

Table of Contents

Preface	XV	3.3 Catalysts	37
Introduction	XIX	3.4 Enzymes as Catalysts	37
		3.5 Rate of Enzyme-Controlled Reactions	38
		3.6 Michaelis–Menten Theory	38
		3.7 Determination of K_m and V_{max}	39
Part I Protein Analytics	1	3.8 Inhibitors	40
1 Protein Purification	3	3.8.1 Competitive Inhibitors	40
1.1 Properties of Proteins	3	3.8.2 Non-competitive Inhibitors	41
1.2 Protein Localization and Purification Strategy	6	3.9 Test System Set-up	41
1.3 Homogenization and Cell Disruption	7	3.9.1 Analysis of the Physiological Function	42
1.4 Precipitation	9	3.9.2 Selecting the Substrates	42
1.5 Centrifugation	11	3.9.3 Detection System	42
1.5.1 Basic Principles	12	3.9.4 Time Dependence	43
1.5.2 Centrifugation Techniques	12	3.9.5 pH Value	43
1.6 Removal of Salts and Hydrophilic Contaminants	15	3.9.6 Selecting the Buffer Substance and the Ionic Strength	43
1.7 Concentration	17	3.9.7 Temperature	44
1.8 Detergents and their Removal	18	3.9.8 Substrate Concentration	44
1.8.1 Properties of Detergents	18	3.9.9 Controls	45
1.8.2 Removal of Detergents	20	Further Reading	45
1.9 Sample Preparation for Proteome Analysis	22		
Further Reading	22	4 Microcalorimetry	47
2 Protein determination	23	4.1 Differential Scanning Calorimetry (DSC)	48
2.1 Quantitative Determination by Staining Tests	25	4.2 Isothermal Titration Calorimetry (ITC)	54
2.1.1 Biuret Assay	26	4.2.1 Ligand Binding to Proteins	54
2.1.2 Lowry Assay	26	4.2.2 Binding of Molecules to Membranes: Insertion and Peripheral Binding	58
2.1.3 Bicinchoninic Acid Assay (BCA Assay)	27	4.3 Pressure Perturbation Calorimetry (PPC)	61
2.1.4 Bradford Assay	28	Further Reading	62
2.2 Spectroscopic Methods	28		
2.2.1 Measurements in the UV Range	29	5 Immunological Techniques	63
2.2.2 Fluorescence Method	31	5.1 Antibodies	63
2.3 Radioactive Labeling of Peptides and Proteins	31	5.1.1 Antibodies and Immune Defense	63
2.3.1 Iodinations	33	5.1.2 Antibodies as Reagents	64
Further Reading	33	5.1.3 Properties of Antibodies	64
		5.1.4 Functional Structure of IgG	66
3 Enzyme Activity Testing	35	5.1.5 Antigen Interaction at the Combining Site	67
3.1 The Driving Force behind Chemical Reactions	35	5.1.6 Handling of Antibodies	68
3.2 Rate of Chemical Reactions	36	5.2 Antigens	69

VI Table of Contents

5.3	Antigen–Antibody Reaction	71	7.4.2	Molecular Vibrations	164
5.3.1	Immunoagglutination	72	7.4.3	Technical aspects of Infrared Spectroscopy	165
5.3.2	Immunoprecipitation	73	7.4.4	Infrared Spectra of Proteins	168
5.3.3	Immune Binding	84	7.5	Raman Spectroscopy	171
5.4	Complement Fixation	94	7.5.1	Basic Principles of Raman Spectroscopy	171
5.5	Methods in Cellular Immunology	95	7.5.2	Raman Experiments	172
5.6	Alteration of Biological Functions	97	7.5.3	Resonance Raman Spectroscopy	173
5.7	Production of Antibodies	98	7.6	Single Molecule Spectroscopy	174
5.7.1	Types of Antibodies	98	7.7	Methods using Polarized Light	175
5.7.2	New Antibody Techniques (<i>Antibody Engineering</i>)	99	7.7.1	Linear Dichroism	175
5.7.3	Optimized Monoclonal Antibody Constructs with Effector Functions for Therapeutic Application	102	7.7.2	Optical Rotation Dispersion and Circular Dichroism	178
5.8	Outlook: Future Expansion of the Binding Concepts	106		Further Reading	180
	Dedication	106	8	Light Microscopy Techniques – Imaging	181
	Further Reading	106	8.1	Steps on the Road to Microscopy – from Simple Lenses to High Resolution Microscopes	181
6	Chemical Modification of Proteins and Protein Complexes	107	8.2	Modern Applications	182
6.1	Chemical Modification of Protein Functional Groups	108	8.3	Basic Physical Principles	183
6.2	Modification as a Means to Introduce Reporter Groups	116	8.4	Detection Methods	189
6.2.1	Investigation with Naturally Occurring Proteins	116	8.5	Sample Preparation	195
6.2.2	Investigation of Recombinant and Mutated Proteins	120	8.6	Special Fluorescence Microscopic Analysis	197
6.3	Protein Crosslinking for the Analysis of Protein Interaction	121		Further Reading	205
6.3.1	Bifunctional Reagents	121	9	Cleavage of Proteins	207
6.3.2	Photoaffinity Labeling	121	9.1	Proteolytic Enzymes	207
	Further Reading	129	9.2	Strategy	208
7	Spectroscopy	131	9.3	Denaturation of Proteins	209
7.1	Physical Principles and Measuring Techniques	132	9.4	Cleavage of Disulfide Bonds and Alkylation	209
7.1.1	Physical Principles of Optical Spectroscopic Techniques	132	9.5	Enzymatic Fragmentation	210
7.1.2	Interaction of Light with Matter	133	9.5.1	Proteases	210
7.1.3	Absorption Measurement and the Lambert–Beer Law	140	9.5.2	Conditions for Proteolysis	215
7.1.4	Photometer	143	9.6	Chemical Fragmentation	216
7.1.5	Time-Resolved Spectroscopy	144	9.7	Summary	217
7.2	UV/VIS/NIR Spectroscopy	146		Further Reading	218
7.2.1	Basic Principles	146	10	Chromatographic Separation Methods	219
7.2.2	Chromoproteins	147	10.1	Instrumentation	219
7.3	Fluorescence Spectroscopy	154	10.2	Fundamental Terms and Concepts in Chromatography	220
7.3.1	Basic Principles of Fluorescence Spectroscopy	154	10.3	Biophysical Properties of Peptides and Proteins	224
7.3.2	Fluorescence: Emission and Action Spectra	156	10.4	Chromatographic Separation Modes for Peptides and Proteins	225
7.3.3	Fluorescence Studies using Intrinsic and Extrinsic Probes	157	10.4.1	High-Performance Size Exclusion Chromatography	227
7.3.4	Green Fluorescent Protein (GFP) as a Unique Fluorescent Probe	158	10.4.2	High-Performance Reversed-Phase Chromatography (HP-RPC)	227
7.3.5	Quantum Dots as Fluorescence Labels	159	10.4.3	High-Performance Normal-Phase Chromatography (HP-NPC)	228
7.3.6	Special Fluorescence Techniques: FRAP, FLIM, FCS, TIRF	160	10.4.4	High-Performance Hydrophilic Interaction Chromatography (HP-HILIC)	229
7.3.7	Förster Resonance Energy Transfer (FRET)	160	10.4.5	High-Performance Aqueous Normal Phase Chromatography (HP-ANPC)	230
7.3.8	Frequent Mistakes in Fluorescence Spectroscopy: “The Seven Sins of Fluorescence Measurements”	161	10.4.6	High-Performance Hydrophobic Interaction Chromatography (HP-HIC)	230
7.4	Infrared Spectroscopy	163	10.4.7	High-Performance Ion Exchange Chromatography (HP-IEX)	232
7.4.1	Basic Principles of IR Spectroscopy	163	10.4.8	High-Performance Affinity Chromatography (HP-AC)	233

10.5	Method Development from Analytical to Preparative Scale Illustrated for HP-RPC	234	12.3.3	Joule Heating	279
10.5.1	Development of an Analytical Method	234	12.3.4	Detection Methods	279
10.5.2	Scaling up to Preparative Chromatography	236	12.4	Capillary Electrophoresis Methods	281
10.5.3	Fractionation	237	12.4.1	Capillary Zone Electrophoresis (CZE)	281
10.5.4	Analysis of Fractionations	238	12.4.2	Affinity Capillary Electrophoresis (ACE)	285
10.6	Multidimensional HPLC	238	12.4.3	Micellar Electrokinetic Chromatography (MEKC)	286
10.6.1	Purification of Peptides and Proteins by MD-HPLC Methods	238	12.4.4	Capillary Electrochromatography (CEC)	288
10.6.2	Fractionation of Complex Peptide and Protein Mixtures by MD-HPLC	239	12.4.5	Chiral Separations	289
10.6.3	Strategies for MD-HPLC Methods	239	12.4.6	Capillary Gel Electrophoresis (CGE)	290
10.6.4	Design of an Effective MD-HPLC Scheme	240	12.4.7	Capillary Isoelectric Focusing (CIEF)	291
10.7	Final Remarks	242	12.4.8	Isotachopheresis (ITP)	293
	Further Reading	242	12.5	Special Techniques	295
11	Electrophoretic Techniques	243	12.5.1	Sample Concentration	295
11.1	Historical Review	244	12.5.2	Online Sample Concentration	295
11.2	Theoretical Fundamentals	245	12.5.3	Fractionation	296
11.3	Equipment and Procedures of Gel Electrophoreses	248	12.5.4	Microchip Electrophoresis	297
11.3.1	Sample Preparation	249	12.6	Outlook	297
11.3.2	Gel Media for Electrophoresis	250		Further Reading	299
11.3.3	Detection and Quantification of the Separated Proteins	251	13	Amino Acid Analysis	301
11.3.4	Zone Electrophoresis	253	13.1	Sample Preparation	302
11.3.5	Porosity Gradient Gels	254	13.1.1	Acidic Hydrolysis	302
11.3.6	Buffer Systems	255	13.1.2	Alkaline Hydrolysis	303
11.3.7	Disc Electrophoresis	255	13.1.3	Enzymatic Hydrolysis	303
11.3.8	Acidic Native Electrophoresis	257	13.2	Free Amino Acids	303
11.3.9	SDS Polyacrylamide Gel Electrophoresis	257	13.3	Liquid Chromatography with Optical Detection Systems	303
11.3.10	Cationic Detergent Electrophoresis	258	13.3.1	Post-Column Derivatization	303
11.3.11	Blue Native Polyacrylamide Gel Electrophoresis	259	13.3.2	Pre-column Derivatization	305
11.3.12	Isoelectric Focusing	259	13.4	Amino Acid Analysis using Mass Spectrometry	309
11.4	Preparative Techniques	263	13.5	Summary	310
11.4.1	Electroelution from Gels	263		Further Reading	311
11.4.2	Preparative Zone Electrophoresis	264	14	Protein Sequence Analysis	313
11.4.3	Preparative Isoelectric Focusing	265	14.1	N-Terminal Sequence Analysis: The Edman Degradation	315
11.5	Free Flow Electrophoresis	266	14.1.1	Reactions of the Edman Degradation	315
11.6	High-Resolution Two-Dimensional Electrophoresis	267	14.1.2	Identification of the Amino Acids	316
11.6.1	Sample Preparation	268	14.1.3	Quality of Edman Degradation: the Repetitive Yield	317
11.6.2	Prefractionation	268	14.1.4	Instrumentation	319
11.6.3	First Dimension: IEF in IPG Strips	269	14.1.5	Problems of Amino Acid Sequence Analysis	322
11.6.4	Second Dimension: SDS Polyacrylamide Gel Electrophoresis	270	14.1.6	State of the Art	325
11.6.5	Detection and Identification of Proteins	270	14.2	C-Terminal Sequence Analysis	325
11.6.6	Difference Gel Electrophoresis (DIGE)	270	14.2.1	Chemical Degradation Methods	325
11.7	Electroblotting	272	14.2.2	Peptide Quantities and Quality of the Chemical Degradation	327
11.7.1	Blot Systems	272	14.2.3	Degradation of Polypeptides with Carboxypeptidases	327
11.7.2	Transfer Buffers	273		Further Reading	328
11.7.3	Blot Membranes	273	15	Mass Spectrometry	329
	Further Reading	273	15.1	Ionization Methods	330
12	Capillary Electrophoresis	275	15.1.1	Matrix Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-MS)	330
12.1	Historical Overview	275	15.1.2	Electrospray Ionization (ESI)	335
12.2	Capillary Electrophoresis Setup	276	15.2	Mass Analyzer	341
12.3	Basic Principles of Capillary Electrophoresis	277	15.2.1	Time-of-Flight Analyzers (TOF)	343
12.3.1	Sample Injection	277			
12.3.2	The Engine: Electroosmotic Flow (EOF)	278			

[illegible]

24.4.5	Lipid Hormones and Intracellular Signaling Molecules	633	26.6	Isolation of RNA	676
24.5	Lipid Vitamins	638	26.6.1	Isolation of Cytoplasmic RNA	677
24.6	Lipidome Analysis	640	26.6.2	Isolation of Poly(A) RNA	678
24.7	Perspectives	642	26.6.3	Isolation of Small RNA	679
	Further Reading	644	26.7	Isolation of Nucleic Acids using Magnetic Particles	679
			26.8	Lab-on-a-chip	680
				Further Reading	680
25	Analysis of Post-translational Modifications: Phosphorylation and Acetylation of Proteins	645	27	Analysis of Nucleic Acids	681
25.1	Functional Relevance of Phosphorylation and Acetylation	645	27.1	Restriction Analysis	681
25.1.1	Phosphorylation	645	27.1.1	Principle of Restriction Analyses	681
25.1.2	Acetylation	646	27.1.2	Historical Overview	682
25.2	Strategies for the Analysis of Phosphorylated and Acetylated Proteins and Peptides	647	27.1.3	Restriction Enzymes	682
25.3	Separation and Enrichment of Phosphorylated and Acetylated Proteins and Peptides	649	27.1.4	<i>In Vitro</i> Restriction and Applications	685
25.4	Detection of Phosphorylated and Acetylated Proteins and Peptides	651	27.2	Electrophoresis	690
25.4.1	Detection by Enzymatic, Radioactive, Immunochemical, and Fluorescence Based Methods	651	27.2.1	Gel Electrophoresis of DNA	691
25.4.2	Detection of Phosphorylated and Acetylated Proteins by Mass Spectrometry	653	27.2.2	Gel Electrophoresis of RNA	697
25.5	Localization and Identification of Post-translationally Modified Amino Acids	653	27.2.3	Pulsed-Field Gel Electrophoresis (PFGE)	698
25.5.1	Localization of Phosphorylated and Acetylated Amino Acids by Edman Degradation	654	27.2.4	Two-Dimensional Gel Electrophoresis	700
25.5.2	Localization of Phosphorylated and Acetylated Amino Acids by Tandem Mass Spectrometry	654	27.2.5	Capillary Gel Electrophoresis	701
25.6	Quantitative Analysis of Post-translational Modifications	659	27.3	Staining Methods	702
25.7	Future of Post-translational Modification Analysis	661	27.3.1	Fluorescent Dyes	702
	Further Reading	661	27.3.2	Silver Staining	704
			27.4	Nucleic Acid Blotting	704
			27.4.1	Nucleic Acid Blotting Methods	704
			27.4.2	Choice of Membrane	704
			27.4.3	Southern Blotting	705
			27.4.4	Northern Blotting	706
			27.4.5	Dot- and Slot-Blotting	707
			27.4.6	Colony and Plaque Hybridization	707
			27.5	Isolation of Nucleic Acid Fragments	708
			27.5.1	Purification using Glass Beads	708
			27.5.2	Purification using Gel Filtration or Reversed Phase	708
			27.5.3	Purification using Electroelution	708
			27.5.4	Other Methods	709
			27.6	LC-MS of Oligonucleotides	709
			27.6.1	Principles of the Synthesis of Oligonucleotides	709
			27.6.2	Investigation of the Purity and Characterization of Oligonucleotides	711
			27.6.3	Mass Spectrometric Investigation of Oligonucleotides	712
			27.6.4	IP-RP-HPLC-MS Investigation of a Phosphorothioate Oligonucleotide	714
				Further Reading	717
			28	Techniques for the Hybridization and Detection of Nucleic Acids	719
			28.1	Basic Principles of Hybridization	720
			28.1.1	Principle and Practice of Hybridization	721
			28.1.2	Specificity of the Hybridization and Stringency	722
			28.1.3	Hybridization Methods	723
			28.2	Probes for Nucleic Acid Analysis	729
			28.2.1	DNA Probes	730
			28.2.2	RNA Probes	731
			28.2.3	PNA Probes	732
			28.2.4	LNA Probes	732
			28.3	Methods of Labeling	733
			28.3.1	Labeling Positions	733
Part IV Nucleic Acid Analytics	663				
26	Isolation and Purification of Nucleic Acids	665			
26.1	Purification and Determination of Nucleic Acid Concentration	665			
26.1.1	Phenolic Purification of Nucleic Acids	665			
26.1.2	Gel Filtration	666			
26.1.3	Precipitation of Nucleic Acids with Ethanol	667			
26.1.4	Determination of the Nucleic Acid Concentration	668			
26.2	Isolation of Genomic DNA	669			
26.3	Isolation of Low Molecular Weight DNA	670			
26.3.1	Isolation of Plasmid DNA from Bacteria	670			
26.3.2	Isolation of Eukaryotic Low Molecular Weight DNA	674			
26.4	Isolation of Viral DNA	674			
26.4.1	Isolation of Phage DNA	674			
26.4.2	Isolation of Eukaryotic Viral DNA	675			
26.5	Isolation of Single-Stranded DNA	676			
26.5.1	Isolation of M13 Phage DNA	676			
26.5.2	Separation of Single- and Double-Stranded DNA	676			

28.3.2	Enzymatic Labeling	735	30.2	Gel-Free DNA Sequencing Methods – The Next Generation	806
28.3.3	Photochemical Labeling Reactions	737	30.2.1	Sequencing by Synthesis	807
28.3.4	Chemical Labeling	737	30.2.2	Single Molecule Sequencing	813
28.4	Detection Systems	738		Further Reading	815
28.4.1	Staining Methods	738	31	Analysis of Epigenetic Modifications	817
28.4.2	Radioactive Systems	738	31.1	Overview of the Methods to Detect DNA-Modifications	818
28.4.3	Non-radioactive Systems	739	31.2	Methylation Analysis with the Bisulfite Method	819
28.5	Amplification Systems	750	31.2.1	Amplification and Sequencing of Bisulfite-Treated DNA	819
28.5.1	Target Amplification	751	31.2.2	Restriction Analysis after Bisulfite PCR	820
28.5.2	Target-Specific Signal Amplification	751	31.2.3	Methylation Specific PCR	822
28.5.3	Signal Amplification	752	31.3	DNA Analysis with Methylation Specific Restriction Enzymes	823
	Further Reading	753	31.4	Methylation Analysis by Methylcytosine-Binding Proteins	825
29	Polymerase Chain Reaction	755	31.5	Methylation Analysis by Methylcytosine-Specific Antibodies	826
29.1	Possibilities of PCR	755	31.6	Methylation Analysis by DNA Hydrolysis and Nearest Neighbor-Assays	827
29.2	Basics	756	31.7	Analysis of Epigenetic Modifications of Chromatin	828
29.2.1	Instruments	756	31.8	Chromosome Interaction Analyses	828
29.2.2	Amplification of DNA	758	31.9	Outlook	829
29.2.3	Amplification of RNA (RT-PCR)	761		Further Reading	829
29.2.4	Optimizing the Reaction	763	32	Protein–Nucleic Acid Interactions	831
29.2.5	Quantitative PCR	763	32.1	DNA–Protein Interactions	831
29.3	Special PCR Techniques	766	32.1.1	Basic Features for DNA–Protein Recognition: Double-Helical Structures	831
29.3.1	Nested PCR	766	32.1.2	DNA Curvature	832
29.3.2	Asymmetric PCR	767	32.1.3	DNA Topology	833
29.3.3	Use of Degenerate Primers	767	32.2	DNA-Binding Motifs	835
29.3.4	Multiplex PCR	767	32.3	Special Analytical Methods	836
29.3.5	Cycle sequencing	768	32.3.1	Filter Binding	836
29.3.6	<i>In Vitro</i> Mutagenesis	768	32.3.2	Gel Electrophoresis	836
29.3.7	Homogeneous PCR Detection Procedures	768	32.3.3	Determination of Dissociation Constants	839
29.3.8	Quantitative Amplification Procedures	769	32.3.4	Analysis of DNA–Protein Complex Dynamics	840
29.3.9	<i>In Situ</i> PCR	769	32.4	DNA Footprint Analysis	841
29.3.10	Other Approaches	769	32.4.1	DNA Labeling	843
29.4	Contamination Problems	770	32.4.2	Primer Extension Reaction for DNA Analysis	843
29.4.1	Avoiding Contamination	770	32.4.3	Hydrolysis Methods	844
29.4.2	Decontamination	771	32.4.4	Chemical Reagents for the Modification of DNA–Protein Complexes	846
29.5	Applications	772	32.4.5	Interference Conditions	848
29.5.1	Detection of Infectious Diseases	772	32.4.6	Chemical Nucleases	849
29.5.2	Detection of Genetic Defects	773	32.4.7	Genome-Wide DNA–Protein Interactions	850
29.5.3	The Human Genome Project	776	32.5	Physical Analysis Methods	851
29.6	Alternative Amplification Procedures	777	32.5.1	Fluorescence Methods	851
29.6.1	Nucleic Acid Sequence-Based Amplification (NASBA)	777	32.5.2	Fluorophores and Labeling Procedures	851
29.6.2	Strand Displacement Amplification (SDA)	777	32.5.3	Fluorescence Resonance Energy Transfer (FRET)	852
29.6.3	Helicase-Dependent Amplification (HDA)	777	32.5.4	Molecular Beacons	853
29.6.4	Ligase Chain Reaction (LCR)	779	32.5.5	Surface Plasmon Resonance (SPR)	853
29.6.5	Q β Amplification	780	32.5.6	Scanning Force Microscopy (SFM)	854
29.6.6	Branched DNA Amplification (bDNA)	782	32.5.7	Optical Tweezers	855
29.7	Prospects	782	32.5.8	Fluorescence Correlation Spectroscopy (FCS)	856
	Further Reading	782	32.6	RNA–Protein Interactions	856
30	DNA Sequencing	785			
30.1	Gel-Supported DNA Sequencing Methods	786			
30.1.1	Sequencing according to Sanger: The Dideoxy Method	789			
30.1.2	Labeling Techniques and Methods of Verification	796			
30.1.3	Chemical Cleavage according to Maxam and Gilbert	800			

32.6.1	Functional Diversity of RNA	856	34.1.1	Overview	895
32.6.2	RNA Secondary Structure Parameters and unusual Base Pairs	857	34.1.2	Nuclease S1 Analysis of RNA	896
32.6.3	Dynamics of RNA–Protein Interactions	857	34.1.3	Ribonuclease-Protection Assay (RPA)	898
32.7	Characteristic RNA-Binding Motifs	859	34.1.4	Primer Extension Assay	901
32.8	Special Methods for the Analysis of RNA–Protein Complexes	860	34.1.5	Northern Blot and Dot- and Slot-Blot	902
32.8.1	Limited Enzymatic Hydrolyses	861	34.1.6	Reverse Transcription Polymerase Chain Reaction (RT-PCR and RT-qPCR)	904
32.8.2	Labeling Methods	861	34.2	Analysis of RNA Synthesis <i>In Vivo</i>	905
32.8.3	Primer Extension Analysis of RNA	862	34.2.1	Nuclear-run-on Assay	905
32.8.4	Customary RNases	862	34.2.2	Labeling of Nascent RNA with 5-Fluoro-uridine (FURd)	906
32.8.5	Chemical Modification of RNA–Protein Complexes	863	34.3	<i>In Vitro</i> Transcription in Cell-Free Extracts	907
32.8.6	Chemical Crosslinking	866	34.3.1	Components of an <i>In Vitro</i> Transcription Assay	907
32.8.7	Incorporation of Photoreactive Nucleotides	867	34.3.2	Generation of Transcription-Competent Cell Extracts and Protein Fractions	908
32.8.8	Genome-Wide Identification of Transcription Start Sites (TSS)	867	34.3.3	Template DNA and Detection of <i>In Vitro</i> Transcripts	908
32.9	Genetic Methods	868	34.4	<i>In Vivo</i> Analysis of Promoter Activity in Mammalian Cells	911
32.9.1	Tri-hybrid Method	868	34.4.1	Vectors for Analysis of Gene-Regulatory cis-Elements	911
32.9.2	Aptamers and the Selex Procedure	869	34.4.2	Transfer of DNA into Mammalian Cells	912
32.9.3	Directed Mutations within Binding Domains	870	34.4.3	Analysis of Reporter Gene Expression	914
	Further Reading	870		Further Reading	916

Part V Functional and Systems Analytics

873

33	Sequence Data Analysis	875	35	Fluorescent <i>In Situ</i> Hybridization in Molecular Cytogenetics	917
33.1	Sequence Analysis and Bioinformatics	875	35.1	Methods of Fluorescent DNA Hybridization	917
33.2	Sequence: An Abstraction for Biomolecules	876	35.1.1	Labeling Strategy	917
33.3	Internet Databases and Services	877	35.1.2	DNA Probes	918
33.3.1	Sequence Retrieval from Public Databases	878	35.1.3	Labeling of DNA Probes	918
33.3.2	Data Contents and File Format	879	35.1.4	<i>In Situ</i> Hybridization	919
33.3.3	Nucleotide Sequence Management in the Laboratory	881	35.1.5	Evaluation of Fluorescent Hybridization Signals	920
33.4	Sequence Analysis on the Web	881	35.2	Application: FISH and CGH	920
33.4.1	EMBOSS	881	35.2.1	FISH Analysis of Genomic DNA	920
33.5	Sequence Composition	882	35.2.2	Comparative Genomic Hybridization (CGH)	921
33.6	Sequence Patterns	882		Further Reading	924
33.6.1	Transcription Factor Binding Sites	884	36	Physical and Genetic Mapping of Genomes	925
33.6.2	Identification of Coding Regions	885	36.1	Genetic Mapping: Localization of Genetic Markers within the Genome	925
33.6.3	Protein Localization	886	36.1.1	Recombination	925
33.7	Homology	887	36.1.2	Genetic Markers	927
33.7.1	Identity, Similarity, Homology	887	36.1.3	Linkage Analysis – the Generation of Genetic Maps	929
33.7.2	Optimal Sequence Alignment	888	36.1.4	Genetic Map of the Human Genome	931
33.7.3	Alignment for Fast Database Searches: BLAST	890	36.1.5	Genetic Mapping of Disease Genes	932
33.7.4	Profile-Based Sensitive Database Search: PSI-BLAST	890	36.2	Physical Mapping	932
33.7.5	Homology Threshold	891	36.2.1	Restriction Mapping of Whole Genomes	932
33.8	Multiple Alignment and Consensus Sequences	891	36.2.2	Mapping of Recombinant Clones	934
33.9	Structure Prediction	892	36.2.3	Generation of a Physical Map	935
33.10	Outlook	893	36.2.4	Identification and Isolation of Genes	937
34	Analysis of Promoter Strength and Nascent RNA Synthesis	895	36.2.5	Transcription Maps of the Human Genome	939
34.1	Methods for the Analysis of RNA Transcripts	895	36.2.6	Genes and Hereditary Disease – Search for Mutations	940
			36.3	Integration of Genome Maps	940

36.4	The Human Genome	942	39.4.1	Two-Dimensional-Gel-Based Proteomics	982
	Further Reading	942	39.4.2	Two-Dimensional Differential Gel Electrophoresis (2D DIGE)	986
37	DNA-Microarray Technology	945	39.4.3	Top-Down Proteomics using Isotope Labels	986
37.1	RNA Analyses	946	39.4.4	Top-Down Proteomics using Intact Protein Mass Spectrometry	987
37.1.1	Transcriptome Analysis	946	39.4.5	Concepts in Intact Protein Mass Spectrometry	987
37.1.2	RNA Splicing	947	39.5	Peptide Based Quantitative Proteome Analysis (Bottom-Up Proteomics)	998
37.1.3	RNA Structure and Functionality	947	39.5.1	Introduction	998
37.2	DNA Analyses	948	39.5.2	Bottom-Up Proteomics	998
37.2.1	Genotyping	948	39.5.3	Complexity of the Proteome	1000
37.2.2	Methylation Studies	948	39.5.4	Bottom-Up Proteomic Strategies	1000
37.2.3	DNA Sequencing	949	39.5.5	Peptide Quantification	1001
37.2.4	Comparative Genomic Hybridization (CGH)	951	39.5.6	Data Dependent Analysis (DDA)	1002
37.2.5	Protein–DNA Interactions	951	39.5.7	Selected Reaction Monitoring	1003
37.3	Molecule Synthesis	952	39.5.8	SWATH-MS	1010
37.3.1	DNA Synthesis	952	39.5.9	Summary	1012
37.3.2	RNA Production	953	39.5.10	Extensions	1012
37.3.3	On-Chip Protein Expression	953	39.6	Stable Isotope Labeling in Quantitative Proteomics	1013
37.4	Other Approaches	954	39.6.1	Stable Isotope Label in Top-Down Proteomics	1013
37.4.1	Barcode Identification	954	39.6.2	Stable Isotope Labeling in Bottom-Up Proteomics	1019
37.4.2	A Universal Microarray Platform	955		Further Reading	1021
37.5	New Avenues	956	40	Metabolomics and Peptidomics	1023
37.5.1	Structural Analyses	956	40.1	Systems Biology and Metabolomics	1025
37.5.2	Beyond Nucleic Acids	956	40.2	Technological Platforms for Metabolomics	1026
	Further Reading	957	40.3	Metabolomic <i>Profiling</i>	1027
38	The Use of Oligonucleotides as Tools in Cell Biology	959	40.4	Peptidomics	1028
38.1	Antisense Oligonucleotides	960	40.5	Metabolomics – <i>Knowledge Mining</i>	1029
38.1.1	Mechanisms of Antisense Oligonucleotides	960	40.6	Data Mining	1030
38.1.2	Triplex-Forming Oligonucleotides	961	40.7	Fields of Application	1032
38.1.3	Modifications of Oligonucleotides to Decrease their Susceptibility to Nucleases	962	40.8	Outlook	1032
38.1.4	Use of Antisense Oligonucleotides in Cell Culture and in Animal Models	964		Further Reading	1032
38.1.5	Antisense Oligonucleotides as Therapeutics	964	41	Interactomics – Systematic Protein–Protein Interactions	1033
38.2	Ribozymes	965	41.1	Protein Microarrays	1033
38.2.1	Discovery and Classification of Ribozymes	965	41.1.1	Sensitivity Increase through Miniaturization – Ambient Analyte Assay	1034
38.2.2	Use of Ribozymes	966	41.1.2	From DNA to Protein Microarrays	1035
38.3	RNA Interference and MicroRNAs	967	41.1.3	Application of Protein Microarrays	1037
38.3.1	Basics of RNA Interference	967		Further Reading	1039
38.3.2	RNA Interference Mediated by Expression Vectors	968	42	Chemical Biology	1041
38.3.3	Uses of RNA Interference	969	42.1	Chemical Biology – Innovative Chemical Approaches to Study Biological Phenomena	1041
38.3.4	microRNAs	970	42.2	Chemical Genetics – Small Organic Molecules for the Modulation of Protein Function	1043
38.4	Aptamers: High-Affinity RNA- and DNA-Oligonucleotides	971	42.2.1	Study of Protein Functions with Small Organic Molecules	1044
38.4.1	Selection of Aptamers	971	42.2.2	Forward and Reverse Chemical Genetics	1046
38.4.2	Uses of Aptamers	973	42.2.3	The Bump-and-Hole Approach of Chemical Genetics	1047
38.5	Genome Editing with CRISPR/Cas9	974	42.2.4	Identification of Kinase Substrates with ASKA Technology	1050
38.6	Outlook	975			
	Further Reading	976			
39	Proteome Analysis	977			
39.1	General Aspects in Proteome Analysis	977			
39.2	Definition of Starting Conditions and Project Planning	979			
39.3	Sample Preparation for Proteome Analysis	980			
39.4	Protein Based Quantitative Proteome Analysis (Top-Down Proteomics)	982			

42.2.5	Switching Biological Systems on and off with Small Organic Molecules	1051	43.2.3	Achievable Spatial Resolution	1065
42.3	Expressed Protein Ligation – Symbiosis of Chemistry and Biology for the Study of Protein Functions	1052	43.2.4	SIMS, ME-SIMS, and Cluster SIMS Imaging: Enhancing the Mass Range	1067
42.3.1	Analysis of Lipid-Modified Proteins	1052	43.2.5	Lateral Resolution and Analytical Limit of Detection	1067
42.3.2	Analysis of Phosphorylated Proteins	1054	43.2.6	Coarse Screening by MS Imaging	1068
42.3.3	Conditional Protein Splicing	1054	43.2.7	Accurate MALDI Mass Spectrometry Imaging	1068
	Further Reading	1055	43.2.8	Identification and Characterization of Analytes	1069
				Further Reading	1070
43	Toponome Analysis	1057	Appendix 1: Amino Acids and Posttranslational Modifications		1073
	“Life is Spatial”	1057			
43.1	Antibody Based Toponome Analysis using Imaging Cycler Microscopy (ICM)	1057	Appendix 2: Symbols and Abbreviations		1075
43.1.1	Concept of the Protein Toponome	1058			
43.1.2	Imaging Cycler Robots: Fundament of a Toponome Reading Technology	1059	Appendix 3: Standard Amino Acids (three and one letter code)		1081
43.1.3	Summary and Outlook	1063			
	Acknowledgements	1063	Appendix 4: Nucleic Acid Bases		1083
43.2	Mass Spectrometry Imaging	1064			
43.2.1	Analytical Microprobes	1064	Index		1085
43.2.2	Mass Spectrometric Pixel Images	1064			