, adsorption of the protein, elution, and regeneration of the affinity matrix. A short description of the activation and coupling is described as follows.

In most studies, the activation process is still performed using the cyanogen bromide method. However, studies on the mechanism of activation with CNBr revealed that the use of this method can cause serious problems. Therefore, new activation methods were developed that gave more stable products. The newer methods have mainly been based on chloroformates, carbonates, such as N-hydroxysuccinimide chloroformate or carbonyl *bis*-imidazole or carbonyl (*bis*-N-hydroxysuccinimide) and hydroxysuccinimide esters, which after reaction with amines result in stable carbamates or amides *(5,6)*. The coupling of ligands or proteins to the activated carrier is usually performed at a pH slightly above neutral. Details regarding subsequent steps can be found in many of the other chapters of this volume.

6. Recognition Fidelity and Analytical Affinity Chromatography

Affinity chromatography is based on the ability of an affinity column to mimic the recognition of a soluble ligand. Such fidelity also has presented a vehicle to analyze. Isocratic elution of a biological macromolecule on an immobilized ligand affinity support under nonchaotropic buffer conditions allows a dynamic equilibrium between association and dissociation. It is directly dependent on the equilibrium constant for the immobilized ligand—macromolecule interaction. Hence, affinity is reflected in the elution volume. The analytical use of affinity chromatography was demonstrated with staphylococcal nuclease *(7)*, on the same kind of affinity support as used preparatively *(1)* but under conditions that allowed isocratic elution. Similar findings have been reported by now in many other systems *(8)*. Of particular note, interaction analysis on affinity columns can be accomplished over a wide range of affinity, as well as size of both immobilized and mobile interactors. This analysis can be achieved on a microscale dependent only on the limits of detectability of the interactor eluting from the affinity column.

7. Automation and Recognition Biosensors

The analytical use of immobilized ligands has been adapted to methodological configurations which allow for automation and expanded information. An early innovation of analytical affinity chromatography was its adaptation to high-performance liquid chromatography. High performance analytical affinity chromatography *(9)* provides a rapid macromolecular recognition analysis at microscale level, using multiple postcolumn monitoring devices to increase the information learned about eluting molecules. Simultaneous multimolecular analysis is also feasible, e.g., by weak analytical affinity chromatography *(10)*.

Years since the development of affinity chromatographic recognition analysis with immobilized ligands followed the evolution of molecular biosensors. Ultimately, a technological breakthrough for direct interaction analysis was the surface plasmon resonance (SPR) biosensor developed by Pharmacia, called BIAcore™, in which the immobilized ligand is attached to a dextran layer on a gold sensor chip. The interaction of macromolecules passing over the chip through a flow cell is detected by changes of refractive index at the gold surface using SPR *(11,12)*.

The SPR biosensor is similar in concept to analytical affinity chromatography: both involve interaction analysis of mobile macromolecules flowing over surface-immobilized ligands. The SPR biosensor also provides some unique advantages. These include (1) access to on- and off-rate analysis, thus providing deeper characterization of molecular mechanisms of biomolecular recognition and tools to guide the design of new recognition molecules; and (2) analysis in real time, thus promising the potential to stimulate an overall acceleration of molecular discovery.

In addition to BIAcore, an evanescent wave biosensor for molecular recognition analysis has been introduced recently by Fisons, called IAsys™ ***(13,14)***. Instead of passing the analyte over the sensor chip through a flow cell, IAsys uses a reinsertable microcuvet sample cell, which contains integrated optics. A stirrer in the cuvette ensures efficient mixing to limit mass transport dependence.

Automation in the analytical use of immobilized ligands seems likely to continue to evolve. Analytical affinity chromatography increasingly is being adapted to sophisticated instrumentation and high-throughput affinity supports. In addition, new methodological configurations with biosensors are being developed. These advances promise to expand greatly the accessibility of both equilibrium and kinetic data for basic and biotechnological research.

8. Conclusions

Looking back, affinity chromatography has made a significant contribution to the rapid progress which we have witnessed in biological science over the last 30 years. Affinity chromatography, due to its interdisciplinary nature, has also introduced organic, polymer and biochemists to the exciting field of solving problems which are purely biological in nature. Thus, affinity chromatography, and the affinity technologies it has inspired, continue to make a powerful impact in fostering the discovery of biological macromolecules and the elucidation of molecular mechanisms of interaction underlying their bioactivities.

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