

CONTENTS

Contributors

xi

Preface

xvii

Section I

ITC

1. Isothermal Titration Calorimetry Measurements of Metal Ions

Binding to Proteins

3

Colette F. Quinn, Margaret C. Carpenter, Molly L. Croteau,
and Dean E. Wilcox

1. Introduction

4

2. Metal Ion Properties Relevant to ITC Measurements

5

3. ITC Measurements Involving Metal Ions

9

4. Fitting ITC Data Involving Metal Ions

12

5. *Post Hoc* Analysis of ITC Data Involving Metal Ions

13

6. Additional Considerations with Metal Ions

18

7. Conclusion

19

Acknowledgments

20

References

20

2. Assessing Coupled Protein Folding and Binding Through Temperature-Dependent Isothermal Titration Calorimetry

23

Debashish Sahu, Monique Bastidas, Chad W. Lawrence, William G. Noid,
and Scott A. Showalter

1. Introduction

24

2. Sample Preparation

27

3. Special Considerations for ITC of Intrinsically Disordered Proteins

29

4. Data Fitting and Analysis

31

5. Summary

41

Acknowledgments

43

References

43

3. Fragment-Based Screening for Enzyme Inhibitors Using Calorimetry

47

Michael I. Recht, Vicki Nienaber, and Francisco E. Torres

1. Introduction

48

2. Required Materials	52
3. Instrumentation	53
4. Fragment Library Selection	56
5. Calorimetric Enzyme Assay: Nanocalorimeter	59
6. Calorimetric Enzyme Assay: Commercial Microcalorimeters	64
7. Summary	66
Acknowledgments	67
References	67
4. Measuring Multivalent Binding Interactions by Isothermal Titration Calorimetry	71
Tarun K. Dam, Melanie L. Talaga, Ni Fan, and Curtis F. Brewer	
1. Introduction	72
2. Determining the Thermodynamics of Lectin Binding to Multivalent Synthetic Analogs	73
3. Determination that the Epitopes of Multivalent Analogs Possess a Gradient of Decreasing Microscopic-Binding Affinities (K_d)	81
4. Direct Calorimetric Measurements of Microscopic Thermodynamic Binding Parameters by Reverse ITC	86
5. ITC Studies of Lectin Binding to Multivalent Glycoproteins	88
6. ITC Studies of Lectin Binding to Polyvalent Mucins	91
Acknowledgments	93
References	94
5. Calorimetric and Spectroscopic Analysis of the Thermal Stability of Short Duplex DNA-Containing Sugar and Base-Modified Nucleotides	97
Kareem Fakhfakh, Curtis B. Hughesman, A. Louise Creagh, Vincent Kao, and Charles Haynes	
1. Introduction	98
2. Melting Thermodynamics from DSC	100
3. Melting Thermodynamics from UV Spectroscopy	112
4. Errors Analysis	121
5. Understanding the Contribution to Duplex Stability of Specific Nucleotide Modifications	122
References	125
6. Calorimetric Quantification of Cyclodextrin-Mediated Detergent Extraction for Membrane-Protein Reconstitution	129
Martin Textor and Sandro Keller	
1. Introduction	130

2. Membrane-Protein Reconstitution	130
3. Practical Approach to CD-Driven Reconstitution	136
4. Concluding Remarks	151
Acknowledgments	153
References	153
7. Extending ITC to Kinetics with kinITC	157
Philippe Dumas, Eric Ennifar, Cyrielle Da Veiga, Guillaume Bec, William Palau, Carmelo Di Primo, Angel Piñeiro, Juan Sabin, Eva Muñoz, and Javier Rial	
1. Introduction	158
2. The Theoretical Basis of <i>kinITC-ETC</i>	160
3. Practical Aspects of the Method	163
4. Results	165
5. Instrument Response Time: Measurement and Important Facts	174
6. Conclusions	176
Acknowledgments	177
Appendix Geometrical Features of an ETC	177
References	179
8. Measuring the Kinetics of Molecular Association by Isothermal Titration Calorimetry	181
Kirk A. Vander Meulen, Scott Horowitz, Raymond C. Trievel, and Samuel E. Butcher	
1. Introduction	182
2. Background	183
3. Procedure and Results	187
4. Method Details	207
Acknowledgments	212
References	212
9. Isothermal Titration Calorimetry to Characterize Enzymatic Reactions	215
Luca Mazzei, Stefano Ciurli, and Barbara Zambelli	
1. Introduction	216
2. Instrumental Setup	218
3. Methods and Theory	219
4. Experimental Conditions	221
5. Data Analysis	224

6. Representative Results	225
7. Conclusions	234
References	234
10. Avoiding Buffer Interference in ITC Experiments: A Case Study from the Analysis of Entropy-Driven Reactions of Glucose-6-Phosphate Dehydrogenase	237
M. Lucia Bianconi	
1. Introduction	238
2. Correcting for Protonation Effects	241
3. Materials and Methods	245
4. Results and Discussion	246
5. Taking Advantage of the Heat Effect of Buffer Protonation/Deprotonation	252
6. Final Remarks	253
Acknowledgments	254
References	254
11. ITC Methods for Assessing Buffer/Protein Interactions from the Perturbation of Steady-State Kinetics: A Reactivity Study of Homoprotocatechuate 2,3-Dioxygenase	257
Kate L. Henderson, Delta K. Boyles, Vu H. Le, Edwin A. Lewis, and Joseph P. Emerson	
1. Introduction	258
2. Required Materials	261
3. Instrumentation and Sample Considerations	261
4. Steady-State ITC Method Applied to HPCD	262
5. Discussion	272
6. Conclusions	276
References	277
Section II	
DSC	
12. Modern Analysis of Protein Folding by Differential Scanning Calorimetry	281
Beatriz Ibarra-Molero, Athi N. Naganathan, Jose M. Sanchez-Ruiz, and Victor Muñoz	
1. Introduction	282
2. Thermodynamics and the DSC Experiment	285

3. Absolute Heat Capacity and DSC Baselines	293
4. Equilibrium Thermodynamics Analysis: Microstates Versus Macrostates	296
5. Statistical Mechanics Analysis of DSC Experiments	299
6. Concluding Remarks	313
Acknowledgments	314
References	314
13. A Guide to Differential Scanning Calorimetry of Membrane and Soluble Proteins in Detergents	319
Zhengrong Yang and Christie G. Brouillette	
1. Introduction	320
2. Detergent Properties	328
3. Detergent Effects on Proteins and Detection by DSC	332
4. Complementary Noncalorimetry Approaches to Study Thermal Unfolding	339
5. Choice of Detergents	340
6. Case Studies	343
7. Experimental Procedures	347
8. Concluding Remarks	352
Acknowledgment	352
References	352
14. Orthogonal Methods for Characterizing the Unfolding of Therapeutic Monoclonal Antibodies: Differential Scanning Calorimetry, Isothermal Chemical Denaturation, and Intrinsic Fluorescence with Concomitant Static Light Scattering	359
Deniz B. Temel, Pavel Landsman, and Mark L. Brader	
1. Introduction	360
2. Therapeutic Monoclonal Antibodies	361
3. Performing and Detecting Protein Unfolding	363
4. Experimental	373
5. Data Interpretation	380
Acknowledgments	387
References	387
15. IATC, DSC, and PPC Analysis of Reversible and Multistate Structural Transition of Cytochrome c	391
Shun-ichi Kidokoro and Shigeyoshi Nakamura	
1. Introduction	392
2. Isothermal Acid-Titration Calorimetry	396

3. Differential Scanning Calorimetry	403
4. Pressure Perturbation Calorimetry with DSC Analysis	408
5. Summary	410
Acknowledgment	411
References	411
16. The Complementarity of the Loop to the Stem in DNA Pseudoknots Gives Rise to Local TAT Base-Triplets	413
Calliste Reiling-Steffensmeier and Luis A. Marky	
1. Introduction	414
2. Materials and Methods	416
3. Results and Discussion	419
4. Conclusions	427
Acknowledgments	429
References	429
Section III	
Calorimetry Facilities	
17. A High-Throughput Biological Calorimetry Core: Steps to Startup, Run, and Maintain a Multiuser Facility	435
Neela H. Yennawar, Julia A. Fecko, Scott A. Showalter, and Philip C. Bevilacqua	
1. Introduction	436
2. Instruments at the Facility	437
3. Running of the Facility	441
4. Science Facilitated	453
5. Conclusions	458
Acknowledgments	459
References	459
<i>Author Index</i>	461
<i>Subject Index</i>	481