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REVIEW ARTICLE

Membranes on nanopores for multiplexed single-transporter analyses

Michael Urban [1] · **Robert Tampé** [1,2]

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Abstract The study of membrane proteins as prime drug targets has led to intensified efforts to characterize their structure and function. With regards to the structural analysis of membrane proteins, there have been considerable technological innovations in cryo-EM and X-ray crystallography, but advancements in the elucidation of membrane protein function, especially on a single-molecule level, have been struggling to bridge from basic science to high-throughput applications. There is a need for advanced biosensor platforms allowing membrane protein-mediated transport and potential suppressor libraries to be characterized. Membrane proteins facilitating the translocation of non-electrogenic substrates particularly suffer from a lack of such techniques to date. Here, we summarize recent developments in the field of membrane protein analysis, with a special focus on micro- and nanostructured platforms for purpose of high-throughput screening using fluorescent read-out systems. Additionally, their use as novel biosensor platforms to elucidate non-electrogenic substrate translocation is described. This overview contains 82 references.

Keywords Biosensors · Membrane transport · Nanopores · Pore-spanning membrane · Suspended lipid bilayer · Membrane protein

✉ Robert Tampé
tampe@em.uni-frankfurt.de

[1] Institute of Biochemistry, Biocenter, Goethe University Frankfurt, Frankfurt, Germany

[2] Cluster of Excellence - Macromolecular Complexes, Goethe University Frankfurt, Frankfurt, Germany

Technological progress in the field of membrane protein research over the last decades focused on the advancement of research on protein function and transmembrane processes in general. Next to structural approaches, the functional characterization of membrane proteins is in particular important due to their potential as drug targets. Understanding and characterizing the mechanisms behind membrane protein activity is the most basic requirement for the development of new drugs specifically targeting this class of proteins.

More than fifty percent of all drugs prescribed today target membrane proteins [1, 2]. This fact underlines their importance for basic and applied research. Yet, the number of novel drugs approved for market release is constantly declining because of high research and developmental expenses and low success rates of potential drug targets [3–5]. Factors leading to this shortcoming are for instance the difficulties and technological deficiencies in the study and screening of membrane proteins, including receptors, transporters, channels, and enzymes. Despite their obvious importance, it is still difficult to prepare membrane proteins in pure and functional state and to obtain sufficient quantities necessary for drug discovery approaches. Substantial efforts have therefore been made in the development of techniques to investigate the function of membrane proteins with the final goal of creating high-throughput screening platforms.

Studies on membrane protein function and transport processes across the membrane barrier struggle with the complexity of native cell membranes. Techniques such as patch clamp are able to directly record ion channel activity in cell membranes or membrane patches [6–8]. However, this method is usually limited to single or sparse numbers of channels at a time. In addition, only ionic fluxes can be recorded. Recent developments have been demonstrated to overcome the limited number of channel recordings per experiment. Techniques such as whole-cell patch clamp or automated

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patch clamp enable systematic screenings for effectors [9–12], but by design this method can still only target proteins translocating ionic substrates.

Alongside the patch clamp methodology, a multitude of biosensor techniques have been developed to implement membranes and membrane proteins into feasible devices. These approaches intend to achieve functional membrane assays at single-transporter level, necessary for drug screening applications and especially important when studying transporters with low turnover numbers and non-electrogenic substrates. Nevertheless, these in vitro systems need to be transferable from the laboratory basic research background to an industrial environment in the end, requiring (i) the ability to be automatable for screening applications, (ii) the use of sparse amounts of membrane protein, which is laborious to isolate, and (iii) the preparation of long-lasting artificial or native-like membranes with high confidence by inexperienced users that allow to perform single- or multiple experimental rounds without losing the sensor interface.

Various platforms for the analysis of membrane protein function and transport processes have been established in different designs. Besides patch clamp, the most widespread method to analyze ion channels includes lipid membranes spanning apertures between two aqueous compartments, so called black lipid membranes (BLMs). Although a successful technique in ion channel research and, by using triblock-copolymer membranes, even applicable for the study of protein facilitated gas transport [13], the shortcomings of this technique are the instability of the membrane (lifetime of only a few minutes to hours) and the requirement of organic solvents.

Recent developments to solve the stability issue of BLMs imply the designed edging or size reduction of apertures [14–16] as well as novel membrane interfaces lacking an aperture [17–21]. The latter approach uses droplet interface bilayers. Here, a high degree of automation and parallelity, a prerequisite for high throughput studies, has been achieved using liquid handling robots or microfluidics [22, 23]. Another approach to increase BLM stability was to encapsulate bilayers into hydrogels. This has proven a feasible method to significantly increase bilayer lifetime, making BLMs a more robust sensor platform [16, 24, 25]. Nevertheless, the remnants of organic solvents in the BLMs, even in trace amounts, may still hinder the integration of most medically relevant, but simultaneously fragile membrane proteins.

In comparison to afore mentioned methodologies, surface based biosensors such as solid-supported lipid bilayers (SLBs) offer an inherent stability and straightforward membrane assembly [26–33]. Their solid-support however is at the same time their main drawback. The limited space between the lipid bilayer and the solid support restricts the functional incorporation and the study of membrane proteins possessing non-membrane-integrated, hydrophilic domains. The minimal aqueous reservoir between the bilayer and the underlying support also restricts the transport of solutes and ions to the proximal side of the membrane. Substrate saturation is rapidly reached, making it very difficult to record electrochemical gradients or transport substrate accumulations [31, 34]. Methodological advances in this field enable the establishment of solid supported membranes (SSMs) on electrode substrates, allowing for electrochemical measurements of inserted membrane proteins [35–40]. Other approaches achieved the decoupling of the bilayer from the underlying solid support, creating so-called tethered lipid bilayers [34, 41–43]. The created space provides incorporation and unrestricted diffusion of membrane proteins, but the aqueous reservoir is still too small to monitor ion or solute transport for longer periods. Further increase of proximal membrane space is possible, using polymer cushions or hydrogels as originally described by Sackmann and Tampé, but their preparation is cumbersome and functional studies are sparse [44–47].

Technological solutions to avoid the membrane/support interface problem include native vesicles or large unilamellar vesicles (LUV) immobilized on a sensor chip surface established by Stamou and Vogel [48–50]. These nanocontainer arrays are already being used as nano-reaction chambers [51, 52], giving single-molecule biophysics researchers a versatile miniaturized toolbox for in vitro assays to study biological processes. Membrane transport processes and signal transduction cascades have been proposed as potential research targets, using the vesicle array technology. It provides a native interface for membrane proteins and can be imaged in a highly parallel manner [53]. A model study demonstrating a successful approach is currently under investigation by Stamou and co-workers. A successful proof-of-concept would drastically increase the applicability of the vesicle array concept towards membrane translocation studies.

Until now however, none of the previously mentioned approaches conjugates all necessary features to study membrane proteins in a highly parallel manner and in a single as well as robust platform [54, 55]. Efficient and feasible techniques for screening applications have to combine convenient handling of membrane formation in a solvent-free environment, membrane stability, and possibly even robustness for commercial applications. Membrane proteins need to be readily insertable and imageable with high sensitivity using either fluorescent or electric readout. Chip arrays are desirable for screening applications as they provide multiple, independent sensing spots in a miniaturized platform, enabling multiplexed sensing and parallel screening via automated readout systems. Finally, the technological platform of choice needs to be cost-efficient compared to already existing, but less informative methods.

To date, a single platform combining all above-mentioned desired features for membrane biosensors is still needed.

Advances in the field of SSMs in combination with nano- and microfabrication techniques however provide a promising approach for the study of membrane transport processes. Micro- and nanoporous sheets or chips bring the fluid membrane interface of black lipid membranes together with the stability of solid supported membranes [32, 56]. Porous supports provide a predominantly solid supported platform. The pore-spanning membrane areas constitute the sensor interface of these platforms, utilizing the transfer of established SSM techniques onto this platform. Both the proximal and distal side of the lipid bilayer are accessible for analysis employing pore-spanning membranes, while their stability is significantly increased through the reduction of aperture size to the nanometer range. Lipid bilayers spanning these nano-apertures have been formed by the Langmuir-Blodgett technique [57], solvent spreading [58], giant unilamellar vesicle (GUV) rupture [59, 60], or LUV fusion and spreading [61–63]. In contrast to the Langmuir-Blodgett technique and solvent spreading, pore-spanning membrane formation through vesicle rupture has the advantage of being a self-organized process with reliable preparative success resulting in solvent-free membranes. The decreased sensor interface dimensions enable higher parallelism of created biosensor platforms for electrogenic and non-electrogenic substrate detection [7, 61, 64–67]. Fluorescence read-out for the analysis of membrane transport processes circumvents the challenging task of further miniaturizing electrical read-out systems.

Nanoporous sheet platforms facilitate the investigation of transport across a membrane barrier between two compartments [68]. Multiple pore-spanning membrane areas contribute in this case to a cumulative translocation effect. This approach however does not allow for single transporter studies, as multiple membrane proteins will be located at the porous sensor interfaces. In contrast, nanoporous chip platforms [14, 61, 69–74] avoid this problem. Developments in this field have been made using aluminum oxide [69, 74, 75] or silicon substrates [60, 61, 76], creating ordered arrays of nanopores. These compact pore arrays are covered by large areas of continuous bilayer membrane. The array design hereby provides multiple parallel signal readout points. Modern microstructuring techniques allow a precise control of solid-state nanopore dimension and arrangement. This becomes important when using multiple sensor substrates in a single experiment or if results have to be reproduced for validation. Experimental conditions, including chip surface and pore properties, have to be reproduced exactly. Consistent quality over multiple production rounds is also a prerequisite for later-on biosensor certification, if used in pharmacological studies.

Recently developed nanoporous substrates demonstrated increased sensor interface stability. Long lasting bilayers spanning micro- and nano-apertures [64, 77] have been produced. In addition, multiple studies have shown that reduction of aperture size and variations in shape are key features in membrane persistence [16, 61, 78]. Compared to microporous supports that rely on pore-spanning membrane formation via painting techniques or rupturing of giant unilamellar vesicles (GUVs), membranes spanning nanopores can be established using large unilamellar vesicles via vesicle rupture and fusion [62]. The benefit of the latter approach resides in the fact that it needs no solvents and allows a straightforward application of membrane proteins reconstituted into LUVs onto the chip surface, which is one of the limiting factors to date.

Further developments of this approach include the design of micro- and nanopore array chips with underlying microcavities [60, 79, 80]. The benefit of these arrayed chips lies in their defined size of the reaction chamber, which specifies the starting conditions via the confinement of a well-defined volume. Read-out of these systems is facilitated via fluorescence, either by pre-loading of the cavities with a fluorophore and subsequent monitoring of the efflux of the dye or via fluorescent changes of ion- or pH-sensitive dyes inside the cavity triggered by substrate translocation (Fig. 1). Enzymatic reactions depending on substrate translocation into the cavities and subsequent activation of a fluorophore can be anticipated, but has not been demonstrated yet. The fluorescent read-out provides the foundation of this technology's parallelism and, using multi-spectral dyes, even multiplex capability. Yet, it simultaneously means the restriction of this approach: the membrane protein of choice is a viable candidate for analysis only if a direct or indirect fluorescent read-out system for the translocation process can be established. The vast amount of labeled, modified, coupled, or substrate depending fluorescent probes spanning the entire spectral regime however provides a rich toolbox to overcome this restriction.

Several approaches already successfully deployed a fluorescent read-out system for the highly parallel study of transmembrane processes using this system. The pore-forming toxin α-hemolysin [61], the mechanosensitive channel of large conductance MscL [64], or the proton transport by the F_0F_1-ATP synthase [81] were investigated, demonstrating the ability of this approach to create high sampling rates due to its parallel and multiplex design, which constitutes a prerequisite for screening applications. An additional profit of nanoporous array chips is the direct application of proteoliposomes to the system, providing their compatibility with most often fragile active transporters. This protein class can especially benefit from this methodological advance, as their most often non-electrogenic transport substrates are not compatible with electrical read-out systems. Further improvements of aluminum oxide or silicon-based chips are so-called glass bottom array designs, where imaging is conducted via inverted air microscopy. Next to its high parallel mode

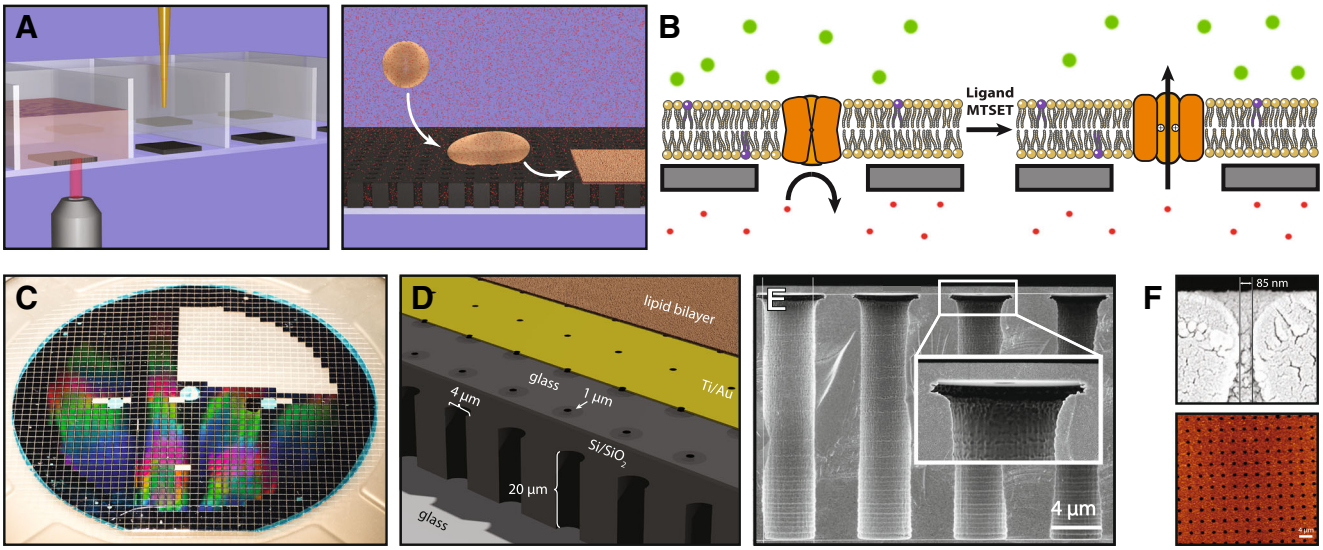

Fig. 1 Principle and design of micro-structured glass bottom chips. **A** Microstructured chips in multi-well sample holders facilitate complete separation of individual chips. The reaction volume is accessed from the top, while data acquisition occurs from below. In combination with motorized microscopes, this setup enables the parallel screening of several chips. **B** Stochastically, only a single membrane protein locates in the pore-spanning region of the membrane across a nanopore forming the biosensor interface. Protein-mediated translocation of a substrate can be followed via fluorescent change. **C** Chip production can be achieved on wafer scale. Approximately 1150 individual chips are fabricated from each wafer with identical properties and quality. **D/E** Chips possess cavity volumes in the femtoliter range and pore diameters ranging from 60 to 1000 nm, depending on the chip type. **F** Nanoporous chip designs possess pores in the lower nm range (60–120 nm). Reprinted, with permission, from [64, 66]

and multiplex capacity, this approach takes advantage from straightforward automation. Motorized microscope setups in combination with contact free air microscopy, as already established for industrial cell culture screening applications, can be readily adapted to the novel sensor platforms. High-throughput membrane translocation studies have been successfully demonstrated for α-hemolysin [61] and MscL protein-mediated translocation [64], viral capsid membrane fusion and cargo release [66], and cell penetrating peptide membrane traversing studies [82], utilizing glass bottom array chips in combination with an automated microscope setup. All of these studies prove the capability of the system to perform highly parallel screening applications with a resolution at single protein or event level, respectively, and thousands of sampling points. Future work will aim at expanding the spectrum of target proteins analyzed on nanopore-spanning membrane chips or arrays, as more complex systems like tripartite transporters and primary as well as secondary active transporters are now within reach.

Surface based sensor platforms appear to be an up-and-coming candidate for the development of functional transmembrane protein activity measurements on the single-molecule level. This technology has the potential to achieve a developmental level making it compatible for screening applications desperately needed in the creation of next generation pharmaceuticals. Platforms using micro- and nano-structured surfaces as sensor interface have taken significant advancement to become a leading technology in the biosensor field. Specifically targeting membrane proteins translocating non-electrogenic substrates inaccessible to most other techniques, they use fluorescent readout to achieve highly parallel data acquisition. Multiple applications have shown the viability of these systems in the characterization of gradually more complex membrane protein facilitated translocation systems. The next phases are the systematic expansion of targets and applications onto these platforms and the first realization of inhibitor screens in combination with automated imaging systems. Further steps to completely bridge from basic to applied research will then be the development of sophisticated software tools to most efficiently analyze the accumulating data, as current systems still rely on the manual evaluation and analysis. Besides the technological advancements of this platform, such are the limiting steps holding back the true power of this approach. This correlation between technological development and data analysis capabilities has already been witnessed in other life science fields, where major breakthroughs always coincided with the simultaneous development of novel software standards to rapidly handle increasing amounts of data most efficiently. The integration of advanced biosensor platforms with improved analysis tools and the continuous extension of applications will hold the key to advance micro- and nanopore technologies towards one of the leading biosensor platforms in basic and pharmaceutical science working on membrane proteins.

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References

1. Yıldırım MA, Goh K-I, Cusick ME, Barabási A-L, Vidal M (2007) Drug—target network. Nat Biotechnol 25(10):1119
2. Arinaminpathy Y, Khurana E, Engelman DM, Gerstein MB (2009) Computational analysis of membrane proteins: the largest class of drug targets. Drug Discov Today 14(23):1130–1135
3. Rawlins MD (2004) Cutting the cost of drug development? Nat Rev Drug Discov 3(4):360–364
4. Nathan C (2004) Antibiotics at the crossroads. Nature 431(7011): 899–902
5. Morgan S, Grootendorst P, Lexchin J, Cunningham C, Greyson D (2011) The cost of drug development: a systematic review. Health Policy 100(1):4–17
6. Hamill OP, Marty A, Neher E, Sakmann B, Sigworth F (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Arch EJP 391(2):85–100
7. Osaki T, Suzuki H, Le Pioufle B, Takeuchi S (2009) Multichannel simultaneous measurements of single-molecule translocation in α-hemolysin nanopore array. Anal Chem 81(24):9866–9870
8. Carrillo L, Cucu B, Bandmann V, Homann U, Hertel B, Hillmer S, Thiel G, Bertl A (2015) High-resolution membrane capacitance measurements for studying endocytosis and exocytosis in yeast. Traffic. doi:10.1111/tra.12275
9. Fertig N, Blick RH, Behrends JC (2002) Whole cell patch clamp recording performed on a planar glass chip. Biophys J 82(6):3056–3062. doi:10.1016/S0006-3495(02)75646-4
10. Stoelzle S, Obergrussberger A, Brüggemann A, Haarmann C, George M, Kettenhofen R, Fertig N (2011) State-of-the-art automated patch clamp devices: heat activation, action potentials, and high throughput in ion channel screening. Front Pharmacol 2
11. Schroeder K, Neagle B, Trezise DJ, Worley J (2003) IonWorks (TM) HT: a new high-throughput electrophysiology measurement platform. J Biomol Screen 8(1):50–64. doi:10.1177/1087057102239667
12. Dunlop J, Bowlby M, Peri R, Vasilyev D, Arias R (2008) High-throughput electrophysiology: an emerging paradigm for ion-channel screening and physiology. Nat Rev Drug Discov 7(4): 358–368
13. Uehlein N, Otto B, Eilingsfeld A, Itel F, Meier W, Kaldenhoff R (2012) Gas-tight triblock-copolymer membranes are converted to CO2 permeable by insertion of plant aquaporins. Sci Rep 2
14. Horn C, Steinem C (2005) Photocurrents generated by bacteriorhodopsin adsorbed on nano-black lipid membranes. Biophys J 89(2): 1046–1054
15. Weiskopf D, Schmitt EK, Klühr MH, Dertinger SK, Steinem C (2007) Micro-BLMs on highly ordered porous silicon substrates: rupture process and lateral mobility. Langmuir 23(18):9134–9139
16. Kalsi S, Powl AM, Wallace BA, Morgan H, de Planque MR (2014) Shaped apertures in photoresist films enhance the lifetime and mechanical stability of suspended lipid bilayers. Biophys J 106(8): 1650–1659
17. Funakoshi K, Suzuki H, Takeuchi S (2006) Lipid bilayer formation by contacting monolayers in a microfluidic device for membrane protein analysis. Anal Chem 78(24):8169–8174
18. Holden MA, Needham D, Bayley H (2007) Functional bionetworks from nanoliter water droplets. J Am Chem Soc 129(27):8650–8655
19. Bayley H, Cronin B, Heron A, Holden MA, Hwang WL, Syeda R, Thompson J, Wallace M (2008) Droplet interface bilayers. Mol Biosyst 4(12):1191–1208
20. Czekalska MA, Kaminski TS, Jakiela S, Sapra KT, Bayley H, Garstecki P (2015) A droplet microfluidic system for sequential generation of lipid bilayers and transmembrane electrical recordings. Lab Chip 15(2):541–548
21. Castell OK, Berridge J, Wallace MI (2012) Quantification of membrane protein inhibition by optical ion flux in a droplet interface bilayer array. Angew Chem Int Ed 51(13):3134–3138
22. Nisisako T, Portonovo SA, Schmidt JJ (2013) Microfluidic passive permeability assay using nanoliter droplet interface lipid bilayers. Analyst 138(22):6793–6800. doi:10.1039/C3AN01314F
23. Poulos JL, Jeon T-J, Damoiseaux R, Gillespie EJ, Bradley KA, Schmidt JJ (2009) Ion channel and toxin measurement using a high throughput lipid membrane platform. Biosensors Bioelectron 24(6): 1806–1810. doi:10.1016/j.bios.2008.08.041
24. Jeon T-J, Malmstadt N, Schmidt JJ (2006) Hydrogel-encapsulated lipid membranes. J Am Chem Soc 128(1):42–43. doi:10.1021/ja056901v
25. X-f K, Cheley S, Rice-Ficht AC, Bayley H (2007) A storable encapsulated bilayer chip containing a single protein nanopore. J Am Chem Soc 129(15):4701–4705. doi:10.1021/ja068654g
26. Tamm LK, McConnell HM (1985) Supported phospholipid bilayers. Biophys J 47(1):105–113
27. Sackmann E (1996) Supported membranes: scientific and practical applications. Science 271(5245):43–48
28. Cremer PS, Boxer SG (1999) Formation and spreading of lipid bilayers on planar glass supports. J Phys Chem B 103(13): 2554–2559
29. Castellana ET, Cremer PS (2006) Solid supported lipid bilayers: from biophysical studies to sensor design. Surf Sci Rep 61(10): 429–444
30. Tanaka M, Sackmann E (2006) Supported membranes as biofunctional interfaces and smart biosensor platforms. Phys Status Solidi A Appl Res 203(14):3452–3462
31. Janshoff A, Steinem C (2006) Transport across artificial membranes—an analytical perspective. Anal Bioanal Chem 385(3): 433–451
32. Reimhult E, Kumar K (2008) Membrane biosensor platforms using nano- and microporous supports. Trends Biotechnol 26(2):82–89. doi:10.1016/j.tibtech.2007.11.004
33. Suzuki H, Takeuchi S (2008) Microtechnologies for membrane protein studies. Anal Bioanal Chem 391(8):2695–2702
34. He L, Robertson JW, Li J, Kärcher I, Schiller SM, Knoll W, Naumann R (2005) Tethered bilayer lipid membranes based on monolayers of thiolipids mixed with a complementary dilution molecule. 1. Incorporation of channel peptides. Langmuir 21(25): 11666–11672
35. Purrucker O, Hillebrandt H, Adlkofer K, Tanaka M (2001) Deposition of highly resistive lipid bilayer on silicon–silicon dioxide electrode and incorporation of gramicidin studied by ac impedance spectroscopy. Electrochim Acta 47(5):791–798
36. Rhoten MC, Burgess JD, Hawkridge FM (2002) The reaction of cytochrome c from different species with cytochrome c oxidase immobilized in an electrode supported lipid bilayer membrane. J Electroanal Chem 534(2):143–150
37. Bin X, Zawisza I, Goddard JD, Lipkowski J (2005) Electrochemical and PM-IRRAS studies of the effect of the static electric field on the structure of the DMPC bilayer supported at a Au (111) electrode surface. Langmuir 21(1):330–347
38. Devadoss A, Palencsár MS, Jiang D, Honkonen ML, Burgess JD (2005) Enzyme modification of platinum microelectrodes for

detection of cholesterol in vesicle lipid bilayer membranes. Anal Chem 77(22):7393–7398
39. Bazzone A, Costa WS, Braner M, Călinescu O, Hatahet L, Fendler K (2013) Introduction to solid supported membrane based electrophysiology. J Vis Exp (75)
40. Mager T, Braner M, Kubsch B, Hatahet L, Alkoby D, Rimon A, Padan E, Fendler K (2013) Differential effects of mutations on the transport properties of the Na+/H+ antiporter NhaA from Escherichia coli. J Biol Chem 288(34):24666–24675
41. Wagner ML, Tamm LK (2000) Tethered polymer-supported planar lipid bilayers for reconstitution of integral membrane proteins: silane-polyethyleneglycol-lipid as a cushion and covalent linker. Biophys J 79(3):1400–1414
42. Naumann CA, Prucker O, Lehmann T, Rühe J, Knoll W, Frank CW (2002) The polymer-supported phospholipid bilayer: tethering as a new approach to substrate-membrane stabilization. Biomacromolecules 3(1):27–35
43. Naumann R, Schiller SM, Giess F, Grohe B, Hartman KB, Kärcher I, Köper I, Lübben J, Vasilev K, Knoll W (2003) Tethered lipid bilayers on ultraflat gold surfaces. Langmuir 19(13):5435–5443
44. Kuhner M, Tampe R, Sackmann E (1994) Lipid mono- and bilayer supported on polymer films: composite polymer-lipid films on solid substrates. Biophys J 67(1):217–226. doi:10.1016/S0006-3495(94)80472-2
45. Hillebrandt H, Wiegand G, Tanaka M, Sackmann E (1999) High electric resistance polymer/lipid composite films on indium-tin-oxide electrodes. Langmuir 15(24):8451–8459
46. Tanaka M, Sackmann E (2005) Polymer-supported membranes as models of the cell surface. Nature 437(7059):656–663
47. Purrucker O, Gönnenwein S, Förtig A, Jordan R, Rusp M, Bärmann M, Moroder L, Sackmann E, Tanaka M (2007) Polymer-tethered membranes as quantitative models for the study of integrin-mediated cell adhesion. Soft Matter 3(3):333–336
48. Stamou D, Duschl C, Delamarche E, Vogel H (2003) Self-assembled microarrays of attoliter molecular vessels. Angew Chem Int Ed 115(45):5738–5741
49. Christensen SM, Stamou D (2007) Surface-based lipid vesicle reactor systems: fabrication and applications. Soft Matter 3(7):828–836
50. Christensen SM, Stamou DG (2010) Sensing-applications of surface-based single vesicle arrays. Sensors 10(12):11352–11368
51. Bolinger P-Y, Stamou D, Vogel H (2004) Integrated nanoreactor systems: triggering the release and mixing of compounds inside single vesicles. J Am Chem Soc 126(28):8594–8595
52. Christensen SM, Bolinger P-Y, Hatzakis NS, Mortensen MW, Stamou D (2012) Mixing subattolitre volumes in a quantitative and highly parallel manner with soft matter nanofluidics. Nat Nanotechnol 7(1):51–55
53. Christensen AL, Lohr C, Christensen SM, Stamou D (2013) Single vesicle biochips for ultra-miniaturized nanoscale fluidics and single molecule bioscience. Lab Chip 13(18):3613–3625
54. Dekker C (2007) Solid-state nanopores. Nat Nanotechnol 2(4):209–215
55. Howorka S, Siwy Z (2009) Nanopore analytics: sensing of single molecules. Chem Soc Rev 38(8):2360–2384
56. Basit H, Gaul V, Maher S, Forster RJ, Keyes TE (2015) Aqueous-filled polymer microcavity arrays: versatile & stable lipid bilayer platforms offering high lateral mobility to incorporated membrane proteins. Analyst 140(9):3012–3018
57. Simon A, Girard-Egrot A, Sauter F, Pudda C, D'Hahan NP, Blum L, Chatelain F, Fuchs A (2007) Formation and stability of a suspended biomimetic lipid bilayer on silicon submicrometer-sized pores. J Colloid Interface Sci 308(2):337–343
58. Han X, Studer A, Sehr H, Geissbühler I, Di Berardino M, Winkler FK, Tiefenauer LX (2007) Nanopore arrays for stable and functional free-standing lipid bilayers. Adv Mater 19(24):4466–4470
59. Schmidt C, Mayer M, Vogel H (2000) A chip-based biosensor for the functional analysis of single ion channels. Angew Chem Int Ed 112(17):3267–3270
60. Buchholz K, Tinazli A, Kleefen A, Dorfner D, Pedone D, Rant U, Tampé R, Abstreiter G, Tornow M (2008) Silicon-on-insulator based nanopore cavity arrays for lipid membrane investigation. Nanotechnology 19(44):445305
61. Kleefen A, Pedone D, Grunwald C, Wei R, Firnkes M, Abstreiter G, Rant U, Tampé R (2010) Multiplexed parallel single transport recordings on nanopore arrays. Nano Lett 10(12):5080–5087
62. Wu H-L, Chen P-Y, Chi C-L, Tsao H-K, Sheng Y-J (2013) Vesicle deposition on hydrophilic solid surfaces. Soft Matter 9(6):1908–1919
63. Kumar K, Isa L, Egner A, Schmidt R, Textor M, Reimhult E (2011) Formation of nanopore-spanning lipid bilayers through liposome fusion. Langmuir 27(17):10920–10928. doi:10.1021/la2019132
64. Urban M, Kleefen A, Mukherjee N, Seelheim P, Windschiegl B, Vor der Brüggen M, Koçer A, Tampé R (2014) Highly parallel transport recordings on a membrane-on-nanopore chip at single molecule resolution. Nano Lett 14(3):1674–1680
65. Kaufeld T, Steinem C, Schmidt CF (2015) Microporous device for local electric recordings on model lipid bilayers. J Phys D Appl Phys 48(2):025401
66. Kusters I, Van Oijen AM, Driessen AJ (2014) Membrane-on-a-chip: microstructured silicon/silicon-dioxide chips for high-throughput screening of membrane transport and viral membrane fusion. ACS Nano 8(4):3380–3392
67. Frese D, Steltenkamp S, Schmitz S, Steinem C (2013) In situ generation of electrochemical gradients across pore-spanning membranes. RSC Adv
68. Danelon C, Perez J-B, Santschi C, Brugger J, Vogel H (2006) Cell membranes suspended across nanoaperture arrays. Langmuir 22(1):22–25
69. Hennesthal C, Drexler J, Steinem C (2002) Membrane-suspended nanocompartments based on ordered pores in alumina. Chemphyschem 3(10):885–889
70. Drexler J, Steinem C (2003) Pore-suspending lipid bilayers on porous alumina investigated by electrical impedance spectroscopy. J Phys Chem B 107(40):11245–11254
71. Römer W, Lam YH, Fischer D, Watts A, Fischer WB, Göring P, Wehrspohn RB, Gösele U, Steinem C (2004) Channel activity of a viral transmembrane peptide in micro-BLMs: Vpu1-32 from HIV-1. J Am Chem Soc 126(49):16267–16274
72. Römer W, Steinem C (2004) Impedance analysis and single-channel recordings on nano-black lipid membranes based on porous alumina. Biophys J 86(2):955–965
73. Schmitt EK, Vrouenraets M, Steinem C (2006) Channel activity of OmpF monitored in nano-BLMs. Biophys J 91(6):2163–2171
74. Steltenkamp S, Müller MM, Deserno M, Hennesthal C, Steinem C, Janshoff A (2006) Mechanical properties of pore-spanning lipid bilayers probed by atomic force microscopy. Biophys J 91(1):217–226
75. Hennesthal C, Steinem C (2000) Pore-spanning lipid bilayers visualized by scanning force microscopy. J Am Chem Soc 122(33):8085–8086
76. Stutzmann M, Garrido JA, Eickhoff M, Brandt MS (2006) Direct biofunctionalization of semiconductors: a survey. Phys Status Solidi A Appl Res 203(14):3424–3437
77. Korman CE, Megens M, Ajo-Franklin CM, Horsley DA (2013) Nanopore-spanning lipid bilayers on silicon nitride membranes that seal and selectively transport ions. Langmuir 29(14):4421–4425. doi:10.1021/la305064j
78. Lazzara TD, Carnarius C, Kocun M, Janshoff A, Steinem C (2011) Separating attoliter-sized compartments using fluid pore-spanning lipid bilayers. ACS Nano 5(9):6935–6944. doi:10.1021/nn201266e

79. Watanabe R, Soga N, Yamanaka T, Noji H (2014) High-throughput formation of lipid bilayer membrane arrays with an asymmetric lipid composition. Sci Rep 4
80. Sumitomo K, McAllister A, Tamba Y, Kashimura Y, Tanaka A, Shinozaki Y, Torimitsu K (2012) Ca2+ ion transport through channels formed by α-hemolysin analyzed using a microwell array on a Si substrate. Biosensors Bioelectron 31(1):445–450. doi:10.1016/j.bios.2011.11.010
81. Watanabe R, Soga N, Fujita D, Tabata KV, Yamauchi L, Kim SH, Asanuma D, Kamiya M, Urano Y, Suga H (2014) Arrayed lipid bilayer chambers allow single-molecule analysis of membrane transporter activity. Nat Commun 5
82. Kolesinska B, Podwysocka DJ, Rueping MA, Seebach D, Kamena F, Walde P, Sauer M, Windschiegl B, Meyer-Ács M, Vor der Brüggen M (2013) Permeation through phospholipid bilayers, skin-cell penetration, plasma stability, and cd spectra of α-and β-oligoproline derivatives. Chem Biodivers 10(1):1–38