Novel Functional Assay Approaches for GPCR Ligand Discovery

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Basel, Switzerland

Screening Europe Barcelona
February 20-21, 2007

These slides were taken, courtesy of Rochdi Bouhelal, from a presentation given at Screening Europe in February 2007.

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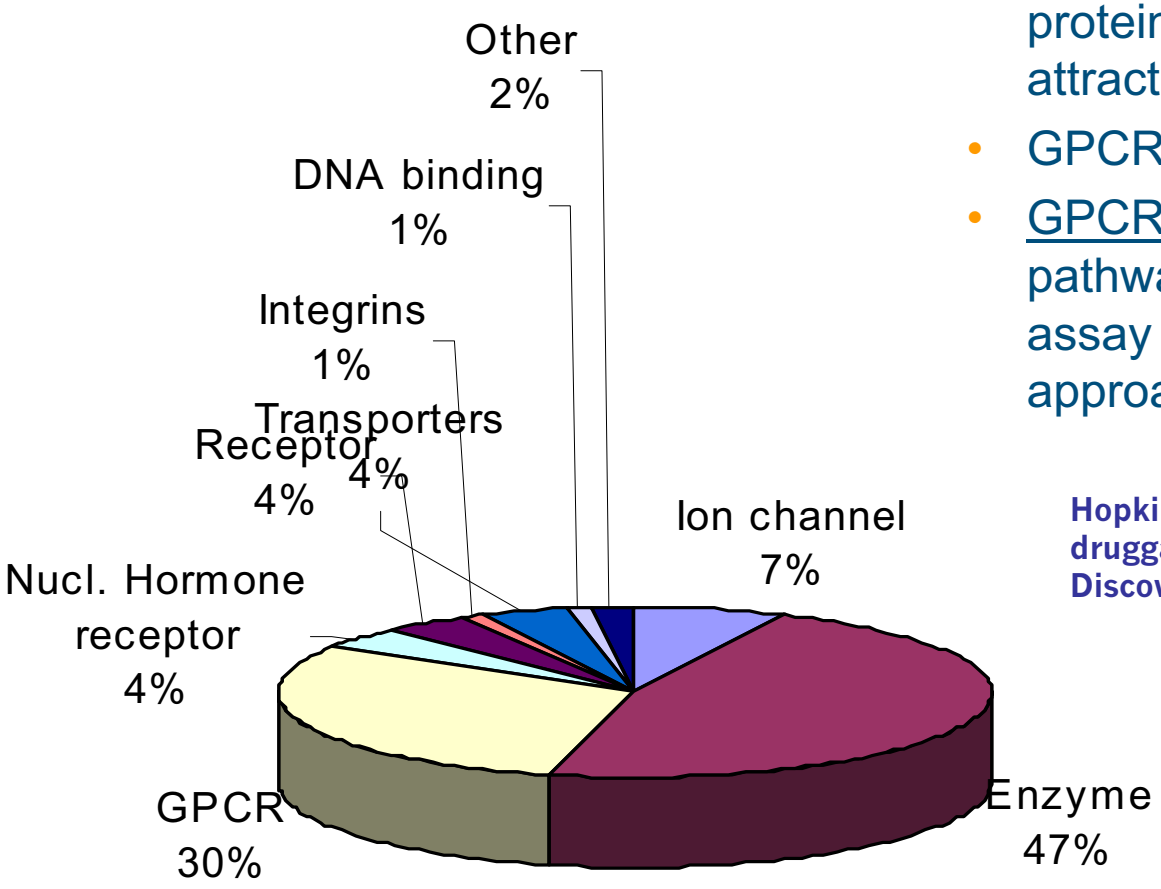
Drug targets for existing medicines



- GPCR the most studied membrane protein and amongst the most attractive drug targets
- GPCR's déjà vu ?
- GPCR's renaissance: Novel pathways, molecular properties and assay technologies and HTS approaches are increasingly studied

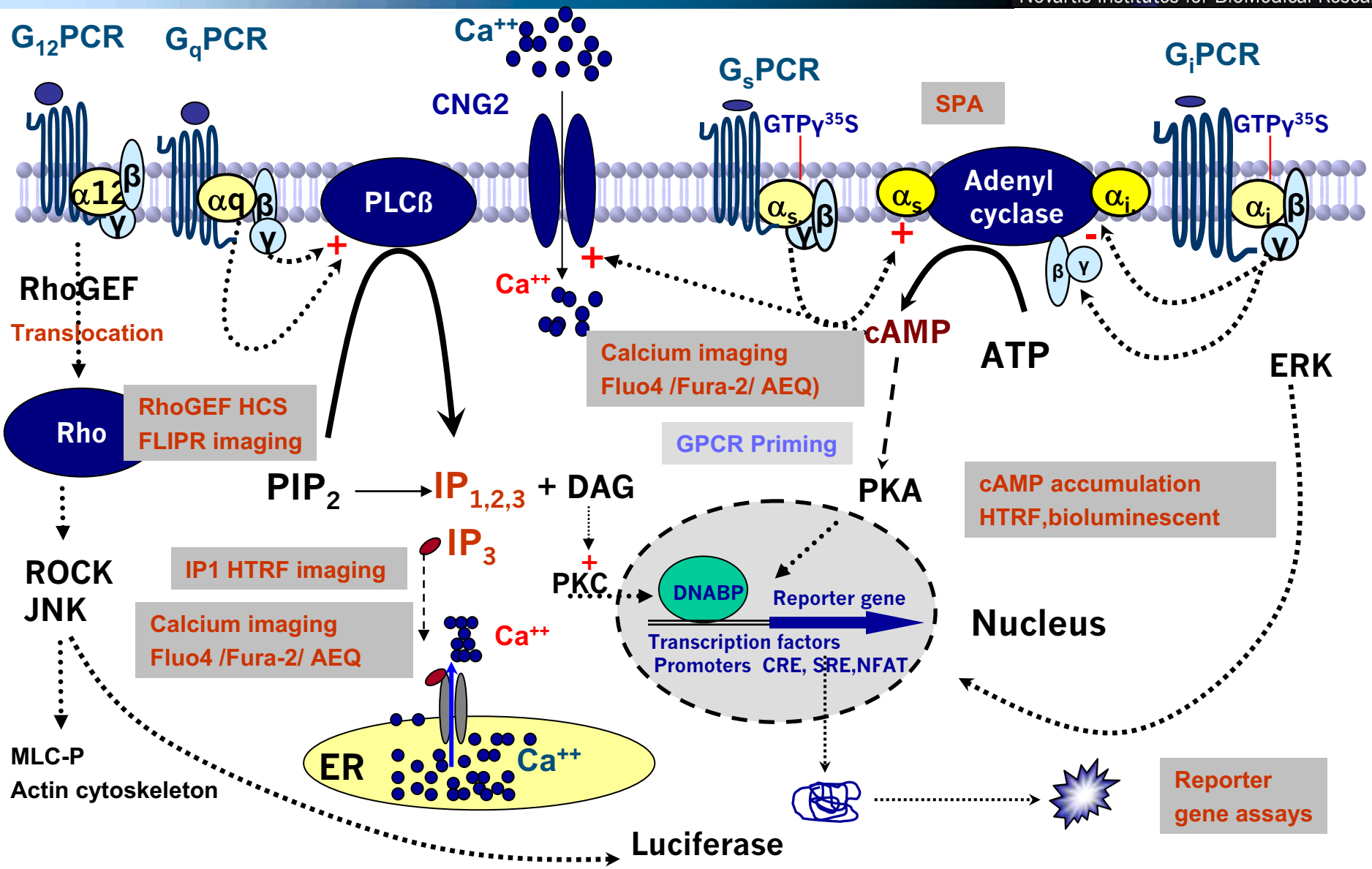
Hopkins et al NRDD 1, 727-730 (2002). The druggable genome": Nature Reviews, Drug Discovery, Vol.1, September 2002

Monitoring GPCR activation: some facts & basic principles

- **Functional & ligand binding GPCR assays**
  - The best approach to ligand discovery ?
  - **Ligand binding assay**
    - ⇒ in general straight forward but poor information content
    - ⇒ In general 1st choice for medicinal chemists
  - **Functional assays**
    - ⇒ Less simple to configure, high hit rates, false positives
    - ⇒ Access to the GPCR biology , Uncovers novel compound mechanisms
    - ⇒ May offer rich information output
- **Try to remain proximal to the receptor**
  - However reporter gene assays can be used in some circumstances
- Imaging is used to enhance throughput
  - One single screening platform for orphan receptors and for HTS
- **Use non–invasive assays**
  - Try to bring all receptors to signal through calcium
    - ⇒ Assay multiplexing (selectivity, several pathways)
    - ⇒ Allows to align more easily HTS data
  - Rich pharmacological data output including kinetic data analysis
    - ⇒ Helps excluding non-specific compounds

GPCR signaling pathways and applied technologies is lead discovery

Novartis Institutes for BioMedical Research



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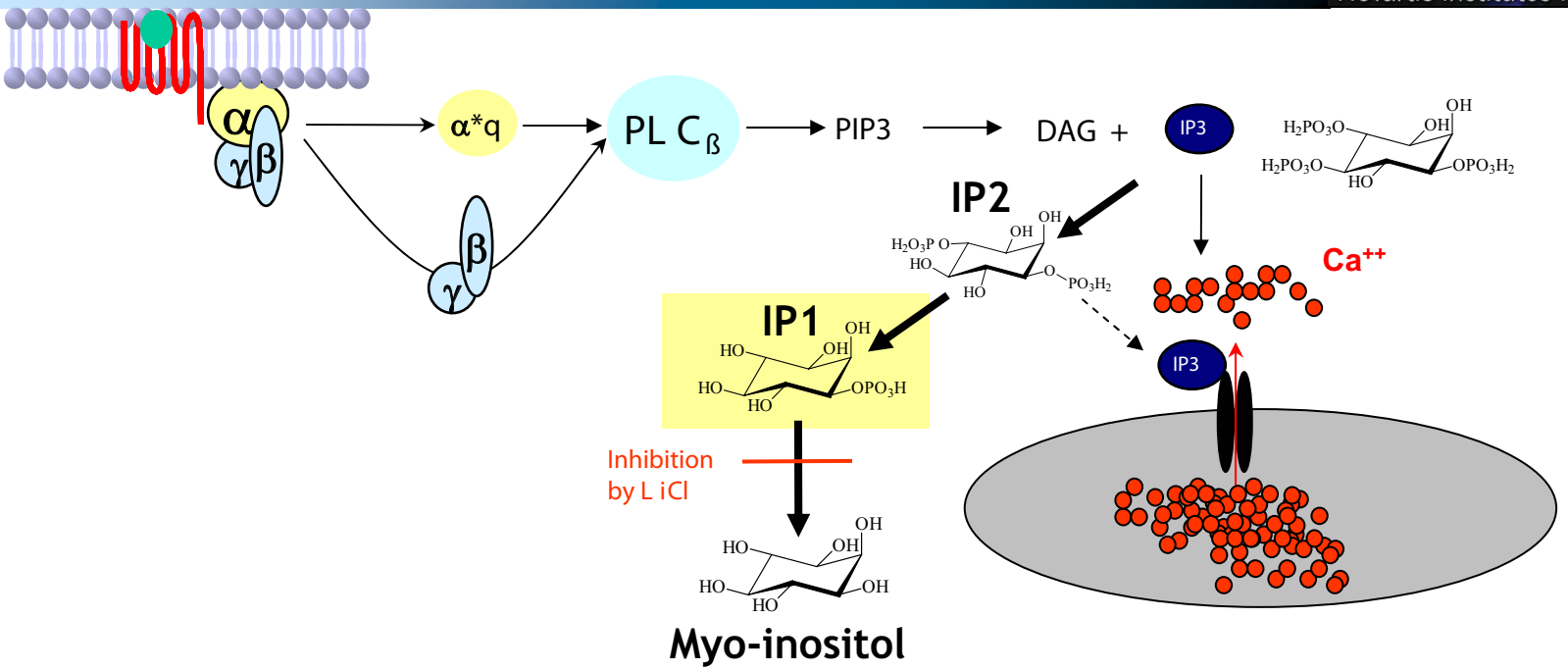
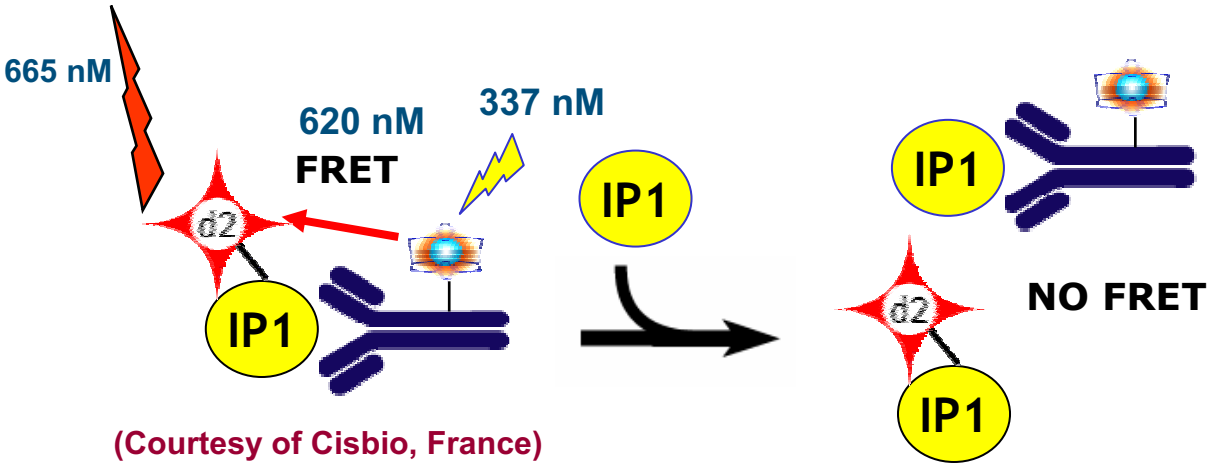
Inositol phosphate 1 for activation Gq coupled receptors

IP1 accumulation HTRF assay





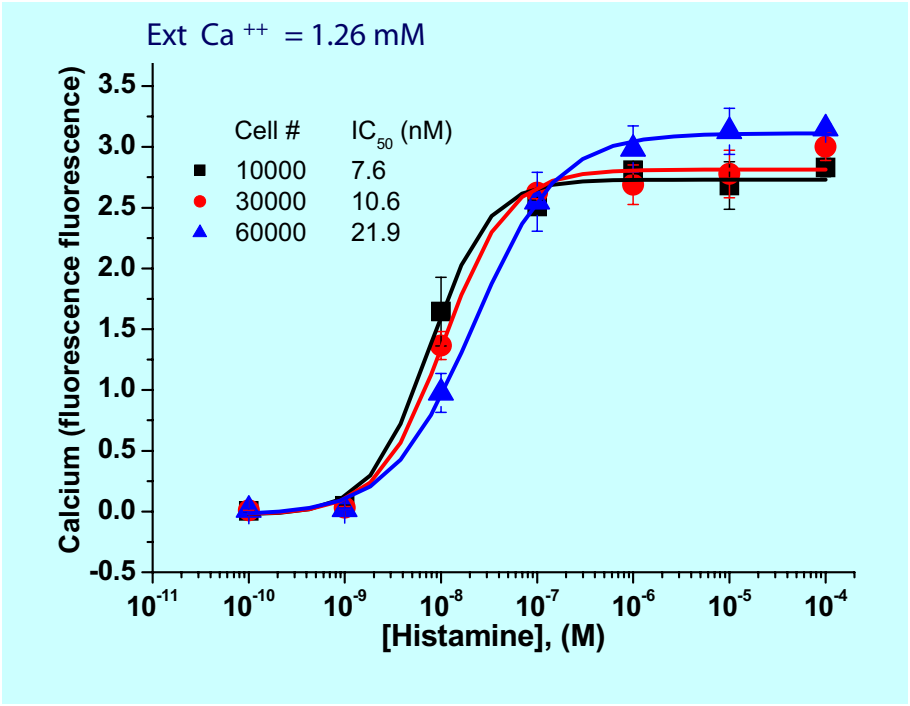
(Courtesy of Cisbio, France)

fluorescent analog of IP1

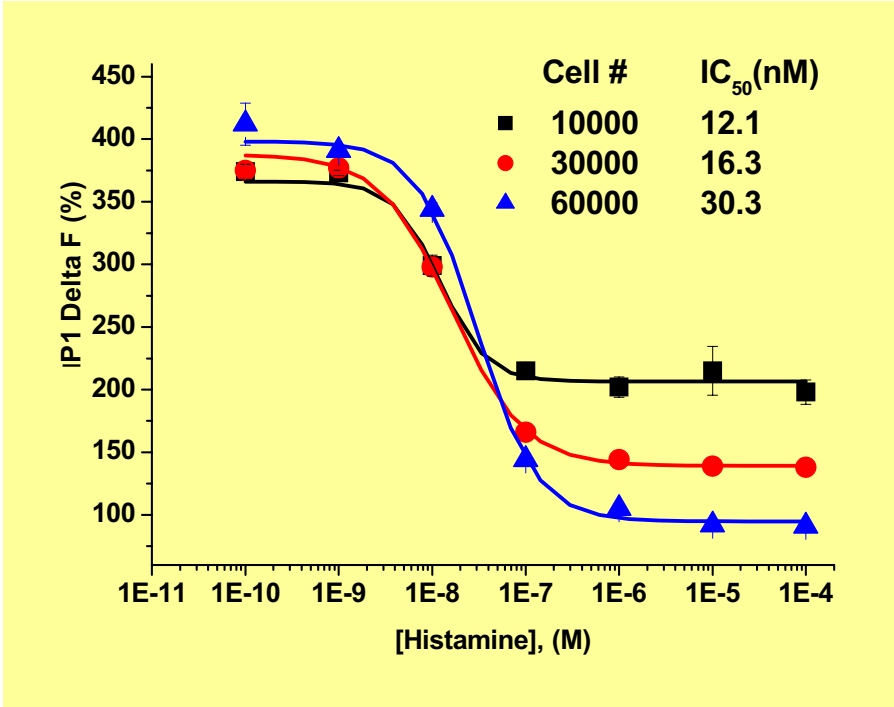
monoclonal antibody against IP1

IP1 accumulation: Histamine responses in CHOK1-H1R

Calcium FLIPR



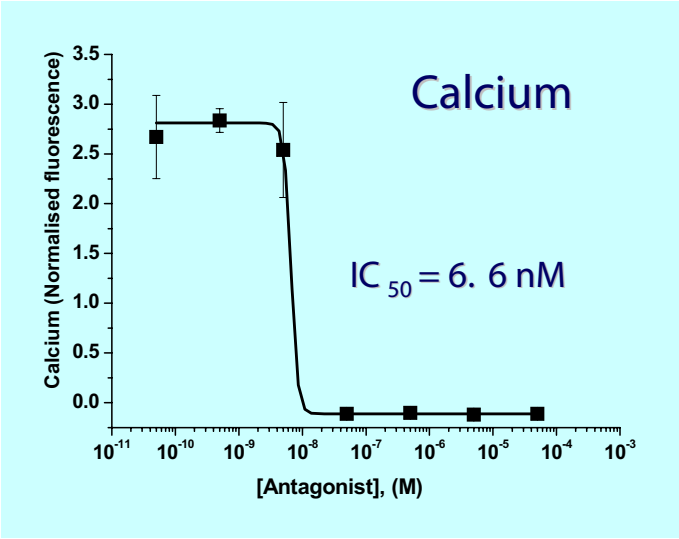
IP1 HTRF

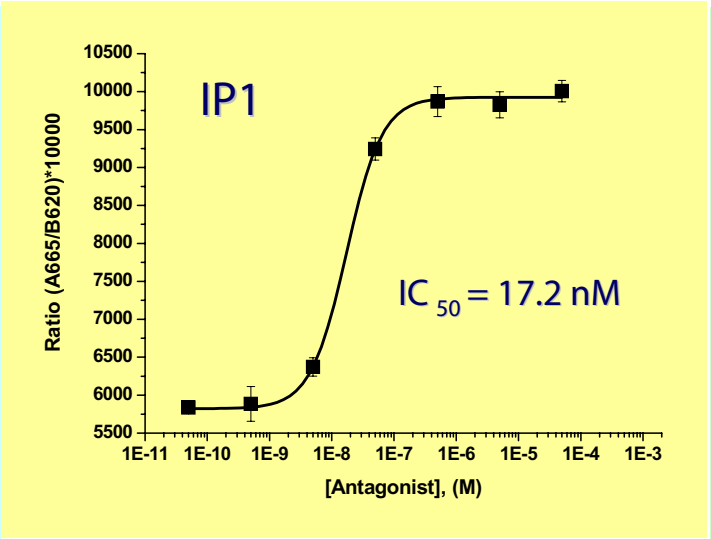


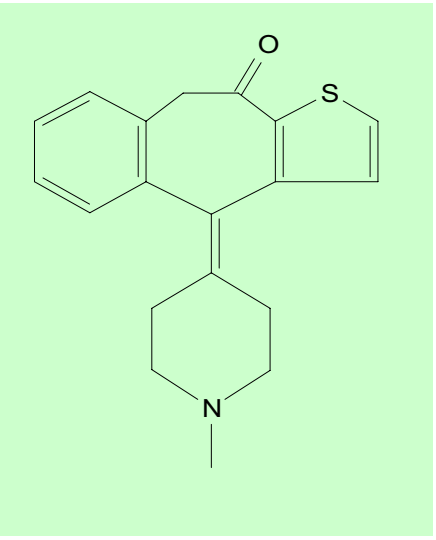
IP1 accumulation: Histamine H1 receptor in CHOK1 cells: antagonist effects

	IC_{50} (nM) Ca^{++} (FLIPR)			IC_{50} (nM) IP1 (HTRF)			
Compound	**exp 1**	**exp 2**	**mean**	**exp 1)**	**exp 2**	**mean**	$IC_{50,\ Ca++}$ / $IC_{50\ IP1}$
Cetirizine	925	1450	**1188**	86	167	**127**	9.38
Ketotifen	6.4	6.6	**6.5**	5.2	17.2	**11**	0.58
Astemizole	460	458	**459**	375	865	**620**	0.74
Loratadine	3910	2350	**3130**	635	939	**787**	3.98
Clemastine	48.5	24.4	**36**	15.7	46.3	**31**	1.18
Doxepin	13.0	33.6	**23**	7.5	13.4	**10**	2.23
Mirtazapine	12.4	7.9	**10**	7.0	12.4	**10**	1.05

Antagonism by Ketotifen







IP1 accumulation for Secondary Screening & HTS

- Alternative format for G_q-coupled receptors evaluated recently (see review June 06)
- Homogeneous format (HTRF)
- Applied for secondary screening of GPR40

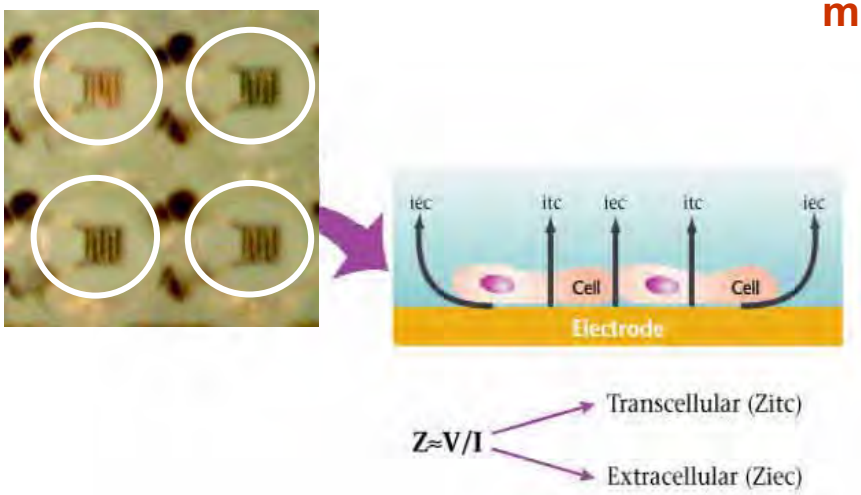
← FLIP R / Ca ++ → ← HTRF / IP1 →

	CHOK1-C4-GPR40 Ca^{++}		CHOK1-C4-GPR40 Ca^{++}		CHOK1-C4-V1a Ca^{++}		CHOK1-C4-GPR40 IP1	
Agonist	C18:2		synthetic agonist		Arg-vasopressine		NVP-BJX437	
Compound	IC_{50} (µM)	Max. inh. (% of control)	IC_{50} (µM)	Max. inh. (% of control)	IC_{50} (µM)	Max. inh. (% of control)	IC_{50} (µM)	Max. inh. (% of control)
RBAS1	>30	20	>30	20	>30	0	>30	31
RBAS2	>30	0	>30	0	>30	0	>30	0
RBAS3	3.6	100	3.0	100	>30	0	5.6	116
RBAS4	6.8	100	2.6	100	>30	0	7.2	109
RBAS5	2.1	60	4.8	62	6	32	>30	0

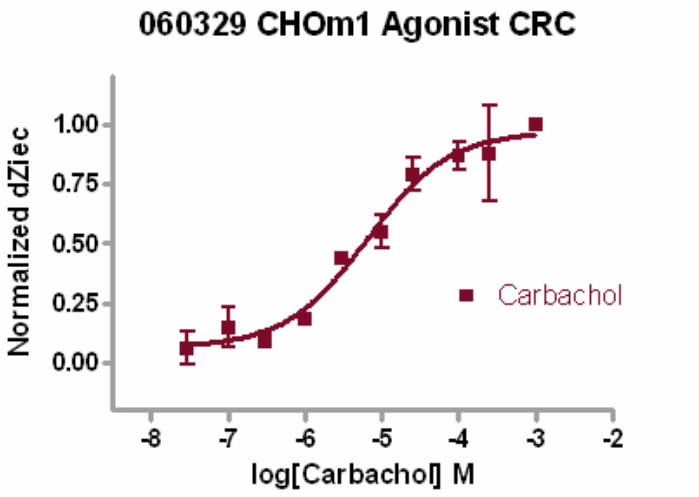
- Good correlation obtained between Ca^{++} and IP1 data
- "Frequent hitters" from FLIPR screens readout can be excluded

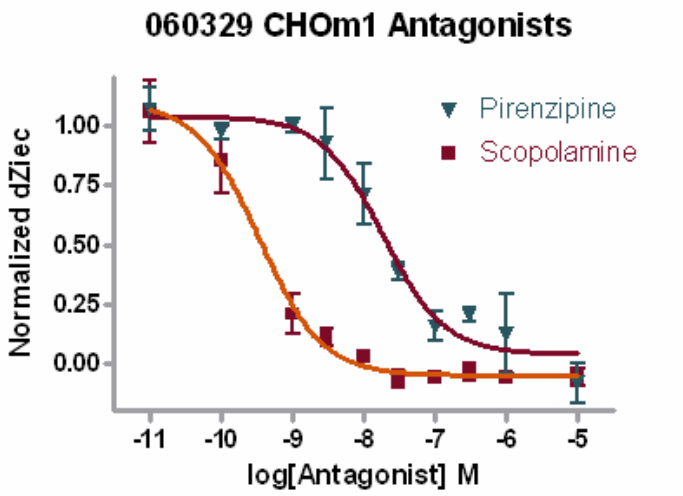
CellKey™ System: Assay Principle

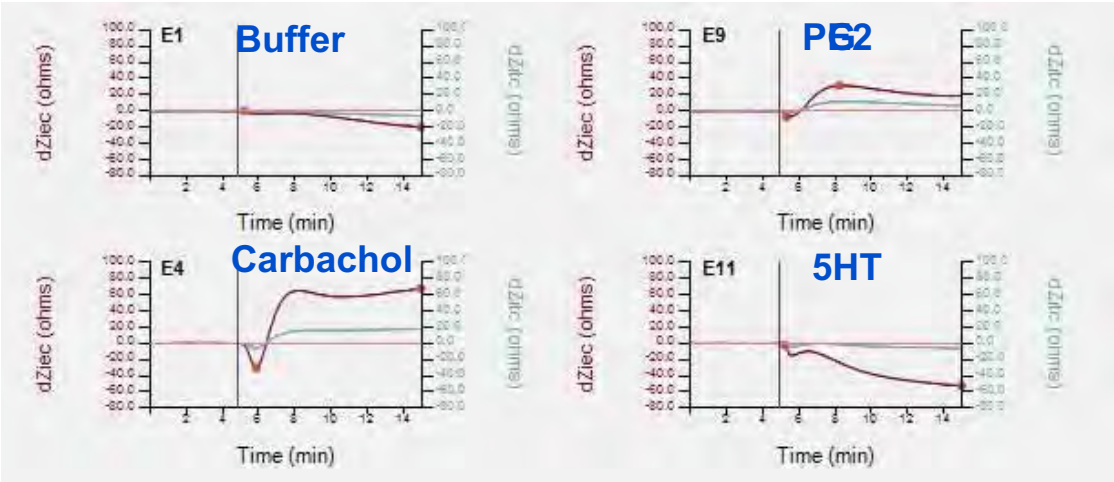
- Impedance based measurements
- Real-time, kinetic measurements across all 96 wells
- Automated fluid handling
- Simultaneous compound addition and read of all wells
- Monitors cell shape changes following receptor activation
- May constitute a phenotype assay reflecting cell migration



CellKey™ System: CHOm1 Muscarinic Agonist & Antagonist Effects





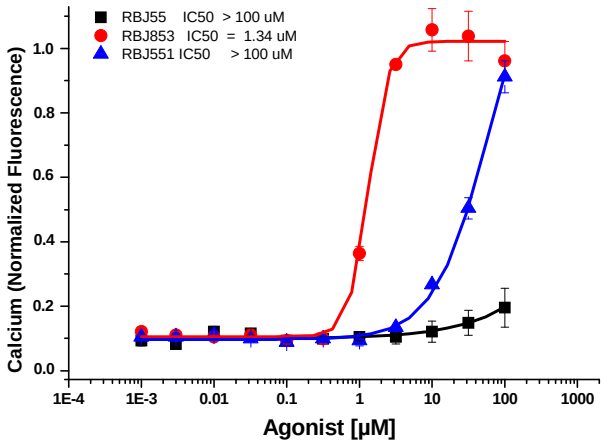


- Monitors activity and kinetic shape
- Shape determines GPCR coupling mode
- Good correlation with FLIPR data

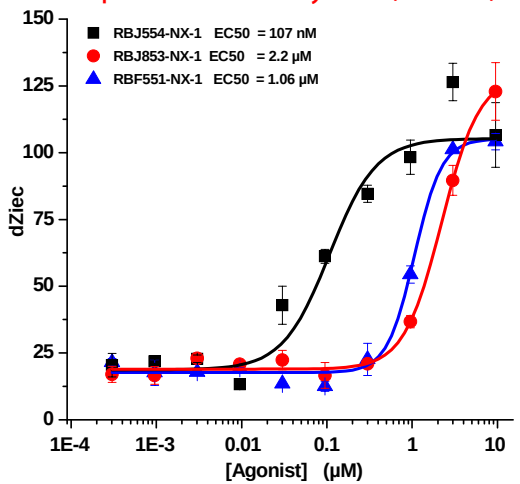
Different potencies with three technologies

Slow acting agonists in three GPCR assay formats

Calcium / FLIPR (3min)

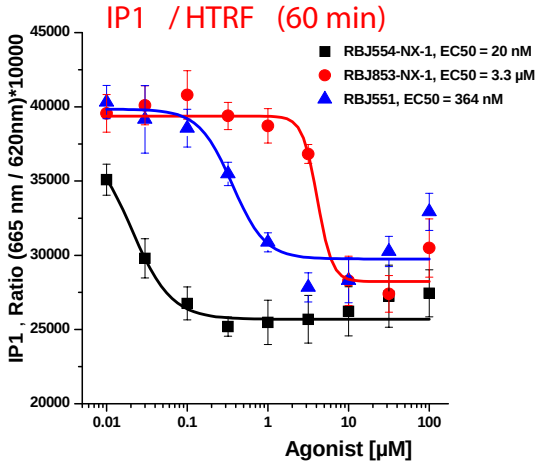


Impedance/CellKey (30 min)



Compound	Calcium (FLIPR)	IP1 (HTRF)	Impedance (CDS)
RBJ551	>100	0.36	1.1
RBJ554	>100	0.020	0.11
RBJ853	1.3	3.3	2.2

IP1 / HTRF (60 min)



Conclusions / summary

Coupling	Ligand binding	G-protein activation	Signaling	2nd mess.	technology / Instrument	Comment
		← FUNCTIONNAL ASSAYS →				
G_q	SPA	-	PLC-?	Ca^{++}	Fluo4 /FLIPR	multiplexing possible
			PLC-?	IP1	HTRF / Viewlux imaging	Temporal multiplexing
G_s	SPA	SPA GTP?S	AC activation	cAMP	HTRF / Viewlux imaging	Temporal multiplexing
G_s			AC activation	cAMP / Ca^{++} CNG2	Fluo4 /FLIPR	cAMP duplex mode possible, Gs /Gq duplex possible
G_s			PLC-?	Ca^{++}	Fluo4 /FLIPR	Ca^{++} obtained via GPCR priming
$G_?16$ & chimeric				Ca^{++}	Fluo4 /FLIPR	valid for ca 70 % Gi or Gs couped receptors
G_i	SPA	SPA GTP?S	AC inhibition	cAMP	HTRF / Viewlux imaging	
G_i			PLC-?	Ca^{++}	Fluo4 /FLIPR	Ca^{++} obtained via GPCR priming
$G_{12/13}$			RHO			
G_s ,G_q ,G_i , & $G_{12/13}$			PKC / PKA ?	role unknow	Cell key	Non-invasive free-label technology

- Novel generic assays being developped:
  - DiscoverX arrestin assays & sensigen assays