

Recombinant Gene Expression: Reviews and Protocols, Second Edition, edited by Paulina Balbas and Argelia Johnson, 2004

Genomics, Proteomics, and Clinical Bacteriology: Methods and Reviews, edited by Neil Woodford and Alan Johnson, 2004

RNA Interference, Editing, and Modification: Methods and Protocols, edited by Jonatha M. Gott, 2004

Protein Arrays: Methods and Protocols, edited by Eric Fung, 2004

Flow Cytometry, Second Edition, edited by Teresa S. Hawley and Robert G. Hawley, 2004

Genetic Recombination Protocols, edited by Alan S. Waldman, 2004

Protein-Protein Interactions: Methods and Applications, edited by Haian Fu, 2004

Mobile Genetic Elements: Protocols and Genomic Applications, edited by Wolfgang J. Miller and Pierre Capi, 2004

Receptor Signal Transduction Protocols, Second Edition, edited by Gary B. Willars and R. A. John Challiss, 2004

Gene Expression Profiling: Methods and Protocols, edited by Richard A. Shimkets, 2004

mRNA Processing and Metabolism: Methods and Protocols, edited by Daniel R. Schoenberg, 2004

Bacterial Artificial Chromosomes, Volume 2: Functional Studies, edited by Shaying Zhao and Marvin Stodolsky, 2004

Bacterial Artificial Chromosomes, Volume 1: Library Construction, Physical Mapping, and Sequencing, edited by Shaying Zhao and Marvin Stodolsky, 2004

Germ Cell Protocols, Volume 2: Molecular Embryo Analysis, Live Imaging, Transgenesis, and Cloning, edited by Heide Schatten, 2004

Germ Cell Protocols, Volume 1: Sperm and Oocyte Analysis, edited by Heide Schatten, 2004

Ribozymes and siRNA Protocols, Second Edition, edited by Mouldy Sioud, 2004

HPLC of Peptides and Proteins: Methods and Protocols, edited by Marie-Isabel Aguilar, 2004

MAP Kinase Signaling Protocols, edited by Rony Seger, 2004

Cytokine Protocols, edited by Marc De Ley, 2004

Protein-Protein Interactions

Methods and Applications

Edited by

Haian Fu

*Department of Pharmacology
Emory University School of Medicine, Atlanta, GA*

Humana Press  Totowa, New Jersey

- 261 -

E4107

© 2004 Humana Press Inc.
999 Riverview Drive, Suite 208
Totowa, New Jersey 07512

www.humanapress.com



All rights reserved. No part of this book may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, microfilming, recording, or otherwise without written permission from the Publisher. Methods in Molecular Biology™ is a trademark of The Humana Press Inc.

The content and opinions expressed in this book are the sole work of the authors and editors, who have warranted due diligence in the creation and issuance of their work. The publisher, editors, and authors are not responsible for errors or omissions or for any consequences arising from the information or opinions presented in this book and make no warranty, express or implied, with respect to its contents.

This publication is printed on acid-free paper. ∞
ANSI Z39.48-1984 (American Standards Institute)

Permanence of Paper for Printed Library Materials.

Cover illustration: Foreground—Figure 3 from Chapter 1, R. C. Liddington. Background—Figure 3, Chapter 28, I. Remy and S. W. Michnick.

Cover design by Patricia F. Cleary.

For additional copies, pricing for bulk purchases, and/or information about other Humana titles, contact Humana at the above address or at any of the following numbers: Tel.: 973-256-1699; Fax: 973-256-8341; E-mail: humana@humanapress.com; or visit our website: www.humanapress.com

Photocopy Authorization Policy:

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by Humana Press Inc., provided that the base fee of US \$25.00 per copy is paid directly to the Copyright Clearance Center at 222 Rosewood Drive, Danvers, MA 01923. For those organizations that have been granted a photocopy license from the CCC, a separate system of payment has been arranged and is acceptable to Humana Press Inc. The fee code for users of the Transactional Reporting Service is: [1-58829-120-0/04 \$25.00].

Printed in the United States of America. 10 9 8 7 6 5 4 3 2 1

Library of Congress Cataloging in Publication Data

Protein-protein interactions : methods and applications / edited by Haian Fu.

p. ; cm. — (Methods in molecular biology ; 261)

Includes bibliographical references and index.

ISBN 1-58829-120-0 (alk. paper) eISBN 1-59259-762-9

1. Protein-protein interactions—Laboratory manuals.

[DNLM: 1. Protein Binding—physiology—Laboratory Manuals. 2. Proteins—metabolism—Laboratory Manuals. QU 25 P96695 2004] I. Fu, Haian. II. Series: Methods in molecular biology (Clifton, N.J.) ; v. 261.

QP551.5.P76 2004

572'.6—dc22

2003020640

Contents

Preface	v
Contributors	xiii

PART I. OVERVIEW

1 Structural Basis of Protein-Protein Interactions <i>Robert C. Liddington</i>	3
2 Quantitative Analysis of Protein-Protein Interactions <i>Keith D. Wilkinson</i>	15

PART II. IN VITRO TECHNIQUES

3 Characterization of Protein-Protein Interactions by Isothermal Titration Calorimetry <i>Adrian Velazquez-Campoy, Stephanie A. Leavitt, and Ernesto Freire</i>	35
4 Circular Dichroism Analysis for Protein-Protein Interactions <i>Norma J. Greenfield</i>	55
5 Protein-Protein Interaction Analysis by Nuclear Magnetic Resonance Spectroscopy <i>Guanghua Gao, Jason G. Williams, and Sharon L. Campbell</i>	79
6 Measuring Rhodopsin-G-Protein Interactions by Surface Plasmon Resonance <i>John Northup</i>	93
7 Using Light Scattering to Determine the Stoichiometry of Protein Complexes <i>Jeremy Mogridge</i>	113
8 Sedimentation Equilibrium Studies <i>Ian A. Taylor, John F. Eccleston, and Katrin Rittinger</i>	119
9 Analysis of Protein-Protein Interactions by Simulation of Small-Zone Gel Filtration Chromatography <i>Rosemarie Wilton, Elizabeth A. Myatt, and Fred J. Stevens</i>	137
10 Fluorescence Gel Retardation Assay to Detect Protein-Protein Interactions <i>Sang-Hyun Park and Ronald T. Raines</i>	155

11	Fluorescence Polarization Assay to Quantify Protein-Protein Interactions Sang-Hyun Park and Ronald T. Raines	161
12	Studying Protein-Protein Interactions via Blot Overlay or Far Western Blot Randy A. Hall	167
13	Glutathione-S-Transferase-Fusion Based Assays for Studying Protein-Protein Interactions Haris G. Vikis and Kun-Liang Guan	175
14	Affinity Capillary Electrophoresis Analyses of Protein-Protein Interactions in Target-Directed Drug Discovery William E. Pierceall, Lixin Zhang, and Dallas E. Hughes	187
15	Mapping Protein-Ligand Interactions by Hydroxyl-Radical Protein Footprinting Nick Loizos	199
16	Use of Phage Display and Polyvalency to Design Inhibitors of Protein-Protein Interactions Michael Mourez and R. John Collier	213
PART III. DETECTING PROTEIN-PROTEIN INTERACTIONS IN HETEROLOGOUS SYSTEMS		
17	A Bacterial Two-Hybrid System Based on Transcriptional Activation Simon L. Dove and Ann Hochschild	231
18	Using the Yeast Two-Hybrid System to Identify Interacting Proteins John Miller and Igor Stagljar	247
19	Analysis of Protein-Protein Interactions Utilizing Dual Bait Yeast Two-Hybrid System Ilya G. Serebriiskii and Elena Kotova	263
20	The Split-Ubiquitin Membrane-Based Yeast Two-Hybrid System Safia Thaminy, John Miller, and Igor Stagljar	297
21	Reverse Two-Hybrid Techniques in the Yeast <i>Saccharomyces cerevisiae</i> Matthew A. Bennett, Jack F. Shern, and Richard A. Kahn	313
22	Mammalian Two-Hybrid Assay for Detecting Protein-Protein Interactions In Vivo Jae Woon Lee and Soo-Kyung Lee	327
23	Co-Immunoprecipitation from Transfected Cells Shane C. Masters	337

quantify	
es	161
via Blot Overlay	
.....	167
sed Assays for Studying	
.....	175
ses of Protein-Protein Discovery	
d Dallas E. Hughes	187
y Hydroxyl-Radical	
.....	199
r to Design Inhibitors	
.....	213
TIONS IN HETEROLOGOUS SYSTEMS	
on Transcriptional Activation	
.....	231
Identify	
.....	247
s Utilizing Dual Bait	
a	263
Yeast Two-Hybrid System	
Stagljar	297
e Yeast	
and Richard A. Kahn	313
ecting Protein-Protein	
.....	327
ted Cells	
.....	337

PART IV: PROBING PROTEIN-PROTEIN INTERACTIONS IN LIVING CELLS

24 Microscopic Analysis of Fluorescence Resonance Energy Transfer (FRET)	
Brian Herman, R. Venkata Krishnan, and Victoria E. Centonze	351
25 Monitoring Molecular Interactions in Living Cells Using Flow Cytometric Analysis of Fluorescence Resonance Energy Transfer	
Francis Ka-Ming Chan	371
26 Fluorescence Correlation Spectroscopy: A New Tool for Quantification of Molecular Interactions	
Keith M. Berland	383
27 Confocal Microscopy for Intracellular Co-Localization of Proteins	
Toshiyuki Miyashita	399
28 Mapping Biochemical Networks with Protein-Fragment Complementation Assays	
Ingrid Remy and Stephen W. Michnick	411
29 In Vivo Protein Cross-Linking	
Fabrice Agou, Fei Ye, and Michel Véron	427

PART V. PROTEOMICS-BASED APPROACHES

30 Computational Prediction of Protein-Protein Interactions	
John C. Obenauer and Michael B. Yaffe	445
31 Affinity Methods for Phosphorylation-Dependent Interactions	
Greg Moorhead and Carol MacKintosh	469
32 Two-Dimensional Gel Electrophoresis for Analysis of Protein Complexes	
Karin Barnouin	479
33 Sample Preparation of Gel Electrophoretically Separated Protein Binding Partners for Analysis by Mass Spectrometry	
Rainer Cramer, Malcolm Saxton, and Karin Barnouin	499
34 Quantitative Protein Analysis by Solid Phase Isotope Tagging and Mass Spectrometry	
Huilin Zhou, Rosemary Boyle, and Ruedi Aebersold	511
35 Internet Resources for Studying Protein-Protein Interactions	
Shane C. Masters	519
Index	525