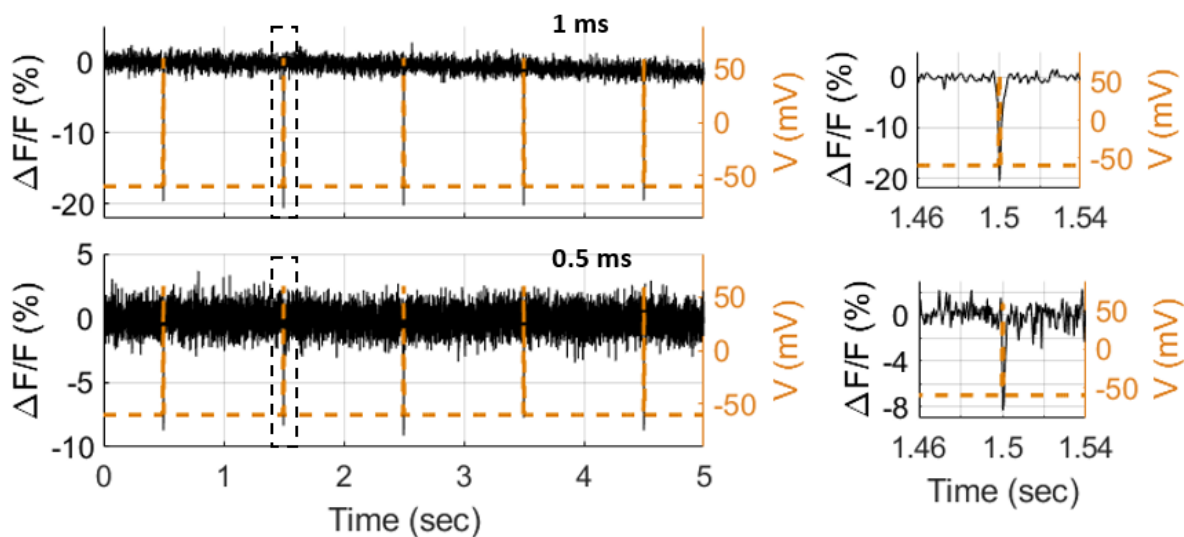
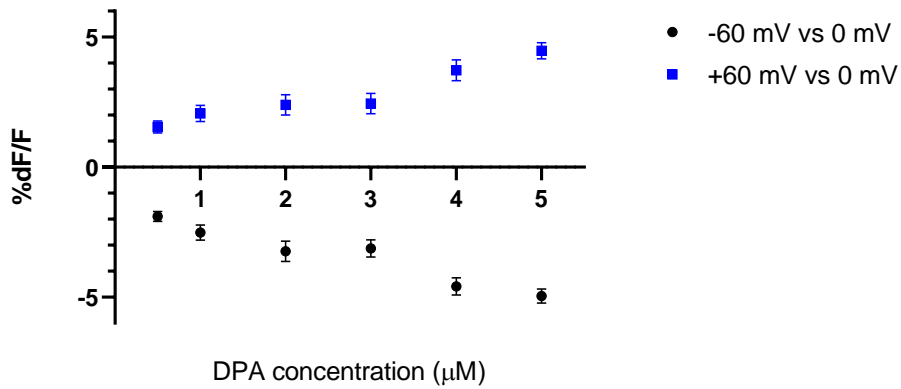


## Optical Probing of Local Membrane Potential with Fluorescent Polystyrene Beads

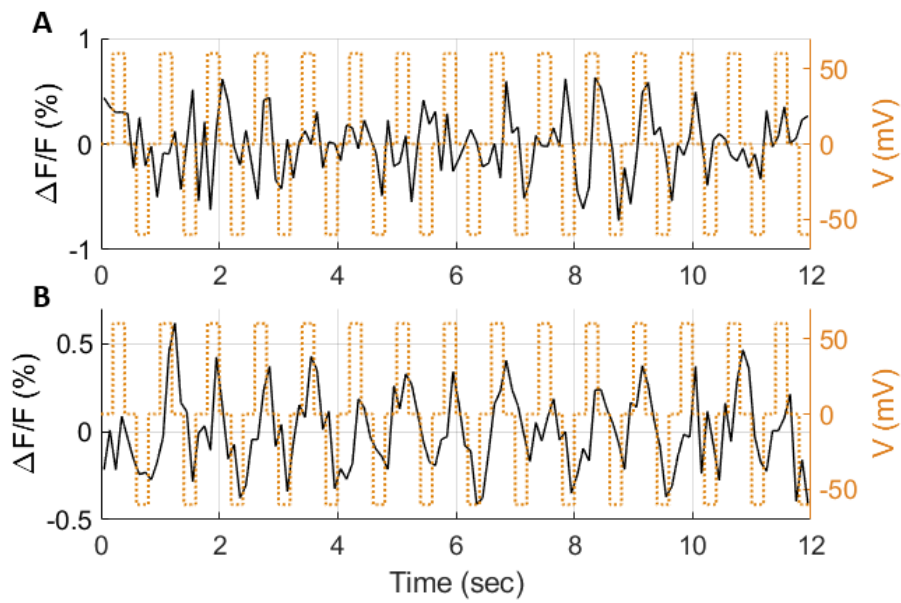
### Supplemental Information



**Figure SI1. Response kinetics of DiO/DPA FRET pair to changes of membrane voltage in HEK cells.** HEK cells were labeled with 10  $\mu\text{M}$  DiO, and the wh and voltage-clamped in the presence of 2  $\mu\text{M}$  DPA to a holding potential of -60 mV, with transient polarization pulses to +60 mV at different durations. **(B)** The changes in fluorescence for 1 and 0.5 ms pulses, imaging at a frame rate of 1 and 2 kHz, respectively.



**Figure SI2. The averaged fluorescence response of FPS beads/DPA to membrane voltage in the presence of different concentrations of DPA.** HEK cells were labeled with 0.5 nM beads, and the bath solution was applied with 0.5 to 5 μM DPA. Whole-cell patch clamp was carried out and the membrane voltage was stepped as described in Fig.2C. The averaged %ΔF/F at +60 mV and -60 mV are shown. Error bars correspond to SD. 5 μM DPA, n=6; 4 μM DPA, n=6; 3 μM DPA, n=6; 2 μM DPA, n=11; 1 μM DPA, n=5; 0.5 μM DPA, n=7.



**Figure SI3. Control fluorescence response measurements in HEK cells.** Representative optical response (black, solid) to changes in membrane potential (orange, dashed), for cells which were (A) exposed to 2  $\mu$ M DPA only, without the addition of beads, and (B) labeled with 0.5 nM beads without the addition of DPA. The voltage protocol is as described in fig. 2C. The average change in fluorescence emission  $\Delta F/F$  for DPA-only and bead-only labeling was  $0.2 \pm 0.5$  % and  $0.5 \pm 0.2$  % per 120 mV, respectively.