METHODS IN MOLECULAR BIOLOGY

Series Editor
John M. Walker
School of Life and Medical Sciences
University of Hertfordshire
Hatfield, Hertfordshire, AL10 9AB, UK

For further volumes:
http://www.springer.com/series/7651

Glyco-Engineering

Methods and Protocols

Edited by

Alexandra Castilho

University of Natural Resources and Life Sciences, Vienna, Austria

Humana Press

Editor
Alexandra Castilho
University of Natural Resources and Life Sciences
Vienna, Austria

ISSN 1064-3745 ISSN 1940-6029 (electronic)
Methods in Molecular Biology
ISBN 978-1-4939-2759-3 ISBN 978-1-4939-2760-9 (eBook)
DOI 10.1007/978-1-4939-2760-9

Library of Congress Control Number: 2015941740

Springer New York Heidelberg Dordrecht London

Printed on acid-free paper

Humana Press is a brand of Springer
Springer Science+Business Media LLC New York is part of Springer Science+Business Media (www.springer.com)

Preface

Glycosylation is a critical modification that confers and controls a range of properties to a protein. The diverse function of glycoproteins is a direct result of their structure, and the majority of the currently approved protein pharmaceutics rely on proper glycosylation to exhibit optimum efficacy. Glycosylation is considered to be one of the main causes for protein heterogeneity and is seen as a critical quality attribute by regulatory agencies. Specific glycosylation patterns obtained through glyco-engineering are expected to play a central role in future bio-manufacturing processes. The fact that glycoproteins, particularly therapeutic proteins, are in great demand have stimulated an extensive interest and search for new, better, and faster sources for recombinant protein production. However, depending on its source, the glycosylation pattern of a recombinant protein may vary greatly.

Controlling glycosylation in vivo through modulation of glycan biosynthesis can be a hurdle since the process has no known template and is dictated by many factors such as the availability, activity, and correct subcellular localization of particular substrates and enzymes. Also, the expression host and production conditions are determinants to the profile of a particular glycoprotein that may include a heterogeneous collection of glycan structures due to differences in processing.

In the past years, we have been witnessing outstanding achievements in glyco-engineering. Glyco-engineering strives to attend to the urgent need of glyco-designed proteins. These can be usable in studies of structure-function relationship and in obtaining "human-like" glycosylation profiles optimized for efficiency, particularly relevant in proteins intended for therapy. The majority of human therapeutic proteins are *N*-linked glycoproteins, and therefore the chosen host for its production must be equipped with the machinery to perform the required protein modification. Engineering expression hosts to express glycoproteins with "tailor-made" glycosylation has been attempted in both prokaryotic and eukaryotic cells, and many of its successes are reported in the extensive literature.

In this series of *Methods in Molecular Biology*, on the subject of Glyco-engineering, I endeavored to select protocols for a wide range of methods being developed to control the composition of carbohydrates and the properties of proteins through manipulations on the production host rather than in the protein itself. The first five parts deal with host-specific glyco-engineering and contain chapters that provide protocols for modifications of the glycosylation pathway in bacteria, yeast, insect, plants, and mammalian cells. Due to limitations in space, the volume focuses mainly on the modification of the *N*-linked glycosylation machinery.

Each part is initiated by a review chapter intended to provide an overview of the most significant approaches for engineering the glycosylation pathways in a specific host. The review chapter is then followed by chapters where experts in the field provide protocols for a specific method/technique used for (or involved in the process of) altering the way that a particular host glycosylates proteins.

Bacteria, particularly *E. coli*, are a well-suitable and preferred expression platform for the production of un-glycosylated proteins or for proteins that do not require posttranslational modifications to attend their biological function. Due to its inability to perform *N*-glycosylation, most heterologous proteins produced in bacteria fail to reach a correct and active conformation. However, engineering for humanized *N*-glycans in bacteria has made significant progress over recent years with improvements still needed to establish a reliable system.

Yeast-based expression platforms are one of the systems of choice by the research community. There are, nevertheless, drawbacks on their wide application mostly related to their non-human-like glycosylation. Most of the engineering attempts have made remarkable accomplishments and have highlighted the power to use glyco-engineered yeast in the production of proteins with defined *N*-glycans.

Although insect systems are widely used as recombinant protein production platforms, no insect-based system is capable of synthetizing human-type glycans, and some insect cell systems produce *N*-glycans with immunogenic epitopes. These problems have been addressed by efforts to glyco-engineer insect-based expression systems introducing the capacity to produce complex type and eliminating the ability to produce immunogenic *N*-glycans.

Despite the highly conserved *N*-glycosylation pathway in plants and mammals, recombinant proteins derived from plants carry glycans that significantly differ from those present in humans including immunogenic epitopes. Moreover, due to the limited repertoire of enzymes, plants cannot produce highly complex structures. Significant progresses were already achieved through glyco-engineering, and plants are able to produce recombinant proteins with highly complex fully humanized *N*-glycosylation. Further improvements are still needed so transgenic plants are seen as a reliable production platform for glycoproteins carrying homogenous humanized *N*-glycosylation profile.

Finally, the most common host for the production of glycoproteins is non-human mammalian cells, and, within these, the Chinese hamster ovary (CHO) cells are prevalently used. When expressed in these cell lines, glycoproteins are close to human ones although critical differences have been identified between humans and most other mammals. Also, due to the vast repertoire of glycosylation enzymes, mammalian glycoproteins are often produced as a collection of glycoforms, and one of the goals of mammalian cell glyco-engineering is to make proteins carrying more defined glycans by controlling and altering the biosynthetic pathway.

Glyco-engineering of host expression systems is undoubtedly booming and the achievements are impressive, but the complete control of the machinery to truly homogenize glycoproteins remains demanding. Part VI presents alternative approaches to host glyco-engineering. Homogeneous glycosylation of recombinant protein is achieved by in vitro glyco-engineering.

The last part of this volume provides selected protocols for the analysis of the *N*-glycans and glyco-profiling by mass spectrometry.

This book was conceived with the intention of providing the reader with an array of strategies and technologies currently in use for glyco-engineering distinct living organisms. The milestones in the glyco-engineering field are laid out and discussed in review chapters, and thus the book offers vast options to help with the choice of the expression system and approach that best suits intended protein research or applications. Obviously, the chapters compiled here have only dipped into the vast collection of strategies for protein glyco-engineering, and I apologize in advance if we missed the technique or approach that best suits your goal.

I would like to pay compliments to those who contributed to this book and took their time to share with all of us their expertise. Also my deepest thanks to the *Methods in Molecular Biology* series editor, Prof. John Walker, for the constant support and helpful advice. In addition, I extend my appreciation to all my colleagues for coping with my stress during the production of the book. Finally, I thank five amazing women—Wanda, Bila, Augusta, Guida, and Ana—for believing in me and keeping me going.

This book is dedicated to my two wonderful children.

Vienna, Austria ***Alexandra Castilho***

Contents

Contributors

Hiroko Abe • *Health Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Takamatsu, Kagawa, Japan*

Markus Aebi • *Department of Biology, Institute of Microbiology, ETH Zurich, Zurich, Switzerland*

Kathirvel Alagesan • *Department of Biomolecular Systems, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany*

Friedrich Altmann • *Department of Chemistry, University of Natural Resources and Life Sciences, Vienna, Austria*

Alexey A. Belogurov • *Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry of the RAS, Moscow, Russia; Kazan Federal University, Kazan, Russia; Institute of Gene Biology of the RAS, Moscow, Russia*

Michael J. Betenbaugh • *Department of Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD, USA*

Katrin Braasch • *Department of Microbiology, University of Manitoba, Winnipeg, MB, Canada*

Michael Butler • *Department of Microbiology, University of Manitoba, Winnipeg, MB, Canada*

Nico Callewaert • *Unit for Medical Biotechnology, Medical Biotechnology Center, VIB-UGent, Ghent, Belgium; Department of Biochemistry and Microbiology, Laboratory for Protein Biochemistry and Biomolecular Engineering, Ghent University, Ghent, Belgium; Medical Biotechnology Center, VIB-UGent, Ghent, Belgium*

Alexandra Castilho • *Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Vienna, Austria*

Hyung Joon Cha • *Department of Chemical Engineering, Pohang University of Science and Technology, Pohang, Korea*

Yi Chai • *School of Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY, USA*

Kah Fai Chan • *Bioprocessing Technology Institute (BTI), Agency for Science, Technology and Research (A*STAR), Singapore, Singapore*

Veronica T. Chang • *Radcliffe Department of Medicine and MRC Human Immunology Unit, John Radcliffe Hospital, University of Oxford, Oxford, UK*

Max Crispin • *Department of Biochemistry, Oxford Glycobiology Institute, University of Oxford, Oxford, UK*

Simon J. Davis • *Radcliffe Department of Medicine and MRC Human Immunology Unit, John Radcliffe Hospital, University of Oxford, Oxford, UK*

Eva L. Decker • *Plant Biotechnology, Faculty of Biology, University of Freiburg, Freiburg, Germany*

Matthew P. DeLisa • *School of Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY, USA*

Sergey M. Deyev • *Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry of the RAS, Moscow, Russia; Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia*

Martina Dicker • *Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Vienna, Austria*

Mario F. Feldman • *Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada*

Kazuhito Fujiyama • *The International Center for Biotechnology, Osaka University, Osaka, Japan*

Alexander G. Gabibov • *Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry of the RAS, Moscow, Russia; Kazan Federal University, Kazan, Russia; Institute of Gene Biology of the RAS, Moscow, Russia; Chemistry Department Moscow State University, Moscow, Russia*

Christina E. Galuska • *Institute of Biochemistry, Faculty of Medicine, University of Giessen, Giessen, Germany*

Sebastian P. Galuska • *Institute of Biochemistry, Faculty of Medicine, University of Giessen, Giessen, Germany*

Yuan Gao • *Department of Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD, USA*

Christoph Geisler • *GlycoBac, LLC, Laramie, WY, USA*

Dmitry D. Genkin • *R&D Laboratories and Business Development Office, Xenetic Biosciences plc, London, UK*

John P. Giddens • *Department of Chemistry and Biochemistry, University of Maryland, College Park, MD, USA*

Christoph Gmeiner • *Research Area Biochemical Engineering, Institute of Chemical Engineering, Vienna University of Technology, Vienna, Austria*

John S.Y. Goh • *Bioprocessing Technology Institute (BTI), Agency for Science, Technology and Research (A*STAR), Singapore, Singapore*

Clemens Gruber • *Department of Chemistry, University of Natural Resources and Life Sciences, Vienna, Austria*

Stephen R. Hamilton • *GlycoFi, (A Wholly Owned Subsidiary of Merck & Co., Inc.) Biologics Discovery, Merck Research Laboratories, Lebanon, NH, USA*

Stephen R.P. Jaffé • *Department of Chemical and Biological Engineering, ChELSI Institute, University of Sheffield, Sheffield, UK*

Donald L. Jarvis • *GlycoBac, LLC, Laramine, WY, USA; Department of Molecular Biology, University of Wyoming, Laramie, WY, USA*

Hiroyuki Kajiura • *The International Center for Biotechnology, Osaka University, Osaka, Japan*

Yeon Kyu Kim • *Department of Chemical Engineering, Pohang University of Science and Technology, Pohang, Korea*

Daniel Kolarich • *Department of Biomolecular Systems, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany*

Bram Laukens • *Unit for Medical Biotechnology, Medical Biotechnology Center, VIB-UGent, Ghent, Belgium; Department of Biochemistry and Microbiology, Laboratory for Protein Biochemistry and Biomolecular Engineering, Ghent University, Ghent, Belgium*

Andreas Loos • *Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Vienna, Austria*
Kai Maass • *Institute for Organic Chemistry, Faculty of Biology and Chemistry, University of Giessen, Giessen, Germany*
Hideaki Mabashi-Asazuma • *Department of Molecular Biology, University of Wyoming, Laramie, WY, USA*
Sylvestre Marillonnet • *Department of Cell and Metabolic Biology, Leibniz-Institut für Pflanzenbiochemie, Halle, Germany*
Aline Mongis • *Synthetic Protein Chemistry and Glyco-Engineering Group, Centre de Biophysique Moléculaire (CNRS UPR 4301), Orléans, France*
Andreas Naegeli • *Department of Biology, Institute of Microbiology, ETH Zurich, Zurich, Switzerland*
Ken-ichi Nakayama • *Research Institute for Sustainable Chemistry, National Institute of Advanced Industrial Science and Technology (AIST), Higashi-Hiroshima, Hiroshima, Japan*
Someet Narang • *Cell Culture and Fermentation Sciences, MedImmune, Gaithersburg, MD, USA*
Anne A. Ollis • *School of Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY, USA*
Dieter Palmberger • *Department of Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria*
Jagroop Pandhal • *Department of Chemical and Biological Engineering, ChELSI Institute, University of Sheffield, Sheffield, UK*
Friedrich Piller • *Synthetic Protein Chemistry and Glyco-Engineering Group, Centre de Biophysique Moléculaire (CNRS UPR 4301), Orléans, France*
Véronique Piller • *Synthetic Protein Chemistry and Glyco-Engineering Group Centre, Centre de Biophysique Moléculaire (CNRS UPR 4301), Orléans, France*
Dubravko Rendic • *Department of Chemistry, University of Natural Resources and Life Sciences, Vienna, Austria*
Ralf Reski • *Plant Biotechnology, Faculty of Biology, University of Freiburg, Freiburg, Germany; TIP Trinational Institute for Plant Research and Plant Biotechnology, Faculty of Biology, University of Freiburg, Freiburg, Germany; BIOSS Centre for Biological Signalling Studies, Freiburg, Germany; FRIAS Freiburg Institute for Advanced Studies, Freiburg, Germany*
Jennifer Schoberer • *Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Vienna, Austria*
Flavio Schwarz • *Glycobiology Research and Training Center, Department of Medicine, La Jolla, CA, USA; Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA, USA*
Ivan V. Smirnov • *Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry of the RAS, Moscow, Russia; Kazan Federal University, Kazan, Russia*
Zhiwei Song • *Bioprocessing Technology Institute (BTI), Agency for Science, Technology and Research (A*STAR), Singapore, Singapore*
Oliver Spadiut • *Research Area Biochemical Engineering, Institute of Chemical Engineering, Vienna University of Technology, Vienna, Austria*
Robert A. Spooner • *Life Sciences, University of Warwick, Coventry, UK*

Herta Steinkellner • *Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Vienna, Austria*
Richard Strasser • *Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Vienna, Austria*
Benjamin Strutton • *Department of Chemical and Biological Engineering, ChELSI Institute, University of Sheffield, Sheffield, UK*
Mark Stuczynski • *Department of Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD, USA*
Ulrike Vavra • *Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Vienna, Austria*
Carina Villacrés • *Department of Microbiology, University of Manitoba, Winnipeg, MB, Canada*
Karthik Viswanathan • *Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA*
Ivan I. Vorobiev • *Centre "Bioengineering" of the RAS, Moscow, Russia*
Nicolas F. Vozza • *Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada*
Charlot De Wachter • *Unit for Medical Biotechnology, Medical Biotechnology Center, VIB-UGent, Ghent, Belgium; Department of Biochemistry and Microbiology, Laboratory for Protein Biochemistry and Biomolecular Engineering, Ghent University, Ghent, Belgium*
Lai-Xi Wang • *Department of Chemistry and Biochemistry, University of Maryland, College Park, MD, USA*
Qiong Wang • *Department of Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD, USA*
Stefan Werner • *Nomad Bioscience GmbH, Halle, Germany*
Gertrud Wiedemann • *Plant Biotechnology, Faculty of Biology, University of Freiburg, Freiburg, Germany; TIP Trinational Institute for Plant Research and Plant Biotechnology, Faculty of Biology, University of Freiburg, Freiburg, Germany*
Markus Windwarder • *Department of Chemistry, University of Natural Resources and Life Sciences, Vienna, Austria*
Phillip C. Wright • *Department of Chemical and Process Engineering, ChELSI Institute, University of Sheffield, Sheffield, UK*
Dongxing Zha • *Institute for Applied Cancer Science, The University of Texas MD Anderson Cancer Center, Houston, TX, USA*