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Poster Presentation

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Expression and purification of the D2 dopamine receptor and the Neurokinin A receptor

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Background

The D2 dopamine receptor (D2DR) and the Neurokinin A receptor (NK2R) are G-protein-coupled receptors (GPCRs). The first one is a major target of antipsychotics and Parkinson's disease drugs while the second is involved in smooth muscle contractions. GPCRs, also known as seven transmembrane receptors (7TMRs), represent by far the largest family of plasma membrane receptors, comprising approximately 1000 members in the human genome. They regulate virtually all known physiological processes in mammals including the senses of smell, taste and vision. Indicative of their central importance in current clinical therapeutics is the very large number of drugs that target these receptors, either directly or indirectly. Although much progress has been made in the pharmacological characterization of a large number of GPCRs, the only three-dimensional structure available is that of bovine rhodopsin. A 3D-structure of D2DR or NK2R would increase our understanding of its molecular mechanism and of the signal transduction of all GPCRs.

Results

In order to produce the large and homogenous receptor preparations required for structural studies, heterologous production procedures have been established using the methylotrophic yeast *Pichia pastoris* (D2DR) and SFV-infected mammalian cell lines (NK2R). The receptors, produced as a fusion protein with both a Flag-tag and a His_{10} tag at their Nterminus and a biotinylation domain at their C-terminus for immuno-detection and purification, shows specific binding activity with their selective antagonists (Spiperone for D2DR and SR48,968 for NK2R). Preliminary purification procedures in a scalable fashion were designed using a mixture of sugarbased detergents, Cholesteryl-HemiSuccinate and other lipids (POPC, POPE, POPG).