Methods of Molecular Analysis in the Life Sciences

Delivering fundamental insights into the most popular methods of molecular analysis, this text is an invaluable resource for students and researchers. It encompasses an extensive range of spectroscopic and spectrometric techniques used for molecular analysis in the life sciences, especially in the elucidation of the structure and function of biological molecules.

Covering the range of up-to-date methodologies from everyday mass spectrometry and centrifugation to the more probing X-ray crystallography and surface-sensitive techniques, the book is intended for undergraduates starting out in the laboratory and for more advanced postgraduates pursuing complex research goals. The comprehensive text has a strong emphasis on the background principles of each method, including equations where they are of integral importance to the individual techniques. With sections on all the major procedures for analysing biological molecules, this book will serve as a useful guide across a range of fields, from new drug discovery to forensics and environmental studies.

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FOREWORD

Contemporary scientific research is in large parts an interdisciplinary effort, especially when it comes to the investigation of processes in living organisms, the so-called life sciences. It has thus become an essential requirement to have an appreciation of methodologies that neighbour one's own area of expertise. In particular areas, such as for example modern structural biology, understanding of a variety of different analytical methods that used to be the core domain of other disciplines or specialised research areas is now a mandatory requirement.

The core focus of this text is on properties of molecules and the study of their interactions. Within the life sciences, spanning diverse fields from analysis of elements in environmental or tissue samples to the design of novel drugs or vaccines, the molecules of interest thus span different orders of magnitude as well – from inorganic ions or gases as molecules with only few atoms, over small organic molecules, natural products and biomolecules, up to macromolecules such as proteins and DNA.

The methods covered in this text are featured in other textbooks, mainly in two different ways. On the one hand, many texts aimed at students contain a brief overview of particular methodologies, and mostly this is just enough to whet the appetite. On the other hand, there are authoritative in-depth treatises where the amount and level of detail in many cases exceeds the absorbing capacity of a non-expert.

The authors of this book, in contrast, have compiled a text that delivers the fundamental insights into the most popular methods of molecular analysis in a concise and accessible fashion.

This book should appeal to researchers in the area of life sciences who are not necessarily expert in all the different methodologies of molecular analysis. It should also be useful to students of chemistry and biochemistry disciplines, in particular to those studying the interactions between molecules. Teachers may find this an auxiliary text for courses in chemistry, biochemistry and biophysical chemistry, as well as forensics and environmental studies. And certainly anyone interested in the understanding of fundamental molecular analytical methods should find this text a useful and accessible introduction.

Professor Dr Robert Huber
Martinsried, 18 March 2013

PREFACE

The life sciences, comprising the study of living organisms, is the most prominent example of modern interdisciplinary research where complex processes are investigated by means of particular scientific disciplines. Important contributions are made by disciplines that study molecular structure, interactions and their implications for function.

This text is meant for everyone who studies or has an interest in molecular aspects of the life sciences. It aims to provide the background for tools and methodologies originating from the core disciplines of chemistry and physics applied to investigation of problems relevant to the life sciences.

With this text, we attempt to fill a gap by presenting relevant methodologies in a manageable volume, but with strong emphasis on describing the fundamental principles for the individual methods covered. Deliberately, we have chosen to include mathematical formulas where we found them to be of integral importance for the matter discussed. A powerful feature of mathematical equations is their ability to capture relationships between different parameters that can be complicated when described in words. Not least, almost all formulas are an essential part of the work and analysis in a scientific project and are thus a tool used in real-life applications. We hope that the combination of discussion, illustration and mathematical expressions deliver a representation of a phenomenon from different aspects, helping to form an understanding of the methodologies, rather than just a memory.

This book is in large parts based on lectures we developed at The University of Edinburgh, Griffith University, University of Lyon, and the University of Queensland. Consciously or unconsciously, many colleagues we have learned from have made contributions. Data for many figures and tables in this book have been obtained from experiments conducted particularly for this book. We are very grateful to Dr Michelle Colgrave (CSIRO, Brisbane), Dr Nien-Jen Hu (Imperial College London) and Lawren Sullivan (Griffith University) for providing experimental data used in various figures. Manuscript and figures for this book have been compiled entirely with open source and academic software under Linux, and we would like to acknowledge the efforts by software developers and programmers who make their products freely available.

Recommendations for further reading and websites of interest have been compiled based on popular acceptance as well as the authors' preferences; however, the selections evidently are not exhaustive. In cases where commercial supplier websites are listed, these have been included based purely on educational value; the authors have not received any benefit from those companies in this context.

We are particularly grateful to Professor Lindsay Sawyer (The University of Edinburgh) for many helpful suggestions and critical reading of the manuscript, and Professor Robert Huber (Max-Planck-Institute for Biochemistry, Martinsried) for his guiding advice.

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UNITS AND CONSTANTS

Decimal factors.

Factor	Prefix	Symbol	Factor	Prefix	Symbol
10^{-1}	deci	d	10	deka	da
10^{-2}	centi	c	10^{2}	hekto	h
10^{-3}	milli	m	10^{3}	kilo	k
10^{-6}	micro	µ	10^{6}	mega	M
10^{-9}	nano	n	10^{9}	giga	G
10^{-12}	pico	p	10^{12}	tera	T
10^{-15}	femto	f	10^{15}	peta	P
10^{-18}	atto	a	10^{18}	exa	E

SI base parameters and units.

Symbol	Parameter	Unit	Name
I	Electric current	A	Ampere
I	Light intensity	cd	Candela
l	Length	m	Metre
m	Mass	kg	kilogram
n	Molar amount	mol	Mol
t	Time	s	second
T	Temperature	K	Kelvin

Important physico-chemical parameters and units.

Symbol	Parameter	Unit	Name
B	Magnetic induction	$1\ \mathrm{T} = 1\ \mathrm{kg\ s^{-2}\ A^{-1}} = 1\ \mathrm{V\ s\ m^{-2}}$	Tesla
c	Molar concentration	$1\ \mathrm{mol\ l^{-1}}$	
C	Electric capacity	$1\ \mathrm{F} = 1\ \mathrm{kg^{-1}\ m^{-2}\ s^{4}\ A^{2}} = 1\ \mathrm{A\ s\ V^{-1}}$	Farad
E	Energy	$1\ \mathrm{J} = 1\ \mathrm{kg\ m^{2}\ s^{-2}}$	Joule
ε	Molar extinction coefficient	$1\ \mathrm{l\ mol^{-1}\ cm^{-1}}$	
F	Force	$1\ \mathrm{N} = 1\ \mathrm{kg\ m\ s^{-2}} = 1\ \mathrm{J\ m^{-1}}$	Newton
Φ	Magnetic flux	$1\ \mathrm{Wb} = 1\ \mathrm{kg\ m^{2}\ s^{-2}\ A^{-1}} = 1\ \mathrm{V\ s}$	Weber
G	Electric conductivity	$1\ \mathrm{S} = 1\ \mathrm{kg^{-1}\ m^{-2}\ s^{3}\ A^{2}} = 1\ \Omega^{-1}$	Siemens
H	Enthalpy	$1\ \mathrm{J} = 1\ \mathrm{kg\ m^{2}\ s^{-2}}$	Joule
L	Magnetic inductivity	$1\ \mathrm{H} = 1\ \mathrm{kg\ m^{2}\ s^{-2}\ A^{-2}} = 1\ \mathrm{V\ A^{-1}\ s}$	Henry
M	Molar mass[a]	$1\ \mathrm{g\ mol^{-1}} = 1\ \mathrm{Da}$	(Dalton)
ν	Frequency	$1\ \mathrm{Hz} = 1\ \mathrm{s^{-1}}$	Hertz
p	Pressure	$1\ \mathrm{Pa} = 1\ \mathrm{kg\ m^{-1}\ s^{-2}} = 1\ \mathrm{N\ m^{-2}}$	Pascal

(*cont.*)

Symbol	Parameter	Unit	Name
P	Power	$1\ W = 1\ kg\ m^2\ s^{-3} = 1\ J\ s^{-1}$	Watt
Q	Electric charge	$1\ C = 1\ A\ s$	Coulomb
ρ	Density	$1\ g\ cm^{-3}$	
ρ^*	Mass concentration	$1\ mg\ ml^{-1}$	
θ	Temperature	1 °C	Celsius
R	Electric resistance	$1\ \Omega = 1\ kg\ m^2\ s^{-3}\ A^{-2} = 1\ V\ A^{-1}$	Ohm
S	Entropy	$1\ J\ K^{-1}$	
U	Electric potential (voltage)	$1\ V = 1\ kg\ m^2\ s^{-3}\ A^{-1} = 1\ J\ A^{-1}\ s^{-1}$	Volt
V	Volume	1 l	
V_m	Molar volume	$1\ l\ mol^{-1}$	
v	Partial specific volume	$1\ ml\ g^{-1}$	
x	Molar ratio	1	

[a] Note that the molecular mass is the mass of one molecule given in atomic mass units. The molar mass is the mass of 1 mol of molecules and thus has the unit of g mol^{-1}.

Important physico-chemical constants.

Symbol	Constant	Value
c	Speed of light *in vacuo*	$2.99792458 \times 10^8\ m\ s^{-1}$
e	Elementary charge	$1.6021892 \times 10^{-19}\ C$
$\varepsilon_0 = (\mu_0\ c^2)^{-1}$	Electric field constant	$8.85418782 \times 10^{-12}\ A^2\ s^4\ m^{-3}\ kg^{-1}$
$F = N_A$	Faraday's constant	$9.648456 \times 10^4\ C\ mol^{-1}$
g	Earth's gravity near surface	$9.81\ m\ s^{-2}$
$g_e = 2\ \mu_e/\mu_B$	Landé factor of free electron	2.0023193134
γ_p	Gyromagnetic ratio of proton	$2.6751987 \times 10^8\ s^{-1}\ T^{-1}$
h	Planck's constant	$6.626176 \times 10^{-34}\ J\ s$
$k = k_B = R/N_A$	Boltzmann's constant	$1.380662 \times 10^{-23}\ J\ K^{-1}$
m_e	Mass of electron	$9.109534 \times 10^{-31}\ kg$
m_n	Mass of neutron	$1.6749543 \times 10^{-27}\ kg$
m_p	Mass of proton	$1.6726485 \times 10^{-27}\ kg$
μ_0	Magnetic field constant	$4\pi \times 10^{-7}\ m\ kg\ s^{-2}\ A^{-2}$
$\mu_B = eh/(4\pi m_e)$	Bohr magneton	$9.274078 \times 10^{-24}\ J\ T^{-1}$
μ_ε	Magnetic moment of electron	$9.284832 \times 10^{-24}\ J\ T^{-1}$
$\mu_N = eh/(4\pi m_p)$	Nuclear magneton	$5.050824 \times 10^{-27}\ J\ T^{-1}$
N_A, L	Avogadro's (Loschmidt's) constant	$6.022045 \times 10^{23}\ mol^{-1}$
p^0	Normal pressure	$1.01325 \times 10^5\ Pa$
R	Gas constant	$8.31441\ J\ K^{-1}\ mol^{-1}$
R_∞	Rydberg's constant	$1.097373177 \times 10^7\ m^{-1}$
θ_0	Zero at Celsius scale	273.15 K
$v^0 = RT^0/p^0$	Molar volume of an ideal gas	$22.41383\ l\ mol^{-1}$

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Conversion factors for energy.

	1 J	1 cal	1 eV
1 J	1	0.2390	$6.24150974 \times 10^{18}$
1 cal	4.184	1	2.612×10^{19}
1 eV	$1.60217646 \times 10^{-19}$	3.829×10^{-20}	1

Conversion factors for pressure.

	1 Pa	1 atm	1 mm Hg (Torr)	1 bar
1 Pa	1	9.869×10^{-6}	7.501×10^{-3}	10^{-5}
1 atm	1.013×10^{5}	1	760.0	1.013
1 mm Hg (Torr)	133.3	1.316×10^{-3}	1	1.333×10^{-3}
1 bar	10^{5}	0.9869	750.1	1

Molar masses of amino acids, free and within peptides.

Amino acid			M (g mol^{-1})	M – M(H_2O) (g mol^{-1})
A	Ala	Alanine	89	71
C	Cys	Cysteine	121	103
D	Asp	Aspartic acid	133	115
E	Glu	Glutamic acid	147	129
F	Phe	Phenylalanine	165	147
G	Gly	Glycine	75	57
H	His	Histidine	155	137
I	Ile	Isoleucine	131	113
K	Lys	Lysine	146	128
L	Leu	Leucine	131	113
M	Met	Methionine	149	131
N	Asn	Asparagine	132	114
P	Pro	Proline	115	97
Q	Gln	Glutamine	146	128
R	Arg	Arginine	174	156
S	Ser	Serine	105	87
T	Thr	Threonine	119	101
V	Val	Valine	117	99
W	Trp	Tryptophan	204	186
Y	Tyr	Tyrosine	181	163