A DNA-based voltmeter for organelles

Anand Saminathan[1,2], John Devany[3], Aneesh T. Veetil[1,2], Bhavyashree Suresh[1,2], Kavya S. Pillai[1], Michael Schwake[4] and Yamuna Krishnan*[1,2].

[1]Department of Chemistry, The University of Chicago, IL, 60637, USA
[2]Grossman Institute of Neuroscience, Quantitative Biology and Human Behavior, The University of Chicago, Chicago, IL 60637, USA
[3]Department of Physics, The University of Chicago, IL, 60637, USA
[4]Department of Neurology, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA
*Correspondence to: yamuna@uchicago.edu

Abstract:
Membrane potential is a key property of all biological membranes and underlies how membranes respond to electrical impulses and transduce chemical signals. It is therefore a fundamental signaling cue in all cells[1]. The role of membrane potential in most intracellular organelles remains unexplored because of the lack of suitable tools. We describe a fluorescent DNA-nanodevice that reports absolute membrane potential and can be targeted to specific organelles in live cells[2]. It is equipped with a voltage sensitive fluorophore, a reference fluorophore for ratiometric quantification, and acts as an endocytic tracer (Fig 1A). Further, one can display molecular trafficking motifs on DNA nanodevices and localize the latter within sub-cellular organelles[3–5] (Fig 1B). We could thereby measure the membrane potential of different intracellular organelles *in situ* in live cells, which has not been possible previously (Fig 1C). By quantitatively reporting electrical properties at the biotic-abiotic interface, *Voltair* can potentially guide the rational design of biocompatible electronics[6]. Additionally, our understanding of how membrane potential regulates organelle biology is poised to expand through this new technology.

References:

1. Alberts, B. *et al.* Ion Channels and the Electrical Properties of Membranes. (2002).
2. Saminathan, A. et al. A DNA-based voltmeter for organelles. *Nat. Nanotechnol.* **16**, 96–103 (2021).
3. Saha, S., Prakash, V., Halder, S., Chakraborty, K. & Krishnan, Y. A pH-independent DNA nanodevice for quantifying chloride transport in organelles of living cells. *Nat. Nanotechnol.* **10,** 645–651 (2015).
4. Modi, S., Halder, S., Nizak, C. & Krishnan, Y. Recombinant antibody mediated delivery of organelle-specific DNA pH sensors along endocytic pathways. *Nanoscale* **6,** 1144–1152 (2014).
5. Modi, S., Nizak, C., Surana, S., Halder, S. & Krishnan, Y. Two DNA nanomachines map pH changes along intersecting endocytic pathways inside the same cell. *Nat. Nanotechnol.* **8,** 459–467 (2013).
6. Acarón Ledesma, H. *et al.* An atlas of nano-enabled neural interfaces. *Nat. Nanotechnol.* **14,** 645–657 (2019).

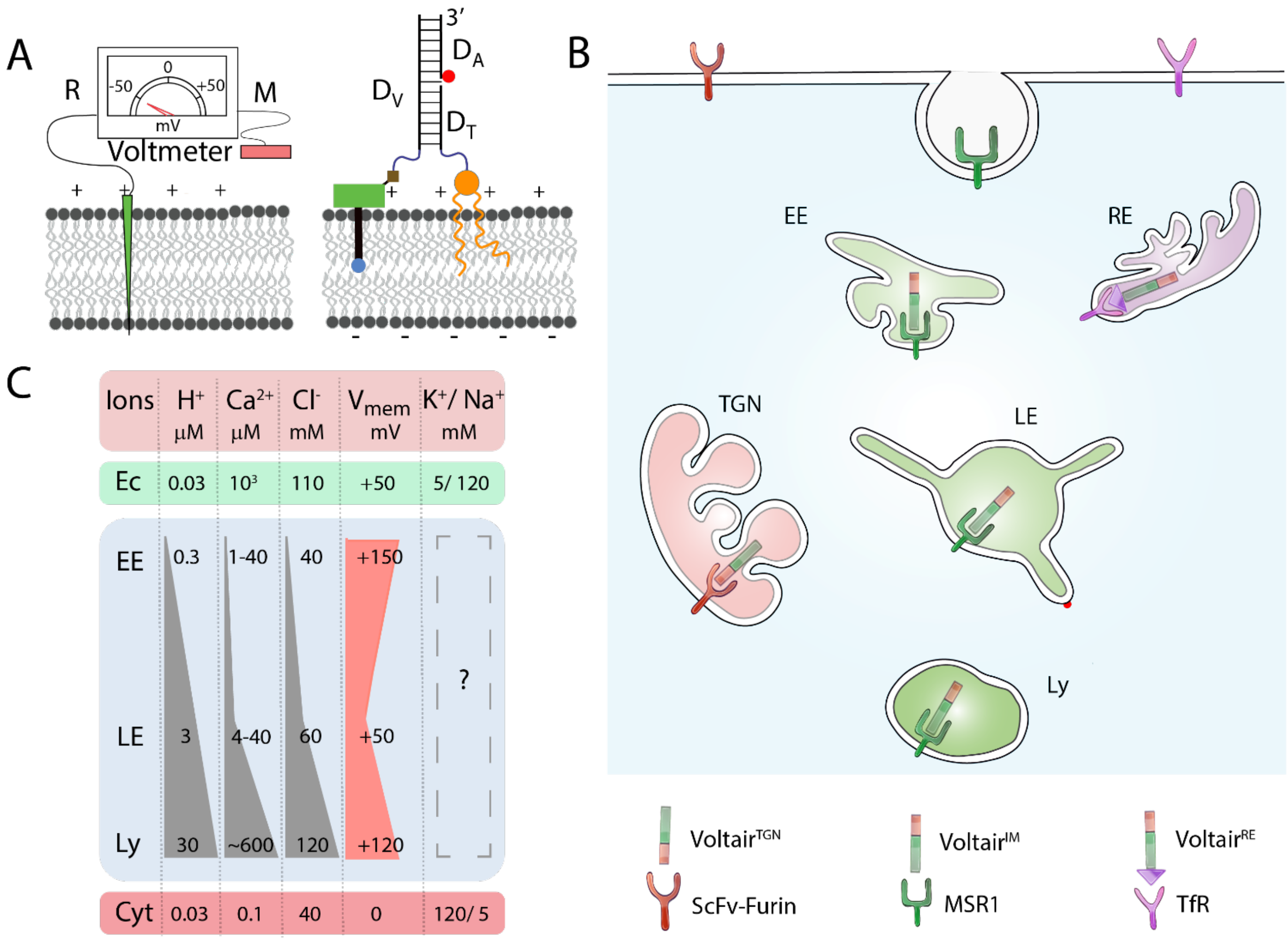

Figure 1: (A) Schematic of the working principle of DNA voltmeter *Voltair*: Measuring probe (M, Green) is a voltage sensitive dye (RVF) conjugated to a DNA duplex that is membrane-tethered by attachment to a lipid anchor (POPE). Reference probe (R, red) is DNA duplex with a reference dye (Atto647N, red sphere) that together with RVF reports membrane potential ratiometrically. (B) Schematic of targeting strategy: *Voltair^{IM}* undergoes scavenger receptor mediated endocytosis by binding scavenger receptors. Endocytosed *Voltair^{IM}* traffics in a time-dependent manner from the plasma membrane to the early endosome, the late endosome and then the lysosome. Modified *Voltair* probes, *Voltair^{TGN}* and *Voltair^{RE}* access the retrograde and recycling pathways and measure the membrane potential of these respective organelles. (C) Tabular column summarizing approximate ionic concentration and voltage difference in organelles along the endosomal pathway. Cytosolic (Cyt), extracellular (Ec) and organellar concentrations of main ions that maintain membrane potential.