Parkinsons-2015 Meeting. October 11-13 , Frankfurt , Germany

The impact of A2A-D2 heteroreceptor complexes and their receptor-receptor interactions on Parkinson's disease and its treatment

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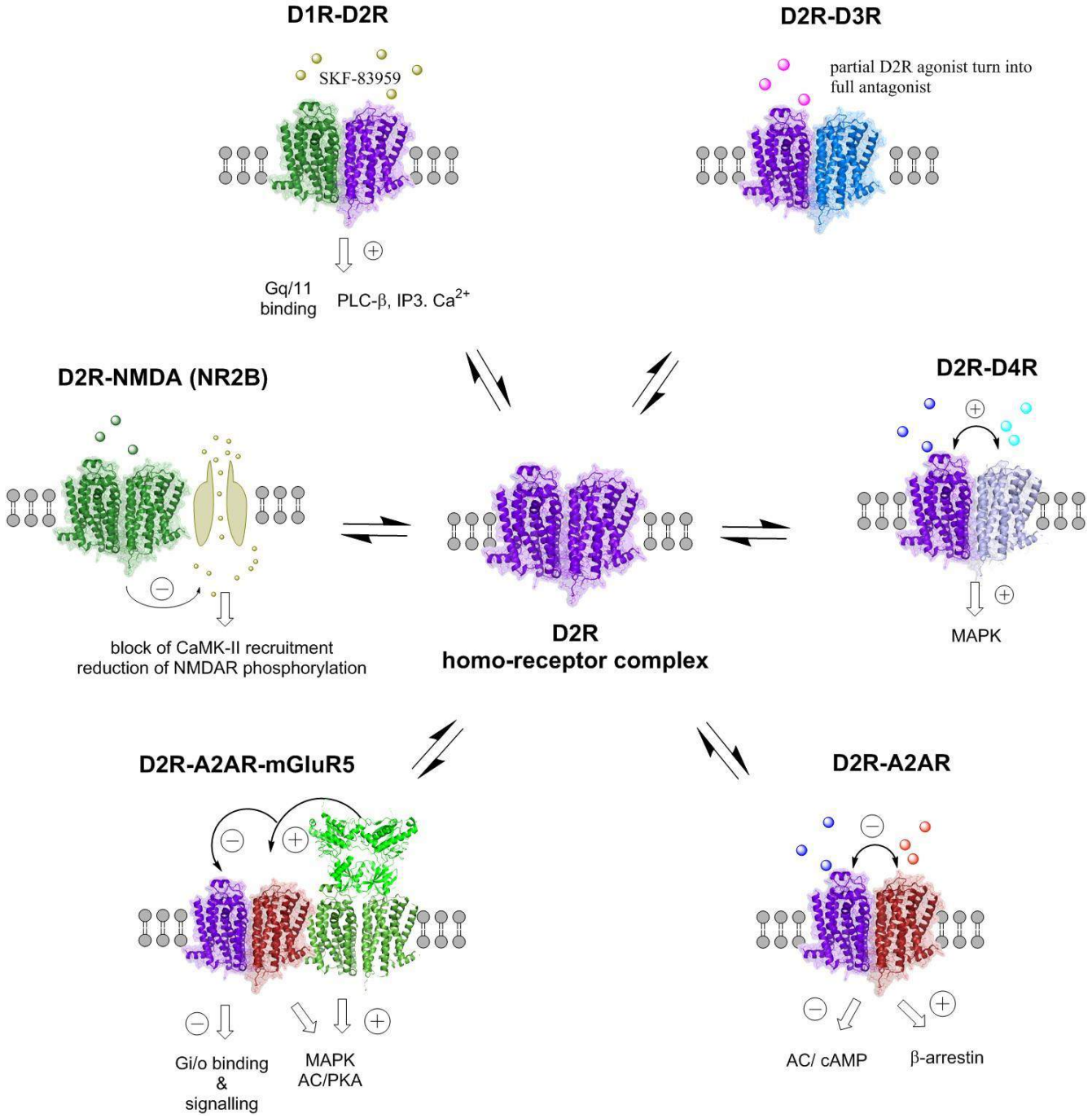
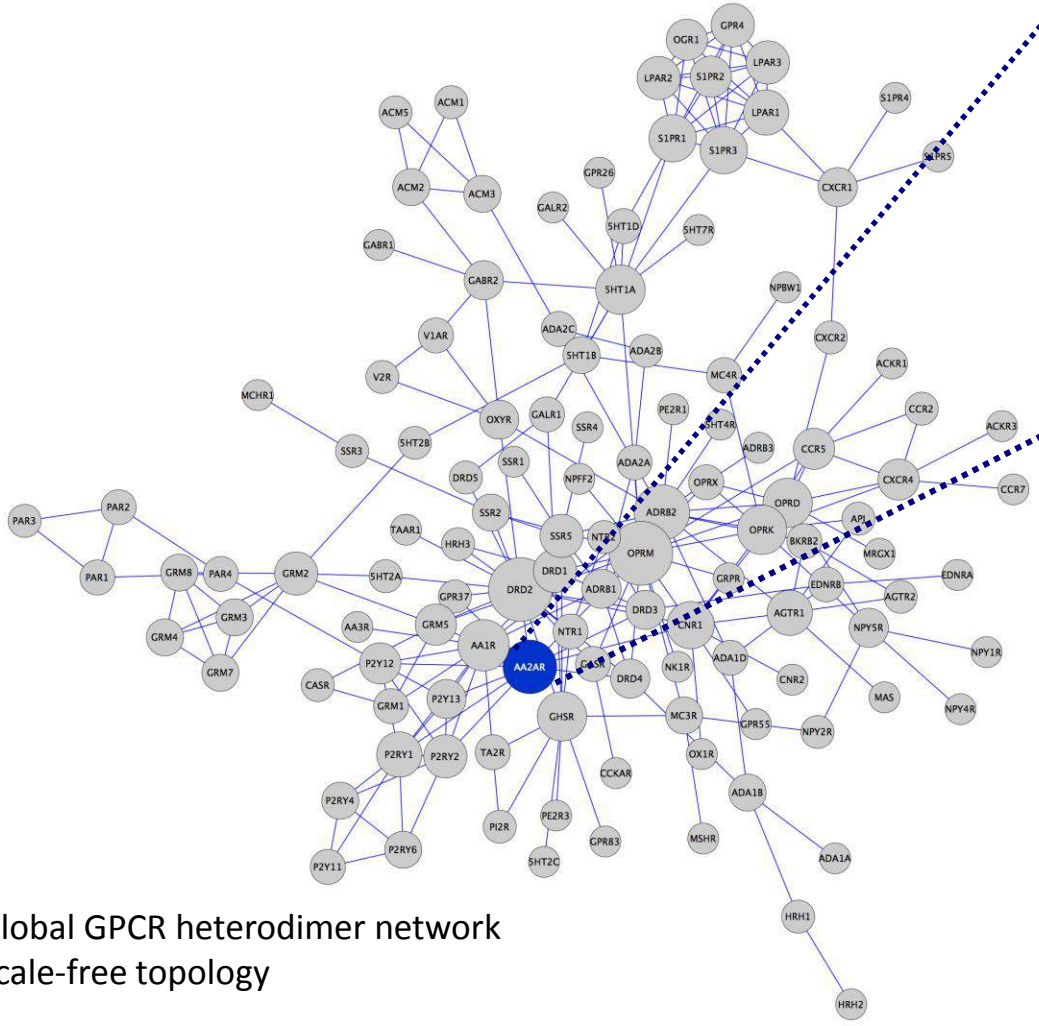
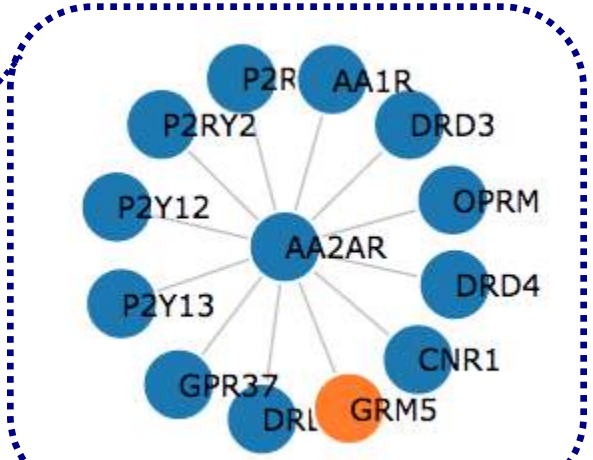

D1R-D2R
SKF-83959
Gq/11
binding
PLC-β, IP3. Ca2+
D2R-D3R
partial D2R agonist turn into
full antagonist
D2R-NMDA (NR2B)
block of CaMK-II recruitment
reduction of NMDAR phosphorylation
D2R
homo-receptor complex
D2R-D4R
MAPK
D2R-A2AR-mGluR5
Gi/o binding
&
signalling
MAPK
AC/PKA
D2R-A2AR
AC/ cAMP
β-arrestin

Different types of A2A heteroreceptor complexes

Experimental data from the String and Scopus databases and using Cytoscape software Implemented in Java



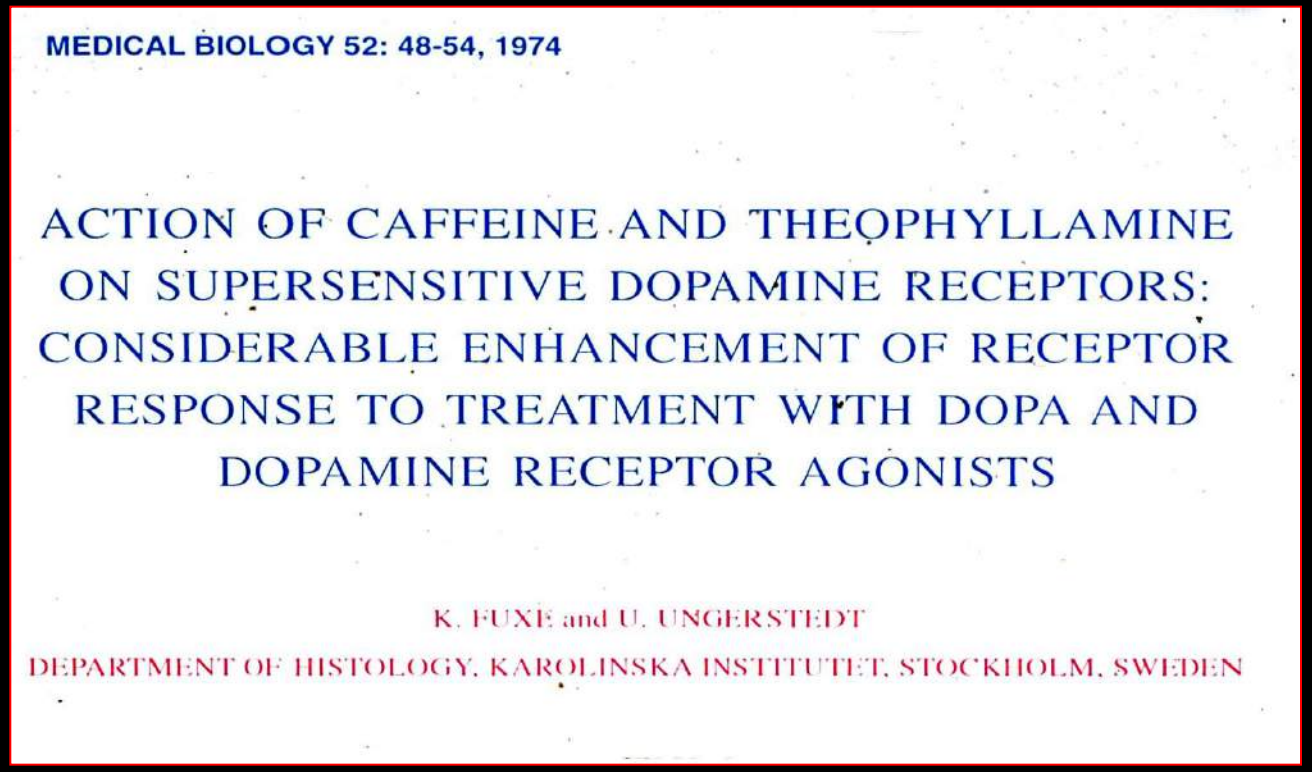
Global GPCR heterodimer network
Scale-free topology



- ✓ A2AR-A1R, A2AR-A2BR, A2AR-A3R
- ✓ A2AR-D2R, A2AR-D3R, A2AR-D4R
- ✓ A2AR-GPR37
- ✓ A2AR-CB1R
- ✓ A2AR-MOR
- ✓ A2AR-P2RY1, A2AR-P2RY2
- ✓A2AR-P2RY12 and A2AR-P2RY13
- ✓**A2AR is a hub receptor**

GPCR-HetNET: *www.gpcr-hetnet-com*
Borroto-Escuela et al.2014

FIRST INDICATIONS OF ADENOSINE AND DOPAMINE INTERACTIONS IN THE BASAL GANGLIA



MEDICAL BIOLOGY 52: 48-54, 1974

ACTION OF CAFFEINE AND THEOPHYLLAMINE ON SUPERSENSITIVE DOPAMINE RECEPTORS: CONSIDERABLE ENHANCEMENT OF RECEPTOR RESPONSE TO TREATMENT WITH DOPA AND DOPAMINE RECEPTOR AGONISTS

K. FUXE and U. UNGERSTEDT

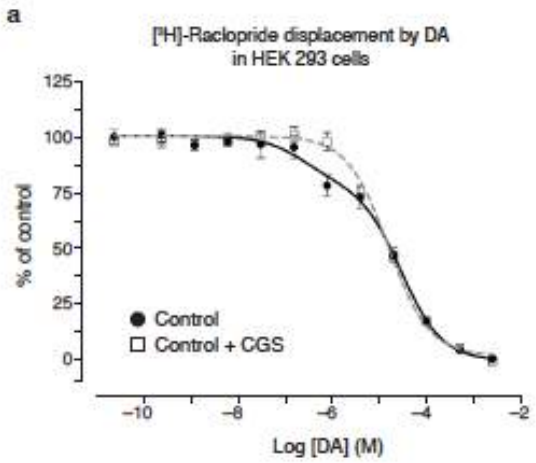
DEPARTMENT OF HISTOLOGY, KAROLINSKA INSTITUTET, STOCKHOLM, SWEDEN

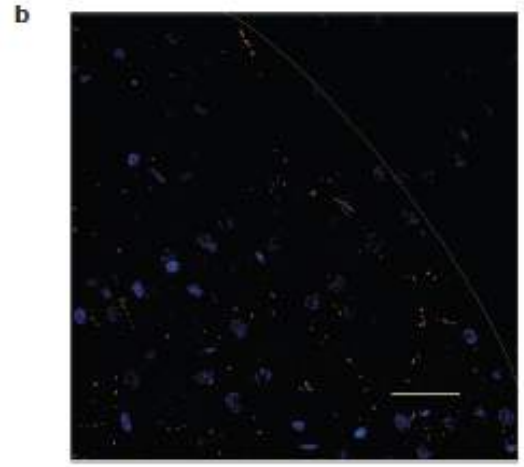
Eur J Pharmacol. 1976 38:31-8.

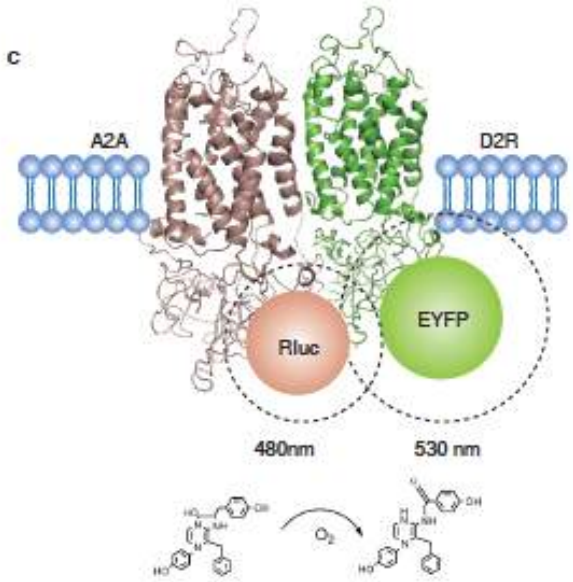
Effect of some phosphodiesterase inhibitors on central dopamine mechanisms

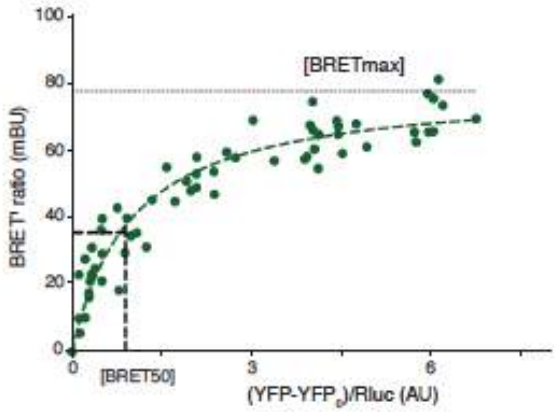
B.Fredholm, K.Fuxe and L.F. Agnati

Receptor-receptor interactions in A2A-D2 heteroreceptor complexes



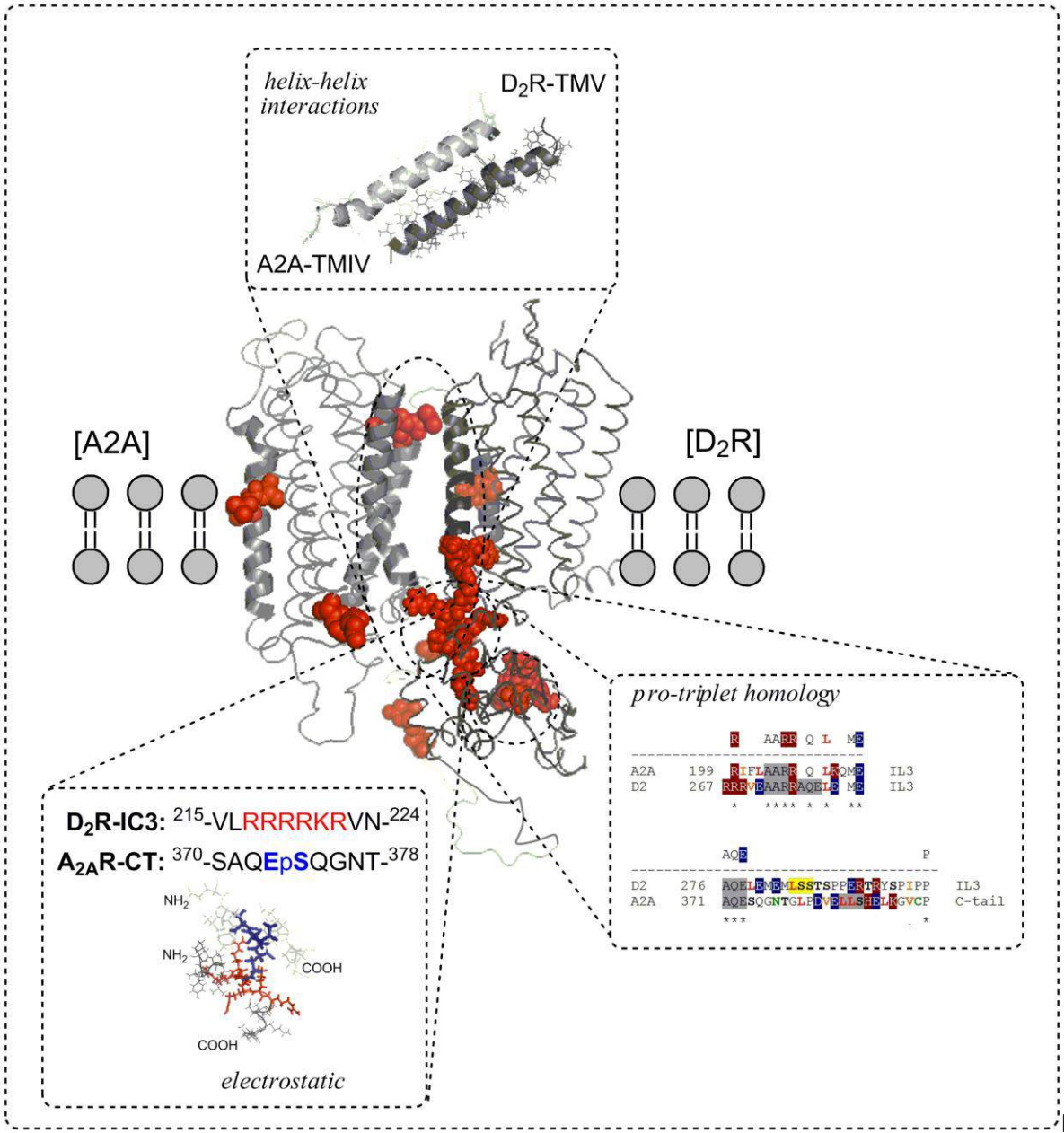






Hillion et al.2002,Fuxe et al.2003,2013

The receptor interface in GPCR: The A2AR-D2R example



Theory of protriplet aminoacid homologies as part of the receptor interface Tarakanov and Fuxe 2010

Fuxe et al.2012, Borroto-Escuela et al. 2010

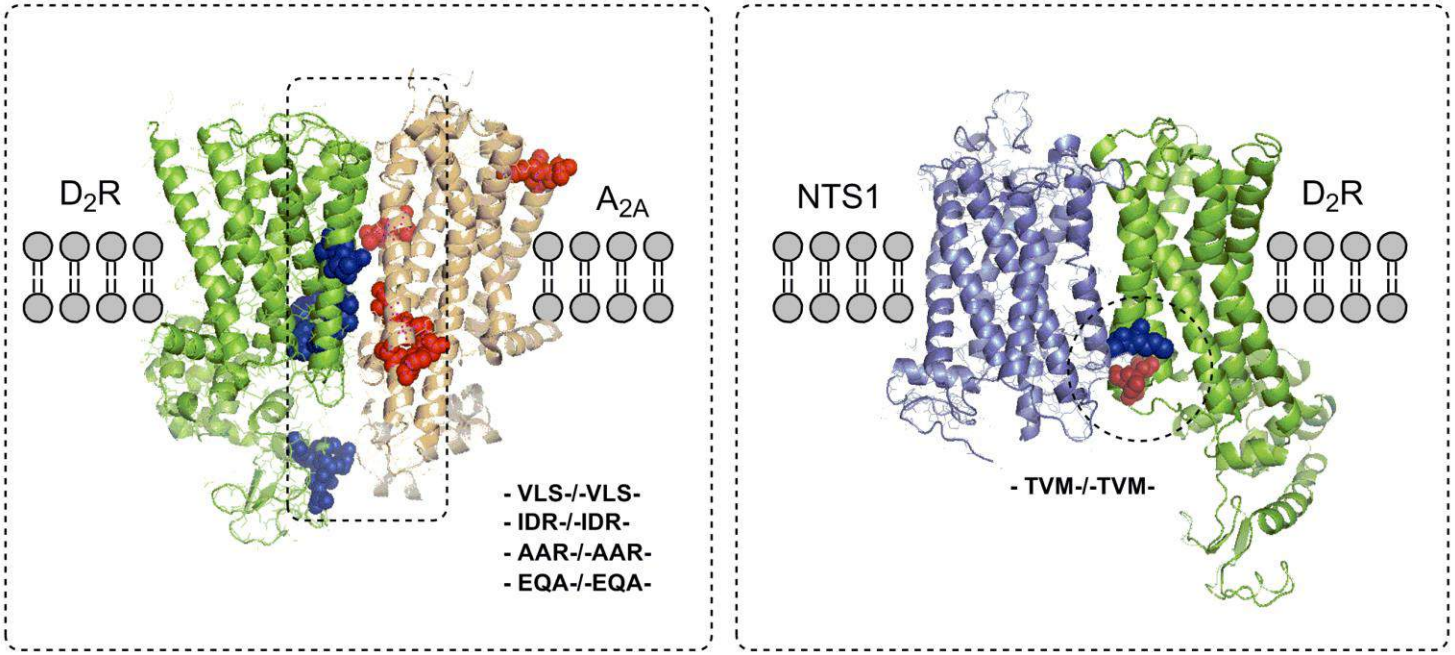
The triplet Puzzle: Homologies of amino acid protriplets in Receptor Heteromers

Rank	Triplets
40 pro-triplets (for heterodimers)	
8	LNL LVI LPF AIV IDR
7	LLT VLS ILS TSL LIL ILI APL LYS AAR
6	LLI LVA TLV LSL SSL ASI IIY TAV ITL PFF AAV
5	LLP LLE LLG LVL KSL NSL IIL VIL ATL FNI FGN SVS VAI VFV AIS
4 contra-triplets (against heterodimers)	
2	IMG IIN VTN GNT

Through a mathematical approach, we have deduced, based on 48 pairs of receptors that form or not form heteromers, sets of triplet amino acid homologies that may participate in receptor interface.

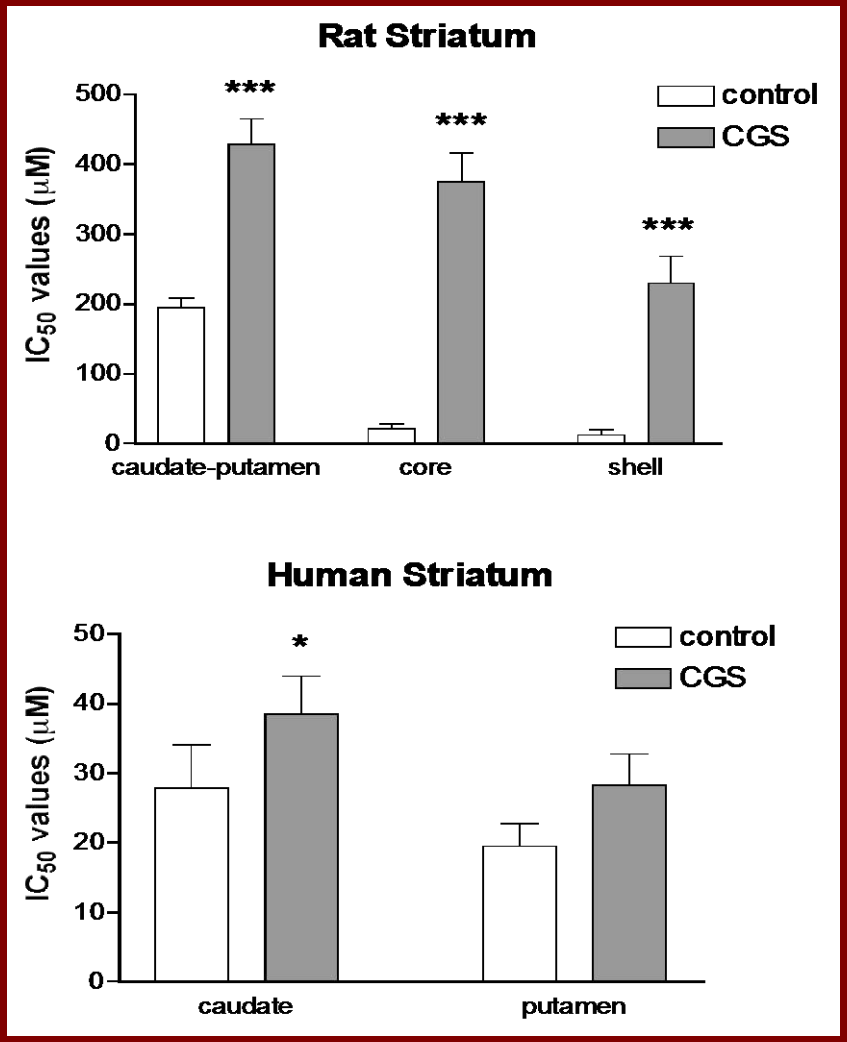
They give a kind of code that help determine which receptors should or should not form heterodimers .

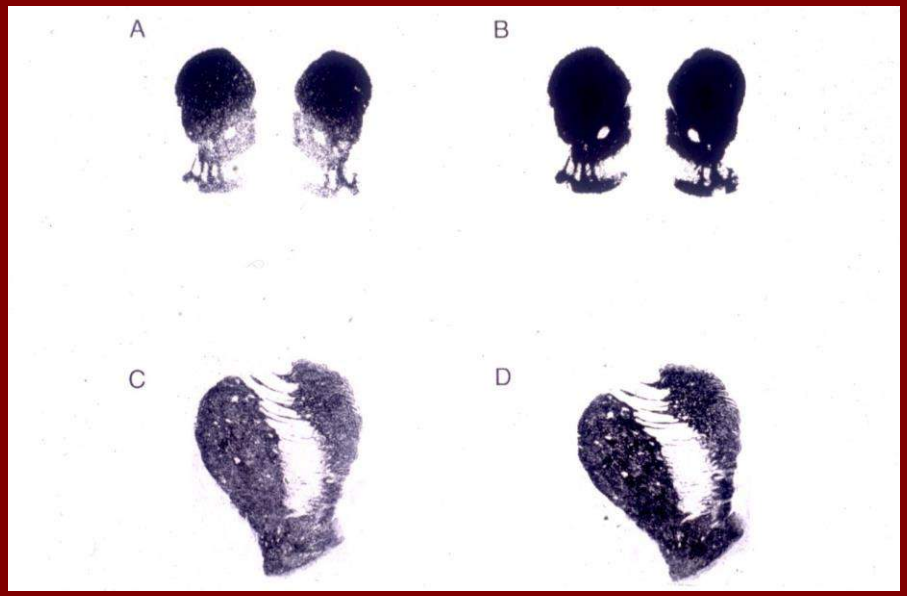
Illustration of homology protriplets in A2A-D_{2L}R and D_{2L}R-NTS1 heteromers



We propose a 'guide-and clasp' manner for receptor–receptor interactions where 'adhesive guides' may be the triplet homologies.

Tarakanov and Fuxe 2010

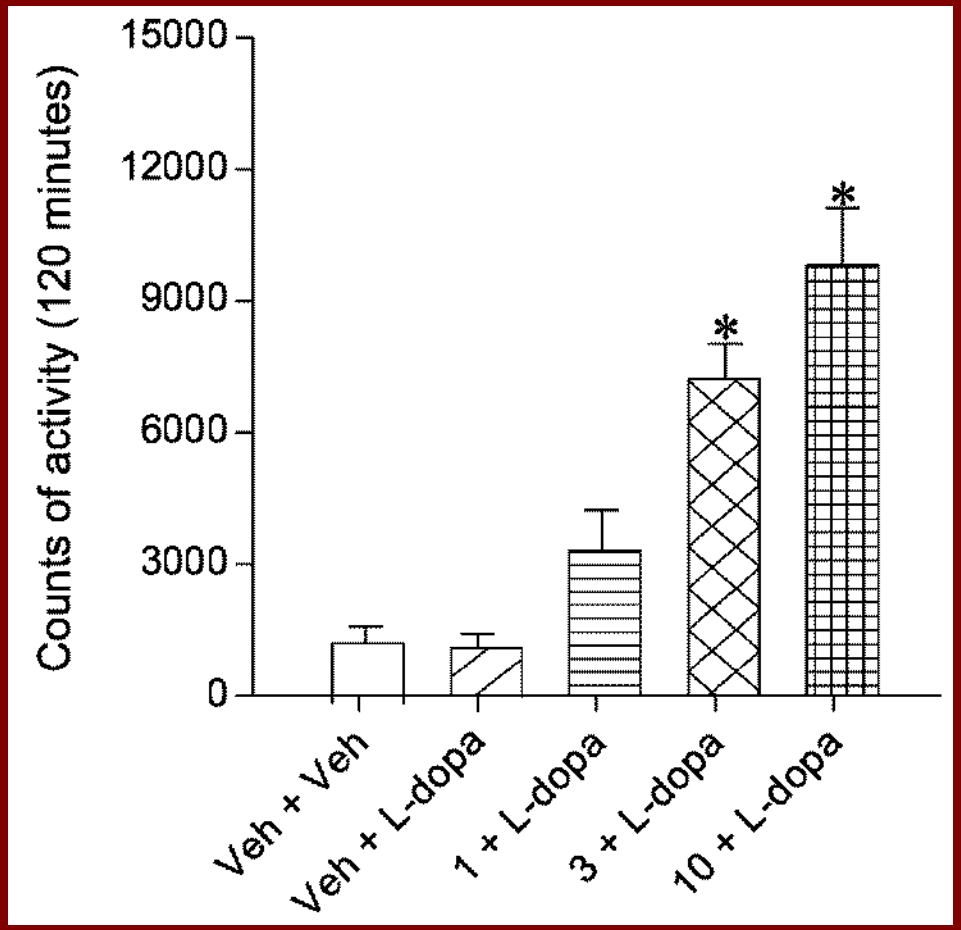




Effect of the $A_{2A}R$ agonist CGS 21680 on competitive inhibition experiments using the D_2R antagonist [^{125}I]-iodosulpiride *vs* dopamine in rat striatal sections (top graph) and in human striatal sections (bottom graph).

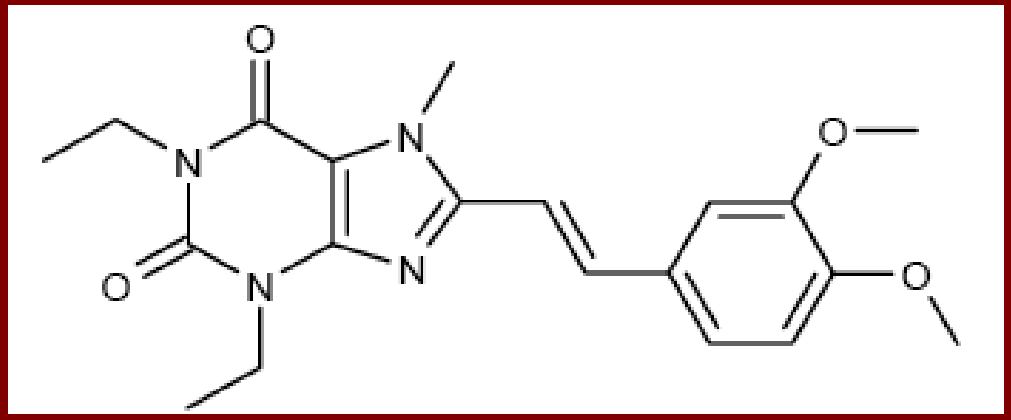
Diaz Cabiale et al., 2001

SCH 58261 increases locomotor activity in reserpinized mice after a sub-threshold dose of L-dopa



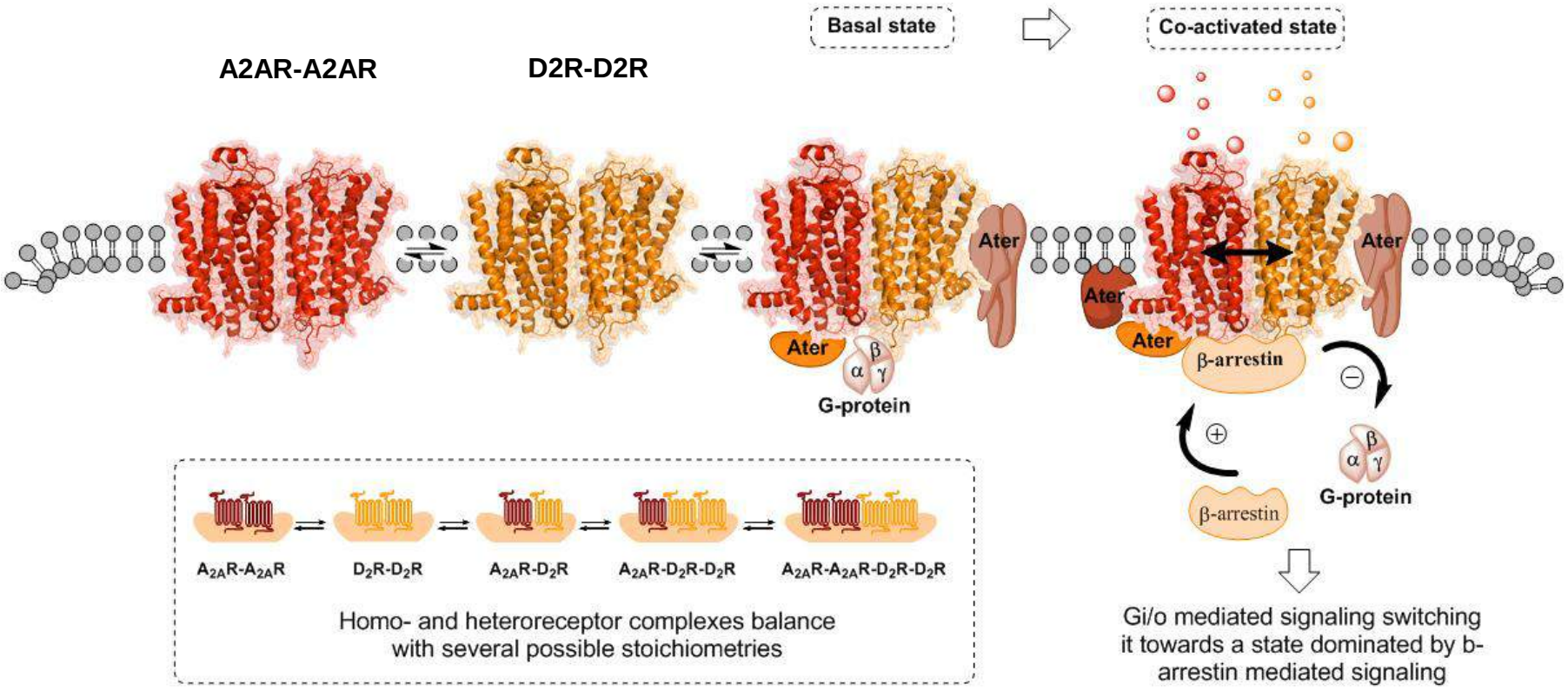
Tanganelli et al.2004

Development of A_{2A} antagonists for the treatment of Parkinson's disease based on antagonstic A_{2A}/D_2 receptor interaction in RMs of the dorsal striato-pallidal GABA pathway



Istradefylline

The balance of A2A-D2 heteromers vs D2 and A2A monomers/homomers



Antonelli et al.2006; Fuxe et al.2007,2010, Fuxe & Borroto-Escuela, NPP 2015

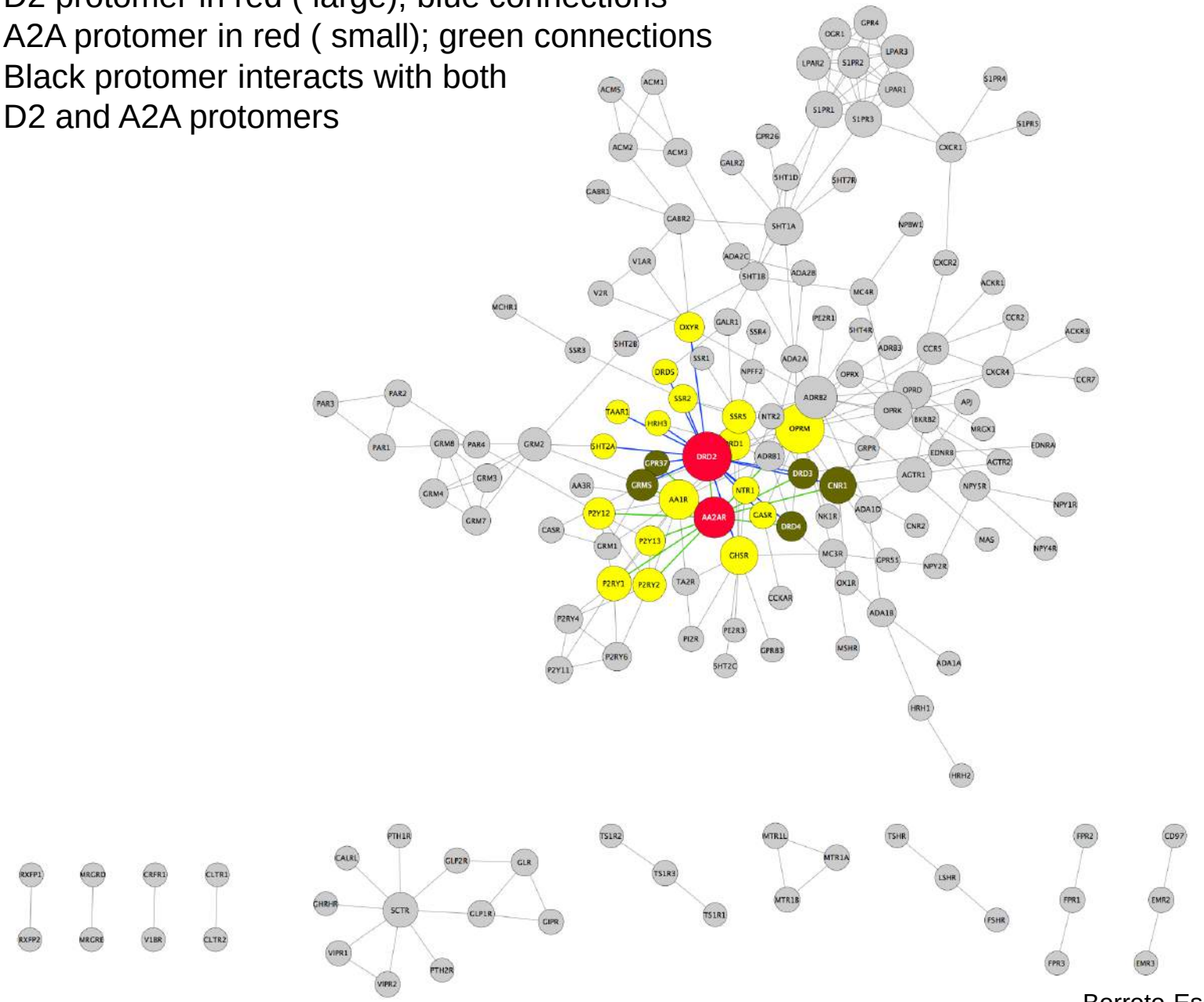
Multiple D2 and A2A heterodimers in the global heterodimer network

D2 protomer in red (large); blue connections

A2A protomer in red (small); green connections

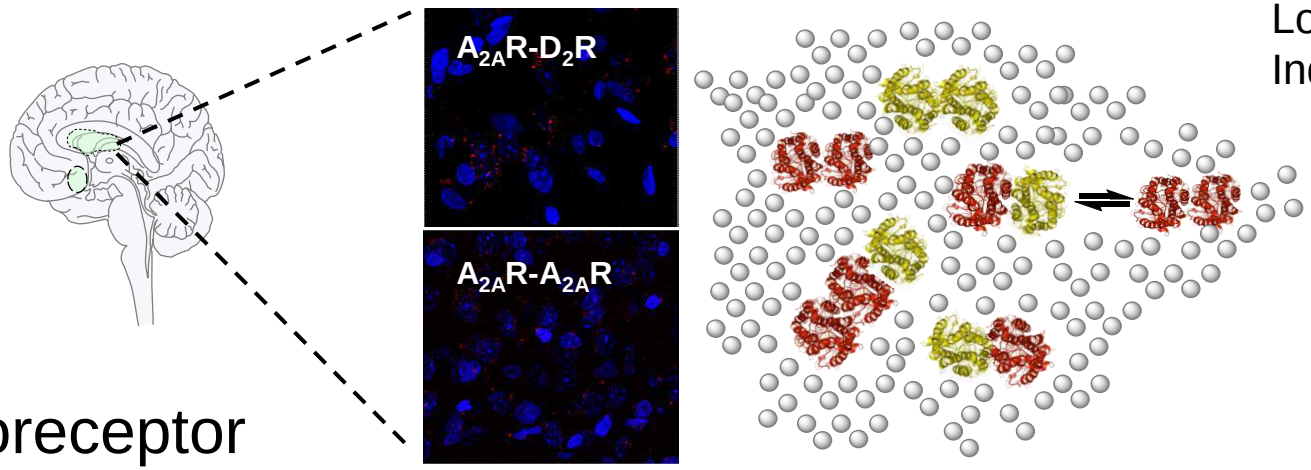
Black protomer interacts with both

D2 and A2A protomers



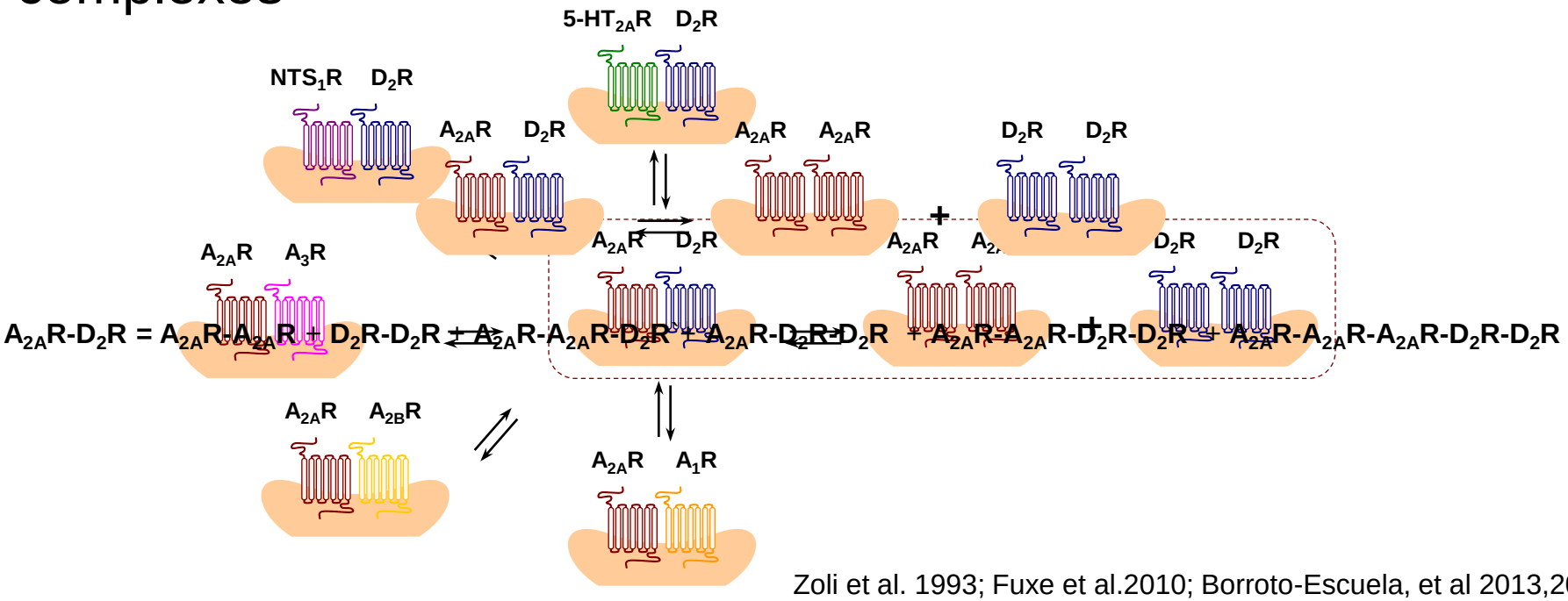
Borroto-Escuela et al.2015

Understanding the complexity of the balance between A2AR-D2R and its homomers, iso A2AR- and iso D2R-heteromers and other A2A- and D2R-heteromers



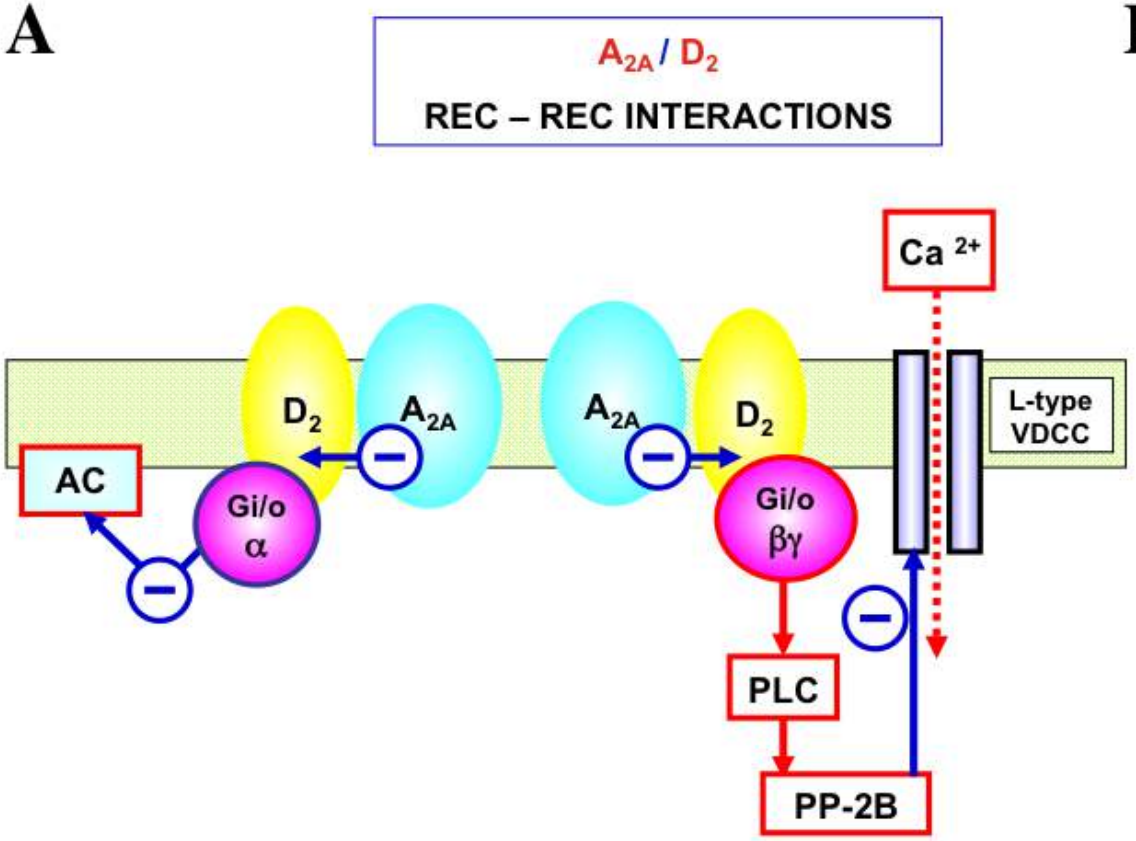
Local network vs Individual cell

Heteroreceptor complexes



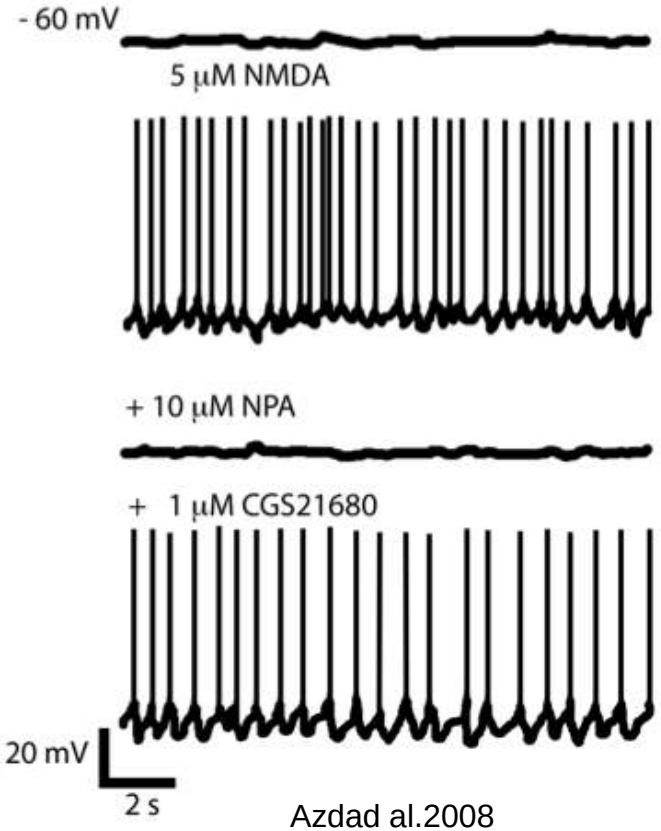
Zoli et al. 1993; Fuxe et al.2010; Borroto-Escuela, et al 2013,2015

A



A_{2A}-REC ACTIVATION REDUCES D_2-REC MEDIATED DECODING MECHANISM VIA AN INTRAMEMBRANE PROT-PROT INTERACTION

B



Azdad al.2008

The D2 mediated suppression of NMDA-induced depolarized plateau potential is reversed by A2A receptor activation

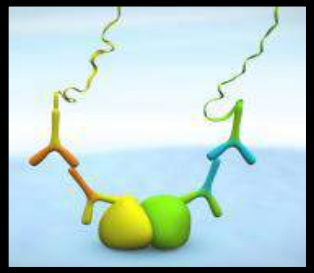
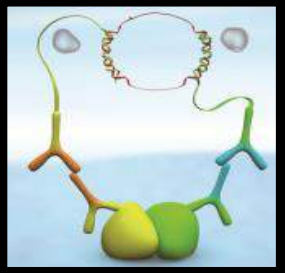
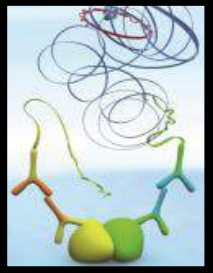
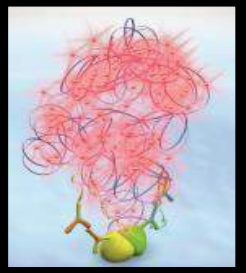
Conjugation. A pair of primary antibodies bind to the proteins to be detected

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

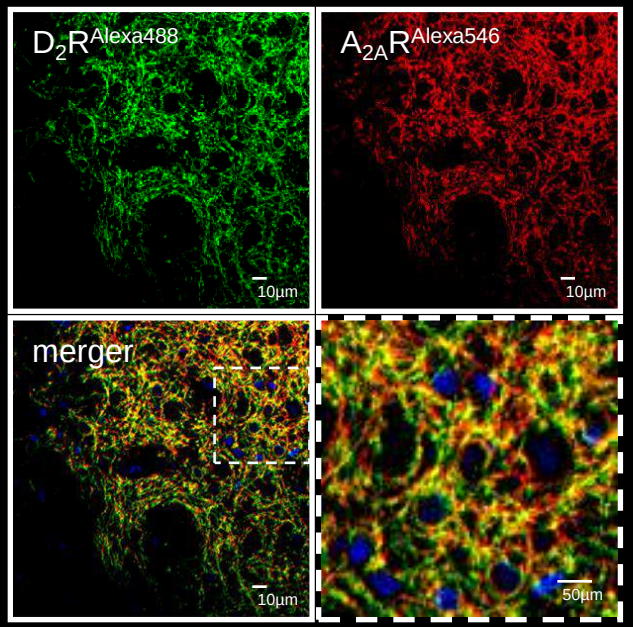
Two connector oligo nucleotides are joined to form a circular molecule by a ligase

A polymerase replicates the circle, producing an concatemeric product

Detection of hybridization by oligonucletide tagged with fluorescent compound.

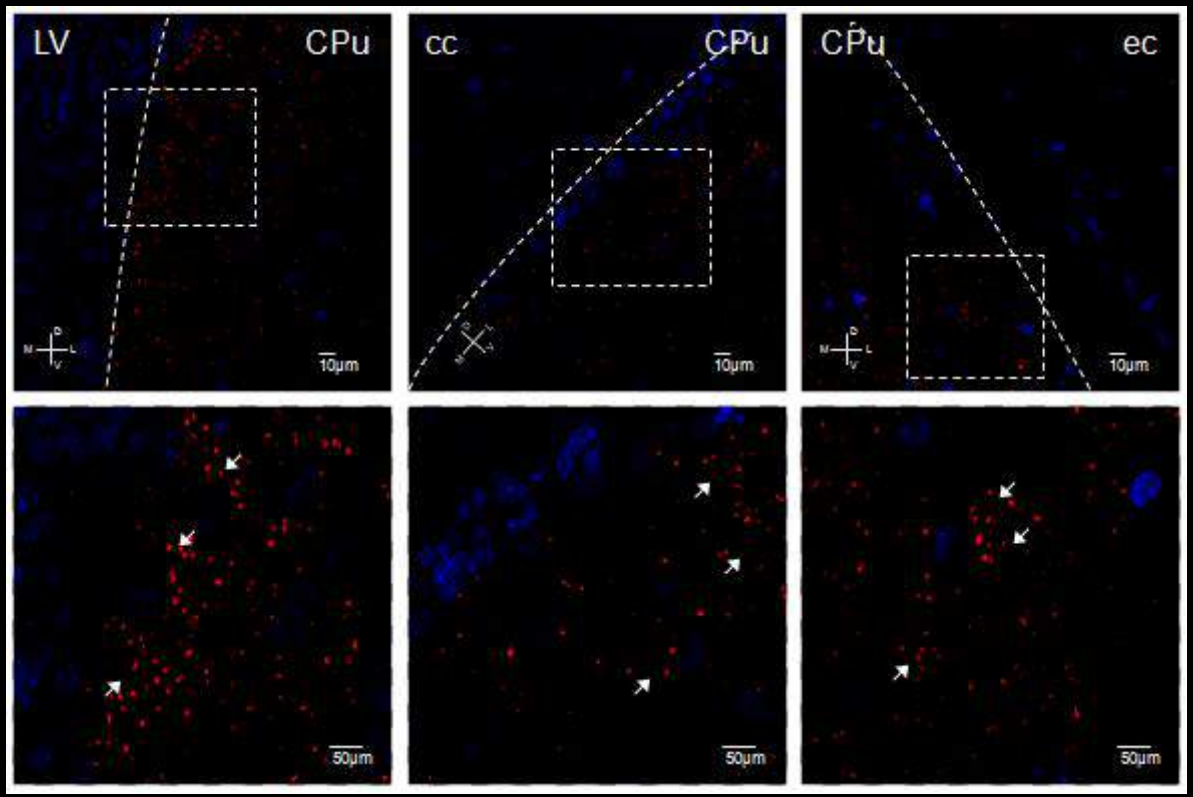






Colocation of A_{2A}-D_2 IR



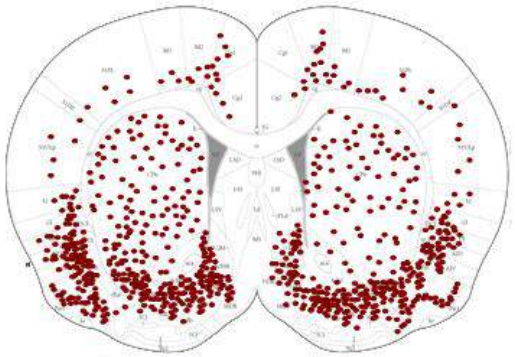
Borroto-Escuela et al.2013 ; see Trifilieff et al. 2011

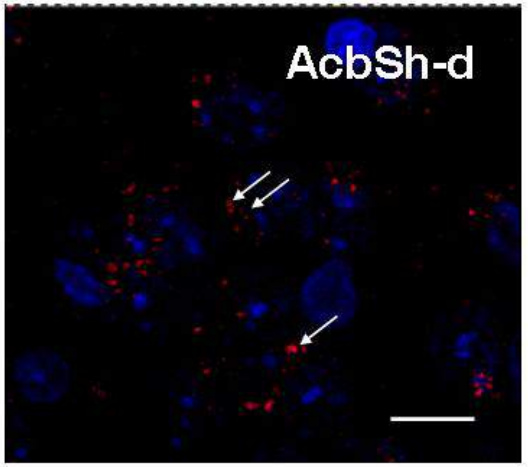
$A_{2A}R$-D_2R heteromers In situ PLA

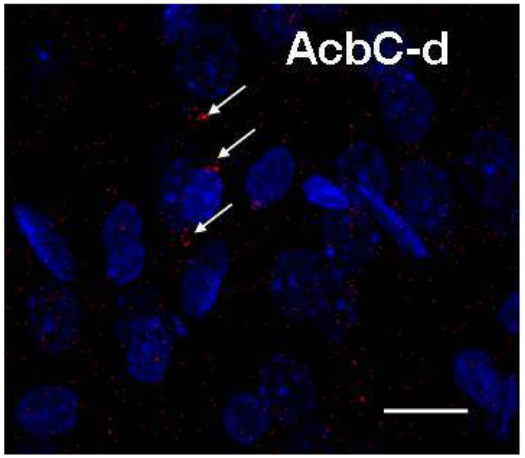


Co-distribution of D2R-Sigma 1R and the A2AR-D2R heteroreceptor complexes in the CPU, AcbC and AcbSh region of the rat brain

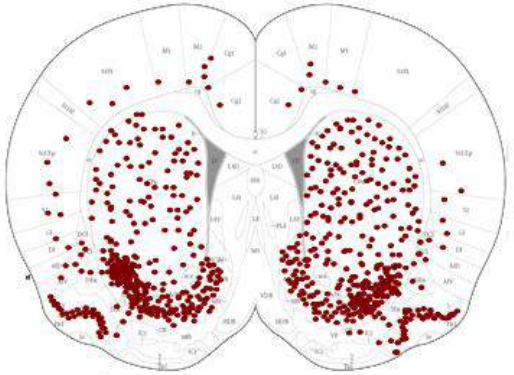
D_2R-Sigma1R

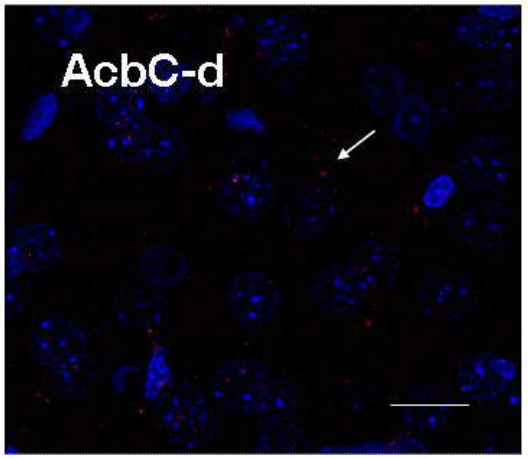


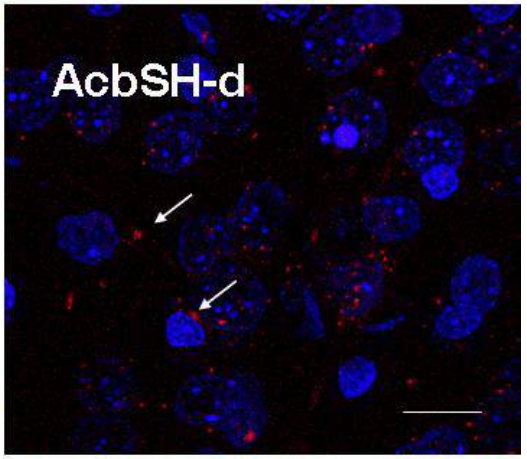




D_2R-$A_{2A}R$

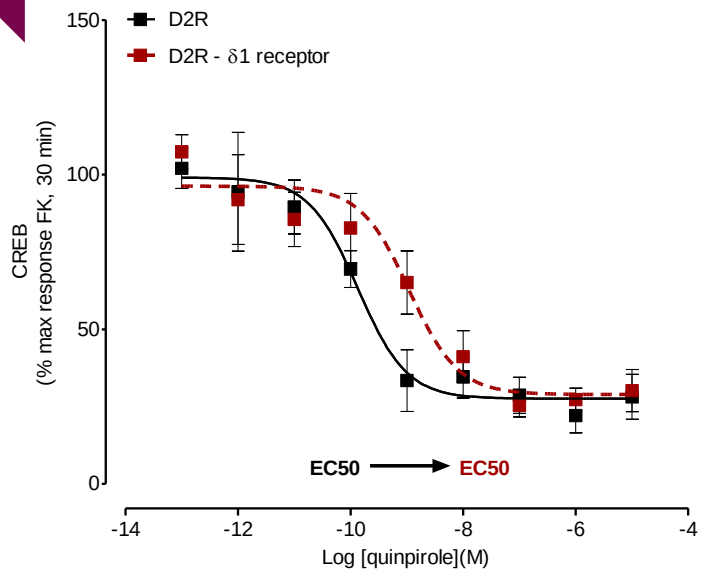


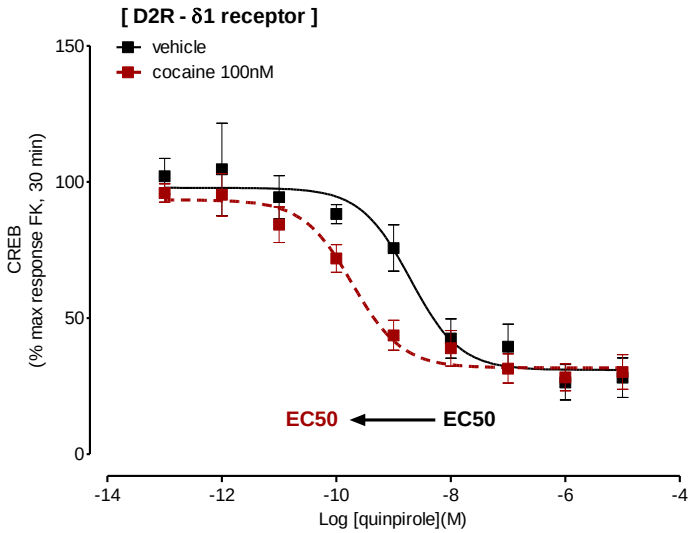


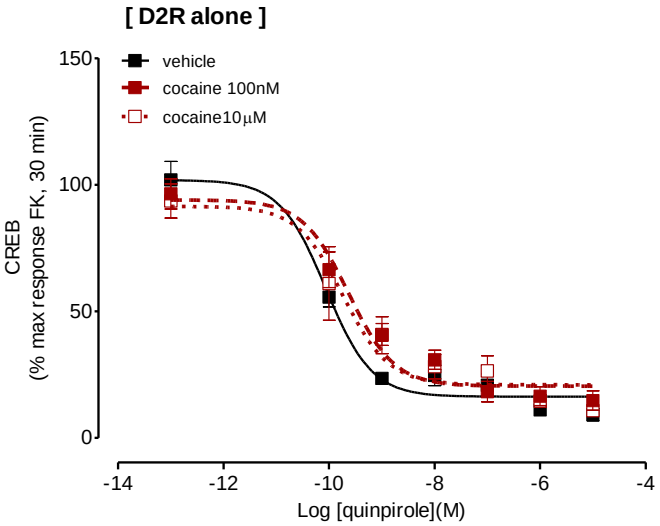


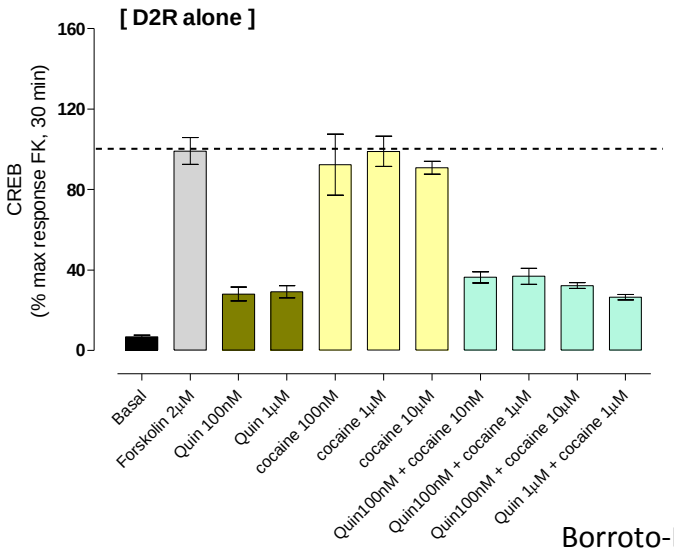
Borroto-Escuela et al.2015

Actions of quinpirole and cocaine on D2 protomer signaling in D2-Sigma 1 complexes using CREB luciferase reporter gene assay



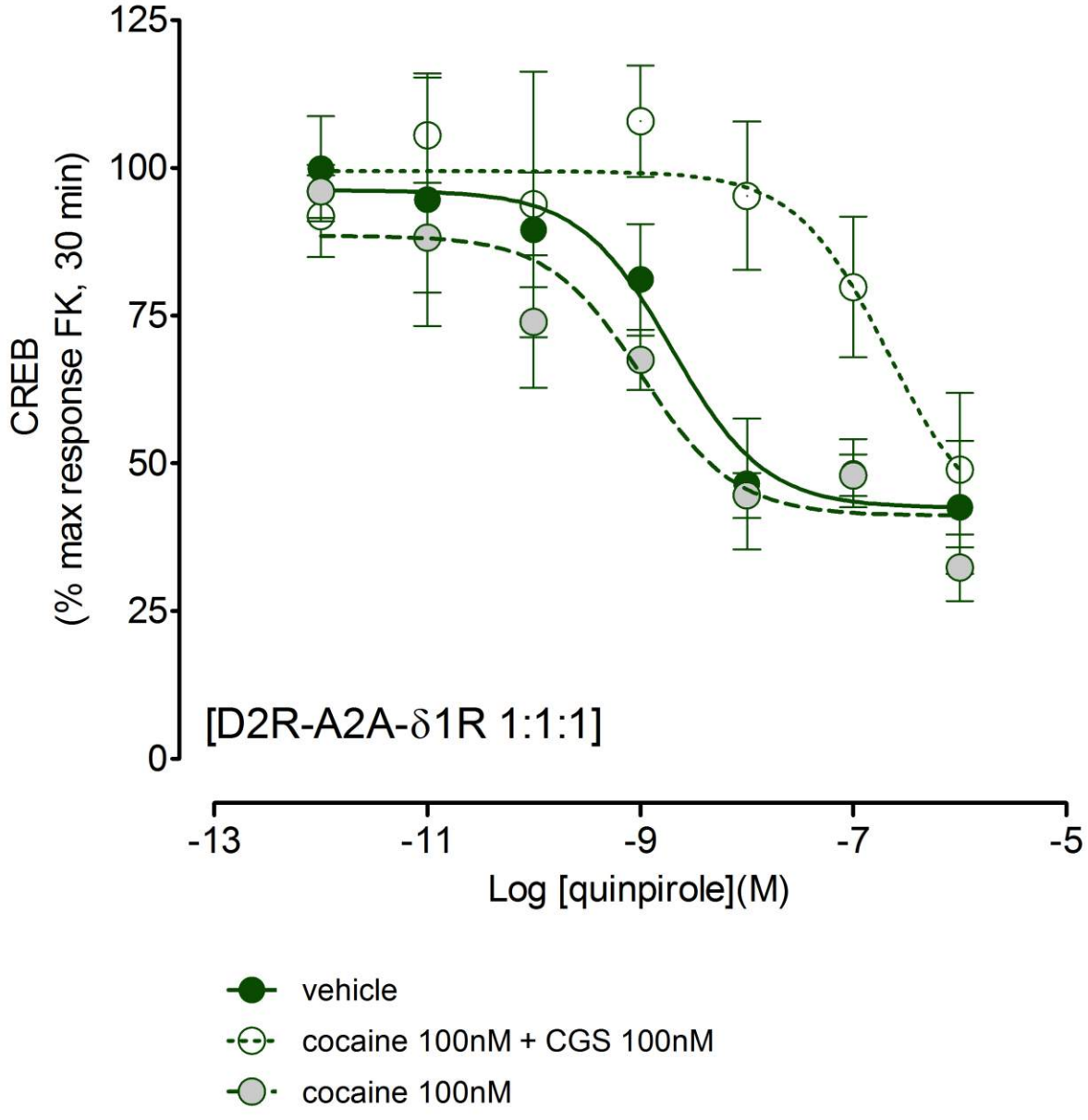






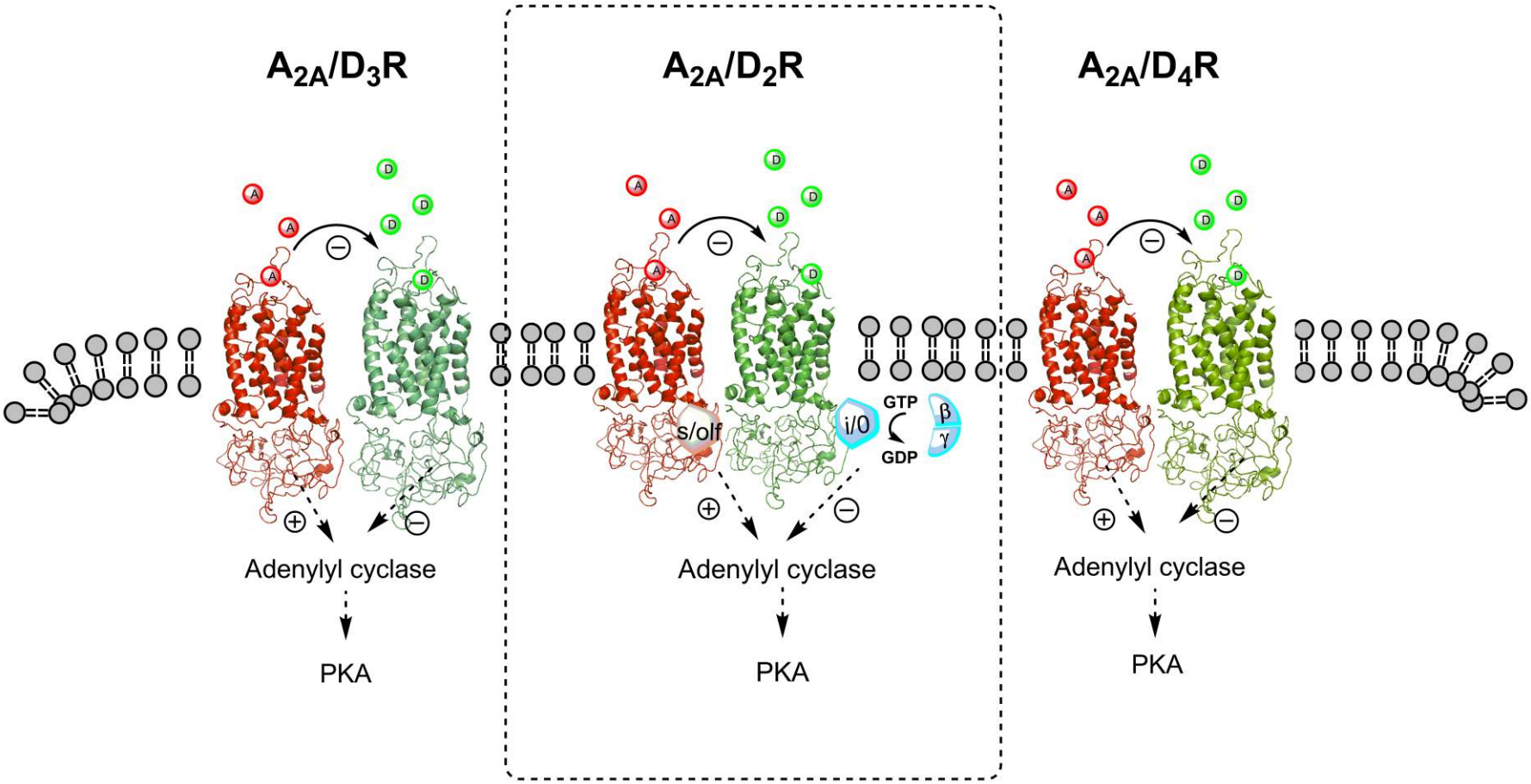
Borroto-Escuela et al.

Strong inhibitory effects of CGS21680 on D2 protomer signaling in putative D2-A2A-Sigma 1 heterotrimeric complexes of HEK cells in the presence of cocaine



Borroto-Escuela et al

Demonstration of arginine-rich epitopes in the N-terminal part of the third cytoplasmic loop of D_2R, D_3R and D_4R



D_3R-3IL: ^{216}-KQRRRKRI-^{223}

D_2R-3IL: ^{215}-VLRRRRKRVN-^{224}

D_4R-3IL: ^{377}-TRRRRRAKI-^{385}

A_{2A}-CT: ^{370}-SAQEpSQGNT-^{378}

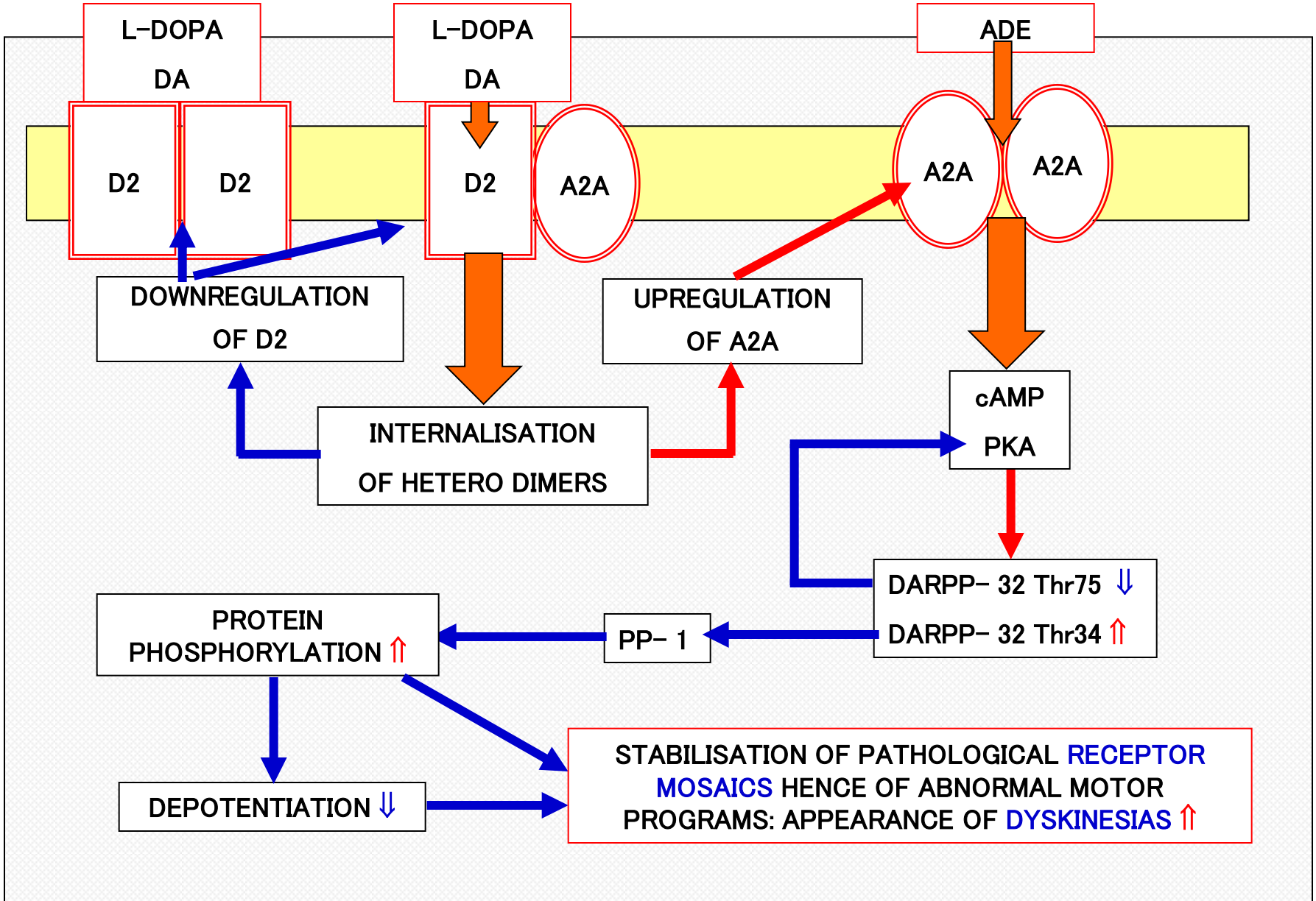
A_{2A}-CT: ^{388}-HELKGVCPEPPGLDDPLAQDGAGVS-^{412}

Torvinen *et al,* 2005

Fuxe *et al,* 2005

POSTULATED DISRUPTION OF A BALANCE BETWEEN A2A HOMOMERS VS A2A/D2HETEROMERS and D2 HOMOMERS IN L- DOPA DYSKINESIAS (Antonelli, et al.2006)

D2 increases through multiple mechanisms phospho-CREB formation activating CRE in the A2A promotor



A_{2A}/D_2 heteroreceptor complexes and A2A mono-homodimers: Targets for A_{2A} antagonists in Parkinson's disease

- The demonstrated modest anti-parkinsonian effects of A_{2A} antagonists in animal models and clinical studies have given support to the concept that blocking antagonistic ***allosteric A_{2A} - D_2 receptor-receptor interactions*** in heteroreceptor complexes can contribute to the development of novel therapies of PD.

- The A_{2A} receptor protomer in the ***A_{2A}/D_2*** heteromer in the dorsal striato-pallidal GABA neurons with antagonistic A_{2A}/D_2 receptor-receptor interactions may be a significant target for ***A_{2A} antagonists*** with regard to antiparkinsonian effects.

- The A_{2A} mono-homodimer may also be a significant target for A_{2A} antagonists with regard to antiparkinsonian and especially possible antidyskinetic actions and the wearing off phenomena of the therapeutic effects of L-DOPA. Monotherapy with A2A antagonist may be recommended in early and should be continued as PD progresses

The MitoPark mouse model

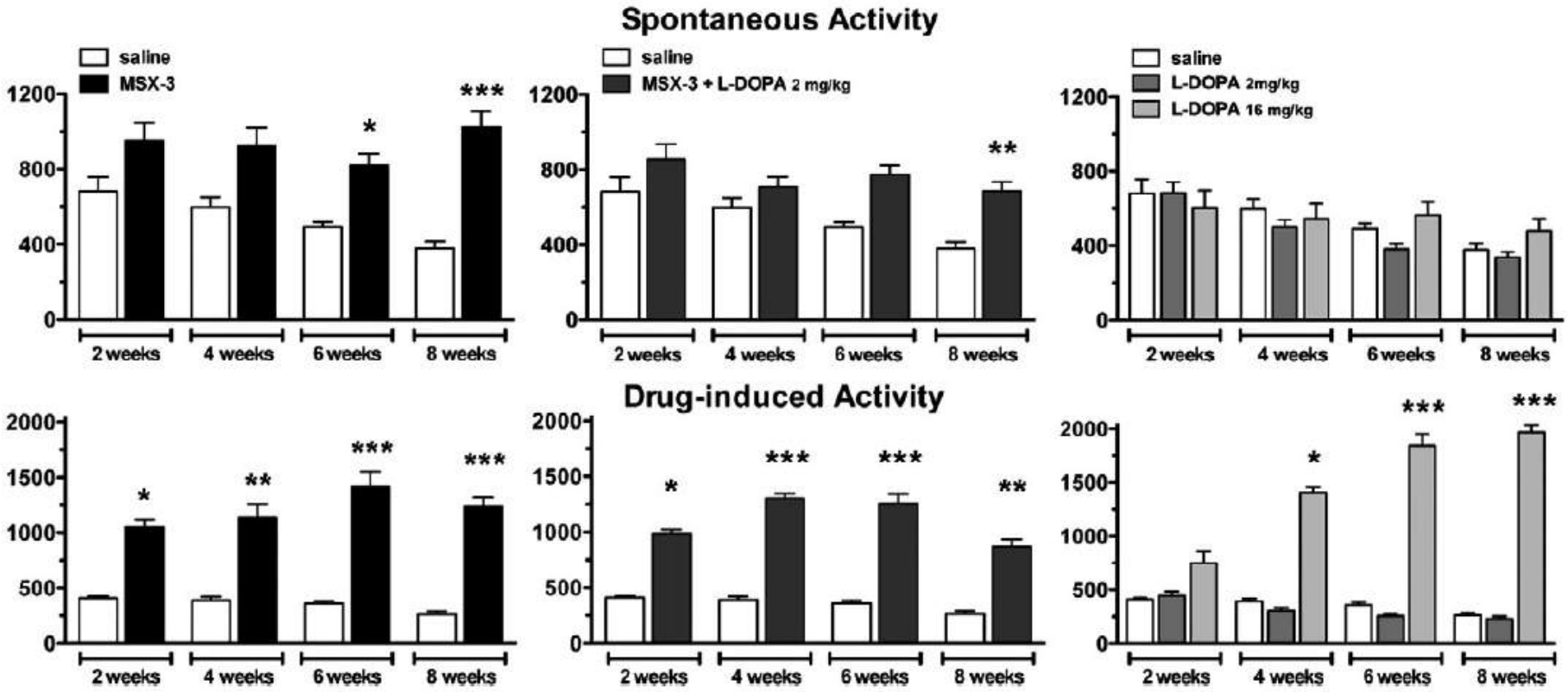
There exist encouraging results from A2AR antagonist treatments in toxin-induced PD models and the disappointing results from a clinical trial of istradefylline as monotherapy in early PD. A genetic PD model, the MitoPark mouse was therefore used. In this model DA neurons undergo a slow and progressive degeneration due to the cell-type specific induction of mitochondrial dysfunction in midbrain DA neurons

Ekstrand et al., 2007,PNAS: Progressive parkinsonism in mice with respiratory-chain-deficient dopamine neurons. Conditional knockout mice with disruption of the gene for mitochondrial transcription factor A (Tfam) in DA neurons.

These mice display gradual development of motor dysfunction, reproducing progressive stages of PD without the potential side effects of toxins used in other animal models (Galter et al., 2009). As A2AR antagonist we used MSX-3, a prodrug of the water-soluble, highly specific A2AR antagonist MSX-2, which is hydrolyzed by cellular phosphatases and exhibits more than a 100-fold higher affinity to A2AR than A1R and is almost completely inactive at A2BR andA3R (Sauer et al., 2000).

Locomotion of MitoPark mice during 8 weeks of daily treatment with saline, MSX-3, MSX-3 plus L-DOPA, or two doses of L-DOPA

Chronic monotherapy with A2A antagonists may help counteract PD by blocking the reorganization of A2A heteroreceptor complexes with progressive DA cell degeneration.



(Validated by Smith et al.2014)

Marcellino et al.2010

A Critical Evaluation of Behavioral Rodent Models of Motor Impairment Used for Screening of Antiparkinsonian Activity: The Case of Adenosine A2A Receptor Antagonists

Annalisa Pinna and Micaela Morelli 2014

-Rodent models of PD suggested that A2A receptor antagonists might have symptomatic therapeutic efficacy in PD.

-Clinical trials evaluated their efficacy in the ‘‘ON/OFF’’ of PD patients with motor complications, showing a limited efficacy of this class of drug.

Treatment of motor fluctuations in Parkinson’s disease: recent developments and future directions

Adolfo Ramirez-Zamora and Eric Molho 2014

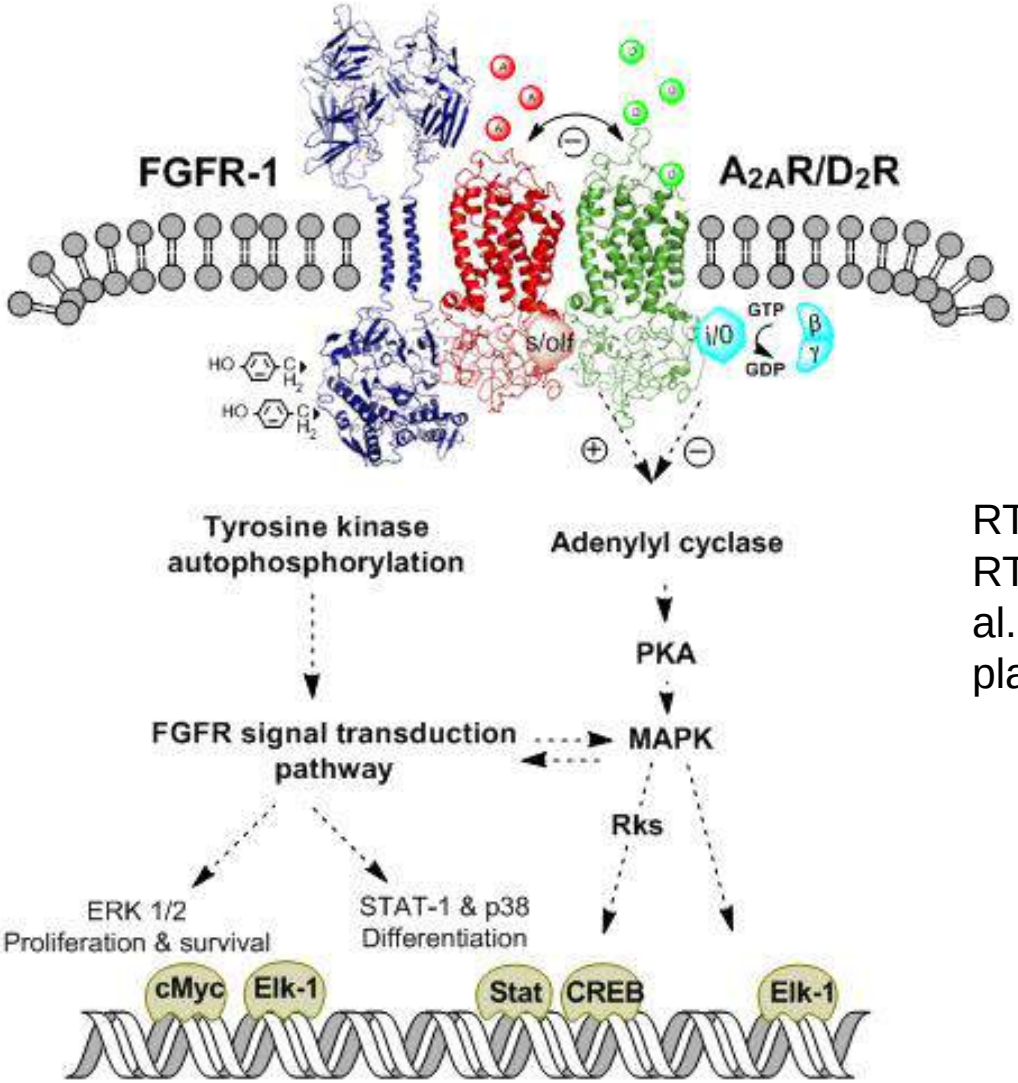
-Adenosine A2A receptors including istradefylline and preladenant have been shown to improve motor disability without inducing dyskinesia in animal models of PD and in small clinical trials

Adenosine A2A Receptor Antagonists in Parkinson's Disease: Progress in Clinical Trials from the Newly Approved Istradefylline to Drugs in Early Development and Those Already Discontinued.

Pinna A. 2014

- phase II and III trials also demonstrate that A2A antagonists are effective in reducing off-time, without worsening troublesome dyskinesia, and in increasing on-time with a mild increase of non-troublesome dyskinesia, in patients at an advanced stage of PD treated with L-DOPA. Early findings suggest that A2A antagonists can be efficacious as monotherapy in patients at an early stage of PD.

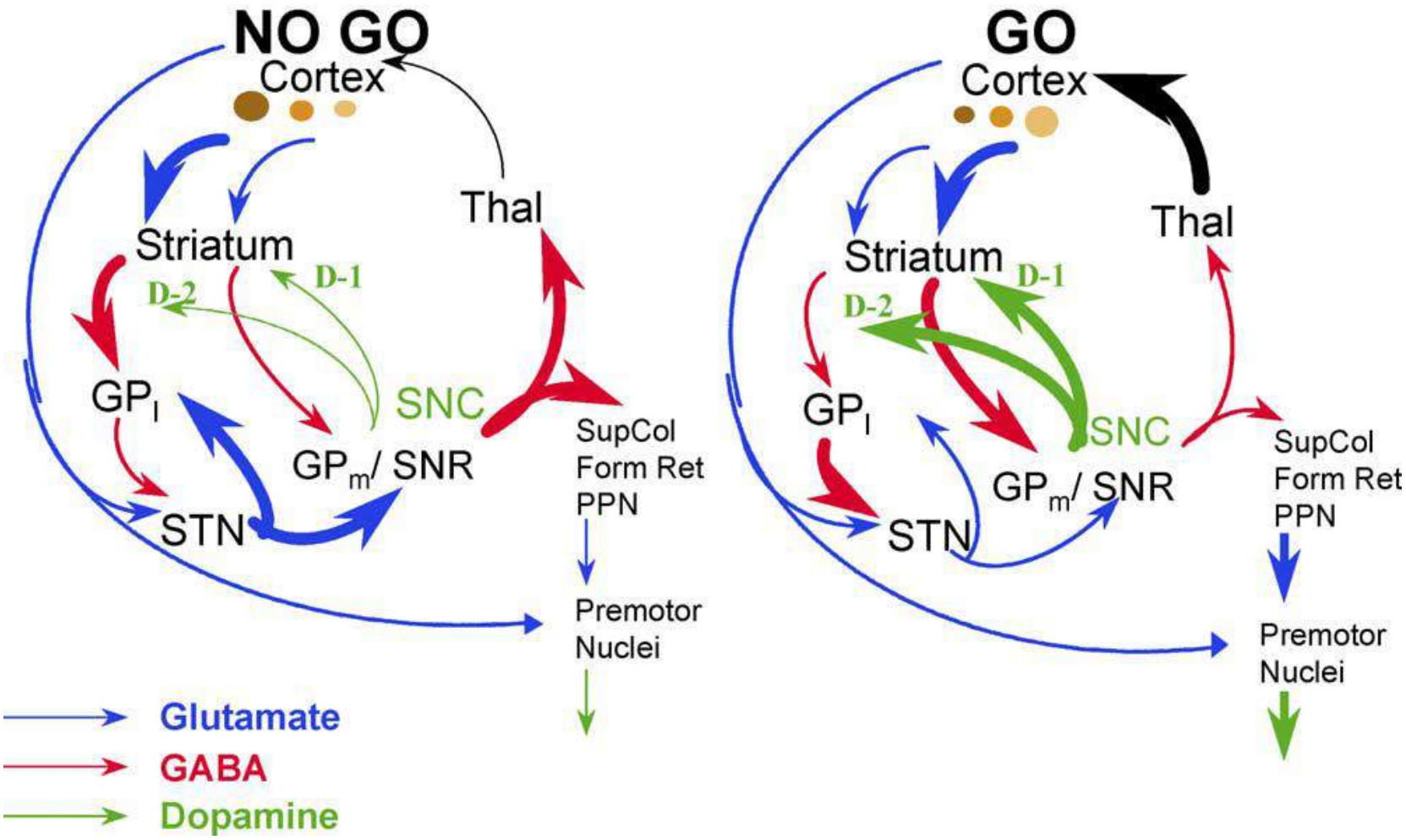
Postulated striatal FGFR1-A2A-D2 heteroreceptor complex



RTK transactivation via direct GPCR-RTK receptor interactions (see Fuxe et al.2007) can lead to effects on neuronal plasticity

Flajolet et al.2008; Borroto-Escuela et al. 2013

The changes in the activity of basal ganglia circuits in Parkinson's Disease ('NO GO') are indicated vs the normal state ('GO').



Now is the time to understand and remove the brakes on the D1 receptor signaling in the D1 heteroreceptor complexes of the direct pathway in PD

Tanganelli et al.2004

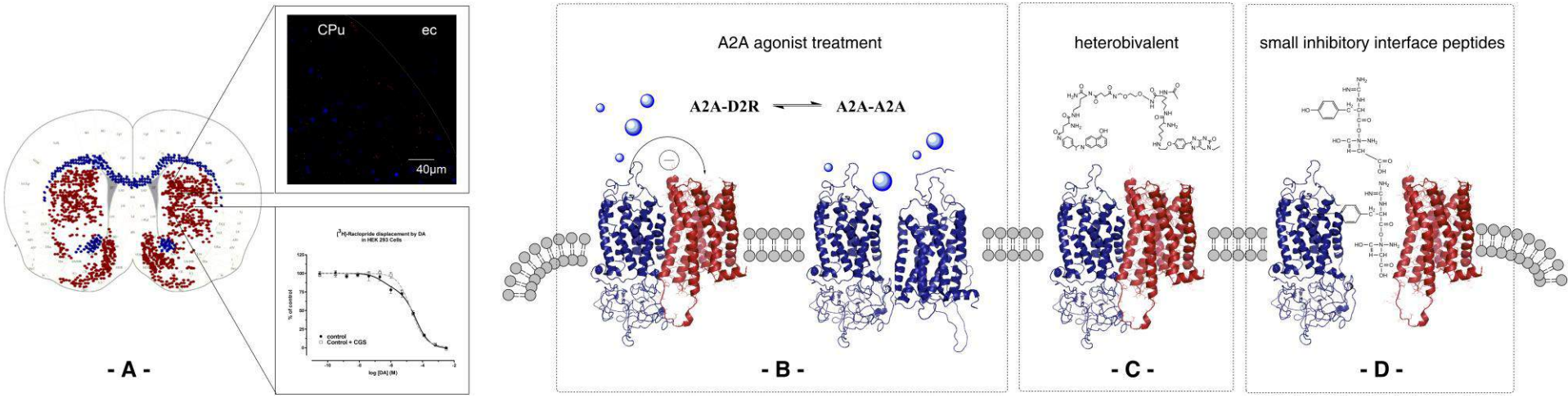
A_{2A}/D_2 heteroreceptor complexes and A2A mono-homomers : Targets for A_{2A} antagonists in Parkinson's disease

- The demonstrated modest anti-parkinsonian effects of A_{2A} antagonists in animal models and clinical studies have given support to the concept that blocking antagonistic allosteric A_{2A}- D_2 receptor-receptor interactions in heteroreceptor complexes can contribute to the development of novel therapies of PD.

- The A_{2A} receptor protomer in the A_{2A}-D_2 heteroreceptor complex in the dorsal striato-pallidal GABA neurons with antagonistic A_{2A}/D_2 receptor-receptor interactions may be a significant target for A_{2A} antagonists with regard to antiparkinsonian effects.

- The A_{2A} mono-homomer may also be a significant target for A_{2A} antagonists with regard to antiparkinsonian and especially antidyskinetic actions and the wearing off phenomena of the therapeutic effects of L-DOPA. Monotherapy with A2A antagonist is recommended in early PD

Illustration of pharmacological approaches to target striatal A2A-D2like heteroreceptor complexes for treatment of Parkinson's disease



A2A antagonists: So far, they target both A2A homomers and A2A-D2like heteroreceptor complexes.
Preferential A2A antagonists for A2A protomer? Preferential D2 agonists for the D2 protomer? Multitarget drugs

Heterobivalent drugs : A2A antagonist pharmacophor linked to a D2 agonist pharmacophor may preferentially target A2A-D2 like heteroreceptor complexes.High affinity for A2A-D2 heterocomplex

Small inhibitory interface peptides targeting the A2A-D2 interface: So far, only used to block the formation of A2A-D2. Can be used also to modulate antagonistic allosteric communication over the A2A-D2 interface

Borroto-Escuela et al.2015