Biomembranes in Bioelectronic Sensing

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Abstract

Cell membranes are integral to the functioning of the cell and are therefore key to drive fundamental understanding of biological processes for downstream applications. Here we review the current state-of-the art with respect to biomembrane systems and electronic substrates, with a view of how the field has evolved towards creating biomimetic conditions and improving detection sensitivity. Of particular interest are conducting polymers, a class of electroactive polymers, which

have the potential to create the next step-change for bioelectronics devices. Lastly, we discuss the impact these types of devices could have for biomedical applications.

1. The significance of the cell membrane

The cell membrane compartmentalises the interior of a cell, separating it from the external environment, serving key roles in cellular homeostasis, metabolism, energy harvesting and growth [1–3]. Cell membranes across all living kingdoms; animal, plant and microbial, serve as a protective barrier against invasion or toxicity. Studies of pathogen and toxin interactions with cells also rely on an understanding of their point of entry, at the external membrane surface. Cell membranes have a molecularly complex surface, comprised of lipids, proteins and glycans, all essential for function. Glycans, for example, are well known for their importance in mediating cell-cell adhesion, macromolecule interactions and being subverted for pathogen invasion [4]. Cell membranes being the first point of contact, play an important role in the absorption, distribution, metabolism, and excretion (ADME) of a drug. Initial cell-drug interactions can dictate the fate of the administered drug [5]. ~ 60% of approved drugs target the cell membrane either via membrane associated proteins or through direct interaction with the membrane lipids. G-protein-coupled receptors (GPCRs) are membrane proteins, accounting for ~27% of the global market share of therapeutic drugs [6]. Targets are not restricted to proteins; local anaesthetic drugs may exert nerve-blocking effect primarily via lipids in the membranes [7]. Despite their significance, the molecular mechanics of cell membranes are not well understood with respect to outside influences, owing to i) the complexity of the native membrane and the challenges in recapitulating this while preserving **transmembrane protein** (TMP, see Glossary) structure/function and ii) the lack of suitable technologies for efficient, low-cost and broad spectrum studies [8].Recent advances in receptor pharmacology, alongside important discoveries in structural biology, have opened new

and exciting avenues in drug discovery, however high-throughput and high content manner technologies are required to assess interactions at the first point of contact, the cell membrane, to facilitate the drug development process.

Upon conducting a literature search, it was observed the number of studies on biomembrane based bioelectronic systems experienced an exponential increase since the inception of the field in the !960s. The exponential increase in the volume of studies points to the significance of the plasma membrane as an important target for drug discovery and development [9], with the majority of studies focusing on improving the membrane model and its fidelity. To elevate this field and increase impact, focus needs to be placed on improving the sensing capabilities for these models as well as their translational capabilities, areas where bioelectronic materials and technologies are anticipated to play a major role [10,11]. Indeed, as recently mentioned in a progress report in Nature Materials, "cell membranes with their complex mixture of proteins, lipids and sugars could be extracted and transferred onto electronic sensors, a 'wolf in sheep's clothing' approach to sensing and transduction" [12].

This review focuses on emerging bioelectronic technologies, used to interface with cell membranes. Applications range from biosensing and drug discovery, to membrane biophysics studies (e.g. transport through **ion channels** [See Glossary] and understanding host-pathogen interactions). We discuss the latest advances in the development of biomimetic cell membrane models, with respect to their biotechnological relevance and their integration with compatible electronic transducers. Finally, we highlight **conducting polymers (CPs, see Glossary)**, an emerging class of electroactive materials, as a promising avenue to overcome limitations observed in traditional inorganic electrode materials, with a view to developing high-throughput sensing technologies for plasma membrane studies.

2. Models of the cell membrane: Increasing complexity and "authenticity" of the model

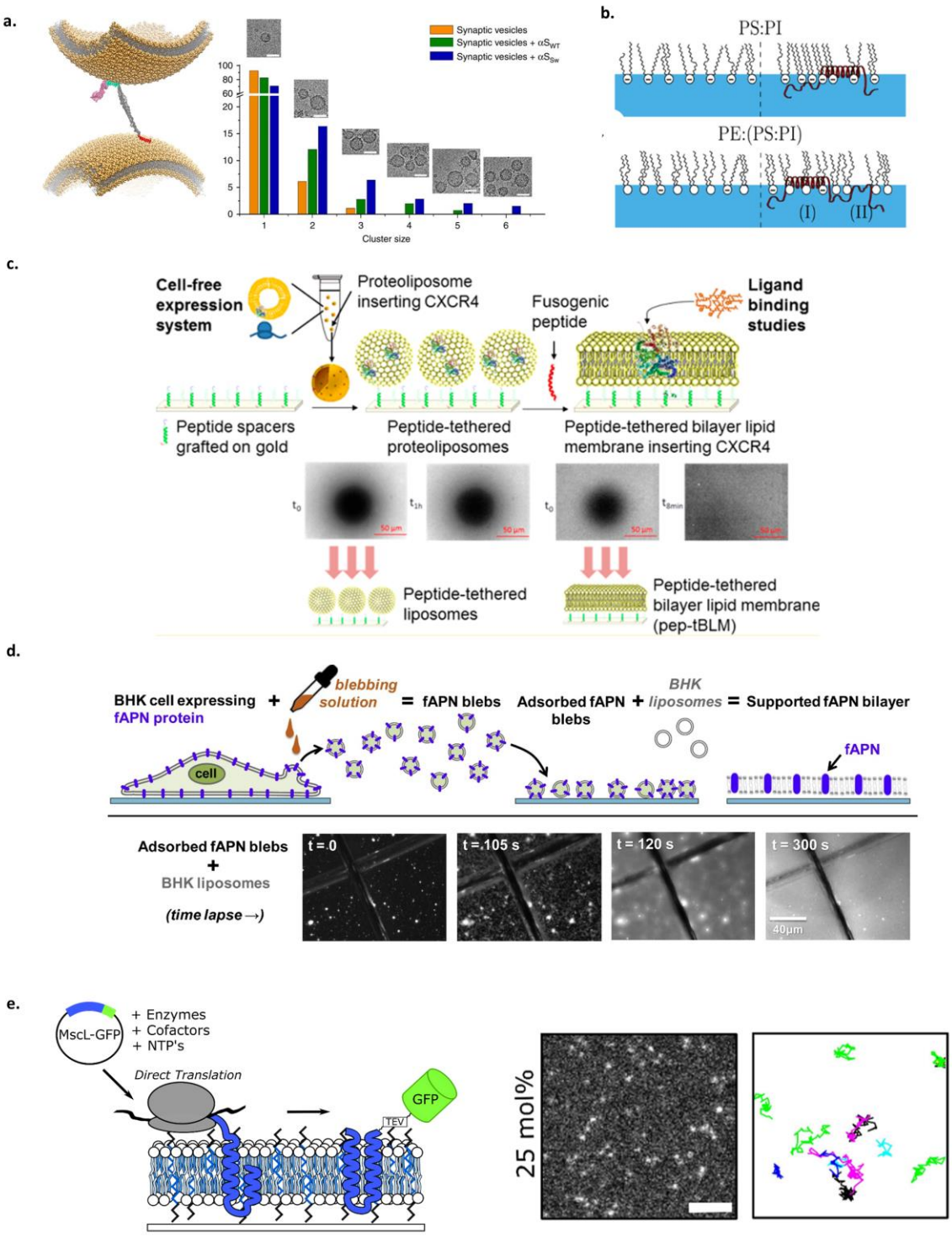

Fig.1. Membrane models used for investigation of biological processes. a. (Left) Schematic diagram highlighting vesicle curvature observed in small unilamellar vesicles (SUVs) and corresponding interactions with protein; (Right) Graph showing clustering of SUVs upon

interacting with α-synuclein wild type (αSwT) and α-synuclein swapped sequence (αSSw) based on super resolution microscopy data [13]. Inset shows cryo-EM images of corresponding vesicles. b. Schematic diagram highlighting adsorption of myelin basic protein (MBP) into lipid monolayers of varying lipid compositions [14]. Reprinted with permission from [14]. Copyright 2018 American Chemical Society, c. Schematic diagram showing principle of formation of peptide-based tethered bilayer membrane (pep-tBLM) [24] Reprinted with permission from [24]. Copyright 2018 American Chemical Society, d. (Top) Schematic diagram showing expression of feline amino-peptidase N (fAPN) blebs derived from baby hamster kidney (BHK) cells followed by formation of supported lipid bilayer (SLB) through vesicle fusion, (bottom) sequence of formation of SLB from blebs over time [36] Reprinted from [36], with permission from Elsevier; e. (Left) Schematic diagram of cell-free hybrid-supported lipid bilayer (HSLB) incorporating a large conductance mechanosensitive channel tagged with green-fluorescent protein (MscL-GFP), (right) tracking of MscL-GFP protein in 25 mol% poly(ethylene oxide)-b-polybutadiene (PEO-b-PBD) through microscopy and associated trajectories (Scale bar is 5 μm [38], Reprinted with permission from [38], Copyright 2021 American Chemical Society.

Given the high degree of complexity and diversity of natural membranes, a plethora of biomimetic membrane models have been developed, the choice of which have mainly been dictated by the biological phenomenon at study. The majority of cell membrane mimics consist of lipid vesicles free in solution [13] **(Figure 1A)** or self-assembled into a mono-/bilayer format [14] **(Figure 1B)**, or indeed surface-tethered lipids (**Box 1**). Langmuir monolayers have typically been adapted for investigation of compound interactions with lipid head groups [15] while vesicles and lipid bilayers are potent models for drug/compound permeability studies [8,16]. Despite the simplicity of lipid monolayers and liposomes, the majority of biomembrane sensors use planar lipid bilayers, which, as we discuss later allows facile integration with multiple sensing modalities, provided the *in vivo* like fluidity and mobility can be maintained. Lipid bilayers supported on solid substrates have become increasingly popular to study fundamental properties of biological membranes and their constituent lipid and protein molecules, as well as other applications including pathogen diagnostics, energy harvesting and drug discovery.

Recent trends in planar lipid bilayer models

The preparation of high quality planar lipid bilayers with lipid compositions that span from single component lipid bilayers to complex systems containing native membrane components have made great strides in recent years **(Box 2)** [17]. Emerging applications of supported lipid bilayers (SLBs) rely increasingly on the incorporation of functional membrane proteins via reconstitution for controlled studies of cellular phenomena. However, the reconstitution process makes it difficult to control protein orientation and there is always a concern about possible denaturation or disruption of essential protein-lipid interactions necessary for preservation of protein activity and function [8,17,18]. In addition, the proximity of the typically rigid solid supports to the lipid bilayer precludes the incorporation of large TMPs with bulky extracellular domains, restricts their mobility (known to be necessary for interaction with key membrane components) and ultimately, impacts their function [19,20]. To circumvent this, several strategies have been adopted including the formation of suspended membrane systems such as **black lipid membranes** (BLM, see Glossary) [21], polymer cushioned lipid bilayers and **tethered lipid bilayers** (see Glossary) [22–24] (**see Box 1**) (**Figure 1C**). These systems come with their own challenges however, and researchers need to consider the goal of the study while selecting an appropriate system.

Polymer cushioned SLBs have successfully incorporated protein molecules with improved mobility compared to non-cushioned SLBs [25,26]. However, the difficulty to control the morphology and density of the polymer cushions [22], alongside their electrically insulating nature [27] impedes their use for ion channel studies, limiting studies to diffusion, orientation, and binding events via conventional optical analysis. Tethered SLBs provide a hydrated sub-membrane domain between the substrate and the membrane to accommodate soluble domains of

incorporated membrane proteins by using a suitable linker between the substrate and the membrane [23,28]. This results in improved bilayer fluidity, long-term stability and a high degree of electrical sealing. Tethered SLBs have thus served as highly flexible platforms for biosensing applications. Two examples of this include the incorporation of a functional plant transporters and GPCR in a tethered SLB using cell free approaches, nicely compatible with sensing techniques such as EIS and SPR [28,29]. A drawback of using tethered SLBs comes however from the fact that the membranes are usually limited in terms of lipid composition, tending to be composed primarily of synthetic lipids. Yet another approach involves formation of SLBs via the Langmuir Blodgett technique, on microcavity arrays, combining the advantages of substrate sensing, with fluidity provided from liquid filled cavities thus yielding a versatile platform to understand drug-membrane interactions and binding studies via optical and electrochemical measurements [30,31].

Vesicle fusion is a simple and attractive way to create SLBs; first demonstrated on glass [32], its use has been expanded to certain other substrates such as titanium dioxide, but this can be challenging and may be limited to a narrow range of supported surfaces and lipid compositions [33]. To extend the utilization of SLBs as a model lipid bilayer with complex lipid compositions such as high cholesterol content, on a variety of substrates, **solvent-assisted lipid bilayers** (SALB, see Glossary) are an interesting alternative, compatible with a wide range of lipid compositions and material supports [34]. This is particularly relevant for bioelectronic devices because the electrical components are not always easily interfaced with biological membranes formed by vesicle fusion, creating possibilities for new applications with more diverse substrates in the future. The disadvantage with the SALB approach however is that functional, correctly-oriented proteins are not easily incorporated due to solvent incompatibility.

Up to now, we have mainly focused on parameters such as bilayer fluidity and protein mobility, however the approaches highlighted are largely synthetic (or reconstituted) in terms of the membrane composition. As summarised in Box 2, blebbing of membranes from live cells has been a major landmark towards native membrane composition in SLBs. Researchers have developed methods to create SLBs using native cell membranes that maintain mobility and orientation of transmembrane proteins [25,35,36] **(Figure 1C, Figure 1D),** an encouraging method for fabrication of biologically complex membranes and a much-needed intermediate between very simple bilayer platforms and whole cell readouts. A major advantage is that native membrane components are captured with the lipid, protein and glycan species found in the live cell [37]. Recently, progress towards cell-free protein synthesis in SLB systems has been made [38] **(Figure 1E)**. The integration of SLBs and cell-free synthesis of mobile transmembrane proteins will greatly facilitate the development of protein-based biomembrane sensors. Maintaining both protein and lipid mobility in SLBs is important as membrane lipids can regulate the distribution and localization of peripheral proteins and then participate in numerous important cellular activities such as protein function, cell adhesion and cell signalling [39,40].

3. Coupling bioelectronics with cell membranes

As mentioned above, SLBs have a major advantage over vesicles in solution, in that they facilitate integration with surface based techniques and microscopy due to their planar configuration. For the same reason, they are also preferred for interfacing with planar electronic devices. The choice of the substrate to form the membrane largely dictates the compatibility of techniques to characterise the SLB (see **Box 3**).

Surface sensitive analytical methods to characterize SLBs

Various techniques for SLB studies have been developed to date, including but not limited to, optical microscopy, **atomic force microscopy** (see Glossary), **surface plasmon resonance** (SPR, see Glossary), **quartz crystal microbalance** (QCM, see Glossary), and electrical/electrochemical spectroscopy, each displaying advantages and disadvantages. In contrast to the large portion of biological assays that involve the use of external probes (e.g. chromophores/fluorophores), many of the techniques listed above are label-free, thus preserving the authenticity of the system at study.

Another consideration for the choice of technique moving forward, will be compatibility with high throughput studies, e.g. automation through integration with fluidics and parallelization, facilitating drug and pathogen screening. In many cases, an authentic, functional membrane can be thought of as an alternative to a live cell, providing a robust and scalable alternative to cell-based assays. Traditionally, electrical characterization of cell membranes (in whole cell-studies) has been performed using patch-clamp electrophysiology, also used to study pore-forming behaviour of drugs, proteins and other molecules [41,42]. Patch clamp in whole cells suffers in terms of time-efficiency, robustness, and the need for sterility for cell culture, limiting the applications of this method. Patch clamp can be applied to SLBs [43,44], however the intricate, technically challenging nature of the method still limits implementation outside of the research laboratory environment. The integration of cells with planar patch clamp devices has been shown, potentially overcoming throughput issues, however the use of live cells remains problematic [45,46].

Measurement of electrical properties of membranes provides a wealth of information on their function. The homeostasis of cells requires control over the ion flux across the membrane and loss of membrane potential leads inevitably to cell death. The highly insulating nature of lipids, combined with mediated flux of different ions by membrane proteins, means that electrical measurements can assay the function of these components. Membrane targeting drugs, toxins and pathogens exert their effects through blocking ion channels, inserting pores, binding to receptors in the membrane, all actions that result in electrical changes in the membrane. Electrical properties of cell membranes can be assessed through formation of SLBs on electrically conducting substrate using electronic methods including EIS [47]. The following sections focus on the biotechnological applications of such bioelectronic biomembrane sensors.

Conventionally used substrates in biomembrane-based bioelectronics devices

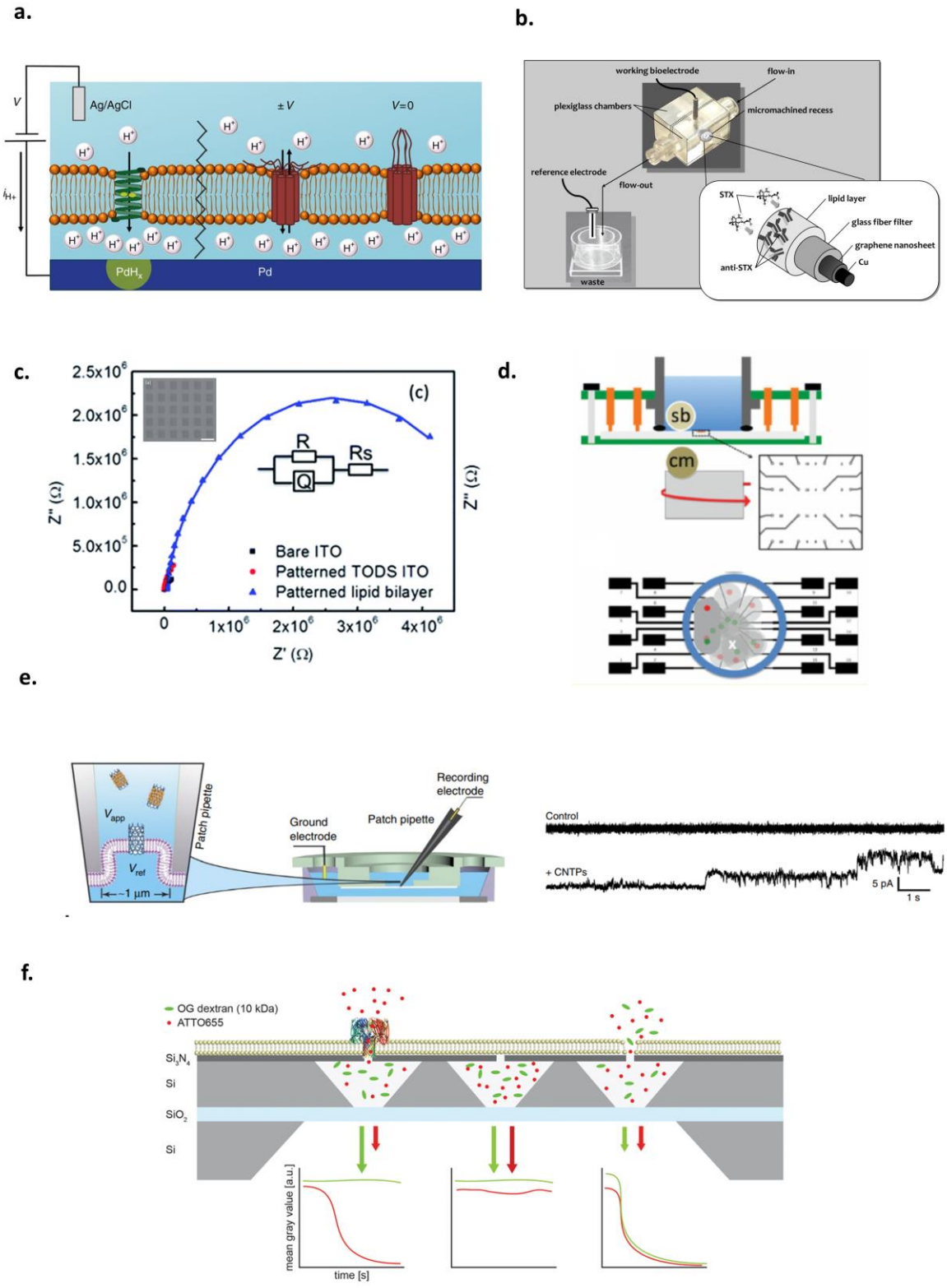

Fig.2. Biomembrane-based bioelectronic platforms using emerging techniques and substrates. a. (Left) Schematic of biotic-abiotic bioprotonic device developed to control and measure H+ transport across a bilayer in the presence of ion channels [56], b. Schematic diagram of graphene-based bioelectronic sensor developed to monitor saxitoxin levels [86]. Reproduced with permission from John Wiley & Sons, c. Microelectrode ITO arrays with SLBs depicting (inset) microscopy image of patterned arrays (scale bar 100 μm) and corresponding EIS measurements depicting formation of bilayer on ITO arrays [59]. Reprinted with permission from RSC, d. Schematic diagram of membrane-based biosensor based on gold microelectrode array [62]. Reprinted with permission from John Wiley & Sons, e. (Left) schematic diagram showing incorporation of CNT porin in lipid membrane and experimental setup, (right) representative conductance recording observed upon CNT porin incorporation in membrane [63]. Reprinted by permission from Springer Nature: Nature Protocols [63], Copyright (2016).
f. (Left) schematic diagram showing possible scenarios of transport experiment in nanopores (with and without incorporation of α-haemolysin) and corresponding readout [66]. Reprinted with permission from [66], Copyright 2018 American Chemical Society.

The integration of lipid bilayers with electronic devices has followed the same trends highlighted in Boxes 1, 2 with respect to format (e.g. BLM, suspended, tethered etc.) and complexity. A variety of electrode materials have been used along with a number of device formats including simple electrodes, microfabricated electrode arrays and transistors.

Early examples of electrical studies on planar lipid bilayers mainly focused on the interrogation of gramicidin insertion into BLMs, using simple electrode systems [48]. BLMs allow for easy integration between two electrodes or even between the gate and channel of a transistor, as demonstrated with an **organic electrochemical transistor (**OECT, see Glossary) [49]. This device demonstrated the effect of an ion impermeable layer on the gating efficiency of the device which partially recovered when gramicidin channels were added. However, the BLM system is limited to certain classes of lipids and additionally faces stability issues.

Gold is a commonly used electrode material, however, issues with roughness and hydrophobicity have challenged the formation of bilayers on the surface. The flat (hard) surface of gold poses additional challenges. Bilayer quality is acutely affected by substrate energetics [50]. One remedy

is to alter the surface, e.g. via template stripping [51]. Another option is to functionalise the surface with lipids. In an early attempt used to study ion channel transport using bioelectronics, tethered lipid bilayers on gold substrates (bioconjugating thiolipids on gold) were used to monitor K+ ion channel interactions with valinomycin [52]. This study offered an insight into how the sensor substrate affects electrical measurement sensitivity, particularly impedance limitations [53], and paved way for the usage of PEDOT:PSS to overcome the challenge. A similar tethered membrane was used to study the function of reconstituted nicotinic acetylcholine receptors, implicated in various disorders such as epilepsy and cystic fibrosis, via EIS [54].

Palladium and silicon oxide have also been used as substrate materials in the case of bioprotonic devices, which measure and control H+ transport through gramicidin A and alamethicin channel-forming peptides respectively, upon the application of bias on a lipid bilayer [55,56] **(Figure 2A)**. The possibility of inducing voltage gated switching in the alamethicin system and correlating proton transport with membrane permeability has also been demonstrated.

Despite important advances, key issues around bilayer fluidity and protein mobility remain in conventional bioelectronic supports such as gold and silicon dioxide. The proximity of the lipid bilayer to the solid support may inhibit the diffusion of the membrane components through the fluid bilayer [57]. Efforts to improve the fluidity/mobility of the membrane include the incorporation of polymer cushions [27], albeit at the expense of sensitivity.

Emerging technologies and substrates in biomembrane-based bioelectronic devices

Issues around robust SLB formation, sensitivity, scalability and reliability have urged the quest for alternative materials and techniques, as summarised below.

Alternative materials for biomembrane-based bioelectronics devices include indium tin oxide (ITO) and graphene. The optical transparency of graphene **(Figure 2B)** and ITO **(Figure 2C)** enables simultaneous optical and electrical measurements further enhancing the capabilities of the devices. Miniaturisation and parallelisation have also been demonstrated in recent studies [58,59]. However, such substrates need further optimisation if they are to be used in sensors manufactured in industrial settings. In the case of graphene, there are challenges in terms of obtaining large defect-free sheets, while in the case of ITO, the higher capacitance commonly observed in comparison to expected membrane capacitances, indicating the presence of defects in the SLBs [58,60]. Transistor configurations bearing those materials have showed improved characteristics given their inherent amplification of signal transduction, however issues related to protein function on flat rigid surfaces remain [61].

Microfabricated electrodes can provide advantages for biomembrane sensing, in terms of miniaturisation, introduction of nanoscale topography and of course throughput via device arrays. Examples include development of lipid arrays patterned on gold microelectrodes for ion transport studies [62] **(Figure 2D)** and incorporation of carbon nanotube-based **porin** (see Glossary) mimics with ion-selective properties into supported lipid bilayers [63,64] (**Figure 2E)**. Nanotechnology-based techniques such as nanopore sensing, able to capture protein conformation upon binding [65], have been also developed, offering the ability to study TMPs behaviour on a single molecule level [66] **(Figure 2F)**. These studies not only allow for the possibility to build high-throughput systems and screen various experimental conditions, but also probe pore dynamics and protein-lipid interactions at the microscopic level.

Electroactive polymers have recently come to the fore as a highly promising electrode material providing a biomimetic, hydrogel-like environment thus merging the critical parameters of “cushioning” and “sensing” in one substrate. They also have lower impedance, which results in higher sensitivity coupled with improved tolerance to SLB defects. In the following section, we will highlight how a class of electroactive material, also known as organic electronic materials have been proven to meet key challenges in the biomembrane sensing arena, specifically with respect to supported lipid bilayers, with future promise for a breadth of applications.

4. Organic bioelectronics: bridging the signalling and biotic-abiotic compatibility gap

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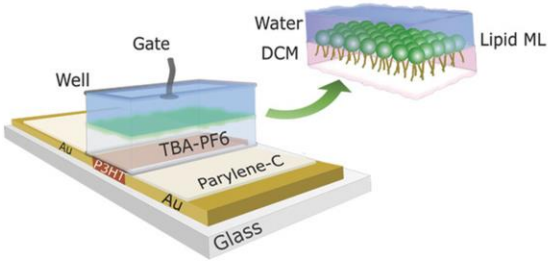

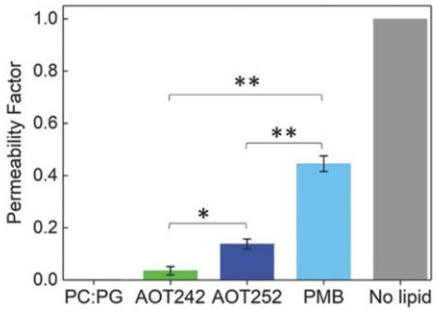

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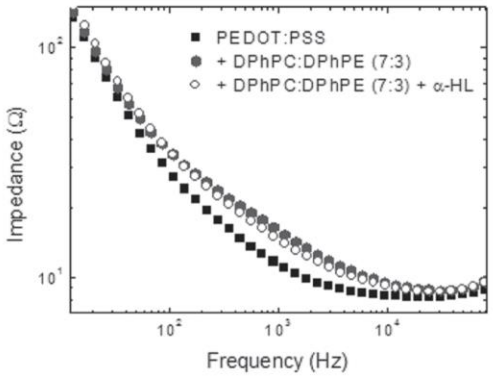

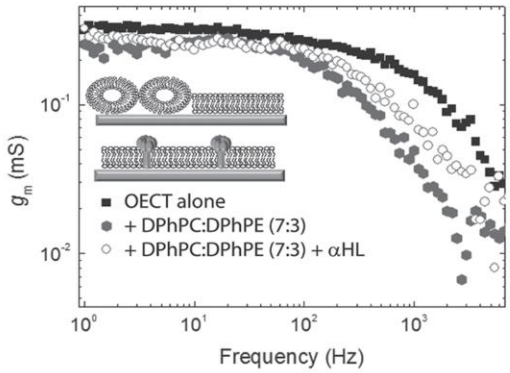

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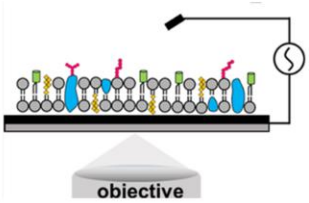

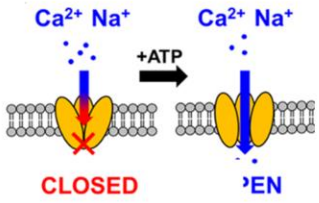

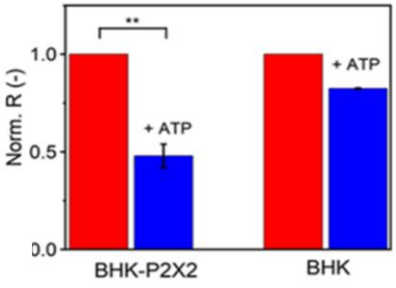

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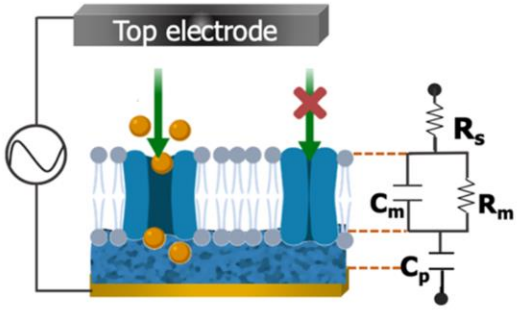

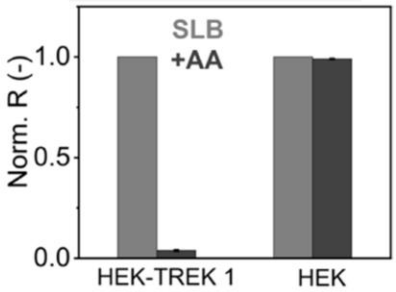

Fig.3. Membrane-based bioelectronic platforms using conducting polymers. a. (Left) Lipid monolayer-based device to study membrane disruption in the presence of an antimicrobial peptide polymyxin B (PMB), (right)) Relative ion permeability of tested compounds polymyxin B (PMB) and antibacterial amine-based oligothiotheramides (AOTs) on lipid monolayer [77]. Reprinted with permission from John Wiley & Sons. b. Bilayer-based PEDOT:PSS transistor device: (left) Formation of lipid bilayer on PEDOT: PSS monitored through EIS upon vesicle rupture of DPhPC:DPhPE vesicles and addition of α-haemolysin respectively, (right) transconductance vs frequency graph of formation of bilayer and subsequent addition of α-haemolysin on an organic electrochemical transistor (OECT) [82]. Reprinted with permission from John Wiley & Sons, c. Biosensor comprising of native mammalian membranes incorporated with functional transmembrane proteins: (Left) Schematic diagram highlighting mechanism of biosensor functioning, (Right) Differences in membrane resistance observed in BHK-P2X2 membrane post interaction with ATP on PEDOT:PSS [85] Reprinted (adapted) with permission from [85] Copyright 2017 American Chemical Society., d. (Left) Schematic diagram of membrane-based biosensor, with HEK-TREK as the TMP and orange molecules indicating cation transport, (right) activation of TREK-1 channels by arachidonic acid (AA) resulting in decrease in membrane resistance [86]. Reprinted (adapted) with permission from [86]. Copyright 2020 American Chemical Society,

Organic electronic materials have proven to be superior to traditional inorganic materials, in improving the communication with biological tissues by providing a more mechanically compliant surface to interface with soft biological structures [67–69]. **Conjugated polymers** (see Glossary) conduct both ionic and electronic currents thereby offering direct ionic (of biological origin) to electronic transduction [70]. Their bulk ionic conductivity, hence low electrical impedance, endows them with high sensitivity in transducing ion fluxes through cell membranes as successfully shown previously with cell layers [71]. Moreover, the inherent optical transparency allows multi-modal transduction, retaining the optical measurement component which is key for biologists. Finally, their versatility in synthesis and post-synthesis processing enables their adaptation in various form-factors, integration in different substrates and device architectures, and incorporation of microfluidics. As such, micro-engineered organic electronic devices can be easily designed to meet the length and time scales of the biological systems in question [72].

Organic electronic devices were initially integrated with lipid bilayers in the context of biosensing, first as a polyaniline-based device for glucose sensing [73] and later, by coupling the channel of a field-effect transistor with a phospholipid bilayer acting as the biorecognition element for biosensing purposes [74], or as a testbed for testing the effect of volatile anaesthetics [75]. Recently, a novel device concept, the **liquid:liquid phase separated** (LiPS, see Glossary) OECT [76], was used to stabilize a lipid monolayer to assess the disruption of a bacterial cell membrane model by an antimicrobial peptide [77] **(Figure 3A),** and subsequently to study the interaction of a mammalian cell membrane model with lidocaine, a local anaesthetic [78]. This system presented a means to study ion flux through the impermeable lipid monolayer as a result of disruption or destabilization, by modulating the electronic current passing through the device. This device platform offers a very robust and highly sensitive measurement system, even sensitive to packing of lipids, likely due to the amplification afforded by the OECT [79], however, the use of single leaflet membrane models limits the studies to membrane processes that do not rely on membrane proteins.

Early attempts to directly interface SLBs with the CP PEDOT:PSS, configured both as electrode and OECT used archaeal lipids allowing the activity of a typical pore forming toxin, **α-haemolysin** (see Glossary), to be monitored, despite the relatively poor quality (in terms of membrane electrical properties) SLB formed. Expanding the library of organic electronic materials, a newly synthesized conjugated polymer based on a naphthalene 1,4,5,8-tetracarboxylic diimide bithiophene backbone functionalised with lysine-based side chains, was shown to facilitate assembly of the zwitterionic lipid vesicles into an SLB [80]. The latter illustrating the ability of organic materials to be designed specifically for purpose. However, both the latter and the former studies highlight that although organic materials bear advantages in terms of mechanical

compliance and volumetric capacitance, their sometimes rough and inhomogeneous surface (dependent on solution/film processing conditions) [81], could limit the adhesion force between the vesicles and the substrate, restricting their ability to rupture and spread spontaneously upon adsorption, as they would optimally do in smooth rigid surfaces [82] **(Figure 3B)**. On the other hand, PEDOT:PSS, as most CPs, provides a hydrogel-like interface owing to its swelling capacity in aqueous electrolytes which favours lipid and protein mobility acting as a cushion. In order to improve SLB formation and yield, SALB, highlighted in an earlier section, has been employed as a universal method that facilitates SLB formation irrespective of the surface charge of the substrate or the vesicles. Both mammalian and bacterial SLBs using phospholipids and cholesterol have been successfully formed on conducting polymer surfaces for drug discovery applications [83,84], as well as for the electrical detection of membrane-binding events by using phospholipids bearing protein recognition elements [84]. Although SALB is seen to be surface agnostic, the solvents used pose limitations around protein functionality and stability restraining protein related studies.

Although SLBs represent a good platform to incorporate functional TMPs, they still pose certain drawbacks in terms of maintaining the complexity of the plasma membrane as described in section 2. Biologically complex cell membranes (described in Box 2) have now been incorporated with organic electronic devices. The major challenge here was triggering rupture of extracellular vesicles on CP substrates, allowing for TMP studies in their native membrane environment. The Daniel and Owens groups have pioneered the integration of these authentic plasma membranes with PEDOT:PSS devices, demonstrating protein mobility *and* functionality. The resulting "authentic" cell membranes on chip have been successfully used for ion channel activity studies [85] **(Figure 3C)** providing a viable alternative to patch clamp whole cell experiments. In this example, an ATP-gated ion channel, P2X2 was overexpressed in baby hamster kidney cells and

the resulting plasma membrane was formed on PEDOT:PSS electrodes preserving orientation and function of the ion-channels as demonstrated both optically and electrically. This represents the first dual mode optical-electrical operation combined with demonstration of ion-channel activity in a native environment, read out using an electronic device [85]. In a follow up study, the effect of two pharmacological compounds on the K+ ion channel, TREK-1, was assessed electrically on native plasma membranes derived from human embryonic kidney cells **(Figure 3D)** [86]. The results were confirmed using two types of electrical measurement, EIS and transistor-based, while simultaneously performing optical studies on the same devices. These studies represent a step change in cell membrane based biosensing, showing true biomimetic communication between biology and device that **i)** facilitates the natural mobility of TMPs, **ii)** maintains their biological activity and response to external stimuli, and **iii)** achieves a native-like membrane environment. These features enhance the native structural and functional properties of the proteins and our ability to sense them and transduce them into observable signals.

Future Outlook

The possibility of integrating native membranes with bioelectronic sensors has opened up a world of opportunities. The fundamentals interactions between components of the cell membrane, and subsequent applications, can now be sensitively characterised, avoiding **difficulties related to live cells**. The journey of biomembrane based sensing has seen two parallel strands evolve; the increased authenticity of cell membranes and the increased biomimicry of the transducer surface, two aspects which converge on highly effective sensing of these complex biological systems. The ability to conduct simultaneous optical and electrical measurements using transparent substrates allows for correlation with established techniques favoured by biologists. Moreover, there is

tremendous potential for scale-up of these miniaturised devices into massive arrays fed by microfluidics.

Although the applications described here are largely for biomedical purposes, this technology is compatible with any mammalian cell type tested so far and now expanding to bacteria [87] and plants (in progress). The latter opens up opportunities for applications beyond drug discovery, in agritech, energy harvesting and environmental applications.

Upcoming work by our team will focus not just on integration of devices with cell membranes from established cell lines, but also from primary human cells (e.g. neurons) for monitoring ion channel function. Antibiotic and phage screening is also being studied using the outer membranes from clinical strains of drug resistant bacteria. In keeping with the times, the detection of enveloped virus fusion with cell membranes is the subject of current studies, using optical methods to track single particle fusion to SLBs [88]. Here, one can imagine a new way to leverage these devices for label-free virus detection. Parallels between enveloped viruses and **extracellular vesicles** (EVs, see Glossary) can also be drawn and so be applied to the devices in a similar fashion [89]. EVs play a role in cell-cell communication, transmitting information and even pathologies [90], and can be subverted as therapies or drug delivery vehicles [91], so their study has become an exciting line of research, with extension to use in CP devices.

The highlighted features of biomembrane-based bioelectronics described in this review will have a significant impact on the research and development phase of **drug discovery**, as thousands of membrane compositions and protein expression combinations, representing conditions on a single-cell level, can be screened in parallel. The **high-throughput nature** of such sensing could also have a positive impact on public health, particularly in the context of pandemics such as Covid-

19, where the possibility of screening thousands of samples simultaneously can better inform epidemiological models and decision making.

In summary, the general integration of electronic (and other types) of sensors with cell membranes is a growing trend fostered by the promise of new translational capabilities. We have highlighted efforts to seamlessly integrate sensing modalities with membranes maintaining native compositions and proportions, providing the most accurate and faithful mimic of a live cell, without compromising on function. Future efforts in this area will use such platforms to enhance our knowledge on how cell membranes interact with outside factors in health and disease that may ultimately aid the translation of this knowledge into clinical actions **(see Outstanding Questions)**

Outstanding Questions

1. Will biomembrane-based bioelectronic devices contribute to the development of highly personalised medicine for chronic conditions such as cancer and neurodegenerative disorders?

2. How quickly will this technology translate into clinical practice and directly impact patient outcomes?

3. How can researchers continue to improve sensitivity while maintaining biological functionality?

4. Can conducting polymers completely replace traditional substrates or will it continue to be a combination of both?

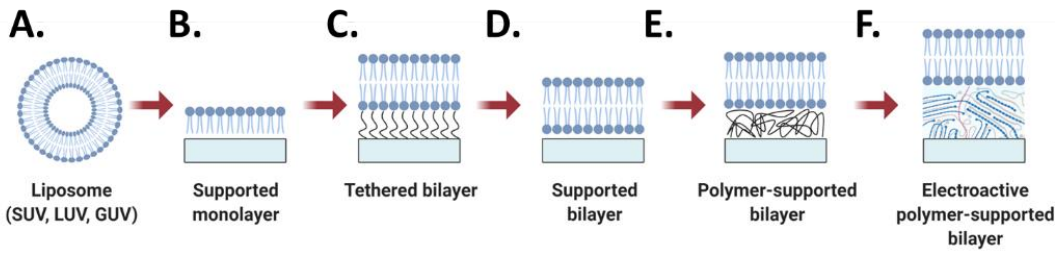

Figure I. Schematic diagram showing process of bilayer fabrication from liposomes highlighting the differences between different types of bilayers

Box 1. Description of the different cell membrane models

Unilamellar vesicles (liposomes, Figure IA, see Glossary) of varying sizes can be formed by a variety of techniques such as extrusion, sonication, freeze-thawing, or electroformation [92–95]. Small unilamellar vesicles (SUVs) are typically less than 100 nm and present highly curved membranes that have been used, for instance, to study the assembly of synaptic vesicles [13]. The next class of vesicles, in terms of size, are large unilamellar vesicles (LUVs) with diameters between 100 nm and 1 μm. LUVs have been used to study various biological processes that occur around this length scale, such as the antimicrobial properties of cationic peptides interacting with model bacterial membranes [96]. The largest and most commonly used liposome models of the plasma membrane, have a diameter ranging anywhere from 1 μm to 200 μm, typically referred to as giant unilamellar vesicles (GUVs). Because of their size, single liposomes are easily observed using optical microscopy, making them convenient for studying membrane dynamics [97]. The large size of GUVs allows direct visualization of various regions within the membrane, enabling membrane biophysics studies such as lipid and protein phase separation [98].

Lipid Monolayers (see Glossary, Figure IB) result from decoupling the two leaflets of the bilayer to study biological interactions with an individual lipid layer. Since biological membranes are compositionally asymmetric, lipids provide a way to overcome some of the constraints of other model membranes, which cannot accurately reproduce this asymmetry. Lipid monolayers can be formed by the Langmuir-monolayer technique at a bi-phasic interface, such as air/water or oil/water [99] or can be tethered on a surface using a linker [34]. Lipid monolayers formed at water-lipid interfaces have been used to study phase separation of proteins [99] as well as interactions of drugs with lipid head groups [14].

Planar Lipid Bilayers (Suspended and Supported, Figure IC-IE) are an extension of the lipid monolayer system and are considered to be a better mimic of the native plasma membrane. Black lipid membranes developed by Muller and Rudin in the 1960s [100] were the first planar lipid bilayers reported and are well-known for single ion channel measurements in combination with advanced electrophysiological techniques [25]. Despite their high electrical sealing properties, these "free-standing", hence unsupported, membranes suffer in terms of robustness and long-term stability [100]. Therefore, solid **supported lipid bilayers** (SLBs, see Glossary), pioneered in the 1980s [32], have emerged as a more stable model system and have been increasingly accepted as the experimental models of choice for biomolecular interaction studies. SLBs are often produced using Langmuir-Blodgett-Schaeffer transfer [32], vesicle fusion [34], or solvent-assisted lipid bilayer formation methods [17]. The hallmark of planar lipid bilayers is that they can be supported on a solid substrate, which in turn can offer a level of tunability by choice of the suitable substrates, an additional advantage for the purpose of biosensing. The majority of the methods to form and subsequently study SLBs rely either on methods that tether the lipids to the surface with a linker to allow the protein to extend below the bilayer, thus limiting the diffusivity of the bilayer, or involve vesicle fusion to surfaces unable to accommodate many functional intracellular domains. The incorporation of proteinaceous or polymeric cushions between the lipids and the substrate has been sought as a way to preserve protein fluidity and functionality. The use of electroactive polymeric substrates has therefore emerged as a very promising alternative to seamlessly interface cell membranes providing a label-free means to directly assess function in a biomimetic environment.

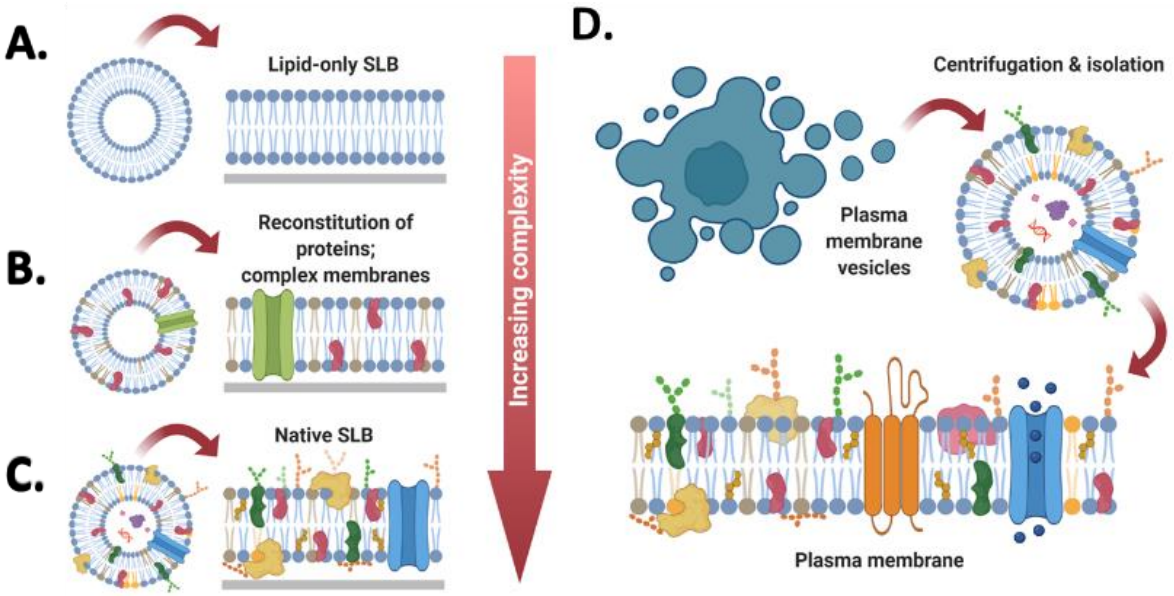

Figure II. Schematic diagram highlighting the cell membrane and the planar lipid bilayer model with increasing complexity

Box 2. The complexity of the cell membrane and the planar lipid bilayer models with increasing complexity

Since their first demonstration SLBs have been widely used in a variety of biotechnological applications and have evolved significantly increasing their biological complexity. The common strategies for incorporation of membrane proteins into SLBs usually involve detergent-based extraction, followed by reconstitution into liposomes [18,101] (Figure IID). However, reconstitution and incorporation approaches in model membranes may influence orientation, and lipid-protein associations that are present in the native membranes (Figure IIB). Additionally, purified phospholipids used in most SLB formulations are unable to capture native lipid-protein associations that are present in cellular membranes. Cell-derived vesicles (blebs) are a promising alternative to study native membrane properties and membrane associated cellular events in a model system [97]. These native membrane vesicles contain a full host of receptors, negating the need to purify and reconstitute membrane proteins. Recently, formation of SLBs using such naturally occurring membranous vesicles, via vesicle fusion, has enabled studies on "authentic" cell membranes that preserve the structure and composition of the cell membrane (Figure IIC).

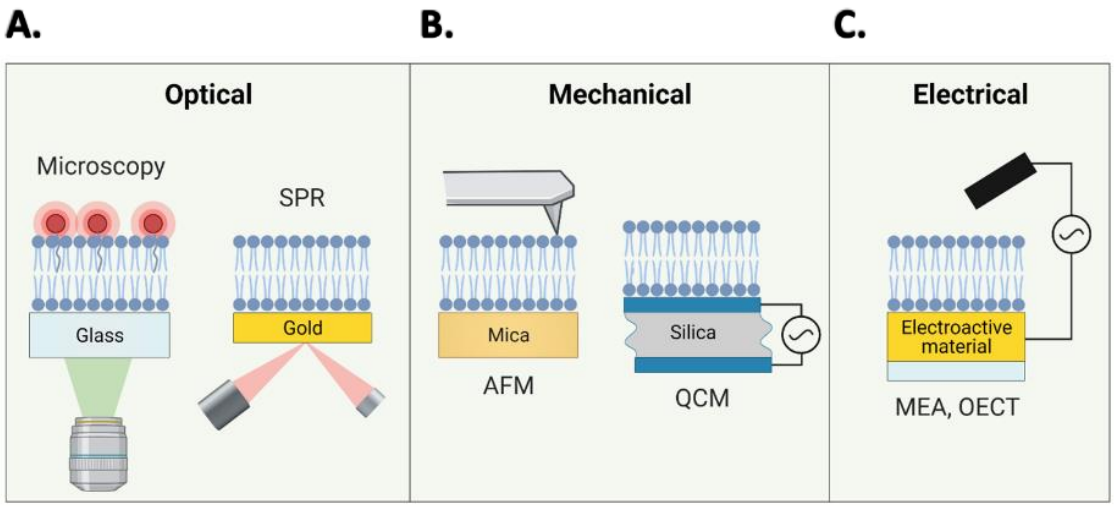

Figure III. Schematic diagram highlighting optical, mechanical and electrical readout mechanisms that can be used to study biological interactions with SLBs

Box 3: Surface- based analytical characterization of supported lipid bilayers

Planar lipid membranes have generally been characterised with a variety of surface-sensitive techniques including atomic force microscopy (AFM), **fluorescence recovery after photo bleaching** (FRAP, see Glossary), total internal reflection fluorescence microscopy (TIRF), quartz crystal microbalance (QCM), and surface plasmon resonance (SPR). The technique used typically dictates the choice of the underlying material/ substrate for interfacing the planar lipid membranes. The most commonly used substrates to date are glass and mica.

Glass (Figure IIIA): Optical techniques like Fluorescence Recovery after Photo bleaching (FRAP) can be carried out on glass, measuring the bilayer fluidity of SLBs [102] while Total Internal Reflection Fluorescence Microscopy (TIRF) is another optical technique that has been used to probe the ordering of lipids in bilayers, of paramount importance in enabling representative protein-membrane interactions in model systems [103]. Although these techniques are crucial in ensuring appropriate quality, coverage and orientation of bilayers, they provide largely qualitative analysis. Moreover, glass as a substrate poses challenges in the formation of SLBs from negatively charged lipids due to electrostatic interactions [104].

Mica (Figure IIIB): Mica, an atomically flat hydrophilic substrate, was used to form SLBs using methods such as Quartz Crystal Microbalance (QCM) and Atomic Force Microscopy (AFM). Mica has occasionally been used in a multitude of characterisation techniques to understand how SLB formation varied based on lipid composition and charge, although mostly for surface characterisation [105].

Electroactive materials (Figure IIIC): Methods such **as electrochemical impedance spectroscopy** (EIS, see Glossary), **cyclic voltammetry** (CV, see Glossary) and transistor-based measurements have been also used to study planar lipid bilayers. A variety of electrically active substrates have been used to interface biomembranes, including inorganic (i.e., gold, silicon oxide, aluminium oxide and indium tin oxide (ITO)) [27,106–108]and organic materials (i.e., graphene, conducting polymers). The latter, in conjunction with surface patterning methods and microfluidics incorporation [109,110], are being increasingly adopted in biosensing and basic biophysical studies. CV and EIS are commonly used for membrane electrical characterisation [111,112], with the latter bearing the advantage of quantifying the electrical changes in the membrane, using the appropriate circuit modelling, upon specific interactions [113]. CV, on the other hand, is particularly useful for understanding electron transport mechanisms in bio-sensing [114]. More recently, lipid bilayers have also been incorporated in transistor setups.

Other complementary analytical techniques: Alongside the aforementioned techniques there is a breadth of analytical techniques that can provide information regarding both composition and structure of the plasma membrane of study which can be complementary to the existing methods. Examples include; X-ray reflectivity/scattering, neutron reflectometry, Raman spectroscopy, and secondary ion mass spectrometry [115–118]. Neutron reflectometry and X-ray can capture bilayers in their "native", fluid state. Surface-enhance Raman spectroscopy (SERS) requires a specific SERS-active substrate. Bilayers remain in their "native", fluid state during the measurements. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) requires freeze-drying samples for analysis.

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Glossary

1. **α-hemolysin:** A pore-forming toxin derived from *Staphylococcus aureus*
2. **Atomic Force Microscope (AFM):** A type of scanning probe microscopy which provides information about surface morphology and mechanical properties of materials
3. **Black Lipid Membrane (BLM)**: An early type of biomimetic bilayer system whose formation is carried out using a solvent such as hexane and which is opaque upon optical observation; the BLM configuration affords the possibility of introducing electrodes on both leaflets of the bilayer.
4. **Conducting polymer (CP):** A class of organic materials that possess the ability to conduct electricity; PEDOT:PSS, mentioned in the paper, is a conducting polymer
5. **Conjugated polymers**: A type of polymer that has alternating single and double bonds in its chemical structure
6. **Cyclic Voltammetry (CV):** An electrochemical technique where the potential of the working electrode is varied with respect to time and the corresponding current response is recorded
7. **Electrochemical Impedance Spectroscopy (EIS):** An electrochemical technique where an AC voltage is applied to a system and the corresponding current is measured in order to calculate the impedance of the system; impedance is regarded as being more complex than resistance.
8. **Extracellular Vesicles (EVs):** Vesicles bound by lipid membranes excreted by cells and non-replicative in nature
9. **Fluorescence Recovery after Photobleaching (FRAP)**: An optical technique where high intensity light from a laser, incident upon a fluorescently labelled region, causes bleaching and subsequent recovery through migration of surrounding fluorescent molecules through diffusion; this is commonly used to monitor the fluidity of lipid bilayers.
10. **Ion channels:** A class of membrane proteins that allow for selective permeation of ions in and out of the cell
11. **Lipid Monolayer**: Biomimetic membrane system representing only the outer lipid leaflet of the bilayer
12. **Liquid Liquid Phase Separation (LiPS):** A phenomenon where a homogeneous liquid demixes into a condensate phase and a dilute phase
13. **Organic Electrochemical Transistor (OECT)**: A thin-film transistor that resembles a field effect transistor (FET) except for the presence of an electrolyte between the channel and gate components
14. **Porins:** A class of membrane proteins that allow for intercellular transport of molecules such as water
15. **Quartz Crystal Microbalance (QCM)**: A characterisation technique that detects change in mass using a quartz crystal; it is commonly used to track SLB formation.
16. **Solvent-assisted Lipid Bilayer (SALB) formation:** A method to form SLBs which involves dissolving the lipid in a solvent such as isopropanol and performing a series of buffer exchange steps to facilitate bilayer formation
17. **Supported Lipid Bilayer (SLB):** Biomimetic membrane system comprising of both the outer and inner lipid leaflets, assembled directly on a substrate such as glass, gold, mica etc.
18. **Surface Plasmon Resonance (SPR):** A label-free optical technique which uses the change in refractive indices upon incidence of light to monitor molecular interactions
19. **Tethered Lipid Bilayer:** Biomimetic membrane system comprising of both the outer and inner lipid leaflets, supported by a connecting molecule such as an oligopeptide or polymer which is further connected to a solid substrate
20. **Transmembrane Protein (TMP):** A protein that spans across both leaflets of the bilayer; these proteins often act as receptors or signalling molecules
21. **Unilamellar Vesicles**: Spherical bodies bounded by one bilayer of lipid headgroups, often enclosing an aqueous liquid

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