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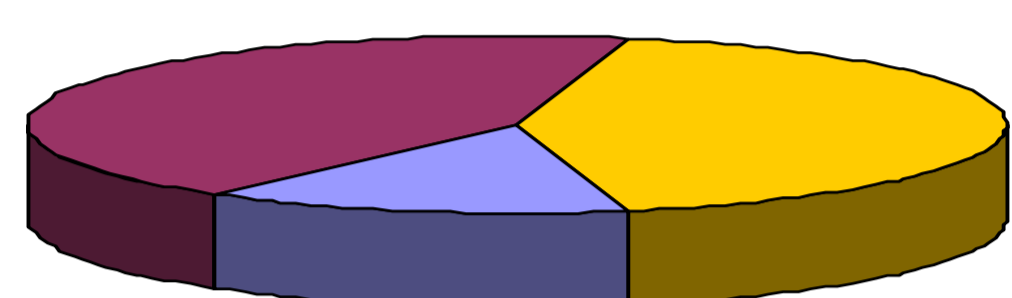
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INTRODUCTION

GPCRs account for the major proportion of drug targets but still suffer from lack of adequate technologies for the study of challenging members such as orphans or for the detection of allosteric modulators, a new class of ligands with unprecedented opportunities. **DTect-All™** is a unique and proprietary technology allowing the identification of ligands interacting specifically with the 7-TM part of GPCRs. This technology, validated with the three GPCR families, is well suited for difficult targets thanks to an unique **collection of GPCR fluorescent probes**. In the course of our screening campaigns, we found that binders presenting no functional activity, hereafter named **SAMs** for **Silent Allosteric Modulators**, were systematically identified and represent a useful source of molecules for the discovery of novel families of modulators. In fact, slight chemical modifications of these SAMs can switch them to functionally active PAMs (Positive) or NAMs (Negative). This approach constitutes an innovative way to make accessible a broader chemical space for allosteric modulator identification. We present here two case studies on GLP-1R and group II mGluRs.

1. GPCRs

- 50% of current marketed drugs with more than \$30B in sales annually
- ~950 GPCRs in the human genome, ~400 being therapeutic targets:

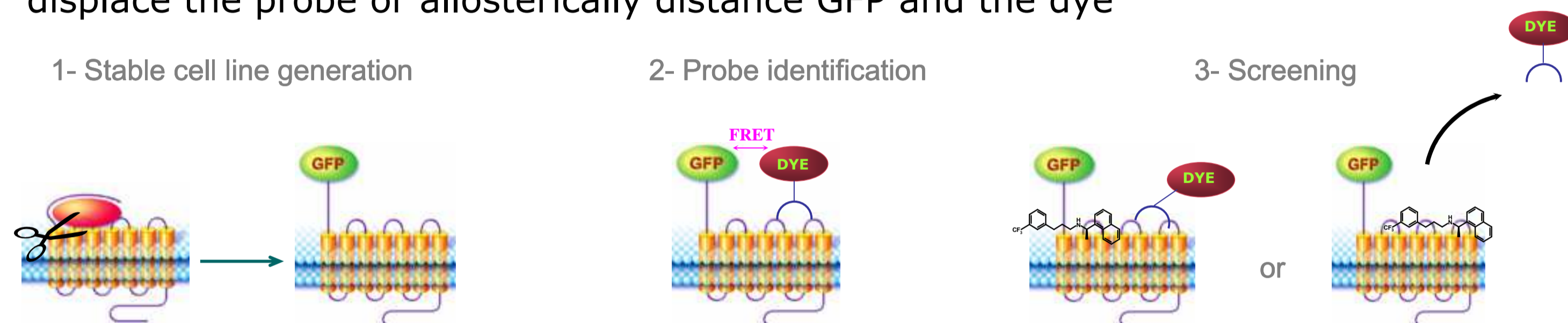


- GPCRs targeted by marketed drugs (50)
- Orphan GPCRs (~150)
- Insufficiently explored GPCRs (~150)

- Lack of technologies to study **orphans** or **challenging GPCRs** (peptide, lipide ...)
- Lack of technologies to explore **new chemical spaces** (linked to the use of assays originally developed for orthosteric ligand identification)
- Lack of technologies to selectively identify **allosteric modulators (AM)**, new class of ligands presenting numerous advantages over orthosteric ligands

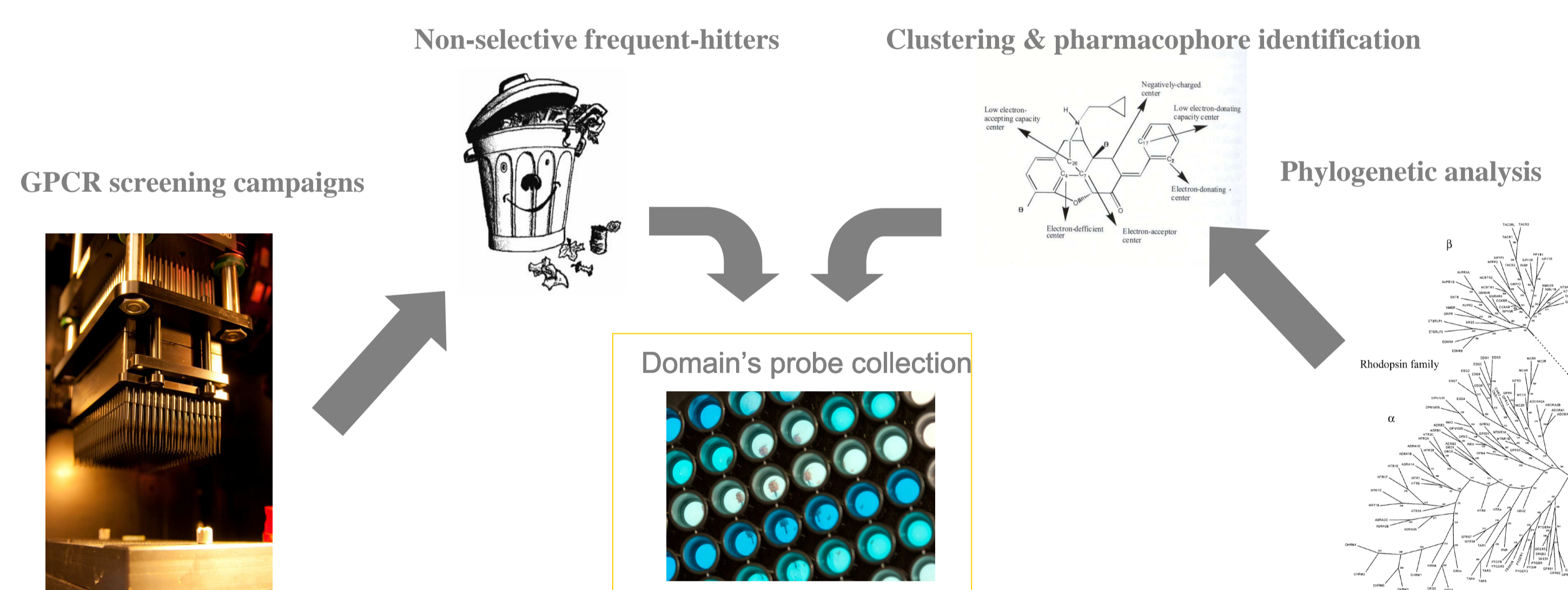
2. DTect-All™

- **DTect-All™**: proprietary FRET-based binding assay used for primary screening
- N-ter truncation can orphanize the GPCR and direct identification of **AM** only
- Collection of GPCR frequent-hitter probes for **orphans** and **challenging GPCRs**
- Validated for every GPCR types (**orphans**, classes 1, 2, 3)
- **Less false positive** vs functional due to double labeling: on-target hits identified
- **Higher sensitivity** vs radioactive binding assays as hits can either competitively displace the probe or allosterically distance GFP and the dye



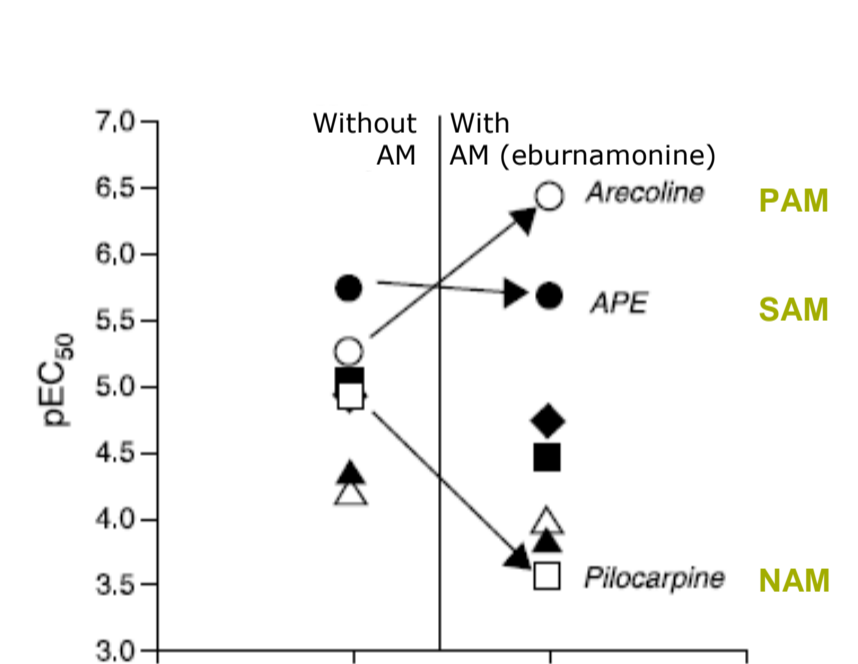
3. GPCR probe collection

- Represents one of the **competitive advantages** of our technology
- Unique proprietary collection of 4000 non-selective fluorescent compounds
- Successfully used for every GPCR families (A, B and C) including several orphans
- Designed from **GPCR frequent-hitter** identified in several screening campaigns
- Continuously enriched with libraries focused on specific GPCRs or GPCR clusters



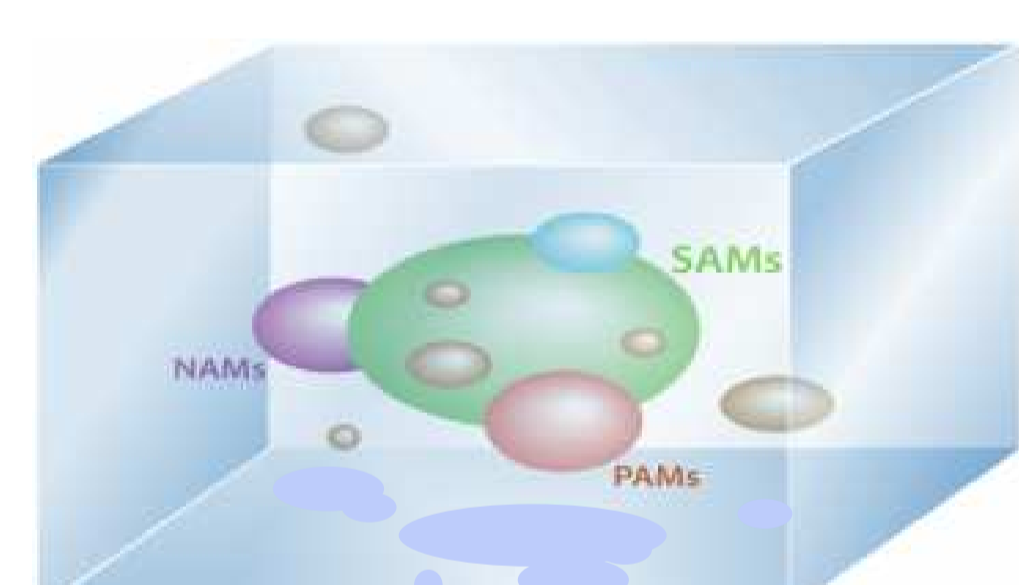
4. Silent Allosteric Modulators

- DTect-All™ enables detection of orthosteric ligands or allosteric modulators that can either be positive (PAMs), negative (NAMs) or **silent (SAMs)**
- SAMs are binders devoid of activity **in a specific functional test**
- SAMs can be **false negatives in standard functional assays** but can turn out to be PAMs or NAMs under different assay conditions
- SAMs are very close to PAMs or NAMs: **source for new chemical diversity**
- At Domain, identified SAMs are further modified by **medicinal chemistry** to switch them into PAMs or NAMs



Name	DFB	DMeOB	DCB
R	-F	-OMe	-Cl
Activity	PAM	NAM	SAM

GPCR chemical space representation



Kenakin T. Molecular Interventions 2004, 222-9.

O'Brien JA et al. Mol Pharm 2003, 731-40.

Adapted from Lipinski C and Hopkins A. Nature 2004, 855-61.

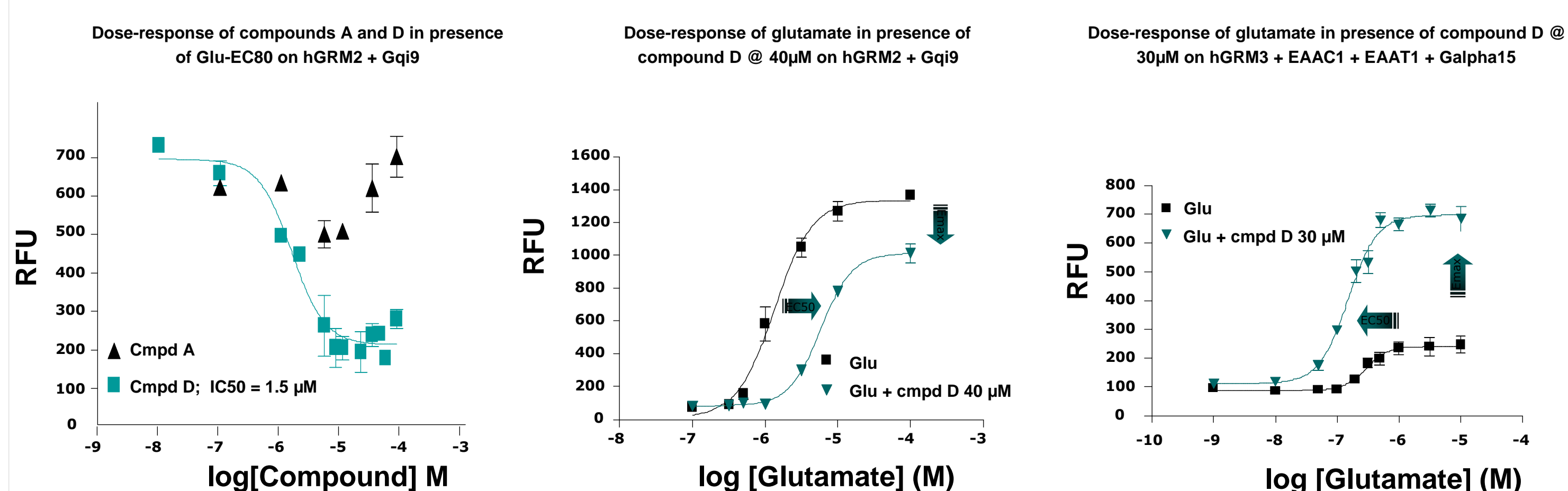
5. Example with mGluR2/3 (family C)

- A FRET assay was developed with a truncated mGluR2 together with two functional assays with full length mGluR2 and mGluR3
- Screening leads to identification of SAMs such as compound A
- Slight chemical modifications of A lead to **mGluR2 NAMs / mGluR3 PAMs**
- This is the first report of a family of compounds having opposite activities on the two group II mGluR subtypes

PHCC	R	mGluR2		mGluR3
		FRET	Ca ⁺⁺	Ca ⁺⁺
PHCC	H	No binding up to 30µM	No activity	Not done
A	F	Ki = 6.6µM	SAM (up to 100µM)	« SAM » (up to 100µM)
B	Cl	Ki = 1µM	NAM IC ₅₀ = 0.8µM	PAM EC ₅₀ = 13.4µM
C	OMe	Ki = 0.8µM	NAM IC ₅₀ = 1µM	PAM EC ₅₀ = 10.4µM
D	Me	Ki = 0.7µM	NAM IC ₅₀ = 1.5µM	PAM EC ₅₀ = 8.9µM

Functional characterization on mGluR2

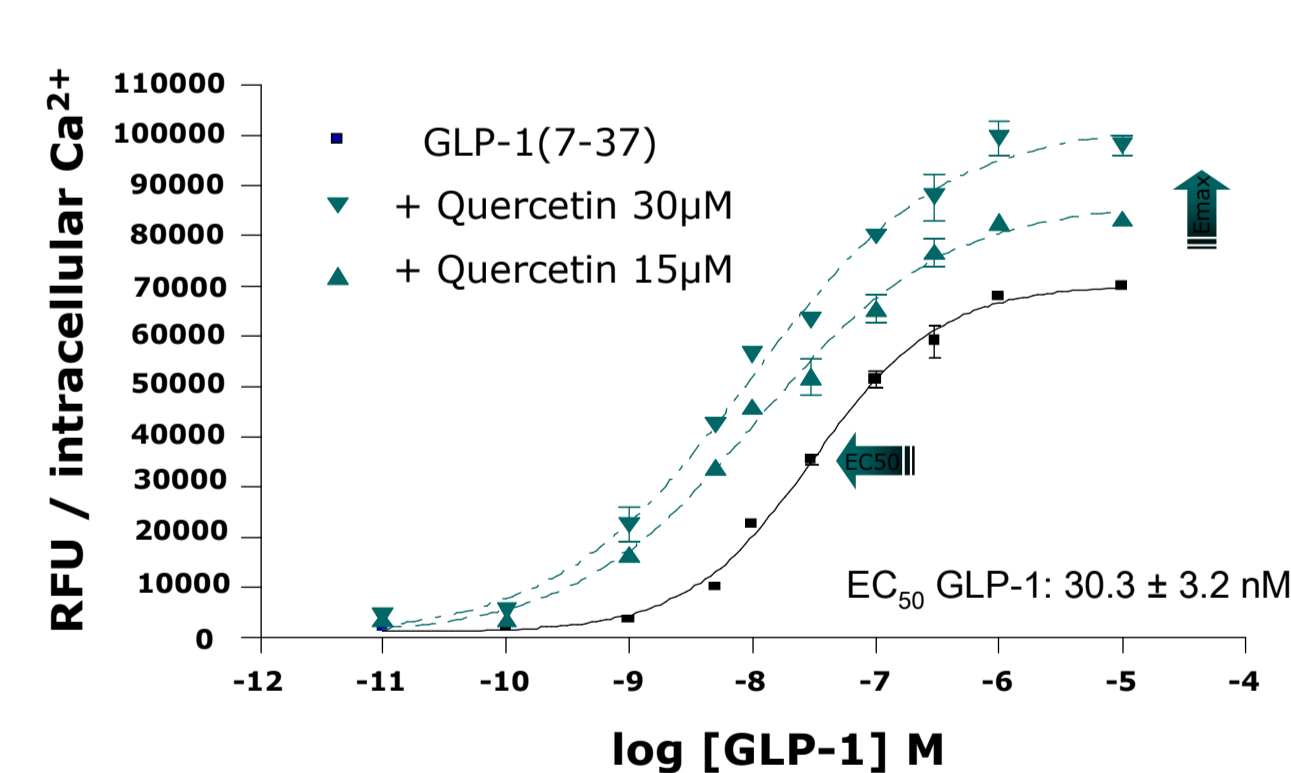
Functional characterization on mGluR3

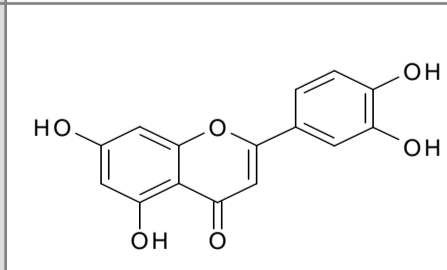
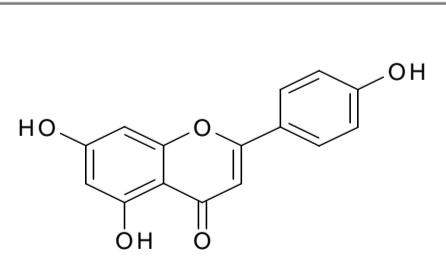
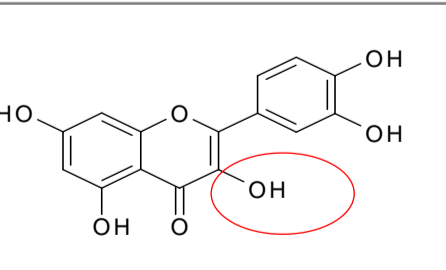
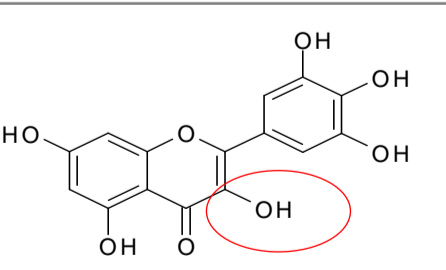
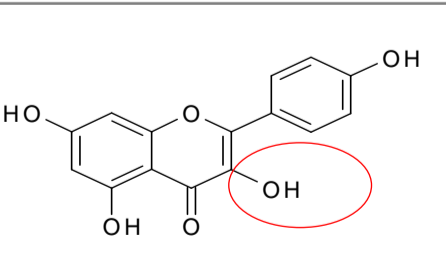


6. Example with GLP-1R (family B)

- A binding FRET assay and a Ca²⁺ functional assay were developed for GLP-1 receptor
- PAMs and SAMs were identified in a same flavonoid chemical family
- Rapid SAR study reveals the crucial role of 3-OH group for PAM activity

Calcium assay
GLP-1 response with Quercetin on GLP-1 Full length cells



Compound	Luteolin	Apigenin	Quercetin	Myricetin	Kaempferol
FRET	Ki = 5.5µM	Ki = 17.3µM	Not done	Ki = 2.8µM	Ki = 9.9µM
Ca ⁺⁺	SAM Up to 30µM	SAM Up to 100µM	PAM @ 30µM Shift GLP-1 EC ₅₀ by 3.2±0.7 fold Shift GLP-1 Emax by 1.5±0.3 fold	PAM @ 30µM Shift GLP-1 EC ₅₀ by 1.9±0.2 fold Shift GLP-1 Emax by 1.1±0.1 fold	PAM @ 30µM Shift GLP-1 EC ₅₀ by 1.7±0.5 fold Shift GLP-1 Emax by 1.1±0.1 fold
Structure					

CONCLUSION

DTect-All™ is an innovative binding technology applicable to every GPCR thanks to **Domain's probe libraries**. It enables the identification of novel families of ligands that cannot be detected by standard functional assays. Amongst these ligands, **SAMs** turn to be a new pharmacological class of binders devoid of functional activity. They represent a unique source of novel chemical scaffolds for medicinal chemistry optimization or pharmacological characterization.

We have recently made **DTect-All™** available to the Industry as illustrated by our main research agreement signed with TAKEDA.