

CONTENTS

<i>Contributors</i>	<i>ix</i>
<i>Preface</i>	<i>xiii</i>
<i>Volumes in Series</i>	<i>xv</i>
1. A Thermodynamic Approach for the Targeting of Nucleic Acid Structures Using Their Complementary Single Strands	1
Hui-Ting Lee, Caroline Carr, Hollie Siebler, Lela Waters, Irine Khutsishvili, Fany Iseka, Brian Domack, Chris M. Olsen, and Luis A. Marky	
1. Introduction	2
2. Materials and Methods	4
3. Results and Discussion	8
4. Conclusions	23
Acknowledgments	24
References	24
2. Thermodynamics of Biological Processes	27
Hernan G. Garcia, Jane Kondey, Nigel Orme, Julie A. Theriot, and Rob Phillips	
1. Introduction: Thermodynamics is Not Just for Dead Stuff	28
2. States and Weights from the Boltzmann Rule	29
3. Binding Reactions and Biological Thermodynamics	33
4. The Unreasonable Effectiveness of Random-Walk Models	52
5. Conclusions	55
Acknowledgments	56
References	56
3. Protein Stability in the Presence of Cosolutes	61
Luis Marcelo F. Holthauzen, Matthew Auton, Mikhail Sinev, and Jörg Rösgen	
1. Introduction	62
2. Isothermal Folding/Unfolding of Protein in the Presence of Stabilizing/Denaturing Osmolyte	64
3. Isothermal Protein (Un)Folding in the Presence of Osmolyte Mixtures	78

4. Osmolyte-Induced Unfolding at Variable Temperature	86
5. Thermal Unfolding in the Presence of Osmolytes	92
6. Where Do the Little Equations Come From?	101
Acknowledgment	121
References	121
 4. Small-Angle X-ray Scattering Studies of Peptide-Lipid Interactions Using the Mouse Paneth Cell α-Defensin Cryptdin-4	 127
Abhijit Mishra, Kenneth P. Tai, Nathan W. Schmidt, André J. Ouellette, and Gerard C. L. Wong	
1. Introduction	128
2. X-Rays as Structural Probes of Biological Systems Under Biomimetic Conditions	132
3. Preparation of Peptide-Lipid Complexes for X-Ray Measurements	137
4. Summary	145
Acknowledgments	145
References	145
 5. Synergy of Molecular Dynamics and Isothermal Titration Calorimetry in Studies of Allostery	 151
Rebecca Strawn, Thomas Stockner, Milan Melicherik, Lihua Jin, Wei-Feng Xue, Jannette Carey, and Rüdiger Ettrich	
1. Allostery	152
2. Arginine Repressor	154
3. Preparation for Simulations	157
4. Sampling of States	159
5. Equilibration	160
6. Observing System Motions	163
7. Correlated Motions	163
8. Structural Features of Correlated Motions	164
9. Arg Residues Promote Rotation and Oscillation	167
10. Structural Correlates of Rotational Oscillation	169
11. Single-Arginine Simulations	169
12. Rotational Ensembles	170
13. Energetic Contributions	173
14. Reconciliation with Crystallographic Data	177
15. Complementarity and Synergy of MD and ITC	179
16. Prospects	184
Acknowledgement	186
References	186

6. Using Tryptophan Fluorescence to Measure the Stability of Membrane Proteins Folded in Liposomes	189
C. Preston Moon and Karen G. Fleming	
1. Introduction	190
2. Issues with Managing Light Scattering from Liposomes	191
3. Using Tryptophan Spectral Properties to Monitor Membrane Protein Folding into Liposomes	200
4. Choosing an Appropriate Tryptophan Spectral Property to Measure the Thermodynamic Stabilities of Folded Membrane Proteins	205
5. Conclusions	207
6. Materials and Methods	209
Acknowledgments	210
References	210
7. Non-B Conformations of CAG Repeats Using 2-Aminopurine	213
Natalya N. Degtyareva and Jeffrey T. Petty	
1. Introduction	214
2. Materials and Methods	216
3. Structure and Thermodynamics of Isolated and Integrated (CAG) ₈	223
4. Conclusions	229
Acknowledgments	229
References	229
8. Disulfide Bond-Mediated Passenger Domain Stalling as a Structural Probe of Autotransporter Outer Membrane Secretion <i>In Vivo</i>	233
Jonathan P. Renn and Patricia L. Clark	
1. Protein Secretion: An Essential Component of Bacterial Virulence	234
2. The Autotransporter Secretion Pathway	234
3. Overview of Cys-Loop Stalling	236
4. Architecture and Processing of AT Passenger Domains	236
5. Heterologous Passenger Domain Secretion	239
6. Selecting a Model Autotransporter for Cys-Loop Stalling	240
7. Disulfide Mediated Passenger Domain Stalling	241
8. Methods to Measure OM Secretion and Folding of the Stalled AT Passenger	244
9. Applications: Using Cys-Loop Stalling to Define the Mechanism of AT OM Secretion	248
References	249

9. Strategies for the Thermodynamic Characterization of Linked Binding/Local Folding Reactions Within the Native State: Application to the LID Domain of Adenylate Kinase from *Escherichia coli* **253**

Travis P. Schrank, W. Austin Elam, Jing Li, and Vincent J. Hilser

1. Introduction	254
2. A Mutation Strategy to Amplify Locally Unfolded States	256
3. Thermodynamic Properties of Linked Folding and Binding Reactions	260
4. Strategies for Quantitative Interpretation of Measured Enthalpies for a Linked Folding and Binding System	265
5. Interplay of Local Mutational Effects, Global Stability, and Binding Affinity	268
6. Success of the Strategy in Preserving Structure	273
7. Comparison of Interaction Versus Entropy Based Mutation Strategy	274
8. How Similar Are Local and Global Unfolding?	276
9. Summary	279
References	280

10. Fluorescence-Detected Sedimentation in Dilute and Highly Concentrated Solutions **283**

Jonathan S. Kingsbury and Thomas M. Laue

1. Overview of AUC	284
2. Fluorescence Optics for the Ultracentrifuge	285
3. Advantages of AU-FDS	287
4. Sample Requirements for Fluorescence Detection	288
5. Applications of AU-FDS	293
6. Current Challenges for AU-FDS	298
7. Conclusion	301
References	302

<i>Author Index</i>	305
<i>Subject Index</i>	317