Detection of lipid bilayer and peptide pore formation at gigahertz frequencies

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A coplanar waveguide defined on a borosilicate glass chip is integrated with a microfluidic cartridge to allow for characterization of lipid bilayer membranes at gigahertz frequencies. The membranes are deposited on the waveguide using a vesicle fusion method. The deposition of the bilayers results in a significant change in the high-frequency transmission properties of the waveguide. We also embed alamethicin, an ion channel forming peptide, in the bilayers. The influence of the pore forming peptides can be clearly detected in the transmission signal. © *2006 American Institute of Physics*. [DOI: 10.1063/1.2159571]

Dielectric spectroscopy is an important and widely used technique for the characterization of biological matter.[1] It provides a comparatively simple electronic way to gain information about such systems without the need for fluorescence labeling or other chemical modifications. In addition, electronic methods are compatible with other sensor techniques and hold the potential for parallelization and integration. Dielectric spectroscopy is routinely performed to characterize the dielectric response of materials in the mHz to GHz regime.[2] In this frequency range, one can discern between different types of dielectric behavior. The so-called α-dispersion relates to the influence of small ions on the dielectric properties of a sample, whereas β-dispersion arises from distortions of macromolecules and membranes. The dipolar relaxation of the solvent molecules themselves is the origin of the γ-(or Debye) dispersion.[1]

As model systems for biological cell membranes, artificial lipid bilayers are among the most interesting systems amenable to such an analysis. Lipid membranes define interfaces in biological systems over which communication and transport is regulated by membrane-bound macromolecules. In this context, ion channels and pores are of particular interest as they naturally transduce molecular into electrical, i.e., ionic signals.[3] On lipid bilayer systems, conventional dielectric spectroscopy has been performed in the frequency range from a few mHz up to 100 kHz.[4–7] The spectra can be well understood in terms of equivalent circuits which model the membrane as a parallel connection of a resistance and a capacitance. The membrane capacitance per unit area is typically on the order of $C=1\ \mu\mathrm{F\,cm^{-2}}$, resistances R vary between 1 $\mathrm{k\Omega\,cm^2}$ and 1 $\mathrm{M\Omega\,cm^2}$. Effects like the polarization of the membrane-solvent interface therefore occur on a timescale RC which is in the millisecond to second range.

In contrast, we here perform GHz frequency transmission experiments on waveguides supporting lipid bilayer membranes in the absence and presence of pore-forming peptides. GHz frequencies have been previously used for the characterization of DNA or protein bulk solutions, and also of bacterial samples.[8–10] In these experiments, processes occurring on much shorter timescales are probed than in conventional impedance experiments. GHz transmission data may therefore contain important additional information about fast processes occurring in a system, e.g., small molecular rearrangements and distortions. Furthermore, due to the simplicity of the experimental setup, transmission experiments may be used for fast and inexpensive sample characterization.

High-frequency (HF) experiments were performed with coplanar waveguides (CPWs) lithographically defined on glass substrates (see Fig. 1). To contain and exchange reagent

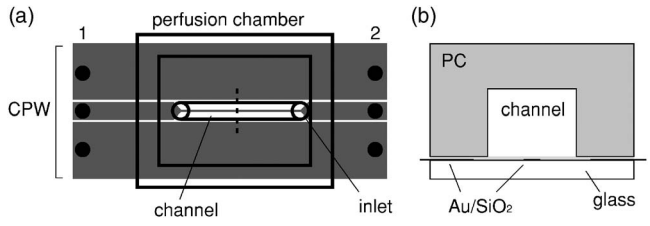

FIG. 1. (a) Top view of the CPW defined on a borosilicate glass slide with size $24\times40\ \mathrm{mm^2}$. The conductor layers are 200 nm Ti/Au covered with 300 nm SiO_2. Glued on top of the CPW is a polycarbonate microfluidic chamber. The inlets of the chamber and the channel where fluid is in contact with the CPW are indicated. The dimensions of the CPW were chosen in order to match its impedance to the 50 Ω inputs of the network analyzer. The impedance was calculated from the distributed capacitances of the waveguide, for which estimates for the effective permittivity of its surroundings (air, water, polycarbonate) were used. These effective values were obtained for the static case from numerical calculations with a self-written Laplace solver. Outside the channel area, the width of the center conductor is 210 μm and the distance of the ground lines is 300 μm. Below the channel, the width of the inner conductor is reduced to 6 μm. The CPW is contacted with microwave probes (indicated by black spots) and transmission is measured from Port 1 to 2. (b) Cross section of the chamber at the position indicated by the dashed line in (a). The cross section area of the channel is $0.5\times0.75\ \mathrm{mm^2}$.

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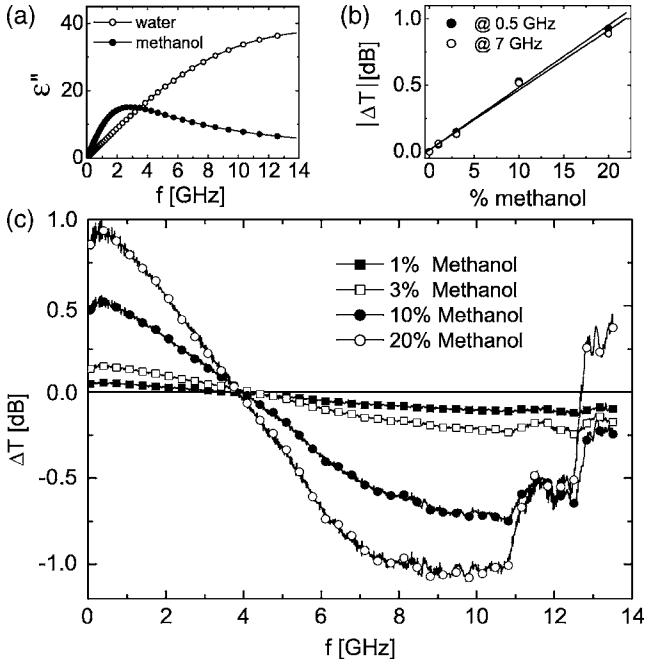

FIG. 2. (a) Negative imaginary part ϵ'' of the dielectric function of water and methanol in the frequency range covered by the experiments. (b) Linear dependence of the absolute value of ΔT from part (c) of this figure at 0.5 GHz and at 7 GHz on the methanol fraction. (c) The transmission of the waveguide changes significantly when the chamber is loaded with solutions with increasing methanol fraction. From all signals, the transmission of the waveguide in the presence of pure PBS buffer has been subtracted.

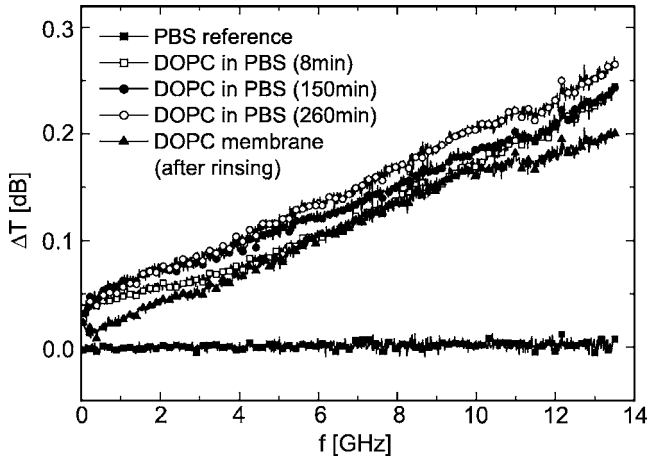

FIG. 3. Signal change due to the formation of a DOPC bilayer. The reference signal is obtained with pure PBS buffer solution. To form the bilayer, lipid vesicles in PBS are injected into the perfusion chamber. A clear signal change is observed a few minutes after introduction of the vesicle suspension. This signal slightly increases over a period of several hours. Extensive subsequent rinsing with buffer solution does not change the signal significantly, indicating that most of the change in HF transmission is caused by the formation of a supported lipid bilayer membrane.

and buffer solutions, a polycarbonate microfluidic chamber (NPC©, Nanion Technologies GmbH) was glued on top of the chips. The HF transmission of the waveguide was then determined in the frequency range from 50 MHz to 13.5 GHz using a network analyzer (HP 8719A). The CPW was contacted using HF probes on a semiconductor probe station (Karl Suess PM5/40 GHz-$|Z|$ probes). For calibration experiments, the microfluidic chamber was initially filled with buffer solution [phosphate buffered saline (PBS), *p*H 7.0]. Transmission signals obtained from pure buffer solutions were used as reference signals for the following measurements. The experiments were carried out in an air-conditioned lab and the solutions were allowed to equilibrate to room temperature (θ=20 °C) before injection.

As a test for the sensitivity of our setup the response to a change in the dielectric properties of the environment of the CPW was checked. To this end, mixtures of methanol (MeOH) and PBS with various fractions of the alcohol were added to the perfusion chamber. The result of these measurements is shown in Fig. 2(c). The transmission signal changes $\Delta T = T_{\text{sample}}/T_{\text{ref}}$ of the MeOH/PBS mixtures are given relative to a calibration solution with pure PBS, without MeOH. With increasing MeOH fraction, ΔT systematically and characteristically varies [Fig. 2(b)]. These changes reflect the different dielectric behavior of methanol as compared to water: At 20 °C, MeOH has a static permittivity $\epsilon(0)=34.8$,[11] while water has $\epsilon(0)=80.3$.[12] The dipolar relaxation time for water is $\tau=9.6$ ps,[12] whereas MeOH relaxes with $\tau=56$ ps.[11] Hence, the dielectric relaxation of methanol occurs at much lower frequencies and within the frequency range investigated [Fig. 2(a)]. Apart from dissipative effects associated with the dipolar relaxation, other mechanisms may contribute to the observed ΔT: Power reflection due to variations in the impedance and radiative power loss. Conductivity contributions, in contrast, can be neglected at GHz frequencies.[13] Owing to the complexity of the system, the relative magnitude of the contributing effects cannot be easily extracted from the transmission data. Above approximately 10.5 GHz, strong signal variations are visible. In this frequency range, the corresponding wavelength approaches characteristic dimensions of the setup and the signal is dominated by interference effects.

The preparation of a supported lipid bilayer on the waveguide was accomplished using the vesicle fusion method.[14] For this method, lipid vesicles from the neutral phospholipid dioleoylphosphocholine (DOPC) (Avanti Polar Lipids; c=25 mM) were ultrasonically prepared in buffer solution. The resulting vesicle suspension was filled into the NPC chamber. Vesicles then settled on the chip surface and fused to form a continuous lipid bilayer system covering the whole surface. The formation and the fluidity of the bilayers—which is important for the incorporation of peptides and ion channels—was checked independently using fluorescence bleaching experiments. For these, fluorescently labeled lipids (BODIPY C_5-HPC, Molecular Probes) were intermixed to 0.1% with DOPC before sonication.

With the CPW setup, the formation of the bilayers can also be directly monitored in the HF transmission T of the waveguide. This is demonstrated in Fig. 3. The introduction of the vesicle suspension and subsequent bilayer formation leads to a significant ΔT. The signal stabilizes after a few minutes and further increases only slowly over a period of several hours. Even after extensive rinsing of the chamber with buffer the signal is only slightly reduced. This demonstrates that the signal change is mainly due to the bilayer itself and not due to the lipid vesicles in suspension above the waveguide.

Compared to the experiment with MeOH/H_2O mixtures, the bilayer signal allows for a simpler interpretation. The relaxation of the dipolar phosphocholine lipid head groups occurs at frequencies as low as 100 MHz (Ref. 13) with only little variation in the GHz frequency range. By contrast, wa-

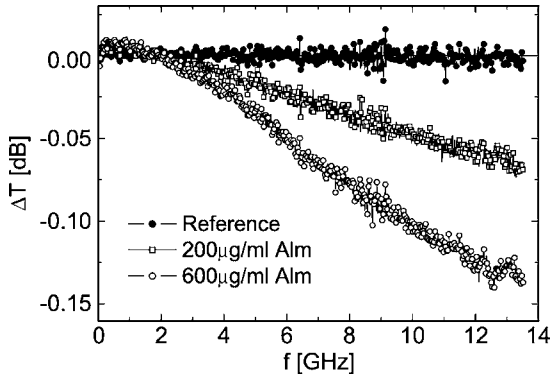

FIG. 4. Transmission of the CPW with DOPC membrane after addition of alamethicin (Alm). The reference signal is obtained before the addition of alamethicin.

ter displays increasing absorption with increasing frequency in the investigated range [Fig. 2(a)]. As the lipid bilayer displaces water above the waveguide, the influence of the HF absorption by water on the transmission is reduced accordingly. The fact that the signal stays almost unaltered even after rinsing the chamber suggests that the setup is indeed more sensitive at the waveguide surface than at other locations in the sample chamber.

For experiments on nanopores, the pore forming peptide alamethicin (Alm) was used.[15] Alm was dissolved in MeOH at various concentrations and subsequently diluted in PBS. The final fraction of MeOH was 4% in each case. After formation of a lipid bilayer in the NPC chamber as described above the buffer was exchanged with a 4% MeOH/PBS solution and a new reference signal was recorded. Then Alm/MeOH/PBS solutions were injected into the chamber. As shown in Fig. 4, addition of Alm results in a clear transmission change ΔT.

Presumably, this change in the transmission signal reflects the reversal of the previous effect. By incorporation of alamethicin, "holes" are introduced into the membrane through which the buffer solution can be sensed by the waveguide. Hence, the signal change is opposite to the change that was observed after bilayer formation. In fact, if the Alm concentration is increased to values as high as 1.6 mg/ml the transmission signal closely resembles the signals obtained for the MeOH/PBS mixtures discussed above (not shown). This indicates that at excessive Alm concentrations the bilayer is no longer intact and only the buffer solution is measured.

The sensitivity of our setup depends on the resolution limit of the network analyzer and its temporal drift. With our setup, peak-to-peak noise was on the order of 10 mdB at 10 GHz. In the case of Fig. 4, this corresponds to a sensitivity on the influence of an alamethicin concentration of about 50 μg/ml. This sensitivity could be significantly enhanced if the measurement setup was optimized, e.g., for (mechanical) drift stability or small frequency bands.

In summary, our measurements show that lipid bilayer formation can be monitored in the transmission of a coplanar waveguide at GHz frequencies. The measured signal is surface-sensitive and reflects the reduced influence of the dielectric properties of the buffer solution on the waveguide transmission. Accordingly, it is shown that also the change in dielectric response caused by a pore-forming peptide, such as alamethicin can be monitored with this technique.

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[1] E. H. Grant, R. J. Sheppard, and G. P. South, *Dielectric Behaviour of Biological Molecules in Solution* (Clarendon, Oxford, 1978).
[2] E. Barsoukov and J. R. MacDonald, *Impedance Spectroscopy* (Wiley, Hoboken, 2005).
[3] B. Hille, *Ion Channels of Excitable Membranes* (Sinauer Associates, Sunderland, 2001).
[4] P. Diao, D. L. Jiang, X. L. Cui, D. P. Gu, R. T. Tong, and B. Zhong, Bioelectrochem. Bioenerg. **45**, 173 (1998).
[5] G. Wiegand, K. R. Neumaier, and E. Sackmann, Rev. Sci. Instrum. **71**, 2309 (2000).
[6] O. Purrucker, H. Hillebrandt, K. Adlkofer, and M. Tanaka, Electrochim. Acta **47**, 791 (2001).
[7] G. Wiegand, N. Arribas-Layton, H. Hillebrandt, E. Sackmann, and P. Wagner, J. Phys. Chem. B **106**, 4245 (2002).
[8] J. Hefti, A. Pan, and A. Kumar, Appl. Phys. Lett. **75**, 1802 (1999).
[9] G. R. Facer, D. A. Notterman, and L. L. Sohn, Appl. Phys. Lett. **78**, 996 (2001).
[10] M. K. Choi, K. Taylor, A. Bettermann, and D. W. van der Weide, Phys. Med. Biol. **47**, 3777 (2002).
[11] B. P. Jordan, R. J. Sheppard, and S. Szwarnowski, J. Phys. D **11**, 695 (1978).
[12] R. Buchner, J. Barthel, and J. Stauber, Chem. Phys. Lett. **306**, 57 (1999).
[13] U. Kaatze, K.-D. Göpel, and R. Pottel, J. Phys. Chem. **89**, 2565 (1985).
[14] J. M. Johnson, T. Ha, S. Chu, and S. G. Boxer, Biophys. J. **83**, 3371 (2002).
[15] G. A. Woolley and B. A. Wallace, J. Membr. Biol. **129**, 109 (1992).