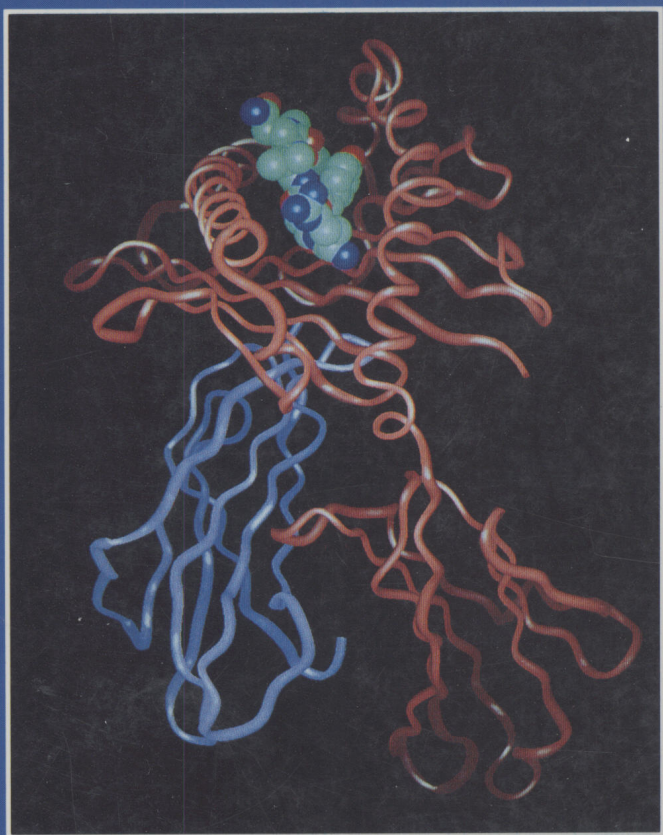


# TECHNIQUES IN PROTEIN CHEMISTRY IV



*Published under the Auspices of  
The Protein Society*

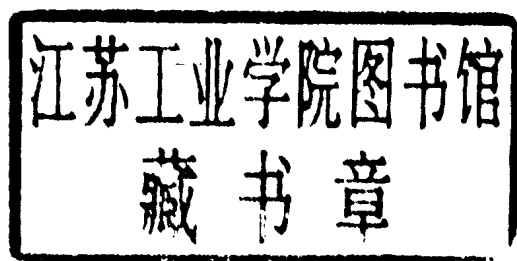
Edited by Ruth Hogue Angeletti

# TECHNIQUES IN PROTEIN CHEMISTRY IV

Edited by

Ruth Hogue Angeletti

Albert Einstein College of Medicine  
Bronx, New York



ACADEMIC PRESS, INC.  
*Harcourt Brace & Company*  
San Diego New York Boston  
London Sydney Tokyo Toronto

## Academic Press Rapid Manuscript Reproduction

This book is printed on acid-free paper. (∞)

Copyright © 1993 by ACADEMIC PRESS, INC.

All Rights Reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording, or any information storage and retrieval system, without permission in writing from the publisher.

**Academic Press, Inc.**

1250 Sixth Avenue, San Diego, California 92101-4311

*United Kingdom Edition published by*

**Academic Press Limited**

24-28 Oval Road, London NW1 7DX

International Standard Book Number: 0-12-058757-2 (Hardcover)

International Standard Book Number: 0-12-058758-0 (Papercover)

PRINTED IN THE UNITED STATES OF AMERICA

93 94 95 96 97 98 EB 9 8 7 6 5 4 3 2 1

## Foreword

For most ventures it would be premature to assert that a fourth volume of the series "carries on a distinguished tradition," but a case can certainly be made for such a description of this volume. This is the fifth book based on abstracts selected from those presented at a Protein Society Symposium (the fourth to carry the title "Techniques in Protein Chemistry"). They were selected from an all-time record of 615 abstracts submitted to this Sixth Protein Society Symposium. The task of the associate editors was a difficult one indeed.

We would have been overwhelmed by the flood of abstracts had it not been for our friends in the Association of Biomolecular Resource Facilities (ABRF) who meet with the Protein Society Symposia. They volunteered to organize the poster sessions and did a superb job. All agreed that the posters were of very high quality. I am pleased that they have once again volunteered their services for next year.

The Seventh Symposium will be held July 24–28, 1993, in the Town and Country in San Diego. This site will not only increase poster space, but also the time in which they can be examined, so we look forward to an even bigger and more interactive meeting.

My most sincere thanks on behalf of the Protein Society to Ruth Hogue Angeletti and her associate editors for their dedicated work on this volume. Their efforts and the top quality contributions by the authors of the articles do indeed "carry on a distinguished tradition."

*Mark Hermodson*  
President  
The Protein Society

## Preface

The president of the Protein Society has declared that this series of volumes is now a “distinguished tradition.” However, this tradition has a dynamic quality, for the “Techniques in Protein Chemistry” series must embody change. In the first volume, advances in the organic chemistry of proteins—sequencing, fragmentations, separations—formed the core of the book. In this volume, the rapid developments in mass spectrometry of proteins dominate. Breakthroughs in technology enable new insights into protein structure and function. It is our job as editors and associate editors to put aside our parochial interests and continue to ensure that areas of rapid development are well represented.

The associate editors are at the forefront of identifying important contributions. This year they were John Crabb, Sheenah Mische, Al Smith, John Stults, Wesley Stites, and Leonard Spicer. Once again, the staff of Academic Press has ensured that the book will be published rapidly and as error free as possible. The members of the society are responsible for the continued high quality of this volume.

*Ruth Hogue Angeletti*

# Acknowledgments

The Protein Society acknowledges with thanks the following organizations who through their support of the Society's program goals contributed in a meaningful way to the sixth annual symposium and thus to this volume.

AAA Laboratory  
Amersham  
Amgen, Inc.  
Applied Biosystems, Inc.  
Beckman Instruments, Inc.  
Biosym Technologies, Inc.  
Boehringer Mannheim Corporation  
*Digital Equipment Corporation*  
Dionex Corporation  
Du Pont Biotechnology Systems  
EM Science  
Finnigan MAT  
Genentech, Inc.  
Hewlett-Packard  
Jasco, Inc.  
Lilly Research Laboratories  
Merck Sharp & Dohme Research  
Laboratories  
Michrom BioResources, Inc.  
MilliGen, Inc.

Millipore Corporation  
Molecular Dynamics, Inc.  
Molecular Simulations, Inc.  
Monsanto Co.  
National Biomedical Research  
Foundation  
Oxford Molecular, Inc.  
PerSeptive Biosystems, Inc.  
Pharmacia LKB Biotechnology  
Pickering Laboratories, Inc.  
Promega Corporation  
Rainin Instrument Co., Inc.  
Savant Instruments, Inc.  
Scientific Analysis Laboratories Ltd.  
SCIEX  
TosoHaas, Inc.  
Tripos Associates, Inc.  
The Upjohn Co.  
Vestec Corporation  
VYDAC, The Separations Group, Inc.

# Contents

<i>Foreword</i>	xiii
<i>Preface</i>	xv
<i>Acknowledgments</i>	xvii

## Section I

### Mass Spectrometry of Proteins and Peptides

Mass Spectrometry in Protein Sequence and Structural Investigations	3
<i>A. L. Burlingame</i>	
Elucidation of Covalent Modifications and Noncovalent Associations in Proteins by Electrospray Ionization Mass Spectrometry	23
<i>Joseph A. Loo, Rachel R. Ogorzalek Loo, David R. Goodlett, Richard D. Smith, Alfred F. Fuciarelli, David L. Springer, Brian D. Thrall, and Charles G. Edmonds</i>	
Identification of Conserved Protein Surface Metal-Binding Sites in Related Proteins by Mass Spectrometry	33
<i>T. William Hutchens, Tai-Tung Yip, and Randall W. Nelson</i>	
Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry of Membrane Proteins: The Scrapie Prion Protein	41
<i>Michael A. Baldwin, Rong Wang, Keh-Ming Pan, Rolf Hecker, Neil Stahl, Brian T. Chait, and Stanley B. Prusiner</i>	
Characterization of the Proteins c-kit Ligand and DHFR by Electrospray Mass Spectrometry	47
<i>Hanno Langen, Bernadette Sander, Francis Vilbois, and Hans-Werner Lahm</i>	
Rapid Purification, Separation, and Identification of Proteins and Enzyme Digests Using Packed Capillary Perfusion Column LC and LC/MS	55
<i>D. B. Kassel, M. A. Luther, D. H. Willard, S. P. Fulton, and J. -P. Salzmänn</i>	

**Sample Immobilisation Protocols for Matrix Assisted Laser Desorption Mass Spectrometry 65**

*K. K. Mock, C. W. Sutton, A. Keane, and J. S. Cottrell*

**Assessing the Multimeric States of Proteins by Matrix-Assisted Laser Desorption Mass Spectrometry: Comparison with Other Biochemical Methods 73**

*Terry B. Farmer and Richard M. Caprioli*

**N-Terminal Modification of Malarial Antigens from *E. coli* 83**

*Jelle Lahnstein, Shanny L. Dyer, Neil H. Goss, Mark Duncan, and Raymond S. Norton*

**Mass Spectrometric Characterisation of C-terminal ‘‘Ragged Ends’’ in Peanut Agglutinin 91**

*Pierre Thibault, David C. Watson, Makoto Yaguchi, and N. Martin Young*

**Accurate Determination of Molecular Mass for Proteins Isolated from Polyacrylamide Gels 99**

*T. N. Asquith, T. W. Keough, R. Takigiku, M. P. Lacey, M. P. Purdon, and D. L. Gauggel*

## Section II

### Analysis of Glycoproteins

**Carbohydrate Characterisation of a Glycoprotein by Matrix Assisted Laser Desorption Mass Spectrometry 109**

*C. W. Sutton, A. C. Poole, and J. S. Cottrell*

**Post-translational Modifications of Recombinant Proteins Determined by LC/Electrospray Mass Spectrometry and High Performance Tandem Mass Spectrometry 117**

*Katalin F. Medzihradszky, Christine A. Settineri, David A. Maltby, and A. L. Burlingame*

**Identification and Characterization of Glycosylation Sites in Carcinoembryonic Antigen (CEA) by Mass Spectrometry 127**

*Kristine M. Swiderek, Constance S. Pearson, and John E. Shively*

**Monosaccharide and Oligosaccharide Analysis of Recombinant Erythropoietin Electro-transferred onto Polyvinylidene Fluoride Membranes 135**

*Michael Weitzhandler, Douglas Kadlecsek, Nebojsa Avdalovic, and R. Reid Townsend*



- A Practical Approach for Isolation and Characterization of Glycosylation Sites of Glycoproteins Bearing N- and/or O-Linked Carbohydrate Chains 143

*Kuo-Liang Hsi, Ling Chen, and Pau-Miau Yuan*

- Location of the Keratan Sulfate Attachment Sites in the Hyaluronate Binding Region of the Proteoglycan, Aggrecan 153

*Peter J. Neame and Frank P. Barry*

- Site Specific Heterogeneity of N-Linked Oligosaccharides on Recombinant Human Erythropoietin 161

*Patricia L. Derby, Thomas W. Strickland, and Michael F. Rohde*

### Section III Phosphorylated Proteins

- Analysis of Serine, Threonine, and Tyrosine Phosphorylation Sites with Mass Spectrometry 171

*John W. Crabb, Charles Johnson, Karen West, Janina Buczylo, Krzysztof Palczewski, Jinzhao Hou, Kerstin McKeehan, Mikio Kan, Wallace L. McKeehan, Michael J. Huddleston, and Steven A. Carr*

- The Identification of Phosphorylation Sites in Large Membrane Proteins Following Their Isolation by SDS-PAGE 179

*Karen S. De Jongh, Eric I. Rotman, and Brian J. Murphy*

- Modification of Thiophosphorylated Proteins with Extrinsic Probes 187

*Keven C. Facemyer, Mark R. Tibeau, and Christine R. Cremo*

- A Novel Method of Identifying Phosphorylation Sites Using a Thiophosphorylated Peptide and ESI-MS 193

*David R. Stover and Kenneth A. Walsh*

- Protein Phosphorylation: Sequence-Specific Identification of *In Vivo* Phosphorylation Sites by MALDI-TOF Mass Spectrometry 201

*Tai-Tung Yip and T. William Hutchens*

- Synthetic Phosphopeptide Epitope Mapping with Mass Spectrometric Verification 211

*William T. Moore, Wan-Kyng Liu, Shu-Hui Yen, Frederick L. Hall, and Richard M. Caprioli*

## Section IV

### Peptide Synthesis

Evaluation of Peptide Synthesis as Practiced in 53 Different Laboratories	229
---	-----

*Gregg B. Fields, Steven A. Carr, Daniel R. Marshak, Alan J. Smith, John T. Stults, Lynn C. Williams, Ken R. Williams, and Janis D. Young*

Synthesis, Purification, and Interactions of Casein Signal Peptides	239
---	-----

*C. Creuzenet, G. Korshunova, H. Naharisoa, H. Nedev, and T. Haertlé*

The Systematic Development of Peptides Having Potent Antimicrobial Activity Against <i>E. coli</i> through the Use of Synthetic Peptide Combinatorial Libraries	249
---	-----

*Richard A. Houghten, Kim Thy Dinh, David E. Burcin, and Sylvie E. Blondelle*

Synthesis of Proteins by Chemical Ligation of Unprotected Peptide Segments: Mirror-Image Enzyme Molecules, D- and L-HIV Protease Analogs	257
--	-----

*R. C. de Lisle Milton, Saskia C. F. Milton, Martina Schölzer, and Stephen B. H. Kent*

Difficult Sequences in the Synthesis of G-Protein Fragments That Harbor a Putative Adhesion Motif	269
---	-----

*Assaf Steinschneider*

## Section V

### Amino Acid Analysis

Cysteine and Tryptophan Amino Acid Analysis of ABRF92-AAA	279
---	-----

*Daniel J. Strydom, Thomas T. Andersen, Izydor Apostol, Jay W. Fox, Raymond J. Paxton, and John W. Crabb*

Compositional Protein Analysis Using 6-Aminoquinolyl-N-Hydroxysuccinimidyl Carbamate, a Novel Derivatization Reagent	289
--	-----

*Steven A. Cohen, Kathryn DeAntonis, and Dennis P. Michaud*

Sensitive Analysis of Cystine/Cysteine using 6-Aminoquinolyl-N-Hydroxysuccinimidyl Carbamate (AQC) Derivatives	299
--	-----

*Daniel J. Strydom and Steven A. Cohen*

- Amino Acid Analysis and Multiple Methylation of Lysine Residues in the Surface Protein Antigen of *Rickettsia prowazeki* 307

*W. -M. Ching, H. Wang, J. Davis, and G. A. Dasch*

- Chirality Determination of *D*- and *L*- Amino Acids of Synthetic and Naturally Occurring Peptides 315

*Brenda L. Purcell and Muriel S. Doleman*

## Section VI

### Protein Separations

- Development of Separation Strategies for Proteins by Capillary Electrophoresis 323

*Judith A. Nolan and Richard Palmieri*

- High Resolution Full-Range ( $pI = 2.5$  to  $10.0$ ) Isoelectric Focusing of Proteins and Peptides in Capillary Electrophoresis 333

*Shiaw-Min Chen and John E. Wiktorowicz*

- Purification of Different Biologically Active Forms of Mouse  $\beta$ -Nerve Growth Factor by Immobilized Metal Ion Affinity Chromatography 343

*Giuseppe Corona, Steven D. Skaper, and Lanfranco Callegaro*

- Increasing the Antigen Binding Capacity of Immobilized Antibodies 353

*Alvin S. Stern and Frank J. Podlaski*

## Section VII

### Peptide Mapping

- Micropreparative Capillary Electrophoresis (MPCE) and Micropreparative HPLC of Protein Digests 363

*James W. Kenny, J. I. Ohms, and Alan J. Smith*

- Peptide Mapping of 2-D Gel Proteins by Capillary HPLC 371

*Susan C. Wong, Christopher Grimley, Allan Padua, James H. Bourell, and William J. Henzel*

- Internal Peptide Sequence of Proteins Digested In-Gel after One- or Two-Dimensional Gel Electrophoresis 379

*Pascual Ferrara, Jorge Rosenfeld, Jean Claude Guillemot, and Joel Capdevielle*

- In Situ* Tryptic Digestion of Proteins Separated by SDS-PAGE: Improved Procedures for Extraction of Peptides Prior to Microsequencing 389

*Tim Tetaz, Esther Bozas, Jerry Kanellos, Ian Walker, and Ian Smith*

- In Situ* Proteolytic Digestions Performed on Proteins Bound to the Hewlett-Packard Hydrophobic Sequencing Column 399

*William Burkhart*

## Section VIII

### Protein Sequencing

- Routine Protein Sequence Analysis Below Ten Picomoles: One Sequencing Facility's Approach 409

*Donna Atherton, Joseph Fernandez, Michael DeMott, Lori Andrews, and Sheenah M. Mische*

- Successful Peptide Sequencing with Femtomole Level PTH-Analysis: A Commentary 419

*Hediye Erdjument-Bromage, Scott Geromanos, Amy Chodera, and Paul Tempst*

- On-Line Microbore HPLC Detection of Femtomole Quantities of PTH-Amino Acids 427

*Russell W. Blacher and John H. Wieser*

- Single Syringe-Pump Solid-Phase Protein Sequencer 435

*Mark Stolowitz and Leroy Hood*

- Analysis of Complex Protein Mixtures on the HP-G1000A Sequencer 443

*T. K. Slattery and R. N. Harkins*

- Protein Sequencing of Post-Translationally Modified Peptides and Proteins: Design, Characterization and Results of ABRF-92SEQ 453

*Sheenah M. Mische, K. Ümit Yüksel, Liane M. Mende-Mueller, Paul Matsudaira, Dan L. Crimmins, and Philip C. Andrews*

- Carboxy-terminal Protein Sequence Analysis Using Carboxypeptidase P and Electrospray Mass Spectrometry 463

*Christine E. Smith and Keven L. Duffin*

*Protein Ladder Sequencing: A Conceptually Novel Approach to Protein Sequencing Using Cycling Chemical Degradation and One-Step Readout by Matrix-Assisted Laser Desorption Mass Spectrometry* 471

*Rong Wang, Brian T. Chait, and Stephen B. H. Kent*

## Section IX

### Protein Conformation, Folding, and Modeling

*The Use of Molecular Modeling to Delineate B-Cell and T-Cell Epitopes of Human Sperm-Specific LDH-C<sup>4</sup>* 481

*Patricia A. O'Hern and Erwin Goldberg*

*Hydrophobic Contact Density Distribution Functions* 491

*Wayne J. Becktel and Lorraine M. Rellick*

*Construction and Functional Selection of a T4 Lysozyme Gene Library Randomly Mutagenized at Five Specific Sites* 499

*Enoch Baldwin, Jian Xu, and Omid Hajiseyedjavadi*

*Horse Heart Ferricytochrome c: Anions, Buffers, and Stability* 509

*Yash P. Myer*

*Measurement of Amide Hydrogen D/H Fractionation Factors in Proteins by NMR Spectroscopy* 517

*Stewart N. Loh and John L. Markley*

*A Model for the Molten Globule State of CTF Generated Using Molecular Dynamics* 525

*Valerie Daggett*

*Urea and Guanidine-HCl Yield Different Unfolding Free Energies for CheY: Which Denaturant Provides the Most Reliable Free Energy Values?* 533

*Gregory T. DeKoster, Andrew D. Robertson, Ann M. Stock, and Jeffry B. Stock*

*Learning an Objective Alphabet of Amino Acid Conformation in Protein* 541

*Robert T. Miller, Richard J. Douthart, and A. Keith Dunker*

*$\Delta\Delta G^\circ$ (WT-mut) for BPTI Hydrophobic Core Mutants Measured by Hydrogen Isotope Exchange* 549

*Feng Tao, James Fuchs, and Clare Woodward*

**Thermodynamics of Side Chain Internal Rotations — Effects on Protein Structure and Stability**    557

*Andrew J. Doig, Mark Gardner, Mark S. Searle, and Dudley H. Williams*

**Section X**

**NMR Analysis of Protein and Peptide Structure**

**Evaluation of NMR Based Structure Determination of Flexible Peptides: Application to Desmopressin**    569

*Jianjun Wang, Frank D. Sönnichsen, Robert Boyko, Robert S. Hodges, and Brian D. Sykes*

**Paramagnetic Proton NMR Methods Used in Studying the Hemeprotein Subunit of *Escherichia coli* Sulfite Reductase**    577

*Jeffrey Kaufman, Lewis M. Siegel, and Leonard D. Spicer*

**One- and Two-Dimensional  $^{19}\text{F}$ -NMR Methods for Proteins and Nucleic Acids**    585

*Fraydoon Rastinejad, Pam Artz, and Ponzy Lu*

**“Active Site” Dynamics: A Comparison of the Wild Type and Single Tryptophan Variants of *E. coli* Thioredoxin**    595

*Marvin D. Kemple, Kenneth E. Nollet, Peng Yuan, and Franklyn G. Prendergast*

**Proton Nuclear Magnetic Resonance Studies of Non-Covalent Complexes of Yeast Cytochrome *c* Peroxidase with Cytochromes *c***    605

*Qian Yi, Steve Alam, Yihong Ge, James D. Satterlee, and James E. Erman*

**The Application of Chemical Shift Calculation to Protein Structure Determination by NMR**    615

*Timothy S. Harvey and Wilfred F. van Gunsteren*

**Toward the Structure of Mosaic Proteins: Expression, Purification, and Structural Analysis of a Pair of Fibronectin Type 1 Modules**    623

*Michael J. Williams, Isabelle Phan, Robin T. Applin, Martin Baron, and Iain D. Campbell*

**Index**    633

# SECTION I

---

## Mass Spectrometry of Proteins and Peptides





# Mass Spectrometry in Protein Sequence and Structural Investigations

A. L. Burlingame

Department of Pharmaceutical Chemistry  
The Mass Spectrometry Facility and the Liver Center  
University of California, San Francisco, California

## I. Overview

The growing realization of the importance of mass spectrometry in protein science is based upon quite recent recognition of the relative ease with which classically difficult or often intractable questions in protein biology may now be readily and successfully addressed. The progression of events which underpin the present state of the art was triggered by discoveries of revolutionary new ionization techniques, viz. LSIMS or FAB, electrospray and matrix-assisted laser desorption, able to handle polar, labile macromolecules mass spectrometrically and the subsequent development of new instruments utilizing these techniques. These methods are virtually ideally suited to deal effectively with the challenges of protein sequencing and structural analysis at the picomole level. Currently active “export” of some of this new instrumental capability to the biochemistry laboratories themselves is under way due to the burgeoning availability of more user-friendly, relatively low cost commercial instrumentation. The most recent examples are instruments designed to permit exploitation of the two newest ionization methods, namely electrospray (ES) (1) and matrix-assisted laser desorption (maLD) (2) techniques.

Over the last decade a number of mass spectrometry laboratories have been key participants in establishing the unique power and versatility of these techniques in solving previously tedious and often impossible problems. There is a growing literature of examples of questions which have been tackled successfully representing increasingly more difficult biological problems (See recent reviews 3-6). The present excitement about mass spectrometry in the protein biochemistry community is not due to any *recently* recognized need to have a better structural understanding of the machinery of cells, but rather to a growing awareness that the tools are now in hand with which to discover and dissect even minute structural details with high accuracy and fidelity through application of these mass spectrometric techniques. Indeed, the time is ripe to exploit these new methodologies, both to gain a deeper