Dynamic quantitative detection of intracellular sodium using FLIM and CoroNaGreen

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Fluorescence lifetime imaging microscopy (FLIM) with chemical ion indicators enables the quantitative detection of ion concentrations based on the lifetime of the fluophore. In contrast to intensity-based imaging, FLIM-based ion detection is not affected by changes in the dye concentration, photo-bleaching or a drift in focus. Therefore, we tested the suitability of CoroNaGreen for FLIM-based determination of the sodium concentration inside cells ($[Na^+]$). *In vitro* measurements confirmed that fluorescence lifetimes of CoroNaGreen (τ_{AVG}) increased with increasing $[Na^+]$. Fluorescence decay curves derived from time-correlated single-photon counting (TCSPC) were fitted bi-exponentially. *In situ* calibrations performed in HEK cells revealed an apparent K_d of ~80 mM (also see Fig. 1B). Based on these calibrations, a $[Na^+]$ of 17.6 mM was determined in the cytosol. Nuclear $[Na^+]$ was significantly lower (13.0 mM), while $[Na^+]$ in perinuclear regions was significantly higher (26.5 mM) as compared to the cytosol. To increase the temporal resolution for dynamic measurements, a "ratio FLIM approach" (Zheng et al., Neuron 2015/88: 277-288) was employed (Fig. 1A). This enabled detection of a sequential increase in $[Na^+]$ upon inhibition of the Na^+/K^+-ATPase by removal of extracellular K^+ in the three different cellular compartments (Fig. 1C). Taken together, our results show that CoroNaGreen is suitable for dynamic, FLIM-based determination of the intracellular $[Na^+]$ (Meyer J., et al. (2019), J. Gen. Phys. 151.11 1319-1331).

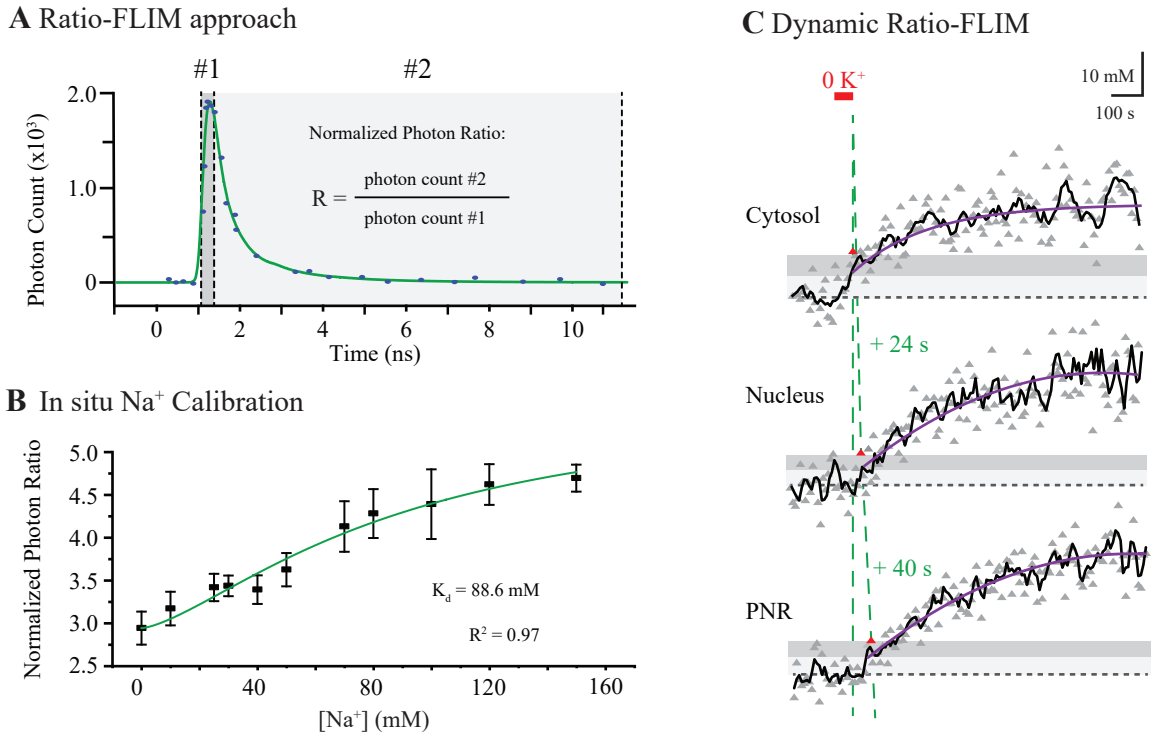

Figure 1 A: Schematic illustration of the Ratio FLIM approach **B**: Plot of the normalized photon ratio against the [Na+] (means ± SD; n=18). **C**: Ratio FLIM of CoroNaGreen in a HEK cell line, rendering changes in $[Na^+]$ upon perfusion with nominally K^+-free solution (green bar) for one minute in the Cytosol, Nucleus and perinuclear region (PNR). Note the latency in the Na^+-increase of the nucleus (24 s) and PNR; 40 s in comparison to the cytosol.

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