Methods for Studying Nucleic Acid/Drug Interactions

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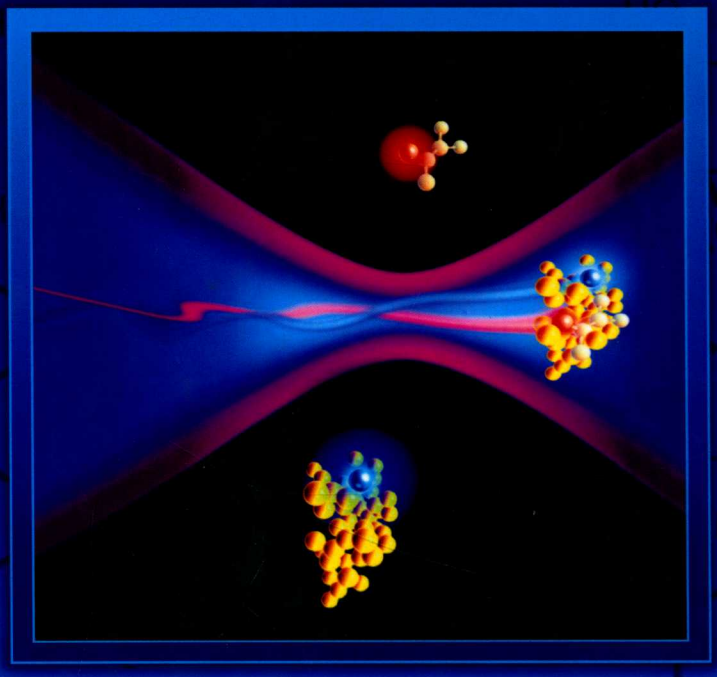

Preface by Ada Yonath, 2009 Nobel Laureate in Chemistry

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Foreword

Although at first viewed only as molecules of heredity, our contemporary understanding of nucleic acids and the flow of information from DNA to RNA to proteins suggests much more complex genetic and regulatory roles for these biomolecules. The relatively recent discovery of RNA interference, where noncoding RNA sequences engage in gene silencing, serves as a case in point. Adding to the complexity of cellular-based processes involving nucleic acids is their intimate relationship with endogenous and exogenous small molecules. Shedding light on structural and biochemical features of nucleic acids and their ligand-binding characteristics is therefore more important than ever.

By focusing on a selection of novel and emerging techniques, Wanunu and Tor provide a remarkable overview of biophysical and computational advances in structure-based investigations of the interactions between nucleic acids and small-molecule ligands. An impressive collection of approaches is described: analytical biophysical techniques alongside novel exploitation of chemical and computational tools. Indeed, the smart combination of results obtained by classical methods with state-of-the-art biophysical approaches reveals astonishing insights, thus paving the new research avenues in this central area of research.

The biophysical methods presented in this book include capillary electrophoresis, Fourier transform infrared spectrometry (FTIR), ultraviolet (UV)–visible and circular dichroism (CD) spectroscopy, real-time surface plasmon resonance (SPR), optical tweezers, mass spectrometry (including electrospray ionization), fluorescence correlation spectroscopy (FCS), atomic force microscopy (AFM), and electron paramagnetic resonance spectroscopy (EPR). The addition of chemical approaches yields hybrid procedures, such as microarray-based two-dimensional combinatorial screening, the use of nanopore ion microscopes for single-molecule analysis, electrochemistry, relaxation kinetics analysis, and design of novel fluorescent nucleoside analogs. The inclusion of theoretical perspective and molecular modeling provides unique means for monitoring RNA–drug interactions and for multidisciplinary insights into one of the most fundamental issues in life sciences.

The vast amount of information documented in this book illustrates the changing landscape of modern nucleic acids research, where instrumental and computational advances drive the evolution of new technology. It provides younger researchers with a glimpse into nascent tools and, in many respects, inspires them to look into the future. By focusing on small-molecule binders, this book uncovers new routes that can facilitate the improvement of existing therapeutic agents and discovery of new drugs. As such, it

raises the expectations for the rational design and synthesis of innovative nucleic acid–targeting drugs. Although only a handful of the techniques described in this book are likely to find their way into mainstream biophysics and drug-screening technology, this compilation is nevertheless a celebration of human creativity!

Ada Yonath
Weizmann Institute of Science
2009 Nobel Laureate in Chemistry

Introduction

Nucleic acids contain a linear sequence of nucleobases, which encode, to a first approximation, a living being's unique features. A well-regulated information transfer process dictates the function of living cells from DNA to RNA and ultimately to proteins. Defects, or interruptions in this delicate flow process, have been implicated in a wide array of disorders. From pathogenic infections to genetic disorders, nucleic acids have been identified as drug targets. Small molecules that target these biopolymers in key regions of interest can mediate cellular processes and determine a cell's fate, thereby providing therapeutic avenues. The discovery and analysis of drugs based on sequence-specific nucleic acid binders has therefore become the goal of both academia and the pharmaceutical industry.

Since most therapeutic efforts have been predominantly focused on pharmaceuticals that target proteins, there is an unmet need to develop drugs that intercept cellular pathways that critically involve nucleic acids. Progress in the discovery of nucleic acid-binding drugs naturally relies on the availability of analytical methods that assess the efficacy and nature of interactions between nucleic acids and their putative ligands. This can tremendously benefit from new methods that probe nucleic acid/ligand interactions both rapidly and quantitatively. Since a variety of novel methods for these studies have emerged in recent years, this book is intended to highlight new and nonconventional methods for exploring nucleic acid/ligand interactions. It is partly designed to present drug-developing companies with a survey of possible future techniques, as well as to highlight their drawbacks and advantages with respect to commonly used tools. Perhaps more importantly, however, this book is designed to inspire young scientists to continue and advance these methods into fruition, especially in light of current capabilities for assay miniaturization and enhanced sensitivity using microfluidics and nanomaterials.

To put new and emerging methods in perspective and provide the appropriate background, this book commences with a survey of established techniques commonly used for the study of nucleic acid/ligand interactions. Studying metal complexes as prototypical nucleic acid binders, Chapter 1 introduces several classical techniques, including crystallography, nuclear magnetic resonance (NMR) and mass spectroscopy, and optical (absorption, emission, circular dichroism (CD), and linear dichroism (LD)) and calorimetry-based techniques (ITC). Over the years, such techniques, both individually and in combination, have provided profound insight into the covalent- and noncovalent-binding modes of nucleic acid binders. Further insight into the utility of established techniques is provided in Chapter 2, which focuses on the interactions of biogenic polyamines, a family of well-studied naturally occurring ligands, with nucleic acids. In addition to spectroscopy-based techniques, the reader is exposed to electrophoresis-based techniques and computational modeling. Accessibility of many of these more classical tools to researchers in the field makes them extremely attractive for the investigation of nucleic acid/ligand interactions, and a bulk of scientific literature is available. Until new techniques as the ones

described in this book slowly transition into mainstream biophysics, classical techniques will continue to serve this community.

Chapter 3 describes in detail advancements in electrospray mass spectrometry (ESI-MS) techniques for discovering and studying complexes formed between small molecules and nucleic acids. In addition to high-throughput screening, the chapter, focusing on RNA/ligand interactions, illustrates the utility of such sensitive techniques for the determination of binding constants and identification of ligand-binding sites. These techniques have proven particularly useful for identifying and analyzing the binding of low-molecular-weight ligands to bacterial and viral RNA targets. Specifically, the chapter looks at the binding of naturally occurring and synthetic ligand to the bacterial-decoding site and the hepatitis C virus (HCV) internal ribosomal entry site (IRES) element, two important drug targets of contemporary interest.

Researchers have long relied on fluorescence-based techniques to shed light on the ligand recognition features of biomolecules. While many proteins contain fluorescent aromatic amino acids, nucleic acids present a challenge, as they are practically nonemissive. This requires either labeling with established fluorophores or the development of new nucleic acid-specific probes. Not surprisingly, this book includes several chapters that detail diverse aspects of powerful fluorescence-based techniques. Chapter 5 describes the theory behind fluorescence correlation spectroscopy, a tool that can be used to study nucleic acid/ligand interactions in solution. The chapter focuses on measuring diffusion times of fluorescently labeled nucleotides at the single-molecule level, which provides insight into complex formation in extremely small volumes. Bright and photostable fluorophores are critical for the success of such experiments. As case studies the authors investigate aptamers, single-stranded oligonucleotides selected as high affinity and selective binders of either small ligands or high-molecular-weight biopolymers.

Other single-molecule techniques for investigating nucleic acid binders have been recently developed. Chapter 6 reports on the use of optical tweezers to pull on individual DNA molecules and measure their force trajectories, yielding rich and unique information on the effect of ligands on the thermodynamics and kinetics of ligand binding. Chapter 8 reports on investigations of the structure of individual DNA molecules using atomic force microscopy (AFM), a relatively young technique that has over the past three decades found its way into biology. In Chapter 12, the use of nanopores as ion microscopes that scan individual nucleic acids and detect ligand binding is described, a technique that enables label-free detection of single molecules at high throughput. The primary advantage of probing single molecules is that structural and topological features that are masked by ensembles can be observed. In addition, the ability to probe a small sample is attractive for future drug-screening applications.

End labeling of oligonucleotides is frequently employed for the analysis of nucleic acid systems. However, such approaches do not typically provide information at the nucleotide level. Minimally perturbing fluorescent nucleoside analogs that judiciously replace selected native nucleosides can provide significant insight into otherwise spectroscopically silent events such as nucleic acid dynamics, recognition, and damage. Chapter 7 discusses the development of such "isomorphic" fluorescent

nucleobases and their application for the development of discovery assays of medical and diagnostic potential. Intriguingly, tuning the photophysical characteristics of novel nucleobases to match the spectral features of other established fluorophores also facilitates the implementation of FRET-based assemblies. The chapter discusses the implementation of such tools for the development of screening assays for new antibacterial and antiviral drugs.

Complementary techniques that do not rely on tagging and labeling have evolved in recent years. The most useful ones utilize surface plasmon resonance (SPR) detection. When oligonucleotides are immobilized to thin gold surfaces creating sensor chips, ligand binding can be detected as a result of mass changes and alteration of the surface refractive index, which in turn alters the SPR angle. Chapter 4 discusses the theoretical and practical aspects of this important tool. As one chip can contain several channels, and both association and dissociation rate constants can be determined, this technique is rather effective at providing a wealth of kinetic and thermodynamic information.

Electrochemical techniques are discussed in Chapter 11, where the authors employ metal and glassy carbon electrodes to measure the diffusion properties of drugs that bind to nucleic acids. Since ligand binding to the nucleic acid reduces its mobility, the electrochemical signatures of several redox active ligands can determine their bound state from free state. Also included in this book is the use of electron paramagnetic resonance (EPR) in Chapter 10, which discusses how continuous wave (CW) EPR can be used to study RNA structural dynamics, that is, how information about motion can give insight into RNA/small molecule and RNA/protein interactions.

Approaching the problem of RNA recognition from drastically different angle, Chapter 9 describes a microarray-based method that identifies RNA motifs that bind to specific small molecules. The approach, described as two-dimensional combinatorial screening (2DCS), relies on an immobilized library of small molecules that is hybridized to an RNA library, which displays discrete secondary structural elements. By simultaneously screening the RNA and ligands spaces and statistically analyzing the results, RNA motif/ligand interactions are identified. These motifs can then be used to mine cellular RNA sequences for potential drug targets. This complements rational design approaches for RNA-friendly small molecules, which are rather challenging due to our incomplete understanding of RNA/ligand interactions. The chapter then demonstrates the utility of this approach for the development of ligands that bind tightly to RNA sequences that cause myotonic muscular dystrophy.

Finally, virtually no experimental effort is unaccompanied by a theoretical counterpart, and the two have gone hand in hand throughout scientific progress. In Chapters 13 through 15, various theoretical developments that are useful for analyzing and interpreting experimental data are discussed. Chapter 13 investigates chemical kinetics with great detail, and in particular, aspects that pertain to dye binding to nucleic acid structures. Chapter 14 gives a theoretical perspective on DNA/drug interactions that discusses various developments in the field. Finally, Chapter 15 reports on a wide array of molecular dynamics studies that investigate the interactions of various ligands with nucleic acids.

We would like to thank all the authors who have taken the time and worked hard to contribute to this book and adhere to its format. Our hope is that students, amateur scientists, and perhaps established scientists will find inspiration within the pages of this book and continue to develop new and clever ways to investigate biomolecular systems. As much as we look forward to the realization of the currently emerging tools described in this book, our hopes are that future discoveries will reshape our approaches to the study of biomolecules and provide new insights into our understanding of nucleic acid/drug interactions.

Editors

Meni Wanunu completed his PhD in 2005 at the Weizmann Institute of Science, where he specialized in supramolecular chemistry, self-assembly, and nanomaterials science. Later, he was a postdoctoral fellow at Boston University and a research associate at the University of Pennsylvania, where he developed ultrasensitive synthetic nanopores for nucleic acid analysis at the single-molecule level. Currently, he is an assistant professor at the Department of Physics and the Department of Chemistry and Chemical Biology in Northeastern University, Boston. His research interests include developing chemical approaches for investigating biomolecular structure and behavior, nucleic acid mechanics and dynamics, and probing biological processes at the single-molecule level.

Yitzhak Tor completed his PhD in 1990 at the Weizmann Institute of Science. After a postdoctoral study at the California Institute of Technology (1990–1993), he joined as a faculty at the University of Chicago. In 1994, he moved to the University of California, San Diego, where he is currently a professor of chemistry and biochemistry and the Traylor Scholar in organic chemistry. His research interests are diverse and include chemistry and biology of nucleic acids, the discovery of novel antiviral and antibacterial agents, as well as the development of cellular delivery agents and fluorescent probes. He is currently the editor-in-chief of *Perspectives in Medicinal Chemistry* (http://la-press.com/journal.php?journal_id=25) and *Organic Chemistry Insights* (http://www.la-press.com/organic-chemistry-insights-journal-j104). Apart from chemistry, his interests are predominantly in music, playing, recording, and producing his own instrumental CDs (http://www.guitormusic.net).

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