SPRINGER BRIEFS IN APPLIED SCIENCES AND TECHNOLOGY · FORENSIC AND MEDICAL BIOINFORMATICS

Krishna Mohan Poluri
Khushboo Gulati

Protein Engineering Techniques

Gateways to Synthetic Protein Universe

Springer

SpringerBriefs in Applied Sciences and Technology

Forensic and Medical Bioinformatics

More information about this series at http://www.springer.com/series/11910

Krishna Mohan Poluri · Khushboo Gulati

Protein Engineering Techniques

Gateways to Synthetic Protein Universe

Springer

Krishna Mohan Poluri
Department of Biotechnology and Center for Nanotechnology
Indian Institute of Technology Roorkee
Roorkee, Uttarakhand
India

Khushboo Gulati
Department of Biotechnology
Indian Institute of Technology Roorkee
Roorkee, Uttarakhand
India

ISSN 2191-530X ISSN 2191-5318 (electronic)
SpringerBriefs in Applied Sciences and Technology
ISSN 2196-8845 ISSN 2196-8853 (electronic)
Springer Briefs in Forensic and Medical Bioinformatics
ISBN 978-981-10-2731-4 ISBN 978-981-10-2732-1 (eBook)
DOI 10.1007/978-981-10-2732-1

Library of Congress Control Number: 2016954620

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #22-06/08 Gateway East, Singapore 189721, Singapore

Dedicated to all our teachers and mentors for their invaluable guidance and unconditional support throughout our research careers

Krishna Mohan Poluri
Khushboo Gulati

Preface

Proteins are the string of amino acids that play key roles in almost all the physiological and pathological events that are occurring in the body. Functionality of proteins is directly related to the structure they attain, which ultimately depends on the composition of their unique amino acid sequence. Protein engineering is a fledging field and a creative process to design the target proteins or signaling networks with desirable structure and functions. The idea of protein engineering is to utilize the unexplored sequence space in order to design/rewire the proteins as per human wish and for the well-being of the society. Numerous protein engineering methods have been developed that are aiding in various research and technological applications. Indeed, protein engineering has been a powerful tool in bioengineering for last couple of decades and has generated vast number of useful enzymes/proteins that have phenomenal therapeutic and industrial potential. Protein engineering field started gaining its approval for last couple of years: In 2012, the market is $56 billion and expected to increase to 168 billion in 2017, at a Compound Annual Growth Rate (CAGR) of 10.9 % from 2012 to 2017.

In this brief, we primarily focus on most popular experimental methods and several computational programs that are being widely used under the categories of directed evolution, rational and de novo designing of proteins and their biotechnological/biomedical applications. Further, the brief sheds light on the advantages and pitfalls of the existing methodologies and their future perspectives. The book is divided into five chapters: Chapter 1 describes the fundamental aspects of protein structure–function–folding relationships and an introduction to the field of biomolecular and protein engineering techniques. Chapter 2 summarizes various experimental techniques that are used for generating novel proteins under the framework of directed evolution. Chapter 3 provides an overview of all the computational methods that aids us in understanding structure–function relationships through a rational approach. Chapter 4 highlights the protocols of de novo protein designing and combinatorial or computer-aided directed evolutionary approaches for engineering novel scaffolds. Chapter 5 provides a glimpse of several biotechnological and biomedical applications of the engineered proteins that are generated using the techniques described in Chaps. 2–4.

We anticipate that this brief will provide a broader perspective about the field of protein engineering to all the researchers planning to gain comprehensive understanding and an up-to-date knowledge about the fundamental techniques and their biotechnological/biomedical applications.

Roorkee, India

Krishna Mohan Poluri
Khushboo Gulati

Acknowledgements

KMP greatly acknowledges the support DBT-IYBA fellowship (BT/07/IYBA/2013-19), SERB Young Scientist grant (SERB - SB/YS/LS-380/2013), and MHRD-IITR Faculty Initiation Grant (FIG-BIO-637), from Government of India (GoI). KG acknowledges the support MHRD-Assistantship for pursuing Ph.D. at IIT-Roorkee.

Contents

Chapter 1
World of Proteins: Structure-Function Relationships and Engineering Techniques

Abstract Proteins are the key biomolecules in almost all the physiological and pathological processes that are occurring in the cell. Functionality of proteins is related to its conformation, which is ultimately dictated by their unique amino acid sequence. In the current chapter, a brief overview will be provided on the nature of amino acids, structural characteristics and functional versatility of proteins. A glimpse on various interactions present in stabilizing the protein fold and the methods for unraveling their atomic level structures are also discussed. Finally, we present the role of engineered synthetic proteins for the welfare of humanity with huge potential in research and industrial sectors including biotechnological and biomedical fields under the emerging concepts of biomolecular/protein engineering.

Keywords Transcription · Translation · Protein structural organization · Ramachandran map · Protein folding · Energy landscape · Non-covalent interactions · Biomolecular engineering

1.1 Introduction

Proteins are known to be the major workhorse macromolecules in the cell. They play a lead role in almost each and every cellular event. Large varieties of proteins are available ranging from small to big, monomeric to oligomeric, globular or fibrillar, hydrophobic or hydrophilic, existing alone or in conjunction with other proteins/biomolecules like carbohydrates, nucleic acids etc, with fixed conformations or with a range of dynamic alterations as per the demand. Such a diversified nature of proteins can be attributed to the differential arrangement of amino acids in a polypeptide chain. Further, decoration of protein strings by the molecular events like co-translational modifications and post translational modifications, adds up another layer of diversification to these entities. All such variations in proteins lead to differential 3D structures that ultimately make them versatile towards their functional characteristics [1].

K.M. Poluri and K. Gulati, *Protein Engineering Techniques*,
Springer Briefs in Forensic and Medical Bioinformatics,
DOI 10.1007/978-981-10-2732-1_1

1.2 Functional Characteristics of Proteins

Proteins perform wide variety of functions in cells including structural organization, maintenance of cell shape, synthesis of biomolecules, acts as molecular sensors, aids in cellular movement, interaction with other cells and biomolecular counterparts, provides defense against infectious agents etc. Proteins with diversified functions have been summarized in the following paragraphs.

Structural proteins provide shape and mechanical strength to the cell like network of filaments including microtubules, actin filaments, intermediate filaments that together forms cytoskeletons thus providing platform for wide variety of cellular functions including organelles movement, vesicles transport [2]. For example; **collagen**, a triple helical protein, present as stress bearing agent in various connective tissues like in cartilage, bone, tendons, ligaments etc [3]. Similarly, **keratin**, found in hair, nails, and skin gives structural strength and protects the epithelial cells from stress and damage. ***Contractile proteins*** like actin and myosin are the force generators that aids in muscle contraction and movement. ***Transport Proteins*** such as dynein and kinesin transport organelles/bio-molecules within the cell. Proteins like hemoglobin present in red blood cells carries oxygen from respiratory organs to the rest of the body.

Enzymes catalyze and speed up various biochemical reactions occurring in the cell. For example, kinases and phosphatases catalyze the post translational events such as phosphorylation and dephosphorylation. There are six different classes of enzymes that are present in the cell. They are Oxidoreductases, Transferases, Hydrolases, Lyases (syntheses), Isomerases, and Ligases (Synthetases) [4].

Membrane Proteins are located within the membranes or else interact with membranes. They are broadly divided into two classes. (a) Integral membrane proteins, also known as intrinsic proteins that anchor the membranes through the phospholipid bilayer. Transmembrane proteins are also intrinsic proteins that spans across the membrane and flank their domains toward extracellular and cytoplasmic regions of the bilayer membrane. (b) Peripheral membrane proteins are the extrinsic proteins which do not interact with hydrophobic environment of lipid bilayer but is present on the surface of membranes by interacting with integral membrane proteins or interacts with polar head groups of the lipid bilayer. Membrane proteins play diversified roles as some of them act as receptors that receive signals from the outside environment, and initiates downstream signaling pathways. Transporters transfer nutrients or other small molecules (Ion channels) across the cells. Membrane proteins are also involved in maintaining the cell shape, cell-cell recognition (especially glycoproteins), cell-cell adhesion to make different kinds of junctions (tight junctions or gap junctions) [5].

Antibodies, also known as immunoglobulins, provide protection against numerous infectious agents. Five major classes of immunoglobulins are present (IgG, IgM, IgA, IgD, IgE). They differ on the basis of the type of heavy chain present in their structure and thereby exhibit different immunological functions [6].