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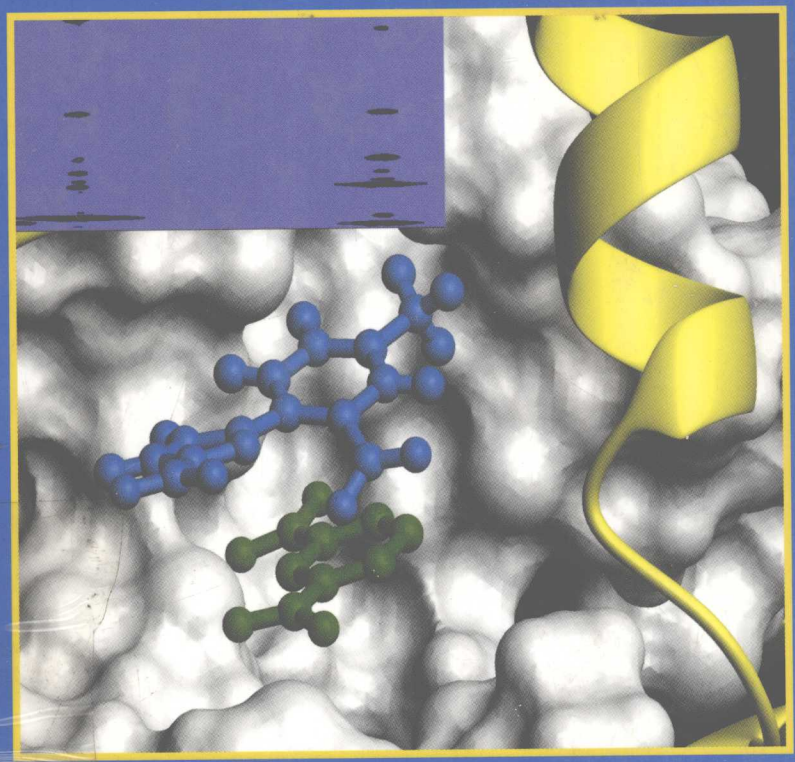
BioNMR in Drug Research

Edited by Oliver Zerbe



Methods and Principles in Medicinal Chemistry

Volume 16

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Preface

Some decades have gone since NMR spectroscopy first hit the analytical scene, and yet its capabilities and applications continue to evolve. Originally designed as a way to verify the structure of relatively small compounds, the technology of NMR boomed and became a valuable means for studying protein structure. Traditionally, X-ray crystallography has been used for solving the structure of proteins; however, it is useful only for those that can be coaxed into a crystalline state. The development of multidimensional NMR and more powerful instruments opened the door for solving the structure of proteins and peptides in an aqueous environment, as they exist in biological systems. NMR allows one to observe the physical flexibility of proteins and the dynamics of their interactions with other molecules, a huge advantage when studying the biochemical function of proteins. The structural information, achieved from NMR studies, can be used to understand the function, mechanism of action, and binding specificity of these proteins.

In addition, NMR can be used to design high affinity ligands for proteins using the SAR by NMR approach (Structure Activity Relationships by Nuclear Magnetic Resonance) introduced by Stephen Fesik. Using this method, small organic molecules that bind to proximal subsites of a protein are identified, optimized, and linked together. The method reduces the amount of chemical synthesis and time required for the discovery of high affinity ligands.

These few remarks highlight that NMR spectroscopy has adapted a central role in drug discovery and design. It is the intention of the present volume to document this role in adequate detail. Accordingly, the book is divided into four larger sections. A methodological section summarizes the technical state of the art including general aspects of spectroscopy; the following section deals in detail with spectroscopic techniques for structure determination of commonly encountered classes of biomolecules. NMR techniques for investigating drug-receptor complexes as well as strategies for drug development using NMR are the topics of the remaining two sections.

The series editors would like to thank the authors and in particular Oliver Zerbe as the volume editor that they devoted their precious time to compiling and structuring the comprehensive information on NMR techniques in drug research. Last, but not least we want to express our gratitude to Frank Weinreich and Gudrun Walter from Wiley-VCH publishers for the fruitful collaboration.

August 2002

Raimund Mannhold, Düsseldorf
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Foreword

Two decades ago, NMR in pharmaceutical industry was mainly used as an analytical tool to validate and identify compounds that were synthesized by medicinal chemists. Following the rapid developments in biomolecular NMR that resulted in the first polypeptide structure being published by the Wüthrich group in the mid eighties, it has thereafter increasingly been used to also determine structures of biomolecules. During the late eighties/early nineties triple-resonance experiments and isotope labeling methods were established which helped to increase the molecular size limits remarkably, and, even more importantly, allowed to more rapidly assign the proton frequencies. While the development of spectroscopic tools still continued, characterized by the advent of TROSY techniques and the use of dipolar couplings, investigations into interactions between drugs or lead candidates and their corresponding receptors were initiated. Early work from Feeney and Roberts in that area was followed by the systematic use of these techniques by the Abott group lead by S. Fesik. In particular, detection of low-affinity ligands has proven to be invaluable in the early development phase of drugs. Accordingly, this book is intended to present an update both in the area of spectroscopic techniques as well as on methodology for screening.

The book is separated into five major sections: One short section on general aspects of spectroscopy, molecular biology and data evaluation is followed by an introduction into the NMR of commonly encountered classes of biomolecules. Thereafter, recent developments in spectroscopic techniques are highlighted. The next section describes experiments and practical aspects useful for the characterization of protein-ligand interactions. The final section presents an account on strategies for drug development using NMR written by experts from pharmaceutical industry.

It is also intended to present techniques which are not routinely applied nowadays but which have the potential to become very useful. Membrane-bound proteins such as G-protein coupled receptors, for example, are important pharmaceutical targets but have so far been very difficult to study by solution-state NMR or single crystal diffraction, the latter mainly due to the enormous difficulties in preparing crystal suitable for diffraction, and the more involved molecular biology to produce them. Solid-state NMR is a technique which may contribute much to that field, and the present progress both in the field of molecular biology as well as in solid-state NMR methodology will certainly stimulate groups to tackle membrane proteins in the future. To provide those readers, which lack experience in that field, with the necessary background, a more detailed introduction to the concepts and applications of solid-state NMR is provided.

The chapters are aiming at providing an overview into a particular field. For a more rigorous description of the experiments, the reader is usually referred to the original literature for which the references are included. A particular emphasis has been placed on practical aspects of work, which originated from the authors' experience with the experiments. As part of this effort, a short chapter on trouble shooting of hardware has been included, in order to make sure that no precious measuring time is wasted. In general, these practical hints are intended for those that decide to move into the field and to provide them with the knowledge necessary to successfully use the tools but should also help to recognize limitations of the techniques.

Biomolecular NMR is a complex technique, which is still rapidly evolving, and people have to learn how the strength of NMR can be exploited best. The editor hopes that the readers will be lucky in choosing the right targets together with the most efficient strategies and wishes that the chapters will help them to do so. Rational drug development is certainly strongly depending upon the input of experimental structural data and hence it is the editor's firm belief that NMR will always play an important role in the drug development process and that new methodology will help to even increase it.

I am deeply grateful to all authors who have patiently responded to my numerous requests for additions and modifications. I would like to especially thank Drs. Marcel Blommers, Wolfgang Jahnke, Werner Klaus, Alfred Ross and Hans Senn for very helpful discussions during the early phase of the book. I would also like to thank Frank Weinreich from Wiley-VCH for the competent help during this project and various publishers for giving the permission to reproduce figures from the original publications. Finally, I would like to thank my wife Katja and my sons Yannick and Benjamin who had to be without me on so many weekends and evenings while I was editing this book. I dedicate this book in love to them.

Zürich, July 2002 *Oliver Zerbe*

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List of Abbreviations

ADME	absorption, distribution, metabolism, excretion
BHK	baby hamster kidney
CD	circular dichroism
CHO	chinese hamster ovary
CMV	cytomegalovirus
COSY	correlation spectroscopy
CSA	chemical shift anisotropy
CSM	chemical shift modulation
DHFR	dihydrofolate reductase
DD	dipole-dipole
DFT	density functional theory
DQ	double quantum
DQC	double-quantum coherence
E.COSY	exclusive correlation spectroscopy
EGF	epidermal growth factor
EPL	expressed protein ligation
FHSQC	fast heteronuclear single-quantum coherence
FIDS	fitting of doublets from singlets
GDP	guanosine diphosphate
HEK	human embryonic kidney
HETCOR	heteronuclear correlation
HMBC	heteronuclear multiple-bond correlation
HMQC	heteronuclear multiple-quantum correlation
HMQC	heteronuclear multiple-quantum correlation
HSQC	heteronuclear single-quantum correlation
HTS	high-throughput screening
IPL	intein-mediated protein ligation
MD	molecular dynamics
MQ	multiple quantum
NOE	nuclear Overhauser effect
PK	pharmacokinetic
PCA	principal component analysis
RDC	residual dipolar coupling

RMSD	root mean square deviation
SAR	structure-activity relationship
SQ	single quantum
TOCSY	total correlation spectroscopy
TROSY	transverse relaxation-optimized spectroscopy
ZQ	zero quantum
ZQC	zero-quantum coherence

Contents