Novel Red Fluorescent Calcium Probes for Functional Analysis of GPCRs and Calcium Channel Targets

Qin Zhao, Haitao Guo, George Yi, Jinfang Liao & Zhenjun Diwu
AAT Bioquest, 520 Mercury Drive, Sunnyvale, CA 94085

AAT Bioquest®
Advancing Assay & Test Technologies

Introduction

The intercellular calcium flux assay is widely used for monitoring GPCRs and calcium channels. In our previous work, Cal-520™ AM has been developed as a new green fluorescent dye with a significantly improved signal to noise ratio and better intracellular retention than Fluo-3 AM and Fluo-4 AM. In this study, two new red fluorescent calcium indicators, Cal-590™ AM and Cal-630™AM, have been developed for monitoring calcium ions in GFP cell lines or multiplexed with green-fluorescent dyes. Cal-590™ AM and Cal-630™AM are much more sensitive than rhodamine calcium dyes (such as Rhod-2, AM). Instead of located mostly in mitochondria as for Rhod-2, Cal-590™ and Cal-630™ are retained in cytoplasm. When stimulated with bioactive compounds, the red fluorescence of Cal-590 and Cal-630 are greatly enhanced when binding intracellular calcium with no overlap with green fluorescence.

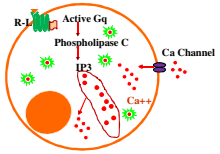
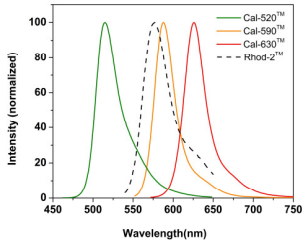


Figure 1. [Ca^{2+}] Increase via Gq or calcium channel is measured by calcium dyes.

Experiments

1. CHO-K1 and CHO-GFP cells were seeded overnight in 50,000 cells per 100 µL per well in a 96-well black wall/clear bottom costar plate at 37 ºC incubator.
2. Take out growth medium. Add 100 µL of 5 µg/ml Cal-520™ AM, Cal-590 ™ AM, Cal-630 ™ AM, Rhod-2 AM, or Fluo-4 AM with different dose of probenecid (PBC) to cells. Incubate the cells at 37 ºC for 1 hour, then remove the dye loading buffer and replace with 200 uL HH, at room temperature for 15 min.
3. Add ATP (50µL/well) with FlexStation (Molecular Devices) to achieve the final indicated concentrations. Run calcium efflux experiments on FlexStation or take images with fluorescence microscope (Olympus IX71).



Calcium Dye	Ex/Em (nm)
Cal-520™	492/514
Cal-590™	573/588
Cal-630™	608/626
Fluo-4	490/516
Rhod-2	549/578

Figure 2. Emission Spectra of Cal-520, Cal-590, Cal-630, and Rhod-2 (calcium bound).

Imaging with Cal-520, Cal-590 and Cal-630

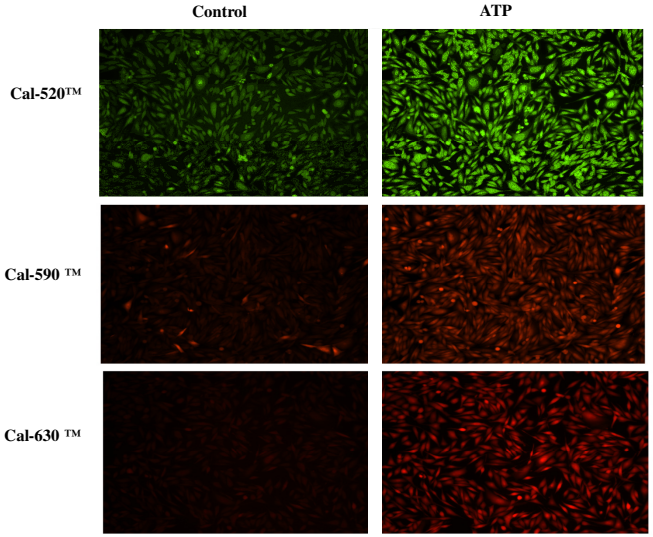


Figure 3. Response of endogenous P2Y receptor to ATP in CHO-K cells. Images were recorded with a fluorescence microscope (Olympus IX71) before and after adding 10 µM ATP (final in the well) using FITC channel (Cal-520™ AM), TRITC channel (Cal-590™ AM) and Texas Red Channel (Cal-630 ™ AM).

FlexStation Assay with GFP Cell Lines

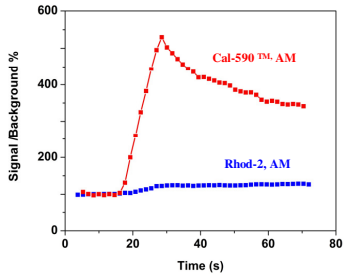


Figure 4. ATP-stimulated calcium response on CHO-GFP cells incubated with Cal-590™ AM, Rhod-2 AM under the same conditions.10 µM ATP (final concentration in the well) was added by FlexStation (Molecular Devices).

FlexStation Assay with Cal-520, Cal-590 & Cal-630

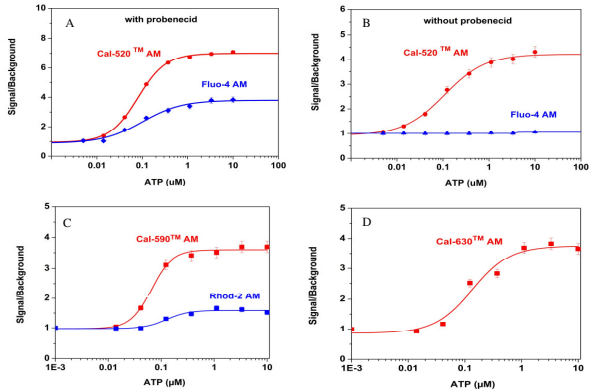


Figure 4. ATP-stimulated calcium response of endogenous P2Y receptor in CHO-K1 cells incubated with different Ca^{2+} indicators under the same conditions. ATP (50 µL/well) was added by FlexStation (Molecular Devices) to achieve the final indicated concentrations. (A: Cal-520™ AM with 1.0 mM PBC and Fluo-4 AM with 2.5 mM PBC; B: Cal-520™ AM and Fluo-4 AM without PBC; C: Cal-590™ AM with 1.0 mM PBC and Rhod-2 AM with 2.5 mM PBC; D: Cal-630™ AM with 1.0 mM PBC)

Summary

Cal 520™ AM, Cal-590™AM and Cal-630™ AM have been developed for evaluating GPCR and calcium channel targets, as well as for screening their agonists and antagonists. They have the following features:

- **High S/N ratio: significantly higher S/N ratio than any other commercially available fluorescent Ca^{2+} indicators.**
- **Enable multicolor detection from green to red fluorescence.**
- **Improved intracellular retention: Minimal probenecid is required.**
- **Located in cytoplasm with minimal distribution in organelles.**

For Reprints Please Visit Booth #266