Radio-frequency response of single pores and artificial ion channels

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Abstract. Intercellular communication relies on ion channels and pores in cell membranes. These protein-formed channels enable the exchange of ions and small molecules to electrically and/or chemically interact with the cells. Traditionally, recordings on single-ion channels and pores are performed in the dc regime, due to the extremely high impedance of these molecular junctions. This paper is intended as an introduction to radio-frequency (RF) recordings of single-molecule junctions in bilipid membranes. First, we demonstrate how early approaches to using microwave circuitry as readout devices for ion channel formation were realized. The second step will then focus on how to engineer microwave coupling into the high-impedance channel by making use of bio-compatible micro-coaxial lines. We then demonstrate integration of an ultra-broadband microwave circuit for the direct sampling of single α-hemolysin pores in a suspended bilipid membrane. Simultaneous direct current recordings reveal that we can monitor and correlate the RF transmission signal. This enables us to relate the open–close states of the direct current to the RF signal. Altogether, our experiments lay the ground for an RF-readout technique to perform real-time in vitro recordings of pores. The technique thus holds great promise for research and drug screening applications. The possible enhancement of sampling rates of single channels and pores by the large recording bandwidth will allow us to track the passage of single ions.

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1. Introduction

Information processing in biological systems is a highly complex task that relies heavily on parallel processing. Due to this complexity, signal transduction times of cellular systems can afford to be fairly slow, e.g. with 1–100 ms for action potentials [1], as compared with current state-of-the-art semiconductor transistors operating in the sub-ns regime. This comparison can be pushed further by analyzing how cellular currents are gated via ion channels or pores embedded in cellular membranes [2]. The typical ionic currents flowing through channels and pores are of the order of some pA to the nA range. This translates via the average current $\langle I \rangle = \langle n \rangle e/\tau_d = \langle n \rangle e f$ to frequencies in the range of 1 MHz–1 GHz. The quantity $\langle n \rangle$ is the average number of ions transported, while $\tau_d$ is the dwell time of the ions within the channel. Hence, we find that fast switching events occur on the nanoscale in biological systems. This is in stark contrast to the common belief that cellular communication is inherently slow. Consequently, it is desirable to have circuitry available that facilitates radio-frequency (RF) spectroscopy of ion transport. This will yield real-time in vitro information on ion channel operation and might ultimately prove useful in enhancing the throughput of chip-based drug screening applications [3]. In addition, the bio-transistors, as these molecular junctions might be called, are sensitive not only to electrical gating, but also to chemical gating effects and mechanical stress on the nanoscale. Determining the exact time evolution of these processes and their interaction is extremely important.

Here, we present measurements of the local interaction of an RF signal with single-ion channels and pores. We found relatively good coupling of the RF power to the channels and pores embedded in suspended bilipid membranes. While commonly either the dc or the RF response is recorded, we carry out the first measurements combining truly microwave excitations with dc spectroscopy. When investigating the interaction of electromagnetic signals with bio-matter the first consideration is to compare the Debye screening length of the protein under investigation in aqueous solution to the frequency of operation. For RF signals, this results in very strong attenuation due to the large dielectric constant of water ($\epsilon \sim 80$). This in turn requires higher signal power, heating the biological system. Hence, heating and rupture of membranes were found in previous work studying the effects of radio- and microwave frequencies on ion channels in membranes [4–6]. Another hindrance is the low temporal
resolution of probing single-pore signals with bandwidths of some 10 kHz. This is due to large access resistances ($R$) and membrane capacitance ($C$), leading to low time constants $\tau = RC$. We finally overcame these limitations by using micro-coaxial lines for delivering and retrieving the RF signal. The proteins we use to form channels and pores are alamethicin (ALA) forming artificial ion channels [7] and $\alpha$-hemolysin ($\alpha$-HL) creating pores upon insertion into a bilipid membrane [8].

In figure 1(a), a diagram of our microwave circuit with a characteristic impedance $Z_0$ is given. The transmission line (TL) is driven by a synthesizer and coupled to a junction circuit with a characteristic impedance ($Z_j$). This junction is required in order to couple the 50 $\Omega$ TL impedance to the actual high-impedance molecular pore with the load (L) impedance $Z_L$. Essentially, the junction can be thought of as an impedance matching unit, which enables the coupling of electromagnetic power into and out of the load. One could assume that $Z_L$ is purely real, since the typical ion channel or pore dc resistance ($R_L$) is of the order of several G$\Omega$. However, these ‘bio-transistors’ possess a finite capacitance $C_{bt}$ and are embedded in a comparatively large cellular or bilipid membrane ($C_m$). Both these capacitances alter the impedance via the reactance $X_L = -1/(\omega C_L) = -1/(\omega(C_{bt} + C_m))$, i.e. $Z_L = R_L + jX_L$. Consequently, the matching condition $Z_j = \alpha Z_L$ or, more precisely, its physical realization is key to this work and will be addressed in detail.

2. Broader scope of nanopore radio-frequency (RF)-sampling techniques

For the experienced physiologist, the general question arises as to why RF sampling of nanopores is relevant. The straightforward answer is that by being able to extend the dynamic range of classical patch-clamp measurements from the kHz to the GHz-range, we are able to observe a host of new phenomena. These include intermediate opening states of ion channels, the influence of noise, and possibly conformational changes resulting in current fluctuations. This certainly will have an impact on high-throughput screening (HTS) methods, since a GHz bandwidth enables data collection rates that are orders of magnitude faster than existing methods. As this paper will show, the RF methods developed here lend themselves for on-chip integration. Hence, they can be directly implemented in commercial HTS.
In the following, we will first reconsider early attempts to funnel electromagnetic power to ion channels at GHz frequencies. The experiment was mainly performed by combining conventional micro-strip lines with painted bilipid layers. Although the approach is straightforward, a lack of capacitive coupling prevents a direct readout of single-channel events. In the subsequent parts, we will focus on using micro-coaxes for delivering RF power to pores and artificial ion channels embedded in bilipid membranes. Furthermore, we present RF transmission measurements on single pores and relate this response to conventional dc recordings. This method enables us to couple RF power to single channels and pores, probed via (i) rectification and (ii) direct modulation of the transmitted RF signal. Finally, we discuss a possible scenario of on-chip integration of RF micro-probes for screening applications.

3. Initial studies on transmission line circuits

Early work on applying TLs for sampling protein binding and conformational changes [9–11] featured the use of simple micro-strip lines onto which an analyte was added, thus changing the overall impedance of the device. The underlying idea was that the high speed of the propagating RF signal could be employed to extract temporal information on the analyte interaction on top of the TL. Typically, a phospholipid membrane is placed on the TL onto which the analytes act. The membranes themselves are ideal model systems, since they contain only phospholipids and no other proteins, which makes them more stable for voltage/current recordings over a long period of time. The resulting interaction should then somehow specifically alter the effective dielectric constant $\epsilon_e$ and thus the characteristic impedance ($Z_0$) of the line in the following way: $Z_0 = \sqrt{\epsilon_e}/(cC)$, where $c$ is the velocity of light in the medium and $C$ the total capacitance of the TL. Consequently, the general aim of these measurements is to maximize the change in capacitance $\delta C$, induced by the interaction of biological analytes in close proximity to the TL. A major drawback of this method is the low sensitivity; that is, a single molecular event $n$ causes only a minimal capacitive change of $\delta C_n$. Commonly, it is then argued that one can effectively sum over many events, i.e. $\delta C = \sum_n \delta C_n$, thus obtaining a rather ‘global’ picture and a fair signal-to-noise ratio (SNR). This then implies temporal averaging over many probably interrelated events, which in turn strongly reduces the resolution of the method. It is thus desirable to enhance the coupling capacitance to the microwave circuit that in turn provides the required speed. In the ideal case, one would achieve capacitive coupling to single events via $\delta C_n$, tracing the resulting impedance variations. Before addressing this highly sensitive coupling of single pores and channels to microwave coaxes, we want to first discuss how to combine the ‘classical’ TL bio-analyte probing with conventional dc spectroscopy.

By combining the TL-based approach (‘global’ analysis) with an on-chip patch-clamp unit (local probe), a direct relation between the microwave spectra and the molecular events can be established. The overall setup is sketched in figure 2(a): a miniature on-chip patch-clamp unit with an embedded glass chip is placed on top of TL defined on glass as well. Although as TL metal we have chosen silver, there exists no direct electrical contact between the solution in the lower chamber and the RF signal line. The coupling to the TL is purely capacitive. The on-chip unit is assembled from two PDMS chambers sandwiching a thin glass chip, which contains a micron-sized aperture. The aperture is fabricated in such a way that phospholipid bilayers can be spanned. The micro-strip line geometry is shown in an aerial view in figure 2(c), which is used as the basis for finite-element simulations of this particular TL [12]. The central area marked by...
Figure 2. (a) Schematic view of the first experimental setup: an on-chip patch-clamping unit is placed on a very thin electrically insulating plastic sheet, which is bonded to a micro-strip line fabricated on a glass slide. (b) Exploded view of the setup: the ‘upper chamber’ (i) is glued on a micromachined glass chip (ii), which in turn is placed on the ‘lower chamber’ (iii). This on-chip patch-clamping unit is placed on a very thin electrically insulating plastic sheet (iv). The whole device is finally fixed on to a micro-strip line fabricated on a glass slide. (c) Circuit structure of the micro-strip line (yellow) with additional grounding pads for the SMA connectors (short yellow stubs). The back of the circuit is covered by a thin metal layer serving as ground potential. Standard finite-element packages are used to solve for the electromagnetic modes supported by this TL. (d) The two SMA connectors on the top and bottom enable quick connection to the standard microwave gear, while the dc connectors are directly dipped into the solution.

gray boxes indicates the region where the on-chip unit is positioned. Naturally, the PDMS will dominate the electromagnetic response in this part of the TL.

The micron-sized pore drilled through the glass chip had a diameter of 150 µm, since we applied a conventional painting technique to place and span the bilayer over the aperture [13]. The on-chip unit’s cis- and trans-sides corresponded to the upper and lower chambers and contained 1 M KCl and 10 mM HEPES (pH = 7.3) in aqueous solution. The lipid bilayer was made of 1,2-diphytanoyl phosphatidyl choline in decane. In figure 2(b), the assembly of the setup is shown in an exploded view: the cis- and trans-chambers sandwiched the glass-chip with the pore. This whole on-chip unit was then placed on top of the micro-strip line.

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Figure 3. Transmission line measurements on the sandwiched micro-stripline and the on-chip patch-clamping unit. The $S_{21}$ parameter versus the RF range of 100 MHz–1 GHz is given. The uppermost (black) trace shows the background when no bilipid is painted on the glass chip. The colored traces below indicate the change once a bilayer forms for different dc bias voltages. The inset gives the dc recording from the integrated on-chip unit, revealing that pores do indeed penetrate the membrane. At about 40 mV, an $\alpha$-HL is inserted into the membrane. The resulting current corresponds to exactly one $\alpha$-HL being inserted. Note that the background is not calibrated, i.e. the visible resonances in transmission are due to interference and reflection of the RF signal.

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that can be identified are due to resonances within the circuit and are evidently not caused by the phospholipids. It would have been possible to calibrate the whole setup and thus ‘remove’ these resonances. However, this would have led to the impression that a painted bilayer actually causes the rich spectrum, as given in the colored traces. From a comparison of the original uncalibrated data set, we can conclude that the fine structure in the spectrum is most likely caused by interference of different TE and TM modes on the micro-strip line.

Nevertheless, there is a strong variation in overall signal attenuation once the bilayer forms over the aperture, i.e. the $S_{21}$ amplitude is strongly reduced towards 1 GHz. This formation we can follow in simply recording the dc resistance of the aperture using the on-chip patch-clamp setup. The relevant change in the microwave signal is its enhanced attenuation towards the 1 GHz mark. This is to be expected, since the presence of the phospholipid also enhances the dielectric losses. As noted before, we performed simultaneous dc recordings of the aperture resistance. From this, we were able to trace bilayer formation and the insertion of $\alpha$-HL pores into the membrane. As an example the inset of figure 3 shows the dc versus time of a blocking event, i.e. we added a sugar component ($\beta$-cyclodextrin) to observe a modulation of the current through the pore. Apparently, we are able to monitor pore blocking events in the dc signal at 40 mV (see the inset of figure 3). This is possible since we are simultaneously recording the dc signal while tracing the ac response of the entire circuit. We can conclude that the blocking of $\alpha$-HL pores leaves a signature in the overall ac response. However, this gradual shift in the global response happens to be only gradual.

Evidently, it is possible to combine an RF circuit with a dc circuit in order to correlate local single-pore events with ‘global’ TL characteristics. This approach is suitable as a testing ground for RF components as high-throughput screening tools. However, it only delivers a correlation but does not entail the full temporal domain. For obtaining a large bandwidth that finally allows real-time recordings with sub-microsecond resolution, the coupling efficiency of the bio-molecular interaction to the RF-circuit component has to be drastically enhanced. One possible approach to achieve this is described in the following, where we present first measurements of single channels and pores using specially fabricated micro-coaxes.

4. Micro-coax probe station

The measurement setup employing micro-coaxes is depicted in figure 4(a): the chamber combines a standard planar bilayer recording setup with a delrin cuvette for ion transport measurements with micro-coaxial lines. The chamber has cis- and trans-sides corresponding to drain and source contacts containing, as before, 1 M KCl and 10 mM HEPES with pH $= 7.3$ in the total aqueous solution. The delrin cuvette has a $\sim 200 \mu$m pore onto which the lipid bilayer is painted. This technique leads to the formation of 4 nm thick phospholipid bilayer in the center of the pore spanning approximately 5 $\mu$m. Under optical inspection with a microscope, the thicker rim can be clearly identified [13].

As before, the dc contacts (Ag/AgCl wires) are simply dipped into the solution (cis-side grounded), similar to a standard membrane patch measurement. The ac contacts (RF micro-coaxes) are brought in from the sides on optical reels to allow for exact positioning close to the suspended bilipid membrane. The alignment procedure is critical for this measurement, since it will determine the coupling efficiency of the coax line. The RF generator used was a Hewlett-Packard synthesizer (HP 83650A) generating a sinusoidal continuous wave excitation. In figure 4(b), a magnified image of a typical micro-coax is shown: the outer conductor is

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Figure 4. Advanced experimental setup: (a) the measurement chamber within the Faraday cage. We use two coaxial lines entering from the top into the trans- and cis-chambers. The micro-coaxes are labeled RF in/out. Simultaneously, we probe the direct current through the ion channels and pores in the membrane via Ag/AgCl wires (dc port-in and port-out). (b) Experimental configuration: the two micro-coaxes are placed as close as possible to the suspended membrane. The membrane is painted on a substrate and spans the aperture. This is sketched as a red layer with thicker edges due to the painting process. (c) Close-up of one of the micro-coax tips: showing the insulating layer and inner and outer conductors. For bio-compatibility the whole micro-coax is coated with a parylene. (d) Circuit diagram of the measurement chamber with the dc ports linked by bias-tees for suppressing RF crosstalk to the head-stage amplifier.

separated by a standard dielectric from the inner conductor, which tapers down to a tip of radius just below 1 µm. The whole coax tip, which is immersed into the ionic solution of the cis-side chamber, is covered by parylene to avoid surface potentials interfering with the dc measurement. Finally, the coax tip is mounted and positioned close to the bilipid membrane, about 10–15 µm away, as sketched in figures 4(b) and (c). The thicker edges and thin center of the membrane indicate the typical thinning process of painted membranes. In figure 4(d), an equivalent circuit is given: as seen, the two RF lines are dc-blocked, allowing the measurement of direct RF transmission. The whole chamber is mounted in a Faraday cage to achieve optimal shielding from spurious electromagnetic radiation. In the following, we will first make use of only one of the micro-coaxes. That is, we will feed an RF signal and trace the response in the direct current through single channels and pores. Only in the final stage of the experiment will we focus on direct RF transmission from one micro-coax to the other.
5. Results on single-ion channels

The measurements with the micro-coaxes were carried out in a fashion that we first defined a stable bilayer and in a second step took current recordings of the insertion of the channel-forming proteins. Only then the RF signal was switched on at a specific frequency and power level. As before, we used two types of membrane proteins: ALA and α-HL. For calibration purposes we first painted a bilipid membrane across the \(200\mu m\) aperture and recorded the direct current versus time trace shown in figure 5(a). The insulation resistance of the pure membrane is of the order of \(16\, \Omega\) (a bias of \(40\, mV\) is applied). Switching the RF source on, we find a strong cross-talk signal, which decays with a typical time constant of about \(1\, s\). With a sinusoidal RF signal at \(800\, MHz\), the membrane resistance is reduced to about \(8\, G\Omega\), as seen on the right-hand side of figure 5(a). The time constant corresponds to the discharging time of the membrane; taking the membrane resistance as of now \(R_m = 8\, G\Omega\) in the on-state and a calculated membrane capacitance of \(C_m = 137\, pF\), we find a time constant of \(\tau_m = R_mC_m \sim 1\, s\). For calculations, we assumed the membrane dielectric constant to be \(\epsilon_m \sim 2\) [13] and a circular geometry of the

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Figure 5. (a) Calibration measurement with a suspended bilipid membrane painted over a \(200\mu m\) opening. Shown is the current versus time recording before and after the RF signal at \(800\, MHz\) is turned on. This indicates a very good electrical insulation of \(cis\)- and \(trans\)-sides and minimal heating. (b) Sketch of six ALA peptides inserted into the suspended lipid bilayer. These peptides conjugate and form channels of different sizes. (c) Recording under \(20\, mV\) dc bias and \(800\, MHz\) applied. RF is rectified and enhances the formation and current level of ALA channels, i.e. ions are more effectively pumped through the membrane.
membrane layer with diameter 200\,µm. After this initial spike the current relaxes to a nonzero value, already indicating an effective bias across the membrane. Although the power of the RF signal is comparatively high with about $P = -15$ dBm, we find only minimal heating effects as is evident from the increase in the dc current signal noise, indicated by the pairs of arrows left and right, as seen in the broadening of the current versus time trace. Also, the data presented in figure 5(a) indicate that the bilipid membrane stays intact. After the initial signal spike, due to capacitive crosstalk, the current level almost completely falls back to the high-impedance value before the RF signal is applied. We have to note that this procedure is repeatable, as the data in figure 5(c) show, where the RF signal is first switched on and then off.

The initial measurements were carried out on ALA: these peptides were added to the cis-chamber and can be inserted into the membrane by a small dc bias voltage, due to their dipole moment of $\approx 75$ D. In the membrane, ALAs can combine in different numbers and form ion channels, as sketched in figure 5(b). Depending on the number of peptides, the current through the ion channel scales accordingly to the cross-sectional surface. These channels are only temporarily stable, i.e. their formation depends on the Brownian motion of the peptides in the membrane. The resulting current under a bias voltage of $-20$ mV is shown in figure 5(c). On the left side, the typical ALA recording is found with predominantly smaller channels forming. After switching the RF signal on at $\approx 800$ MHz and relaxation of the current spike, we find a strongly enhanced current level. This is already a clear indication of an effective increase in dc bias by the RF voltage. This voltage enhances the current through the membrane by pumping the ions more effectively and increasing channel formation. We found several frequencies (between 750 and 850 MHz) at which the current through the pores could be increased, but the best coupling was achieved at a frequency of 788 MHz. This frequency selectivity is most likely due to the geometry of the conducting elements and the dielectrics of the measurement cell, i.e. the location of the dc lines, the coaxes, the chip, the pore and membrane and the aqueous solution in the chamber. Importantly, the RF signal at $\approx 800$ MHz is faster and able to reverse (or pump) the flow of ions through the channels and pores with dwell times of $(1/100–1/200)\,\text{MHz}^{-1}$.

In figure 6, the measurements are repeated for different dc bias values: in (a), the current is traced without bias. At zero bias, no ALAs are inserted into the membrane and consequently no current spikes are observed. Switching the RF signal on induces ion channel formation with an average channel current corresponding to an effective bias of about 25 mV. In figure 6(b), the conditions are identical except that the bias is inverted to +20 mV. The ALA peptides are inserted into the membrane and show the typical current spikes. However, when the RF signal is turned on, ion channel formation is knocked off. Only after the excitation is turned off do the ALA channels form again with the typical current trace. So, therefore we can conclude that the application of an appropriate RF-modulation corresponds to an effective dc voltage, which is applied to the membrane. This can be interpreted as rectification of the RF at the highly resistive bilipid membrane junction.

Conventionally, the term ‘rectification’ refers to the conversion of an electromagnetic ac signal into a dc voltage across a junction. The junctions’ $IV$ characteristic has to be nonlinear of some form, which is the result of asymmetric barrier resistances and capacitances such as in a Schottky diode. In dc measurements on ALA it was shown by Woolley et al [14] that the $IV$ relation of ALA is non-ohmic due to the electrostatic potential profile within this ion channel. This is also termed ‘rectification’. In more recent work by Siwy et al [15–17], asymmetric artificial nano-pores were fabricated in thin membranes. A similar rectification of ionic currents was found, being related to the asymmetry of the surface charges inside the
nano-pore. Heins et al [18] have finally shown that this rectification effect can be enhanced by placing asymmetric molecular groups at the nano-pore. This so-called chemical rectification leads to a further increase of the non-Ohmic response. In summary, we can state that applying an RF signal leads to rectification, which in turn effectively pumps ions through the artificial ion channels.

6. Rectification on single pores

Obviously, the asymmetric potential along the ion channel or nano-pore determines the transport properties, i.e. the rectification effects are reflected in the response to an RF excitation. This appears to be the case for the ion channel ALA, as we discussed previously. In order to determine how the potential and geometrical shape of the protein-forming channel influences this rectification process and the resulting pumping of ions, other proteins have to be considered. Hence, we also employed the porin α-HL for the second line of experiments, which in contrast to ALA acts more like a dc resistor once inserted into the bilipid membrane [7]. This is related to the fact that pores allow a constant flux of ions after insertion. There is no gating mechanism as for the ion channel ALA. Consequently, pores or porins, as they are sometimes called, lend themselves as a perfect calibration set from the electrical engineering perspective. Furthermore, it is important to note that α-HL is a protein which possesses a highly asymmetric molecular structure once inserted into the membrane (see figure 7(a)). The opening of the pore on the

Figure 6. Bias variation on ALA under excitation: (a) RF triggers channel insertion at zero bias applied, i.e. rectification. Both results indicate rectification of the RF signal, since an effective dc voltage is present. (b) At +20 mV bias the RF knocks off channel activity, leading to an effective pumping of ions against the bias.
Figure 7. Measurements on a protein pore: (a) single $\alpha$-HL after insertion into the bilipid membrane from the cis-side. The mushroom-like head of the protein faces the incoming RF signal. (b) Circuit diagram with membrane resistance and capacitance, $R_m$ and $C_m$, respectively, and the $\alpha$-HL as a lumped resistor with $R_{\alpha-HL} \approx 1.1$ G$\Omega$. (c) Current through a single $\alpha$-HL pore with the standard current level at $-35$ pA, which is enhanced to about $-55$ pA under RF pumping at $-40$ mV bias.

cis-side has a diameter of 2.9 nm, which widens into a mushroom-shaped vestibule of 4.2 nm diameter and again narrows to an opening of 2 nm on the trans-side.

In figure 7(a), a simplified circuit diagram is shown: in parallel with the membrane resistance and capacitance, we place a single $\alpha$-HL pore with a resistance of $R_{\alpha-HL} = 1.1$ G$\Omega$. Once $\alpha$-HL is added to the cis compartment of the setup, it is inserted into the membrane with the head of the protein facing the RF signal (see figure 7(b)) and the current level rises to $-35$ pA under a bias of $-40$ mV. In figure 7(c) the direct current through the pore is shown with and without the RF signal. Evidently, switching on the RF results in a current spike, but with a reduced relaxation time as compared with the ALA measurements (see figures 5 and 6). This is related to the initial resistance of the system being an order of magnitude smaller, $R_{\alpha-HL} \approx 1$ G$\Omega < 9$ G$\Omega \sim R_{ALA}$ and consequently $\tau_m \sim 100$ ms. During RF emission the current through the pore is enhanced by 20 pA, indicating pumping of ions through the pore at a rate of $\tau = f^{-1} = 1/800$ MHz$^{-1}$. This shows the possibility of real-time spectroscopy, i.e. the passage of single ions can now be resolved in the time domain. The current level of 20 pA then translates to $\langle n \rangle = \langle I \rangle / (ef) = 0.16$ ions on average, which are pumped through the pore in each cycle of the RF signal ($f = 800$ MHz). The increase by almost a factor of two in current again shows pumping of ions by the RF voltage. It is accompanied by a moderate increase in noise level. In addition to the rectification on single-ion channels, the single pore rectification indicates that micro-coaxes can be reliably used for delivering RF power to nanoscale structures [19].

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7. Direct RF transmission

We now make use of the full potential of the measurement setup depicted in figure 4 by employing one micro-coax as an RF emitter and the other previously passive coax as an RF detector. As before, we record the direct current simultaneously. The experimental conditions were similar to those described in the previous section. The RF micro-coaxes are now brought in from both sides on optical rails to allow for exact positioning close to the suspended bilipid membrane. The alignment procedure is critical for this measurement, since it determines the coupling efficiency of the coax line. We were able to bring the coaxes as close as 10 µm to the suspended bilipid membrane. The RF source generates a sinusoidal continuous wave excitation. Both coax tips, which are immersed into the ionic solution of each side of the chamber, are covered with parylene to avoid surface potentials interfering with the dc measurement.

As before, the measurements are then carried out in such a fashion that we first define a stable bilayer and in a second step took recordings of the insertion of the channel-forming proteins, as sketched in figure 8(a). Only then was the RF signal switched on at a specific
frequency and power level. In these experiments, the mushroom-shaped top of α-HL is facing the cis-side. For calibration purposes of the absolute seal resistance, we first painted a bilipid membrane across the 200 µm aperture and recorded the direct current versus time. The insulation resistance of the pure membrane is of the order of 16 GΩ (a bias of 40 mV is applied). The membrane capacitance was found to be of the order of $C_m = 137 \text{ pF}$.

For α-HL no direct gating mechanism exists as for ion channels. The classical method to modulate the current through α-HL is to add a small molecule, such as β-cyclodextrin (β-CD). This sugar component is ring-shaped with an outer and an inner diameter of $\sim 1.66 \text{ nm}$ and $\sim 0.7 \text{ nm}$, respectively; see figure 8(a). Hence, β-CD can propagate into the pore, but is finally blocked at the neck of the α-HL, thus reducing the total current through the pore, as shown in the direct current blocking in figure 8(b). For the measurements, we used the configuration with a single α-HL pore embedded into the membrane from the cis-side, being blocked by β-CD from the trans-side. In this blocking mode, the current through the pore is reduced to about $-5 \text{ pA}$. In the traces of figure 8(c), the same recordings are shown with the microwave transmission of one micro-coax tip to the other via the single pore. The measurements are taken with a bandwidth of 3 MHz as compared to the dc data for which we reduced the filter width to 10 kHz. Hence, the RF-data trace had to be strongly filtered. The averaged RF signal is finally represented by the red line. The capacitive coupling we estimate to be of the order of $\delta C_{\text{pore}} \approx 50 \text{ aF}$. As seen, we find a correlation of the blocking events in the direct current and the RF transmission.

Similar to standard dc recordings we traced the opening/blocking events shown in figures 8(b) and (c) over longer time scales for analysis. Obviously, the SNR for the conventional recordings is still superior to the RF traces. Nevertheless, we are able to pick up the small changes in capacitance once the molecular pores are blocked and thus can directly record single-pore action at microwave frequencies. Upon improving this coupling capacitance by reducing the micro-coax dimensions and enhancing the positioning accuracy, we are confident to broaden the coaxes’ bandwidth dramatically. A summary of the measurements is depicted in the histogram of figure 9: it shows bins of open and blocked distributions for the case of dc recordings $I$ and microwave transmission $V_{\text{mw}}$. As seen, the dc counts for the open channel show the expected Gaussian distribution. In contrast to this, the RF counts obtained at a bandwidth of 3 MHz show a Gaussian with a much narrower distribution for the blocked state as well as for the open state. At the current resolution this is attributed to the filtering required for retrieving the RF signal. In any case, we can state that the method is operational and delivers statistically reliable data.

8. Summary and outlook

In summary, we demonstrated the efficient coupling of RF signals to single pores embedded in bilipid membranes. In the first set of experiments, we demonstrated the combination of TLs with an on-chip single-channel recording unit. In further measurements, we presented how to improve such a passive TL, by implementing bio-compatible micro-coaxes for direct RF delivery. The simultaneous direct current recordings show a good seal resistance, which will enable RF recordings with a superior SNR due to the reduction of $1/f$-noise at RFs. Using the micro-coaxes, we find rectification of the RF power with a resulting voltage. This effect can be applied for pumping ions through the channels. Finally, we have shown that the combination of two micro-coaxes can also be used for picking up RF signals modulated by molecular pores. We find that the direct transmission modulation occurs in clear coincidence with direct
**Figure 9.** A histogram summarizing the total measurements presented in figure 8. The solid black line shows a Gaussian distribution of the open and blocked events for the current bins. The red line presents the RF signal bins for the corresponding events, also as a Gaussian. Note that the current $I$ is on the lower side of the graph and the microwave voltage $V_{mw}$ is on the upper side.

**Figure 10.** Integrated micro-coax for purely capacitive reflection measurements. The channels and pores are situated on top of a silicon probe tip fabricated in the doped substrate, so that the analyte flow can be traced capacitively at high speed.

current recording. This method provides evidence for the potential of real-time spectroscopy on single-molecular channels and points toward future applications in high-throughput drug screening applications [3].

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A drawback of the current setup is that both micro-coaxial lines have to be carefully placed very close to the suspended bilayer. If this is not the case, RF coupling is minimal and the transmitted signal vanishes in the noisy background. A possible remedy to this shortcoming is a change in the microwave circuit layout, as shown in figure 10. Instead of relying on positioning two micro-coaxes, we envision integration of the coax-port into the substrate. The channels and pores can be anchored within the bilayers, which are placed on top of an adhesive monolayer. The proximity of channel/pore and coax tip maximizes the achievable SNR. Apart from optimizing the coupling capacitance and thus increasing the readout frequency, such a geometry enables the addition of ever more versatile high-speed microwave components into the substrate. The obvious next step is to design resonant circuits, i.e. tank circuit, etc, for probing analyte flow and possibly protein conformational changes. This geometry is also suitable for on-chip integration and, hence, is of potential use in high-throughput screening.

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