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# Protein Secretion

## Methods and Protocols

Edited by

**Anastassios Economou**

*Institute of Molecular Biology and Biotechnology, Foundation for Research  
and Technology - Hellas, Heraklion, Greece*

*Editor*

Anastassios Economou  
Foundation for Research &  
Technology – Hellas (FORTH)  
Institute of Molecular Biology &  
Biotechnology and Department  
of Biology  
University of Crete  
Nikolaou Plastira 100  
700 13 Iraklion  
Greece  
aeconom@imbb.forth.gr

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## Preface

The elucidation of the complete information content in hundreds of genomes has brought with it a surprising realization. More than a third of all the proteins in any given proteome are comprised of non-cytoplasmic polypeptides. These can be resident membrane proteins such as channels and receptors or secretory proteins such as hydrolytic enzymes and toxins. Membrane biogenesis and protein trafficking and secretion are central to the biology and pathology of the cell. Optimal protein trafficking is essential for cell viability, communication, and programmed death, for cells to modulate and yield metabolic goods from their environment, for pathogens to attack, and for hosts to fend them off.

Since all polypeptides in prokaryotes and most in eukaryotes are synthesized by cytoplasmic ribosomes, the cell has acquired tools that enable it to accurately and efficiently sort exported proteins from the cytoplasmic residents. Various specialized chaperones, pilots, and ushers have evolved to correctly recognize secretory and membrane proteins and in several instances this recognition prevents or stalls folding reactions. Moreover, this chaperone-mediated “face-control” effectively sorts extra-cytoplasmic from cytoplasmic proteins and then delivers them to complex membrane-associated cellular nanomachines. These catalyze the transmembrane crossing of the targeted polypeptides. Exported proteins come in different functional and structural flavors and are destined for residency of different subcellular compartments or the outside world. Some of them are even savvy enough to cross several prokaryotic and eukaryotic membranes before they reach their final destination. Hence we are now aware that various secretory proteins carry different export signals that act as specific address tags. In many instances, the features of these export signals are well understood and have hence predictive value when a new genome is deciphered through the use of biocomputing.

The study of protein secretion comes with some challenging biochemistry since a large part of the reactions take place at or in the membrane. Elegant genetic and biochemical approaches have been combined over the past 30 years in order to dissect the ways by which the membranes are negotiated so that the exported protein lands on the other side. Overexpression systems have allowed the purification of the subunits involved in large amounts, and this in turn facilitated structural studies. Such is the progress in this approach that, in many of the newer secretion systems, the structures of the components precede the description of biochemical functions. This is less true for the structural elucidation of membrane proteins. Despite recent progress, <200 membrane protein structures are known. In recent years, other powerful cell biology tools that can even offer real-time observation of the secretion process have become available. Finally, organism-wide proteomics is providing insight into how protein secretion is incorporated in the whole metabolic reaction network of the cell and in many instances has revealed interesting links with the rest of the cell’s physiology.

The purpose of *Protein Secretion: Methods and Protocols* is to provide some examples of the multiplicity of tools that have been developed to study protein sorting, membrane targeting, transmembrane crossing, and secretion across multiple membranes. A wide variety of methods are covered that range from bioinformatics and proteomics to fundamental

enzymology and genetics to cell biology, structural analyses, and biophysics. This only reflects the highly multidisciplinary nature one expects from a mature field. It is hoped that the study of the various systems and the tools developed to decipher their secrets will provide users with inspiration in finding ways to tackle problems encountered in their research. The multiplicity of protein export systems discovered to date suggests that we are nowhere near a complete inventory. I chose to focus on well-characterized paradigms so that the reader can benefit from robust, well-established protocols in which many of the experimental wrinkles have been ironed out. Several systems have been chosen from both prokaryotic and eukaryotic organisms. The book is aimed at the biochemist, geneticist, or biologist (cell, molecular, or structural) who is a protein secretion novice and also at seasoned protein secretion experts who wish to incorporate new experimental tools in their studies. The book is also aimed at researchers who want to explore the immense biotechnological potential of secretion systems in the manipulation of protein export pathways for the production of heterologous proteins (be they biopharmaceuticals or industrial enzymes) as well as their use to develop vaccines and anti-microbials. The reader may gain insight from the difficulties encountered in the more established systems and use this rationally in the dissection of less characterized protein secretion machines, in less characterized organisms, or other cell biology and membrane-related questions.

I would like to thank the authors who have contributed to this work for their enthusiastic response and efforts; to John Walker, the series editor, for his constant help, encouragement, and vigilant eye; to Georgia Houlaki for her expert secretarial help and exceptional organizational skills; and to the staff at Humana Press who helped produce this volume.

*Tassos Economou*

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## Contributors

SUHILA APPATHURAI • *Cell Biology and Metabolism Program, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA*

LAURENT AUDRY • *Institut Pasteur, Groupe “Dynamique des interactions hôte-pathogène”, Paris, France*

DIBYENDU BHATTACHARYA • *Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL, USA*

DOMINIQUE BELIN • *Department of Pathology and Immunology, University Medical Center, University of Geneva, Geneva, Switzerland*

BEN C. BERKS • *Department of Biochemistry, Oxford University, Oxford, UK*

GEERT VAN DER BOGAART • *Department of Membrane Enzymology, Groningen Biomolecular Sciences and Biotechnology Institute and Zernike Institute for Advanced Materials, University of Groningen, Groningen, The Netherlands*

MIKHAIL BOGDANOV • *Department of Biochemistry and Molecular Biology, University of Texas Medical School, Houston, TX, USA*

BETTINA BÖLTER • *Department Biologie I-Botanik, Ludwig-Maximilians-Universität, Planegg-Martinsried and Munich Center for Integrated Protein Science, CiPSM, Ludwig-Maximilians- Universität, Munich, Germany*

JEFFREY L. BRODSKY • *Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA, USA*

NERMIN CELIK • *Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia*

AGNIESZKA CHACINSKA • *Institut für Biochemie und Molekularbiologie, ZBMZ, and Centre for Biological Signalling Studies (bioss), Universität Freiburg, Freiburg, Germany*

JENNINE M. CRANE • *Department of Biochemistry, University of Missouri, Columbia, MO, USA*

MICHAEL DAGLEY • *Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia*

KUSH DALAL • *Department of Biochemistry and Molecular Biology, Life Sciences Institute, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada*

ROSS E. DALBEY • *Department of Chemistry, The Ohio State University, Columbus, OH, USA*

PAVEL DOLEZAL • *Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic*

WILLIAM DOWHAN • *Department of Biochemistry and Molecular Biology, University of Texas Medical School, Houston, TX, USA*

ARNOLD DRIESSEN • *Department of Molecular Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute and Zernike Institute for Advanced Materials, University of Groningen, Groningen, The Netherlands*

FRANCK DUONG • *Department of Biochemistry and Molecular Biology, Life Sciences Institute, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada*

ANASTASSIOS ECONOMOU • *Department of Biology, University of Crete and Institute of Molecular Biology and Biotechnology-Foundation for Research and Technology Hellas, Heraklion, Crete, Greece*

JOST ENNINGA • *Institut Pasteur, Groupe “Dynamique des interactions hôte-pathogène”, Paris, France*

JAN-WILLEM DE GIER • *Center for Biomembrane Research, Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden*

BENJAMIN S. GLICK • *Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL, USA*

GIORGOS GOURIDIS • *Department of Biology, University of Crete and Institute of Molecular Biology and Biotechnology-Foundation for Research and Technology Hellas, Heraklion, Crete, Greece*

ADAM T. HAMMOND • *Institute for Biophysical Dynamics, The University of Chicago, Chicago, IL, USA*

PHILIP N. HEACOCK • *Department of Biochemistry and Molecular Biology, University of Texas Medical School, Houston, TX, USA*

RAMANUJAN S. HEGDE • *Cell Biology and Metabolism Program, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA*

STEPHEN HIGH • *Faculty of Life Sciences, University of Manchester, Manchester, UK*

I. BARRY HOLLAND • *Institut de Genetique et Microbiologie, UMR 8621 CNRS, Universite de Paris-Sud, Orsay, France*

SPYRIDOULA KARAMANOU • *Institute of Molecular Biology and Biotechnology-Foundation for Research and Technology Hellas, Heraklion, Crete, Greece*

MIRJAM KLEPSCH • *Center for Biomembrane Research, Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden*

MARINA KOUKAKI • *Institute of Molecular Biology and Biotechnology-Foundation for Research and Technology Hellas, Heraklion, Crete, Greece*

VIKTOR KRASNIKOV • *Department of Optical Condensed Matter Physics, Zernike Institute for Advanced Materials, University of Groningen, Groningen, The Netherlands*

ANDREAS KUHN • *Institute of Microbiology and Molecular Biology, University of Hohenheim, Stuttgart, Germany*

ILJA KUSTERS • *Department of Molecular Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute and Zernike Institute for Advanced Materials, University of Groningen, Groningen, The Netherlands*

VLADIMIR A. LIKIC • *Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, Victoria, Australia*

ANGELA A. LILLY • *Department of Biochemistry, University of Missouri, Columbia, MO, USA*

TREVOR LITHGOW • *Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia*

MALAIYALAM MARIAPPAN • *Cell Biology and Metabolism Program, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA*

CHRIS MEISINGER • *Institut für Biochemie und Molekularbiologie, ZBMZ, and Centre for Biological Signalling Studies (bioss), Universität Freiburg, Freiburg, Germany*

MATTHIAS MÜLLER • *Institute of Biochemistry and Molecular Biology, ZBMZ, University of Freiburg, Freiburg, Germany*

KUNIO NAKATSUKASA • *Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA, USA*

SHIN-ICHIRO NARITA • *Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo, Japan*

TRACY PALMER • *Division of Molecular Microbiology, College of Life Sciences, University of Dundee, Dundee, Scotland*

SASCHA PANAHANDEH • *Institute of Biochemistry and Molecular Biology, ZBMZ, University of Freiburg, Freiburg, Germany*

NIKOLAUS PFANNER • *Institut für Biochemie und Molekularbiologie, ZBMZ, and Centre for Biological Signalling Studies (bioss), Universität Freiburg, Freiburg, Germany*

BERT POOLMAN • *Department of Membrane Enzymology, Groningen Biomolecular Sciences and Biotechnology Institute and Zernike Institute for Advanced Materials, University of Groningen, Groningen, The Netherlands*

LINDA L. RANDALL • *Department of Biochemistry, University of Missouri, Columbia, MO, USA*

SHRUTI RASTOGI • *Department of Biochemistry and Molecular Biophysics, Columbia University and Columbia University Center for Computational Biology and Bioinformatics (C2B2), New York, NY, USA*

FERNANDA RODRIGUEZ • *Biozentrum, Growth and Development, University of Basel, Basel, Switzerland*

BURKHARD ROST • *Department of Biochemistry and Molecular Biophysics, Columbia University and Columbia University Center for Computational Biology and Bioinformatics (C2B2) and NorthEast Structural Genomics Consortium (NESG) & New York Consortium on Membrane Protein Structure (NYCOMPS), New York, NY, USA*

FRANK SARGENT • *Division of Molecular Microbiology, College of Life Sciences, University of Dundee, Dundee, Scotland*

ANNE-KATHRIN SCHUBERT • *Institute of Microbiology and Molecular Biology, University of Hohenheim, Stuttgart, Germany*

AJAY SHARMA • *Cell Biology and Metabolism Program, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA*

SUSAN SCHLEGEL • *Center for Biomembrane Research, Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden*

DIONISIA P. SIDERIS • *Department of Biology, University of Crete and Institute of Molecular Biology and Biotechnology-Foundation for Research and Technology Hellas, Heraklion, Crete, Greece*

NANDI SIMPSON • *Institut Cochin, Universite Paris Descartes, CNRS (UMR 8104) Inserm, U567, Paris, France*

OLIVER SCHMIDT • *Institut für Biochemie und Molekularbiologie, ZBMZ, and Centre for Biological Signalling Studies (bioss), Universität Freiburg, Freiburg, Germany*

JÜRGEN SOLL • *Department Biologie I-Botanik, Ludwig-Maximilians-Universität, Planegg-Martinsried and Munich Center for Integrated Protein Science, CiPSM, Ludwig-Maximilians-Universität, Munich, Germany*

ANNE SPANG • *Biozentrum, Growth and Development, University of Basel, Basel, Switzerland*

NATALIE STIEGLER • *Institute of Microbiology and Molecular Biology, University of Hohenheim, Stuttgart, Germany*

PENELOPE STRITTMATTER • *Department Biologie I-Botanik, Ludwig-Maximilians-Universität, Planegg-Martinsried and Munich Center for Integrated Protein Science, CiPSM, Ludwig-Maximilians-Universität, Munich, Germany*

STEVEN M. THEG • *Department of Plant Biology, University of California, Davis, CA, USA*

KOSTAS TOKATLIDIS • *Department of Materials Science and Technology, University of Crete and Institute of Molecular Biology and Biotechnology-Foundation for Research and Technology Hellas, Heraklion, Crete, Greece*

HAJIME TOKUDA • *Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo, Japan*

F.-NORA VÖGTLE • *Institut für Biochemie und Molekularbiologie, ZBMZ, and Centre for Biological Signalling Studies (bioss), Universität Freiburg, Freiburg, Germany*

SAMUEL WAGNER • *Center for Biomembrane Research, Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden*

PENG WANG • *Department of Chemistry, The Ohio State University, Columbus, OH, USA*

DAVID WICKSTRÖM • *Center for Biomembrane Research, Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden*

CORNELIA M. WILSON • *Faculty of Medicine, University of Limoges, Limoges, France*

JANNY DE WIT • *Department of Molecular Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute and Zernike Institute for Advanced Materials, University of Groningen, Groningen, The Netherlands*

JIJUN YUAN • *Department of Chemistry, The Ohio State University, Columbus, OH, USA*