

Immobilized Biomolecules in Analysis

A PRACTICAL APPROACH

Edited by

T. CASS and F. S. LIGLER

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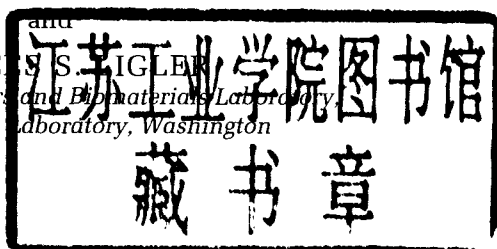
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Immobilized Biomolecules in Analysis

The Practical Approach Series

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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 it 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

Preface

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.

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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 <i>N</i> -hydroxysuccinimide
BPF	bandpass filter
BSA	bovine serum albumin
B-sulfo-NHS	biotinyl <i>N</i> -hydroxy-sulfo-succinimide ester
BxHZ	biotinyl ϵ -aminocaproyl hydrazide
BxNHS	biotinyl ϵ -aminocaproyl <i>N</i> -hydroxysuccinimide
Bx-sulfo-NHS	biotinyl ϵ -aminocaproyl <i>N</i> -hydroxy-sulfo-succinimide ester
CDI	carbonyldiimidazole
CMEC	1-cyclohexyl-3-(2-morpholinoethyl)carboxydiimide
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	glycidoxypopyltrimethoxysilane
GOx	glucose oxidase
HBS	hepes-buffered saline
HRP	horseradish peroxidase
HSA	human serum albumin
iPA	isopropyl alcohol
LDPE	low density polyethylene

Abbreviations

M ₂ C ₂ H	4-(<i>N</i> -maleimidomethyl)-cyclohexane-1-carboxylhydrazide-HCl
MAb	monoclonal antibody
MBDD	12-mercapto (8-biotinamide-3,6-dioxacytl)dodecanamide
Mb	myoglobin
MbCO	carbonyl myoglobin
MbO ₂	oxymyoglobin
2-MEA	2-mercaptoethylamine
MeOH	methanol
MPB	maleimidopropionyl biocytin
MPC	2-methacryloyl oxyethyl phosphoryl choline
MPM	multifrequency phase modulation
MPTS	mercaptopropyltrimethoxysilane
NAS	<i>N</i> -acryloxysuccinimide
NHS	<i>N</i> -hydroxysuccinimide ester
NIPAAm	<i>N</i> -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(<i>N</i> -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

Contents

<i>List of contributors</i>	xv
-----------------------------	----

<i>Abbreviations</i>	xxii
----------------------	------

1. Silane-modified surfaces for biomaterial immobilization	1
---	---

Lisa C. Shriver-Lake

1. Introduction	1
2. General concepts	2
Silanization	2
Solid support surfaces	2
Cross-linkers	3
Biomolecules	3
3. Methods for preparing surfaces for silanization	4
4. Immobilization procedures using mercapto-terminal silanes	6
5. Immobilization procedures using amino-terminal silanes	9
6. Immobilization procedures using epoxy-terminal silanes	11
7. Conclusion	12
References	14

2. Avidin–biotin immobilization systems	15
--	----

Meir Wilchek and Edward A. Bayer

1. The avidin–biotin system	15
2. Biotinylation of the binder	17
3. Immobilization of avidin to solid supports	25
4. Modified avidins	29
Acknowledgements	33
References	34

3. Antibodies as immobilization reagents	35
<i>Kamal L. Egodage and George S. Wilson</i>	
1. Introduction	35
2. Methods of antibody immobilization	36
Antibody immobilization through sulfhydryl moiety of the Fab' fragment	37
Preparation of antibody Fab' fragments	39
Antibody coupling through carbohydrate moieties	42
Specific antibody immobilization using a capture antibody	43
Antibody immobilization through an avidin–biotin linkage	44
3. Optimization of antibody immobilization to matrices	46
Study of the activity of the immobilized antigen over time	49
4. Other factors that are important for the high capture capacity of the immobilized antibodies	50
Study of the relationship between capture capacity of immobilized antibodies and the size of antigen	50
Relationship between selection of antibodies for the immobilization of enzymes for bioanalytical application	51
5. Conclusion	52
References	52
 4. Bioanalytical applications of self-assembled monolayers	 55
<i>Bo Liedberg and Jonathan M. Cooper</i>	
1. Introduction	55
2. A historical perspective	56
3. Preparation of alkanethiolate SAMs	60
Deposition of alkanethiolate SAMs	62
Preparation of alkanethiolate SAMs on gold	63
The preparation of mixed alkanethiolate SAMs on gold	64
Biological self-assembly at mixed self-assembled monolayers for protein immobilization	68
Immobilization of antibodies on gold	69
4. Preparation of silane SAMs on silicon-based substrates	72
The preparation of substrates	72
Silanization of silicon or glass substrates	73
The immobilization of biological molecules on silane layers	75
5. Conclusion	77
References	78

5. Protein adsorption: friend or foe? 79

Erika Johnston and Buddy D. Ratner

1. Introduction	79
2. Strategies to inhibit protein adsorption	80
General concepts	80
Surface physical interpenetrating network (SPIN)	81
Grafting of adsorbed molecules using RF plasmas	83
Plasma deposited PEO-like films	85
Self-assembled monolayers	87
Phospholipid layers	87
3. Directing protein adsorption and retaining protein on a surface	88
General concepts	88
Fluoropolymer films deposited from glow discharge plasma environments	89
Allylamine films deposited from glow discharge plasma environments	91
Glow discharge plasma deposited films from acetone, methanol, and formic acid	92
Acknowledgements	93
References	93

6. Micropatterning cell adhesiveness 95

Peter Clark

1. Introduction	95
2. Basic photolithography	95
Overview	95
Primary pattern definition	97
3. Patterning organosilanes	99
4. Cell culture on patterned substrata	103
Basic cell culture	103
Patterning adhesion by adsorption of attachment factors	104
Patterning adhesion by covalent immobilization of proteins	106
5. Photopatterning cell-attachment molecules	108
6. Other approaches	109
Acknowledgements	110
References	111

7. Sol-gel matrices for protein entrapment 113

Bakul C. Dave, Bruce Dunn, Joan S. Valentine, and Jeffrey I. Zink

1. Introduction	113
2. Overview of the sol-gel process	114
Reaction chemistry	115
Processing	118
3. Sol-gel encapsulation of biomolecules	120
Sol-gel encapsulated myoglobin	121
Cytochrome <i>c</i> in sol-gel thin films	123
4. Sol-gel encapsulation of enzymes	126
Sol-gel encapsulated glucose oxidase	126
Sol-gel encapsulated oxalate oxidase	127
5. Sol-gel-based biosensor elements	128
Biosensor element for dissolved oxygen	129
Biosensor element for glucose	132
6. Concluding remarks	133
Acknowledgements	133
References	134

8. Immobilization of 'smart' polymer-protein conjugates 135

Patrick S. Stayton and Allan S. Hoffman

1. Introduction	135
2. Applications of stimuli-responsive polymers and gels	136
Stimuli-responsive hydrogels	136
Stimuli-responsive polymer-protein conjugates	136
Site-specific stimuli-responsive polymer-protein conjugates	139
3. Synthesis of stimuli-responsive polymers and gels	141
Temperature-sensitive PNIPAAm	141
Temperature- and pH-sensitive polymers and gels	141
Thiol-reactive polymers	142
4. Conjugation of stimuli-responsive polymers to proteins	143
Conjugation to protein amino groups	143
Site-specific conjugation to engineered proteins	144
Characterization of conjugates by mass spectrometry	146
References	146