Multiplexing Calcium Mobilization and Membrane Potential Assays Using the FDSS6000

Abstract

The FDSS6000 from Hamamatsu Photonics Systems uses Xenon lamps, enabling the use of fluorescent dyes ranging from UV to visible excitation wavelengths. FDSS6000 optics can read up to two excitation wavelengths and two emission wavelengths in the same assay.
Current high throughput screening (HTS) strategies use only one dye per assay, such as Fura-2-AM, Fluo-3-AM, or Fluo-4-AM (calcium mobilization), or DisBAC$_2$(3) (membrane potential).
We present a novel multiplexing method for HTS Screening: Loading the same cells using both the UV dye Fura-2 AM and a no wash Membrane Potential Dye.
Multiplexing assays measure orthogonal cell responses (calcium mobilization and membrane potential) using one volume of valuable library compound.

Introduction

Several groups report results using double dye loaded cells (Figure 1) for studying either calcium channels or G Protein Coupled Receptors, including Fura-2AM/DisBac$_2$(3)[1], Indo-1AM/DisBac$_2$(3)[2], Fura-2AM/DiBac$_4$(3)[3], and Fluo-4AM/Di4 ANEPPS[4]. These assays use single channel Spectrofluorometers for readout.
In this technical note we report the results of a multiplexed assay in a 384 well format.

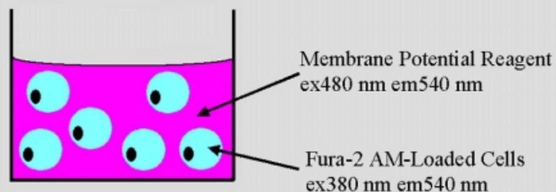


Fig. 1: Multiplex Theory

Materials and Methods

Adherent cells were washed and left with a 30 µL residue. Fura-2 AM, 1.2 µM final concentration, was incubated with the cells for 1 h, RT. Following cell washing (30 µL residual volume) 15 µL No Wash Membrane Potential Dye was added to 0.25 X. After 15 min incubation cells were read on the FDSS6000.
FDSS6000 optics were set to sequentially read ex 380 nm/em 540 nm for calcium mobilization (Fura-2 AM) and ex 480 nm/em 540 nm for membrane potential, in a 1.2 sec cycle time.

Results

A recombinant cell line expressing Receptor A was used in the study. Cells were agonized against Ligand 1 and Ligand 2 (Figure 2).
Cells show calcium mobilization (decrease in fluorescence at ex 380 nm) following exposure to both Ligand 1 and 2 (Figure 2(a)). Figure 2(b) shows Ligand 1 induces membrane potential hyperpolarization while Ligand 2 induces depolarization.

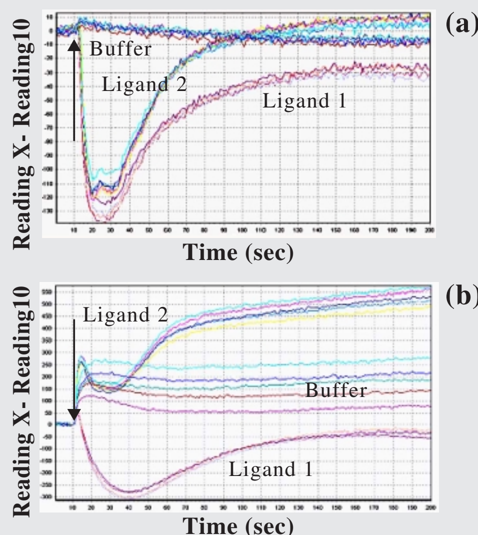


Fig. 2: Fluorescence of calcium mobilization (a) and membrane potential (b).
Ligands (arrow) were added at 10 sec.

Dose response curves measuring calcium mobilization and membrane hyperpolarization were built using Ligand 1 (Figure 3).

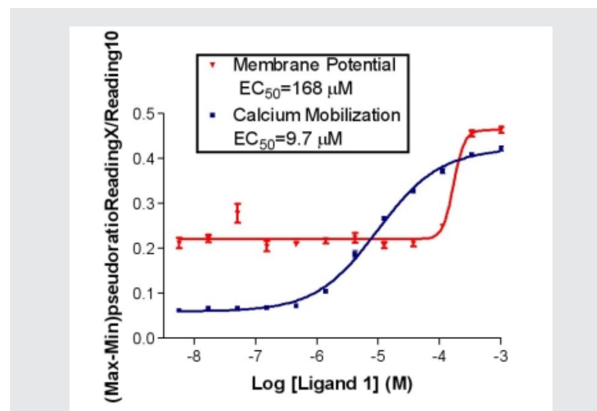
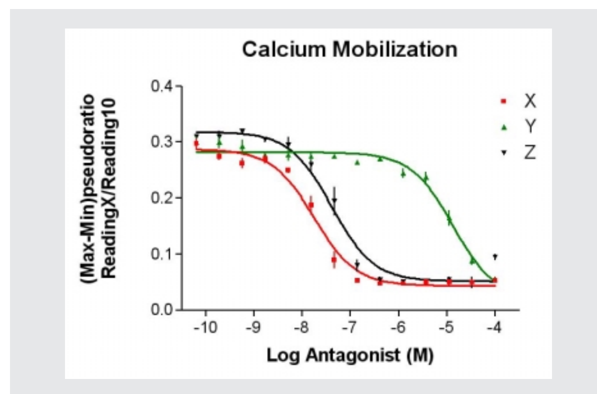


Fig. 3: Dose response curves expressing calcium mobilization and membrane hyperpolarization using Ligand 1 (n=6/group error bars=SEM).

Three antagonists against Receptor A were tested for potency (Figure 4). Note the difference in potency of compound Z; calcium mobilization inhibition is detected yet little membrane potential inhibition is shown. By contrast compound X inhibits both calcium mobilization and membrane potential with equivalent potency.



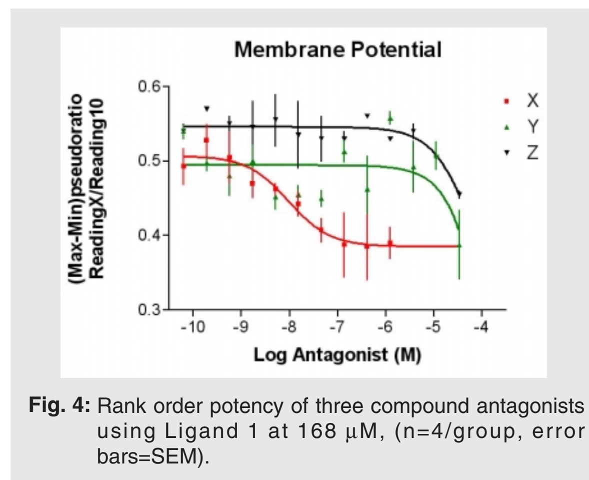


Fig. 4: Rank order potency of three compound antagonists using Ligand 1 at 168 μM, (n=4/group, error bars=SEM).

Summary

Multiplexing, simultaneously measuring changes in calcium mobilization and membrane potential, is the next step in the evolution of cell based HTS. This report shows how multiplexing further categorizes compounds, i.e. concomitant inhibition of calcium mobilization and membrane potential versus calcium mobilization inhibition only.

References

1. Montero, M. J. Garcia-Sancho, J. Alvarez. Activation by Chemotactic Peptide of a Receptor-operated Ca^{2+} entry Pathway in differentiated HL60 cells. J. Biol. Chem. 269: 29451-29456. 1994.

2. Kremer, S.G., W. Zeng, K. Skorecki. Simultaneous fluorescence measurement of calcium and membrane potential responses to endothelin. Am. J. Physiol. 263 (Cell Physiol. 32): C1302-C1309. 1992.

3. Laskey, R.E., D.J. Adams, M. Cannell, C. van Breeman. Calcium entry-dependent oscillations of cytoplasmic calcium concentration in cultured endothelial cell monolayers.Proc. Natl. Acad. Sci. 89:1690-1694. 1992.

4. Johnson, P.L., W. Smith, T.C. Baynham, S.B Knisley. Errors caused by combination of Di-4 ANEPPS and Fluo 3 / 4 for simultaneous measurements of transmembrane potentials and intracellular calcium. Ann. Biomed. Eng. 27:563-571. 1999.

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