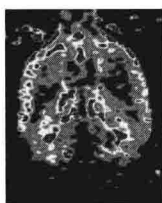


Molecular Imaging

Basic Principles and Applications
in Biomedical Research

••• Markus Rudin

Imperial College Press



Molecular Imaging

Basic Principles and Applications
in Biomedical Research

... **Markus Rudin**

University of Zürich, Switzerland

Published by

Imperial College Press
57 Shelton Street
Covent Garden
London WC2H 9HE

Distributed by

World Scientific Publishing Co. Pte. Ltd.
5 Toh Tuck Link, Singapore 596224

USA office: 27 Warren Street, Suite 401-402, Hackensack, NJ 07601

UK office: 57 Shelton Street, Covent Garden, London WC2H 9HE

Library of Congress Cataloging-in-Publication Data

Molecular imaging : principles and applications in biomedical research / by Markus Rudin.
p. cm.

Includes bibliographical references and index.

ISBN 1-86094-528-7 (alk. paper)

1. Molecular probes. 2. Diagnostic imaging. 3. Imaging systems in medicine. I. Rudin, M. (Markus), 1953-

QP519.9.M64M638 2005
616'.027--dc22

2005047432

British Library Cataloguing-in-Publication Data

A catalogue record for this book is available from the British Library.

Copyright © 2005 by Imperial College Press

All rights reserved. This book, or parts thereof, may not be reproduced in any form or by any means, electronic or mechanical, including photocopying, recording or any information storage and retrieval system now known or to be invented, without written permission from the Publisher.

For photocopying of material in this volume, please pay a copying fee through the Copyright Clearance Center, Inc., 222 Rosewood Drive, Danvers, MA 01923, USA. In this case permission to photocopy is not required from the publisher.

Typeset by Stallion Press
Email: enquiries@stallionpress.com

Printed in Singapore by Mainland Press

Molecular Imaging

Basic Principles and Applications
in Biomedical Research

To Verena

Foreword

Many scientists state that *molecular imaging is not a new discipline*; nuclear imaging approaches such as positron emission tomography and single-photon emission computer tomography have used molecular imaging concepts since more than a decade to visualize the biodistribution of labeled compounds including analyses of receptor occupancy. In these studies image contrast is not governed by the anatomical features of the sample but rather by the local concentration of the radio-labeled reporter compound, i.e., by a molecular property.

Other scientists claim that *molecular imaging is a new scientific area merging concepts of molecular biology with noninvasive imaging technologies*. This allows the study of biological processes in a noninvasive manner. These concepts go beyond labeling of reporter ligands. They involve the development of a battery of reporter assays that are used to probe specific biological questions: Is the expression of a receptor modified under specific pathological conditions? Does the receptor exert its biological activity; does it activate its associated signaling cascade? Can these molecular markers be used as early indicators of a pathological transformation?

The last years experienced rapid growth in the field of molecular imaging. Many of the assay systems originally developed for studying biochemical systems in homogeneous solutions or isolated cells have been successfully translated to the *in vivo* situation allowing the testing of a biomedical hypothesis in the context of the intact

organism. Molecular or target-specific imaging potentially provides a holistic view on a biological system in contrast to the reductionistic approaches of classical molecular biology and biochemistry. Despite the rapid progress that has been achieved in the last couple of years the field is still in its infancy and major developments are still required. This relates to the imaging technologies, which should provide high sensitivity, high temporal and spatial resolution, and most importantly quantitative data, as well as to the probe design. Highly specific probe constructs should provide the desired information with little interference from nonspecific background signals. Multiplexed probes that allow the monitoring of several biological processes simultaneously would be of outmost relevance.

There are considerable expectations associated with molecular imaging techniques. First, the study of molecular events in the intact organism will *enhance our basic molecular biological knowledge*. For instance, specific receptor systems or signal transduction pathways can be studied in their biological context. Second, tissue functional or structural aberrations are always preceded by molecular events such as abnormal cellular signaling. It is reasonable to assume that *molecular markers might serve as early indicators of a disease process*, long before a pathomorphological or pathophysiological transformation of tissue occurs. It may furthermore improve the specificity of diagnosis and may help to select the patient population that is most likely to respond to a specific therapy. Third, modern pharmaceutical drugs are designed to interact with a well-characterized molecular target. As clinical drug development is becoming increasingly expensive (today the development of a drug to reach the market will cost approximately 800 million USD), early information on clinical efficacy is of crucial importance. Molecular imaging might provide a *direct proof that a therapeutic concept is valid* also in man; e.g., the target enzyme is in fact inhibited by the drug candidate or inhibition of a specific receptor shuts down the associated molecular signaling cascade. For these reasons, molecular imaging will soon become an indispensable tool in biomedical research in particular for drug discovery and development.

This book is aimed to give the reader an introduction into this fascinating and dynamically evolving field. It discusses basic aspects of molecular imaging technology and probe design, which are illustrated

with numerous selected examples from the literature. It does, however, not provide a comprehensive review of the current activities in the field. This was never intended as such a book would probably never be finished: new exciting applications are being reported almost on a daily basis.

The first part of the book discusses technological aspects of molecular imaging. In Chapter 2, the various imaging modalities such as X-ray computer tomography (CT), magnetic resonance imaging (MRI), single photon emission computer tomography (SPECT), positron emission tomography (PET), fluorescence and bioluminescence imaging, as well as ultrasound imaging are discussed in some detail. Chapter 3 describes the various reporter systems that are being used for the various imaging approaches, from short-lived radionuclides to microbubbles suited for ultrasound studies. The design of reporter constructs for molecular imaging applications is the topic of Chapter 4. Important aspects to consider are target-specificity, delivery of the probe to the target site, and signal amplification. Most molecular targets are expressed at low concentration (nano- to femtomolar); hence, strategies to enhance the signals produced by the reporter system are essential.

The second part deals with applications of molecular imaging in biomedical research. Knowledge of the drug biodistribution and pharmacokinetics (PK) is highly relevant for drug development as many drug candidates fail due to inadequate PK properties. Chapter 5 deals with drug imaging using PET techniques. In Chapter 6, methods to visualize the expression of the drug targets are being discussed. Levels of transcription products can be probed using labeled anti-sense molecules, receptor imaging relies on the availability of small molecular probes or labeled antibodies. Enzymatic drug targets are attractive for imaging as the enzyme activity can be exploited to activate or trap a reporter substrate thus yielding high degrees of signal amplification and minimal interference by background signals. A different approach to visualize gene expression is the use of the reporter genes, which allows unique questions to be addressed. Drug-target interaction will initiate a series of downstream processes. Measurement of these *effector readouts* are the topic of Chapter 7, which discusses methods to visualize protein-protein interactions or apoptotic activity as an example. Further downstream a ligand

receptor interaction will initiate a physiological response that can be visualized using conventional imaging approaches such as the measurement of glucose utilization, energy turnover, tissue perfusion, or second messenger turnover. Chapter 8 describes techniques for monitoring of cell trafficking. Myeloid and lymphoid cells are important mediators of inflammation and visualization of the infiltration of these cells into inflamed tissue are sensitive indicators of the disease process. Novel therapeutic approaches in degenerative diseases try to exploit the pluripotency of stem cells for tissue repair. Obviously, such therapy concepts will benefit from the ability to monitor the fate of such cells under *in vivo* conditions.

While many of the techniques and applications described will undergo further development in the coming years, most of the basic concepts outlined in this book will remain valid and will be applicable also to these advanced procedures. In this regard, the book is designed as a textbook summarizing basic principles and potential applications of molecular imaging in biomedical research.

Zurich, June 2005

Markus Rudin

Acknowledgements

Many colleagues and friends contributed directly and indirectly to this book. Since my early days in imaging I had always the privilege to work with partners both NMR/MRI imagers and spectroscopists and biologists, who were eager to discuss not only new ideas, but also to challenge the novel nonvalidated approaches provided by *in vivo* imaging. Together, we have distilled many concepts of imaging trying to understand what we were looking at on our computer screens.

In particular I would like to thank all the actual members of the imaging group, with whom I had many discussion concerning molecular imaging and its application to the drug discovery process: Peter Allegrini, Nicolau Beckmann, Hans-Ulrich Gremlich, Rainer Kneuer, Didier Laurent, Martin Rausch, Markus Stöckli, and Jeffrey Tsao. I am also grateful for the support by Research management of the Novartis Institutes for Biomedical Research and in particular to Rene Amstutz heading Discovery Technologies. His support and his challenge were essential for the implementation of molecular imaging approaches into our imaging portfolio.

I had the opportunity to discuss many aspects of molecular imaging with the protagonists in the field. These people had made significant contribution to the development of molecular imaging. My special thanks go to Ronald Blasberg (Sloan Kettering Memorial Cancer Center, New York), Sam (Sanjiv) Gambhir (Stanford University, San Francisco), Allan Johnson (Duke University, Durham), Ralph

Weissleder (*Massachusetts General Hospital, Boston*). I am also grateful to Joachim Seelig (*Biocenter University of Basel*), who was my tutor and advisor throughout my years in imaging.

Finally, I would like to thank my wife Verena. Without her tolerance and also her support, it would have not been possible to free the time to write this book.

Zurich, June 2005

Contents

<i>Foreword</i>	xvii
<i>Acknowledgements</i>	xxi
1. Introduction	1
1.1 Biomedical Research: Elucidating Molecular Mechanisms of Disease	1
1.1.1 Tumorigenesis as a result of multiple mutations	2
1.1.2 Pathogenesis in Alzheimer-type dementia . . .	3
1.1.3 Multiple factors causing disease: Multiplexed diagnostic tools	5
1.2 The Drug Discovery Process: From Target Validation to Proof-of-Concept in Man	6
1.3 Imaging in Biomedical Research	9
1.3.1 Diagnostic imaging	9
1.3.1.1 Contrast-to-noise ratio and spatial resolution	9
1.3.1.2 Diagnostic imaging: Detection of pathology using structural and functional readouts	14
1.3.1.3 Quantification	14
1.3.2 Target-specific or molecular imaging	16
1.3.2.1 Definition: Molecular imaging	16
1.3.2.2 Imaging targets	17

1.3.2.3 Prerequisites for molecular imaging: Reporter constructs	19
1.3.2.4 Prerequisites for molecular imaging: Imaging modalities	21
1.3.2.5 Molecular imaging modalities	25
1.3.2.6 Comparison of imaging modalities . .	36
1.4 Summary	37
References	38

PART 1: METHODOLOGIES 43

2. Imaging Techniques	45
2.1 X-Ray Computerized Tomography	45
2.1.1 Basic principles of X-rays	45
2.1.2 Principles of X-ray CT	46
2.1.3 Image representation, spatial resolution, contrast-to-noise ratio	51
2.1.4 Animal CT scanners	53
2.2 Magnetic Resonance Imaging	54
2.2.1 Interaction of a nuclear magnetic moment with a static magnetic field	54
2.2.2 Classical description of NMR: Bloch equations and relaxation	56
2.2.3 The NMR experiment	60
2.2.4 Measurement of relaxation rates in FT-NMR	62
2.2.4.1 Measurement of the transverse relaxation rate R_2	62
2.2.4.2 Measurement of the longitudinal relaxation rate R_1	64
2.2.5 Principle of magnetic resonance imaging . . .	66
2.2.5.1 Spatial encoding	66
2.2.5.2 The k -space concept	68
2.2.5.3 Slice selection	69
2.2.5.4 Some basic image acquisition modules	71

2.2.6	Contrast in MR images, signal enhancement by contrast agents	74
2.2.7	<i>In vivo</i> magnetic resonance spectroscopy . . .	77
2.2.8	Animal MRI scanners	81
2.3	Nuclear Imaging: Gamma Scintigraphy, Single Photon Emission Computer Tomography	82
2.3.1	The gamma camera: Projection images	83
2.3.2	3D gamma imaging: Single photon emission computer tomography	88
2.3.3	SPECT scanners for animal imaging	88
2.4	Positron Emission Tomography	89
2.4.1	Physical principles of PET	90
2.4.2	Image reconstruction	94
2.4.2.1	Filtered backprojection	94
2.4.2.2	Statistical image reconstruction	95
2.4.2.3	3D reconstructions procedures	97
2.4.3	High-resolution PET instrumentation	99
2.5	Optical Imaging	100
2.5.1	Photon propagation in scattering media	100
2.5.1.1	The diffusion equation for light propagation in tissue	100
2.5.1.2	Solutions of the diffusion equation	103
2.5.2	Planar imaging/reflectance imaging	104
2.5.3	Diffuse optical tomography	107
2.5.4	Noncontact optical tomography	114
2.5.5	Instrumentation	116
2.5.5.1	Planar imaging systems	116
2.5.5.2	Optical tomography systems	118
2.5.5.3	Intravital microscopy	118
2.6	Ultrasound Imaging	120
2.6.1	Principles of ultrasound imaging	120
2.6.1.1	Introduction, definitions	120
2.6.1.2	Attenuation	121
2.6.1.3	Reflection and refraction	122
2.6.1.4	Scattering	122
2.6.2	Axial and angular resolution, frame rate . . .	123

2.6.2.1	Spatial resolution	124
2.6.2.2	Temporal resolution, frame rate	128
2.6.3	Contrast enhancement	128
2.7	Summary	130
	References	132
3.	Molecular Reporter Systems	141
3.1	X-ray Contrast Agents	141
3.1.1	Introduction	141
3.1.2	Iodine-based contrast agents	142
3.2	MRI Contrast Agents	144
3.2.1	Factors determining the relaxivity of contrast agents	144
3.2.2	Gadolinium-based contrast agents	148
3.2.3	Magnetite nanoparticles	152
3.2.4	Liposomes/micelles/microemulsions	156
3.3	SPECT Radioisotopes	161
3.3.1	Radioisotopes for gamma scintigraphy/ SPECT imaging	161
3.3.2	Technetium-99m	161
3.3.3	Indium-111	164
3.3.4	Iodine-123 and iodine-131	165
3.4	PET Radioisotopes	166
3.4.1	Production of radionuclides, lifetimes	166
3.4.2	Carbon-11-labeled compounds	167
3.4.3	Fluorine-18-labeled compounds	169
3.4.4	Other PET nuclei	173
3.5	Optical Probes: Fluorescence and Bioluminescence	174
3.5.1	Principles of fluorescence	174
3.5.2	Fluorescent proteins	177
3.5.3	Fluorescent dyes	179
3.5.4	Fluorescent nanocrystals, quantum dots	182
3.5.5	Fluorescence energy transfer (FRET), quenching	186
3.5.6	Bioluminescence	189
3.6	Contrast Agents for Ultrasound Imaging	191
3.6.1	Microbubbles	191

3.6.2	Non-microbubble-based contrast agents . . .	195
3.7	Summary	196
	References	200
4.	Design of Molecular Imaging Probes	210
4.1	Design of Target-specific Probes	210
4.1.1	Low molecular weight probes	210
4.1.2	Macromolecular probes: Antibodies, oligonucleotides	211
4.1.3	Activatable probes, “smart” probes	213
4.1.3.1	Change in fluorescent properties . . .	214
4.1.3.2	Change in magnetic relaxation rates .	218
4.2	Probe Delivery, Cell Penetration	225
4.2.1	Cell penetrating peptides	226
4.2.2	Receptor-mediated endocytosis	230
4.2.2.1	Asialoglycoprotein receptor-mediated endocytosis	231
4.2.2.2	Transferrin receptor-mediated endocytosis	231
4.2.2.3	Integrin (CD11c)-mediated endocytosis	233
4.2.3	Transfection agents	234
4.3	Signal Amplification	236
4.3.1	Increasing the payload of reporters per target	236
4.3.1.1	Branched linker groups: Avidin–biotin	236
4.3.1.2	Polymers with multiple reporter groups	239
4.3.2	Trapping of reporters	241
4.3.3	Enzymatic probe activation	243
4.4	Physiological Amplification	244
4.5	Summary	245
	References	247

PART 2: APPLICATIONS	257
5. Drug Imaging	259
5.1 Drug Biodistribution and Pharmacokinetics	259
5.1.1 Classical approaches in rodents: Autoradiographic studies	259
5.1.2 <i>In vivo</i> studies using nuclear imaging approaches	262
5.1.2.1 Labeling by radioisotopes to preserve pharmacokinetic properties	262
5.1.2.2 Dose linearity	263
5.1.2.3 Correction for plasma contribution	264
5.1.2.4 Examples: CNS drugs	264
5.2 Receptor Occupancy Studies	265
5.2.1 Receptor binding in a homogeneous compartment	266
5.2.2 Compartment models for estimation of tracer concentrations	268
5.2.3 Indirect imaging or drug-receptor interactions	273
5.2.4 Receptor occupancy studies: Examples	275
5.3 Summary	279
References	281
6. Imaging Gene Expression	284
6.1 Visualizing Transcription: Targeting Messenger RNA Using Labeled Antisense Oligonucleotides	286
6.2 Direct Target Imaging Using Receptor-Specific Ligands	292
6.2.1 Small molecular ligands	292
6.2.1.1 Neuroendocrine tumors expressing somatostatin receptors	293
6.2.1.2 Neurotransmitter systems	296
6.2.1.3 Small molecule probes to study Alzheimer's disease	301
6.2.2 Antibodies and antibody fragments	307