A Novel Ratiometric Fluorescence Calcium Indicator for Functional Analysis of GPCRs and Calcium Channel Targets

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Introduction

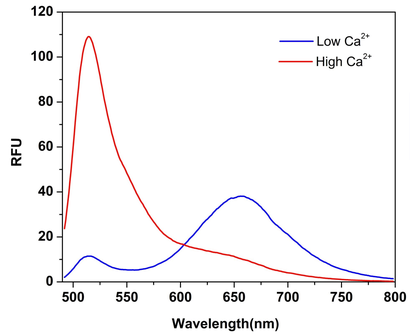
The intracellular calcium flux assay is a widely used method for monitoring the activities of GPCRs and calcium channels. To quantify the intracellular calcium concentration, ratiometric fluorescent calcium indicators are preferred because the ratio is directly related to the calcium concentration and independent of the cell numbers and dye loading concentration. However, the most popular ratiometric calcium indicators (such as Fura-2 and Indo-1) have certain limitations such as lower sensitivity, UV excitation, and not compatible with screnning filter set.

Cal Red R525/650 has been developed as a new 488 nm-excitable ratiometric fluorescence calcium indicator. Cal Red R525/650 is weakly fluorescent, and once enters cells, the lipophilic AM blocking groups are cleaved by intracellular esterase, resulting in a negatively charged fluorescent dye retained well in cells with excitation close to 488 nm and two emissions at 525 nm and 650 nm. When cells are stimulated with a bioactive compound, the receptor initiates the release of intracellular calcium, which is chelated by Cal Red R525/650. The emission signal is increased at 525 nm and decreased at 650 nm when excited at 488 nm. The excitation and emission wavelength of Cal Red R525/650 are compatible with common filter sets with minimal damage to cells, making it a robust tool for evaluating and screening GPCR agonists and antagonists as well as calcium channel targets.

Experiments

1. CHO-K1 cells were seeded overnight in 60,000 cells per 100 µL per well in a 96-well black wall/clear bottom costar plate at 37 ºC , 5% CO_2 incubator.
2. Take out growth medium. Add 100 µL of 5 µg/ml Cal Red R525/650, AM, Fura Red ™, AM or Fura-2, AM with probenecid (PBC) to cells. Incubate the cells at 37 ºC, 5% CO_2 incubator for 1 hour, then remove the dye loading buffer and replace with 200 µL HH with probenecid (PBC) at room temperature for 15 min.
3. Run calcium efflux experiments on FlexStation 3 ((Molecular Devices) by adding ATP (50µL/well) with FlexStation 3 to achieve the final indicated concentrations.

Emission Spectra of Cal Red R525/650



Signal (High Ca^{2+}) / Signal (0 Ca^{2+}) :
~ 30 folds in pH=7.2 Buffer

Figure 1: Emission Spectra of Cal Red R525/650 (calcium bound).

FlexStation Assay with CHO-K1 Cell Line

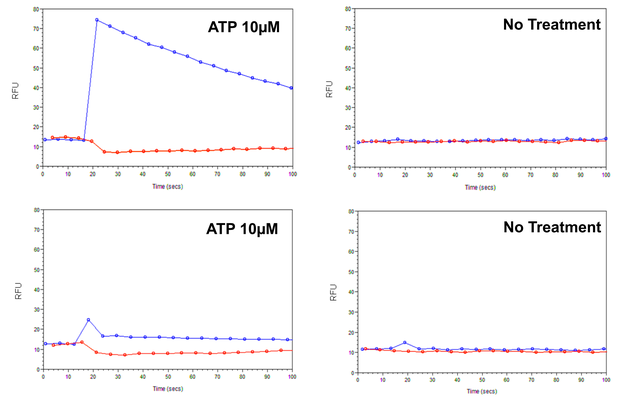


Figure 2. ATP-stimulated calcium response on CHO-K1 cells incubated with Cal Red R 525/650, AM and Fura-Red™ AM. 10 µM ATP (final concentration in the well) was added by FlexStation 3 (Molecular Devices).

Cal Red R525/650 vs. Fura-Red™ and Fura-2

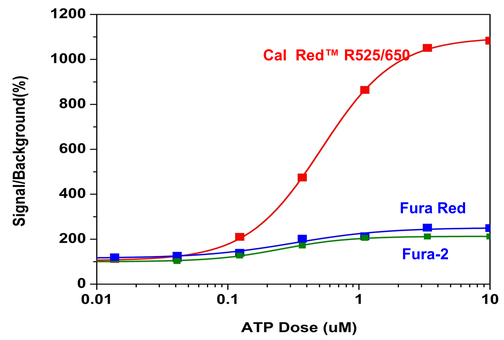


Figure 3. ATP-stimulated calcium response of endogenous P2Y receptor in CHO-K1 cells incubated with different Ca^{2+} indicators under the same conditions. ATP (50 µL/well) was added by FlexStation 3 (Molecular Devices) to achieve the final indicated concentrations. (Red: Cal Red R525/650, AM; Blue: Fura Red, AM; Green: Fura-2, AM)

Summary

Cal Red R525/650 is a new ratiometric fluorescence calcium indicator excitable with visible light (~488nm), and its emission increases at 525nm and decrease at 650 nm with the rise of intercellular Ca^{2+} concentration. This ratiometric fluorescence indicator has the following features:

- The excitation and emission wavelength are compatible with common filter sets, making it a robust tool for evaluating and screening GPCR agonists and antagonists as well as calcium channel targets.
- Compared to the Fura Red, Cal Red R525/650 has significantly higher S/N ratio.
- Compared to the UV-excitable Fura-2, the visible light-excitable Cal Red R525/650 has minimal damage to cells and is more photostable.