A genetically encoded biosensor platform for assessing GPCR dimer formation and modulation

Adam Mosswood[1,2], Cassandra J Hatzipantelis[1,3], David E Olson[1,3,4,5]*

[1]Institute for Psychedelics & Neurotherapeutics, University of California, Davis (UCD), Davis

[2]Biochemistry, Cellular, Molecular and Developmental Biology Graduate Program, UCD, Davis

[3]Department of Chemistry, UCD, Davis

[4]Center for Neuroscience, UCD, Davis

[5]Department of Biochemistry & Molecular Medicine, UCD[5], Sacramento

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***Abstract*:**

The advent of cpGFP-based conformational biosensors has enabled the reporting of receptor activation through direct conformational change, independent of downstream transducer coupling. This approach ensures rapid, pathway-agnostic detection of ligand–receptor interactions that has been harnessed for in vivo target engagement studies and high throughput screening. We sought to leverage this conformational biosensor technology to develop a modular biosensor platform capable of detecting GPCR dimerisation and dimer-induced changes in constitutive and ligand-dependent receptor activation. We engineered three cpGFP-based biosensors, psychLight (5-HT2A), GRAB5-HT (5-HT2C) and nLight (α-1A), and eight putative GPCR dimer partners (A1, 5-HT2A, 5-HT2C, KOR, CB1, mGlu2, D2 and α-1A) to create dimerLight, a system enabling concurrent optical detection of dimer formation and biosensor activation. This platform successfully yielded 24 homo- and heterodimer combinations, revealing both known and previously undescribed dimer pairs. Notably, the 5-HT2A–mGlu2 dimer (dimerLight$_{5\text{-HT2A–mGlu2}}$) selectively enhanced the maximal response to hallucinogenic 5-HT2A agonists, but not non-hallucinogenic agonists, an effect abolished when mGlu2 was replaced with a non-dimerising ΔTM4 variant. dimerLight therefore provides a genetically encoded strategy for identifying GPCR dimers and quantifying ligand-dependent, dimer-specific modulation of receptor activation. This offers a framework for dissection allosteric interactions and uncovering previously inaccessible dimensions of GPCR pharmacology.

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***Contact Information for Presenting Author**: cjhatzipantelis@ucdavis.edu*