

Methods in Molecular Biology™

Volume 76

# GLYCOANALYSIS PROTOCOLS

*Second Edition*

*Edited by*  
**Elizabeth F. Hounsell**



Humana Press

# Glycoanalysis Protocols 2nd Edition

**Anthony P. Corfield**



## **Glycoanalysis Protocols 2nd Edition:**

**Glycoanalysis Protocols** Elizabeth F. Hounsell, 2008-02-02 Glycoanalysis Protocols Second Edition makes available to all protein scientists and particularly those working in today's pharmaceuticals industry the most advanced and reproducible glycoanalysis techniques. These detailed up to date and proven analytical methods cover the areas of glycoprotein macromolecular structural analysis, oligosaccharide profiling, lipid conjugate characterization, microorganism structure determination and proteoglycan function. They also include advanced analytical techniques in biotechnology during the production of recombinant glycoproteins and other therapeutics. These protocols will well serve anyone starting work on the analysis of glycoproteins as well as experienced investigators seeking to carry their expertise to higher levels of accomplishment.

**2-D Proteome Analysis Protocols** Andrew J. Link, 2008-02-02 With the completion of sequencing projects and the advancement of analytical tools for protein identification, proteomics, the study of the expressed part of the genome, has become a major region of the burgeoning field of functional genomics. High resolution 2-D gels can reveal virtually all proteins present in a cell or tissue at any given time, including posttranslationally modified proteins. Changes in the expression and structure of most cellular proteins caused by differentiation or external stimuli can be displayed and eventually identified using 2-D protein gels. 2-D Proteome Analysis Protocols covers all aspects of the use of 2-D protein electrophoresis for the analysis of biological problems. The contributors include many of the leaders in the fields of biochemistry and analytical chemistry who were instrumental in the development of high resolution 2-D gels, immobilized pH gradients, computer analysis and mass spectrometry based protein identification methodologies. This book is intended as a benchtop manual and guide both for novices to 2-D gels and for those aficionados who wish to try the newer techniques. Any group using protein biochemistry, especially in the fields of molecular biology, biochemistry, microbiology and cell biology should find this book eminently useful. 2-D Proteome Analysis Protocols takes the researcher through the complete process of working with 2-D protein gels from making the protein tract to finally identifying the proteins of interest. It includes protocols for generating 2-D protein extracts from most of the standard model organisms including bacteria, yeast, nematode, *Drosophila*, plants, mouse and human.

**Glycoprotein Methods and Protocols** Anthony P. Corfield, 2007-10-26 The mucins, mucus glycoproteins, have long been a complex corner of glycoprotein biology. While dramatic advances in the separation, structural analysis, biosynthesis and degradation have marked the progress in general glycoprotein understanding, the mucins have lagged behind. The reasons for this lack of progress have always been clear and are only now being resolved. The mucins are very large molecules; they are difficult to separate from other molecules present in mucosal secretions or membranes; they are often degraded owing to natural protective functions or to isolation methodology; and their peptide and oligosaccharide structures are varied and complex. Understanding these molecules has demanded progress in several major areas. Isolation techniques that protect the intact mucins and allow dissociation from other adsorbed but discrete molecules needed to be

developed and accepted by all researchers in the field Improved methods for the study of very large molecules with regard to their aggregation and polymerization were also needed Structural analysis of the peptide domains and the multitude of oligosaccharide chains was required for smaller sample sizes for multiple samples and in shorter time In view of these problems it is perhaps not surprising that the mucins have remained a dilemma of obvious biological importance and interest but very difficult to analyze

**Lipase and Phospholipase Protocols** Mark Doolittle, 2008-02-02 The lipases and phospholipases represent a diverse group of enzymes that are expressed in animals plants fungi and bacteria Their ubiquitous distribution among all species is a testament to the essential roles played by these enzymes in lipid storage mobilization and metabolism membrane homeostasis and remodeling endocrine and immune functions and signal transduction In humans lipases and phospholipases are also thought to contribute to complex diseases such as atherosclerosis obesity arthritis and cancer as well as to single gene defects such as Wolman's disease and Type I hyperlipoproteinemia Enzymatically the lipases are unique since they hydrolyze substrates that are either insoluble or only partly soluble in aqueous solvents thus enzyme catalysis takes place at a lipid water interface The interface comprises at least two and often more discrete bulk and surface phases in which the enzyme substrate and products of lipolysis disperse among these phases based on their physical properties Furthermore the distribution of these components changes continuously as lipolysis proceeds Thus the lipases and phospholipases are fundamentally different from any other enzyme because of the physical complexity of the environment in which catalysis occurs

**Integrin Protocols** Anthony R. Howlett, 2008-02-03

**Electron Microscopy Methods and Protocols** M. A. Nasser Hajibagheri, 2008-02-02 Electron Microscopy Methods and Protocols is designed for the established researcher as a manual for extending knowledge of the field It is also for the newcomer who wishes to move into the field A wide range of applications for the examination of cells tissues biological macromolecules molecular structures and their interactions are discussed We have tried to gather together methods that we consider to be those most generally applicable to current research in both cell and molecular biology Each chapter contains a set of related practical protocols with examples provided by experts who have first hand knowledge of the techniques they describe The individual chapters are grouped according to similarities in their specimen preparation and methodology Methods are presented in detail in a step by step fashion using reproducible protocols the authors have personally checked During the last decade the scientific literature describing the use of colloidal gold as an immunocytochemical marker has increased at an exponential rate and this trend is expected to continue We have included a large number of variations on the immunogold labeling technique In both the negative staining and cryo chapters authors emphasize the immunological applications in order to correlate as fully as possible with the emphasis on immunogold labeling in the other chapters Electron Microscopy Methods and Protocols commences with the routine preparation of biological material for classical transmission electron microscopy involving tissue fixation embedding and sectioning Chap 1

**Cytochrome P450 Protocols** Ian R. Phillips, Elizabeth A.

Shephard, 2008-02-02 In *Cytochrome P450 Protocols* Ian Phillips and Elizabeth Shephard assemble a comprehensive collection of cutting edge techniques for the investigation of cytochromes P450. Described in detail by hands on experimentalists for easy reproducibility these methods include spectral analysis purification and enzymatic assays expression in heterologous systems and the production and use of antibodies as well as methods for quantification of gene expression transfection of hepatocytes and for the investigation of DNA protein interactions and genetic polymorphisms. In addition because of the growing importance of in vitro systems in pharmacological toxicology the book contains techniques for the culture of rodent and human hepatocytes and human epidermis. Cytochrome P450 induction as a biomarker for environmental pollution and the generation of mice with targeted gene disruptions complete this exhaustive collection of core techniques. *Cytochrome P450 Protocols* includes in one volume both state of the art and classic methods that have not been superseded but remain extremely useful. The collection provides both novice and experienced researchers across many fields toxicology pharmacology environmental biology biochemistry and molecular biology all the tools needed to elucidate the crucial biological role played by cytochromes P450 in the metabolism of therapeutic drugs chemical carcinogens and environmental pollutants.

**Protein Lipidation Protocols** Michael H. Gelb, 2008-02-03 In *Protein Lipidation Protocols* Michael Gelb brings together a collection of readily reproducible techniques for studying protein lipidation the covalent attachment of lipids to proteins. These cutting edge methods many never published before in a hands on format deal with glycosyl phosphatidylinositol GPI containing compounds protein fatty acylation and protein prenylation. Included are novel techniques for determining the chemical structure of GPI anchors for radiolabeling the prenyl groups of protein in eukaryotic cells a tool for developing inhibitors of the protein farnesyltransferase and for an exciting lysosomal enzyme that cleaves fatty acyl groups from proteins the first fatty acylase discovered. *Protein Lipidation Protocols* offers biochemists cell and molecular biologists medicinal chemists and pharmaceutical researchers state of the art tools for understanding the complex biochemistry of protein lipidation as well as catalyzing the development of many important new biopharmaceuticals including anticancer drugs.

**Protein Kinase Protocols** Alastair D. Reith, 2008-02-02 As key components of many cell signaling pathways protein kinases are implicated in a broad variety of diseases including cancers and neurodegenerative conditions and offer considerable potential as tractable targets for therapeutic intervention. In *Protein Kinase Protocols* a panel of highly skilled laboratory investigators describe both basic and more sophisticated methods for the analysis of kinase mediated signaling cascades with emphasis on the identification of proteins according to their interactive relationships and the analysis of their functional properties. Described in step by step detail these readily reproducible techniques offer novices quick access to a complicated field and provide more experienced investigators many novel time saving ploys. Emphasis is given to the critical technical steps that are often omitted from methods published in the primary literature. There are also tips on potential pitfalls and copious notes on how to adjust the protocols to work in related systems. Broad in its range of

techniques and thoroughly detailed to help ensure experimental success Protein Kinase Protocols offers both novice and experienced investigators powerful tools for understanding the functional roles of specific protein kinases within signaling cascades and for identification and evaluation of novel therapeutic targets

**T Cell Protocols** Kelly P. Kearse, 2008-02-02 The purpose of T Cell Protocols Development and Activation is to collect a series of protocols particularly those that have been developed within the past few years to help investigators master new techniques or improve existing ones for the study of T cell Biology Invariably in putting together a book like this it is difficult to decide which methods to include and which to leave out To this end methods were selected from a variety of disciplines including cellular immunology biochemistry and molecular biology to try to provide something of interest for everyone who works on T cell development and activation I would like to mention that my primary reason for agreeing to put this book together is that when I was a graduate student I purchased a copy of Selected Methods in Cellular Immunology by Mishell and Shigii which proved a tremendous help in learning the basics of one and two dimensional gel techniques and other methods The cover has long since fallen off but it still remains one of my most valued reference books for the laboratory It is my hope that T Cell Protocols Development and Activation will prove similarly useful to current and future scientists wishing to learn new methods for exploring the development and activation of T cells

*Plant Hormone Protocols* Gregory A. Tucker, Jeremy A. Roberts, 2008-02-04 Established investigators from around the world describe in step by step detail their best techniques for the study of plant hormones and their regulatory activities These state of the art methods include contemporary approaches to identifying the biosynthetic pathways of plant hormones monitoring their levels characterizing the receptors with which they interact and analyzing the signaling systems by which they exert their effects Comprehensive and fully detailed for reproducible laboratory success Plant Hormone Protocols offers plant biologists an indispensable compendium of today's most powerful methods and strategies to studying plant hormones their regulation and their activities

*Adhesion Protein Protocols* Elisabetta Dejana, Monica Corada, 2008-02-03 Adhesion molecules are of fundamental importance in the regulation of immunity inflammation tissue remodeling and embryonic development They comprise different families of homologous proteins such as selectins integrins cadherins and immunoglobins In addition beyond these groups other structures with adhesive properties such as proteoglycans occludin and CD44 have been characterized recently An understanding of the type and characteristics of adhesive molecules expressed by the different cell types and the possibility of manipulating their activity promises considerable clinical potential Antibodies small peptidic and nonpeptidic molecules have recently been used to inhibit thrombosis by blocking platelet aggregation or inflammation through inhibition of leukocyte infiltration and adhesion Inhibitors of adhesive molecules are used in experimental systems for the study of tumor growth and dissemination Among major goals in the field are the identification of new members of the known adhesive protein families and of independent new adhesive structures After structural characterization even more demanding is the study of the biological activity of the new

proteins and the development of simple rapid tests for the screening of possible inhibitors In this regard the production of such reagents as fragments and antibodies would help define the structure function relationship of individual proteins Data available in the literature show the complexity of the adhesive process and how different molecular epitopes might contribute to the adhesive properties of a single structure Finally a new area of investigation is the characterization of the intracellular signaling cascade triggered by the engagement of transmembrane adhesive proteins

Natural Killer Cell Protocols Kerry S. Campbell, Marco Colonna, 2008-02-03 In Natural Killer Cell Protocols Cellular and Molecular Methods Kerry S Campbell and Marco Colonna have assembled a comprehensive collection of readily reproducible methods designed to study natural killer NK cells from the broadest variety of viewpoints These include not only classic techniques but also new approaches to standard methods newly evolved techniques that have become valuable for specific applications and unique models for manipulating and studying NK cells Among the advanced methods covered are those for in vitro transendothelial migration in vivo detection of cells migrating into tumors immunofluorescence staining of intracellular cytokines and in vitro NK cell development Valuable techniques for specific applications include vaccinia virus protein expression soluble KIR Fc fusions for HLA class I binding assays calcium mobilization in cell conjugates and identification of heterodimeric receptor complexes using cDNA library expression cloning No less important are accounts of such classic methods as hybrid resistance ADCC viral defense target cell cytotoxicity assays cloning and culturing tumor immunotherapy and generation of HLA class I transfected target cells Natural Killer Cell Protocols Cellular and Molecular Methods offers immunologists cancer researchers virologists and cell biologists today's most comprehensive collection of both established and cutting edge techniques methods that will contribute significantly to advancing our understanding of this fascinating and critically important class of cells

Protein Structure Prediction David Webster, 2008-02-03 The number of protein sequences grows each year yet the number of structures deposited in the Protein Data Bank remains relatively small The importance of protein structure prediction cannot be overemphasized and this volume is a timely addition to the literature in this field Protein Structure Prediction Methods and Protocols is a departure from the normal Methods in Molecular Biology series format By its very nature protein structure prediction demands that there be a greater mix of theoretical and practical aspects than is normally seen in this series This book is aimed at both the novice and the experienced researcher who wish for detailed information in the field of protein structure prediction a major intention here is to include important information that is needed in the day to day work of a research scientist important information that is not always decipherable in scientific literature Protein Structure Prediction Methods and Protocols covers the topic of protein structure prediction in an eclectic fashion detailing aspects of prediction that range from sequence analysis a starting point for many algorithms to secondary and tertiary methods on into the prediction of docked complexes an essential point in order to fully understand biological function As this volume progresses the authors contribute their expert knowledge of protein structure prediction to many disciplines

such as the identification of motifs and domains the comparative modeling of proteins and ab initio approaches to protein loop side chain and protein prediction **NMDA Receptor Protocols** Min Li,2008-02-03 Min Li and a panel of hands on experimentalists detail state of the art molecular techniques for studying NMDA ligand gated ion channels and developing assays for nontherapeutic lead selection The topics range from cDNA cloning to in vitro and in vivo investigation of the channel complex in the mammalian brain Additional topics include the biochemical analysis of the channel protein and the construction of various heterologous systems for both basic research and high throughput screens HTS for pharmaceutical chemicals Although the focus is on NMDA receptors the methods are applicable to other ligand gated ion channels and with some modification may be extended to related membrane signaling receptors NMDA Receptor Protocols offers today s scientists powerful methods for basic research on NMDA receptor structure and function as well as enormous opportunities for clinical investigation toward the development of novel bioactive compounds **Molecular Methods in Developmental**

**Biology** Matt Guille,2008-02-03 The process whereby a single cell the fertilized egg develops into an adult has fascinated for centuries Great progress in understanding that process has been made in the last two decades when the techniques of molecular biology have become available to developmental biologists By applying these techniques the exact nature of many of the interactions responsible for forming the body pattern are now being revealed in detail Such studies are a large and it seems ever expanding part of most life science groups It is at newcomers to this field that this book is primarily aimed A number of different plants and animals serve as common model organisms for developmental studies In Molecular Methods in Developmental Biology Xenopus and Zebrafish a range of the molecular methods applicable to two of these organisms are described these are the South African clawed frog Xenopus laevis and the zebrafish Brachydanio rerio The embryos of both of these species develop rapidly and externally making them particularly suited to investigations of early vertebrate development However both Xenopus and zebrafish have their own advantages and disadvantages Xenopus have large robust embryos that can be manipulated surgically with ease but their pseudotetraploidy and long generation time make them unsuitable candidates for genetics This disadvantage may soon be overcome by using the diploid Xenopus tropicalis and early experiments are already underway The transparent embryos of zebrafish render them well suited for in situ hybridization and immunohistochemistry and good for observing mutations in genetic screens **Confocal Microscopy** Stephen W.

Paddock,2008-02-03 **RNA-Protein Interaction Protocols** Susan R. Haynes,2008-02-03 The molecular characterization of RNA and its interactions with proteins is an important and exciting area of current research Organisms utilize a variety of RNA protein interactions to regulate the expression of their genes This is particularly true for eukaryotes since newly synthesized messenger RNA must be extensively modified and transported to the cytoplasm before it can be used for protein synthesis The realization that posttranscriptional processes are critical components of gene regulation has sparked an explosion of interest in both stable ribonucleoprotein RNP complexes and transient RNA protein interactions RNA is



conformationally flexible and can adopt complex structures that provide diverse surfaces for interactions with proteins The fact that short RNA molecules aptamers see Chapter 16 can be selected to bind many different types of molecules is evidence of the structural variability of RNA RNA molecules are rarely entirely single or double stranded but usually contain multiple short duplexes interrupted by single stranded loops and bulges in some RNAs such as tRNAs the short duplexes stack on each other Further variability is generated by the presence of non Watson Crick base pairs modified nucleotides and more complex structures such as pseudoknots and triple strand interactions      **Eicosanoid Protocols** Elias A Lianos,2008-02-03

Polyamine Protocols David M. L. Morgan,2008-02-02 A unique collection of hands on enzyme assay techniques to study polyamines and their function The techniques range from assay methods for enzymes of polyamine biosynthesis and catabolism to measurements of polyamines polyamine transport and polyamine effects on cell growth The methods are presented by leading researchers who have perfected them to a high art and include clear step by step instructions with numerous hints and tips to ensure readily reproducible results

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