

# **DRUG-DNA INTERACTIONS**

---

# **DRUG–DNA INTERACTIONS**

## **STRUCTURES AND SPECTRA**

---

**Kazuo Nakamoto**

Wehr Professor Emeritus of Chemistry  
Marquette University

**Masamichi Tsuboi**

Emeritus Professor of Pharmaceutical Science  
University of Tokyo

**Gary D. Strahan**

 **WILEY**

**A JOHN WILEY & SONS, INC., PUBLICATION**

*About the Cover:* The cover was created by G. Strahan, and is composed of the circular dichroism (CD) spectrum and a molecular model of a complex formed when the drug Distamycin binds as a dimer to an AT-rich DNA sequence (see Chapter 3). The molecular model was generated by the program AMBER9, using the methods outlined in Chapter 1 and Appendix 2, and visualized by the program UCSF Chimera.

Copyright © 2008 by John Wiley & Sons, Inc. All rights reserved

Published by John Wiley & Sons, Inc., Hoboken, New Jersey  
Published simultaneously in Canada

No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, scanning, or otherwise, except as permitted under Section 107 or 108 of the 1976 United States Copyright Act, without either the prior written permission of the Publisher, or authorization through payment of the appropriate per-copy fee to the Copyright Clearance Center, Inc., 222 Rosewood Drive, Danvers, MA 01923, (978) 750-8400, fax (978) 750-4470, or on the web at [www.copyright.com](http://www.copyright.com). Requests to the Publisher for permission should be addressed to the Permissions Department, John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030, (201) 748-6011, fax (201) 748-6008, or online at <http://www.wiley.com/go/permission>.

**Limit of Liability/Disclaimer of Warranty:** While the publisher and author have used their best efforts in preparing this book, they make no representations or warranties with respect to the accuracy or completeness of the contents of this book and specifically disclaim any implied warranties of merchantability or fitness for a particular purpose. No warranty may be created or extended by sales representatives or written sales materials. The advice and strategies contained herein may not be suitable for your situation. You should consult with a professional where appropriate. Neither the publisher nor author shall be liable for any loss of profit or any other commercial damages, including but not limited to special, incidental, consequential, or other damages.

For general information on our other products and services or for technical support, please contact our Customer Care Department within the United States at (800) 762-2974, outside the United States at (317) 572-3993 or fax (317) 572-4002.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic formats. For more information about Wiley products, visit our web site at [www.wiley.com](http://www.wiley.com).

***Library of Congress Cataloging-in-Publication Data:***

Nakamoto, Kazuo

Drug-DNA interactions : structures and spectra / Kazuo Nakamoto, Masamichi Tsuboi, Gary D. Strahan.

p. ; cm.

Includes index.

ISBN 978-0-471-78626-9 (cloth)

1. DNA-drug interactions—Research—Methodology. 2. DNA—Spectra. 3. Drugs—Spectra. I. Tsuboi, Masamichi, 1925- II. Strahan, Gary D. III. Title.

[DNLM: 1. DNA—drug effects. 2. Chemistry, Analytical—methods. 3.

DNA—chemistry. 4. Pharmaceutical Preparations—chemistry. 5. Structure-Activity Relationship. QU 58.5 N163d 2008]

QP624.75.D77N35 2008

572.8'6—dc22

2007050457

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

*The authors wish to dedicate this book to their wives,  
Kimiko Nakamoto, Yo Tsuboi, and Kellie Hom.*

# CONTENTS

---

<b>Preface</b>	<b>xi</b>
<b>Introduction</b>	<b>xiii</b>
<b>1. DNA Structures and Spectra</b>	<b>1</b>
1.1. DNA Structures	2
1.1.1. Primary (Chemical) Structure	2
1.1.2. Bond Lengths and Bond Angles	5
1.1.3. Canonical Structure of DNA: B-DNA	9
1.1.4. Deoxyribose Moiety as a Flexible Joint	13
1.1.5. Variations of B-DNA Structure	15
1.1.6. Participation of Water Molecules in B-DNA Structure	16
1.1.7. Partially Dehydrated Structure: A-DNA	17
1.1.8. Internal Dihedral Angles	19
1.1.9. Left-Handed Duplex: Z-DNA	21
1.1.10. Superhelical Form of DNA	23
1.1.11. Other Duplex Structures	25
1.1.12. Higher-Order DNA Structures	29
1.2. Electronic Spectra	34
1.2.1. Ultraviolet Absorption Spectra	34
1.2.2. Fluorescence Spectra	37
1.2.3. Linear and Circular Dichroism	38
1.3. Vibrational Spectra	42
1.3.1. Infrared Spectra of Bases	42
1.3.2. Raman Spectra of Bases	49
1.3.3. Vibrational Spectra of Nucleosides	54
1.3.4. Vibrational Spectra of Nucleotides	57
1.3.5. Resonance Raman Spectra	59
1.3.6. Vibrations of the Phosphodiester Linkage	59
1.3.7. Infrared Spectra of DNA	61
1.3.8. Raman Spectra of B-DNA	63
1.3.9. Comparison of Raman Spectra of A- and B-DNA	67
1.3.10. Raman Spectra of Z-DNA	70

1.4. NMR Spectra	72
1.4.1. NMR Signals and Magnetization Relaxation	73
1.4.2. Proton NMR Spectra of Bases, Nucleosides, and Nucleotides	76
1.4.3. Correlation Analysis, or “Spin–Spin Coupling”	80
1.4.4. Nuclear Overhauser Effect	85
1.4.5. Residual Dipolar Coupling	94
1.5. Electron Spin Resonance Spectra	95
1.6. X-Ray Crystallography	96
1.6.1. Bragg Diffraction	97
1.6.2. Diffraction of Helical DNA	98
1.7. Molecular Modeling and Molecular Mechanics	99
1.7.1. Molecular Modeling	99
1.7.2. Modeling of Experimental Data	100
References	102
<b>2. Intercalating Drugs</b>	<b>119</b>
2.1. Acridine Dyes	122
2.1.1. X-Ray Diffraction Studies of Acridine–Dinucleoside Monophosphate Crystals	122
2.1.2. Complexes with Longer DNAs: X-Ray and Modeling Analyses	128
2.1.3. Unwinding Angle Measurements	131
2.1.4. NMR Studies	134
2.1.5. Fluorescence Spectra	137
2.1.6. Flow Linear Dichroism Spectra/Studies	140
2.1.7. Circular Dichroism Studies	141
2.1.8. Biochemical Implications—a Few Examples	142
2.2. Ethidium Bromide	143
2.2.1. X-Ray and Modeling Analyses of Ethidium Bromide–Dinucleoside Monophosphate Crystals	143
2.2.2. Fiber X-Ray Diffraction Studies	145
2.2.3. Visible Absorption Spectra	148
2.2.4. Circular Dichroism Spectra	150
2.2.5. Fluorescence Spectra	152
2.2.6. Raman Spectra	154
2.2.7. Electrophoresis Studies of DNA Unwinding	159
2.2.8. Atomic Force Microscopy	161
2.3. Aclacinomycin	165
2.3.1. Absorption Spectroscopy	165
2.3.2. <sup>31</sup> P NMR	167
2.3.3. Exchangeable <sup>1</sup> H NMR Spectra	168

2.3.4. Nonexchangeable $^1\text{H}$ NMR Spectra	170
2.3.5. Raman Spectroscopy	175
2.3.6. Unwinding	176
2.4. Sequence Preference	178
2.5. Bis- and Tris- Intercalators	194
References	199
<b>3. Groove-Binding Drugs</b>	<b>209</b>
3.1. Netropsin and Distamycin	210
3.2. Derivatives of Netropsin and Distamycin	217
3.3. Hoechst 33258, SN6999, and Their Derivatives	224
3.4. Chromomycin, Mithramycin, and Other GC Binders	230
3.5. Groove Binding and Intercalation	234
References	240
<b>4. Covalent Bonding Drugs</b>	<b>245</b>
4.1. (+)CC1065 and Related Drugs	245
4.2. Anthramycin and Tomaymycin	254
4.3. Ecteinasidins	256
4.4. Mitomycins	259
4.5. Intercalating Alkylators	264
References	271
<b>5. Strand-Breaking Drugs</b>	<b>275</b>
5.1. Bleomycins	275
5.1.1. Metal-Free Bleomycins	275
5.1.2. Iron Complexes of Bleomycin	279
5.1.3. Cobalt and Zinc Complexes of Bleomycin	283
5.1.4. Model Compounds of Bleomycins	288
5.2. Eneidyne Antibiotics	290
5.2.1. Calicheamicins	291
5.2.2. Esperamicins	294
5.2.3. Neocarzinostatin	296
References	299
<b>6. Metal-Containing Drugs</b>	<b>303</b>
6.1. Cisplatin	303
6.1.1. Intrastrand Crosslinking Complexes	304
6.1.2. Interstrand Crosslinking Complexes	316
6.2. Cisplatin Derivatives	316

6.2.1. Oxaliplatin and Carboplatin	316
6.2.2. Platinum Complexes of Monofunctional Ligands	318
6.2.3. Platinum Complexes of Chelating Ligands	321
6.3. Transplatin and Derivatives	327
6.4. Monofunctional Platinum Complexes	332
6.5. Polynuclear and High-valent Platinum Complexes	336
6.6. Complexes Containing Other Metals	345
6.6.1. Palladium(II) and Gold(III)	346
6.6.2. Other Metals	347
References	347
<b>Appendixes</b>	<b>357</b>
A.1. Raman Tensor-Depolarization Ratio	357
A.2. NMR Spectroscopy	359
A.2.1. Origins of the Phenomenon	359
A.2.2. Chemical Shift	361
A.2.3. Magnetization Relaxation Processes	362
A.3. Molecular Mechanics	364
References	366
<b>Index</b>	<b>367</b>

## **PREFACE**

---

Drug discoveries are made serendipitously or systematically. In the latter case, a large number of derivatives of a candidate drug molecule are prepared and screened first by cytotoxicity tests *in vitro* since no definitive structure–activity relationships are available. This process is inefficient and time-consuming, in spite of the recent advent of combinatorial chemistry and laboratory automation. Hence, the ultimate goal of this book is to contribute to our knowledge of the structure–activity relationship found in drug–DNA interaction by coalescing much of the basic structural information, thereby improving the efficiency of the drug discovery process.

First, however, we must discuss the structures of drug–DNA complexes that were determined by a variety of physicochemical and biochemical techniques. Although some of these structural studies have already been reviewed by other authors, they tend to focus on structural information on the atomic level. In this book, we have emphasized global information obtained by a variety of spectroscopic techniques. It is hoped that this book will serve as a guide for students in chemistry, biochemistry, and pharmacology who are interested in learning the nature of drug–DNA interactions and as a reference book for academic and industrial researchers who are doing research in this field.

The authors would like to express their sincere thanks to the staffs of John Raynor Library of Marquette University for their help in collecting references, to Professor Jung-Ja Kim of Medical College of Wisconsin for her critical review of our manuscript, and to many authors and publishers who kindly gave us permission to reproduce their figures.

KAZUO NAKAMOTO  
MASAMICHI TSUBOI  
GARY D. STRAHAN

## INTRODUCTION

---

Cellular DNA is the major target of most antitumor drugs, as well as many antiviral and antibacterial agents. Depending on the mode of interaction, these drugs can be classified as intercalating, groove-binding, covalent bonding, and strand-breaking drugs. They can distort the double-helix structure of DNA, alkylate or cleave a DNA strand, thereby inhibiting DNA replication and transcription, which are the necessary preconditions for cell division. Previously, the nature of the drug–DNA interaction was investigated extensively using a variety of physicochemical and biochemical techniques. The results obtained from these studies have begun to shed light on the underlying principles of the structure–activity relationship, which is indispensable for understanding the mechanism of the drug–DNA interaction and for designing new and more effective drugs with fewer side effects.

Although many review articles on drug–DNA interactions (e.g., see Refs. G1–G8 listed at the end of this Introduction) have been published, comprehensive reviews combining structural and spectroscopic studies have not yet been available. The aim of this book is to provide such information using examples chosen from a large number of references. Cytotoxicity data are quoted whenever available and appropriate. It is hoped that any unbalanced presentation may be compensated by the review articles cited throughout the book. Drug–DNA interactions on the cellular and clinical levels and drug–protein interactions are not included because detailed structural and bonding information on these systems is limited by their complexity.

In this book, the terms *DNA* and *drug* are used loosely to cover a wide range of chemicals. Thus, *DNA* includes its components such as purine and pyrimidine bases, nucleosides as well as synthetic oligonucleotides, and natural DNA. Likewise, a *drug* (or a ligand) refers to any (natural and synthetic) chemical with potential pharmacological activity. Most of these are anticancer drugs, although antiviral, antibacterial drugs and a wide range of other chemicals such as biological stains and metal complexes are included.

This book focuses largely on structural and bonding information obtained by physicochemical methods, namely, X-ray crystallography, NMR spectroscopy, ESR spectroscopy, and optical spectroscopy such as UV–visible absorption, fluorescence, circular dichroism (CD), flow linear dichroism (FLD), and infrared (IR) and Raman (R) spectroscopy. Among these methods, X-ray crystallography and NMR spectroscopy are the most important when atom-level structural information is desired. However, X-ray crystallography is limited to drugs bound to relatively short oligonucleotides *in the crystalline state*, because it becomes more difficult to obtain single

crystals of diffraction quality as the nucleotide sequence becomes longer. Although multidimensional NMR spectroscopy coupled with computer-aided molecular modeling techniques can provide structural information on the atomic level *in solution*, this method is also limited to drug molecules bound to relatively short nucleotides since NMR signals tend to overlap in longer oligonucleotides. It should be noted that the molecular structure determined in the crystalline state suffers from the lattice effect, which could influence the molecular conformation. Although the solution structure obtained by NMR spectroscopy may be less precise than X-ray structure, it is closer to the structure in a biological environment and can provide insight into molecular flexibility. Furthermore, it is possible to study the effects of changing the pH, temperature, and drug–DNA mixing ratio on the drug–DNA interaction in solution and even dynamic equilibria between different conformers.

In contrast, the other spectroscopic techniques mentioned above are applicable to drug molecules complexed to long oligonucleotides such as poly(dG-dC)<sub>2</sub>, poly(dA-dT)<sub>2</sub>, and natural DNA, and provide structural and bonding information on the molecular level. It should be noted, however, that detailed structural information obtained by X-ray and NMR studies may not always be directly applicable to those with long oligonucleotides, because the structure of the latter is affected by the flexibility and base-pair sequence of their strands.

Since the main objective of this book is to review structural and spectroscopic information obtained by the aforementioned techniques, we provide only a very limited discussion of their basic theories and experimental methods. This information is found mainly in Chapter 1, with some expanded details presented in the appendices. More thorough information on these topics may be found in many monographs and review articles cited (e.g., see Refs. (G9–G14).

As stated earlier, and discussed at greater length in the following chapters, the modes of the drug–DNA interaction can be classified into the categories of *intercalation*, *groove binding*, *covalent-bonding* and *strand breaking* as well as a variety of their combinations. Furthermore, their bindings are often reinforced by hydrogen bonding, Coulombic forces, and van der Waals interactions. These are discussed throughout this book, which consists of six chapters.

Chapter 1 provides basic knowledge about the structures and spectra of bases, nucleosides, and nucleotides, and is vital to the understanding of the nature of drug–DNA interactions discussed in later chapters. It explains all of the conventional nomenclatures regarding DNA structures for the reader who is not familiar with DNA structures. For more information, the reader should consult those books and review articles on DNA structures that are abundantly available. Chapter 1 also provides an overview of how this structural information can be derived from the experimental methods.

Chapter 2 deals with drugs bound to DNA *via intercalation*, which was discovered in 1961 and is the best known mode of drug–DNA binding. Acridines, aclacinomycins, actinomycins, and ethyidium bromide are typical examples. More than 20 other intercalating drugs are listed together with their sequence preferences. Recently, a variety of bis and tris intercalating drugs have been prepared to attain stronger binding and better selectivity of binding sites, and their modes of binding are discussed.

Chapter 3 focuses on *groove-binding* drugs such as netropsin and distamycin, which interact with AT-rich region of DNA in the minor groove via hydrogen bonding and Coulombic and van der Waals forces. Sequence-specific drugs have been prepared by arranging pyrrole and imidazole rings in a specific order since pyrrole binds to A/T base whereas imidazole prefers to interact with the G base. Some derivatives of netropsin and distamycin exhibit antitumor and antiviral activities much stronger than those of distamycin. Chromomycin, mithramycin, and their derivatives represent another type of *groove-binding* drugs that interact with the GC-rich region of DNA. A variety of hybrid drugs in which groove binders are connected to intercalators have been synthesized and their modes of interactions with DNA investigated.

Chapter 4 describes *covalent bonding* drugs (e.g., alkylating agents) such as (+) CC-1065, anthracycline, ecteinascidins, and mitomycins. Active-carbon atoms of these drugs form *covalent bonds* with the N3 atom of adenine or guanine, and with the N7 atom and exocyclic NH<sub>2</sub> (N2 amine) group of guanine. Hedamycin and atromycin B are known as *intercalating alkylators*. Although metal-containing drugs such as cisplatin and its derivatives also form *covalent bonds* with DNA, they are discussed separately in Chapter 6, because their interactions with DNA have been studied much more extensively than other covalent bonding drugs.

Chapter 5 deals with *strand-breaking* drugs. Bleomycins and enediyne antibiotics such as calicheamicins, esperamicins, and neocarzinostats are able to cleave either single- or double-stranded DNA. Bleomycins consist of a DNA-binding domain and a metal-binding domain that are connected by a linker. The former brings the latter close to the DNA strand, while the latter, in the presence of the Fe(II) ion and O<sub>2</sub>, produces “activated BLM,” which is responsible for DNA *strand breaking*. Green BLM-Co(III)-OOH serves as an ideal model of the “activated BLM” because it is a stable, diamagnetic compound and the structure of its complex with a short oligonucleotide can be determined by NMR spectroscopy. Enediyne antibiotics contain highly strained enediyne rings that produce phenylene biradicals in the presence of a nucleophile such as thiol. Simultaneous cleavage of both strands of DNA by these biradicals is responsible for their strong antitumor activities.

Chapter 6 covers a large number of metal-containing drugs. Among them, cisplatin is best known, and its binding to DNA has been studied most extensively. The major products of cisplatin–DNA reaction are *1,2-intrastrand crosslinking* adducts formed by *covalent bonding* of the Pt atom with the N7 atom of guanine or adenine. The minor products are *1,3-intrastrand* and *1,2-interstrand crosslinking* adducts between two guanine bases. Thus, the main interest of research is on *1,2-intrastrand crosslinking* adducts, particularly those involving two guanine bases. To search for more potent drugs with fewer side effects, a large number of cisplatin analogs have been synthesized, and their cytotoxicities have been tested. These include cisplatin derivatives in which its NH<sub>3</sub> ligands are replaced by a variety of monodentate and bidentate (chelating) ligands, and transplatin and monofunctional complexes such as [Pt(NH<sub>3</sub>)<sub>3</sub>Cl]<sup>+</sup>. Dinuclear platinum complexes can form *1,4-interstrand crosslinks* with DNA, and some of them are highly cytotoxic and more effective against cisplatin-resistant cell lines. Other metal-containing drugs

included are those of Pt(IV), Pd(II), Au(III), Ru(II,III), Rh(III), Cu(II), and Zn(II), the cytotoxicities of which are reported.

Appendixes include discussion of several topics on Raman and NMR spectroscopy and molecular modeling that are not described in the main text of Chapter 1. This appendix material is intended to help the interested reader understand some of the more technical aspects of these methodologies.

## GENERAL REFERENCES

### Drug–DNA Interaction

- G1. Wilman, D. E. V., ed, *The Chemistry of Antitumour Agents*, Blackie & Sons, London, 1990.
- G2. Kallenbach, N. R. ed, *Chemistry and Physics of DNA-Ligand Interactions*, Adenine Press, Schenectady, NY, 1990.
- G3. Probst, C. L. and Perun, T. J. eds., *Nucleic Acid Targeted Drug Design*, Marcel Dekker, New York, 1992.
- G4. Krush, T. R. "Drug-DNA interactions," *Curr. Opin. Struct. Biol.* **4**: 351–364 (1994).
- G5. Hurley, L. H. and Chaires, J. B. eds., *Advances in DNA Sequence Specific Agents*, Vol. 2, JAI Press, London, 1996.
- G6. Yang, X.-L. and Wang, A. H.-J., "Structural studies of atom-specific anticancer drugs acting on DNA," *Pharm. Ther.* **83**: 181–215 (1999).
- G7. Chaires, J. B. and Waring, M. J., eds., "Drug-nucleic acid interactions," in *Methods in Enzymology*, Vol. 340, Academic Press, San Diego, 2001.
- G8. Demeunynck, M., Bailly, C., and Wilson, W. D., eds., *DNA and RNA Binders: From Small Molecules to Drugs*, Vols. 1 and 2, Wiley-VCH, Weinheim, Germany, 2003.

### Experimental Methods

- G9. Cantor, C. R. and Schimmel, P. R., *Biophysical Chemistry, Part II. Techniques for the study of Biological Structure and Function*, W. H. Freeman, San Francisco, 1980.
- G10. Campbell, J. D. and Dwek, R. D., *Biological Spectroscopy*, Benjamin/Cumming, Menlo Park, CA, 1984.
- G11. Sauer, K., ed., Biochemical Spectroscopy, in *Methods in Enzymology*, Vol. 246, Academic Press, San Diego, 1995.
- G12. James, T. L., ed., "Nuclear magnetic resonance and nucleic acids," in *Methods in Enzymology*, Vol. 261, Academic Press, San Diego, 1995.
- G13. Perun, T. J. and Probst, C. L. eds., *Computer-Aided Drug Design: Methods and Applications*, Marcel Dekker, New York, 1989.
- G14. Sheehan, D., *Physical Biochemistry: Principles and Applications*, Wiley, New York, 2000.