Hardware module for single Ion channel spectroscopy with 100ps time resolution (Nano-patch-clamp)

Simon Grall,[1] F. Kienberger,[2] T. Fujii,[3] Nicolas Clement,[3] Georg Gramse[1*]

[1]Institute of Biophysics, Johannes Kepler University, 4020 Linz, Austria;
[2]Keysight Labs Austria, Keysight Technologies, 4020 Linz, Austria;
[3]LIMMS-CNRS/IIS, Institute of Industrial Science, University of Tokyo, Tokyo 153–8505, Japan
*Corresponding author: Georg.gramse@jku.at

ABSTRACT

Electrochemical recording of signals at nanoscale remains one of the most important challenges in electrochemistry and life science, with the promise to better understand biology at nanoscale and to develop new biosensor technology. Here, we show that the use of high-frequency hardware allows for detection of local electrochemical reactions associated with ultra-small currents in sub-fA range. The module developed in the framework of this EU-attract project that combines advantages of unprecedented electrical sensitivity and compatibility with Internet of Things hardware technology could be implemented in any scanning probe microscope, and potentially more broadly in lab-on a-chip devices in the near future.

Keywords: High-frequency hardware, nanoelectrochemistry, Ion channels.

1. INTRODUCTION

- Detection of ultra-small ionic and electrochemical currents is required in many R&D fields starting from surface electro-chemistry, over energy materials and ion channels.
- The ion-channels are of fundamental importance for the function of biological processes in humans and therefore the target of almost a quarter of all currently available drugs for medical treatment.
- Patch-clamp allows the measurement of ionic currents, however, due to physical and technical limitations, only in a time domain down to 1µs.
- This project aims to develop a high temporal resolution patch-clamp device, able to measure from the microsecond to a few hundreds of picoseconds the small current (pA) flowing through ion channels.

Reconstituted ion channels immobilized on gold nanodots embedded on a silicon chip[1,2] allows for fast application of an electrochemical potential to the ion channel at nanoscale. A scanning probe microscope (SPM) can get in very short distance from the ion channel, resulting in a high capacitive coupling between the probe and the nanodot electrode. A pump-probe detection setup using dedicated high frequency electronics and adequate cabling will allow application and sensing of ultra-sharp potential pulses to SPM probe and nano-electrode substrate.

A first implementation is studied using a scanning tunnelling microscope (STM) on an electroactive self-assembled monolayer (SAM) on gold. A vector network analyzer (VNA) is connected to the tip for high frequency measurements. This setup allows measurements of capacitance (C) and conductance (G) as well as their derivatives during the STM topography measurements. The VNA allows fine measurements of the impedance at the end of the tip, insulated everywhere except for insertion and at its apex. The capacitance corresponding to faradic current is measured at several GHz which leads to unprecedented high current sensitivity (<fA). A potentiostat is used to maintain a constant bias between the STM tip and the sample and to vary the potential on the gold surface[3]. Cyclic voltammograms (CV) are recorded locally with a chemical spatial definition of ~100 nm[3].

2. STATE OF THE ART

The current technology for detection of ionic currents through ion channels and pores is based on the patch-clamp technique, which consist of a micropipette-electrode configuration to clamp the channel containing cell and a dedicated high gain and low noise current amplifier to sense ionic current between both sides of the channel as low as sub-pA. The temporal resolution of commercial patch clamp devices is under optimal conditions about 1ms at >0.1pA noise level.

Here we propose a hardware-module that can be connected to a commercial scanning probe microscope and waveform generator to allow much faster measurements of 100 ps.

The current patch-clamp technology is limited 1) by the gain-bandwidth-product of the electronics and 2) physically by access resistance and coupling/stray capacitance between the electrodes on both sides of the channel. Recent technical developments in this field achieved high-end electronics which offers input noise in range of 0.1pA at 10µs temporal resolution (see Fig. 1). While this is a significant improvement of the temporal resolution, it still not allows access to the interesting nanosecond domain.

In this project we develop a device that overcomes the fundamental barriers by shrinking the sizes of the sensing and probing electrode by 4 orders of magnitude in a way that in our new configuration we reduce the RC time constant to the picosecond range what will effectively remove the physical limitation of the temporal resolution. By careful RF-design of the module we enable application of electric fields with frequencies up to ~10 GHz

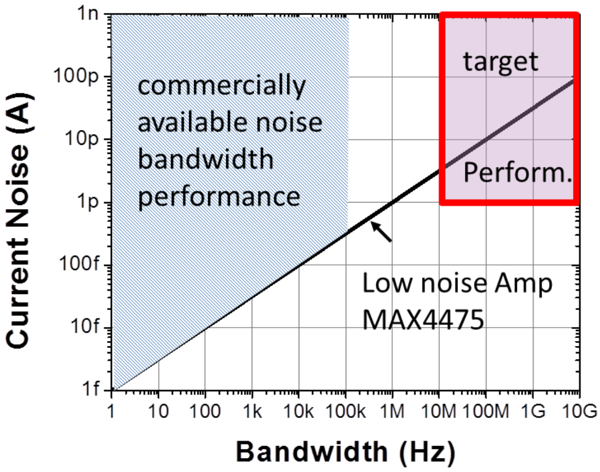

Fig. 1. Figure of merit of state-of-the-art patch clamp electronics versus technological solution developed in this project.

3. BREAKTHROUGH CHARACTER OF THE PROJECT

Nano-bioelectricity is currently an extremely active field of research which may help to elucidate many of the still unknown mechanisms in which directed and selective charge transport happens in the smallest and most complex machinery of ion channels.

Ion channels and pores, as the gates to the inside of our cells and responsible for energy synthesis and information transport play hereby a fundamental role and have been successfully investigated using the patch-clamp technique. This technique has become also one of the major tools for the development of new drugs or treatments of which 25% target ion channels. Nevertheless, the technique, which remained largely unchanged for the last 30 years, is limited in temporal resolution and cannot resolve dynamic processes faster than ~1µs. Since the passage of ions through channels and parts of the gating process are clearly faster (t=10-100ns for passage of ion through a potassium channel) the extension of the temporal resolution of this technique to the ns and ps domain would be a great benefit for the field.

First of all, researchers could investigate the dynamics of the gating and conduction process with unprecedented temporal resolution which may resolve still unknown intermediate steps and resonant effects that are postulated in literature but not measurable. Ultimately it would close the temporal gap between molecular dynamics simulations and experiments and such allow comparison with theory and experiment in Biophysics. Secondly, in the long term, pharmacologists would benefit from this tool which would allow investigation of fast kinetics of the channels in presence of specific drugs.

4. PROJECT RESULTS

The prototype of the electrochemical RF-scanning probe microscope has been developed. It consists of a modified scanning tunneling microscopy (STM) scanner interfaced with a vector network analyzer (VNA), that allows probing the local impedance between the apex of a nano-metric probe and sample on the substrate at GHz frequencies (see Fig. 2). The VNA measures the S11 reflection parameter and allows from this the estimation of local conductance, G, capacitance, C, and its respective derivatives dG/dV and dC/dV simultaneously with topography. By integration of a potentiostat the DC electrochemical potentials on the probe and substrate can be controlled individually with respect to the reference electrode. Standard electrochemical methods like cyclic voltammetry (CV) can be carried out.

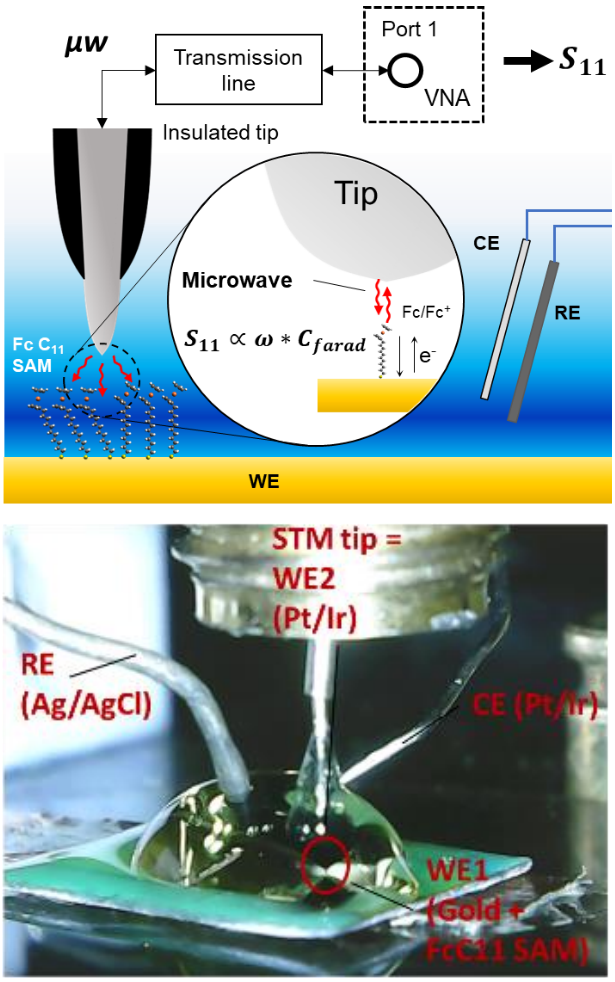

Fig. 2 Schematics and photo of the experimental setup. An isolated STM tip is coupled through an impedance matched transmission line to the VNA. Potentiostat controls electrochemical DC potential of probe and sample. VNA measures high frequency conductance and capacitance below the probe.

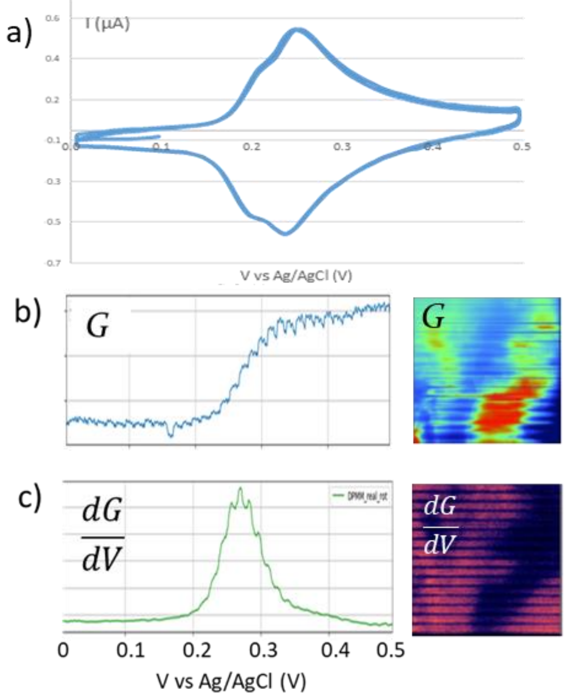

Fig. 3 Cyclic voltamograms (CV) at DC and GHz frequencies and corresponding images. a) Standard CV of FcC11 immobilized on a gold surface (electode area 0.25 cm^2, electrolyte concentration 10mM $KClO_4$). b,c) G and dG/dV measured during cyclic voltammograms with SMM/STM tip. Measurement frequency 2.72 GHz. Corresponding images on the right show the locality of the electrochemical measurement (image size is 2um x 2um).

Additionally with the STM probe the RF tunneling & electron transfer on electroactive species (redox reactions) of few ferrocene molecules are measured at high frequency (1-10 GHz). Typically CV curves on self-assembled monolayers anchored through

a sulfuric bridge to the surface are shown in Fig. 3a. The corresponding high frequency G and dG/dV voltamograms are visualized in Fig. 3b and Fig. 3c, respectively, and show the change of the oxidation state of the ferrocene. The corresponding images proof the locality of the technique and the good lateral resolution of ~100nm.

The operation principle of high-frequency nano-electrochemistry has been successfully demonstrated. Importantly this harware is fully compatible with any scanning probe microscope. Exploiting the sensitivity-amplification coming from the measurement of faradic conductances and capacitances at high frequency instead of DC, allows for detection of faradic processes that would correspond to currents on the order of sub-fA.

After showing that the technique is capable of detecting current levels corresponding to the transfer of just a few 100 electrons at bandwidths of 1 kHz, we are currently working on the integration of specifically optimized nano-electrodes as a replacement of the full gold substrates with the aim to further reduce the background capacitance. Finally, the current work focuses on the adaptation of the measurement protocol for high temporal resolution detection of ionic transport through ionic membrane pores. The results of this work will be reported elsewhere.

5. FUTURE PROJECT VISION

5.1. Technology Scaling

In Attract I our experimental concept has been proven to allow for detection of ultra-small (<fA) ionic and electrochemical currents in electrolyte environment for a well-known electrochemical test system. Next steps will consist in maturing the technology as a tool for research and development in the SPM market. Additionally we will proof the high speed detection capabilities of the technique. We are currently involved in an EMPIR project on this topic, where also one of the partners is an AFM manufacturer. Integration of the RF-SPM as an add-on into an AFM product, which is envisaged at the end of an Attract II project will lift the TRL to the desired level.

In addition, the technological concept will be translated to a Lab-on-the-chip environment in order to enable parallel readout, while reducing instrument cost and making our technology more applicable to a broader community of experts in the life-science sector, beyond the AFM market. Specifically we aim to develop a technology combining nanoelectrodes, lab-on chips and the currently developed but fully integrated hardware, for detecting the binding of cells/viruses and secreted proteins in bilayer membranes with our project partners as detailed below. By measuring the specific capture of the cells also additional release of specific proteins can be measured label-free, simultaneously in real-time. By placing many sensor elements in parallel, the effect of pharmaceuticals and experimental conditions on lipids, sectretome and ion channels can be measured with multiple modes: SPR, dielectric spectroscopy and electrochemistry. This will allow for ultra-detailed, high throughput screening.

5.2. Project Synergies and Outreach

We want to exploit the synergy of Attract I participants and develop a Lab-on-the-chip device that, that is based on the distinct expertise of the partners in the iSLICE projects 236 and 1181 and the nano-patch-clamp project 1107 and is capable of sensing multiple electrochemical and optical parameters of reconstituted ion channels .

The JKU/CNRS developed nano-electrochemical spectroscopy technology will be combined with the new SPR imaging instrument technology. ECsens and CNRS will develop a nanoscale sensor array with integrated in-situ bilayer formation functionality, where both the SPR functionality and the JKU technology will be integrated.

Well known ion channel receptors on cell membranes can be revealed using specific antibodies. These anti-CD antibodies will be spotted on the surface of an SPR-imaging sensor. By measuring the specific capture of the cells also additional release of specific proteins can be measured label-free, simultaneously in real-time. By placing many sensor elements in parallel, the effect of pharmaceuticals and experimental conditions on lipids, sectretome and ion channels can be measured with multiple modes: SPR, dielectric spectroscopy and electrochemistry. This will allow for ultra-detailed, high throughput screening.

5.3. Technology application and demonstration cases

Next steps in an Attract II project will involve the establishment of the developed technique in different research communities. We will take advantage of the involvement in other EU projects in order to gain visibility of the developed technology in the following industrial and research networks on the topics of:

- Nano-electrochemistry (MC-ITN Sentinel). The developed RF-SPM technology, which features a >1000 fold higher sensitivity than conventional electrochemical SPM techniques will be demonstrated as a new tool for research in Nano-electrochemistry, relevant both for R&D on energy materials and life-science applications. (Benefits in: health, green transport, environment)

- Battery technology (H2020-NMBP Nanobat). The Nanobat project involves many industrial partners and pilot lines active interested and active in the field of battery research. The RF-SPM technique with its high electrical and lateral resolution will be employed as a highly beneficial tool to study/optimize the just a few nm thick solid-electroyte-interface layer (SEI) in Lithium ion batteries (LIB), which is highly relevant for the performance of LIB. (Benefits in: green transport, environment, resource efficiency)

- Ion and proton channels (MC-ITN Proton). Both the optimzed RF-SPM and the jointly developed Lab-on-the-chip device will be presented to the partners of the Proton project, which are research organizations or SME active in applied electrophysiology or electrochemistry. (Benefits in: health)

Past and current involvement in EMPIR projects of the European Metrology institutes on electrical SPM technologies will bring benefit to the Research Infrastructure communities in Europe.

5.4. Technology commercialization

Both outstanding performance and technological relevance can be the key drivers for development and successful commercialization of new technologies in research and development. We are convinced that our technology with the singular capability of a >1000 fold increased detection sensitivity for ionic and electrochemical currents and the above mentioned applications are currently highly relevant topics.

We note that SPM has a market value of about 300 Mio $ per year and patch clamp of about 200 Mio $ per year. Even if the developed add-on would be of interest for only 10% of the costumers it would generate a market potential of 50 Mio $ and therefore have a clear impact on industry.

Considering the commercialisation of the *Lab-on-the-chip device* we are collaborating in the ITN project SENTINEL with various small and medium companies active in the field of instrumentation for electrophysiology and electrochemical measurement (Elements SRL, Uniscan). Additionally, we have a close collaboration with Agilent Technologies and NTT, which are large international technology companies, active in life science instrumentation. Especially the current need for fast and cost efficient lab-on-the-chip technologies will make this technology relevant for the global market.

5.5. Envisioned risks

High risk
From the experience of the last month we consider travel and work restrictions to be the highest risks that may lead to delays and difficulties in the collaboration, especially in the multi-partner part of the project. It is still not fully predictable when the situation will change again.

- *Mitigation:* Organizational meetings can be done online, technical meetings that require direct meetings can be shifted to a later point.
- *Contingency:* The parts of the project that do not require direct interaction between partners are planed in the beginning of the project.

Medium risk
The technical integration of multiple technologies into one Lab-on-the chip device may be complex. Although, the partners have extensive experience with this topic, the high frequency matching is partly new.

- *Mitigation:* Additional expertise on signal line matching for low and GHz frequencies can be obtained from Keysight.
- *Contingency:* The frequency range for the detection will be reduced to still obtain good enough S/N.

Low risks
The RF-SPM technique does not show enough electrical sensitivity for application in ion channels or on battery surfaces.

- *Mitigation:* Preliminary results show that sensitivity can be still improved by a factor of 5-10 by optimizing the RF coupling.

- *Contingency:* We will show viability of the approach for research in nano-electrochemistry which has been shown to be successful and the lab-on-the chip application.

5.6. Liaison with Student Teams and Socio-Economic Study

JKU, Keysight and CNRS are currently involved in 3 Marie Curie ITNs related to this topic. These ITNs are training networks for PhD students that facilitate the learning and exchange of technologies between different labs. The project partners will take advantage of the experience and the explanation materials developed in these projects on the technology. Additionally, we plan to create a direct overlap specifically with the projects SENTINEL and PROTON. The student teams will be liaised within these projects by joining the Attract and ITNs-PhDs experiments and during one or more training events that are held at different labs. By this we hope to stimulate a dynamic exchange between the Attract and ITN students on socio-economic aspects that can be potentially also fed back into the ITNs.

6. ACKNOWLEDGEMENT

This project has received funding from the ATTRACT project funded by the EC under Grant Agreement 777222. Additional support by Stefan Muellegger with the ERC project SARF under Grant Agreement 771193 is acknowledged.

7. REFERENCES

[1] N. Clement et al., Large-array of sub-10 nm single-grain Au nanodots for use in Nanotechnology, Small 7, 2607 (2011)
[2] J. Trasobares et al., A 17 GHz Molecular Rectifier, Nature Communications, 7, 12850 (2016)
[3] S. Grall et al., A microwave scanning microscope for study local electrochemistry at the nanoscale. In preparation, (2020)