FRET-based Voltage Sensor dyes for Drug Screening

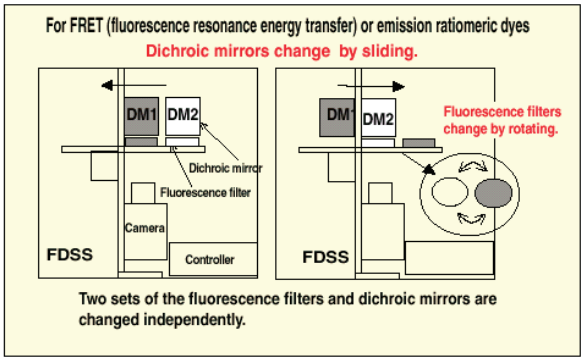
Abstract

Voltage-sensitive FRET offers the several significant advantages over traditional voltage-sensitive dyes for biological and pharmaceutical screening applications. The voltage sensitive FRET sensor is composed of two fluorescent components[1),2),3)]. The first is the highly fluorescent, negatively charged hydrophobic ion that "senses" the trans-membrane electrical potential. It rapidly redistributes between two binding sites on opposite sites of the plasma membrane in the Nernstain manner. In response to a membrane potential charge, the hydrophobic ions translocate across the membrane and establish a new equilibrium corresponding to the new membrane potential. The voltage dependent redistribution is transduced into a ratio metric fluorescent reedout by using second fluorescent molecule that binds specifically to one face of the plasma membrane and functions as a FRET partner to the mobile voltage-sensing ion. A variety of the oxonols and fluorescent membrane-bound molecules have been shown to the function as FRET partners that result in voltage-sensitive FRET. Different dye combinations have different voltage-sensitivity, temporal response, and wavelengths. Currently Panvera supports two oxonols ($DiSBAC_2$[3)] and $DiSBAC_4$[3)]) and one fluorescent phospholipid FRET donors (CC2-DMPE).
In this application note, we report that voltage-sensitive FRET dye can monitor the fast response of membrane potential[4),5)] and FRET-based functional assays are now adapted for HTS of drug lead of ion channel receptor.

FDSS option (A8472/FRET optical system)

FDSS has dichroic filter changer and emission filter changer in front of the detector for FRET dye.

● **Emission filter and dichroic mirror changer (A8472-03)**



The time of emission filter change: approx. 1.3 s
The time of dichroic filter change: approx. 1.4 s
The minimum interval time: approx. 1.7 s (including the data transfer time)

Optical requirements (filters)

In order to attain the maximum performance of the FRET voltage sensor dyes, one must selectively excite the FRET donor and detect the emission of the both donor and acceptor probes.
We recommend an exciattion filter of 400 nm ±15 nm for CC2-DMPE. For FDSS applications, the exciting and emitte1d light is separated with a dichroic mirror, we recommend a 430 nm long-pass dichroic with the steep onset of transmission. We suggest 460 nm and 565 nm long-pass emmission filters for the coumarin lipid donor and oxonol respectively.

Materials and Methods

Loading conditions

HEK cell transfeced by HERG channel receptor were tripsinized and plated in 96 or 384well plate. Generally, cells are loaded sequentially with the coumarin lipid loaded first followed by oxonol. Dye loading concentrations vary with different cell types and must be empirically determined for the given cell type. Optimal loading conditions generally range between 1-20 μM final dye concentration. The following is an example for loading CC2-DMPE at max 40 μM final concentration. A typical buffer solution is Hank balance salt solution with 10 mM HEPES and 2 g/L glucose added. The pH of the buffer solution shoud be adjusted to pH 7.2-7.4. After staining, the excess dye is removed by washing the extracellular solution with serum-free buffer. $DiSBAC_2$[3] is loaded at max 10 μM final concentration for 30 minutes. The stock solution is diluted with of a serum-free buffer with the vortex. The cells are now ready for optical recording. $DiSBAC_4$[3] has a different loading procedure. $DiSBAC_4$[3] is loaded with Pluronic at 2-3 μM concentration and the excess dye is washed away prior to FDSS optical recording.

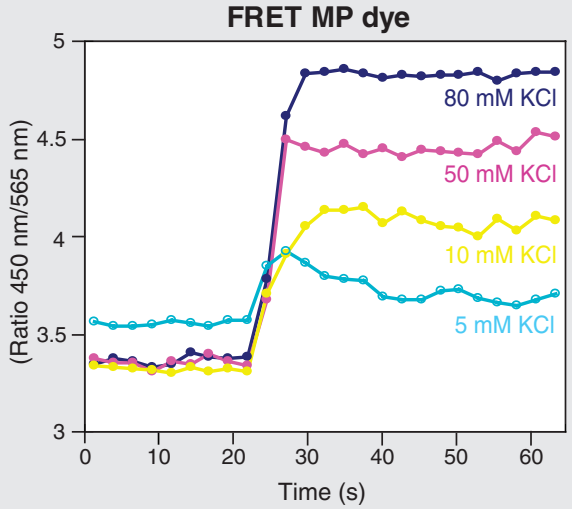


Fig. 1: HEK cells were transfected with HERG channel vector. Above signal is the change in fluorescence due to addition of KCl.
* All data not shown for lack of space.

Results and Discussion

To affirm the Na^+ response of CoroNa red, we used the veratridine/ 3-Veratroylveracevine (Sigma V5754). Veratridine is one of the neurotoxin recognize the -subunit (site2) of voltage gated Na^+ channels[4]. As expected, fluorescent ratios were increased immediately after stimulation (Figure. 1). These results are basically consistent with previous data. We present the first data for the use of sodium indicator CoroNa Red with the FDSS. The longer-wavelength absorption of the CoroNa Red indicator results in reduction of the potential for photodamage to the cell because the energy of the excitation light is lower than that of the UV light required for excitation of SBFI. The high Kd for Na^+ of ~200 mM, approximating physiological ionic strength, CoroNa Red indicator will become the useful dye for imaging the intracellular sodium influxes.

Consumable

Invitrogen (http://www.invitrogen.com/) now offers Voltage Sensor Probes (VSPs) for FRET-based screening of Ion Channels.

References

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