# Small molecule permeation across membrane channels: Chemical modification to quantify transport across OmpF

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**ABSTRACT** Biological channels facilitate the exchange of small molecules across membranes, but surprisingly there is a lack of general tools for the identification and quantification of transport (i.e., translocation and binding). Analyzing the ion current fluctuation of a typical channel with its constriction region in the middle does not allow a direct conclusion on successful transport. For this, we created an additional barrier acting as a molecular counter at the exit of the channel. To identify permeation, we mainly read the molecule residence time in the channel lumen as the indicator whether the molecule reached the exit of the channel. As an example, here we use the well-studied porin, OmpF, an outer membrane channel from *E. coli*. Inspection of the channel structure suggests that aspartic acid at position 181 is located below the constriction region (CR) and we subsequently mutated this residue to cysteine, where else cysteine free and functionalized it by covalent binding with 2-sulfonatoethyl methanethiosulfonate (MTSES) or the larger glutathione (GLT) blockers. Using the dwell time as the signal for transport, we found that both mono-arginine and tri-arginine permeation process is prolonged by 20% and 50% respectively through $OmpF_{E181C}$MTSES, while the larger sized blocker modification $OmpF_{E181C}$GLT drastically decreased the permeation of mono-arginine by 9-fold and even blocked the pathway of the tri-arginine. In case of the hepta-arginine as substrate, both chemical modifications led to an identical 'blocked' pattern observed by the dwell time of ion current fluctuation of the $OmpF_{wt}$. As an instance for antibiotic permeation, we analyzed norfloxacin, a fluoroquinolone antimicrobial agent. The modulation of the interaction dwell time suggests possible successful permeation of norfloxacin across $OmpF_{wt}$. This approach may discriminate blockages from translocation events for a wide range of substrates. A potential application could be screening for scaffolds to improve the permeability of antibiotics.

## Introduction

During recent years a new field called nanopore emerged taking advantages of the confinement to detect biomolecules with its most prominent example of DNA sequencing,[1-3] protein interaction or small molecule sensing.[4-5] The detection signal is the change in ion current fluctuation in absence or presence of analysts. Enhanced sensitivity is often achieved by modifying the channel surface via mutagenesis or chemical modification. A particular attractive case is the possible reversible modification via cysteine bridges as shown by Bayley and Jayasinghe.[6-7] In an earlier work Miedema and coworker[8-9] modified the constriction region by cysteine chemistry and achieved to alter the selectivity. While most of the current nanopore research effort is focusing on sensing, here our goal is to quantify permeation of small often uncharged molecules.

A natural function of biological channel is to facilitate the uptake of small molecules and the molecular selectivity is given by the channel size and a narrow selection filter. In contrast to characterization of the selection filter, a general method to quantify translocation of small molecules is lacking. In biological pores the expected flux is typically less than a few thousand molecules per seconds. Such few translocated molecules are simply not enough to be detected in a direct manner by standard techniques. A promising method to provide information on transport is electrophysiology. Penetration and binding of the substrate often leads to significant ion current fluctuations from which we may extract on and off rates for the binding site. However recognition and binding doesn't provide directly information on *bona fide* transport.[10] More recently it was shown that for ion selective channel the application of an external electric field induce electroosmotic flows which itself drives molecules either faster or lead to longer blockage times depending inasmuch the molecule may permeate or not. Nevertheless transport detection depend inasmuch the permeation of the molecule of interest reduced the ion current enough to be detected.[10]

For small charged molecules recently we could integrate the net flux of ions and read out the so-called zero-current membrane potential. Application of a concentration gradient causes fluxes of both ions. Depending on the ion pair the differences in the flux results in excess charges and lead to a potential gradient.[11] Here we focus on an approach to distinguish permeated from reflected molecules by introducing a second barrier at the exit to slow down the translocation time or even block the pathway. If the blocker is placed far enough from the constriction region, we may conclude from the difference in noise spectra on possible transport. As a proof of concept, we chose OmpF from *E. coli* and its substrates including antibiotics.

OmpF is a general porin located in the outer membrane of Gram-negative bacteria, *E. coli*. Biologically, OmpF is involved in the nutrient uptake, also later studies suggested its role in the

antimicrobial agent pathways.[12-13] The structure of OmpF[14] is composed by 16 anti-parallel β-sheets that span in the membrane domain. From extracellular side, there are long loops connecting the neighboring β-sheets and typically the loop 3 (L3) folds back nearly half way of the porin lumen, forming the narrowest constriction region (CR) that screens the approaching molecules. The CR of OmpF has a size of 7x11 Å which is the narrowest part in the whole porin lumen. Previous electrophysiology studies[14-15] reconstituted purified OmpF into artificial lipid bilayer indicated the biophysical properties of OmpF having slightly cation selectivity and the molecule extrusion limitation less than 600 Da.

To distinguish the permeability of molecules through OmpF, placing a molecular sieve at the periplasmic side allows to identify whether the analyte could overcome the CR and reach the exit of the permeable pathway. For this goal, we combined site-directed cysteine mutagenesis with chemical modification by bulky cysteine-specific reagents.[8-9] After the introduction of the cysteine residue at position 181 in the otherwise cysteine-free $OmpF_{wt}$, the mutants were chemically modified by the commonly used negatively charged methanethiosulfonate-based (MTS) reagents, i.e., MTSES (2-sulfonatoethyl methanethiosulfonate), and a relatively larger size glutathione (GLT). Below we show that this approach allows to detect even very fast events which previously could not be analyzed. Note that this approach allows to work under physiological concentration (100 mM salt, micro-molar concentration for the antibiotic) and the setup can be miniaturized down to 40 μl.

In this study we first probe translocation of a putative natural substrate for which we have a second method to quantify translocation as a control. In a second series of measurement we apply our knowledge to quantify norfloxacin as an example for a zwitterionic antibiotic. Currently we transfer this approach to the most prominent channel in *E. coli, Acinetobacter baumannii* or *Pseudomonas aeruginosa* with the ultimate goal to screen molecular pattern with good permeability, an information which may help to optimize the membrane permeability of antibiotics in Gram negative bacteria.

# Material and Methods

The short cationic peptides used in this work are L-arginine (Sigma-Aldrich, USA) as well as synthetic peptides tri-arginine (H–Arg–Arg–Arg–OH acetate salt Mw: 486.54 Da) and hepta-arginine (H–Arg–Arg–Arg–Arg–Arg–Arg–Arg–OH trifluoroacetate salt, Mw: 1111.33 Da). These synthetic peptides were ordered from Bachem AG, Switzerland. Norfloxacin was obtained from Sigma (Sigma-Aldrich, USA).

**Chemical modification**

The chemical modification of cysteine mutant OmpF was carried out as previously described,[9].Mutant $OmpF_{E181C}$ (0.2mg/ml)[8-9] was incubated overnight with 20 mM both MTS-based label MTSES (2-sulfonatoethyl methanethiosulfonate) (Anatrace Products, LLC, USA) and GLT (Glutathione reduced form) (Sigma-Aldrich, USA), in a 200 mM sodium phosphate buffer at 7.5 containing 1 mM n-octyl-poly-oxyethylene detergent (OPOE). To improve the modification efficient by keeping the cysteine reduced, the protein was pretreated with 1 mM DTT (1,4-dithiothreitol) (Carl Roth GmbH, Germany) at room temperature for 1 hour.[7] The electrophysiological characterization was performed without any further purification of the chemically modified protein.

**Bilayer formation**

Horizontal artificial lipid bilayer has been formed using 5 mg/ml DPhPC/Octane (1,2-diphytanoyl-sn-glycero-3-phosphocholine from Avanti polar lipids, Inc, USA, Octane from Sigma-Aldrich, USA) on Orbit mini (Nanion Technologies GmbH, Germany) along with 50~150 µm MECA chip (Ionera Technology GmbH, Germany).[16-17] The Purified protein, as previously described,[18] or chemically modified protein samples were diluted $10^4$-fold using OPOE detergent. 0.5 µL sample was directly added to the measuring chamber from the ground (*cis*) side. The sudden change in the bilayer conductance has been regarded as a successful reconstitution of protein into the artificial lipid bilayer. The asymmetric conductance and current behavior of $OmpF_{wt}$ at different voltage polarities have been considered for the protein reconstitution orientation.[19-20] To control the number of functional channels inserted, we used the known voltage gating (closure of the channel) above 120 mV transmembrane applied voltages. The current behavior has been characterized by running a voltage ramp protocol from -200 mV to +200 mV elapsed 2 min (Figure S1). Both modified proteins showed gating spikes especially the GLT modified cysteine mutant OmpF has much higher gating probabilities above ± 100 mV.

The electrophysiology measurement has been monitored and recorded using *Element Data Recorder* software (Element s.r.l., Italy) and further analyzed using *Clampfit 10.7* software (Axon Instrument Ltd., USA). All the electrophysiology experiments are performed under 20 kHz sampling rate and filtered at 10 kHz under 1 M KCl, 20 mM MES, pH 6.0, room temperature if no further indication.

**Charged molecules permeation flux calculation**

In the simple case of a 1:1 electrolyte (e.g. KCl) this can be obtained by applying a concentration gradient and recording the voltage needed to zero net ion current. [11, 21] The Goldman-Hodgkin-Katz (GHK) provides a simple relation which allows us to obtain the permeability ratio:

$$V_m = \frac{RT}{zF}\ln(\frac{P_{Na}[Na^+]_{cis} + P_{Cl}[Cl^-]_{trans}}{P_{Na}[Na^+]_{trans} + P_{Cl}[Cl^-]_{cis}})$$

Measuring the zero-current transmembrane voltage $V_m$ at different concentration gradients gives an approximate permeability ratio.

In case of multivalent ions, we rather use the GHK equation for the ion current and solve the permeability ratio numerically. The total ion current *I* is then given by the sum of all individual ion current $I_x$

$$I_x(V, P_x, z, c_{cis}, c_{trans}) = P_x z^2 \frac{VF^2}{RT} \cdot \frac{(c_{x,cis} - c_{x,trans} \cdot \exp(\frac{-zVF}{RT}))}{1 - \exp(\frac{-zVF}{RT})}$$

where $V$ is the transmembrane voltage, $P_x$ the permeability for the ion $x$, $z$ is the valence, $F$ (Faraday constant) = 9.6x10$^4$ A·s·mol$^{-1}$, and $R$ (gas constant) = 8.3 J·mol$^{-1}$·K$^{-1}$. The experimental input $c_{x,cis}$ and $c_{x,trans}$ are the ion concentration on the respective sides of the membrane.

**Single channel noise spectra calculation**

To analyze the ion current fluctuation with respect to interaction we use the following model[22]:

$$\begin{array}{ccccc} & k_{on}' & & k_{on}'' & \\ \text{open channel + substrate} & <=> & \text{closed channel} & <=> & \text{open channel + substrate} \\ & k_{off}' & & k_{off}'' & \end{array}$$

Whereas the on-rate $k_{on}$ (association rate) is obtained by counting the number of ion-current blockage events per time *n* [$s^{-1}$] divided by the concentration *[c]* of the substrate. Furthermore, OmpF is a trimer and the ion-current blockages may originate independently from one of the three monomers.

$$k_{on} = \frac{\mathrm{n}}{3 \cdot [\mathrm{c}]}$$

On the other hand, the re-opening ($k_{off}'$, $k_{off}''$) is a statistical event and correlated to the strength of the affinity. A channel which is closed at t = 0 will have the probability R(t) to open at time t. Within the aforementioned simple binding model, this leads to an exponentially decaying function $\mathrm{R(t)} = \exp(-\mathrm{t}/\tau)$. Fitting the life-time distribution of the closed channel by an exponential parameter τ (dwell time) yields the off-rate (dissociation rate) $k_{off} = 1/\tau$.[23] The equilibrium binding constant K [$M^{-1}$] of the substrates to the porin is given by the ratio of the rates $K = k_{on}/k_{off}$.

For such analysis we should note that in our experiment we work under dilute concentrations ($\mathrm{K} \cdot [\mathrm{c}] \ll 1$) and thus, the dissociation rate is independent to the substrate concentration.

# Results and discussion

**Characterization of chemically modified OmpF**

The orientation of the channel can be visualized by the asymmetry in ion conductance as a function of voltage. Asymmetric current behavior of both chemically modified porin $OmpF_{E181C}$MTSES and $OmpF_{E181C}$GLT were observed akin $OmpF_{wt}$[20] indicating the identical orientation and this type of reconstitution were observed most of the time (n>20). More sub-gating noise especially at negative polarities (Figure S2) were recorded.

The measured single open channel conductance for MTSES and GLT modified cysteine mutant $OmpF_{E181C}$ was 4.0 ± 0.2 nS and 3.6 ± 0.4 nS respectively at 1 M KCl, pH 6.0. Comparing to the conductance of $OmpF_{wt}$,[24] the MTSES modification did not vary the conductance, while the GLT modification resulted in 10% reduction. Considering the spatial volume of the modification molecules, the GLT occupies 0.31 $nm^3$ which is three times more than the volume taken by MTSES (0.09 $nm^3$)[9] from the periplasmic side after covalent binding to the cysteine. Nonetheless, the biophysical properties are not drastically altered as the position of the molecule is beyond the constriction zone of the porin.

A sensitive test for the functionality of the main constriction zone is to measure the ion selectivity. The ion selectivity of both porins were measured following the GHK equation. Briefly,

asymmetric KCl salt gradient ($\frac{[KCl]_{cis}}{[KCl]_{trans}} = \frac{400\ mM}{100\ mM}$) was created on both sides of the porin reconstituted membrane. The zero-current membrane potential (Figure S3) was recorded and the ion selectivity was calculated using the GHK equation.

*Table 1. Ion selectivity measurement using $OmpF_{wt}$ and two chemically modified $OmpF_{E181C}$ porin (MTSES and GLT) 100 mM KCl, 20 mM MES, pH 6.0 was used as the initial salt solution. The salt concentration on the electrical ground (cis) side was raised to 400 mM KCl. (average of more than 3 sets of experiments).*

| **Protein** | **Conductance** (nS) | $P_k/P_{cl}$ (100/400 mM KCl) |
|---|---|---|
| wt OmpF | 4.0 [24] | 4 : 1 |
| $OmpF_{E181C}$ MTSES | 4.0±0.2 | 4 : 1 |
| $OmpF_{E181C}$ GLT | 3.6±0.4 | 4 : 1 |

Previously,[25] we investigated the permeation of arginine across OmpF. We have shown that tri-arginine (486 Da) may permeate through $OmpF_{wt}$ whereas, hepta-arginine (1111 Da) was too bulky reaching the empirical exclusion limit of OmpF (~600 Da[26]). Even though the hepta-arginine is highly positively charged at pH 6.0, the external trans-membrane voltages below 100 mV did not allow significant permeation. Mono-arginine (chloride salt), whose molecular weight is 174 Da has also been tested with the porins. Using the reversal potential characterization on the permeation of charged molecules[11, 27] the flux rate of small molecules could be calculated. Thus, we take the mono-arginine, tri-arginine and hepta-arginine in our study to demonstrate inasmuch we may differentiate binding from translocation.

**Arginine interaction with OmpF – Cysteine mutants**

Mono-arginine, tri arginine and hepta arginine of 0.5 µM each were added to the cis side of the membrane in independent experiments to study the interaction. The current trace of the interaction between mono-arginine and single OmpF porin is plotted in Figure 1 (first row). In the case of interaction between mono-arginine molecules with MTSES and GLT modified porins, the current baseline shifted 2 pA and 10 pA respectively, as indicated in the histogram in Figure 1 insets. Specifically, fast interactions (dwell times $\tau < 100\ \mu s$) will not be sampled properly and induce a baseline shift towards the close state.[28-29] Though the interaction was too fast to

be properly determined in single channel measurements, another method previously developed for small charged molecules has been used and analyzed below.

Figure 1 (2nd row) shows ion current in presence of tri-arginine. At pH 6.0 arginine being positively charged, can be pulled inside the channel via application of negative applied voltages only causing distinguishable single monomer blockages. In the third row of Figure 1, we show typical recording in presence of hepta-arginine. It is more often to observe two-thirds or even whole trimer conductance closure during the interaction between hepta-arginine and OmpF porins suggesting the relative strong interaction.

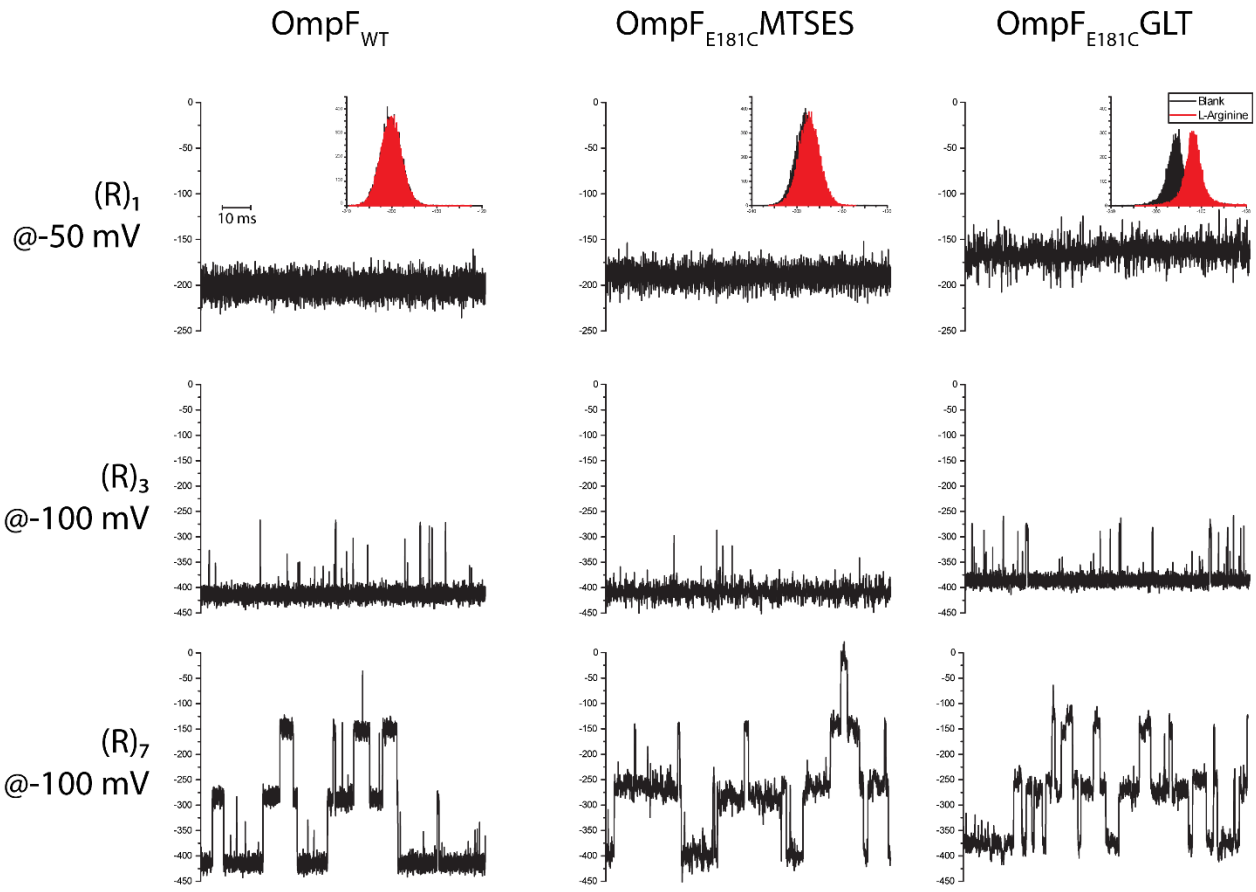


*Figure 1 Interaction between different arginine oligomers (mono-arginine, tri-arginine, hepta-arginine, 0.5 µM, cis addition) and OmpF$_{wt}$ as well as OmpF$_{E181C}$MTSES, OmpF$_{E181C}$GLT. Insets indicate the current histogram of mono-arginine interaction (red) and blank recording (black, trace not shown). 1 M KCl, 20 mM MES, pH 6.0 was used as buffer, the applied voltage is indicated from the left side. Buffer temperature of 20°C was maintained during the whole experiment session.*

In order to quantify tri- and hepta-arginine permeation we quantified the interaction kinetics (*Material and methods*). In Figure 2 we summarized the analysis. The interaction of tri-arginine with OmpF$_{wt}$ is highly voltage dependent. The association rate increased from $5x10^6$ to $50x10^6$ $M^{-1}s^{-1}$, one order of magnitude, when the transmembrane voltage was increased from -

25 mV to -125 mV. The exponential increase of association rate terminated when the voltage further increased to -150 mV due to the association activation enthalpy saturates to the constant value.[30] In contrast, the dwell time of the interaction with $OmpF_{wt}$ was linearly decreased from 100 μs to 60 μs without the alteration at higher voltages.

Regarding the chemically modified $OmpF_{E181C}$, when the tri-arginine enters the modified porins, the association rate ($k_{on}$) increased from $0.5x10^6$ to $18.0x10^6$ $M^{-1}s^{-1}$ interfered by MTSES blocker. The dwell time of the interaction decreased from 140 μs to 130 μs when the voltage increased at negative polarity. The dwell time for tri-arginine enhances on average by more than 50% in MTSES modified $OmpF_{E181C}$ compared to $OmpF_{wt}$. When the relatively larger size GLT is bound to the cysteine site, the $k_{on}$ increased from $8.0x10^6$ to $23.0x10^6$ $M^{-1}s^{-1}$ and the dwell time of tri-arginine alternatively increased from 160 μs to 200 μs completely different to the $OmpF_{wt}$. Since the modified porins start gating frequently especially $OmpF_{E181C}$GLT unable to distinguish between gating spikes and interaction events even beyond -100 mV, the voltage dependence was characterized until this limitation.

Hepta-arginine has more than the double molecular weight compared to tri-arginine. When the transmembrane voltage applied from -25 mV to -100 mV, the $k_{on}$ between hepta-arginine with the $OmpF_{wt}$, and MTSES as well as GLT modified $OmpF_{E181C}$ increased from $2.6x10^6$ to $85.0x10^6$ $M^{-1}s^{-1}$, $14.0x10^6$ to $100.0x10^6$ $M^{-1}s^{-1}$, $5.5x10^6$ to $130.0x10^6$ $M^{-1}s^{-1}$ respectively. As expected for cationic molecules the association rate of hepta-arginine is higher than the tri-arginine and increases with increasing negative voltages.

More conclusion with respect to translocation can be drawn from the voltage dependence of dwell time, contrast to the tri-arginine, the hepta-arginine reside much longer time inside the porin than tri-arginine. Importantly, the engineered porins both do not alter the trend of the voltage dependence of the hepta-arginine dwell time, i.e. the dwell time of the interaction increased from 250 μs to 2.5 ms suggesting that hepta-arginine cannot exit at the engineered periplasmic side.

Further measurements of both tri- and hepta-arginine from *trans* addition also suggest the expected orientation as well as the effectiveness of MTSES and GLT (Figure S3).

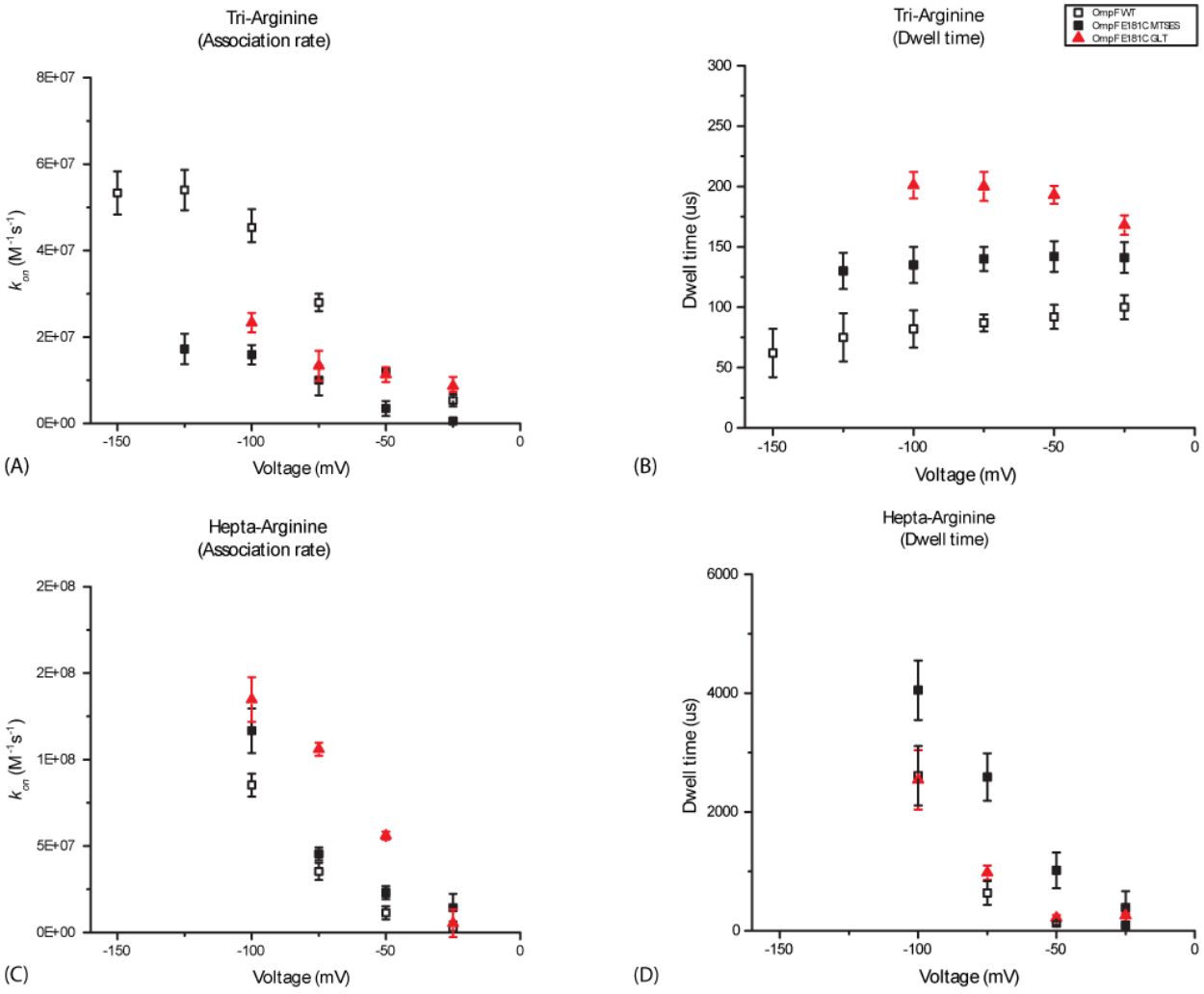


*Figure 2 Interaction kinetics of tri-arginine and hepta-arginine (electrical ground cis side addition) with WT OmpF, MTSES modified and GLT modified OmpF. No significant detectable interaction has been observed at positive polarities. 1 M KCl, 20 mM MES, pH 6.0. (average of more than 3 sets of experiments).*

**Detection limit**

In contrast to poly-arginine, the mono-arginine (chloride salt) was previously too fast to be detected. Here we applied a concentration gradient and recorded the so-called reversal potential originating from the difference in electrophoretic mobility of arginine and chloride [11, 27]. In detail, we created a bi-ionic arginine-Cl condition (i.e. 100 mM arginine-Cl) at both sides of the porin reconstituted bilayer. Raising the solute concentration at *cis* (ground side) of the bilayer to 400 mM concentration, a concentration gradient was then generated and the inducing zero-current membrane potential was recorded. By applying simplified GHK equation to this monovalent bi-ionic condition, we calculated the permeability ratio of arginine-Cl across the $OmpF_{wt}$, $OmpF_{E181C}$MTSES and $OmpF_{E181C}$GLT are 1:2.8, 1: 3.4 and 1:250 respectively.

Following the trend that the larger size of the blocker attached to the periplasmic side, the permeability of arginine over chloride ion decreased 20% by MTSES modification and 90 times less caused by GLT modification. According to the permeability determination, the mono-arginine though permeable through both modified OmpF porin, the GLT present at the periplasmic side decreased the rate drastically.

*Table 2 Ion permeability of arginine-Cl across $OmpF_{wt}$ as well as MTSES and GLT modified $OmpF_{E181C}$ porin. (average of more than 3 sets of experiments).*

| **Protein** | **[Arginine-Cl]$_{trans}$ (mM)** | **[Arginine-Cl]$_{cis}$ (mM)** | **$V_{rev}$ (mV)** [1] | **$P_{arginine}$ : $P_{Cl}$** | **Ion flux** [2] | |
|---|---|---|---|---|---|---|
| | | | | | $Arginine^+$ | $Chloride^-$ |
| $OmpF_{wt}$ | 100 | 400 | -15 (n = 3) | 1 : 2.75 | 5,200 | 14,300 |
| $OmpF_{E181C}$ MTSES | 100 | 400 | -17.75 (n =4) | 1 : 3.40 | 2,500 | 9,000 |
| $OmpF_{E181C}$ GLT | 100 | 400 | -36.5 (n = 2) | 1 : 250.00 | 55 | 14,700 |

[1] number of experiments indicated in brackets
[2] flux at 1mM Arginine-Cl gradient was determined
*trans* = active electrode; *cis* = ground electrode

**Sensing small antimicrobial molecules with chemically modified OmpF**

In a further series of measurements, we tested the possibility to discriminate permeability from blockage for a zwitterionic antibiotic. In order to verify the capability of the biosensor detecting the label-free substrates, we tested norfloxacin, an antibiotic derivative from nalidic acid which belongs to the synthetic fluoroquinolone group. The charge distribution of norfloxacin is zwitterionic at pH 7.0.

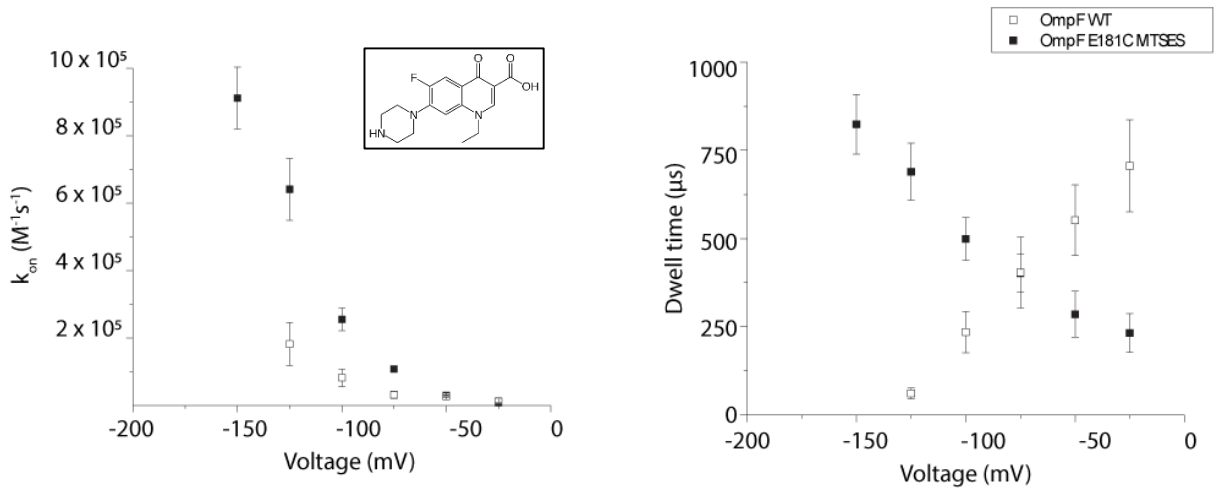


*Figure 3 Interaction kinetics of 0.2 mM norfloxacin (inset figure) from cis (ground) side with MTSES modified OmpF. No observation of interaction been observed at positive polarities, only the negative voltages dependence is indicated. 1 M KCl, 10 mM HEPES, pH 7.0. (average of more than 3 sets of experiments).*

Single channel measurement was carried out in presence of 0.2 mM norfloxacin. In a similar analysis we obtained the voltage dependent association rate and dwell time, the increase of the negative polarity transmembrane voltages induced $k_{on}$ in $OmpF_{wt}$ increases from $1.3x10^4$ to $18x10^4$ $M^{-1}s^{-1}$ exponentially whereas the dwell time decreased from 700 µs to 60 µs linearly when experiencing a transmembrane voltage from -25 mV to -125 mV.

The $OmpF_{E181C}$MTSES gave a similar trend of the voltage dependence of the association rate ($10^4$ to $10^7$ $M^{-1}s^{-1}$). In contrast the MTSES modification completely reversed the dwell time trend (an increase from 250 µs to 830 µs).

The unchanged association rate in both WT and modified porin confirmed that the porin entrance for norfloxacin was not significantly modified. The strongly altered dwell time from interaction between norfloxacin and $OmpF_{E181C}$MTSES suggested that MTSES already created high enough barrier that the molecule could not overcome when approaching the periplasmic/exit side. Consequently, the norfloxacin has the capability to overcome the constriction region in $OmpF_{wt}$, however, the introduction of the MTSES at periplasmic side successfully blocked the pathways of norfloxacin and make it impermeable through the chemical engineered porin.

# Conclusion

Here we show that introducing a second barrier at the exit of a channel allows to conclude on the identification and quantification of molecule translocation process. In our model, any molecule permeable through $OmpF_{wt}$, once approached at the constriction regionsees the molecule-counter at the exit. Depending on the barrier created by this molecule counter which is determined by the steric extrusion in most of the cases, the permeable molecules either been slowed down or even been blocked making the interaction dwell time different. In contrast impermeable molecules that could not overcome the constriction region will not be drastically affected by the molecule counter thus inducing no difference of the interaction dwell time. We read the interaction dwell time as a signal for making conclusion from single channel recordings.

We tested our approach with poly-arginine where a clear difference in the signal could be observed via single channel biophysical characterization. In contrast, monomeric arginine is too fast to be resolved, consequently, we determined the transport process via a simple bi-ionic reversal potential characterization. Norfloxacin as an example of antibiotic molecule which has been reported several antibiotic resistance clinical relevant isolates is tested. According to our model, the norfloxacin could successfully take the OmpF as one of the pathways across bacterial outer membrane. A particular advantage of this method that we may work at biological relevant concentrations and moreover this approach can be parallelized to allow high throughput.

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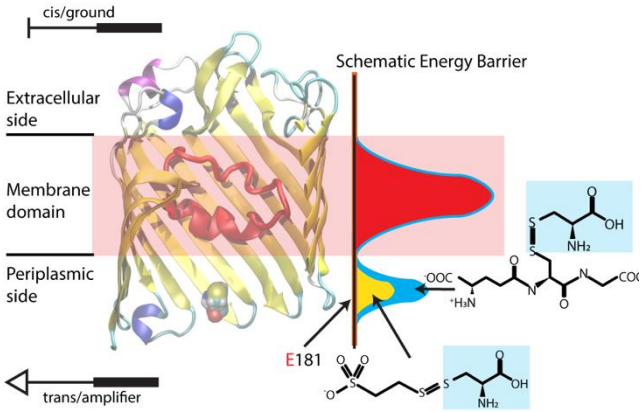
TOC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Membrane domain
Periplasmic side
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trans/amplifier