# STUDYING DOPAMINE $D_1$ RECEPTORS WITH FLUORESCENCE ANISOTROPY BASED ASSAY

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G-protein-coupled receptors (GPCRs) have become very important drug targets and therefore ligand binding assays for these receptors are an essential part of drug discovery. Here we have applied fluorescence anisotropy (FA) method, which allows to measure receptor-ligand interactions in real time. However, in case of this assay system high receptor expression levels are required. For that we have used budded baculoviruses, which are like rod-shaped nanoparticles that display GPCRs on their surfaces. This system has many advantages, for example good signal to noise ratio, homogeneity of the receptor, high expression levels and long-term stability of the receptor preparation [1].

Here we present data about applying this approach for the characterization of ligand binding to dopamine $D_1$ receptors. Dopamine receptors are involved in a wide variety of physiological processes, which makes these proteins important drug targets for diseases like schizophrenia, Parkinson's disease, depression and many others. We have used fluorescently labelled dopamine $D_1$ receptor antagonist BodipyFL-SKF83566 as a reporter ligand. This ligand binds to dopamine $D_1$ receptors reversibly and with high affinity. In addition, kinetic properties of the ligand were described. Association as well as dissociation of BodipyFL-SKF83566 was rapid with an apparent half-life of $t_{1/2}$ = 38.5 ± 0.3 s for association (2 nM) and $t_{1/2}$ = 73.4 ± 3.8 s for dissociation. All studied non-labeled dopaminergic ligands inhibited binding of BodipyFL-SKF83566 in a concentration-dependent manner. Obtained apparent potencies of these ligands were in good agreement with the data obtained from the radioligand [$^3$H]SCH23390 binding experiments performed with the same baculovirus preparation. These results indicate that fluorescence anisotropy based assay is applicable for studying interactions between dopamine receptors and their ligands.

References

1.) Veiksina,S., Kopanchuk,S., Rinken, A, 2014, *Biochimica et Biophysica Acta*, *1838*, 372-381.

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