

CONTENTS

<i>Contributors</i>	<i>xiii</i>
<i>Preface</i>	<i>xix</i>
<i>Volumes in Series</i>	<i>xxi</i>

1. Using NMR-Detected Backbone Amide ^1H Exchange to Assess Macromolecular Crowding Effects on Globular-Protein Stability **1**

Andrew C. Miklos, Conggang Li, and Gary J. Pielak

1. Introduction	2
2. Globular Protein Stability	3
3. Mechanism and Limits of Amide ^1H Exchange	3
4. Requirements for Candidate Systems	6
5. Preliminary Experiments	8
6. A Protocol for Amide ^1H Exchange	14
7. Summary and Future Directions	15
Acknowledgments	16
References	16

2. Fluorescence Spectroscopy in Thermodynamic and Kinetic Analysis of pH-Dependent Membrane Protein Insertion **19**

Alexey S. Ladokhin

1. Introduction: Co-Translational Versus Post-Translational Membrane Protein Insertion	21
2. Challenges of Thermodynamic Analysis of Membrane Protein Folding/Insertion	22
3. FCS and Protein-Membrane Interactions	23
4. Thermodynamic Schemes for Analysis of Membrane Partitioning	28
5. Kinetic Analysis of Membrane Protein Insertion	32
6. Perspectives: Annexin B12 as a Model for Thermodynamic and Kinetic Analysis of Membrane Protein Insertion, Folding and Misfolding	38
Acknowledgments	40
References	40

3. Evaluating the Energy-Dependent "Binding" in the Early Stage of Protein Import into Chloroplasts	43
Mitsuru Akita and Hitoshi Inoue	
1. Introduction	44
2. The <i>In Vitro</i> Chloroplastic Import Assay Using Recombinant Precursor Proteins	45
3. Limited Proteolysis of Docked Precursor Proteins	53
4. The Behavior of Transit Peptide During the Transition	59
5. Conclusions	62
Acknowledgments	62
References	62
 4. Use of DNA Length Variation to Detect Periodicities in Positively Cooperative, Nonspecific Binding	 65
Manana Melikishvili, Lance M. Hellman, and Michael G. Fried	
1. Introduction	66
2. Protein and DNA Preparations	68
3. Stoichiometry Analyses	68
4. Affinity and Cooperativity as Functions of DNA Length	75
Acknowledgments	78
References	78
 5. The Impact of Ions on Allosteric Functions in Human Liver Pyruvate Kinase	 83
Aron W. Fenton and Aileen Y. Alontaga	
1. Introduction	84
2. General Strategy to Assess Allosteric Coupling	86
3. PYK Assay	88
4. Buffers	91
5. Divalent Cation	93
6. Monovalent Cation	95
7. Anion	100
8. Concluding Remarks	103
Acknowledgments	105
References	105

6. Conformational Stability of Cytochrome <i>c</i> Probed by Optical Spectroscopy	109
Reinhard Schweitzer-Stenner, Andrew Hagarman, Daniel Verbaro, and Jonathan B. Soffer	
1. Introduction	110
2. Basic Theory of Absorption and Circular Dichroism Spectroscopy	113
3. Secondary Structure Analysis of Cytochrome <i>c</i> Using Ultra-Violet Circular Dichroism Spectroscopy	117
4. Visible CD and Absorption Spectroscopy of Native Cytochrome <i>c</i>	121
5. Nonnative States of Ferricytochrome <i>c</i> Probed by Visible CD and Absorption Spectroscopy	131
6. Summary and Outlook	148
References	149
7. Examining Ion Channel Properties Using Free-Energy Methods	155
Carmen Domene and Simone Furini	
1. Introduction	156
2. Free-Energy Calculations	157
3. Thermodynamic Integration	159
4. Free-Energy Perturbation	160
5. Umbrella Sampling	162
6. Adaptive Biasing Force	164
7. Metadynamics	167
8. Applications of Free-Energy Methods to Study Ion Channel Properties	169
9. Conclusions and Future Outlook	174
Acknowledgments	175
References	175
8. Examining Cooperative Gating Phenomena in Voltage-Dependent Potassium Channels: Taking the Energetic Approach	179
Ofer Yifrach, Nitzan Zandany, and Tzilhav Shem-Ad	
1. Introduction	180
2. High-Order Thermodynamic Mutant Cycle Coupling Analysis	181
3. The Voltage-Activated Potassium Channel Allosteric Model System	188
4. Deriving a Hill Coefficient for Assessing Cooperativity in Voltage-Dependent Ion Channels	193
5. Direct Analysis of Cooperativity in Multisubunit Allosteric Proteins	196
6. Long-Range Energetic Coupling Mediated Through Allosteric Communication Trajectories	202
7. Concluding Remarks	207
Acknowledgments	207
References	207

9. Thermal Stability of Collagen Triple Helix	211
Yujia Xu	
1. Introduction	212
2. Methods	214
References	231
10. Electrostatic Contributions to the Stabilities of Native Proteins and Amyloid Complexes	233
Sarah R. Sheftic, Robyn L. Croke, Jonathan R. LaRochelle, and Andrei T. Alexandrescu	
1. Introduction	234
2. Practical Aspects of pK_a Measurements by NMR	236
3. Interpreting pK_a Values in Terms of Stability	240
4. Importance of the Reference (Unfolded) State	240
5. Results from Globular Proteins	240
6. Results from Coiled Coils	241
7. Comparison of NMR and Crystallographic Results	242
8. Comparison of NMR and Mutagenesis: Nonadditivity of Ion Pairs	243
9. Improving Structure-Based Modeling of pK_a Values	244
10. Results with Micelle-Bound Proteins	245
11. Results from Fibrillization Kinetics	249
12. Conclusion	253
Acknowledgments	254
References	254
11. Kinetics of Allosteric Activation	259
Enrico Di Cera	
1. Linkage	259
2. Allosteric Activation at Steady State	261
3. Different Types of Activation (Type Ia, Type Ib, and Type II)	266
4. Concluding Remarks	269
Acknowledgment	270
References	270
12. Thermodynamics of the Protein Translocation	273
Alexej Kedrov, Tanneke den Blaauwen, and Arnold J. M. Driessen	
1. Introduction	274
2. Example 1: SecA Nucleotide Binding	278
3. Example 2: Probing SecB:Substrate Interactions	283
4. Concluding Remarks	288
References	289

13. Thermodynamic Analysis of the Structure-Function Relationship in the Total DNA-Binding Site of Enzyme-DNA Complexes	293
Włodzimierz Bujalowski and Maria J. Jezewska	
1. Introduction	294
2. Thermodynamic Bases of Quantitative Equilibrium Spectroscopic Titrations	296
3. Anatomy of the Total DNA-Binding Site in the PriA Helicase-ssDNA Complex	302
4. Structure-Function Relationship in the Total ssDNA-Binding Site of the DNA Repair Pol X From ASFV	317
Acknowledgments	322
References	322
14. Equilibrium and Kinetic Approaches for Studying Oligomeric Protein Folding	325
Lisa M. Gloss	
1. Introduction	326
2. Methods to Monitor Folding and Association	327
3. Equilibrium Studies	336
4. Kinetic Studies	343
Acknowledgments	354
References	354
15. Methods for Quantifying T cell Receptor Binding Affinities and Thermodynamics	359
Kurt H. Piepenbrink, Brian E. Gloor, Kathryn M. Armstrong, and Brian M. Baker	
1. Introduction	360
2. Isothermal Titration Calorimetry of TCR-Peptide/MHC Interactions	362
3. Surface Plasmon Resonance Studies of TCR-Peptide/MHC Interactions	367
4. Fluorescence Anisotropy as a Tool for Characterizing TCR-Peptide/MHC Interactions	373
5. Concluding Remarks	378
Acknowledgments	378
References	378

16. Thermodynamic and Kinetic Analysis of Bromodomain–Histone Interactions	383
Martin Thompson	
1. Introduction	384
2. Fluorescence Anisotropy Theory and Concepts	384
3. Developing Binding Models for the Analysis of Fluorescence Anisotropy Data	386
4. Experimental Considerations in Designing Fluorescence Anisotropy Assays	390
5. Preparation of Histone and Bromodomain Samples	391
6. Fluorescence Anisotropy Measurements	392
7. Kinetic Analysis	395
8. Determination of Thermodynamic Parameters	399
9. Thermodynamic Measurements	400
10. Developing a Binding Model	403
11. Concluding Remarks	405
Acknowledgments	405
References	405
17. Thermodynamics of 2-Cys Peroxiredoxin Assembly Determined by Isothermal Titration Calorimetry	409
Sergio Barranco-Medina and Karl-Josef Dietz	
1. Introduction	410
2. Dimer–Decamer Equilibrium	412
3. Isothermal Titration Calorimetry—General Concepts	415
4. ITC Dilution Experiments	416
5. Material and Instruments	418
6. Experimental Procedure	418
7. Results, Data Analysis, and Discussion	423
8. Conclusions	428
Acknowledgments	428
References	428
18. Protein–Lipid Interactions: Role of Membrane Plasticity and Lipid Specificity on Peripheral Protein Interactions	431
Jesse Murphy, Kristofer Knutson, and Anne Hinderliter	
1. Introduction	432
2. Defining Protein–Lipid Interactions	433
3. Selective Partitioning and Lipid Activities	434
4. Protein–Protein Interactions at the Membrane Surface	435
5. Measuring Protein–Lipid Interactions	437

6. Modeling of Protein–Lipid Interactions	439
7. Synopsis	448
Acknowledgments	450
References	451
19. Predicting pK_a Values with Continuous Constant pH Molecular Dynamics	455
Jason A. Wallace and Jana K. Shen	
1. Introduction	456
2. Theoretical Methods for pK_a Predictions	457
3. Predicting Protein pK_a s with REX–CPHMD Simulations	465
4. Conclusions	470
Acknowledgment	471
References	471
20. Unfolding Thermodynamics of DNA Intramolecular Complexes Involving Joined Triple- and Double-Helical Motifs	477
Irine Khutsishvili, Sarah Johnson, Hui-Ting Lee, and Luis A. Marky	
1. Introduction	478
2. Materials and Methods	481
3. Results and Discussion	485
4. Conclusions	499
Acknowledgments	499
References	500
21. Thermodynamics and Conformational Change Governing Domain–Domain Interactions of Calmodulin	503
Susan E. O'Donnell, Rhonda A. Newman, Travis J. Witt, Rainbo Hultman, John R. Froehlig, Adam P. Christensen, and Madeline A. Shea	
1. Introduction	504
2. Overexpression and Purification of rCaM Fragments	507
3. Calcium-Binding Properties of N-Domain CaM Fragments	507
4. Tertiary Constraints of N-Domain CaM Fragments	512
5. Tertiary Conformation of N-Domain CaM Fragments	516
6. High-Resolution Studies of N-Domain CaM Fragments	519
7. Conclusions	522
Acknowledgments	524
References	525

22. Use of Pressure Perturbation Calorimetry to Characterize the Volumetric Properties of Proteins	527
Katrina L. Schweiker and George I. Makhatadze	
1. Introduction	528
2. Determination of the Coefficient of Thermal Expansion (α_{PT}) Using PPC	531
3. Sample Preparation	533
4. Derivation of a Two-State Model for Analysis of PPC Data	535
5. Practical Considerations	539
6. Implications of Two-State Model for Future PPC Experiments	545
References	545
23. Solvent Denaturation of Proteins and Interpretations of the m Value	549
J. Martin Scholtz, Gerald R. Grimsley, and C. Nick Pace	
1. Introduction	549
2. Protein Unfolding or Denaturation	550
3. Linear Extrapolation Method	555
4. $\Delta G(H_2O)$: Conformational Stability	556
5. The m Value	558
6. Concluding Remarks	562
Acknowledgments	563
References	563
24. Measuring Cotranslational Folding of Nascent Polypeptide Chains on Ribosomes	567
Patricia L. Clark and Krastyu G. Ugrinov	
1. Introduction	568
2. Translation and the Ribosome:Nascent Chain (RNC) Complex	570
3. General Approaches for Generating Stalled RNC Complexes	572
4. Methods for Preparing RNC Complexes	577
5. Biophysical Studies with RNC Complexes	579
6. Measuring Nascent Chain Cotranslational Folding and Rigidity by Limited Protease Digestion	583
7. Future Directions	584
References	585
<i>Author Index</i>	591
<i>Subject Index</i>	603