BIOLOGICAL PHOTORECEPTORS FOR QUANTUM TECHNOLOGIES

Ilaria Cardace[1,2], Luisa De Marco[2], Adriano Cola[2,3], Lorenzo Dominici[2], Vincenzo Ardizzone[2], Giuseppe Gigli[1,2], Daniele Sanvitto[2]

1, Università del Salento, Dipartimento di Matematica e Fisica "E. De Giorgi", Lecce (LE)
2, CNR Nanotec, Istituto di Nanotecnologia, Lecce (LE)
3, CNR IMM, Istituto per la microelettronica e microsistemi, Lecce (LE)

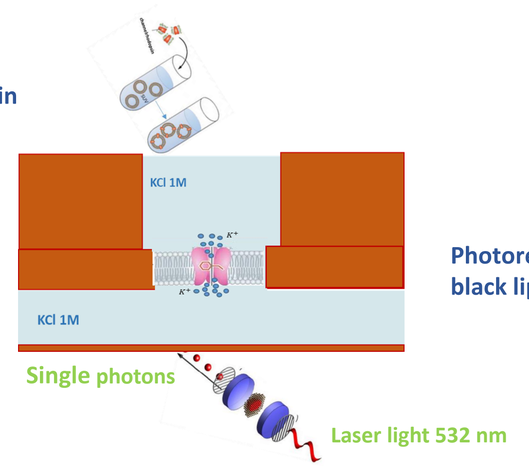
OBJECTIVE

The present project is based on the idea of exploiting natural photoreceptors to generate single-photon detectors. The use of genetically modified cells or molecules would allow the conversion of single photons in electrical signals with efficiency and size advantages (Carter & Lecea, 2012).

WHY?

- **Conversion of a single photon in current**
- **Substantial reduction of the device size**
- **Possibility of array formation**
- **Operation at room temperature**

Photoreceptors reconstituted in liposomes or nanodiscs



Photoreceptors inserted in a black lipid membrane (BLM)

Fig. 1 *Schematic representation of a microfluidic device to be used for the project.*

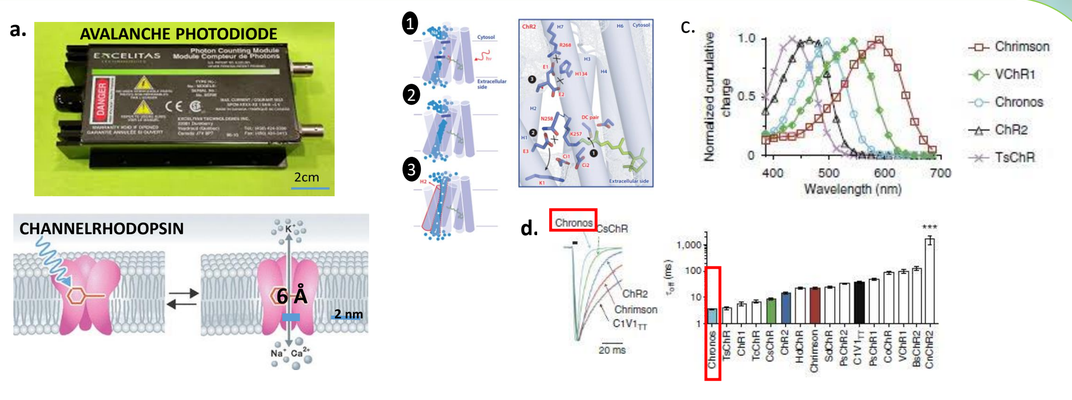
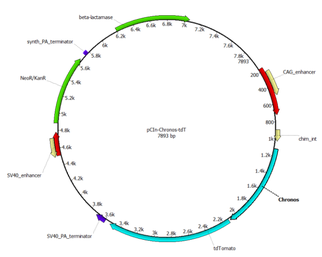


Fig. 2a. *Comparison of the size of an avalanche photodiode to a photoreceptor molecule.* ***b.*** *schematic representation of the operation of a Channelrhodopsin photoreceptor: a single photon absorption (1) leads to the isomerization of the retinal group and a breakage of hydrogen bonds (2), this is followed by a movement of the H2 helix away from the molecule axis, the breakage of a second hydrogen bond and channel opening (3) (*Schneider et al., 2015). ***c.*** *Typical action spectra and* ***d.*** *kinetic responses of Channelrhodopsin variants measured in cultured neurons* (Klapoetke *et al.*, 2014).

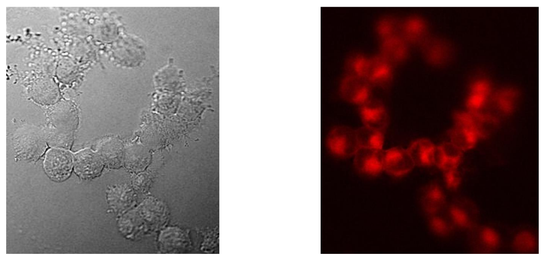
METHODS

Protein expression and integration in nanostructures

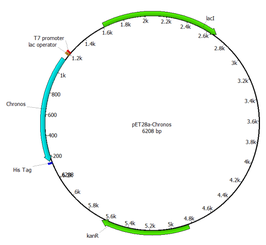
a. **In a eukaryotic expression vector (pCI-neo)**



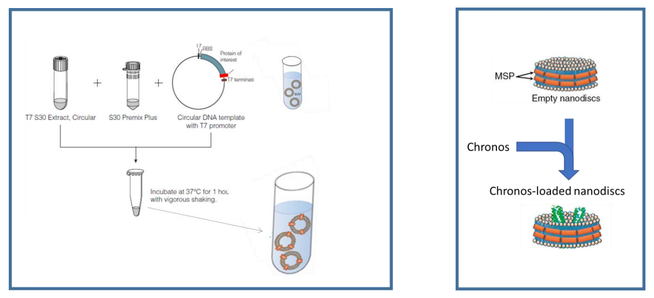
b.



c. **In a prokaryotic expression vector (pET-28a)**

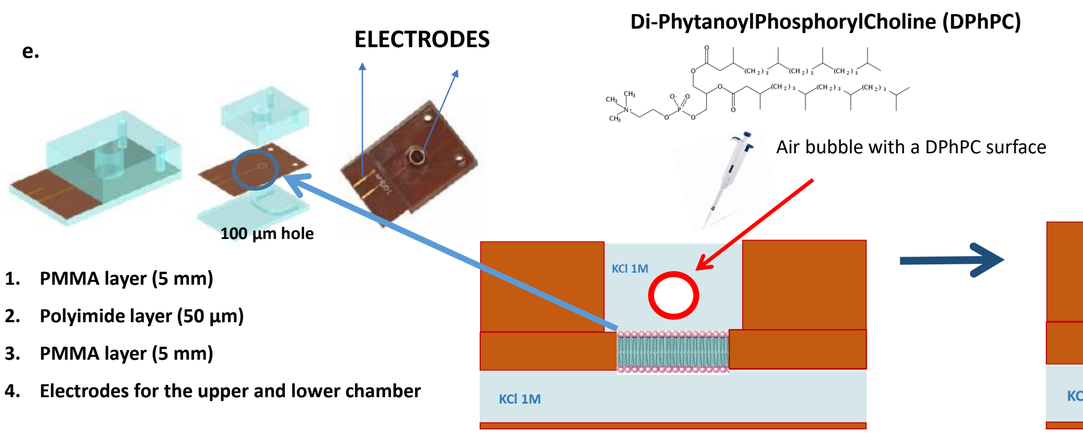


d. **CELL-FREE EXPRESSION + LIPOSOMES OR NANODISCS**



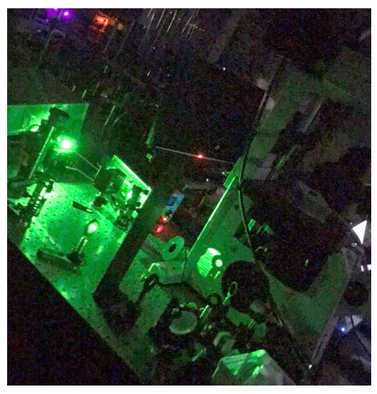
Photoreceptor protein incorporation in an artificial membrane in a microfluidic device and photoelectric measurement setup

e.



1. **PMMA layer (5 mm)**
2. **Polyimide layer (50 µm)**
3. **PMMA layer (5 mm)**
4. **Electrodes for the upper and lower chamber**

f.



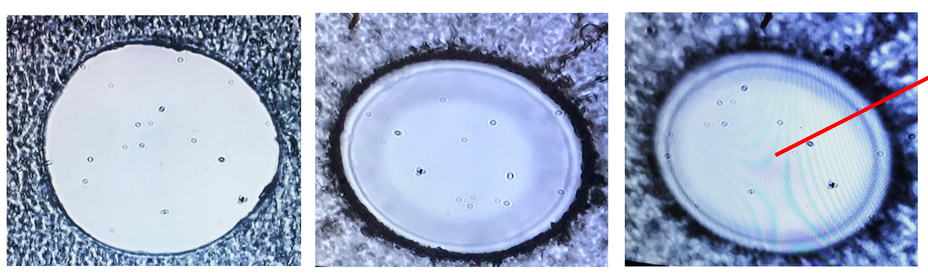
- **Green laser**
- **Optical microscope**
- **V-generator + A-meter**

Fig 3. A Channelrhodospin (Chronos) coding sequence fused to a red fluorescent protein was obtained from Addgene (pAAV-CAG-FLEX-rc, https://www.addgene.org/84484***)*** and recloned in both a eukariotic (pCI-neo, **a**) and a procaryotic (pET-28) expression vector (**c**)**.** The former gives good expression in HEK293 (**b**), whereas no expression was obtained with the latter in *E.coli* (not shown), hence a bacterial cell-free system was used, supplemented with liposomes or nanodiscs (**d**). ***e.*** schematic representation of the microfluidic device (https://elements-ic.com/) used for artificial membrane formation, protein incorporation and photoelectric measurement, **f.** Picture of the setup used for optical stimulation and electric measurement.

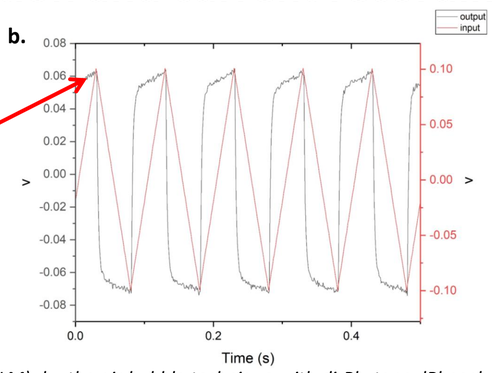
RESULTS

Artificial membrane formation and current recording from a single control channel

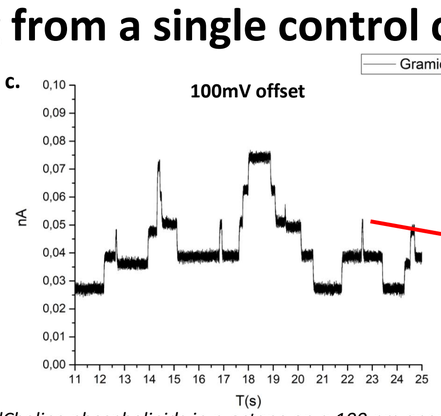
a.



b.



c.



d.

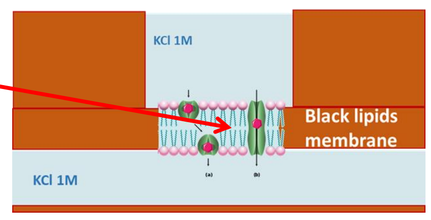


Fig 4 a. *Optical microscopic (transmission) images of a progressive formation of the black lipid membrane (BLM) by the air bubble technique with di-PhytanoylPhosphorylCholine phospholipids in n-octane on a 100 nm aperture in the microfluidic device,* ***b.*** *Current readings (dark graph, output signal), from across the BLM on application of a triangular voltage waveform (100mV, 10Hz) (red graph, input signal). The almost rectangular current response to the triangular voltage stimulus corresponds to that of a ~40 pF capcitor as expected of a properly formed BLM.* ***C.*** *Graph of the current obtained with a BLM incorporating gramicidine A at a constant voltage of 100mV. The gramicidine A channel is formed by the alignment of 2 molecules inserted on opposite sides of the membrane (****d****). The current steps visible in (****c****) correspond to the current carried by single gramicidine A channels.*

CHANNELRHODOSPIN EXPRESSION IN A BACTERIAL CELL-FREE SYSTEM

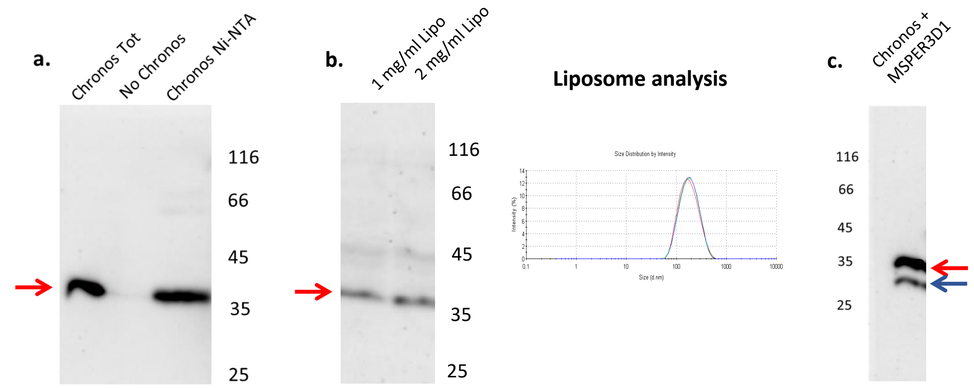


Fig 5. Cell-free expression of Chronos in the presence of liposomes or Membrane Scaffold Proteins (MSP) and lipids (DMPC) for nanodiscs formation: ***a.*** *Cell-free expression of Chronos with a commercial lysate (Expressway™ mini kit). The procaryotic expression vector including the Chronos coding sequence was added to the E. Coli slyD- extract with all-trans retinal and incubated for 6h. Chronos expression was assessed by SDS-PAGE and Western blotting both in the total reaction mix and after purification on Ni-Nitrilotriacetic acid agarose, which binds the hexahistidine sequence engineered at the COOH of Chronos.* ***b.*** *Liposomes at concentrations of 1-2 mg/ml in the cell-free reaction mix inhibit Chronos expression. Liposomes were prepared from asolectin and their size following sonication and ultrafiltration were determined by dynamic light scattering.* ***c.*** *Cell-free co-expression of Chronos and MSPE3D1 with Di-MyristoylPhosphorylCholine results in good expression of both molecules. The red arrow points to the position of the Chronos band, the blue arrow to that of MSPE3D1.*

Next steps

- **Chronos incorporation in the microfluidic device**
- **Optimization of the optical excitation pattern**
- **Determination of photocurrents given by single Channelrodhopsin excitation**

ACKNOWLEDGEMENTS

The authors gratefully thank Dr. Franco Calabi for scientific support and useful discussions.
This research was funded by Apulia Region, project "Progetto Tecnopolo per la Medicina di precisione", grant number: Deliberazione della Giunta Regionale n. 2117 del 21/11/2018

REFERENCES

Schneider et al. Ann. Rev. Biophy 44(1): 167-186, 2015

Klapoetke, N. C., Murata, Y., Kim, S. S., Pulver, S. R., Birdsey-benson, A., Cho, Y. K., … Boyden, E. S. (2014). Independent optical excitation of distinct neural populations. *Nature Methods*, (January). https://doi.org/10.1038/nmeth.2836

Carter, M. E., & Lecea, L. De. (2012). Optogenetic investigation of neural circuits in vivo. *Trends Mol Med. 2011 April ; 17(4): 197– 206*, *17*(4), 197–206. https://doi.org/10.1016/j.molmed.2010.12.005.Optogenetic