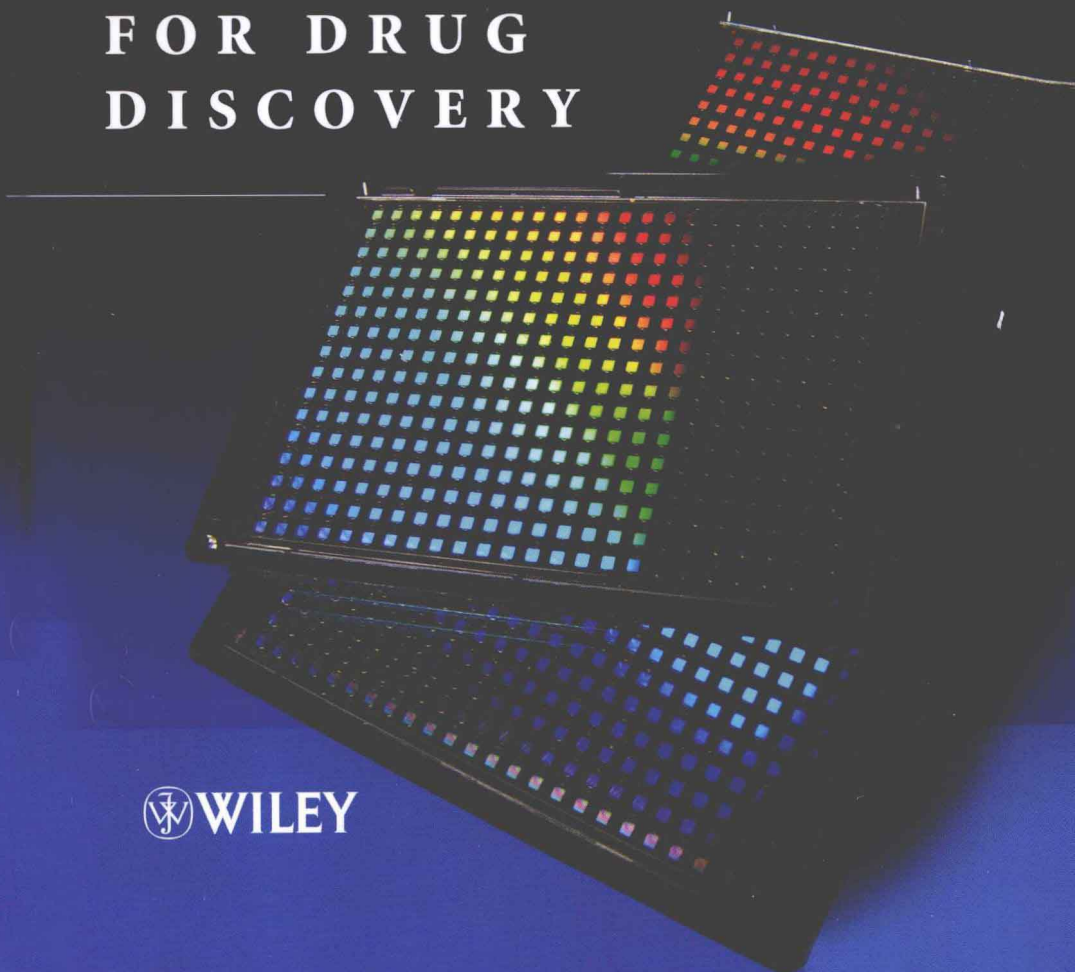
Editors
MATTHEW COOPER
LORENZ M. MAYR

Label-Free Technologies

FOR DRUG DISCOVERY



WILEY

Label-Free Technologies For Drug Discovery

Editors

Matthew Cooper
Institute for Molecular Bioscience, University of Queensland, Australia

Lorenz M. Mayr
Biology Unit, Protease Platform, Novartis Pharma AG, Basel, Switzerland

WILEY
A John Wiley and Sons, Ltd., Publication

This edition first published 2011

Registered office
John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, United Kingdom

Library of Congress Cataloging-in-Publication Data

Label-free technologies for drug discovery / editors, Matthew Cooper, Lorenz M. Mayr.
p. ; cm.
Includes bibliographical references and index.
ISBN 978-0-470-74683-7 (cloth)
1. Drug development. I. Cooper, M. A. (Matthew A.) II. Mayr, M. Lorenz.
[DNLM: 1. Drug Discovery. 2. Biosensing Techniques. 3. Drug Design. QV 744]
RM301.25.L33 2011
615'.19–dc22

2010042191

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

Print ISBN: 9780470746837
ePDF ISBN: 9780470979136
oBook ISBN: 9780470979129
ePub ISBN: 9781119990277

Typeset in 10.5/13pt Sabon by Aptara Inc., New Delhi, India.
Printed and bound in Singapore by Markono Print Media Pte Ltd

Preface

In the 1980s, surface plasmon resonance (SPR) and related techniques exploiting evanescent waves were first applied to the interrogation of biological and chemical interactions. These techniques allowed us to study the interaction between immobilized receptors and analytes in real time and without labelling of the analyte; leading to the term 'label-free'. While initially intended as a method of determining affinities, the use of a microfluidic delivery system to the sensor interface allowed kinetics (on and off rates of binding) to be measured. This, in turn, allowed new questions on compound action to be addressed and new compound optimization strategies to be explored. Today it is generally accepted that observed binding rates and binding levels can be interpreted to provide information on the specificity, kinetics and affinity of a drug–receptor interaction that relate to compound mode of action. This builds on the most often quoted maxim used in selecting bioactive compounds; *'Corpora non agunt nisi fixate'*: a drug will not work unless it is bound (Paul Ehrlich; 1854–1915). This now axiomatic statement guided Ehrlich through many scientific discoveries covering haematology, immunology, bacteriology and early chemotherapy. In the drug discovery process, we are now having to consider not just equilibrium-based, static descriptors of drug–receptor interactions (e.g. IC_{50} and EC_{50}), but also descriptors of the *dynamic* nature of drug action. For example, similarly structured molecules can bind to a target with similar affinity. However, only one may have a slow enough off rate to effectively block action of an endogenous ligand; only one may bind in an orientation suitable for a catalytic reaction; only one may induce a conformational change in the receptor. At the extreme, two similarly structured molecules may bind with similar affinity, but one may initiate the receptor response (an agonist), whereas the other may block the response (an antagonist). Optimal binding

mechanisms can thus define the therapeutic index and the utility of a drug. Label-free techniques can hence help us understand and optimize these parameters, particularly with respect to predictive pharmacodynamics, competition/interaction with endogenous ligands, binding to side effect profiling targets and metabolic enzyme, and many other attributes that lead to differentiation of a drug candidate from competitor compounds.

Since the development of the first commercial label-free biosensors in the late 1980s, their use in research and development has been described in over 5000 scientific publications covering most disciplines found in the pharmaceutical and diagnostic industries. Traditional solution-based thermodynamic techniques, such as isothermal titration calorimetry (ITC), have evolved from cumbersome, labour-intensive techniques to automated systems with much lower requirements for reagents and reduced (200 μl) sample volume. Nonfluorescent (white light) high content cell-based assays have been developed together with automated microscope systems combining rapid auto-focusing, automated stage movement and dedicated analysis software capable of batch processing large numbers of images from 96 and 384 well plates. Mass spectrometry combined with high throughput size exclusion and solid phase extraction methods now allows quantification of free and bound species in minutes. Mass spectrometry is thus now a powerful label-free technique that has transitioned from an analytical quality control tool to a mainstream compound profiling and screening platform. In a similar manner, nuclear magnetic resonance (NMR) has evolved from a method to confirm compound structure to a powerful screening tool for identifying low molecular weight drug 'fragment' binders, and even elucidate the specific target binding site of a fragment or lead compound. This approach was pioneered by scientists at Abbott Laboratories, who identified hits from changes in NMR chemical shifts (^{15}N-^{1}H HSQC) and Vertex, who relied on detecting changes in the NMR relaxation properties of the fragments themselves when bound to a protein target.

Finally, in the last five years, the advent of 384- and 1536-well screening systems based on resonant waveguide patterned microtitre plates and electrical impedance 96- and 384-plates has led to an explosion in the application of label-free to GPCR screening. This is highly significant for drug discovery, as at least 800 distinctive human G-protein coupled receptors (GPCRs) are known, with ~350 being estimated to be useful drug targets. Although only ~7% of GPCRs are currently targeted by drugs, this accounts for ~35% of blockbuster pharmaceuticals. Here label-free can really challenge current screening paradigms. It has emerged that

different ligands (agonists or antagonists) that bind to the same GPCR, even the same subtype, can display profoundly different biological properties (ligand directed signalling) arising through different regulation of intracellular pathways (e.g. IP3 flux, ERK 1/2 phosphorylation, cAMP activation, Ca^{2+} release, β-arrestins, etc.). GPCR-mediated pathways, initially thought to be independent, are now known to cross-communicate with other activation paths. For instance, GPCR stimulation can lead to activation of 'traditional' tyrosine kinase pathway components such as Raf, MEK and ERK.

Drug candidate screening paradigms typically involve selection of a transfected cell line, over-expressing the target GPCR. This cell line is then used with one, or a variety, of downstream markers of receptor activation, such as Ca^{2+}, cAMP, inositol phosphate and diacylglyercol flux. Standard assay development can be summarized as: (i) compound selection and synthesis, (ii) cell line selection and (iii) downstream reporter assay selection, all of which lead to a data set predicated by cell line and assay format chosen in the first instance. Although this standard approach has become well-accepted for compound screening and pharmacological characterization, it is *fundamentally limited in scope* in profiling target-related response outcomes. An 'agonist' or 'antagonist' may only be so in the specific screen used; a response in a transfected or transduced cell line may not be the same as that found in the disease relevant endogenous cell. In contrast, label-free screening, which can be carried out using parental cell lines, is thought to be indicative of ligand binding induced changes in cell morphology and holistic behaviour. The readout is noninvasive, temporal, cumulative and most importantly, *signalling pathway independent*. Kinetic responses or 'fingerprints' elicited by a compound are mechanistically informative, and profiles for particular G-protein coupling can be determined. Hence, the combination of label-free, pathway independent receptor and whole cell profiling with standard reporter and pathway dependent screening should provide new insight into compound mode of action, in addition to identifying new hits that could be missed by traditional assays.

Label-free continues to grow from a niche technology with a user base comprised of early adopters, towards a mainstream, easy to use (but sometimes not easy to understand) technology. We hope the reader finds this compendium of chapters describing label-free technologies and case studies both useful and thought provoking.

Matt Cooper and Lorenz M. Mayr
Brisbane, Australia and Basel, Switzerland, July 2010

List of Contributors

Yama A. Abassi
ACEA Biosciences Inc., 6779 Mesa Ridge Rd, San Diego, CA 92121, USA

J. Bradley
Pfizer Global Research and Development, Sandwich, Kent CT13 9NJ, UK

Tsafrir Bravman
Bio-Rad Laboratories, Inc., Gutwirth Park, Technion, Haifa 32000, Israel

Vered Bronner
Bio-Rad Laboratories, Inc., Gutwirth Park, Technion, Haifa 32000, Israel

Jason Brown
Neurosciences Centre of Excellence in Drug Discovery, GlaxoSmith-Kline, New Frontiers Science Park, Harlow, CM19 5AW, UK

Richard Brown
GE Healthcare, MicroCal Products Group, 22 Industrial Drive East, Northampton, MA, USA

Xun Chen
FAST (Facility for Automation & Screening Technologies), Merck Research Laboratories, Rahway, NJ, USA

Yen-Wen Chen
Molecular Devices, Inc., 1311 Orleans Drive, Sunnyvale, CA 94089, USA

Mike Chin
Novartis Institutes for Biomedical Research, 4560 Horton Street, Emeryville, CA 94608, USA

Bernard K. Choi
FAST (Facility for Automation & Screening Technologies), Merck Research Laboratories, Rahway, NJ, USA

Steven S. Choi
Micro and Nanotechnology Laboratory, Department of Electrical and Computer Engineering, University of Illinois at Urbana-Champaign, 1406 West Green Street, Urbana, IL 61801 USA

Chun-Wa Chung
GlaxoSmithKline, New Frontiers Science Park, Stevenage, Essex CM19 5AW, UK

Brian T. Cunningham
Micro and Nanotechnology Laboratory, Department of Electrical and Computer Engineering, University of Illinois at Urbana-Champaign, 1406 West Green Street, Urbana, IL 61801 USA

Mike Doyle
Novartis Institutes for Biomedical Research, 4560 Horton Street, Emeryville, CA 94608, USA

Claude Dufresne
FAST (Facility for Automation & Screening Technologies), Merck Research Laboratories, Rahway, NJ, USA

J. Gary Eden
Laboratory for Optical Physics and Engineering, Department of Electrical and Computer Engineering University of Illinois at Urbana-Champaign, 1406 West Green Street, Urbana, IL 61801 USA

E. Fairman
Pfizer Global Research and Development, Sandwich, Kent CT13 9NJ, UK

Paul Feucht
Novartis Institutes for Biomedical Research, 4560 Horton Street, Emeryville, CA 94608, USA

Ernesto Freire
Department of Biology, Johns Hopkins University, Baltimore, MD 21218, USA

Debra L. Gallant
Molecular Devices, Inc., 1311 Orleans Drive, Sunnyvale, CA 94089, USA

E. Gbekor
Pfizer Global Research and Development, Sandwich, Kent CT13 9NJ, UK

Chun Ge
Micro and Nanotechnology Laboratory, Department of Electrical and Computer Engineering, University of Illinois at Urbana-Champaign, 1406 West Green Street, Urbana, IL 61801 USA

Neil S. Geoghagen
FAST (Facility for Automation & Screening Technologies), Merck Research Laboratories, Rahway, NJ, USA

Robert Graves
GE Healthcare Life Sciences, 800 Centennial Avenue, Piscataway, New Jersey 08855-1327, USA

P. Hayter
Pfizer Global Research and Development, Sandwich, Kent CT13 9NJ, UK

Tom G. Holt
Facility for Automation & Screening Technologies (FAST), Merck Research Laboratories, Rahway, NJ, USA

Walter Huber
F.Hoffmann-La RocheAG, Pharma Research Basel, Grenzacherstrasse, 4070 Basel, Switzerland

Kristian K. Jensen
FAST (Facility for Automation & Screening Technologies), Merck Research Laboratories, Rahway, NJ, USA

Jeffrey C. Jerman
Molecular Discovery Research, 1–3 Burtonhole Lane, London NW7 1AD, UK

Maxine Jonas
BioTrove, Inc., Woburn, MA, USA

William A. LaMarr
BioTrove, Inc., Woburn, MA, USA

Lukas Leder
Novartis Institutes for Biomedical Research, Lichtstrasse 35, CH-4056, Basel, Switzerland

Melanie Leveridge
GlaxoSmithKline, New Frontiers Science Park, Stevenage, Essex CM19 5AW, UK

Meng Lu
Micro and Nanotechnology Laboratory, Department of Electrical and Computer Engineering, University of Illinois at Urbana-Champaign, 1406 West Green Street, Urbana, IL 61801 USA

Ming-Juan Luo
FAST (Facility for Automation & Screening Technologies), Merck Research Laboratories, Rahway, NJ, USA

Qi Luo
FAST (Facility for Automation & Screening Technologies), Merck Research Laboratories, Rahway, NJ, USA

Lorraine Malkowitz
FAST (Facility for Automation & Screening Technologies), Merck Research Laboratories, Rahway, NJ, USA

Eric Martin
Novartis Institutes for Biomedical Research, 4560 Horton Street, Emeryville, CA 94608, USA

Julio Martin
GlaxoSmithKline, Centro de Investigacion Basica, Parque Tecnologico de Madrid, 28760 Tres Cantos, Spain

Ryan P. McGuinness
Molecular Devices, Inc., 1311 Orleans Drive, Sunnyvale, CA 94089, USA

Marco Meyerhofer
Novartis Institutes for Biomedical Research, Lichtstrasse 35, CH-4056, Basel, Switzerland

David G. Myszka
Center for Biomolecular Interaction Analysis, University of Utah, Salt Lake City, UT 84132, USA

Oded Nahshol
Bio-Rad Laboratories, Inc., Gutwirth Park, Technion, Haifa 32000, Israel

Thomas Neumann
Graffinity Pharmaceuticals GmbH, INF 518, 69120 Heidelberg, Germany

Ronan O'Brien
GE Healthcare, MicroCal Products Group, 22 Industrial Drive East, Northampton, MA, USA

Johannes Ottl
Novartis Institute for BioMedical Research, Centre for Proteomic Chemistry, Forum 1, Novartis Campus, CH-4056, Basel, Switzerland

Can C. Ozbal
BioTrove, Inc., Woburn, MA, USA

John M. Proctor
Molecular Devices, Inc., 1311 Orleans Drive, Sunnyvale, CA 94089, USA

S. Ramsey
Pfizer Global Research and Development, Sandwich, Kent CT13 9NJ, UK

Rebecca L. Rich
Center for Biomolecular Interaction Analysis, University of Utah, Salt Lake City, UT 84132, USA

Magalie Rocheville
Molecular Discovery Research, GlaxoSmithKline, New Frontiers Science Park, Harlow, CM19 5AW, UK

Renate Sekul
Graffinity Pharmaceuticals GmbH, INF 518, 69120 Heidelberg, Germany

Kevin Shoemaker
Novartis Institutes for Biomedical Research, 4560 Horton Street, Emeryville, CA 94608, USA

Alexander Sieler
Roche Diagnostics GmbH, BP-C1 Nonnenwald 2, 82377 Penzberg, Germany

F. Stuhmeier
Pfizer Global Research and Development, Sandwich, Kent CT13 9NJ, UK

David C. Swinney
iRND3, Institute for Rare and Neglected Diseases Drug Discovery, 1514 Ridge Road, Belmont, CA 94002, USA

Blisseth Sy
Novartis Institutes for Biomedical Research, 4560 Horton Street, Emeryville, CA 94608, USA

H. Roger Tang
Molecular Devices, Inc., 1311 Orleans Drive, Sunnyvale, CA 94089, USA

Georg C. Terstappen
Faculty of Pharmacy, University of Siena, Via Fiorentina 1, 53100 Siena, Italy

Trisha A. Tutana
Molecular Devices, Inc., 1311 Orleans Drive, Sunnyvale, CA 94089, USA

Clark J. Wagner
Laboratory for Optical Physics and Engineering, Department of Electrical and Computer Engineering, University of Illinois at Urbana-Champaign, 1406 West Green Street, Urbana, IL 61801 USA

John Wang
Novartis Institutes for Biomedical Research, 4560 Horton Street, Emeryville, CA 94608, USA

Jun Wang
FAST (Facility for Automation & Screening Technologies), Merck Research Laboratories, Rahway, NJ, USA

Xiaobo Wang
ACEA Biosciences Inc., 6779 Mesa Ridge Rd, San Diego, CA 92121, USA

Bob Warne
Novartis Institutes for Biomedical Research, 4560 Horton Street, Emeryville, CA 94608, USA

Charles Wartchow
FortéBio, Inc., 1360 Willow Road, Suite 201, Menlo Park, CA 94025-1516, USA

Trevor Wattam
GlaxoSmithKline, New Frontiers Science Park, Stevenage, Essex CM19 5AW, UK

Manfred Watzele
Roche Diagnostics GmbH, BP-C1 Nonnenwald 2, 82377 Penzberg, Germany

Glyn Williams
Astex Therapeutics Ltd, 436 Cambridge Science Park, Milton Road, Cambridge CB4 0QA, UK

Donna L. Wilson
Molecular Devices, Inc., 1311 Orleans Drive, Sunnyvale, CA 94089, USA

Yusheng Xiong
FAST (Facility for Automation & Screening Technologies), Merck Research Laboratories, Rahway, NJ, USA

Xiao Xu
ACEA Biosciences Inc., 6779 Mesa Ridge Rd, San Diego, CA 92121, USA

Kelly Yan
Novartis Institutes for Biomedical Research, 4560 Horton Street, Emeryville, CA 94608, USA

Danfeng Yao
FortéBio, Inc., 1360 Willow Road, Suite 201, Menlo Park, CA 94025-1516, USA

Jiamin Yu
Novartis Institutes for Biomedical Research, 4560 Horton Street, Emeryville, CA 94608, USA

Isabel Zaror
Novartis Institutes for Biomedical Research, 4560 Horton Street, Emeryville, CA 94608, USA

Contents