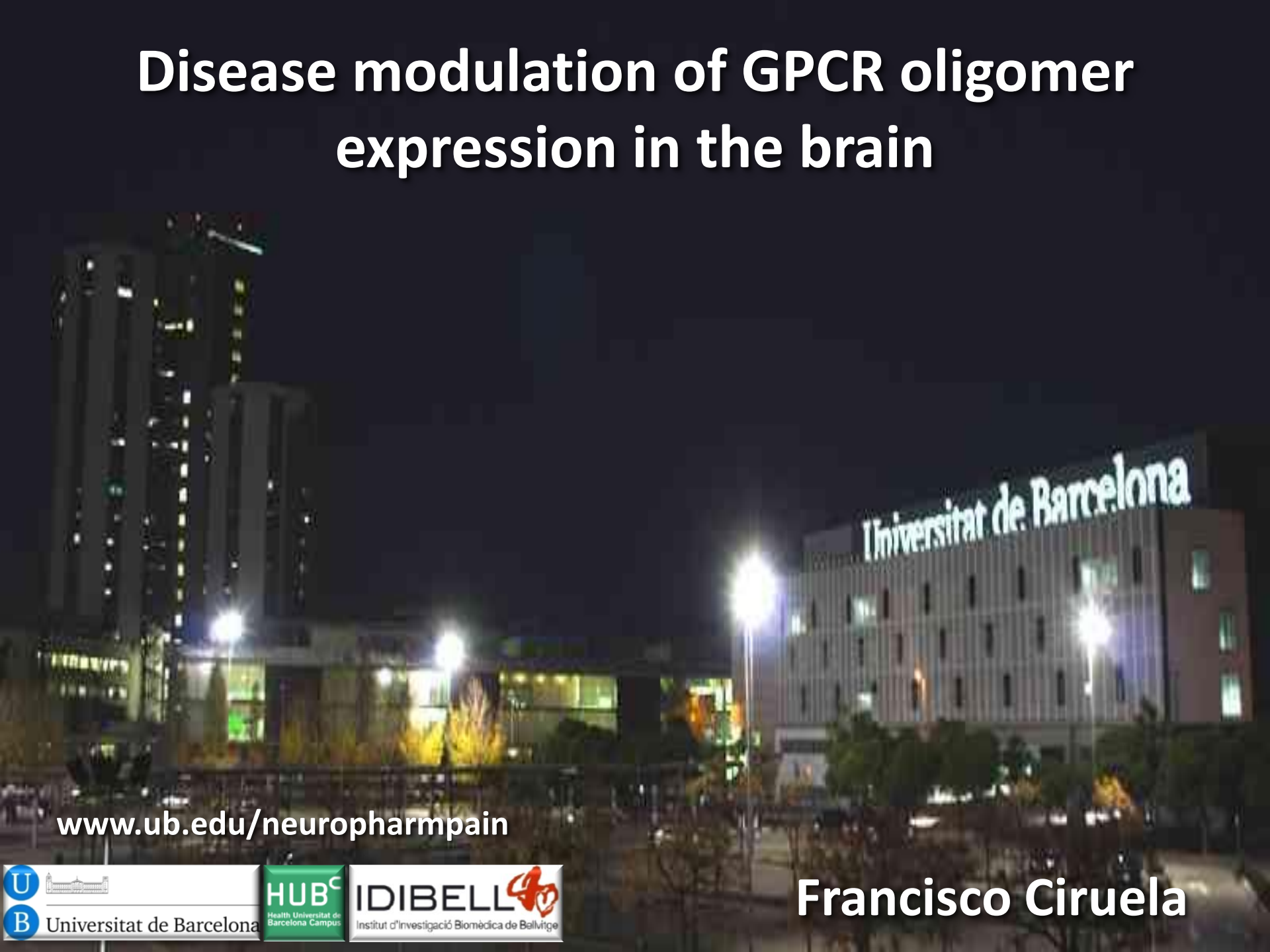
Disease modulation of GPCR oligomer expression in the brain



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Universitat de Barcelona
HUB Health Universitat de Barcelona Campus
IDIBELL Institut d'Investigació Biomèdica de Bellvitge

Francisco Ciruela

Outline

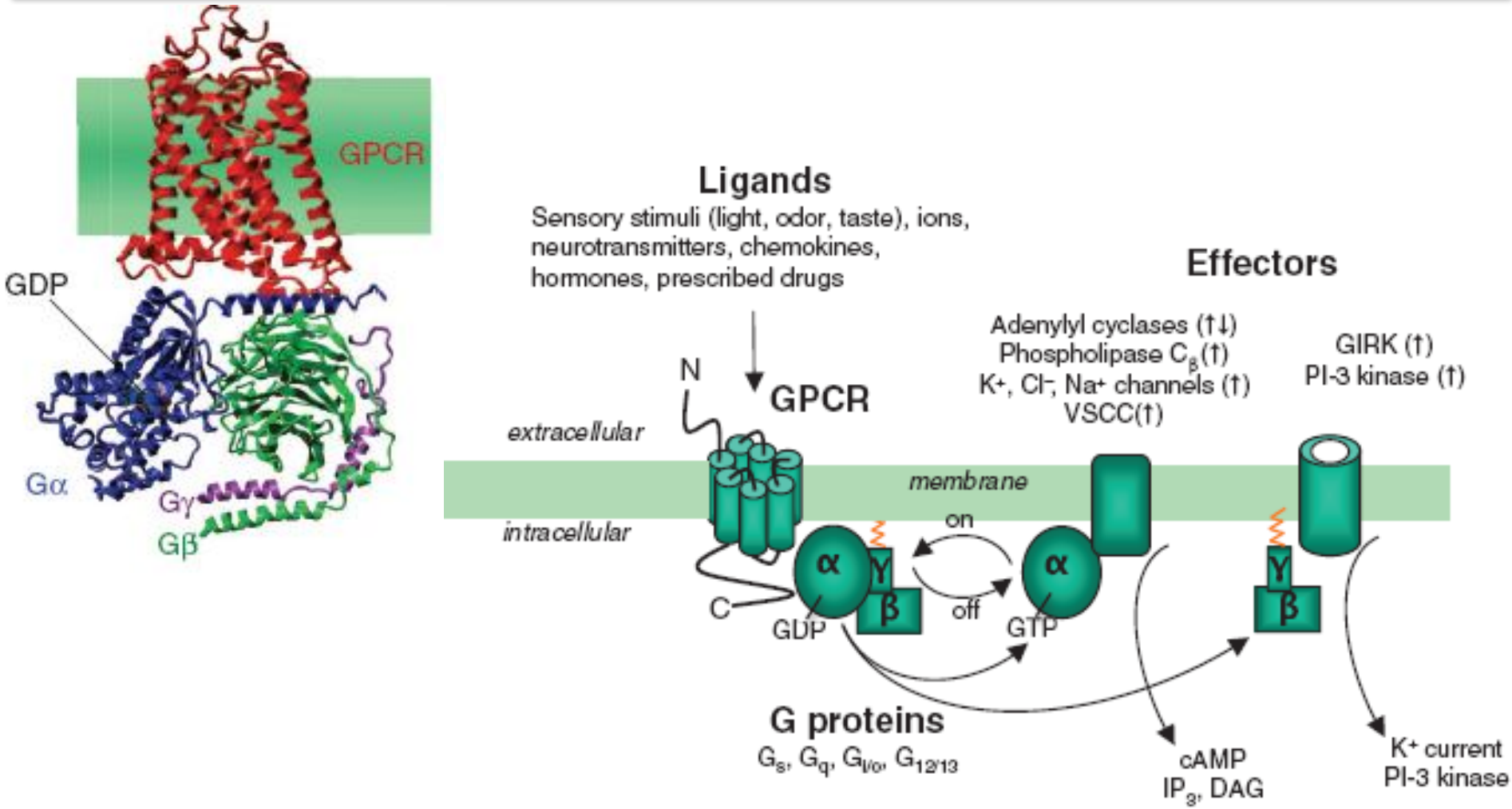
1. Introduction: G protein-coupled receptors oligomerization

2. Visualizing adenosine/dopamine receptors oligomers in native tissue (i.e. striatum)

3. Potential impact of adenosine and dopamine receptors oligomerization in Parkinson's disease therapeutics

4. Overall conclusion

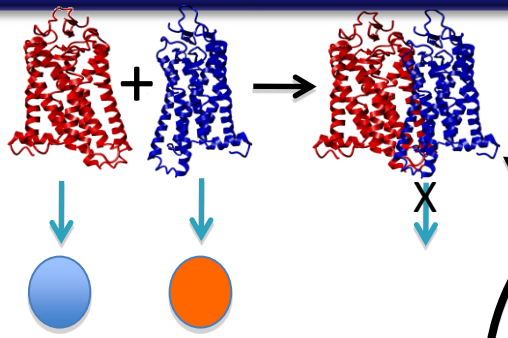
General principle of the GPCR signalling system



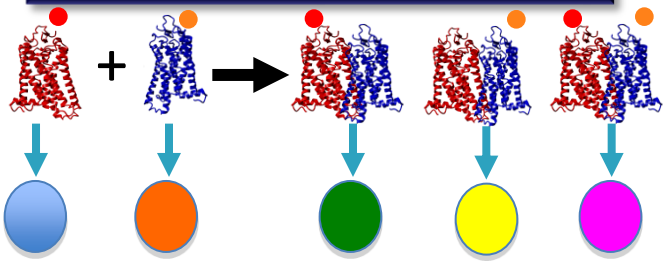
Vilardaga et al., 2010. J. Cell. Sci. 123, 4215-4220.

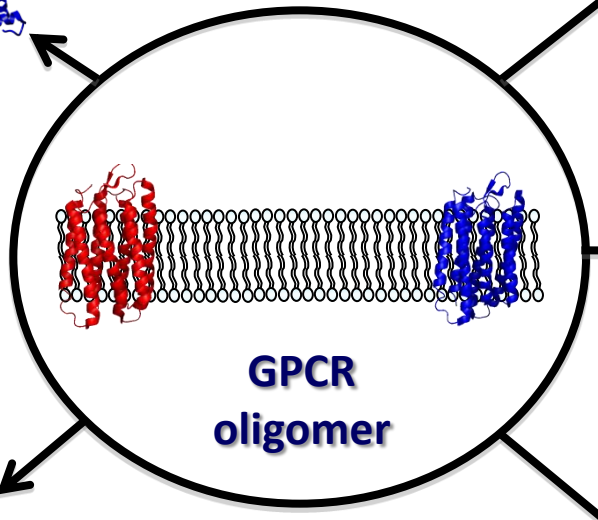
GPCR oligomerization

Modulation of G protein activation



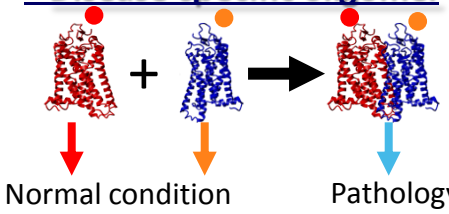
Modulation of G protein selectivity



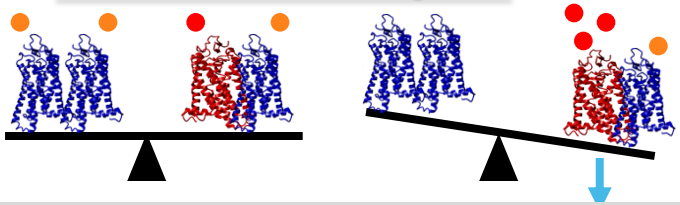


Role in pathophysiology

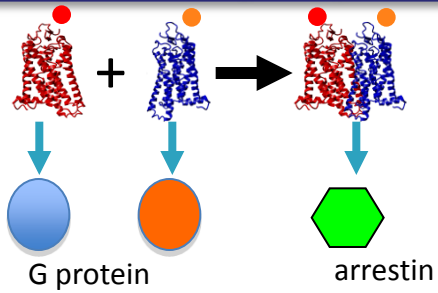
- **Disease-specific oligomer**



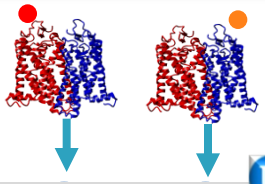
- **Disease-unbalanced oligomer**



Modulation of signaling cascades

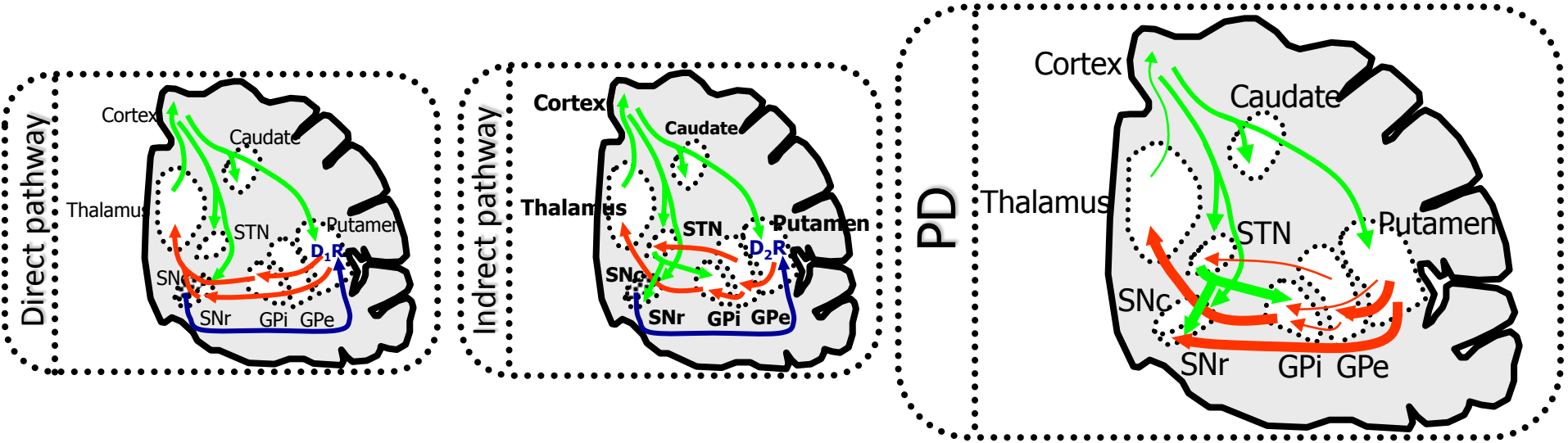


More allosteric sites for modulation



Question

There is any relationship between Parkinson's disease and $A_{2A}R/D_2R$ oligomerization state?

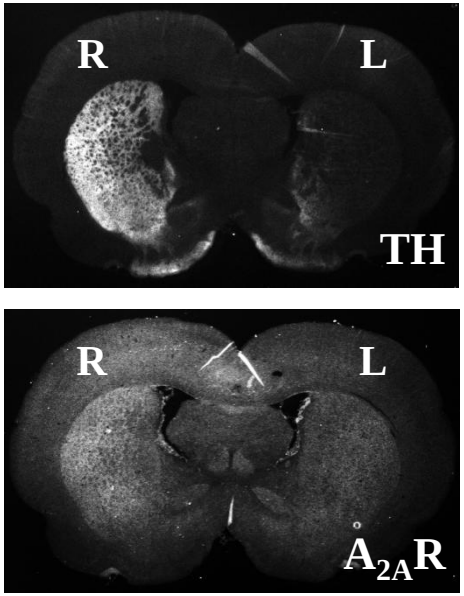


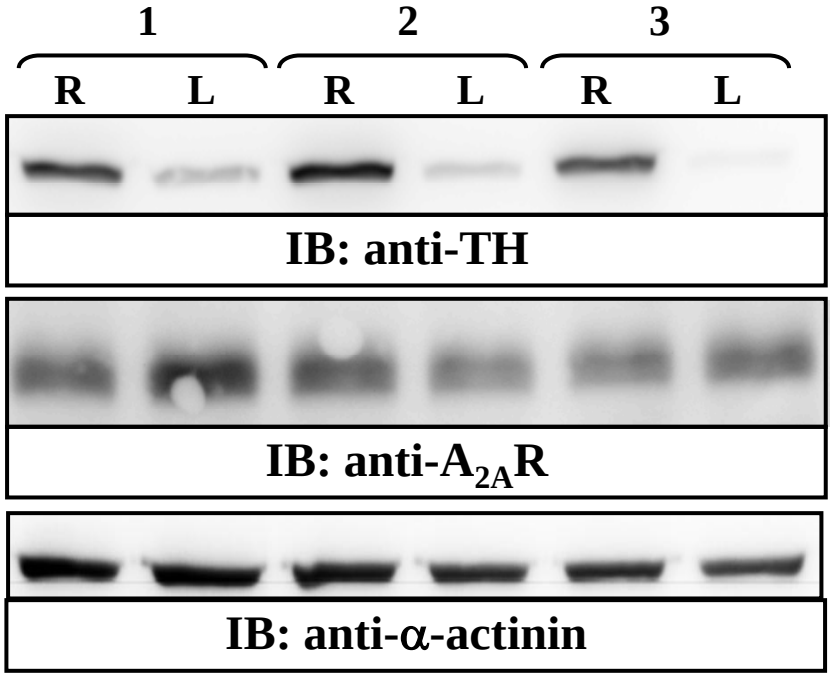
Basal ganglia circuitry and PD

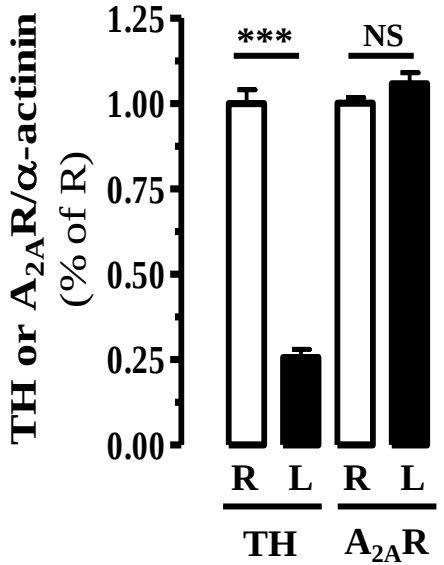
Vallano et al., 2013. CNS & Neurol. Dis.-Drug Targets. 12(8):1128-42.

Detection of striatal $A_{2A}R/D_2R$ oligomer in a PD animal model

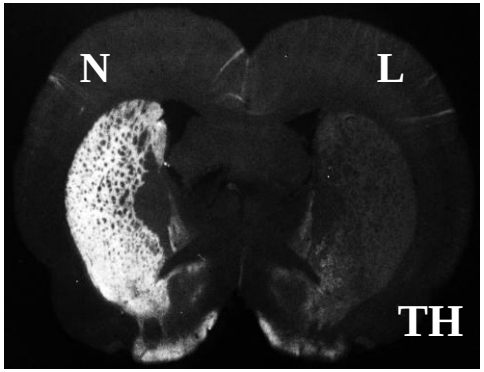
The 6-OHDA-lesioned rat: a validated animal model of PD

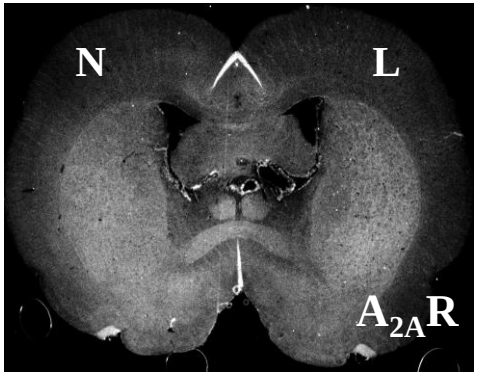


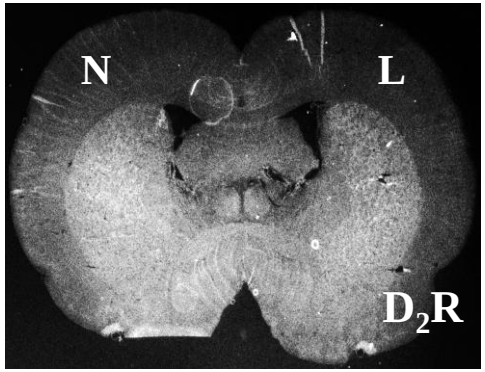




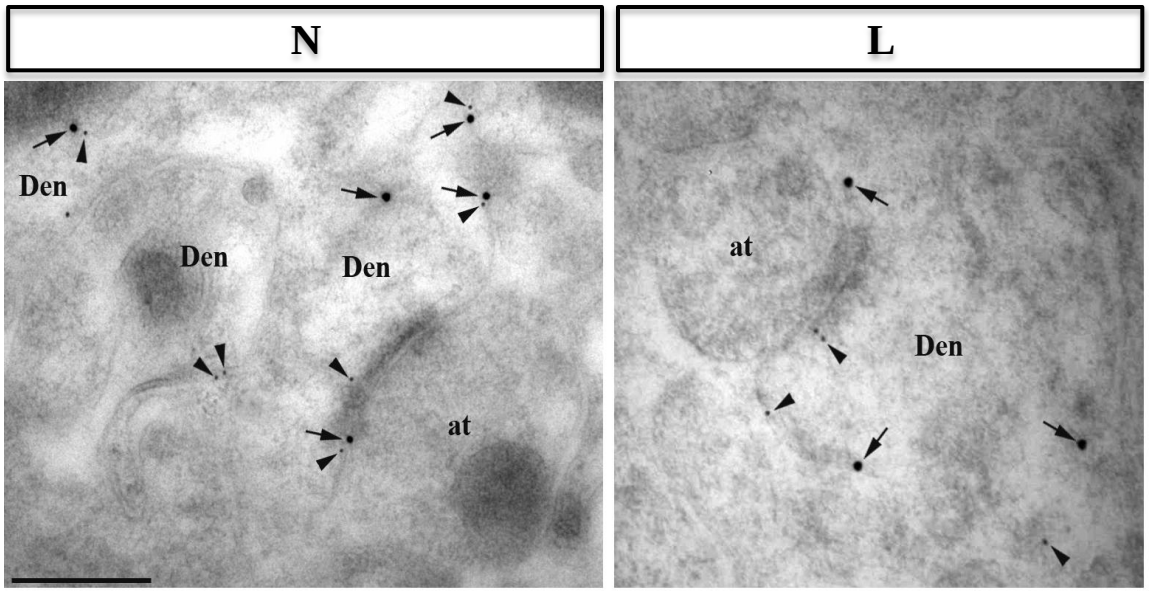
D_2R-$A_{2A}R$ co-clustering in the striatum of normal and 6-OHDA-lesioned rats

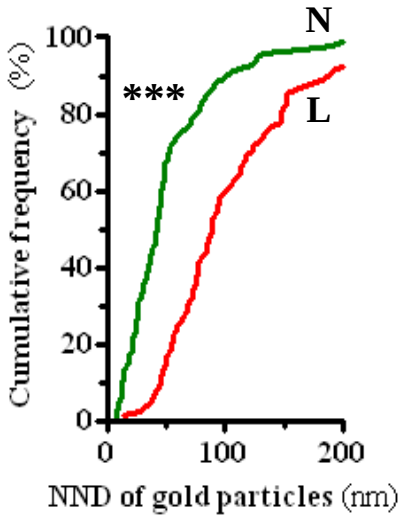






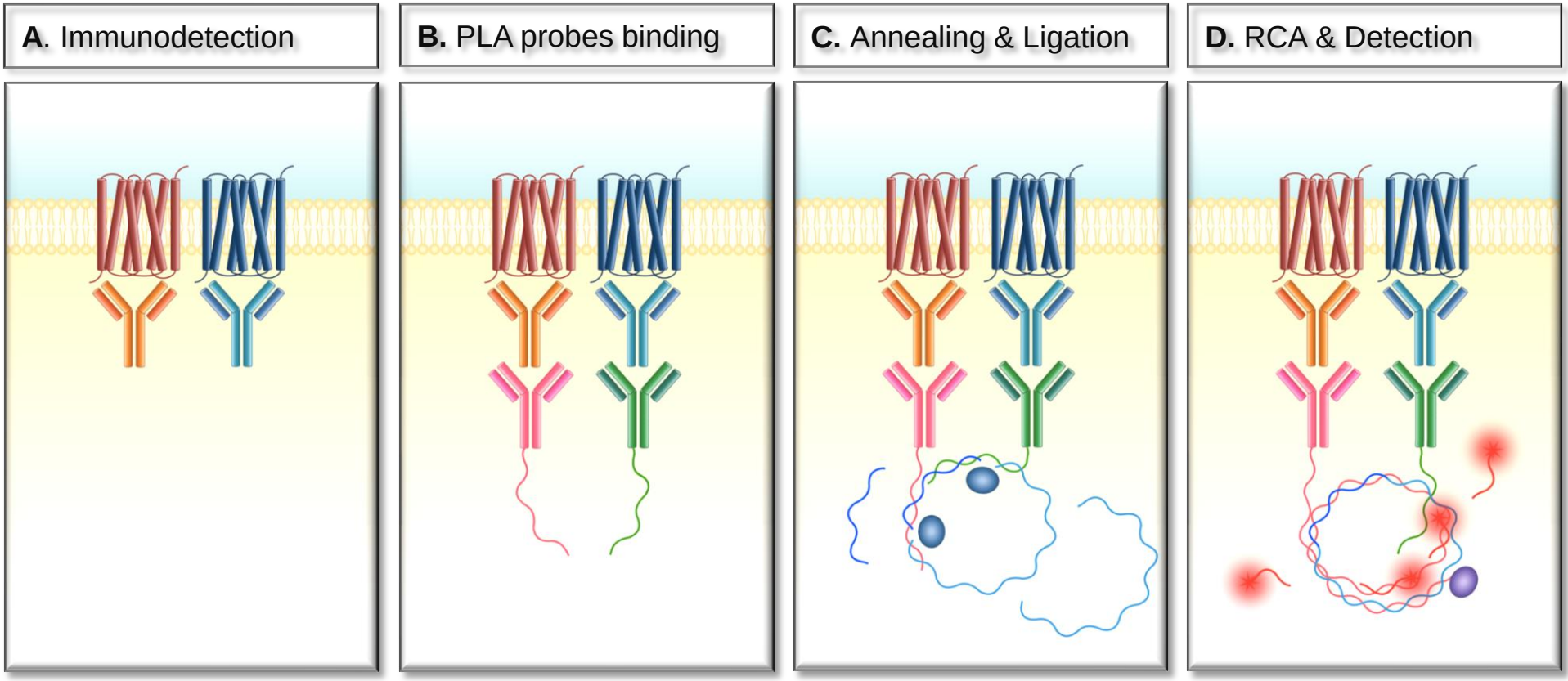
►$A_{2A}R$
→D_2R





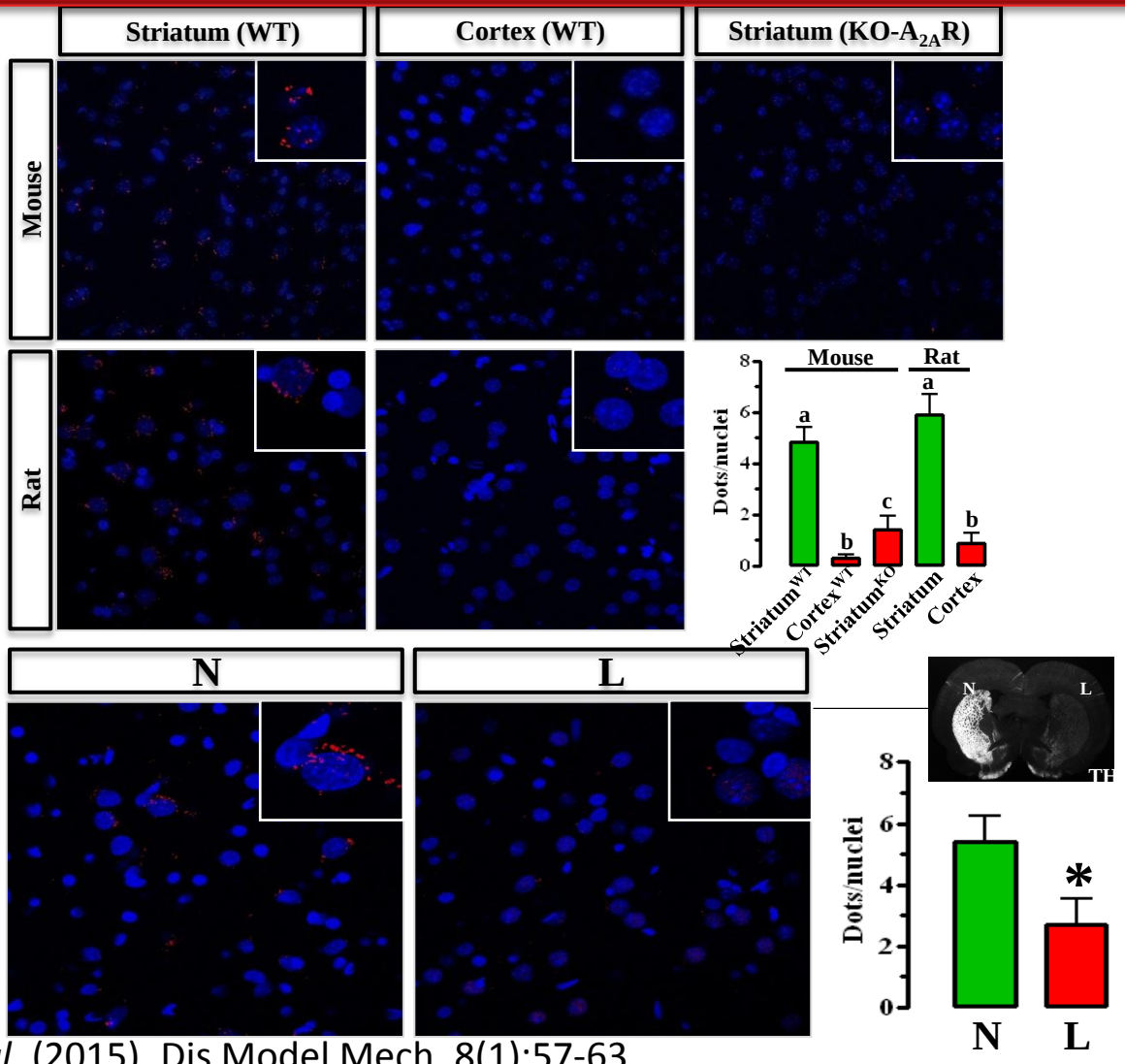
Fernández-Dueñas *et al*. (2015). Dis Model Mech. 8(1):57-63.

The PLA technique



Taura *et al*. (2015). Current Protocols in Cell Biology **67**, 17.17.1-17.17.16.

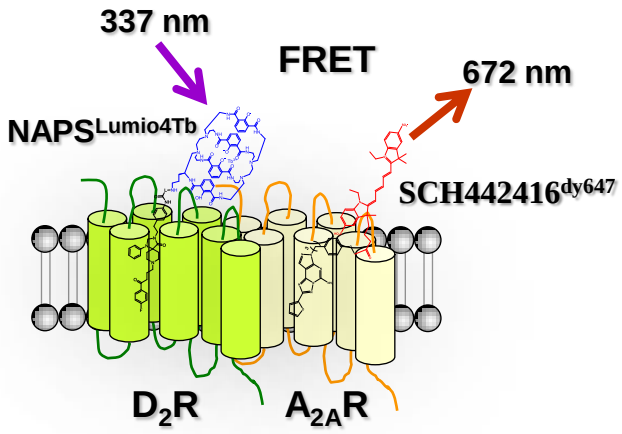
Detection of D_2R and $A_{2A}R$ proximity in normal and 6-OHDA-lesioned rat striatal sections



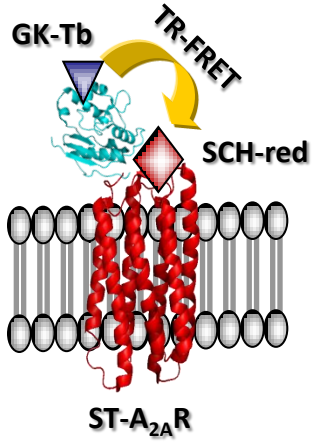
Fernández-Dueñas *et al*. (2015). Dis Model Mech. 8(1):57-63.

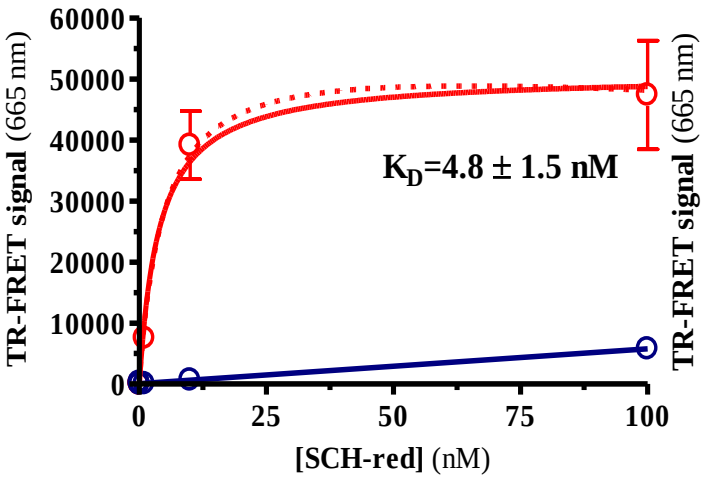
Detection of D_2R and $A_{2A}R$ proximity in normal and 6-OHDA-lesioned rat striatal sections

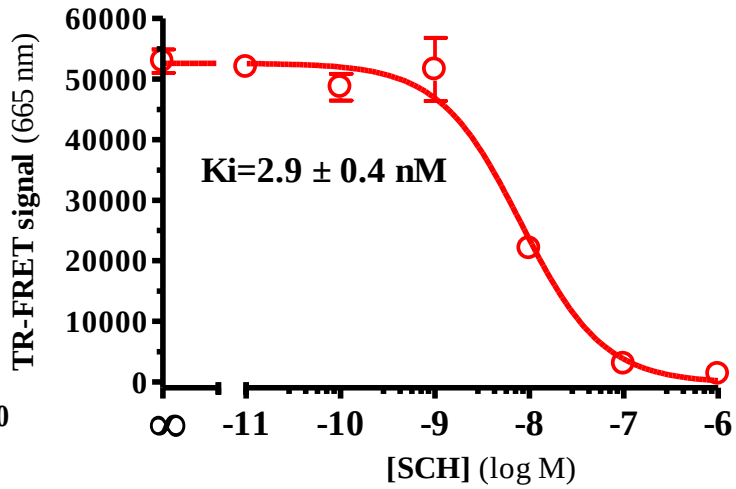
TR-FRET using fluorescent adenosine and dopamine receptor antagonists in striatal membranes

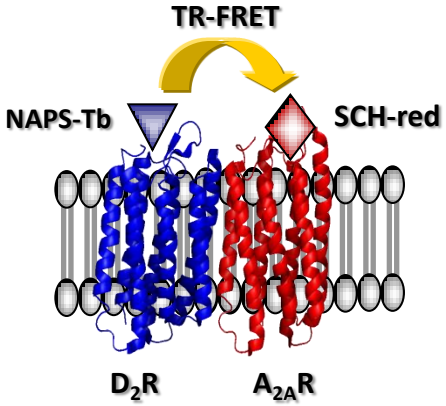


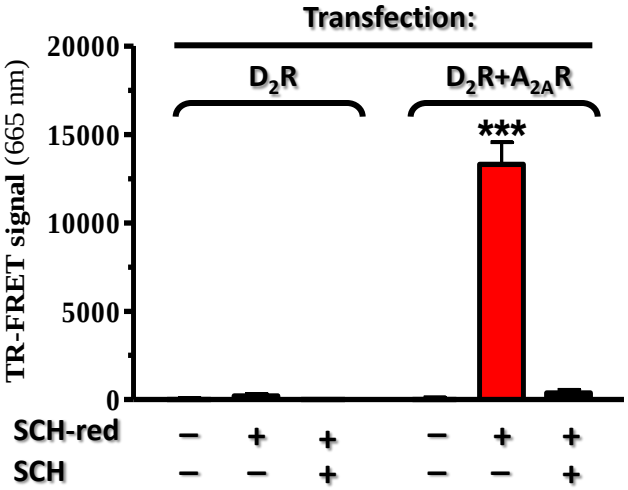
Detection of adenosine receptor containing oligomers in HEK cells

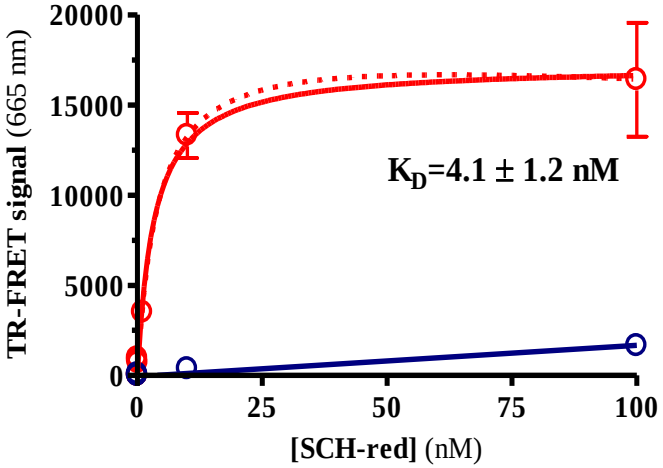






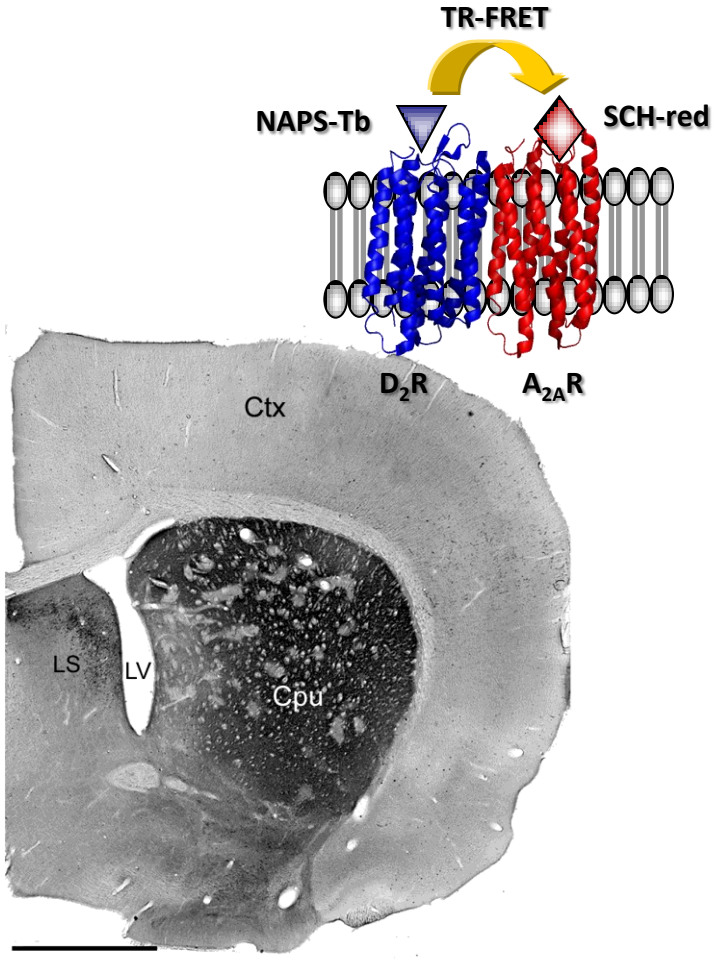


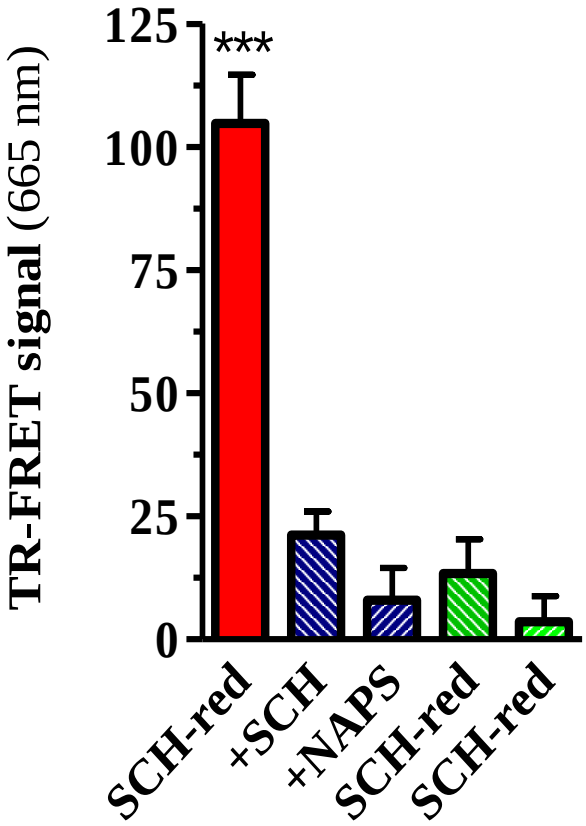




Fernández-Dueñas *et al*. (2015). Dis Model Mech. 8(1):57-63.

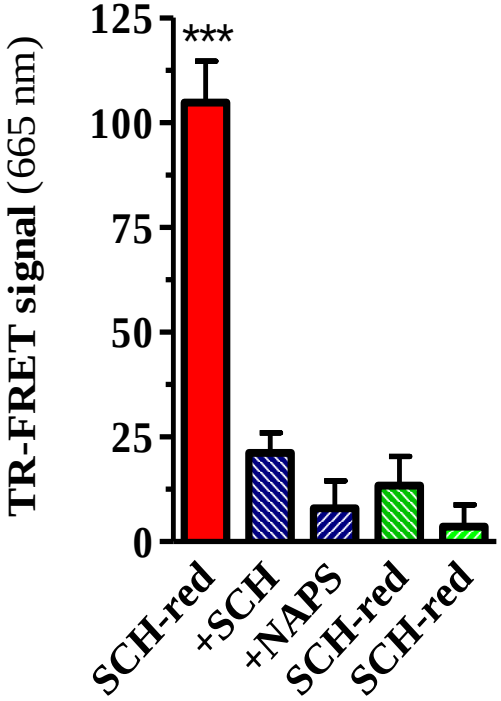
Detection of adenosine receptor containing oligomers in native tissue

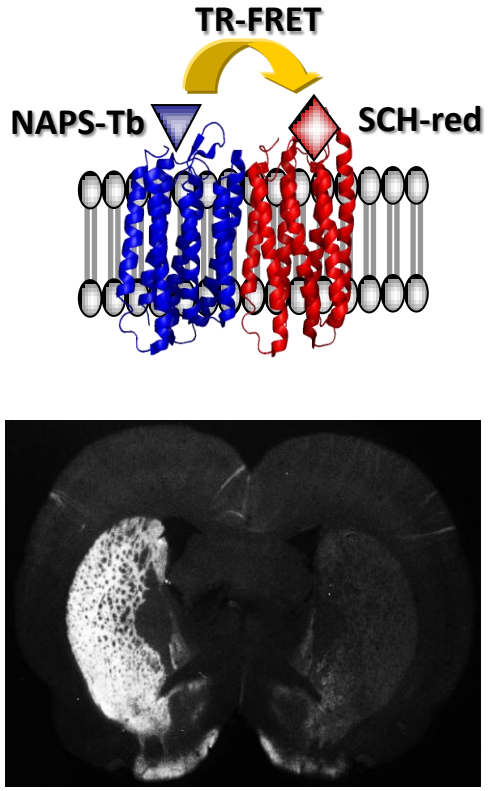


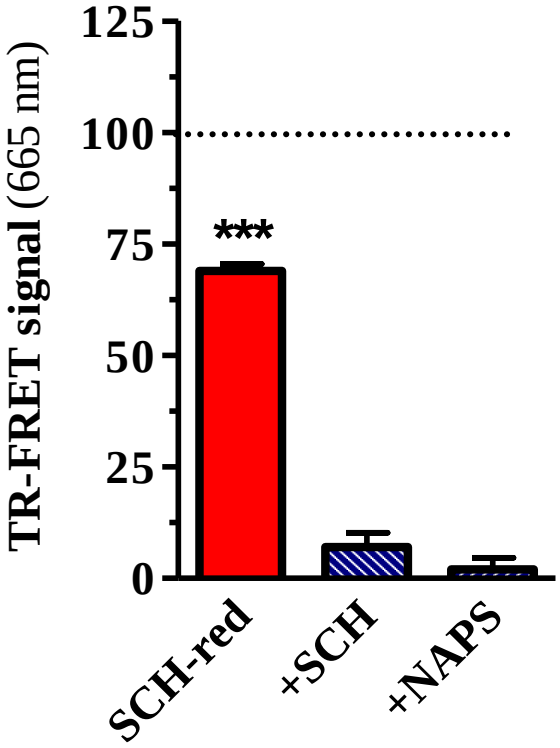


Fernández-Dueñas *et al*. (2015). Dis Model Mech. 8(1):57-63.

Detection of the $A_{2A}R/D_2R$ oligomer in the striatum of the PD animal model



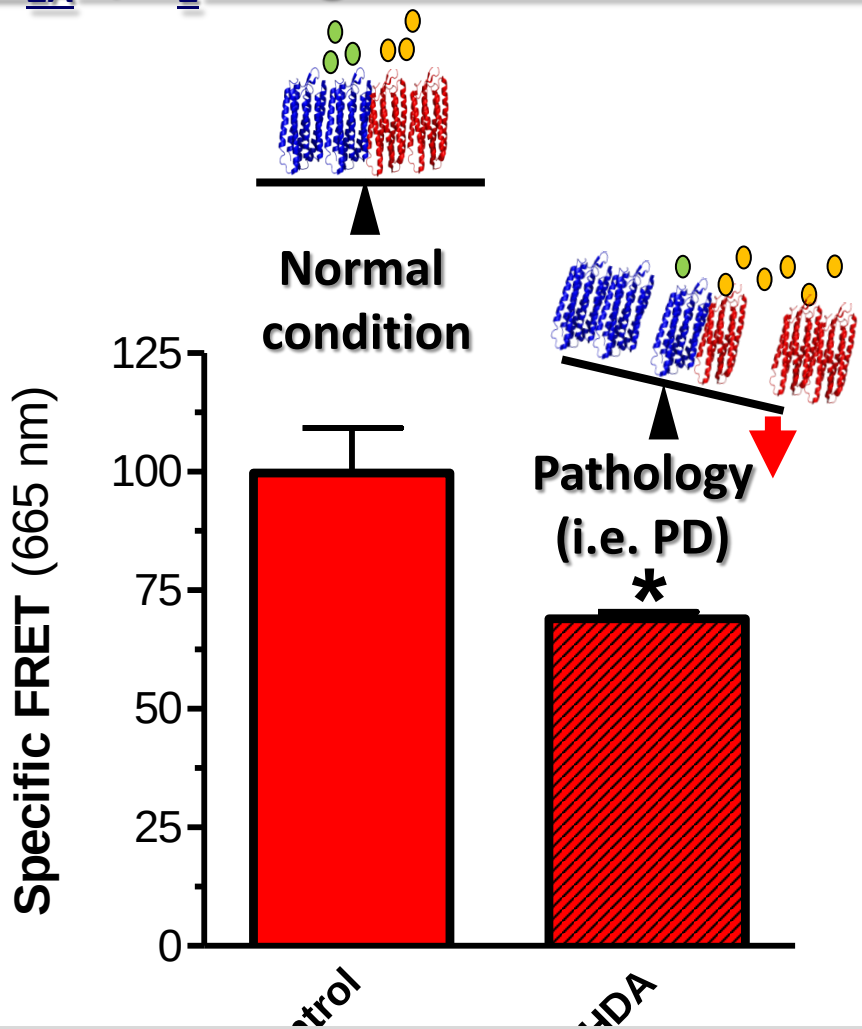


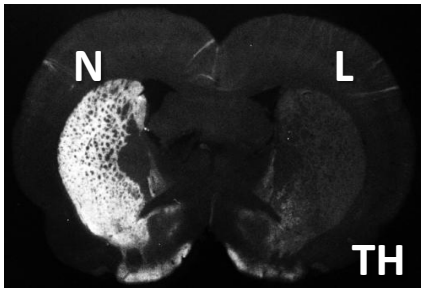


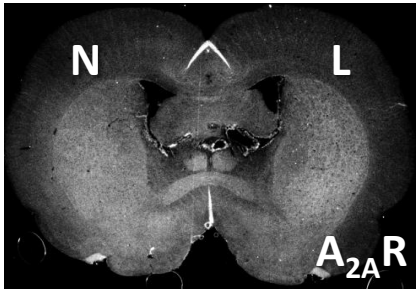
Fernández-Dueñas *et al*. (2015). Dis Model Mech. 8(1):57-63.

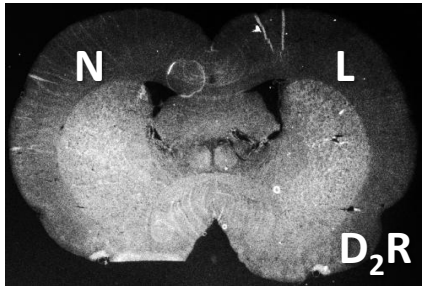
Conclusion

- $A_{2A}R/D_2R$ oligomer is unbalanced in experimental parkinsonism





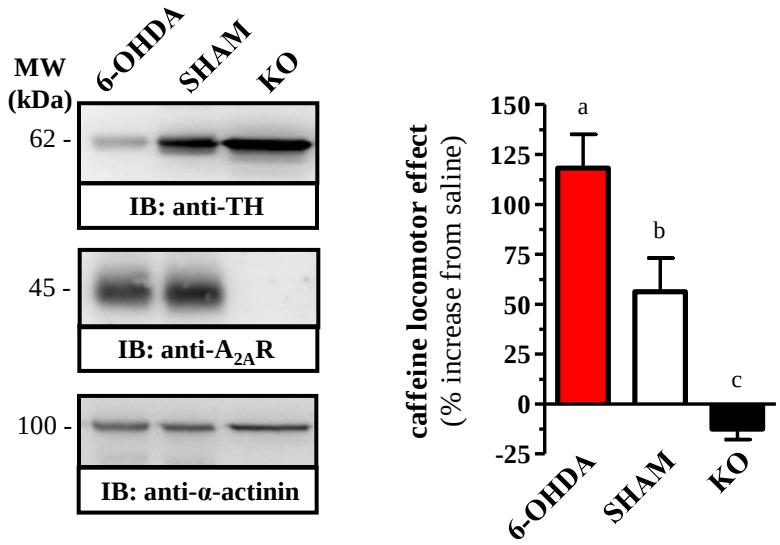




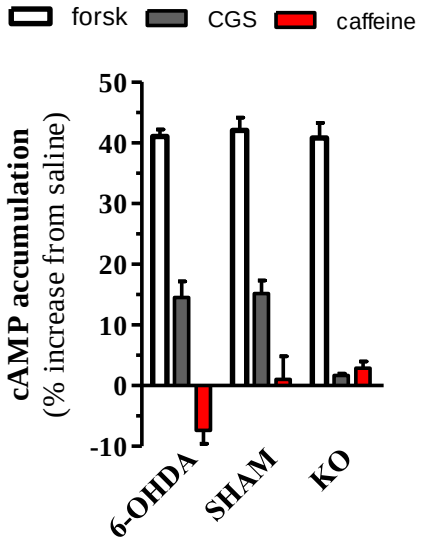
Functional impact of oligomer down-regulation

The striatal $A_{2A}R$ shows constitutive activity in experimental parkinsonism

- **Caffeine-evoked locomotor activity in 6-OHDA lesioned mice**

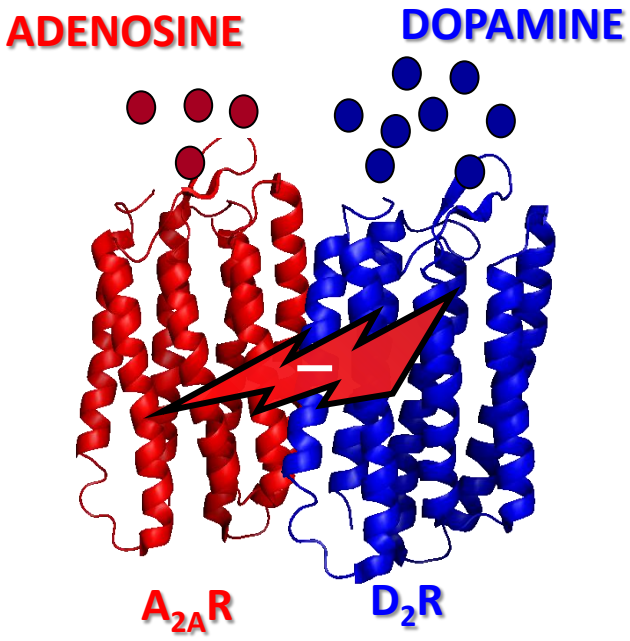


- **cAMP measurement in striatal synaptosomes**



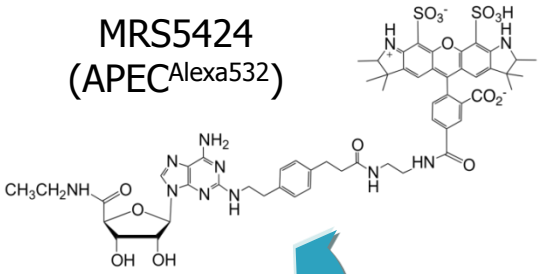
Fernández-Dueñas *et al*. (2014). ACS Chem. Biol. 9 (11), 2496-2501.

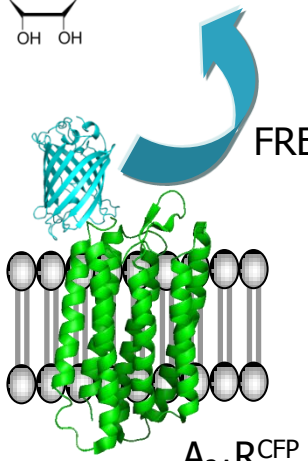
Which should be the profile of an anti-PD drug?



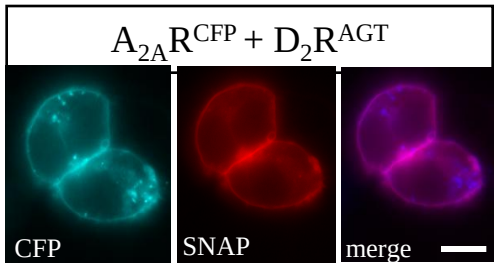
Effect of D_2R challenge on real-time $A_{2A}R$ agonist binding

MRS5424
(APEC^{Alexa532})





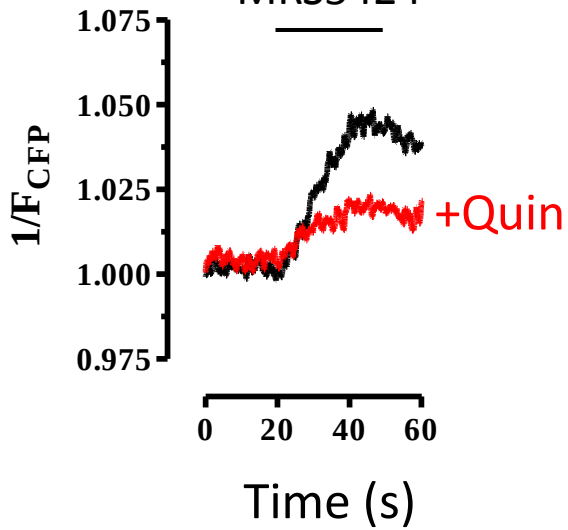
Transfection

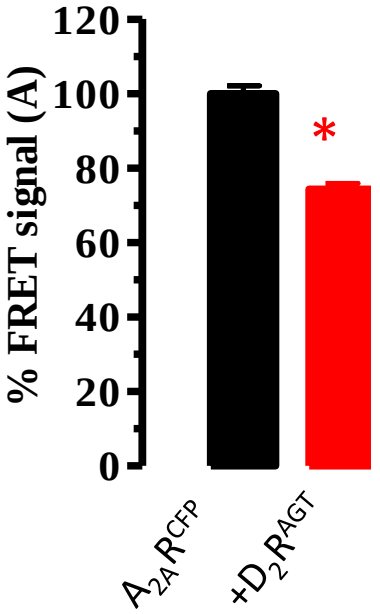


Transfection: $A_{2A}R^{CFP}$

+ D_2R^{AGT}

MRS5424





Fernández-Dueñas et al., 2012. J. Neurochem. 123, 373-384.

Effect of D_2R challenge on real-time $A_{2A}R$ agonist binding

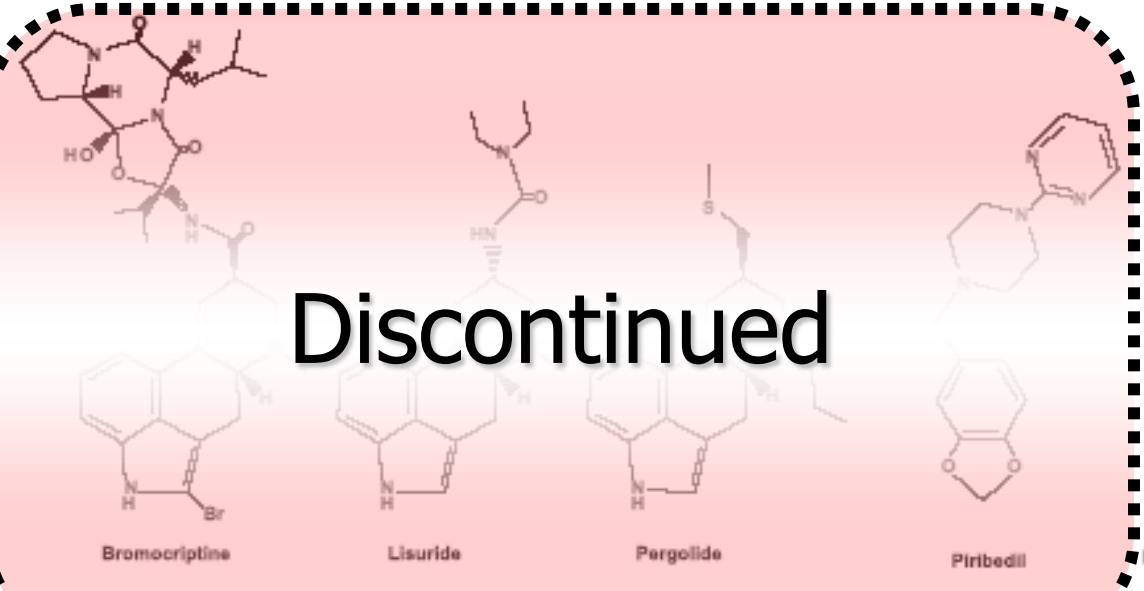
D_2R agonists are negative allosteric modulators (NAMs) of $A_{2A}R$ binding and this phenomenon is $A_{2A}R/D_2R$ oligomer dependent

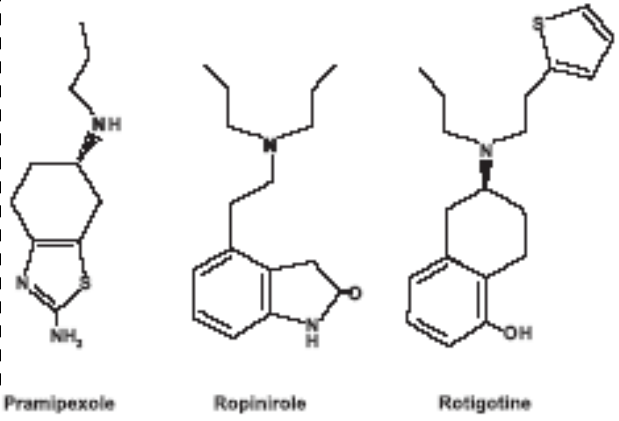
Parkinson's disease context

Effect of D_2R challenge on real-time $A_{2A}R$ agonist binding

Screening for more efficacious D_2R agonists to be used in PD management

Discontinued

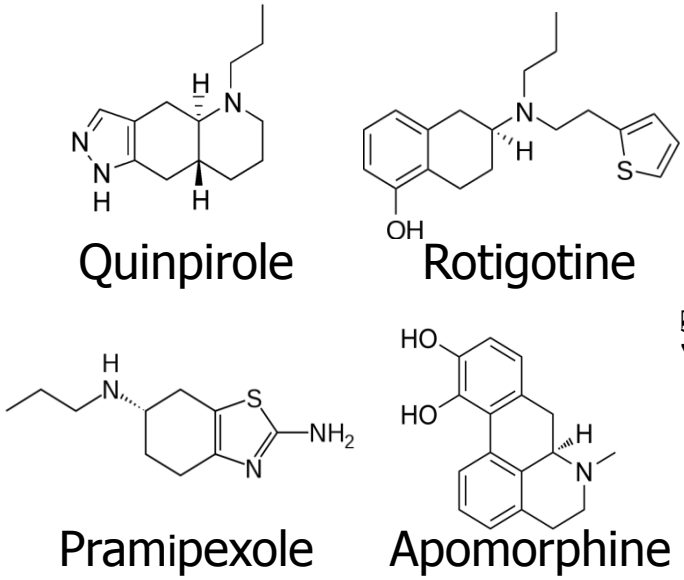


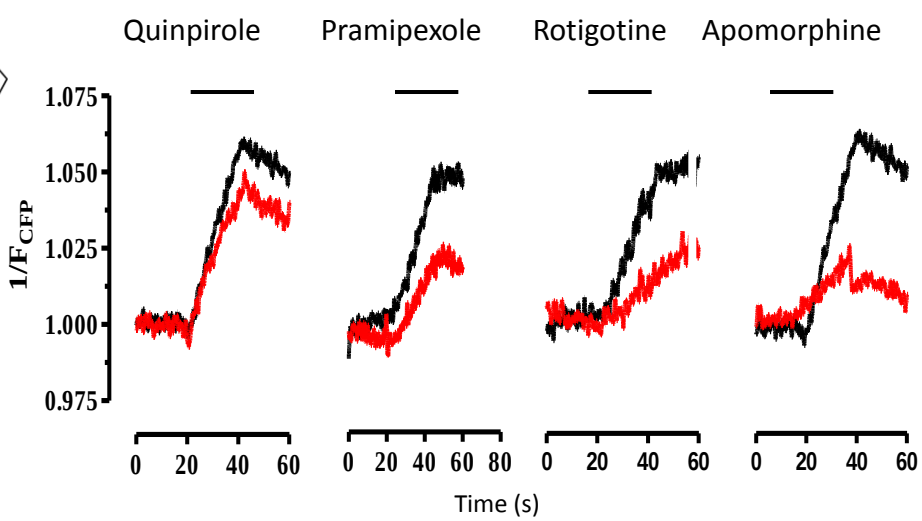


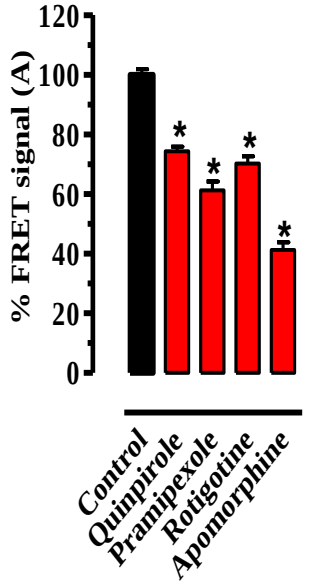
Fernández-Dueñas *et al*. (2013). Neurochem. Int. 63 (1), 42-46..

Effect of D_2R challenge on real-time $A_{2A}R$ agonist binding

Testing the $A_{2A}R$ NAM activity of most common therapeutic D_2R agonists



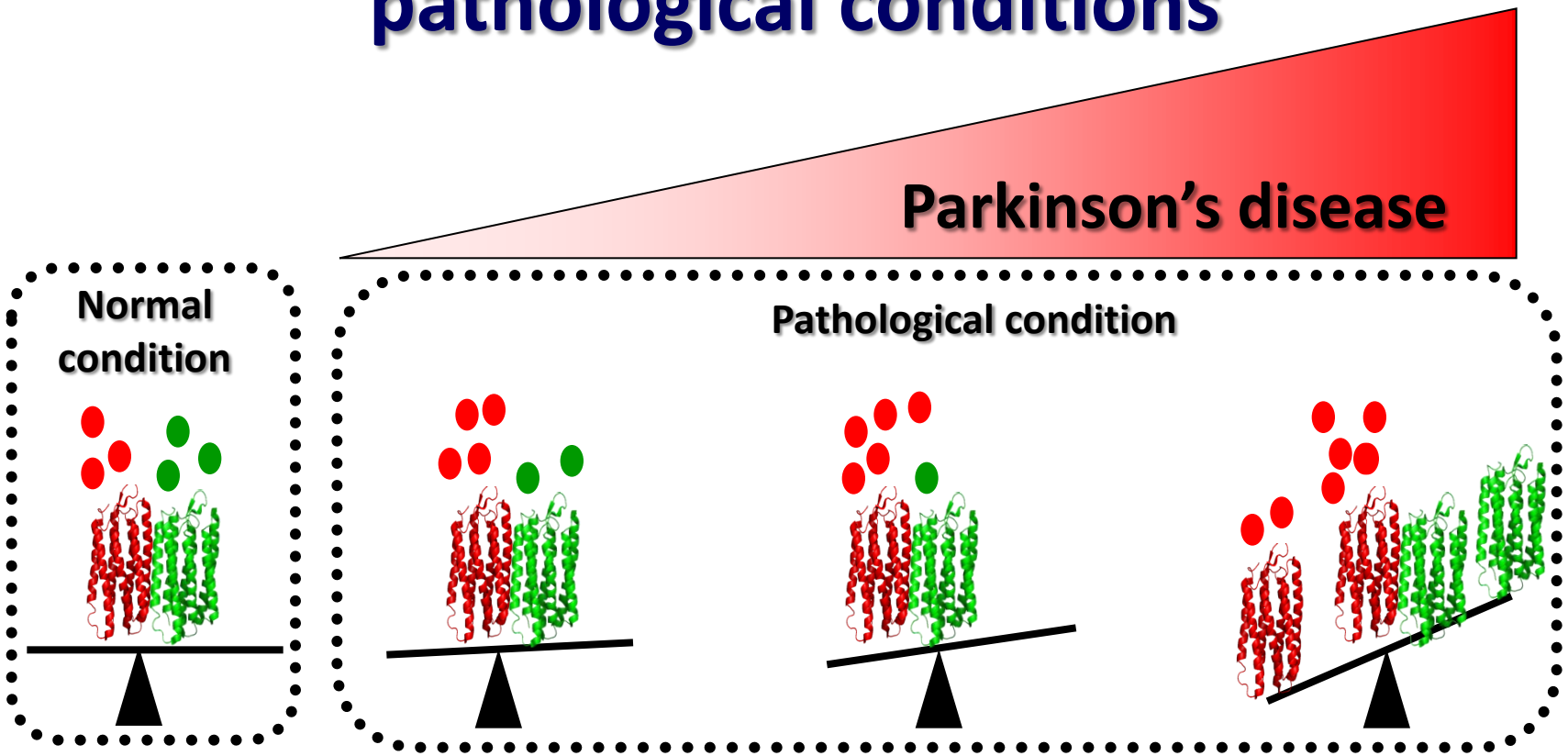




Fernández-Dueñas *et al*. (2013). Neurochem. Int. 63 (1), 42-46.

Conclusion

Ontogeny of $A_{2A}R/D_2R$ oligomer in normal and pathological conditions



Overall Conclusion

- ✓ We demonstrate the existence of the $A_{2A}R/D_2R$ oligomers in native tissue (i.e. striatum)
- ✓ The striatal dopaminergic denervation induces a down-regulation of the $A_{2A}R/D_2R$ oligomer
- ✓ The down-regulation of the striatal $A_{2A}R/D_2R$ oligomer correlates well with an increase in $A_{2A}R$ constitutive activity
- ✓ D_2R agonists behave as $A_{2A}R$ NAMs in an oligomer-dependent manner. Thus, this might be interesting in the design, screening and usage of D_2R-based drugs for PD pharmacotherapy

Thanks to our collaborators!

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Ken Jacobson
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Kjell Fuxe
Karolinska Institutet, Sweeden

Veli-Pekka Jaakola
Oulu University, Finland

Igor Stagljar
Toronto University, Canada

JM Arnau
A Vallano
C Pedrós
S Sánchez
L Cuffí
L Carbonell
V Fernández
X Morató
J Taura
M Gómez
M López
G García
M Valle
S Sánchez

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Hokkaido University School of Medicine, Japan

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