Contents

List of Figu	ıres			xix
List of Tab	oles			xxix
List of Box	kes			xxxi
Preface	face xx anges in Second Edition xx nowledgments hors x sical Quantities and Constants x of Acronyms xl apter 1 Introduction 1.1 IMPORTANCE OF PROTEINS IN LIVING ORGANISMS 1.1.1 Life, proteins and mysterious forces 1.1.2 Molecular organization of living organisms	xxxii		
Changes i	n Second	d Edition		xxxi
Acknowle	dgments			xli
Authors				xliii
Physical C	Quantities	s and Cons	tants	xlv
List of Acr	onyms			xlvii
Chapter	1 • Int	roduction		1
1.1	IMPOR	TANCE OF	PROTEINS IN LIVING ORGANISMS	1
	1.1.1	Life, pro	teins and mysterious forces	1
	1.1.2	Molecula	ar organization of living organisms	2
	1.1.3	Proteins	have numerous biological roles	6
		1.1.3.1	Catalysis of metabolic processes	6
		1.1.3.2	Energy transfer	8
		1.1.3.3	Gene expression	11
		1.1.3.4	Transport of solutes across biological membranes	13
		1.1.3.5	Cellular communication	13
		1.1.3.6	Molecular recognition	16
		1.1.3.7	Defense	17
		1.1.3.8	Forming intracellular and extracellular structures	19
		1.1.3.9	Cell- and tissue-specific functions	20
	1.1.4	Physiolo	gical and evolutionary importance of proteins	22
	1.1.5	Medical,	industrial, and social importance of proteins	22
		1.1.5.1	Proteins as drug targets	22
		1.1.5.2	Proteins as toxin targets	23
		1.1.5.3	Industrial applications of proteins	24
1.2	STRUC	TURAL CC	MPLEXITY AND ITS EFFECT ON PROTEIN FUNCTION	25

1.3	NONC	OVALENT	INTERACTIONS BETWEEN ATOMS IN BIOMOLECULES	29
	1.3.1	Electrost	tatic interactions	31
		1.3.1.1	Introduction	31
		1.3.1.2	Basic principles	32
		1.3.1.3	Hydrogen bonds	44
		1.3.1.4	Other types of electrostatic interactions	45
	1.3.2	Van der	Waals interactions	50
	1.3.3	Nonpola	r interactions and hydrophobic effect	53
	1.3.4	Conclusi	ions	55
1.4	SUMM	ARY		56
1.5	ORGAN	NIZATION	OF BOOK	56
EXERO	CISES			57
REFER	RENCES			57
Chapter	2 • Pro	otein Struc	ture	65
2.1	INTRO	DUCTION		65
2.1	2.1.1		y in protein structure	65
	2.1.2		nes and prosthetic groups	66
2.2		RY STRUC		71
	2.2.1	Amino a	cids and their properties	72
		2.2.1.1	Amino acid structure	72
		2.2.1.2	Configurations of amino acids	77
		2.2.1.3	Side chain properties	79
		2.2.1.4	Amino acid derivates in proteins	104
	2.2.2	Peptide l	oond	110
2.3	SECON	DARY STR	UCTURE	113
	2.3.1	α-helix		122
		2.3.1.1	Geometry	122
		2.3.1.2	Intramolecular interactions	122
		2.3.1.3	Amphipathic α -helices	123
	2.3.2	Non-α-h	relices	124
		2.3.2.1	3 ₁₀ -helix	124
		2.3.2.2	π -helix	125
		2.3.2.3	Type II polyproline helix (PPII)	126
	2.3.3	β confor	mation	129
	2.3.4	Why hel	ices and sheets?	130

	2.3.5	Reverse t	turns	133	
		2.3.5.1	eta-turn	133	
		2.3.5.2	Loops	134	
	2.3.6	Secondar	ry structure preferences of amino acids	135	
		2.3.6.1	α -helix	135	
		2.3.6.2	β conformation	137	
2.4	TERTIA	RY STRUC	TURE	139	
	2.4.1	Basic pro	operties of tertiary structure	141	
		2.4.1.1	Structural properties required for complex function	141	
		2.4.1.2	Core versus surface	141	
		2.4.1.3	Stabilizing forces	143	
	2.4.2	Architec	ture of proteins	143	
		2.4.2.1	Simple folding motifs	143	
		2.4.2.2	Complex folds	150	
		2.4.2.3	Domains	161	
		2.4.2.4	Protein classification	167	
		2.4.2.5	Knotted proteins	173	
	2.4.3	Evolution	nary conservation of structure and function in proteins	174	
		2.4.3.1	Interests of individual versus those of species	174	
		2.4.3.2	Structure conservation: evolutionary mechanisms	176	
		2.4.3.3	Evolution of function	179	
	2.4.4	Water m	olecules inside proteins	180	
2.5	QUATERNARY STRUCTURE				
	2.5.1	Introduction			
	2.5.2	Characte	eristics	183	
		2.5.2.1	Dimensions and complexity	183	
		2.5.2.2	Symmetry	183	
		2.5.2.3	Subunit interactions	185	
	2.5.3	Advantag	ges of quaternary structure	186	
2.6	POST-T	RANSLATI	ONAL MODIFICATIONS	188	
	2.6.1	Introduc	tion	188	
	2.6.2	Phospho	rylation	191	
	2.6.3	Glycosyl	ation	193	
	2.6.4	Acylation	n	195	
		2.6.4.1	ε - N -acetylation	195	
		2.6.4.2	N'-myristoylation and S -palmitoylation	196	
		2.6.4.3	Ubiquitination and SUMOylation	197	

		2.6.5	Alkylatio	n	198
			2.6.5.1	Methylation	198
			2.6.5.2	S-prenylation	199
			2.6.5.3	Adenylation	199
		2.6.6	Hydroxyl	lation and oxidation	199
		2.6.7	Proteolys	is	200
		2.6.8	Amidatio	on	200
		2.6.9	Addition	of metal ions	200
			2.6.9.1	Stabilization of protein structure	201
			2.6.9.2	Ligand binding	201
			2.6.9.3	Electron transport	201
			2.6.9.4	Enzymatic catalysis	202
		2.6.10	Mixed m	odifications	204
		2.6.11	Patholog	ical aspects of post-translational modifications	205
			2.6.11.1	Cancer	205
			2.6.11.2	Age-related illnesses	207
		2.6.12	Identifyii	ng post-translational modifications	208
	2.7	FIBROU	JS PROTEII	NS	209
		2.7.1	Fiber-bas	sed structures inside and outside cells	209
			2.7.1.1	Mechanical support	209
			2.7.1.2	Tissue organization and cell-environment communication	214
			2.7.1.3	Motion	216
			2.7.1.4	External structures	218
			2.7.1.5	Other roles	219
		2.7.2	Fiber-for	ming versus fibrous proteins	221
		2.7.3	Structura	ll differences between globular and fibrous proteins	221
		2.7.4	Structure collagen	e-function relationships in helical proteins α -keratin and	223
			2.7.4.1	α-Keratin	223
			2.7.4.2	Collagen	224
	2.8	SUMMA			232
	EXERO	CISES			233
		ENCES			235
Ch	napter	3 ■ Me	thods of S	tructure Determination and Prediction	259
	3.1	INTROI	DUCTION		259
	3.2	DIFFRACTION AND SCATTERING METHODS			260

	3.2.1	X-ray di	ffraction and scattering	261
		3.2.1.1	Principles	261
		3.2.1.2	Steps of procedure	262
		3.2.1.3	Information obtained from crystallography	263
		3.2.1.4	Problems of method	266
		3.2.1.5	X-ray scattering	267
	3.2.2	Neutron	scattering	270
		3.2.2.1	Principles	270
		3.2.2.2	Advantages and shortcomings	272
	3.2.3	Electron	microscopy (EM)	273
		3.2.3.1	Principles	273
		3.2.3.2	Advantages and shortcomings	277
3.3	SPECTI	ROSCOPIC	EMETHODS	278
	3.3.1	Nuclear	magnetic resonance (NMR) spectroscopy	278
		3.3.1.1	Principles	278
		3.3.1.2	Steps in protein structure determination by NMR spectroscopy	280
		3.3.1.3	Advantages and shortcomings	282
	3.3.2	Electron	paramagnetic resonance (EPR) spectroscopy	283
	3.3.3	Informat	tion derived from other methods	284
		3.3.3.1	Fluorescent spectroscopy	284
		3.3.3.2	Circular dichroism spectroscopy	285
		3.3.3.3	Mass spectrometry	286
3.4	COMP	UTATIONA	AL METHODS FOR STRUCTURE PREDICTION	291
	3.4.1	Introduc	ction	291
	3.4.2	Ab initio	(physical) approach	292
		3.4.2.1	Calculating total potential energy of system	292
		3.4.2.2	Sampling configurational space of system	294
		3.4.2.3	Limitations and partial solutions	297
	3.4.3	Template	e-based (comparative) approach	307
		3.4.3.1	Introduction	307
		3.4.3.2	Homology modeling	308
		3.4.3.3	Fold recognition via threading	315
	3.4.4	Integrati	ve and fragment-based methods	317
	3.4.5	Prediction	on assessment and verification	324
3.5	EXPERI	MENTALLY	GUIDED COMPUTATIONAL PREDICTION	325
	3.5.1	Introduc	ction	325

	3.5.2	Applicati	ons and tools	326
3.6	CONCI	LUSIONS		329
3.7	PROTE	IN DATA B	ANK (PDB)	329
3.8	SUMM	ARY		333
EXERO	CISES			334
REFER	RENCES			335
Chapter	4 ■ Ene	ergetics an	d Protein Stability	355
4.1	BASIC I	PRINCIPLE:	S OF THERMODYNAMICS	355
	4.1.1	Introduc	tion	355
	4.1.2	Free ener	gy and spontaneous processes	356
	4.1.3	Enthalpy	, entropy, and molecular thermodynamics	358
		4.1.3.1	Enthalpy	358
		4.1.3.2	Entropy	363
		4.1.3.3	Computational approaches focus on individual interactions	364
	4.1.4	Thermod	lynamics and protein structure	365
4.2	PROTE	in stabili	TY AND FORCES INVOLVED	365
	4.2.1	How stab	ple are proteins?	365
	4.2.2	Dominar	nt driving forces	366
		4.2.2.1	Nonpolar interactions (ΔG_{np})	367
		4.2.2.2	Configurational entropy effect $(-T\Delta S_{con})$	369
	4.2.3	Electrost	atic interactions (ΔG_{elec})	371
	4.2.4	van der V	Vaals interactions (ΔG_{vdW})	375
	4.2.5	Summar	y and conclusions	375
4.3	PROTE	in denatu	URATION AND ADAPTATION TO EXTREME CONDITIONS	377
	4.3.1	Denatura	ation as experimental tool	377
		4.3.1.1	Temperature-dependent denaturation	378
		4.3.1.2	pH-dependent denaturation	379
		4.3.1.3	Pressure-induced denaturation	379
		4.3.1.4	Chemical denaturation	379
	4.3.2	Adaptatio	on of proteins to extreme environments	380
	4.3.3	Conclusi	ons	382
4.4		ity enhan eering	NCEMENT OF INDUSTRIAL ENZYMES USING PROTEIN	383
	4.4.1	Enzymes	in industry	383
	4.4.2	•	engineering	384
	4.4.3	·	engineering of enzymes for increased stability	384
			· · · · · · · · · · · · · · · · · · ·	

4.5	SUMM	ARY		387
EXER	S Protein Dynamics INTRODUCTION PROTEIN FOLDING 5.2.1 Kinetic aspects 5.2.1.1 Levinthal's paradox and energy landscape theory 5.2.1.2 Folding models and mechanisms 5.2.2 In vivo folding 5.2.2.1 In vivo factors that complicate folding 5.2.2.2 Assisted folding FOLDED STATE DYNAMICS 5.3.1 Spontaneous dynamics 5.3.1.1 Proteins are conformational ensembles 5.3.1.2 Statistical-thermodynamic view of protein dynamics 5.3.1.3 Dynamics of disordered proteins 5.3.1.4 Biological significance of thermally induced conformational changes 5.3.1.5 Effects on protein dynamics 5.3.2 External effects on protein dynamics 5.3.2.1 Ligand-induced dynamics and allostery 5.3.2.2 Dynamics induced by environmental changes 5.3.2.3 Enzyme-mediated protein dynamics METHODS FOR STUDYING PROTEIN DYNAMICS 5.4.1 Tools for studying slow (ms-sec) to intermediate (ns-µs) motions 5.4.1.1 Tools for studying rapid motions (fs-ps) SUMMARY CISES RENCES 6 ■ Intrinsically Unstructured Proteins	388		
REFER	RENCES			388
Chapter	5 • Pro	otein Dyna	amics	397
5.1				397
5.2				400
	5.2.1	Kinetic a		400
		5.2.1.1	Levinthal's paradox and energy landscape theory	400
		5.2.1.2	Folding models and mechanisms	403
	5.2.2	In vivo fo	olding	405
		5.2.2.1	In vivo factors that complicate folding	405
		5.2.2.2	Assisted folding	416
5.3	FOLDE	ED STATE D	DYNAMICS	425
	5.3.1	Spontan	eous dynamics	426
		5.3.1.1	Proteins are conformational ensembles	426
		5.3.1.2	Statistical-thermodynamic view of protein dynamics	426
		5.3.1.3	Dynamics of disordered proteins	428
		5.3.1.4	,	428
		5.3.1.5	Effects of solvents on protein dynamics	433
	5.3.2	External	effects on protein dynamics	434
		5.3.2.1	Ligand-induced dynamics and allostery	434
		5.3.2.2	Dynamics induced by environmental changes	456
		5.3.2.3	Enzyme-mediated protein dynamics	456
5.4	METHO	ODS FOR S	STUDYING PROTEIN DYNAMICS	457
	5.4.1	Tools for	studying slow (ms-sec) to intermediate (ns-\mu s) motions	458
		5.4.1.1	Tools for studying rapid motions (fs-ps)	460
5.5	SUMM	ARY		461
EXER	CISES			462
REFER	RENCES			463
Chapter	6 ■ Int	rinsically (Unstructured Proteins	477
6.1	INTRO	DUCTION		477
	6.1.1	Molecula	ar recognition	479
	6.1.2	Entropic	chain activity	482

6.2	SEQUE	NCE AND	STRUCTURAL ORGANIZATION OF TUPS AND IDRS	487
6.3	STRUC	TURE-FUN	NCTION RELATIONSHIP	489
	6.3.1	IUP bind	ding to target proteins	489
		6.3.1.1	IUPs are designed for fast protein binding and release	489
		6.3.1.2	Mechanism and kinetics of binding-folding coupling in IUPs	492
		6.3.1.3	Significance of PPII helix in IUPs	493
		6.3.1.4	Disorder can be used for regulation	494
	6.3.2	Entropy	assistance-related roles	494
6.4	IUPs IN	N VIVO		495
6.5	SUMM	ARY		495
EXERO	CISES			496
REFER	RENCES			496
Chapter	7 ■ M€	embrane-E	Bound Proteins	503
<i>7</i> .1	INTRO	DUCTION		503
7.2			O ORGANIZATION OF BIOLOGICAL MEMBRANES	506
	7.2.1	General	structure and properties	506
	7.2.2		ition of lipid bilayer	508
		7.2.2.1	Glycerophospholipids	508
		7.2.2.2	Sphingolipids	508
		7.2.2.3	Sterols	511
		7.2.2.4	Ethers	511
		7.2.2.5	Variability	511
	7.2.3	Lipid pro	operty effects on membranes	514
		7.2.3.1	Amphipathicity	514
		7.2.3.2	Asymmetry	514
		7.2.3.3	Degree of order and thickness	515
		7.2.3.4	Curvature	516
7.3	PRINCI	IPLES OF M	membrane protein structure	518
	7.3.1	Overviev	W	518
	7.3.2	Structure	es of integral membrane proteins	519
		7.3.2.1	Primary structure	521
		7.3.2.2	Secondary structure	530
		7.3.2.3	Tertiary structure	532
	7.3.3	Peripher	ral membrane proteins	552
7.4	PROTE	IN-MEMBF	rane interaction	553

	7.4.1	Lipid bil	ayer effects on membrane proteins	553
		7.4.1.1	Effects of general bilayer properties	553
		7.4.1.2	Effects of specific bilayer lipids	558
	7.4.2	Effects o	f membrane proteins on lipid bilayer properties	565
		7.4.2.1	Decrease in mobility	565
		7.4.2.2	Deformation and curvature changes	565
7.5	G PRO	tein-cou	PLED RECEPTORS	568
	7.5.1	Introduc	ction	568
	7.5.2	GPCR si	gnaling	569
		7.5.2.1	General view	569
		7.5.2.2	G-protein mechanisms and regulation	572
	7.5.3	GPCR st	tructure	575
		7.5.3.1	General features	575
		7.5.3.2	Structural variations among GPCRs	578
	7.5.4	GPCR a	nd G-protein activation	588
		7.5.4.1	Structural changes in GPCRs upon activation	589
		7.5.4.2	Agonist effect and G-protein activation	593
	7.5.5	GPCR d	esensitization	600
	7.5.6	GPCRs o	of other classes	601
		7.5.6.1	Class B GPCRs	601
		7.5.6.2	Class C GPCRs	607
		7.5.6.3	Class F GPCRs	608
	7.5.7	GPCR-ta	argeting drugs	609
7.6	SUMM	ARY		613
EXERO	CISES			614
REFER	RENCES			616
Chapter	8 - Pro	tein-Ligar	nd Interactions	637
8.1	INTRO	DUCTION		637
8.2	THEOR	IES ON PE	ROTEIN-LIGAND BINDING AND DYNAMICS	638
8.3	PROTE	IN-LIGANI	D BINDING ENERGETICS	641
	8.3.1	Total bir	nding free energy	641
		8.3.1.1	Protein-ligand binding displays diverse affinities	641
		8.3.1.2	Calculating absolute binding free energy	643
		8.3.1.3	Calculating relative binding energies	647
	8.3.2	Thermo	dynamic determinants of binding energy	648

8.4	LIGANI	D-BINDING	G SITES	650
	8.4.1	Overviev	N	650
	8.4.2	Geometr	ric complementarity	650
	8.4.3	Electrost	tatic complementarity	652
	8.4.4	Binding	specificity and promiscuity	654
8.5	PROTE	IN-PROTEI	n interactions	665
	8.5.1	Overviev	N	665
	8.5.2	Protein-	protein binding domains	666
	8.5.3	Structure	e-function relationships	667
		8.5.3.1	Protein-protein interface	667
		8.5.3.2	PPII helices in protein-protein interactions	676
	8.5.4	Effect of	molecular crowding on protein-protein interactions	677
8.6	PROTE	IN-LIGAN[D INTERACTIONS IN DRUG ACTION AND DESIGN	679
	8.6.1	Involven	nent of proteins in disease	679
	8.6.2	How pha	armaceutical drugs work	680
		8.6.2.1	Principal modes of action	680
		8.6.2.2	Selectivity and side effects	684
	8.6.3	Drug de	velopment and design	685
		8.6.3.1	General sources of pharmaceutical drugs	685
		8.6.3.2	Drug development process	686
		8.6.3.3	Principal steps in rational drug design	687
		8.6.3.4	Rational drug design case study: ACE inhibitors	700
8.7	SUMM	ARY		713
EXERO	CISES			714
REFER	RENCES			715
Chapter	9 • Enz	zymatic Ca	atalysis	729
9.1	INTRO	DUCTION		729
	9.1.1	Metaboli	ic needs of cells	729
	9.1.2	Cellular	processes must be catalyzed in order to sustain life	729
	9.1.3	Why wei	re enzymes selected as biocatalysts?	737
	9.1.4	Why is it	t important to understand enzyme action?	739
	9.1.5	Enzyme	classification	739
		9.1.5.1	Oxidoreductases (EC 1)	744
		9.1.5.2	Transferases (EC 2)	753
		9.1.5.3	Hydrolases (EC 3)	766

		9.1.5.4	Lyases (EC 4)	774			
		9.1.5.5	Isomerases (EC 5)	778			
		9.1.5.6	Ligases (EC 6)	779			
		9.1.5.7	Catalytic promiscuity	781			
9.2	ENZYM	ME KINETIC	S	783			
	9.2.1	Basic cor	ncepts	784			
	9.2.2	Michaeli	s-Menten model	786			
	9.2.3	Use of M	ichaelis-Menten kinetic parameters for enzyme analysis	791			
		9.2.3.1	Enzyme-substrate affinity	791			
		9.2.3.2	Enzyme efficiency and specificity	792			
		9.2.3.3	Enzyme proficiency	794			
	9.2.4	Limitatio	ons of M–M formalism	794			
9.3	HOW I	HOW DO ENZYMES CATALYZE REACTIONS?					
	9.3.1	Overviev	V	795			
	9.3.2	Binding	specificity and selectivity	796			
	9.3.3	Catalysis		799			
		9.3.3.1	Substrate confinement	802			
		9.3.3.2	Electrostatic preorganization and noncovalent stabilization of transition state	803			
		9.3.3.3	Covalent catalysis and electronic polarization of substrate bonds	807			
		9.3.3.4	Metal ion catalysis	814			
		9.3.3.5	General acid-base catalysis	817			
		9.3.3.6	Mechanisms related to protein dynamics	821			
9.4	ENZYM	1E COFACT	ORS	824			
	9.4.1	Overviev	V	824			
	9.4.2	Chemica	l characteristics of organic cofactors	830			
	9.4.3	Function	al characteristics	833			
9.5	ENZYM	ie inhibiti	ION	833			
	9.5.1	Overviev	V	833			
	9.5.2	Modes of	f enzyme inhibition	834			
		9.5.2.1	Reversible inhibition	835			
		9.5.2.2	Irreversible inhibition	845			
9.6	INDUS	TRIAL USE	S OF ENZYMES	848			
	9.6.1	Medical	uses of enzymes	848			
		9.6.1.1	Drugs and drug targets	848			
		9.6.1.2	Diagnostic roles	849			

		9.6.2	Use of enzymes as industrial catalysts	850		
		9.6.3	Limitations and solutions	853		
	9.7	SUMMA	ARY	855		
	EXERCISES 85					
	REFER	rences		868		
	APPE	ndix: en	ZYME NOMENCLATURE RECOMMENDATIONS OF THE NC-IUBMB	881		
Inc	dex			891		