Calcium-Binding Protein Protocols

Volume 1: Reviews and Case Studies

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Humana Press Totowa, New Jersey
Dedication

This book is dedicated to the memory of Dr. J. David Johnson (Columbus, OH) whose untimely death on January 21, 2000 has deeply shocked all his colleagues and friends. David has made numerous excellent contributions to our understanding of calcium-binding proteins. His insight and enthusiasm will be sadly missed.

Hans J. Vogel, PhD
Calcium plays an important role in a wide variety of biological processes. This divalent metal ion can bind to a large number of proteins; by doing so it modifies their biological activity or their stability. Because of its distinct chemical properties calcium is uniquely suited to act as an on–off switch or as a light dimmer of biological activities. The two books entitled Calcium-Binding Protein Protocols (Volumes I and II) focus on modern experimental analyses and methodologies for the study of calcium-binding proteins. Both extracellular and intracellular calcium-binding proteins are discussed in detail. However, proteins involved in calcium handling (e.g., calcium pumps and calcium channels), fall outside of the scope of these two volumes. Also, calcium-binding proteins involved in bone deposition will not be discussed, as this specific topic has been addressed previously. The focus of these two books is on studies of the calcium-binding proteins and their behavior in vitro and in vivo. The primary emphasis is on protein chemistry and biophysical methods. Many of the methods described will also be applicable to proteins that do not bind calcium.

Calcium-Binding Protein Protocols is divided into three main sections. The section entitled Introduction and Reviews provides information on the role of calcium in intracellular secondary messenger activation mechanisms. Moreover, unique aspects of calcium chemistry and the utilization of calcium in dairy proteins, as well as calcium-binding proteins involved in blood clotting, are addressed. The second section entitled Calcium-Binding Proteins: Case Studies provides a wealth of information about protein purification and characterization strategies, X-ray crystallography and other studies that are focused on specific calcium-binding proteins. Together, these two sections comprise Volume I of this series. By introducing the various classes of intra- and extracellular calcium-binding proteins and their modes of action, these two sections set the stage and provide the necessary background for the third section. The final section entitled Methods and Techniques to Study Calcium-Binding Proteins makes up Volume II of Calcium-Binding Protein Protocols. Here the focus is on the use of a range of modern experimental techniques that can be employed to study the solution structure, stability, dynamics, calcium-binding properties, and biological activity of calcium-binding proteins in general. As well, studies of their ligand-binding properties and their distribution in cells are included. In addition to enzymatic assays and more routine spectroscopic and protein chemistry techniques, particular attention has been paid in the second volume to modern NMR approaches, thermodynamic analyses,
kinetic measurements such as surface plasmon resonance, strategies for amino acid sequence alignments, as well as fluorescence methods to study the distribution of calcium and calcium-binding proteins in cells. In preparing their chapters, all the authors have attempted to share the little secrets that are required to successfully apply these methods to related proteins. Together the two volumes of Calcium-Binding Protein Protocols provide the reader with a host of experimental methods that can be applied either to uncover new aspects of earlier characterized calcium-binding proteins or to study newly discovered proteins.

As more and more calcium-binding proteins are being uncovered through genome sequencing efforts and protein interaction studies (e.g., affinity chromatography, crosslinking or yeast two-hybrid systems) the time seemed right to collect all the methods used to characterize these proteins in a book. The methods detailed here should provide the reader with the essential tools for their analysis in terms of structure, dynamics, and function. The hope is that these two volumes will contribute to our understanding of the part of the proteome, which relies on interactions with calcium to carry out its functions.

In closing, I would like to thank Margaret Tew for her invaluable assistance with the editing and organization of these two books. Finally, I would like to thank the authors of the individual chapters, who are all experts in this field, for their cooperation in producing these two volumes in a timely fashion.

Hans J. Vogel, PhD
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