SPIROCHROME
Probes for Bioimaging

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Product information: Flipper-TR® (SC020)

Live Cell Membrane Tension Probe

Introduction

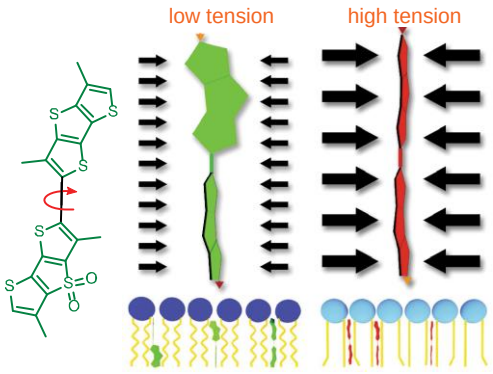
Flipper-TR® is a fluorescent probe that specifically targets the plasma membrane of cells and reports membrane tension changes through its fluorescence lifetime changes. It is the most advanced member of the Flipper probes family[2,3], which sense changes of the organization of lipid bilayer membranes through changes of the twist angle and polarization between the two twisted dithienothiophenes of the mechanophore. Flipper-TR® spontaneously inserts into the plasma membrane of cells and is only fluorescent when inserted in a lipid membrane. It has a broad absorption and emission spectrum, excitation can be commonly performed with a 488nm laser, while emission is collected between 575 and 625nm. Flipper-TR® works on a wide range of organisms including bacteria, yeast and mammalians.

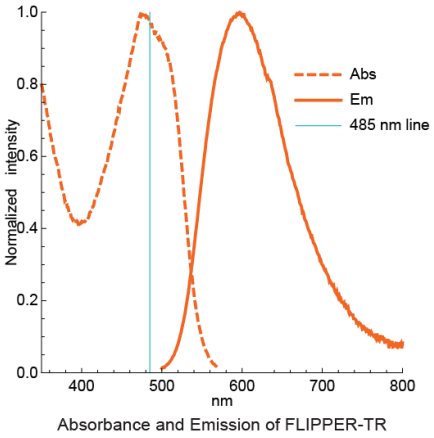
Storage & Handling

Store the probe at -20°C upon receipt. Prepare solutions of the probe using new and anhydrous DMSO (as old and wet DMSO can strongly reduce the shelf life of the probe). Store solutions of the probe at -20°C after use. Vials should be allowed to warm to room temperature before opening. When stored properly, the probe in solution should be stable for about 3 months. Note: DMSO solutions should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. Dispose of these reagents in compliance with all pertaining local regulations.

Photopysical properties

λ_{Abs}	480 nm
λ_{Em}	600 nm
ε_{max}	$1.66 \cdot 10^4$ $mol^{-1} \cdot cm^{-1}$ (DMSO)
lifetime	2.8 - 7 ns
QY	30% (AcOEt)





Absorbance and Emission of FLIPPER-TR

Labelling Protocol

Note: This protocol was optimized using HeLa cells adhering to coverslips and has been confirmed in other common cell lines. Recommendations for experimental protocols should be used as a starting point, and optimal labeling conditions for each cell type should be determined empirically.

Prepare 1 mM stock solution. Dissolve the content of the vial of Flipper-TR® in 50 µL of anhydrous DMSO to make a 1 mM stock solution. This solution should be stored at -20°C or below. **Do not divide the solution into small aliquots**, they will decay faster and the compound is not altered by multiple freeze-thaw cycles. When stored properly, this stock solution should be stable for three months or more. (Optional) If the concentration of the stock solution needs be accurately determined, dilute 1 µl of 1 mM stock solution in 99 µl of DMSO. Measure the absorbance at 425 nm. Calculate the concentration using the extinction coefficient given above.

Prepare staining solution. Dilute Flipper-TR® to the desired concentration (start with 1 µM) in cell culture medium shortly before applying to the cells (do not keep staining solution for hours before using it). NOTE: when using a cell culture media with Fetal Calf Serum (FCS) or other serum, the efficiency of labelling may be reduced compared to media devoid of serum. If a low signal is observed, the probe concentration can be increased up to 2 µM.

Cell preparation and staining. Grow cells on coverslips, glass bottom dish or glass bottom multi-well plates as usual. When cells have reached the desired density, replace the culture medium by the **staining solution** ensuring that all the cells are covered with solution. Place the cells in the incubator at 37°C in a humidified atmosphere containing 5% CO_2 for 15 minutes before imaging. After 30 minutes or more, a labelling of endosomal structures may visible due to membrane turnover. Staining of other membrane

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ontaining organelles (ER, golgi) is however limited. Endosome structures usually have a lower lifetime. Optionally, the medium containing the probe can be removed, and cells washed once in fresh media. As the probe is fluorescent only in membranes, the probe does not need to be removed, especially in cases where the media contains serum when long term imaging (>24h) is planned. No impact on cell viability has been observed after 2-3 days of incubation with the probe at concentrations below 1 μM.

FLIM imaging. Cells are imaged with standard FLIM microscopes using a 485 or 488 nm pulsed laser for excitation and collecting photons through a 600/50 nm bandpass filter. We recommend optimizing the labeling procedure as well as the image acquisition settings to minimize photodamage induced by the 488nm excitation light on live samples. To extract lifetime information, the photon histograms from ROI or single pixels (accumulate sufficient counts to ensure good statistics) are fitted with a double-exponential, and two decay times, $\tau 1$ and $\tau 2$ are extracted. The longest lifetime with the higher fit amplitude $\tau 1$ is used to report membrane tension and varies between 2.8 and 7.0 ns. Longer lifetime means more tension in the membrane. $\tau 2$ with a smaller value (between 0.5 and 2 ns) and a small fit amplitude is less suited to study membrane tension. The lifetime can be correlated to absolute membrane tension using the calibration procedure given in Reference 1.

Important notes:

- Membrane tension measurements can only be performed by FLIM microscopy, fluorescence intensity or wavelength is not reliably reporting on membrane tension.

- Systems where the lipid composition changes over time may also induce a change of Flipper-TR® lifetime.

-FLIM imaging is an advanced microscopy technique requiring a commercial or custom built FLIM microscopy system with the adequate excitation lasers, photon counting systems and emission filters. Customers are advised to consult their instrument responsible person or contact the microscope manufacturer to ensure that their system is able to image Flipper-TR® fluorescence and lifetime.

References:

1) Colom A, *et al*: A fluorescent membrane tension probe. *Nat Chem*, 2018, **10**:1118–1125 ().

2) Dal Molin M, *et al*: Fluorescent flippers for mechanosensitive membrane probes. *JACS,* 2015, **137**:568-571.

3) Soleimanpour S, *et al*: Headgroup engineering in mechanosensitive membrane probes. *Chem Commun (Camb)* 2016, **52**:14450-14453.

Flipper-TR® probe is distributed by Spirochrome under an exclusive license from the University of Geneva, Switzerland and was developed by the NCCR Chemical Biology.