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ß-barrel Channel Proteins as Tools in Nanotechnology

Biology, Basic Science and Advanced Applications

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Contents

Abbreviations

ABC	ATP-binding cassette
AFM	Atomic force microscopy
ATP	Adenosine triphosphate
Bam	β-Barrel assembly machine
BCA	Bicinchoninic acid
BNPA	2-Bromo-2-(2-nitrophenyl)acetic acid
CC	Coupled cluster
CD	Circular dichroism
CG	Course grained
CLSM	Confocal laser scanning microscopy
CM	Chemical modification
cmc	Critical micellar concentration
COSY	Correlation spectroscopy
CPU	Central processing unit
CRAMPS	Combined rotation and multiple pulse spectroscopy
Da	Dalton
DAGK	Diacylglycerol kinase
DDAO	Dimethyldecylamine-N-oxide
DFT	Density functional theory
Dh	Hydrodynamic diameters
DHPC	1,2-Dihexanoyl-sn-glycero-3-phosphocholine
DLS	Dynamic light scattering
DMPC	Dimyristoyl-phosphatidylcholine
DNA	Deoxyribonucleic acid
DNPC	1,2-Dinervonyl-sn-glycero-3-phosphocholine
dNTP	Deoxyribonucleotide triphosphate
DOPC	1,2-Dioleoyl-sn-glycero-3-phosphocholine
DTT	Dithiothreitol
ECD	Electronic circular dichroism
EDTA	Ethylenediamine-tetra-acetic acid
EE	Ethylethylene
EIA	Enzyme immunoassays
EM	Electron microscopy
em	Electromagnetic
EO	Ethyleneoxide
ER	Endoplasmic reticulum
ESEM	Environmental scanning electron microscopy

FCS	Fluorescence correlation spectroscopy
FET	Field-effect transistor
FhuA	Ferric hydroxamate uptake component A
FT-NMR	Pulse-Fourier-transformation-NMR
GdmCl	Guanidinium chloride
GF	Gel filtration
GFP	Green fluorescent protein
GPI	Glycosylphosphatidylinositol
GST	Glutathione-S-transferase
GTP	Guanosine triphosphate
GUV	Giant unilamellar vesicle
HF	Hartree-Fock
hIMP	Human integral membrane protein
HMBC	Heteronuclear multiple-bond correlation spectroscopy
HMM	Hidden Markov model
HPH	High pressure homogenisator
HRP	Horse radish peroxidase
HSQC	Heteronuclear single-quantum correlation spectroscopy
IBI	Iterative Boltzmann inversion
IDP	Intrinsically disordered protein
IEXC	Ion exchange chromatography
igG	Immunoglobulin G
IMAC	Immobilized metal-affinity chromatography
IPTG	Isopropyl β-D-1-thiogalactopyranoside
kDa	Kilo Dalton
L	Loop
LDAO	Lauryldimethyl amine oxide
LMPG	1-Myristoyl-2-hydroxy-sn-glycero-3-[phosphorac-(1-glycerol)]
LPS	Lipopolysaccharides
LUV	Large unilamellar vesicles
MAD	Multiwavelength anomalous dispersion
MAS	Magic angle spinning
MBP	Maltose binding protein
MD	Molecular dynamics
MM	Molecular mechanics
MP	Membrane protein
MP	Møller-Plesset
MRE	Mean residue ellipticity
mRNA	Messenger ribonucleic acid
MRW	Mean residue weight
ms	Millisecond
MSP	Membrane scaffold protein
NHS	N-hydroxysuccinimide
NLP	Nano-lipoprotein particles
NMR	Nuclear magnetic resonance
NN	Neural network
NOE	Nuclear Overhauser effect
NOESY	Nuclear Overhauser effect spectroscopy

NRMSD	Normalised root mean square deviation
nS	Nanosiemens
NVOC-Cl	6-Nitroveratryloxycarbonyl chloride
NW	Nanowire
octyl-POE	n-Octylpolyoxyethylene
OES	n-Octyl-2-hydroxyethyl sulfoxide
OG	Octyl-β-D-glucoside
OM	Outer membrane
OMP	Outer membrane protein
OmpA	Outer membrane protein A
ORF	Open reading frame
P2	Poly(Pro)II type structure
PBD-PEO	Polybutadiene-polyethyleneoxide
PCDDB	Protein circular dichroism data bank
PCM	Polarizable continuum model
PCR	Polymerase chain reaction
PCS	Photon correlation spectroscopy
PDB	Protein data bank
PDI	Polydispersity index
PDMS	Poly(dimethylsiloxane)
PEG	Poly(ethylene glycol)
PEtOz	Poly(2-ethyl-2-oxazoline)
PH	Pleckstrin homology
PIB	Polyisobutylene
PME	Particle mesh-Ewald summation
PMF	Proton motive force
PMOXA	Poly(2-methyloxazoline)
POTRA	Polypeptide translocation associated
ps	Picosecond
PS-b-PAA	Polystyrene-block-poly(acrylic acid)
PSI	Protein structure initiative
Pyr	3-(2-Pyridyldithio)-propionic-acid
QELS	Quasi-elastic light scattering
QM	Quantum mechanics
RA	Rotational alignment
RBS	Ribosome binding site
RF	Radio frequency
rHDLs	Reconstituted high density lipoprotein particles
RMSD	Root mean square deviation
RMSF	Root mean square fluctuation
ROESY	Rotating frame nuclear Overhauser effect spectroscopy
SCF	Self-consistent field
SCRF	Self-consistent reaction field
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SIMPLS	Simultaneous partial least squares
SiNW	Silicon nanowires
SLS	Static light scattering

SPP	Single protein production
SR	Signal recognition particle receptor
SRCD	Synchrotron radiation in circular dichroism
SRP	Signal recognition particle
SSM	Site-specific mutagenesis
ssNMR	Solid-state NMR
STREP	Streptavidin-binding peptide
SUV	Small unilamellar vesicles
SVM	Support vector machines
T	Turn
TAT	Twin arginine translocon
TEM	Transmission electron microscopy
TEV	Tobacco etch virus
TFE	2,2,2-trifluoroethanol
THF	Tetrahydrofuran
TIC	Chloroplast inner membrane translocon
TMB	3,3′,5,5′-Tetramethylbenzidine
TMP	Transmembrane protein
TOC	Chloroplast outer membrane translocon
Toc	Translocase of the chloroplast outer membrane
TOCSY	Total correlation spectroscopy
Tom	Translocase of the mitochondrial outer membrane
TRCD	Time resolved CD spectroscopy
ULV	Unilamellar vesicle
UV	Ultraviolet
VDAC	Voltage dependent ion channel
WT	Wild type
X-FEL	X-ray free electron laser

Introduction 1

Though there is some dispute about whether barrels were first invented by the Egyptians, or rather by Greeks and Romans (some even claim the achievement for the Celts), earliest finds have been dated back to as early as 2500 B.C. and there is no discussion on that the barrel geometry facilitates transport by maximizing cube utilization allowing tight loading of ships and wagons [1] and that even more importantly casks generally hold good things, such as wine, oil, beer, honey or in case of a "barrel of laughs" also fun.

Keeping in mind the macroscopic wooden barrel's great transport and packing-component potentials, as well as its importance for the civilization and technology development, this book will call to the reader's attention a completely different kind of barrel: the nano-sized so-called β-barrel membrane protein (see Fig. 1.1).

β-barrel membrane proteins that regardless of their nanoscopic size have as great potential as their large wooden counterparts These potentials lie within their structure that forms channels in hydrophobic membranes and that it is for a protein exceptionally robust imparting outstanding nano-material properties.

1.1 Nano-technology – An Overview

Originally the term nano-technology was used for anything technologically applicable and smaller than microscopic. More recently the term is associated with the bottom-up construction of nano-scale components purposefully built to be assembled to form nano-materials. Nano-technology thus operates at the first level of organization, means at the level of atoms and molecules (10^{-9} m) and it promises the ability to build precise machines and components at the molecular size scale. In theory the feasibility of nano-technology was envisioned and prophesied by the physicist Richard Feynman [2] as early as 1959 in his famous talk entitled "There's plenty of room at the bottom" [3] (available online at http://www.zyvex.com/nanotech/feynman.html).

As nano-structures can be either derived from non-biological or biological components and as nano-materials can be applied to a broad range of fields like electronics including opto-electronics, medicine, pharmaceutical drug development, water-purification, food-technology to name but a few, nano-technology research necessitates the cooperation of scientists from various disciplines, such as physicists, engineers, chemists, and biologists.

1.2 Biological Nano-materials

The aim of nano-technology is as mentioned the design of new functional materials and devices through controlling their organization at the atomic and molecular level. One common strategy when designing a new nano-device is to survey naturally occurring biological structures with the ability to perform the desired process and use them as nano-material components or

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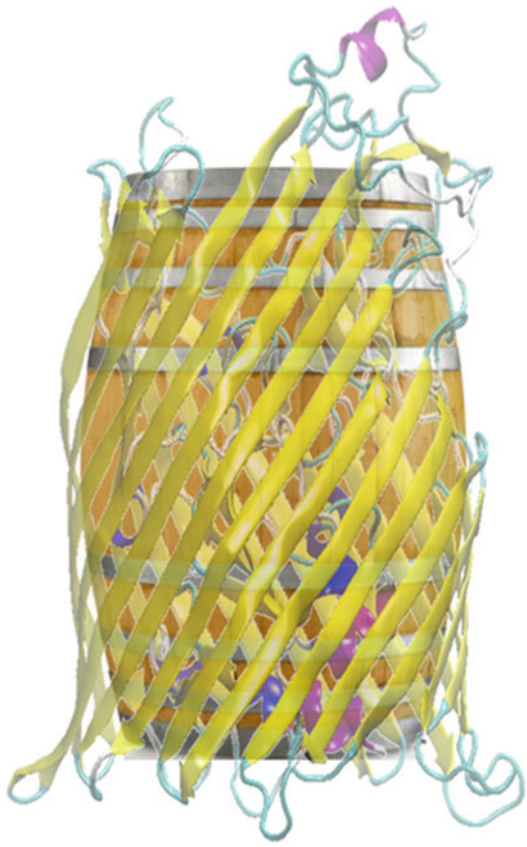

Fig. 1.1 β-barrel shaped membrane protein "in the light of" its macroscopic geometrical counterpart

scaffolds based on which to build the new nano-construct. This approach opens the promising field of bio-nanomaterial design.

In this sense the intrinsic nano-scale architecture and rich chemistry of proteins, as well as their catalytic activity in the case of enzymes, may be exploited to build a wide array of specific components in sophisticated nano-sized devices such as nano-motors, nano-reactors or stochastic nano-sensors. The protein based nano-material development is backed up by the vast progress that has been made in the molecular biology and biotechnology field, specifically in the development and optimization of advanced genetic engineering techniques allowing a tailoring of proteins towards a specific technical application [4].

Many of the possible applications of protein nano-materials (*i.e.* nano-reactors, functionalized nano-compartments, nano-sensors, drug-release systems) require channel shaped nano-components that allow the controlled transport of matter or the detection and analysis of an analyte that interacts with the channel interior by channel conductance measurements (nano-sensing elements).

Having said this, a rather obvious protein choice is the class of transmembrane proteins that reside within the various biological membranes, as many of these proteins form channels and pores to facilitate the passive or active transport of solutes, nutrients or cellular waste over the membrane. From the two types of channel shaped transmembrane protein classes, *i.e.* α-helical bundle and β-barrel proteins, the β-barrel structure stands out due to its versatility, flexibility, exceptional robustness and stability. Moreover β-barrel membrane proteins have the ability to refold *in vitro* and to reconstitute or insert into artificial lipid and polymer flat membranes or lipid and polymer vesicle (*i.e.* liposome and polymersome) membranes.

Due to the mentioned robustness of the β-barrel membrane proteins, they are easily modified by genetic engineering without loss of overall structure or function allowing the resulting protein nano-channels to be adapted to the non-biological synthetic polymer environment, rendering them competitive with artificial non-biological nano-pores. An example of the conception of a β-barrel nano-channel employing polymersome release system is given in Fig. 1.2.

Therefore the β-barrel and its serviceability for the nano-material design will be the chief topic of the present book and on the example of the *Escherichia coli* FhuA (ferric hydroxamate uptake component A) the design of a set of protein nano-channels with tailored geometry (diameter, length), conductance and functionality will be reported as a case study.

In order to make this book a valuable source of information for both biotechnologists and other

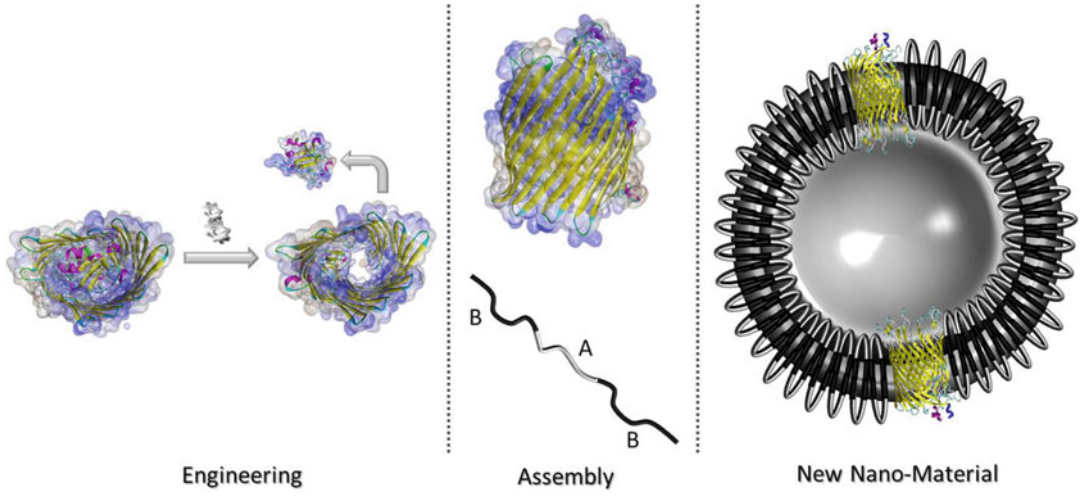

Fig. 1.2 Schematic conception work-flow of a hybrid bio-polymer nano-system on the example of a synthetic block copolymer based (*A* – hydrophophilic block, *B* – hydrophobic block) polymersome functionalized with a tailored β-barrel nano-channel for controlled compound release; involving protein engineering, protein polymer assembly and the resulting finished nano-release system

scientists interested in bio-nanotechnology an overview of the different steps involved in the nano-channel protein design and production will be reported, including concept design, theoretical considerations, genetic engineering and large scale production, as well as the system assembly and biophysical characterization.

In the following section a brief outline on the individual chapters will be given.

1.3 Outline

1.3.1 Chapter 2

To enter the membrane protein nano-channel material design topic, Chap. 2 will introduce to the biological basics of natural membranes and membrane proteins in general.

It will give some elementary information on the lipid bilayer composition and function, the main models to describe the bilayer membrane as well as on the lipids that assemble to form the membrane.

The various classes of membrane proteins (*i.e.* integral *vs.* peripheral) and their key differences will be mentioned. The two main structural classes of integral membrane proteins (*i.e.* α-helical and β-barrel) will be presented with regard to their biological origin, functional and structural features (considering primary, secondary, tertiary and quaternary sequence/structure), their biogenesis and their usability as bio-based nano-material components. For both protein classes several well-known and well-studied literature examples will be given.

In the light of the present book's title: *β-Barrel Channel Proteins as Tools in Nano-technology*, the main focus however will be on the characteristics and unique structural and functional properties of the β-barrel outer membrane proteins (OMPs). Their relevance for the nano-channel material design will be emphasized by presenting recent examples of the nano-technological use of lipid or polymer reconstituted β-barrel channel proteins.

The *E. coli* outer membrane iron transporter FhuA, which is a member of the TonB protein-dependent transporters and is one of the largest known β-barrel membrane proteins, will be introduced. Its unique biological characteristics (regarding structure and function) will be outlined. Since the FhuA and its engineered variants have been successfully employed as a model for the

transformation of a β-barrel outer membrane protein into a custom-made nano-channel, Chap. 2 aims to use the FhuA example to view β-barrel proteins not only within their biological context but to already introduce the reader to the relevance of members belonging to this membrane protein class for the nano-material sciences and specifically to their use for the design of biological nano-channels that can be reconstituted into artificial lipid or polymer membranes.

1.3.2 Chapter 3

The biophysical characterization of β-barrel outer membrane proteins (OMPs) opens the master way for the OMP behavioral understanding under the alien conditions they experience in a new environment such as a polymersome membrane.

The OMP characterization, furthermore, grounds the basics for a rational protein engineering necessary to solve the problems related to their functional reconstitution and widens their use and applications in technologies such as drug delivery, stochastic sensors and bio-nanoelectronics (see Chap. 6).

The main tools for the structural characterization of channel proteins and OMPs in particular is given in Chap. 3, where the state of the art of the main biophysical techniques used in β-barrel membrane protein analysis will be introduced, specifically mentioning X-ray crystallography, Circular Dichroism (CD) and Nuclear Magnetic Resonance (NMR).

The main problems, difficulties and limitations for each of the single techniques will be reported and most importantly, their alliances with other sophisticated techniques *i.e.* synchrotron radiation, to obtain structural, kinetic as well as thermodynamical informations will be reported.

Obviously the chapter is not intended as an exhaustive description of the single techniques, where existing reviews and books have been cited alongside the text, but has the aim to show how the X-ray diffraction measurements of protein crystals, CD spectroscopy and NMR have been and are used to study channel proteins further illustrating the direction toward which modern methodology and applications are facing.

However in the reported case of studies, many of the characterization problems associated with channel proteins and OMPs are intertwined with the difficulties deriving from the expression and purification methods that can be, quite often, the determining step in membrane proteins studies.

A particular stress will be given to Circular Dichroism. CD is a qualitative undervalued technique which gives general content in secondary structure of a protein and, in some cases, clues on the tertiary/quaternary structural changes, with no atomistic detail. However its use due to its quite user friendly experimental procedure and rapid data acquisition and processing, can rapidly uncover basic information on the protein stability within different environments not always easily obtainable or even impossible to obtain for example, by NMR analysis.

The obvious aim of each scientist working in the channel protein field is to obtain complete knowledge on the system with which he/she is working, however the fast development and number of bio-technologically modified β-barrel proteins to be, for example, embedded in liposomes or polymersomes, result in a need of a rapid characterization, a need that CD can promptly answer to, giving first clues on the obtained mutant secondary structure and stability.

1.3.3 Chapter 4

Chapter 4 will describe different theoretical and computational approaches that are useful to model and design new nano-systems based on β-barrel membrane proteins. Computational power in terms of continuously growing hardware facilities together with the continuous development of theoretical methodologies makes biomolecular modeling and bioinformatics a basic tool to complement experiments in many ways. In this sense computational simulations can provide valuable information on the specific system under study. Chapter 4 will moreover stress on the importance of computational simulations and bioinformatic

tools to interpret experimental observables, to rationally guide further improvements providing data unaccessible or difficult to access by experimental studies.

The chapter has been divided in two parts. The first part aims to provide the reader with a broad scope about the most common and useful simulation tools in biomolecular modeling stressing on the advantages and limitations of each methodology. At first will be presented the basis of Quantum Mechanics (QM) based methods, including an analysis on the case studies to which QM can be fruitfully applied.

Even though QM represents matter at the most accurate level of description, QM based methods are computationally very expensive and their practical application in the context of the present book will be limited only to small parts of the system. In this sense the present chapter will illustrate the importance of QM methods to design new functional groups that can be used to functionalize β-barrel membrane proteins for nano-technological applications. Secondly atomistic Molecular Dynamics (MD) simulations and their suitability to simulate β-barrel membrane proteins will be presented. Atomistic MD simulations represent a system in the Molecular Mechanics framework, where atoms are represented as spheres and bonds as harmonic springs, and allow the simulation of systems to be analyzed at the nanosecond timescale with a resolution at the atomistic level.

However many important processes such as refolding or self-assembly of lipid/copolymer bilayers occur at the microsecond timescale, far beyond the timescale reachable by atomistic MD simulations. To that end will be introduced a third level of biomolecular simulations, *i.e.* the so-called Coarse Grained (CG) simulations. In CG simulations many atoms are grouped into a single bead and consequently the total number of particles is drastically reduced compared to the total number of particles present in a full atomistic description, which allows reaching longer timescales.

The second part of the chapter will briefly introduce some common bioinformatic structure prediction tools used to perform a first 2D and 3D structure prediction of the β-barrel membrane proteins focusing on the challenges and problems one might encounter when applying these tools to predict the structure of non-natural (engineered) β-barrel membrane proteins. The basic logic algorithms used by these tools will be summarized and reviewed.

1.3.4 Chapter 5

Chapter 5 will assist the experimentalist that plans to work with membrane proteins and in particular with bacterial β-barrel outer membrane proteins in the context of the development of new nano-material components (*i.e.* nano-channels). It will offer an overview on genetic engineering methods as well as expression, extraction and purification procedures considering standard techniques, new alternatives as well as methods compatible with scale-up and high yield production purposes, including difficulties that the beginner might encounter and tips and suggestions on how to overcome these difficulties.

In a narrower sense Chap. 5 will explain in detail how to practically transform a β-barrel membrane protein into a nano-channel with desired geometrical and functional features, starting from the gene design, when planning an entirely novel protein variant. Site-specific mutagenesis for slight changes or the introduction of amino acid residues suitable for chemical modification purposes will be furthermore discussed. An overview on commonly used protein chemical modifications (useful to introduce triggers and switches to OMP channels) will be given focusing on outer membrane protein examples. Here the FhuA protein of *E. coli* will serve as the main example, as it has great potentials as a starting-template to develop a set of engineered nano-channels with tailored geometry and controllable channel function and as the reader will see considerable steps toward this goal have been already made during the last decade.

Chapter 5 will then summarize the conventional means of production and purification of bacterial OMPs (outer membrane proteins), stressing on the problems and challenges of