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G-protein coupled receptors, neurotransmitters, allosteric modulators


## Research topics

We aim to describe principles of activation of G-protein-coupled receptors (GPCRs) for major neurotransmitters. The research is focused on the structure-function relationships of these receptors and molecular machinery that regulates their signalling properties. The metabotropic glutamate (mGlu) receptors that belong to family 3 GPCRs are composed of two identical subunits. The relevance of dimerization of these receptors in respect to activation of the transmembrane heptahelical domain (HD) of each subunit is of our particular interest. Using the mutagenesis approach combined with a functional expression system we showed that within the homodimeric structure only one HD reaches active state. Interestingly, this situation is very similar to that observed in GABAb receptor. Within the GABAb receptor that is composed of two different proteins, only one of them activates G-proteins. The activation process of these family 3 GPCRs is thus asymmetrical. Currently, we take use of this observation to reveal the mechanism of action of allosteric modulators on these receptors. To this aim we analyse energy transfer deviations upon activation and/or modulation of the receptors tagged with different fluorochromes at distinct portions of the receptors.

Regulation of the receptor activity on the cell surface is examined by search for associated proteins that interact mainly with the intracellular C-termini. This project is focused on signalling of cannabinoid receptor 1. It is approached by molecular biology means combined with biochemical tools including yeast two-hybrid technology, *in vivo* introduction of tagged "bites" into living animal brains followed up by the pull-down method for isolation of the interactors and their successive identification.

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### Selected recent papers


1. Hlavackova V, Kniazeff J, Goudet C, Zikova A, Maurel D, Vol C, Trojanova J, Prézeau L, Pin J-P, Blahos J. Evidence for a single heptahelical domain being turned on upon activation of a dimeric GPCR. **EMBO J.** 2005;24,499–509.
2. Goudet C, Kniazeff J, Hlavackova V, Malhaire F, Maurel D, Acher F, Blahos J, Prezeau L, Pin J-P. Asymmetric functioning of dimeric metabotropic glutamate receptors disclosed by positive allosteric modulators. **J Biol Chem.** 2005;280:24380-5.
3. Sinagra M, Verrier D, Frankova D, Korwek KM, Blahos J, Weeber EJ, Manzoni O, Chavis P. Reelin. Very-low-density lipoprotein receptor, and apolipoprotein E receptor 2 control somatic NMDA receptor composition during hippocampal maturation *in vitro*. **J Neurosci.** 2005;25:6127-36.
4. Brock C., Boudier L, Maurel D, Blahos J, Pin J-P. Assembly-dependent surface targeting of the heterodimeric GABAB receptor is controlled by COPI, but not 14-3-3. **Mol Biol Cell.** 2005;16:5572–5578.
5. Bertaso F, Lill Y, Airas JM, Espeut J, Blahos J, Bockaert J, Fagni L, Betz H, El-Far O. MacMARCKS interacts with the metabotropic glutamate receptor type 7 and modulates G protein-mediated constitutive inhibition of calcium channels. **J Neurochem.** 2006;99:288-98.


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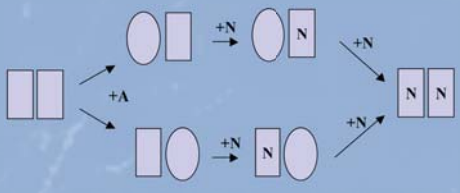
*In dimeric Metabotropic Glutamate receptor, upon competitive agonist binding only one of two identical subunits reaches activated state. Thus, modulation of a single heptahelical domain by allosteric enhancer is sufficient to exert full effect. Accordingly, both heptahelical domains have to be blocked by a negative allosteric modulator.*




*Heptahelical domain (HD) activation of mGlu receptor, schematically. Upon activation of the receptor by an agonist (A) either heptahelical domain can reach the active state (square=inactive state, oval=active HD conformation). Interaction of the negative allosteric modulator (N) with a single HD results in no apparent change in the receptor activity, as the adjacent subunit is still capable to activate G-proteins. The inhibitory effect of such compound is reached only when both HDs are occupied. The conformational changes of the HDs and association of the adjacent G-proteins upon various pharmacological treatments are currently studied using the FRET (Forster's Resonance Energy Transfer) approach.*