

# Prokaryotic cell membrane-based protein technologies (Review)

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**Abstract.** The biological cell membranes play a crucial role in living tissues through a heterogeneity of metabolic processes, such as apoptosis, necrosis, autophagy, cell signalling processes and metabolic disorders. Due to the sensitive nature of cell membranes and proteins, the utilization of specialized instruments for carrying out biological experiments such as extraction, isolation, gene expression and protein expression analyses is required. Currently, there are several instruments available for performing biological experiments. The present study selectively discusses the protein bioanalytical techniques, including electrophoresis techniques, chromatographic techniques, sequencing approaches and computational approaches that have been created as a result of difficulties in researching membrane protein and glycoproteomic techniques. Since these techniques have provided an efficient strategy with which to enrich and characterize membrane and plasma-membrane proteomes, some of the recent advancements are also discussed. In addition, the present study focuses particularly on prokaryotic organisms for a better understanding of cell membrane protein technologies. Hopefully, a summary of these studies will help future researchers to understand the gaps behind the technique and its advances.

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## 1. Introduction

One of the most fundamental parts of a functioning cell is the cellular membrane, where proteins closely related to the membrane mediate important functions, such as selective material mobility and information transmission between the cell and its surroundings (1). Of note >75% of the total membrane proteins are present in the mitochondria and the remaining 25% are present in myelin membranes (2). Membrane proteins can be broadly classified into two types, extrinsic and intrinsic, based on their function and are also classified based on their position attached to the lipid membrane including integral, lipid anchored and peripheral (3). Integral proteins are intrinsic and have a cytoplasmic and extracellular domain; they are transmembrane proteins found in an embedded form with membrane-spanning domains, such as  $\alpha$ -helices and multiple  $\beta$ -strands (4). The integral proteins can be otherwise termed as single-pass transmembrane and multi-pass transmembrane proteins based on the polypeptide chain crossing the lipid bilayer (5). Bacteriorhodopsin (single-pass transmembrane protein) and aquaporins (multi-pass transmembrane proteins) are examples of integral proteins. Mostly they are glycosylated and located in the extracellular space, and they are involved in endocytic and secretory pathways (6,7). Peripheral membrane proteins are extrinsic and have hydrophilic domains that transduce intracellular signalling. G protein-coupled receptor (GPCR), receptor tyrosine kinases, protein channels and transporters are some examples of peripheral membrane proteins, and these are expressed in downstream signalling pathways and are involved in cellular changes (8,9). Lipid-anchored proteins are water soluble proteins attached covalently and function either on one side of the cytoplasmic phase or extracellular phase (10). One group of proteins are anchored to the membrane by fatty acyl chain, which are covalently linked to the N-terminal of glycine residue (acylation), a second group of proteins are anchored to the membrane by a hydrocarbon chain attached to the cysteine residue in the C terminus (prenylation), and a third group (glycophosphatidylinositol

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anchored proteins) bind to the cell surface and are specialized proteins in the exoplasmic phase of membranes (11).

When proteins are synthesized by pre-existing membranes, they are inserted into the plasma membrane asymmetrically and are distributed across the lipid bilayer. An inside and outside asymmetry distribution of lipid bilayer occurs by the external location of carbohydrates (oligosaccharide) attached to the membrane proteins (12). Oligosaccharide chains are linked via the H-bonds with the glycolipids attached on the extracellular surface. Phospholipid-binding proteins recognize specific phospholipids and transfer them from the plasma membrane to mitochondria and peroxisomes (13). The present review discusses the topics behind protein studies step by step in an aim to provide an understanding of strategies used to perform experiments based on the type of proteins and how they can be processed.

## 2. Protein separation and purification

Protein molecules can be separated based on the charge, affinity, solubility, size and adsorption properties. Electrophoresis and chromatography principles are the most popular separation technique for protein molecules and some examples of this include ion-exchange chromatography, affinity chromatography, dialysis, ultrafiltration, size-exclusion chromatography (14) and electrophoresis [including capillary electrophoresis, isoelectric focusing and sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE)] utilized to isolate protein samples (15).

During the biological experimentation process to separate and purify protein samples, several detergent solutions are used. The detergents utilized for protein separation can be categorized into denaturing and non-denaturing detergents. Denaturing detergents are polar molecules that contain charges (anionic or cationic); one such widely used anionic detergent is SDS and cationic detergents include cetyltrimethylammonium bromide. Similarly, non-denaturing detergents were also utilized and are classified based on the material and components used; these include non-ionic detergents, zwitterionic detergents and bile salts. Some popular non-denaturing detergents include Triton X-100, CHAPS and cholate (16).

In a recent study, Zhou *et al* (17) established with a protocol for isolating mitochondrial proteins. They utilized cultured 293T cells for demonstration in which the mitochondrial fraction was isolated from cultured 293T cells in the initial phase and membrane proteins were localized by two methods, sonication and the sodium carbonate method. The sodium carbonate method is utilized to extract integral proteins and the sonication method is used to extract both integral and peripheral proteins from the soluble protein. By contrast, the non-ionizing detergent (Triton-X) and proteolytic enzyme (proteinase K) are used to separate the outer membrane proteins (17).

## 3. Protein identification and mapping

Protein appears in a 3-dimensional structure and to determine its structure, mapping is performed to identify hot-spot interactions and various other potential functions to recognize functional binding sites and their roles. This can be performed

experimentally using various computer aided platforms, as well bioinstrumentation. One such extraordinary analytical technique invention for the identification of unknown proteins is matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MS). It functions on the principle of peptide mass fingerprinting (PMF) in which the unidentified protein molecules are cleaved into small peptides and absolute masses are accurately measured. Finally, the NIST mass spectral libraries is utilized with the PMF data for the identification of anonymous proteins (18). This technique can be applied to various fields, such as microbiology, biotechnology, food chemistry and environmental sciences to uncover the hidden protein from the large population (19).

Nuclear magnetic resonance (NMR) spectroscopy is another technique widely used to obtain structural information of protein, nucleic acids and other biomolecules. It is performed in a very lower energy frequency (radio frequency region of the electromagnetic spectrum) and is based on nuclear spin interactions with a magnetic field; the structure of a molecule is predicted. It is a technique widely used by chemists to predict a chemical structure of an unknown molecule. Chemical shifts and J-couplings (internal nuclear spin interactions) are two key parameters exclusively used by chemists to predict a chemical structure. Processing samples in NMR should be diluted with deuterated solvent, such as chloroform, deuterium oxide and other solvents based on the nature of the material. Acetone, methanol, trichloromethyl, nitrogen gas and a vacuum are some of the substances used to cleanse the NMR tubes. This technique is widely applied in pharmaceutical sciences, metabolomics research and food chemistry (20-22). In addition, reversed-phase-high pressurized liquid chromatography (RP-HPLC) has been utilized in anti-platelet drug determination (ticagrelor) in human blood plasma and in the determination of the dosage form of chemical compounds in pharmaceuticals (23,24). By contrast, for the diagnostic and monitoring parameters in healthcare, enzyme-linked immunosorbent assay is utilized as a significant approach to measure certain types of biomarkers responsible for the cause of disease (25).

Additionally, some of the recent advancements in protein identification include the identification of phosphoprotein and signatures (cyclin D1, ERa and AR S650) using laser capture microdissection-reverse phase protein microarrays by Gallagher *et al* (26).

## 4. Evolution of sequencing approaches and proteomic studies

The evolution of determining the protein sequence began in the early 1950s with the sequencing of insulin using the Edman degradation method. Edman degradation is the first used method to sequence the amino acids in a peptide and is performed without breaking the peptide bonds between other amino acid residues, amino-terminal residue is labelled and separated from the peptide. This method is also known as N-terminal sequencing, and is utilized to identify unknown proteins, and the quality and identity of proteins can also be determined. The main advantage of this method is that it does not damage the protein entity and a disadvantage is that it is not able to identify multiple proteins simultaneously. Advancements to the Edman method led to the use

of nucleic acids and enzymes to sequence the protein, and sequencing known as the Sanger method. The application of the Edman method is widely applied to product development in chemical and pharmaceutical industries by the development of enzyme/bio-catalyst for large scale production, whereas the Sanger method is used in biological molecules (DNA and RNA) for performing variant studies (27).

A chemical reagent known as phenyl-isothiocyanate is a widely used in HPLC, whereas in the Edman degradation method, the same reagent is also called the Edman reagent (28). In the Edman method, only amino the acid sequence in the peptide can be determined; hence, researchers devised the addition of protein digestion and fractionization protocols to determine the whole sequence of proteins. Protein digestion can be performed with various enzymes, such as proteinase K and fractionization is performed using HPLC, a chromatographic method used to analyse and separate the digested protein samples in liquid form. Later in the 1990s (29), following the discovery of MS, researchers used MS combined with the HPLC technique as an alternative to Edman degradation for the effective identification and for the analysis of protein molecules (30).

The unidentified/unknown proteins from the large sample population can be identified based on two approaches, the bottom-up approach and top-down approach. In the bottom-up approach, the protein is digested into peptides and peptides are separated using MS techniques to determine the protein sequences. The bottom-up approach is also known as shotgun proteomics and HPLC combined with MS are utilized to determine the protein sequences. In the top-down approach, protein is directly separated using MS and other protocols remains the same, as in Shotgun proteomics to determine protein sequences (31,32).

Moreover, recently, proteomic techniques have been explored with several other potential proteins, including heat shock proteins, metabolic enzymes, oxidative proteins, structural proteins, and cell death and signalling regulators that can be utilized in the application of drug or biomarker discovery (33). The applications of proteomics are widely employed by several industry sectors. These include post-translational modification, targeted protein quantification, protein-protein interaction analysis, chemical proteomics, and protein expression profiling (34,35). By contrast, recent developments in proteomics include the monitoring of post-translational modifications by the capillary and microchip electrophoresis techniques (36).

### 5. *In silico* analysis in proteomics

Various computer-aided platforms have also made analysis easier by performing the majority of the experiment *in silico*. Diverse software and applications have been utilized based on the objective of the study; these make the analysis faster, accurate, as well as time and cost-effective, in comparison to wet-lab experiments. Schrödinger (<https://www.schrodinger.com/>) is one such widely utilized software for *in silico* experiments and the data can be retrieved using a number of open-source platforms, such as Ensembl (<https://asia.ensembl.org/index.html>), NCBI (<https://www.ncbi.nlm.nih.gov/>), EMBL (<https://www.embl.org/>), ExPasy (<https://www.expasy.org/resources/uniprotkb-swiss-prot>) and DDBJ (<https://www.ddbj.nig.ac.jp/index-e.html>). Some experiments performed using *in silico* analysis involve variant studies (37), molecular docking (38), critical assessment of structure prediction (CASP) experiments (39), homology modelling (40), pharmacophore modelling, quantitative structure activity relationship (QSAR) modelling (41), *ab initio* methods (42), phylogenetic analysis, sequence similarity searches, primer designing, and computational fluid dynamics (43-45). Over the past decade, bioinformatics has also played a crucial role in computer programming approaches by algorithms (46). Hence as a state of the art, the development of graph algorithms based on protein sequencing and protein identification issues by dynamic programming would be a future goal.

**6. Focus on bacterial or fungal cell membrane proteins**

Cell membrane proteins and integral components can be studied and are more extensively understood in prokaryotes (bacteria and fungi) when compared to eukaryotic organisms. In bacteria, cell division and synthesis occur with the influence of cell membrane proteins. One such key protein includes, filamenting temperature-sensitive mutant Z (FtsZ) and it is encoded by the FtsZ gene (47). FtsZ is a complex pinpoint in which all cytosolic and membrane proteins are detached. Researchers have previously demonstrated that surfactant-free membrane protein complex separation can be achieved by the presence of FtsZ within penicillin-binding protein (PBP)2/2a nanoparticles by utilizing anti-FtsZ antiserum for the purification of membrane proteins by immuno-affinity chromatography. It was found that FtsZ, PBP2 and PBP2a were captured by styrene-co-maleic acid-lipid particles (SMALP) using an anti-FtsZ antibody, illustrating the ability of the technique to remove significant protein complexes (48).

### 6. Focus on bacterial or fungal cell membrane proteins

RodA is a protein belonging to the shape, elongation, division and sporulation (SEDS) family that plays a crucial, yet ambiguous role in cell wall biosynthesis throughout growth, division, and sporulation (49). RodA, a crucial core component of the Rod complex that is highly conserved and serves as a dynamic peptidoglycan-synthesizing tool that mediates the elongation of rod-shaped bacteria. Meeske *et al* (49), performed several biotechnological experiments to prove that SEDS proteins constitute a family of peptidoglycan polymerases and the revelation that SEDS family proteins are peptidoglycan glycosyltransferases (PGTs) with extra cytoplasmic catalytic centres opens an alluring new option to design antibiotics that specifically target this pathway.

Complex regulatory systems ensure that bacteria have the required level of  $\beta$ -barrel outer membrane proteins (OMPs) to facilitate habitat adaptation. The OMP islands are comprised of the Bam complex, which catalyses the insertion of OMPs in the outer membrane, and are distributed throughout the cell (50). The study by Rassam *et al* (51) entrenched a mechanism of binary OMP partitioning by utilizing fluorescent colicins as OMP-specific probes, along with *in vivo* and *in vitro* ensemble and single-molecule fluorescence microscopy, as well as molecular dynamics simulations, to uncover the process underpinning OMP turnover in *Escherichia coli*.

The presence of two adjacent folded subdomains with an IgG-like structure distinguishes the family of proteins known

as the microbial surface components recognising adhesive matrix molecules (MSCRAMMs). The 'dock-lock-latch' mechanism used to bind fibrinogen or the 'collagen hug' mechanism used to bind collagen are two examples of how these promote binding to ligands through procedures that involve significant conformational changes. MSCRAMMs function based on the clumping factors A and B, which play a key role in the pathogenesis of *Staphylococcus aureus* infections (52).

As the incidence of fungal diseases is increasing in developed countries, it is critical to understand the pathological mechanisms of fungi. The stimulation of host defences, including phagocytosis and mediators of humoral immunity, as well as tissue adherence, immune escape mechanisms and host defences are all mediated by cell wall molecules. Endohydrolases, fucosyl transferase, glucuronosyl transferase, chitinases, 1,3- $\beta$ -glucan synthase, chitin synthase and deacetylase, sialoglycoproteins and uronic acid-containing glycoproteins are some of the enzymes attached to the cell wall and need to be further investigated for understanding the cell mechanisms and functions in fungi (53). To produce antifungal medications against pathogenic fungus including *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus*, protein kinase-C (PKC) is a viable target. Thus, the growing body of research on enzymes from various species has shown a keen interest in fungal PKCs (54).

## 7. Proteins in structural biology

As numerous cell-membrane proteins are utilized in therapeutics, it is important to understand the 3D structure of proteins. X-ray crystallography is one such widely used method for the characterization of the 3D structure of proteins. Effective protein extraction, solubilization, stabilization and crystallization are necessary for this method to be successful. For those working to crystallize membrane proteins, each of these processes may provide significant difficulties (55). Pre-crystallization screening needs to be carried out after protein extraction, solubilization and purification to evaluate the stability of proteins. The possibility of crystallization and higher-resolution diffraction improves with the discovery of factors that make pure proteins more stable. A rapid, high-throughput technique utilized to assess the thermostability of solubilized proteins is the thermal denaturation experiment (56).

Dynamic light scattering (DLS) is a rapid and sensitive alternative technique with proven effectiveness in identifying membrane proteins in solution. DLS measures light scattered by the macromolecules in solution, and depending on the rate of light scattering fluctuations, it may assess the size, homogeneity, and stability of samples (57). Based on the protein environment, a precise method of crystallization is applied; this includes vapor diffusion crystallization, *in meso* crystallization and chaperone crystallization (58).

## 8. Challenges and future perspectives

The electrophoresis protocols are the most commonly used assays for the separation of protein molecules. Among these, SDS-PAGE is generally performed in all cases of separation of biomolecules, whereas in multilevel proteomics, the determination of the peptide, proteoform and protein complex is achieved

by capillary electrophoresis utilized with MS (59). In recombinant protein therapeutics to perform quality assessment and unravel protein molecules, capillary isoelectric focusing-MS can be utilized and charge variant analysis can also be performed, demonstrated with bispecific antibody (60). NMR and MS are two techniques widely used to study and determine biological molecules. However, in the field of metabolomics and flux-omics, NMR has certain drawbacks as its resolution, sensitivity and accessibility are low with multiple analytical technical approaches (61). Other than NMR in predicting structure, cryo-electron microscopy also plays a crucial role in predicting the 3D structure of biomacromolecules and these approaches will become advanced in future research applications in analysing the peptide (62). In determining the quality of food components, the majority of food testing laboratories and industries use NMR and MS for quality control; however, to date, there is no NMR database for foodstuffs for comparative analysis and this has yet to be developed (63).

MS methods serve as effective tools for diagnosis and prognosis by differentiating healthy vs. abnormal samples (blood, urine or cerebrospinal fluid). Spatial metabolomics in omics studies enable biomolecule localization, such that it can be utilized with MS for generating imaging MS, and artificial intelligence applications such as deep learning and machine learning will also be utilized as a tool for image analysis (64,65). In the development and advancement of MS in multi-disciplinary studies, the study by Kuo *et al* (66) described five future directions that include constructing a public data repository, creating a future automated platform for usage in a robotic laboratory, moving towards on-site tests, broadening outreach and blindly unravelling biomolecules in routine analysis. Various *in silico* tools and projects based on web developments can also be performed to advance the techniques bioanalytically.

However, beyond all the developed protein technologies (for purification, detection, labelling and fractionation), the major loophole that still exists is the unknown complexity of protein structures in biological cells that can be used against the novel therapeutic technology and this can be answered by the development of RNA-sequencing techniques and omics approaches (67). In the recent decade, numerous sequencing techniques for analysing RNA and proteins brought advances to the therapeutic technology against various diseases. Some of the recent ones include Illumina and ion-torrent barcoding technologies (68).

Cell membrane proteins including the protein kinases, GPCR, B-cell lymphoma and chaperones can be utilized in the development of diagnostic and therapeutic products (69-71). Likewise, cell membrane-coated nanoparticles have played a crucial role in nanomedicine, vaccination and targeted drug delivery in various diseases such as cancer, and metabolic disorders (72,73). The various types of bioanalytical analyses used for protein characterization are depicted in Fig. 1, along with a focus on prokaryotic organisms (Table I) for a better understanding of cell membrane complexes and proteins.

## 9. Conclusion

The present review mainly focused on bacterial and fungal cell membrane proteins, which have been utilized in multiple

Table I. Studies on prokaryotic cell membranes (published over the past 5 years).

| Prokaryotic cell membrane proteins                   | Authors/(Refs.)   |
|--|---|
| <i>Acinetobacter baumannii</i>                       | Nie <i>et al</i> (74)   |
| <i>Pichia pastoris</i>                               | Chen <i>et al</i> (75)  |
| <i>Beauveria bassiana</i>                            | Ding <i>et al</i> (76)  |
| <i>Akkermansia muciniphila</i>                       | Wang <i>et al</i> (77)  |
| <i>Aspergillus flavus</i>                            | Manju Devi <i>et al</i> (78)  |
| <i>Lasiodiplodia theobromae</i>                      | Peng <i>et al</i> (79)  |
| Bacterial outer membrane protein assembly            | Nie <i>et al</i> (74), Doyle and Bernstein (80), Oluwole <i>et al</i> (81), Peterson <i>et al</i> (82), Sun <i>et al</i> (83), and Tomasek and Kahne (84) |
| Bacterial respiratory membrane protein complexes     | Muras <i>et al</i> (85)   |
| Bitopic membrane proteins in bacterial cell division | den Blaauwen and Luirink (86), and Nguyen <i>et al</i> (87)   |
| Bacteriophage derived proteins                       | Grabowski <i>et al</i> (88) and Sharma <i>et al</i> (89)  |
| Bacterial membrane protein biogenesis                | Hegde and Keenan (6), Avila-Calderón <i>et al</i> (90), and McDowell <i>et al</i> (91)  |
| Fungal membrane protein biogenesis                   | Diederichs <i>et al</i> (92), and Lübeck and Lübeck (93)  |

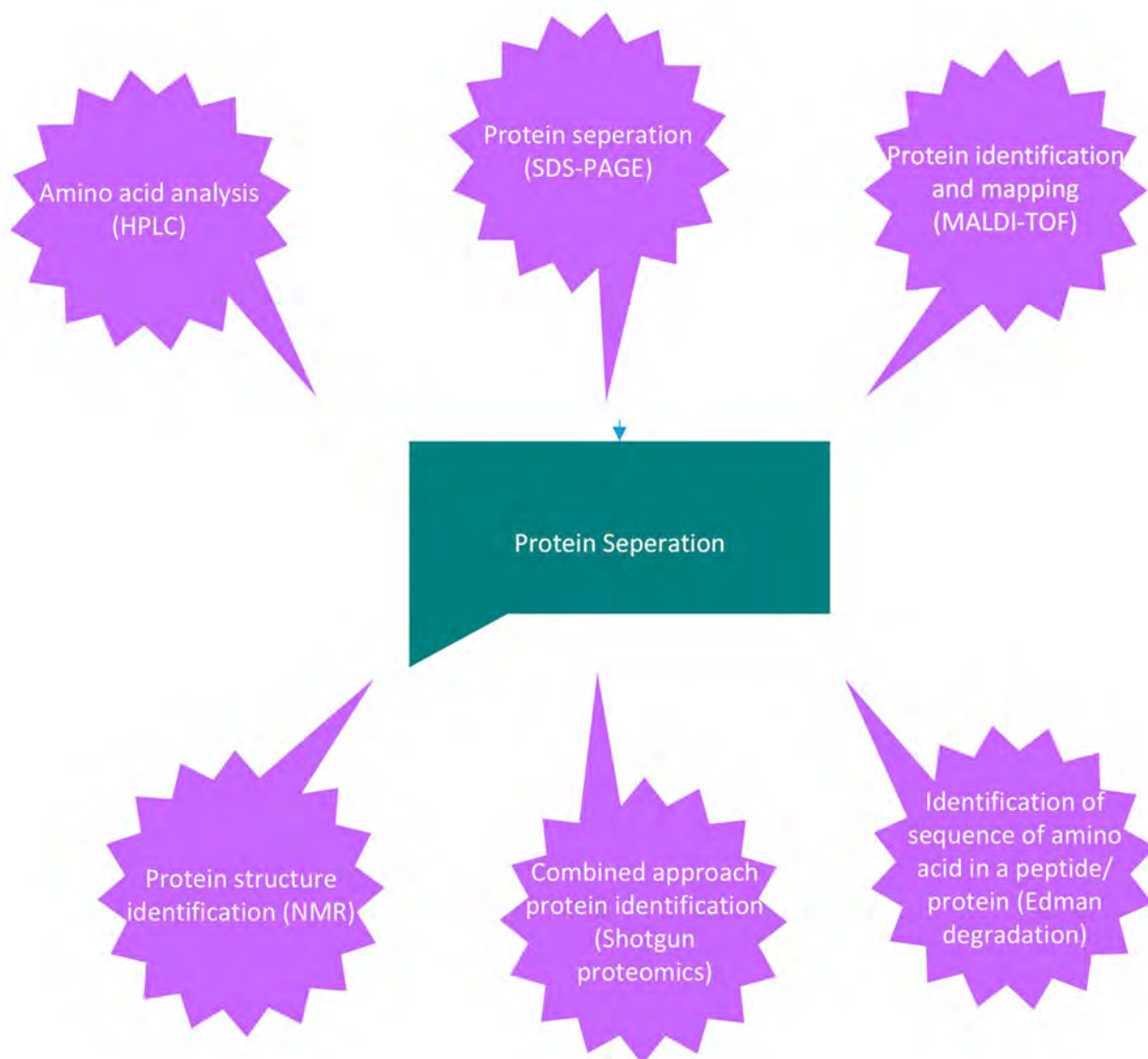


Figure 1. Bioanalytical techniques used for protein characterization. HPLC, high-performance liquid chromatography; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; MALDI-TOF, matrix-assisted laser desorption/ionization-time of flight; NMR, nuclear magnetic resonance.

aspects of pharmaceuticals to produce therapeutic products. Overall, the bioanalytical techniques, principles behind them, and computer-aided platforms were also discussed in the present study. For researchers who have a very keen interest in proteomics and cell membrane proteins, these studies give the proposal and clarification by helping in the development of problem statements, novelty and applications.

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### Authors' contributions

MSAH and IRO were involved in the conceptualisation of the study and in the editing of manuscript. MSAH performed the literature search. KR and IRO reviewed the study. IRO was involved in the submission of final manuscript and in correspondence. All authors have read and approved the final manuscript. Data authentication is not applicable.

### Ethics approval and consent to participate

Not applicable.

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### Competing interests

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### References

- Watson H: Biological membranes. *Essays Biochem* 59: 43-69, 2015.
- Gopalakrishnan G, Awasthi A, Belkaid W, De Faria O Jr, Liazoghli D, Colman DR and Dhaunchak AS: Lipidome and proteome map of myelin membranes. *J Neurosci Res* 91: 321-334, 2013.
- Muller MP, Jiang T, Sun C, Lihan M, Pant S, Mahinthichaichan P, Trifan A and Tajkhorshid E: Characterization of lipid-protein interactions and lipid-mediated modulation of membrane protein function through molecular simulation. *Chem Rev* 119: 6086-6161, 2019.
- Wardhan R and Mudgal P: *Membrane Proteins in Textbook of Membrane Biology*. Springer, Singapore, pp49-80, 2017.
- Bai L and Li H: Structural insights into the membrane chaperones for multi-pass membrane protein biogenesis *Curr Opin Struct Biol* 79: 102563, 2023.
- Hegde RS and Keenan RJ: The mechanisms of integral membrane protein biogenesis. *Nat Rev Mol Cell Biol* 23: 107-124, 2022.
- Tzen JTC: Integral proteins in plant oil bodies. *ISRN Botany* 2012: 1-16, 2012.
- Che T: Advances in the treatment of chronic pain by targeting GPCRs. *Biochemistry* 60: 1401-1412, 2021.
- Li Y, Zhang H, Kosturakis AK, Cassidy RM, Zhang H, Kennamer-Chapman RM, Jawad AB, Colomand CM, Harrison DS and Dougherty PM: MAPK signaling downstream to TLR4 contributes to paclitaxel-induced peripheral neuropathy. *Brain Behav Immun* 49: 255-266, 2015.
- Müller GA and Müller TD: (Patho)Physiology of glycosylphosphatidylinositol-anchored proteins I: Localization at plasma membranes and extracellular compartments. *Biomolecules* 13: 855, 2023.
- Lee IH, Imanaka M, Modahl EH and Torres-Ocampo AP: Lipid raft phase modulation by membrane-anchored proteins with inherent phase separation properties. *ACS Omega* 4: 6551-6559, 2019.
- Henderson JC, Zimmerman SM, Crofts AA, Boll JM, Kuhns LG, Herrera CM and Trent MS: The power of asymmetry: Architecture and assembly of the gram-negative outer membrane lipid bilayer. *Ann Rev Microbiol* 70: 255-278, 2016.
- Lorent JH, Levental KR, Ganesan L, Rivera-Longworth G, Sezgin E, Doktorova M, Lyman E and Levental I: Plasma membranes are asymmetric in lipid unsaturation, packing and protein shape. *Nat Chem Biol* 16: 642-652, 2020.
- Coskun O: *Separation techniques: Chromatography*. North Clin Istanbul 3: 156-160, 2016.
- Jorin-Novo JV, Komatsu S, Sanchez-Lucas R and de Francisco LE: Gel electrophoresis-based plant proteomics: Past, present, and future. Happy 10th anniversary *Journal of Proteomics!*. *J Proteomics* 198: 1-10, 2019.
- Orwick-Rydmark M, Arnold T and Linke D: The use of detergents to purify membrane proteins. *Curr Protoc Protein Sci* 84: 4.8.1-4.8.35, 2016.
- Zhou D, Zhong S, Han X, Liu D, Fang H and Wang Y: Protocol for mitochondrial isolation and sub-cellular localization assay for mitochondrial proteins. *STAR Protoc* 4: 102088, 2023.
- Webster J and Oxley D: Protein identification by MALDI-TOF mass spectrometry. *Methods Mol Biol* 2012: 227-240, 2012.
- Santos IC, Hildenbrand ZL and Schug KA: Applications of MALDI-TOF MS in environmental microbiology. *Analyst* 141: 2827-2837, 2016.
- Crook AA and Powers R: Quantitative NMR-based biomedical metabolomics: Current status and applications. *Molecules* 25: 5128, 2020.
- Hatzakis E: Nuclear magnetic resonance (NMR) spectroscopy in food science: A comprehensive review. *Compr Rev Food Sci Food Saf* 18: 189-220, 2019.
- Li M, Xu W and Su Y: Solid-state NMR spectroscopy in pharmaceutical sciences. *Trends Anal Chem* 135: 116152, 2021.
- D'cruz D, Babu A and Joshy E: Bioanalytical method development and validation of ticagrelor by rp-Hplc. *Int J App Pharm* 9: 51, 2017.
- Aneesh TP, Radhakrishnan R, Sasidharan APM and Choyal M: RP-HPLC method for simultaneous determination of losartan and chlorthalidone in pharmaceutical dosage form. *Int Res J Pharm* 6: 453-457, 2015.
- Sobsey CA, Ibrahim S, Richard VR, Gaspar V, Mitsa G, Lacasse V, Zahedi RP, Batist G and Borchers CH: Targeted and untargeted proteomics approaches in biomarker development. *Proteomics* 20: 1900029, 2020.
- Gallagher RI, Wulfkuhle J, Wolf DM, Brown-Swigart L, Yau C, O'Grady N, Basu A, Lu R, Campbell MJ, Magbanua MJ, *et al*: Protein signaling and drug target activation signatures to guide therapy prioritization: Therapeutic resistance and sensitivity in the I-SPY 2 trial. *Cell Rep Med* 4: 101312, 2023.
- Mann M: The rise of mass spectrometry and the fall of edman degradation. *Clin Chem* 62: 293-294, 2016.
- Matsudaira P: *A Practical Guide to Protein and Peptide Purification for Microsequencing*. Matsudaira P (ed). 2nd edition. Academic Press, San Diego, CA, pp1-13, 1993.
- Pomerantz SC and McCloskey JA: Analysis of RNA hydrolyzates by liquid chromatography-mass spectrometry. *Methods Enzymol* 193: 796-824, 1990.
- Su D, Chan CT, Gu C, Lim KS, Chionh YH, McBee ME, Russell BS, Babu IR, Begley TJ and Dedon PC: Quantitative analysis of ribonucleoside modifications in tRNA by HPLC-coupled mass spectrometry. *Nat Protoc* 9: 828-841, 2014.
- Cupp-Sutton KA and Wu S: High-throughput quantitative top-down proteomics. *Mol Omics* 16: 91-99, 2020.
- Gao Y and Yates JR III: Protein analysis by shotgun proteomics. In: *Mass Spectrometry-Based Chemical Proteomics*. John Wiley & Sons, Ltd., Hoboken, NJ, pp1-38, 2019.

33. Rozario LT, Sharker T and Nila TA: In silico analysis of deleterious SNPs of human MTUS1 gene and their impacts on subsequent protein structure and function. *PLoS One* 16: e0252932, 2021.
34. Huang C, Hou C, Ijaz M, Yan T, Li X, Li Y and Zhang D: Proteomics discovery of protein biomarkers linked to meat quality traits in post-mortem muscles: Current trends and future prospects: A review. *Trends Food Sci Technol* 105: 416-432, 2020.
35. Yokota H: Applications of proteomics in pharmaceutical research and development. *Biochim Biophys Acta Proteins Proteom* 1867: 17-21, 2019.
36. Zhao Y, Sun Q and Huo B: Focal adhesion regulates osteogenic differentiation of mesenchymal stem cells and osteoblasts. *Biomater Transl* 2: 312-322, 2021.
37. de Oliveira Garcia FA, de Andrade ES and Palmero EI: Insights on variant analysis in silico tools for pathogenicity prediction. *Front Genet* 13: 1010327, 2022.
38. Deshpande RR, Tiwari AP, Nyayanit N and Modak M: In silico molecular docking analysis for repurposing therapeutics against multiple proteins from SARS-CoV-2. *Eur J Pharmacol* 886: 173430, 2020.
39. Cramer P: AlphaFold2 and the future of structural biology. *Nat Struct Mol Biol* 28: 704-705, 2021.
40. Cavasotto CN and Phatak SS: Homology modeling in drug discovery: Current trends and applications. *Drug Discov Today* 14: 676-683, 2009.
41. Taha MO, Atallah N, Al-Bakri AG, Paradis-Bleau C, Zalloum H, Younis KS and Levesque RC: Discovery of new MurF inhibitors via pharmacophore modeling and QSAR analysis followed by in-silico screening. *Bioorg Med Chem* 16: 1218-1235, 2008.
42. Huang YZ, Chen SY and Deng F: Well-characterized sequence features of eukaryote genomes and implications for ab initio gene prediction. *Comput Struct Biotechnol J* 14: 298-303, 2016.
43. Dereeper A, Audic S, Claverie JM and Blanc G: BLAST-EXPLORER helps you building datasets for phylogenetic analysis. *BMC Evol Biol* 10: 8, 2010.
44. Meglécz É, Pech N, Gilles A, Dubut V, Hingamp P, Trilles A, Grenier R and Martin J: QDD version 3.1: A user-friendly computer program for microsatellite selection and primer design revisited: Experimental validation of variables determining genotyping success rate. *Mol Ecol Resour* 14: 1302-1313, 2014.
45. Jess R, Ling T, Xiong Y, Wright CJ and Zhao F: Mechanical environment for in vitro cartilage tissue engineering assisted by in silico models. *Biomater Transl* 4: 18-26, 2023.
46. Wu YW, Simmons BA and Singer SW: MaxBin 2.0: An automated binning algorithm to recover genomes from multiple metagenomic datasets. *Bioinformatics* 32: 605-607, 2016.
47. Xiao J and Goley ED: Redefining the roles of the FtsZ-ring in bacterial cytokinesis. *Curr Opin Microbiol* 34: 90-96, 2016.
48. Paulin S, Jamshad M, Dafforn TR, Garcia-Lara J, Foster SJ, Galley NF, Roper DI, Rosado H and Taylor PW: Surfactant-free purification of membrane protein complexes from bacteria: Application to the staphylococcal penicillin-binding protein complex PBP2/PBP2a. *Nanotechnology* 25: 285101, 2014.
49. Meeske AJ, Riley EP, Robins WP, Uehara T, Mekalanos JJ, Kahne D, Walker S, Kruse AC, Bernhardt TG and Rudner DZ: SEDS proteins are a widespread family of bacterial cell wall polymerases. *Nature* 537: 634-638, 2016.
50. Noinaj N, Kuszak AJ, Gumbart JC, Lukacik P, Chang H, Easley NC, Lithgow T and Buchanan SK: Structural insight into the biogenesis of  $\beta$ -barrel membrane proteins. *Nature* 501: 385-390, 2013.
51. Rassam P, Copeland NA, Birkholz O, Tóth C, Chavent M, Duncan AL, Cross SJ, Housden NG, Kaminska R, Seger U, *et al*: Supramolecular assemblies underpin turnover of outer membrane proteins in bacteria. *Nature* 523: 333-336, 2015.
52. Foster TJ: The MSCRAMM family of cell-wall-anchored surface proteins of gram-positive cocci. *Trends Microbiol* 27: 927-941, 2019.
53. Lecointe K, Cornu M, Leroy J, Coulon P and Sendid B: Polysaccharides cell wall architecture of mucorales. *Front Microbiol* 10: 469, 2019.
54. Heinisch JJ and Rodicio R: Protein kinase C in fungi-more than just cell wall integrity. *FEMS Microbiol Rev* 42: 10.1093/femsre/fux051, 2018.
55. Kermani AA: A guide to membrane protein X-ray crystallography. *FEBS J* 288: 5788-5804, 2021.
56. Alexandrov AI, Mileni M, Chien EYT, Hanson MA and Stevens RC: Microscale fluorescent thermal stability assay for membrane proteins. *Structure* 16: 351-359, 2008.
57. Kwan TOC, Reis R, Siligardi G, Hussain R, Cheruvara H and Moraes I: Selection of biophysical methods for characterisation of membrane proteins. *Int J Mol Sci* 20: 2605, 2019.
58. Kermani AA, Aggarwal S and Ghanbarpour A: Chapter 11 - Advances in X-ray crystallography methods to study structural dynamics of macromolecules. *Advanced Spectroscopic Methods to Study Biomolecular Structure and Dynamics* 2023: 309-355, 2023.
59. Chen D, McCool EN, Yang Z, Shen X, Lubeckyj RA, Xu T, Wang Q and Sun L: Recent advances (2019-2021) of capillary electrophoresis-mass spectrometry for multilevel proteomics. *Mass Spectrom Rev* 42: 617-642, 2023.
60. Wu G, Yu C, Wang W, Du J, Xu G, Fu Z and Wang L: Charge variants analysis of a bispecific antibody using a fully automated one-step capillary isoelectric focusing-mass spectrometry method. *Curr Pharm Anal* 18: 860-870, 2022.
61. Giraudeau P: NMR-based metabolomics and fluxomics: Developments and future prospects. *Analyst* 145: 2457-2472, 2020.
62. Hiroaki H: Molecular mechanisms of amyloid- $\beta$  peptide fibril and oligomer formation: NMR-based challenges. *Biophys Physicobiol* 20: e200007, 2023.
63. Sobolev AP, Thomas F, Donarskic J, Ingallinad C, Circid S, Marincolae FC, Capitania D and Mannina L: Use of NMR applications to tackle future food fraud issues. *Trends Food Sci Technol* 91: 347-353, 2019.
64. Alexandrov T: Spatial metabolomics and imaging mass spectrometry in the age of artificial intelligence. *Ann Rev Biomed Data Sci* 3: 61-87, 2020.
65. Silanteyev AS, Falzone L, Libra M, Gurina OI, Kardashova KS, Nikolouzakis TK, Nosyrev AE, Sutton CW, Mitsias PD and Tsatsakis A: Current and future trends on diagnosis and prognosis of glioblastoma: From molecular biology to proteomics. *Cells* 8: 863, 2019.
66. Kuo TH, Dutkiewicz EP, Pei J and Hsu CC: Ambient ionization mass spectrometry today and tomorrow: Embracing challenges and opportunities. *Anal Chem* 92: 2353-2363, 2020.
67. Kroll AV, Fang RH and Zhang L: Biointerfacing and applications of cell membrane-coated nanoparticles. *Bioconjug Chem* 28: 23-32, 2017.
68. Ahmad R and Budnik B: A review of the current state of single-cell proteomics and future perspective. *Anal Bioanal Chem* 415: 6889-6899, 2023.
69. Khan WA: Chapter 3-Whole-exome and whole-genome sequencing in the molecular diagnostic laboratory. In: *Diagnostic Molecular Pathology*. Coleman WB and Tsongalis GJ (eds). 2nd edition. Academic Press, San Diego, CA pp27-38, 2024.
70. Wang F, Guo J, Wang S, Wang Y, Chen J, Hu Y, Zhang H, Xu K, Wei Y, Cao L, *et al*: B-cell lymphoma-3 controls mesenchymal stem cell commitment and senescence during skeletal aging. *Clin Transl Med* 12: e955, 2022.
71. Al Huq Mohammed S and Rajamani K: Role of chaperones and endoplasmic reticulum stress in protein complexity associated with dyslipidemia: A future perspective to novel therapeutics (Review). *World Acad Sci J* 6: 1-9, 2024.
72. Kingsak M, Maturavongsadit P, Jiang H and Wang Q: Cellular responses to nanoscale substrate topography of TiO<sub>2</sub> nanotube arrays: Cell morphology and adhesion. *Biomater Transl* 3: 221-233, 2022.
73. Xue X, Liu H, Wang S, Hu Y, Huang B, Li M, Gao J, Wang X and Su J: Neutrophil-erythrocyte hybrid membrane-coated hollow copper sulfide nanoparticles for targeted and photothermal/anti-inflammatory therapy of osteoarthritis. *Composites Part B: Engineering* 237: 109855, 2022.
74. Nie D, Hu Y, Chen Z, Li M, Hou Z, Luo X, Mao X and Xue X: Outer membrane protein A (OmpA) as a potential therapeutic target for *Acinetobacter baumannii* infection. *J Biomed Sci* 27: 26, 2020.
75. Chen Z, Wang Y, Cheng Y, Wang X, Tong S, Yang H and Wang Z: Efficient biodegradation of highly crystallized polyethylene terephthalate through cell surface display of bacterial PETase. *Sci Total Environ* 709: 136138, 2020.
76. Ding JL, Hou J, Feng MG and Ying SH: Transcriptomic analyses reveal comprehensive responses of insect hemocytes to mycopathogen *Beauveria bassiana*, and fungal virulence-related cell wall protein assists pathogen to evade host cellular defense. *Virulence* 11: 1352-1365, 2020.

77. Wang J, Xu W, Wang R, Cheng R, Tang Z and Zhang M: The outer membrane protein Amuc\_1100 of *Akkermansia muciniphila* promotes intestinal 5-HT biosynthesis and extracellular availability through TLR2 signalling. *Food Funct* 12: 3597-3610, 2021.
78. Devi SM, Raj N and Sashidhar RB: Efficacy of short-synthetic antifungal peptides on pathogenic *Aspergillus flavus*. *Pestic Biochem Physiol* 174: 104810, 2021.
79. Peng J, Wu L, Zhang W, Zhang Q, Xing Q, Wang X, Li X and Yan J: Systemic identification and functional characterization of common in fungal extracellular membrane proteins in *Lasiodiplodia theobromae*. *Front Plant Sci* 12: 804696, 2021.
80. Doyle MT and Bernstein HD: Bacterial outer membrane proteins assemble via asymmetric interactions with the BamA  $\beta$ -barrel. *Nat Commun* 10: 3358, 2019.
81. Oluwole A, Shutin D and Bolla JR: Mass spectrometry of intact membrane proteins: shifting towards a more native-like context. *Essays Biochem* 67: 201-213, 2023.
82. Peterson JH, Doyle MT and Bernstein HD: Small molecule antibiotics inhibit distinct stages of bacterial outer membrane protein assembly. *mBio* 13: e0228622, 2022.
83. Sun J, Rutherford ST, Silhavy TJ and Huang KC: Physical properties of the bacterial outer membrane. *Nat Rev Microbiol* 20: 236-248, 2022.
84. Tomasek D and Kahne D: The assembly of  $\beta$ -barrel outer membrane proteins. *Curr Opin Microbiol* 60: 16-23, 2021.
85. Muras V, Toulouse C, Fritz G and Steuber J: Respiratory membrane protein complexes convert chemical energy. *Bacterial Cell Walls and Membranes* 2019: 301-335, 2019.
86. den Blaauwen T and Luirink J: Checks and balances in bacterial cell division. *MBio* 10: e00149-e00119, 2019.
87. Nguyen HTV, Chen X, Parada C, Luo AC, Shih O, Jeng US, Huang CY, Shih YL and Ma C: Structure of the heterotrimeric membrane protein complex FtsB-FtsL-FtsQ of the bacterial divisome. *Nat Commun* 14: 1903, 2023.
88. Grabowski L, Łeppek K, Stasiłojć M, Kosznik-Kwaśnicka K, Zdrojewska K, Maciąg-Dorszyńska M, Węgrzyn G and Węgrzyn A: Bacteriophage-encoded enzymes destroying bacterial cell membranes and walls, and their potential use as antimicrobial agents. *Microbiol Res* 248: 126746, 2021.
89. Sharma U, Vipra A and Channabasappa S: Phage-derived lysins as potential agents for eradicating biofilms and persisters. *Drug Discovery Today* 23: 848-856, 2018.
90. Avila-Calderón ED, Ruiz-Palma MDS, Aguilera-Arreola MG, Velázquez-Guadarrama N, Ruiz EA, Gomez-Lunar Z, Witonsky S and Contreras-Rodríguez A: Outer membrane vesicles of gram-negative bacteria: An outlook on biogenesis. *Front Microbiol* 12: 557902, 2021.
91. McDowell MA, Heimes M and Sinning I: Structural and molecular mechanisms for membrane protein biogenesis by the Oxal1 superfamily. *Nat Struct Mol Biol* 28: 234-239, 2021.
92. Diederichs KA, Pitt AS, Varughese JT, Hackel TN, Buchanan SK and Shaw PL: Mechanistic insights into fungal mitochondrial outer membrane protein biogenesis. *Curr Opin Struct Biol* 74: 102383, 2022.
93. Lübeck M and Lübeck PS: Fungal cell factories for efficient and sustainable production of proteins and peptides. *Microorganisms* 10: 753, 2022.



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