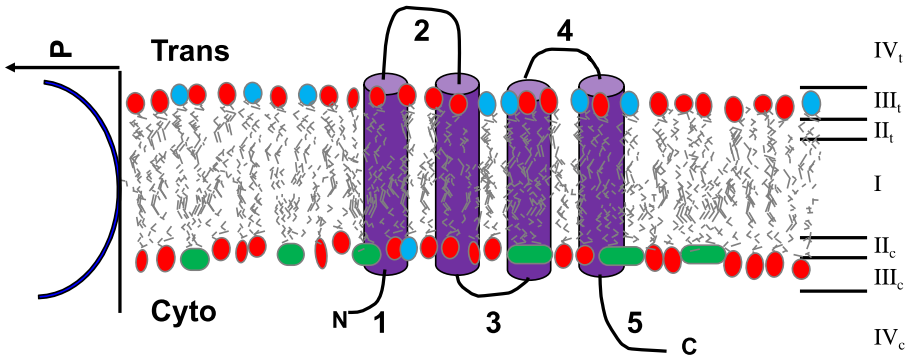
A CLOSER LOOK AT MEMBRANE PROTEINS

By

Dragana Robajac, Miloš Šunderić, Nikola Gligorijević, Olgica Nedić, Mark Tingey, Steven J. Schnell, Yichen Li, Samuel Junod, Wenlan Yu, Weidong Yang, Nilay K. Roy, Oliver Beckstein, Fiona Naughton, Min Lu, Katherine Si-Jia Lu, Manjusha Lekshmi, Nicholas Wenzel, Sanath H. Kumar, Manuel F. Varela, Raymond J. Turner, Irshad Ahmad, Pikyee Ma, Nighat Nawaz, David J. Sharples, Peter J. F. Henderson, Simon G. Patching (Ed.)



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CONTRIBUTORS

(Alphabetical order)

Irshad Ahmad	University of Leeds, UK
Oliver Beckstein	Arizona State University, USA
Nikola Gligorijević	University of Belgrade, Serbia
Peter J. F. Henderson	University of Leeds, UK
Samuel Junod	Temple University, USA
Sanath H. Kumar	ICAR-Central Institute of Fisheries Education, India
Manjusha Lekshmi	ICAR-Central Institute of Fisheries Education, India
Yichen Li	Temple University, USA
Katherine Si-Jia Lu	Rosalind Franklin University of Medicine & Science, USA
Min Lu	Rosalind Franklin University of Medicine & Science, USA
Pikyee Ma	University of Leeds, UK
Fiona Naughton	Arizona State University, USA
Nighat Nawaz	Islamia College Peshawar, Pakistan
Olgica Nedić	University of Belgrade, Serbia
Simon G. Patching (Ed.)	University of Leeds, UK
Dragana Robajac	University of Belgrade, Serbia
Nilay K. Roy	Northeastern University, USA
Steven J. Schnell	Temple University, USA
David J. Sharples	University of Leeds, UK
Miloš Šunderić	University of Belgrade, Serbia
Mark Tingey	Temple University, USA
Raymond J. Turner	University of Calgary, Canada
Manuel F. Varela	Eastern New Mexico University, USA
Nicholas Wenzel	Eastern New Mexico University, USA
Weidong Yang	Temple University, USA
Wenlan Yu	Temple University, USA

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PREFACE

Membrane proteins are coded by up to 30% of the open reading frames in know genomes. They have pivotal roles in many biological processes including: transport of ions and molecules, control of transmembrane potential, generation and transduction of energy, signal recognition and transduction, cell-cell communication, enzymatic activity, structural roles. Mutations in membrane proteins are linked with various human diseases including: Alzheimer's disease, Brugada syndrome, cancer, cystic fibrosis, heart disease, hypothyroidism, lysosomal storage disease, nephrogenic diabetes insipidus, retinitis pigmentosa. Membrane proteins are the molecular targets for around 50-60% of validated drugs and they remain a principal target for new drug discovery. Despite all this, the number of structures of membrane proteins is less than 1% of total protein structures in the Protein Data Bank due to various challenges associated with applying the main biophysical techniques for high-resolution protein structure determination: X-ray crystallography, electron microscopy, NMR spectroscopy. There is an infinite amount of information and understanding yet to be obtained about the structure, function and molecular mechanism of membrane proteins and their ligands.

This book "A Closer Look at Membrane Proteins" brings together recent developments in the structures, molecular mechanisms and roles of some different types of membrane proteins using various computational and experimental methods, and also views on the challenges around expression and purification of membrane proteins and a successful demonstration of how these challenges can be overcome.

Chapter One considers insulin-like growth factor receptors and their roles in initiating mitogenic and metabolic pathways involved in cell growth and proliferation and energy metabolism, and also their roles in cell apoptosis. Information on the receptors is related to normal and abnormal tissue growth and development, using placental and colorectal tissues as examples. ***Chapter Two*** demonstrates how transmembrane protein transport across the nuclear envelope can be imaged at high-resolution using dynamic single-molecule microscopy; especially how the technique can be used to interrogate different proposed models for the mechanism of membrane protein transport: diffusion-retention, ATP-dependent, nuclear localization signal–mediated, sorting motif–mediated.

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Computer simulation provides a way to study the structure and function of membrane proteins, alternative to using laboratory techniques, and this is the subject of ***Chapter Three***. The focus is on large scale molecular dynamics (MD) simulations with special emphasis on scalable parallel methods, and how correctly relating molecular structures to the physiological properties of proteins is a major challenge in the field. ***Chapter Four*** consolidates general principles of secondary active transporter function, which catalyse transport of ions and small molecules across cell membranes against electrochemical gradients. It considers thermodynamics and molecular mechanism and how these transporters cycle between inward- and outward-facing conformations. Also how experimental structural data and MD simulations indicate that transporters can be understood as gated pores. A unified picture emerges in which symporter, antiporter and uniporter function are extremes in a continuum of functionality.

Following recent high-resolution X-ray crystal structures of substrate-bound proteins, ***Chapter Five*** reviews emerging structural insights about multidrug recognition and extrusion by MATE (Multidrug and Toxic Compound Extrusion) and MFS (Major Facilitator Superfamily) secondary active transporters, which provide a mechanism of resistance to therapeutic drugs. In addition to providing a better understanding about the underlying mechanism of multidrug extrusion, this chapter engenders new ideas about how to curtail efflux-mediated multidrug resistance. A myriad of membrane proteins in the pathogenic bacterium *Vibrio cholerae* are described in ***Chapter Six*** that contribute to its physiology, virulence and antimicrobial resistance. These include outer membrane proteins and efflux pumps of the RND (Resistance-Nodulation-Division) family and MFS. The chapter emphasises how inhibition of efflux pumps can reduce virulence of *V. cholerae* and restore susceptibility to conventional antibiotics, and demonstrates how a complex network involving quorum sensing, efflux pumps and virulence gene expression regulates physiology and virulence.

The challenges around expression and purification of integral membrane proteins and performing laboratory experiments to study their structure and function are well recognised. In this respect, ***Chapter Seven*** gives a personal view on “The commandments of studying integral membrane proteins”. These commandments consider integral membrane protein expression and purification, biochemistry, functionality studies and high-resolution structures. It is possible to overcome the challenges for expression and purification of integral membrane proteins, especially by those who are suitably experienced and have longevity of success. This is demonstrated in ***Chapter Eight*** by the amplified expression, functional characterisation and purification of a cytosine transporter of the NCS1 (Nucleobase Cation Symporter-1) family from the bacterium *Vibrio*

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parahaemolyticus. The gene was cloned into plasmid pTTQ18 along with a sequence for introducing a C-terminal hexahistidine-tag to aid purification and amplified expression achieved in *Escherichia coli* BL21(DE3). The secondary structure and stability of the purified protein was analysed by circular dichroism spectroscopy and the protein was confirmed as a cytosine transporter by radiolabelled transport measurements in whole cells.

NOTE TO THE READER

In the interest of advancement in scientific research, the authors and publisher have made this eBook Open Access so that it reaches the widest readership without barriers and so that no individual(s) or organization(s) receive direct financial profit. All individual chapters in this book were separately subjected to single-blinded peer review.

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ABBREVIATIONS

7TMIR	7-TM inverted repeat
ABC	ATP–binding cassette (transporter)
AbgT	p-Aminobenzoyl-glutamate transporter
ADP	Adenosine diphosphate
AMBER	Assisted model building with energy refinement
aMD	Accelerated molecular dynamics
AMP	Antimicrobial peptide
APC	Amino acid-polyamine-organocation (transporter)
AQP	Aquaporin
ARM	Armadillo repeat motif
ATP	Adenosine triphosphate
BAF	Barrier-to-autointegration factor
BCA	Bicinchoninic acid
BCCT	Betaine-choline-carnitine-transporter
BD	Brownian dynamics
BHK	Baby hamster kidney
cAMP	Cyclic adenosine monophosphate
CCCP	Carbonyl cyanide *m*-chlorophenyl hydrazone
CD	Circular dichroism (spectroscopy)
CDG	Congenital disorders of glycosylation
CGMD	Coarse grained molecular dynamics
CHARMM	Chemistry at Harvard Macromolecular Mechanics
CMC	Critical micellar concentration
CMT	Critical micellular concentration
CMM-CG	Center for Molecular Modeling Coarse-Grained
Co-IP	Co-immunoprecipitation
CT	Cholera toxin
DAPI	4′,6-Diamidino-2-phenylindole
DDM	*n*-Dodecyl β-*D*-maltoside
DEER	Double electron-electron resonance (spectroscopy)
DHA1	Drug/H^+ antiporter-1
DinF	DNA damage-inducible protein F
DM	Diabetes mellitus
DMPC	Dimyristoylphosphatidylcholine

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DMT	Drug/metabolite transporter
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DPPC	Dipalmitoylphosphatidylcholine
DSSP	Define secondary structure of proteins
DXC	Deoxycholate
EC	Extracellular
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
EFPA	Enhancing functional protein accumulation
EGF	Epidermal growth factor
EM	Electron microscopy
EMT	Epithelial–tesenchymal transition
ER	Endoplasmic reticulum
EVB	Empirical valence bond
FOXO	Forkhead family box O (transcription factors)
FRAP	Fluorescence recovery after photobleaching (microscopy)
FRB	FKBP12/rapamycin-binding
FRET	Förster resonance energy transfer
FSM	Flexible surface model
GCMC	Grand canonical Monte Carlo
GDM	Gestational diabetes mellitus
GFP	Green fluorescent protein
GH	Growth hormone
GLUT	Glucose transporter
gp210	Glycoprotein-210 (antibody)
GPCR	G–Protein coupled receptor
Grb2	Growth factor–bound protein 2
GROMOS	GROningen MOlecular Simulation
GSK3 β	Glycogen synthase kinase 3β
HILO	Highly inclined and laminated optical sheet (microscopy)
HIV	Human immunodeficiency virus
HLB	Hydrophile-lipophile balance
HPr	Heat-stable protein
HyR	Hybrid receptor
IC	Intracellular

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IF	Inward facing (conformation)
IGF	Insulin-like growth factor
IGFBP	Insulin-like growth factor binding protein
IMAC	Immobilised metal affinity chromatography
IMP	Integral membrane protein
IPTG	Isopropyl β-*D*-1-thiogalactopyranoside
INM	Inner nuclear membrane
INM-SM	Inner nuclear membrane sorting motif
IR	Insulin receptor
IRS	Insulin receptor substrate
IUGR	Intrauterine growth restriction
KEGG	Kyoto Encyclopedia of Genes and Genomes
KASH	Klarsicht, ANC-1, syne homology
LB	Luria-Bertani
LBR	Lamin B receptor
LDAO	Lauryldimethylamine oxide
LEM	Lamin-associated protein [LAP]2, emerin, MAN1
MATE	Multidrug and toxic compound extrusion
MD	Molecular dynamics
MDR1	Multidrug resistance protein 1
MES	2-(*N*-Morpholino)ethanesulfonic acid
MFS	Major facilitator superfamily
MMP	Matrix metalloproteinase
MRE	Mean residue ellipiticity
MS-CG	Multiscale coarse grained
MtlA	Mannitol-specific enzyme IICBA
MtlD	Mannitol-1-phosphate dehydrogenase
MWCO	Molecular weight cut off
NAT	Nucleobase ascorbate transporter
NBD	Nucleotide binding domain
NCS1	Nucleobase-cation-symporter 1
NE	Nuclear envelope
NETs	Nuclear envelope pransmembrane proteins
NLS	Nuclear localization signal
NMR	Nuclear magnetic resonance
NPC	Nuclear pore complexes
NSS	Neurotransmitter sodium symporter
Nup	Nucleoporin

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ODV	Occlusion derived virus
OF	Outward facing (conformation)
OHS	Oligosaccharide/H^+ symporter
ONM	Outer nuclear membrane
PACE	Proteobacterial antimicrobial compound efflux
PBC	Periodic boundary conditions
PC	Phosphatidylcholine
PCR	Polymerase chain reaction
PDB	Protein Data Bank
PE	Preeclampsia
PEP	Phosphoenol pyruvate
PG	Phosphatidylglycerol
POPE	1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylethanolamine
PTEN	Phosphatase and tensin
PTM	Post–translational modification
PTS	Phosphoenolpyruvate-dependent phospotransferase
QM	Quantum mechanical
RDF	Radial distribution functions
RMSF	Root mean square fluctuations
RNAi	RNA interference
RND	Resistance-nodulation-division
ROS	Reactive oxygen species
S6K	S6 kinase
SAXS	Small-angle X-ray scattering
SBGP	Single binding center gated (model)
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SHC	Src homology collagen
SMA	Styrene maleic acid
SMALP	Styrene maleic acid lipid particle
smFRAP	Single-molecule fluorescence recovery after photobleaching
SNR	Signal to noise ratio
SP	Sugar porter
SPEED	Single-point edge-excitation sub-diffraction (microscopy)

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SREBP1c	Sterol regulatory element binding protein 1c
SSS	Solute/sodium symporter
SUN	Sad1p, UNC-84
TCP	Toxin co-regulated pilus
TIRF	Total internal reflection (microscopy)
TM	Transmembrane
TMH	Transmembrane helices
TPP	Tetraphenylporphyrin
TSC2	Tuberous sclerosis 2
UV	Ultraviolet
VcBMC	*V. cholerae* biofilm matrix cluster
VEGF	Vascular endothelial growth factor
VPI	*V. cholerae* pathogenicity island
VPS	Vibrio polysaccharide

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