Visualisation of metabotropic glutamate receptor by single particle cryoEM

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Metabotropic Glutamate receptor (mGlu) belongs to the G Protein-Coupled Receptors family (GPCRs) and more specifically to class C GPCRs. Class C GPCRs that also include taste receptors, $GABA_B$ receptor and Calcium-sensing receptor, are unusual in terms of their molecular architecture and allosteric regulation. They all form obligate dimers, dimerization being fundamental for their function. mGlu receptors are composed of a large extracellular domain (ECD), that includes the Venus Fly trap domains (VFT), in which the glutamate binds and the Cystein Rich Domain (CRD) that links the VFT to the 7TM. To date, there is no structure of an intact full-length class C receptor dimer either alone or bound to a signalling complex. As a first step toward structure determination, we have thermostabilised the $mGlu_5$ receptor bounds to negative allosteric modular (NAM). Thermostabilised $mGlu_5$ receptor is fully functional and displays an increased thermal stability by 20ºC compared to the WT $mGlu_5$ receptor. Single particle electron cryomicroscopy (cryoEM) is a powerful approach for investigating its quaternary structure. However, the $mGlu_5$ receptor dimer is highly flexible and dynamic specimen and has a molecular weight of approximately 200 KDa. Here we present high quality images of $mGlu_5$ receptor dimer in ice that allowed us to visualised isolated single particle of the receptor dimer. The proportion of dimeric receptor is low, with a large proportion of what we suspect to be receptor monomers dissociating during the process of purification and or freezing. 2D classification revealed the presence of dimeric single particle of the receptor. Sample optimisation is currently on going in our lab for stabilising the receptor dimer. Understanding the structural basis of mGlu receptor dimer signalling will represent a landmark achievement and pave the way for structural investigation of GPCR dimer signalling in general. Structural information will open new avenues for structure-based drug design.