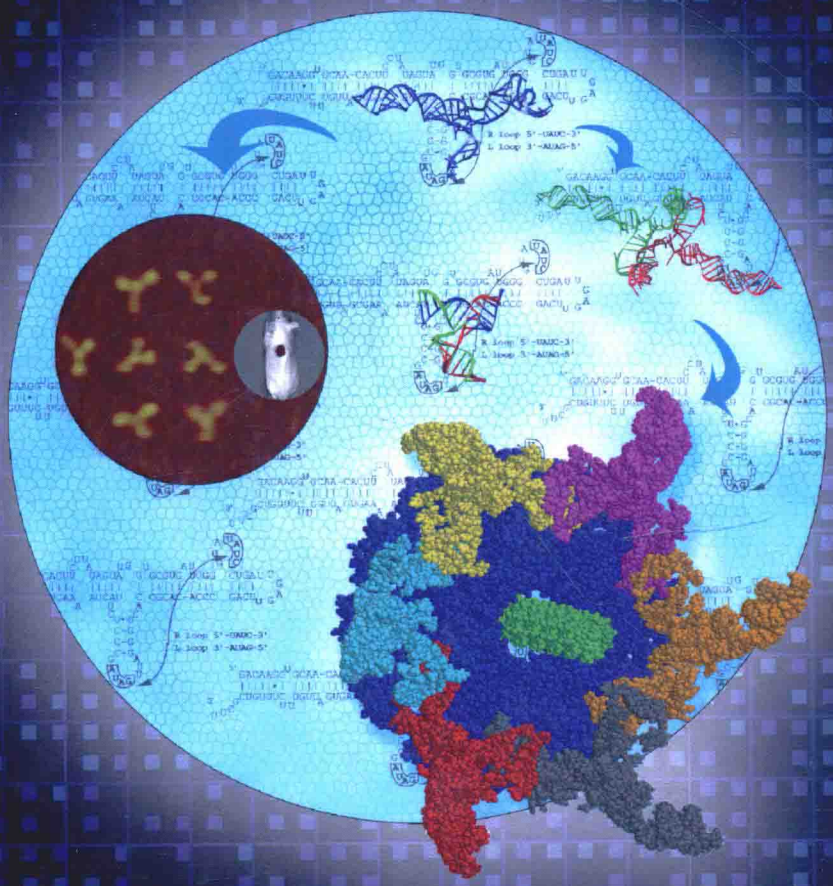
RNA Nanotechnology and Therapeutics



Edited by
Peixuan Guo
Farzin Haque

CRC Press
Taylor & Francis Group

RNA Nanotechnology and Therapeutics

Edited by
Peixuan Guo
Farzin Haque

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Printed on acid-free paper
Version Date: 20130315

International Standard Book Number-13: 978-1-4665-0566-7 (Hardback)

Library of Congress Cataloging-in-Publication Data

RNA nanotechnology and therapeutics / edited by Peixuan Guo and Farzin Haque.
p. ; cm.
Includes bibliographical references and index.
ISBN 978-1-4665-0566-7 (hardcover : alk. paper)
I. Guo, Peixuan, editor of compilation. II. Haque, Farzin, editor of compilation.
[DNLM: 1. Nanotechnology--methods. 2. RNA--therapeutic use. 3. Nanomedicine--methods. 4. Nanoparticles. QT 36.5]

QP619.A45
572.8'86--dc23 2013009491

Visit the Taylor & Francis Web site at
http://www.taylorandfrancis.com

and the CRC Press Web site at
http://www.crcpress.com

Preface

Nanotechnology deals with the creation and application of materials at the nanometer scale, using either top-down approaches or bottom-up assembly. Macromolecules of DNA, RNA, and proteins have defined features and the perfect size to serve as powerful building blocks for the bottom-up fabrication of nanostructures and nanodevices. However, nanotechnology must fulfill certain criteria: (1) products should have a defined size and structure at the nanometer scale; (2) the nanoparticles can be purified to homogeneity or relatively homogeneous; and (3) the nanoparticles can be characterized or visualized by either chemical, physical, biophysical, or optical procedures. For example, molecular biology studies of DNA are not nanotechnology; however, the use of DNA as a nanomaterial to build homogeneous structures by bottom-up assembly to generate products with defined physical, chemical, and biophysical properties of the DNA structures is DNA nanotechnology, and this pioneering concept has created an unexpected paradigm of materials engineering and synthetic structural biology. As an alternative to DNA, RNA has recently been catapulted into place as a nanotechnology platform due to its diversity in both structure and function. RNA is unique in comparison to DNA by virtue of its higher thermodynamic stability, canonical and noncanonical base pairing ability, as well as a variety of single-stranded loops suitable for inter- and intramolecular interactions, base stacking, and distinct *in vivo* attributes. Previously, the sensitivity of RNA to RNase degradation had been the biggest hurdle in the production of RNA for use as a construction material. Recently, simple chemical modifications, such as that with 2′-fluorine, have led to the generation of certain RNAs resistant to degradation that have retained their folding property and even their function in certain cases. The robust production of stable RNA has now moved the dream of RNA nanotechnology into a reality. However, simply conjugating functional RNA modules to gold, liposome, dendrimer, or polymer-based nanoparticles does not constitute RNA nanotechnology; rather, RNA nanotechnology is a bottom-up approach to assemble nanometer-scale particles with its main constituent composed of RNA.

The studies on RNA structure and folding are dated decades ago. However, RNA nanotechnology is a unique, emergent field that is distinct from the classical studies of RNA structure and folding. Besides intramolecular interaction and folding, the special knowledge of intermolecular interaction is necessary. In 1998, the pioneering work of Peixuan Guo demonstrated that RNA dimer, trimer, and hexamer nanoparticles can be assembled using re-engineered RNA fragments derived from pRNA (packaging RNA), a vital component that gears the bacteriophage phi29 DNA packaging motor. This finding was published in *Molecular Cell* (Guo et al., 1998) and was featured in *Cell* (Hendrix, 1998), cementing the concept of RNA nanotechnology. Since then, pRNA nanoparticles have been used successfully as polyvalent vehicles to deliver a variety of therapeutic molecules, and for the construction of RNA arrays. Over the last few years, investigations into the folding and structure of RNA motifs, as well as advances in RNA 3D computation from the traditional intramolecular interactions to intermolecular interactions, have laid a solid foundation for further development in RNA nanotechnology.

RNA nanotechnology is a vigorous and rapidly progressing new field of science, as evidenced by the explosion of publications on RNA nanostructures that have been published over the last five years. These have come from diverse fields such as chemistry,

biochemistry, structural biology, microbiology, cancer biology, cell biology, biophysics, pharmacy, and nanomedicine. This innovative area of study is truly an interdisciplinary one that involves professionals with multiple backgrounds and skills. At this point in time, it is imperative to compile a book to serve as the first comprehensive collection of basic research that includes real-world applications pertinent to nanotechnology. The intended style of this book is one that can engage researchers from undergraduates all the way to postdoctoral researchers and professors, in engineering and other sciences, to further enhance this field. It is imperative that we work together to propel it to all that it can be and more.

This text strives to assemble information, engage its readers, and inspire scientists all over the world. It covers a wide range of topics, including the principles and fundamentals of RNA nanotechnology (Chapters 1 and 2); RNA folding, structure, and motifs in RNA nanoparticle assembly (Chapters 3 through 6); RNA computation and structure prediction for RNA nanoparticle construction (Chapters 7 and 8); nucleotide chemistry for nanoparticle synthesis, conjugation, and labeling (Chapters 9 through 11); single-molecule and biophysical techniques in RNA nanostructure analysis (Chapters 12 and 13); methods for the assembly of RNA nanoparticles (Chapters 14 through 16); and RNA nanoparticles for therapy and diagnostic applications (Chapters 17 through 29).

We would not have been able to accomplish our goal without the tremendous efforts of leading experts in the field who have taken the time to contribute a chapter. Foremost, we express our gratitude to them and the time that they have put into this text. We sincerely thank the staff at CRC Press/Taylor & Francis Group, LLC. In particular, we thank Michael Slaughter, the executive editor who convinced us to undertake this project; Laurie Schlags, the project coordinator; Ed Curtis, the project editor; and Amor Nanas, the project manager. Without their valuable contributions, this book would have never come to fruition. Finally, without the support of our devoted wives, this endeavor would never have been possible. We are delighted to present this book and hope that readers will find it very resourceful and as exciting as we do.

Peixuan Guo
Farzin Haque
University of Kentucky

Editors

Dr. Peixuan Guo is the William Farish Endowed Chair of Nanobiotechnology and the director of the Nanobiotechnology Center at the University of Kentucky. He also directed one NIH National Nanomedicine Development Center from 2006 to 2011 and currently directs the National Cancer Institute (NCI) Cancer Nanotech Platform Partnership Program with a focus on RNA nanotechnology for cancer therapy.

He earned a PhD from the University of Minnesota in 1987 and a postdoctoral degree at NIH before joining Purdue as an assistant professor in 1990. He tenured in 1993, became a full professor in 1997, and has been honored as a Purdue Faculty Scholar since 1998. He moved to the University of Cincinnati's endowed chair in biomedical engineering in 2007 and moved to the University to Kentucky as endowed chair and center director in 2012.

He constructed the phi29 DNA packaging motor (*PNAS*, 1986), discovered phi29 motor pRNA (*Science*, 1987), assembled infectious dsDNA viruses (*J Virology*, 1995), discovered the pRNA hexamer (*Mol Cell*, 1998), and pioneered RNA nanotechnology (*Mol Cell*, 1998; *JNN*, 2003; *Nano Lett*, 2004, 2005; *Nat Nanotechnol*, 2010). His laboratory built a dual imaging system to detect single fluorophores (*EMBO J*, 2007; RNA, 2007) and incorporated the phi29 motor channel into a lipid membrane (*Nat Nanotechnol*, 2009) for single-molecule sensing with potentials for high-throughput dsDNA sequencing.

He received the Pfizer Distinguished Faculty Award in 1995; the Purdue Faculty Scholar Award in 1998; the Purdue Seed Award in 2004, 2005, and 2007; the Lions Club Cancer Research Award in 2006; the honor of being a COV Distinguished Alumni of the University of Minnesota in 2009; and the University of Cincinnati Distinguished Research Award in 2011. He is an editor or board member of five nanotech journals. His work has been reported hundreds of times on radio and television, such as in ABC and NBC, and has been featured in the newsletters or websites of NIH, NSF, MSNBC, NCI, and ScienceNow, among others. He was a member of two prominent national nanotech initiatives by NIST, NIH, NSF, and the National Council of Nanotechnology and a member of the NIH Nanomedicine Development Center Steering Committee from 2006 to 2010.

Dr. Farzin Haque is a research assistant professor in the University of Kentucky College of Pharmacy, Department of Pharmaceutical Sciences. He earned a BA in biochemistry and mathematics (2004) from Lawrence University and a PhD in chemistry (2008) from Purdue University. He held a postdoctoral appointment (2009–2011) at the University of Cincinnati with Professor Peixuan Guo. Dr. Haque's scholarly interest broadly focuses on nanoscience and nanotechnology in biology and medicine.

An excellent emerging scientist in the field of RNA nanotechnology, Dr. Haque has significant expertise in constructing RNA nanoparticles harboring functional modules for therapeutic and diagnostic applications (*Nano Today*, 2012; *Nat Nanotechnol*, 2011). In addition, he has expertise in a variety of fields, including lipid–lipid and lipid–protein interactions (*J Phys Chem B*, 2010; *Biophys J*, 2008), and protein nanopore development for single-molecule detection and sensing of chemicals and biopolymers (*ACS Nano*, 2012; *Nat Protoc*, 2013; *Nano Lett*, 2010).

Contributors

Sherine Abdelmawla
Kylin Therapeutics
West Lafayette, Indiana

Clara Alfaro-Cervello
Department of Clinical Neurosciences
Cambridge Centre for Brain Repair and Stem Cell Institute
University of Cambridge
Cambridge, United Kingdom

Donald E. Bergstrom
Department of Medicinal Chemistry and Molecular Pharmacology and Bindley Bioscience Center
Purdue University
West Lafayette, Indiana

Daniel W. Binzel
Department of Pharmaceutical Sciences
College of Pharmacy
University of Kentucky
Lexington, Kentucky

Longxin Chen
Laboratory of Molecular Biology
Zhengzhou Normal University
Henan, China

Chiara Cossetti
Department of Clinical Neurosciences
Cambridge Centre for Brain Repair and Stem Cell Institute
University of Cambridge
Cambridge, United Kingdom

Peter Daldrop
Cancer Research UK Nucleic Acid Structure Research Group
University of Dundee
Dundee, United Kingdom

Subha R. Das
Department of Chemistry and Center for Nucleic Acids Science and Technology
Carnegie Mellon University
Pittsburgh, Pennsylvania

Baoquan Ding
National Center for Nanoscience and Technology, China
Beijing, China

Feng Ding
Department of Biochemistry and Biophysics
School of Medicine
University of North Carolina at Chapel Hill
Chapel Hill, North Carolina

Nikolay V. Dokholyan
Department of Biochemistry and Biophysics
School of Medicine
University of North Carolina at Chapel Hill
Chapel Hill, North Carolina

Hiroyuki Furuta
Department of Chemistry and Biochemistry
Graduate School of Engineering
Kyushu University
Fukuoka, Japan

Katherine Germer
Department of Cancer and Cell Biology
Vontz Center for Molecular Studies
University of Cincinnati College of Medicine
Cincinnati, Ohio

Bin Guo
Department of Pharmaceutical Sciences
College of Pharmacy
North Dakota State University
Fargo, North Dakota

Peixuan Guo
Department of Pharmaceutical Sciences
College of Pharmacy
University of Kentucky
Lexington, Kentucky

Brent Hallahan
Department of Pharmaceutical Sciences
College of Pharmacy
University of Kentucky
Lexington, Kentucky

Paul Hanson
Department of Pathology and Laboratory Medicine
University of British Columbia
Vancouver, British Columbia, Canada

Farzin Haque
Department of Pharmaceutical Sciences
College of Pharmacy
University of Kentucky
Lexington, Kentucky

Maged Hemida
Department of Pathology and Laboratory Medicine
University of British Columbia
Vancouver, British Columbia, Canada

Lin Huang
Cancer Research UK Nucleic Acid Structure Research Group
University of Dundee
Dundee, United Kingdom

Zhen Huang
Department of Chemistry
Center for Neuroscience Research
University at Albany, SUNY
Albany, New York

Zhen Huang
Department of Chemistry
Georgia State University
Atlanta, Georgia

Yoshiya Ikawa
Department of Chemistry and Biochemistry
Graduate School of Engineering
Kyushu University
Fukuoka, Japan

Tan Inoue
Graduate School of Biostudies
Kyoto University
Kyoto, Japan

Nunzio Iraci
Department of Clinical Neurosciences
Cambridge Centre for Brain Repair and Stem Cell Institute
University of Cambridge
Cambridge, United Kingdom

William Jaremko
Department of Chemistry
Center for Neuroscience Research
University at Albany, SUNY
Albany, New York

Wojciech Kasprzak
Basic Science Program, SAIC-Frederick, Inc.
Center for Cancer Research Nanobiology Program
Frederick National Laboratory for Cancer Research
Frederick, Maryland

Emil F. Khisamutdinov
Department of Pharmaceutical Sciences
College of Pharmacy
University of Kentucky
Lexington, Kentucky

Thomas Kissel
Department of Pharmaceutics and Biopharmacy
Philipps-Universität Marburg
Marburg, Germany

Brian M. Laing
Department of Medicinal Chemistry and Molecular Pharmacology and Bindley Bioscience Center
Purdue University
West Lafayette, Indiana

Markos Leggas
Department of Pharmaceutical Sciences
College of Pharmacy
University of Kentucky
Lexington, Kentucky

Ping Lei
Department of Immunology
Tongji Medical College
Huazhong University of Science and Technology
Hubei, China

Neocles B. Leontis
Department of Chemistry
Bowling Green State University
Bowling Green, Ohio

Hui Li
Department of Pharmaceutical Sciences
College of Pharmacy
University of Kentucky
Lexington, Kentucky

Runting Li
Laboratory of Molecular Biology
Zhengzhou Normal University
Henan, China

David M. J. Lilley
Cancer Research UK Nucleic Acid Structure Research Group
University of Dundee
Dundee, United Kingdom

Chi-yen Lin
Department of Chemistry
Center for Neuroscience Research
University at Albany, SUNY
Albany, New York

Yuri L. Lyubchenko
Department of Pharmaceutical Sciences
University of Nebraska Medical Center
Omaha, Nebraska

Runlin Z. Ma
Institute of Genetics and Developmental Biology
Chinese Academy of Sciences
Beijing, China

Olivia M. Merkel
Department of Pharmaceutical Sciences
Wayne State University
Detroit, Michigan

Li Niu
Department of Chemistry
Center for Neuroscience Research
University at Albany, SUNY
Albany, New York

Hirohisa Ohno
Graduate School of Biostudies
Kyoto University
Kyoto, Japan

Eriko Osada
Center for iPS Cell Research and Application
Kyoto University
Kyoto, Japan

Eduardo Paredes
Department of Chemistry and Center for Nucleic Acids Science and Technology
Carnegie Mellon University
Pittsburgh, Pennsylvania

Fengmei Pi
Department of Pharmaceutical Science
College of Pharmacy
University of Kentucky
Lexington, Kentucky

Stefano Pluchino
Department of Clinical Neurosciences
Cambridge Centre for Brain Repair and Stem Cell Institute
University of Cambridge
Cambridge, United Kingdom

Ye Qiu
Department of Pathology and Laboratory Medicine
University of British Columbia
Vancouver, British Columbia, Canada

Randall Reif
Department of Biochemistry
St. Mary's College of Maryland
St. Mary's City, Maryland

Chris Richards
Department of Chemistry
University of Kentucky
Lexington, Kentucky

John J. Rossi
Department of Molecular and Cellular Biology
Beckman Research Institute of City of Hope
Duarte, California

Hirohide Saito
Center for iPS Cell Research and Application
Kyoto University
Kyoto, Japan

Peter M. Schön
Materials Science and Technology of Polymers
MESA+ Institute for Nanotechnology
University of Twente
Enschede, The Netherlands

Kersten T. Schroeder
Cancer Research UK Nucleic Acid Structure Research Group
University of Dundee
Dundee, United Kingdom

Bahar Seremi
Department of Physics
Texas Tech University
Lubbock, Texas

Bruce A. Shapiro
Center for Cancer Research Nanobiology Program
National Cancer Institute
Frederick, Maryland

Guanxin Shen
Department of Immunology
Tongji Medical College
Huazhong University of Science and Technology
Hubei, China

Hua Shi
Department of Biological Sciences and the RNA Institute
University at Albany, SUNY
Albany, New York

Luda S. Shlyakhtenko
Department of Pharmaceutical Sciences
University of Nebraska Medical Center
Omaha, Nebraska

Dan Shu
Department of Pharmaceutical Sciences
College of Pharmacy
University of Kentucky
Lexington, Kentucky

Yi Shu
Department of Pharmaceutical Sciences
College of Pharmacy
University of Kentucky
Lexington, Kentucky

Ka-To Shum
Department of Molecular and Cellular Biology
Beckman Research Institute of City of Hope
Duarte, California

Jayden A. Smith
Department of Clinical Neurosciences
Cambridge Centre for Brain Repair and Stem Cell Institute
University of Cambridge
Cambridge, United Kingdom

Matilde Stefanini
Department of Clinical Neurosciences
Cambridge Centre for Brain Repair and Stem Cell Institute
University of Cambridge
Cambridge, United Kingdom

Huiyan Sun
Department of Chemistry
Georgia State University
Atlanta, Georgia

Takahiro Tanaka
Department of Chemistry and Biochemistry
Graduate School of Engineering
Kyushu University
Fukuoka, Japan

Jia Wang
Cancer Research UK Nucleic Acid Structure Research Group
University of Dundee
Dundee, United Kingdom

Shaoying Wang
Department of Pharmaceutical Sciences
College of Pharmacy
University of Kentucky
Lexington, Kentucky

Zhen-Gang Wang
National Center for Nanoscience and Technology, China
Beijing, China

Decheng Yang
Department of Pathology and Laboratory Medicine
University of British Columbia
Vancouver, British Columbia, Canada

Xin Ye
Department of Pathology and Laboratory Medicine
University of British Columbia
Vancouver, British Columbia, Canada

Hui Zhang
Department of Pharmaceutical Sciences
College of Pharmacy
University of Kentucky
Lexington, Kentucky

Mary Zhang
Department of Pathology and Laboratory Medicine
University of British Columbia
Vancouver, British Columbia, Canada

Xiaoting Zhang
Department of Cancer and Cell Biology
Vontz Center for Molecular Studies
University of Cincinnati College of Medicine
Cincinnati, Ohio

Zhengyi Zhao
Department of Pharmaceutical Sciences
College of Pharmacy
University of Kentucky
Lexington, Kentucky

Mengyao Zheng
Department of Pharmaceutics and Biopharmacy
Philipps-Universität Marburg
Marburg, Germany

Jiehua Zhou
Department of Molecular and Cellular Biology
Beckman Research Institute of the City of Hope
Duarte, California

Contents

Section IV RNA Chemistry for Nanoparticle Synthesis, Conjugation, and Labeling

Section V Single-Molecule and Biophysical Techniques in RNA Nanostructure Analysis

Section VI Methods for the Assembly of RNA Nanoparticles

Section VII RNA Nanoparticles for Therapy of Cancer, Viral Infections, and Genetic Diseases

Section XI Application of siRNAs in RNA Nanotechnology and Therapeutics

Section I

Introduction: Principles and Fundamentals of RNA Nanotechnology