Multimodal approaches applied to membrane receptors signaling using µCELL Kinetic Image plate reader (Hamamatsu) available on the ARPEGE facility

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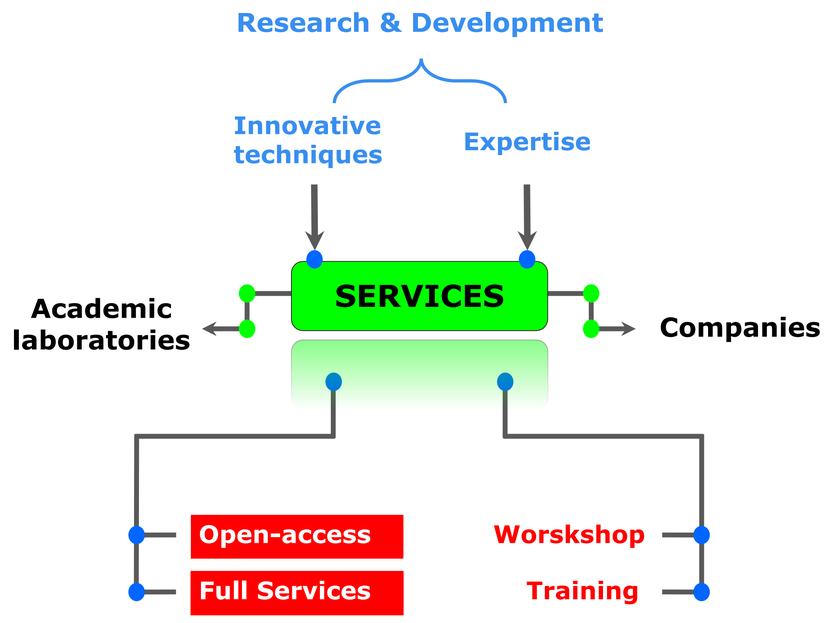
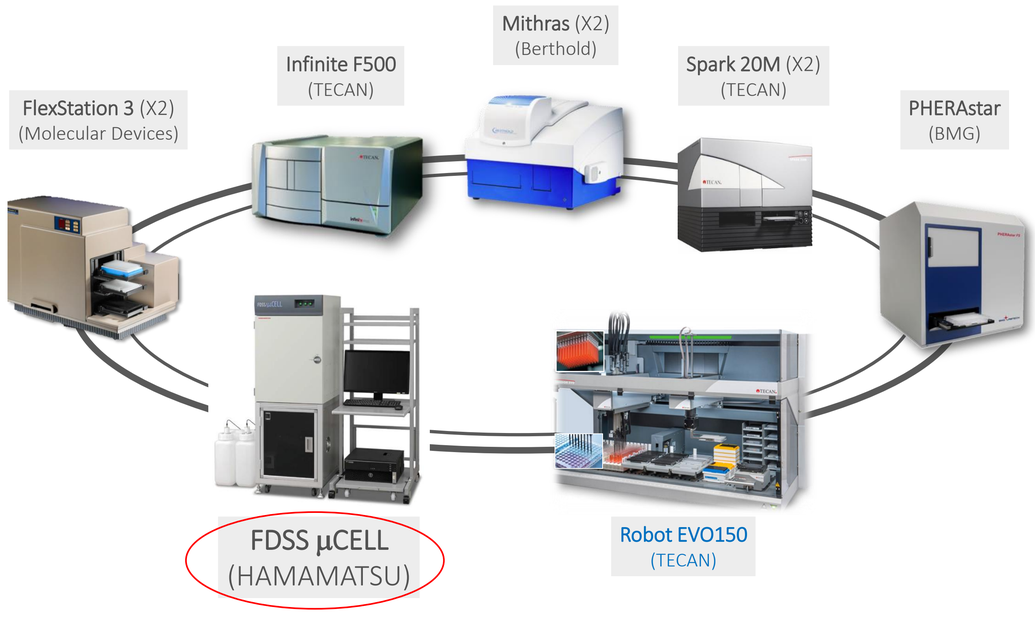
1 ARPEGE FACILITY



The ARPEGE facility allows extensive analysis of the structural and functional features of signaling protein complexes. It permits the characterization of all kinds of specific interactions either between ligand and target or between protein partners in signaling protein complexes.

2 AVAILABLE PLATE READERS

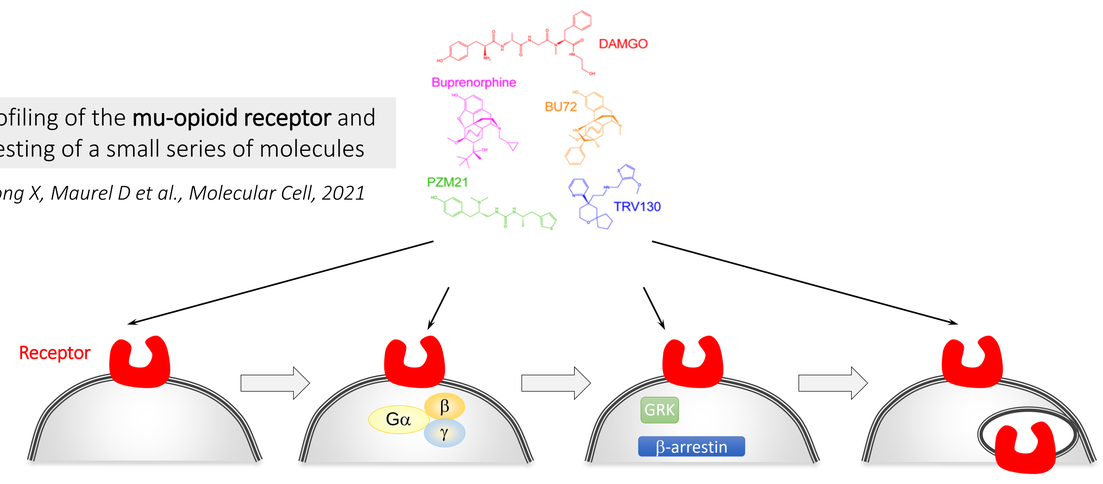


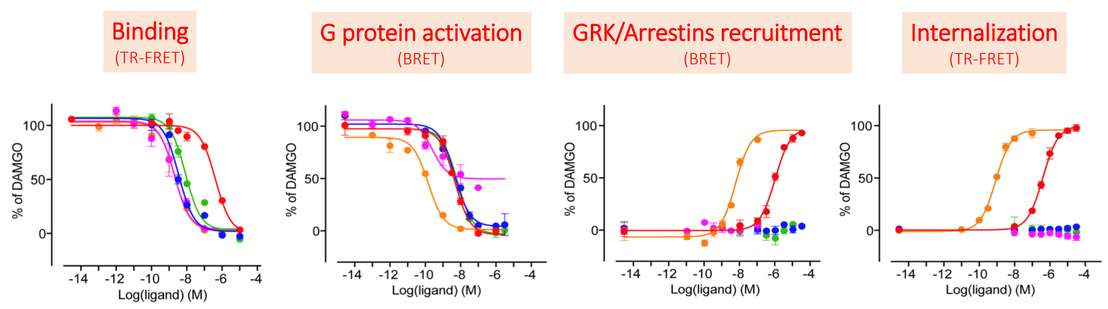
The available equipment includes a selection of the most efficient and sensitive plate readers including the FDSS/µCELL plate reader (Hamamatsu) adapted to luminescence/fluorescence assays in medium-throughput format.

3 SCREENING ON SIGNALING PATHWAYS

Profiling of the **mu-opioid receptor** and testing of a small series of molecules

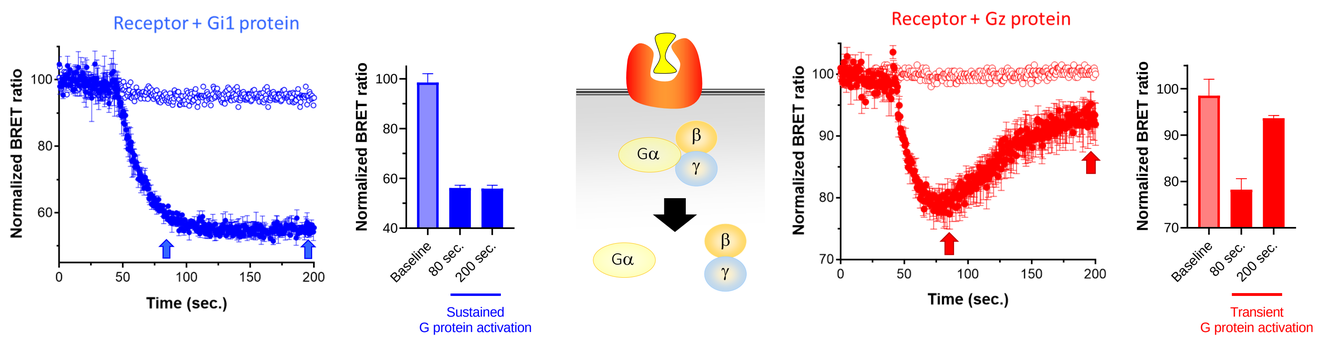
Cong X, Maurel D et al., Molecular Cell, 2021





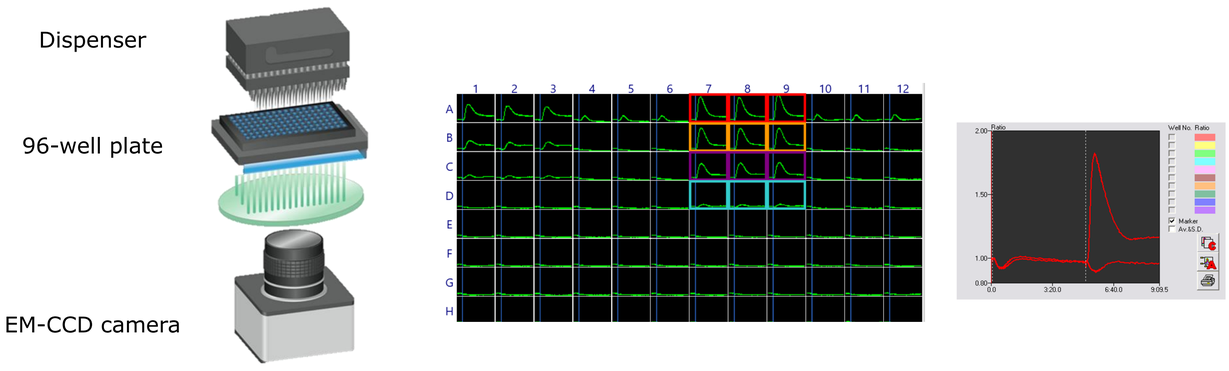
The ARPEGE facility performs multiple cell-based assays in order to extensively analyze transmembrane protein target features like G proteins activation (Gi1,2,3, GoA,B, Gz, Gq), GRK (GRK2,3,5), Arrestins (arrrestin 1,2) recruitment or second messengers production as cAMP or calcium.

4 KINETIC VS ENDPOINT MEASUREMENT



5 FDSS/µCELL: A CAMERA-BASED KINETIC PLATE READER

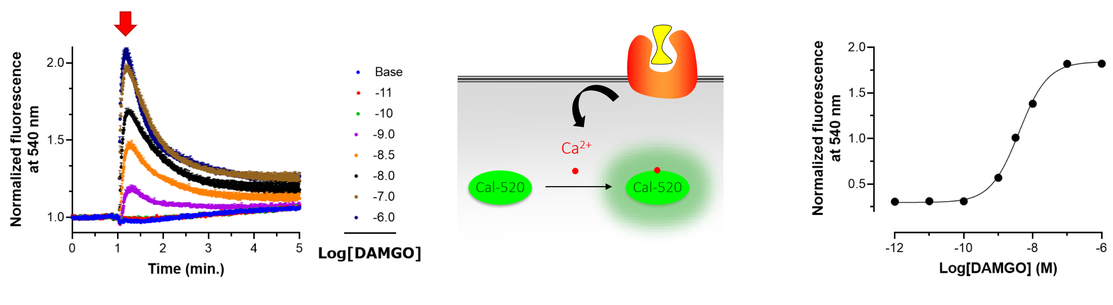
Addition → Detection → Analysis



In contrast to conventional luminescence readers, the FDSS/µCELL plate reader enables 96 samples to be measured simultaneously, enabling rapid kinetic measurements of cell signaling.

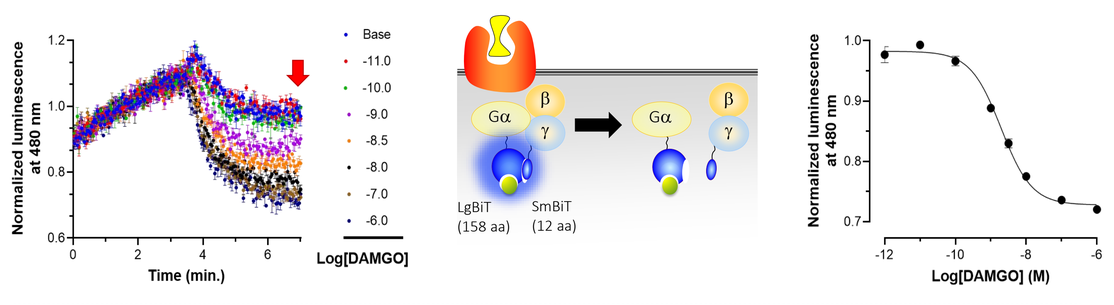
6 FDSS/µCELL A MULTIMODE PLATFORM FOR DRUG SCREENING IN REAL TIME

Fluorescence (calcium release)



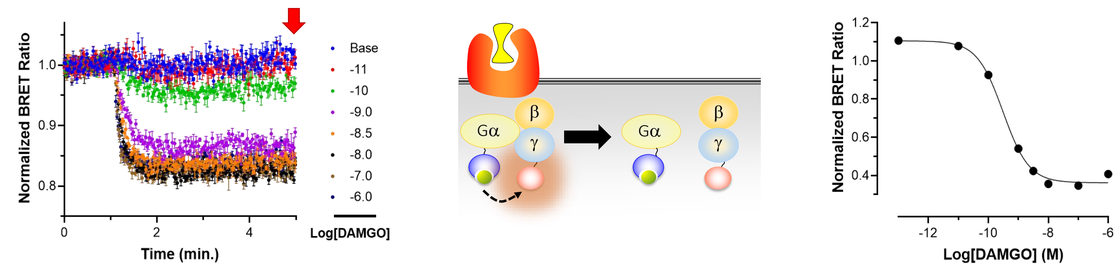
Mu-opioid receptor function was performed by monitoring the calcium signal generated by the coupling of receptors with a chimeric G protein, GqTop (Gq chimeric protein wherein C-terminal amino-acids were substituted with the corresponding sequence from the C-terminal domain of Gi). Stimulated receptors coupled to the chimeric G protein induce an intracellular calcium release, causing the calcium sensitive dye (here Cal-520) to fluoresce (fluorescence increase).

Luminescence (G protein dissociation)



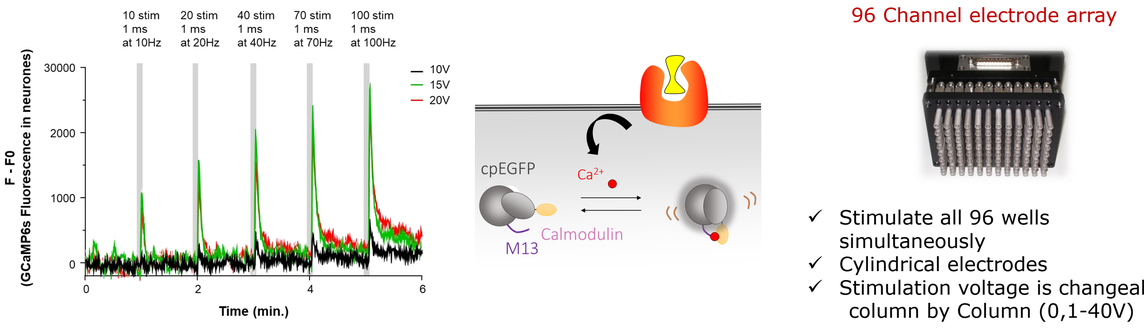
Heterotrimeric G protein complex dissociation was monitored by NanoLuc binary technology (NanoBiT, Promega). The Gα protein dissociation is monitored with reversible changes of luminescence (signal decrease) of the NanoLuc luciferase split into two parts: the Large-BiT to Gα and the Small-BiT to β-1.

BRET: Bioluminescence Resonance Energy Transfer (G protein dissociation)



Heterotrimeric G protein complex dissociation was monitored by BRET (Bioluminescence Resonance Energy Transfer). The Gα protein is fused to Renilla Luciferase8 (donor) and γ-2 to Venus (acceptor). Activation of receptor coupled to G protein complex promotes the dissociation of the Gαβγ protein complex resulting in the BRET signal decay.

Electric Field Stimulation (calcium release)



- ✓ Stimulate all 96 wells simultaneously
- ✓ Cylindrical electrodes
- ✓ Stimulation voltage is changeable column by Column (0,1-40V)

Neurons were transduced with lentivirus in order to express GCaMP6s sensor. GCamp6s signal variation was measured upon electric stimulation (FDSS/µCell/EFS) of neurons with variable voltages and frequencies. The increase of frequency from 10Hz to 100Hz induce the increase of Ca^{2+} signal of Neuronal cells (data kindly provided by Dr. V. Compan)

The FDSS/µCELL kinetic image plate reader is a high-performance instrument enabling the simultaneous acquisition of 96 cell samples at a time, with no measurement delay between samples. This reader can be used in different modes (fluorescence, luminescence) compatible with a wide range of cell signaling sensors (calcium, cAMP, G-protein…). The electrical stimulation head completes the instrument's portfolio, enabling electrical stimulation of voltage-sensitive cells. **In conclusion, the FDSS/µCELL plate reader is an instrument perfectly suited to a service facility offering its customers cell-based assays in a wide range of measurement modes.**