

01 **Nanobiotechnology of Biomimetic**
02 **Membranes**
03

04
05
06
07
08
09
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45

01
02
03
04
05
06
07
08
09
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45

01 **Nanobiotechnology**
02
03 **of Biomimetic Membranes**
04
05

06
07
08
09
10
11
12
13

14 **DONALD K. MARTIN**
15

16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42

43  **Springer**
44
45

01 *Editor:*
02 Donald K. Martin
03 Associate Professor
04 Faculty of Science
05 University of Technology, Sydney
06 Broadway N.S.W. 2007
07 Australia
08 donm@uts.edu.au

08
09
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24

25 Library of Congress Control Number: 2006931774

26
27 ISBN-10: 0-387-37738-7
28 ISBN-13: 978-0-387-37738-4

29
30 e-ISBN-10: 0-387-37740-9
31 e-ISBN-13: 978-0-387-37740-7

32 Printed on acid-free paper.

33
34 © 2007 Springer Science+Business Media, LLC

35
36 All rights reserved. This work may not be translated or copied in whole or in part without the written
37 permission of the publisher (Springer Science+Business Media, LLC, 233 Spring Street, New York,
38 NY 10013, USA), except for brief excerpts in connection with reviews or scholarly analysis. Use in
39 connection with any form of information storage and retrieval, electronic adaptation, computer
40 software, or by similar or dissimilar methodology now known or hereafter developed is forbidden.
41 The use in this publication of trade names, trademarks, service marks and similar terms, even if
42 they are not identified as such, is not to be taken as an expression of opinion as to whether or not
they are subject to proprietary rights.

43 10 9 8 7 6 5 4 3 2 1

44
45 springer.com

01
02
03
04
05
06
07
08
09
10
11
12
13**PREFACE**14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45

This book is focussed on the lipid membrane, since that structure is a key component of the way that living cells are able to maintain and organise their functions. Unlocking the secrets of those membranes provides important lessons that are valuable in guiding the construction of devices to be used for medical applications. That philosophy is a central theme for scientists and engineers working in the field of biomimetics. Indeed, throughout this book we emphasise that approach in order to define the discipline of nanobiotechnology.

We define nanobiotechnology to be an interdisciplinary field of research and development that integrates engineering, physical sciences, and biology through the development of very small physical and biological devices using biomimetically inspired nano-fabrication techniques. In that sense, biomimetically-inspired means that the fabrication processes are based on the way the natural systems are constructed, usually by self-assembly of molecules in an aqueous environment.

That approach is often termed bionanotechnology, rather than nanobiotechnology. However, it is more appropriate to term the discipline that we support in the pages of this book as being nanobiotechnology. We emphasise that a significant research outcome is to exploit an understanding of biological processes in order to guide and influence the creation of devices and processes for use in biomedical applications, and this is usually defined as biotechnology. The nano prefix is necessary to accurately describe the scale of the manipulations required of the proteins, lipids and other molecules in order to create those biomedical devices and processes.

We have not broadly included the myriad of aspects of nanobiotechnology that are often included in other books that describe this discipline. We deliberately focus on the lipid membrane due to its importance in the function of the natural cells of the living organisms. Indeed, the targets of the majority of drugs and pharmaceuticals are membrane-incorporated proteins. That targeting is not by chance, since nature utilises the membrane and membrane-incorporated proteins as key components in maintaining organisation and function in the body. The separation and compartmentalisation of electrolyte concentrations within the body is maintained by the lipid membranes

vi Preface

01 and the membrane-incorporated proteins. Amongst other vital functions, that
02 separation of electrolyte concentrations provides the electrochemical driving
03 force for propagation of electrical “action potential” signalling in nerves and
04 muscles. Biomedical devices based around biomimetic lipid membranes will
05 allow improved biocompatibility and connection of the devices with the natural
06 cells of living organisms. Perhaps the realisation that Drexler’s robots will not
07 be built from metal and plastic, but rather from biomimetic components utilising
08 the principles of lipid membrane nanobiotechnology described in this book.

09 The book develops the principles of membrane nanobiotechnology by
10 discussing methods to produce lipid membranes, methods of characterising lipid
11 membranes, and the application of membranes to produce biosensors. We have
12 addressed those topics in some depth in order to produce a reference book that
13 is useful for researchers and senior undergraduates. The chapters have been
14 written by friends and colleagues who are expert in the disciplines of physics,
15 engineering, chemistry, and biology. Nanobiotechnology is the interdisciplinary
16 glue that unites us, and I am indebted to those friends and colleagues who have
17 generously and enthusiastically contributed the ideas and concepts described
18 within the pages of this book. On many occasions they have forgiven my indul-
19 gences with time.

20 I must extend a special acknowledgement to the International Science Linkages
21 program under the Australian government’s innovation statement Backing
22 Australia’s Ability. That program had the foresight to fund the OzNano₂Life
23 program (www.ambafrance-au.org/oznano2life) which has provided the inter-
24 national collegiality and “glue-funding” that has allowed nanobiotechnology
25 research programs to flourish between Australian and international laboratories.
26 That program of research-without-borders has been assisted significantly by the
27 support of the Embassy of France in Australia, and notably by successive Science
28 Attachés, M. Alain Moulet, and Professor Robert Farhi.

29 *Donald K. Martin*
30 *Sydney, Australia*
31 *August, 2006*

32
33
34
35
36
37
38
39
40
41
42
43
44
45

01		
02		
03	CONTENTS	
04		
05		
06		
07		
08		
09		
10		
11		
12		
13		
14	Contributors	xi
15		
16	1. The Significance of Biomimetic Membrane Nanobiotechnology	
17	to Biomedical Applications	1
18	Donald K. Martin	
19	1.1. Introduction.....	1
20	1.2. Interaction of Lipid Membranes	
21	with Transport Proteins.....	3
22	1.3. Reaction of Eukaryotic Cells	
23	to the Physical Environment.....	4
24	1.3.1. Example of the Influence of Membrane Ion Channels	
25	on the Biology of Endothelial Cells	5
26	1.3.2. Mechanical Transduction of Stress in Lipid Bilayers.....	8
27	1.4. What is the Relevance of Lipid Bilayer Membranes	
28	to Nanotechnology?	10
29	1.5. Can Biosensor Technology Benefit from Biomimetic	
30	Membrane Nanobiotechnology?	13
31	1.6. Does Biomimetic Membrane Nanobiotechnology Assist	
32	in Drug Delivery?	15
33	1.7. Can Implants Benefit from Biomimetic Membrane	
34	Nanobiotechnology?.....	16
35	1.8. Concluding Remarks.....	17
36		
37	2. Langmuir-Blodgett Technique for Synthesis of Biomimetic Lipid	
38	Membranes	23
39	Agnès P. Girard-Egrot and Loïc J. Blum	
40		
41	2.1. Introduction.....	23
42	2.2. Langmuir Monolayer Formation	25
43	2.2.1. Surface Tension.....	26
44	2.2.2. Surfactants	27
45	2.2.3. Surface Pressure	30

viii Contents

01	2.2.4. Surface Pressure (π) – Area (A) Isotherms	33
02	2.2.5. Monolayer Stability.....	37
03	2.3. Langmuir-Blodgett Technique.....	39
04	2.3.1. Vertical Film Deposition Principles	39
05	2.3.1.1. Transfer Process Energy.....	41
06	2.3.1.2. Contact Angle Values	42
07	2.3.1.3. Deposition Ratio.....	43
08	2.3.1.4. Advantages and Caution	43
09	2.3.2. Elaboration of Organised Lipidic LB Films	44
10	2.3.3. Phospholipid LB Films	47
11	2.3.4. Free Supported Phospholipid LB Films	52
12	2.3.5. Asymmetric Phospholipid LB Bilayers.....	54
13	2.4. Functionalisation of Lipidic LB Films: Specific Features.....	57
14	2.4.1. Protein Association with the Floating Monolayer before	
15	LB Deposition	57
16	2.4.2. Protein Association onto Preformed-Lipidic LB Films	59
17	2.4.3. Oriented Protein Association in Lipidic LB Films	60
18	2.5. Trends and Prospects	62
19		
20		
21	3. Liposome Techniques for Synthesis of Biomimetic	
22	Lipid Membranes	75
23	Stella M. Valenzuela	
24	3.1. Introduction.....	75
25	3.2. Applications and Uses of Liposomes.....	75
26	3.3. Liposome Structure is Influenced by its Phospholipid	
27	Composition	76
28	3.4. Common Terminology Used in the Description of Liposome	
29	Structure	77
30	3.5. Liposome Preparation.....	77
31	3.5.1. Preparation of Multilamellar Vesicles.....	78
32	3.5.2. Preparation of Unilamellar Vesicles.....	79
33	3.5.2.1. Ultrasonication.....	79
34	3.5.2.2. Extrusion through Polycarbonate Filters	79
35	3.5.2.3. Freeze – Thawing	79
36	3.5.2.4. Ethanol Injection	81
37	3.5.2.5. Detergent Method.....	81
38	3.5.2.6. Preparation of Sterile Large Unilamellar Vesicles	81
39	3.5.3. Preparation of Giant Unilamellar Liposomes.....	82
40	3.5.3.1. Electroformation.....	82
41	3.5.3.2. Rapid Preparation of Giant Liposomes.....	82
42	3.5.3.3. Giant Unilamellar Liposomes Prepared in	
43	Physiological Buffer.....	83
44	3.5.4. Modified Liposomes	83
45	3.5.5. Purification of Liposomes.....	85

01	4. Characterization and Analysis of Biomimetic Membranes	89
02	Adam I. Mechler	
03	4.1. Important Properties of Biomimetic Membranes.....	89
04	4.2. Methods of Characterization and Analysis	91
05	4.2.1. A Few Thoughts.....	91
06	4.2.2. Atomic Force Microscopy	92
07	4.2.3. Quartz Crystal Microbalance.....	96
08	4.2.4. Surface Force Apparatus.....	96
09	4.2.5. Ellipsometry	97
10	4.2.6. Surface Plasmon Resonance	98
11	4.3. Coverage and Mass.....	99
12	4.4. Morphology and Mechanical Properties	104
13	4.4.1. Imaging and a Few Common Artefacts	104
14	4.4.2. Surface Forces and Continuum Mechanics;	
15	AFM Simulation.....	107
16	4.4.3. Mechanical Properties.....	118
17	4.5. A Brief Outlook.....	122
18		
19		
20	5. Biomimetic Membranes in Biosensor Applications	127
21	Till Böcking and J. Justin Gooding	
22		
23	5.1. Introduction.....	127
24	5.2. Biosensors	129
25	5.2.1. Classes of Biosensors.....	129
26	5.2.2. Why Biomimetic Membranes for Biosensing Applications? ..	130
27	5.3. Biomimetic Membranes for Biosensor Applications.....	133
28	5.3.1. Hybrid Bilayer Lipid Membranes (Supported Lipid	
29	Monolayers).....	134
30	5.3.2. Solid Supported “Floating” Bilayer Lipid Membranes.....	134
31	5.3.3. Tethered Bilayer Lipid Membranes.....	137
32	5.3.3.1. Surface Attachment via Low Molecular Weight	
33	Tethers.....	137
34	5.3.3.2. Phytanyl Lipid Derivatives for Highly	
35	Insulating Membranes	138
36	5.3.3.3. Surface Attachment via Functionalised Polymers.....	140
37	5.3.4. Laterally Structured Bilayer Lipid Membranes.....	140
38	5.4. Catalytic and Affinity Biosensors Fabricated using Supported	
39	Bilayer Lipid Membranes	141
40	5.4.1. Catalytic Biosensors based on Supported BLMs.....	141
41	5.4.2. Affinity Biosensors	143
42	5.4.2.1. Immunosensors based on Supported BLMs	143
43	5.4.2.2. DNA Modified BLMs	143
44	5.4.2.3. Detection of Toxins using Hybrid BLMs,	
45	Supported BLMs and Vesicles.....	143

x Contents

01	5.4.3. General Remarks on Supported BLMs for Biosensing	
02	Applications.....	147
03	5.5. Membrane Biosensors Based on Ion Channel Gating	148
04	5.5.1. Signal Transduction via Ion Channels.....	148
05	5.5.1.1. Criteria for the Biomimetic Membrane	148
06	5.5.1.2. Measurement of Membrane Conductance	149
07	5.5.1.3. Gating of Ion Channels Incorporated into	
08	Tethered BLMs.....	149
09	5.5.1.4. Gating of Ion Channels Incorporated into	
10	Membranes on a Sensor Chip	150
11	5.5.2. Taking Biosensors a Step Further: The AMBRI Ion	
12	Channel Switch Biosensor	150
13	5.6. Concluding Remarks.....	154
14		
15	Index	167
16		
17		
18		
19		
20		
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
31		
32		
33		
34		
35		
36		
37		
38		
39		
40		
41		
42		
43		
44		
45		

01
02
03
04
05
06
07
08
09
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45

CONTRIBUTORS

Loïc J. Blum

Laboratoire de Génie Enzymatique
et Biomoléculaire
EMB2/UMR 5013 - CNRS/UCBL
Université Claude Bernard Lyon 1
43 Bd du 11 novembre 1918
F-69622 Villeurbanne Cedex
France

Till Böcking

School of Chemistry and School of
Physics
The University of New South Wales
Sydney, NSW 2052
Australia

Agnès P. Girard-Egrot

Laboratoire de Génie Enzymatique
et Biomoléculaire
EMB2/UMR 5013 - CNRS/UCBL
Université Claude Bernard Lyon 1
43 Bd du 11 novembre 1918
F-69622 Villeurbanne Cedex
France

J. Justin Gooding

School of Chemistry
The University of New South Wales
Sydney, NSW 2052
Australia

Donald K. Martin

Faculty of Science
University of Technology Sydney
Broadway, N.S.W. 2007
Australia

Adam I. Mechler

Monash University
School of Chemistry
Clayton, VIC 3800
Australia

Stella M. Valenzuela

Faculty of Science
University of Technology Sydney
Broadway, N.S.W. 2007
Australia

01
02
03
04
05
06
07
08
09
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45