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Protein Purification
Micro to Macro

Editor
Richard Burgess

Protein Purification

Micro to Macro

Proceedings of a Cetus-UCLA Symposium
Held at Frisco, Colorado, March 29–April 4, 1987

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Preface

The Cetus-UCLA Symposium on *Protein Purification: Micro to Macro* was held at Frisco, Colorado, March 29–April 4, 1987. This meeting was attended by about 239 scientists who came together with a common interest in learning more about protein purification. The meeting presentations were focused on several areas including micropurification and analysis, protecting proteins during purification and storage, precipitation and phase partitioning methods, chromatography, overproduction of proteins in bacteria and other hosts, coping with problems of insolubility and proteolysis, and scale-up considerations. This book contains a sampling of the topics presented at plenary sessions and poster sessions.

The interest in protein purification has increased dramatically as numerous enzymes of research, pharmaceutical, and industrial importance are identified. In many cases, the technology for cloning the genes for these proteins has developed more rapidly than the technology for purifying the expressed gene product. In addition, the need to produce some of these proteins at the kilogram to ton level has required a collaborative effort between biochemists and chemical engineers. This meeting was organized to bring together a wide spectrum of researchers (theoretical and practical, academic, governmental and industrial, microscale, laboratory scale and very large scale, protein biochemists, genetic engineers, and chemical engineers) to review together the state of protein purification, to share new concepts and techniques, and to pinpoint theoretical and practical problems yet to be solved.

At a time when the bulk of the advances in large scale protein purification and process design are occurring in biotechnology companies, it is crucial that the growing body of knowledge find its way to people who will teach the next generation of protein purifiers and who will be needed to fuel future innovation in separations technology. Dr. Robert Scopes, whose excellent textbook on protein purification has contributed greatly to this teaching process, was an appropriate keynote speaker.

Micropurification of proteins at the microgram level was reported using a variety of methods including HPLC, affinity chromatography, and elution from SDS polyacrylamide gels and renaturation.

Numerous examples of overproduction of cloned gene products were presented. Although overexpression in *E. coli* was the most common method, other host systems reported included yeast, filamentous fungi, mammalian cell culture, and insect cells.

Enzymes destined for pharmaceutical use pose special considerations. Products have to be consistent, well-defined, and highly purified, in some cases exceeding 99.99% purity. This requires high resolution final "polishing" steps and the need for very high sensitivity methods to detect trace impurities. An important problem was protein heterogeneity; either naturally occurring, or introduced due to production in heterologous hosts or post-harvest modification.

In designing a large-scale protein purification process new considerations arise such as viscosity, heat transfer rates, phase separations, and concentration of dilute solutions. For non-clinical commercial enzymes, cost becomes the prime design consideration. It was encouraging to see the biochemists and chemical engineers working together to try to learn and appreciate each other's perspective.

Overall, there was a sense of excitement and satisfaction generated by this meeting: excitement at the new knowledge gained, the rapid progress of the field, and the new personal connections established; satisfaction at the opportunity to participate in a meeting where the main focus was protein purification, not the protein being purified. Most of us thoroughly enjoyed being part of a meeting where protein purification emerged as a discipline rather than merely as a means to an end.

Special thanks are due Cetus Corporation for the generous Sponsorship of this meeting. I also gratefully acknowledge additional gifts from Cambridge Bioscience Corporation; Celltech Limited; Pharmacia, Inc., Biotechnology Group; Millipore Corporation; The Upjohn Company; Boehringer Mannheim GmbH; Bristol-Myers Company, Pharmaceutical Research & Development Division; DNAX Research Institute; and Interferon Sciences. I wish to thank the UCLA Symposia staff, especially Robin Yeaton, who played a crucial role in helping me organize the meeting, and Bill Coty, who skillfully saw to the details at the meeting so that I could relax and enjoy the meeting myself.

Richard Burgess

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CLASSICAL AND MODERN TECHNIQUES IN PROTEIN PURIFICATION

Robert K. Scopes

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Our understanding of the structure and function of proteins has progressed so rapidly over the past few decades that we tend to forget that scientists were studying them over two centuries ago. My first reference - incompletely documented for unavoidable reasons - dates from the year of the French Revolution, 198 years ago [1], and it describes the purification of coagulable substances from plants having similar properties to egg albumen, a protein already well known at that time. Of course, none of these substances were anything like as "pure" as a journal editor would like us to demonstrate these days, but they were recognised as being distinct from each other; by the early 19th century the words gelatin, albumen, fibrin, casein, gluten, gliadin, zein and legumin had been invented. The origin of the word "protein" is attributed to Berzelius in about 1838 [2] and publicised by Mulder [3,4] who was working on the concept of a "proto-radical" which was thought to be the building block from which the proteins were constructed. It was to be nearly 100 years before any further great strides in the understanding of protein structure were made. Nevertheless, proteins were not only being isolated from a wide variety of plants and animal tissues, but were already being obtained in crystalline form as early as 1859 [5]. 100 years ago egg albumen was crystallized by Hofmeister [6], these days better known for his series, but not until 1926 were crystals of a bioactive protein, the enzyme urease, obtained [7]. For the most part these early proteins were isolated from plant sources in which they existed at a high concentration, so that a relatively simple process such as acidification, or simply letting stand, might suffice to cause crystallization from the water-extract of the raw material. Genuine fractionation