CROSSTALK

Rebuttal from Michel Bouvier and Terence E. Hébert

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Is the jury really out?

Arguments made by Nevin Lambert and Jonathan Javitch (2014) are not terribly distant from our own positions in that we agree additional work is necessary to settle the question of GPCR dimers *in situ*, but we believe that cell-based and *in vitro* data largely support the existence of class A GPCR oligomers (see also Ferre *et al.* 2014). They argue that GPCRs are less stable than other oligomeric membrane proteins such as ion channels. Given the conformational flexibility required to serve their distinct functions in the cell, this is likely to be an important structural feature of GPCRs in the context of allosteric machines dedicated to sensing the environment and translating signalling events into cellular actions requiring interactions with many distinct effectors. Multiple, weak interfaces allow for allostery and the requirements of distinct organizational designs as needed by the cell. This would be lost in attempts to purify receptors for structural studies and probably explains why dimers are not always seen in crystal structures. This transience may not be reflected *in vivo* where receptors and signalling partners are wired into larger metastable arrays. This could explain why so few conserved interfaces have been identified in GPCR dimers. Monomeric receptors may signal when forced into proteoliposomes, but relevance to GPCR biology remains an open question.

We agree regarding the necessity in going beyond current approaches to understand the nature and role of GPCR oligomers in the cellular context. However, we maintain that measuring individual affinities between partners may be irrelevant in a larger array of signalling molecules held together by numerous interactions and may not be an improvement over current proximity-based assays. Studies of cooperativity in native systems still represent the most consistent evidence for allostery between GPCRs both in a homo- and heterodimer context. Observations made while investigating potential roles of hetero-oligomerization may be explained by crosstalk downstream of receptors despite significant efforts to exclude such effects. However, studies demonstrating that activation of one protomer can promote internalization of another unliganded partner point to the existence of hetero-oligomers (reviewed in Milligan, 2010). Studies which show transience of receptor dimers based on fluorescent ligands often ignore that negative cooperativity might report as a monomer, when simply ligand binding to dimers is affected. To exclude allostery mediated through the agency of shared G proteins, direct measures of allostery between two GPCRs in the presence and the absence of G proteins must be demonstrated (Pellissier *et al.* 2011).

Call for comments

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References

Ferre S, Casado V, Devi LA, Filizola M, Jockers R, Lohse MJ, Milligan G, Pin JP & Guitart X (2014). G protein-coupled receptor oligomerization revisited: functional and pharmacological perspectives. *Pharmacol Rev* **66**, 413–434.

Lambert NA & Javitch JA (2014). CrossTalk opposing view: Weighing the evidence for class A GPCR dimers, the jury is still out. *J Physiol* **592**, 2443–2445.

Milligan G (2010). The role of dimerisation in the cellular trafficking of G-protein-coupled receptors. *Curr Opin Pharmacol* **10**, 23–29.

Pellissier LP, Barthet G, Gaven F, Cassier E, Trinquet E, Pin JP, Marin P, Dumuis A, Bockaert J, Baneres JL & Claeysen S (2011). G protein activation by serotonin type 4 receptor dimers: evidence that turning on two protomers is more efficient. *J Biol Chem* **286**, 9985–9997.

Additional information

Competing interests

None declared.

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