Celtarys RESEARCH

Innovative Chemistry to

Illuminate Biology

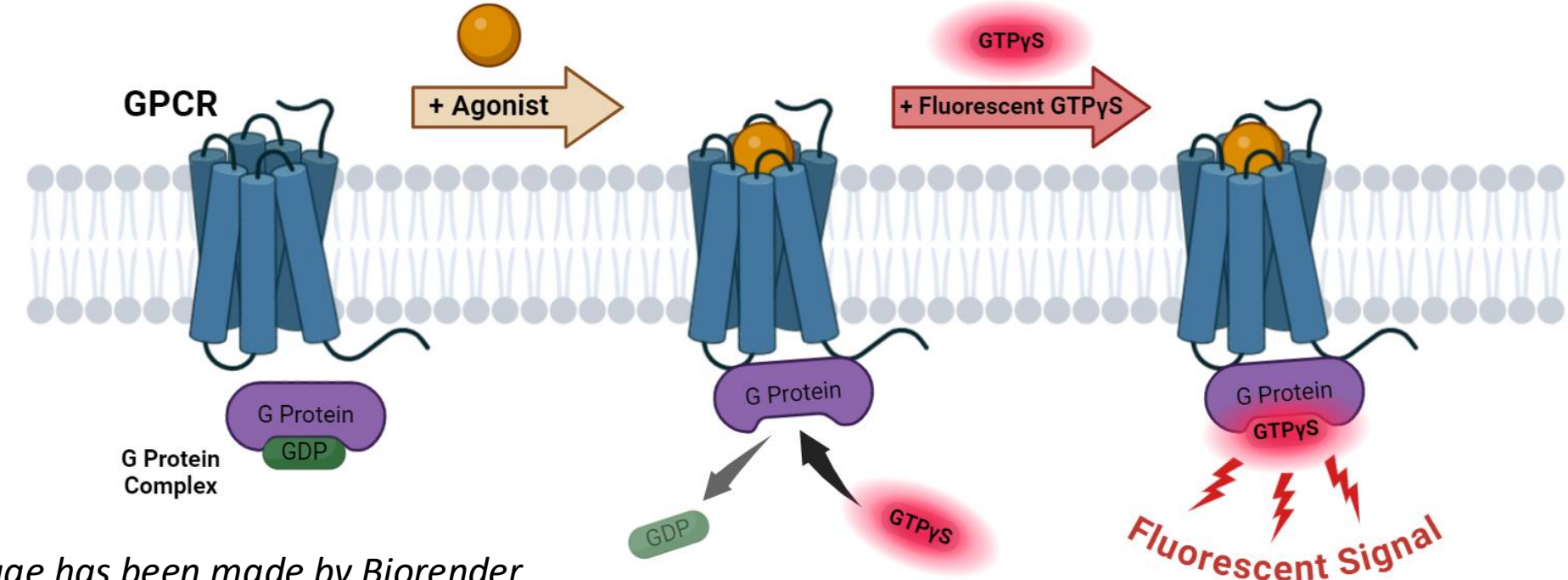
Fluorescent GTPγS

GPCR Functional assay

GPCR functional activity data are **extremally important** in Drug Discovery programs, both for monitoring therapeutical effects or off target side effects.

A reliable and efficient assay is based on the use of *no-hydrolysable GTP derivative, GTPγS, which allows to detect the activation of G protein after ligand binding.*

Compared to the radioactive [^{35}S]GTPγS, **fluorescent GTPγS enables assays to be performed with the same sensitivity and robustness** while allowing the **use of conventional facilities and equipment**



This image has been made by Biorender

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Fluorescent GTPγS

In the frame of a collaboration between Celtarys and Arcoscreen, we developed a **Fluorescent GTPγS (CELT-503)** emitting the in red region of the spectra (589/616).

The developed fluorescent probe was validated by Arcoscreen in its own platform for GPCRs functional screening.

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arcoscreen

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Advantages of Fluorescent GTPγS vs conventional radioactive GTPγS

	[^{35}S]GTPγS	Fluorescent GTPγS
No-hydrolysable GTP derivative	✓v	✓v
Detect the direct activation of G protein, not second messengers	✓v	✓v
Special handling	YES (Radioactive)	NO
Compatible with common equipment	×x	✓v
Compatible with High Throughput Screening (HTS)	×x	✓v
Costs per assay	↑	↓

As a radioactive substance, [^{35}S]GTPγS requires additional precautions, including **trained personnel and specific safety measures**, as well as **specialized facilities for handling radioactive materials like sulfur-35.** Additionally, the radioactive waste generated during the assays must be managed and disposed of appropriately. Furthermore, the **[^{35}S] isotope has a relatively short half-life of 87 days**, compared to the 12-year half-life of tritium.

These challenges are effectively addressed by replacing radioactivity with fluorescence (CELT-503), offering a safer and more practical alternative.

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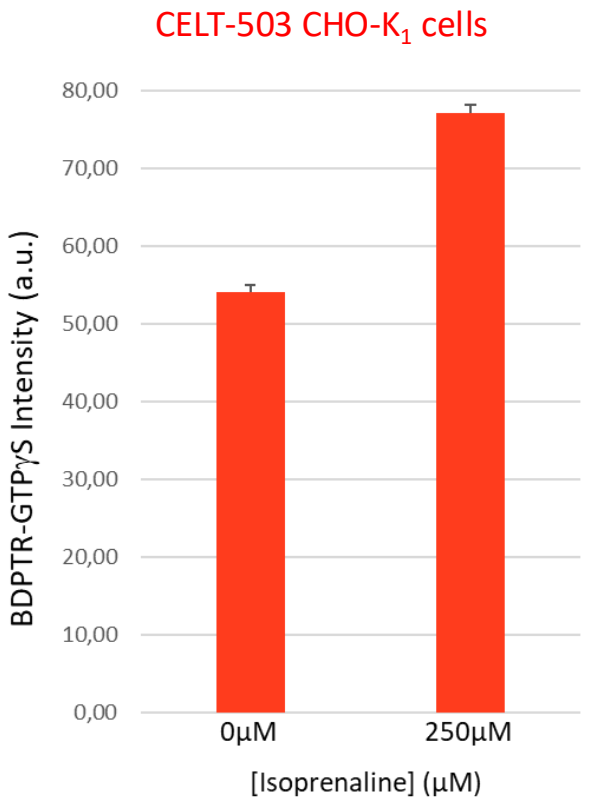
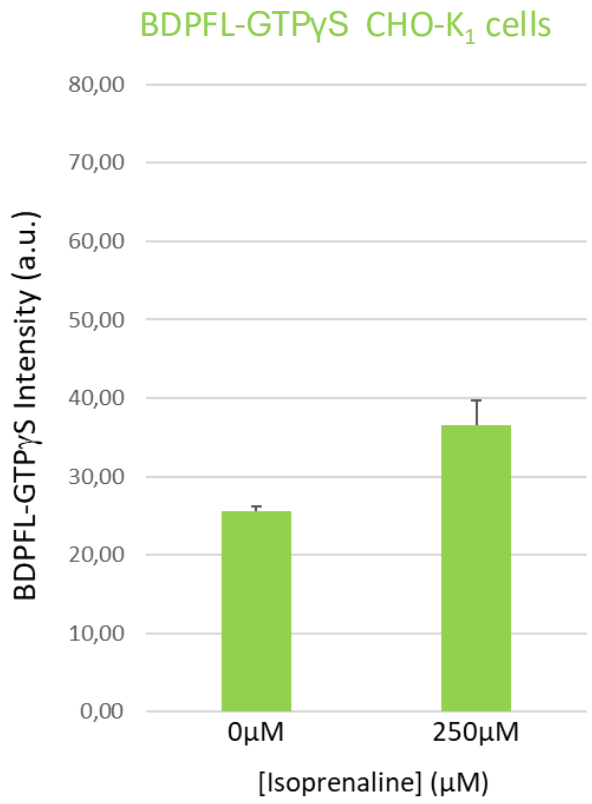
GPCRs Functional assay

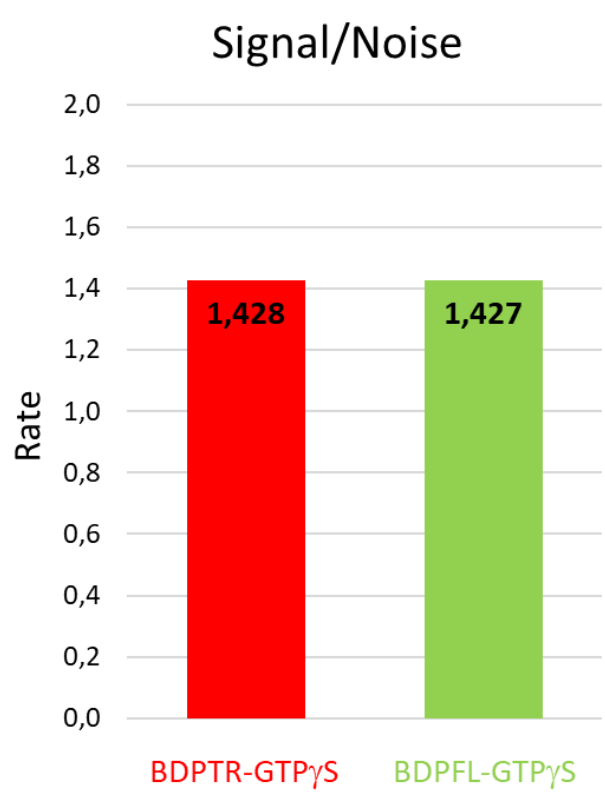
α-adrenergic receptors

Experiment performed in triplicate in CHO-K1 cells (passage 12) expressing endogenously adrenoreceptors.
Experiment read-out is the *zeiss fluorescent microscope axiovert 7* with the following parameters:
-CELT-503: ex. 555 nm, em 614 nm , 50% intensity light source, 150ms exposure time
-BDPFL-GTPγS: ex. 475 nm, em 509 nm , 50% intensity light source, 500ms exposure time

Compared to the BDPFL-GTPγS (actual market available), the CELT-503 (Celtarys) is 2x brighter with **3 times less exposure time**, **while keeping the same ratio Signal/Noise**. As it emits in the red spectrum, **it is compatible with most multiplexing experiments**, as most fluorophores emit in the green spectrum.







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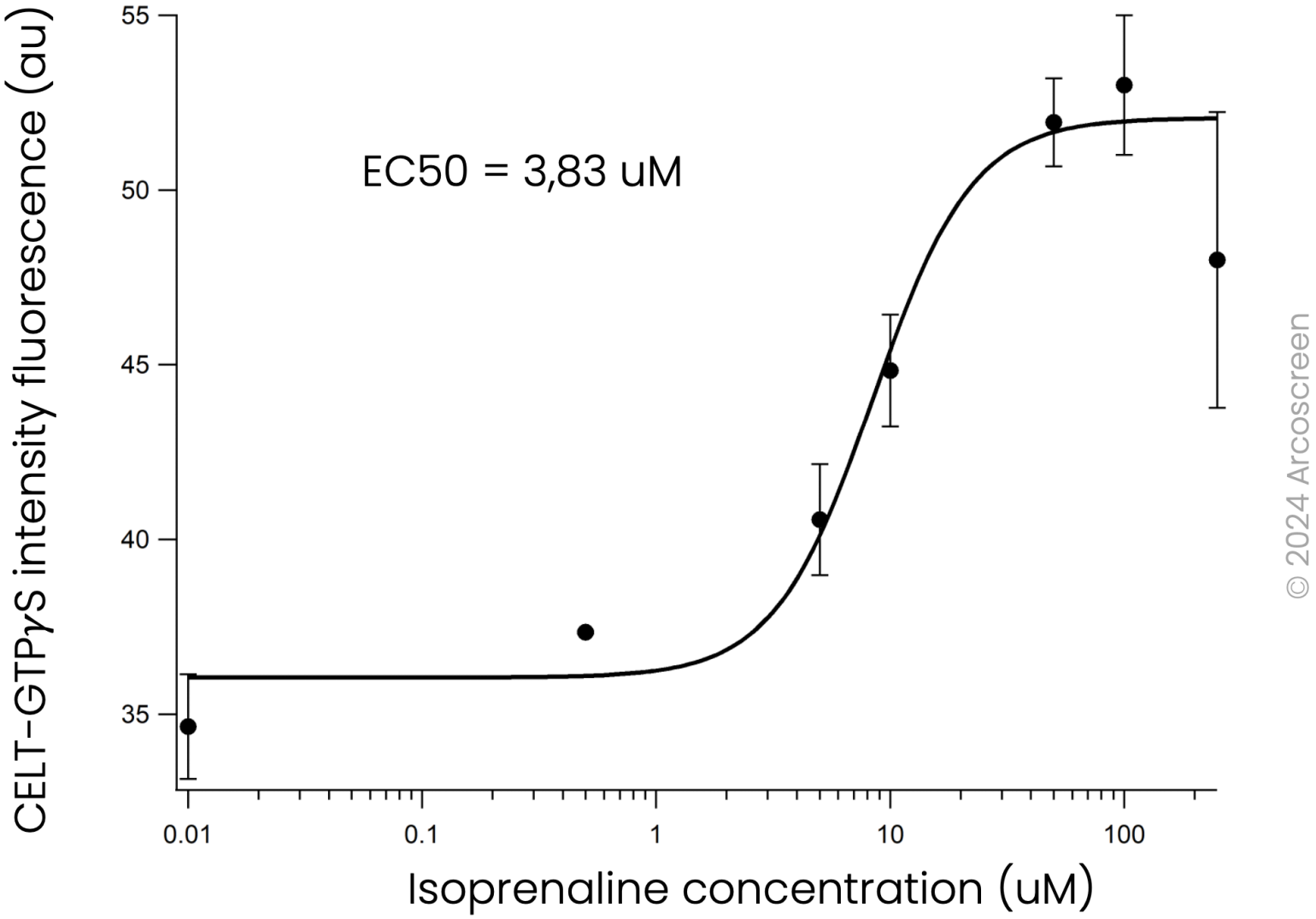
GPCR Dose-response curve

The EC_{50} of isoprenaline was measured using **CELT-503.** The experiments were performed in triplicates in CHO-K1 cells endogenously expressing adrenoreceptors.



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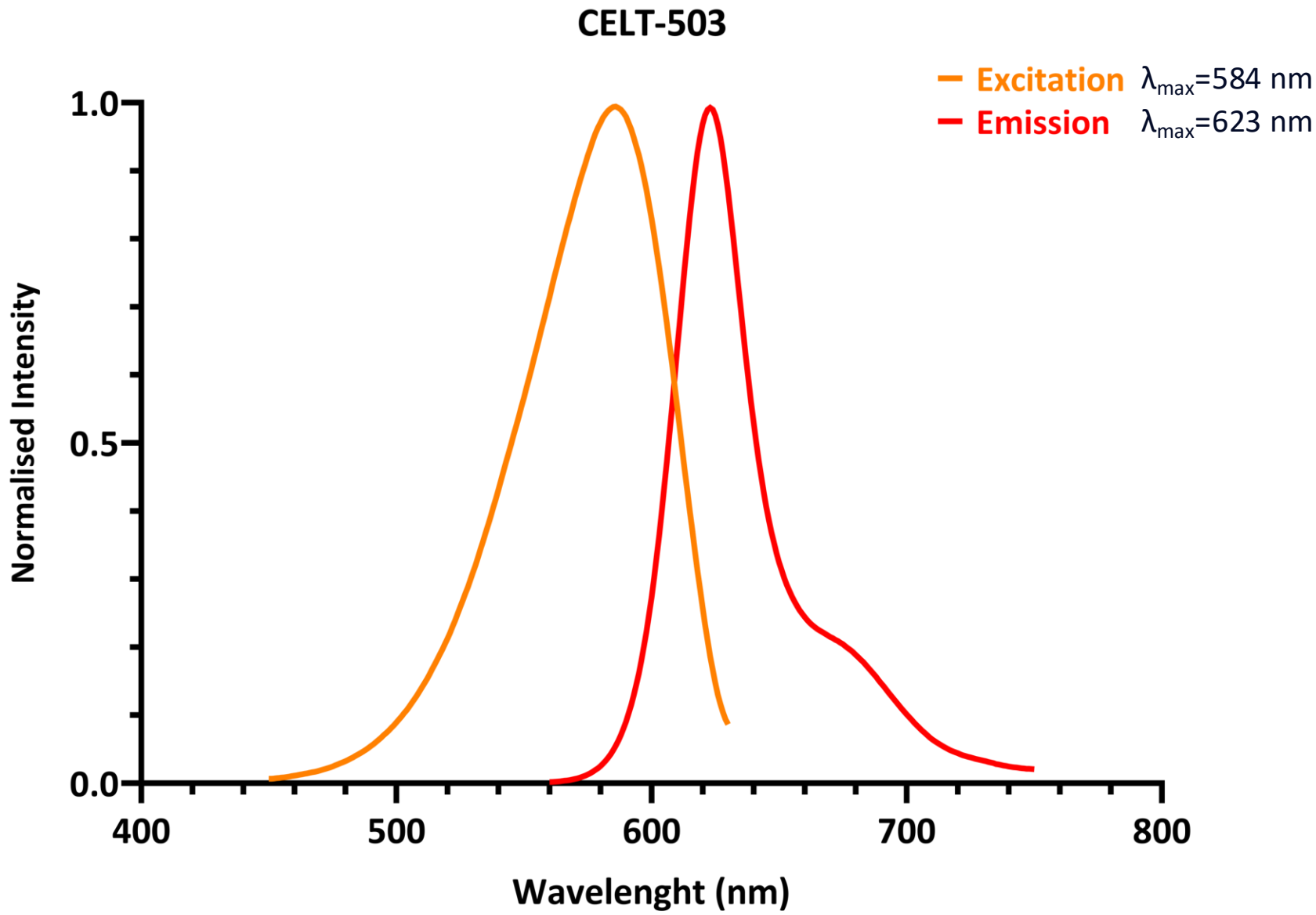
CELT-503

excitation emission spectra in water



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Thank you

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