Membrane-Suspended Nanopores in Microchip Arrays for Stochastic Transport Recording and Sensing

Tim Diederichs and Robert Tampé*

Institute of Biochemistry, Biocenter, Goethe University Frankfurt, Frankfurt, Germany

The transport of nutrients, xenobiotics, and signaling molecules across biological membranes is essential for life. As gatekeepers of cells, membrane proteins and nanopores are key targets in pharmaceutical research and industry. Multiple techniques help in elucidating, utilizing, or mimicking the function of biological membrane-embedded nanodevices. In particular, the use of DNA origami to construct simple nanopores based on the predictable folding of nucleotides provides a promising direction for innovative sensing and sequencing approaches. Knowledge of translocation characteristics is crucial to link structural design with function. Here, we summarize recent developments and compare features of membrane-embedded nanopores with solid-state analogues. We also describe how their translocation properties are characterized by microchip systems. The recently developed silicon chips, comprising solid-state nanopores of 80 nm connecting femtoliter cavities in combination with vesicle spreading and formation of nanopore-suspended membranes, will pave the way to characterize translocation properties of nanopores and membrane proteins in high-throughput and at single-transporter resolution.

Keywords: DNA nanopores, solid-state nanopores, membrane proteins, membrane transport, silicon chips, biosensors, single-molecule analyses

INTRODUCTION

Nanopores are holes with diameters of a few nanometers that already found multiple applications in basic research and industry facilitating our daily life. They are used for water desalination (O’Hern et al., 2015; Amadei et al., 2017), filtering of ions (Walker et al., 2017; Caglar et al., 2020), gas separation (Dong et al., 2016; Park et al., 2017), biological analysis (Miles et al., 2013), DNA/RNA sensing (Wanunu et al., 2010; Riedl et al., 2015; Xi et al., 2016; Shang et al., 2018), and detection of diverse analytes of biomedical and environmental relevance (Litvinchuk et al., 2007; Howorka and Siwy, 2009; Movileanu, 2009; Reiner et al., 2012; Miles et al., 2013; Stoloff and Wanunu, 2013; Wang et al., 2013). The commercialization of DNA sequencing by Oxford Nanopore Technologies extended the field significantly (Cherf et al., 2012; Manrao et al., 2012; Deamer et al., 2016; Quick et al., 2016).

Nanopores can be classified into two groups based on their material and fabrication procedure: 1) membrane-embedded pores and 2) solid-state pores. The latter can be fabricated with tunable sizes in materials such as silicon derivatives (Li et al., 2003; Chang et al., 2004; Bafna and Soni, 2016; Deng et al., 2016), graphene (Merchant et al., 2010), molybdenum disulfide (MoS2) (Feng et al., 2015), as well as various polymers (Choi et al., 2019). In contrast, membrane-embedded nanopores are assemblies spanning biological or semifluid membranes, typically with diameters below 10 nm.
They are compatible with cell, organelle, and liposome applications and possess a great potential for biological functions and interactions (Banghart et al., 2004; Volgraf et al., 2006; Howorka, 2017). Nevertheless, the number of channels per square area cannot be controlled precisely, and they lack the chemical and environmental stability of their solid-state counterparts (Wanunu et al., 2010; Wang et al., 2011; Yusko et al., 2011; Wei et al., 2012a; Traversi et al., 2013; Heerema and Dekker, 2016; Ding et al., 2020). By reducing the bilayer patches (Urban et al., 2014) and using polymerized lipids (Daly et al., 2006; Heitz et al., 2010) or amphiphilic polymers (Meier et al., 2000; Nardin et al., 2000), the stability of membrane nanopores can be increased.

Membrane-embedded nanopores can either be engineered from biological channels and peptides or can be composed of building blocks of synthetic organic materials (Bayley and Jayasinghe, 2004; Gokel and Negini, 2013; Montenegro et al., 2013; Sakai and Matlile, 2013; Vargas Jentszsch et al., 2013; Zhao et al., 2013). Protein-based nanopores are the current flagships in single-molecule studies and sequencing (Howorka and Siwy, 2009; Quick et al., 2016). They were fine-tuned by genetic engineering to enhance stability (Soskine et al., 2012), remove unnecessary moieties (Chen et al., 2008), and add new functional modules and structures (Moreau et al., 2008), or were modified by specific reactions to reduce current flickering (Spicer and Davis, 2014). Pioneering work in sequencing and sensing was performed with the heptameric pore α-hemolysin. However, the Mycobacterium smegmatis porin A (MspA) (Faller et al., 2004) and the Escherichia coli outer membrane curli secretion protein G (CsgG) (Goyal et al., 2014) replaced the original toxin pore in many applications in the field of DNA sequencing. Other channels, such as the outer membrane proteins OmpG and OmpF (Cowan et al., 1992; Liang and Tammi, 2007; Chen et al., 2008; Fahie et al., 2016; Perez-Rathke et al., 2018), aerolysin (Cao et al., 2016), or cytotoxic cytolsin A (ClyA) (Mueller et al., 2009; Bo et al., 2018), can also be used.

Protein pores cannot be easily engineered from scratch as the folding of a polypeptide chain in the quaternary structure is highly complex, and defined pores of >5 nm are very rare or experimentally challenging. For example, the release of proteins from therapeutic vesicles is yet not possible with biological channels (Mahendran et al., 2017). Here, the recently engineered DNA origami-based nanopores constitute a prosperus alternative, featuring the characteristics of simple self-assembly in a bottom-up fashion (Langecker et al., 2012; Burns et al., 2013; Seifert et al., 2015; Burns et al., 2016; Chichchob et al., 2016; Göpfrich et al., 2016a; Göpfrich et al., 2016b; Howorka, 2016; Krishnan et al., 2016; Diederichs et al., 2019; Thomsen et al., 2019; Chen et al., 2020). DNA nanopores can be precisely designed due to the predictable and programmable folding scheme based on simple base-pairing rules of DNA (Douglas et al., 2009a; Douglas et al., 2009b). De novo design generates structures of >20 nm (Rothenmund, 2006; Seeman, 2010; Pinheiro et al., 2011; Chen et al., 2015; Jones et al., 2015), which are assembled by simple heating and cooling protocols in the classical scaffold and staple approach, according to which a long scaffold strand is folded with shorter staple nucleotides into a DNA structure (Rothenmund, 2006). DNA nanostructures with controlled curvature and twists were generated (Dietz et al., 2009). The first membrane-embedded DNA nanopore was developed by the Simmel group (Langecker et al., 2012) (Figure 1A), directly followed by a six-helix bundle pore of the Howorka lab (Burns et al., 2013) (Figure 1B), likely indicating an independent parallel development. Both structures share a core of six hexagonally arranged DNA duplexes, differing in wall thickness and height (Langecker et al., 2012; Burns et al., 2013). However, also combinations of DNA origami structures or pores with solid-state nanopores or glass capillaries were developed (Bell et al., 2012; Wei et al., 2012b; Hernández-Ainsa et al., 2013; Hernández-Ainsa et al., 2013). For a detailed description see references (Bell and Keyser, 2014; Göpfrich and Keyser, 2019). Different pore widths were constructed, ranging from 0.5 to 9.6 nm (Krishnan et al., 2016; Göpfrich et al., 2016b; Thomsen et al., 2019), even allowing the translocation of folded proteins (Diederichs et al., 2019) or enabling the interaction with tumor cells (Guo et al., 2017). Nanopores with pore diameters of ~4.0 and ~7.5 nm are illustrated in Figures 1C,D (Krishnan et al., 2016; Diederichs et al., 2019). Lipid insertions were achieved by introducing anchors of cholesterol (Langecker et al., 2012; Burns et al., 2013; Burns et al., 2016), porphyrin (Burns et al., 2014; Seifert et al., 2015), or tocopherol (Krishnan et al., 2016). Despite successful incorporation in bilayers, aggregations of multiple pores were observed after attachment of lipophilic groups (Burns and Howorka, 2019; Ohmann et al., 2019). Other modifications such as the removal of negative charges at the backbone (Burns et al., 2013; Burns et al., 2014) or the replacement with neutral peptide nucleic acids were established (Ackermann and Falumok, 2013). By using complementary single strands acting as lock and key at the nanopore entrance, the efflux of small molecules through a DNA nanopore can be controlled, peaking in the discrimination of cargos based on their charges (Burns et al., 2016). The disadvantages of DNA as building blocks are the flexibility and loose packing of the structures so that ions can diffuse through their walls (Maingi et al., 2015; Yoo and Aksimentiev, 2015), which can be partially improved by thickening of the nanopore scaffold (Langecker et al., 2012). It is also reported that small water-filled channels can be formed at the interface between nanopore and membrane, allowing the passage of small molecules (Göpfrich et al., 2016a). The strengths and weaknesses of solid-state and membrane-embedded nanopores are summarized in Figure 2.

Microchip Arrays Suspended By Nanopore-Embedded Biological Membranes

Nanopores are often combined with microchips to ensure parallelization, high-throughput, and single-molecule resolution. Here, micro- or nanocavities are connected via solid-state nanopores with a second aqueous compartment. These nanostructures are fabricated in top-down or bottom-up approaches by photolithography or electron-beam lithography in combination with etching techniques, thermal oxidation, spin coating, and sputtering (Sharma et al., 2006; Buchholz et al., 2008; Herzer et al., 2010; Liebest-Peer et al., 2016; Xu et al., 2019). They
provide benefits of miniaturized sizes, high reliability, parallelization, and biocompatibility (Jeon et al., 2010; Ho et al., 2013; Meng et al., 2014; Santos et al., 2015). By spanning lipid bilayers across these solid connections, translocation processes of membrane-embedded nanopores or membrane proteins became observable. These chip architectures are ideal for fluorescent-based high-throughput screenings as exemplified by α-hemolysin and the mechanosensitive channel of large conductance (MscL) (Kleefen et al., 2010; Urban et al., 2014; Diederichs et al., 2018). Membrane coverage across the nanoportures is achieved by techniques such as lipid painting from organic solvents (Han et al., 2007), vesicle spreading in physiological buffers (Schmidt et al., 2000; Buchholz et al., 2008; Kleefen et al., 2010; Kumar et al., 2011; Wu et al., 2013), or shear-driven membrane formation (Jönsson et al., 2009). The self-organized vesicle spreading approach enabled the use of proteoliposomes resulting in solvent-free membranes harboring complex proteins. Here, the vesicle diameter is critical to successfully ensure coverage by simultaneously avoiding lipid intrusions (Urban et al., 2014; Janshoff and Steinem, 2015). The suspended membrane combines the advantages of increased stability provided by the solid support and high membrane fluidity created by the free-standing patches (Sun et al., 2020). Nevertheless, a tight seal for small ions could not be demonstrated so far. In contrast, painting requires the use of organic solvents, which can harm the function of membrane proteins, however, supports a tight membrane seal impermeable for small ions (Watanabe et al., 2014a).

Femtoliter Cavity-Based Microchips for Optical Recordings

The first cavity-based chips for optical single-channel recordings were developed in the 1990s mainly by the Peters group (Peters et al., 1990; Tschödrich-Rotter et al., 1996; Tschödrich-Rotter and Peters, 1998; Siebrasse and Peters, 2002; Kiskin et al., 2003; Peters, 2003). However, fabrication techniques regarding reliability, accuracy, and parallelism were limited. With the development of advanced fabrication methods, micro- and nanopore arrays with thousands of micro- or nanocavities were constructed and covered by lipid membranes (Buchholz et al., 2008; Sumitomo et al., 2012; Watanabe et al., 2014b). These microchip systems...
allow high-throughput screening applications for individual translocation processes as exemplified by the bacterial pore α-hemolysin (Kleefen et al., 2010; Watanabe et al., 2014a) and MscL (Urban et al., 2014). Applications are not limited to the analysis of channels and transporters, as cell-penetrating peptides (Kolesinska et al., 2013) or viral capsid membrane fusion and cargo release were investigated with cavity-based chips (Kusters et al., 2013). The Noji lab developed carbon fluorine polymer (CYTOP) coated chips by combining microfluidics and sequential liquid injections (Soga et al., 2015; Watanabe et al., 2016). First, an aqueous solution is infused to fill the cavities with substrates, followed by phospholipids dissolved in chloroform, creating a lipid monolayer at the chamber opening due to the hydrophobicity of the coated material. Finally, a second aqueous solution is injected forming a bilayer at the cavity entrance, whereas a monolayer is formed elsewhere (Watanabe, 2020). Asymmetric membranes were generated by introducing a second chloroform-based solution supplemented with different lipid species (Watanabe et al., 2014b). In addition to the bacterial pore α-hemolysin, the F₆F₇-ATP synthase (Watanabe et al., 2014a) and the phospholipid scramblase TMEM16F were examined by this approach (Watanabe et al., 2018b). Based on the advection-diffusion model, the sequential injection of aqueous solutions with various concentrations of analytes allows to produce concentration gradients of encapsulated molecules across the chip (Watanabe et al., 2018a). Recently, the platform was used to measure single-protein activities in high-throughput by encapsulating soluble enzymes in the microchambers (Sakamoto et al., 2020) or creating hybrid cell reactor systems (Moriizumi et al., 2018).

Despite these multiple applications, the use of organic solvent does not allow the investigation of complex and fragile membrane proteins as required for biomedical screenings. In general, the vesicle-spreading method is superior, as delicate transport complexes can be reconstituted beforehand in proteoliposomes followed by SLB formation as shown in Urban et al. (2014). For vesicle spreading, different chip architectures exist, varying in cavity shape, volume, nanopore diameter, and surface roughness. A comparison of two chip designs published by Urban et al. (2014) and Diederichs et al. (2018) is displayed in Figure 3. The latest silicon chip features truncated inverted pyramidal cavities of ~50 fL volumes, which are connected by a solid-state nanopore of 80 nm with a buffer reservoir. The transparent Si₃N₄ top and SiO₂ bottom layers allow readout of both compartments, whereas the second chip architecture displays cylindrical cavities of ~6–15 fL with nanopores ranging in diameter between 70 and 120 nm. A large difference was observed in the surface roughness which is ~3.6 nm for the opaque chip (Figure 3A), whereas the most recent chip design exhibits a surface roughness of ~0.2 nm (Figure 3B). These extremely smooth, low strain surfaces in combination with well-defined and tapered pore edges provide optimal conditions for a high coverage upon membrane spreading.

Membrane-Embedded DNA Nanopores Analyzed by Microchips

The recently designed silicon microcavity array was used to analyze translocation processes of two different DNA nanopores at single-channel resolution in high-throughput. The first investigated DNA pore is composed of six interconnected oligonucleotides forming a channel with a height of ~9.0 nm, a width of ~5.0 nm, and an inner diameter of ~1.8 nm (Diederichs et al., 2021). Cholesterol-functionalized DNA strands were attached to drive membrane insertion. This
six-helix bundle was used to elucidate how translocation is affected by the analytes’ charge and size. Three different organic fluorophores possessing different net charges and sizes were studied. The translocation rate through the negatively charged DNA nanopore decreased with increasing negative net charges of the analytes. The (-1)-charged fluorophore ATTO655 fluxes faster than the (-3)-charged dye FAM, whereas the (-4)-charged complexometric calcium indicator Calcein cannot translocate through the DNA nanopore (Figures 4A,B) (Diederichs et al., 2021). This charge-dependent effect is most likely based on repulsion with the negatively charged pore walls of the DNA backbone. However, only a minor effect of the translocation is assumed for analytes with small numbers of net charges, as the flux of the (-1)-charged ATTO655 is only ~2.5 times faster through the protein pore α-hemolysin, after correction for the cross-sectional area of the pore lumen (Diederichs et al., 2021). Strikingly, the lipid environment affects the translocation properties of the DNA nanopore, as 30 mol% of the negatively charged 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (POPG) limits the
transporters is difficult regarding controllability, stability, and functionalization to pave the way for diagnostic applications. The detection of cancer biomarkers from blood samples derived from DNA-assisted nanopores, which can also be used for the development of drug-delivery systems, is a good example for this (Liu et al., 2018; Ding et al., 2020).

By combining solid-state nanopores with microchip systems, single-molecule resolution can be linked with high-throughput and microfluidics. In future, smaller microchips with drastically reduced volumes will be fabricated allowing to characterize medical relevant transporters or to screen lipid membrane conditions affecting the translocation properties of membrane proteins. A promising direction for optical single-molecule detection with nanopores are zero-mode waveguides (ZMWs). ZMWs are small holes in metal films with sub-wavelength apertures for nanophotonic and sensing applications (Assad et al., 2017; Larkin et al., 2017; Han et al., 2019; Spitzberg et al., 2019) as commercialized by Pacific Biosciences for DNA sequencing (Levene et al., 2003; Foquet et al., 2008; Korlach et al., 2008; Lundyquist et al., 2008; Eid et al., 2009; Metzker, 2010; Uemura et al., 2010). As the wavelength of light is smaller than the nanopore aperture itself, light cannot penetrate in the confined chamber, which results in an exponential decay of the optical field creating a zeptoliter volume (Levene et al., 2003; Foquet et al., 2008). By immobilization of the DNA polymerase at the bottom of these cavities, only photolabeled molecules in the first tens of nanometer can be observed (Korlach et al., 2008; Eid et al., 2009). Sensing applications with ZMWs bear the risk of unspecific binding and biocompatibility, however, protein sensing has recently been demonstrated with palladium ZMWs indicating a promising direction (Klughammer and Dekker, 2021).

**AUTHOR CONTRIBUTIONS**

TD and RT wrote the manuscript. RT conceived and supervised the work.

**FUNDING**

This research was supported by the German Research Foundation (SFB 807, TA 157/12-1, and GRK 1986 to RT), the LOEWE program (DynaMem A3 to RT) and ERC Advanced Grant (789121 to RT).

**ACKNOWLEDGMENTS**

We thank Michael Urban for providing the AFM images of the opaque silicon-on-insulator chip and Elina Nürenberg-Goloub, Ma. Florencia Sanchez, Ralph Wieneke, Andrea Pott, Inga Nold, and all members of the Institute for Biochemistry, Goethe University Frankfurt for discussion and comments.
Metzker, M. L. (2010). Sequencing Technologies - The Next Generation. Frontiers in Nanotechnology | www.frontiersin.org June 2021 | Volume 3 | Article 703673

Merchant, C. A., Healy, K., Wanunu, M., Ray, V., Peterman, N., Bartel, J., et al. (2010). DNA Translocation through Graphene Nanopores. Nano Lett. 10, 2915–2921. doi:10.1021/nl101046e

Metzker, M. L. (2010). Sequencing Technologies - The Next Generation. Nature Rev. Genet. 11, 31–46. doi:10.1038/nrg2626

Miles, B. N., Ivanov, A. P., Wilson, K. A., Dool, J. B. (2013). Single Molecule Sensing with Solid State Novel Nanopore Materials, Methods, and Applications. Chem. Soc. Rev. 42, 15–28. doi:10.1039/c2cs35286a

Montenegro, J., Ghadiri, M. R., and Granja, J. R. (2013). Ion Channel Models Based on Self-Assembling Cyclic Peptide Nanotubes. Acc. Chem. Res. 46, 2955–2965. doi:10.1021/ar400061d

Moreau, C. J., Dupuis, J. P., Revillard, J., Arumugam, K., and Vivaudou, M. (2008). Giant Free-Standing ABA Triblock Copolymer Membranes. Langmuir 24, 1677–1677. doi:10.1021/la801024a

Möleveau, L. (2009). Interrogating Single Proteins through Nanopores: Challenges and Selectivity. Nano Lett. 11, 31

Mohseni, E., Japkung, D., and Edel, J. B. (2012). Disease Detection and Management via Single Nanopore-Based Sensors. Chem. Rev. 112, 6431–6451. doi:10.1021/cr300381m

Riedl, J., Ding, Y., Fleming, A. M., and Burrows, C. J. (2015). Identification of DNA Lesions Using a Third Base Pair for Amplification and Nanopore Sequencing. Nat. Commun. 6, 8807. doi:10.1038/ncomms9807

Rothermund, P. W. K. (2006). Folding DNA to Create Nanoscale Shapes and Patterns. Nature 440, 297–302. doi:10.1038/nature04586

Sakai, N., and Maithe, S. (2013). Synthetic Ion Channels. Langmuir 29, 9031–9040. doi:10.1021/la400716e

Sakamoto, S., Komatsu, T., Watanabe, R., Zhang, Y., Inoue, T., Kagawuchi, M., et al. (2020). Multiplexed Single-Molecule Enzyme Activity Analysis for Counting Disease-Related Proteins in Biological Samples. Sci. Adv. 6, eaay0888. doi:10.1126/sciadv.aay0888

Santos, A., Deen, M. J., and Marsal, L. F. (2015). Low-Cost Fabrication Technologies for Nanostructures: State-Of-The-Art and Potential. Nanotechnology 26, 042001. doi:10.1088/0957-4484/26/4/042001

Schmidt, C., Mayer, M., and Vogel, H. (2000). A Chip-Based Biosensor for the Functional Analysis of Single Ion Channels. Angew. Chem. Int. Ed. 39 (17), 3117–3140. doi:10.1002/1521-3773(20000913)39:17<3117::AID-ANIE3137>3.0.CO;2-d

Seeman, N. C. (2010). Nanomaterials Based on DNA. Annu. Rev. Biochem. 79, 65–87. doi:10.1146/annurev-biochem-060309-102244

Seifert, A., Göpfrich, K., Burns, J. R., Fertig, N., Keyser, U. F., and Howorka, S. (2015). Bilayer-spanning DNA Nanopores with Voltage-Switching between Open and Closed State. ACS Nano 9, 1119–1126. doi:10.1021/nn503943s

Shang, J., Li, Z., Liu, X., Li, D., and Wang, H. (2018). Label-free Sensing of Human α-Oxoguanine DNA Glycosylase Activity with a Nanopore. ACS Sens. 3, 512–518. doi:10.1021/acssensors.7b00954

Sharma, S., Buchholz, K., Luber, S. M., Rant, U., Tornow, M., and Abstreiter, G. (2006). Silicon-On-Insulator Microfluidic Device with Monolithic Sensitivity Integration for µTAS Applications. J. Microelectromech. Syst. 15, 308–313. doi:10.11109/JMEMS.2006.872222

Siebrasse, J. P., and Peters, R. (2002). Rapid Translocation of NTF2 through the Nuclear Pore of Isolated Nuclei and Nuclear Envelopes. EMBO Rep. 3, 887–892. doi:10.1037/embo-reports/klf171

Soga, N., Watanabe, R., and Noji, H. (2015). Attolitre-Sized Lipid Bilayer Chamber Array for Rapid Detection of Single Transporters. Sci. Rep. 5, 11025. doi:10.1038/srep11025

Soskine, M., Biesmans, A., Moeyaert, B., Cheley, S., Bayle, H., and Maglia, G. (2012). An Engineered Clya Nanopore Detects Folded Target Proteins by Selective External Association and Pore Entry. Nano Lett. 12, 4895, 4900. doi:10.1021/nl3024438

Spicer, C. D., and Davis, B. G. (2014). Selective Chemical Protein Modification. Nat. Commun. 5, 4740. doi:10.1038/ncomms5740

Spitzer, J. D., Zrehen, A., van Kooten, X. F., and Meller, A. (2014). Plasmonic-Nanopore Biosensors for Single Molecule-Molecule Detection. Adv. Mater. 31, 1900422. doi:10.1002/adma.201900422

Stoloff, D. H., and Wanunu, M. (2013). Recent Trends in Nanopores for Biotechnology. Curr. Opin. Biotechnol. 24, 699–704. doi:10.1016/j.copbio.2012.11.008

Sumitomo, K., McAllister, A., Tamba, Y., Kashimura, Y., Tanaka, A., Shinohzaki, Y., et al. (2012). Ca2+ Ion Transport through Channels Formed by α-Helical Toxin Pore Reveals its Assembly Mechanism. J. Am. Chem. Soc. 134, 9397–9404. doi:10.1021/ja3053384

Taglialaczuca, M., and Slezifer, I. (2015). Transport Mechanisms in Nanopores and Nanochannels: Can We Mimic Nature? Mater. Today 18, 131–142. doi:10.1016/j.mattod.2014.10.020

Thomson, R. P., Malle, M. G., Ohkholm, A. H., Krishnan, S., Bohr, S.-S.-R., Serensen, R. S., et al. (2019). A Large Size-Selective DNA Nanopore with Sensing Applications. Nat. Commun. 10, 5655. doi:10.1038/s41467-019-13284-1

Traversi, F., Raillo, C., Benamer, S. M., Liu, K., Khlybov, S., Tousun, M., et al. (2013). Detecting the Translocation of DNA through a Nanopore Using Graphene Nanoribbons. Nat. Nanotech 8, 939–945. doi:10.1038/nnano.2013.240

Tschödrich-Rotter, M., Kubitscheck, U., Ugochukwu, G., Buckley, J. T., and Peters, R. (1996). Optical Single-Channel Analysis of the Aerythroid Pore in Erythrocyte Membranes. Biophysical J. 70, 723–732. doi:10.1016/S0006-3495(96)79612-1

Tschödrich-Rotter, M., and Peters, R. (1998). An Optical Method for Recording the Activity of Single Transporters in Membrane Patches. J. Microsc. 192, 114–125. doi:10.1046/j.1365-2818.1998.00411.x
Uemura, S., Atken, C. E., Korfach, J., Flusberg, B. A., Turner, S. W., and Puglisi, J. D. (2010). Real-Time tRNA Transit on Single Translating Ribosomes at Codon Resolution. Nature 464, 1012–1017. doi:10.1038/nature08925

Urban, M., Kleefer, A., Mukherjee, N., Seelheim, P., Windschlegl, B., Vor der Brüggen, M., et al. (2014). Highly Parallel Transport Recordings on a Membrane-On-Nanopore Chip at Single Molecule Resolution. Nano Lett. 14, 1674–1680. doi:10.1021/nl5002873

Vargas Jentszch, A., Hennig, A., Mareda, J., and Matile, S. (2013). Synthetic Ion Transporters that Work with Anion–π Interactions, Halogen Bonds, and Anion-Macródipole Interactions. Acc. Chem. Res. 46, 2791–2800. doi:10.1021/ar400014r

Volgraf, M., Gorostiza, P., Numano, R., Kramer, R. H., Isacoff, E. Y., and Trauner, D. (2006). Allosteric Control of an Ionotropic Glutamate Receptor with an Optical Switch. Nat. Chem. Biol. 2, 47–52. doi:10.1038/nchembio756

Walker, M. I., Ubych, K., Saraswat, V., Chalklen, E. A., Braeuninger-Weimer, P., Volgraf, M., Gorostiza, P., Numano, R., Kramer, R. H., Isacoff, E. Y., and Trauner, D. (2006). Allosteric Control of an Ionotropic Glutamate Receptor with an Optical Switch. Nat. Chem. Biol. 2, 47–52. doi:10.1038/nchembio756

Wang, G., Wang, L., Han, Y., Zhou, S., and Guan, X. (2013). Nanopore Stochastic Detection: Diversity, Sensitivity, and Beyond. Acc. Chem. Res. 46, 2867–2877. doi:10.1021/ar400031x

Wang, Y., Zheng, D., Tan, Q., Wang, M. X., and Gu, L.-Q. (2011). Nanopore-Based Detection of Circulating microRNAs in Lung Cancer Patients. Nat. Nanotechnol. 6, 666–674. doi:10.1038/nnano.2011.147

Wanunu, M., Dadosh, T., Ray, V., Jin, J., McReynolds, L., and Drndić, M. (2010). Rapid Electronic Detection of Probe-Specific MicroRNAs Using Thin Nanopore Sensors. Nat. Nanotechnol. 5, 807–814. doi:10.1038/nnano.2010.202

Watanabe, R., Komatsu, T., Sakamoto, S., Urano, Y., and Noji, H. (2018a). High-Throughput Single-Molecule Bioassay Using Micro-Reactors Arrays with a Concentration Gradient of Target Molecules. Lab. Chip 18, 2849–2853. doi:10.1039/c8lc00535d

Watanabe, R. (2020). Microsystem for the Single Molecule Analysis of Membrane Transport Proteins. Biochim. Biophys. Acta (BBA) - Gen. Subjects 1864, 129330. doi:10.1016/j.bbagenn.2019.03.016

Watanabe, R., Sakuragi, T., Noji, H., and Nagata, S. (2018b). Single-Molecule Analysis of Phospholipid Scrambling by TMEM16F. Proc. Natl. Acad. Sci. USA 115, 3066–3071. doi:10.1073/pnas.1717956115

Watanabe, R., Soga, N., Fujita, D., Tabata, K. V., Yamauchi, I., Hyeon Kim, S., et al. (2014a). Arrayed Lipid Bilayer Chambers Allow Single-Molecule Analysis of Membrane Transporter Activity. Nat. Commun. 5, 4519. doi:10.1038/ncomms5519

Watanabe, R., Soga, N., Harai, M., and Noji, H. (2016). Arrayed Water-In-Oil Droplet Bilayers for Membrane Transport Analysis. Lab. Chip 16, 3043–3048. doi:10.1039/c6lc00155f

Watanabe, R., Soga, N., Yamanaka, T., and Noji, H. (2014b). High-Throughput Formation of Lipid Bilayer Membrane Arrays with an Asymmetric Lipid Composition. Sci. Rep. 4, 7076. doi:10.1038/srep07076

Wei, R., Gatterman, V., Wieneke, R., Tampé, R., and Rant, U. (2012a). Stochastic Sensing of Proteins with Receptor-Modified Solid-State Nanopores. Nat. Nanotechnol. 7, 257–263. doi:10.1038/nnano.2012.24

Wei, R., Martin, T. G., Rant, U., and Dietz, H. (2012b). DNA Origami Gatekeepers for Solid-State Nanopores. Angew. Chem. Int. Ed. 51, 4864–4867. doi:10.1002/anie.20120688

Wu, H.-L., Chen, P.-Y., Chi, C.-L., Tsao, H.-K., and Sheng, Y.-J. (2013). Vesicle Deposition on Hydrophilic Solid Surfaces. Soft Matter 9, 1908–1919. doi:10.1039/c2sm27450g

Xu, Z., Wang, J., Fan, E., You, J., Zhang, S., and Wang, H. (2016). Nanopore-Based Selective Discrimination of microRNAs with Single-Nucleotide Difference Using Locked Nucleic Acid-Modified Probes. Anal. Chem. 88, 10540–10546. doi:10.1021/acs.analchem.6b02620

Xu, Y., Hu, X., Kundu, S., Nag, A., Afsarimanesh, N., Sapra, S., et al. (2019). Silicon-Based Sensors for Biomedical Applications: A Review. Sensors 19, 2908. doi:10.3390/s19122908

Yoo, J., and Aksimentiev, A. (2015). Molecular Dynamics of Membrane-Spanning DNA Channels: Conductance Mechanism, Electro-Osmotic Transport, and Mechanical Gating. J. Phys. Chem. Lett. 6, 4680–4687. doi:10.1021/acs.jpclett.5b01964

Yuuko, E. C., Johnson, J. M., Majd, S., Prangkio, P., Rollings, R. C., Li, J., et al. (2011). Controlling Protein Translocation through Nanopores with Bio-Inspired Fluid Walls. Nat. Nanotechnol. 6, 253–260. doi:10.1038/nnano.2011.12

Zeng, S., Wen, C., Solomon, P., Zhang, S.-L., and Zhang, Z. (2019). Rectification of Protein Translocation in Truncated Pyramidal Nanopores. Nat. Nanotechnol. 14, 1056–1062. doi:10.1038/s41565-019-0549-0

Zhao, Y., Cho, H., Widanapathirana, L., and Zhang, S. (2013). Conformationally Controlled Oligocholate Membrane Transporters: Learning through Water Play. Acc. Chem. Res. 46, 2763–2772. doi:10.1021/ar300337f

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Diederichs and Tampé. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.