

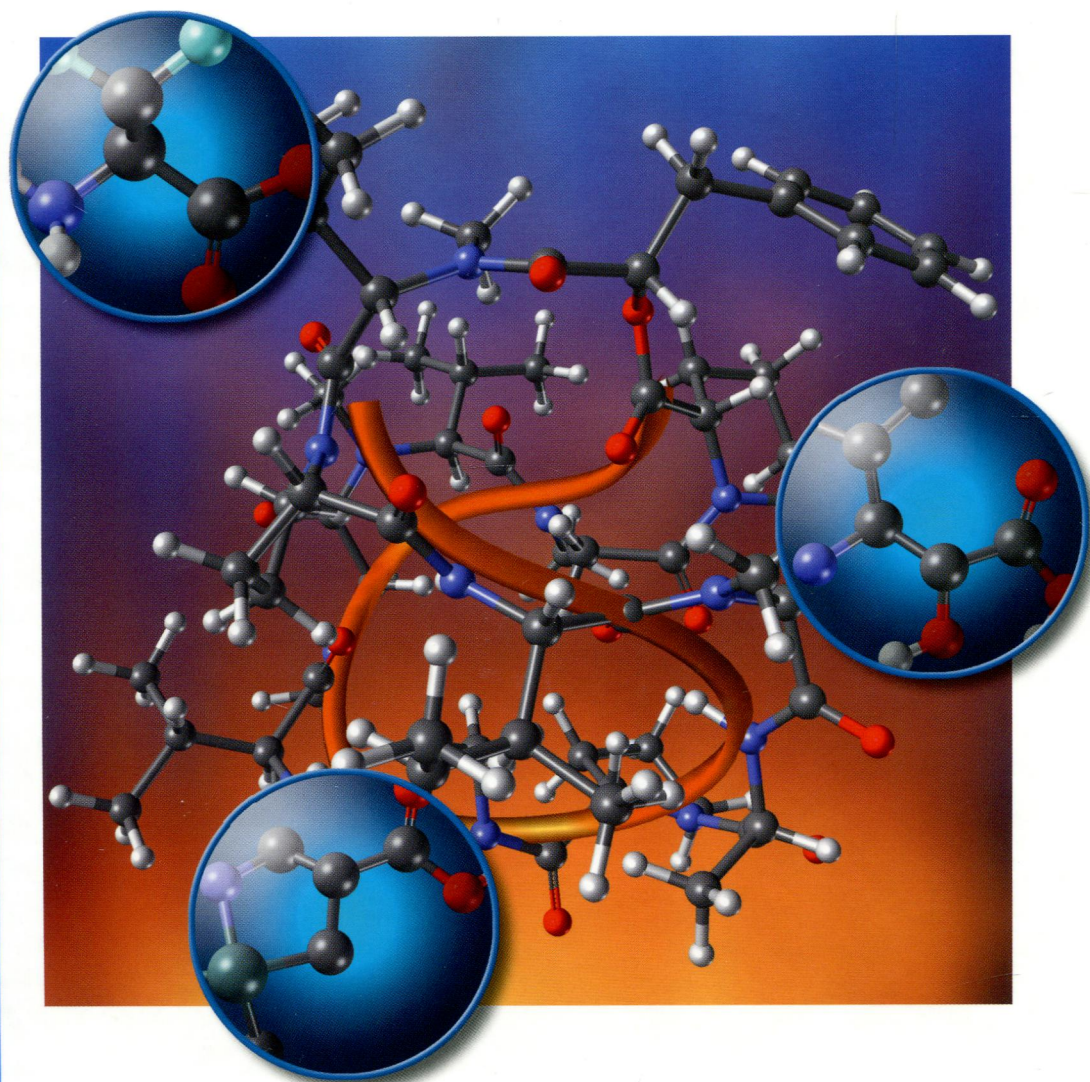
Edited by Andrew B. Hughes

 WILEY-VCH

# Amino Acids, Peptides and Proteins in Organic Chemistry

Volume 5

Analysis and Function of Amino Acids  
and Peptides



*Edited by*  
*Andrew B. Hughes*

# **Amino Acids, Peptides and Proteins in Organic Chemistry**

Volume 5 - Analysis and Function of  
Amino Acids and Peptides



WILEY-VCH Verlag GmbH & Co. KGaA

**The Editor**

**Andrew B. Hughes**  
La Trobe University  
Department of Chemistry  
Victoria 3086  
Australia

All books published by **Wiley-VCH** are carefully produced. Nevertheless, authors, editors, and publisher do not warrant the information contained in these books, including this book, to be free of errors. Readers are advised to keep in mind that statements, data, illustrations, procedural details or other items may inadvertently be inaccurate.

**Library of Congress Card No.:** applied for

**British Library Cataloguing-in-Publication Data**

A catalogue record for this book is available from the British Library.

**Bibliographic information published by the Deutsche Nationalbibliothek**

The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available on the Internet at <http://dnb.d-nb.de>.

© 2012 Wiley-VCH Verlag & Co. KGaA,  
Boschstr. 12, 69469 Weinheim, Germany

All rights reserved (including those of translation into other languages). No part of this book may be reproduced in any form – by photoprinting, microfilm, or any other means – nor transmitted or translated into a machine language without written permission from the publishers. Registered names, trademarks, etc. used in this book, even when not specifically marked as such, are not to be considered unprotected by law.

**Composition** Thomson Digital, Noida, India

**Printing and Binding** betz-druck GmbH, Darmstadt

**Cover Design** Schulz Grafik Design, Fußgönheim

Printed in the Federal Republic of Germany

Printed on acid-free paper

**Print ISBN:** 978-3-527-32104-9

**ePDF ISBN:** 978-3-527-63185-8

**oBook ISBN:** 978-3-527-63184-1

## Contents

### List of Contributors XV

<b>1</b>	<b>Mass Spectrometry of Amino Acids and Proteins</b>	<b>1</b>
	<i>Simin D. Maleknia and Richard Johnson</i>	
1.1	Introduction	1
1.1.1	Mass Terminology	1
1.1.2	Components of a Mass Spectrometer	4
1.1.3	Resolution and Mass Accuracy	6
1.1.4	Accurate Analysis of ESI Multiply Charged Ions	10
1.1.5	Fragment Ions	11
1.2	Basic Protein Chemistry and How it Relates to MS	21
1.2.1	Mass Properties of the Polypeptide Chain	21
1.2.2	<i>In Vivo</i> Protein Modifications	21
1.2.3	<i>Ex Vivo</i> Protein Modifications	26
1.3	Sample Preparation and Data Acquisition	28
1.3.1	Top-Down Versus Bottom-Up Proteomics	28
1.3.2	Shotgun Versus Targeted Proteomics	28
1.3.3	Enzymatic Digestion for Bottom-Up Proteomics	29
1.3.4	Liquid Chromatography and Capillary Electrophoresis for Mixtures in Bottom-Up	30
1.4	Data Analysis of LC-MS/MS (or CE-MS/MS) of Mixtures	32
1.4.1	Identification of Proteins from MS/MS Spectra of Peptides	32
1.4.2	<i>De Novo</i> Sequencing	35
1.5	MS of Protein Structure, Folding, and Interactions	36
1.5.1	Methods to Mass-Tag Structural Features	37
1.6	Conclusions and Perspectives	40
	References	40
<b>2</b>	<b>X-Ray Structure Determination of Proteins and Peptides</b>	<b>51</b>
	<i>Andrew J. Fisher</i>	
2.1	Introduction	51
2.1.1	Light Microscopy	51
2.1.2	X-Rays and Crystallography at the Start	52

2.1.3	X-Ray Crystallography Today	53
2.1.4	Limitations of X-Ray Crystallography	54
2.2	Growing Crystals	55
2.2.1	Why Crystals?	55
2.2.2	Basic Methods of Growing Protein Crystals	55
2.2.3	Protein Sample	59
2.2.4	Preliminary Crystal Analysis	59
2.2.5	Mounting Crystals for X-Ray Analysis	61
2.3	Symmetry and Space Groups	62
2.3.1	Crystals and the Unit Cell	62
2.3.2	Point Groups	65
2.3.3	Space Groups	66
2.3.4	Asymmetric Unit	67
2.4	X-Ray Scattering and Diffraction	67
2.4.1	X-Rays and Mathematical Representation of Waves	67
2.4.2	Interaction of X-Rays with Matter	70
2.4.3	Crystal Lattice, Miller Indices, and the Reciprocal Space	73
2.4.4	X-Ray Diffraction from a Crystal: Bragg's Law	75
2.4.5	Bragg's Law in Reciprocal Space	77
2.4.6	Fourier Transform Equation from a Lattice	79
2.4.7	Friedel's Law and the Electron Density Equation	80
2.5	Collecting and Processing Diffraction Data	82
2.5.1	Data Collection Strategy	82
2.5.2	Symmetry and Scaling Data	83
2.6	Solving the Structure (Determining Phases)	83
2.6.1	Molecular Replacement	83
2.6.2	Isomorphous Replacement	85
2.6.3	MAD	88
2.7	Analyzing and Refining the Structure	90
2.7.1	Electron Density Interpretation and Model Building	90
2.7.2	Protein Structure Refinement	91
2.7.3	Protein Structure Validation	93
	References	94
<b>3</b>	<b>Nuclear Magnetic Resonance of Amino Acids, Peptides, and Proteins</b>	<b>97</b>
	<i>Andrea Bernini and Pierandrea Temussi</i>	
3.1	Introduction	97
3.1.1	Active Nuclei in NMR	98
3.1.2	Energy Levels and Spin States	98
3.1.3	Main NMR Parameters (Glossary)	99
3.1.3.1	Chemical Shift	99
3.1.3.2	Scalar Coupling Constants	100
3.1.3.3	NOE	100
3.1.3.4	RDC	101

3.2	Amino Acids	101
3.2.1	Historical Significance	101
3.2.2	Amino Acids Structure	101
3.2.3	Random Coil Chemical Shift	102
3.2.4	Spin Systems	105
3.2.5	Labile Protons	110
3.2.6	Contemporary Relevance: Metabolomics	112
3.3	Peptides	113
3.3.1	Historical Significance	113
3.3.2	Oligopeptides as Models for Conformational Transitions in Proteins	114
3.3.3	Bioactive Peptides	116
3.3.4	Choice of the Solvent	117
3.3.4.1	Transport Fluids	118
3.3.4.2	Membranes	120
3.3.4.3	Receptor Cavities	122
3.3.5	Ensemble Calculations	125
3.3.6	Selected Examples from the Major Fields of Bioactive Peptides	125
3.3.6.1	Aspartame	125
3.3.6.2	Opioids	126
3.3.6.3	Transmembrane Helices	127
3.3.6.4	Cyclopeptides	128
3.4	Proteins	129
3.4.1	An Alternative to or a Validation of Diffractometric Methods?	129
3.4.2	Protein Spectra	129
3.4.3	Wüthrich's Protocol	130
3.4.3.1	Sample Preparation	131
3.4.3.2	Recording NMR Spectra	131
3.4.3.3	Sequential Assignment	131
3.4.3.4	Conformational Constraints	132
3.4.3.5	Model Building	134
3.4.4	Recent Developments	134
3.4.5	Selected Structures	136
3.4.5.1	Superoxide Dismutases	137
3.4.5.2	Malate Synthase G	137
3.4.5.3	Interactions	138
3.5	Conclusions	145
	References	146
<b>4</b>	<b>Structure and Activity of N-Methylated Peptides</b>	<b>155</b>
	<i>Raymond S. Norton</i>	
4.1	Introduction	155
4.2	Conformational Effects of N-Methylation	157
4.3	Effects of N-Methylation on Bioactive Peptides	159

4.3.1	Thyrotropin-Releasing Hormone	159
4.3.2	Cyclic Peptides	159
4.3.3	Somatostatin Analogs	160
4.3.4	Antimalarial Peptide	161
4.4	Concluding Remarks	162
	References	163
<b>5</b>	<b>High-Performance Liquid Chromatography of Peptides and Proteins</b>	<b>167</b>
	<i>Reinhard I. Boysen and Milton T.W. Hearn</i>	
5.1	Introduction	167
5.2	Basic Terms and Concepts in Chromatography	169
5.3	Chemical Structure of Peptides and Proteins	173
5.3.1	Biophysical Properties of Peptides and Proteins	173
5.3.2	Conformational Properties of Peptides and Proteins	176
5.3.3	Optical Properties of Peptides and Proteins	176
5.4	HPLC Separation Modes in Peptide and Protein Analysis	177
5.4.1	SEC	178
5.4.2	RPC	179
5.4.3	NPC	181
5.4.4	HILIC	181
5.4.5	ANPC	183
5.4.6	HIC	184
5.4.7	IEX	187
5.4.8	AC	188
5.5	Method Development from Analytical to Preparative Scale Illustrated for HP-RPC	189
5.5.1	Development of an Analytical Method	190
5.5.2	Scaling Up to Preparative Chromatography	196
5.5.3	Fractionation	198
5.5.4	Analysis of the Quality of the Fractionation	198
5.6	Multidimensional HPLC	198
5.6.1	Purification of Peptides and Proteins by MD-HPLC Methods	200
5.6.2	Fractionation of Complex Peptide and Protein Mixtures by MD-HPLC	202
5.6.3	Operational Strategies for MD-HPLC Methods	202
5.6.3.1	Off-line Coupling Mode for MD-HPLC Methods	202
5.6.3.2	On-Line Coupling Mode for MD-HPLC Methods	203
5.6.4	Design of an Effective MD-HPLC Scheme	203
5.6.4.1	Orthogonality of Chromatographic Modes	203
5.6.4.2	Compatibility Matrix of Chromatographic Modes	205
5.7	Conclusions	206
	References	207

<b>6</b>	<b>Local Surface Plasmon Resonance and Electrochemical Biosensing Systems for Analyzing Functional Peptides</b>	<b>211</b>
	<i>Masato Saito and Eiichi Tamiya</i>	
6.1	Localized Surface Plasmon Resonance (LSPR)-Based Microfluidics Biosensor for the Detection of Insulin Peptide Hormone	211
6.1.1	LSPR and Micro Total Analysis Systems	211
6.1.2	Microfluidic LSPR Chip Fabrication and LSPR Measurement	212
6.1.3	Detection of the Insulin–Anti-Insulin Antibody Reaction on a Chip	213
6.2	Electrochemical LSPR-Based Label-Free Detection of Melittin	215
6.2.1	Melittin and E-LSPR	215
6.2.2	Fabrication of E-LSPR Substrate and Formation of the Hybrid Bilayer Membrane	215
6.2.3	Measurements of Membrane-Based Sensors for Peptide Toxin	217
6.3	Label-Free Electrochemical Monitoring of $\beta$ -Amyloid (A $\beta$ ) Peptide Aggregation	218
6.3.1	Alzheimer's A $\beta$ Aggregation and Electrochemical Detection Method	218
6.3.2	Label-Free Electrochemical Detection of A $\beta$ Aggregation	219
	References	221
<b>7</b>	<b>Surface Plasmon Resonance Spectroscopy in the Biosciences</b>	<b>225</b>
	<i>Jing Yuan, Yinqiu Wu, and Marie-Isabel Aguilar</i>	
7.1	Introduction	225
7.2	SPR-Based Optical Biosensors	225
7.3	Principle of Operation of SPR Biosensors	226
7.4	Description of a SPR Instrument	228
7.4.1	Sensor Surface	228
7.4.2	Flow System	229
7.4.3	Detection System	230
7.5	Application of SPR in Immunosensor Design	230
7.5.1	Assay Development	232
7.5.1.1	Immobilization of the Analyte to a Specific Chip Surface	232
7.5.1.2	Assay Design	233
7.6	Application of SPR in Membrane Interactions	234
7.6.1	General Protocols for Membrane Interaction Studies by SPR	236
7.6.1.1	Liposome Preparation	236
7.6.1.2	Formation of Bilayer Systems	236
7.6.1.3	Analyte Binding to the Membrane System	237
7.6.1.4	Membrane Binding of Antimicrobial Peptides by SPR	238
7.7	Data Analysis	240
7.7.1	Linearization Analysis	240
7.7.2	Numerical Integration Analysis	241
7.7.3	Steady-State Approximations	242



7.8	Conclusions	243
	References	244
<b>8</b>	<b>Atomic Force Microscopy of Proteins</b>	<b>249</b>
	<i>Adam Mechler</i>	
8.1	Foreword	249
8.1.1	Importance of Asking the Right Question	250
8.2	AFM	250
8.2.1	Principle and Basic Modes of Operation	250
8.2.2	How Does a Tip Tap?	251
8.3	Bioimaging Highlights	253
8.3.1	Protein Oligomerization, Aggregation, and Fibers	253
8.3.2	Membrane Binding and Lysis	255
8.3.3	Ion Channel Activity	257
8.3.4	Protein–DNA-Specific Binding	261
8.4	Issues	261
8.4.1	Resolution	262
8.4.2	Imaging Force	263
8.4.3	Repetitive Stress	264
8.4.4	Artifacts Related to too Low Free Amplitude	265
8.4.5	Transient Force and Bandwidth	266
8.4.6	Accuracy of Surface Tracking	266
8.4.7	Step Artifacts	268
8.5	Force Measurements	269
8.6	Liquid Imaging	269
8.7	Sample Preparation for Bioimaging	272
8.7.1	Adhesion	272
8.7.2	Physical Entrapment	273
8.7.3	Chemical Binding	274
8.8	Outlook	274
	References	275
<b>9</b>	<b>Solvent Interactions with Proteins and Other Macromolecules</b>	<b>277</b>
	<i>Satoshi Ohtake, Yoshiko Kita, Kouhei Tsumoto, and Tsutomu Arakawa</i>	
9.1	Introduction	277
9.2	Solvent Applications	280
9.2.1	Research	280
9.2.2	Precipitation	287
9.2.3	Chromatography	288
9.2.4	Protein Refolding	296
9.2.5	Formulation	297
9.3	Solvent Application for Viruses	300
9.3.1	Isolation and Purification of Viruses	301
9.3.2	Stabilization and Formulation of Viruses	302
9.3.3	Inactivation of Viruses	309

9.4	Solvent Application for DNA	310
9.4.1	Isolation and Purification of DNA	310
9.4.2	Stability of DNA in a Cosolvent System	312
9.5	Mechanism	314
9.5.1	Physical Mechanism	315
9.5.1.1	Hydration	315
9.5.1.2	Excluded Volume	318
9.5.2	Thermodynamic Interaction	322
9.5.2.1	Group Interaction: Model Compound Solubility	322
9.5.3	Preferential Interaction	328
9.6	Protein–Solvent Interactions in Frozen and Freeze-Dried Systems	342
9.6.1	Frozen Systems	342
9.6.2	Freeze-Dried System	345
9.7	Conclusions	348
	References	349
<b>10</b>	<b>Role of Cysteine</b>	<b>361</b>
	<i>Lalla A. Ba, Torsten Burkholz, Thomas Schneider, and Claus Jacob</i>	
10.1	Sulfur: A Redox Chameleon with Many Faces	361
10.2	Three Faces of Thiols: Nucleophilicity, Redox Activity, and Metal Binding	365
10.3	Towards a Dynamic Picture of Disulfide Bonds	371
10.4	Chemical Protection and Regulation via S-Thiolation	374
10.5	“Dormant” Catalytic Sites	378
10.6	Peroxiredoxin/Sulfiredoxin Catalysis and Control Pathway	379
10.7	Higher Sulfur Oxidation States: From the Shadows to the Heart of Biological Sulfur Chemistry	384
10.8	Cysteine as a Target for Oxidants, Metal Ions, and Drug Molecules	388
10.9	Conclusions and Outlook	390
	References	391
<b>11</b>	<b>Role of Disulfide Bonds in Peptide and Protein Conformation</b>	<b>395</b>
	<i>Keith K. Khoo and Raymond S. Norton</i>	
11.1	Introduction	395
11.2	Probing the Role of Disulfide Bonds	396
11.3	Contribution of Disulfide Bonds to Protein Stability	396
11.4	Role of Disulfide Bonds in Protein Folding	397
11.5	Role of Individual Disulfide Bonds in Protein Structure	399
11.6	Disulfide Bonds in Protein Dynamics	401
11.7	Disulfide Bonding Patterns and Protein Topology	403
11.7.1	Conservation and Evolution of Disulfide Bonding Patterns	403
11.7.2	Conservation of Disulfide Bonds	404
11.7.3	Cysteine Framework and Disulfide Connectivity	404
11.7.4	Non-Native Disulfide Connectivities	407

11.8	Applications	408
11.9	Conclusions	409
	References	410
<b>12</b>	<b>Quantitative Mass Spectrometry-Based Proteomics</b>	<b>419</b>
	<i>Shao-En Ong</i>	
12.1	Introduction	419
12.2	Quantification in Biological MS	420
12.2.1	Label-Free Approaches in Quantitative MS Proteomics	423
12.2.2	SIL in Quantitative Proteomics	425
12.3	Identifying Proteins Interacting with Small Molecules with Quantitative Proteomics	430
12.4	Conclusions	433
	References	434
<b>13</b>	<b>Two-Dimensional Gel Electrophoresis and Protein/Polypeptide Assignment</b>	<b>439</b>
	<i>Takashi Manabe and Ya Jin</i>	
13.1	Introduction	439
13.2	Aim of Protein Analysis and Development of 2-DE Techniques	439
13.3	Current Status of 2-DE Techniques	441
13.3.1	Denaturing 2-DE for the Separation of Polypeptides	442
13.3.1.1	Principle	442
13.3.1.2	Procedures	444
13.3.1.3	Specific Features	445
13.3.2	Nondenaturing 2-DE for the Separation of Biologically Active Proteins and Protein Complexes	445
13.3.2.1	Principle	445
13.3.2.2	Procedures	446
13.3.2.3	Specific Features	447
13.3.3	Blue-Native 2-DE for the Detection of Protein–Protein Interactions	448
13.3.3.1	Principle	448
13.3.3.2	Procedures	448
13.3.3.3	Specific Features	449
13.3.4	Visualization of Proteins Separated on 2-DE Gels	449
13.3.4.1	Fixing Before CBB, Silver, or Fluorescent Dye Staining	450
13.3.4.2	CBB Staining	450
13.3.4.3	Silver Staining	450
13.3.4.4	Reverse Staining with Zinc-Imidazole	451
13.3.4.5	Fluorescent Dye Staining	451
13.3.4.6	Quantitation	451
13.4	Development of Protein Assignment Techniques on 2-DE Gels and Current Status of Mass Spectrometric Techniques	452
13.4.1	Development of Protein Assignment Techniques	452

13.4.2	MS-Based Assignment Techniques Utilizing Amino Acid Sequence Databases	454
13.4.2.1	Sample Preparation for MS Analysis	455
13.4.2.2	MALDI-TOF-MS and PMF	456
13.4.2.3	MS/MS and Peptide Sequence Search	459
13.5	Conclusions	460
	References	460
<b>14</b>	<b>Bioinformatics Tools for Detecting Post-Translational Modifications in Mass Spectrometry Data</b>	<b>463</b>
	<i>Patricia M. Palagi, Erik Arhné, Markus Müller, and Frédérique Lisacek</i>	
14.1	Introduction	463
14.2	PTM Discovery with MS	465
14.2.1	Detecting PTMs in MS and MS/MS Data	466
14.2.2	Discovering PTMs in MS or MS/MS Data	468
14.2.3	PTM Prediction Tools	469
14.2.3.1	From MS Data	469
14.2.3.2	From Sequence Data	469
14.3	Database Resources for PTM Analysis	470
14.4	Conclusions	473
	References	473
	<b>Index</b>	<b>477</b>