纳米科学与技术

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方法与操作

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Methods and Protocols

Giampaolo Zuccheri Bruno Samorì

科学出版社

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《纳米科学与技术》丛书序

在新兴前沿领域的快速发展过程中，及时整理、归纳、出版前沿科学的系统性专著，一直是发达国家在国家层面上推动科学与技术发展的重要手段，是一个国家保持科学技术的领先权和引领作用的重要策略之一。

科学技术的发展和应用，离不开知识的传播：我们从事科学研究，得到了“数据”（论文），这只是“信息”。将相关的大量信息进行整理、分析，使之形成体系并付诸实践，才变成“知识”。信息和知识如果不能交流，就没有用处，所以需要“传播”（出版），这样才能被更多的人“应用”，被更有效地应用，被更准确地应用，知识才能产生更大的社会效益，国家才能在越来越高的水平上发展。所以，数据→信息→知识→传播→应用→效益→发展，这是科学技术推动社会发展的基本流程。其中，知识的传播，无疑具有桥梁的作用。

整个 20 世纪，我国在及时地编辑、归纳、出版各个领域的科学技术前沿的系列专著方面，已经大大地落后于科技发达国家，其中的原因有许多，我认为更主要的是缘于科学文化的习惯不同：中国科学家不习惯去花时间整理和梳理自己所从事的研究领域的知识，将其变成具有系统性的知识结构。所以，很多学科领域的第一本原创性“教科书”，大都来自欧美国家。当然，真正优秀的著作不仅需要花费时间和精力，更重要的是要有自己的学术思想以及对这个学科领域充分把握和高度概括的学术能力。

纳米科技已经成为 21 世纪前沿科学技术的代表领域之一，其对经济和社会发展所产生的潜在影响，已经成为全球关注的焦点。国际纯粹与应用化学联合会（IUPAC）会刊在 2006 年 12 月评论：“现在的发达国家如果不发展纳米科技，今后必将沦为第三世界发展中国家。”因此，世界各国，尤其是科技强国，都将发展纳米科技作为国家战略。

兴起于 20 世纪后期的纳米科技，给我国提供了与科技发达国家同步发展的良好机遇。目前，各国政府都在加大力度出版纳米科技领域的教材、专著以及科普读物。在我国，纳米科技领域尚没有一套能够系统、科学地展现纳米科学技术各个方面前沿进展的系统性专著。因此，国家纳米科学中心与科学出版社共同发起并组织出版《纳米科学与技术》，力求体现本领域出版读物的科学性、准确性和系统性，全面科学地阐述纳米科学技术前沿、基础和应用。本套丛书的出版以高质量、科学性、准确性、系统性、实用性为目标，将涵盖纳米科学技术的所有领域，全面介绍国内外纳米科学技术发展的前沿知识；并长期组织专家撰写、编辑出版下去，为我国

纳米科技各个相关基础学科和技术领域的科技工作者和研究生、本科生等，提供一套重要的参考资料。

这是我们努力实践“科学发展观”思想的一次创新，也是一件利国利民、对国家科学技术发展具有重要意义的大事。感谢科学出版社给我们提供的这个平台，这不仅有助于我国在科研一线工作的高水平科学家逐渐增强归纳、整理和传播知识的主动性（这也是科学研究回馈和服务社会的重要内涵之一），而且有助于培养我国各个领域的人士对前沿科学技术发展的敏感性和兴趣爱好，从而为提高全民科学素养作出贡献。

我谨代表《纳米科学与技术》编委会，感谢为此付出辛勤劳动的作者、编委会委员和出版社的同仁们。

同时希望您，尊贵的读者，如获此书，开卷有益！

中国科学院院长

国家纳米科技指导协调委员会首席科学家

2011 年 3 月于北京

Preface

Giorgio Vasari, a painter, architect, and art historian during the Italian Renaissance, is credited with coining the expression "andare a bottega," ("attending the studio") referring to the internship that the apprentice would complete in the master's studio in order to learn what could be uniquely transmitted in person and in that particular environment and that could then lead to making a unique artist of the apprentice.

Nowadays, this same concept holds true in science, and despite the many opportunities for communication and "virtual presence", the real physical permanence in a lab is still the best way for a scientist to learn a technique or a protocol, or a way of thinking. A book of protocols, such as this, humbly proposes itself as the second-best option. Not quite the same as being in person in a lab and witnessing the experts' execution of a protocol, it still holds many more details and hints than the usually brief methods section found in research papers. This book of protocols for DNA nanotechnology was composed with this concept in mind: prolonging the tradition of Methods in Molecular Biology, it tries to simplify researchers' lives when they are putting in practice protocols whose results they have learnt in scientific journals.

DNA is playing a quite important and dual role in nanotechnology. First, its properties can nowadays be studied with unprecedented detail, thanks to the new instrumental nano(bio)technologies and new insight is being gathered on the biological behavior and function of DNA thanks to new instrumentation, smart experimental design, and protocols. Second, the DNA molecule can be decontextualized and "simply" used as a copolymer with designed interaction rules. The Watson–Crick pairing code can be harnessed towards implementing the most complicated and elegant molecular self-assembly reported to date. After Ned Seeman's contribution, elegantly complicated branched structures can be braided and joined towards building nano-objects of practically any desired form.

DNA nanotechnology is somewhat like watching professional tennis players: everything seems so simple, but then you set foot on the court and realize how difficult it is to hit a nice shot. When you see the structural perfection of a self-assembling DNA nano-object, such as a DNA origami, you marvel at how smart DNA is as a molecule and wonder how many different constructs you could design and realize. Among the others, this book tries to show the procedures to follow in order to repeat some of the methods that lead to such constructs, or to the mastering of the characterization techniques used to study them. Many details and procedures are the fruit of the blending of artistry, science, and patience, which are often unseen in a journal paper, but that could be what makes the difference between a winning shot and hitting the net.

Many research groups share their expertise with the readers in this book. For the sake of conciseness, we here mention the group leaders, while it is truly from the daily work of a complete team that the details of a protocol can be worked out. The chapters of this book can be roughly divided into two parts: some deal with the methods of preparing the nanostructures, from the rationale of the operations to the techniques for their handling; some other chapters deal more directly with advanced instrumental techniques that can manipulate and characterize molecules and nanostructures. As part of the first group, Roberto Corradini introduces the reader to the methods and choices for taming helix chirality, Alexander Kotlyar, Wolfgang Fritzsche, Naoki Sugimoto, and James Vesenka

share their different methods in growing, characterizing, and modifying nanowires based on G tetraplexes; Hao Yan and Friedrich Simmel teach all the basics for implementing the self-assembly of branched DNA nanostructures, and then characterizing the assembly. Hanadi Sleiman tells about hybrid metal–DNA nanostructures with controlled geometry. Frank Bier shows the use of rolling circle amplification to make repetitive DNA nanostructures, while, moving closer to technological use of DNA, Arianna Filoramo instructs on how to metalize double-stranded DNA and Andrew Houlton reports on the protocol to grow DNA oligonucleotides on silicon. Also with an eye to the applicative side, Yamuna Krishnan instructs on how to insert and use DNA nanostructures inside living cells. On the instrument side, Ciro Cecconi and Mark Williams introduce the readers to methods for the use of optical tweezers, focusing mainly on the preparation of the ideal molecular construct and on the instrument and its handling, respectively. John van Noort and Sanford Leuba give us protocols on how to obtain sound data from single-molecule FRET and apply it to study the structure of chromatin. Claudio Rivetti teaches the reader how to extract quantitative data from AFM of DNA and its complexes, while Matteo Castronovo instructs on the subtleties of using the AFM as a nanolithography tool on self-assembled monolayers; Jussi Toppari dwelves on the very interesting use of dielectrophoresis as a method to manipulate and confine DNA, while Matteo Palma and Jennifer Cha explain methods for confining on surfaces DNA and those very same types of DNA nanostructures that other chapters tell the reader how to assemble. Aleksei Aksimientev shows the methods for modeling nanopores for implementing DNA translocation, a technique bound to find many applications in the near future.

We hope this book will help ignite interest and spur activity in this young research field, expanding our family of enthusiastic followers and practitioners. There are certainly still many chapters to be written on this subject, simply because so much is happening in the labs at this very moment. There will certainly be room for the mainstreaming of protocols on the use of DNA analogues (starting with the marvelous RNA, of course), for the design and preparation of fully 3D architectures, for the development of routes towards functional DNA nanostructures, which will lead to applications. DNA nanostructures can be "re-inserted" in their original biological context, as microorganisms can be convinced to replicate nanostructures or even code them. And eventually, applications will require massive amounts of the nanostructures to be produced and to be manipulated automatically, possibly with a precision and output rate similar to that of the assembly of microelectronics circuitry nowadays.

Our personal wish is that the next chapters will be written by some of our readers.

Bologna, Italy — *Giampaolo Zuccheri*
Bologna, Italy — *Bruno Samorì*

Contributors

Justin J. Abramson • *Department of Mechanical Engineering, Columbia University, New York, NY, USA*

Aleksei Aksimentiev • *Department of Physics, University of Illinois at Urbana-Champaign, Urbana, IL, USA*

Faisal A. Aldaye • *Department of Systems Biology, Harvard Medical School, Boston, MA, USA*

Frank F. Bier • *Department of Nanobiotechnology & Nanomedicine, Fraunhofer Institute for Biomedical Engineering, Branch Potsdam-Golm, Potsdam, Germany*

Ruth Buning • *Leiden Institute of Physics, Leiden Universiteit, Leiden, The Netherlands*

Carlos Bustamante • *Howard Hughes Medical Institute, Department of Physics, University of California, Berkeley, CA, USA*

Stephane Campidelli • *CEA Saclay, Laboratoire d'Electronique Moléculaire, Gif-sur-Yvette Cedex, France*

Matteo Castronovo • *Department of Biology, MONALISA Laboratory, College of Science and Technology, Temple University, PA, USA*

Ciro Cecconi • *CNR-Istituto Nanoscienze S3, Department of Physics, University of Modena e Reggio Emilia, Modena, Italy*

Jennifer N. Cha • *Department of Nanoengineering, UC San Diego, La Jolla, CA, USA*

Rahul Chhabra • *University of Alberta, National Institute of Nanotechnology, Edmonton, AB, Canada*

Jeffrey R. Comer • *Department of Physics, University of Illinois at Urbana-Champaign, Urbana, IL, USA*

Bernard A. Connolly • *Chemical Nanoscience Laboratory, School of Chemistry, Newcastle University, Newcastle upon Tyne, UK*

Roberto Corradini • *Dipartimento di Chimica Organica e Industriale, Univeristà di Parma, Parma, Italy*

Andrea Csaki • *Institute of Photonic Technology (IPHT), Jena, Germany*

Matt V. Fagerburg • *Departments of Cell Biology and Physiology and Bioengineering, University of Pittsburgh School of Medicine and Swanson School of Engineering, Petersen Institute of Nano Science and Engineering and University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA*

Arianna Filoramo • *CEA Saclay, Laboratoire d'Electronique Moléculaire, Gif-sur-Yvette Cedex, France*

Wolfgang Fritzsche • *Institute of Photonic Technology (IPHT), Jena, Germany*

Alon A. Gorodetsky • *Department of Chemistry, Columbia University, New York, NY, USA*

Mark M. Green • *Dipartimento di Chimica Organica e Industriale, Univeristã di Parma, Parma, Italy*
Graham D. Hamblin • *Department of Chemistry, McGill University, Montreal, Canada*
Ralph Hölzel • *Department of Nanobiotechnology & Nanomedicine, Fraunhofer Institute for Biomedical Engineering, Branch Potsdam-Golm, Potsdam, Germany*
James Hone • *Department of Mechanical Engineering, Columbia University, New York, NY, USA*
Benjamin R. Horrocks • *Chemical Nanoscience Laboratory, School of Chemistry, Newcastle University, Newcastle upon Tyne, UK*
Andrew Houlton • *Chemical Nanoscience Laboratory, School of Chemistry, Newcastle University, Newcastle upon Tyne, UK*
Albert M. Hung • *Department of Nanoengineering, UC San Diego, La Jolla, CA, USA*
Yonggang Ke • *Dana-Farber Cancer Institute & Harvard Medical School, Boston, MA, USA*
Wiepke J.A. Koopmans • *Leiden Institute of Physics, Leiden Universiteit, The Netherlands*
Alexander Kotlyar • *Department of Biochemistry, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Ramat Aviv, Israel*
Yamuna Krishnan • *Biochemistry, Biophysics and Bioinformatics, National Centre for Biological Sciences, Bangalore, India*
Anton Kuzyk • *Lehrstuhl für Bioelektronik, Physik-Department and ZNN/WSI, Technische Universität München, Garching, Germany*
Christian Leiterer • *Institute of Photonic Technology (IPHT), Jena, Germany*
Sanford H. Leuba • *Departments of Cell Biology and Physiology and Bioengineering, University of Pittsburgh School of Medicine and Swanson School of Engineering, Petersen Institute of NanoScience and Engineering, University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA*
Chenxiang Lin • *Dana-Farber Cancer Institute & Wyss Institute at Harvard University, Boston, MA, USA*
Yan Liu • *Department of Chemistry and Biochemistry, The Biodesign Institute, Arizona State University, Tempe, AZ, USA*
Pik Kwan Lo • *Department of Chemistry, McGill University, Montreal, Canada*
Rosangela Marchelli • *Dipartimento di Chimica Organica e Industriale, Univeristà di Parma, Parma, Italy*
Susan Marqusee • *Department of Molecular & Cell Biology, University of California, Berkeley, CA, USA*
Micah J. McCauley • *Department of Physics, Northeastern University, Boston, MA, USA*
Christopher K. McLaughlin • *Department of Chemistry, McGill University, Montreal, Canada*
Daisuke Miyoshi • *Faculty of Frontiers of Innovative Research in Science and Technology (FIRST), and Frontier Institute for Biomolecular Engineering Research (FIBER), Konan University, Kobe, Japan*

Souvik Modi • *Biochemistry, Biophysics and Bioinformatics, National Centre for Biological Sciences, Bangalore, India*
Khoa Nguyen • *CEA Saclay, Laboratoire d'Electronique Moléculaire, Gif-sur-Yvette Cedex, France*
Colin Nuckolls • *Department of Chemistry, Columbia University, New York, NY, USA*
Matteo Palma • *Department of Mechanical Engineering & Applied Physics and Applied Mathematics, Columbia University, New York, NY, USA*
Andrew R. Pike • *Chemical Nanoscience Laboratory, School of Chemistry, Newcastle University, Newcastle upon Tyne, UK*
Edda Reiß • *Department of Nanobiotechnology & Nanomedicine, Fraunhofer Institute for Biomedical Engineering, Branch Potsdam-Golm, Potsdam, Germany*
Claudio Rivetti • *Department of Biochemistry and Molecular Biology, University of Parma, Parma, Italy*
Denis Scaini • *Sincrotrone Trieste, Basovizza, Trieste, Italy*
Stefano Sforza • *Dipartimento di Chimica Organica e Industriale, Univeristà di Parma, Parma, Italy*
Elizabeth A. Shank • *Harvard Medical School, Boston, MA, USA*
Jaswinder Sharma • *Center for Integrated Nanotechnologies, Los Alamos National Laboratory, Los Alamos, NM, USA*
Michael P. Sheetz • *Department of Biological Sciences, Columbia University, New York, NY, USA*
Friedrich C. Simmel • *Physik Department, Technische Universität München, Munich, Germany*
Hanadi F. Sleiman • *Department of Chemistry, McGill University, Montreal, Canada*
Thomas L. Sobey • *Physik Department, Technische Universität München, Munich, Germany*
Naoki Sugimoto • *Faculty of Frontiers of Innovative Research in Science and Technology (FIRST), and Frontier Institute for Biomolecular Engineering Research (FIBER), Konan University, Kobe, Japan*
Tullia Tedeschi • *Dipartimento di Chimica Organica e Industriale, Università di Parma, Parma, Italy*
J. Jussi Toppari • *Department of Physics, Nanoscience Center, University of Jyväskylä, Jyväskylä, Finland*
Päivi Törmä • *Department of Applied Physics, School of science, Aalto University, Aalto, Finland*
John van Noort • *Leiden Institute of Physics, Leiden Universiteit, Leiden, The Netherlands*
James Vesenka • *Department of Chemistry and Physics, University of New England, Biddeford, ME, USA*
David B. Wells • *Department of Physics, University of Illinois at Urbana-Champaign, Urbana, IL, USA*

Mark C. Williams • *Department of Physics, Northeastern University, Boston, MA, USA*
Shalom J. Wind • *Department of Applied Physics and Applied Mathematics, Columbia University, New York, NY, USA*
Hao Yan • *Department of Chemistry and Biochemistry, The Biodesign Institute, Arizona State University, Tempe, AZ, USA*
Hua Yang • *Department of Chemistry, University of British Columbia, Vancouver, Canada*

Contents

Chapter 1

Synthesis and Characterization of Self-Assembled DNA Nanostructures

Chenxiang Lin, Yonggang Ke, Rahul Chhabra, Jaswinder Sharma, Yan Liu, and Hao Yan

Abstract

The past decade witnessed the fast evolvement of structural DNA nanotechnology, which uses DNA as blueprint and building material to construct artificial nanostructures. Using branched DNA as the main building block (also known as a "tile") and cohesive single-stranded DNA (ssDNA) ends to designate the pairing strategy for tile–tile recognition, one can rationally design and assemble complicated nanoarchitectures from specifically designed DNA oligonucleotides. Objects in both two- and three-dimensions with a large variety of geometries and topologies have been built from DNA with excellent yield; this development enables the construction of DNA-based nanodevices and DNA-template directed organization of other molecular species. The construction of such nanoscale objects constitutes the basis of DNA nanotechnology. This chapter describes the protocol for the preparation of ssDNA as starting material, the self-assembly of DNA nanostructures, and some of the most commonly used methods to characterize the self-assembled DNA nanostructures.

Key words: DNA nanotechnology, Self-assembly, Electrophoresis, Atomic force microscopy

1. Introduction

The notion that DNA is merely the gene encoder of living systems has been eclipsed by the successful development of DNA nanotechnology. DNA is an excellent nanoconstruction material because of its inherent merits: First, the rigorous Watson-Crick base-pairing makes the hybridization between DNA strands highly predictable. Second, the structure of the B-form DNA double helix is well-understood; its diameter and helical repeat have been determined to be ~2 and ~3.4 nm (i.e., ~10.5 bases), respectively, which facilitates the modeling of even the most complicated DNA nanostructures. Third, DNA possesses combined

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structural stiffness and flexibility. The rigid DNA double helixes can be linked by relatively flexible single-stranded DNA (ssDNA) to build stable motifs with desired geometry. Fourth, modern organic chemistry and molecular biology have created a rich toolbox to readily synthesize, modify, and replicate DNA molecules. Finally, DNA is a biocompatible material, making it suitable for the construction of multicomponent nanostructures made from hetero-biomaterials.

The field of structural DNA nanotechnology began with Nadrian Seeman's vision of combining branched DNA molecules bearing complementary sticky-ends to construct two-dimensional (2D) arrays (1) and his experimental construction of a DNA object topologically equal to a cube (2). Today, DNA self-assembly has matured with such vigor that it is currently possible to build micro- or even millimeter-sized nanoarrays with desired tile geometry and periodicity as well as any discrete 2D or 3D nanostructures we could imagine (3–8). Modified by functional groups, those DNA nanostructures can serve as scaffolds to control the positioning of other molecular species (9–21), which opens opportunities to study intermolecular synergies, such as protein–protein interactions, as well as to build artificial multicomponent nanomachines (22–24).

Generally speaking, the creation of a novel DNA motif usually requires the following steps: (1) Structural modeling: physical and/or graphic models are used to help the design of a new DNA motif; (2) Sequence design: in this step, specific sequences are assigned to all ssDNA molecules in the model; (3) Experimental synthesis of the DNA nanostructure; and (4) Characterization of the DNA nanostructure. The first two steps are crucial to program the outcome of self-assembly and assisted by computer software (25–30). In this chapter, we are going to describe the experimental protocols involved in steps 3 and 4.

2. Material

All chemicals are purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise noted. All buffer solutions are filtered and stored at room temperature unless otherwise noted.

2.1. Denaturing Polyacrylamide Gel Electrophoresis for the Purification of Synthetic Single-Stranded DNA

1. Synthetic ssDNA (Integrated DNA Techonologies, Coralville, IA) with designated sequences.
2. TBE buffer (1×): 89 mM Tris–boric acid, pH 8.0, 2 mM ethylenediaminetetraacetic acid disodium salt (EDTA-Na_2).
3. 20% urea-acrylamide Mix: 20% acrylamide (19:1 acrylamide:bis, Bio-Rad Laboratories, Hercules, CA), 8.3 M urea in 1× TBE buffer.

4. 0% Urea-acrylamide Mix: 8.3 M Urea in 1× TBE buffer.
5. Ammonium persulfate (APS): prepare 10% water solution and store at 4°C.
6. *N,N,N,N'*-tetramethyl-ethylenediamine (TEMED, Bio-Rad).
7. Bromophenol blue (BB) or xylene cyanole FF (XC) (2×): prepare 0.1% w/v solution of the dye in 90% formamide solution containing 10 mM NaOH and 1 mM Na_2EDTA.
8. Ethidium bromide: prepare 300 mL 0.1 μg/mL solution in a glass tray for gel staining.
9. Elution buffer (1×): 500 mM ammonium acetate, 10 mM magnesium acetate, 2 mM EDTA-Na_2.
10. 1-Butanol and 100% Ethanol.
11. Spin X centrifuge tube filters (Corning, Lowell, MA).

2.2. Self-Assembly of DNA Nanostructures

1. Polyacrylamide Gel Electrophoresis (PAGE) purified ssDNA.
2. TAE-Mg buffer (10×): 0.4 M Tris–acetic acid, pH 8.0, 125 mM magnesium acetate, 20 mM EDTA-Na_2.

2.3. Non-denaturing PAGE for the Characterization of Self-Assembled DNA Nanostructures

1. Self-assembled DNA nanostructures.
2. 40% acrylamide (19:1 acrylamide:bis, Bio-Rad Laboratories, Hercules, CA) solution.
3. Non-denaturing loading buffer (10×): 0.2% w/v bromophenol blue and xylene cyanole FF in 1× TAE-Mg buffer containing 50% v/v glycerol.
4. DNA ladder with suitable size (Invitrogen, Carlsbad, CA).
5. TAE-Mg buffer (1×), TEMED, and 10% APS solution (*vide supra*).
6. Stains-All: prepare 0.01% w/v Stains-All in 45% v/v formamide solution.

2.4. Atomic Force Microscope Imaging of Self-Assembled DNA Arrays

1. Self-assembled DNA nanostructures.
2. TAE-Mg buffer (1×) (*vide supra*).
3. Mica discs (Ted Pella, Inc) and Atomic Force Microscope (AFM) cantilevers of choice with integrated probes (such as NP-S from Veeco, Inc for imaging in liquids).

3. Methods

3.1. Denaturing PAGE Purification of Synthetic ssDNA

With advanced solid state synthesis chemistry, DNA synthesizer can generate DNA strands with designated sequences up to 200-base long. However, a significant yield drop is normally associated with the synthesis of longer DNA strands. For example,