

Methods in Enzymology

Volume 317

RNA-Ligand Interactions

Part A
Structural Biology Methods

EDITED BY

Daniel W. Celandor

LOYOLA UNIVERSITY
CHICAGO, ILLINOIS

John N. Abelson

CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA



ACADEMIC PRESS

San Diego London Boston New York Sydney Tokyo Toronto

Table of Contents

CONTRIBUTORS TO VOLUME 317	ix
PREFACE	xiii
VOLUMES IN SERIES	xv

Section I. Semisynthetic Methodologies

A. RNA Synthetic Methods

1. Advanced 5'-Silyl-2'-Orthoester Approach to RNA Oligonucleotide Synthesis	STEPHEN A. SCARINGE	3
2. Preparation of Specifically ² H- and ¹³ C-Labeled Ribonucleotides	LINCOLN G. SCOTT, THOMAS J. TOLBERT, AND JAMES R. WILLIAMSON	18
3. Base-Modified Phosphoramidite Analogs of Pyrimidine Ribonucleosides for RNA Structure-Activity Studies	LEONID BEIGELMAN, JASENKA MATULIC-ADAMIC, ALEXANDER KARPEISKY, PETER HAEBERLI, AND DAVID SWEEDLER	39

B. Derivatization of RNA

4. Use of T7 RNA Polymerase and Its Mutants for Incorporation of Nucleoside Analogs into RNA	RUI SOUSA	65
5. Phosphorothioate Modification of RNA for Stereochemical and Interference Analyses	L. CLAUS S. VÖRTLER AND FRITZ ECKSTEIN	74
6. Chemical Probing of RNAs by Nucleotide Analog Interference Mapping	SEAN P. RYDER, LORI ORTOLEVA-DONNELLY, ANNE B. KOSEK, AND SCOTT A. STROBEL	92
7. Joining of RNAs by Splinted Ligation	MELISSA J. MOORE AND CHARLES C. QUERY	109
8. Heavy Atom Derivatives of RNA	BARBARA L. GOLDEN	124
9. Site-Specific Cleavage of Transcript RNA	JON LAPHAM AND DONALD M. CROTHERS	132

- | | | |
|---|---|-----|
| 10. Using DNAszymes to Cut, Process, and Map RNA Molecules for Structural Studies or Modification | ANNA MARIE PYLE,
VI T. CHU,
ECKHARD JANKOWSKY, AND
MARC BOUVILLAIN | 140 |
|---|---|-----|

Section II. RNA Structure Determination

A. X-Ray Crystallography

- | | | |
|--|--|-----|
| 11. Purification, Crystallization, and X-Ray Diffraction Analysis of Small Ribozymes | JOSEPH E. WEDEKIND AND
DAVID B. MCKAY | 149 |
| 12. Solving Large RNA Structures by X-Ray Crystallography | JAMIE H. CATE AND
JENNIFER A. DOUDNA | 169 |
| 13. Conventional and Time-Resolved Ribozyme X-Ray Crystallography | WILLIAM G. SCOTT AND
JAMES B. MURRAY | 180 |

B. Nuclear Magnetic Resonance Spectroscopy

- | | | |
|---|---|-----|
| 14. Nuclear Magnetic Resonance Methods to Study RNA-Protein Complexes | PETER BAYER,
LUCA VARANI, AND
GABRIELE VARANI | 198 |
| 15. Filamentous Bacteriophage for Aligning RNA, DNA, and Proteins for Measurement of Nuclear Magnetic Resonance Dipolar Coupling Interactions | MARK R. HANSEN,
PAUL HANSON, AND
ARTHUR PARDI | 220 |
| 16. Biochemical and Nuclear Magnetic Resonance Studies of Aminoglycoside-RNA Complexes | STEPHEN R. LYNCH,
MICHAEL I. RECHT, AND
JOSEPH D. PUGLISI | 240 |

C. Electron Microscopy

- | | | |
|---|---|-----|
| 17. Experimental Prerequisites for Determination of tRNA Binding to Ribosomes from <i>Escherichia coli</i> | FRANCISCO J. TRIANA-ALONSO,
CHRISTIAN M. T. SPAHN,
NILS BURKHARDT,
BEATRIX RÖHRDANZ, AND
KNUD H. NIERHAUS | 261 |
| 18. Three-Dimensional Cryoelectron Microscopy of Ribosomes | JOACHIM FRANK,
PAWEL PENCZEK,
RAJENDRA K. AGRAWAL,
ROBERT A. GRASSUCCI, AND
AMY B. HEAGLE | 276 |
| 19. Preparation of Functional Ribosomal Complexes and Effect of Buffer Conditions on tRNA Positions Observed by Cryoelectron Microscopy | GREGOR BLAHA,
ULRICH STELZL,
CHRISTIAN M. T. SPAHN,
RAJENDRA K. AGRAWAL,
JOACHIM FRANK, AND
KNUD H. NIERHAUS | 292 |

Section III. Techniques for Monitoring RNA Conformation and Dynamics

A. Solution Methods

- | | | |
|--|--|-----|
| 20. Probing RNA Structure and Function by Circular Permutation | TAO PAN | 313 |
| 21. Kinetic Oligonucleotide Hybridization for Monitoring Kinetic Folding of Large RNAs | DANIEL K. TREIBER AND
JAMES R. WILLIAMSON | 330 |
| 22. Time-Resolved Synchrotron X-Ray Footprinting and Its Application to RNA Folding | CORIE Y. RALSTON,
BIANCA SCLAVI,
MICHAEL SULLIVAN,
MICHAEL L. DERAS,
SARAH A. WOODSON,
MARK R. CHANCE, AND
MICHAEL BRENOWITZ | 353 |

B. Electrophoretic and Spectroscopic Methods

- | | | |
|---|---|-----|
| 23. Analysis of Global Conformation of Branched RNA Species Using Electrophoresis and Fluorescence | DAVID M. J. LILLEY | 368 |
| 24. Application of Circular Dichroism to Study RNA Folding Transitions | TOBIN R. SOSNICK,
XINGWANG FANG, AND
VALERIE M. SHELTON | 393 |
| 25. Fluorescence Assays to Study Structure, Dynamics, and Function of RNA and RNA-Ligand Complexes | NILS G. WALTER AND
JOHN M. BURKE | 409 |
| 26. Transient Electric Birefringence for Determining Global Conformations of Nonhelix Elements and Protein-Induced Bends in RNA | PAUL J. HAGERMAN | 440 |

Section IV. Modeling Tertiary Structure

- | | | |
|---|--|-----|
| 27. Structure-Function Relationships of RNA: A Modeling Approach | FABRICE LECLERC,
BELSIS LLORENTE, AND
ROBERT CEDERGREN | 457 |
| 28. Computational Modeling of Structural Experimental Data | MICHAEL A. BADA AND
RUSS B. ALTMAN | 470 |
| 29. Modeling RNA Tertiary Structure from Patterns of Sequence Variation | FRANÇOIS MICHEL,
MARIA COSTA,
CHRISTIAN MASSIRE, AND
ERIC WESTHOF | 491 |
| AUTHOR INDEX | | 511 |
| SUBJECT INDEX | | 533 |