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Xenopus laevis oocyte based biosensor

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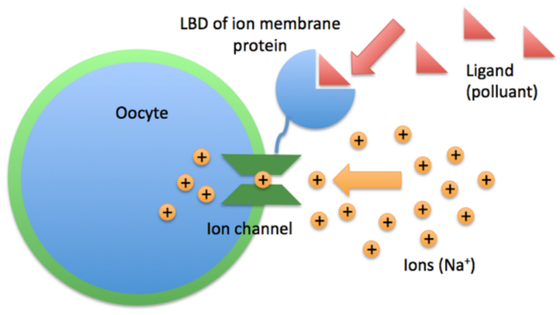
Introduction & Goal

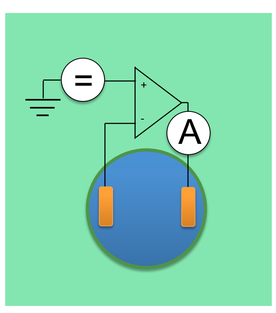
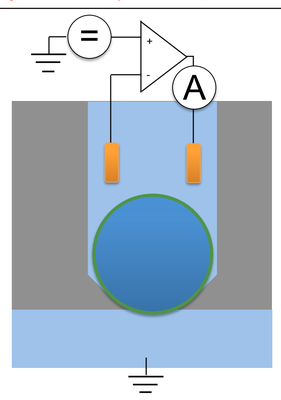
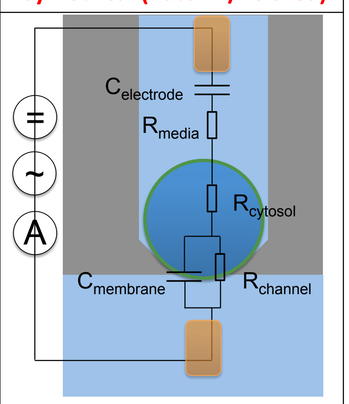
The Envirobot consortium develops a smart aquatic robot integrating sensors that will map water pollutants to find their source. Our research activity focus on a biosensor development based on a *Xenopus Laevis* oocyte expressing specific Ligand Binding Domain Ion Channel sensitive to pollutants. For this purpose, *Xenopus Laevis* Oocytes are microinjected with cRNA coding ligand-gated ion channels (LGCIS). LGICs are transmembrane proteins which modulate ions flux such as Na^+, K^+, Ca^{2+}, or Cl^- in response to the binding of a ligand being the pollutant.



In the context a pollutant sensor embedded in the robot, inherent constrains are long term oocytes measurements as well as operations in vibrating environments. This work consists of developing a non-invasive robot compatible method to perform electrophysiology on a Xenopus Laevis Oocyte allowing to measure ion channel activity evoked by pollutants present in water.

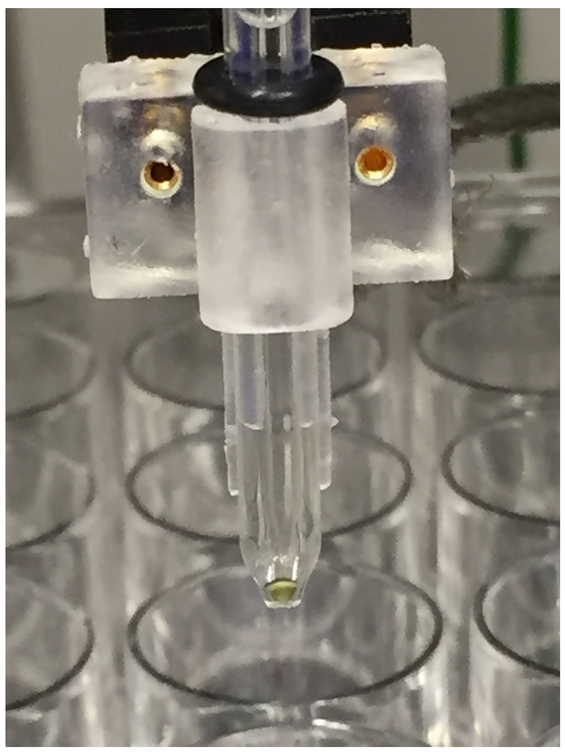
Material & Methods

A first method tested was the Asymetrical Transoocyte Voltage Clamp (ATOVC), a method developed by Schaffhauser et al. [1]. A second and new method that we called ZVC for Impedance Voltage Clamp was developed and tested. This new method is based on a pure non invasive impedance measurement and allows investigation of cytosolic resistance, membrane conductance and capacitance that are parameters affected by the binding of an analyte to the ligand binding domain of ion channels. A setup compatible with both methods ATOVC and ZVC was developed and adapted from an existing automated TEVC (HiClamp, MCS, GE). The oocytes is placed in a glass pipette, two electrodes are placed in the upper compartment and two other in the lower compartment allowing all measurement configuration (ATOVC & ZVC). Additionally an impaling electrode can be simultaneously inserted in the oocyte to monitor the oocyte inner electrical potential as a control value (Fig. 1 a).

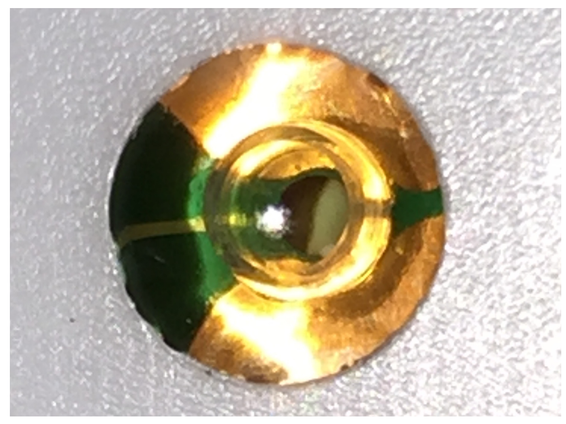
	TEVC	ATOVC	ZVC
measure	Current (inner potential potential imposed)	Current (inner potential potential imposed)	Impedance
setup	TEV200A	TEV200A	Impedance meter
invasive	yes	no	no
Surface	whole cell	Asymetrical (Patch 1/20 area)	Asymetrical (Patch 1/20 area)
Schematic			

Once the impedance measurement was proven with the glass pipette based setup, a second setup suited for impedance measurement was developed for robot integration with smaller dimension and facilitated oocyte placement and aspiration (Fig. 1 b)

a)



b)



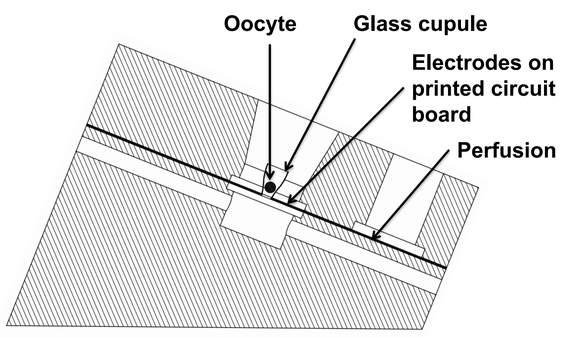


Figure 1: a) Combined ATOVC & ZVC setup based on the MCS HiClamp system, b) ZVC setup developed for robot integration

Results

To demonstrate ion channel activity measurement, oocytes expressing rat Epithelial sodium channel (rENaC) as well as non-injected oocytes selected as control were used. Real part of impedance was measured at 25Hz in the glass pipette based setup. Figure 2 shows an oocyte expressing ENaC (red curve) presenting an increase of impedance when the oocyte is dipped in a solution containing an ENaC blocker (Amiloride). The same figure shows an non-injected oocytes not presenting the impedance increase when dipped in the amiloride solution. Hence, we showed that ENaC channel closing due to amiloride can be monitored by impedance.

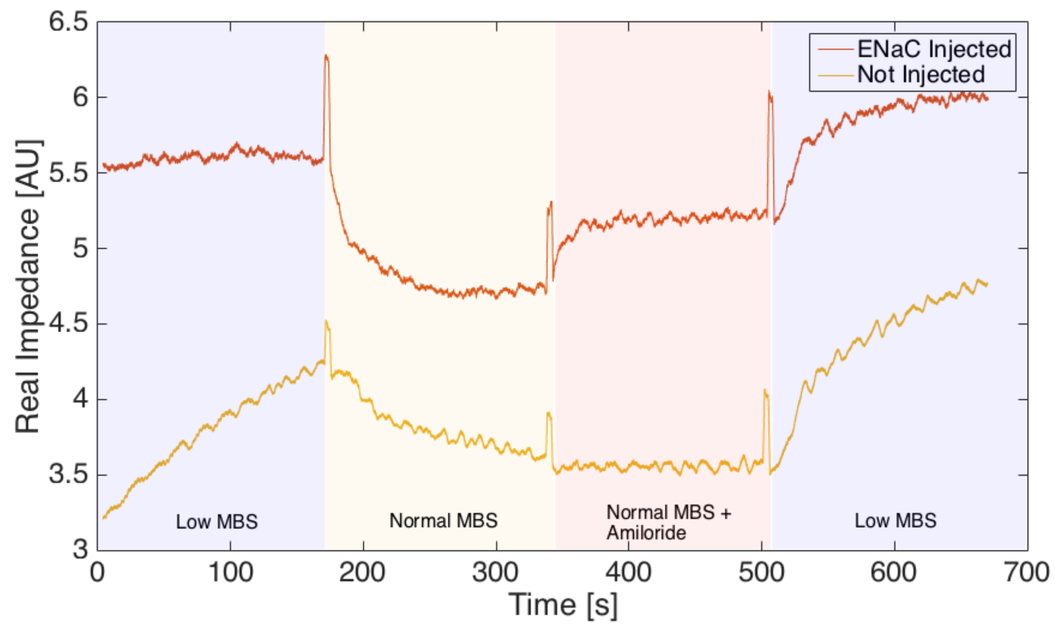


Figure 2: ZVC measurement showing the real part of impedance at low freqency (25Hz) on an oocyte expressing ENaC (red curve) and an oocyte not injected (yellow curve) with and without amiloride in the perfusion.

Conclusion & Outlook

We demonstrated the ability to measure ENaC channels activity by ZVC with impedance changes due to the amiloride channel closing. Using the glass pipette based setup, performances will be compared with ATOVC and standard TEVC methods. Similar meausrements will also be performed on the ZVC setup for robot integration.

References

1. D. F. Schaffhauser, O. Andrini, C. Ghezzi, I. C. Forster, A. Franco-Obregón, M. Egli, and P. S. Dittrich, “Microfluidic platform for electrophysiological studies on Xenopus laevis oocytes under varying gravity levels” *Lab Chip*, vol. 11, pp. 3471–8, 2011.