GPCR Allosteric Modulator Discovery: Silence is Golden

Stephan Schann, Head of Research

3rd RSC/SCI Symposium on GPCRs in Medicinal Chemistry
September 20-22, 2010 – MSD, Oss, The Nederlands

Domain Therapeutics Snapshot

The company

- Based in Strasbourg, France
- Drug discovery and early development

Unique Focus

- GPCRs

Key competitive advantage

- DTect-All™: unique, proprietary GPCR technology

Opportunities

- Challenging GPCRs
- Allosteric Modulators

Business model

- Give access to DTect-All™ to Pharma/Biotech
- Generate partnerships on patented NCEs

Pipeline

Optimized leads

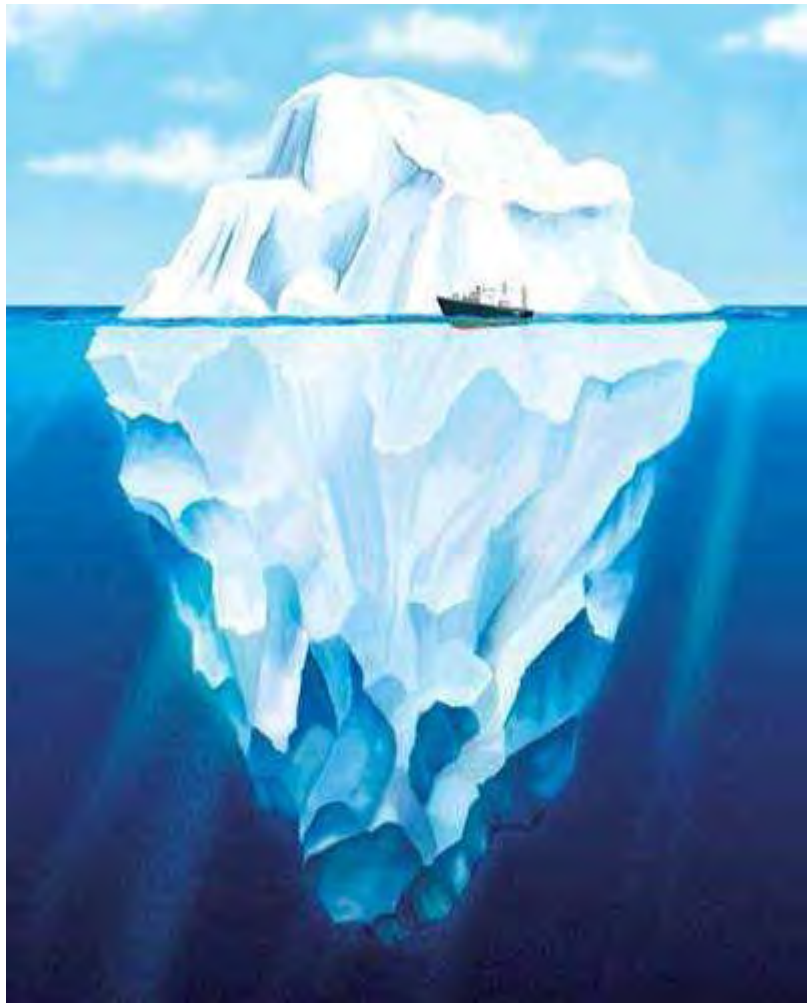
- ✓ ***Nanomolar activity***
- ✓ ***Selectivity (subtype and profiling)***
- ✓ ***Peripheral activity in animal models***
- ✓ ***Clean early ADME-Tox package***
- ✓ ***Composition of matter patent filed***

Project	Hit to Lead	Lead to Optimized Lead	Origin of compound	Indications
DT1133	A2A antagonist		NCE / DTect-All™	Parkinson's disease
DT0738	A3 antagonist		NCE / DTect-All™	Glaucoma
DT1687	mGluR4 PAM		NCE Prestwick Chemical	Parkinson's disease Schizophrenia
DT1876	mGluR2 NAM		NCE / DTect-All™	Alzheimer's disease Depression
GLP1R PAMs			NCE / DTect-All™	Diabetes

Many GPCRs are unexplored

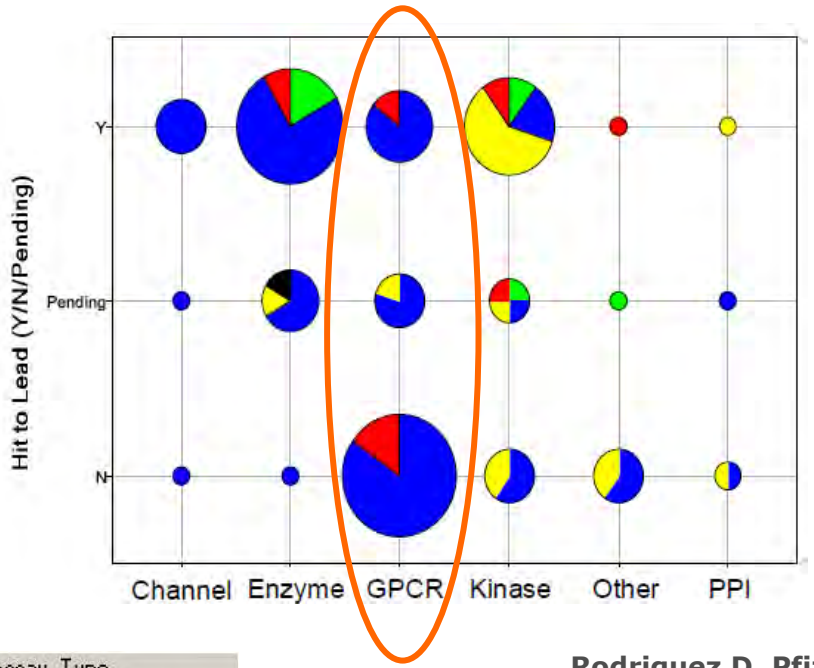
- 50% of current marketed drugs
- >$30B in sales annually
- GPCR-drugs target only 50 GPCRs

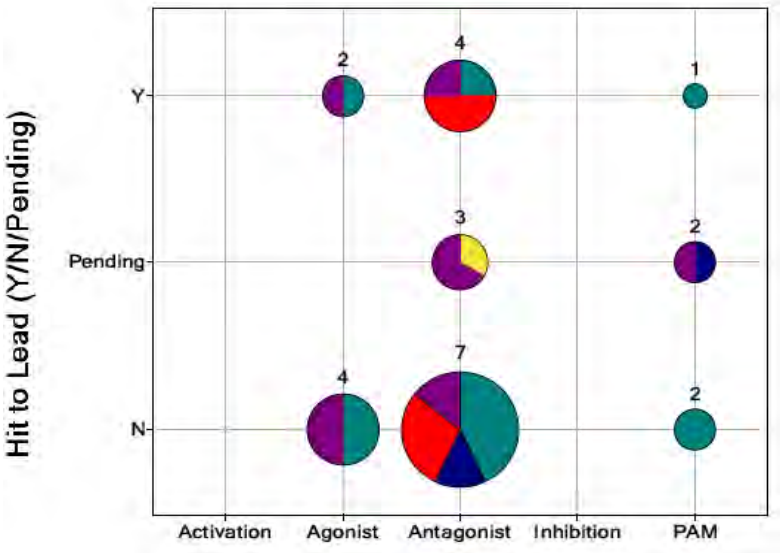
- ~350 therapeutic GPCRs
- ~150 are orphans
- ~150 challenging (selectivity/chemistry)

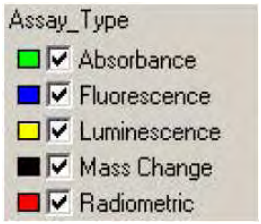


Low success rate of finding GPCR tractable hits

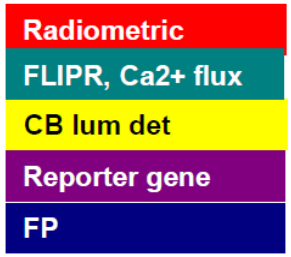
Retrospective analysis of 25 GPCR screening campaigns performed at Pfizer





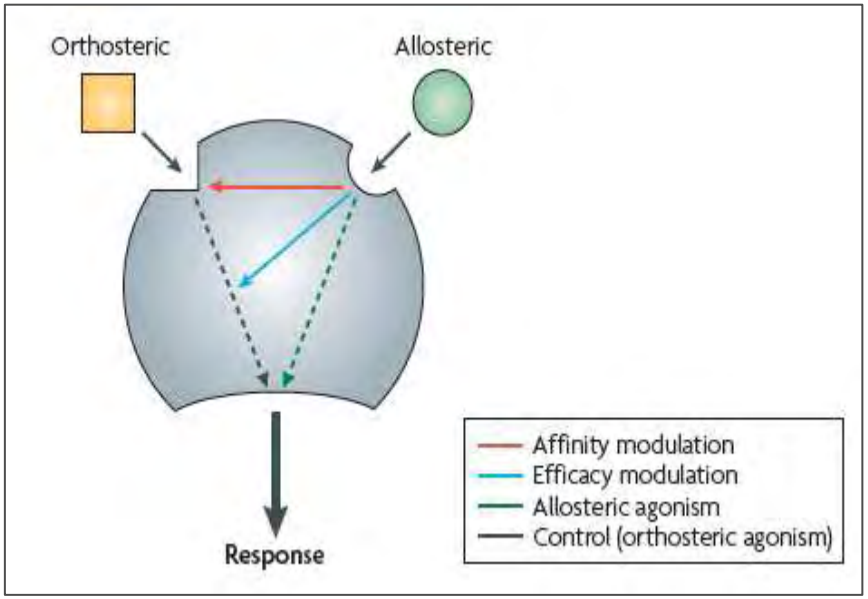


Rodriguez D, Pfizer, poster presentation, ELRIG meeting 2009



DOMAIN THERAPEUTICS

GPCR allosteric modulators



Conn PJ et al, Nat Rev Drug Disco 2009, 41-54.

- Positive Allosteric Modulators (PAMs)
  - Affinity / efficacy / both
- Negative Allosteric Modulators (NAMs)
  - Full / "partial antagonism"
- Ago-allosteric Modulators
- Silent Allosteric Modulators (SAMs)

GPCR allosteric modulators

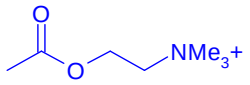
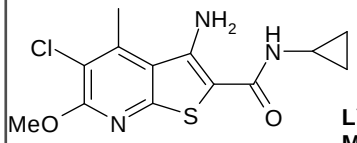
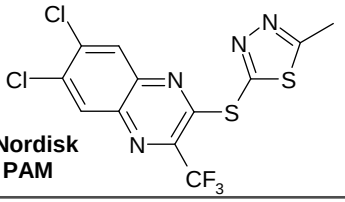
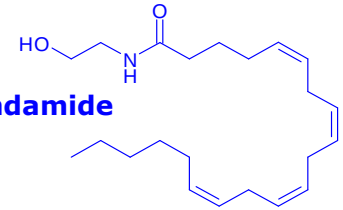
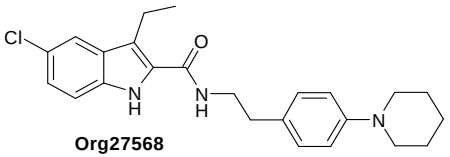
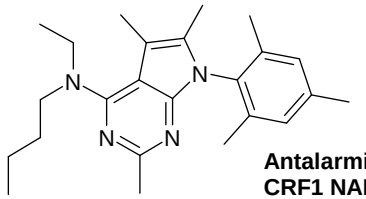
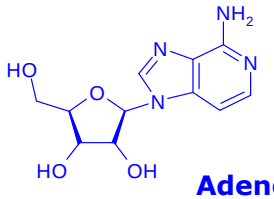
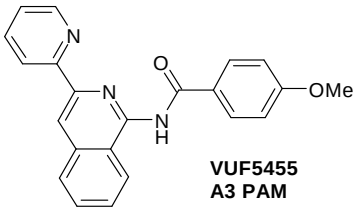
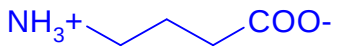
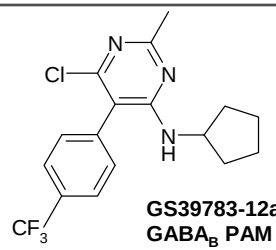
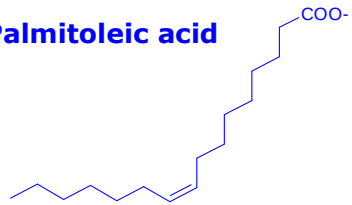
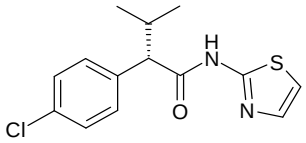
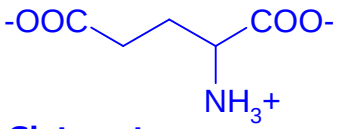
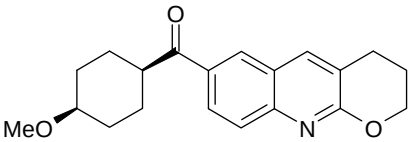
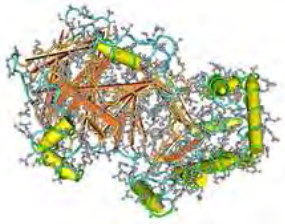
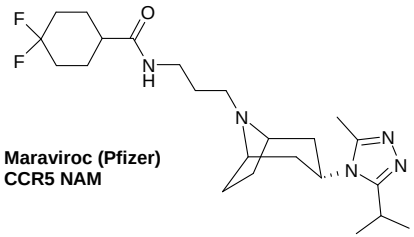
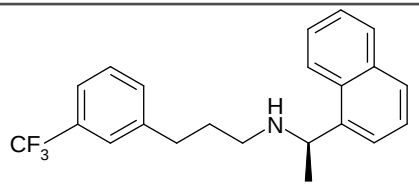
1. Better selectivity of action
2. Prevent the development of tolerance
3. More druggable chemistry
4. Higher subtype selectivity profile

Important features for drug discovery

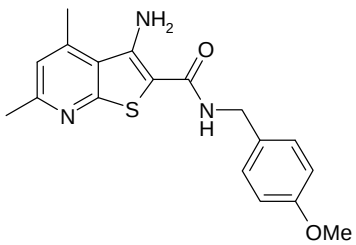
=> 2 products on the market

- ✓ Maraviroc (Pfizer)
- ✓ Cinacalcet (Amgen)

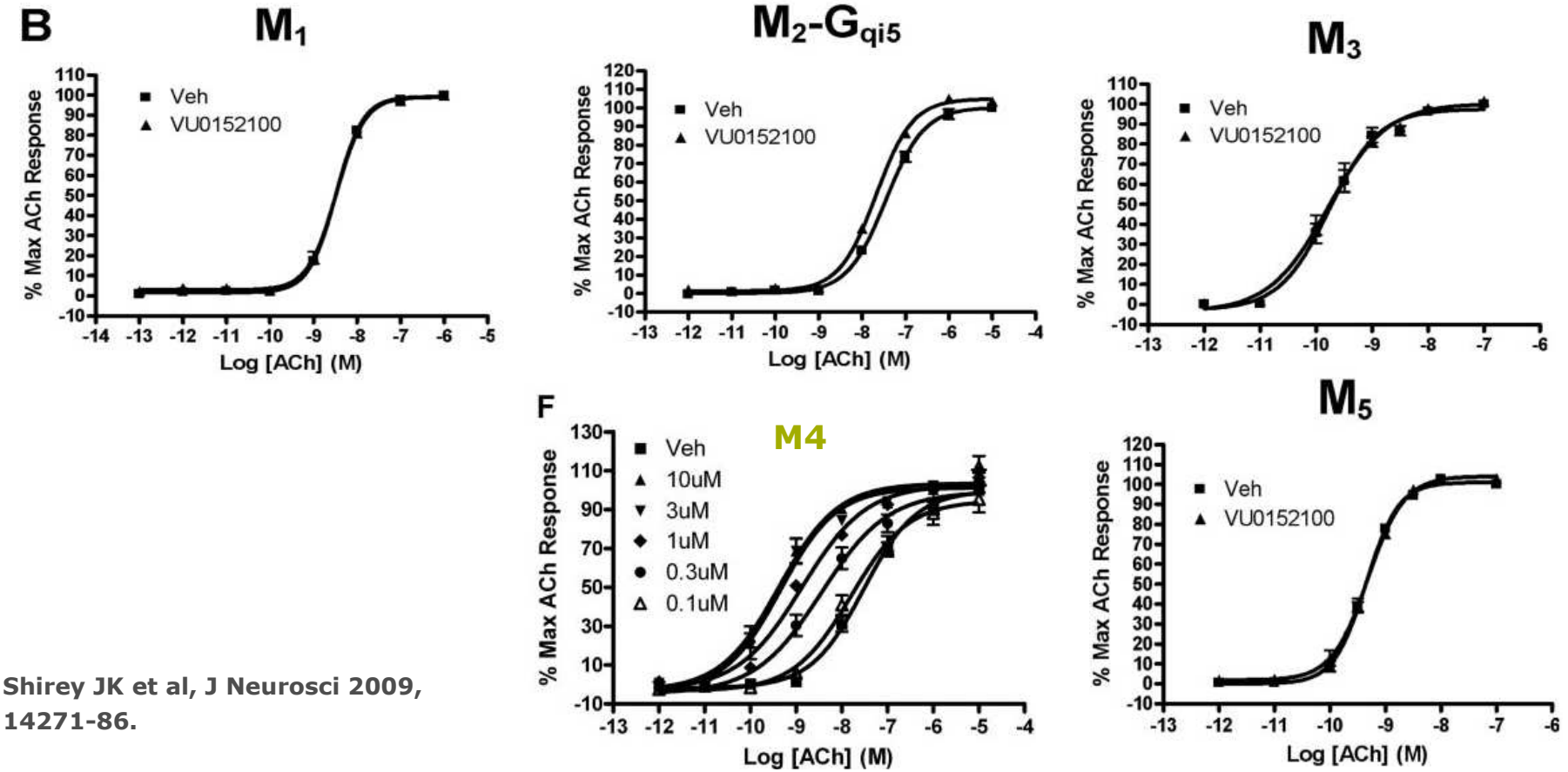
More druggable chemistry

 **Acetylcholine**	 **LY2033928** **M4 PAM**	 **Glucagon-like peptide**	 **NovoNordisk** **GLP1 PAM**
 **Anandamide**	 **Org27568** **CB1 P/NAM**	 **Corticotropin-releasing factor**	 **Antalarmin** **CRF1 NAM**
 **Adenosine**	 **VUF5455** **A3 PAM**	 **GABA**	 **GS39783-12a** **$GABA_B$ PAM**
 **Palmitoleic acid**	 **Amgen 1** **GPR40 Ago Allo**	 **Glutamate**	 **JNJ 162596685** **mGluR1 NAM**
 **RANTES**	 **Maraviroc (Pfizer)** **CCR5 NAM**	**Ca^{2+}**	 **Cinacalcet (Amgen)** **Calcium-sensing receptor PAM**

Higher subtype selectivity profile

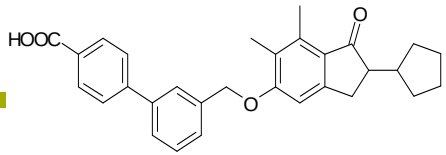


- Exemplify for several GPCRs:
  - ✓ Class A: muscarinic M4 VU0152100 :



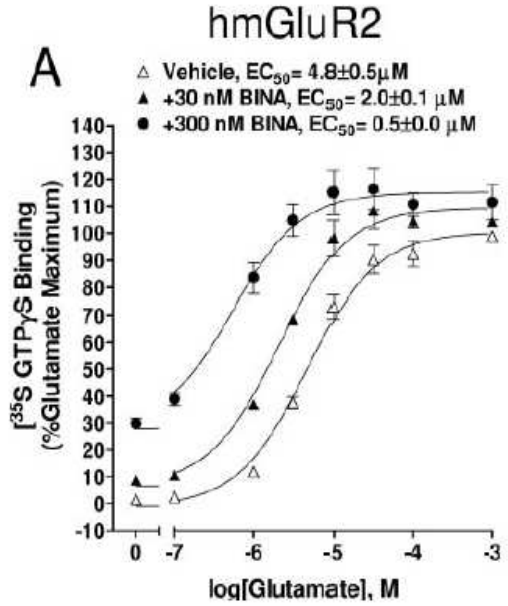
Shirey JK et al, J Neurosci 2009, 14271-86.

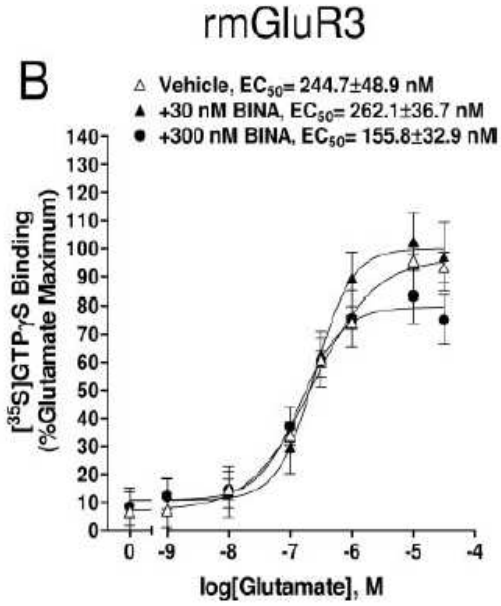
Higher subtype selectivity profile

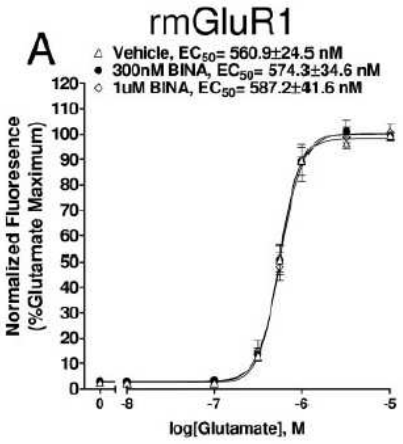


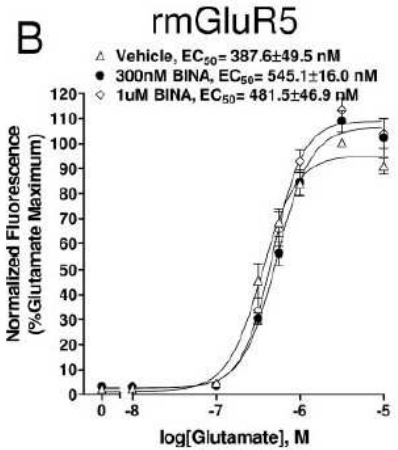
- Exemplify for several GPCRs:
  - ✓ Class C: mGluR2 BINA :

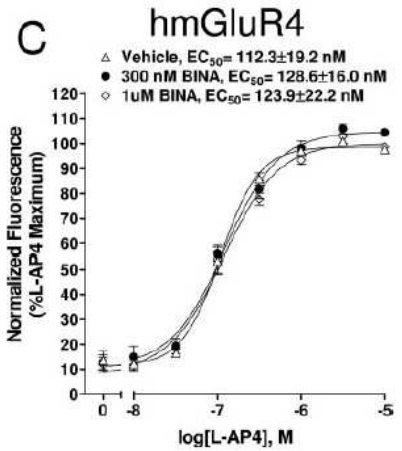
Galici R et al, JPET 2006, 173-85.







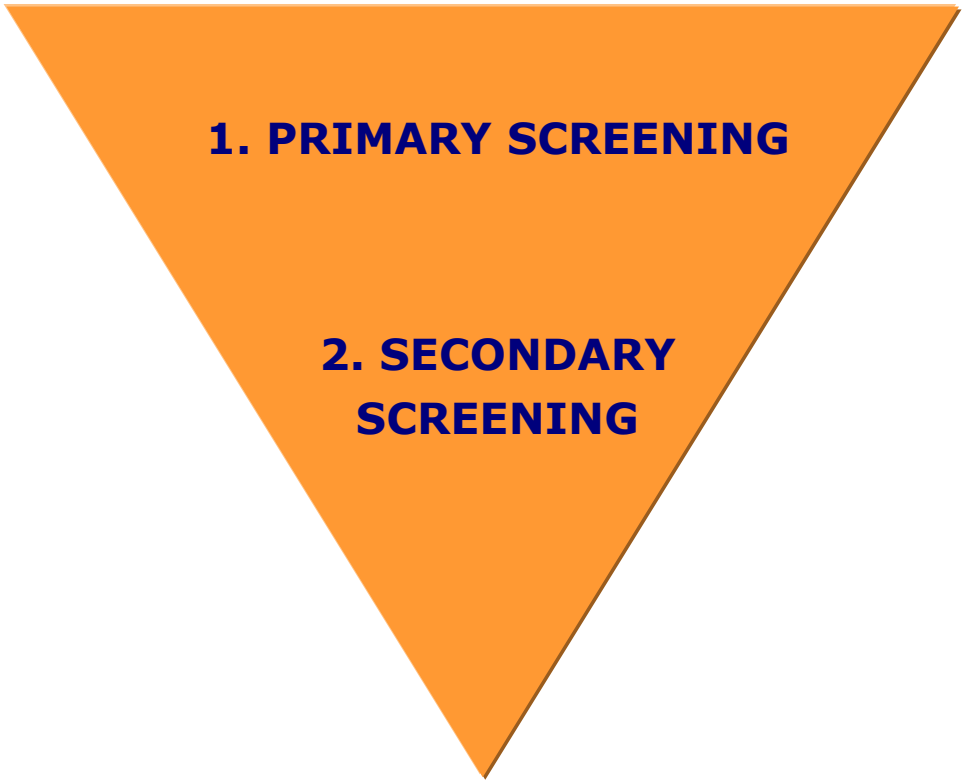




DOMAIN THERAPEUTICS

3rd

GPCR screening

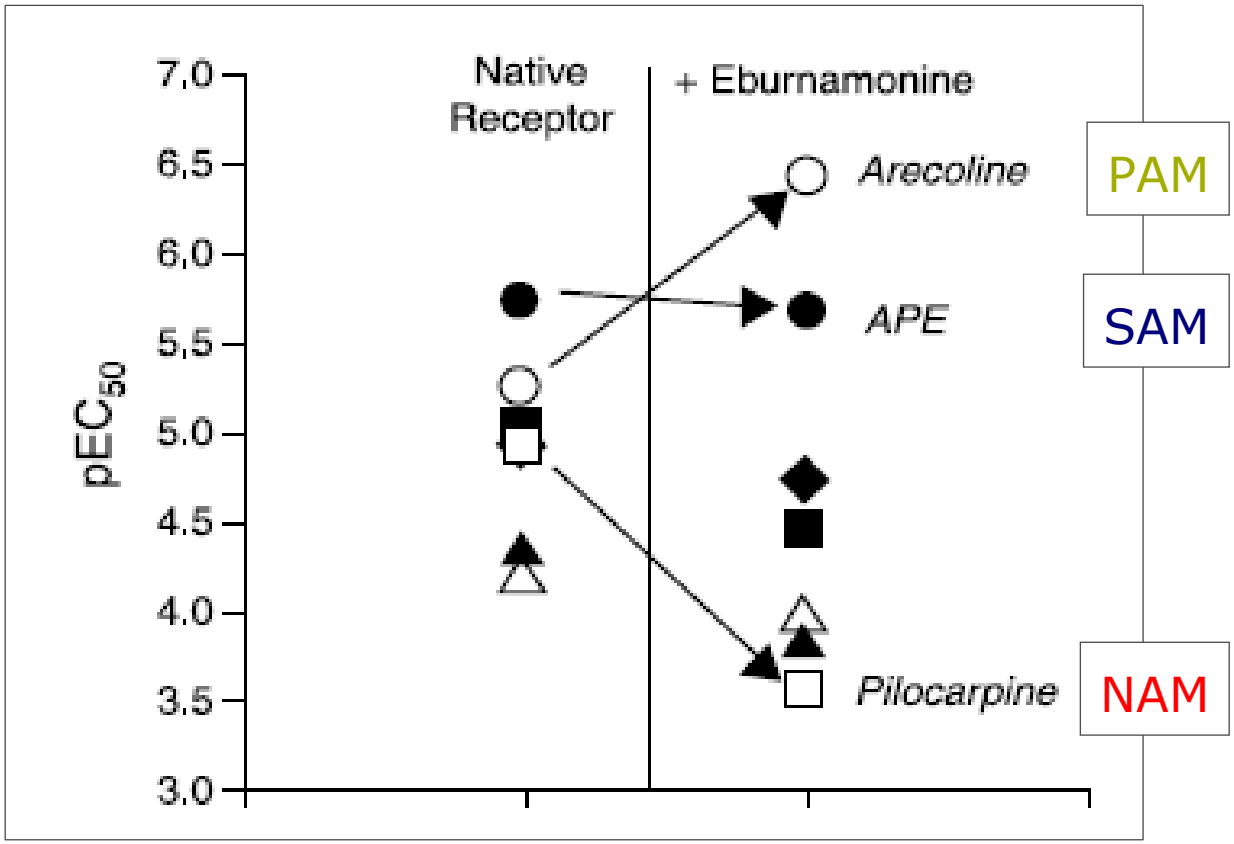


PRIMARY SCREENING
Single functional assay with specific conditions

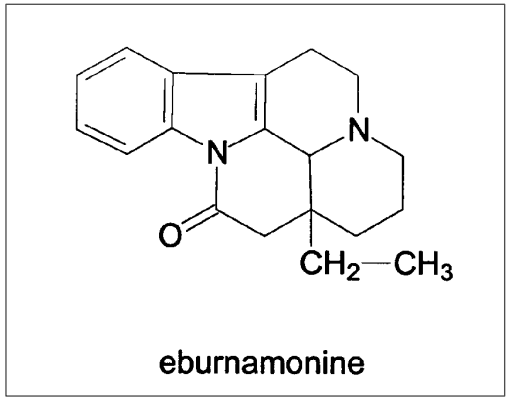
SECONDARY SCREENING
Confirmation, dose-response, selectivity…

Tractable hits for < 50% screened GPCRs !!

Probe dependency

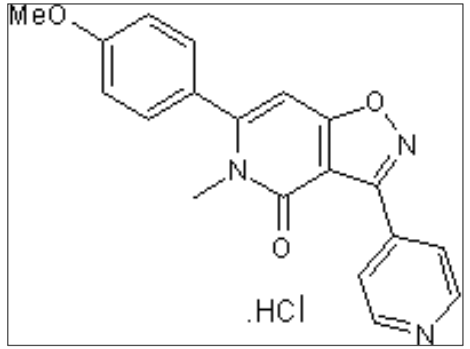


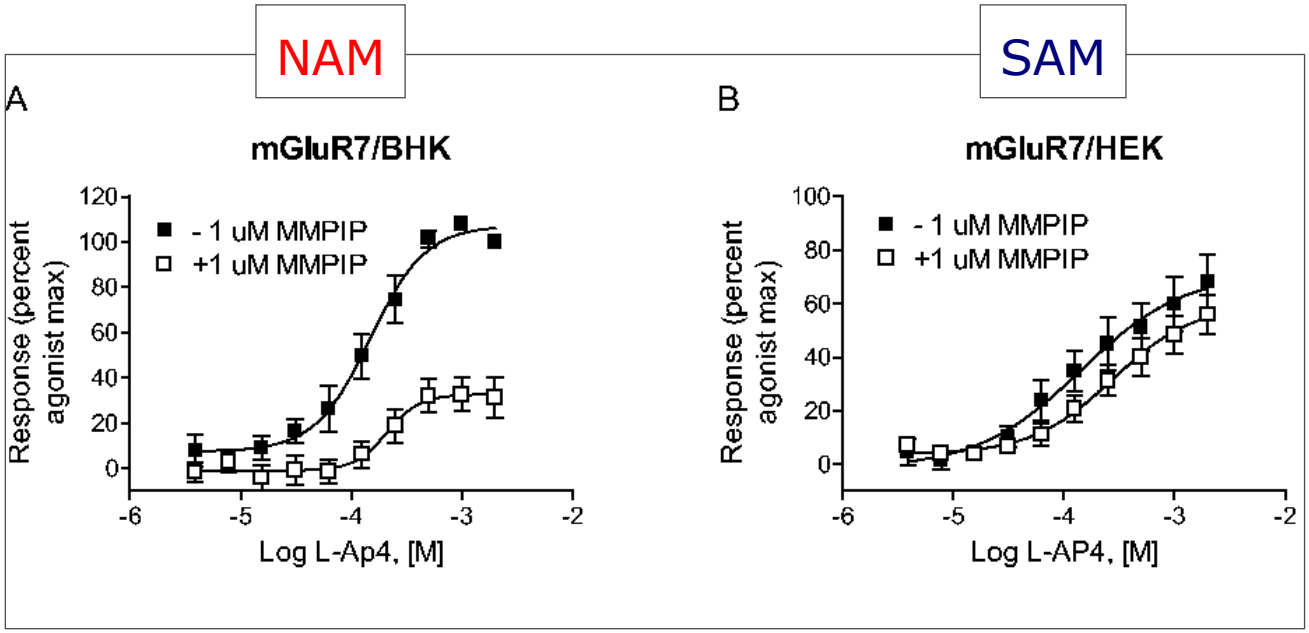
Kenakin T, Mol Interventions, 2004



eburnamonine

Cell system dependency

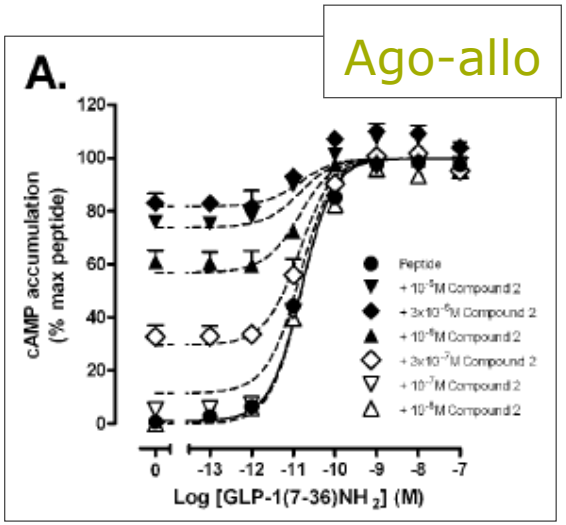




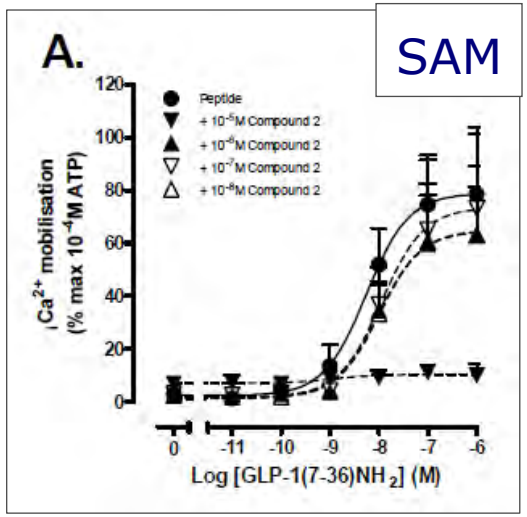
Niswender C et al, Mol Pharm, 2010

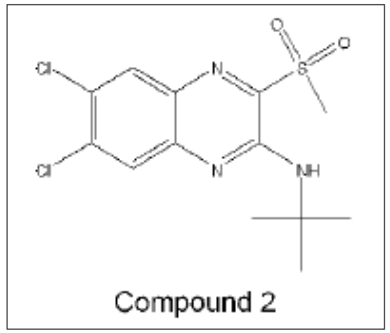
Stimulus dependency

Ago-allo



SAM

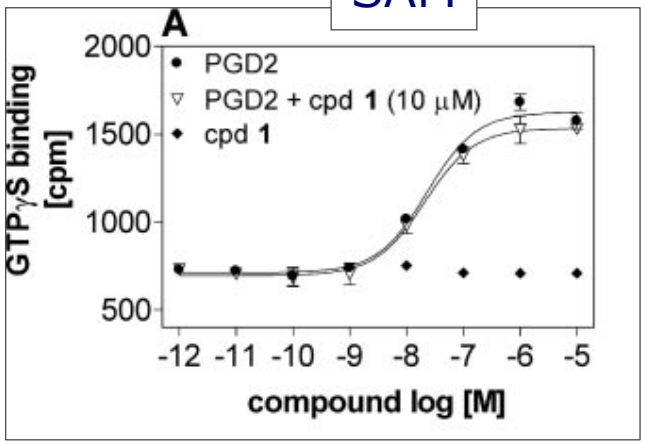




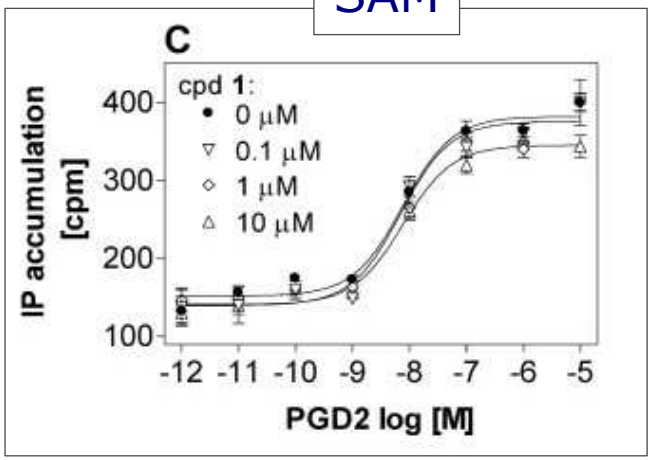
Koole C et al, Mol Pharm, 2010

Stimulus dependency

SAM



SAM



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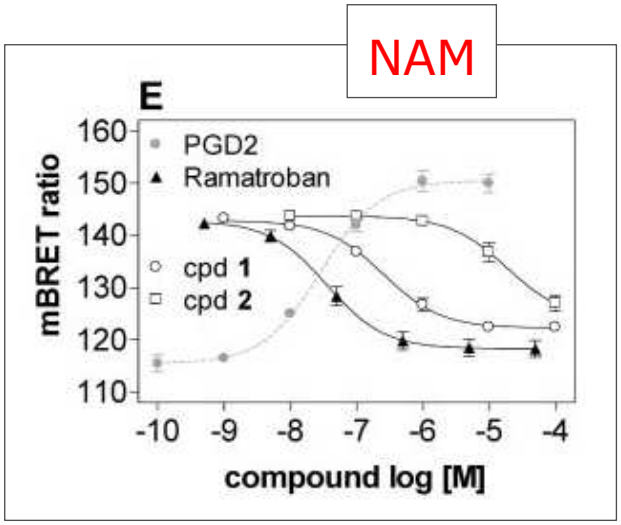
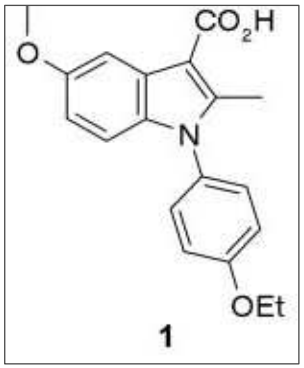


CRTH-2 receptor

A: GTPγS binding

C: IP accumulation

E: beta-arrestin recruitment



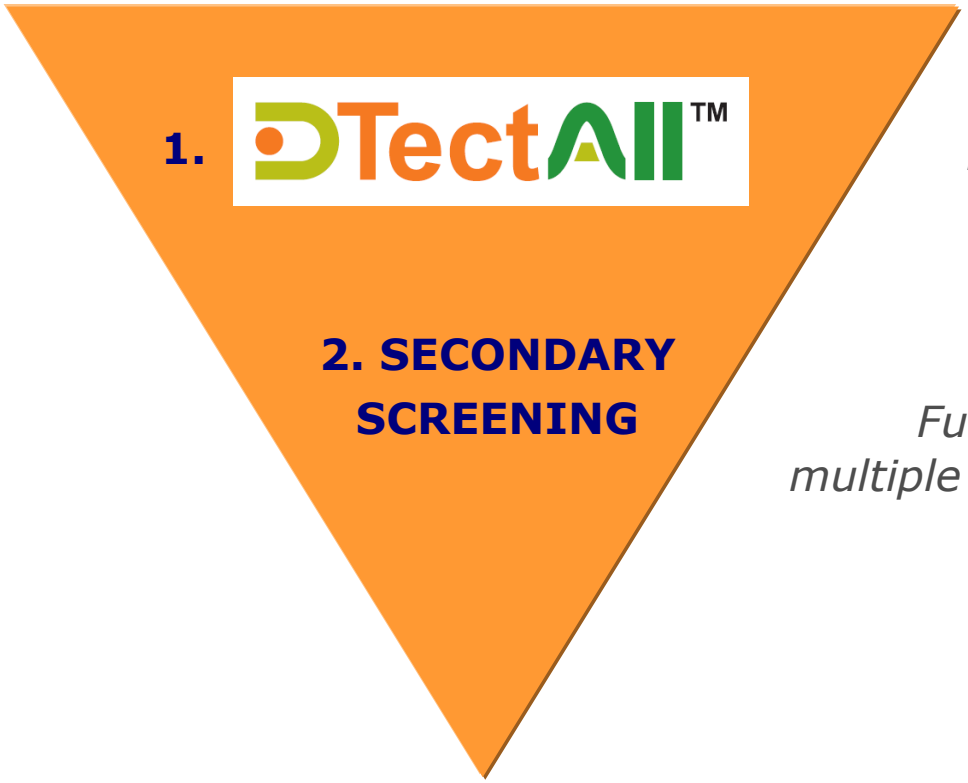
Mathiesen JM et al, Mol Pharm, 2005

Identification of GPCR Allosteric Modulators

As is often the case with new concepts applied to existing technologies and approaches, significant challenges remain. Given the multitude of effects that allosteric modulators can exert, how can we screen for such compounds without missing important pharmacological properties? This is particularly pertinent because, in many cases, we do not know which functional assay is predictive of the desired (patho)physiological endpoint. Although a change in high-throughput-screening methods from binding-based to functional assays within the pharmaceutical industry has enabled better detection of compounds with allosteric properties, a single functional endpoint is still often used to characterize these compounds. If stimulus trafficking and collateral efficacy, whether inherent or allosterically engendered, are likely common pharmacological behaviours, the use of a single screen will undersample the chemical space surrounding potential therapeutic candidates for a chosen GPCR target. Thus, screening needs to be as broad as is feasible.

Leach K, TiPS, 2007

Identification of GPCR AM at Domain



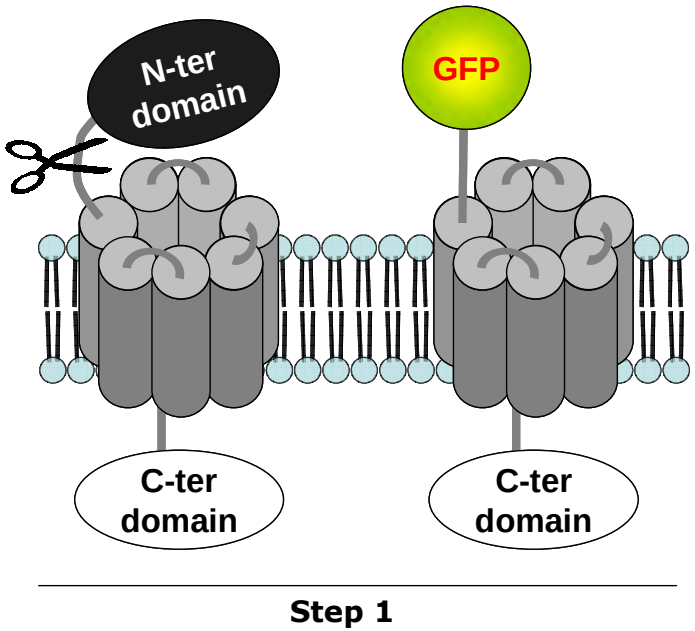
PRIMARY SCREENING
For 7-TM binder identification

SECONDARY SCREENING
Functional characterization with multiple functional tests, selectivity …

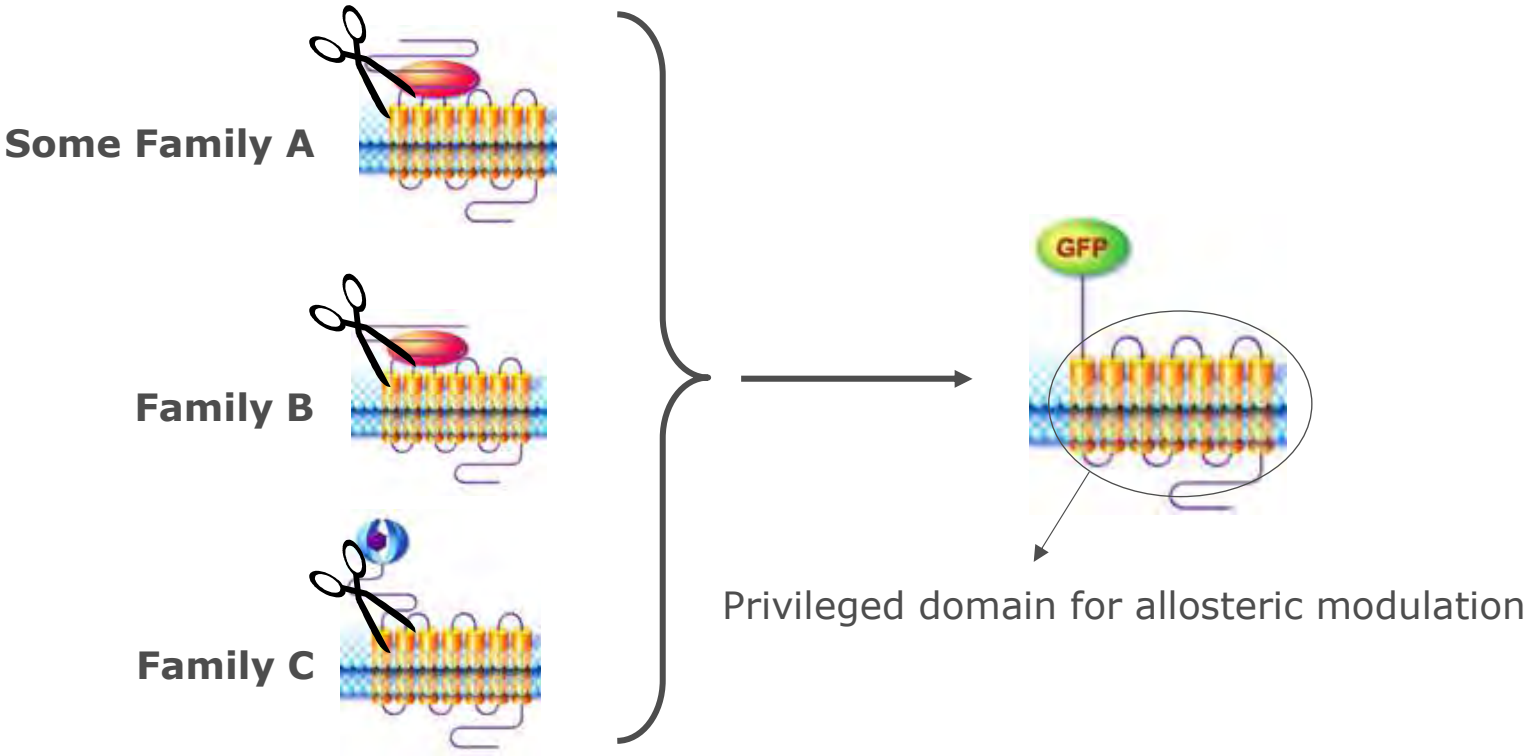
Higher tractable hit rate


DTectAll™

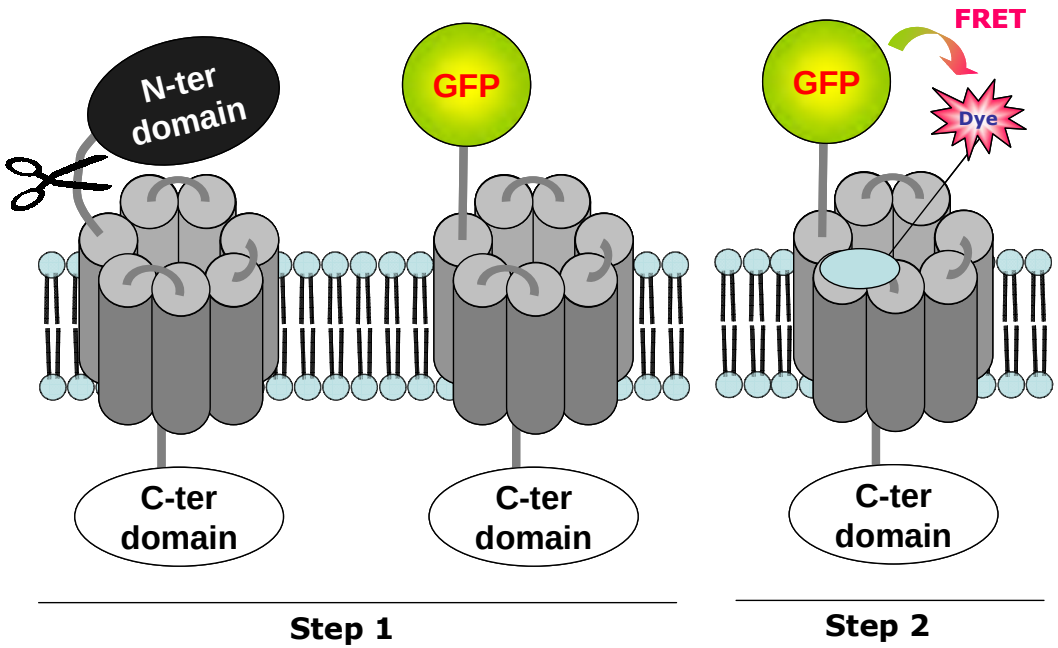
DTectAll™ Step 1



DTectAll™ Step 1

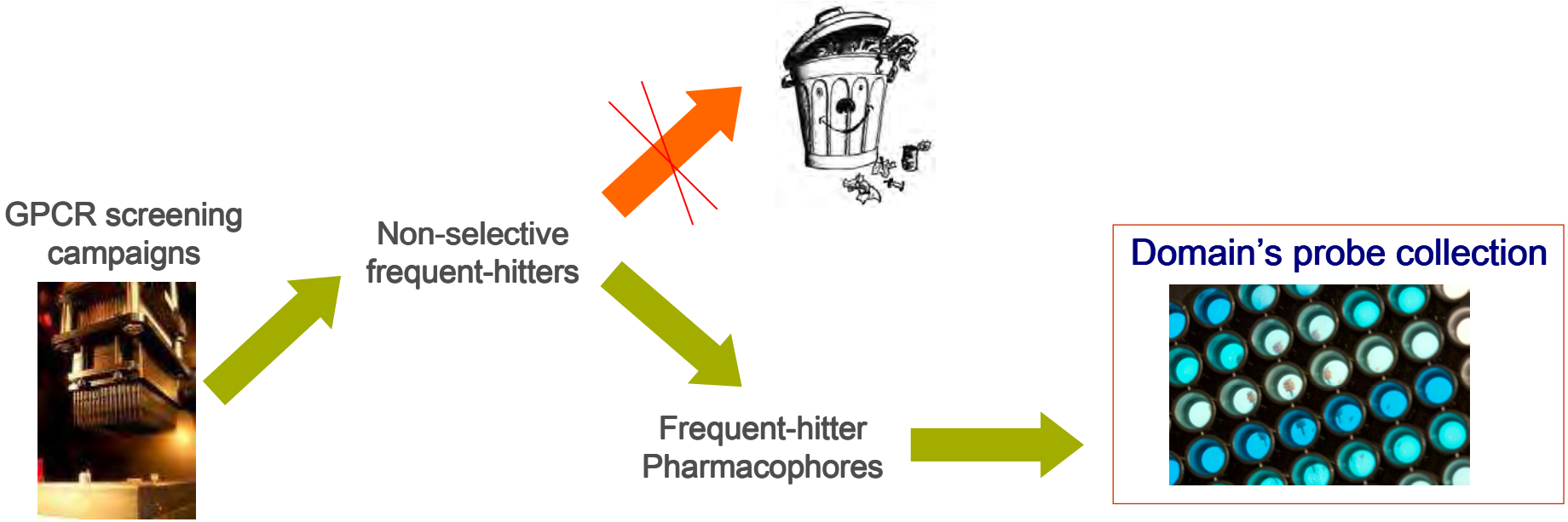


DTectAll™ Step 2

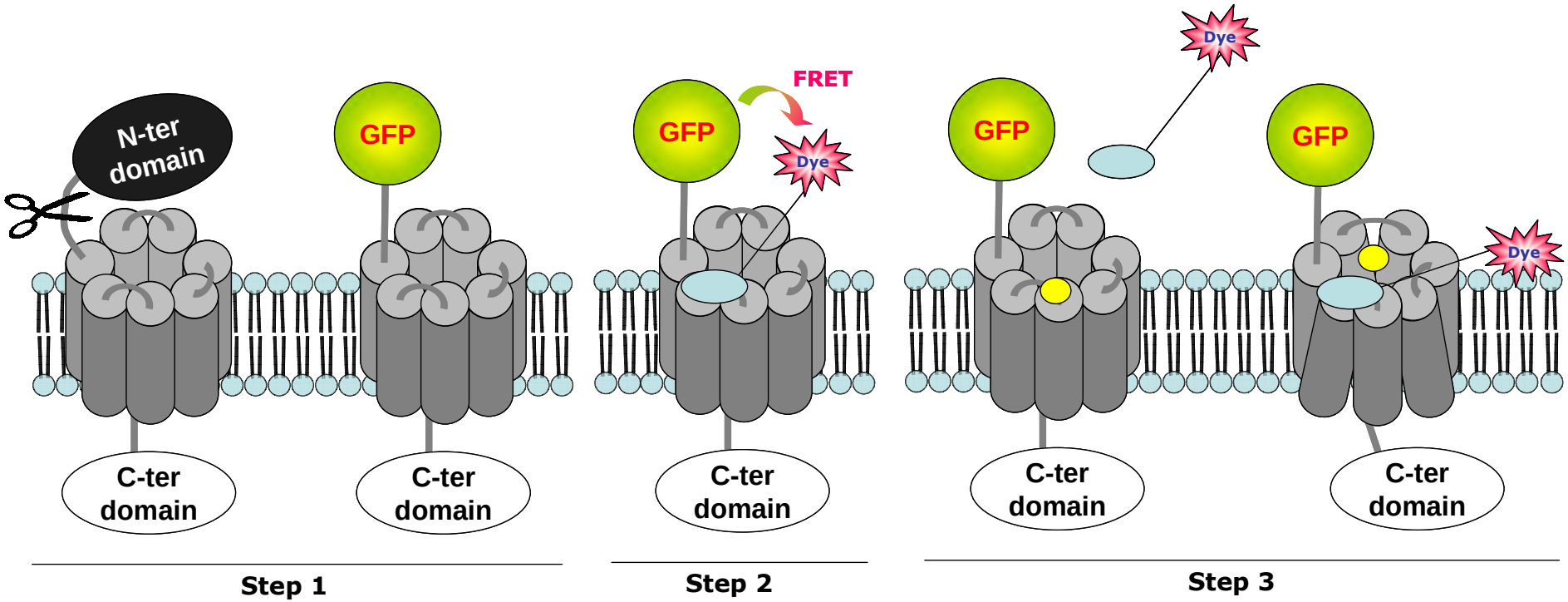


Competitive advantage of DTectAll™

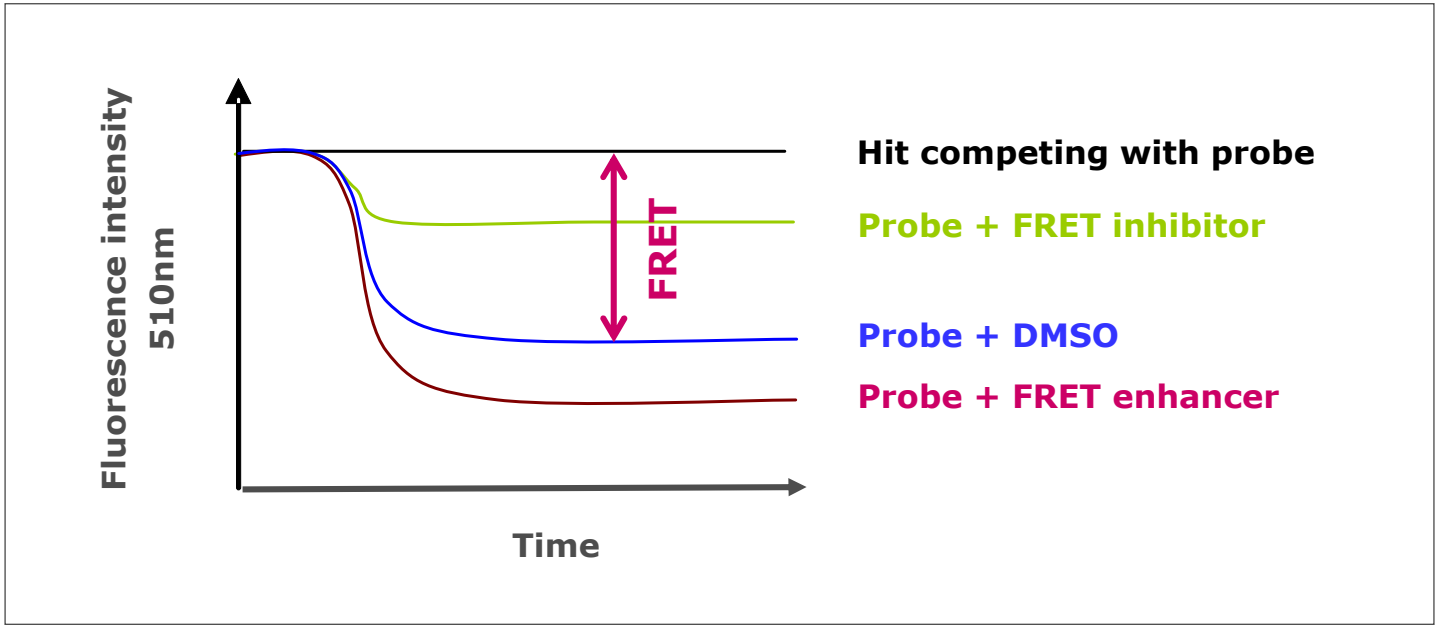
- Unique collection of more than 5000 **non-selective** fluorescent probes
- Patented libraries with WW exclusivity to Domain
- Designed from **GPCR frequent-hitters** and continuously enriched
- Validated for classes A, B, C GPCRs and for **orphans**



DTectAll™ Step 3

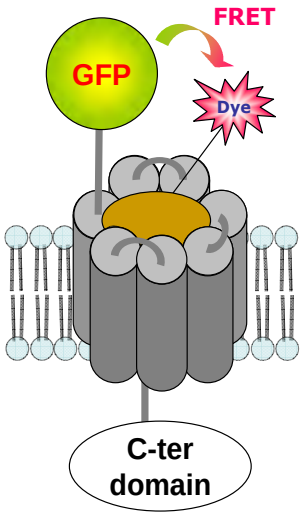


DTectAll™ Step 3

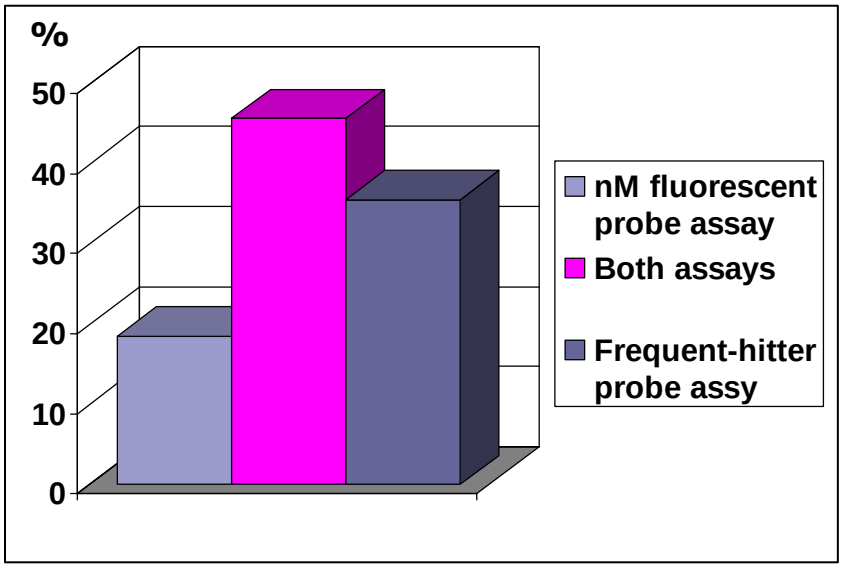


Competitive advantage of DTectAll™

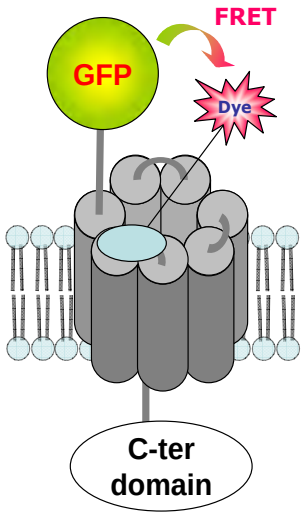
- Screening with frequent-hitter probe leads to higher sensitivity:



nM fluorescent probe assay

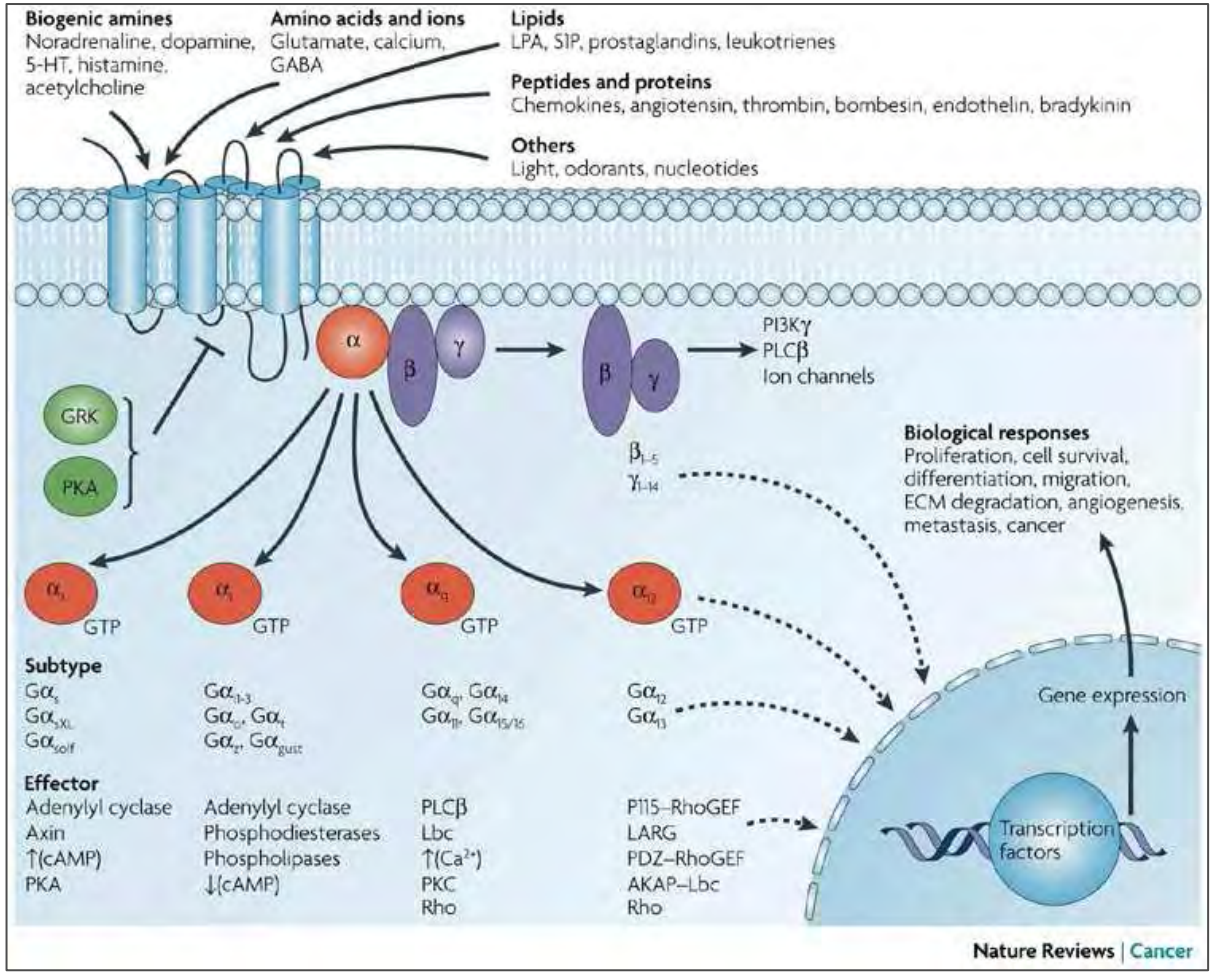


** GPCRs were screened against a 5000 compound library. Values in y-axis correspond to percentage of identified hits. 100% corresponds to total number of hits identified*



Frequent-hitter fluorescent probe assay

DTectAll™ Step 4



Agonist

Antagonist

PAM

NAM

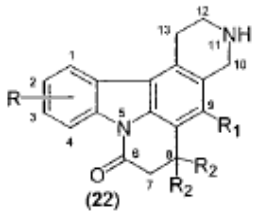
Silent binder

Dorsam RT and Gutkind JS, Nat Rev Cancer, 2007

SAMs to PAMs/NAMs on Class A (literature)

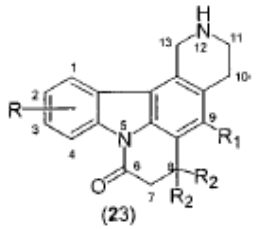
- Slight modifications are switching SAMs into PAMs/NAMs
- Same modification is switching SAMs into PAMs/NAMs for different subtypes

compd	R	R_1	R_2	M_1	M_2	M_3	M_4
22a	H	H	H	+	+	–	+
22b	1-OMe	H	H	–	+	0	0
22c	1-OH	H	H	++	++	0	++
22d	1-Cl	H	H	+	++	0	+
22e	1-COOMe	H	H	–	+	–	–
22f	2-Me	H	H	–	+	–	+
22g	2-OMe	H	H	–	+	–	0
22h	2-OH	H	H	++	++	++	++
22i	2-Cl	H	H	0	++	0	++
22j	2-COOH	H	H	–	++	–	++
22k	2-COOMe	H	H	– –	0	–	–
22l	2-CON$(CH_2CH_2)_2O$	H	H	– –	++	– –	–
22m	2-$CH_2N(CH_2CH_2)_2O$	H	H	–	+	–	0
22n	3-OMe	H	H	+	+	–	+
22o	3-OH	H	H	–	+	0	+
22p	3-Cl	H	H	+	+	0	++
22q	3-COOMe	H	H	–	–	–	–
22r	3-CN	H	H	–	–	–	0
22s	4-OMe	H	H	–	+	–	+
22t	4-OH	H	H	–	+	–	–
22u	2,3-$(OMe)_2$	H	H				
22v	2,3-OCH_2O	H	H	–	+	0	+
22w	1,2,3-$(OMe)_3$	H	H	–	+	–	–
22x	2,3-$(OMe)_2$	COOMe	H	–	0	–	+
22y	2,3-$(OMe)_2$	CH_2OCOMe	H	–	+	–	+
22z	1-$(OMe)_2$	H	Me	0	++	0	++
22aa	1-OH	H	Me	–	+	–	0
23a	H	H	H	0	+	–	+
23b	2-OMe	H	H	–	+	–	+
23c	2-OH	H	H	–	+	–	0
23d	2-COOMe	H	H	0	+	0	+
23e	3-OMe	H	H	–	–	–	0
23f	3-OH	H	H	0	+	–	+
23g	2,3-$(OMe)_2$	H	H	–	0	–	0
23h	H	H	Me	+	++	+	++
23i	2,3-$(OMe)_2$	H	Me	+	+	–	+
				–	–	–	–



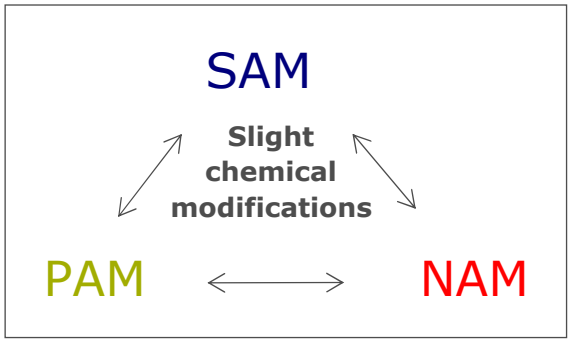
(22)

and



(23)

+ PAM
0 SAM
- NAM



Gharagozloo P et al, J Med Chem, 2002

SAMs to PAMs/NAMs on Class A (literature)

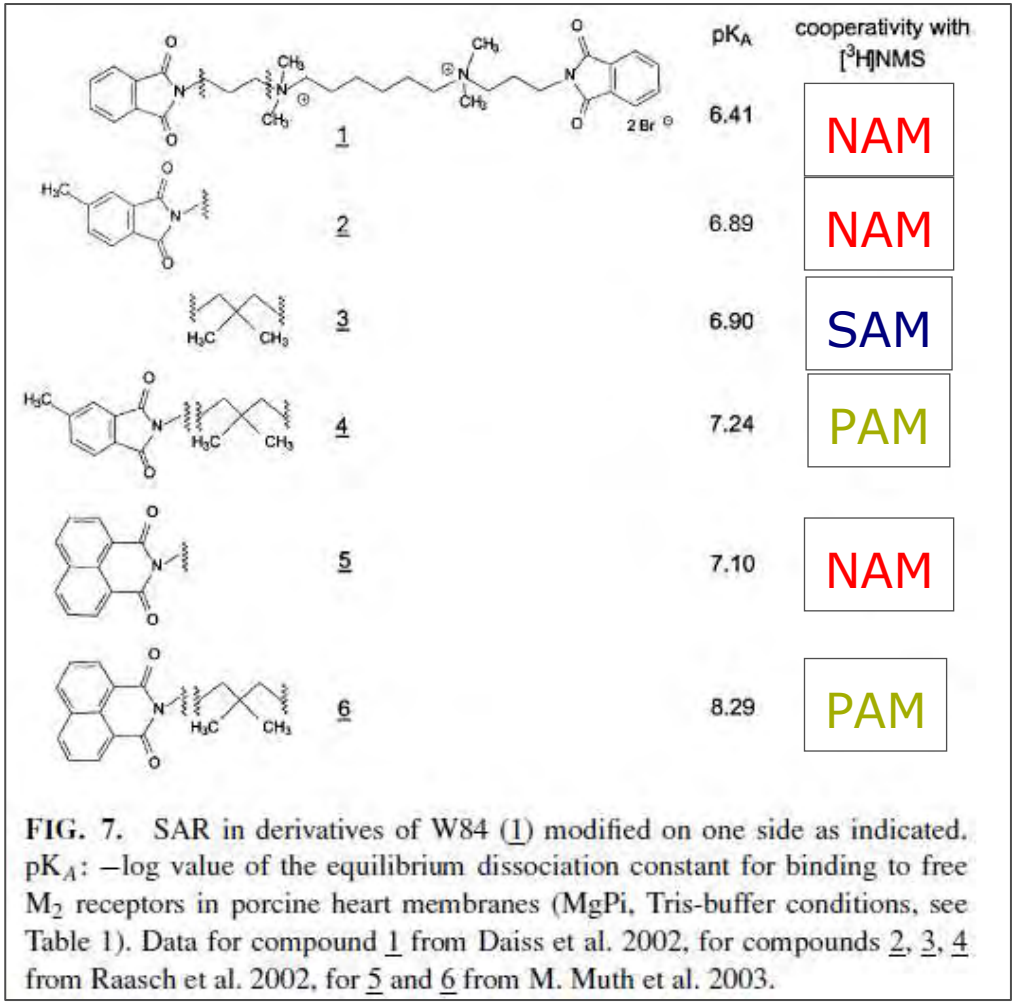
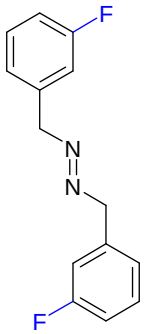
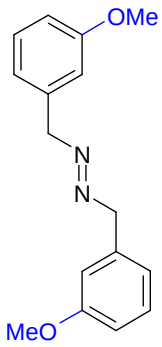
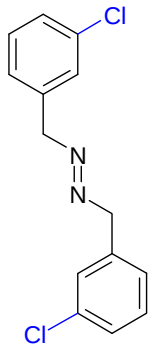


FIG. 7. SAR in derivatives of W84 (1) modified on one side as indicated. pK$_A$: −log value of the equilibrium dissociation constant for binding to free M_2 receptors in porcine heart membranes (MgPi, Tris-buffer conditions, see Table 1). Data for compound 1 from Daiss et al. 2002, for compounds 2, 3, 4 from Raasch et al. 2002, for 5 and 6 from M. Muth et al. 2003.

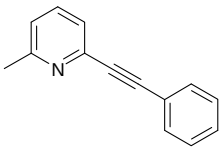
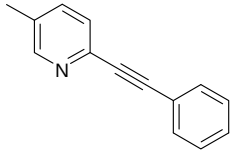
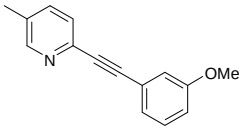
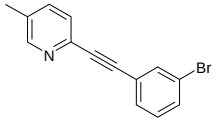
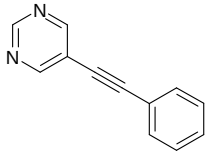
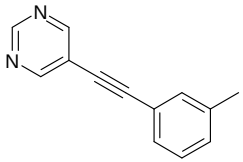
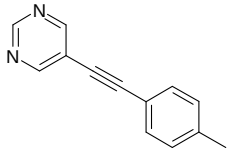
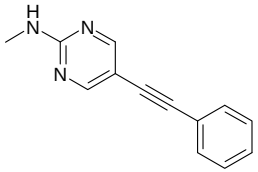
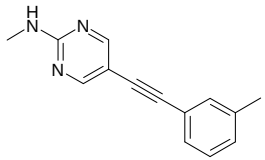
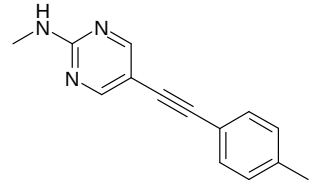
Mohr K et al, Receptors and Channels, 2004

SAMs to PAMs/NAMs on Class C (literature)

			
Name	**DFB**	**DMeOB**	**DCB**
Activity	**PAM**	**NAM**	**SAM**

O'Brien JA et al, Mol Pharm, 2003

SAMs to PAMs/NAMs on Class C (literature)

				
MPEP	5MPEP	M-5MPEP	Br-5MPEPy	**23**
Full NAM	**SAM**	**Partial NAM**	**Partial NAM**	**Partial NAM**
				
24	**25**	**16b**	**16c**	**16f**
Full NAM	**PAM**	**PAM**	**PAM**	**PAM**

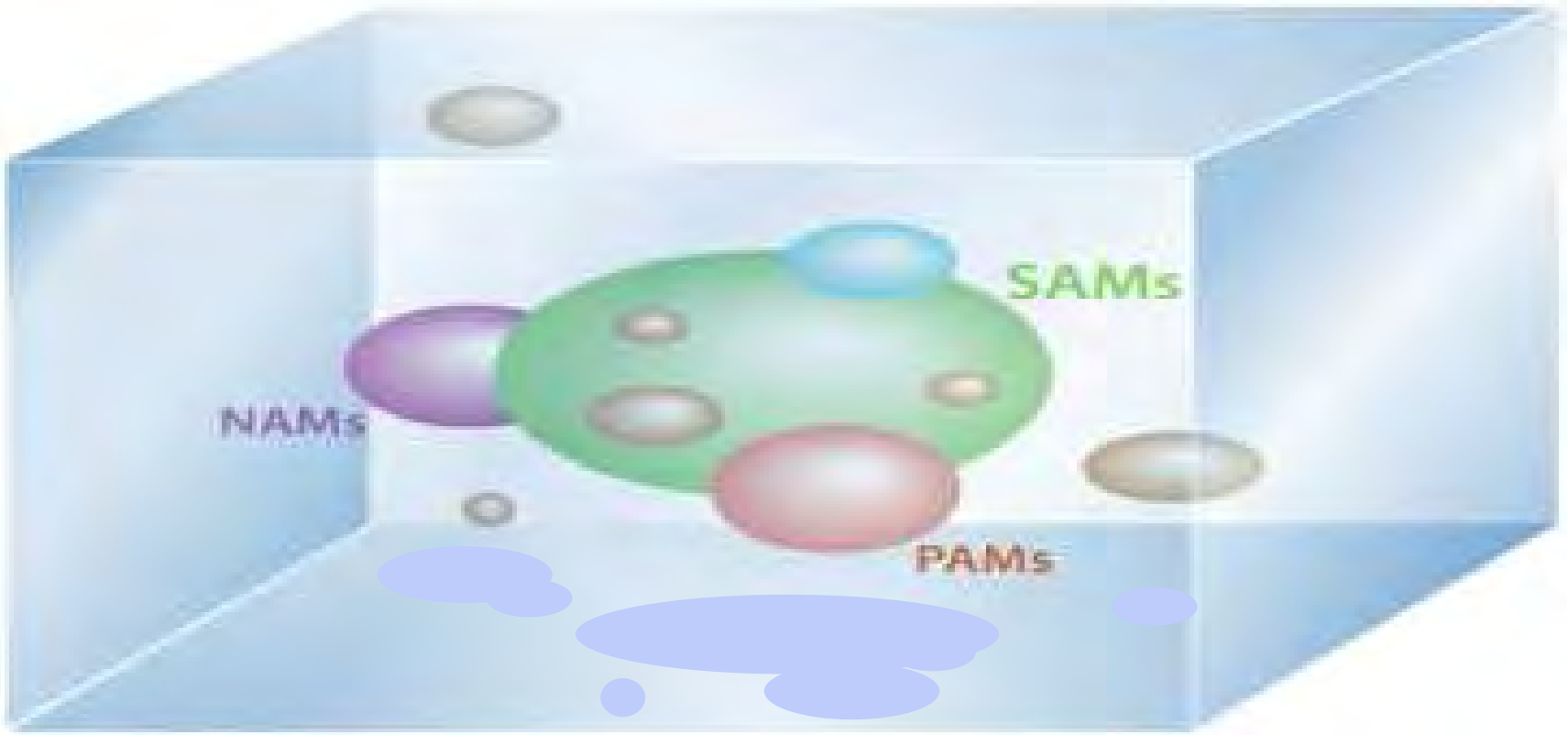
Rodriguez AL, et al.; Mol Pharm 2005, 1793-802
Sharma S, et al.; BMCL 2008, 4098-101
Sharma S, et al.; J Med Chem 2009, 4103-6

SAM definition

1. **Binding to the receptor of interest**
2. **Absence of functional activity in at least one functional test**
3. **Capacity to be transformed into a functionally active compound with minor chemical modifications**

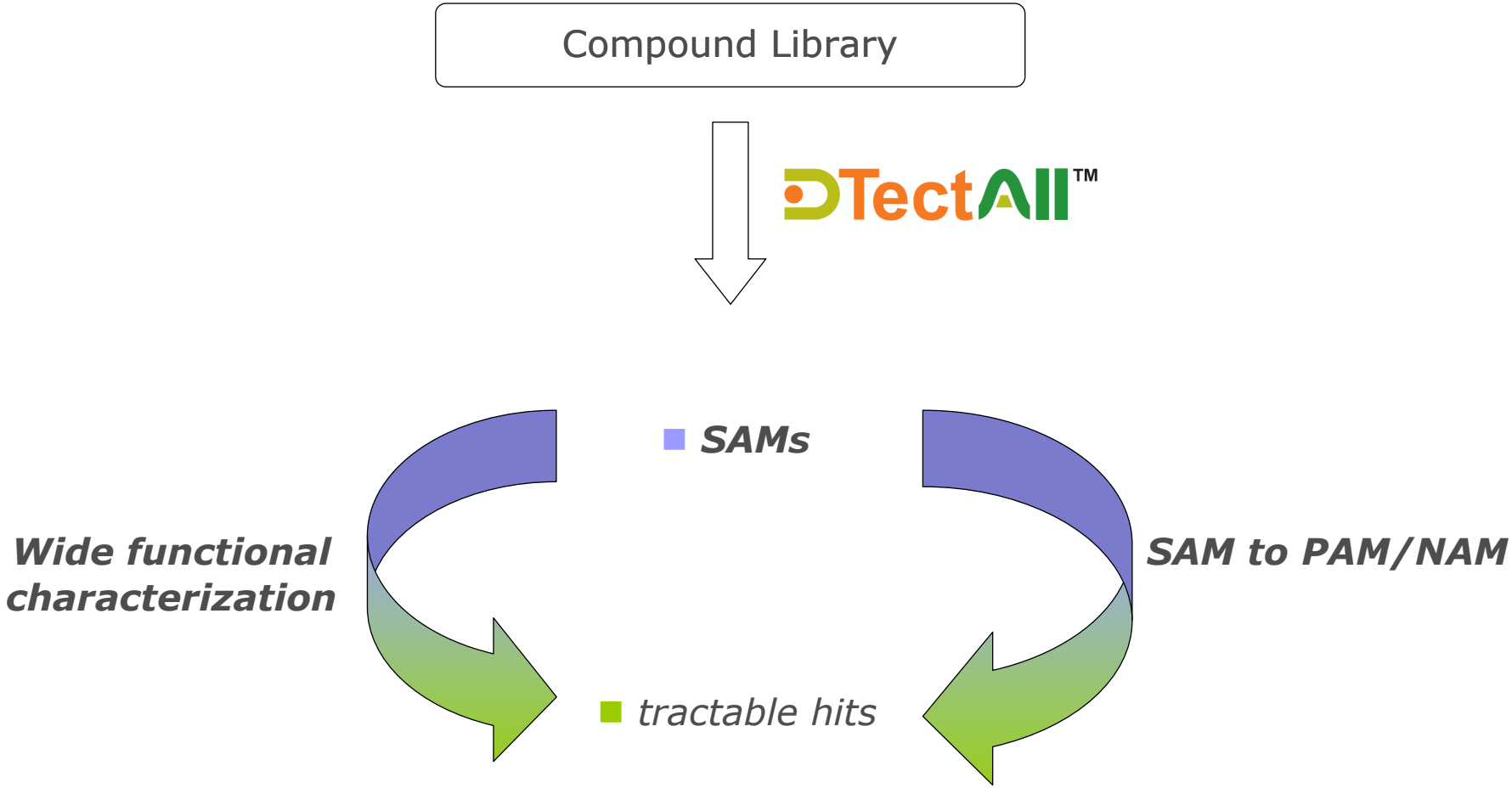
Schann S, et al.; submitted

Silent Allosteric Modulators (SAMs)



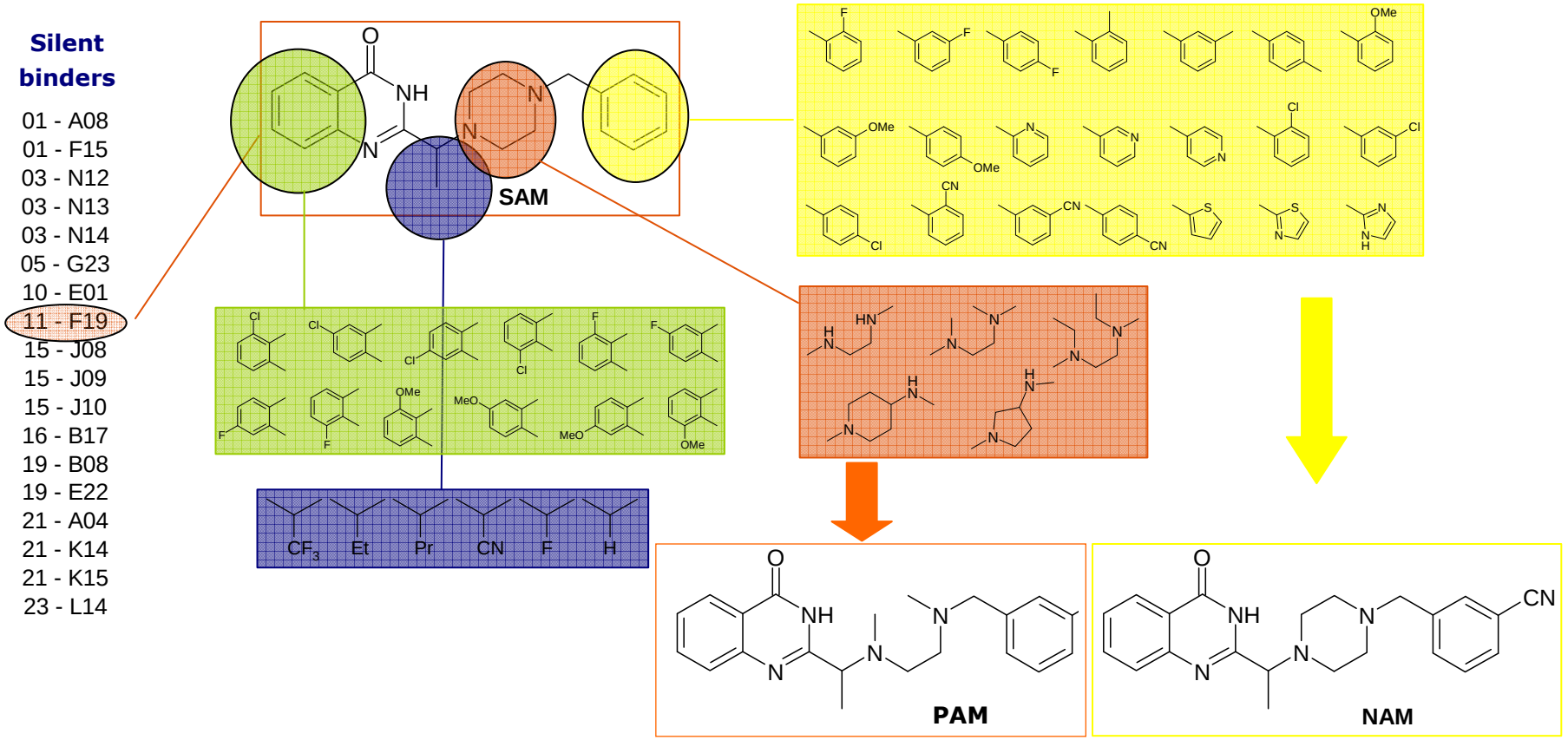
Adapted from Lipinski C and Hopkins A, Nature, 2004.

Screening process at Domain



DTectAll™ SAMs to PAMs/NAMs

- Chemistry efforts on several SAMs to optimize chances of success
- Synthesis of one focused library per SAM with a few dozens of very close analogs (20 to 50) produced by parallel chemistry
- Tested in FRET-binding and functional assays



Takeda collaboration

Domain Therapeutics announces successful completion of the first milestone in its Research Agreement with Takeda Pharmaceutical

Strasbourg, France, November 4, 2009 - Domain Therapeutics, a drug discovery company focused on G Protein-Coupled Receptors (GPCRs), announces today that it has successfully completed the first milestone in its research agreement with Takeda Pharmaceutical. This research agreement initially concerns the use of Domain's DTect-All™ technology for the discovery of novel drug candidates for GPCRs of interest to Takeda.

"Achievement of this milestone is a firm confirmation of the ability of Domain Therapeutics' unique technology to address difficult GPCRs," said Pascal Neuville, CEO of Domain Therapeutics. "We are confident that its continued use will further the goals of this on-going research program."

DTect-All™ is Domain's proprietary technology platform dedicated to identification of GPCR ligands, more specifically allosteric modulators. DTect-All™ addressees every GPCR including challenging ones such as orphan and peptidic receptors.

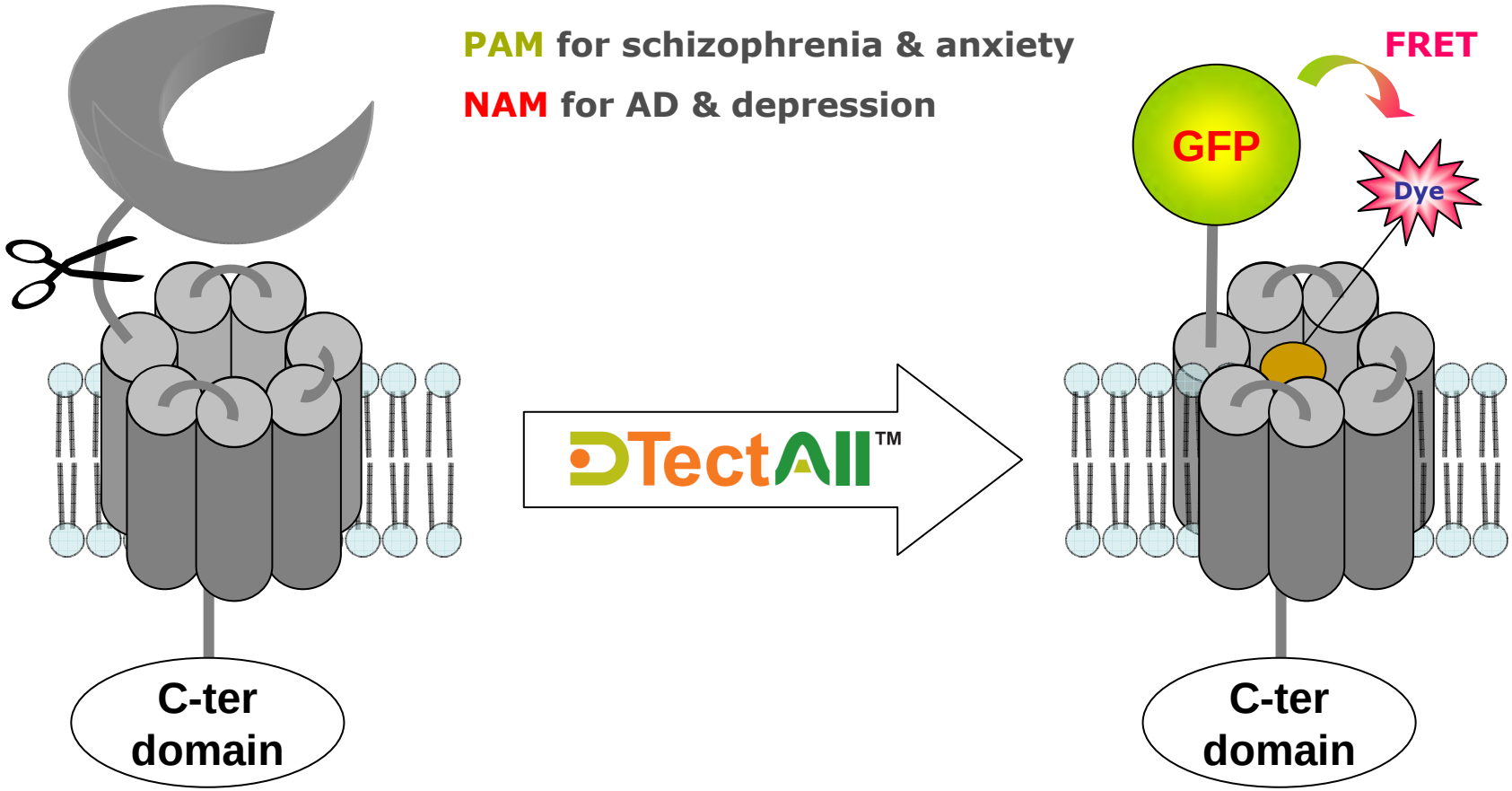
For this milestone, Domain will receive an undisclosed success fee.

DTectAll™ Assay portfolio

Receptor and Class		Frequent-hitter probe	nM ligand-derived probe	Therapeutic area
Adenosine A2A	(A)	✓	✓	CNS
mGluR2	(C)	ongoing	✓	CNS
mGluR3	(C)	ongoing	✓	CNS
mGluR4	(C)	✓		CNS
mGluR5	(C)	TBD	✓	CNS
GPR101	(A)	✓		CNS
Muscarinic M1	(A)	✓		CNS
NTSR1	(A)	✓		CNS
GalR2	(A)	✓		CNS
GPR88	(A)	✓		CNS
GPR85	(A)	✓		CNS
CB1	(A)	TBD	✓	Metabolic disorders
GLP1-R	(B)	✓		Metabolic disorders
GPR40	(A)	✓		Metabolic disorders
GPR43	(A)	✓		Metabolic disorders
GPR10	(A)	✓		Metabolic disorders
GPR119	(A)	✓		Metabolic disorders
GPR120	(A)	✓		Metabolic disorders

: under development

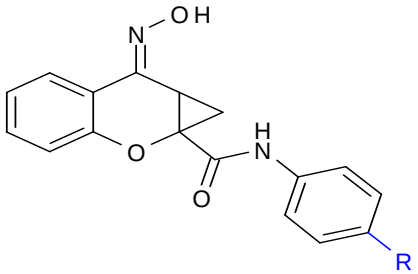
SAMs to PAMs/NAMs on mGluR2/3



Native mGluR2

Truncated GFP-mGluR2

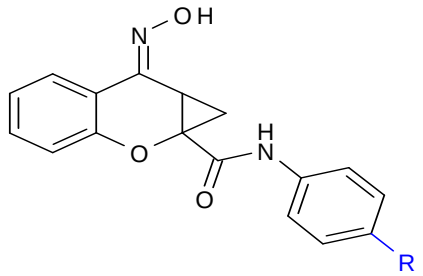
SAMs to PAMs/NAMs on mGluR2/3



	R	mGluR2		mGluR3
		FRET	Ca^{++}	Ca^{++}
A	F	Ki = 6.6μM	**NA** (up to 100μM)	**NA** (up to 100μM)

Schann S, et al.; submitted

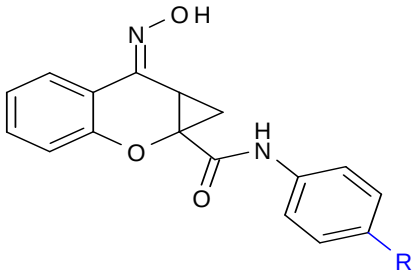
SAMs to PAMs/NAMs on mGluR2/3



	R	mGluR2		mGluR3
		FRET	Ca^{++}	Ca^{++}
A	F	Ki = 6.6μM	**NA** (up to 100μM)	**NA** (up to 100μM)
B	Cl	Ki = 1μM		
C	OMe	Ki = 0.8μM		
D	Me	Ki = 0.7μM		

Schann S, et al.; submitted

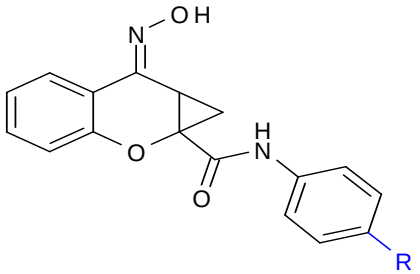
SAMs to PAMs/NAMs on mGluR2/3



	R	mGluR2		mGluR3
		FRET	Ca^{++}	Ca^{++}
A	F	Ki = 6.6µM	**SAM** (up to 100µM)	**NA** (up to 100µM)
B	Cl	Ki = 1µM	**NAM** IC_{50} = 0.8µM	
C	OMe	Ki = 0.8µM	**NAM** IC_{50} = 1µM	
D	Me	Ki = 0.7µM	**NAM** IC_{50} = 1.5µM	

Schann S, et al.; submitted

SAMs to PAMs/NAMs on mGluR2/3



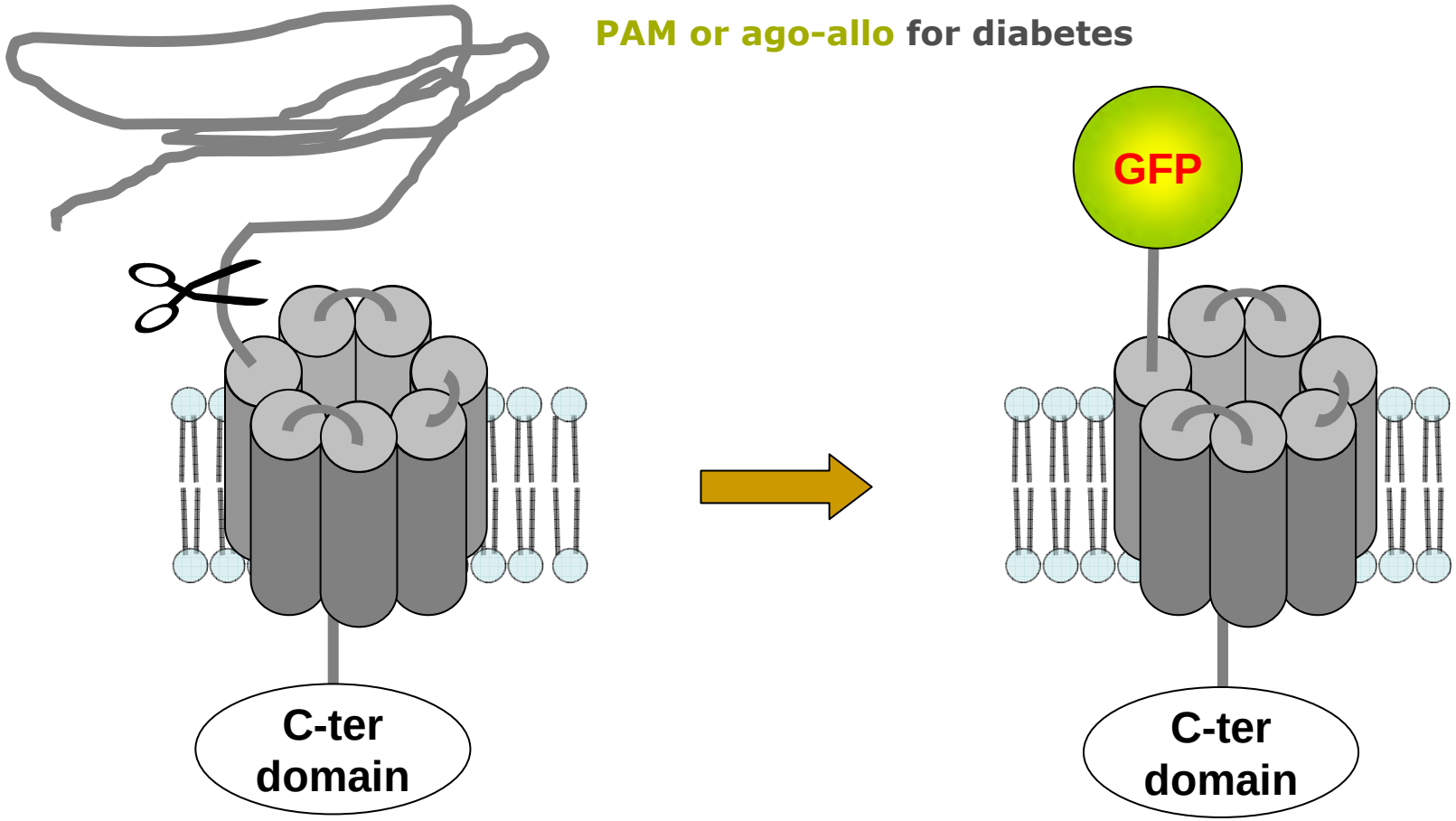
	R	mGluR2		mGluR3
		FRET	Ca^{++}	Ca^{++}
A	F	Ki = 6.6µM	**SAM** (up to 100µM)	**SAM** (up to 100µM)
B	Cl	Ki = 1µM	**NAM** IC_{50} = 0.8µM	**PAM** EC_{50} = 13.4µM
C	OMe	Ki = 0.8µM	**NAM** IC_{50} = 1µM	**PAM** EC_{50} = 10.4µM
D	Me	Ki = 0.7µM	**NAM** IC_{50} = 1.5µM	**PAM** EC_{50} = 8.9µM

Schann S, et al.; submitted

⇨ **Other identified series with SAM / NAM compound under optimization**

SAMs/PAMs/NAMs on GLP-1R

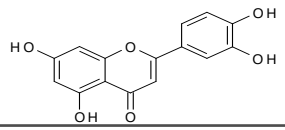
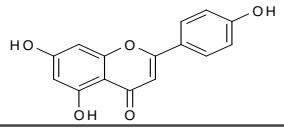
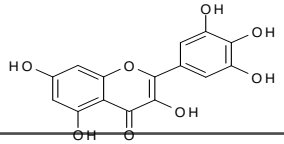
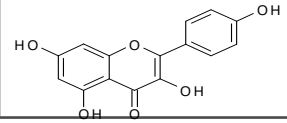
PAM or ago-allo for diabetes



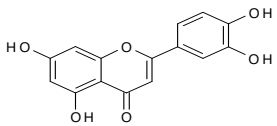
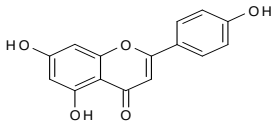
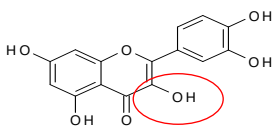
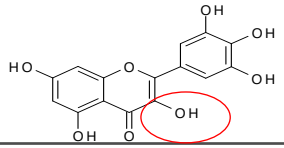
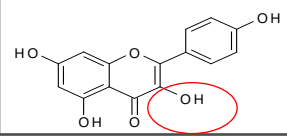
Native GLP-1R

Truncated GFP-GLP-1R

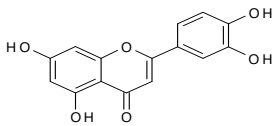
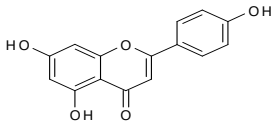
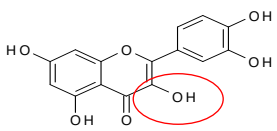
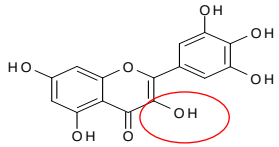
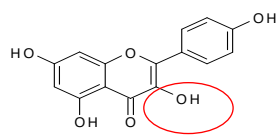
SAMs to PAMs/NAMs on GP-1R

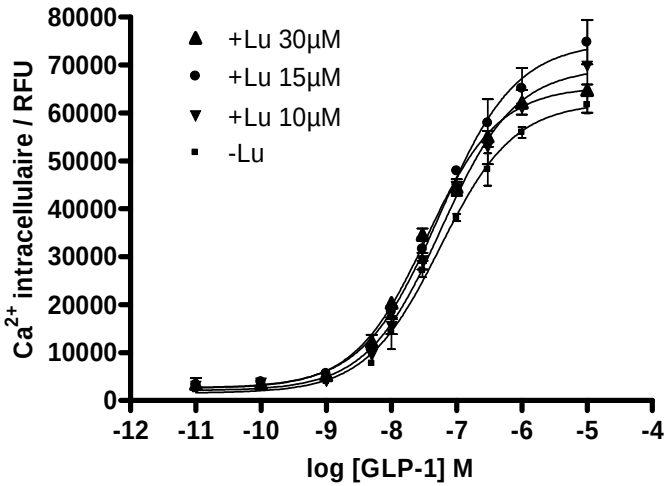
Compound	Luteolin	Apigenin		Myricetin	Kaempferol
FRET	Ki = 5.5μM	Ki = 17.3μM		Ki = 2.8μM	Ki = 9.9μM
Structure					

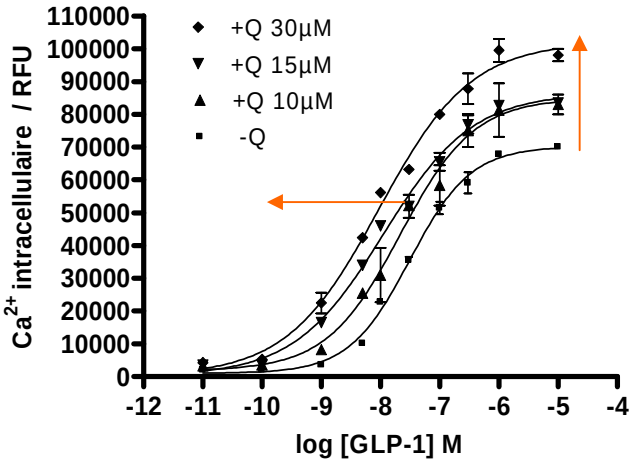
SAMs to PAMs/NAMs on GP-1R

Compound	Luteolin	Apigenin	Quercetin	Myricetin	Kaempferol
FRET	Ki = 5.5μM	Ki = 17.3μM	Not done	Ki = 2.8μM	Ki = 9.9μM
Ca^{++}	**NA** Up to 30μM	**NA** Up to 100μM			
Structure					

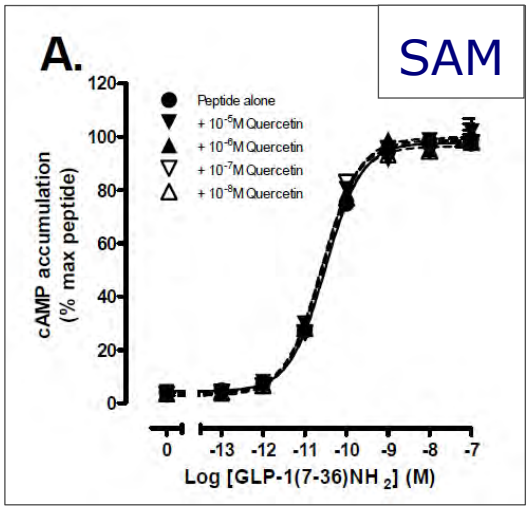
SAMs to PAMs/NAMs on GP-1R

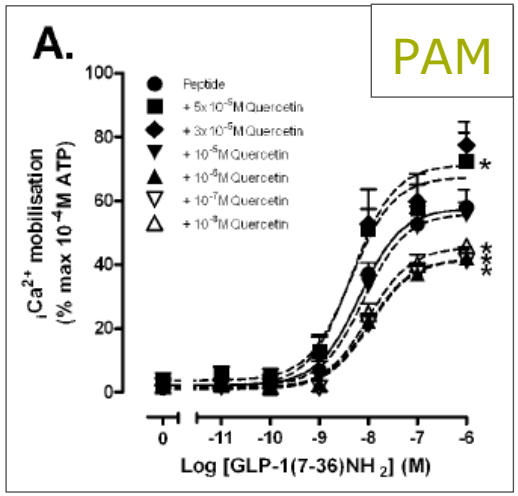
Compound	Luteolin	Apigenin	Quercetin	Myricetin	Kaempferol
FRET	Ki = 5.5μM	Ki = 17.3μM	Not done	Ki = 2.8μM	Ki = 9.9μM
Ca^{++}	**SAM** Up to 30μM	**SAM** Up to 100μM	**PAM** @ 30μM Shift GLP-1 EC_{50} by 3.2 fold Shift GLP-1 Emax by 50%	**PAM** @ 30μM Shift GLP-1 EC_{50} by 1.9 fold Shift GLP-1 Emax by 20%	**PAM** @ 30μM Shift GLP-1 EC_{50} by 1.7 fold Shift GLP-1 Emax by 15%
Structure					

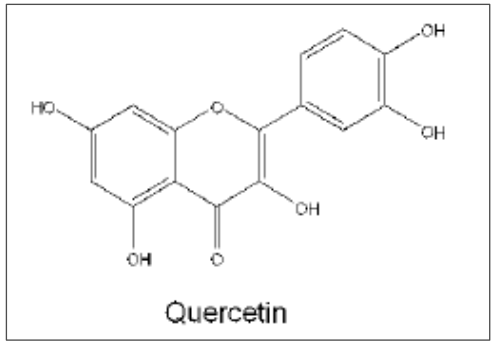




SAMs to PAMs/NAMs on GP-1R







Koole C et al, Mol Pharm, 2010

SAM, PAM or NAM

	GPCRs	PubMed	DOMAIN THERAPEUTICS
Class A	M1 – M4	Gharagozloo, 2002 Mohr, 2004	
	NTSR1		✓
	Undisclosed peptide GPCR		✓
Class B	GLP-1R	Koole, 2010	✓
Class C	mGluR2/3		✓
	mGluR4		✓
	mGuR5	O'Brien, 2003 Rodriguez, 2005 Sharma, 2008 & 2009	

Pascal Neuville
Christel Franchet
Stanislas Mayer
Mélanie Frauli
Edith Steinberg
Camille Amalric
Luc Baron
Lydie Bricka

www.domaintherapeutics.com

sschann@domaintherapeutics.com

Marcel Hibert
Jean-Luc Galzi
Dominique Bonnet
Sophie Gioria
François Debaene

3rd RSC/SCI Symposium on GPCRs in Medicinal Chemistry
September 20-22, 2010 – MSD, Oss, The Nederlands

DTectAll™ screening process

COMPOUND LIBRARY

1 **n=1** screening (10µM)

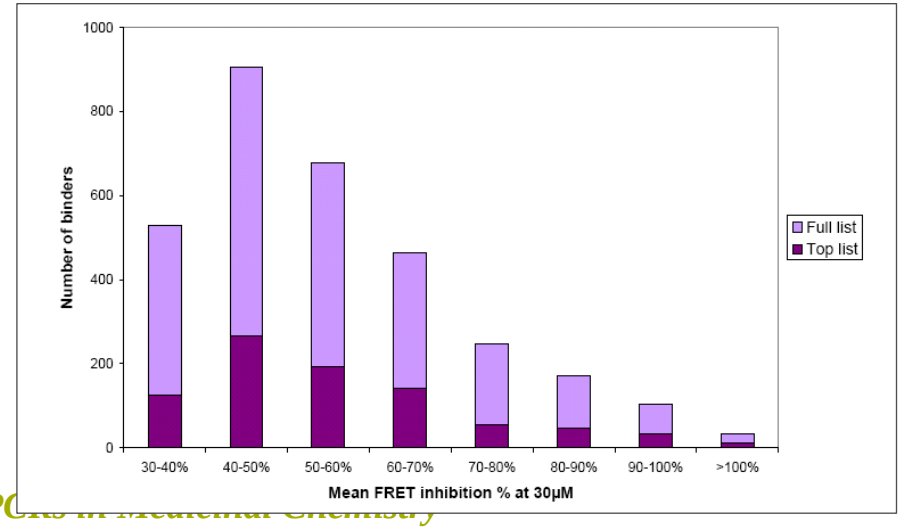
HITS

2 **n=2** confirmation (10µM)

BINDERS

3 **n=3 & 4** @ 3µM
=> Ratio <3 = Top list

HIGH-AFFINITY BINDERS



4 **Ki determination**
10 conc. on a 3 log range

Selectivity
on two FRET assays

5 **Functional characterization**

Agonists / antagonists / NAMs / PAMs / SAMs

REAL BINDERS

DOMAIN THERAPEUTICS