Immobilized Biomolecules in Analysis

A PRACTICAL APPROACH

Edited by
T. CASS and F. S. LIGLER

The Practical Approach Series
Series Editor: B. D. Hames
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OXFORD
UNIVERSITY PRESS

OXFORD
UNIVERSITY PRESS
Great Clarendon Street, Oxford OX2 6DP
Oxford University Press is a department of the University of Oxford
and furthers the University's aim of excellence in research, scholarship,
and education by publishing worldwide in
Oxford New York
Athens Auckland Bangkok Bogotá Buenos Aires Calcutta
Cape Town Chennai Dar es Salaam Delhi Florence Hong Kong Istanbul
Karachi Kuala Lumpur Madrid Melbourne Mexico City Mumbai
Nairobi Paris São Paulo Singapore Taipei Tokyo Toronto Warsaw
and associated companies in Berlin Ibadan
Oxford is a registered trade mark of Oxford University Press
Published in the United States
by Oxford University Press Inc., New York

A catalogue record for this book is available from the British Library
Library of Congress Cataloging in Publication Data
(Data available)
ISBN 0 19 963637 0 (Hbk)
ISBN 0 19 963636 2 (Pbk)
Typeset by Footnote Graphics, Warminster, Wilts
Printed in Great Britain by Information Press, Ltd, Eynsham, Oxon.

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Preface

Biosensors herald the coming of a technology that will explode during the next decade; they demonstrate that we can harness the incredible functions of living molecules and cells, crafted for millennia by nature, for our own, more limited purposes. Nevertheless, to make use of these small wonders, we have to first capture them and restrict them geographically to the artificial space in which we demand that they perform. Moreover, we have to do this without jeopardizing the ability of the relatively fragile molecule or cell to do the desired job.

Over the last decade, we have both attended numerous conferences labelled 'Biosensors' in which over 50% of the papers centred on how to immobilize a biomolecule or cell, i.e. a biomaterial, on a sensing surface. In many cases, the papers presented documented the loss of activity suffered by the biomaterial when subjected to glutaraldehyde or other abusive chemical treatment. In other instances, the loss of activity was simply a function of non-specific interactions of the biomaterial with the surface or steric hindrances placed on the molecule by the method of immobilization. There have been discussions *ad nauseam* about how to immobilize a biomolecule in the 'right orientation', but the subsequent work often seemed to be more driven by cartoons that could be drawn on a computer than by any deep appreciation of how the biomolecule or its function was being affected by the surface to which is was attached.

And what is really happening to these harnessed biomolecules? The portentous words of Enrico Fermi have become a favourite quote for both of us, 'God made the solid state. He left the surface to the Devil'. Consider the poor biomolecule or cell trying to do its job in its new location. It has to cope with a foreign surface, which often—like a hot skillet fries an egg—tries to alter its conformation mainly through hydrophobic interactions and so denature it. It may have to cope with tethers that—like those the Lilliputians used to immobilize Gulliver—tie it down via many small lines. It has to function in a geometrically deranged configuration where solution mass transfer rules usually do not apply. A variety of solutes are present which may affect not only the immobilized cell or molecule but also the analytes with which it is supposed to interact. And it is probably being asked to perform lying down or standing on its head!

Both of us, frustrated with the lack of any recently compiled information on biomolecule immobilization, have been considering such a book as this for several years. Hopefully, it will become obsolete in the next decade as we deepen our understanding of the factors controlling biomolecular interactions with surfaces and biomolecular function at surfaces. However, we have tried to provide the reader with a set of options to guide his choice of how to

immobilize a biomolecule so that it can best perform the desired function. Included are chapters on biomolecule immobilization via adsorption (Johnston and Ratner), entrapment (Dave *et al.*), through a tried-and-true tether (Shriver-Lake), and via site-specific binding (Egodage and Wilson), or avidin–biotin technology (Wilchek and Bayer). Elegant surface chemistries such as self-assembled monolayers (Liedberg and Cooper) or conducting organic polymers (Schuhmann) can be recruited. An exciting new approach describes a method for immobilization that reversibly regulates function (Stayton and Hoffman). Bright describes a method for monitoring the biomolecule once it is immobilized, and Karlsson and Löfås describe how kinetic analysis is important in understanding the behaviour of these systems. The advances in cell patterning which require both spatially and chemically controlled immobilization are described by Clark.

We expect the field of immobilized biomolecules and cells to expand far beyond biosensor applications. Already there are examples of immobilized biomolecules used for pharmaceutical production and processing. Over the coming decades, 'biomaterials' will become a term not limited to the scientific community as immobilized biomolecules and cells are incorporated into 'smart polymers' which can respond to their environment, into filters for remediating toxic pollutants, into artificial tissues for organ replacement, and into silicon chips for advanced computing. Immobilizing the biomolecule in a functional state is the critical starting point.

London T.C.
Washington, DC F.S.L.
May 1998

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Abbreviations

AAc	acrylic acid
Ab	antibody
Ac	acrylodan (6-acryloyl-(dimethylamino)-naphthalene)
AIBN	2,2′-azoisobutyronitrile
AOT	dioctyl sodium sulfosuccinate
APTS	aminopropyltriethoxysilane
BCHZ	biocytin hydrazide
BHZ	biotin hydrazide
BMA	butyl methacrylate
BNHS	biotinyl *N*-hydroxysuccinimide
BPF	bandpass filter
BSA	bovine serum albumin
B-sulfo-NHS	biotinyl N-hydroxy-sulfo-succinimide ester
BxHZ	biotinyl ϵ-aminocaproyl hydrazide
BxNHS	biotinyl ϵ-aminocaproyl *N*-hydroxysuccinimide
Bx-sulfo-NHS	biotinyl ϵ-aminocaproyl *N*-hydroxy-sulfo-succinimide ester
CDI	carbonyldiimidazole
CMEC	1-cyclohexyl-3-(2-morpholinoethyl)carboxdiimide
CNBr	cyanogen bromide
DBB	dibenzoyl biocytin
DMF	dimethylformamide
DMSO	dimethylsulfoxide
dNTP	deoxynucleotide triphosphate
DO	dissolved oxygen
DTT	dithiothreitol
ECM	extracellular matrix
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
EO	ethylene oxide
FITC	fluorescein isothiocyanate
FMP	2-fluoro-1-methylpyridinium toluene-4-sulfonate
GMBS	gamma-maleimidobutrylsuccinimide
GOPS	glycidoxypropyltrimethoxysilane
GOx	glucose oxidase
HBS	hepes-buffered saline
HRP	horseradish peroxidase
HSA	human serum albumin
iPA	isopropyl alcohol
LDPE	low density polythylene

M_2C_2H	4-(*N*-maleimidomethyl)-cyclohexane-1-carboxylhydrazide–HCl
MAb	monoclonal antibody
MBDD	12-mercapto (8-biotinamide-3,6-dioxaocytl)dodecanamide
Mb	myoglobin
MbCO	carbonyl myoglobin
MbO_2	*oxy*myoglobin
2-MEA	2-mercaptoethylamine
MeOH	methanol
MPB	maleimidopropionyl biocytin
MPC	2-methacryloyl oxyethyl phosphoryl choline
MPM	multifrequency phase modulation
MPTS	mercaptopropyltrimethoxysilane
NAS	*N*-acryloxysuccinimide
NHS	*N*-hydroxysuccinimide ester
NIPAAm	*N*-isopropylacrylamide
OF	optical fibre
OTS	octadecyl trichlorosilane
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
4-PDS	4,4′-dithiodipyridine
PEO	poly(ethylene oxide)
PET	poly(ethylene terephthalate)
PMMA	poly(methyl methacrylate)
PNIPAAm	poly(*N*-isopropylacrylamide)
poly HEMA	poly (hydroxyethylmethacrylate)
PU	polyurethane
RF	radiofrequency
RU	resonance units
SAM	self-assembled monolayer
SAW	surface acoustic wave
SPIN	surface physical interpenetrating network
SPR	surface plasmon resonance
TBATos	tetrabutylammonium toluene-4-sulfonate
TCEP	Tris(2-carboxyethyl)phosphine
TFAA	trifluoroacetic acid
THF	tetrahydrofuran
TIRF	total internal reflection fluorescence
TMOS	tetramethyl orthosilicate
TNBS	2,4,6-trinitrobenzenesulfonic acid
TOF	time-of-flight
UHV	ultrahigh vacuum
VS	vinyl sulfone
VTPDMS	vinyl terminated polydimethylsiloxane
XPS	X-ray photoelectron spectroscopy

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