Over the past decade, we have witnessed an explosive development of research dedicated to intrinsically disordered proteins (IDPs), which are also known as natively unfolded proteins among various other names. The existence of biologically active but extremely flexible proteins is challenging the century-old structure-to-function paradigm according to which a rigid well-folded 3D structure is required for protein function. Many structural biologists now recognize that the functional diversity provided by disordered regions complements the functional repertoire of ordered protein regions. The high abundance of IDPs in various organisms, their unique structural features, numerous functions, and crucial associations with different diseases show that there are enough grounds to conclude that these proteins should be considered as a unique entity, an unfoldome.

In comparison with “normal” globular proteins, IDPs possess increased amounts of disorder that can be detected by many physicochemical methods that were originally developed to characterize protein self-organization. On the other hand, due to the highly dynamic nature of IDPs, new and existing experimental methods need to be developed and extended, respectively, for the structural and functional analysis of these IDPs. These methods represent an instrumental foundation for experimental unfoldomics.

Information based on modern protocols is provided herein on virtually every experimental method used both to identify IDPs and to analyze their structural and functional properties. Hence, this book will be of interest to all scientists and students studying IDPs, whether the focus is on an IDP’s (lack of) structure or on its function.

The general audience for this book includes scientists working in the fields of biochemistry, biophysics, molecular medicine, biotechnology, pharmacology and drug discovery, molecular and cellular biology; students of Medical Schools, departments of Biochemistry, Biophysics, Molecular Biology, Biotechnology, and Cell Biology, to name a few. We are aware that many scientists have encountered IDPs in their research, but have shied away from deeper studies due to the lack of knowledge of what to try next. By collecting the current methods for the analysis of IDPs in one place, our goal is to help such scientists further their investigations of these fascinating, dynamic molecules.

Tampa, FL, USA; Moscow Region, Russia
Indianapolis, IN, USA

Vladimir N. Uversky, Ph.D.
A. Keith Dunker, Ph.D.
# Contents

Preface ................................................................. v  
Contributors ........................................................... xi  

## PART I SINGLE MOLECULE TECHNIQUES

1 Immobilization of Proteins for Single-Molecule Fluorescence Resonance Energy Transfer Measurements of Conformation and Dynamics ............... 3  
   *Ucheor B. Choi, Keith R. Weninger, and Mark E. Bowen*

2 Application of Confocal Single-Molecule FRET to Intrinsically Disordered Proteins .................................................... 21  
   *Benjamin Schuler, Sonja Müller-Späth, Andrea Soranno, and Daniel Nettels*

3 Single-Molecule Force Spectroscopy of Chimeric Polyprotein Constructs Containing Intrinsically Disordered Domains .............................. 47  
   *Marco Brucale, Isabella Tessari, Luigi Bubacco, and Bruno Samorì*

4 Visualization of Mobility by Atomic Force Microscopy .......................... 57  
   *Toshio Ando and Noriyuki Kodera*

5 Unequivocal Single-Molecule Force Spectroscopy of Intrinsically Disordered Proteins .................................................... 71  
   *Javier Oroz, Rubén Hervás, Alejandro Valbuena, and Mariano Carrión-Vázquez*

## PART II METHODS TO ASSESS PROTEIN SIZE AND SHAPE

6 Sedimentation Velocity Analytical Ultracentrifugation for Intrinsically Disordered Proteins .................................................... 91  
   *Andrés G. Salvay, Guillaume Communie, and Christine Ebel*

7 Analysis of Intrinsically Disordered Proteins by Small-Angle X-ray Scattering .... 107  
   *Pau Bernadó and Dmitri I. Svergun*

8 Small Angle Neutron Scattering for the Structural Study of Intrinsically Disordered Proteins in Solution: A Practical Guide ............................. 123  
   *Frank Gabel*

9 Dynamic and Static Light Scattering of Intrinsically Disordered Proteins ........ 137  
   *Klaus Gast and Christian Fiedler*

10 Estimation of Intrinsically Disordered Protein Shape and Time-Averaged Apparent Hydration in Native Conditions by a Combination of Hydrodynamic Methods .................................................... 163  
   *Johanna C. Karst, Ana Cristina Sotomayor-Pérez, Daniel Ladant, and Alexandre Chenal*

11 Size-Exclusion Chromatography in Structural Analysis of Intrinsically Disordered Proteins .................................................... 179  
   *Vladimir N. Uversky*
PART III METHODS TO ANALYZE CONFORMATIONAL BEHAVIOR

12 Denaturant-Induced Conformational Transitions in Intrinsically Disordered Proteins .................................................... 197  
Paolo Neyroz, Stefano Ciurli, and Vladimir N. Uversky

13 Identification of Intrinsically Disordered Proteins by a Special 2D Electrophoresis .................................................... 215  
Agnes Tantos and Peter Tompa

14 pH-Induced Changes in Intrinsically Disordered Proteins .................... 223  
Matthew D. Smith and Masoud Jelokhani-Niaraki

15 Temperature-Induced Transitions in Disordered Proteins Probed by NMR Spectroscopy .................................................... 233  
Magnus Kjaergaard, Flemming M. Poulsen, and Birthe B. Kragelund

16 Analyzing Temperature-Induced Transitions in Disordered Proteins by NMR Spectroscopy and Secondary Chemical Shift Analyses ............... 249  
Magnus Kjaergaard, Flemming M. Poulsen, and Birthe B. Kragelund

17 Osmolyte-, Binding-, and Temperature-Induced Transitions of Intrinsically Disordered Proteins ..................................... 257  
Allan Chris M. Ferreon and Ashok A. Deniz

18 Laser Temperature-Jump Spectroscopy of Intrinsically Disordered Proteins ...... 267  
Stephen J. Hagen

19 Differential Scanning Microcalorimetry of Intrinsically Disordered Proteins ...... 283  
Sergei E. Permyakov

20 Identifying Disordered Regions in Proteins by Limited Proteolysis ............. 297  
Angelo Fontana, Patrizia Polverino de Laureto, Barbara Spolaore, and Erica Frare

21 The Effect of Counter Ions on the Conformation of Intrinsically Disordered Proteins Studied by Size-Exclusion Chromatography ................ 319  
Magdalena Wojtas, Tomasz M. Kaplon, Piotr Dobryszczyki, and Andrzej Ożyhar

22 Mean Net Charge of Intrinsically Disordered Proteins: Experimental Determination of Protein Valence by Electrophoretic Mobility Measurements ... 331  
Ana Cristina Sotomayor-Pérez, Johanna C. Karst, Daniel Ladant, and Alexandre Chenal

23 Protein Characterization by Partitioning in Aqueous Two-Phase Systems ...... 351  
Larissa Mikheeva, Pedro Madeira, and Boris Zaslavsky

PART IV MASS-SPECTROMETRY

24 Detection and Characterization of Large-Scale Protein Conformational Transitions in Solution Using Charge-State Distribution Analysis in ESI-MS ........ 365  
Rinat R. Abzalimov, Agya K. Frimpong, and Igor A. Kaltashov

Cedric E. Bobst and Igor A. Kaltashov
PART V  EXPRESSION AND PURIFICATION OF IDPS

28 Identifying Solubility-Promoting Buffers for Intrinsically Disordered Proteins Prior to Purification                  415
   Kelly A. Churion and Sarah E. Bondos

29 Proteomic Methods for the Identification of Intrinsically Disordered Proteins  . 429
   Agnes Tantos and Peter Tompa

30 Selective Isotope Labeling of Recombinant Proteins in Escherichia coli       439
   Kit I. Tong, Masayuki Yamamoto, and Toshiyuki Tanaka

Index                                                                 449
Contributors

RINAT R. ABZALIMOV • Department of Chemistry, University of Massachusetts-Amherst, Amherst, MA, USA

JEFFREY N. AGAR • Chemistry and Volen Center for Complex Systems, Brandeis University, Waltham, MA, USA

TOSHIANDO • Department of Physics and Bio-AFM Frontier Research Center, Kanazawa University, Kanazawa, Japan

JARED R. AUCLAIR • Department of Biochemistry and Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA, USA; Department of Chemistry and Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA, USA; Chemistry and Volen Center for Complex Systems, Brandeis University, Waltham, MA, USA

PAU BERNADÓ • Institute for Research in Biomedicine, Parc Científic de Barcelona, Baldiri Reixac, Spain; Centre de Biochimie Structurale. CNRS UMR-5048, Montpellier, France

CEDRIC E. BOBST • Department of Chemistry, University of Massachusetts-Amherst, Amherst, MA, USA

SARAH E. BONDOS • Department of Molecular and Cellular Medicine, Texas A&M Health Science Center, College Station, TX, USA

MARK E. BOWEN • Department of Physiology and Biophysics, Stony Brook University, Stony Brook, NY, USA

MARCO BRUCALE • Istituto per lo Studio dei Materiali Nanostrutturati (ISMN), Consiglio Nazionale delle Ricerche (CNR), Salaria, RM, Monterotondo

LUIGI BUBACCO • Department of Biochemistry, University of Bologna, Bologna, Italy

MARIANO CARRIÓN-VÁZQUEZ • Instituto Cajal, CSIC, Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED) and IMDEA Nanociencia, Madrid, Spain; Instituto Madrileño de Estudios Avanzados en Nanociencia (IMDEA-Nanociencia), Madrid, Spain

ALEXANDRE CHENAL • Unité de Biochimie des Interactions Macromoléculaires, CNRS UMR 3528, Institut Pasteur, Paris, France; Institut Pasteur, Unité de Biochimie des Interactions Macromoléculaires, Département de Biologie Structurale et Chimie, CNRS UMR 3528, Paris, France

UCHEOR B. CHOI • Department of Physiology and Biophysics, Stony Brook University, Stony Brook, NY, USA

KELLY A. CHURION • Department of Molecular and Cellular Medicine, Texas A&M Health Science Center, College Station, TX, USA

STEFANO CIURLI • Department of Agro-Environmental Science and Technology, University of Bologna, Bologna, Italy

GUILLAUME COMMUNIE • Institut de Biologie Structurale, CEA-CNRS-Université, Grenoble, France; Unit for Virus Host Cell Interactions, UJF-EMBL-CNRS, UMI 3265, Grenoble, France

ASHOK A. DENIZ • Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA, USA
Contributors

PIOTR DOBRYSZYCKI • Department of Biochemistry, Faculty of Chemistry, Wrocław University of Technology, Wrocław, Poland

CHRISTINE EBEL • Institut de Biologie Structurale, Université Grenoble, CNRS, CEA, Grenoble, France

JAMES E. EVANS • Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA, USA; Department of Proteomics and Mass Spectrometry, University of Massachusetts Medical School, Worcester, MA, USA

ALLAN CHRIS M. FERREON • Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA, USA

CHRISTIAN FIEDLER • Institut für Biochemie und Biologie, Physikalische Biochemie, Universität Potsdam, Potsdam, Germany

ANGELO FONTANA • CRIBI Biotechnology Centre, University of Padua, Padua, Italy

ERICA FRARE • CRIBI Biotechnology Centre, University of Padua, Padua, Italy

AGYA K. FRIMPONG • Department of Chemistry, University of Massachusetts-Amherst, Amherst, MA, USA

FRANK GABEL • Institut de Biologie Structurale Jean-Pierre Ebel. UMR 5075 (CNRS, CEA, UJF), Grenoble, France

KLAUS GAST • Institut für Biochemie und Biologie, Physikalische Biochemie, Universität Potsdam, Potsdam, Germany

KARIN M. GREEN • Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA, USA; Department of Proteomics and Mass Spectrometry, University of Massachusetts Medical School, Worcester, MA, USA

STEPHEN J. HAGEN • Physics Department, University of Florida, Gainesville, FL, USA

BASTIAN HENGERER • Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany

RUBÉN HERVÁS • Instituto Cajal, CSIC and Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Madrid, Spain; Instituto Madrileño de Estudios Avanzados en Nanociencia (IMDEA-Nanociencia), Madrid, Spain

MARIUS IONUT IURASCU • Laboratory of Analytical Chemistry and Biopolymer Structure Analysis, Department of Chemistry, University of Konstanz, Konstanz, Germany

MASOUD JELOKHANI-NIARAKI • Department of Chemistry, Wilfrid Laurier University, Waterloo, ON, Canada

IGOR A. KALTASHOV • Department of Chemistry, University of Massachusetts-Amherst, Amherst, MA, USA

TOMASZ M. KAPŁON • Department of Biochemistry, Faculty of Chemistry, Wrocław University of Technology, Wrocław, Poland

JOHANNA C. KARST • Unité de Biochimie des Interactions Macromoléculaires, CNRS UMR 3528, Institut Pasteur, Paris, France; Institut Pasteur, Unité de Biochimie des Interactions Macromoléculaires, Département de Biologie Structurale et Chimie, CNRS UMR 3528, Paris, France

MAGNUS KJAERGAARD • Structural Biology and NMR Laboratory, Department of Biology, University of Copenhagen, Copenhagen, Denmark
BRUNO SAMORI • Department of Biochemistry, University of Bologna, Bologna, Italy
CELIA A. SCHIFFER • Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA, USA
BENJAMIN SCHULER • Department of Biochemistry, University of Zurich, Zurich, Switzerland
STEFAN SLAMNOIU • Laboratory of Analytical Chemistry and Biopolymer Structure Analysis, Department of Chemistry, University of Konstanz, Konstanz, Germany
MATTHEW D. SMITH • Department of Biology, Wilfrid Laurier University, Waterloo, ON, Canada
MOHAN SOMASUNDARAN • Department of Pediatrics, University of Massachusetts Medical School, Worcester, MA, USA
ANDREA SORANNO • Department of Biochemistry, University of Zurich, Zurich, Switzerland
ANA CRISTINA SOTOMAYOR-PEREZ • Unité de Biochimie des Interactions Macromoléculaires, CNRS UMR 3528, Institut Pasteur, Paris, France; Département de Biologie Structurale et Chimie, CNRS UMR 3528, Paris, France
BARBARA SPOLAORE • CRIBI Biotechnology Centre, University of Padua, Padua, Italy
DMITRI I. SVERGUN • European Molecular Biology Laboratory, Hamburg Outstation, Hamburg, Germany
TOSHIYUKI TANAKA • Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan
AGNES TANTOS • Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, Budapest, Hungary
ISABELLA TESSARI • Department of Biochemistry, University of Bologna, Bologna, Italy
PETER TOMPA • Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, Budapest, Hungary
KIT I. TONG • Department of Medical Biochemistry, Tohoku University Graduate School of Medicine, Sendai, Tohoku, Japan
VLADIMIR N. UVERSKY • Department of Molecular Medicine, College of Medicine, University of South Florida, Tampa, FL, USA; Institute for Biological Instrumentation, Russian Academy of Sciences, Pushchino, Moscow Region, Russia
ALEJANDRO VALBUENA • Instituto Cajal, CSIC and Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Madrid, Spain; Instituto Madrileño de Estudios Avanzados en Nanociencia (IMDEA-Nanociencia), Madrid, Spain
CAMELIA VLAD • Laboratory of Analytical Chemistry and Biopolymer Structure Analysis, Department of Chemistry, University of Konstanz, Konstanz, Germany
KEITH R. WENINGER • Department of Physics, North Carolina State University, Raleigh, NC, USA
MAGDALENA WOJTAS • Department of Biochemistry, Faculty of Chemistry, Wroclaw University of Technology, Wroclaw, Poland
MASAYUKI YAMAMOTO • Department of Medical Biochemistry, Tohoku University Graduate School of Medicine, Sendai, Tohoku, Japan
BORIS ZASLAVSKY • Analiza, Cleveland, OH, USA