BRET^1-assay using the FDSS/µCELL imaging plate reader

Introduction

The GPCRs represent the largest family of cell surface receptors and are the main target for drugs available on the pharmaceutical market. To prevent receptors from both acute and chronic overstimulation, GPCR activity is regulated by an intensively studied mechanism called desensitization or internalization [1].
Following ligand exposure, arrestins interact with phosphorylated GPCRs, uncoupling them from their cognate G protein, blocking further activation and promoting endocytosis [2]. Interaction between receptors and β-arrestins is a measurable functional event in the GPCR-mediated signaling cascade. The biophysical technique named Bioluminescence Resonance Energy Transfer (BRET) has been widely used to monitor and quantify agonist-promoted β-arrestin recruitment [3], including high throughput screenings [4]. For the first time, Pierre Fabre Research Institute presents this BRET^1 application on the FDSS/µCELL imaging plate reader (HAMAMATSU PHOTONICS) by monitoring the activity of the dopaminergic D2 receptor (short splice form), a prototypic and well characterized GPCR.

Experimental procedure

Rluc8 tagged-D2s receptor (C-terminal) and eYFP-tagged β-arrestin2 (N-terminal) were transfected into CHO cells, and 36 hours after transfection, cells were starved overnight (16 hours) in serum-free medium. The next day, cells were washed twice with PBS plus glucose 1 g/l, detached with PBS plus 3 mM EDTA and resuspended in PBS plus glucose 1 g/l. A Dc protein measurement assay (Bio-Rad) was performed according to the manufacturer's protocol. For BRET monitoring, cells were then distributed (70 µg per well, about 4x105 cells) in flat and white clear-bottom 96-well microplates (Costar, ref. #3903) and incubated during 10 minutes in the presence or absence of various ligands before signal collection. BRET between Rluc8 and eYFP was measured 9 minutes after the addition of the Rluc8 substrate coelenterazine h (5 µM final) (Promega). The BRET^1 signals were calculated by the ratio of emission of eYFP (516 nm-556 nm) to Rluc8 (460 nm-500 nm). BRET signals were collected by the FDSS/µCELL (HAMAMATSU PHOTONICS), a new imaging plate reader for kinetic cell-based assays .It can be equipped with one injection head (96, 384) and enables simultaneous injection and detection of all the wells (Cf. Fig 1).

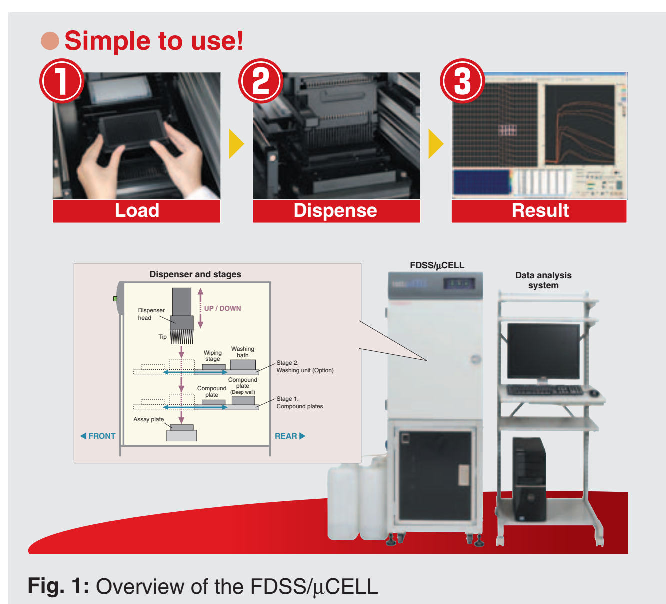

Fig. 1: Overview of the FDSS/µCELL

Pharmacology

The antagonist Haloperidol fully reverses the BRET signal induced by dopamine (a). The stimulation by dopamine is dose-dependent (b). Dopamine efficacy can be discriminated from others compounds activity (c) (Cf. Fig 2).

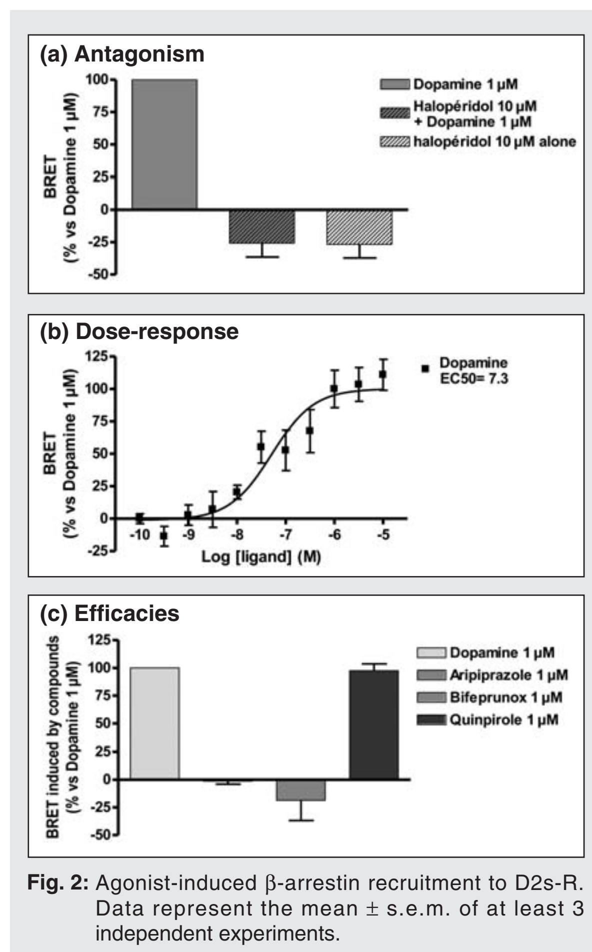

Fig. 2: Agonist-induced β-arrestin recruitment to D2s-R. Data represent the mean ± s.e.m. of at least 3 independent experiments.

Conclusions

This work confirms the ability of FDSS/µCELL to monitor protein-protein interactions, using BRET[1] technology. The main advantages of the FDSS/µCELL are its capability dispensing compounds and reading the light emitted simultaneously from the entire 96 well plate, thus allowing potentially HTS. Moreover, the switch time between the two filters for light emission readings is shorter than for the main other devices, which is a precious advantage for BRET measurements. The next step is to validate BRET[2] screening method, more sensitive and suitable for small distance changes.

Biblio

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HAMAMATSU PHOTONICS K.K., Systems Division
812 Joko-cho, Higashi-ku, Hamamatsu City, 431-3196, Japan, Telephone: (81)53-431-0124, Fax: (81)53-435-1574, E-mail: export@sys.hpk.co.jp

U.S.A.: Hamamatsu Corporation: 360 Foothill Road, Bridgewater. N.J. 08807-0910, U.S.A., Telephone: (1)908-231-0960, Fax: (1)908-231-1218 E-mail: usa@hamamatsu.com
Germany: Hamamatsu Photonics Deutschland GmbH: Arzbergerstr. 10, D-82211 Herrsching am Ammersee, Germany, Telephone: (49)8152-375-0, Fax: (49)8152-2658 E-mail: info@hamamatsu.de
France: Hamamatsu Photonics France S.A.R.L.: 19, Rue du Saule Trapu, Parc du Moulin de Massy, 91882 Massy Cedex, France, Telephone: (33)1 69 53 71 00, Fax: (33)1 69 53 71 10 E-mail: infos@hamamatsu.fr
United Kingdom: Hamamatsu Photonics UK Limited: 2 Howard Court, 10 Tewin Road, Welwyn Garden City, Hertfordshire AL7 1BW, United Kingdom, Telephone: (44)1707-294888, Fax: (44)1707-325777 E-mail: info@hamamatsu.co.uk
North Europe: Hamamatsu Photonics Norden AB: Torshamnsgatan 35 SE-164 40 Kista, Sweden, Telephone: (46)8-509-031-00, Fax: (46)8-509-031-01 E-mail: info@hamamatsu.se
Italy: Hamamatsu Photonics Italia S.r.l.: Strada della Moia, 1 int. 6, 20020 Arese (Milano), Italy, Telephone: (39)02-93581733, Fax: (39)02-93581741 E-mail: info@hamamatsu.it
China: Hamamatsu Photonics (China) Co., Ltd.: B1201 Jiaming Center, No.27 Dongsanhuan Beilu, Chaoyang District, Beijing 100020, China, Telephone: (86)10-6586-6006, Fax: (86)10-6586-2866 E-mail: hpc@hamamatsu.com.cn

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