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INTRINSIC ACTIVITY

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MEETING ABSTRACT

A1.7

Monitoring of ligand binding to receptors using fluorescence anisotropy-based assay

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G protein-coupled receptors (GPCRs) transduce signals into cells via guanosine nucleotide binding regulatory proteins (G proteins). As GPCRs respond to different stimuli and modulate various signal transduction pathways inside the cells, they have become important targets for treatment of many diseases. The ligand binding to the receptor is the first, but often also most crucial step in the signal transduction pathway. During the last decade, in addition to conventional radioligand binding, several fluorescence-based methods have been implemented for characterization of ligand binding to GPCRs. We have implemented fluorescence anisotropy-based assay, which allows on-line monitoring of ligand binding and obtain valuable kinetic data. The ratiometric nature of the assay requires high concentrations of receptors, which we could achieve with implementation of budded baculovirus particles, which display GPCRs on their surfaces [1]. Interpretation of the kinetic results of the non-pseudo first-order reactions is also more demanding and requires special attention. Simple one-step binding schemes and competitive reactions can be solved with analytical algorithms with several simplifications [2], while in most cases integrative global analysis is required to achieve physically meaningful kinetic parameters. We have already achieved working fluorescence anisotropy/baculovirus kinetic assays for monitoring of ligand binding to melanocortin (MC_4R) [3], neuropeptide Y (NPY_1R), serotonin ($5\text{-}HT_{1A}R$) [4], dopamine (D_1DAR) and muscarinic (M_2mAChR) receptors.

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Keywords: fluorescence anisotropy – budded baculoviruses – ligand binding – G protein-coupled receptors – kinetics

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