

PROTEIN-LIPID
INTERACTIONS

PT.1

无
锈



馆

PROTEIN-LIPID INTERACTIONS

蛋白質

0052973

Elsevier Science Publishers B.V.
P.O. Box 211
1000 AE Amsterdam
The Netherlands

Library of Congress Cataloging-in-Publication Data

Protein-lipid interactions / editor, A. Watts.

p. cm. -- (New comprehensive biochemistry ; v. 25)

Includes bibliographical references and index.

ISBN 0-444-81575-9 (alk. paper). -- ISBN 0-444-80303-3 (series)

1. Membrane proteins. 2. Membrane lipids. 3. Lipoproteins.

4. Protein binding. I. Watts, A. II. Series.

[DNLM: 1. Membrane Proteins--metabolism. 2. Membrane Lipids--metabolism. 3. Cell Membrane--metabolism. W1 NE372F v. 25 1993 /

QU 55 P96655 1993]

QD415.N48 vol. 25

574.19'2 s--dc20

[574.19'245]

DNLM/DLC

for Library of Congress

93-1825

CIP

ISBN 0 444 81575 9

ISBN 0 444 80303 3 (series)

©1993 Elsevier Science Publishers B.V. All rights reserved.

No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise without the prior written permission of the publisher, Elsevier Science Publishers B.V., Copyright and Permissions Department, P.O. Box 521, 1000 AM Amsterdam, the Netherlands.

No responsibility is assumed by the publisher for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions or ideas contained in the material herein. Because of the rapid advances in the medical sciences, the publisher recommends that independent verification of diagnoses and drug dosages should be made.

Special regulations for readers in the USA - This publication has been registered with the Copyright Clearance Center Inc. (CCC), Salem, Massachusetts. Information can be obtained from the CCC about conditions under which photocopies of parts of this publication may be made in the USA. All other copyright questions, including photocopying outside the USA, should be referred to the publisher.

Printed on acid-free paper

Printed in the Netherlands

Preface

Protein-lipid interactions as a field of study is now a mature area, and has been reviewed in volumes and single review articles several times in the past decade or so. Over this period there has not been complete agreement in the interpretation of results from a range of methods and systems. Some rationalization has now been achieved and to some degree a level of consensus of opinion and description of the protein-lipid interface (as presented by Mouritsen and Biltonen from a thermodynamic viewpoint, and from a spectroscopic and structural aspect by Marsh) and its all-important relevance to the functional integrity (as described by Sandermann, Duncan, McIntyre and Fleischer) of the system, has been described.

It was thought appropriate that a reflective view could now be presented in a volume with two objectives in mind. Firstly, to look towards the future, and try to envisage how the subject may develop in the near to medium-term future. Secondly, to present contrasting or complementary views on the same system, for example, the acetylcholine receptor is discussed from a predominantly structural aspect by Barrantes and from the kinetic standpoint by Rankin, Raines, Dalton and Miller. Similarly, the (Ca^{2+} - Mg^{2+})-ATPase is considered in the sarcoplasmic reticulum by Thomas and Mahaney, and in reconstituted systems by Lee and East.

Recent new information has been gained about the genetic modulation of membranes and the effect on protein-lipid interactions (as discussed by McGee, Fung and Bankaitis), as well as how proteins and peptide insertion into the membrane could involve the membrane lipids (from de Kroon, de Gier and de Kruijff). An intriguing possibility that M13 bacteriophage infection can involve lipid-protein interactions is discussed by Hemminga, Sanders, Wolfs and Spruijt, where reconstitution and *in vivo* studies of the coat protein (a 50-mer) give information about assembly and association of the protein in the membrane. Peripheral protein-lipid interactions are considered by Sankaram and Marsh, and the effects of cholesterol on lipid-protein interactions in natural membranes are considered by Castuma, Lamy-Freund, Brenner and Schreier. The future possibilities for the use of FT-IR spectroscopy are considered by Arondo and Goñi and, again looking well into the future, the way in which lipid-protein interactions may control 2D array and 3D crystal formation of integral membrane proteins is discussed by Watts, Vénien-Bryan, Sami, Whiteway, Boulter and Sternberg.

It is hoped that this volume not only gives an update on specific aspects of the field, but also shows a way in which the phenomenon of protein-lipid interactions is now seemingly infiltrating many areas of biomembrane research, from recombinant DNA studies, protein insertion and assembly and reconstitution considerations to structural studies of membrane proteins.

A. Watts
December, 1992

List of contributors

José Luis R. Arrondo,

*Department of Biochemistry, University of the Basque Country, P.O. Box 644,
48080 Bilbao, Spain.*

V.A. Bankaitis,

*Department of Cell Biology, University of Alabama at Birmingham, Birmingham,
AL 35294, U.S.A.*

F.J. Barrantes,

*Instituto de Investigaciones Bioquímicas de Bahía Blanca, 8000 Bahía Blanca,
Argentina.*

Rodney L. Biltonen,

*Department of Biochemistry, University of Virginia, Charlottesville, VA 22908,
U.S.A.*

J. Boulter,

*Department of Biochemistry, University of Oxford, South Parks Road, Oxford,
OX1 3QU, UK.*

Rudolfo R. Brenner,

*Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), UNLP-
CONICET, Facultad de Ciencias Medicas, 60 y 120, (1900), La Plata, Argentina.*

Celina E. Castuma,

*Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), UNLP-
CONICET, Facultad de Ciencias Medicas, 60 y 120, (1900), La Plata, Argentina.*

Lauraine A. Dalton,

*Department of Biological Chemistry and Molecular Pharmacology, Harvard
Medical School, Boston, MA 02115, U.S.A.*

J. de Gier,

*Department of Biochemistry of Membranes, Centre for Biomembranes and Lipid
Enzymology, University of Utrecht, Utrecht, The Netherlands.*

A.I.P.M. de Kroon,

*Department of Biochemistry of Membranes, Centre for Biomembranes and Lipid
Enzymology, University of Utrecht, Utrecht, The Netherlands.*

B. de Kruijff,

*Department of Biochemistry of Membranes, Centre for Biomembranes and Lipid
Enzymology, and Institute of Molecular Biology and Medical Biotechnology,
University of Utrecht, Utrecht, The Netherlands.*

- T.M. Duncan,
Department of Biochemistry and Molecular Biology, SUNY Health Science Center, Syracuse, NY 13210, U.S.A.
- J. Malcolm East,
Department of Biochemistry, University of Southampton, Bassett Crescent East, Southampton, SO9 3TU, U.K.
- S. Fleischer,
Department of Molecular Biology, Vanderbilt University, Nashville, TN 37235, U.S.A.
- M.K.Y. Fung,
Department of Cell Biology, University of Alabama at Birmingham, Birmingham, AL 35294, U.S.A.
- Félix M. Goñi,
Department of Biochemistry, University of the Basque Country, P.O. Box 644, 48080 Bilbao, Spain.
- Marcus A. Hemminga,
Department of Molecular Physics, Agricultural University, P.O. Box 8128, 6700 ET Wageningen, The Netherlands.
- M. Teresa Lamy-Freund,
Institute of Physics, Universidade de S. Paulo, C.P. 20516, CEP 01498, S. Paulo, Brazil.
- Anthony G. Lee,
Department of Biochemistry, University of Southampton, Bassett Crescent East, Southampton, SO9 3TU, U.K.
- James E. Mahaney,
Department of Biochemistry, University of Minnesota Medical School, Minneapolis, MN 55455, U.S.A.
- Derek Marsh,
Max-Planck-Institut für biophysikalische Chemie, Abteilung Spektroskopie, Postfach 2841, WD-3400 Göttingen, Fed. Rep. Germany.
- T.P. McGee,
Department of Cell Biology, University of Alabama at Birmingham, Birmingham, AL 35294, U.S.A.
-
- J.O. McIntyre,
Department of Molecular Biology, Vanderbilt University, Nashville, TN 37235, U.S.A.
- Keith W. Miller,
Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, U.S.A.

Ole G. Mouritsen,

*Department of Physical Chemistry, The Technical University of Denmark,
Building 206, DK-2800 Lyngby, Denmark.*

Douglas E. Raines,

*Department of Biological Chemistry and Molecular Pharmacology, Harvard
Medical School, Boston, MA 02115, U.S.A.*

Saffron E. Rankin,

*Department of Biological Chemistry and Molecular Pharmacology, Harvard
Medical School, Boston, MA 02115, U.S.A.*

M. Sami,

*Department of Biochemistry, University of Oxford, South Parks Road, Oxford,
OX1 3QU, UK.*

H. Sandermann Jr.,

*GSF-Forschungszentrum für Umwelt und Gesundheit, GmbH, Institut für
Biochemische Pflanzenpathologie, D-8042 Neuherberg, FRG.*

Johan C. Sanders,

*Department of Molecular Physics, Agricultural University, P.O. Box 8128, 6700
ET Wageningen, The Netherlands.*

Mantripragada B. Sankaram,

*Department of Biochemistry, University of Virginia Health Sciences Center,
Charlottesville, VA 22908, U.S.A.*

Shirley Schreier,

*Department of Biochemistry, Institute of Chemistry, Universidade de S. Paulo,
C.P. 20780, CEP 01498, S. Paulo, Brazil.*

Ruud B. Spruijt,

*Department of Molecular Physics, Agricultural University, P.O. Box 8128, 6700
ET Wageningen, The Netherlands.*

B. Sternberg,

*Abt. für Elektronenmikroskopie, Friedrich-Schiller-Universität Jena, Ziegel-
mühlenweg 1, D-6900 Jena 1, Germany.*

David D. Thomas,

*Department of Biochemistry, University of Minnesota Medical School,
Minneapolis, MN 55455, U.S.A.*

C. Vénien-bryan,

*Department of Biochemistry, University of Oxford, South Parks Road, Oxford,
OX1 3QU, UK.*

A. Watts,

*Department of Biochemistry, University of Oxford, South Parks Road, Oxford,
OX1 3QU, UK.*

C. Whiteway,

*Department of Biochemistry, University of Oxford, South Parks Road, Oxford,
OX1 3QU, UK.*

Cor J.A.M. Wolfs,

*Department of Molecular Physics, Agricultural University, P.O. Box 8128, 6700
ET Wageningen, The Netherlands.*

Contents

<i>Preface</i>	v
<i>List of Contributors</i>	vii
<i>Chapter 1. Protein-lipid interactions and membrane heterogeneity</i>	1
<i>Ole G. Mouritsen and Rodney L. Biltonen</i>	1
Abbreviations	1
1. Perspectives and overview	1
1.1. Lipids, proteins, and the biological membrane	1
1.2. Phase transitions and membrane heterogeneity	3
2. Membrane heterogeneity	4
2.1. Static membrane heterogeneity	4
2.2. Dynamic membrane heterogeneity	4
3. Evidence of heterogeneity in lipid bilayers	6
3.1. What can thermodynamics tell us?	6
3.1.1. Differential scanning calorimetry	7
3.1.1.1. A simple two-state model	8
3.1.2. Volume perturbation calorimetry	12
3.1.3. The effect of anesthetics on the gel-to-fluid transition	16
3.1.2. What can microscopic modelling tell us?	16
4. Effects of proteins on membrane heterogeneity	19
4.1. Perturbation of lipid acyl-chain structure by integral membrane proteins	20
4.2. Lateral distribution of proteins in membranes	21
4.3. Compositional membrane heterogeneity induced by protein-lipid interactions: lipid enrichment and selectivity	25
5. The effect of lipid structure on protein state and functions	27
6. Lipid microheterogeneity and the activation of phospholipase A ₂	29
7. Effects of drugs on protein-lipid interactions and membrane heterogeneity	33
References	34
<i>Chapter 2. The nature of the lipid-protein interface and the influence of protein structure on protein-lipid interactions</i>	41
<i>Derek Marsh</i>	41
1. Introduction	41

2. Molecular modelling and crystal structures	43
3. Lipid chain ordering: NMR and ESR results	46
4. Hydrophobic matching, membrane thickness and protein secondary structure	47
5. Protein conformation and lipid-protein stoichiometry	48
6. Protein secondary structure and lipid-protein interactions	53
7. Selectivity of lipid-protein interaction	55
8. Protein sequence and lipid selectivity	57
9. Conclusions	63
References	64
 <i>Chapter 3. Cooperative regulation of membrane enzymes by lipids</i>	
<i>H. Sandermann Jr., T.M. Duncan, J.O. McIntyre and S. Fleischer . . .</i>	67
 Abbreviations	67
1. Introduction	67
2. Lipid specificity	69
3. Case studies	70
3.1. Cardiolipin synthase	70
3.2. Diacylglycerol kinase	70
3.2.1. Lipid specificity	70
3.2.2. Kinetic cooperativity	72
3.3. R-3-Hydroxybutyrate dehydrogenase	72
3.3.1. Lipid specificity	72
3.3.2. Kinetic cooperativity	73
3.3.3. Basis of the role for phosphatidylcholine	75
3.4. Protein Kinase C	75
3.4.1. Background	75
3.4.2. Proposed mechanisms	76
3.4.3. Trapping model	77
3.4.4. Electrostatic mechanism	79
3.5. Pyruvate oxidase	79
4. Conclusions	82
References	83
 <i>Chapter 4. Lipid-protein interaction in a biological membrane: Effect of cholesterol and acyl chain degree of unsaturation</i>	
<i>Celina E. Castuma, M. Teresa Lamy-Freund, Rudolfo R. Brenner and Shirley Schreier</i>	87
 Abbreviations	87
1. Introduction	87
2. Effect of cholesterol and acyl chain degree of unsaturation on the kinetic properties of UDP-glucuronyl transferase	88
2.1. Effect of in vivo modification of cholesterol content	89
2.2. Effect of in vitro modification of cholesterol content	91
2.3. Effect of in vivo modification of acyl chain degree of unsaturation	91
2.4. Significance of the kinetic data	94

3.	Fluorescence studies of bilayer properties in normal and modified microsomes, and in extracted lipids	95
3.1.	Spectral behaviour of fluorescent probes	95
3.2.	Significance of the fluorescence data	98
4.	Spin label study of the effect of cholesterol on lipid-protein interactions in microsomal membranes	98
4.1.	Spectral behaviour of spin label probes	98
4.2.	Significance of spin label data	102
5.	Possible models for the effect of cholesterol and acyl chain unsaturation on lipid-protein interaction	102
6.	Concluding remarks	103
	Acknowledgements	104
	References	104

Chapter 5. Lipid-peptide interactions in model systems: Membrane insertion and translocation of peptides

	<i>A.I.P.M. de Kroon, J. de Gier and B. de Kruijff</i>	107
--	--	-----

	Abbreviations	107
1.	Introduction	107
2.	Membrane affinity and topology of the peptides	109
3.	Consequences of peptide insertion for structural and dynamic properties of the phospholipid bilayer	112
4.	Peptide translocation across a phospholipid bilayer and ion gradients	115
5.	Biologically active peptides	120
6.	Conclusions	123
	Acknowledgements	124
	References	124

Chapter 6. Protein-lipid interactions with peripheral membrane proteins

	<i>Mantripragada B. Sankaram and Derek Marsh</i>	127
--	--	-----

	Abbreviations	127
1.	Introduction	128
2.	Binding requirements/modes	129
2.1.	Binding	129
2.2.	Functional implications	130
3.	Surface electrostatics	131
3.1.	Binding isotherms	131
3.2.	Strength of binding	133
3.3.	Ionic strength and pH dependence	135
4.	Surface dehydration	137
4.1.	Interfacial pK	137
4.2.	Fluorescence isotope effects	137
5.	Membrane penetration	138
5.1.	Electron spin resonance	139
5.2.	Photochemical crosslinking	140
5.3.	Tryptophan fluorescence	141

6.	Lipid selectivity	142
6.1.	Selectivity sequence	142
6.2.	Functional implications of specificity	145
7.	Phase separation/lipid polymorphism	146
7.1.	Phase separation	146
7.2.	Lipid polymorphism	146
8.	Covalently linked acyl chains	148
8.1.	Membrane attachment	150
8.2.	Protein–protein interactions	151
9.	Protein conformation	151
9.1.	Circular dichroism	151
9.2.	Fourier transform infrared spectroscopy	153
9.3.	Nuclear magnetic resonance	153
10.	Peripheral protein–integral protein interactions	154
10.1.	Specific interactions	155
10.2.	Transmembrane signalling	156
11.	Conclusions	158
	Acknowledgements	158
	References	158

Chapter 7. Genetic studies on the functions of membrane-forming phospholipids

T.P. McGee, M.K.Y. Fung and V.A. Bankaitis 163

	Abbreviations	163
1.	Introduction	163
2.	Genetics of phospholipid biosynthesis	165
2.1.	Phospholipid synthesis in <i>Escherichia coli</i>	165
2.2.	Phospholipid biosynthesis in yeast	167
3.	The genetic case for lipids as cofactors	170
4.	Functions of acidic phospholipids in <i>E. coli</i>	171
4.1.	Acidic phospholipids and secretion	171
4.2.	Cardiolipin and DNA replication	173
5.	Functions of phospholipids in eukaryotes	174
5.1.	The phospholipid composition of the Golgi is critical to secretory function	174
5.1.1.	Identification of the SEC14p and the connection to phospholipids	174
5.1.2.	Testing the PI/PC ratio hypothesis	177
5.1.3.	Genetic distinctions between the CDP-choline pathway and the PE methylation pathway	178
5.1.4.	Why phospholipids and Golgi function?	181
5.2.	Fatty acid unsaturation and mitochondrial inheritance	185
5.3.	Plasmalogen synthesis and peroxisome biogenesis	186
6.	Summary	187
	References	187

Chapter 8. Lipid–protein interactions involved in bacteriophage M13 infection

Marcus A. Hemminga, Johan C. Sanders, Cor J.A.M. Wolfs and Ruud B. Spruijt 191

Abbreviations	191
1. Introduction	191
2. The M13 virion and its reproductive life cycle	192
2.1. M13 bacteriophage	192
2.2. Reproductive cycle	192
2.3. Biological questions	194
3. The major coat protein during the infection process	195
3.1. M13 major coat protein	195
3.2. Effects of phospholipids	195
4. Reconstitution of M13 coat protein	196
4.1. General principles	196
4.2. Basic conformations of transmembrane domains	196
5. The <i>in vitro</i> membrane-bound state of M13 coat protein	198
5.1. The α -helical and β -polymeric state	198
5.2. Putative <i>in vivo</i> state of M13 coat protein	198
5.3. Reconstitution procedures	199
6. The coat protein structure	200
6.1. Secondary structure – transmembrane helix	200
6.2. Secondary structure – the terminal parts	201
6.3. The N-terminal helix	201
6.4. Molecular dynamics	202
7. Lipid order and dynamics in reconstituted systems	203
7.1. Protein packing in the bilayer	203
7.2. Protein aggregation	204
7.3. Model for protein–lipid interaction	204
7.4. The phospholipid headgroup region	206
7.5. Dynamic protein–lipid network	207
8. Concluding remarks	208
Acknowledgements	209
References	209

Chapter 9. Functional aspects of acetylcholine receptor–lipid interactions

Saffron E. Rankin, Douglas E. Raines, Lauraine A. Dalton and Keith W. Miller 213

Abbreviations	213
1. Introduction	213
1.1. Acetylcholine receptor states	214
1.2. Acetylcholine receptor structure	215
2. Information from the lipid–protein interface	218
2.1. Spectroscopic studies of the lipid–protein interface of the nAcChoR	218
2.2. Axial orientation of the transmembrane helices within the bilayer	220

3. The lipid environment of the nAcChoR	221
4. Towards more definitive kinetics of reconstituted systems	224
4.1. The framework for a new approach	224
4.2. The limitations of current work	224
4.3. Introduction of new probes	226
4.4. Other structural approaches	227
References	228
<i>Chapter 10. The lipid annulus of the nicotinic acetylcholine receptor as a locus of structural-functional interactions</i>	
<i>F.J. Barrantes</i>	231
1. Introduction	231
2. AChR ligand sites	231
3. Topographical relationship between AChR and membrane lipids: From structural data to structural-functional correlations	233
4. Structural asymmetry of the AChR-rich membrane. I. The annulus	239
4.1. Early data on immobilized lipid in AChR-rich membranes	239
4.2. Quantitation of annular lipid	240
4.3. Non-annular sites	244
5. Structural asymmetry of the AChR-rich membrane. II. The two leaflets of the bilayer	244
6. Testing the influence of lipid on AChR <i>in situ</i>	245
6.1. Phospholipid polar headgroup substitution	246
6.2. Cholesterol	247
6.3. Fatty acids	249
7. Concluding remarks	252
Acknowledgements	252
References	252
<i>Chapter 11. The (Ca^{2+}-Mg^{2+})-ATPase and other membrane proteins: what reconstitution tells us about the biological membrane</i>	
<i>Anthony G. Lee and J. Malcolm East</i>	259
Abbreviations	259
1. Introduction	259
2. Why should phospholipid structure affect the function of membrane proteins	263
2.1. The membrane and the cell	263
2.2. Selectivity in phospholipid-protein interactions	264
2.3. Effects on enzyme function	269
2.3.1. General principles	269
2.3.2. The (Ca^{2+} - Mg^{2+})-ATPase	277
2.3.3. Other ATPases	288
2.3.4. Other systems	289
2.4. Diffusion in the membrane	291
3. Extrapolation to the biological membrane	293
Acknowledgements	295
References	295

<i>Chapter 12. The functional effects of protein and lipid dynamics in sarcoplasmic reticulum</i>	
<i>David D. Thomas and James E. Mahaney</i>	301
Abbreviations	301
1. Introduction	301
2. Spectroscopic methods for studying membrane molecular dynamics	303
2.1. Electron paramagnetic resonance	303
2.1.1. Conventional EPR	303
2.1.2. Saturation transfer EPR	305
2.2. Time-resolved fluorescence and phosphorescence anisotropy	306
2.3. Relationship between lipid fluidity and protein mobility	307
3. Correlation of molecular dynamics with Ca-ATPase function	309
3.1. Temperature variation	309
3.1.1. EPR studies of temperature effects in SR	310
3.1.2. TPA analysis of temperature dependence in SR	310
3.1.3. Effective temperature change	311
3.2. Perturbation of lipid fluidity	311
3.2.1. Decreased fluidity: Lipid substitution or delipidation	311
3.2.2. Increased fluidity: Diethyl ether	312
3.2.3. Variation of bilayer thickness	312
3.3. Direct perturbation of protein-protein interactions	313
3.3.1. Covalent cross-linking	313
3.3.2. Peptide effects on protein-protein interactions	313
3.4. Cardiac SR	316
4. Conclusions	318
Acknowledgements	319
References	319
<i>✓ Chapter 13. Infrared spectroscopic studies of lipid-protein interactions in membranes</i>	
<i>José Luis R. Arrondo and Félix M. Goñi</i>	321
Abbreviations	321
1. The problem	321
2. The technique	323
2.1. Dispersive versus FT-IR spectroscopy	323
2.2. Data processing	326
2.2.1. Fourier deconvolution and LOMEP	326
2.2.2. Derivation	328
2.2.3. Differential spectroscopy	329
2.3. Technical improvements desired and foreseen	330
3. Recent studies	331
3.1. Lipid components	331
3.1.1. The acyl chain region	333
3.1.2. The interfacial region	335
3.1.3. The phosphate group region	336

3.2. Protein structure	336
3.2.1. Assignment of protein bands	336
3.2.2. Quantification of protein secondary structure by FT-IR spectroscopy	338
3.3. How proteins influence lipid structure	339
3.4. How lipids influence protein structure	342
4. Looking into the future	344
Acknowledgments	345
References	345
 <i>Chapter 14. Lipid–protein interactions in controlled membrane protein array and crystal formation</i>	
<i>A. Watts, C. Vénien-Bryan, M. Sami, C. Whiteway, J. Boulter and B. Sternberg</i>	351
1. Introduction	351
2. Possible involvement of lipids in the formation of arrays or crystals of membrane proteins	352
3. Two-dimensional protein arrays	355
3.1. Bacterial porins	355
3.2. Photosystem I reaction centre	357
3.3. Light-harvesting chlorophyll a/b-protein complex, LHC-II	358
3.4. Na^+, K^+ -ATPase	359
3.5. Bacteriorhodopsin	360
3.6. Other proteins	361
3.7. Summary	362
4. Three-dimensional crystallization of integral membrane proteins	362
4.1. Porins	363
4.2. Bacteriorhodopsin	364
4.3. Band 3	365
4.4. Other proteins	366
4.5. Summary	367
5. Conclusion	368
Acknowledgements	368
References	368
 <i>Index</i>	371