

Contents

Preface V

1	Biochemical literature	1
1.1	Accessing the biochemical literature	1
1.1.1	Textbooks of biochemistry	2
1.1.2	Current reviews of the biochemical literature	3
1.1.3	The primary biochemical literature: scientific journals	4
1.2	Access to the methodologically-based biochemical literature	6
1.2.1	Monographs and series	6
1.2.2	Methods-based biochemical journals	6
1.3	Reference works and handbooks	7
1.3.1	Reference works	7
1.3.2	Handbooks and collected tables	8
1.4	Literature searches	9
1.4.1	Retrospective literature searches	9
1.4.2	Current literature searches	9
1.4.3	The Internet as an information resource	10
1.5	Documentation of practical work	10
1.5.1	The laboratory book	10
1.5.2	The layout of the laboratory book	11
1.6	Literature	11
2	General laboratory procedures	13
2.1	The biochemistry laboratory	13
2.1.1	Apparatus needed in every biochemistry laboratory	13
2.1.2	Apparatus that can be shared between several laboratories	14
2.1.3	Miscellaneous small items	15
2.1.4	Containers: glass, ceramic, metal and plastic in various sizes	15
2.1.5	Disposables	15
2.1.6	Safety equipment	16
2.1.7	Standard reagents	16
2.2	Routine biochemical procedures	17
2.2.1	Safety requirements	17

2.2.2	Cleaning of glass and plastic containers	19
2.2.3	Weighing out solids	20
2.2.4	Pipetting and measuring liquid volumes	20
2.2.5	Preparation and storage of solutions: water quality and the purity of chemical reagents	23
2.2.6	Thermostatting	24
2.2.7	Shaking and stirring	25
2.2.8	Use of pumps	26
2.2.9	Buffers	27
2.2.10	Supplementary reagents (preservatives, chelating agents, SH reagents and detergents)	31
2.2.11	pH determination	32
2.2.12	Conductivity measurements	33
2.3	Working with radioactivity	33
2.3.1	Radioactive isotopes and their decay	34
2.3.2	Measurement of radioactivity	36
2.3.3	Alternatives to radioactivity	43
2.4	Literature	46
3	Sample preparation	49
3.1	Cell and tissue disruption	49
3.1.1	General aspects of protein and nucleic acid isolation	50
3.1.2	Mechanical homogenisation	52
3.1.3	Non-mechanical homogenisation procedures	54
3.2	Solubilisation	55
3.3	Precipitation procedures for proteins and nucleic acids	56
3.3.1	Precipitation of proteins	56
3.3.2	Precipitation of nucleic acids	60
3.4	Dialysis, ultrafiltration and lyophilisation	61
3.4.1	Dialysis	61
3.4.2	Ultrafiltration	63
3.4.3	Lyophilisation	66
3.5	Literature	67
4	Separation methods	69
4.1	Chromatography	69
4.1.1	General principles and definitions	70
4.1.2	Column chromatography	70
4.1.3	Paper and thin layer chromatography	102
4.1.4	Gas–liquid chromatography	104
4.2	Electrophoresis	106
4.2.1	General principles and definitions	106
4.2.2	Cellulose acetate electrophoresis	109
4.2.3	Gel electrophoresis	110
4.2.4	Isoelectric focusing	124

4.2.5	2D electrophoresis	127
4.2.6	Blotting methods	129
4.2.7	Evaluation and documentation of electrophoresis results	131
4.2.8	Capillary electrophoresis	131
4.3	Centrifugation	137
4.3.1	Basic principles	138
4.3.2	Centrifuges and rotors	140
4.3.3	Analytical ultracentrifugation	145
4.3.4	Preparative centrifugation	149
4.4	Literature	151

5 Analytical methods 155

5.1	Protein analysis	155
5.1.1	Determination of protein molar masses	155
5.1.2	Quantitation of proteins	157
5.1.3	Amino acid analysis	162
5.1.4	End group determination	163
5.1.5	Edman degradation	164
5.1.6	Peptide mapping	166
5.1.7	Co- and post-translational modification	171
5.1.8	Chemical modification of proteins	173
5.1.9	Structural analysis of proteins	182
5.1.10	Protein stability	184
5.1.11	Peptide synthesis and in vitro protein synthesis	187
5.2	Nucleic acid analysis	190
5.2.1	Determination of nucleic acid concentration	190
5.2.2	Determination of nucleic acid size	191
5.2.3	Base composition	191
5.2.4	Restriction mapping	191
5.2.5	Detection of specific DNA and RNA sequences by Southern and Northern blotting	193
5.2.6	Detection of specific DNA and RNA sequences by the polymerase chain reaction (PCR)	194
5.2.7	Nucleic acid sequencing	199
5.2.8	Determination of the stability of double stranded nucleic acids	202
5.2.9	Oligonucleotide synthesis	204
5.2.10	Labelling and chemical modification of nucleic acids	207
5.3	Enzymatic analysis	208
5.3.1	Direct determination of metabolite concentrations	208
5.3.2	Determination of metabolite concentrations by coupled measurements	209
5.3.3	Determination of enzyme activity	210
5.4	Literature	212

6	Immunological methods	219
6.1	Antibodies	219
6.1.1	Antibody structure	220
6.1.2	Antibody production	221
6.1.3	Antibody purification	224
6.2	Antibody–antigen interactions	225
6.2.1	Antibody–antigen interactions in solution	226
6.2.2	Antibody–antigen interactions in gels	226
6.2.3	Radioimmunoassay	230
6.2.4	Enzyme-linked immunosorbent assay	230
6.2.5	Western blotting and dot blotting	234
6.2.6	Immunofluorescence and immunogold electron microscopy	235
6.2.7	Fluorescence activated cell sorting	235
6.3	Literature	236
7	Biophysical methods	239
7.1	Spectroscopy	239
7.1.1	Absorption of light	240
7.1.2	Spectrophotometers	243
7.1.3	Fluorescence	248
7.1.4	Vibrational spectroscopy	255
7.1.5	Anisotropic spectroscopy	256
7.1.6	Nuclear magnetic resonance spectroscopy	259
7.1.7	Mass spectrometry	264
7.2	Scattering techniques	267
7.2.1	Light-scattering in solution	267
7.2.2	Scattering with other radiation	273
7.3	Interactions	275
7.3.1	Equilibrium dialysis	275
7.3.2	Binding studies using filtration methods	276
7.3.3	Binding studies using chromatography, electrophoresis and centrifugation	277
7.3.4	Biomolecular interaction analysis	279
7.3.5	Binding studies using protection and interference	280
7.3.6	Calorimetry	281
7.3.7	Kinetics	283
7.4	Determination of structure	286
7.4.1	X-ray structural analysis	286
7.4.2	Structural databases	291
7.5	Literature	292
8	Mathematical Methods	295
8.1	Statistics	295
8.1.1	Observations and variables	296
8.1.2	Errors and mean values	297

8.1.3	Distributions	299
8.2	Quantitative evaluation of experimental results	305
8.2.1	Analysis of binding	306
8.2.2	Enzyme kinetics	307
8.3	Sequence analysis	313
8.3.1	Databases	314
8.3.3	Database searching	315
8.4	Literature	320

9 Quantitative Analysis of Biochemical Data 321

9.1	Introduction	321
9.1.1	General principles of quantitative data analysis	321
9.1.2	Experimental systems	322
9.1.3	Measurement and signals	323
9.1.4	Models	323
9.1.5	Selection of appropriate models	324
9.1.6	Parameters	325
9.1.7	Essential steps in the analysis	325
9.1.8	Fitting data by the method of least squares	326
9.1.9	Global fitting of multiple data sets	329
9.1.10	Introduction to error estimation	329
9.1.11	Introduction to numerical integration	331
9.2	Applications	332
9.2.1	Linear regression	332
9.2.2	Michaelis–Menten kinetics	332
9.2.3	Dissociation kinetics	334
9.2.4	Binding data	335
9.2.5	Independent identical binding sites	336
9.2.6	Analysis of simple binding data	337
9.2.7	Independent non-identical binding sites	337
9.2.8	Cooperative binding	338
9.2.9	Association kinetics	339
9.2.10	Pre-steady state kinetics	340
9.2.11	pH dependence of enzyme catalysed reactions	341
9.2.12	Analysis of competition experiments	343
9.3	Guide to the compact disc	344

Appendix I: SI-Units 345

Appendix II: Conversions into SI-Units 346

Index 347