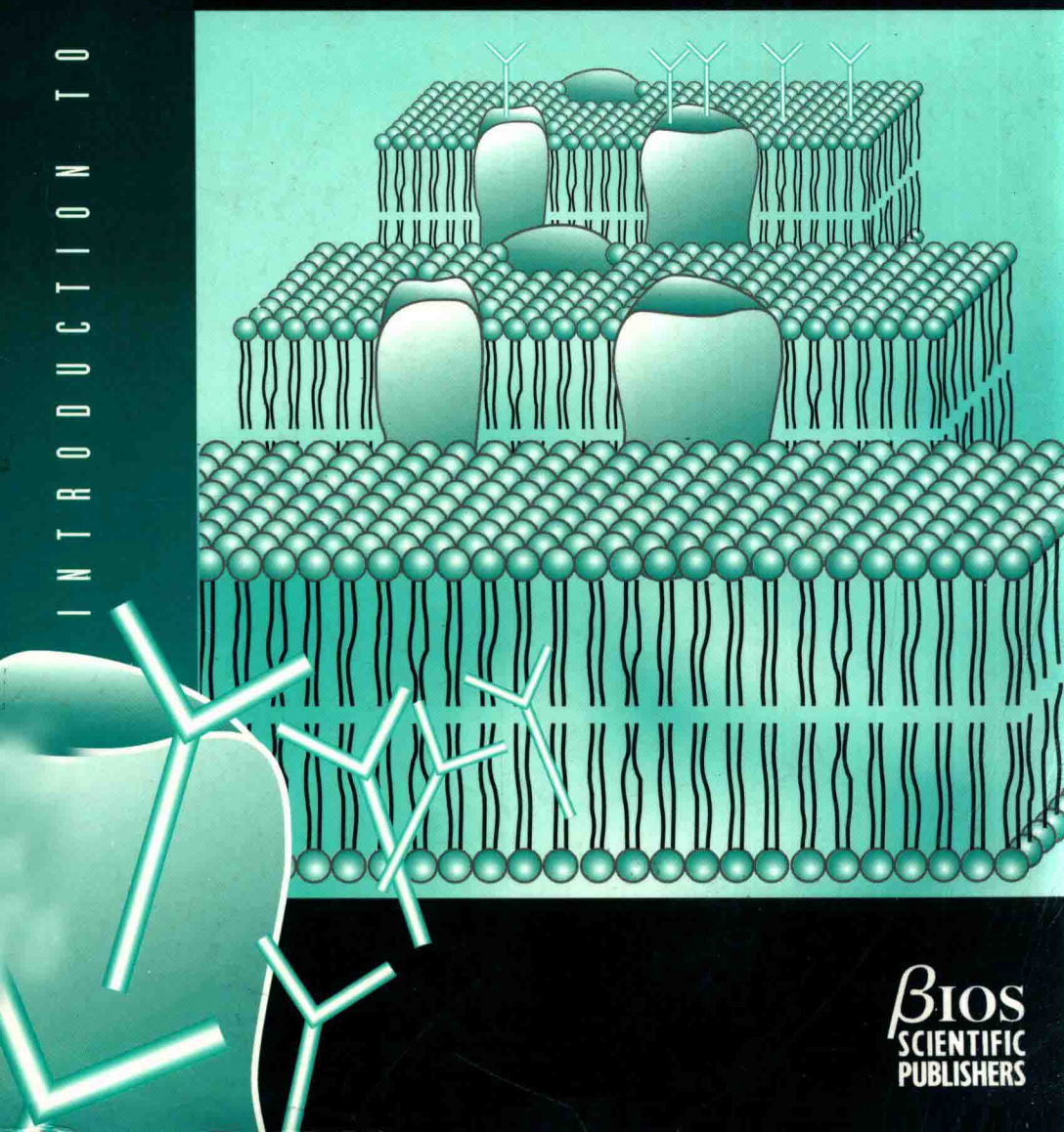
INTRODUCTION TO BIOTECHNIQUES

J.M. GRAHAM & J.A. HIGGINS

Membrane Analysis



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MEMBRANE ANALYSIS

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MEMBRANE ANALYSIS

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Abbreviations

BCA	bicinchoninic acid
BSA	bovine serum albumin
CAT	chloramphenicol acytltransferase
CRD	cross-reacting determinant
DPG	diphosphatidylglycerol
ELISA	enzyme linked immunoassay
ER	endoplasmic reticulum
FAMEs	fatty acid methyl esters
GAG	glycosaminoglycan
GLC	gas-liquid chromatography
HPLC	high performance liquid chromatography
HPTLC	high performance thin-layer chromatography
IEF	isoelectric focussing
LMP	light mitochondrial fraction
LUVs	large unicellular vesicles
MAb	monoclonal antibody
MLVs	multilamellar vesicles
NANS	N-acetylneuraminic acid
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PG	phosphatidylglycerol
PS	phosphatidylserine
PT	phosphatidylinositol
RER	rough endoplasmic reticulum
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SER	smooth endoplasmic reticulum
SM	sphigomyelin
SUVs	small unicellular vesicles
TLC	thin layer chromatography

Preface

A brief examination of the program from any international or national congress in biochemistry, cell biology, molecular biology or immunology, reveals that subcellular membrane isolation and analysis form major elements in a wide range of research interests. Secretion and internalization of physiologically important molecules, ion transport, energy transduction, intracellular and intercellular signalling and the biogenesis of cell components are just a few of the areas of intensive research activity which involve membrane analysis. These exciting areas are underpinned by the continuing and crucial basic research on how membranes are organized and function.

Unlike most other books in the Introduction to Biotechniques series, this text is organized around a broadly based research topic rather than a more defined technical one; therefore the first two chapters provide some background information on membrane composition and membrane types, particularly as these relate to later chapters on fractionation and identification (Chapter 3) and on compositional and structural analysis (Chapter 4). Techniques in these two chapters are prodigious and in an introductory text they cannot be reviewed in detail. The authors' approach to this problem is either to provide a few sample protocols, or to describe only the principles of individual techniques and their applicability and to highlight important practical points. Many of these techniques are subsequently used as part of the methodologies described in Chapters 5–8.

These subsequent chapters on membrane structural/functional relationships have necessarily been rather selective and their choice has been partly influenced, by the authors' own research interests. Nevertheless, the topics covered in Chapters 5–8, protein and lipid topography, protein targetting, membrane trafficking and membrane biogenesis, should be of relevance to a broad spectrum of research workers. Here too, the emphasis is on technical principles, as detailed protocols often have to be tailored to specific situations and sample types and dictated by operational requirements.

This book is aimed primarily at the new research worker, but we hope that it will also be of considerable benefit to more experienced workers who will find the more basic information in Chapters 1–4 a useful source of revision, and the new and advanced technologies described in Chapters 5–8 a valuable aid to their research. In addition to the

usual figures and tables which clarify and expand the text, important points which are relevant to the selection or execution of a particular technique are highlighted in single-line-framed boxes, while the techniques themselves are provided in double-line-framed boxes.

John M. Graham
Joan A. Higgins

Contents

1 General membrane composition and structure

All membranes have an amphipathic nature, i.e. they have a hydrophobic (non-polar) central layer sandwiched between two hydrophilic (polar) surfaces, and the molecules and macromolecules which membranes contain are designed to conform to and maintain this basic structure. The basic unit has a thickness of 5–8 nm. There are three principal components of membranes, lipid, protein and carbohydrate.

1.1 Lipids

1.1.1 General lipid composition

Membranes are composed of a bilayer of amphipathic lipids: although there is evidence from physical studies (NMR spectroscopy and X-ray diffraction) that nonbilayer structures (e.g. hexagonal arrays or micelles) are present, the bilayer structure is the predominant one.

The lipid bilayer:

- is permeable to small nonionized molecules, such as glycerol, urea, water, oxygen and carbon dioxide and to all lipophilic molecules;
- provides a structural framework and the proper environment for the functioning of many membrane proteins.

The total amount of lipid in a membrane is normally expressed as a protein:lipid weight ratio (see *Table 1.1*). Generally this ratio is >1.0 and in the most metabolically active membranes may approach 4.0. Eukaryotic surface membranes, however, tend to have a lower ratio (i.e. more lipid) than do membranes of cytoplasmic organelles or the prokaryotic cytoplasmic membrane, although only in lipid-rich myelin does the weight ratio fall significantly below 1.0.

Table 1.1. Protein:lipid ratios in membranes

Membrane	Protein:lipid	Membrane	Protein:lipid
Animal			
Myelin	0.25	Erythrocyte	1.1
Surface (liver)	1.5	Nuclear (liver)	2.0
Rough ER (liver)	2.5	Smooth ER (liver)	2.1
Inner mit. (liver)	3.6	Outer mit. (liver)	1.2
Golgi (liver)	2.4	Sarcoplasmic reticulum	3.0
Retinal rod	1.0		
Plant			
Surface	0.9	Chloroplast	1.9
Saccharomyces			
Surface	1.2		
Prokaryote surface			
Bacillus	2.8	*Micrococcus*	2.4
Staphylococcus	2.4	*Escherichia coli*	2.8
Outer membrane of Gram-negative bacteria	2.2		

Because protein is much denser than lipid, membranes with different protein:lipid ratios may also have different densities – one of the properties that is used to fractionate membranes by centrifugation (see Chapter 3).

Generally lipids can be divided into four major classes:

- free fatty acids;
- esters of fatty acids, e.g. triacylglycerol and phospholipids;
- isoprenoids, e.g. sterols, sterol esters, dolichol and farnesol;
- glycolipids.

Phospholipids are major components of all membranes and, with the exception of myelin, they represent greater than 50% of the total membrane lipid mass. Glycolipids and cholesterol are usually concentrated in the plasma membrane of mammalian cells, while in plants only sterols (mostly stigmasterol and sitosterol) show this preferential localization and glycolipids predominate in the chloroplast. Bacterial membranes may also be rich in glycolipid but, with the exception of mycoplasmas, they lack sterols, and even in mycoplasmas the sterol is derived almost exclusively from the growth medium. It is only relatively recently that isoprenoids other than sterols (dolichol phosphate and derivatives of farnesol and geranylgeranol) have been shown to be important in membranes. The relative amounts of glycolipid, phospholipid and cholesterol in some examples of membrane types is given in *Table 1.2*.

Table 1.2. Lipid composition of membranes (figures are % of total lipid mass)

Membrane	Phospholipid	Glycolipid	Sterol
Mammalian			
Plasma	50–60	5–17	15–22
Endoplasmic reticulum	70–80	<5	5–10
Mitochondria (inner)	80–90	<5	<5
Mitochondria (outer)	80–90	<5	5–8
Lysosomes	70–80	5–10	10–15
Nuclear	85–90	<5	10–15
Golgi	85–90	<5	5–10
Peroxisomes	90–95	<5	<5
Myelin	50–60	15–25	20–25
Erythrocyte	70–80	5–10	20–25
Plant			
Surface	30–65	10–20	25–50
Mitochondria	90–95	<5	<5
Chloroplast envelope	20–30	65–80	<5
Chloroplast lamellae	35–45	50–70	<5
Endoplasmic reticulum	70–80	5–15	10–20
Bacterial			
Cytoplasmic	50–90	10–50	None

Nonpolar lipids such as cholesterol ester, and triacylglycerol are rarely encountered as membrane components; if they are present in an isolated membrane fraction it is normally within a membrane vesicle as part of a lipoprotein, for example, which is destined for secretion from the cell. The endoplasmic reticulum (ER) which is the site of synthesis of triacylglycerol and cholesterol ester may, however, contain small amounts of these lipids. Free fatty acids are also not considered as discrete membrane components, although they may arise from the degradation of phospholipids, either as part of a biological process, or artificially during isolation of membranes (see Chapter 3) as a result of exposure of the lipids to hydrolytic enzymes which are normally sequestered *in vivo*. Like the farnesyl and geranyl-geranyl isoprenoid residues, however, some fatty acids (e.g. palmitic or myristic acid) may anchor proteins to the lipid bilayer (see Sections 1.1.3, 1.2.3, 5.2.3 and 7.2.11).

Degradation of membrane lipids during fractionation must be limited by careful control of temperature (4°C) and the general avoidance of Ca-containing media which can activate some phospholipases. Free fatty acids cause uncoupling of phosphorylation in mitochondria.

1.1.2 Phospholipids

Derivatives of diacylglycerol-3-phosphate. These are the most common forms of phospholipid; two acyl chains are esterifed onto the 1 and 2 positions of glycerol-3-phosphate (*Figure 1.1*). Diacylglycerol-3-phosphate is commonly called phosphatidate and its phosphate group is linked to one of three nitrogenous bases (choline, ethanolamine or serine) or to one of two polyhydric alcohols (inositol or glycerol) to form phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI) and phosphatidylglycerol (PG), respectively. Diphosphatidylglycerol (DPG) or cardiolipin is a phosphatidate derivative of PG and has four fatty acyl chains (*Figure 1.1*). The phosphate group makes all phospholipid molecules negatively charged overall, except for the choline-containing ones which are zwitterionic.

Other phospholipids. Sphingomyelin (SM) is only found in mammals. The sphingosine molecule (*Figure 1.2*) has one variable acyl group which is linked to the free amine group (amide-linked) to form acylsphingosine (ceramide) whose terminal OH group is linked to phosphorylcholine.

In mammalian membranes small amounts (1–3% of total lipid) of lysophosphatidylcholine and lysophosphatidylethanolamine are present – these are deficient in one acyl group and are probably derived from the parent molecule by phospholipase action.

In ether lipids (plasmalogens) the hydrocarbon chain on the C1 position of the glycerol backbone is linked via an ether group rather than an ester (*Figure 1.2*). Although normally at low levels (1–5% of the total lipid), in certain membranes (e.g. sarcoplasmic reticulum) and in some microorganisms, they can be major components.

Fatty acids of phospholipids. In eukaryotes the acyl chains are derived from unbranched fatty acids of between C14 and C24 (animals) and C16 and C18 (plants). They always have an even C-number (they are built up from the stepwise addition of 2C units) and are often unsaturated or polyunsaturated: in animals up to six *cis* double bonds may occur, in plants rarely more than three. In phospholipids based on diacylglycerol-3-phosphate (from animals) one of the fatty acids is normally saturated and the other unsaturated. Some of the commonly occurring acyl residues in eukaryotes are given in *Table 1.3*.

In prokaryotes C15–C19 fatty acids are the most common: both odd and even C-numbers are possible and they may be branched or be cyclopropane derivatives (*Figure 1.3*). Polyunsaturated acids are