

# On the role of A1-D1 heteroreceptor complexes in the direct pathway in models of 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  
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**Karolinska  
Institutet**



International Conference on  
**Parkinson's Disease & Movement Disorders**

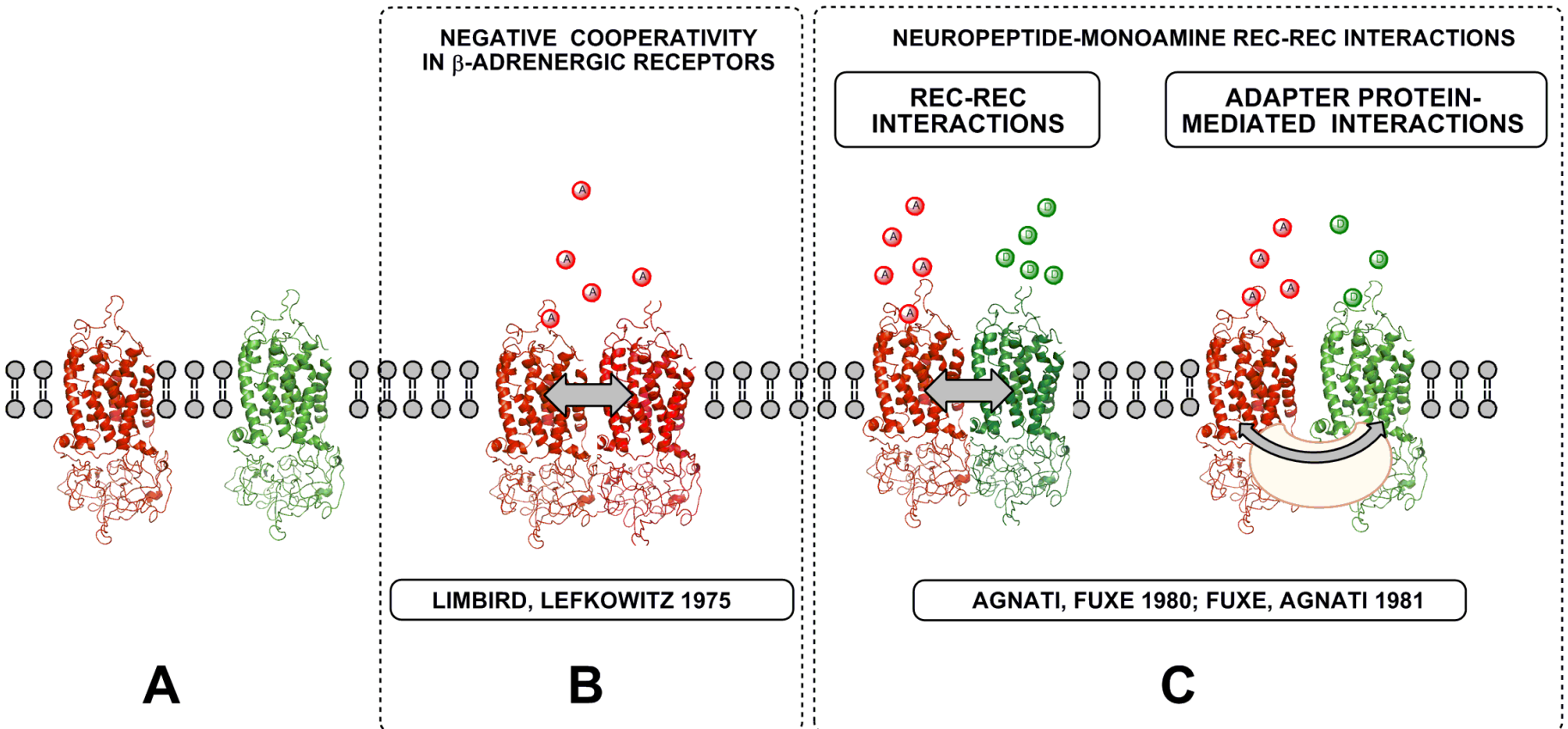
August 11-13, 2015 Frankfurt, Germany

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

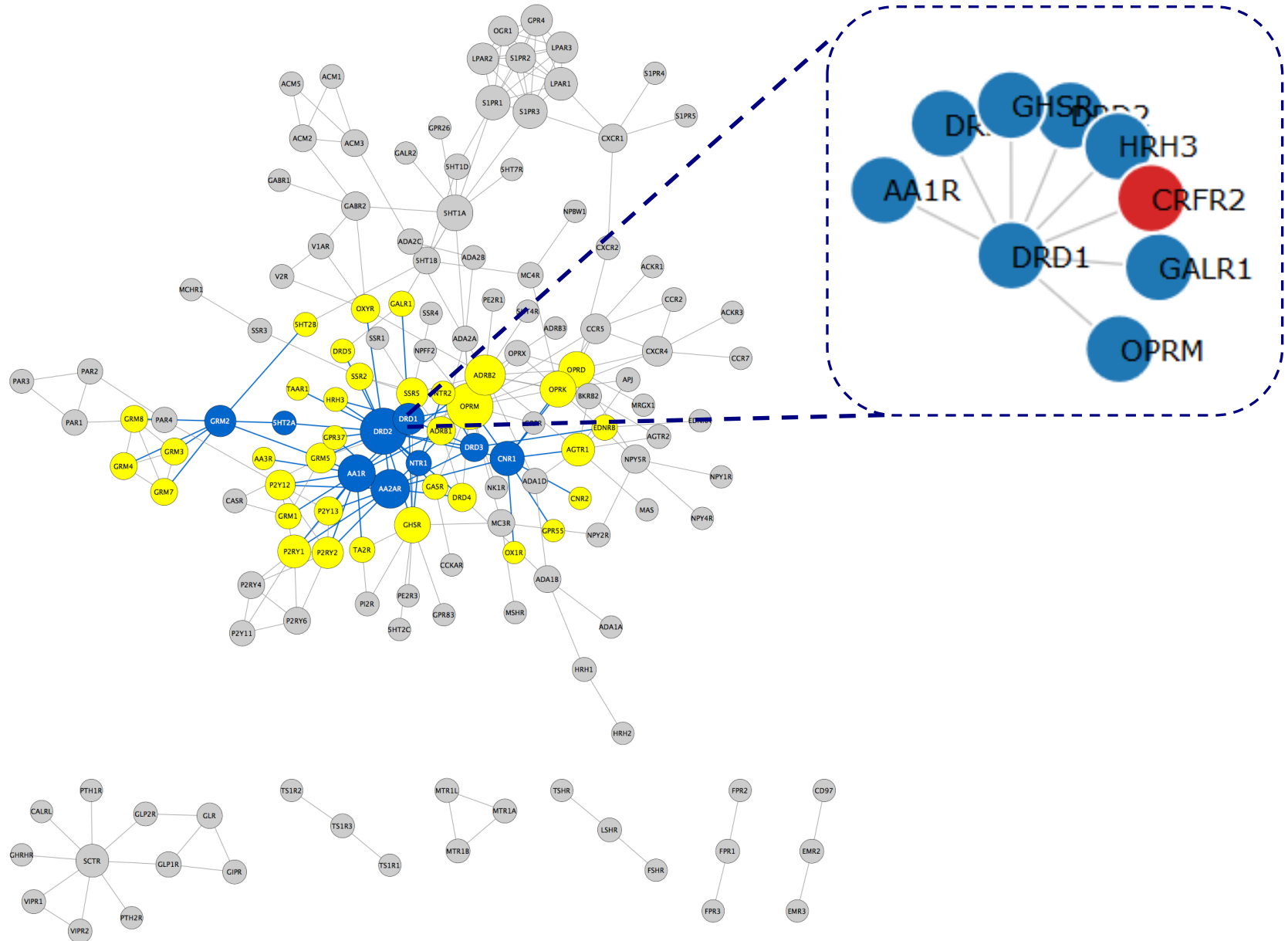
## MONOMERS

## HOMOMERS

## HETEROMERS



# Examples of GPCR heteroreceptor complexes that may be relevant for PD



# A1-D1 heteroreceptor complexes

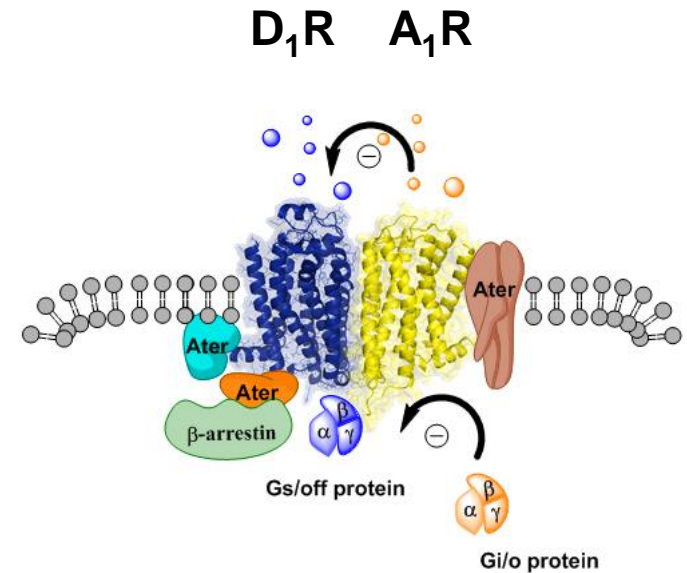
✓ **Heteroreceptor complexes of A1Rs and D1Rs were demonstrated with coimmunoprecipitation in cotransfected Ltk-fibroblast cells and later on in striatum using also this technique. With BRET and FRET, further evidence was later on obtained for their existence in A1R and D1R cotransfected cell lines.**

✓ **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.**

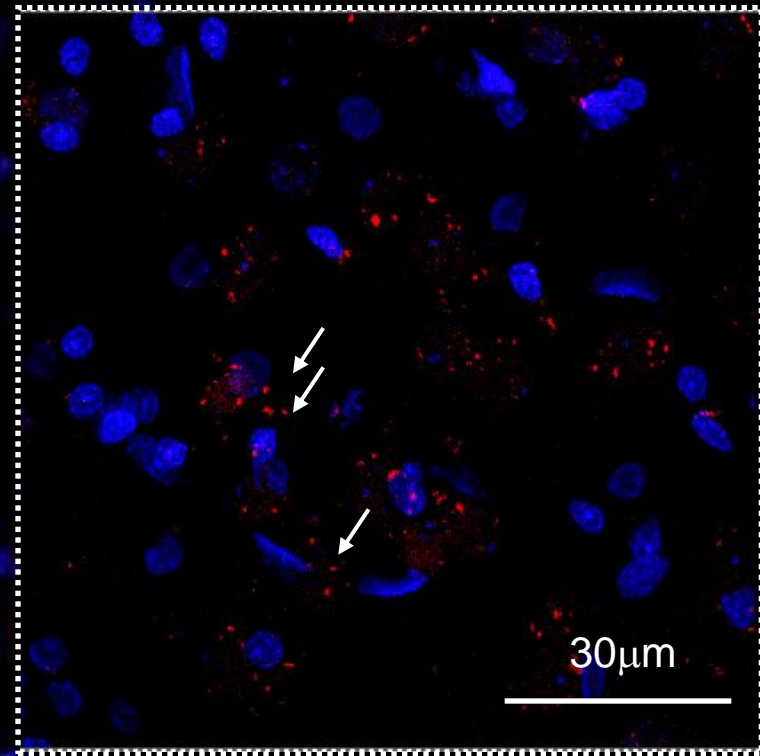
✓ **A1R antagonists enhance motor activity**



## In situ PLA

<http://www.youtube.com/watch?v=KbpUU7jQTF8>

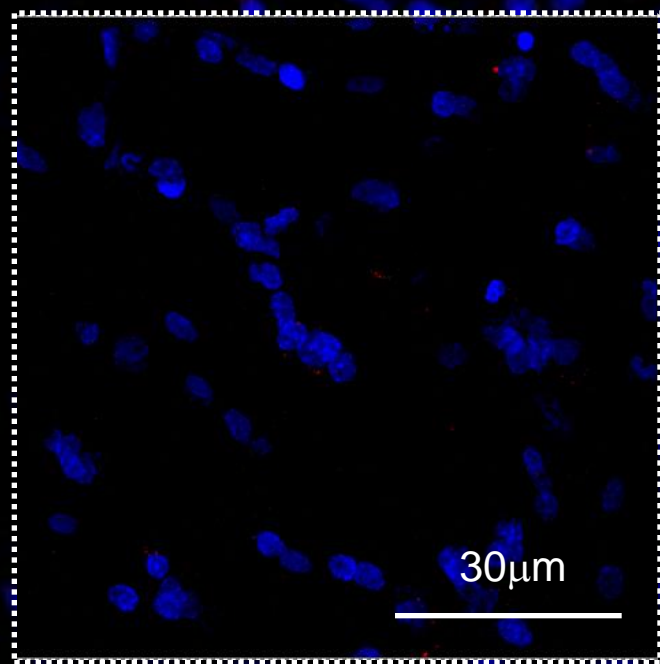
**CPU**



75µm

**A<sub>1</sub>R-D<sub>1</sub>R**

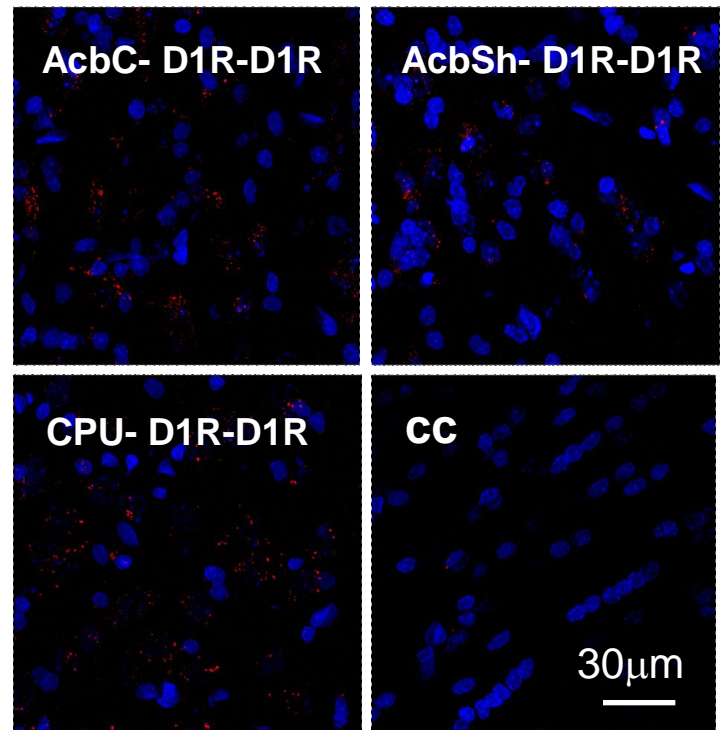
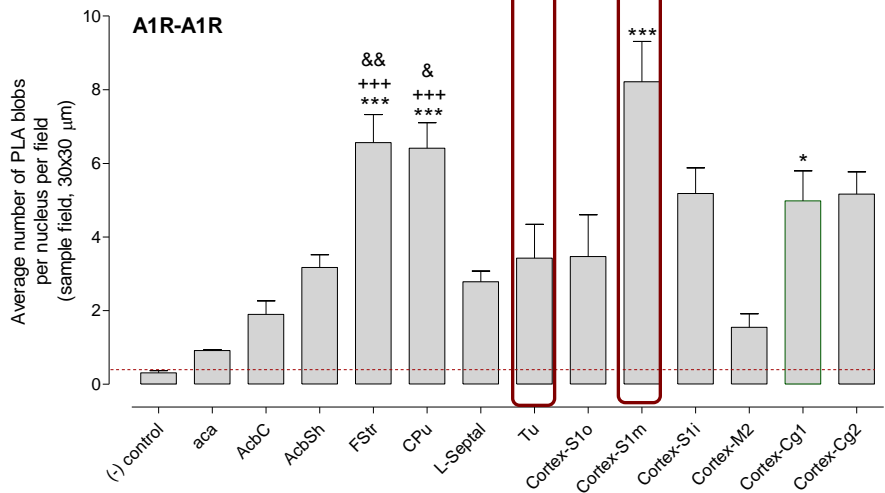
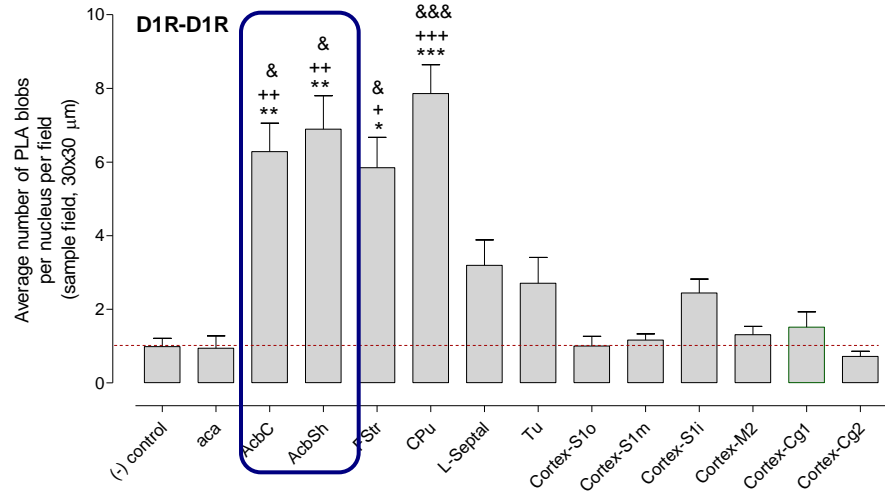
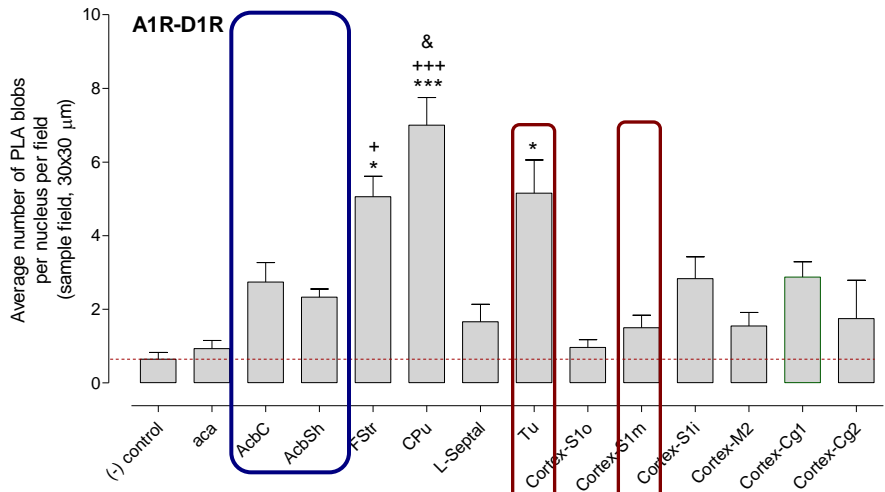
**aca**



75µm

**A<sub>1</sub>R-D<sub>1</sub>R**



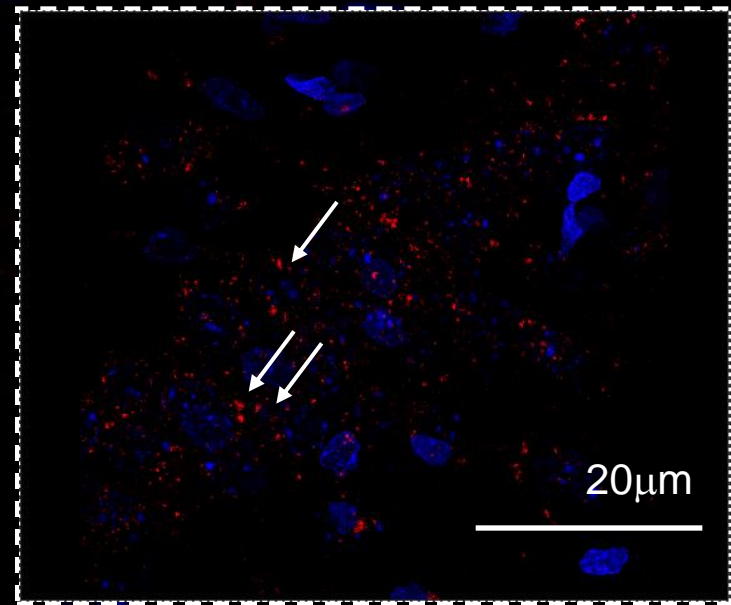




CA3

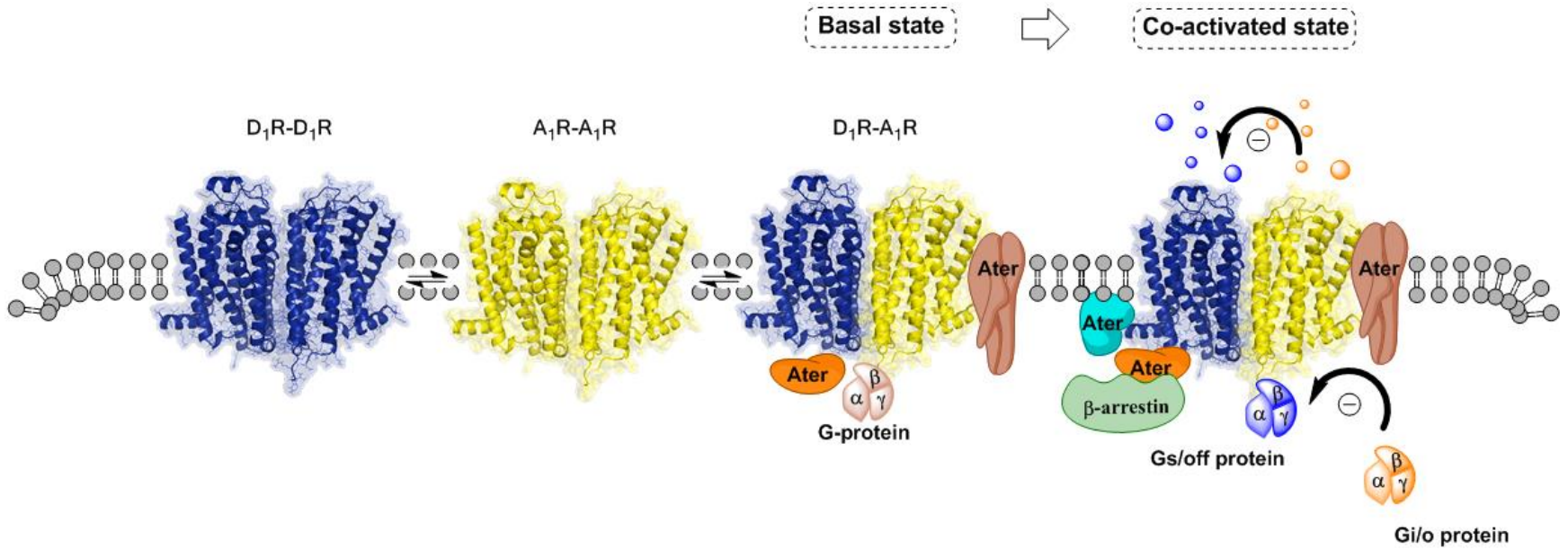
[A1-A1]

50 $\mu$ m

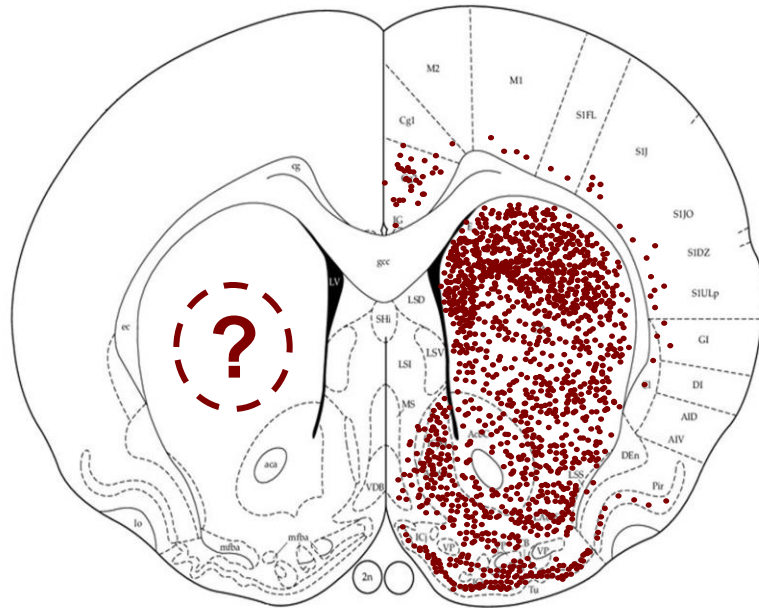


20 $\mu$ m

# The balance of A1-D1 homo and heteroreceptor complexes



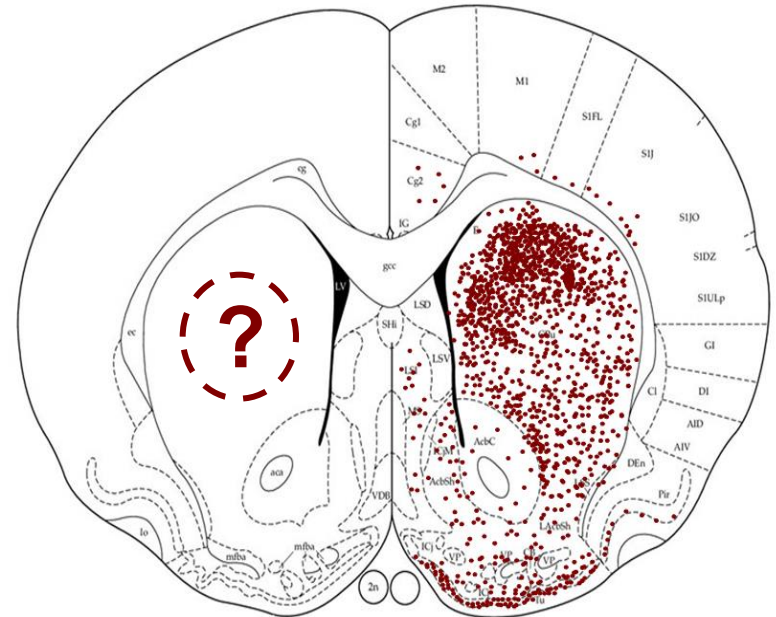
# Conclusions



**D1R-D1R**

**homoreceptor complexes**

Bregma 1.00 mm



**A1R-D1R**

**homoreceptor complexes**

Bregma 1.00 mm

## METHODS

### ➤ Radioligand binding assay

### ➤ Coimmunoprecipitation

➤ Split-ubiquitin Membrane Yeast Two Hybrid system (MYTHS)

### ➤ Fluorescence Cross Correlation Spectroscopy

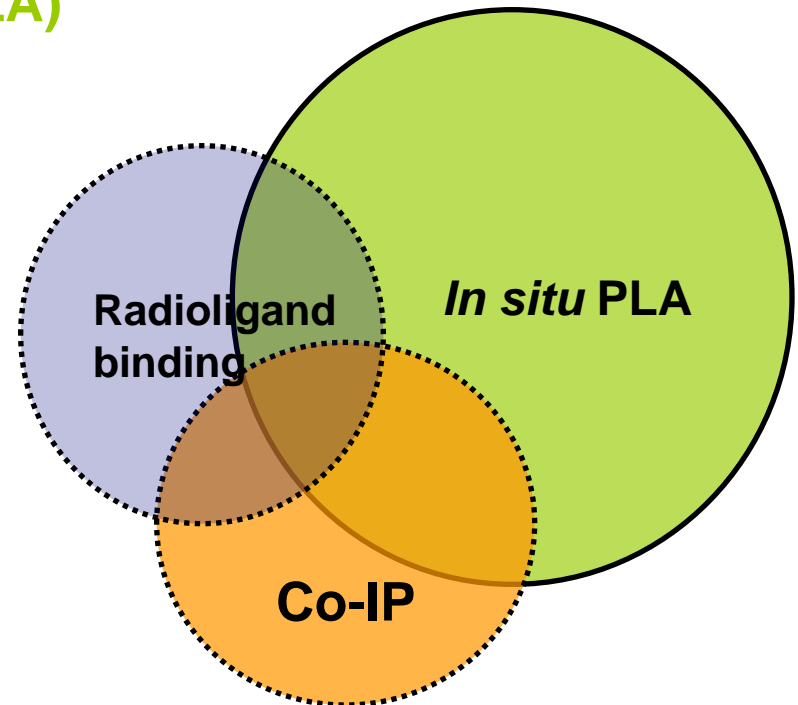
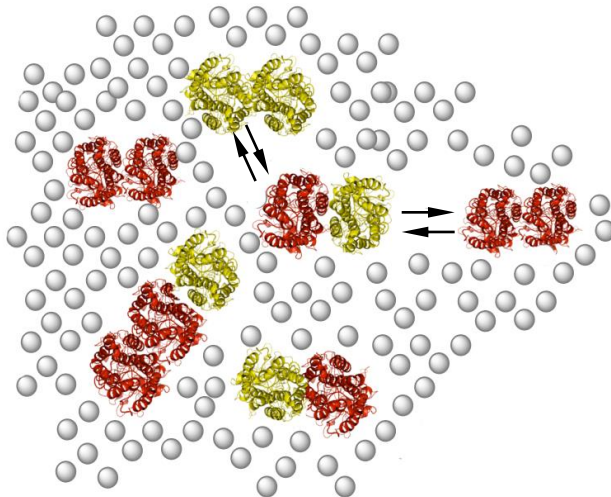
➤ Receptor activation by biased ligands and small interface interfering peptides (SIIP)

➤ Phosphoproteomics

➤ NanoScan PET/MRI scanner

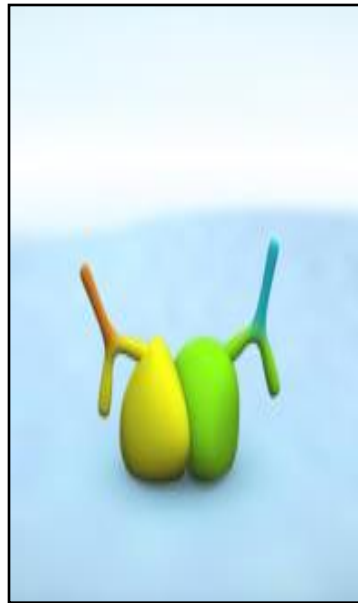
➤ Generation and phenotypical analysis of knock-in rats

### ➤ In situ Proximity Ligation Assays (PLA)



## In situ PLA

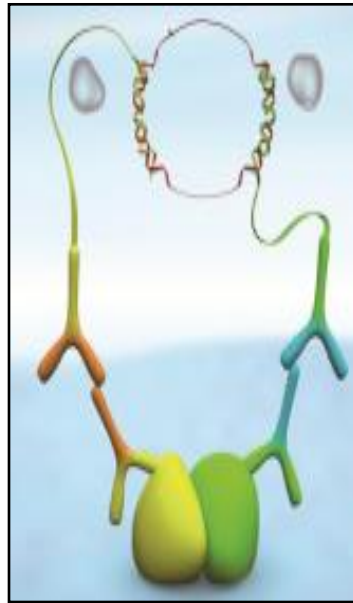
Conjugation. A pair of primary antibodies bind to the proteins to be detected



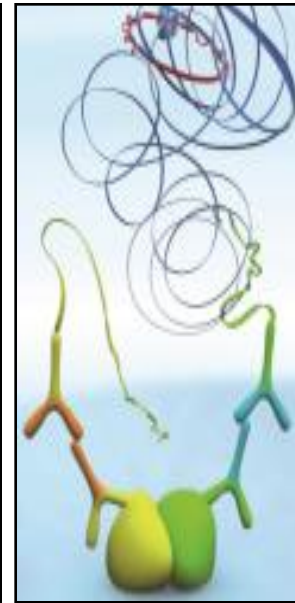
A pair of PLA probes (PLUS and MINUS) bind their respective primary antibody



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 oligonucleotide tagged with fluorescent compound.

