Parkinsons-2015 Meeting. October 11-13 , Frankfurt , Germany
14:30-16:00 **Workshop on:**

On the role of GPCR homo and heteroreceptor complexes balance on Parkinson´s disease

Dasiel O. Borroto-Escuela, Julia Oflijan, Thorsten Schäfer Luca Pinton, Ismel Brito, Manuel Narváez, Kristina Friedland, Luigi F. Agnati, Rafael Franco, Kjell Fuxe

Department of Neuroscience - Karolinska Institutet
Stockholm – Sweden
Frankfurt, August-2015

Karolinska Institutet

OMICS International
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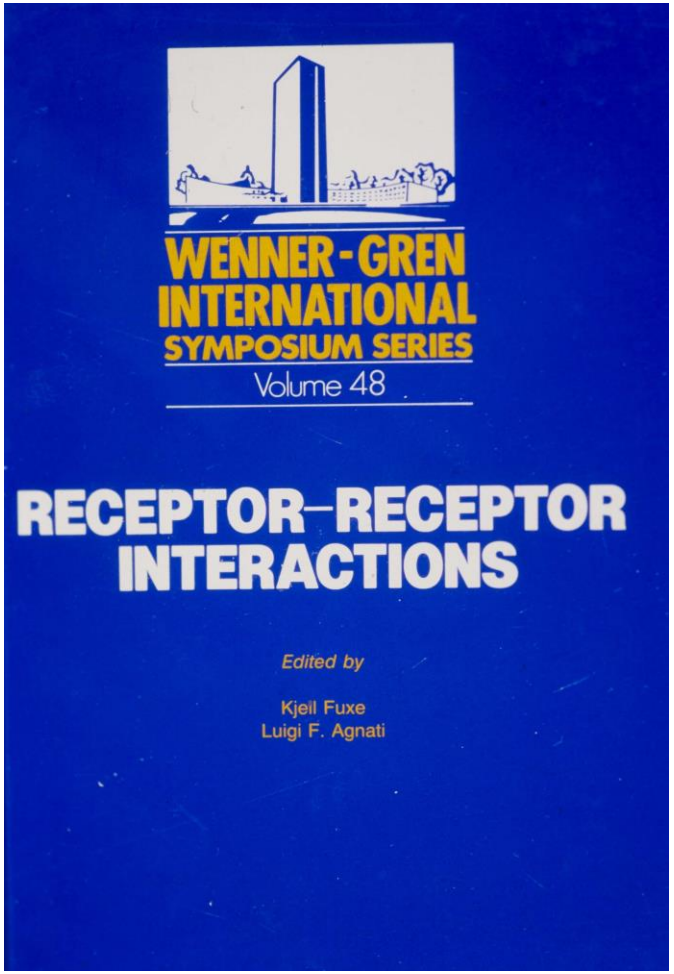
International Conference on
Parkinson's Disease & Movement Disorders
August 11-13, 2015 Frankfurt, Germany

… a little of history and background

Where and how the concept of GPCR receptor-receptor started?

Fuxe/Agnati introduced the hypothesis of receptor-receptor interactions in 1980-1981.

✓ In membrane preparations of various CNS regions they found that neuropeptides could modulate the binding characteristics, especially the affinity of the monoamine receptors in a receptor subtype specific way.



Fuxe et al. 1982, Fuxe et al. 1983, Agnati et al., 1985

Receptor–Receptor Interactions as an Integrative Mechanism in Nerve Cells

Michele Zoli,[2] Luigi F. Agnati,[2] Peter B. Hedlund,[1] Xi Ming Li,[1] Sergi Ferré,[1] and Kjell Fuxe,[1]*

[1]Department of Histology and Neurobiology, Karolinska Institutet, Box 60400, S-10401, Stockholm, Sweden; and [2]Institute of Human Physiology, University of Modena, Via Campi 287, 41100, Modena, Italy

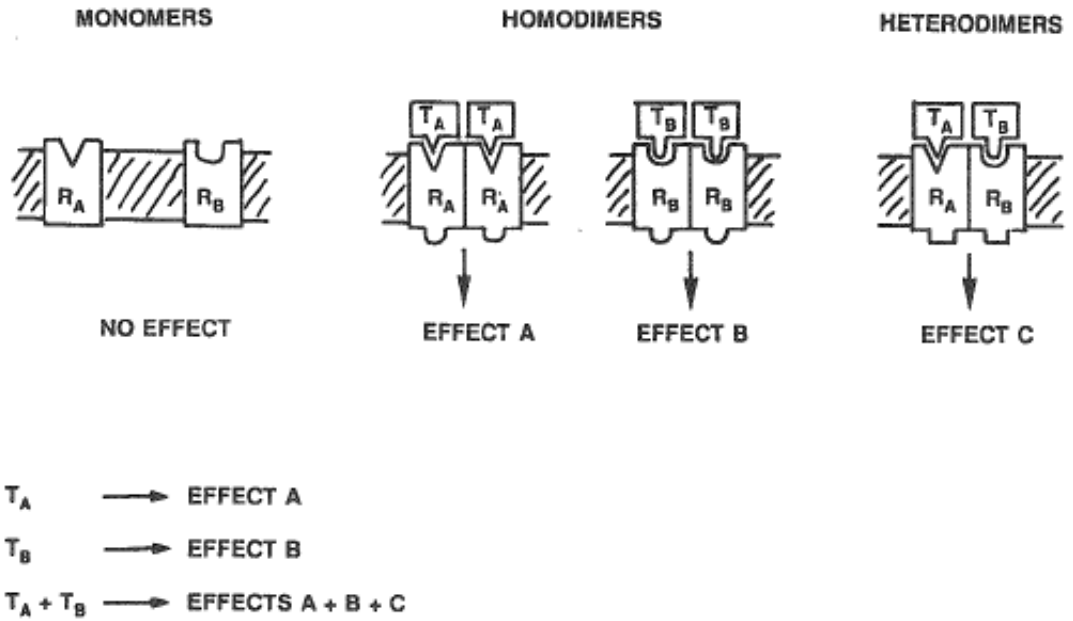


Fig. 4. Homo- and heterodimerization of membrane receptors. Heterodimers induce a cellular effect different from homodimers. The relative proportion of the two transmitters, the concentration of the two receptor populations, and the characteristics of receptor–receptor interactions will determine the amount of homo- vs heterodimers and thus the overall effect on the target cell.

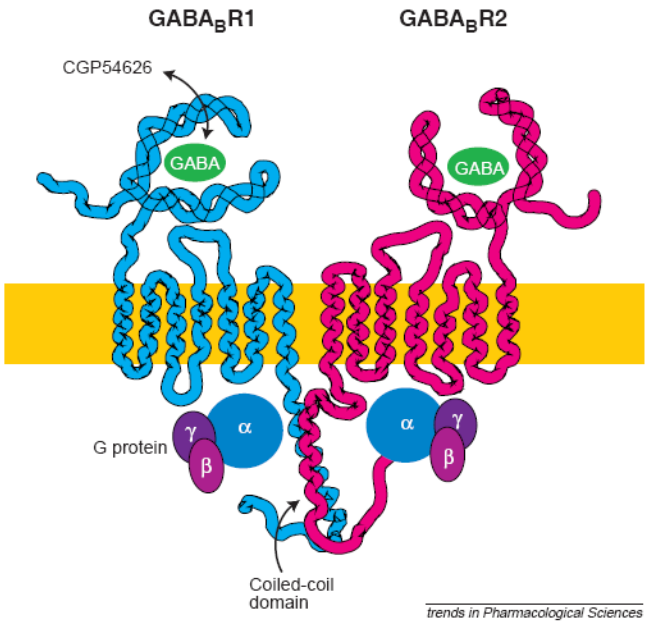
… first molecular experimental confirmation

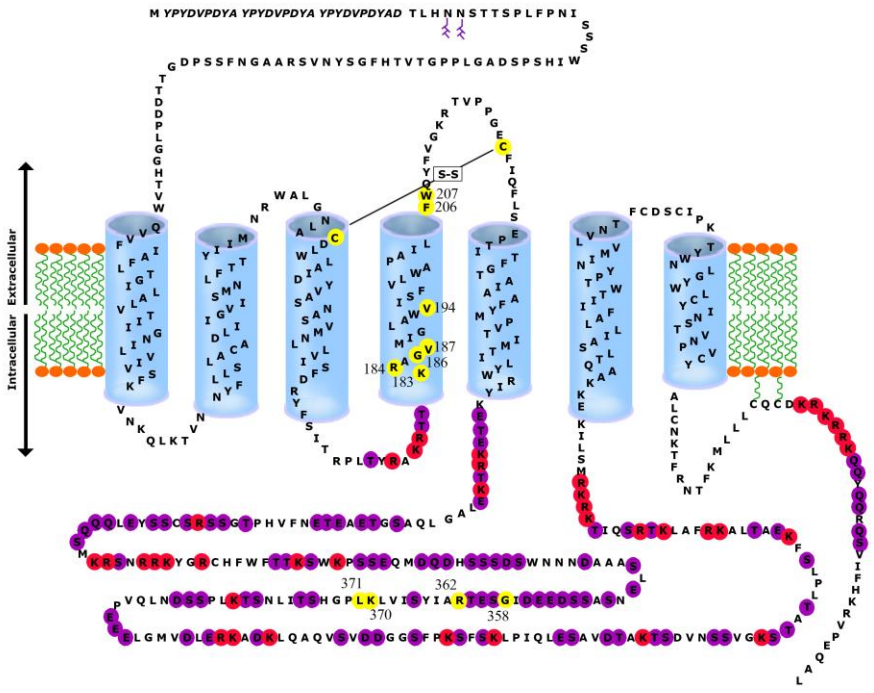
✓ The concept of GPCR heterodimer was later confirmed in an excellent way in 1998-99 by studies reporting that two non-functional GPCR monomers, GABAB1 and GABAB2, can assemble in a signaling heterodimer at the cell surface.

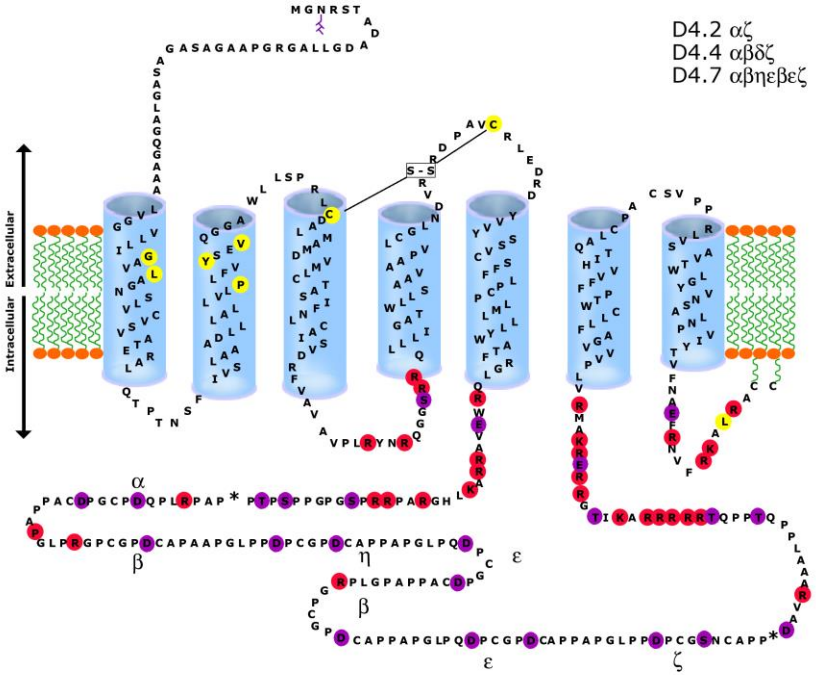
✓ The GABAB receptor belongs to the class C of GPCR with dimerization taking place between the venus flytrap modules and the C terminal coiled-coil domains

$GABA_B$ receptors – the first 7TM heterodimers

Fiona H. Marshall, Kenneth A. Jones, Klemens Kaupmann and Bernhard Bettler



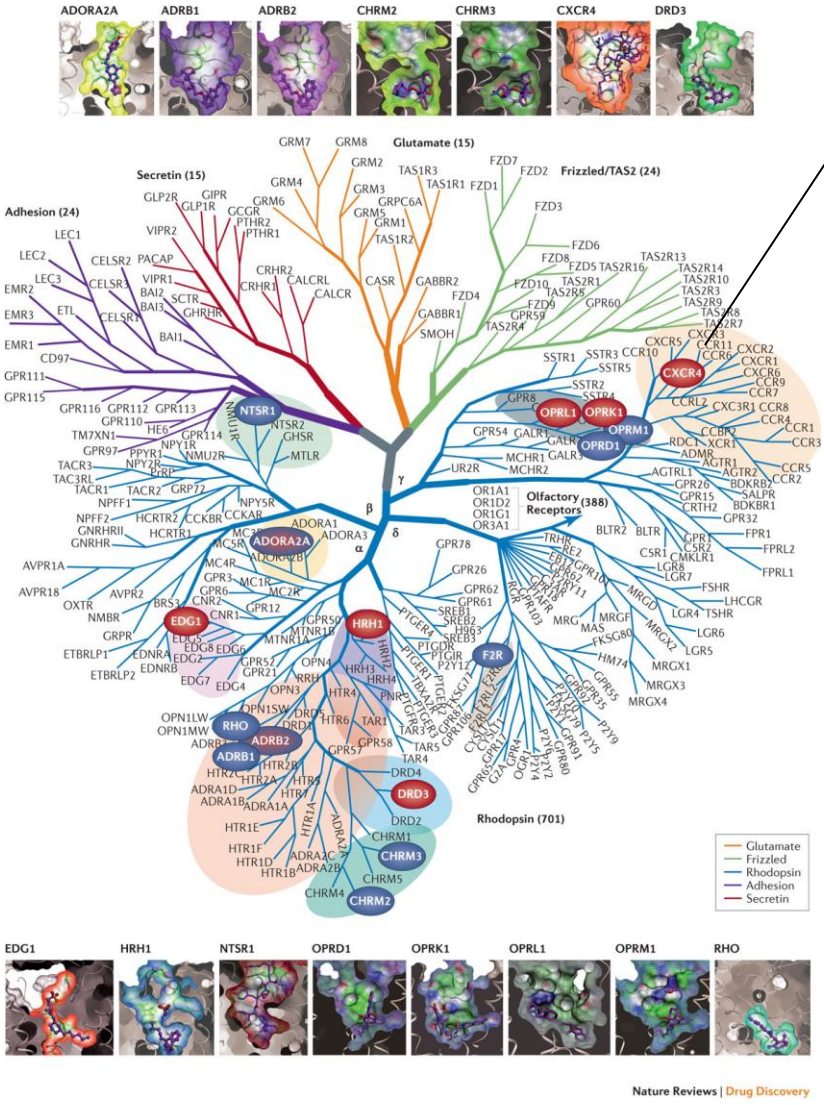




Borroto-Escuela et al. 2010

Phylogenetic tree representation of the human GPCR superfamily.

The GPCR Network: a large-scale collaboration to determine human GPCR structure and function



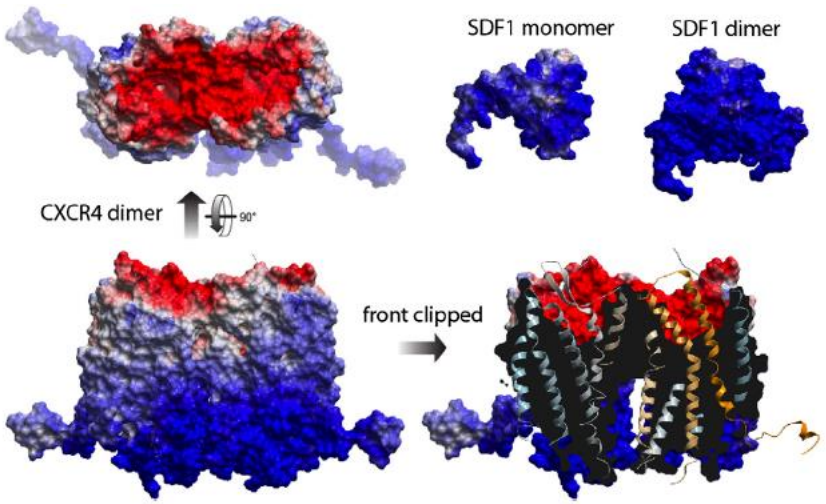
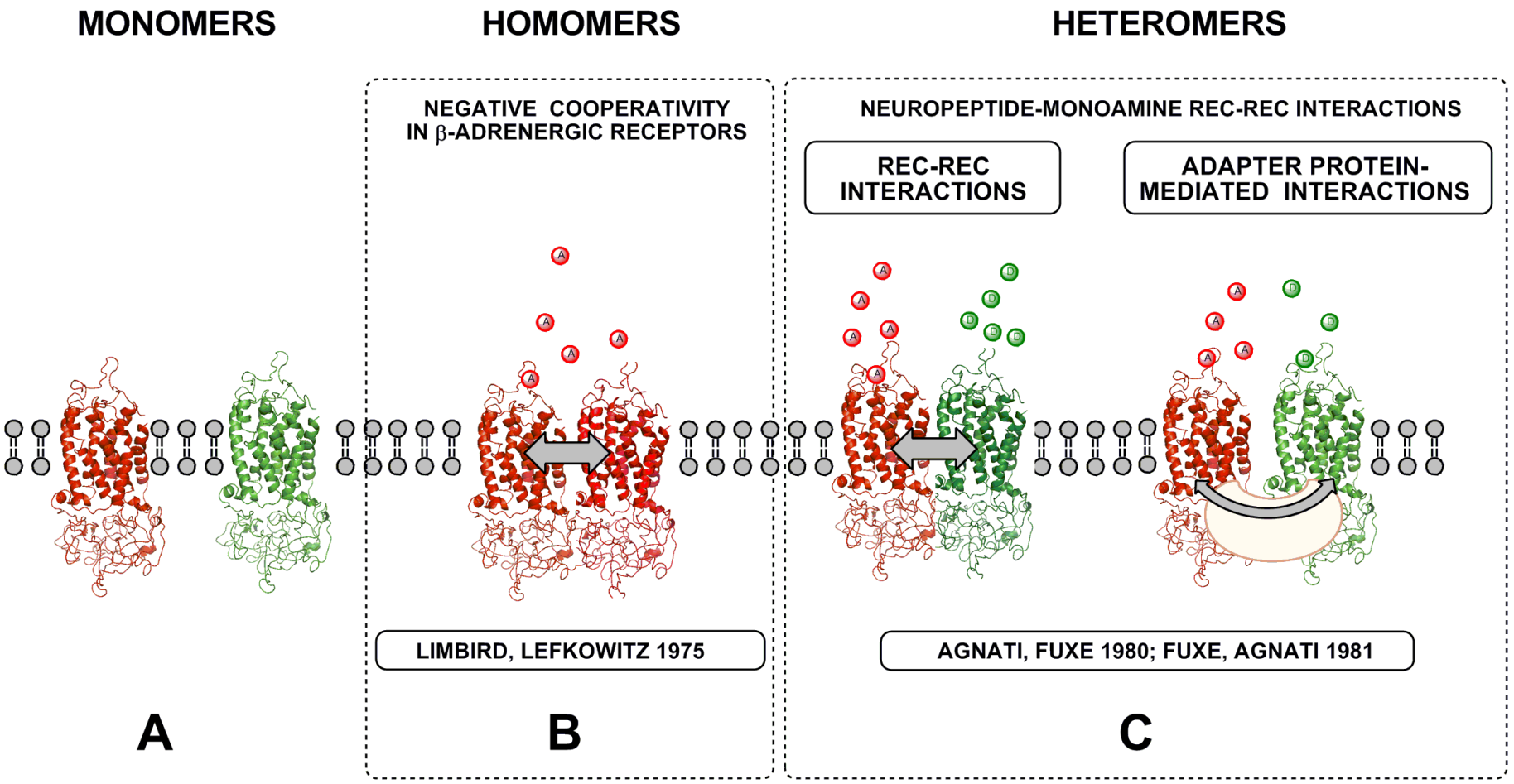


Fig. 5 – Structures of the CXCR4 dimer and the CXCL12 monomer and dimer (PDB ID 2J7Z) colored according to their electrostatic potential from red (negative) to blue (positive), in order to highlight the charge complementarity of these proteins. On the left, the CXCR4 structure is shown in two orientations — on the top looking into the ligand binding pocket and on the bottom, from the side of the dimer. The top right shows the monomer and dimer of CXCL12; the bottom right shows the structure of the CXCR4 dimer, clipped, in order to illustrate the binding pocket and that multiple stoichiometries and orientations of the CXCL12:CXCR4 seem feasible, as described in the text (no orientations are implied in the figure). Figures were prepared using ICM software (www.Molsoft.com)

Structures of the CXCR4 chemokine GPCR with small-molecule and cyclic peptide antagonists.

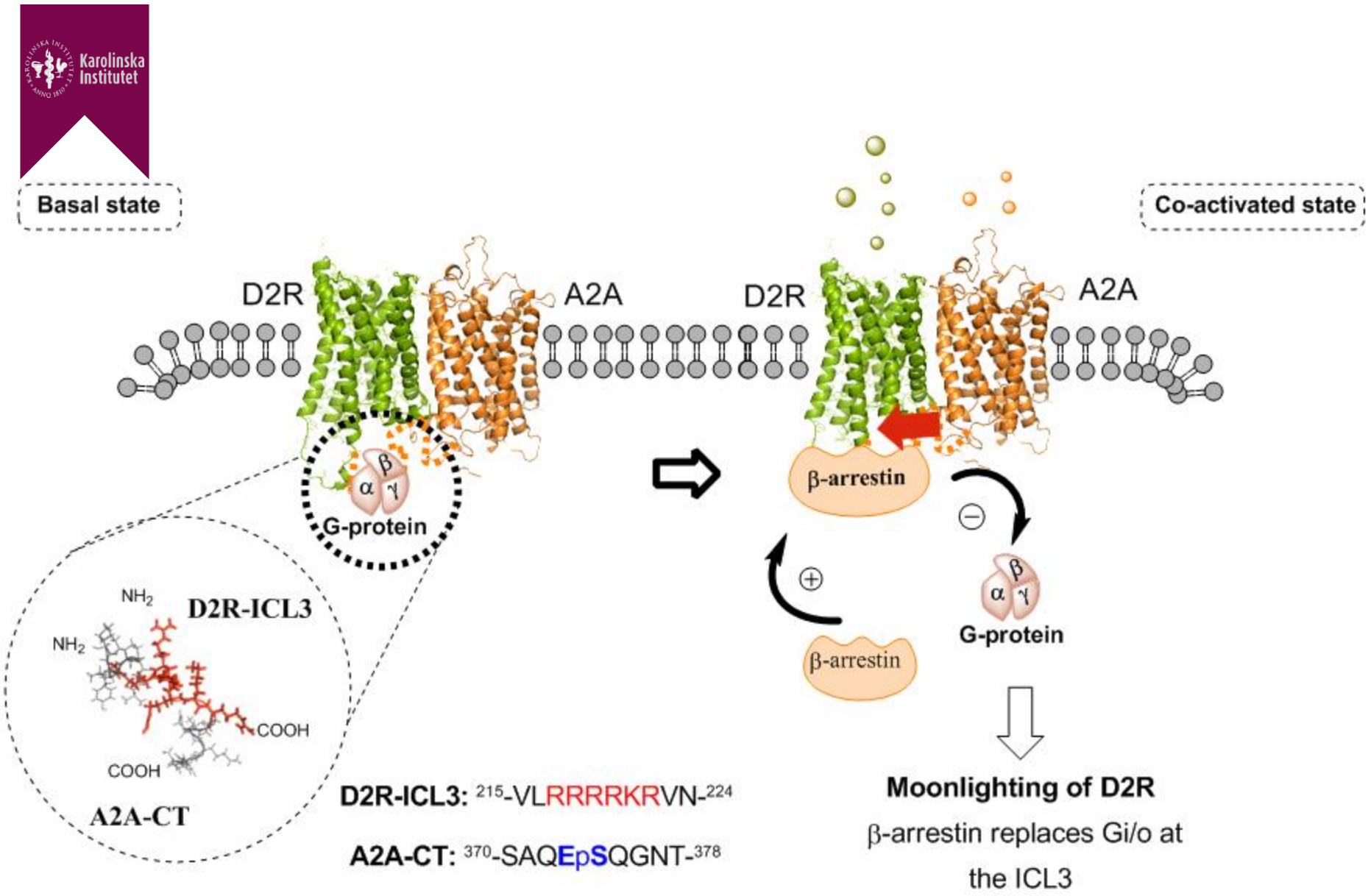
Wu et al. (2010) Science **330:** 1066-1071

RECEPTOR-RECEPTOR INTERACTIONS: A NEW INTEGRATIVE MECHANISM AT MEMBRANE LEVEL



Fuxe et al.2010

… diversity



Fuxe K. *et al* 2013

… promiscuity and specificity

The whole picture…the GPCR heterodimer network

http://www.gpcr-hetnet.com

Borroto-Escuela et al. 2014

… on the balance of homo and heteroreceptor complexes

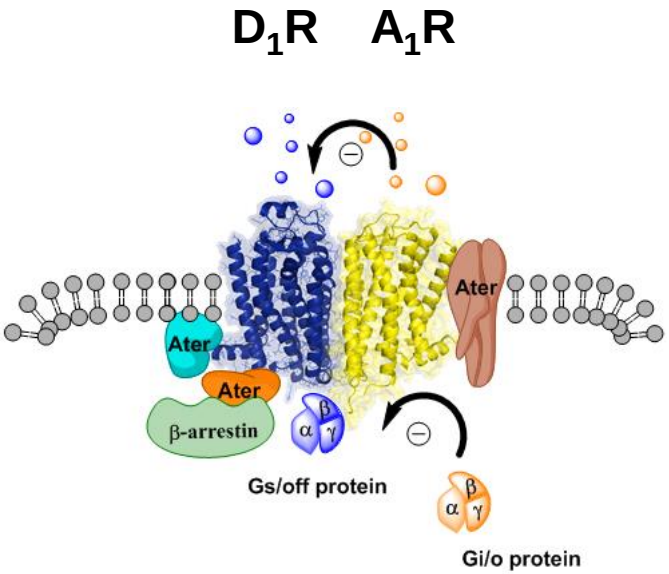
A1-D1 heteroreceptor complexes

✓ **Heteroreceptor complexes of A1Rs and D1Rs were demonstrated with coimmunoprecipitation** in cotransfected Ltk-fibroblast cells and later on in striatum using also this technique. With **BRET and FRET**, further evidence was later on obtained for their existence in A1R an D1R cotransfected cell lines.

✓**Antagonistic allosteric A1R-D1R receptor--receptor interactions** were found in these complexes as seen from the substantial reduction of D1Rs in the high-affinity state induced by A1R agonists in cellular models and in striatal membrane preparations.

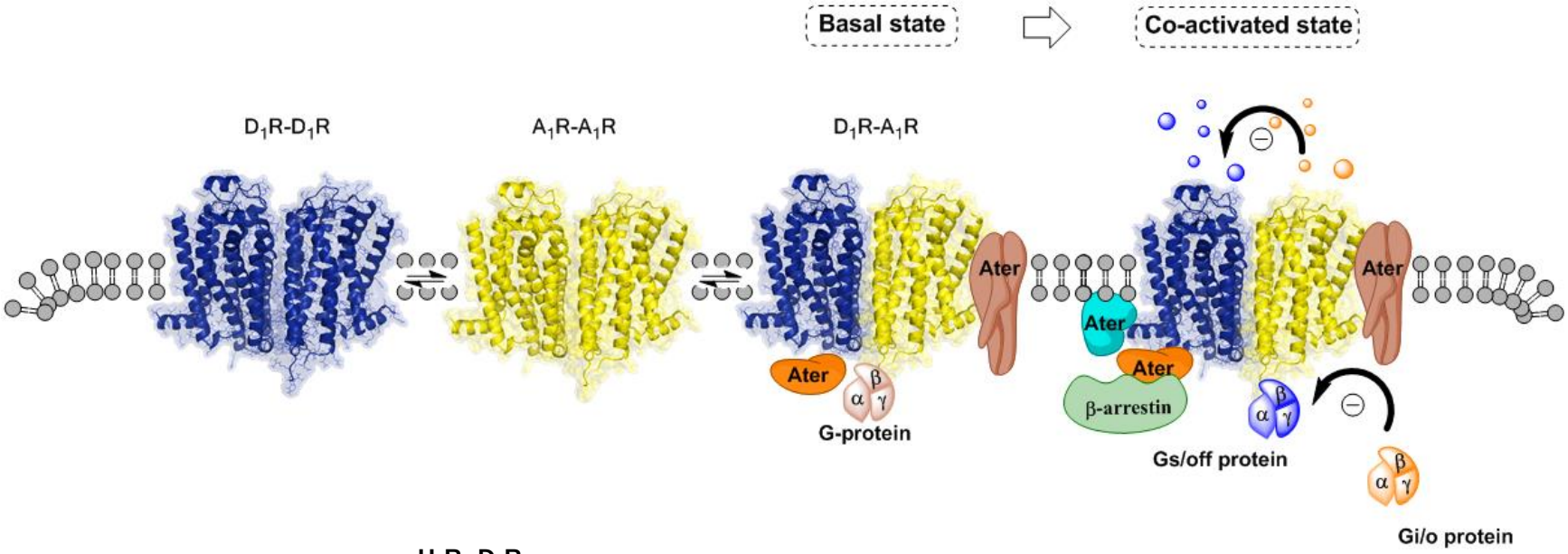
✓ the **Gi/o coupled A1R antagonistically also interact with the Gs/olf coupled D1R** at the AC level.

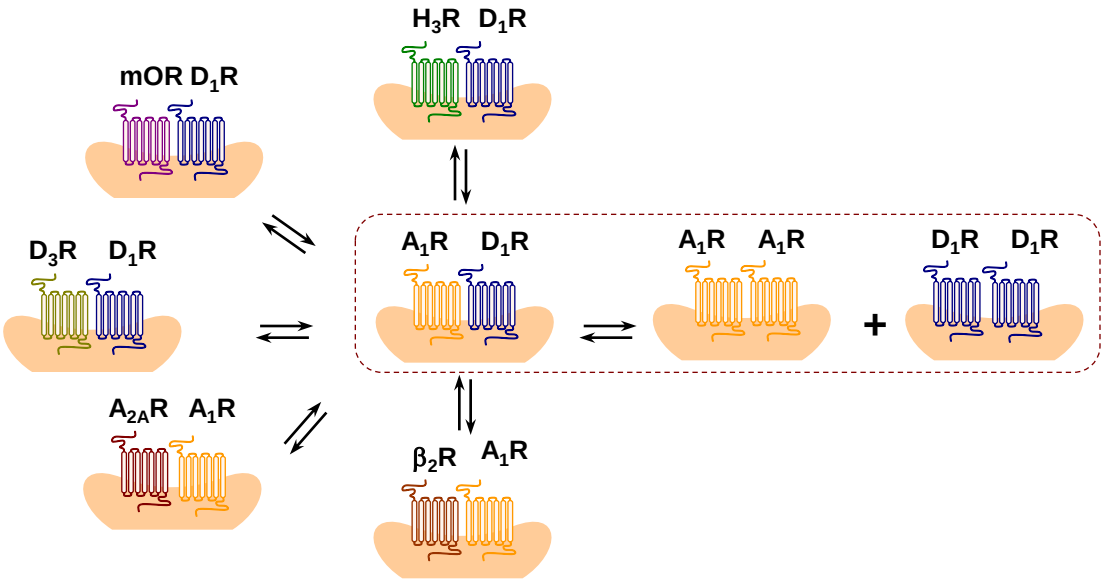
✓ **A1R agonists** in rabbits can **counteract D1R agonist-induced oral dyskinesias**



Gines et al. 2000, Torvinen et al. 2002, Franco et al. 2007

The balance of A1-D1 homo and heteroreceptor complexes





Antonelli et al.2006; Fuxe et al.2007,2010, Borroto-Escuela et al, 2015 a and b

… relevance for Parkinson's disease??

Table 1. Heteroreceptor complexes containing dopamine D1R subtype.

Heteroreceptor complex	Location	Signaling	Potential relevance for PD
D1R-D2R	Subsets of MSNs in accumbens (DYN, ENK, GABA/glutamate) [58]	$G_{q/11}$, Ca^{2+} release via IP_3, recruitment of BDNF via CaMK-II and MeCP2 are blocked by D1R and D2R antagonist. SKF83959 D1R-D2R heteromer agonist	Hyperdopaminergia increases function of D1R-D2R heteromer, mental side effects with L-DOPA and D2R agonist including addiction [60,179]
D1R-D3R	Certain striato-nigral GABA neurons [72]	D3R enhancement of D1R affinity and postsynaptic signaling	D3R enhances D1R-induced locomotion and dyskinesias [68,69]
D1R-A1R	Striato-endopeduncular-nigral GABA pathway and prefrontal cortex [44,138,139]	A1R agonist uncouples the D1R to its Gs/olf protein leading to desensitization. A1R agonist inhibits D1R-induced hyperactivity, EEG arousal, oral dyskinesias. Neuronal A1R increase kynurenic acid	A1R antagonists enhance motor activity. A1R agonists may reduce L-DOPA-induced dyskinesias [44,102,140]
D1R-D3R-A1R			Postulated [110,132]
D1R-NMDAR (NR1)	Hippocampus, striatum (synapse) [145,149]	D1R reduced NMDA currents and excitotoxicity, NMDA increased D1R signaling. D1R activation upregulates NMDA-dependent LTP and promotes working memory and NR1-CaMK-II coupling	Cognitive dysfunction after uncoupling of the receptor complex [180]

BDNF: Brain-derived neurotrophic factor; LTP: Long-term potentiation; PD: Parkinson's disease.

Fuxe et al. 2015

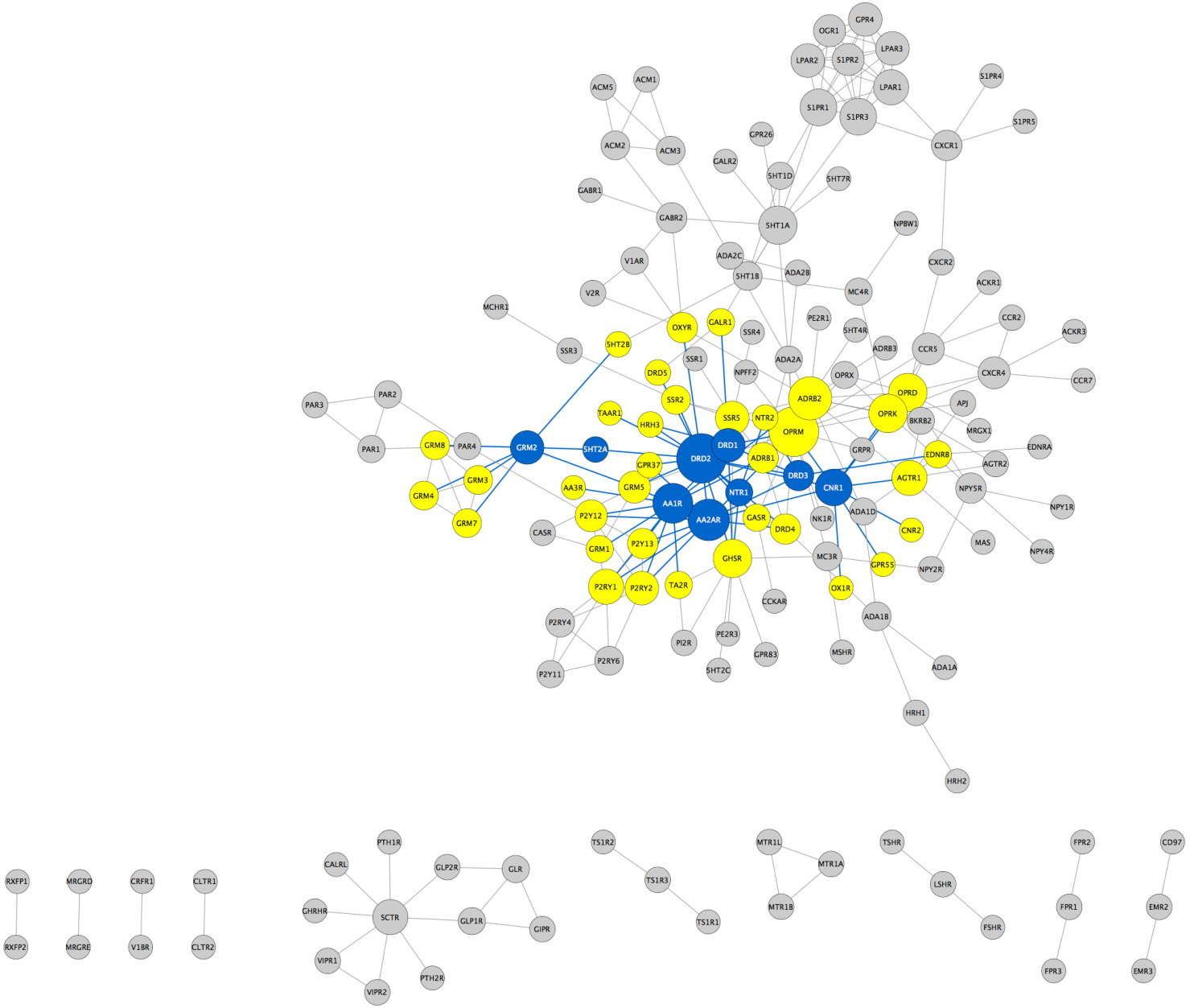
Table 2. Heteroreceptor complexes containing dopamine D2R subtype.

Heteroreceptor complex	Location	Signaling	Potential relevance for PD
D2R-D1R	Subsets of MSNs in accumbens (DYN, ENK, GABA/glutamate) [58]	$G_{q/11}$, Ca^{2+} release via IP_3, recruitment of BDNF via CaMK-II and MeCP2, blocked by D1R and D2R antagonist, inactivation of GSK-3b. SKF83959 D1R-D2R heteromer agonist	Hyperdopaminergia increases function of D1R-D2R heteromer, mental side-effects with L-DOPA and D2R agonist including addiction [60,179]
D2R-D3R	Ventral striatum [83]	Partial D2R agonists turn into D2R antagonists at the D2R-D3R heteromer	Partial D2R agonists in PD treatment have reduced mental side-effects [41,84]
D2R-D4R	Striatum [87,88]	Combined D2R and D4R agonist treatment resulted in potentiating effects on ERK1/2 phosphorylation for D4.2R, D4.4R but not for D4.7R containing heteromers [87]	The synergistic effects on ERK signaling may increase plasticity responses to L-DOPA treatment. The D4.7 variant may be linked to ADHD [87,88]
D2R-NMDAR (NR2B)	Striatal glutamate synapses [133]	This complex blocks CaMK-II-NR2B interaction with reduction of NR2B phosphorylation and NMDAR currents. Disruption of the D2R-NR2B complex reduced cocaine-induced locomotion The complex is increased by cocaine.	Reduction of dopamine VT in PD can reduce the formation of the D2R-NMDAR complex increasing the NMDA-mediated synaptic glutamate drive [133]
D2R-A2AR	Striato-pallidal GABA neurons, striatal cholinergic interneurons [44,46,47,103]	Antagonistic A2AR-D2R receptor–receptor interactions in the heteroreceptor complex and at the AC level. D2R recognition, Gi/o coupling and signaling reduced [45,181]. A2A agonist blocked D2R-induced LTD and restored LTP [109]	A2AR antagonists may significantly target the A2A protomer. They increase locomotion, contralateral turning behavior after subthreshold doses of L-DOPA and D2 like agonists. No worsening of dyskinesia. Antidepressant activity [107,110]
D2R-A2AR-mGluR5	Striato-pallidal GABA neurons.	A2AR-mGluR5 synergize to reduce D2R recognition and Gi/o coupling and signaling [45,120,124]. Interactions also at the level of the signaling cascades: MAPK and CREB-P. A2AR and mGluR5 agonists synergistically increase GABA release in ventral pallidum [125]	MGluR5 antagonists and negative allosteric modulators may significantly target the mGluR5 protomer. They increase locomotion and exert antiparkisonian actions and antidyskinetic actions specially combined with A2A antagonists [126,127,129,130]

AC: Adenylate cyclase; BDNF: Brain-derived neurotrophic factor; LTD: Long term depression; LTP: Long term potentiation; PD: Parkinson's disease; VT: Volume transmission.

Fuxe et al. 2015

Examples of GPCR heteroreceptor complexes relevance for PD.



Fuxe et al. 2015, Borroto-Escuela et al. 2015

Clinical pharmacology

Disease progression

… can be defined in term of changes in disease status as a function of time

In degenerative disorders such as PD, natural disease progression is caused by a continuous degeneration of dopaminergic neurons, which is reflected in such disease status measured as the UPDRS

Drug action

… reflects the effect of a drug on disease status

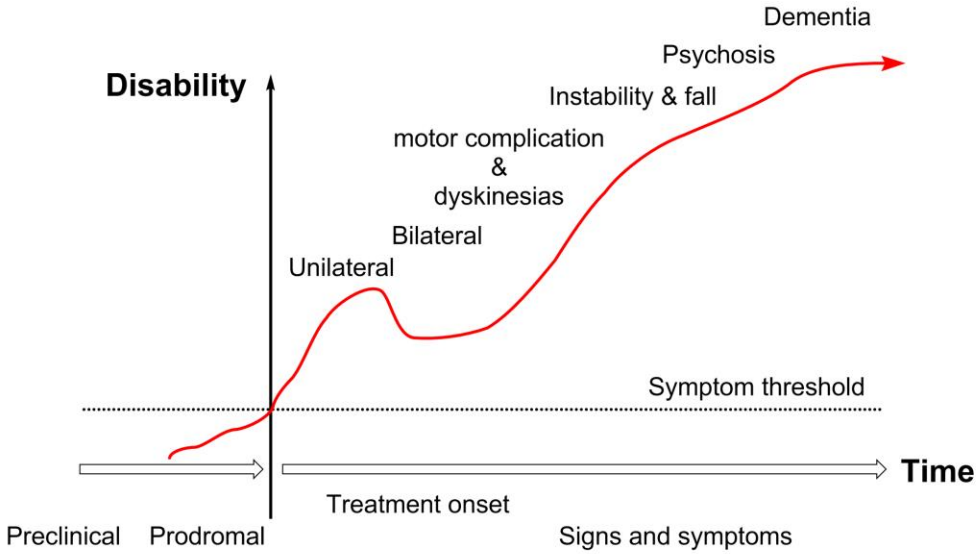
Drug may provide symptomatic benefit without influencing the underlying progression of the disease (**relieve clinical symptoms**)

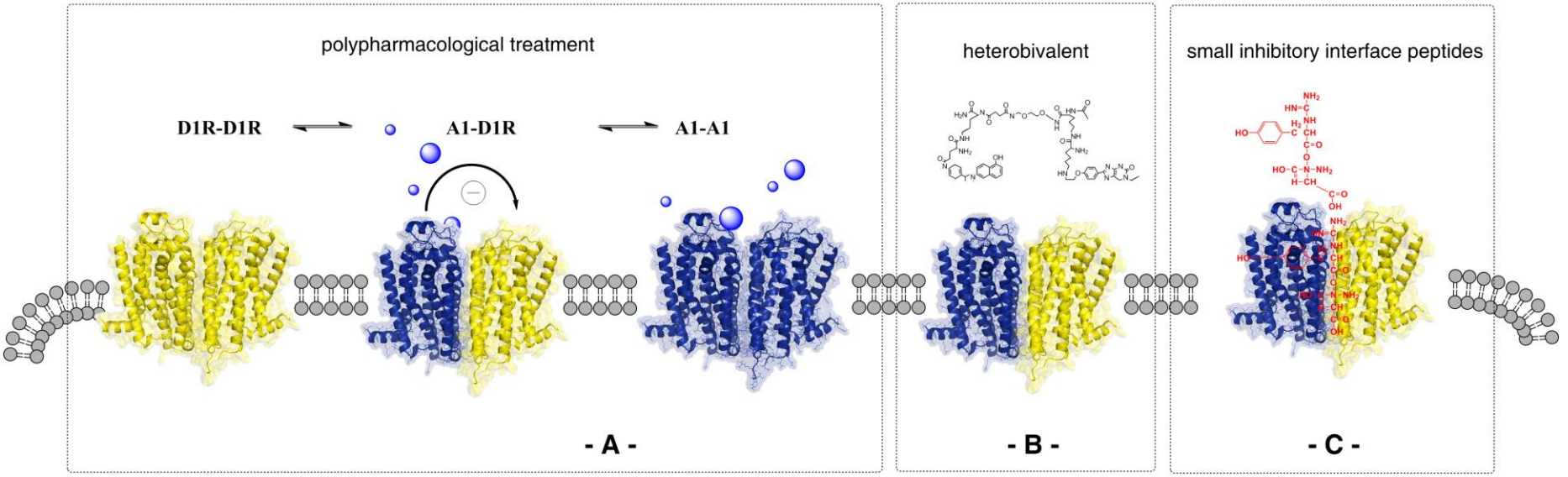
Or

Drug may influence the underlying time course of progression (**slow disease progression**)

We should consider to find out how the time course of levodopa effects might be modified as PD pregresses and how the different relevant dopamine homo and heteroreceptor complexes are altered during each specific disease status.

GPCR homo and heteroreceptor complexes balance on Parkinson´s disease progression and treatment

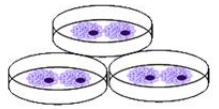


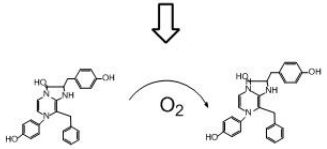


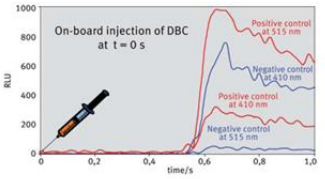
… are we technological ready?

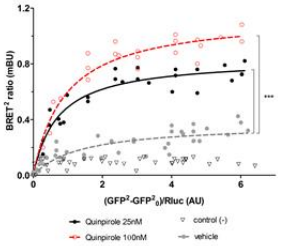
Light Resonance Energy Transfer-based methods

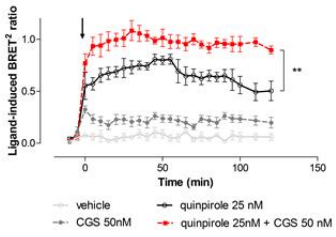
- ✓ Single cell BRET imaging
- ✓ Intra-molecular FRET/BRE
- ✓ TR-FRET
- ✓ BiFC
- ✓New FRET- and BRET-based second messenger sensors

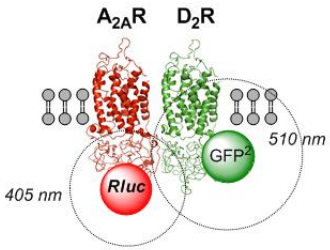


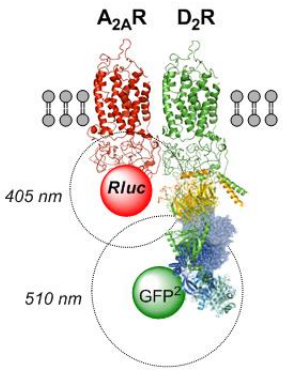


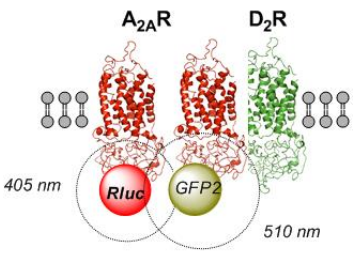








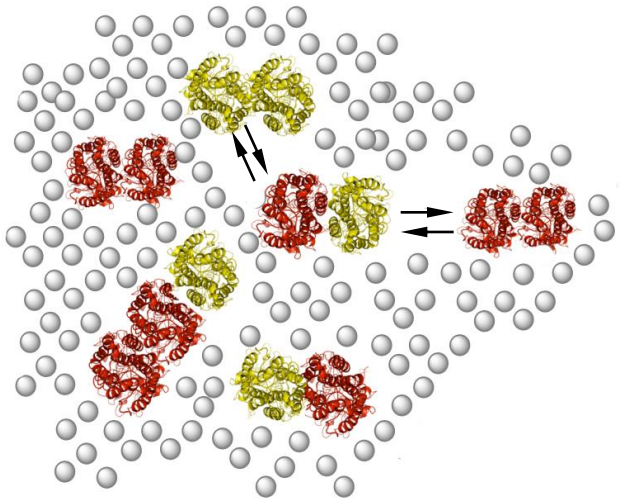


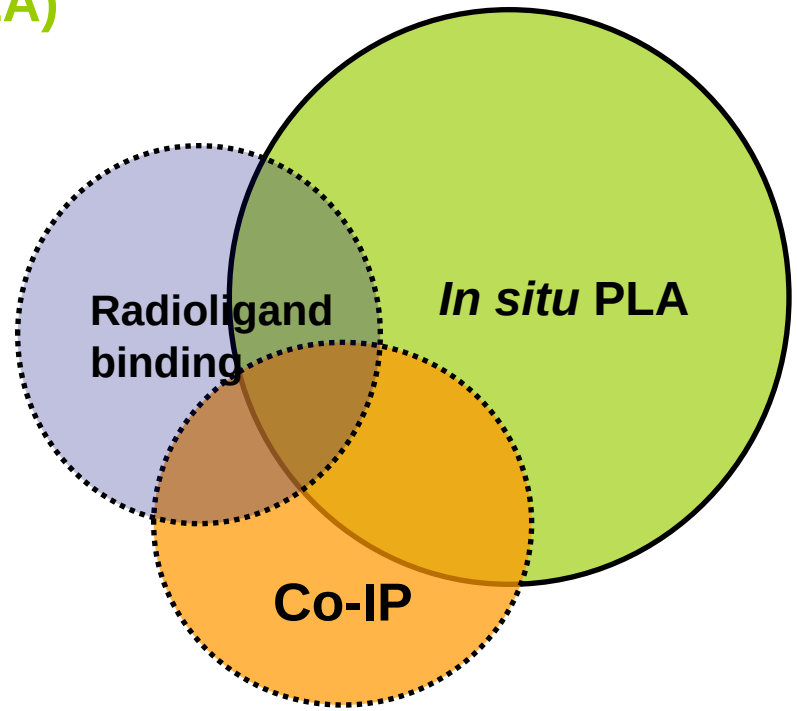


Borroto-Escuela et al. 2013

METHODS

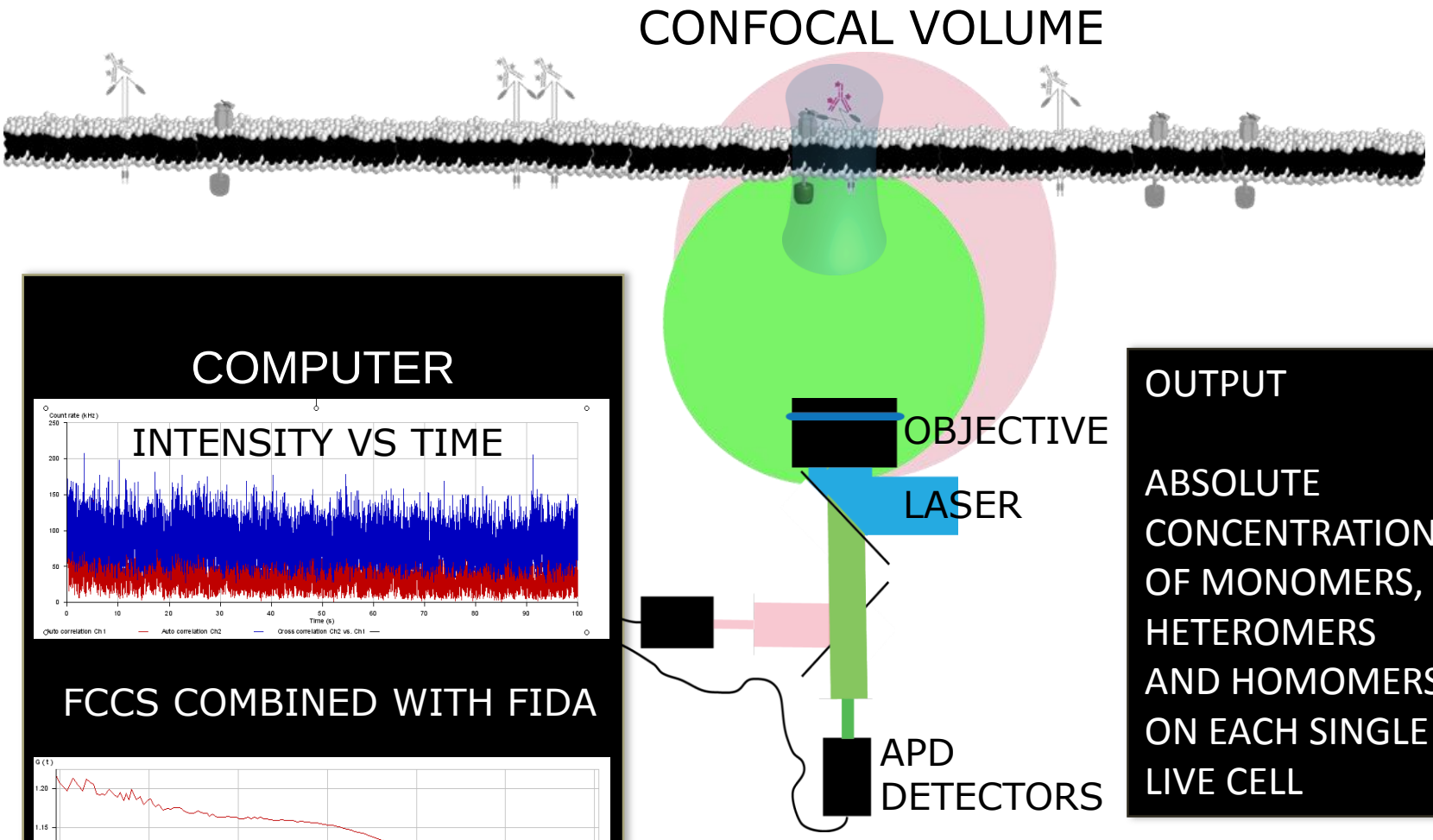
- **Radioligand binding assay**
- **Coimmunoprecipitation**
- Split-ubiquitin Membrane Yeast Two Hybrid system (MYTHS)
- **Fluorescence Cross Correlation Spectroscopy**
- Receptor activation by biased ligands and small interface interfering peptides (SIIP)
- Phosphoproteomics
- NanoScan PET/MRI scanner
- Generation and phenotypical analysis of knock-in rats
- **In situ Proximity Ligation Assays (PLA)**





Borroto-Escuela 2014, 2015, Fuxe 2014, 2015

Fluorescence Cross Correlation Spectroscopy (FCCS)



APICONISIS

In situ PLA

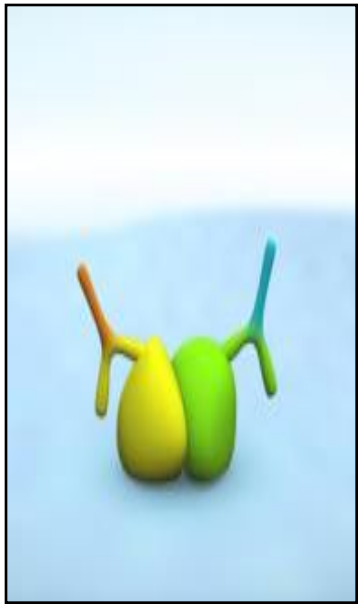
Conjugation. A pair of primary antibodies bind to the proteins to be detected

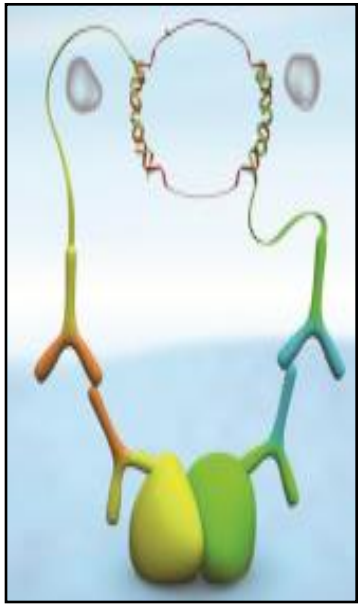
A pair of PLA probes (PLUS and MINUS) bind their respective primary antibody

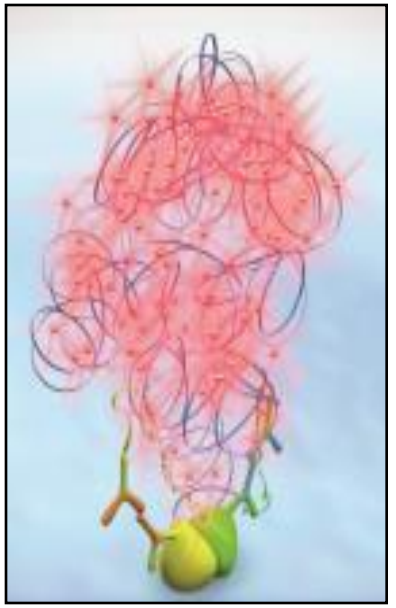
Two connector oligo nucleotides are joined to form a circular molecule by a ligase

A polymerase replicates the circle, producing an concatemeric product

Detection of hybridization by oligonucletide tagged with fluorescent compound.







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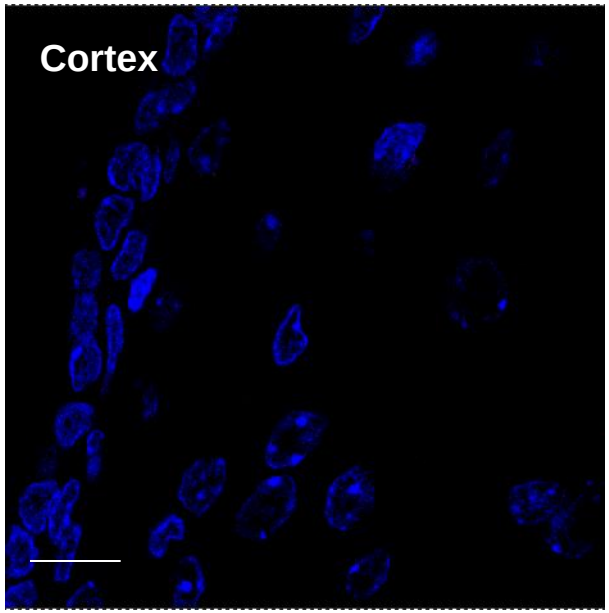
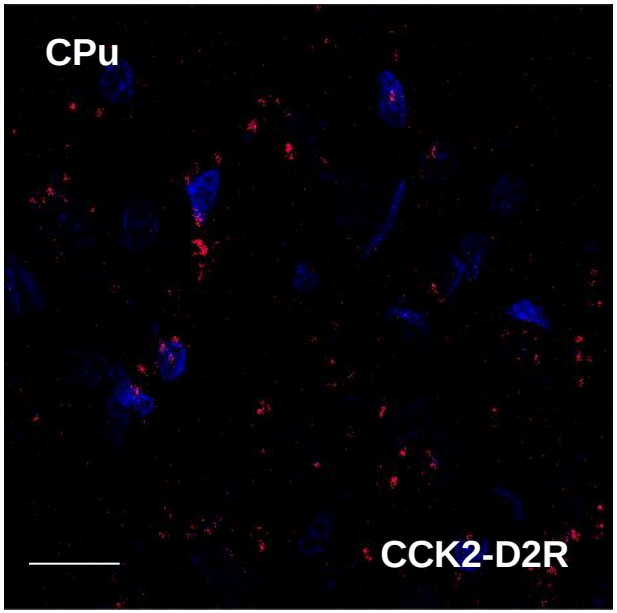
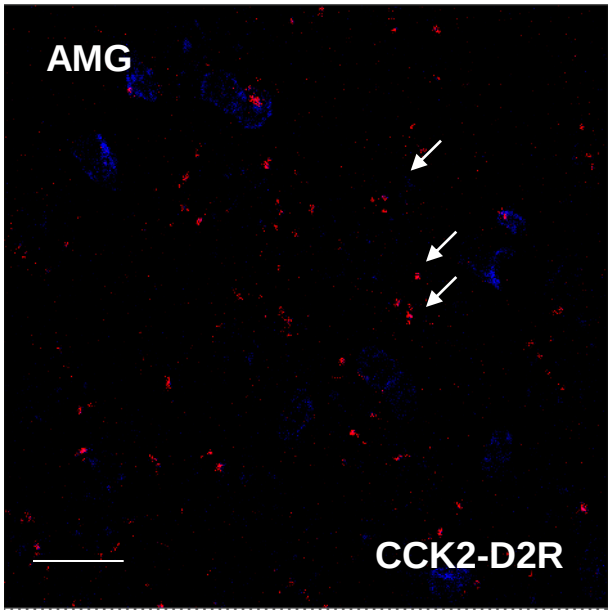
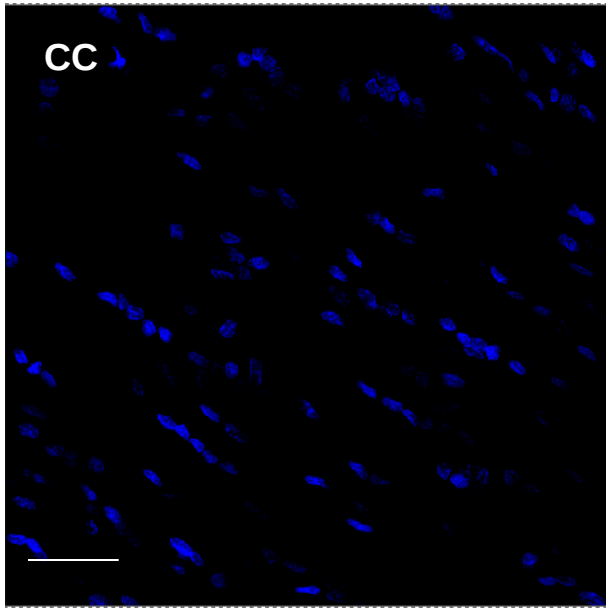
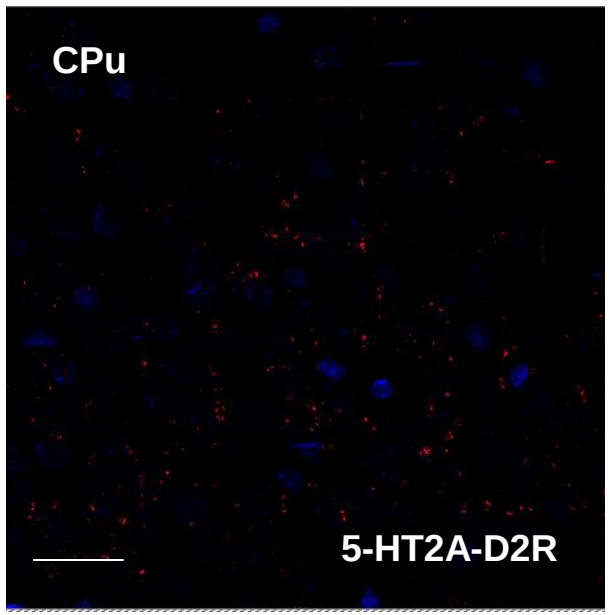
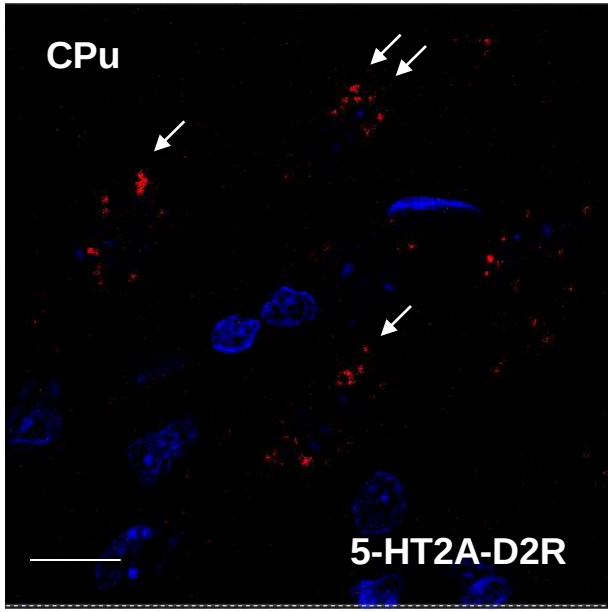

Cortex


CPu
CCK2-D2R

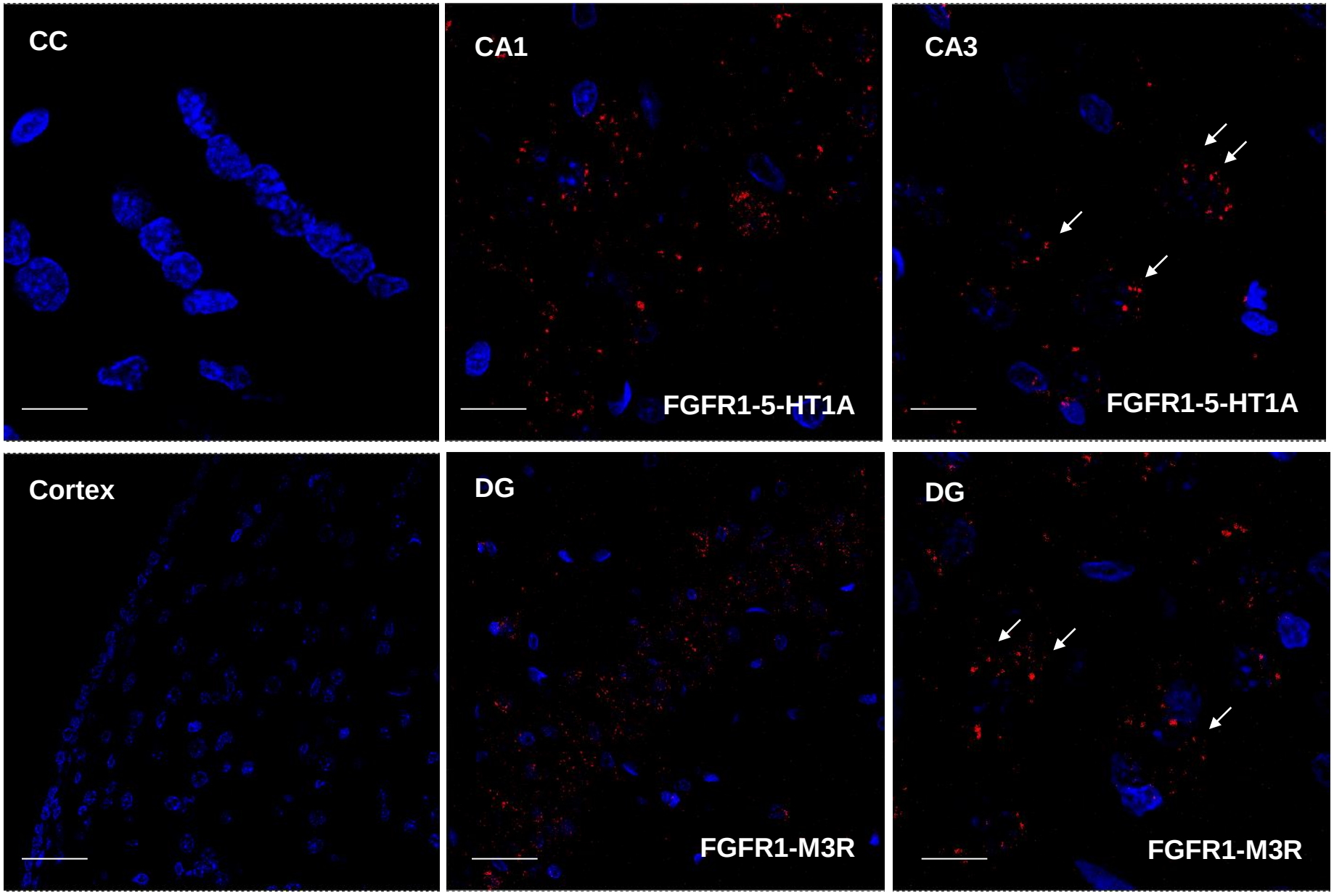

AMG
CCK2-D2R


CC


CPu
5-HT2A-D2R

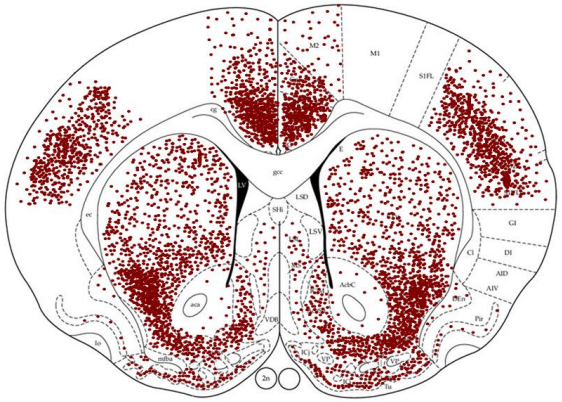

CPu
5-HT2A-D2R

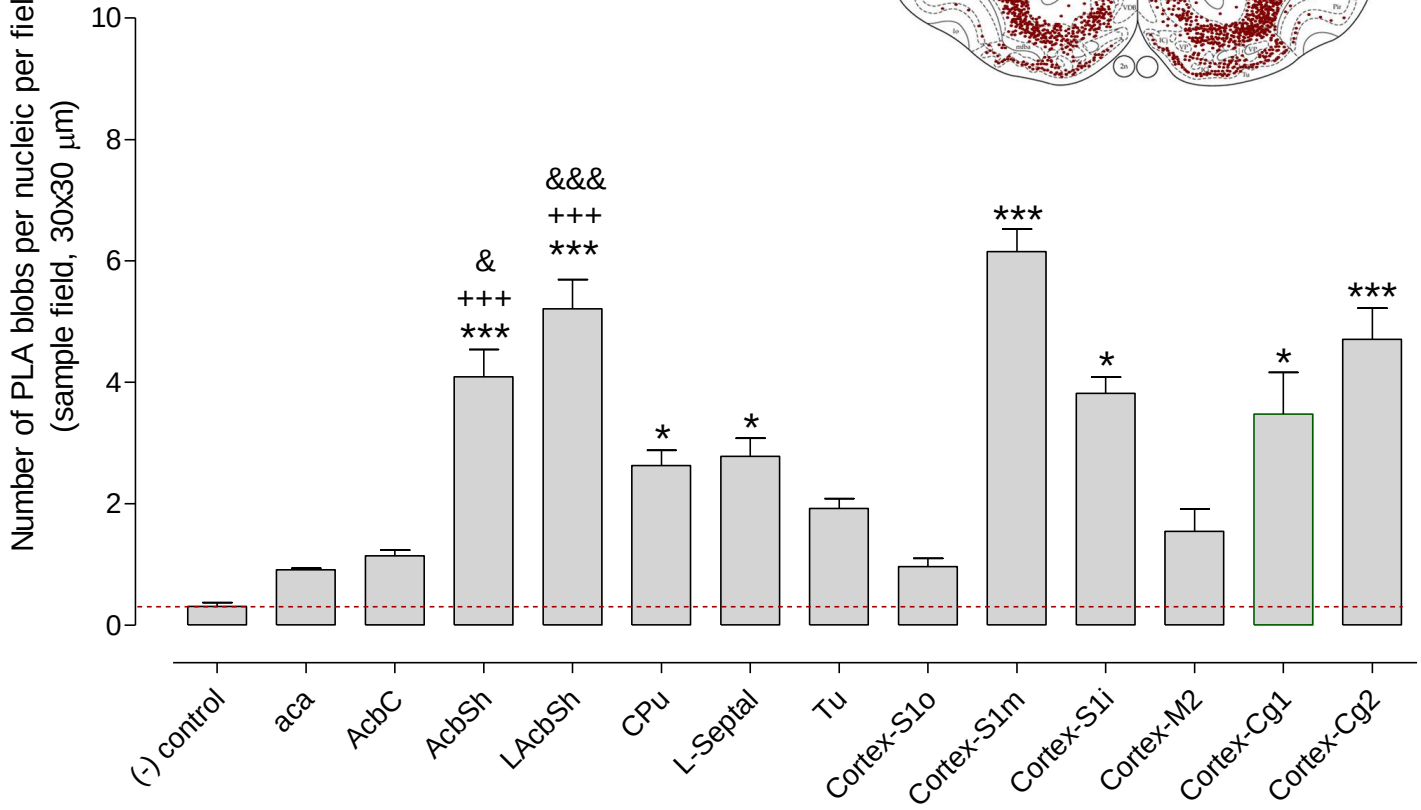
Examples

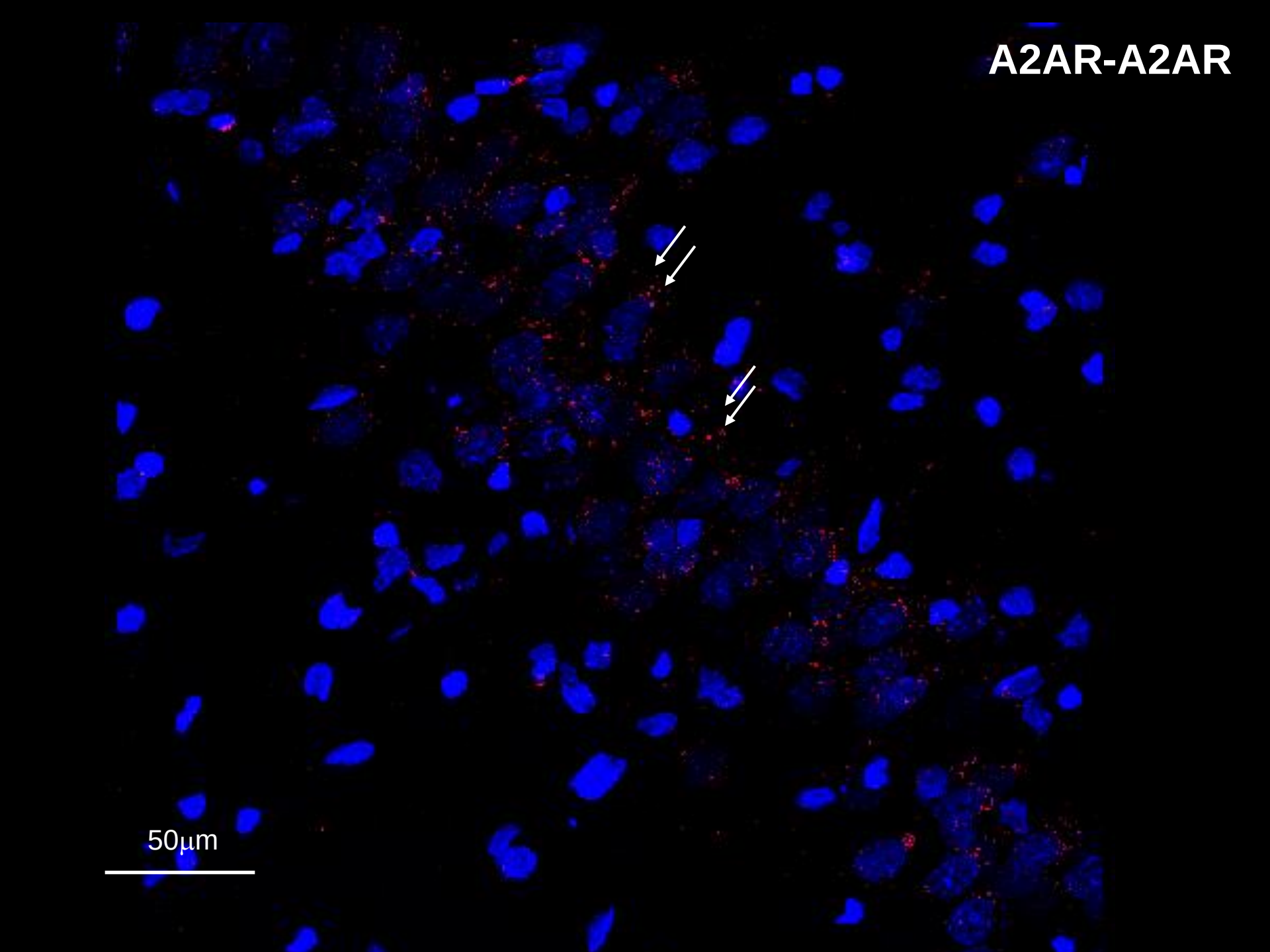

CC
CA1
FGFR1-5-HT1A
CA3
FGFR1-5-HT1A
Cortex
DG
FGFR1-M3R
DG
FGFR1-M3R

A2AR-mGluR5

Bregma 1.00 mm

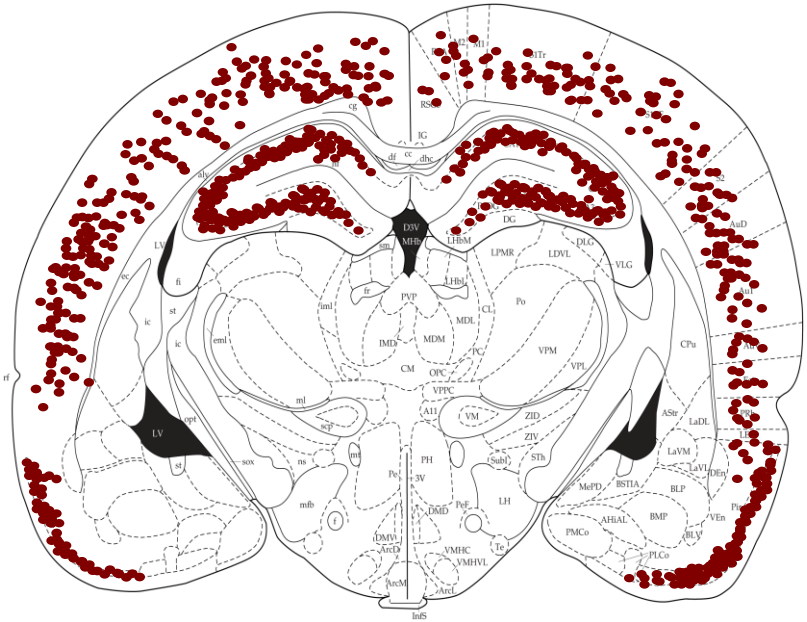


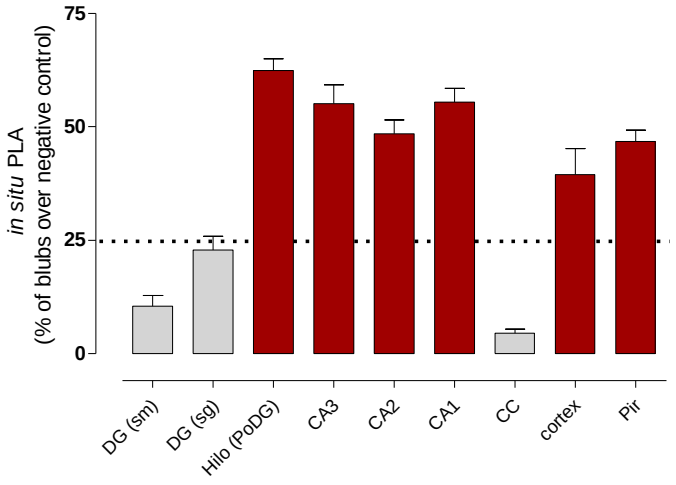



A2AR-A2AR
50μm

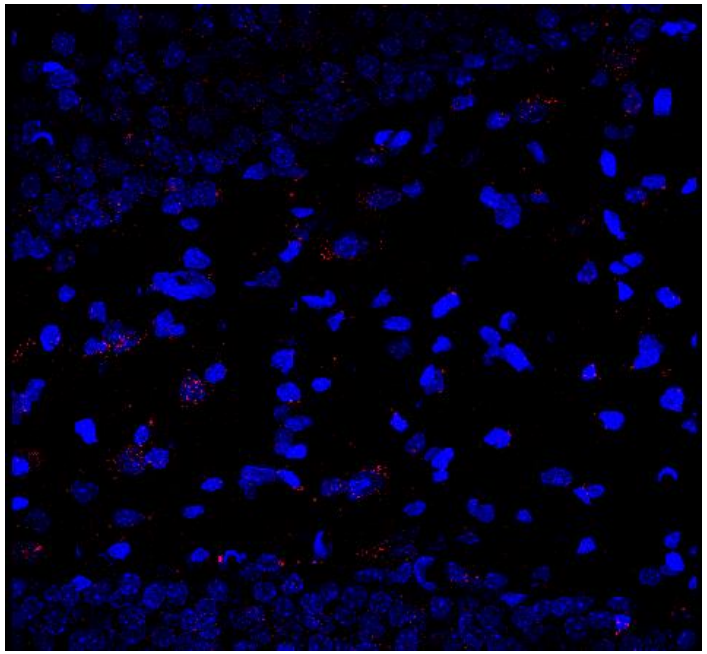
A2AR-A2AR heteroreceptor complexes in the rat cortex and hippocampus

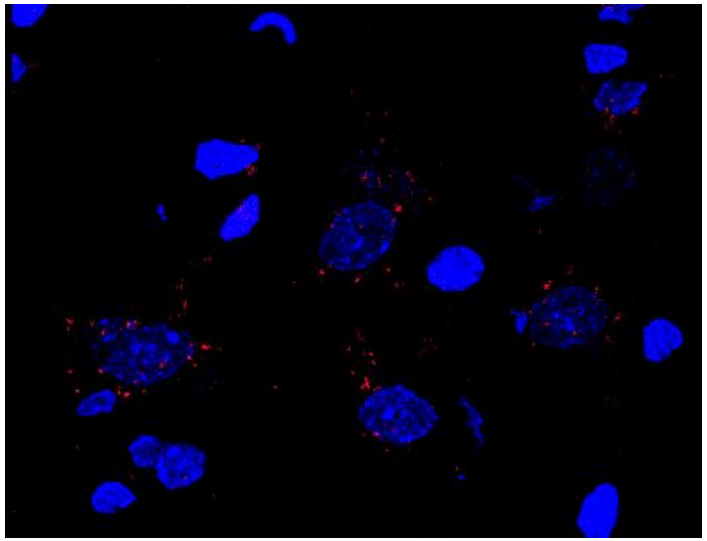
[preliminary results]



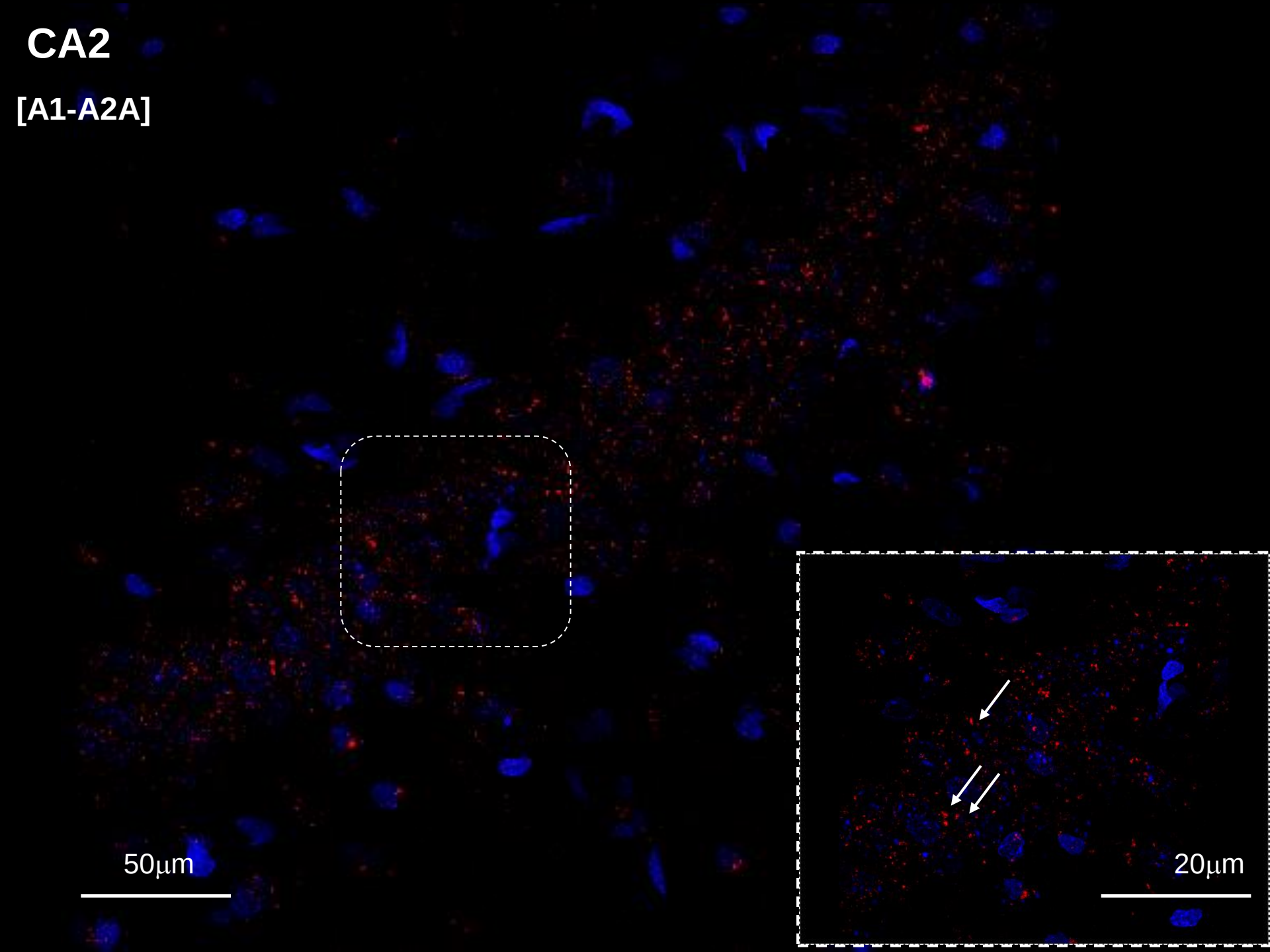


[A2AR-A2AR heteroreceptor complexes **WT**]



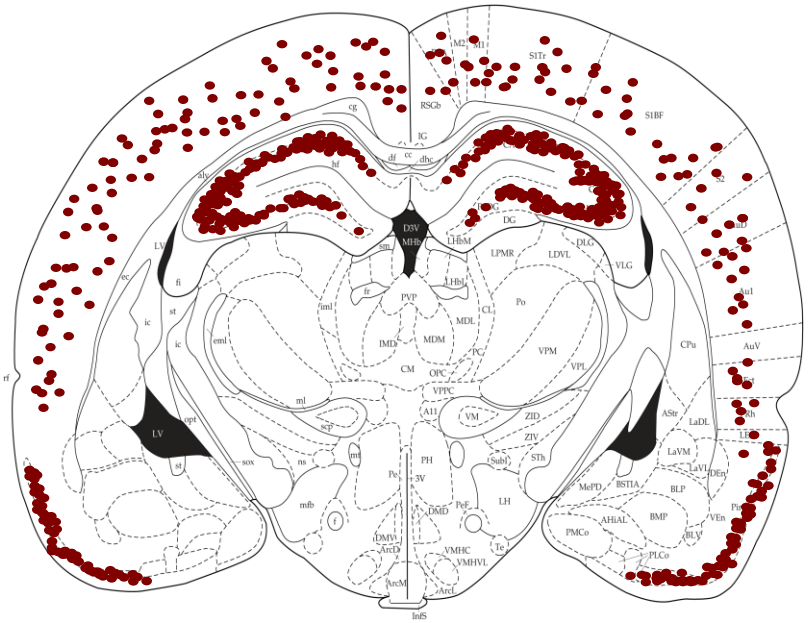


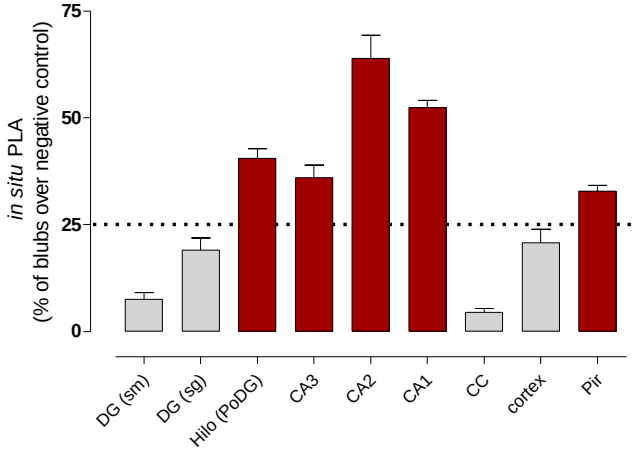
Borroto-Escuela *et al.* 2014


CA2
[A1-A2A]
50μm
20μm

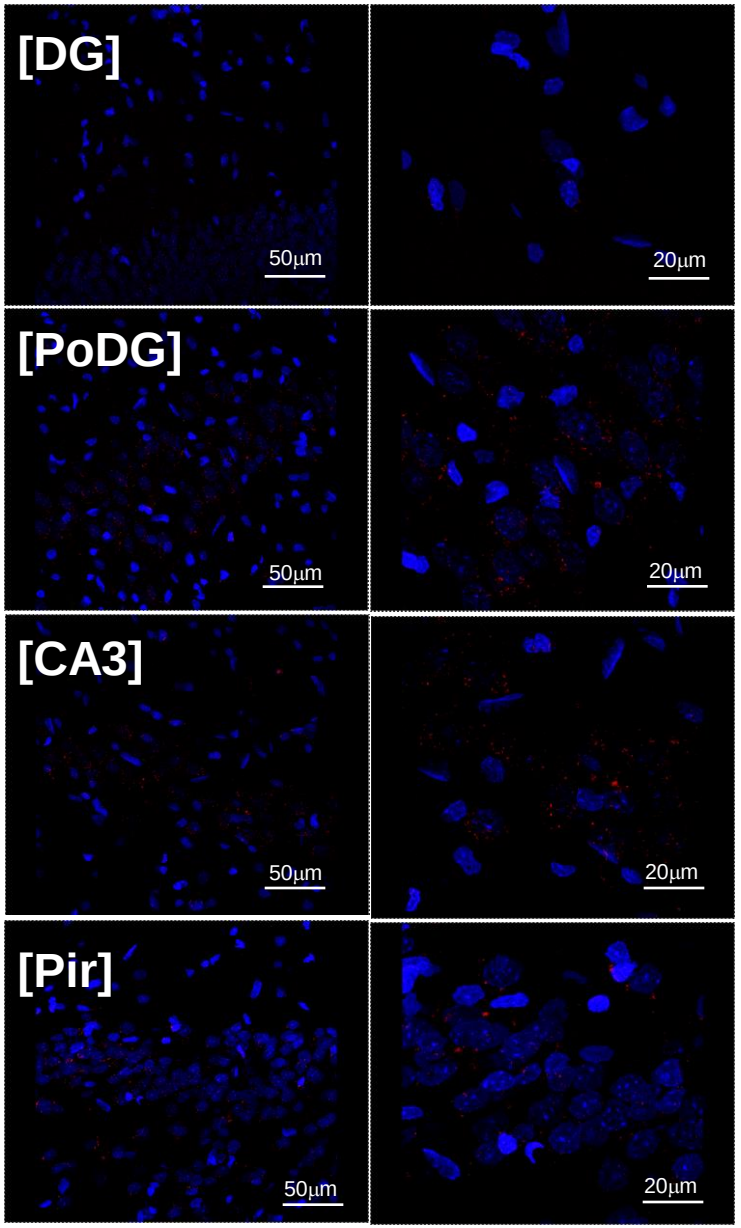
A1R-A2AR heteroreceptor complexes in the rat cortex and hippocampus

[preliminary results]





[A1R-A2AR heteroreceptor complexes **WT**]



Borroto-Escuela *et al.* 2014

Usually four **important parameters** should be kept in mind **for a proper analysis and in situ PLA result interpretation**:

- ❑ the number of DAPI nuclei in the sample field.
- ❑ the number of positive PLA/dots per sample field
- ❑ the total number of positive PLA cells/nuclei per sample field.
- ❑ the diameter sizes of the individual PLA blobs (the diameter may indicate if aggregates (higher order) of receptor complexes exists).

Within these four values it will be possible to get an overall view of the expression/enrichment of GPCR homo/heteroreceptor complexes in the different brain areas analyzed and extract relevant conclusions from the comparisons between brain areas.
Certainly, **we cannot compare or determine directly a balance between the homo- and heteroreceptor complexes populations in the same tissue using the *in situ* PLA approach**, because of a technical limitation of the procedure itself. But the method could help us determine each population independently and compare their relative expression levels after an appropriate numerical analysis.

Borroto-Escuela et al. 2015

Open to Discussions… ¿?

✓ We have proposed **the molecular phenomenon of receptor-receptor interactions** as a fruitful way **to understand how brain function can increase through molecular integration of signals**.

✓ An **alteration in specific receptor-receptor** interactions or their balance/equilibrium (with the corresponding monomers-homomers) **are indeed considered to have a role in the pathogenic mechanisms** that lead to various brain diseases.

✓ Therefore, targeting protomer-protomer interactions in heteroreceptor complexes or **changing the balance with their corresponding homoreceptor complexes** in discrete brain regions **may become an important field for developing novel drugs**, including hetero-bivalent drugs and optimal types of combined treatments.

✓ The analysis of animal or human brain material with *in situ* PLA can reveal if the **relative abundance of specific homo-and heteroreceptor complexes in discrete brain regions is altered in brain diseases** or under certain drug treatments, for instance, chronic L-dopa treatment in Parkinson's disease.

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www.iiia.csic.es/~ismel/GPCR-Nets/index.html

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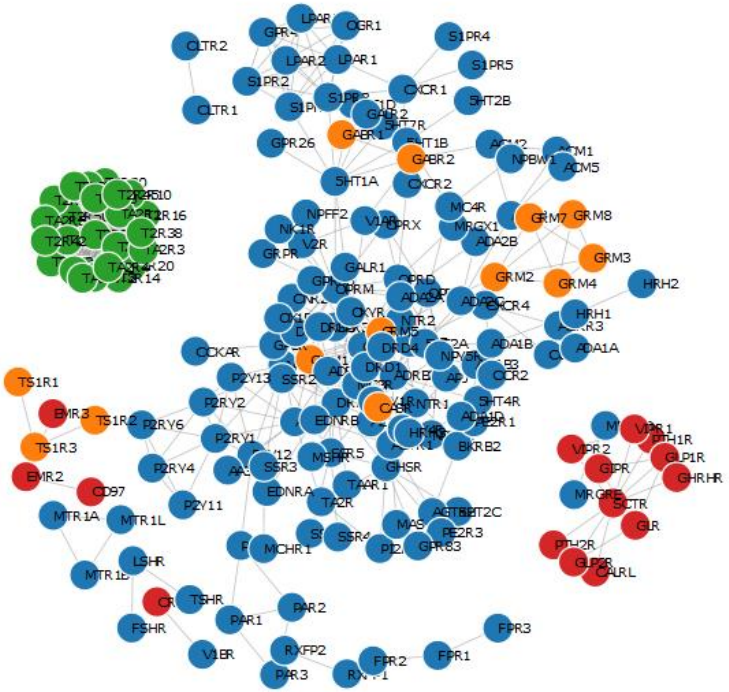
GPCR networks

HetNet NonHetNet

Search protomer by UniProt name



Zoom - +

Protomer families

G-protein coupled receptor 1
G-protein coupled receptor 2
G-protein coupled receptor 3
G-protein coupled receptor T2R

Network topology

Num. of protomers = 181
Num. of interactions = 523
For further info click here