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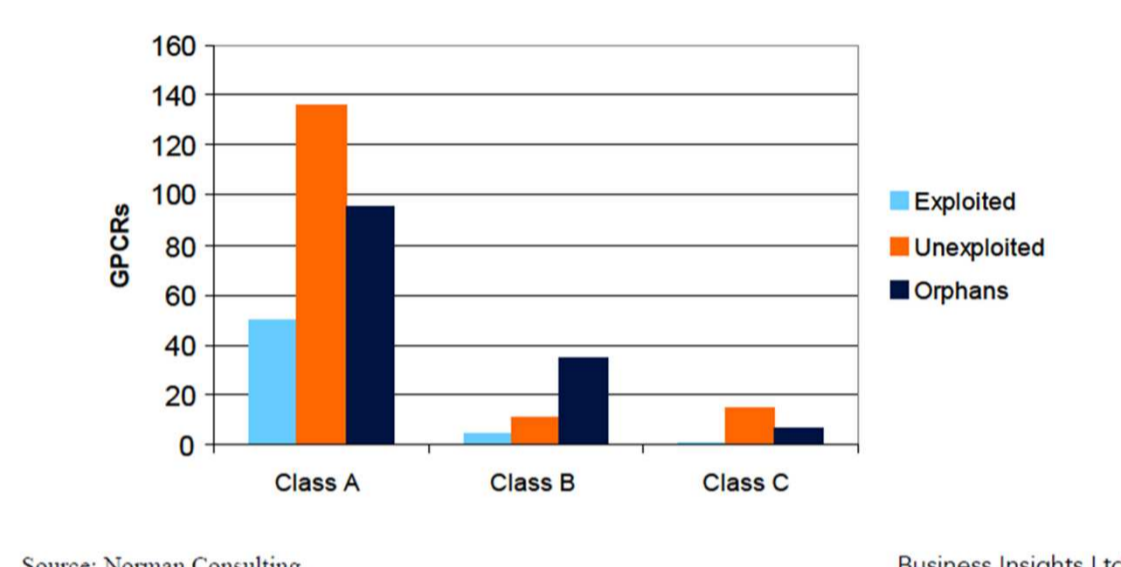
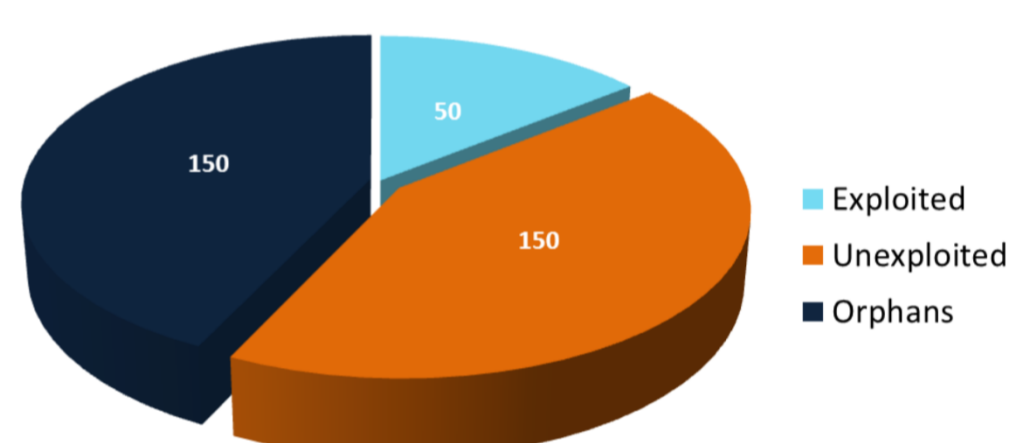
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INTRODUCTION

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 the peptide GPCRs for which allosteric modulation represents a highly promising approach, yet unachievable. Screening using functional assay, which is the standard paradigm for allosteric modulator (AM) identification in the Pharma industry, is limited by an outcome highly dependent on the specific conditions used to setup the assay. This feature leads to numerous false negatives and to the weak success rate of GPCR HTS campaigns. Domain Therapeutics has developed an innovative approach for the identification of AMs consisting in identifying a large diversity of 7-TM binders of the targeted GPCR that are further characterized using several functional assays. **This poster illustrates how this approach was used for the screening of positive AMs of challenging peptidic GPCRs such as the neuropeptide S receptor, the GLP-1R and the galanin 2 receptor.**

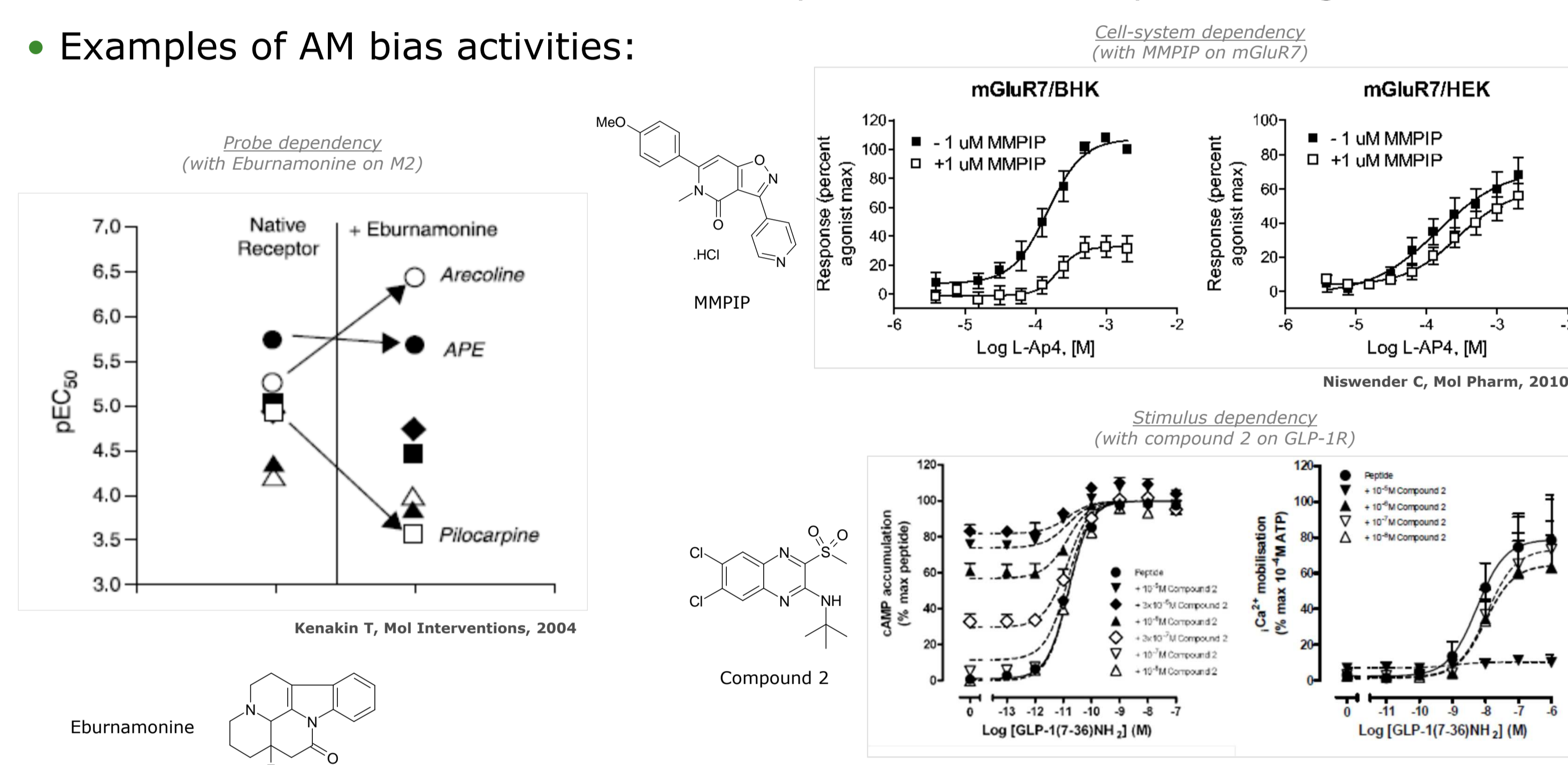
1. Unexploited GPCRs

- GPCRs represent the best drug target family
- Marketed GPCR drugs target **only 50 receptors** out of the 350 druggable ones
- Half of the remaining unexplored are challenging GPCRs for which no orthosteric drugs were successfully developed
- **Allosteric Modulators (AMs)** constitute an innovative approach with major advantages (more amenable chemistry, higher subtype selectivity profile ...)
- **Positive AMs** or **ago-allosteric** compounds are highly promising alternatives for **peptide GPCR** activation, however their identification **remains challenging** using standard screening technologies



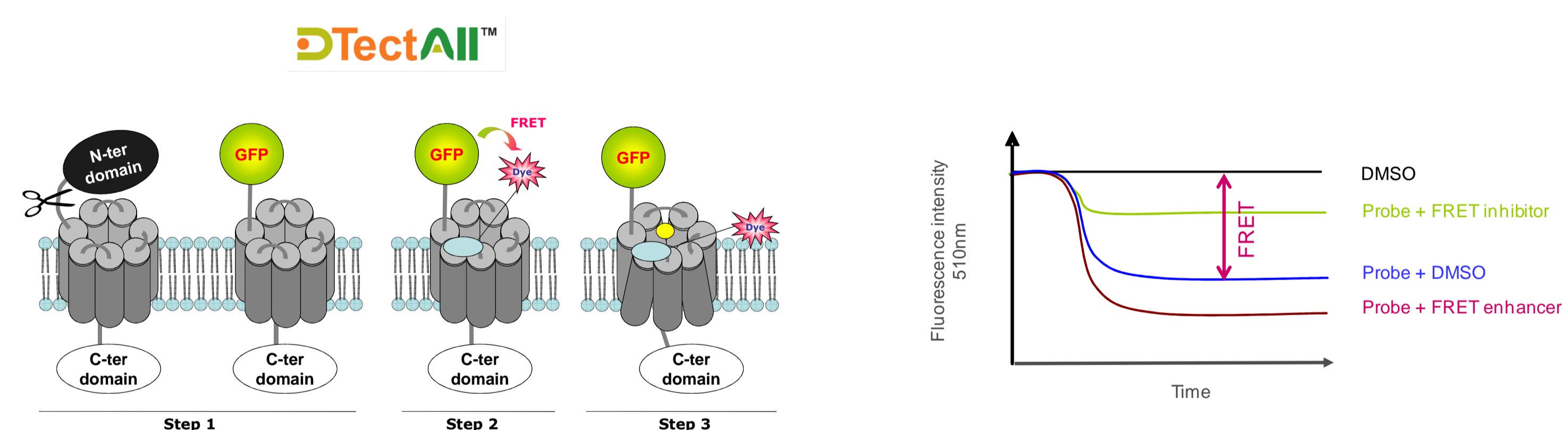
2. Functional HTS bias

- HTS with **single functional endpoint** is still often used for AM identification
- Growing number of reports show that GPCR AM activity is highly dependent of **specific functional test conditions**
- Functional HTS will therefore undersample the chemical space of a given GPCR
- Examples of AM bias activities:



3. DTect-All™

- **DTect-All™**: proprietary FRET-based binding assay used for primary screening
- N-ter truncation (step 1) will orphanize some class A and all class B peptide GPCRs to **focus on AM discovery**
- Applicable to any GPCR thanks to GPCR frequent-hitter probe library for step 2
- Surrogate markers of ligand-induced conformational change of the GPCR
- 7-TM binders can be widely characterized and/or switched into PAM/NAM

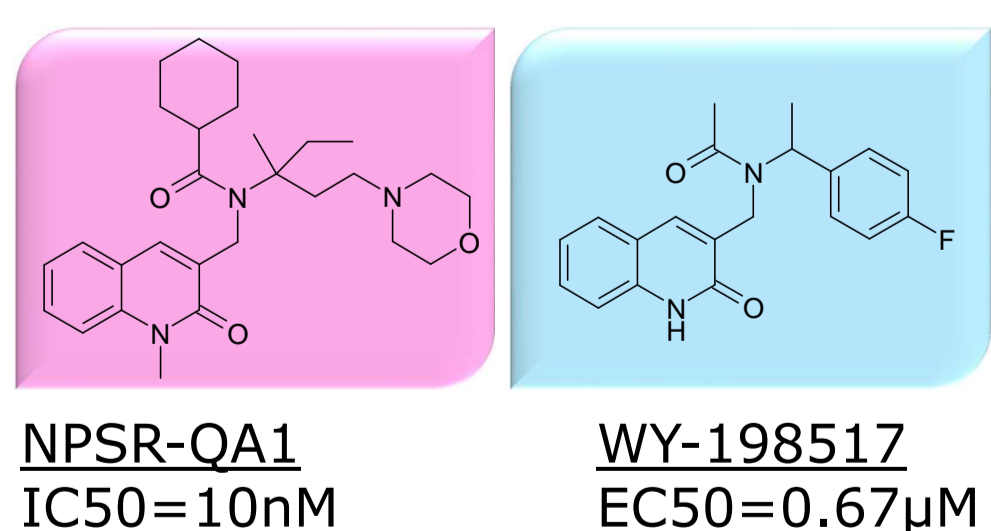
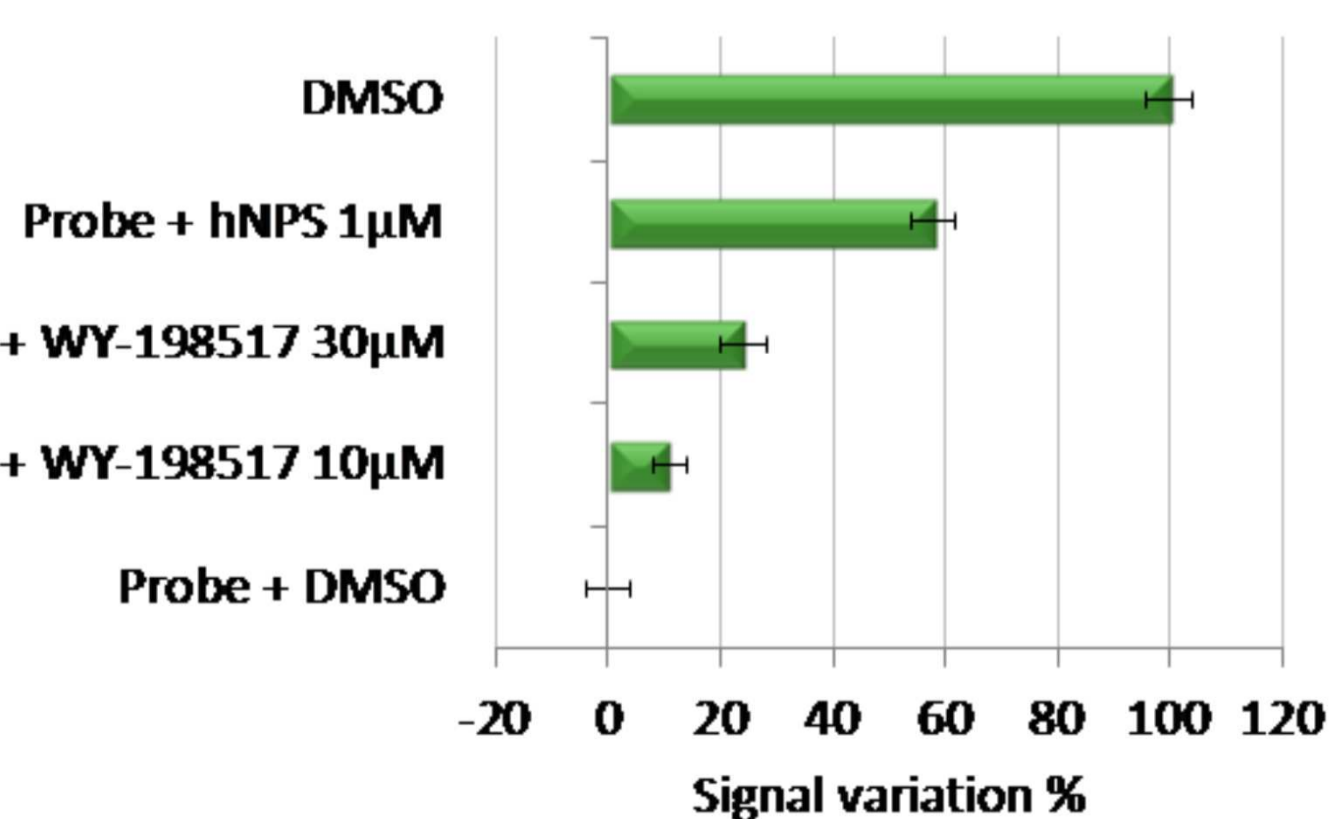


5. Case study II: Neuropeptide S receptor (NPSR)

- Class A GPCR, also known as GPR154, recently deorphanized
- Neuropeptide S is a 20-aa peptide with high potency ($K_d=0.33nM$) on NPSR
- Highly expressed in CNS regions regulating stress, wake and anxiety
- Numerous antagonists / NAMs described, **only 1 PAM family**: quinolinones described in the late 2010 by Wyeth/Pfizer
- PAM shows close homology with antagonists / NAMs reported by Merck

- ⇒ Used in HTS with $Z=0.73 \pm 0.05$ (384 well-plate format)
- ⇒ WY-198517 resynthesized @ Domain
- ⇒ Truncated GFP-NPSR still binds NPS
- ⇒ WY compound detected at 10 μM & 30 μM

Binding signal on HEK GFP-GPR154 Nter truncated cells

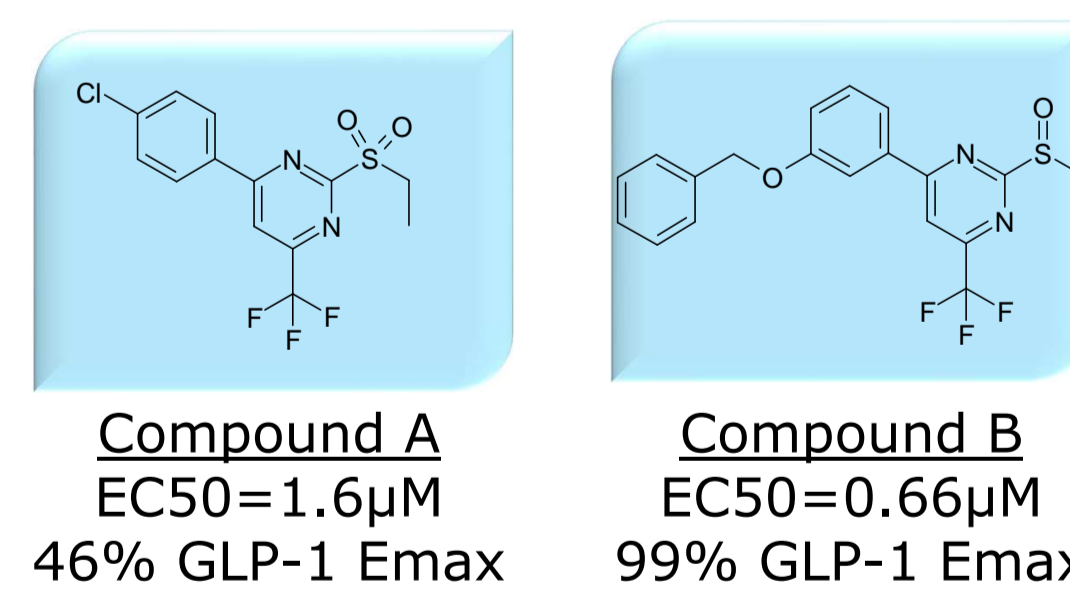


Functional test: HEK-293 cells stably co-expressing hNPSRA – measurement of calcium response with stimulation with a 0.5nM NPS. Leonard SK, 162.17, Annual meeting of the SFR, San Diego, CA, 2010 Melamed JV, BMCL, 2010

4. Case study I: Glucagon-like receptor 1 (GLP-1R)

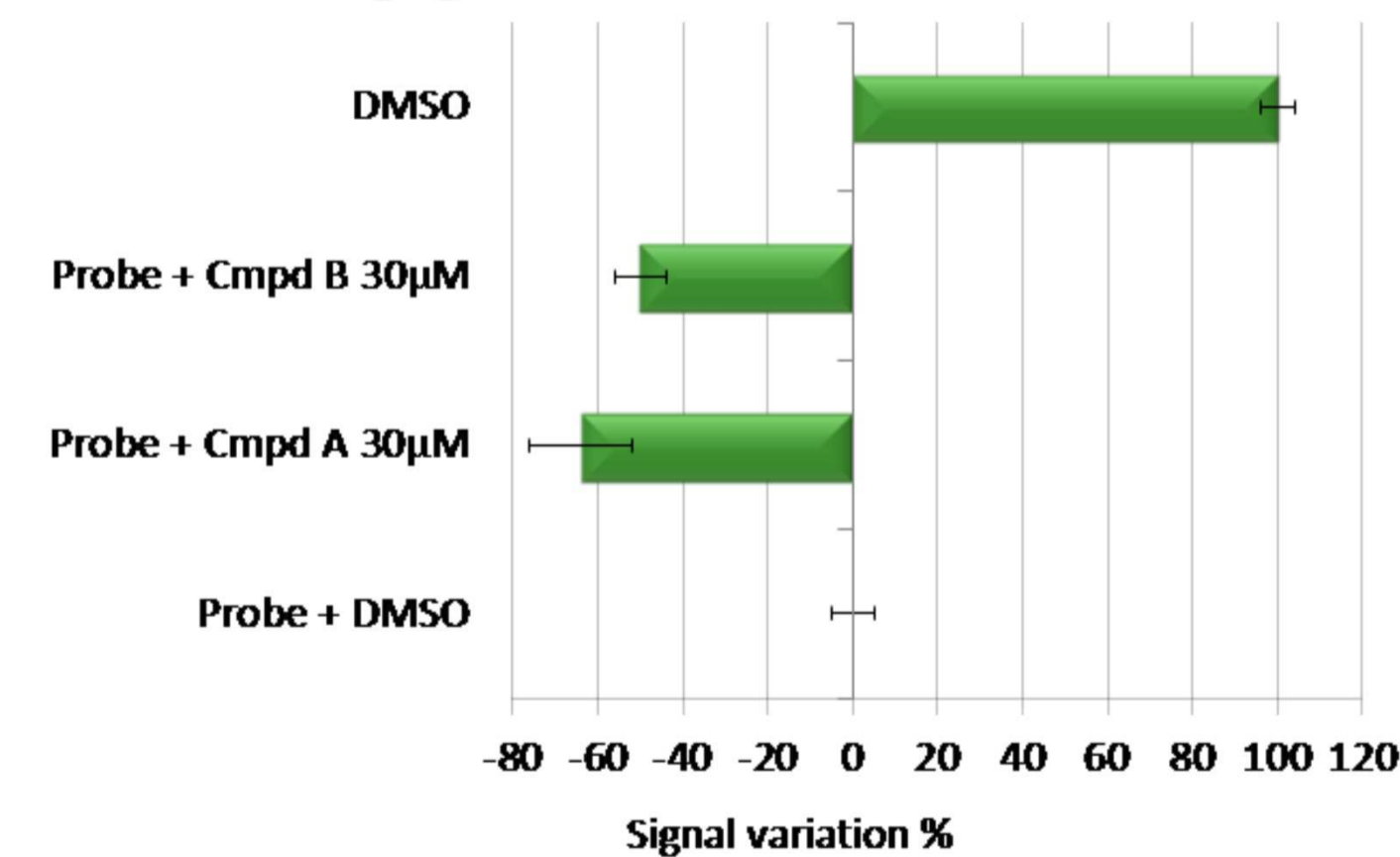
- Class B GPCR, target of peptide **anti-diabetic drugs** (*exenatide, liraglutide*)
- No applicable to oral dosing and associated with pancreatitis
- PAMs and / or ago-allosteric small molecules represent the **“holy grail”**
- GLP-1R turned to be a challenging target for modulator identification
- Very few small molecule modulators described
- Amongst them, pyrimidines described by Lilly in 2010 showed promising *in vitro* and *in vivo* activities as GLP-1R ago-allosterics

- ⇒ Assay developed using DTect-All™
- ⇒ 384 well-plate format
- ⇒ Used in HTS with $Z=0.67 \pm 0.05$
- ⇒ Truncated GFP-GLP-1R no longer binds GLP-1 peptide
- ⇒ A & B detected as FRET enhancers

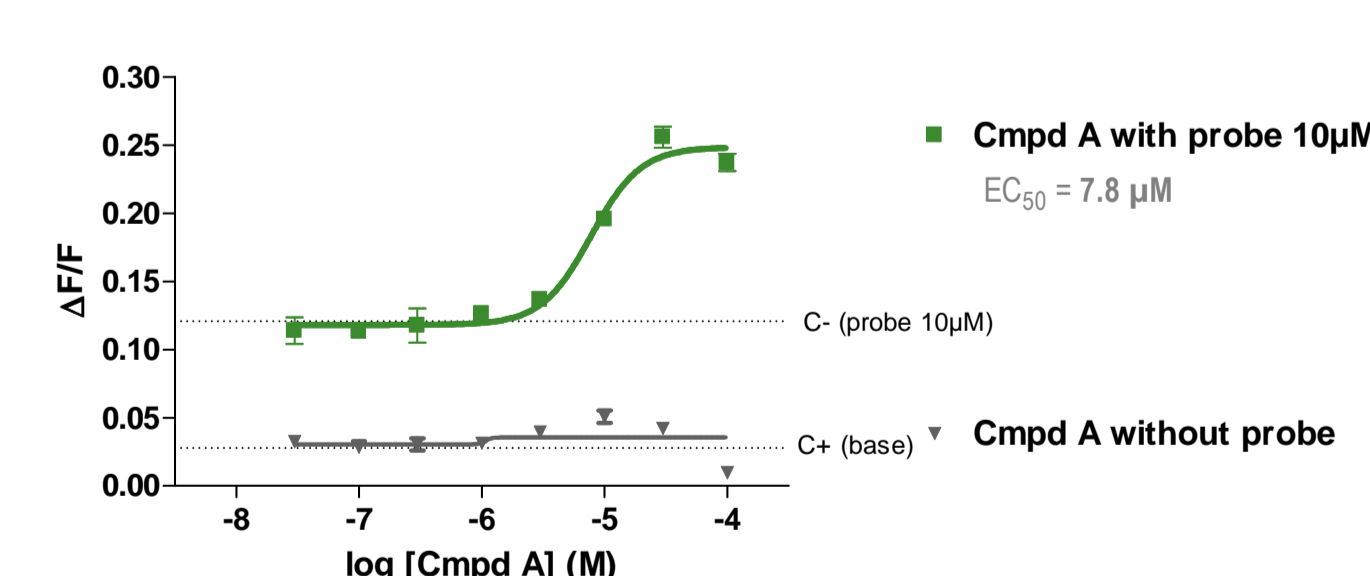


Functional test: HEK-293 cells stably co-expressing GLP-1R and a luciferase reporter system. Sloop KW, Diabetes, 2010

Binding signal on HEK GFP-GLP-1R Nter truncated cells



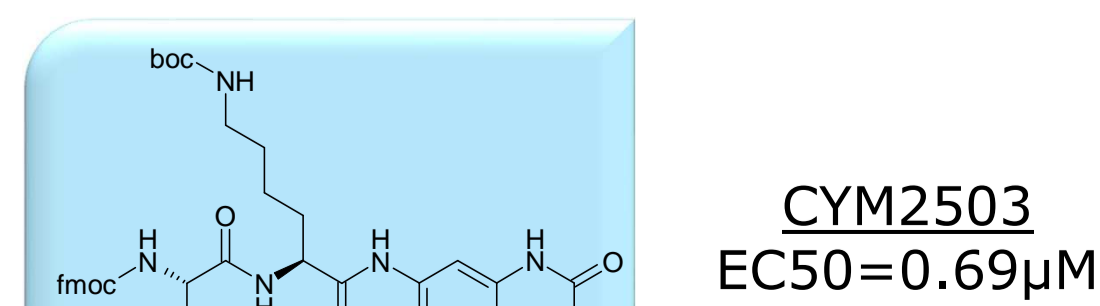
Dose dependent effect of Cmpd A



6. Case study III: Galanin receptor 2 (GalR2)

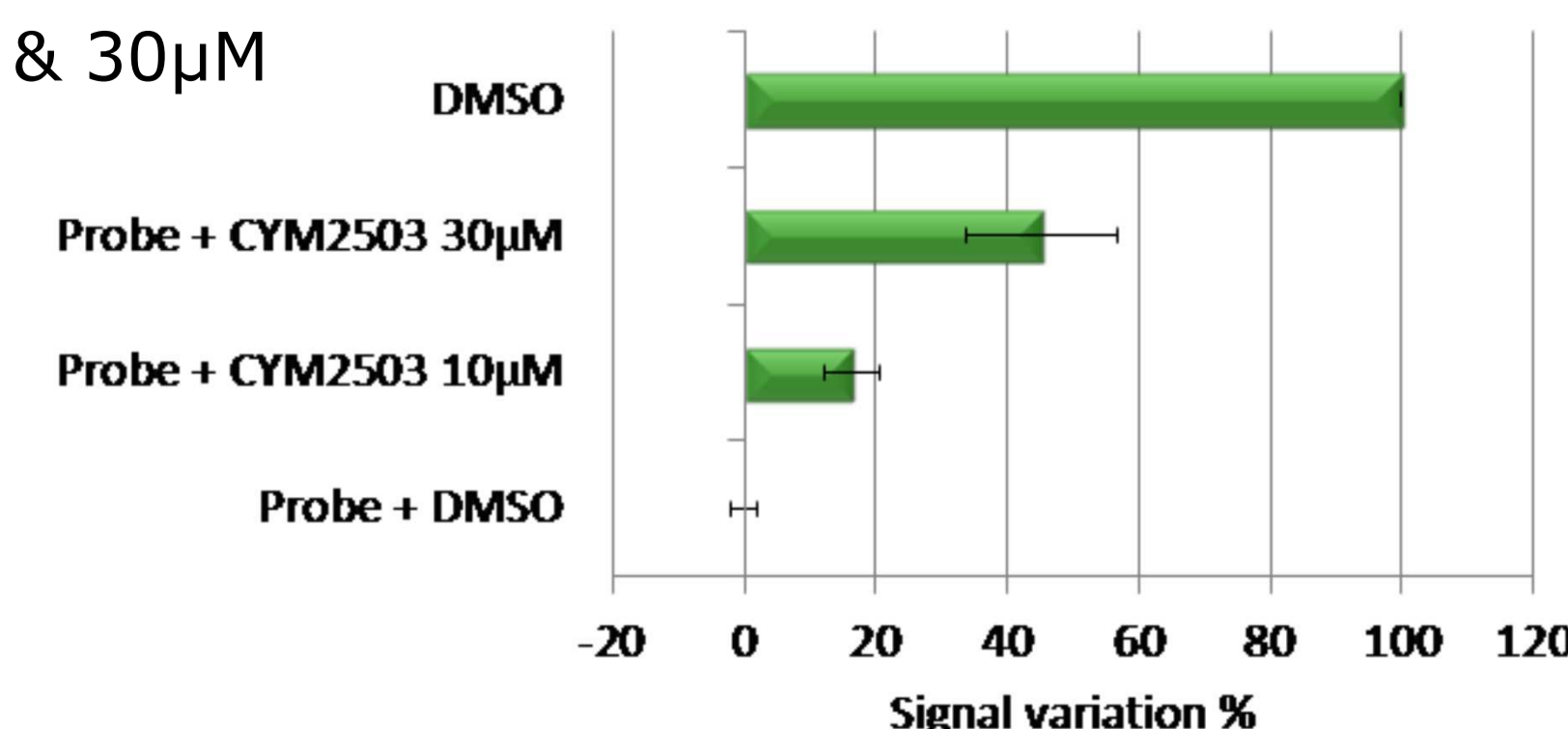
- Class A GPCR, receptor of the neuropeptide galanin
- Expressed in DRG and hippocampus, target for **chronic pain, AD & MS**
- One single small molecule reported as modulator: CYM2503 described as a PAM in 2010
- Peptide agonist showed promising activities *in vivo*

- ⇒ Good assay robustness: $Z=0.81 \pm 0.04$
- ⇒ CYM2503 resynthesized @ Domain
- ⇒ CYM2503 detected at both 10 μM & 30 μM



Functional test: HEK-293 cells stably expressing rat GalR2 – measurement of IP1 production with stimulation with 100nM rat galanin. Lu X, PNAS, 2010

Binding signal on HEK GFP-GalR2 Nter truncated cells



CONCLUSION

Although **PAMs** or **ago-allosterics** constitute a highly promising alternative for **peptide GPCR** modulation, they are still considered as **intractable ligands** as most of the currently screening technologies failed in their identification. At Domain Therapeutics, novel small molecules recently described as PAMs or ago-allosterics of **NPSR**, **GLP-1R** or **GalR2** were identified using corresponding assay developed with the FRET-based binding technology called DTect-All™. These results further validate the strong potential of DTect-All™ for the identification of novel validated hits for challenging GPCRs.