β-arrestin2 conformational fingerprints upon ligand binding to the mu-opioid receptor

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The mu-opioid receptor (MOR) is the GPCR target of commonly used analgesics, such as morphine or fentanyl. The MOR is also a prototypical GPCR where ligand-dependent differences in receptor regulation have been extensively described. Such differences have been observed at the level of receptor phosphorylation and desensitization as well as in the ability of different agonists to induce β-arrestin2 recruitment and receptor internalisation. However, it is still unclear whether, despite the differences in β-arrestin recruitment, MOR agonists are able to engender distinct β-arrestin2 conformations that result in the observed differences in receptor internalisation.

Recently, new biosensors have been developed to monitor conformational changes of β-arrestins. These biosensors were generated by fusing an energy donor at the C terminus and inserting a fluorescein arsenical hairpin (FlAsH) binding motif into different positions at the periphery of the N and C domains of β-arrestin1 and 2. Here, we used these sensors to investigate whether different ligands binding the MOR are able to induce distinct β-arrestin2 conformational fingerprints.

While all sensors were similarly recruited to the MOR upon receptor activation, they showed different sensitivities to MOR stimulation depending on the location of the acceptor moiety within the β-arrestin2 structure. These data are in agreement with a ligand-induced conformational change that leads to β-arrestin2 activation. Interestingly, when we tested MOR ligands with a wide range of intrinsic efficacies, we observed that conformational signatures could be divided according to a threshold of efficacy. Furthermore, using MOR mutants with alanine substitutions in C-tail phosphosites we determined the impact of the different phosphorylation motifs in dictating conformational changes of β-arrestin2 upon MOR activation with a high efficacy agonist. Together, these data highlight the relationship between MOR agonist efficacy and ligand-induced β-arrestin2 recruitment and conformational changes.