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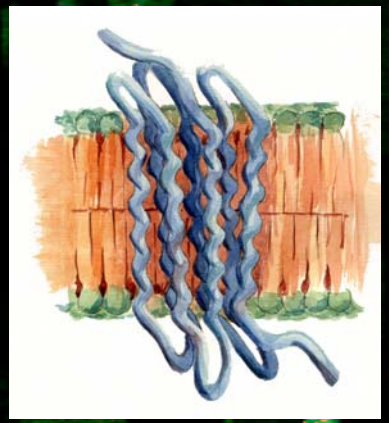
Basic concepts in G-protein-coupled receptor homo- and heterodimerization



NIDA minisymposium. SfN

San Diego. November 2007

www.bq.ub.es/recep/franco.html

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F. Fanelli
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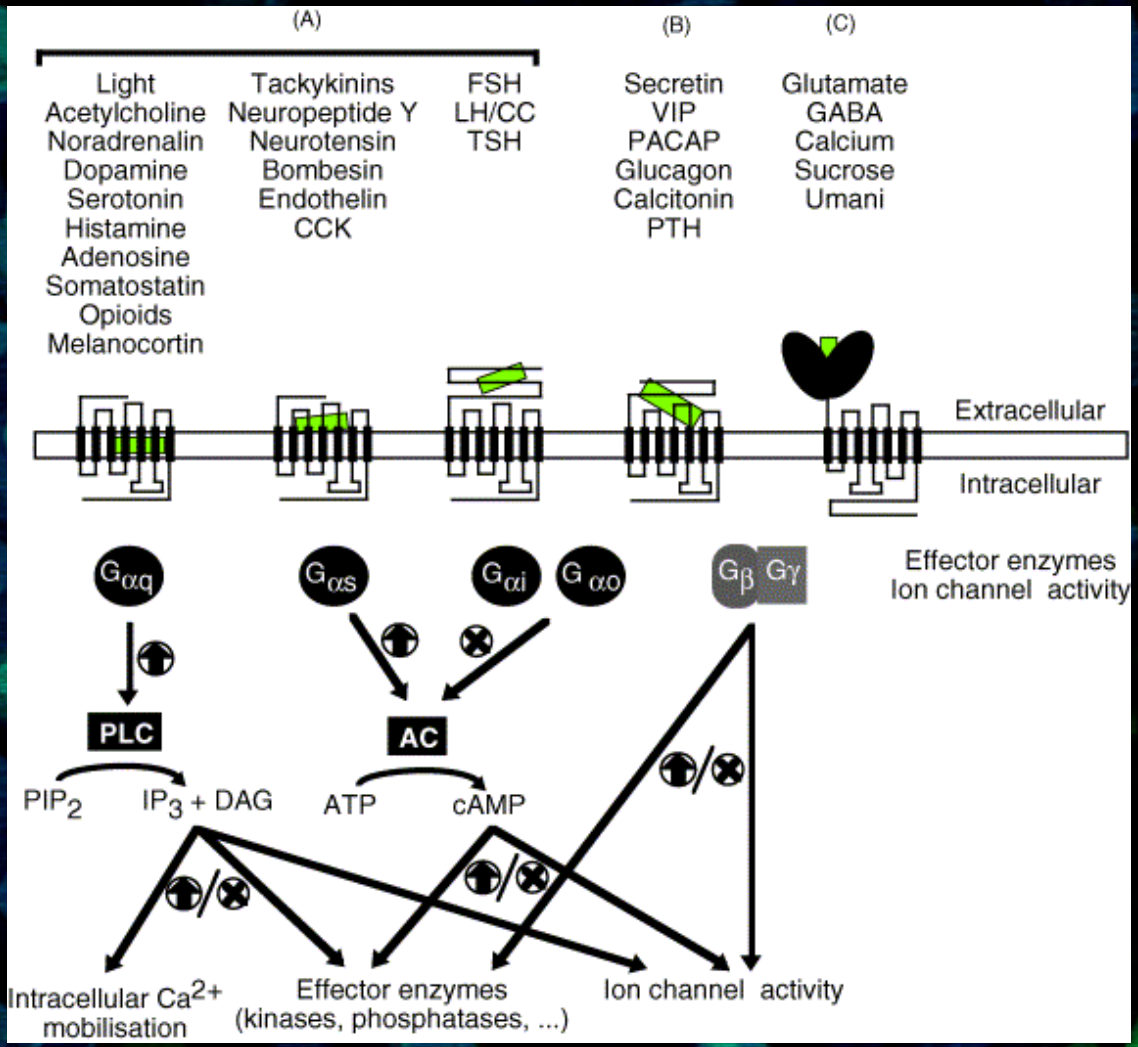
MONTREAL
M. Bouvier

Centro Investigación Médica Aplicada Pamplona

Digna Biotech

GPCR classes



NOMENCLATURE

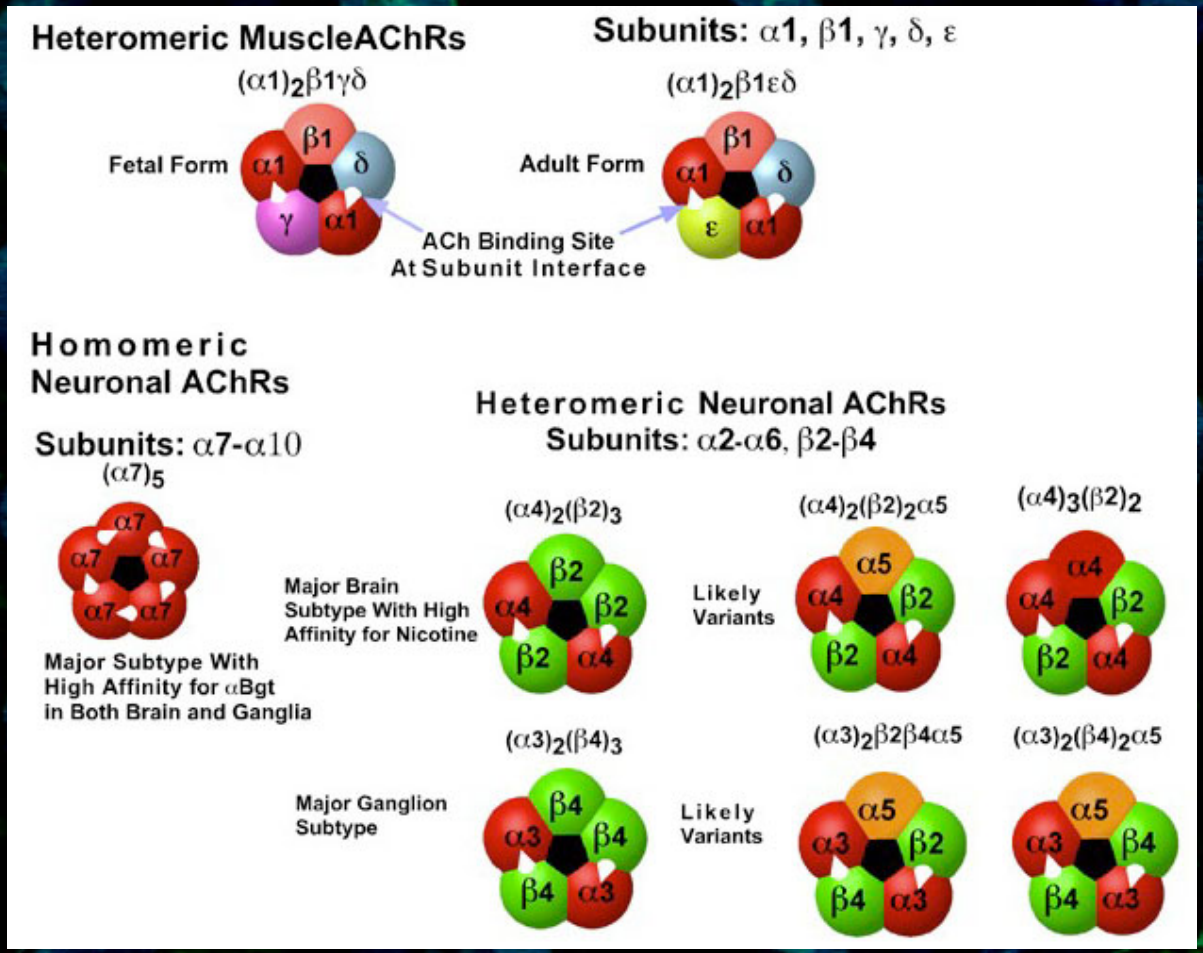
Receptor for Neurotransmitters (including neuromodulators and neuropeptides):
- Ionotropic
- Metabotropic (G-protein-coupled, GPCR)

Heteromeric receptor

Receptor homomer
Receptor heteromer

Ferré, Ciruela, Woods, Lluis & Franco (2007) Trends Neurosci 30 (9) 440-446

Heteromeric receptor: Ach receptor



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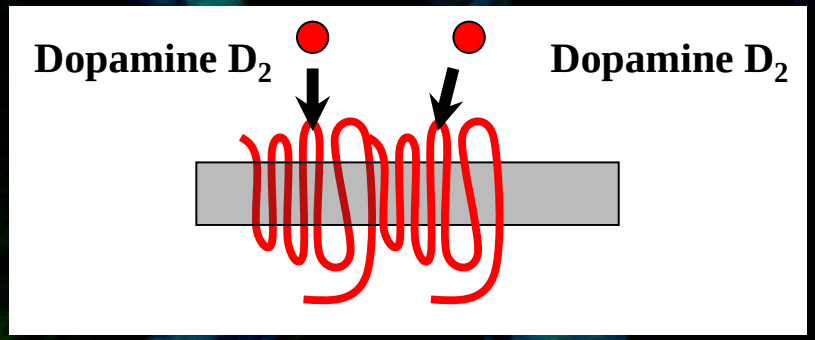
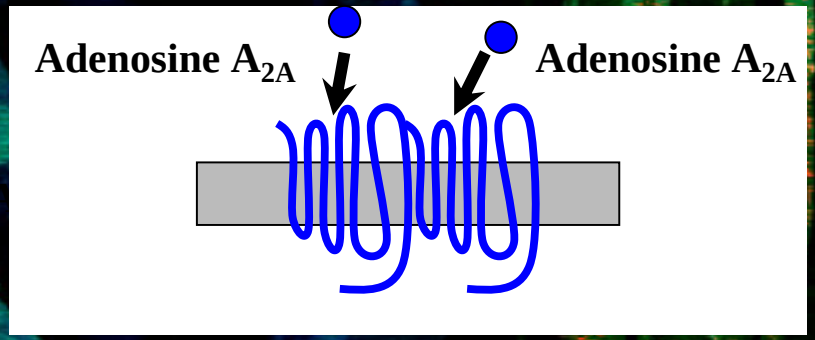
Receptor heteromer

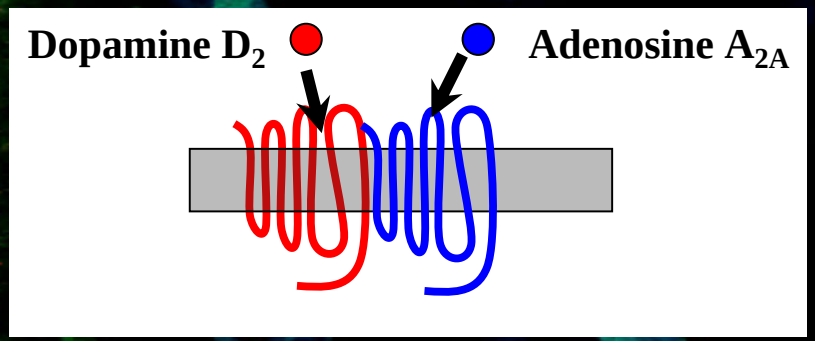
Ferré, Ciruela, Woods, Lluis & Franco (2007) Trends Neurosci 30 (9) 440-446

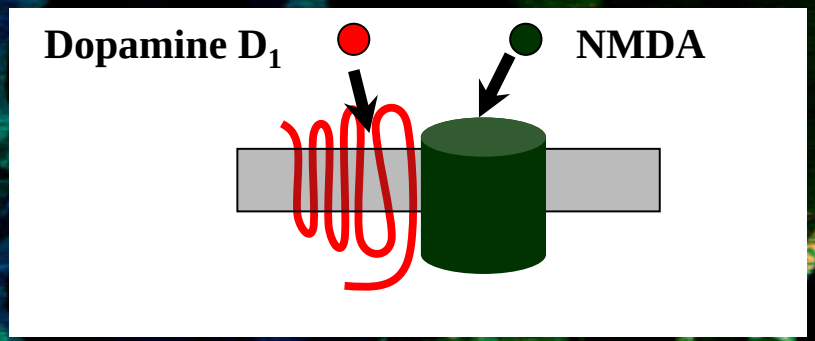
Receptor homomers





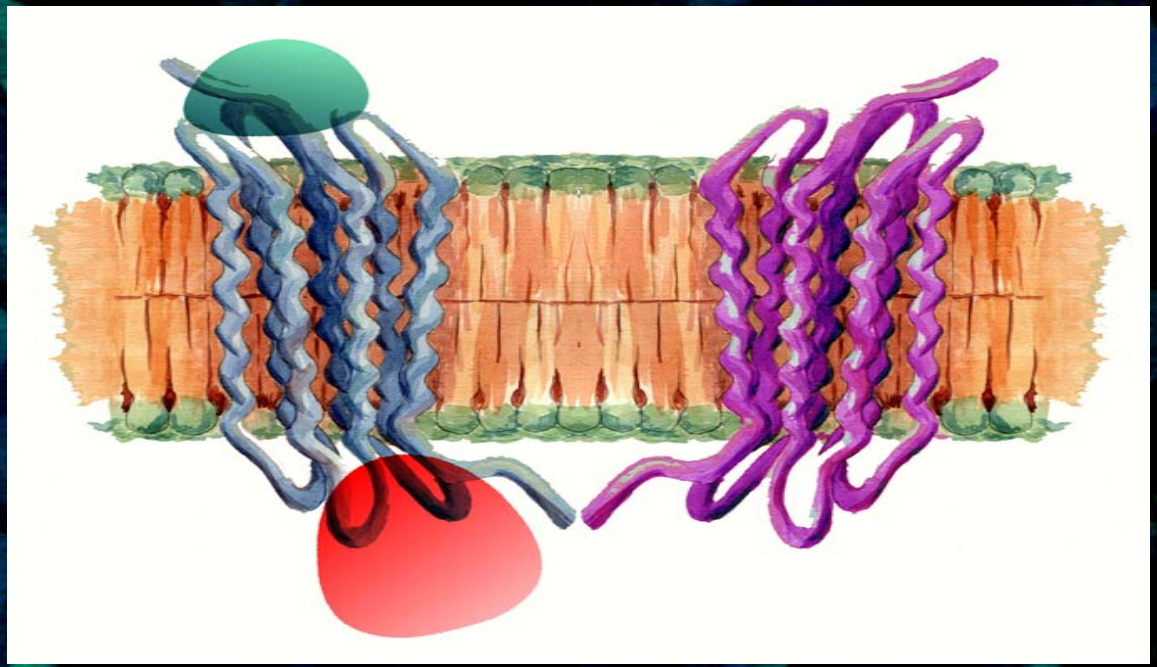
Receptor heteromers





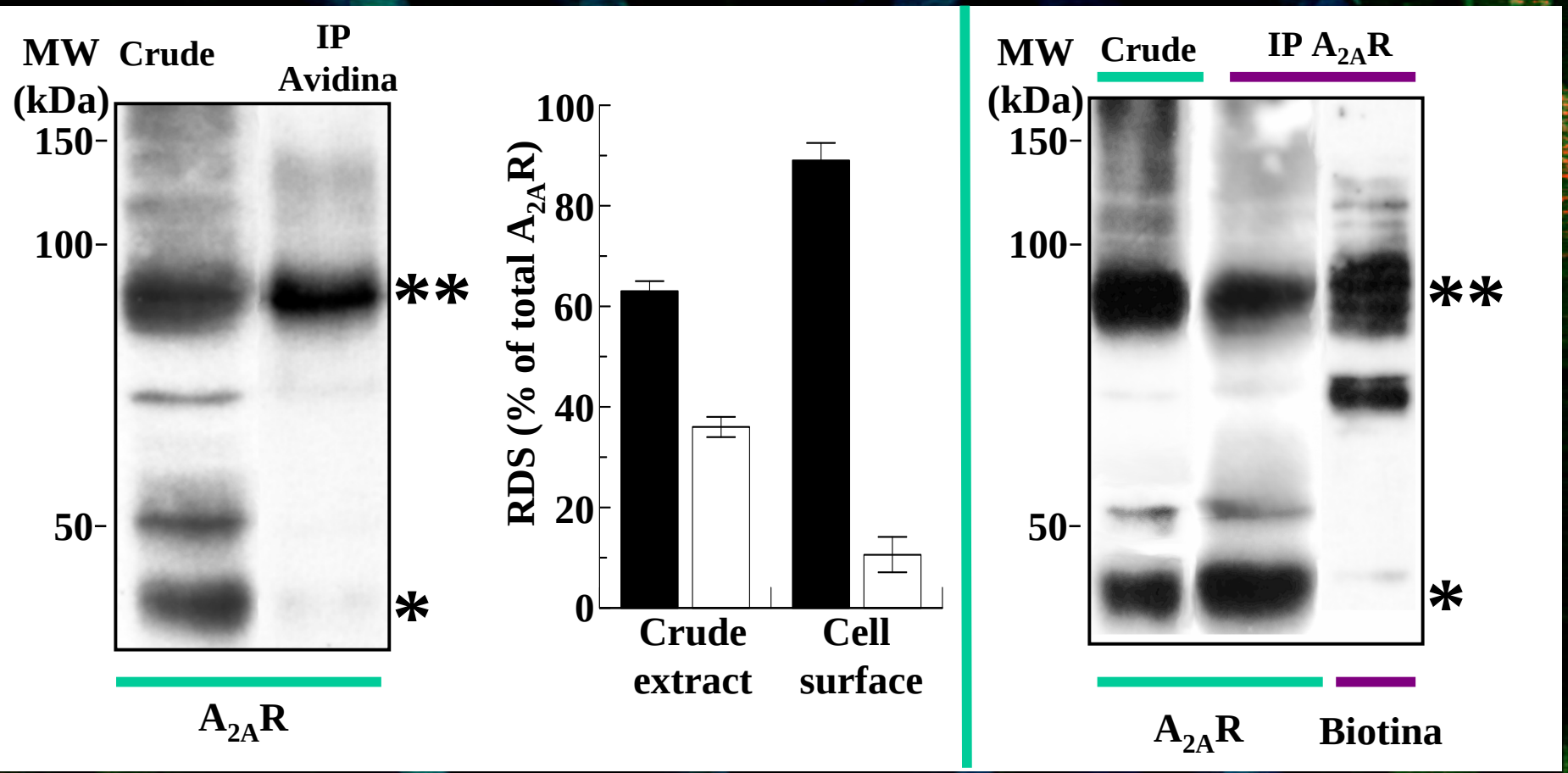
Ferré, Ciruela, Woods, Lluis & Franco (2007) Trends Neurosci 30 (9) 440-446

HETEROMERS AS SENSORS



- 1983: Evidence and formulation of the hypothesis: Agnati et al., Neurosc. Letters
- 1994: DR homomers: Ng et al., Eur. J. Pharmacol. (infected cells)
- 1995: A1R homomers: Ciruela et al., J. Neurosc. Res. (brain extracts)
- Late nineties, early XXI Century: heteromers (opioid, GABA, Dopa/Ado)

The $A_{2A}R$ dimer is the functional specie

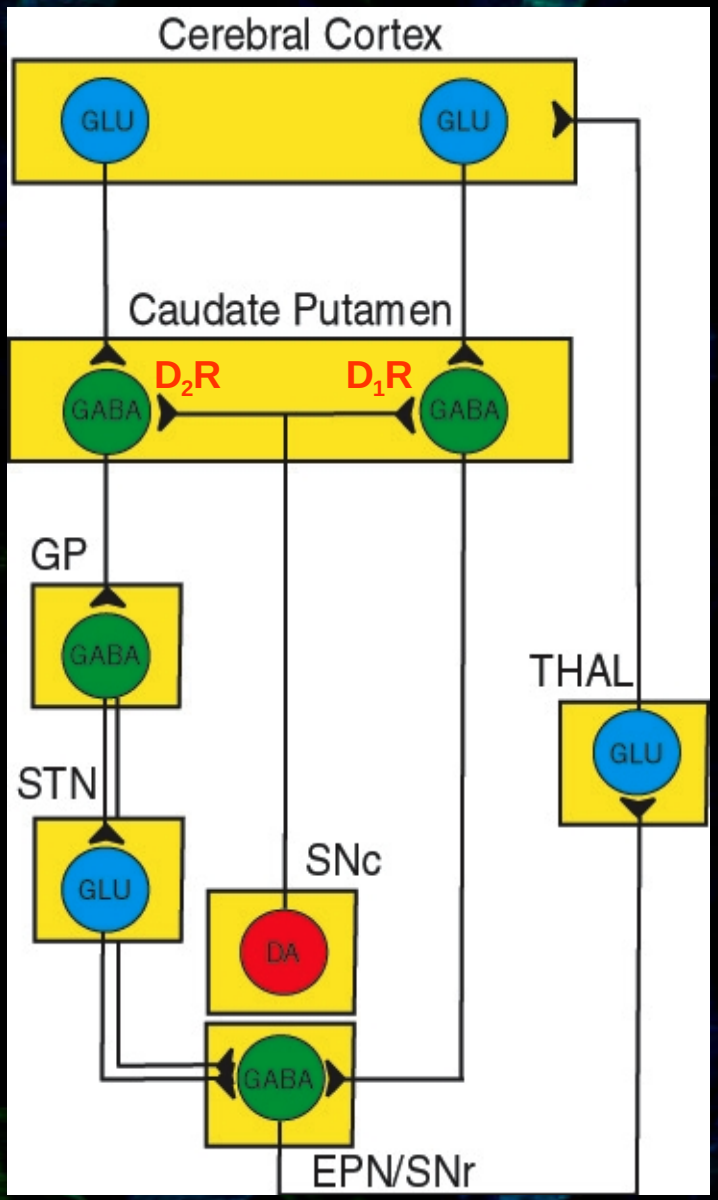


The C terminal tail of $A_{2A}R$ is not required for dimerization

Canals et al (2004) J Neurochem 88, 726-734

HETERODIMERS: DR/AR

D_2R-$A_{2A}R$ receptor heteromers

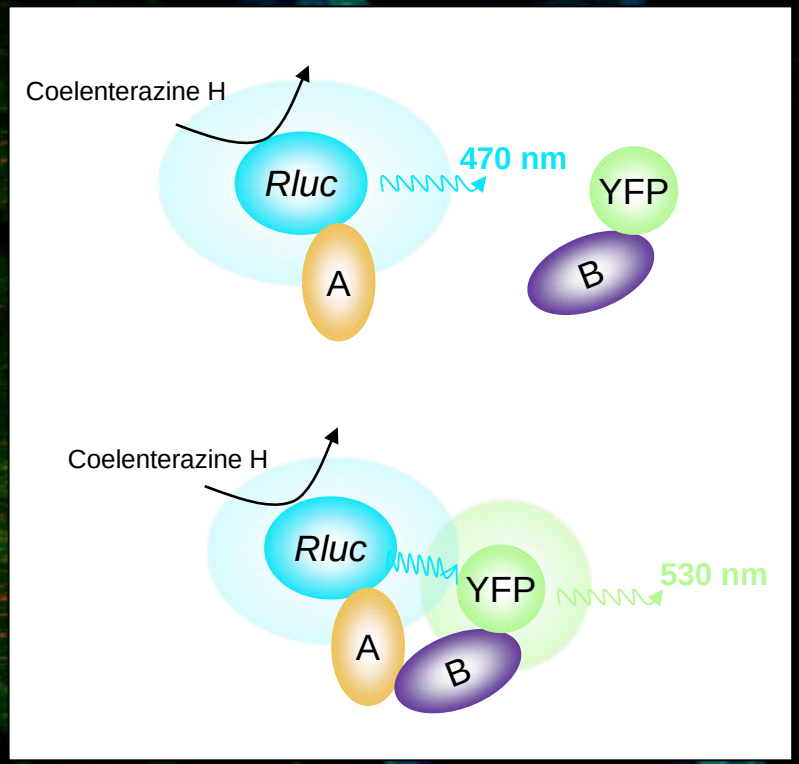


D_1R-A_1R receptor heteromers

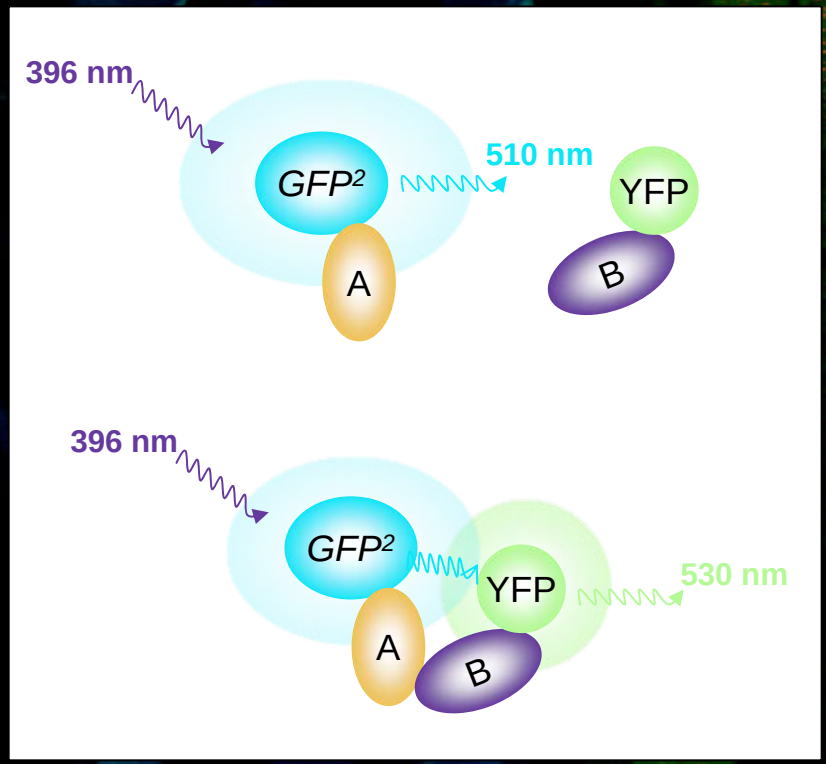
Hillion et al (2002)
J Biol Chem

Gines et al (2000)
PNAS

BRET

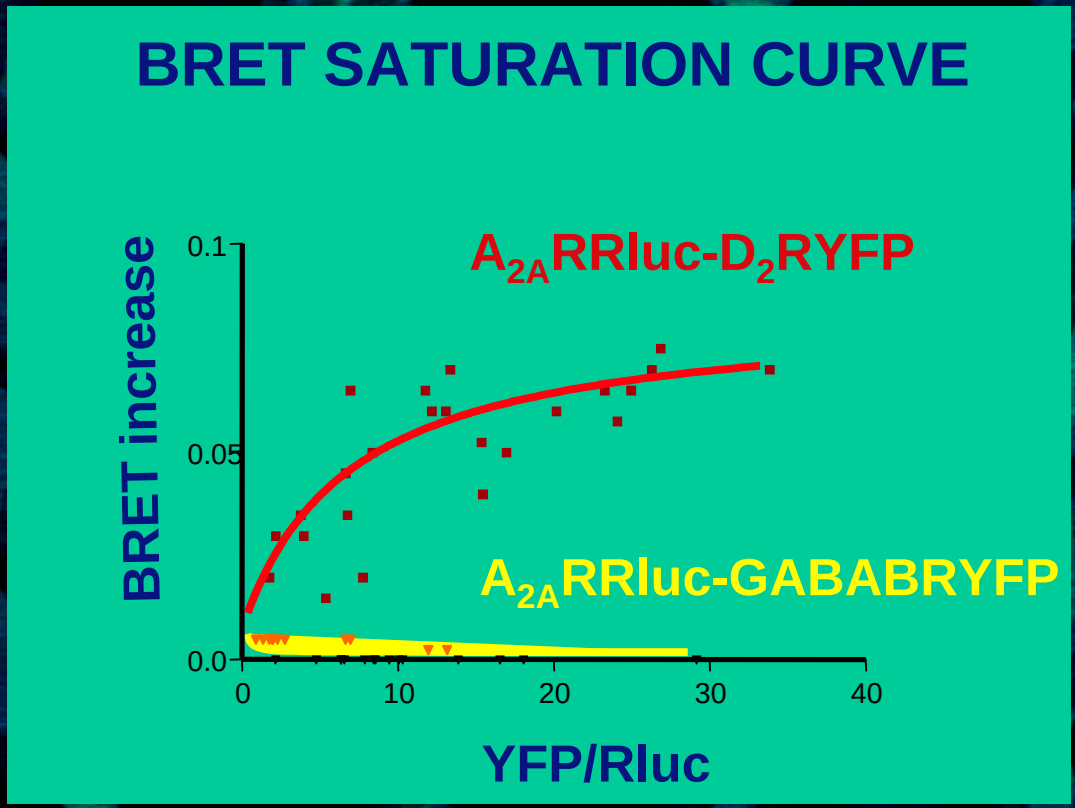

Coelenterazine H
Rluc
470 nm
YFP
A
B
Coelenterazine H
Rluc
YFP
530 nm
A
B

FRET


396 nm
GFP2
510 nm
YFP
A
B
396 nm
GFP2
YFP
530 nm
A
B

The A2AR/D2 homodimers:

BRET: Bioluminiscence Resonance Energy Transfer



Canals et al (2003) J Biol Chem 278: 46741-49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LOOKING FOR THE
BIOCHEMICAL/PHARMACOLOGICAL
“DIMER FINGERPRINT”

IUBCP: Recognition and nomenclature

0031-6997/07/5901-5–13$20.00
PHARMACOLOGICAL REVIEWS
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Pharmacol Rev 59:5–13, 2007

Vol. 59, No. 1
5043/3191655
Printed in U.S.A.

International Union of Basic and Clinical Pharmacology. LXVII. Recommendations for the Recognition and Nomenclature of G Protein-Coupled Receptor Heteromultimers

JEAN-PHILIPPE PIN, RICHARD NEUBIG, MICHEL BOUVIER, LAKSHMI DEVI, MARTA FILIZOLA, JONATHAN A. JAVITCH, MARTIN J. LOHSE, GRAEME MILLIGAN, KRZYSZTOF PALCZEWSKI, MARC PARMENTIER, AND MICHAEL SPEDDING

Alternative nomenclature in:

Ferré, Ciruela, Woods, Lluis & Franco (2007) Trends Neurosci 30 (9) 440-446

1. Evidence for physical association in native tissue or primary cells.
   A. Both subunits that compose the receptor heterodimer must be identified in the same cell and, if possible, within the same subcellular compartment. Coimmunolocalization experiments using antibodies recognizing each of the subunits should be used, if possible, at the electron microscopic level. If the physical interaction is convincingly demonstrated in vivo (see B) the need for colocalization is less important. In contrast, a colocalization study without the physical evidence for interaction is more or less meaningless.
   B. The physical interaction between both subunits should be documented in native tissue. This can be achieved using coimmunoprecipitation experiments from native tissue. However, such an experiment would only demonstrate that both proteins are part of the same multimeric protein complex, but this result cannot be an argument for a direct interaction between the two partners. Alternatively, energy transfer technologies using labeled ligands and/or labeled antibodies or transgenic animals (knockin) expressing physiological levels of recombinant fluorescent proteins could be used to demonstrate close receptor proximity in native tissue. Alternatively, the use of antibodies selective for a specific receptor dimer may be useful (Wager-Miller et al., 2002).
2. A specific functional property for the heterodimeric receptor will be critical to identify such receptors in native tissue. This could include the

3. The use of knockout animals or RNAi technology may also provide key information on the existence of heterodimeric GPCRs in vivo. Indeed, the response mediated by such a unique dimeric receptor should be greatly modified in the absence of either one of the subunits. These results can be meaningfully interpreted only if the dimer has been shown to occur in vivo or if the change in function has been shown to be related to the dimerization in a simpler heterologous expression system in which the dimerization can be more easily documented.

IUBCP: Recognition

2 out of 3:

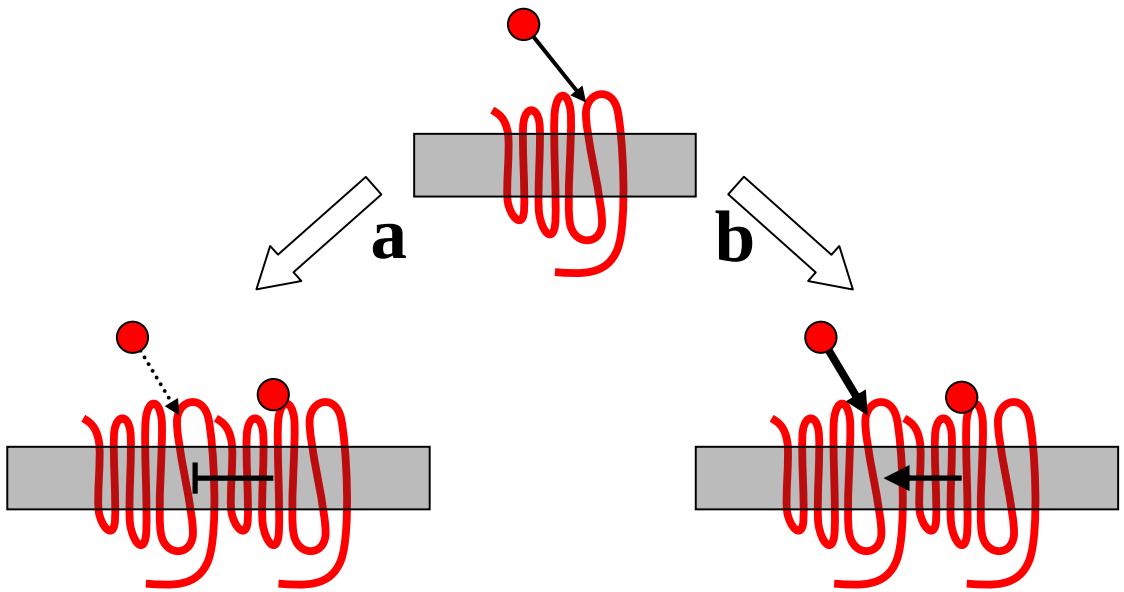
1 A Coimmunolocalization

1 B Coimmunoprecipitation/transgenics

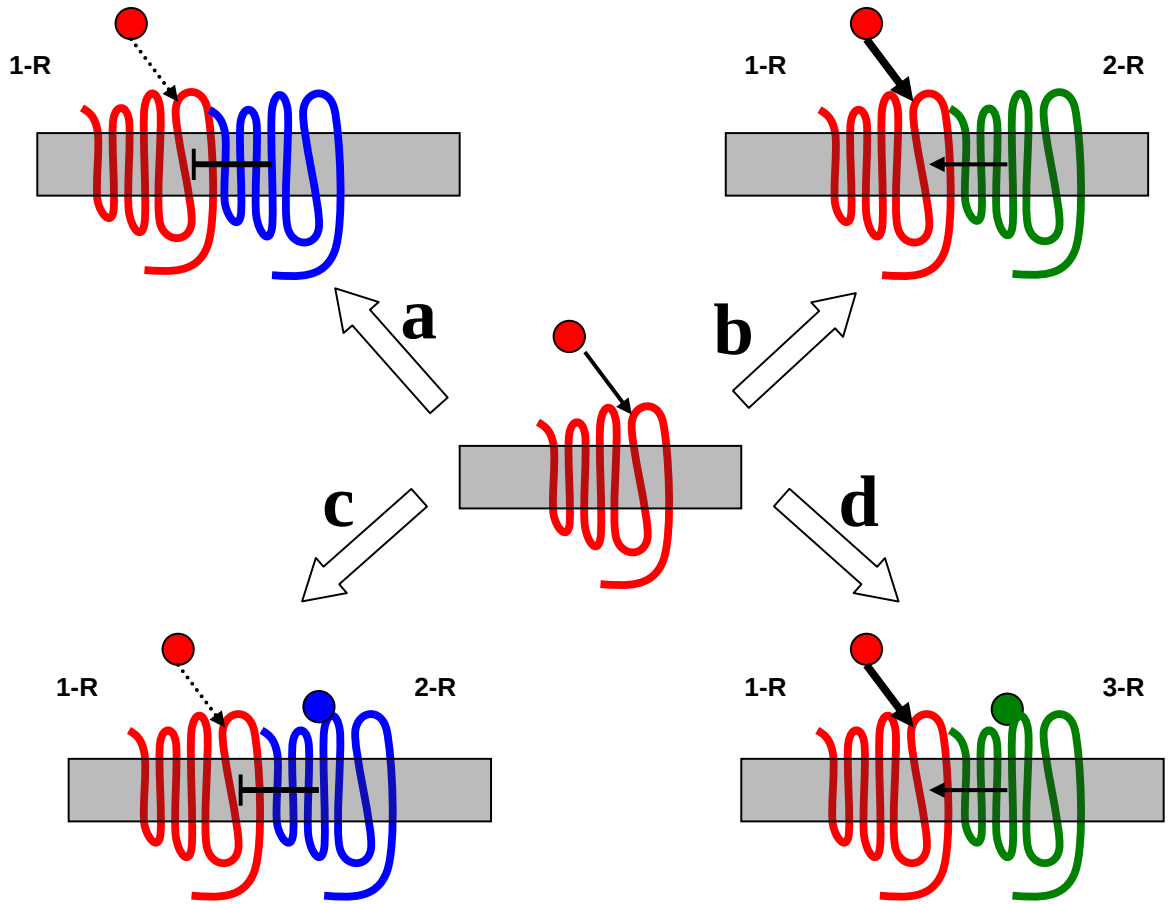
2 Specific functional property (Pharmacology/Signalling)

3 Knockouts: Loss of function!!!??

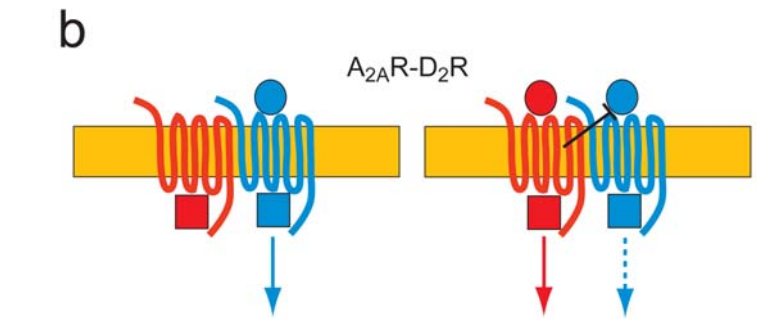
Intramolecular cross-talk in the homomer: Cooperativity

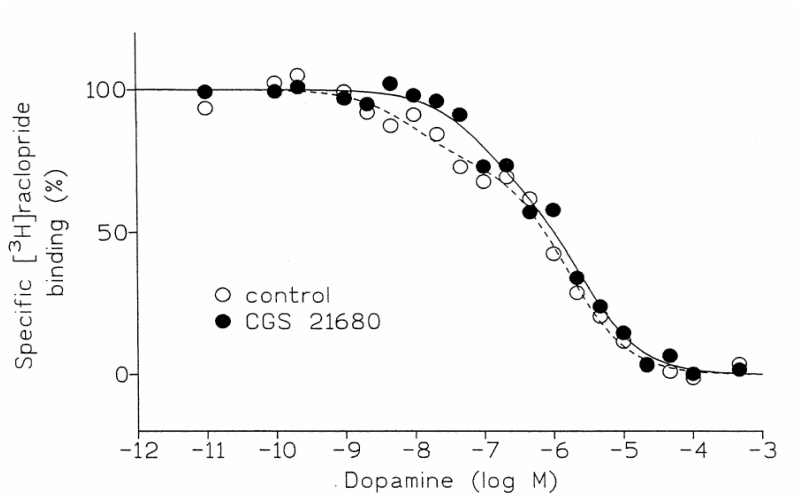


Intramolecular cross-talk in the heteromer: Cooperativity and/or ?



Looking for the “biochemical fingerprint” of a receptor heteromer





Fingerprint lost
in knockout animals

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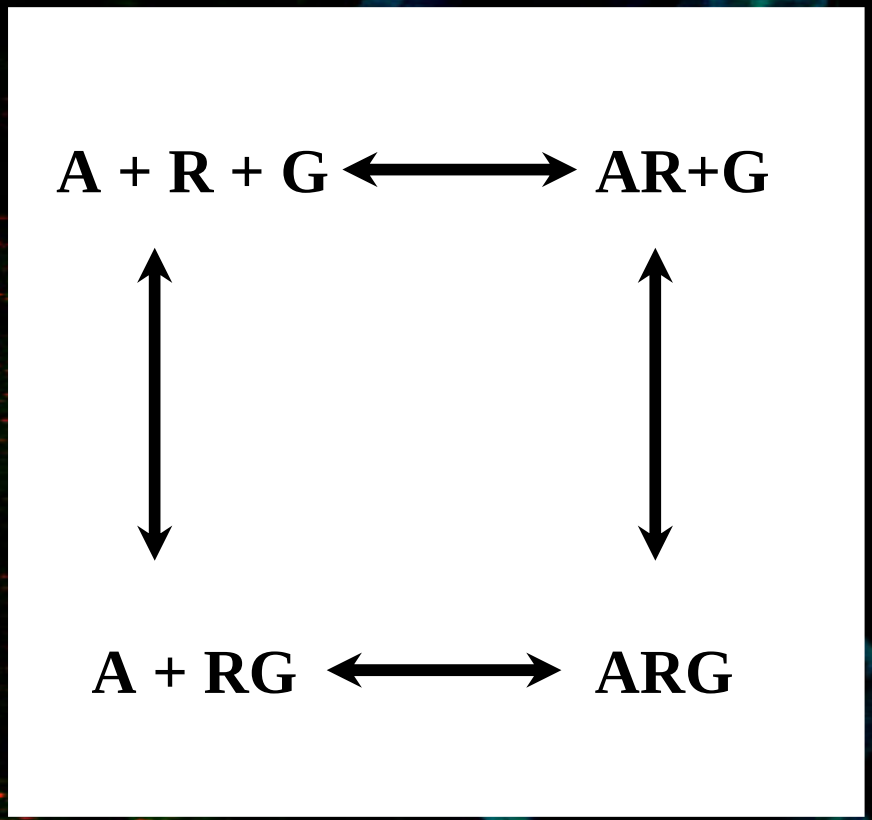
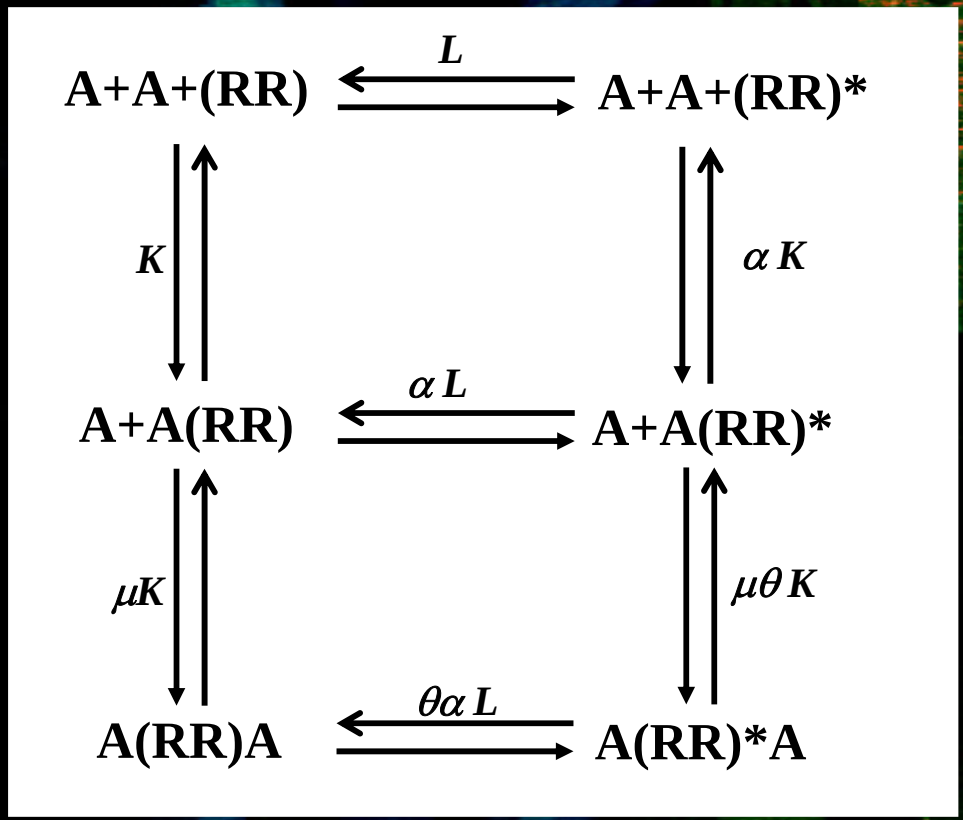
Different affinities for ligands: Caffeine as adenosine antagonist

Transfection	Radioligand	Displacer	K_D
A_1R	[^{3}H]R-PIA	R-PIA	1.9±0.4 nM
		Caffeine	90±20 μM
$A_{2A}R$	[^{3}H]CGS21680	CGS21680	110±30 nM
		Caffeine	7±1 μM
A_1R+$A_{2A}R$	[^{3}H]R-PIA	R-PIA	1.9±0.3 nM
		Caffeine	90±20 μM
	[^{3}H]CGS21680	CGS21680	120±40 nM
		Caffeine	90±20 μM*
$A_{2A}R$+D_2R	[^{3}H]CGS21680	CGS21680	110±40 nM
		Caffeine	5±2 μM

TERNARY MODEL

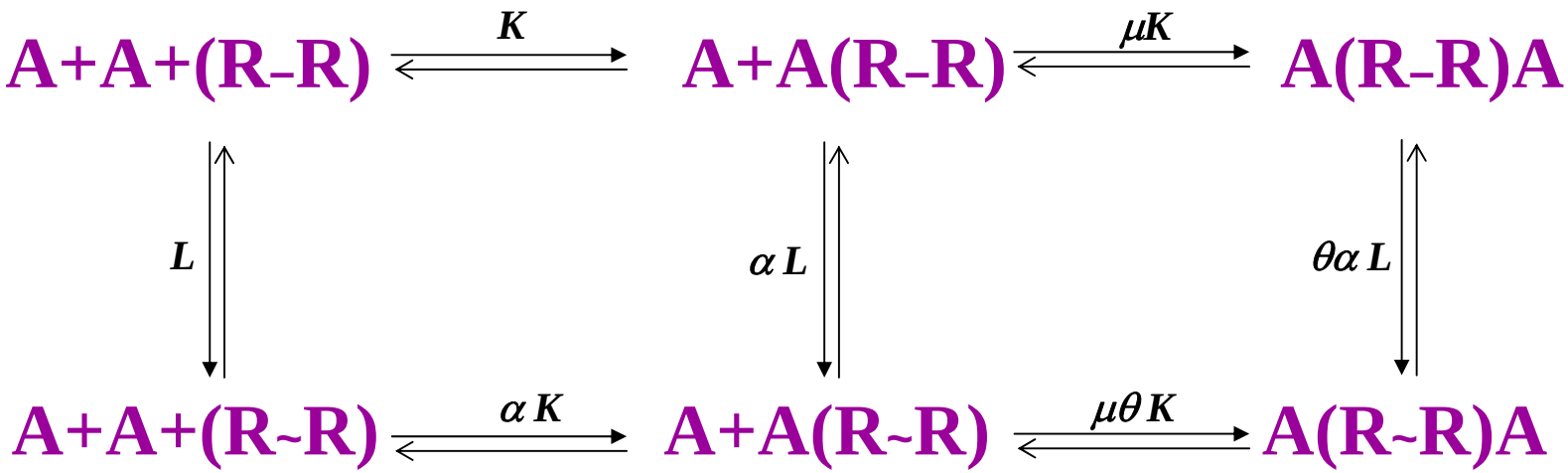


RECEPTOR DIMER MODEL



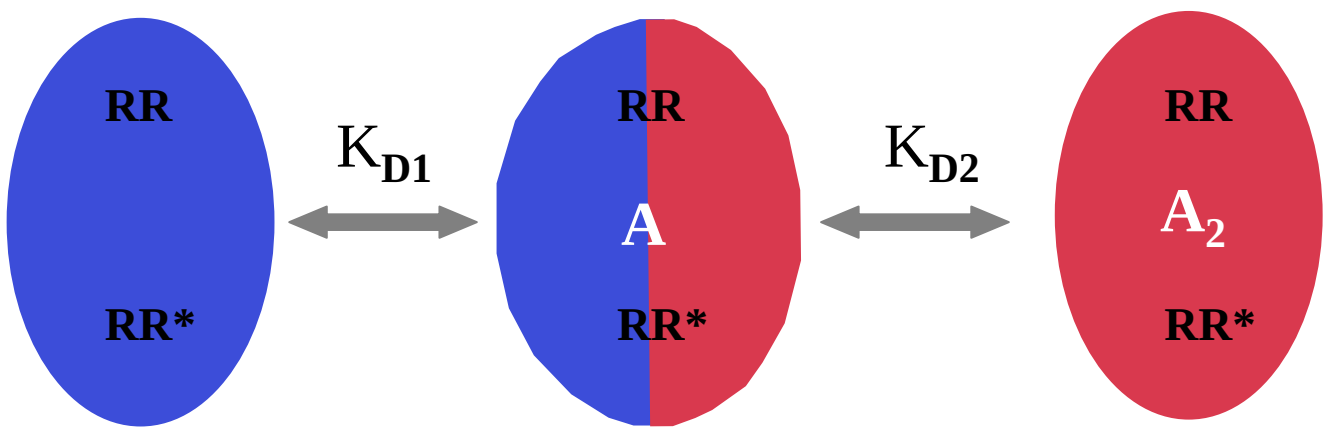
Franco et al (2005) Trends Biochem Sci 30: 360-66

a



$$\frac{[A]_{Bound}}{[R_T]} = \frac{K\cdot(1+\alpha L)\cdot[A]+2K^2\mu\cdot(1+\alpha\theta L)\cdot[A]^2}{1+L+K\cdot(1+\alpha L)\cdot[A]+K^2\mu\cdot(1+\alpha\theta L)\cdot[A]^2}$$

b



$$A_{bound} = (K_{D2}\,A + 2\,A^2)\,R_T\,/(K_{D1}\,K_{D2} + K_{D2}\,A + A^2)$$

Dimer Cooperativity Index, Dc:

It depends on K_{D1} and K_{D2}

Allosterism:

Useful parameter to "measure allosterism"

D_{50} in Drug discovery:

A more meaningful parameter to measure binding potency compared to IC_{50}.

Casadó et al., Pharmacol Ther In the Press (Dec 2007)

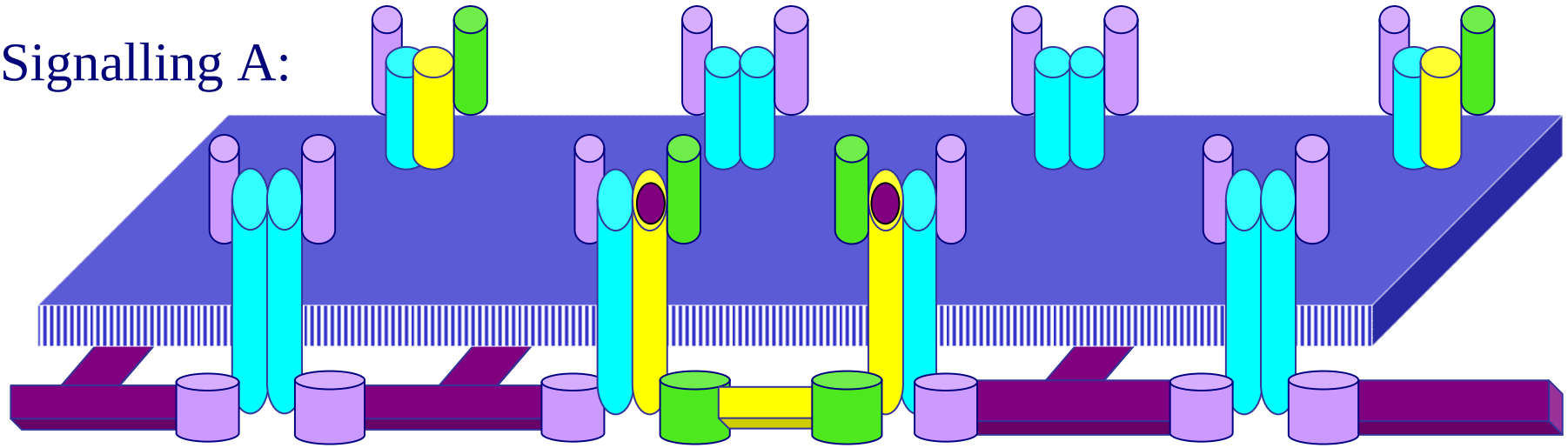

LOOKING FOR THE
FUNCTIONAL
“DIMER FINGERPRINT”

Fig 4

Clustering adaptor/scaffolding proteins

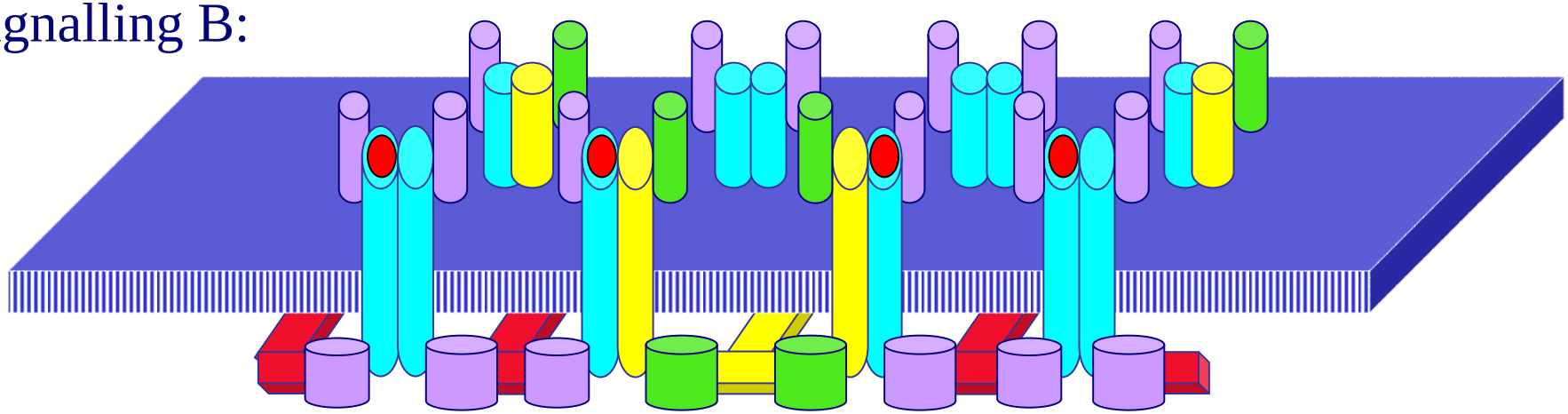
Agonists for the two receptors

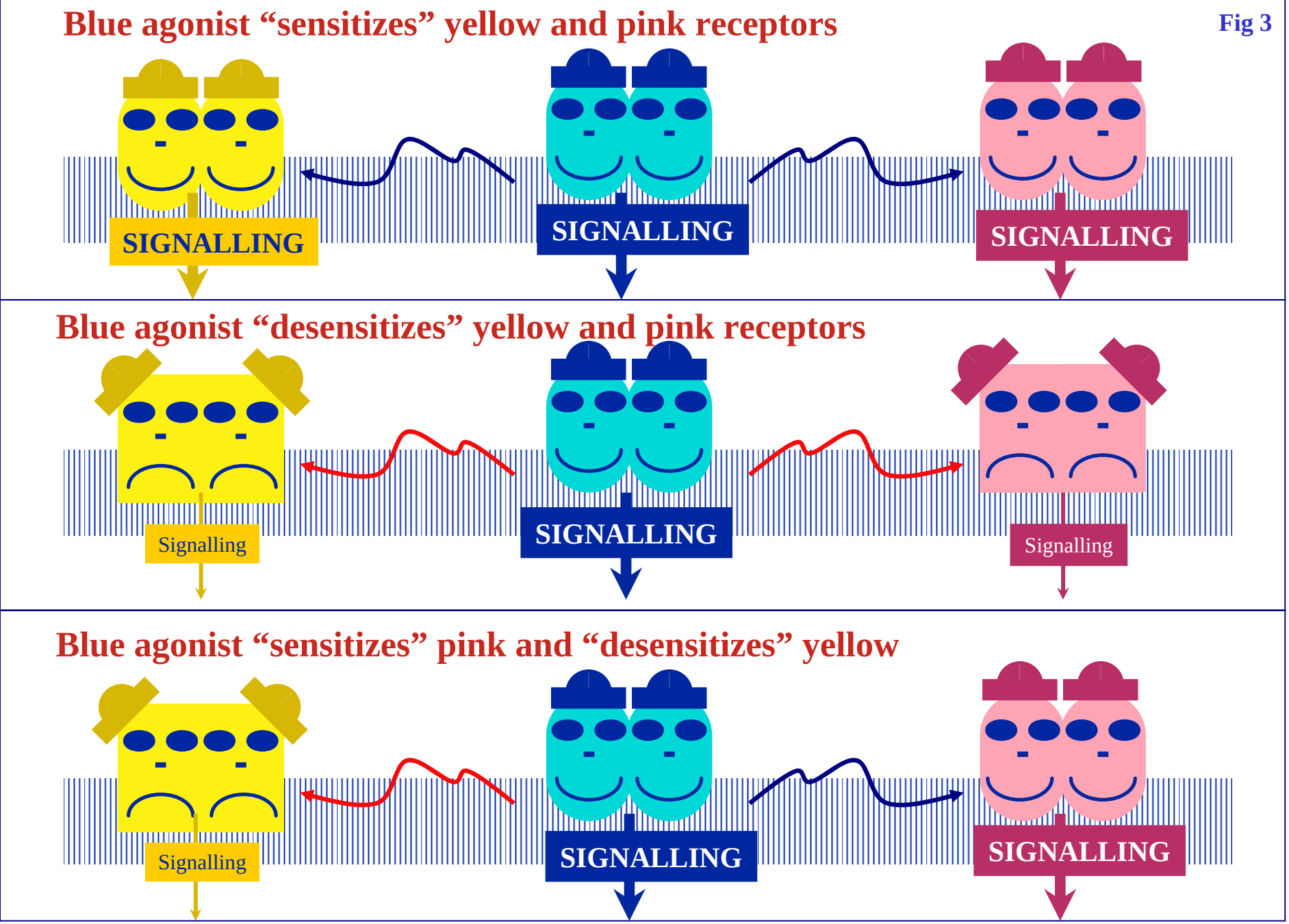
Signalling A:



Franco et al Trends Biochemical Sci 28 (2003) 238-243

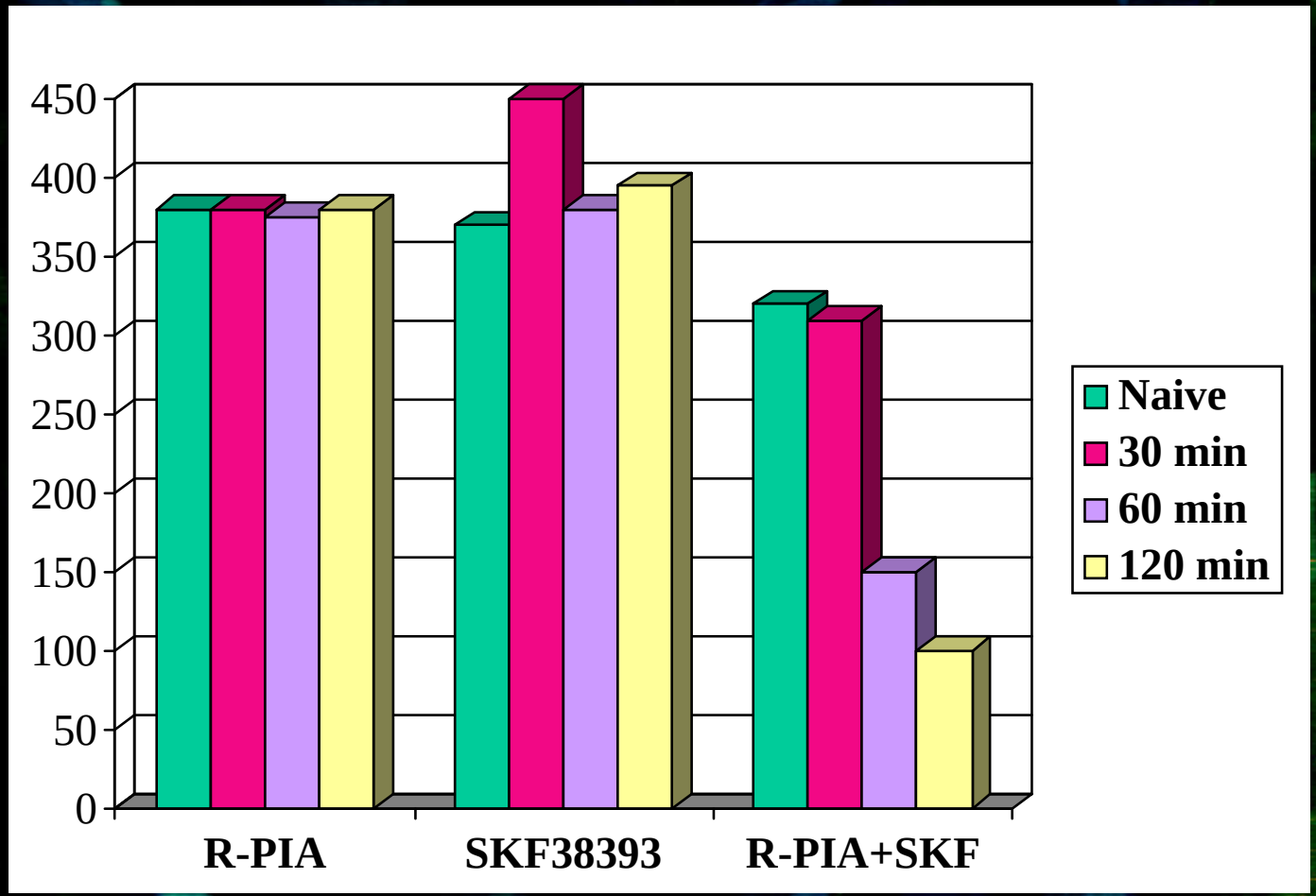
Signalling B:





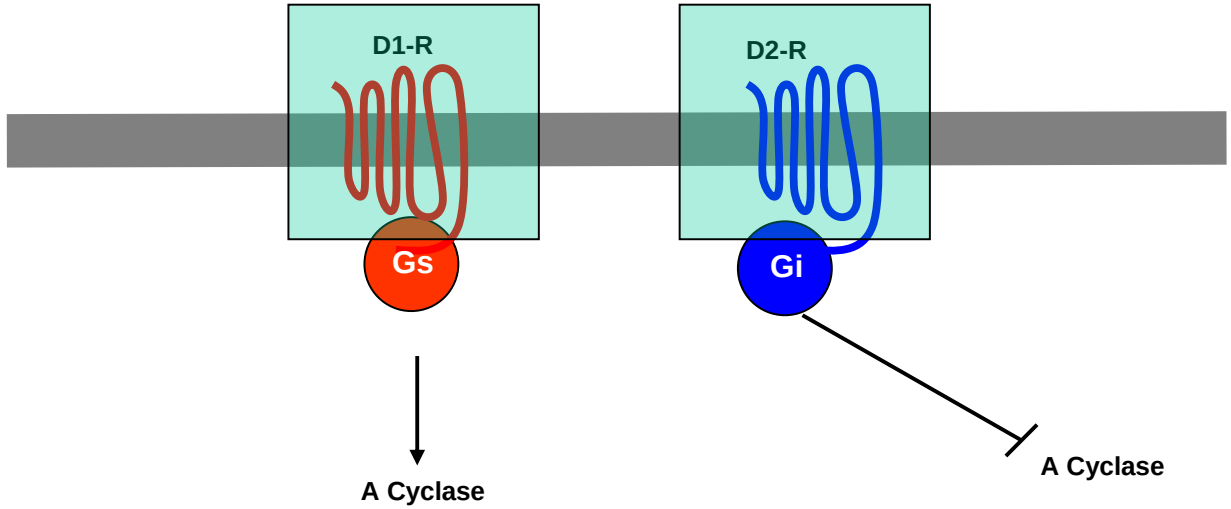
Franco et al Trends Biochemical Sci 28 (2003) 238-243

The intracytoplasmic receptor-receptor cross-talk: ANTAGONISM (A1R/D1R)

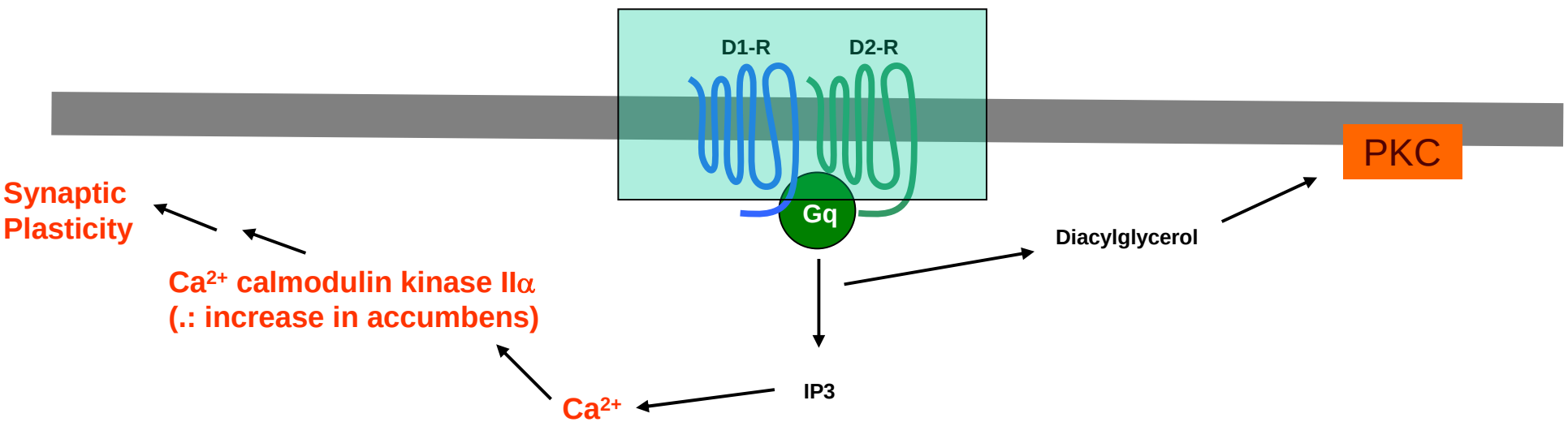


Ginés et al (2000) PNAS 97, 8606-11

cAMP producion via D1R after pretreatment with agonists of A1R and/or D1R
(A1/D1 cotransfected mouse Ltk$^-$ fibroblasts)

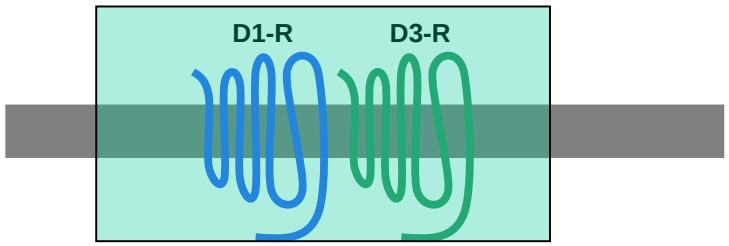


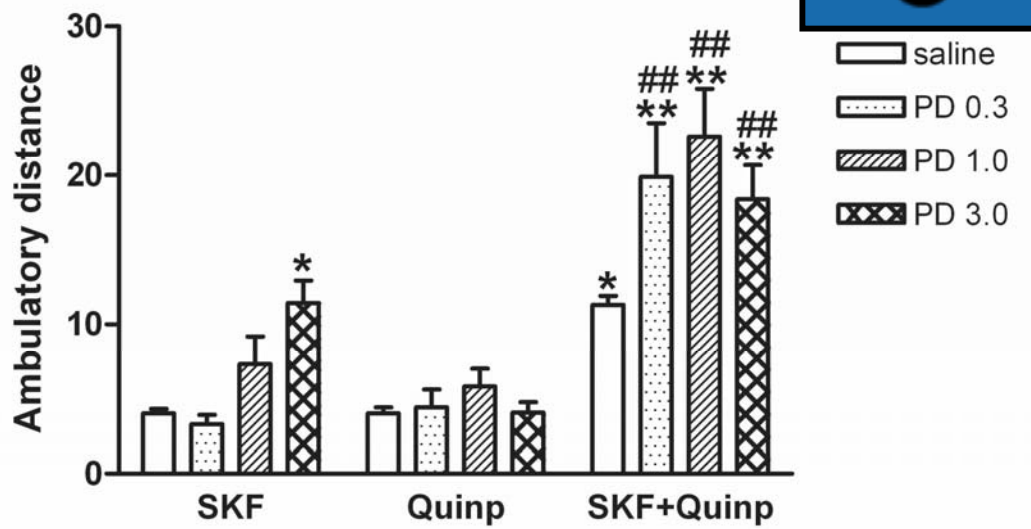
Shift to Gq coupling in the D1-D2 receptor heteromer



Rashid et al. (2007) Proc Natl Acad Sci 104, 654-9

D1-D3 Receptor heteromerization





-FRET
-BRET
-Fingerprint (Intramembrane cross-talk)

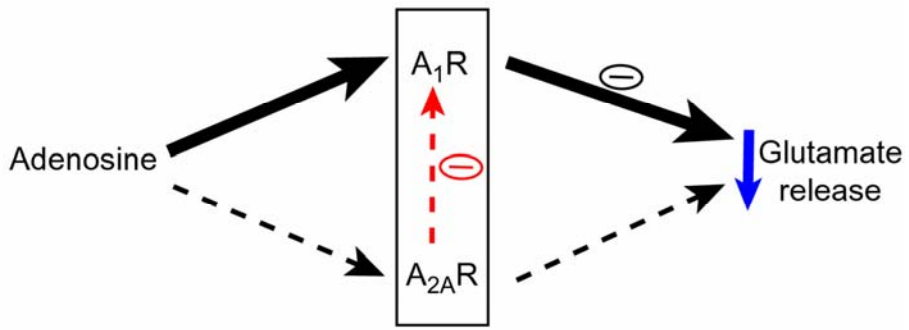
1.-D_1 receptor agonist affinity is enhanced by D_3 agonists. This indicates the existence of a synergistic intramembrane receptor-receptor interaction.

2.-Experiments in reserpinized mice showed that D_3 receptor stimulation potentiates D_1 receptor-mediated behavioral effects by a different mechanism than D_2 receptor stimulation.

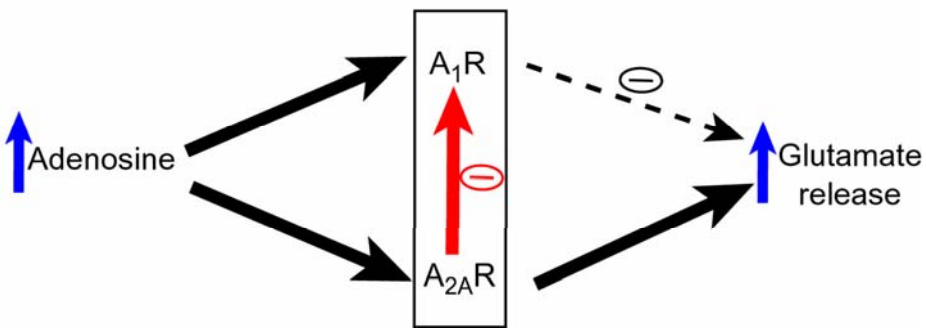
Marcellino et al. (2007) Submitted

Dual regulatory role of Adenosine on Glu release via $A_1R/A_{2A}R$

a. Heteromeric A_1R-$A_{2A}R$, low adenosine



b. Heteromeric A_1R-$A_{2A}R$, high adenosine

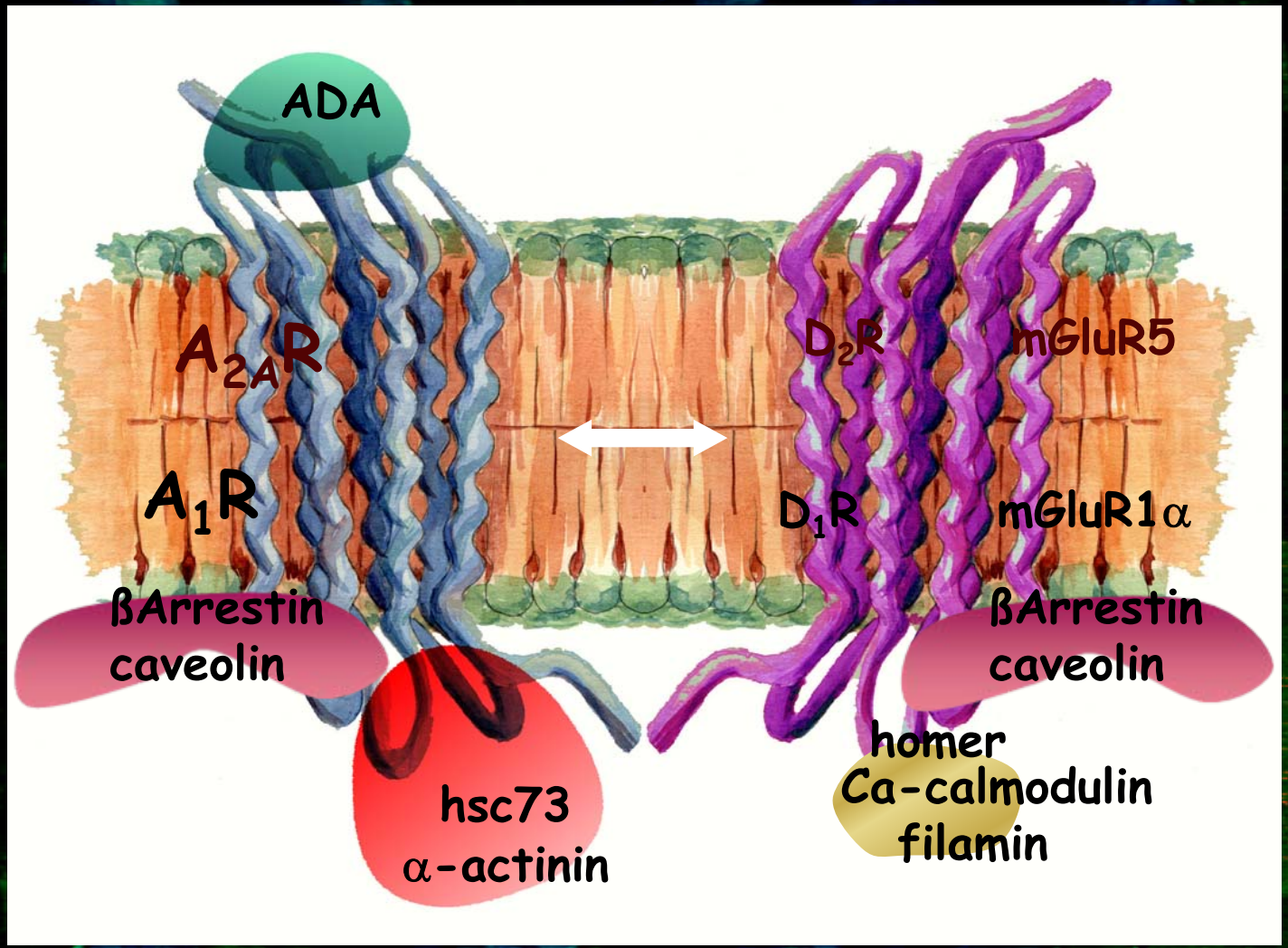


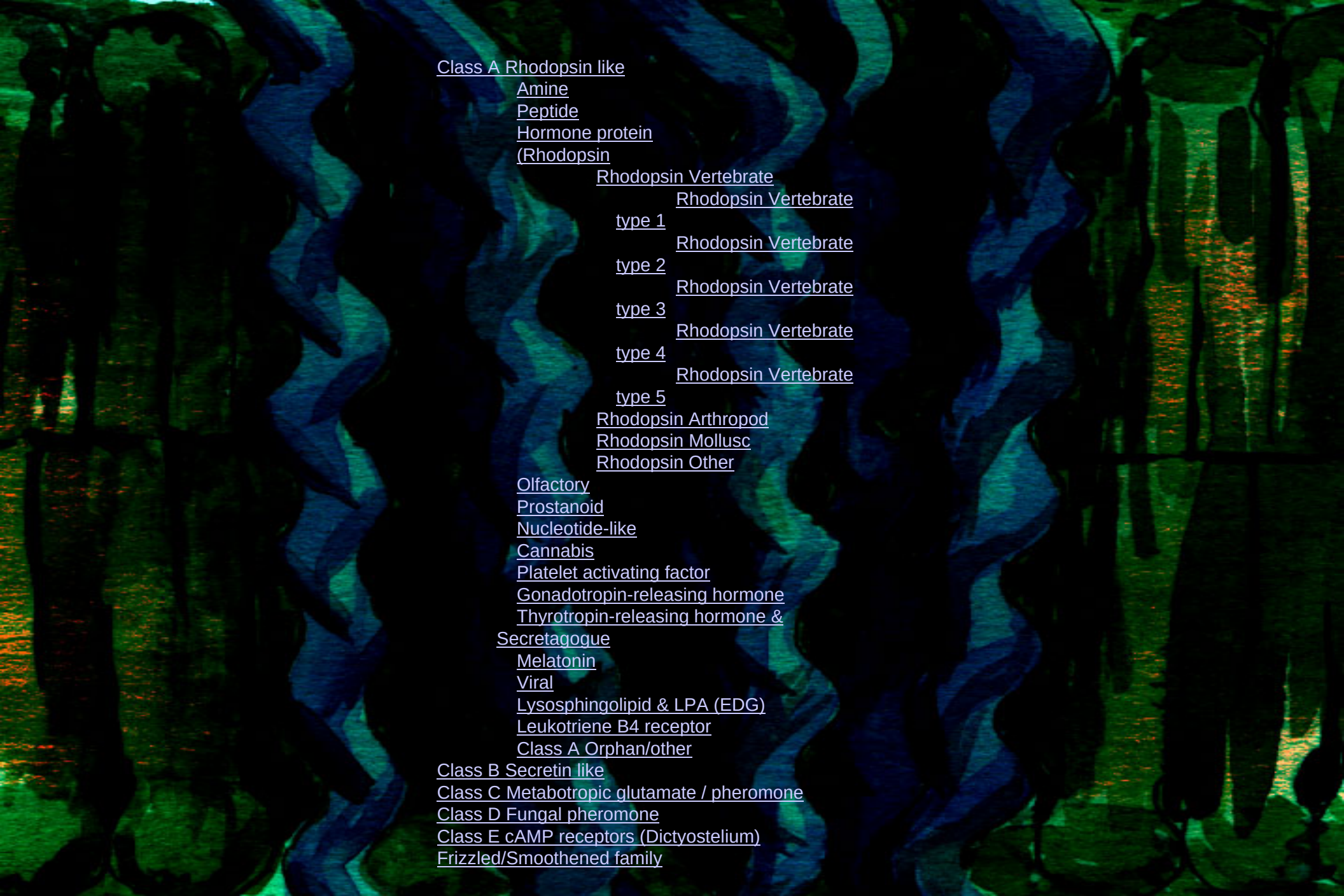
Ciruela et al (2006) J Neurosc 26:2080-87

CONCLUSIONS

1. **Heteromers exist in natural tissues**
2. **Heteromers are sensors for neurotransmitters**
3. **Heteromerization modifies:**
   - **biochemical,**
   - **pharmacological,**
   - **and functional properties of receptors**

FUNCTIONAL MODULES IN THE PLASMA MEMBRANE




Class A Rhodopsin like
Amine
Peptide
Hormone protein
(Rhodopsin
Rhodopsin Vertebrate
Rhodopsin Vertebrate
type 1
Rhodopsin Vertebrate
type 2
Rhodopsin Vertebrate
type 3
Rhodopsin Vertebrate
type 4
Rhodopsin Vertebrate
type 5
Rhodopsin Arthropod
Rhodopsin Mollusc
Rhodopsin Other
Olfactory
Prostanoid
Nucleotide-like
Cannabis
Platelet activating factor
Gonadotropin-releasing hormone
Thyrotropin-releasing hormone &
Secretagogue
Melatonin
Viral
Lysosphingolipid & LPA (EDG)
Leukotriene B4 receptor
Class A Orphan/other
Class B Secretin like
Class C Metabotropic glutamate / pheromone
Class D Fungal pheromone
Class E cAMP receptors (Dictyostelium)
Frizzled/Smoothened family