Protein Purification And Characterization

In humans, the kinesin-related protein Kif2b, a potent regulator of microtubule dynamics, plays an important role in high fidelity chromosome segregation during mitotic progression. As with some other members of the kinesin 13 subfamily, Kif2b is unusual in that its activity is regulated by phosphorylation, and phosphorylation at threonine 125 and serine 204 can regulate the activity and function of Kif2b in microtubule dynamics. Due to the lack of detailed structural information, the molecular basis for regulation by phosphorylation, as well as the mechanism of microtubule depolymerization catalyzed by Kif2b, is unknown. In order to investigate these activities, the primary goal of this project was to determine the high-resolution crystal structure of the Kif2b catalytic domain, as well as the phosphomimetic mutants Kif2b[superscript T125D], Kif2b[superscript S204D] and Kif2b[superscript T125D, S204D]. As bacterial expression and protein purification protocols for of Kif2b have not been described previously, we successfully developed a purification of the Kif2b motor domain involving a three-step protocol including ion-exchange chromatography, affinity chromatography, and size-exclusion chromatography. Characterization using circular dichroism (CD) spectroscopy and differential scanning calorimetry (DSC) illustrated a well-folded Kif2b motor domain with denaturation temperatures between 31 and 34 °C and a robust ATPase activity was confirmed using EnzChek phosphate assay. Although preliminary

crystals were obtained for the wild-type construct, diffraction was not yet observed. Finally, to identity the structural consequences of phosphorylation, the phosphomimetic mutants Kif2b[superscript T125D], Kif2b[superscript S204D] and Kif2b[superscript T125D, S204D] were generated and purified following a similar procedure to the wild-type motor domain.

This book describes the principles and reactions for most techniques of protein extraction, purification, and characterization. The second edition covers modern techniques that are extensively used in protein identification and analyses including NMR, mass spectrometry, recombinant protein, and database search. It incorporates NMR and mass spectrometry for protein identification, use of computers and websites for protein annotation, and identification of commonly modified proteins. The text also expands coverage of the recombinant proteins. Two new chapters address the identification and annotation of the target protein and the identification of other posttranslational modifications.

Protein purification is vital for the characterization of the function, structure, and interactions of a protein.(Berg et al. 2002) Before a specific protein can be identified and characterized, the protein is extracted from a complex mixture and purified. Once the protein is pure, we can determine amino acid sequences, characterize the proteins based on its size, charge, shape, and function, investigate the protein's biological functions, and learn about relationships between proteins and diverse organisms. In

order to purify a protein, there are a series of processes that isolate one or a few proteins from a complex mixture. The protein may only be a small fraction of the starting material. (Berg et al. 2002) A few different factors go into consideration when choosing a purification method such as the initial state of the protein mixture and the required sample size. Some of the different purification methods include: chromatography, centrifugation, sonication, filtration, and gel electrophoresis. In this thesis, two different proteins are purified and analyzed using different spectroscopic techniques. The first protein purification described in this thesis is bZIP23. bZIP23 is a known transcription factor for zinc homeostasis, however, the putative metal binding domain has not been characterized. A typical zinc-finger domain either follows a 3cysteine-1histidine or a 2cysteine-2histidine cluster pattern but bZIP23 does not follow this pattern, which makes it an unusual candidate for zincbinding. To characterize the putative metal binding domain (MBD) of bZIP23, it is purified from a bacterial vector and compared to a truncated version of bZIP23 without the putative MBD. Once both versions are expressed and purified from a bacterial plasmid using affinity capture, they are then characterized to learn about molecular weight, which metals are bound, if the proteins are monomers or dimers, as well as the overall structure of the gene. Methods described in this thesis include: polymerase chain reaction, transformation, protein purification, bicinchoninic assay, sodium dodecyl sulfate polyacrylamide gel electrophoresis, and Western blot. Once the protein has been purified methods such as

fast performance liquid chromatography and inductively coupled plasma mass spectrometry were used to characterize and compare the full-length and truncated versions of bZIP23. The second part of this thesis describes purifying a protein to monitor FRET changes in response to a metabolite, glutathione. Three enzymes known to bind to glutathione were purified and each one was inserted into three separate FRET-cassettes. Florescence was measured before and after the addition of glutathione in hopes that the fluorescence would increase or decrease due to a protein confirmation change once glutathione was added. The purification methods are very similar for both proteins (bZIP23 and FRET sensor). The main difference is that bZIP23 is tagged with a FLAG-tag and the FRET sensor is tagged with a His-tag.

In this new edition of the very successful Protein Purification Protocols (1996), Paul Cutler completely updates the existing protocols to reflect recent advances and adds an enormous new array of proteomic techniques for protein isolation and analysis. These cutting-edge techniques include not only two-dimensional gel electrophoresis for analysis and characterization, but also analytical chromatography for multidimensional separations of proteins and peptides, and mass spectrometry for isolating proteins. With the many recent advances in technology, simple spectrometric detection is no longer the only option for separating proteins, and the authors treat in full detail all the newer methods for these separations. Comprehensive and highly practical, Protein Purification Protocols, Second Edition, brings together all the key methodologies that

both novice and experienced investigators need to carry out successful experimental work on proteins and their functions today.

A Practical Guide to Membrane Protein Purification is written especially for researchers who have some familarity with separation of water-soluble proteins, but who may not be aware of the pitfalls they face with membrane proteins. This guide presents techniques in a concise form, emphasizing the aspects unique to membrane proteins. The book explains the principles of the methods, permitting researchers and students new to this area to adapt these techniques to their particular needs. The second volume in the series, this book is an essential manual for investigations of structure and function of native membrane proteins, as well as for purification of these proteins for immunization and protein sequencing. Separation, Detection, and Characterization of Biological Macromolecules is a new series of laboratory guides. Each volume focuses on a topic of central interest to scientists and students in biomedical and biological research. Introductory chapters are followed by clear, step-by-step protocols that present principles and practice. These concise manuals are designed for optimal understanding of methods as well as for practical benchtop use. Provides general guidelines and strategies for isolation of membrane proteins Describes detailed practical procedures that have been the widest applications, and lowest specialized equipment needs Gives special emphasis to new native and denaturing electrophoresis techniques Explains modifications of techniques used for water-soluble proteins

Knowledge of the three-dimensional structure of a protein is absolutely required for the complete understanding of its function. The spatial orientation of amino acids in the active site of an enzyme demonstrates how substrate specificity is defined, and assists the medicinal chemist in the design of s- cific, tight-binding inhibitors. The shape and contour of a protein surface hints at its interaction with other proteins and with its environment. Structural ana- sis of multiprotein complexes helps to define the role and interaction of each individual component, and can predict the consequences of protein mutation or conditions that promote dissociation and rearrangement of the complex. Determining the three-dimensional structure of a protein requires milligram quantities of pure material. Such quantities are required to refine crystallization conditions for X-ray analysis, or to overcome the sensitivity limitations of NMR spectroscopy. Historically, structural determination of proteins was limited to those expressed naturally in large amounts, or derived from a tissue or cell source inexpensive enough to warrant the use of large quantities of cells. H- ever, with the advent of the techniques of modern gene expression, many p- teins that are constitutively expressed in minute amounts can become accessible to large-scale purification and structural analysis.

Representing one third of the proteins encoded by an organism's genome and 60 % of all human drug targets, membrane proteins are ubiquitous and hold the key to structure-based design of therapeutics. Despite the great interest surrounding membrane proteins, they are grossly underrepresented in the Protein Data Bank

due in large part to the inherent difficulties of working with macromolecules with extensive hydrophobic surfaces. Detergents are typically used to isolate and characterize membrane proteins, but maintenance of the native protein structure in detergent solutions presents a major challenge. Another bottleneck to structural determination of membrane proteins is the production of high-quality, three-dimensional crystals for x-ray diffraction, which remains a difficult and largely empirical task due to the complexity of the crystallizing solutions and the vastness of the multi-dimensional parameter space. Crystallization of membrane proteins is favored near the phase boundaries of surfactant solutions, and we find here that more specific characteristics of the surfactant and polymer phase behavior and resulting surfactant microstructure may play a major role in facilitating the interactions required for crystallization. The objective of this work is to understand the stabilizing role of detergents, while identifying specific detergent properties that are predictive of the effectiveness towards stabilization and crystallization of membrane proteins. The stability of E. coli diacylglycerol kinase was studied in detergent mixtures in order to gain an understanding of the cause of protein instability in detergent solutions. The adapted stability assay revealed that changes in the mixed micelle composition correlate directly with the thermostability of the protein. Excess concentrations of small amphiphiles relative

to the solubilizing detergent can significantly impact micelle composition and structure and adversely affect protein stability. Cryogenic-transmission electron microscopy images of protein-free solutions near several reported crystallization conditions reveal microdispersions consisting of a dense, surfactant-rich phase interspersed within the bulk solvent phase. The microstructure of the surfactant-rich phase varies from elongated micelles arranged in a hexagonal lattice to a randomly branched micellar network. The existence of such microstructures and the intermicellar ordering, which are both reminiscent of the mesophases that are used in the crystallization of membrane proteins in meso, suggest that a similar mechanism may be responsible for 3D crystallization in detergents.

Guide to Protein Purification, designed to serve the needs of the student, experienced researcher and newcomer to the field, is a comprehensive manual that provides all the up-to-date procedures necessary for purifying, characterizing, and handling proteins and enzymes in one source. Key Features * Detailed procedures newly written for this volume * Extensive practical information * Rationale and strategies for protein and enzyme purification * Personal perspectives on enzyme purification by eminent researchers Among the Topics Covered * General methods for handling proteins and enzymes * Extraction, subcellular fractionation, and solubilization procedures *

Comprehensive purification techniques * Specialized purification procedures * Protein characterization * Immunological procedures * Computer analysis of protein structure

Principles and Reactions of Protein Extraction, Purification, and Characterization provides the mechanisms and experimental procedures for classic to cutting-edge techniques used in protein extraction, purification, and characterization. The author presents the principles and reactions behind each procedure and uses tables to compare the different

Cold Spring Harbor Laboratory. Softcover manual of fundamental procedures commonly used in protein biochemistry, for reseachers. Plastic comb spiral binding.

Principles and Reactions of Protein Extraction, Purification, and Characterization provides the mechanisms and experimental procedures for classic to cutting-edge techniques used in protein extraction, purification, and characterization. The author presents the principles and reactions behind each procedure and uses tables to compare the different methods. The book also discusses the development of antibodies and immunochemical techniques as tools for characterizing proteins and modified proteins such as glycoproteins. Helpful illustrations, diagrams, and tables effectively transform theoretical concepts into practical knowledge. Along with methodical working procedures for most techniques, the book also offers useful advice on which technique

to use and when to apply a particular method. Presenting the advantages and disadvantages of the various protein techniques, Principles and Reactions of Protein Extraction, Purification, and Characterization enables students and researchers to master the mechanisms behind the protocols and choose the best method for their purposes.

Protein Biotechnology and Biochemistry is a complete and definitive source of information for all those interested in the area, providing a broad overview of the various medical, diagnostic and industrial uses of proteins. It covers basic biochemical principles as well as providing a comprehensive survey of products currently available or under development. * The new edition has been thoroughly updated with new material. * The key difference is that this new edition will include more "pure" biochemistry. * There are two completely new chapters: Protein Structure - an overview and Novel Proteins from Novel Sources. Chapter 2, Protein Structure, an overview and chapter 3, Protein Purification & Characterisation, make up approximately 30% of the book. These chapters concentrate on the basic biochemical principles of proteins and will lay the foundations for the rest of the book. The remaining chapters focus on protein biotechnology and have been rearranged, updated and expanded.