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Antibiotic translocation through porins studied in planar lipid bilayers using parallel platforms

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A comparative study of the three different techniques Orbit16, Port-a-Patch and BLM applied for the investigation of antibiotic translocation

Abstract

In general, the method of choice to characterize the conductance properties of channel-forming bacterial porins is electrophysiology. Here, the classical method is to reconstitute single porins into planar lipid bilayers to derive functional information from the observed channel conductance. In addition to an estimated pore size, ion selectivity or transport properties in general are of importance. For the latter question measuring the ion current fluctuation can provide some information about the mode of transport of charged molecules penetrating the proteins. For instance, increasing the external voltage modifies the residence time in the channel: charged molecules with the ability to permeate through channels will travel faster whereas non-permeating molecules get pushed to the constriction zone with enhanced residence time. Here, we are interested in the ability of antibiotics to permeate channels and compare different techniques to reveal fast events.

Keywords: single molecule, nanopore, planar lipid bilayer, antibiotic

Introduction

Emergence of bacterial resistance to common antibiotics has become a worldwide problem. The existing antibiotics face more and more resistant strains while there is a dramatic reduction of new antibiotic classes introduced[1,2]. In Gram-negative bacteria, resistance is often correlated with modification of the porin composition in the outer membrane[3–5]. The European Union recognized the problem and launched a private-public platform called 'New Drugs for Bad Bugs'. The subproject "Translocation" is devoted to the elucidation of the molecular origin of the observed low permeability of antibiotics across the cell wall of Gram-negative bacteria. Understanding the permeability across porins might open new routes for the design of tailor-made molecules with optimized permeation rates. Unfortunately, to date there is no direct assay to quantify permeation across single porins. Previously, we have used the ion-current fluctuation in the absence and presence of antibiotics to elucidate on and off rates[6,7]. Inspection of the ion current fluctuation

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allows conclusion on the interaction of sugars, peptides or antibiotics with the channel[8–10]. However, as outlined previously, the temporal resolution of the translocation experiments is rather limited and there is a need for improvement[11,12]. In that regard, characterizing the kinetic rates by studying translocation of antibiotics through porins using different techniques can give useful information for developing new antibacterial molecules. A further step towards automated screening would then be to upscale the validated procedures to a parallel platform with higher throughput.

Previously, we have shown that the kinetic rates of interactions between the constriction zone inside a channel and different antibiotics can be obtained through an analysis of the ion-current fluctuation in the presence of antibiotics[6]. One particular bottleneck for this type of analysis is the limited time resolution, which does not cover the entire range of possible fast diffusion events. To elucidate the optimal setup, we herein provide a comparative study of the different techniques used in a quantitative manner. In particular, we study the interaction of three beta-lactam antibiotics (cefepime, ceftazidime and imipenem) with the bacterial outer membrane protein OmpF and compare the rate constants obtained for three different types of experimental techniques. We were able to show that the translocation experiments performed were comparable in terms of noise and rates obtained among all the three techniques. We also discuss the potentials and draw backs of each technique to measure antibiotic/porin interaction quantitatively.

Material and Methods

In all experiments, 1,2-Diphytanoyl-*sn*-Glycero-3-Phosphocholine (DPhPC) from Avanti polar lipids, Inc. (Alabaster, AL), was used. Cholesterol, Sorbitol, KCl, chloroform, HEPES and KOH were obtained from Roth (Carl Roth GmbH, Karlsruhe, Germany) and *n*-Octylpolyoxyethylene (OPOE) was obtained from Bachem, Bubendorf, Switzerland. Bio-Beads were obtained from Bio-Rad (Bio-Rad Laboratories GmbH, Munich, Germany). All antibiotics were obtained from SeqChem (Sequoia Research Products) except for Ceftazidime (Sigma-Aldrich).

Experiments on a single channel level were carried out using artificial planar lipid bilayer techniques. Lipid membrane bilayers can be made in a variety of ways, such as painting an aperture with dissolved lipids in an organic solvent[13] (black lipid membrane, BLM), or by slowly rising the buffer level of each half chamber after a lipid monolayer has been formed at the water/air interface (folded bilayer technique). The monolayers were made either from an organic solvent-based mixture of phospholipids[14], or by self-assembly from solvent-free lipid vesicles[15]. These techniques require extra equipment necessary for the high resolution recordings, such as a Faraday cage, an anti-vibration table or an acoustic chamber. An alternative method involves the formation of lipid bilayers on a glass substrate with a µm sized aperture[16]. Stable lipid bilayers are formed by the spreading of giant unilamellar vesicles (GUVs) or proteo-GUVs on the glass surface which form a free-standing portion of lipid bilayer with protein already reconstituted above the hole[16–18]. The latter method was found to be a robust platform which provides a first step towards screening, the next step towards a rapid automated screening would then be to significantly increase the throughput of these experiments. In a recent study, a chip-based microarray (MECA) offered the possibility to perform parallel high-resolution single-molecule analysis[19,20]. This could lead to a potential method to screen antibiotic interactions with the porin and this information eventually could be used for development of new antibiotics.

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In this report, we describe the reconstitution of the outer-membrane channel-forming protein OmpF from Escherichia coli into planar lipid bilayers made by classic folded bilayer technique across a a ≈50 µm aperture (planar lipid bilayer technique) or by bursting GUV's on a glass chip with an about 1µm sized aperture (Port-a-Patch).We were also able to show that specific translocation experiments can easily be upscaled using the micro electrode array (MECA) chips up to sixteen-fold (Orbit 16).

Planar lipid bilayer (BLM) assays

In the so-called solvent-free method suggested by Montal-Mueller[14], the lipids are spread with an organic solvent on top of an aqueous buffer. The lipid bilayer is generated from two monolayers over a Teflon hole with a diameter of 50-100 µm. The Teflon hole is pre-painted with an organic solvent which typically is hexadecane. The advantage of this technique is that the content of each lipid monolayer can be controlled. This gives the possibility to form an asymmetric membrane by spreading a second lipid layer on the other compartment and raising both water levels after spreading of the lipids. After lipid membrane formation, reconstitution is usually initiated by adding very small amounts of protein from stock solutions, which contain a detergent at a concentration that is higher than the critical micelle concentration (CMC). Single channel reconstitution is reproducibly obtained by optimizing the concentration of the protein in a stock solution. Obviously, the larger the lipid membrane area is, the higher is the probability that a protein will be incorporated. OmpF was reconstituted by adding 0.1 µl of purified protein into the *cis* chamber containing 250 µL electrolyte solution (1M KCl, 20 mM MES pH 6) at a final concentration of 60 ng/ml. There is a strong indication that when the protein is added to the *cis* side the OmpF porin inserts with periplasmic loop first, i.e the extracellular loops face the *cis* side (reference/grounded side) and the periplasmic loops face the trans side of the chamber (live side; potential applied on this electrode). The orientation is determined based on asymmetry of the channel conductance at positive and negative voltages.

Microelectrode cavity array, MECA16 chips

MECA16 chips were prepared following the protocol described in Baaken et al. [20,21]. Briefly, to define the metal structures (gold contact pads, strip lines and electrodes) an image reversal photoresist (AZ 5214E, Microchemicals GmbH, Ulm, Germany) was lithographically patterned on a glass substrate, followed by deposition of a 20 nm chromium adhesion layer and a gold-layer of 200 nm using electron-beam evaporation. Subsequently a standard lift-off process was performed to remove excess metal. SU-8 3025 (MicroChem, Newton, MA, USA) was spun onto the substrate to a thickness of 20 µm and lithographically structured to form cavities at the position of the electrodes and insulate all other gold layers except the contact pads. Unless stated otherwise cavities had a diameter of 50 µm. Ag was electrochemically deposited on the electrodes from an $AgNO_3$ solution. A layer of AgCl was formed by anodizing in 150 mM KCl.

Bilayer formation was performed as described in Del Rio et al.[19]. Briefly, 150µl of electrolyte solution (1 M KCl, 20 mM MES, pH6) was added to the measurement chamber of the Orbit 16 System (Nanion Technologies GmbH, Munich, Germany). For the automated formation of bilayers in parallel on the 16 cavities, a small amount (approx. 0.1 µl of DPhPC at 5 mg/ml in octane) was applied beside the micro cavities to the chip surface. Subsequently, the counter magnet was turned repeatedly (up to 10 times) at a speed of 45-180°/s to evenly spread the lipid-solvent mixture over the surface, leading to an increasing fraction of the cavities being electrically sealed. Using the MECA chips the

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orientation can be controlled in the same way as the BLM technique. When using the MECA chip, one or two microliter of OmpF porin micelle suspension from 2 ng/ml with 0.5% octyl-POE is added on top of the chip (the grounded side) and the incorporation is achieved by applying a transmembrane voltage of ±200 mV.

Free standing bilayers on microstructured borosilicate glass chip

Planar lipid bilayers were obtained from Giant Unilamellar Vesicles (GUVs) prepared by using the electroformation method[17] in an indium-tin oxide (ITO) coated glass chamber connected to the Nanion Vesicle Prep Pro setup (Nanion Technologies GmbH, Munich, Germany). The purified OmpF (150 µg/ml) in 1% Octyl-POE was then reconstituted into GUVs as described previously[11,17]. Briefly, the GUVs prepared by electroformation were incubated with the porins solubilized in detergent at a concentration of 1 to 5 µg/ml. After incubation the detergent Octyl-POE was removed using Biobeads® SM-2 (Bio-Rad) at 40 mg/ml. The Bio-Beads were discarded after centrifugation and the protein containing GUVs could be used immediately.

For formation of a planar lipid bilayer containing proteins, 5 µL of the proteoliposomes solution (1-5 µg/ml) was pipetted in 5 µL of electrolyte solution containing 1M KCL, 20 mM MES pH 6 onto the microstructured glass chip (grounded side) with an aperture of approximately 1 µm in diameter. The vesicles burst when they touch the glass surface of the chip forming a planar lipid bilayer and additional suction was applied to patch GUV's in this aperture. The orientation of OmpF in lipid bilayer in this case is not very clear since the proteo-GUV's bursting on the aperture could contain OmpF in both orientations.

Data recording and analysis

Kinetic parameters can be derived from ion current fluctuation as described previously[6,22] . Briefly, at low concentration (c), $c \ll k_{off}/k_{on}$, the characteristic time is close to the average residence time of the drug τ thus allowing us to use the following equations: $\tau \approx \tau_r = k_{off}^{-1}$, k_{off} being the dissociation rate constant and the inverse of the dwell time τ_r and $k_{on} = \nu/(3[c])$, k_{on} being the association rate constant where ν is the number of binding events and $[c]$ is the antibiotic concentration. As the data were filtered with a Bessel filter at a cut-off frequency (-3 dB attenuation) of f_c = 10 kHz and hence a filter rise time of $\tau_{rise} = 0.33\ f_c^{-1} = 33$ µs[23], only events displaying a dwell time of τ = 30-50 µs or more were taken into consideration for further evaluation. (this cut off is based on the type of events observed, analysis parameters are chosen where there is no underestimation of amplitude of events observed)

Here k_{on}^{cis} is the association rate for antibiotic being added to the *cis* side (extracellular side of protein) and the k_{on}^{trans} is the association rate obtained for antibiotic being added to the *trans* side of the protein.

Planar lipid bilayer assays (BLM)

Ion current was recorded using an Axopatch 200B (Axon instruments) amplifier and the signal was filtered by a low-pass 4-pole Bessel filter at 10 kHz and sampled at a frequency of 50 kHz. The analysis was done by counting single events using the Clampfit 10 software as described above. The

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experiments were performed in 1 M KCl, 20 mM MES pH 6 solution for all the antibiotics unless otherwise specified. Antibiotic stock solutions were made up to 30 mM in 1 M KCl, 20 mM MES pH 6 and diluted into chamber in a concentration dependent manner.

Port-a-Patch

Patch clamp experiments were performed with the Port-a-Patch automated patch clamp system (Nanion Technologies GmbH, Munich, Germany), using borosilicate glass chips. Experiments were done in symmetric solutions of 1 M KCl, 20 mM MES pH 6. Application of all solutions was achieved using an automated external and internal perfusion system. Currents were recorded with an Axopatch 200B (Axon instruments) amplifier and the signal was filtered by a low-pass 4-pole Bessel filter at 10 kHz and sampled at a frequency of 50 kHz. The analysis was done by counting of single events using the Clampfit 10 software as described above.

MECA:

A single channel amplifier (Axopatch 200B, Molecular Devices, Sunnyvale, CA, USA) was connected to the multiplexer electronics port of the Orbit 16 system. The 16 bilayers on the MECA chip were scanned for insertions of porins. Bilayers which showed a successful reconstituted single protein were then recorded at a sampling rate of 50 kHz and filtered at 10 kHz using a 4-pole Bessel filter integrated in the amplifier. Data were analysed using Clampfit (Molecular Devices, Sunnyvale, CA, USA).

Results

To elucidate the information content of each platform with respect to antibiotic translocation we selected OmpF, the most abundant porin in *E. coli*'s outer membrane. This channel is well investigated and exhibits a slight conductance asymmetry allowing to conclude on the probability for an oriented insertion. We chose commonly used antibiotics: imipenem, a member of the carbapenem class of antibiotics, cefazidime and cefepime, members of the third and the fourth generation antibiotics cephalosporins respectively to study their interaction with the porin and further compare all three techniques.

Planar lipid bilayers were formed following optimized respective protocols as described in materials and methods section. To ensure the comparability of the data recorded, all experiments were carried out using the same conditions (as specified in materials and methods) in each platform.

The best signal to noise ratio is obtained on single channel recordings and thus allows the best comparison. In a first series of measurements we determined the conductance of OmpF in 1 M KCl to be G_{OmpF} = 4 nS which decreases at transmembrane potentials exceeding V_m = ±150 mV in a stepwise manner corresponding to successive monomer closures. The characteristic of OmpF, conductance (4 ± 0.2 nS), critical voltage gating (150 -199 mV) , subconductances (at –ve voltage very short flickering were observed as observed in figure 2, did not change significantly in "virtual" solvent free (BLM), solvent free (microstructured glass chip) and solvent containing bilayers (MECA chip) as described previously [24].

A detailed look on respective techniques reveals an overall root-mean-square (RMS) noise (calculated from standard deviation of mean of the ion current) level of the open pore at a

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transmembrane voltage of U_m=50 mV of 2.6 pA (figure 2a) for BLM technique compared to the microstructured glass chip (Port-a-Patch), which was calculated to be 1.6 pA at 10 kHz Bessel filter (figure 2b). The RMS noise in the parallel automated bilayers using the MECA chips, was calculated to be 2.3 pA at 10 kHz Bessel filter (figure 2c).

In a second series of measurements we were interested in the kinetics of antibiotic penetration. Application of a transmembrane voltage establishes an ion current through the channel and, in the absence of antibiotics, no visible current blockages are detected (figure 2). Addition of antibiotics causes fluctuation in the ion current reflecting the possible channel-drug interactions. Addition of Imipenem, a zwitterionic carbapenem antibiotic, caused rapid blockages of the ionic current through the monomer (figure 3). The number of blockages increased with increasing concentration of the antibiotic. The three platforms showed similar kinetics for imipenem in the OmpF pore (within the statistical error) calculated and reported in the Table 1. Apart from observing ion current flickering we also observed a huge increase in noise in presence of imipenem (figure 4B). The noise level was calculated to be 5.4, 5.8 and 5.7 pA for the BLM technique, the microstructured glass chip and the MECA chip containing 16 bilayers in parallel, respectively. Figure 4A shows a single event caused by imipenem blocking the channel at zoomed in time scale for BLM and the corresponding dwell time histogram for the determination of the dwell time t (inset). Both Orbit 16 and Port- a-Patch displayed similar noise levels and parameters of interaction in presence of imipenem with OmpF channel as seen in power spectrum from both systems compared to BLM (Supplementary figure 1)

Interactions of OmpF with cefepime, a zwitterionic cephalosporin (s. Fig. 5 a), were also detected, but the blockage events were shorter and less frequent than those caused by imipenem when antibiotic was added to the *cis* side (electrical ground). Table 1 shows the kinetics calculated for cefepime in OmpF for all three platforms. In contrast, the addition of cefepime to the *trans* side lead to an increase of k_{on} of about a factor of 30 (s.Fig. 5b) compared to the addition to the *cis* side. This asymmetry in the kinetics was used in further experiments as an indicator for the orientation of the porin in the lipid membrane. As a result, in 80% of the cases (n=6) in the BLM experiments the pore was inserted in the lipid membrane with an orientation leading to rather low k_{on} rates. In comparison, this orientation was observed in 50% of the experiments with the MECAs and in 60% of the cases with the Port-a-Patch, indicating a slightly different distribution of porin orientation in between the BLM and the two other systems. Subsequent addition of cefepime to the same channel on the *trans* side caused well resolved ion current fluctuations with all bilayer forming methods. For MECA chips the kinetics where calculated for the cases of ion channel orientation exhibiting high current fluctuation in presence of cefepime.

Addition of ceftazidime to a single OmpF channel causes partial ion current blockages in all the three platforms (Figure 6). Figure 6 shows a scatter plot of the amplitudes of all the single blocking events counted over the dwell time derived from measurements with the three systems. BLM and Port-a-Patch show similar characteristics where both full monomer blockages about 60 pA amplitude events and short events around 30 pA amplitude events are observed in contrast to Orbit 16 where only these short events were observed. In this case as well, the rate constants obtained in all three platforms were comparable. Within all the experiments reported here the minimum residence time of the antibiotic in the porin which could be measured was τ=30 µs, due to the limited availability of diverse filter settings in the range from >10 kHz to <100 kHz of the single channel amplifiers used. Table 1 summarizes the kinetics for 3 antibiotics (cefepime, ceftazidime and imipenem).

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Discussion

Electrophysiology is a technique to quantify antibiotic-pore interaction at the single molecule level by measuring fluctuations in the ion current caused by antibiotic molecules residing within a single pore[6,11,25]. In this study we use OmpF, an outer membrane protein of *E. coli*, as a model porin to investigate the interactions of beta-lactam antibiotics and compare the kinetics obtained from three experimental platforms and methods of bilayer formation respectively: Solvent free lipid bilayer technique (BLM), Port-a-Patch and Orbit 16. We obtained kinetic parameters of cefepime, ceftazidime and imipenem with the OmpF channel. Here we observed that experiments conducted on the three different platforms gave rise to comparable data sets (Table 1). Addition of imipenem to a single OmpF channel causes an increase of ion current noise and well resolved monomer blockages (figure 3 and 4B). The latter suggests that at least 50% of the events correspond to translocation events, which correlates well to a report by Yoshimura *et al.* in which high diffusion rates of imipenem through OmpF in permeation assays were observed[26]. In contrast, in a recent study with OmpPst1 porin, a homologue of *E. coli* OmpF porin from *Providencia stuartii*, no well-resolved events were observed[9], although the addition of imipinem resulted in a similar increase in noise and reduction of average conductance found with OmpF and imipenem . A liposome permeation assay was employed in case of OmpPst1, which suggested binding/poor permeation of imipenem through this porin. This indicates strongly that the increased noise in the ion current and the reduction of conductance observed with OmpF after addition of imipinem are a result of an interaction of imipenem with the protein. As yet, this cannot be correlated with the effectivity of permeation or translocation of imipinem through the porins. For future experiments, the origin of the noise increase is an interesting problem to solve, which might require a better method for quantitative analysis, apart from single event analysis employed here. However, the main purpose of this study was to compare the kinetics/interaction of imipenem with OmpF pore in different systems to see whether an improved signal to noise ratio (e. g. as obtained with the Port-a-Patch) would give rise to different kinetics, but as it can be seen from table 1 this was not the case.

In case of cefepime, we observed asymmetric kinetics where a prominent effect was observed with *trans* addition compared to *cis* addition (figure 5). BLM membrane technique is taken as a reference for the orientation of the porin (as described in results section) and asymmetric kinetics observed by asymmetric cefepime addition due to accessibility of antibiotic addition on both sides of the membrane. On the Port-a-Patch, although providing accessibility to both sides of the membrane, the orientation of the porin seems to be random which is also observed for MECA chips. In this context, the orientation of the porin seems to play an important role in concluding on the kinetics which is probably physiologically relevant (k_{on}^{cis}; as described in methods section) and not misinterpreted because of a different orientation in lipid bilayer. Another parameter which could change the absolute rate constants are the parameters used in Clampfit for single event analysis e.g. a significant change in kinetics can be obtained due to different dead time/ignore duration used. Table 1 has been calculated by using same parameters in all the three platforms (details in materials and methods) for single event counting, changing the ignore duration/ dead time (from 30 µs to 50 µs) led to different kinetic parameters especially the association rates by factor of 3 irrespective of the system/platform (supplementary table 1). This highlights at least in case of cefepime that many events below 50 µs of residence time were observed and were missed in calculation in supplementary Table 1 and 30 µs dead time seems to be more optimum in this case. Although we

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present the kinetics mainly at an ignore time of 30 µs since this can vary mainly to count the un-attenuated events that mostly reach the full amplitudes [23].

Apart from cefepime and imipenem measured with OmpF, we investigated and compared the effect of ceftazidime on OmpF in all three systems. For ceftazidime, since we observed partial ion current blockages (Figure 6), probably using Port-a-Patch, we could get a better signal to noise ratio and hence a slightly better resolution due to small sized aperture about 1 µm compared to ≥50 µm apertures in BLM. As seen in table 1, the kinetics obtained using single event analysis for all the three systems are identical in the error range, leaving the scope for an instrument needed with a much higher resolution to catch all these fast events. As seen in Figure 6, we observe a scatter of events with amplitude about 30 pA to 60 pA in Port-a-Patch as well as BLM, unlike Orbit 16 where the distribution does not have these full monomer blockages. This might be a result of a slightly higher temperature in the Orbit device (27°C) leading to faster kinetics and, thus, shorter dwell times. One way to catch the fast events could be measuring antibiotic kinetics at lower temperature, BLM[27] and Port-a-Patch offer such a possibility (Table 2). Port-a-Patch/Orbit 16 in this sense also provide a more stable platform in terms of high stability in lipid bilayers formed compared to BLM (Table 2).

Another issue we address in this study is the possibility to increase the throughput of the experiments to enable screening of antibiotics to suggest a trend on efficiency of translocation through porins. Micro-electrode array (MECA) chips offer such a prospect to upscale the system sixteen-fold with a throughput on average up to *n*=7-8 bilayers with single pore, making it a medium throughput platform compared to BLM or Port-a-Patch, which are low throughput platforms (Table 2). Another requirement for successful screening of several compounds is the usage of very little of material, in this respect Port-a-Patch provides promising platform compared to BLM (Table 2). Another aspect for drug screening is the portability of instrument , where Port-a-Patch /Orbit 16 do not require other accessories such as a Faraday cage to record high resolution traces The BLM apparatus requires tedious building of a Faraday cage on an anti-vibration table , acoustic isolation and proper grounding of the instrument (figure 1).

Conclusion

A single porin reconstituted into a planar lipid bilayer embedded into an electrophysiological setup can be used to study the interactions between the protein and single antibiotic molecules. Three different methods are frequently used for the formation of such a lipid bilayer: painting from lipid solution, the Montal-Mueller technique and vesicle fusion to a hydrophilic surface. In this study, three experimental setups: a black lipid membrane workstation, the Port-a-Patch and the Orbit16 (both from Nanion Technologies), each of them using one particular method of bilayer formation, are compared in regard of the quantitative information yield derived from antibiotic – porin experiments. From studies investigating interaction of 3 beta-lactam antibiotics imipenem, cefepime and ceftazidime with the model pore OmpF using BLM, Port-a-Patch and Orbit 16 (figure 1), we observe that the RMS noise values of the open pore ion currents are comparable: BLM: 2.6 pA; Port-a-Patch: 1.6 pA and Orbit 16: 2.3 pA. The rate constants obtained from the three different platforms are similar, revealing an equivalent time and current resolution of all the setups at a filter cut off frequency of 10 kHz. An increase of the bandwidth could possibly resolve the current fluctuations obtained with ceftazidime more precisely. A drastic reduction of the bilayer size (Ø 1µm, Port-a-Patch) compared to larger bilayers (Ø50µm, Orbit 16 and BLM) does not improve significantly the

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signal to noise ratio and the resolution of the blocking events at the filter frequencies used here. However smaller diameters most probably will play a significant role in future experiments at higher bandwidths which could also facilitate further investigations regarding the increased noise in the ion current of OmpF in presence of Imipenem. However it is more likely that an alternate analysis method to quantify this phenomenon is needed. Aspects like orientation of protein in relation to the side of addition of the antibiotics play an important role in obtaining relevant kinetics (antibiotic flux from extracellular to periplasmic side); a point which is highlighted in case of cefepime.

We conclude that BLM and Port-a-Patch are viable platforms for the quantification of antibiotic-pore interactions at lower experimental throughput. The Orbit 16 is suitable for medium experimental throughput and has the potential for further upscaling towards a higher throughput screening device.

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Tables

Table 1: Kinetic properties found for the interaction of a single OmpF protein and three different antibiotics. (Analysis performed with dead time/ignore duration as 30 µs: conditions: 1M KCl, 20mM MES pH 6, at -50 mV for cis addition and +50mV for trans addition) 1 Calculated from -50 mV (no much effect at +50mV)

Antibiotic	Method	k_{on}^{cis} [M^{-1} s^{-1}]	k_{on}^{trans} [M^{-1} s^{-1}]	k_{off} [s^{-1}]
Cefepime	BLM	300	9.600 ± 200	20.000
	Orbit 16	100*	6.400 ± 600	13.000
	Port-a-Patch	1.200 ± 400	9.500 ± 700	15.300
Ceftazidime	BLM	1.500 ± 300	200	25.000
	Orbit 16	1.800 ± 600	220 ± 100	20.000
	Port-a-Patch	2100 ± 300	350 ± 100	17.000
Imipenem	BLM	1.100 ± 100	600 ± 60	16.700
	Orbit 16	1.600 ± 200	300 ± 100	22.000
	Port-a-Patch	2.800 ± 600	700 ± 200	19.000

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Table 2: Comparison of the systems

System	RMS noise (open pore)	Throughput	Resolution	Volume	Accessibility	Membrane stability	Portability	Temperature control
BLM	2.6	N=1	Filter @ 10 KHz; rise time 33 µs	250 µL or 2.5 mL	both sides of protein (extracellular and periplasmic side)	200 – 250 mV	low	Yes
Port a patch	1.7	N=1	Filter @ 10 KHz; rise time 33 µs	5 µL	Both sides of protein	More than 300 mV	high	Yes
Orbit 16	2.3[a]/2.1[b]	N=16	Filter @ 10 KHz; rise time 33 µs	150 µL	Only one side of the protein	More than 300 mV	medium	No

[a] MECA-chips with 50µm cavity diameter. [b] MECA-chips with 16µm cavity diameter

Figure legends

Figure 1: The 3 different planar lipid bilayer techniques a) BLM b) Port-a-Patch c) Orbit16

Figure 2: Ion current trace of single OmpF channel without antibiotic in a) BLM planar lipid bilayer (open pore current: 210 ± 12 pA) b) Port a patch (open pore current: 208 ± 7 pA) c) Orbit 16 (open pore current: 202 pA ± 12 pA). Conditions: 1M KCl, 20mM MES pH 6, at -50 mV.

Figure 3: Steady state frequency of translocation events of Imipenem (c = 5 mM) through a single OmpF channel observed at a transmembrane potential of Um = -50 mV on a) BLM planar lipid bilayer b) the Port-a-Patch® c) Orbit16. Conditions: 1M KCl, 20mM MES pH 6, at -50 mV

Figure 4: Detail of the current trace shown in Fig. 3. A. At high magnification single translocation events can clearly be identified. (Inset) the dwell time of these events τ is then used for the calculation of kinetic rate constants B. Power spectrum of single OmpF pore in presence and absence of 5mM Imipenem. Conditions: 1M KCl, 20mM MES pH 6, at -50 mV

Figure 5: Ion current trace of single trimeric OmpF recorded in BLM with Cefepime added to *cis* side (- 50 mV) and *trans* (+ 50 mV) side. Conditions: 1M KCl, 20 mM MES pH 6, at ±50 mV.

Figure 6. Scatter plot of events in presence of Ceftazidime through a single trimeric OmpF channel measured through BLM (black squares), Port a patch (blue triangle) and Orbit 16 (red circles). Conditions: 1M KCl, 20mM MES pH 6, at -50 mV.

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Figure 1

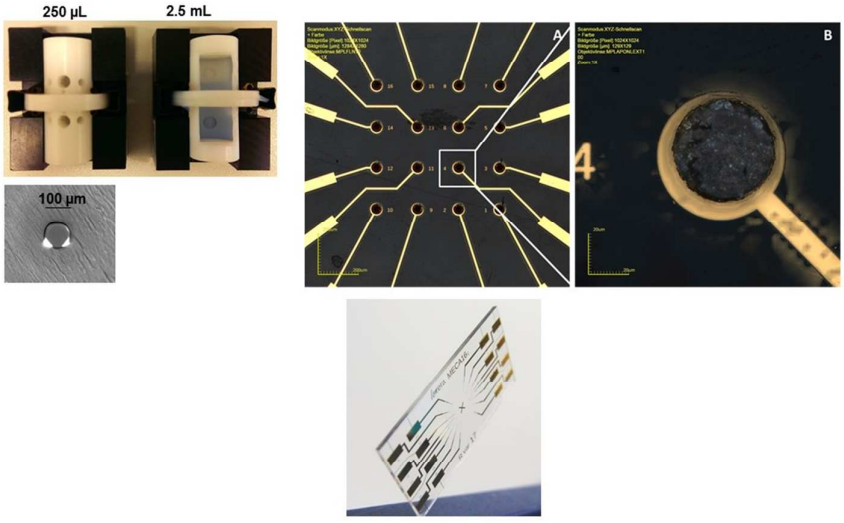

Figure 2

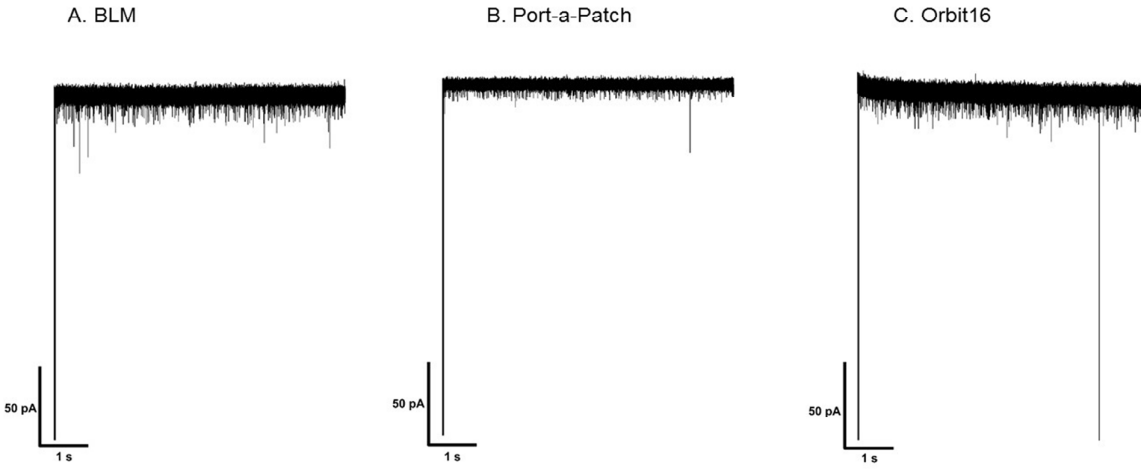

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Figure 3

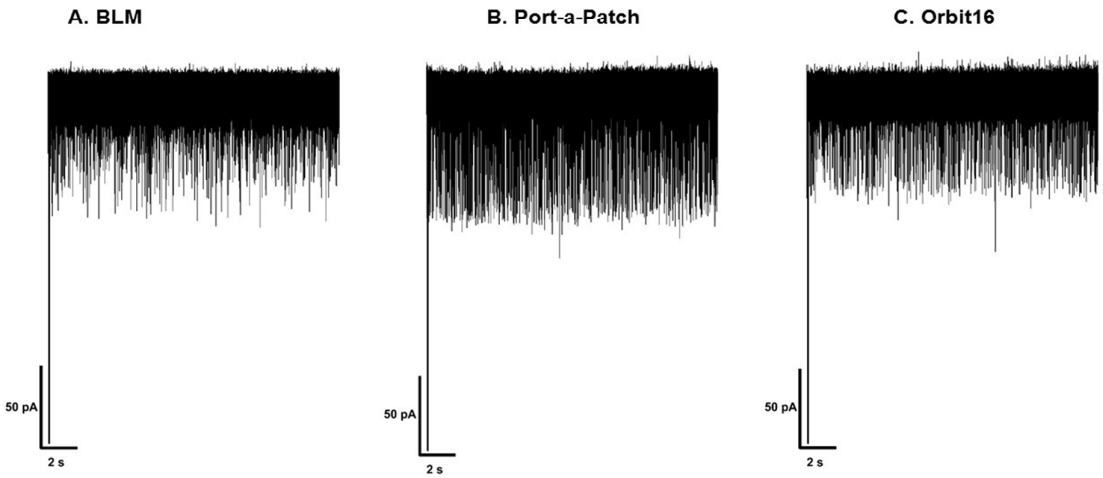

Figure 4

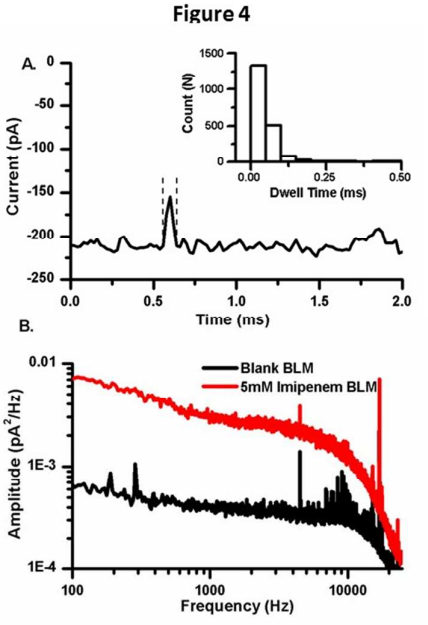

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Figure 5

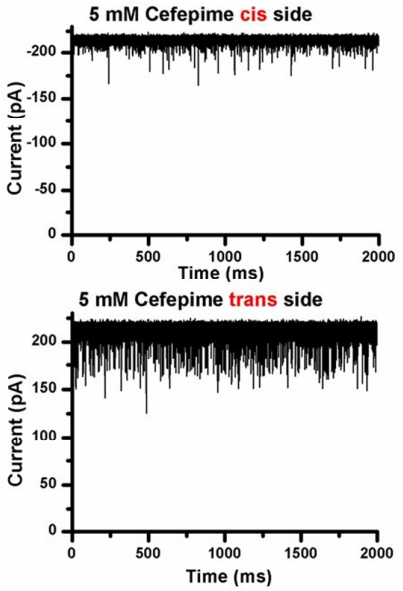
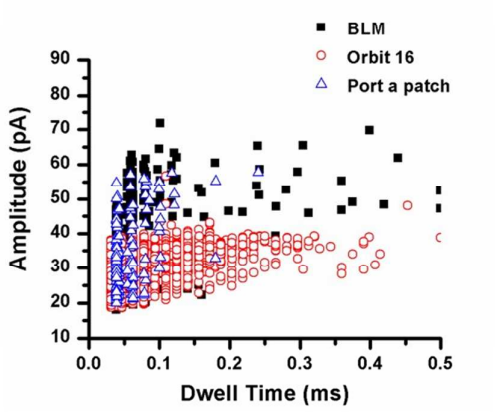


Figure 6



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